Polydorid species (Annelida: Spionidae) associated with commercially important oyster shells and their shell infestation along the coast of Normandy, in the English Channel, France

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Abstract

Polydorid species (Annelida, Spionidae), which inhabit the shells of the commercially important oyster *Crassostrea gigas*, were investigated along the coast of Normandy, France. Eight species, including five new records for Normandy (*Polydora onagawaensis*, *Polydora websteri*, *Boccardia pseudonatrix*, *Boccardia proboscidea*, and *Boccardiella hamata*) and two first records in European waters (*P. onagawaensis* and *B. pseudonatrix*), were identified based on morphological, molecular biological, and ecological characteristics. *Polydora onagawaensis*, which belongs to the *Polydora ciliata/websteri* complex, was discovered in the shells of wild and suspended cultured oysters, as well as in limestone substrates. In the phylogenetic analysis of mitochondrial COI gene sequences, specimens of *P. onagawaensis* collected from Normandy were grouped together with specimens from the USA into a single clade and were distinguished from the other three lineages that comprised Japanese and USA specimens. *Polydora websteri* inhabited shells of suspended cultured oysters. *Polydora hoplura*, *Dipolydora giardi*, and *Dipolydora* sp. were observed in shells from the sandy oyster culture grounds. *Boccardiella hamata* has been found in wild oyster shells from muddy oyster culture grounds. *Boccardia pseudonatrix* was observed in the shells of both the wild and cultured oysters. Adult and juvenile *Boccardia proboscidea* were observed in coralline algae, as well as in suspended cultured oysters. Mud tubes were observed to protrude from the outer surface of the shells, and abnormal black and calcareous deposits were secreted on the inner surface of the shells against polydorid penetration.

Keywords *Crassostrea gigas* · Shell infestation · *Polydora* · Annelida · Normandy · English Channel

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Introduction

The family Spionidae Grube, 1850, is found in a wide variety of marine environments worldwide. It is one of the most abundant polychaete groups in Annelida in terms of the number of species and biomass. Within the family Spionidae, the so-called polydorid species comprises nine genera: *Polydora* Bosc, 1802; *Dipolydora* Verrill, 1881; *Pseudopolydora* Czerniavsky, 1881; *Boccardia* Carazzi, 1893; *Polydorella* Augener, 1914; *Tripolydora* Woodwick, 1964; *Boccardiella* Blake & Kudenov, 1978; *Carazziella* Blake & Kudenov, 1978; and *Amphipolydora* Blake, 1983; each of them has the common morphological characteristics of specially modified major spines in the fifth chaetigerous segment (Blake 1996). They are widely distributed in coastal benthic environment. A variety of habitat types are observed depending on the species, that is, some species are known to create mud tubes in the bottom sediment, utilize sand or mud deposits in crevices of various substrates (e.g., rocks and mollusk shells), to inhabit the surface of various organisms (e.g., sponges, brachiopods, and mollusk shells), inside of various organisms (e.g., calcareous algae and sponges), and some excavate burrows in calcareous substrates, such as limestone, mollusk shells, and corals (Blake and Evans 1973; Simon and Sato-Okoshi 2015; Abe et al. 2019a; Abe and Sato-Okoshi 2020). Many have been reported to have symbiotic associations with other marine benthic invertebrates (Martin and Britayev 1998; 2018), and new symbiotic relationships continue to be discovered (Abe and Sato-Okoshi 2020; Abe et al. 2022). Polydorids feed on suspended organic particles or deposited organic matter by exposing a pair of long palps from mud tubes (Dauer et al. 1981). In cases where symbiotic relationships have been observed, it is assumed that they compete with their hosts for food (Abe and Sato-Okoshi 2020).

Some genera in polydorids have long been well known and reported, especially in fisheries (Takahashi 1937; Evans 1969; Kent 1979; Okoshi and Sato-Okoshi 1996; Mortensen et al. 2000; Lleonart et al. 2003; Simon et al. 2006; Sato-Okoshi et al. 2008). For species inhabiting the shells of commercially important mollusk species, whether they excavate the shells (boring) or just inhabit the shells as interstitial or epifaunal (non-boring), a significant infestation can negatively affect the host shells. They often reduce the commercial value of mollusks by damaging their shells, decreasing the growth rate and meat yield, and causing heavy mortality in some cases (Okoshi and Sato-Okoshi 1996; Sato-Okoshi et al. 2008; 2012; 2013; Simon and Sato-Okoshi 2015). With the globalization of the aquaculture industry, commercially important mollusks are constantly transported between countries. Some mollusk-associated organisms, such as boring and non-boring polydorid species, are unintentionally introduced to newer, non-native regions. (Elton 1958; Carlton 1975; Bailey-Brock 2000; Sato-Okoshi et al. 2008; Simon and Sato-Okoshi 2015). Therefore, polydorids have established invasive populations in the waters where they have been introduced (Sato-Okoshi et al. 2012). For species that cause damage to cultured shells, it is necessary to determine the species accurately and suggest preventive measures or control such infestations (Diggles et al. 2002; Simon et al. 2010), but this problem often remains unresolved. Moreover, these unintentionally introduced species are not only a source of economic concern but may also pose a threat ecologically as they can infest indigenous mollusk species in their invasive ranges if they disperse from aquaculture facilities (Elton 1958; Carlton 1975; Cohen and Carlton 1998; Bailey-Brock 2000). Ecological disturbances caused by invasive organisms associated with cultured mollusks, such as oysters, are currently a major cause of diversity losses worldwide (Mack et al. 2000; Miura 2007).

The similarity of morphological characteristics in polydorids has led to confusion in species identification (Sato-Okoshi 1999; 2000; Sato-Okoshi and Takatsuka 2001; Radashevsky and Pankova 2006; 2013; Read 2010; Sato-Okoshi and Abe 2012; 2013). Several species complexes within the polydorid group are morphologically indistinguishable, whereas others show high intraspecific morphological variations. Some species previously thought to be the same include more than one species (Abe et al. 2016; Kondoh et al. 2017; Simon et al. 2019), and cases have been reported in which two species thought to be different were the same (Sato-Okoshi et al. 2017; Malan et al. 2020). Thus, the identification of these species, based on morphology, is often difficult and complex. Accurate species identification is necessary to elucidate the means by which they are transported and to assess the severity of their impact as exotic invasive species.

The Japanese or Pacific oyster *Crassostrea gigas* (Thunberg, 1793) was introduced in European countries owing to the decline in native populations of the European flat oyster *Ostrea edulis* Linnaeus, 1758 (Robert et al. 1991) and the Portuguese oyster *Crassostrea angulata* (Lamarck, 1819) (Grizel and Héral 1991). In France, oyster production is thriving, and oysters have been cultured for many years. *Crassostrea gigas* oysters have been exported from Japan since 1880. After the disease of *C. angulata* (1969–1971), large numbers of juvenile (spat) and adult *C. gigas* were introduced into France at the beginning of 1970, coming directly from Japan and also from British Colombia and Canada (Grizel and Héral 1991). Many polydorid species probably accompanied such voluntary introduction and have since been reported in cultured shells (Ruellet 2004; Royer et al. 2006). Polydorids are not the only annelids to be transported with cultured mollusks. Recently, *Marphysa victori* (Eunicidae) was reported to have accompanied oysters to France (Abe et al. 2019b; Lavesque et al. 2020).

Successive inventories have reported polydorids in the English Channel (Fauvel 1927; Dauvin et al. 2003; Ruellet 2004; Gully and Cochu 2020; Le Mao et al. 2020). In recent years, it has become necessary to distinguish the species of morphologically similar polydorids, especially the so-called *Polydora ciliata/websteri* complex (Blake 1996); however, this has not yet been sufficiently re-examined in France. Therefore, species identification using molecular biological methods as well as morphology is required in this region. In this study, we attempted to identify the polydorid species inhabiting commercially important oyster shells along the coast of Normandy using both morphology and molecular biology. Shell infestation by polydorids was investigated not only in oyster shells but also in other calcareous substrates from the Normandy intertidal zone in March 2018. Here, we report the species and their habitat conditions of polydorids 50 years after the mass introduction of Pacific oysters into France, which began in the 1970s.

Materials and methods

Sampling of calcareous substrates and polydorid species

Sampling was conducted along the coast of Normandy in the English Channel from March 19 to 21, 2018 (Fig. 1). *Crassostrea gigas* oysters were collected from four oyster culture sites: Blainville-sur-Mer (sandy oyster culture ground dotted with rocks together) from the western coast of Cotentin, Saint-Vaast-La-Hougue (muddy oyster culture ground), Quineville (suspended culture) from the eastern coast of Cotentin, and Asnelles (suspended culture) along the Calvados coast. In Normandy, which is currently
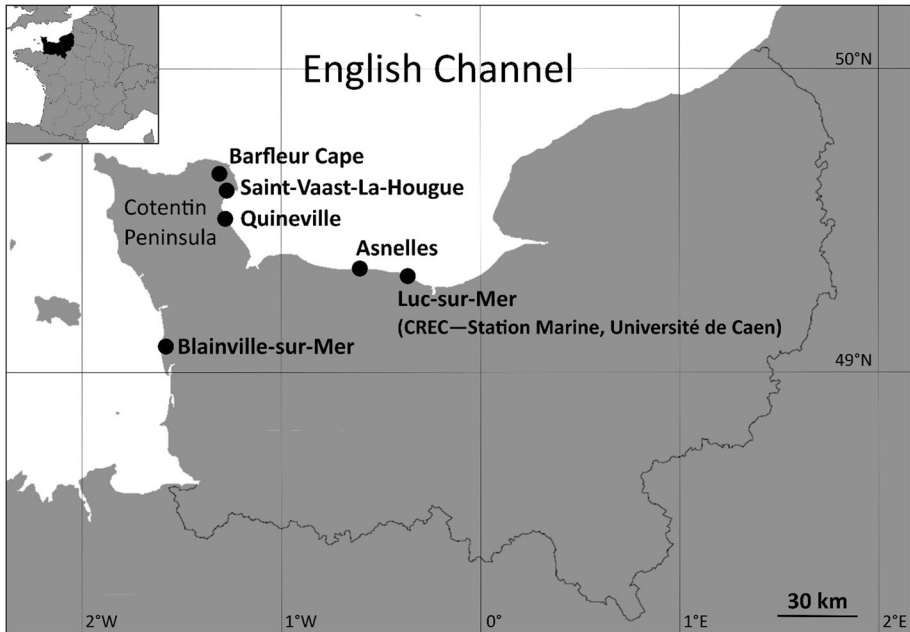
Fig. 1 Map of sampling sites along the coast of Normandy, northern France, in the English Channel

the most important center of oyster production in France, there has been a rapid increase in the number of oyster farms just after the introduction of the Japanese oyster *C. gigas*, in two main places of the Cotentin Peninsula: Blainville-sur-Mer on the western coast and Saint-Vaast-La-Hougue on the eastern coast. Blainville-sur-Mer has an extended intertidal zone of up to 5 km at low tide during spring tide. Here, the oyster farms were located between 1 and 3 km from the seashore, and the tables were placed on heterogeneous sediment from sand to gravel. Saint-Vaast-La-Hougue is another important hot spot for oyster production in Normandy, with a moderate intertidal zone of up to 2 km, on muddy and sandy sediments, and the tables occupy the entire intertidal zone. Twenty cultured oysters that fell out of the net bags and 20 wild oysters that were attached to rocks in Blainville-sur-Mer were randomly collected during the March spring tide. Twenty wild oysters from muddy oyster beds in Saint-Vaast-La-Hougue were randomly collected at sites during the March spring tide. Twenty cultured oysters each, from Quineville and Asnelles, were obtained randomly from harvested ones cultured in cages suspended in the intertidal zone. Additional samples were 20 harvested scallops *Pecten maximus* collected by dredging near Asnelles. Calcareous algae and clumps of limestone and concrete blocks covered with barnacles and mussels were also sampled from intertidal zones in Barfleur Cape, northeast Cotentin, and Luc-sur-Mer (Calvados), respectively, to identify polydorid species that inhabit wild calcareous substrates.

