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

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ABSTRACT

Invasions by the spionid polychaete *Polydora websteri* have resulted in the collapse of oyster aquaculture industries in Australia, New Zealand, and Hawaii. These worms burrow into the shells of bivalves, 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 mud 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 [COI]) 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 cryptogenic species in Washington State. The presence of this notorious invader could threaten the sustainability of oyster aquaculture in Washington, which currently produces more farmed bivalve shellfish than any other US state.

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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" (Lauckner 1983), which bores into the shells of molluscs (Blake 1969). By creating unsightly blisters on the shells of their commercially important hosts, these pests have led to significant economic losses for shellfish aquaculture worldwide (Shinn et al. 2015). *P. websteri* can infest a variety of mollusc hosts (see reviews in Blake and Evans 1973, Martin and Britayev 1998, Simon and Sato-Okoshi, 2015), including oysters (Royer et al. 2006, Ogburn et al. 2007, Read 2010, Rice et al. 2018), mussels (Kent 1979, 1981, Read and Handley 2004), scallops (Evans 1969, Bergman et al. 1982, Mori et al. 1985), and abalone (Hahn 1989).

P. websteri and related polydorids 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 (Whitelegge 1890, Roughley 1922, 1925, Nell 2007, Ogburn et al. 2007). When oyster transplants from Kaneohe Bay brought *P. websteri* to Kakuku, Hawaii, the introduction caused extensive damage to shellfish production (Bailey-Brock and Ringwood 1982, Bailey-Brock 1987). 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 (Lunz 1940, 1941, Loosanoff and Engle 1943). In addition, high mortalities of the Japanese scallop *Patinopecten yessoensis* in British Columbia, Canada were attributed to *P. websteri* (Bower et al. 1992). These examples attest to the ability of

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P. websteri to successfully invade new locations and, once established, to significantly alter 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 (Blake 1969, Nell 2007, Clements et al. 2018). The worm then forms a U-shaped burrow with two exterior openings (Loosanoff and Engle 1943, Hopkins 1958). 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 (Korringa 1954, Haigler 1969, Blake and Evans 1973, Zottoli and Carriker 1974, Rice et al. 2018). 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 (Haigler 1969, Handley and Bergquist 1997). Blisters can be irregular in shape and darkly colored, compromising the presentation of oysters served on the half-shell (Morse et al. 2015). Moreover, if a blister is nicked during oyster shucking, the mud and feces will foul the oyster meat, rendering it inedible (Shinn et al. 2015). 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 (Sato-Okoshi et al. 2017). 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 (Buschbaum et al. 2007). Pacific oysters (*Crassostrea gigas*) infested by

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the polydorids *P. hoplura*, *P. cornuta,* and *Boccardia semibranchiata* grow more slowly and have poorer body condition than do uninfested oysters (Chambon et al. 2007). Glycogen, protein, and lipid content relative to the shell cavity volume are lower in infested compared to uninfested *Crassostrea* spp. oysters (Lunz 1940, Wargo and Ford 1993, Royer et al. 2006). Additionally, polydorids have been shown to increase mortality rates in Pacific oysters that are heavily infested (Owen 1957, Dinamani 1986). These negative effects on growth and survivorship may be caused by the energetic demands of worm-induced nacre production (Kojima and Imajima 1982, Wargo and Ford 1993, Lleonart et al. 2003, Royer et al. 2006, Simon 2011, Boonzaaier et al. 2014); 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 (Handley 1998). Given these impacts on host vital rates, *P. websteri* outbreaks may affect more than just the bottom line of the shellfish industry; they may also compromise the important ecosystem services provided by filter-feeding shellfish species (Coen et al. 2007).

Polydora websteri has been reported from locations all over the world (see reviews in Ruellet 2004, Walker 2011, 2013), but due to its complex taxonomic history (see Radashevsky and Williams 1998, Radashevsky 1999, ICZN 2001), many records remain to be confirmed. Some historical reports of *P. ciliata* (a non-burrowing species) have been re-identified as other, burrowing polydorid species, including *P. websteri* (Read 2010), and more such erroneous historical reports might exist. *P. 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 (Rice et al. 2018). Although *P. websteri* has been predicted to be present in Washington, USA (Hobson and Banse

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1981) based on records of its presence north in British Columbia (Bower 1990, Bower et al. 1992) and south in Oregon and California (e.g., Hartman 1954, 1961, 1969, Blake 1996), it has never before been described from Washington. This 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 (Washington Sea Grant 2015). Of Washington State's cultured shellfish production, Pacific oysters (*Crassostrea gigas*) contribute 38% by weight and 38% by revenue (Washington Sea Grant 2015). Pacific oysters are also culturally important to local communities, Native American tribes, family-owned farms, and recreational farmers and collectors (Washington Shellfish Initiative 2016). 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 (Washington Sea Grant 2015). 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 mud blisters on the valves of Pacific oysters (*Crassostrea gigas*) grown in Puget Sound (Figure 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. Worms were recovered from shell blisters and burrows, and identified to species using morphological traits, as well as mitochondrial COI and nuclear 18S gene

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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.