Polydorid species that bore into or inhabit the shells, limestone, and coralline algae were extracted by fracturing calcareous substrates with cutting pliers. Five to thirty individuals of each species of extracted worms were examined to determine their morphological characteristics, state of sexual maturity, and presence and conditions of oocytes and egg capsules under a stereomicroscope (SZX16, Olympus, Tokyo, Japan) and a biological microscope (BX53F, Olympus, Tokyo, Japan), in live individuals and specimens fixed in 10%

formalin in seawater. For molecular analysis, one–eight worms per species were directly preserved in 99.5% ethanol.

Shell infestation was investigated by observing outer and inner shell surfaces and taking photographs using a digital camera (Tough TG-5, Olympus, Tokyo, Japan). Abnormal shell formation, along with the presence of black organic or calcareous deposits, indicates worm infestation. Worms, if present, were extracted from such shells and their species-level identification was determined. The number of shells with the abnormal formation due to polydorid invasion was examined for each species and valued as the occurrence rate of abnormal shell formation.

Specimens were deposited at the French National Natural History Museum, Paris, France, and the National Museum of Nature and Science, Tsukuba, Japan.

SEM observation

Six specimens of *Polydora onagawaensis* Teramoto et al., 2013 were used for scanning electron microscopy (SEM) (Hitachi SU8000, Hitachi, Japan). Each fixed specimen in 99.5% ethanol was freeze-dried in t-butyl alcohol, coated with platinum palladium, and viewed using an SEM equipped with a digital camera.

Molecular analysis

Two individuals each of *Boccardia proboscidea* Hartman, 1940, *Boccardia pseudonatrix* Day, 1961, *Boccardiella hamata* (Webster, 1879), *Dipolydora giardi* (Mesnil, 1893), *Polydora hoplura* Claparède, 1868, and *Polydora websteri* Hartman in Loosanoff & Engle, 1943, one individual of *Dipolydora* sp., and eight individuals of *P. onagawaensis* collected in the present study were used for DNA analysis. Additional samples of *P. onagawaensis* collected from Japan and China and *Polydora* sp. 3 sensu Abe and Sato-Okoshi (2021) collected from Japan were used for DNA analysis for sequence comparison (Table 1).

Genomic DNA was extracted from palps or a small piece of tissue from ethanol-preserved specimens by grinding and heating at 95 °C for 20 min in 50 µL of TE buffer (pH 8.0) with 10% Chelex 100 (Bio-Rad; Richlen and Barber 2005). Undiluted or tenfold diluted extracted DNA in TE buffer was used as a template for the polymerase chain reaction (PCR). Partial sequences of nuclear 18S and 28S rRNA, mitochondrial 16S rRNA, and COI genes were amplified by PCR using the primer pairs 18S-1F1/18S-1R632, 18S-2F576/18S-2R1209, and 18S-3F1129/18S-R1772 for 18S, D1R/D2C for 28S, 16Sar /16Sbr for 16S, and Dorid COI.3F/Dorid COI.1R for COI (Table 2). We performed the PCR in a 20 µL reaction mixture containing 1.0–2.0 µL of template DNA, 8.8 µL of sterilized water, 10 µL of 2×KOD One PCR Master Mix (TOYOBO, Osaka, Japan), 0.1 µL of 50 µM forward and reverse primers. The PCR cycling conditions were 36 cycles at 98 °C for 10 s, 60 °C (18S and 28S), 50–56 °C (16S), or 45 °C (COI) for 5 s, and 68 °C for 1 s. PCR products were purified using ExoSAP-IT (Affymetrix, Cleveland, OH) and sequenced by Eurofins Genomics (Tokyo, Japan). Forward and reverse complementary sequences and contigs were assembled using GeneStudio ver. 2.2.0.0 (GeneStudio, Inc. Suwanee, GA, USA). All sequences generated in the present study were deposited in the DDBJ/ENA/GenBank nucleotide sequence database under accession numbers LC682679–LC682767 (Table 1).

Gene sequences obtained in the present study were aligned using the MAFFT online service ver. 7 using the L-INS-i algorithm (Katoh et al. 2017) to reconstruct the molecular phylogeny with the sequences of other *Polydora*, *Dipolydora*, *Boccardiella*, and *Boccardia* species available in the DDBJ/ENA/GenBank databases (Table 1). For

Table 1 DNA sequences of polydorid species and their DDBJ/EMBL/GenBank accession numbers used in the phylogenetic analyses. The sequences obtained in the present study are indicated in boldface

Genus	Species	Country	DDBJ/ENA/GenBank accession number				Reference
			18S	28S	16S	COI	
Boccardia	*Boccardia perata* (Chlebovitsch, 1959)	Russia	–	MH493018	MH493047	–	Radashevsky et al. (2019)
	Boccardia polybranchia (Haswell, 1885)	South Africa	KY677891	–	–	–	Williams et al. (2017)
	Boccardia proboscidea Hartman, 1940	France	**LC682679, LC682680**	**LC682700, LC682701**	**LC682723, LC682724**	–	**This study**
		Japan	LC107607	AB973944	LC595721	–	Abe et al. (2016), Simon et al. (2019), Abe & Sato-Okoshi (2021)
	Boccardia pseudonatrix Day, 1967	France	**LC682681, LC682682**	**LC682702, LC682703**	**LC682725, LC682726**	–	**This study**
		Japan	LC545892	–	LC595723	–	Abe & Sato-Okoshi (2021)
	Boccardia sp. 1 sensu Abe & Sato-Okoshi (2021)	Japan	LC545894	–	LC595725	–	Abe & Sato-Okoshi (2021)
	Boccardia sp. 2 sensu Abe & Sato-Okoshi (2021)	Japan	LC545895	–	LC595726	–	Abe & Sato-Okoshi (2021)
Boccardiella	*Boccardiella hamata* (Webster, 1879)	France	**LC682683, LC682684**	**LC682704, LC682705**	**LC682727**	–	**This study**
		Japan	LC107608	–	LC595727	–	Abe et al. (2016), Abe & Sato-Okoshi (2021)

Table 1 (continued)

Genus	Species	Country	DDBJ/ENA/GenBank accession number				Reference
			18S	28S	16S	COI	
Dipolydora	*Dipolydora armata* (Langerhans, 1880)	Japan	LC545897	–	LC595729	–	Abe & Sato-Okoshi (2021)
	Dipolydora bidentata (Zachs, 1933)	Japan	LC107609	–	LC595730	–	Abe et al. (2016), Abe & Sato-Okoshi (2021)
		Russia	JX228065	JX228085	JX228103	–	Radashevsky & Pankova (2013)
	Dipolydora capensis (Day, 1955)	South Africa	KY677897	–	–	–	Williams et al. (2017)
	Dipolydora cardalia (E. Berkeley, 1927)	Russia	JX228073	JX228093	JX228113	–	Radashevsky & Pankova (2013)
	Dipolydora carunculata (Radashevsky, 1993)	Russia	JN048711	JN048724	JN048698	–	Radashevsky & Pankova (2013)
	Dipolydora cf. *commensalis* (Andrews, 1891)	Japan	LC545899	–	LC595732	–	Abe & Sato-Okoshi (2021)
	Dipolydora giardi (Mesnil, 1893)	France	**LC682685, LC682686**	**LC682706, LC682707**	**LC682728, LC682729**	–	**This study**
		Japan	LC545900	–	LC595733	–	Abe & Sato-Okoshi (2021)
	Dipolydora quadrilobata (Jacobi, 1883)	Russia	–	–	MH493041	–	Radashevsky et al. (unpubl.)
	Dipolydora cf. *socialis* (Schmarda, 1861)	Japan	LC545902	–	LC595735	–	Abe & Sato-Okoshi (2021)
	Dipolydora sp.	France	**LC682687**	**LC682708**	**LC682730**	–	**This study**

Table 1 (continued)

Genus	Species	Country	DDBJ/ENA/GenBank accession number				Reference
			18S	28S	16S	COI	
	Dipolydora sp. sensu Abe & Sato-Okoshi (2021)	Japan	LC545904	–	LC595737	–	Abe & Sato-Okoshi (2021)
Polydora	*Polydora aura* Sato-Okoshi, 1998	Japan	AB705409	LC500923	LC500931	–	Sato-Okoshi & Abe (2012), Abe & Sato-Okoshi (2020)
	Polydora brevipalpa Zachs, 1933	Japan	AB705407	–	LC595738	–	Sato-Okoshi & Abe (2012), Abe & Sato-Okoshi (2021)
	Polydora calcarea (Templeton, 1836)	Japan	AB705403	–	LC595740	–	Sato-Okoshi & Abe (2013), Abe & Sato-Okoshi (2021)
	Polydora cornuta Bosc, 1802	Japan	LC541483	LC541485	LC541484	–	Abe & Sato-Okoshi (2020)
	Polydora cf. *glycymerica* Radashevsky, 1993	Japan	LC545907	–	LC595742	–	Abe & Sato-Okoshi (2021)
	Polydora hoplura Claparède, 1868	France	**LC682688, LC682689**	**LC682709, LC682710**	**LC682731, LC682732**	–	**This study**
		Japan	LC101841	LC101854	LC101870	–	Sato-Okoshi et al. (2017)
		South Africa	LC101850	LC101863	LC101881	–	Sato-Okoshi et al. (2017)
	Polydora lingshuiensis Ye, Tang, Wu, Su, Wang, Yu & Wang 2015	China	KF562240	KF562246	KF562233	–	Ye et al. (2015)

Table 1 (continued)

Genus	Species	Country	DDBJ/ENA/GenBank accession number				Reference
			18S	28S	16S	COI	
	Polydora lingulicola Abe & Sato-Okoshi, 2020	Japan	LC500909	LC500916	LC500924	–	Abe & Sato-Okoshi (2020)
	Polydora neocaeca Williams & Radashevsky, 1999	Japan	AB705404	–	LC595744	–	Sato-Okoshi & Abe (2013), Abe & Sato-Okoshi (2021)
		China	KF562241	KF562248	KF562234	–	Ye et al. (2015)
	Polydora onagawaensis Teramoto et al., 2013	France	LC682690–LC682697	LC682711–LC682718	LC682733–LC682740	LC682743–LC682745	**This study**
		Japan	AB691768	LC682719	LC595745	LC682746–LC682762	Teramoto et al. (2013), Abe & Sato-Okoshi (2021), **This study**
		China	AB820870	–	–	LC682763–LC682765	Sato-Okoshi et al. (2013), **This study**
		USA	MN822032–44	–	–	MN856839–50	Silverbrand et al. (2021)
		USA	MT857758–64	–	–	MT858706–12	Rodewald et al. (2021)
	Polydora cf. *nuchalis* Woodwick, 1953	South Africa	KY677903	–	–	–	Williams et al. (2017)
	Polydora cf. *spongicola* Berkeley & Berkeley, 1950	Japan	LC545910	–	LC595747	–	Abe & Sato-Okoshi (2021)
	Polydora triglanda Radashevsky & Hsieh, 2000	Taiwan	JN048718	JN048731	JN048705	–	Radashevsky & Pankova (2013)

Table 1 (continued)

Genus	Species	Country	DDBJ/ENA/GenBank accession number				Reference
			18S	28S	16S	COI	
	Polydora websteri Hartman in Loosanoff & Engle, 1943	France	**LC682698, LC682699**	**LC682720, LC682721**	**LC682741, LC682742**	**LC682766, LC682767**	**This study**
		Japan	AB705402	–	LC595749	–	Sato-Okoshi & Abe (2013), Abe & Sato-Okoshi (2021)
	Polydora sp. 1 sensu Abe & Sato-Okoshi (2021)	Japan	LC545912	–	LC595750	–	Abe & Sato-Okoshi (2021)
	Polydora sp. 2 sensu Abe & Sato-Okoshi (2021)	Japan	LC545914	–	LC595752	–	Abe & Sato-Okoshi (2021)
	Polydora sp. 3 sensu Abe & Sato-Okoshi (2021)	Japan	LC545915	**LC682722**	LC595753	–	Abe & Sato-Okoshi (2021), **This study**
Pseudopolydora (outgroup)	*Pseudopolydora paucibranchiata* (Okuda, 1937)	Japan	LC019991	LC019995	LC595758	–	Abe et al. (2016), Abe & Sato-Okoshi (2021)

Table 2 PCR and sequence primers used in the present study

Gene/region	Primer	Sequence (5′–3′)	Direction	Usage	Reference
18S	18S-1F1	AACCTGGTTKATCCTGCCAG	Forward	PCR/sequencing	Nishitani et al. (2012)
	18S-1R632	ACTACGAGCTTTTTAAACYGCARC	Reverse	PCR/sequencing	Nishitani et al. (2012)
	18S-2F576	GGTAATTCCAGCTCYAATRG	Forward	PCR/sequencing	Nishitani et al. (2012)
	18S-2R1209	AAGTTTYCCCGTGTTGARTC	Reverse	PCR/sequencing	Nishitani et al. (2012)
	18S-3F1129	GCTGAAACTTAAAGRAATTGACGG	Forward	PCR/sequencing	Nishitani et al. (2012)
	18S-R1772	TCACCTACGGAAACCTTGTTACG	Reverse	PCR/sequencing	Nishitani et al. (2012)
28S	D1R	ACCCGCTGAATTTAAGCATA	Forward	PCR/sequencing	Scholin et al. (1994)
	D2C	CCTTGGTCCGTGTTTCAAGA	Reverse	PCR/sequencing	Scholin et al. (1994)
16S	16Sar	CGCCTGTTTATCAAAAACAT	Forward	PCR/sequencing	Palumbi et al. (1991)
	16Sbr	CCGGTCTGAACTCAGATCACGT	Reverse	PCR/sequencing	Palumbi et al. (1991)
COI	Dorid COI.3F	AAGGWATACCTACACAGAAAAARATACC	Forward	PCR/sequencing	Williams et al. (2017)
	Dorid COI.1R	CTGTGAATAGRGGRAATCAGTTTAT	Reverse	PCR/sequencing	Williams et al. (2017)

phylogenetic analyses of the 18S, 28S, and 16S rRNA gene regions, the gene sequences of *Pseudopolydora paucibranchiata* (Okuda, 1937), obtained from DDBJ/ENA/Gen-Bank, were used as outgroup taxa, according to Abe and Sato-Okoshi (2021). Phylogenetic analysis of the COI gene was performed to investigate the geographical genetic variation in *P. onagawaensis*, and the *P. websteri* sequence obtained in the present study was used as the outgroup. Ambiguously aligned regions were eliminated using Gblocks ver. 0.91b (Castresana 2000) with less stringent settings. The final lengths of the alignments were 1769 (18S), 766 (28S), 471 (16S), and 656 (COI) bp for the multiple sequence alignment. Two phylogenetic trees were constructed based on the concatenated sequences of 18S, 28S, and 16S, and the sequences of the COI gene region by maximum likelihood (ML) analyses performed using IQ-TREE (Nguyen et al. 2015) implemented in PhyloSuite v.1.2.2 (Zhang et al. 2020) under an edge-linked partition model. The $TIM2e + I + G4$, $TNe + R2$, $GTR + F + I + G4$, and $TPM + F + G4$ models were selected for the 18S, 28S, 16S rRNA, and COI gene regions, respectively, as the best substitution model by ModelFinder (Kalyaanamoorthy et al. 2017) as implemented in IQ-TREE under the Bayesian information criterion. The robustness of the ML trees was evaluated using the Shimodaira–Hasegawa–like approximate likelihood-ratio test (SH-aLRT) with 5000 replicates (Guindon et al. 2010), approximate Bayes (aBayes) test (Anisimova et al. 2011), and ultrafast bootstraps (UFBoot) with 5000 replicates (Hoang et al. 2018). SH-aLRT $\geq 80\%$, aBayes ≥ 0.95, and UFBoot $\geq 95\%$ were defined as robust statistical supports.

Results

Identification of polydorid species

Based on morphology and gene sequence results, a total of eight species were identified: three *Polydora*, *P. onagawaensis*, *P. hoplura*, and *P. websteri*; two *Dipolydora*, *D. giardi*, and *Dipolydora* sp.; two *Boccardia*, *B. proboscidea*, and *B. pseudonatrix* Day, 1961; and one *Boccardiella*, *B. hamata*. The sampling site, host species or associated substrate, culture or wild condition, habitat type, and condition of shell infestation and other calcareous substrates by each species are summarized in Table 3.

Molecular phylogenetic analysis of concatenated sequences of nuclear 18S and 28S and mitochondrial 16S rRNA gene sequences of polydorid species obtained in the present study and from the DDBJ/EMBL/GenBank database supported the morphological identification results. The species identified in the present study were genetically close to the same species previously reported from other localities (Fig. 2).

Molecular phylogenetic analyses based on the mitochondrial COI gene sequences obtained in the present and previous studies showed that the individuals identified as *P. onagawaensis* were divided into four lineages (Fig. 3). One lineage consisted of individuals from Japan and China, two lineages consisted of individuals from the USA and Japan, and the remaining lineage consisted of individuals from the USA and France. The Japanese and USA specimens each contained three lineages, while the French specimens were of a single lineage.

Table 3 Polydorid species extracted from oyster shells and other various calcareous substrates and their infestation condition

Polydorid species	Sampling locality	Host shell/sub-strate	Culture or wild	Habitat type	*Occurrence rate of abnormal shell formation	Condition of shell infestation	Museum registration number
Polydora hoplura	Blainville-sur-Mer	*Crassostrea gigas*	Culture	Shell boring	10/20	Heavy shell infestation, presence of multiple abnormal black organic deposits or calcareous deposits on the inner surface of the shell	
	Blainville-sur-Mer	*Crassostrea gigas*	Wild	Shell boring	20/20	Heavy shell infestation, presence of multiple abnormal black organic deposits or calcareous deposits on the inner surface of the shell	MNHN-IA-PNT 133 NSMT-Pol 113470
	Quineville	*Crassostrea gigas*	Culture	Shell boring	2/20	Shell infestation, presence of abnormal black organic deposits or calcareous deposits on the inner surface of the shell	
Polydora websteri	Quineville	*Crassostrea gigas*	Culture	Shell boring	20/20	Heavy shell infestation, presence of multiple abnormal black organic deposits or calcareous deposits on the inner surface of the shell	
	Asnelles	*Crassostrea gigas*	Culture	Shell boring	13/20	Shell infestation, presence of abnormal black organic deposits or calcareous deposits on the inner surface of the shell	MNHN-IA-PNT 134 NSMT-Pol 113471

Table 3 (continued)

Polydorid species	Sampling locality	Host shell/sub-strate	Culture or wild	Habitat type	*Occurrence rate of abnormal shell formation	Condition of shell infestation	Museum registration number
Polydora onagawaensis	Saint-Vaast-La-Hougue	*Crassostrea gigas*	Wild	Shell boring	12/20	Heavy shell infestation, presence of multiple abnormal black organic deposits or calcareous deposits on the inner surface of the shell	MNHN-IA-PNT 132 NSMT-Pol 113469
	Quineville	*Crassostrea gigas*	Culture	Shell boring	20/20	Heavy shell infestation, presence of multiple abnormal black organic deposits or calcareous deposits on the inner surface of the shell	
	Asnelles	*Crassostrea gigas*	Culture	Shell boring	7/20	Shell infestation, presence of abnormal black organic deposits or calcareous deposits on the inner surface of the shell	
	Luc-sur-Mer	Limestone	Wild	Boring		Many burrows all over the surface	
		Calcareous substrates on concrete block	Wild	Boring		Mud tubes all over the surface	
Dipolydora giardi	Blainville-sur-Mer	*Crassostrea gigas*	Culture	Shell boring	0/20	Many burrows in multi-layers of the shell	MNHN-IA-PNT 135 NSMT-Pol 113472
Dipolydora sp.	Blainville-sur-Mer	*Crassostrea gigas*	Culture	Shell boring	0/20	Burrows visible	

Table 3 (continued)

Polydorid species	Sampling locality	Host shell/sub-strate	Culture or wild	Habitat type	*Occurrence rate of abnormal shell formation	Condition of shell infestation	Museum registration number
Boccardia proboscidea	Asnelles	*Crassostrea gigas*	Culture	Inhabit mud tubes in the crevices of the shell	1/20	Mud tubes protruding from the crevices of the shell	MNHN-IA-PNT 137 NSMT-Pol 113474
	Point de Barfleur	Coralline algae	Wild	Inhabit mud tubes in the crevices of the coralline algae		Mud tubes all over the surface	
Boccardia pseudonatrix	Saint-Vaast-La-Hougue	*Crassostrea gigas*	Wild	Inhabit mud tubes in the crevices of the shell	0/20	Mud tubes protruding from the crevices of the shell	MNHN-IA-PNT 136 NSMT-Pol 113473
	Quineville	*Crassostrea gigas*	Culture	Inhabit mud tubes in the crevices of the shell	0/20	Mud tubes protruding from the crevices of the shell	
Boccardiella hamata	Saint-Vaast-La-Hougue	*Crassostrea gigas*	Wild	Inhabit mud tubes in the crevices of the shell	8/20	Shell infestation, presence of abnormal black organic deposits or calcareous deposits on the inner surface of the shell	MNHN-IA-PNT 138 NSMT-Pol 113475