METHODS

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", Figure 2, Table 1), and 114 individuals were from Totten Inlet (47° 9' 43.09", -122° 59' 19.62", Figure 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 (Washington Sea Grant 2015). 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 tissue was removed. We observed right and left valves under a stereo-microscope 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

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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).

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.

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 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).

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 (Radashevsky

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and Pankova 2006, Rice et al. 2008, Sato-Okoshi and Abe 2013). A more fruitful approach is through nuclear 18S rRNA analysis (Williams et al. 2017). We followed the protocol of Williams et al. (2017) 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 and the mitochondrial cytochrome c oxidase I [COI]. For the 18S rRNA gene, three regions were amplified: 18S-1F1/18S-1R632, 18S-2F576/18S-2R1209, and 18S-3F1129/18S-R1172 (Nishitani et al. 2012). For COI, we amplified one region: Dorid_COI.3F/Dorid_COI.1R (Williams et al. 2017). The expected length of the fragments is between 680 and 780 bp.

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). COI mtDNA 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

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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 and COI genes using Geneious (version 11.0.5). Initially, the majority of 18S rRNA sequences were 660 bp in length. We manually reduced the sequence alignment to 615 bp for analysis. Only the first of the three regions for 18S (18S-1F1/18S-1R632) was used for analysis because the other two were not well resolved. COI sequences were initially 680 bp in length and were manually reduced to 540 bp for analysis. After sequences were trimmed, we aligned partial sequences of 18S and COI genes with sequences of related species from the *Polydora* and *Boccardiella* genera obtained from GenBank. We reconstructed phylogenetic trees using the maximum-likelihood method based on Tamura 3-parameter model in Molecular Evolutionary Genetics Analysis software (MEGA version 7.0.26), using *Pseudopolydora dayii* (KY677807) as an outgroup.

RESULTS

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.

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Morphological identification

Specimens from both Washington (Figure 3) and New York (Figure 4) matched the taxonomically important features of *Polydora websteri* in the original description (Hartman in Loosanoff and Engle 1943), redescription (Radashevsky 1999) and more recent reports (Read 2010, Sato-Okoshi et al. 2013, Ye et al. 2017, Rice et al. 2018; 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).

Molecular identification

Most worms collected from Oakland Bay were identified as *P. websteri* from their DNA sequences (Table 1). 18S sequences of 12 worms from Oakland Bay clustered with *P. websteri* sequences obtained from GenBank (Figure 5). Eight of these worms also clustered with *P. websteri* in the COI tree (Figure 6, Table 1). Worms collected from Totten Inlet were more diverse and formed a separate branch from Oakland Bay for both 18S rRNA and COI genes. Eleven 18S rRNA sequences from Totten Inlet formed a close sister group to *P. websteri* sequence entries obtained from GenBank (Figure 5). Two individuals from Totten Inlet (TI 13 and 14) clustered separately from all known species in both 18S and COI (Figures 5 and 6). In summary, both 18S and COI 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 2.

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DISCUSSION

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 polydorid species may also be present in local oysters. This work indicates that Washington State waters host several cryptogenic, shell-boring polydorid parasites, all of which may pose a danger to the region's valuable oyster aquaculture industry.

Nearly all worms from Oakland Bay and the majority of worms from Totten Inlet were molecularly identified as *P. websteri* based on 18S and COI (Table 1). Based on detailed morphological analysis, specimens of *P. websteri* from Washington (Figure 3) matched previous descriptions and the newly collected material from near the type locality (Figure 4); the same specimens that we morphologically identified were also sequenced, and morphological and molecular diagnoses agreed (Table 1). We therefore confirm the presence of *P. websteri* 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. Extensive exchange of shell and live oysters among oyster-growing regions continues to the present day, and to such an extent that *P. websteri* populations are genetically homogenous across broad swathes of their

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contemporary range (Rice et al. 2018). Washington State has a long history of exchange with other oyster-growing regions (Fofonoff et al. 2003) and polydorid pelagic larvae may also have been introduced through ballast water (Carlton and Geller 1993; Drake and Lodge 2004). Although it is likely that *P. websteri* is native to Asia and exotic to North America (Rice et al. 2018), we suggest that *P. websteri* be considered cryptogenic in Washington State (Carlton 1996) until further research can resolve its origins. It is possible that the species is native to Washington and that it has never before been described because it was present only at very low prevalence until recently. The prevalence of *P. websteri* is sensitive to environmental change. For example, increasing siltation can increase the susceptibility of *Crassostrea virginica* to *P. websteri* (Clements et al. 2017a). In contrast, reducing pH actually decreases susceptibility to infestation (Clements et al. 2017b). Because *P. websteri* can recruit to both live and dead oyster shells (Clements et al. 2018), the expansion of the oyster aquaculture industry, oyster restoration, and increased density of oysters in beds across the state might have promoted an increase in transmission and prevalence. Whatever their origin, the blister-forming polychaetes we document here are a new challenge for Washington State oyster growers and the government agencies charged with management of shellfish stocks.