*Occurrence rate of abnormal shell formation: number of shells which had abnormal shell formation in response to the species invasion/number of shells examined

Morphological characteristics of the specimens collected from Normandy

Polydora onagawaensis Teramoto et al., 2013 (Fig. 4)

Adult morphology. Thirty individuals of *Polydora onagawaensis* were examined. After fixation, the maximum body length (95 chaetigers) was 15 mm, and the maximum width was 680 μm (at the 5th chaetiger). The body coloration of the live specimen was light tan. Body pigmentation in live and preserved specimens was weak; weak black pigmentation was present on and along the caruncle. Occasionally, intense black pigmentation was present on peristomium around the base of both palps (Fig. 4A–D). Palp pigmentation was either entirely absent or a faint black (Fig. 4D). Black pigment was occasionally present on the dorsal and lateral sides of the posterior chaetigers (Fig. 4E). The pygidium had no pigmentation or black pigmentation along the edge or on the side of the disc-like pygidium (Fig. 4E, F). The peristomium is wide and short. The prostomium was weakly incised on the anterior margin. The eyes were absent or up to four black eyes and were arranged in a trapezoid, with the anterior pair more widely spaced than the posterior pair (Fig. 4C). The caruncle extended posteriorly to the end of chaetigers 1 to 4, unrelated to body size, without a median antenna (Fig. 4G).

Chaetiger 1 had noto- and neuropodia; notochaetae were absent, and short capillary neurochaetae were present. Parapodial lobes and postchaetal lamellae were well-developed on anterior chaetigers, except chaetiger 5, gradually reducing on posterior chaetigers. Neuro- and notochaetae on chaetiger 2–4 and 6 were winged capillaries. The number of capillaries per fascicle and wings in the notopodia of succeeding chaetigers diminished gradually. Hooded hooks on neuropodia began from chaetiger 7 and were not accompanied by capillaries. Hooks were bidentate, with the main fang at a right angle to the shaft and an acute angle to the apical tooth. The shaft was slightly curved, with a constriction in the upper part. The size and number of hooks gradually diminished from anterior to posterior. Approximately six hooks were present in a series.

The modified chaetiger 5 was longer than adjacent chaetigers 4 and 6 (Fig. 4G) and possessed curved horizontal rows of up to six major spines alternating with pennoned companion chaetae. Both dorsal and ventral tufts of several short and winged capillaries were present. The major spines were falcate with lateral flanges or sheaths, and unworn spines were tooth-like (Fig. 4H).

Branchiae began from chaetiger 7, continuing to 1/2 to a maximum of 3/5 of the body. Nototrochs began from chaetiger 7 onwards.

Pygidium was white and disc-shaped, with a dorsal gap (Fig. 4E, F). Gizzard-like structures were absent in the digestive tract. Glandular pouches began with chaetiger 7.

Juvenile morphology. Twenty chaetigers juveniles were examined (Fig. 4I, J). The prostomium was rounded anteriorly. Conspicuous black pigmentation occurred on peristomium around the base of the palps. Dorsal pigmentation consisted of two rows of melanophores from chaetiger 2 with those of the first six band-shaped pairs, which were then replaced by ramified melanophores in posterior chaetigers. Lateral pigments were found in chaetigers 1–3. Dorsolateral pigments at the base of the parapodia started from chaetiger 7. A pair of black pigments appeared on the pygidium. The ventral pigment was absent. Modified chaetae developed in chaetiger 5.

Reproduction. Only a few large individuals had oocytes in their coeloms. Egg capsules were absent; however, numerous juveniles with approximately 20 chaetigers,
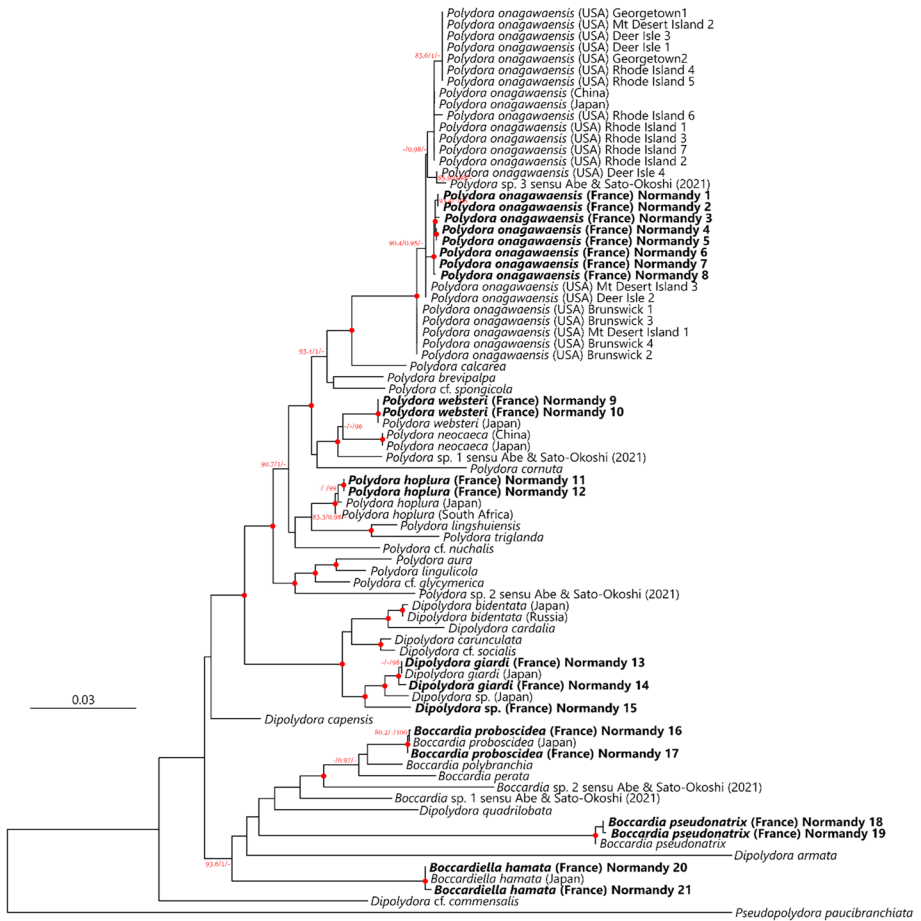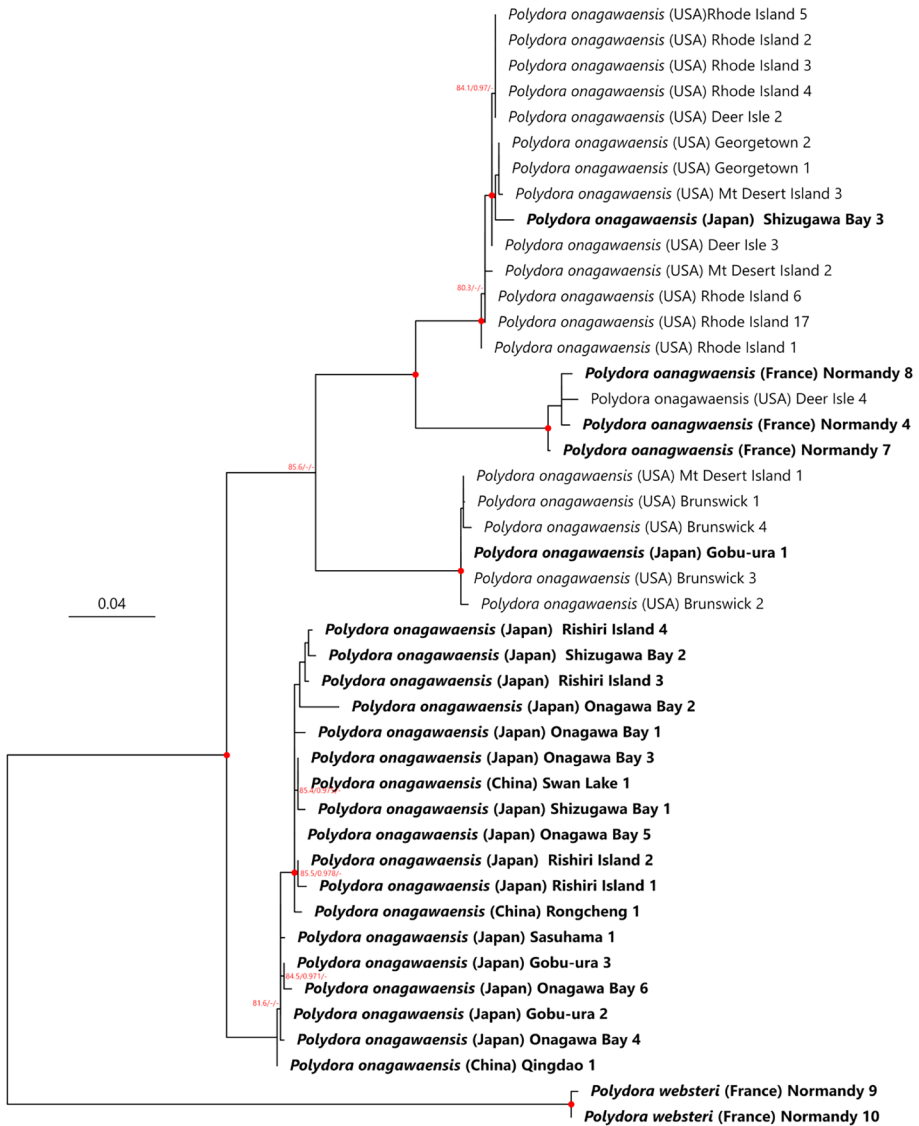
Fig. 2 Maximum likelihood tree inferred from concatenated sequences of nuclear 18S, 28S, and mitochondrial 16S rRNA gene sequences of polydorid species obtained in the present study and from the DDBJ/EMBL/GenBank database (Table 1). The gene sequences obtained in this study are highlighted in bold. SH-aLRT/approximate Bayes support/ultrafast bootstrap support values of ≥ 80%/≥ 0.95/≥ 95% are shown beside the respective nodes in red. Nodes with red circles indicate triple high support values of SH-aLRT ≥ 80, approximate Bayes support ≥ 0.95, and ultrafast bootstrap support ≥ 95. Scale bar represents the number of substitutions per site. The sequence of *Pseudopolydora paucibranchiata* was used for outgroup rooting

representing characteristic larval pigmentation on the dorsal side, were observed in early March (Fig. 4I).

Remarks. *Polydora onagawaensis* resembles *P. websteri* and *P. hoplura* in that all three possess similar chaetal morphology, arrangement, and body pigmentation variation. However, *P. onagawaensis* can be distinguished from *P. websteri* by its small maximum size, few consecutive branchial chaetigers, wide and short peristomium, pygidium disc-like morphology rather than flared, and more pigment variations rather than only black pigment along the edge of the palps. Moreover, although body pigmentation resembles each other, *P. onagawaensis* can be distinguished from *P. hoplura* by its smaller body size, both in length and width, smaller pygidium, absence of a median

Fig. 3 Maximum likelihood tree inferred from mitochondrial COI gene sequences of *Polydora onaga-waensis* obtained in the present study and from the DDBJ/EMBL/GenBank database (Table 1). The gene sequences obtained in this study are highlighted in bold. SH-aLRT/approximate Bayes support/ultrafast bootstrap support values of ≥80%/≥0.95/≥95% are shown beside the respective nodes in red. Nodes with red circles indicate triple high support values of SH-aLRT ≥80, approximate Bayes support ≥0.95, and ultrafast bootstrap support ≥95. Scale bar represents the number of substitutions per site. Sequences of *Polydora websteri* were used for outgroup rooting

antenna, and absence of posterior hooks in notopodia. The morphology of specimens from Normandy can be slightly distinguished from those from Japan (Teramoto et al. 2013), where the species was originally described by occasional intense black pigmentation around the base of both palps.
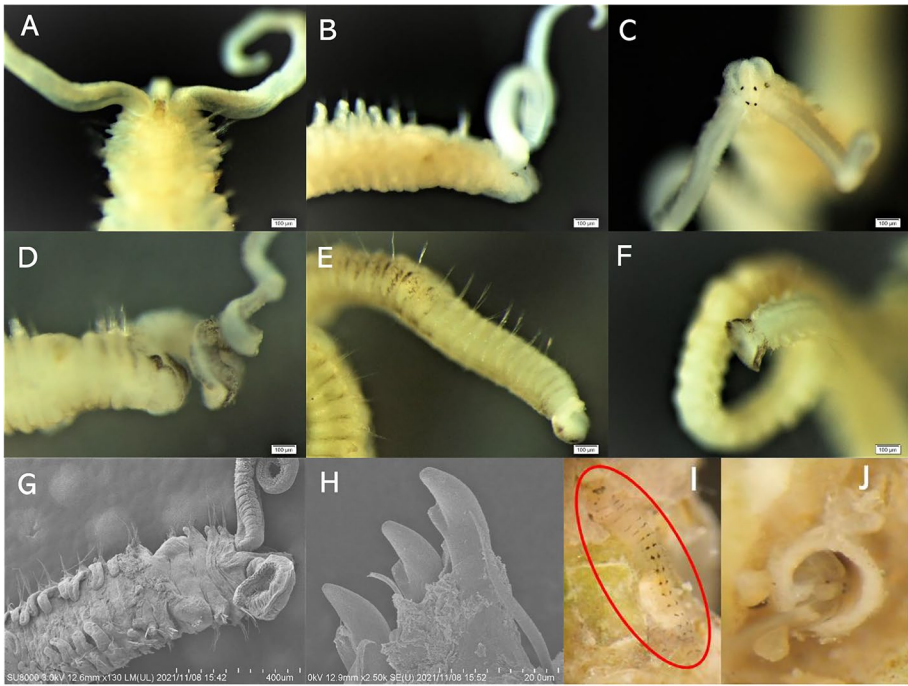
Fig. 4 *Polydora onagawaensis*. Photographs showing morphological characteristics focused on pigmentation after fixation (**A–F**), SEM (**G–H**), and juveniles in live (**I–J**). **A** Black pigmentation on caruncle and dorsal side of anterior chaetigers. **B** Black pigmentation near the base of palp from lateral view. **C** Black pigmentation near the base of both palps from dorsal view. **D** Black pigmentation on prostomium, around the base of palp, and on palp from lateral view. **E** Black pigmentation on posterior chaetigers and along the edge of pygidium from lateral view. **F** Black pigmentation on lateral side of pygidium. **G** Anterior dorsal view. **H** Major falcate spines with lateral flange or sheath of modified chaetiger 5. **I** Juvenile with black pigmentation on its dorsal side. **J** Juvenile in mud tube

Polydora websteri Hartman in Loosanoff & Engle, 1943 (Fig. 5A, B)

Twenty individuals of *P. websteri* were examined in this study. The maximum recorded body length after fixation was 18 mm. *Polydora websteri* from Normandy showed similar morphological characteristics to previous descriptions worldwide (Read 2010; Sato-Okoshi and Abe 2013; Rodewald et al. 2021). Palps had weak-to-clear black pigmentation along the margin (Fig. 5A). Branchiae began from chaetiger 7, and were fairly long throughout, continuing almost till the posterior end. The pygidium was flared outward (Fig. 5B).

Polydora hoplura Claparède, 1868

Twenty individuals of *P. hoplura* were examined in this study. The maximum recorded body length after fixation was 35 mm Morphological characteristics of *P. hoplura* from Normandy were similar to those previously described worldwide (Sato-Okoshi and Abe 2013; Walker 2013; Radashevsky et al. 2017; Sato-Okoshi et al. 2017). Black pigmentation

on the palps, prostomium, and peristomium was feebly visible or entirely absent. A median antenna was present. Special recurved hooks were present on the posterior notopodia.

Dipolydora giardi (Mesnil, 1893)

We examined 20 individuals of *Dipolydora giardi* in this study. The maximum recorded body length after fixation was 5 mm. The morphological characteristics of *D. giardi* from Normandy were similar to those previously described in Asia (Sato-Okoshi 1999; Sato-Okoshi et al. 2012) and Australia (Sato-Okoshi et al. 2008; Walker 2013). Pigmentation was entirely absent on the body. Notochaetae were present on chaetiger 1, branchiae began from chaetiger 9, and capillaries were accompanied by hooded hooks.

Dipolydora sp.

The following description of *Dipolydora* sp. is based on an incomplete specimen. Branchiae began at chaetiger 9. While detailed morphological characteristics were unavailable, genetic data suggested that this species was different from *D. giardi*.

Boccardia pseudonatrix Day, 1961 (Fig. 5C, D)

We examined five individuals of *Boccardia pseudonatrix* in this study. The maximum recorded body length after fixation was 18 mm. The morphological characteristics of *B. pseudonatrix* from Normandy matched its previous descriptions from South Africa (Simon et al. 2010) and Australia (Sato-Okoshi et al. 2008, as *B. knoxi*: see Sato-Okoshi et al. 2015; Walker 2013). Palps were transparent with irregular colorless spots crossing transversely, giving the appearance of being crossed by white bars in the lateral view. Conspicuous black pigmentation was observed in the prostomium (Fig. 5C, D). A mid-dorsal ridge appeared from chaetiger 5 to the middle of chaetiger 8 (Fig. 5D).

Boccardia proboscidea Hartman, 1940

Twenty individuals of *Boccardia proboscidea* were examined in this study. The maximum recorded body length after fixation was 18 mm. The morphological characteristics of *B. proboscidea* from Normandy matched well with previous descriptions from Japan (Sato-Okoshi 2000), Australia (Blake and Kudenov 1978; Sato-Okoshi et al. 2008; Walker 2013), South Africa (Simon et al. 2006), USA (Bailey-Brock 2000), and Canada (Sato-Okoshi and Okoshi 1997). Black pigmentation was present on both sides of the caruncle. Their body color in life was greenish. The pygidium was divided into lobes.

Boccardiella hamata (Webster, 1879) (Fig. 5E, F)

We examined 20 individuals of *Boccardiella hamata*. The maximum recorded body length after fixation was 38 mm. The morphological characteristics of *B. hamata* from Normandy matched those of *B. hamata* from North America (Dean and Blake 1966) and Asia (Sato-Okoshi 2000; Sato-Okoshi et al. 2012; 2013). Pigmentation was entirely absent on
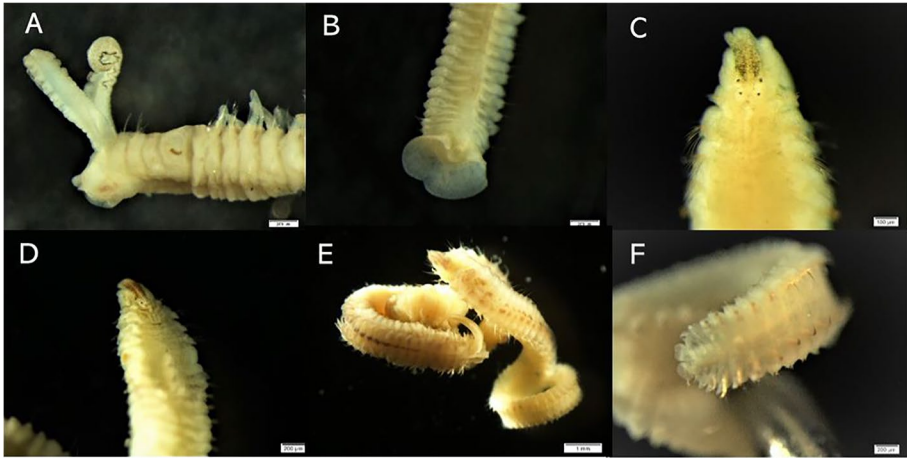
Fig. 5 Photographs showing morphological characteristics focused on pigmentation of newly recorded species in the English Channel; *Polydora websteri* (**A**, **B**), *Boccardia pseudonatrix* (**C**, **D**), and *Boccardiella hamata* (**E**, **F**) after fixation. **A** Black pigmentation along the edge of palp from anterior lateral view. **B** Flared pygidium without pigmentation. **C** Black pigmentation on prostomium from anterior dorsal view. **D** Black pigmentation on prostomium and on the dorsal side of anterior chaetigers. Mid-dorsal ridge can be observed from chaetiger 5 to 8. **E** No particular pigmentation on the whole body. **F** Special hooks on posterior chaetigers and pygidium from dorsal view

the body (Fig. 5E). Special recurved hooks were present in posterior notopodia (Fig. 5F). Pygidium had two broad ventral lappets, each with a short process (Fig. 5F).

Shell infestation by polydorids

The conditions of shell infestation by each polydorid species at each sampling site are shown in Table 3. Shell infestation was determined by the occurrence rate of abnormal shell formation, and the degree of abnormal shell formation observed on the inner surface of the shell. The presence of abnormal black organic deposits or calcareous deposits is a direct response of the host mollusks to cover and defend the polydorid excavation and repair after their penetration. The degree "heavy infestation" was used only when multiple abnormal shell formations were observed per shell. We used the term "infestation" only when a host oyster formed an abnormal shell in response to an invasion, irrespective of the number of worms inhabiting the shell.