Because *P. websteri* is a generalist pest (Korringa 1954, Haigler 1969, Read 2010), it may impact other shellfish species of ecological, economic, and cultural importance to Washington State. An important example is the Olympia oyster (*Ostrea lurida)*, an overexploited native species that is the focus of intensive restoration efforts (White et al. 2009). Mussels (Kent 1979, 1981, Read and Handley 2004), scallops (Evans 1969, Bergman et al. 1982, Mori et al. 1985), and abalone (Hahn 1989; see review in Blake and Evans 1973) are also at risk. Given the

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important ecosystem services provided by filter-feeding shellfish species (Coen et al. 2007), a polydorid outbreak could affect more than just the bottom line of the shellfish industry; ecosystem function is also at risk.

In addition to *P. websteri,* our data suggest the presence of other unidentified polydorids. Polydorids have a long history of being misidentified, because the morphological differences between some species are subtle or even absent (Radashevsky and Pankova 2006, Read 2010). For example, Rice et al. (2008) 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* and *P. cornuta,* 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 polydorids were not developed until recently (Williams et al. 2017), so reference material may soon be available for resolving this phylogeny.

In this work, we positively identify the notorious shell-boring polydorid, *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

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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.

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Tables

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

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	-	Oakland Bay 1	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	-	Oakland Bay 2				-	MH891521	MK188731
Polydora websteri	-	Oakland Bay 3				-	MH891520	MK188732
Polydora websteri	-	Oakland Bay 4				-	MH891519	MK188733
Polydora websteri	-	Oakland Bay 5				-	MH891517	MK188734
Polydora websteri	-	Oakland Bay 6				-	MH891514	-
Polydora websteri	-	Oakland Bay 7				-	MH891515	MK188735
Polydora websteri	-	Oakland Bay 8				-	MH891516	MK188736
Polydora websteri	-	Oakland Bay 9				-	MH891513	-
Polydora websteri	*Polydora websteri*	-			15 Aug 2018	H1-61-1(SEM)	MK695999	-
Polydora websteri	-	-				H1-61-3(EtOH)	-	-
Polydora websteri		-				H1-61-4(SEM)	-	-
Polydora websteri	*Polydora websteri*	Oakland Bay 10				H1-61-5(EtOH)	MK696002	-
Polydora websteri	*Polydora websteri*	Oakland Bay 11/ Oakland Bay 9 in COI				H1-61-6(SEM)	MK696000	MK696586
Polydora websteri	*Polydora websteri*	Oakland Bay 12/ Oakland Bay 10 in COI				H1-61-7(SEM)	MK696001	MK696587
Polydora websteri	*Polydora websteri*	Oakland Bay 13/ Oakland Bay 13 in COI				H1-61-8(SEM)	MK696003	MK696588
Polydora websteri	-	-				H1-61-9(SEM)	-	-
Polydora websteri	-	Totten Inlet 1	Totten Inlet, Washington State, USA; from shells of *Crassostrea gigas*	47° 9' 43.09", –122° 59' 19.62"	18 Sep 2017	-	MH891524	MK188738
Polydora websteri	-	Totten Inlet 2				-	MH891525	MK188739
Polydora websteri	-	Totten Inlet 3				-	MH891527	MK188740
Polydora websteri	-	Totten Inlet 4				-	MH891530	-
Polydora websteri	-	Totten Inlet 5				-	MH891528	-
Polydora websteri	-	Totten Inlet 6				-	MH891536	-

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Polydora websteri	-	Totten Inlet 7				-	MH891534	-
Polydora websteri	-	Totten Inlet 8				-	MH891531	-
Polydora websteri	-	Totten Inlet 9				-	MH891532	-
Polydora websteri	-	Totten Inlet 10				-	MH891523	-
Polydora websteri	-	Totten Inlet 11				-	MH891535	-
Polydora websteri	*Polydora websteri*	-	North Sea Harbor, Long Island, New York, USA; from shells of *Crassostrea virginica*	40° 56' 24.13"N, 72° 25' 3.97"W	12 Sep 2018	P1-109-2a, b	MK369933	MK696582
Polydora websteri	*Polydora websteri*	-				P1-109-3a, b	MK369934	MK696583
Polydora websteri	*Polydora websteri*	-				P1-109-4a, b	MK369935	MK696584
Polydora websteri	*Polydora websteri*	-				P1-109-5a, b	MK369936	MK696585

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Table 2. Sequences alignment statistics for 18S and COI sequences. Values were calculated using MEGA7.0.26.

Variable	18S	COI
Sample size	27	17
Final length of aligned sequences	615	540
No. samples from Oakland Bay	13	11
No. nucleotides Oakland Bay	1768	600
No. variable nucleotides Oakland Bay	22/1768	185/660
No. samples from Totten Inlet	14	6
No. nucleotides Totten Inlet	1768	660
No. variable nucleotides Totten Inlet	22/1768	185/660
Haplotype diversity	0.009	0.135
Trasitions/Transversions	1.02	1.31

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1 **Figures**

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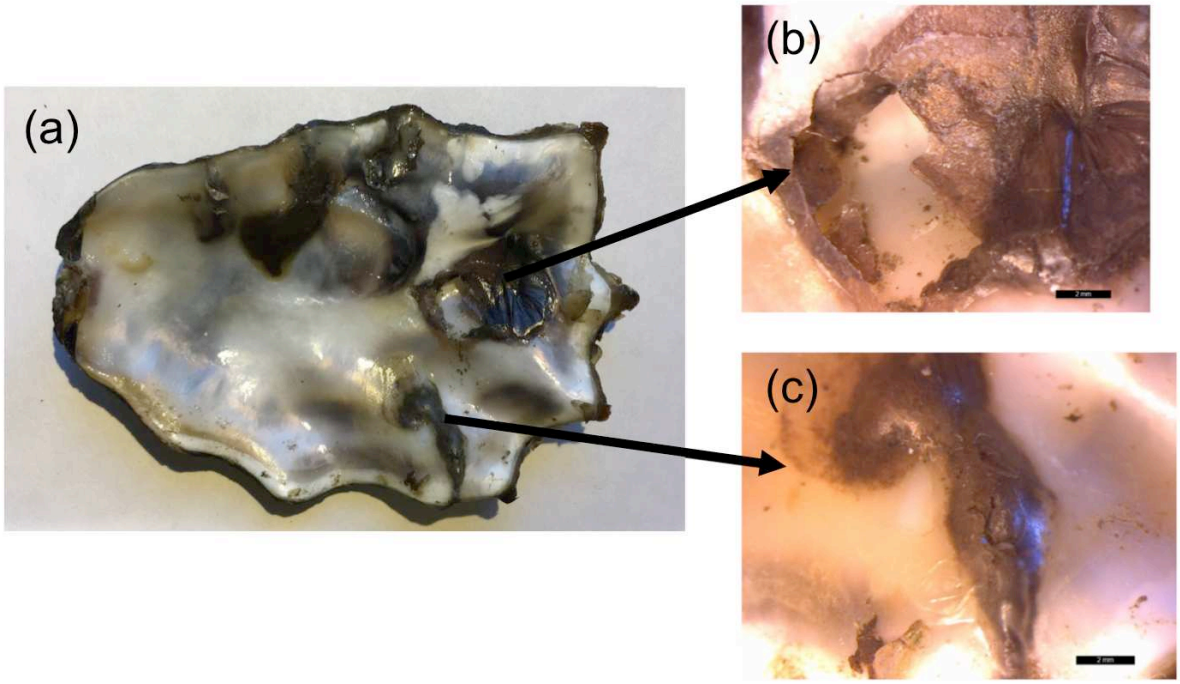

4

5 ***Figure 1***. Infested *Crassostrea gigas* collected from Oakland Bay, WA. Pathology shown is
6 associated with shell-boring mud worms. (a) Inner surface of an infested valve, (b) opened mud
7 blister, and (c) closed mud blister filled with mud, detritus, and worm feces. In (b) and (c), scale
8 bar indicates 2 mm.