Sandy areas, including some rocks, were spread across Blainville-sur-Mer (Fig. 6A). Cultured Japanese oyster *C. gigas* that fell out of the net bags (Fig. 6B), along with wild Japanese oyster (Fig. 6C) and European flat oyster *O. edulis* (Fig. 6D) were collected and observed. They exhibited a large shell width (Fig. 6C–E) with calcareous layers (Fig. 6F). Shell chambers (French word: chambrage) are constructed with thin paper shells filled with jelly-like materials. Chalky deposits (Okoshi et al. 1987) or mud were observed in both shell valves. Small-sized *D. giardi* and large-sized *P. hoplura* were observed to inhabit the shells. Mud blisters, which were the responses against large-sized *P. hoplura*, were also confirmed. The number of individuals per shell fluctuated among the examined oysters, ranging from several to several tens of worms per shell. Although high densities of *D. giardi* (approximately 4 inds. /cm^3 in aggregation) were detected in the shells of cultured oysters that fell from the net bags, the damage to the shells was
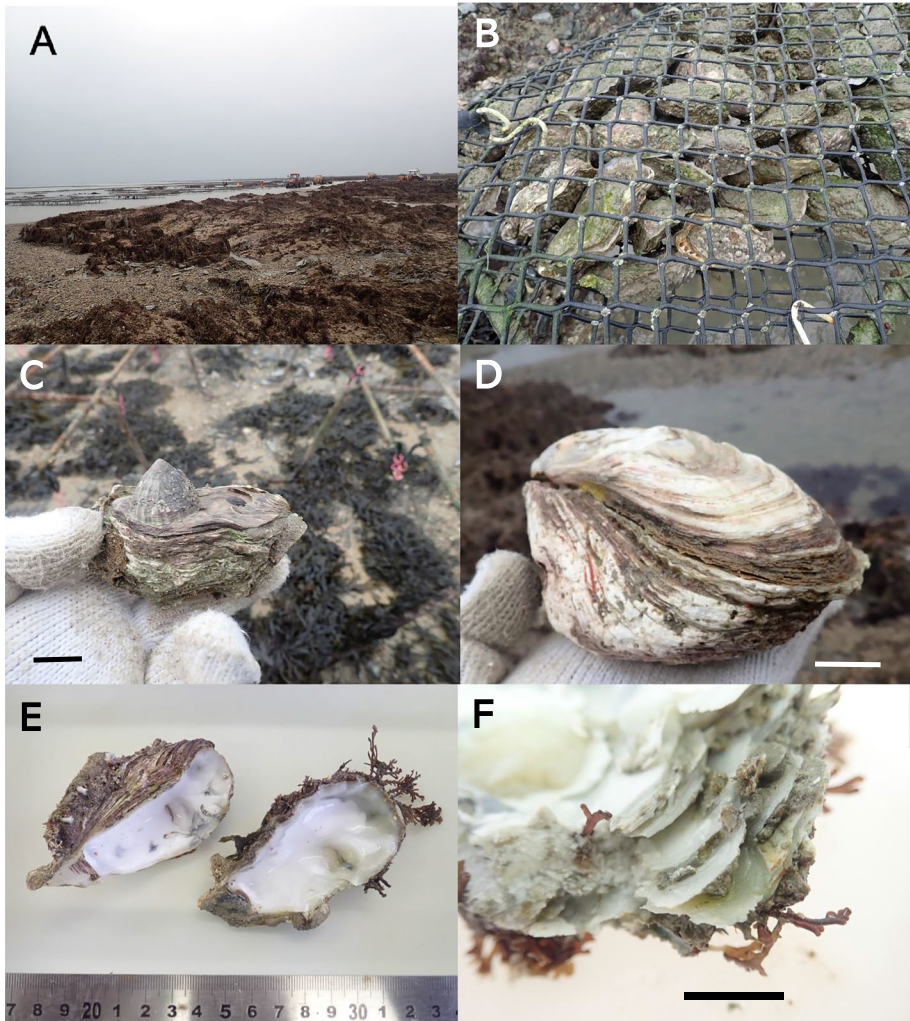
Fig. 6 Wild and cultured oysters from Blainville-sur-Mer. **A** A large sandy oyster culture ground, including some rocky areas. **B** Cultured oysters in a mesh net. **C** Wild *Crassostrea gigas* attached to a rock. A limpet was attached to the right valve. The left valve is cup-shaped and thick. Scale bar: 2 cm. **D** Old and cup-shaped *Ostrea edulis* collected on the beach. Scale bar: 3 cm. **E** An oyster with a large shell width. **F** Fracture surface of right valve with multi- calcareous layers. Scale bar: 1 cm

less owing to their smaller body size. Large, aged *O. edulis* shells collected from the bottom showed traces of polydorid burrows, but no live individuals were found.

Wild oyster shells from oyster beds in the muddy ground at Saint-Vaast-La-Hougue (Fig. 7A–C) were inhabited by *P. onagawaensis*, *B. hamata*, and *B. pseudonatrix*. Mud tubes were observed in, on, and within the shell crevices (Fig. 7D, E). The burrows created inside the shell are visible on the side of the inner surface of the shell (Fig. 7F, G). The number of individuals per shell fluctuated, ranging from several to several tens of worms per shell in *P. onagawaensis*.
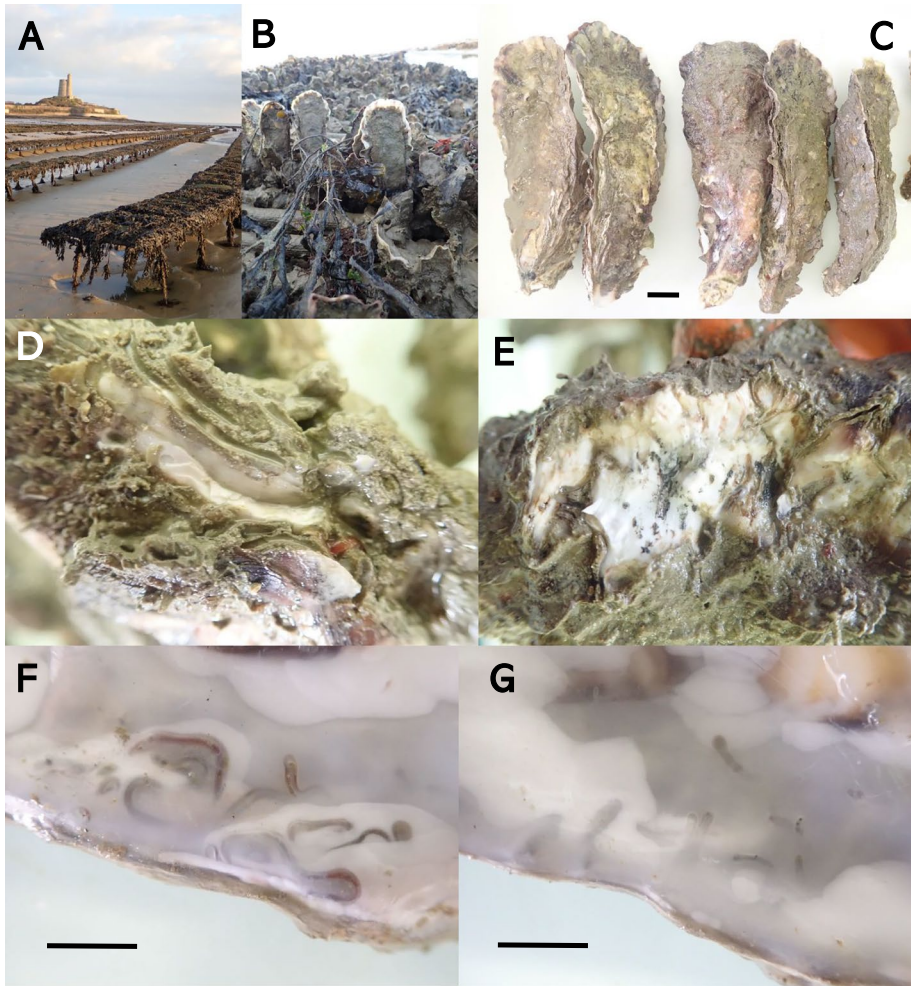
Fig. 7 Wild oysters from Saint-Vaast-La-Hougue. **A** Muddy culture ground with continuous oyster shelves. **B** Oyster bed. **C** Oysters with elongated shells collected from oyster bed. Scale bar: 2 cm. **D** Numerous mud tubes formed interstitially on the surface of the shell. **E** Mud tubes and burrows observed in a cross-section after peeling away the shells from each other. **F**, **G** Numerous burrows of different sizes visible from the inner surface of the shell. Scale bars: 1 cm

The number of worms extracted per shell (valve) was especially high (up to 135 individuals) in suspended cultured oysters from Quineville (Fig. 8A, B). Numerous mud tubes of *P. onagawaensis* and *P. websteri* protruded from the edge and near the umbo of the outer surface of the shell (Fig. 8C). The palps were observed waving from their tubes (Fig. 8D). The outer surface of the shell was peeled off, and a large number of burrows were found inside (Fig. 8E). Further, mud blisters were observed near the attachment site of the adductor muscle on the inner surface of the shell (Fig. 8F). Few cracks were observed along the burrows (Fig. 8G, H). Black or brown organic deposits and calcareous shell material were secreted against worm penetration on the inner surface of the shells (Fig. 8B, F–H).
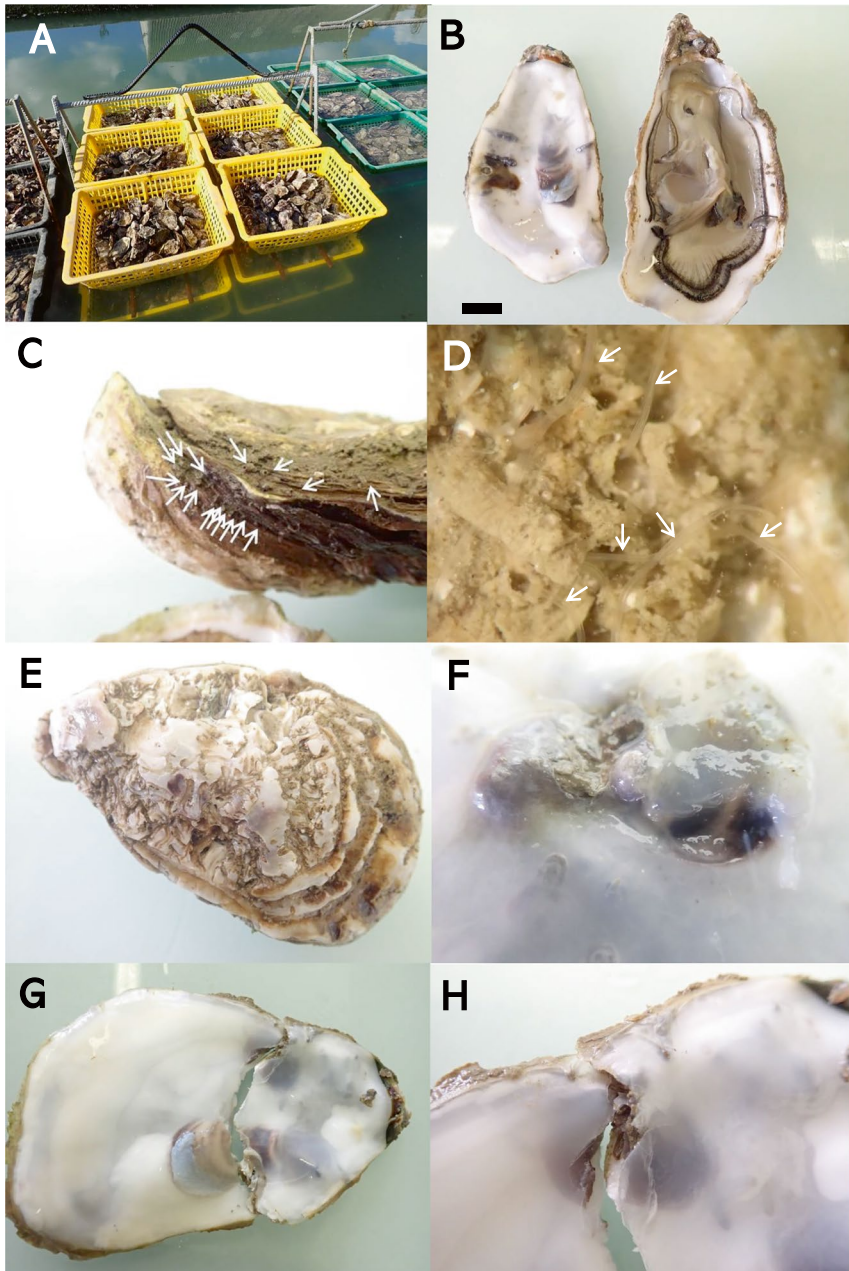
Fig. 8 Cultured oysters from Quineville. **A** Cultured oysters placed in mesh cages and suspended in culture pond. **B** Inner surface of the left and right valves, respectively, left valve with soft body. Brown deposits and calcareous shell materials were observed to secrete against polydorid infestation on the inner surface of the right valve. **C** Each arrow shows the mud tube protruding from along the edge of the valves in a triploid oyster. **D** Each arrow shows polydorid palp waving from its tube. **E** Outer surface of the shell revealing many polydorid burrows which were formed inside after the shell surface peeling off. **F** Mud blister near the attachment site of the adductor muscle. **G** Crack observed running across the polydorid burrow over the adductor muscle attachment and mud blister. **H** Enlarged view of a part of a crack in **G**