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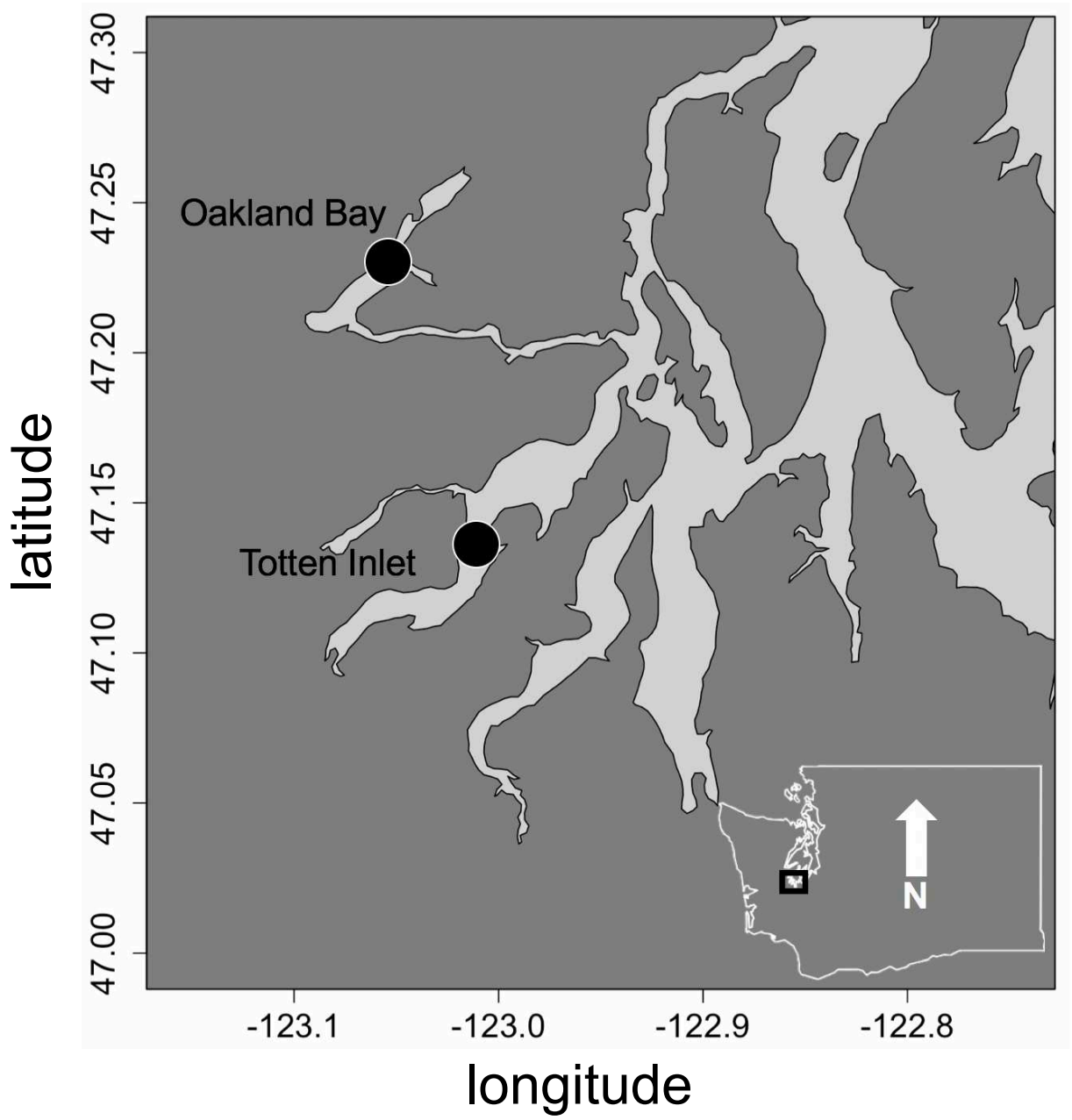

Figure 2. Map of sampling sites in Southern Puget Sound, Washington state. Oysters were grown in Oakland Bay (n = 72) and Totten Inlet (n = 114).

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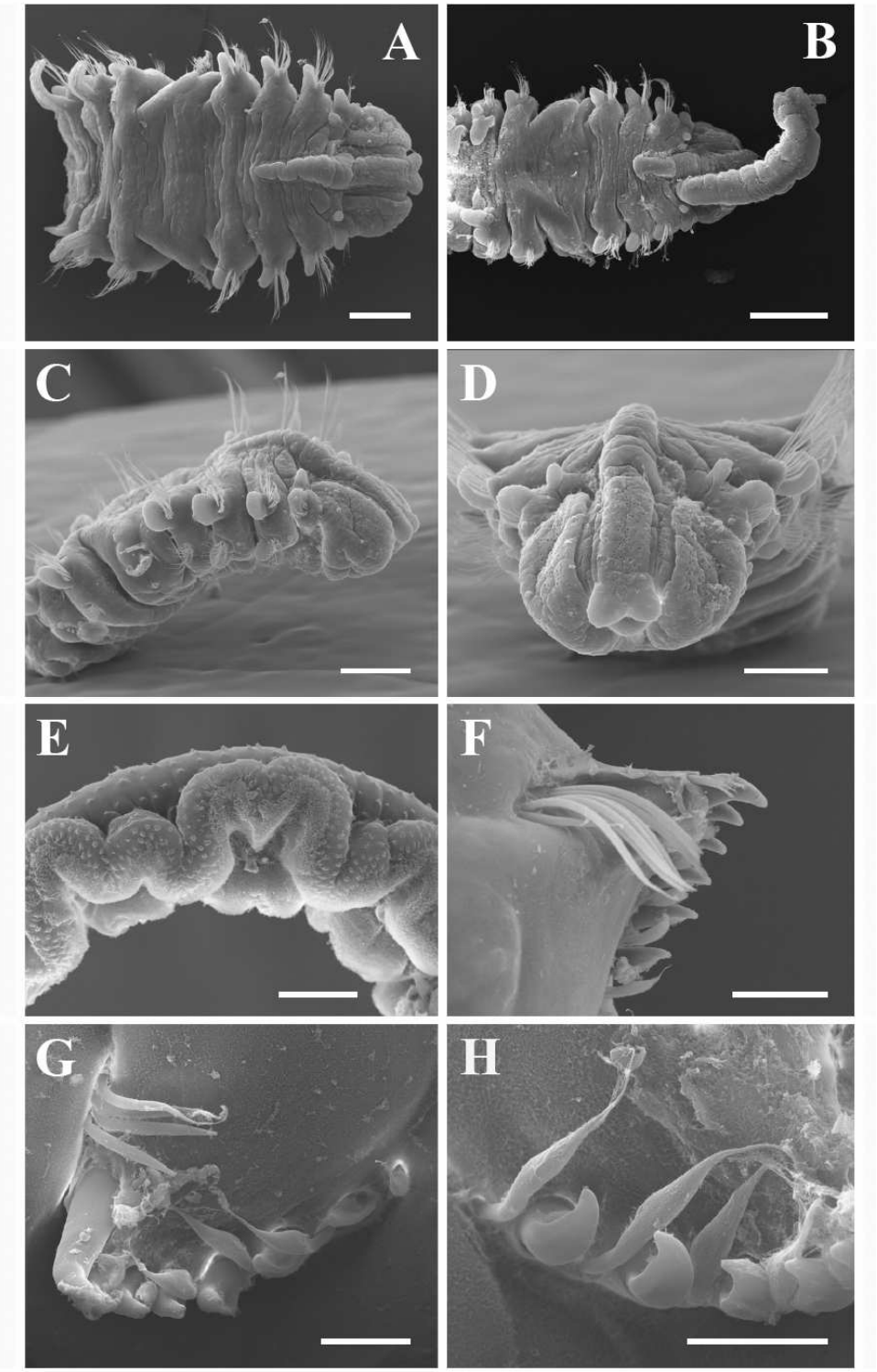

Figure 3. *Polydora websteri* from Oakland Bay, Washington extracted from *Crassostrea gigas*. (A) Anterior dorsal view of specimen lacking palps (USNM 000000 H3-61-8). (B) Anterior dorsal view of specimen with basal portion of right palp attached (USNM 000000 H3-61-4). (C)

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Anterior, right lateral view, same specimen as in A (USNM 000000 H3-61-8). (D) *En face* view of specimen showing anterior end of prostomium, same specimen as in A (USNM 000000 H3-61-8). (E) Lateral view of middle portion of palp, palp removed from specimen shown in B (USNM 000000 H3-61-8). (F) Dorsal view of fifth chaetiger spines (USNM 000000 H3-61-1). (G) Dorsal view of fifth chaetiger spines, same specimen as in B (USNM 000000 H3-61-4). (H) Lateral view of fifth chaetiger spines, close-up, same specimen as in B (USNM 000000 H3-61-4). Scale bars A-C = 250 µm, D = 200 µm, E = 100 µm, F = 50 µm, G, H = 25 µm.

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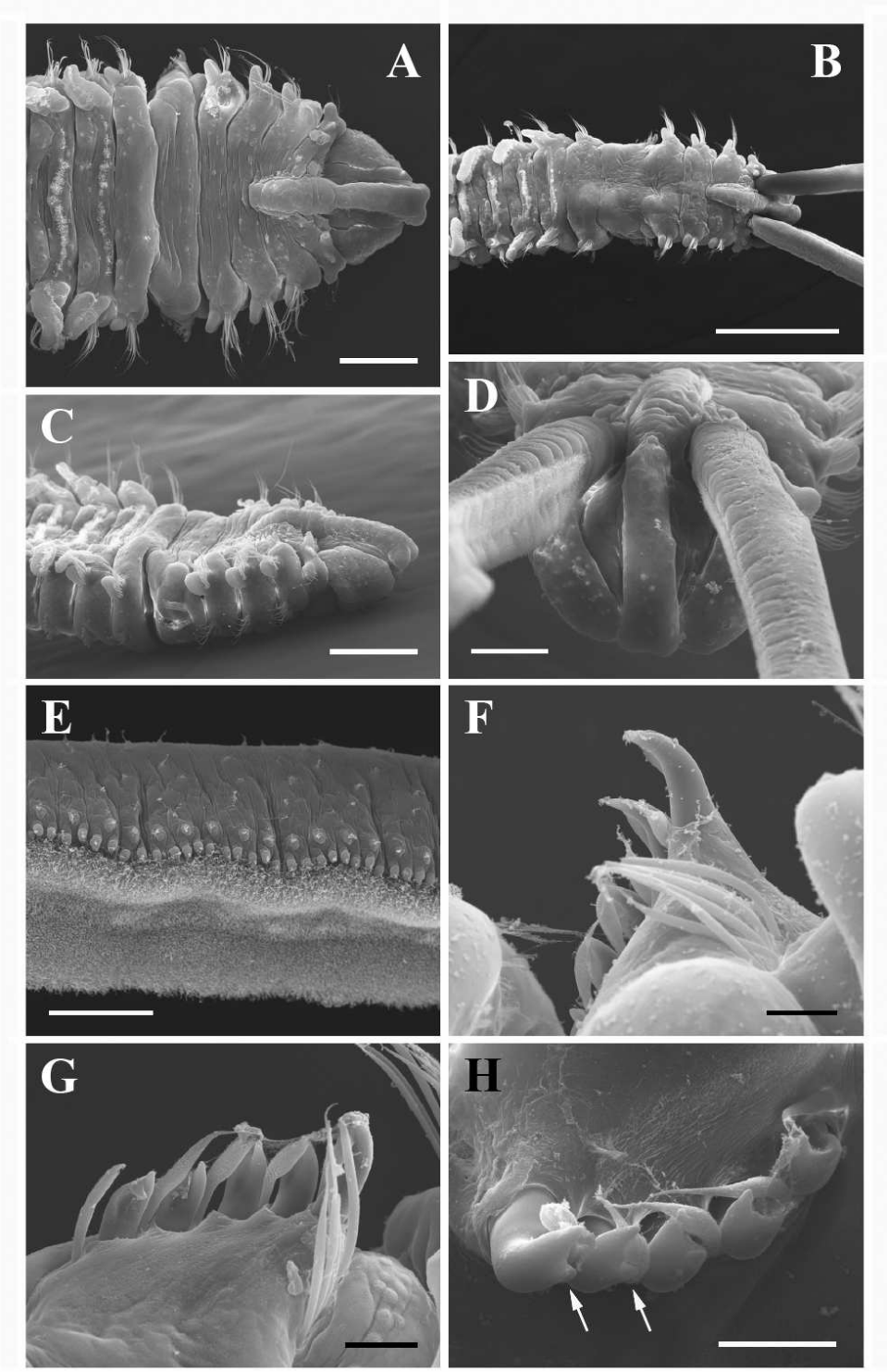