From the cultured oyster shells of Asnelles (Fig. 9A–D), three species, *P. onagawaensis*, *P. websteri*, and *B. proboscidea*, were documented. Mud blisters were also observed on the inner surface of the shells (Fig. 9D). Harvested scallops that were dredged near Asnelles had *Cliona* (Porifera) infestation; however, no polydorid infestations were observed on the shells.

In the wild, numerous burrows of *P. onagawaensis* were observed on the surface of limestone (ca. 2 burrows/cm²), which covered the intertidal sea bottom in Luc-sur-Mer (Fig. 10A–D). Juveniles presenting larval pigmentation (Fig. 4I, J) were found from the shallow burrows on the surface of the concrete block covered with barnacles and mussel shells, which were installed during another study (Fig. 10E) (Dauvin et al. 2021).

High densities of approximately 1 ind./cm² of *B. proboscidea* were observed to inhabit coralline algae that covered the entire surface of the intertidal rocks at Point de Barfleur (Fig. 11A–D). They created mud tubes in the crevices of coralline algae. Adults with egg capsules in their tubes were obtained (Fig. 11E, F).

Discussion

Diversity of environment along the coast of Normandy and calcareous substrates as a habitat

Eight species of polydorids were discovered during the present survey of the western half of the Normandy coast along approximately 450 km of the English Channel. In this study,
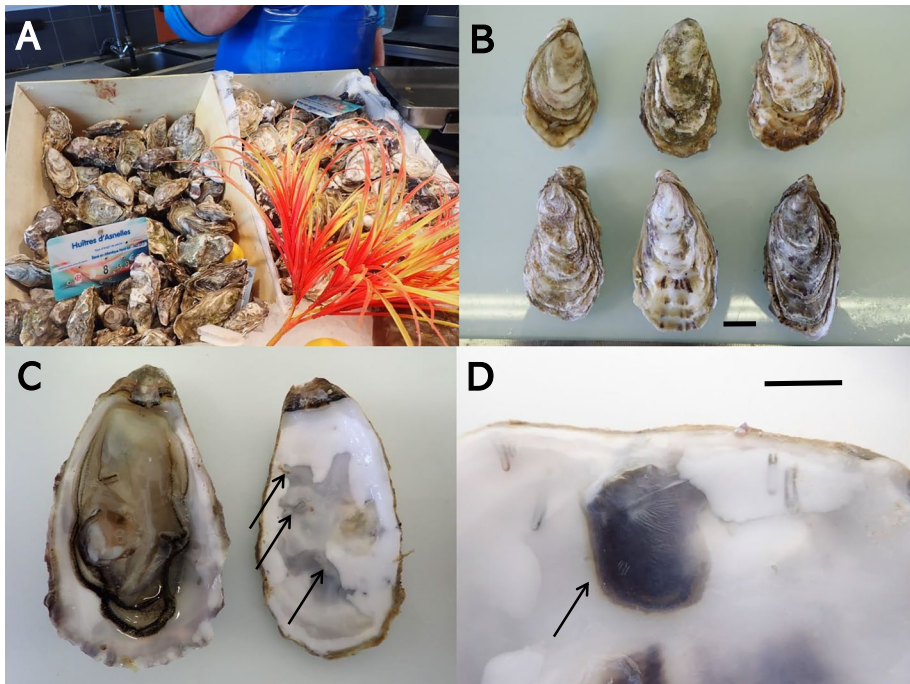

Fig. 9 Cultured oysters from Asnelles. **A** Cultured oysters displayed in the market. **B** Harvested cultured oysters. Scale bar: 2 cm. **C** Inner surface of the left and right valves, respectively, left valve with soft body. Each arrow shows the polydorid burrows. **D** Mud blister and polydorid burrows visible from the inner surface of the right valve shell. Scale bar: 1 cm
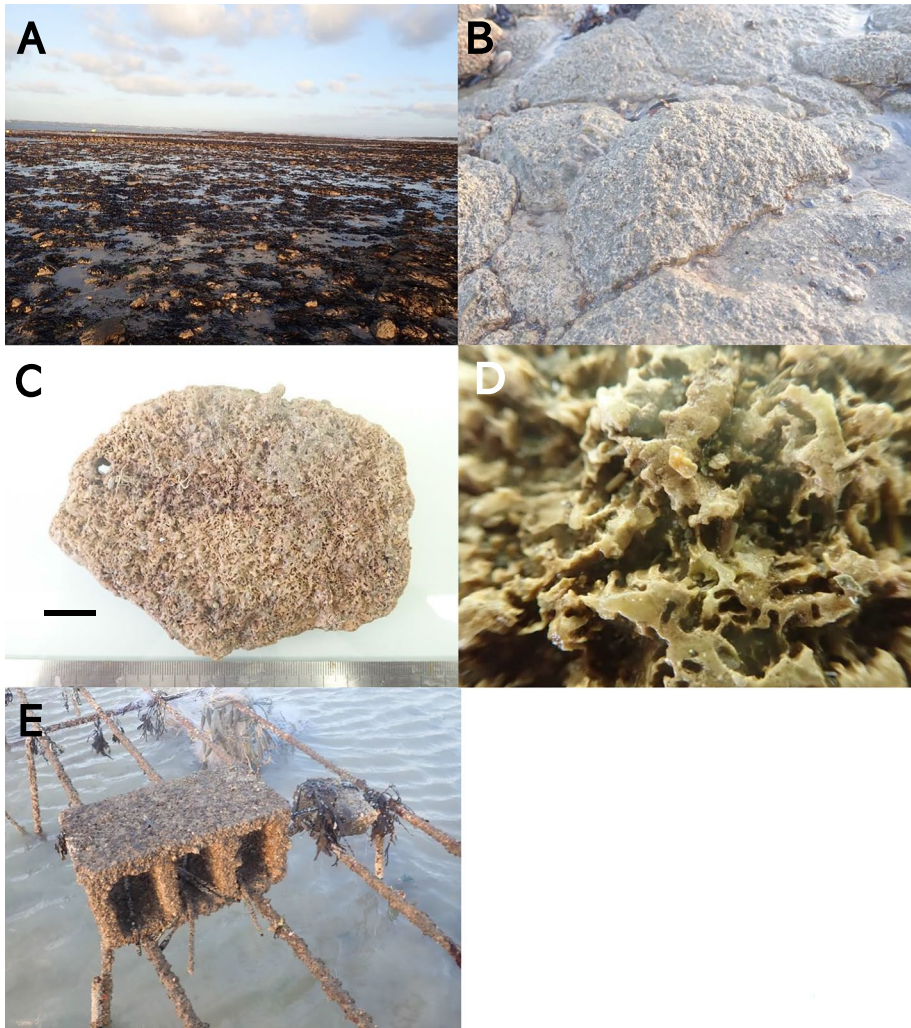
Fig. 10 Limestone and concrete block in Luc-sur-Mer. **A**, **B** Sea bottom covered with limestone. **C** A piece of limestone with numerous burrows on the surface. Scale bar: 2 cm. **D** Enlarged view of limestone surface. **E** Concrete block covered with barnacles and mussel shells which *P. onagawaensis* inhabits

we investigated two large oyster culture grounds and two suspended culture sites in different environments and the surrounding natural calcareous substrates.