Figure 4. *Polydora websteri* from Long Island, New York extracted from *Crassostrea virginica*. (A) Anterior dorsal view of specimen lacking palps (USNM 000000 P1-109-2a). (B) Anterior dorsal view of specimen with palps (USNM 000000 P1-109-3a). (C) Anterior, right lateral view, same specimen as in A (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

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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, G, H = 25 µm.

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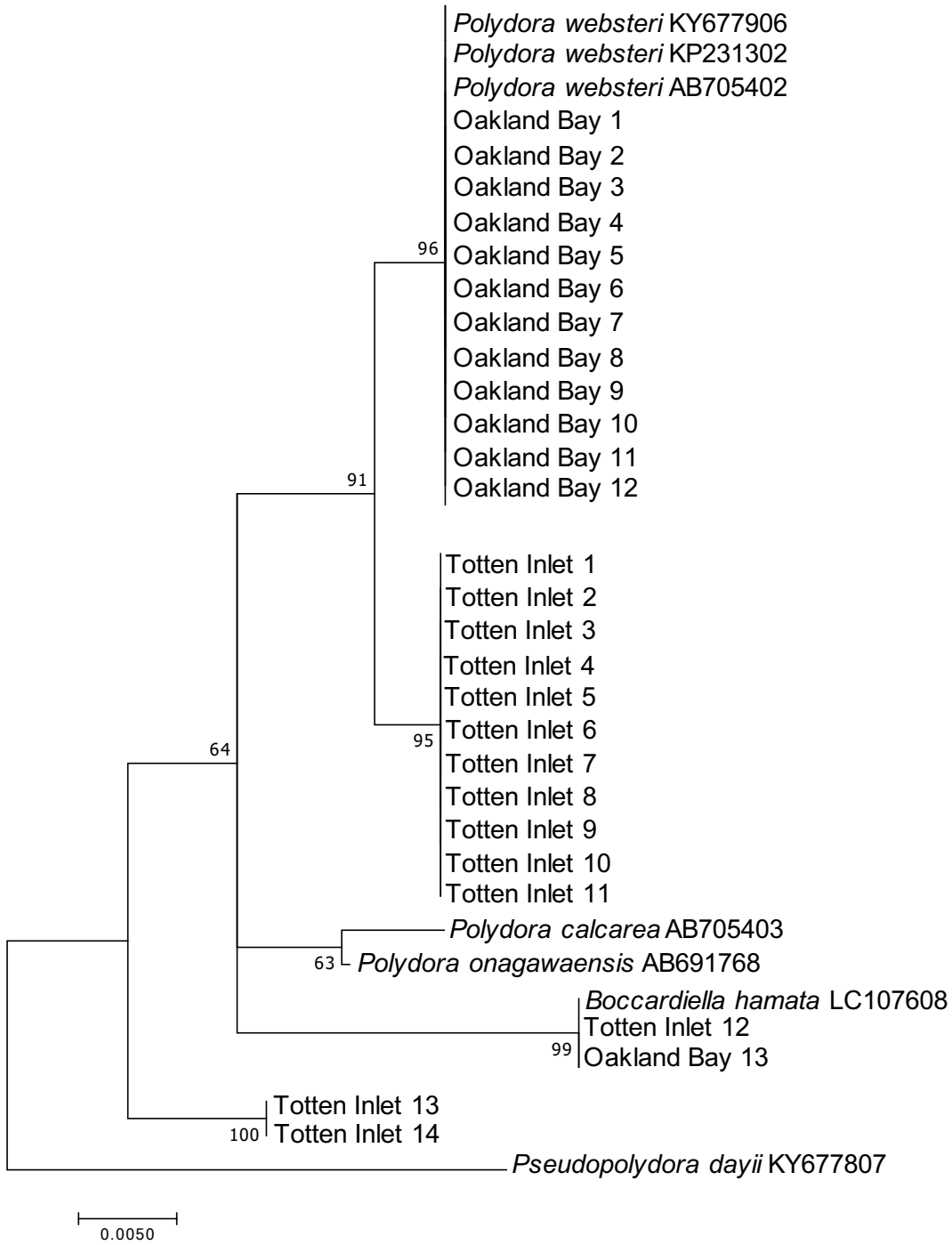

Figure 5. Maximum likelihood phylogeny based on Tamura 3-parameter distances using trimmed 18S1 rRNA sequences (500 replicates). *Pseudopolydora dayii* (KY677807) was used as an outgroup. Entries accompanied with accession number were acquired from GenBank.

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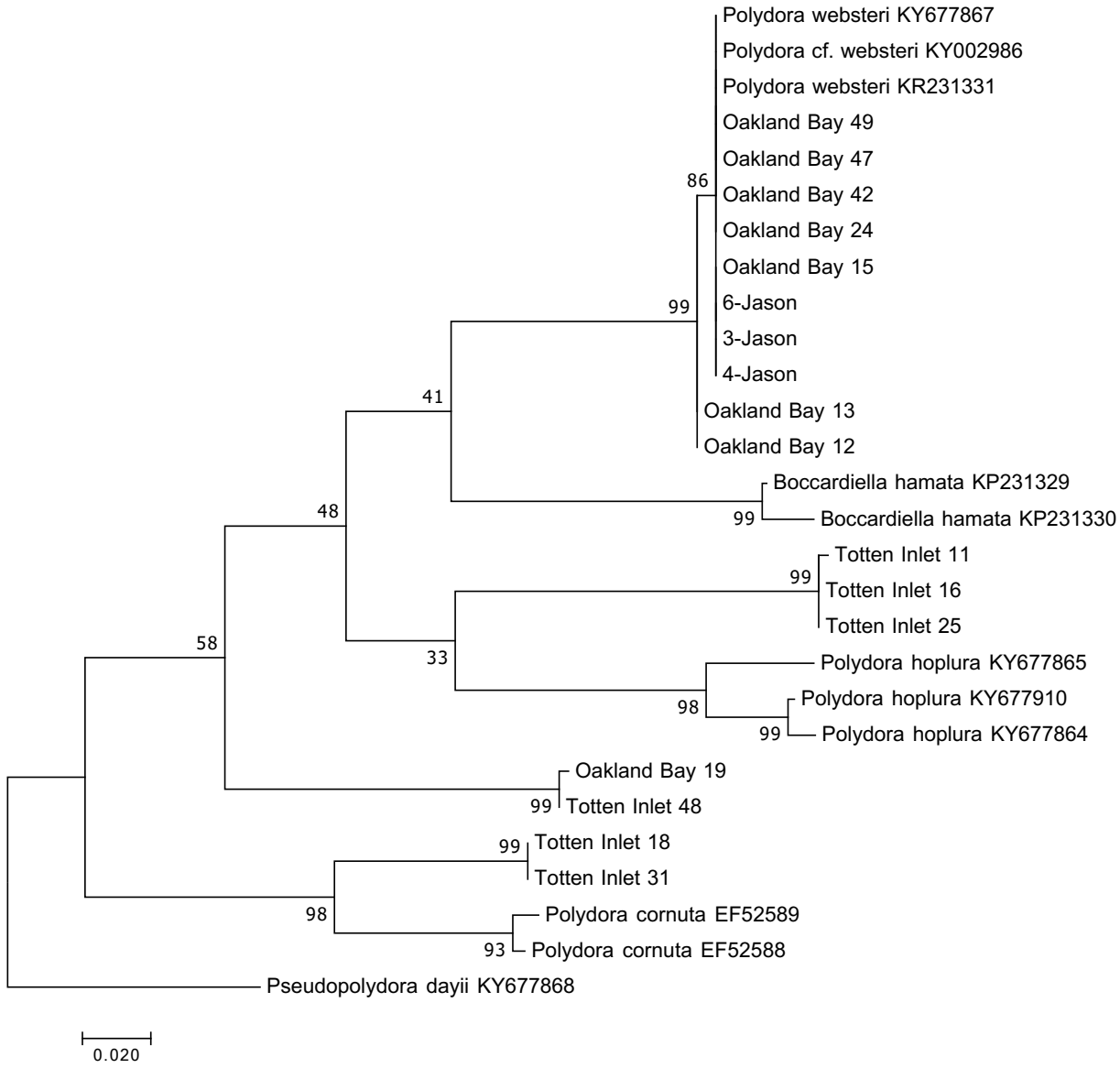

Figure 6. Maximum likelihood phylogeny based on Tamura 3-parameter method using trimmed COI sequences (500 replicates). Phylogeny consists of nine sequenced worms from Oakland Bay, WA, and six sequenced worms from Totten Inlet, WA (bold text). *Pseudopolydora dayii* (KY677868) was used as an outgroup. Entries accompanied with accession number were acquired from GenBank.

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