The existence of varied habitats, such as the different environments at oyster farms, the diverse natural environments of mud, sand, and rocky areas, as well as the existence of artificial environments such as oyster farming facilities and structures, could be the reason for the coexistence of many of the eight species found in Normandy area. We also observed different morphological characteristics of shells between wild and cultured oysters. For example, all oysters cultivated in mesh bags were "single oysters" and were not stuck to each other. In contrast, the wild oysters that live on the muddy bottom in Saint-Vaast-La-Hougue were attached to each other (Fig. 7B). Crevices between individual oysters and mud accumulates created a conducive habitat for polydorids.
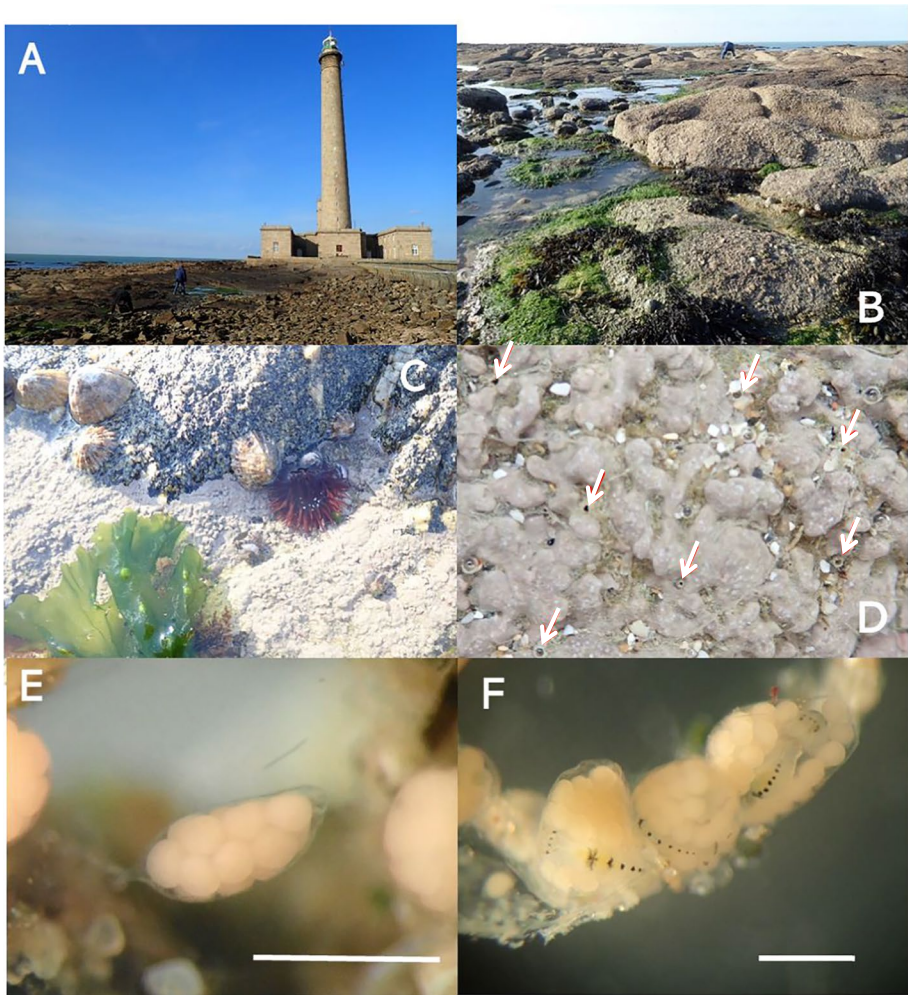
Fig. 11 Coralline algae at Point de Barfleur. **A–C** Surface of the rocks covered with coralline algae. **D** Many burrow openings (arrows) observed on the surface of coralline algae. **E, F** Egg capsules with nurse eggs and developing larvae of *Boccardia proboscidea*. Scale bars: 500 μm

Moreover, at Saint-Vaast-La-Hougue, oyster shells that live on the muddy bottom grow longitudinally upward and are thin to avoid burial under the mud (Fig. 7B, C). Since oysters with thin shells are less suitable as long-term habitats for large numbers of polydorid individuals, the abundance of mud provides a suitable alternate for species that build mud tubes to live in (Fig. 7D, E).

Oyster shells cultured in mesh bags on gravel and sandy ground farms and a wild single oyster grown on rocks or coarse sand tended to be thicker in Blainville-sur-Mer (Fig. 6B–E) compared to wild oyster shells in Saint-Vaast-La-Hougue. The cross-section of the shell was thicker (Fig. 6F), and a large number of shell chambers (Okoshi et al. 1987) were also observed. Owing to the thickness of the shell and the tendency for mud to collect in the shell chamber, it is inhabitable by more polydorid individuals. Triploid-cultured oysters were characterized by a cup-shaped valve on the left (Fig. 8C). In this case, the surface area of the shell

increases, becoming a substrate on which more individuals can live. Thus, it is speculated that these microhabitats can be created, and multiple species can coexist within them.

Polydorid species inhabiting the coast of Normandy and the English Channel

In the past, 13 polydorid species, *Polydora ciliata* (Johnston, 1838), *P. hoplura*, *Dipolydora giardi*, *D. coeca* (Örsted, 1843), *D. flava* (de Claparède, 1870), *D. quadrilobata* (Jacobi, 1883), *D. caulleryi* (Mesnil, 1897), *D. armata* (Langerhans, 1880), *Boccardia polybranchia* (Haswell, 1885), *B. semibranchiata* Guérin, 1990, *Boccardiella ligerica* (Ferronnière, 1898), *Pseudopolydora antennata* (Claparède, 1868), and *Ps. pulchra* (Carazzi, 1893) has been reported in this vicinity along the coast of the English Channel in France (Fauvel 1927; Dauvin et al. 2003; Ruellet 2004; Le Mao et al. 2020). Of these, Fauvel (1927) reported three shell-boring polydorids, *D. coeca* (*Polydora*), *D. flava* (*Polydora*), and *P. hoplura* from *Ostrea edulis* Linnaeus, 1758, and Ruellet (2004) recently reported five polydorids, *P. hoplura*, *P. ciliata*, *B. polybranchia*, *B. semibranchiata*, and unidentified *Boccardia* from *Crassostrea gigas* east and west of the Cotentin Peninsula in Normandy. Among the eight Normandy species identified in this survey, only two, *P. hoplura* and *D. giardi*, have been previously reported. The remaining species that remained undocumented during this survey may have moved away from this area or could have been misidentified. Future work is needed to confirm each of the previously reported species individually. *Polydora ciliata* is known to be a non-borer. Species in the *P. ciliata/websteri* complex are morphologically very similar and may be misidentified as *P. websteri* (see discussion by Blake and Kudenov 1978) or other morphologically similar species. In contrast, *B. semibranchiata* was described from the Mediterranean coast of France in 1990 (Guérin 1990) and subsequently from the Atlantic coast of Spain (Martinez et al. 2006). This species has been reported in oyster shells in Veys Bay (Ruellet 2004). *Boccardia semibranchiata* is morphologically very similar to *B. pseudonatrix*, and further species elucidation is required. It has been suggested that *B. polybranchia* may also be morphologically confusing (Simon et al. 2010). Six species, *P. onagawaensis*, *P. websteri*, *Dipolydora* sp., *B. proboscidea*, *B. pseudonatrix*, and *B. hamata*, were added to Normandy in the present survey. Of these, three species, namely *P. websteri*, *B. proboscidea*, and *B. hamata*, have been recently reported in nearby waters (Kerckhof and Faasse 2014; Spilmont et al. 2018; Waser et al. 2020; Gully and Cochu 2020).

Interestingly, the species composition differed between the west and east across the Cotentin Peninsula. While from Blainville-sur-Mer, which is a gravel and sandy oyster culture ground located in the west, *P. hoplura* and *D. giardi*, both species were already known to be distributed in this locality, were observed to inhabit the oyster shells. In contrast, the two species were not reported from the east except Quineville. However, *P. onagawaensis* and *P. websteri*, both species previously unknown from this locality, abundantly inhabited muddy oyster beds in Saint-Vaast-La-Hougue and suspended culture oysters from Quineville and Asnelles, respectively, located in the east. None of these two species was observed on the west side of the peninsula during the present survey. Differences in species composition between the west and the east may be related to differences in habitat environment conditions, such as coastal current, sediment type, and sea water temperature, which was higher in the eastern part of the Cotentin (Dauvin 2019). At the same time, it is crucial to clarify the origin and distribution route of oysters brought to each farm since cultured oysters are transported and moved manually.

In this study, a newly recorded species, *P. onagawaensis*, was found to be distributed on the east side of the Cotentin Peninsula from wild and cultured oyster shells and limestone

substrates. Notably, *P. websteri* was extracted only from suspended cultured oysters, and not from the wild. Further, our previous survey showed that *P. websteri* did not inhabit the shells of cultured oysters from Arcachon Bay, located along the Bay of Biscay, south of Normandy, in 2017 (Sato-Okoshi et al. *in prep*). *Polydora websteri* was recently reported to be abundant in the shells of cultured oysters and naturalized oyster reefs in the Wadden Sea, Netherlands, and Germany (Waser et al. 2020). These results suggest the possibility of new transportation of the species to the east through anthropogenic factors. Further studies on the distribution of *P. onagawaensis* and *P. websteri* along the western European coast, based on accurate species identification, are required.

Specimens of a newly recorded species, *P. onagawaensis*, reported for the first time in Normandy, were compared to the type locality in northeastern Japan and USA (Silverbrand et al. 2021). *Polydora onagawaensis* in France was grouped with a USA specimen in a single clade and was differentiated from three other lineages containing specimens from Japan and the USA (Fig. 3). Moreover, the morphological characteristics of French *P. onagawaensis* differed slightly from those of Japanese *P. onagawaensis*, as described in the remarks section. In the future, the *P. onagawaensis* complex should be studied in more detail by examining its genetic information, morphology, and ecology from additional habitat areas.

Boccardia proboscidea was originally described on the Pacific coast of North America (Hartman 1940), but the number of reports of this species from new locations globally continues to increase (Sato-Okoshi 2000; Sato-Okoshi et al. 2008; Simon and Sato-Okoshi 2015; Jaubet et al. 2018; Spilmont et al. 2018; Radashevsky et al. 2019). *Boccardia proboscidea* was found in coralline algae and the shells of cultured oysters during the present survey. Moreover, it was recently reported along the French coast of the English Channel, on the Opal Coast in northern France in 2014 (Spilmont et al. 2018), and in 2018 in North Brittany (Gully and Cochu 2020).

Boccardiella hamata was also reported for the first time in the English channel. This species was originally described from the Atlantic coast of North America (Webster 1879) and is now commonly known from the Pacific coast, for example, Vancouver Island, Canada (Sato-Okoshi and Okoshi 1997), Japan (Sato-Okoshi 2000), China (Zhou et al. 2010), Korea (Sato-Okoshi et al. 2012), and recently near Belgium and the North Sea (Kerckhof and Faasse 2014). The species has been reported from muddy environments, particularly sandstones (Sato-Okoshi and Okoshi 1997) and rigid man-made substrates such as coastal defense structures on sandy coasts (Kerckhof and Faasse 2014). *Boccardiella hamata* was also recently found to inhabit sponges (Abe et al. 2019a).

Boccardia pseudonatrix, which has never been reported in European waters, was found in the shells of wild and cultured oysters. *Boccardia pseudonatrix* was originally described in South Africa (Day 1967; Simon et al. 2010), from which it may have been artificially transferred to rest of the world by aquaculture or ship ballast water. This species has recently been found in Australia (Sato-Okoshi et al. 2008 (as *B. knoxi*), 2015; Walker 2013) and Japan (Sato-Okoshi et al. 2015; Abe et al. 2019a) and is likely to be found worldwide after accurate identification in the future.

It was unclear from this survey whether each of the eight species inhabiting this water area was introduced as non-indigenous or misidentified due to confusion in species identification. Since the life history of polydorids has been reported to range from less than a year to several years, it can be assumed that the species extracted from the shells of cultured and wild oysters from the oyster farms of Saint-Vaast-La-Hougue and Blainville-sur-Mer could include both the species that originally inhabited the water areas and those that were transported to the areas accompanying the host shells.

Notably, all species of polydorids were identified to the species level in the present study (except *Dipolydora* sp.), which are also found in Japan, especially along the Pacific coast of Tohoku District (Sato-Okoshi 1999; 2000; Abe and Sato-Okoshi 2021). Considering the historical background of cultured oysters in France and Japan, Japanese oysters were abundantly exported from the Pacific coast of Tohoku District of Japan to France (Koike 2015). Future discussions on the species of polydorids inhabiting the waters should consider the anthropogenic transfer of oysters, which could be their hosts and species genetic information.

Analyzing historical trends may be challenging; however, considering the future range expansion of these species, their larval developmental patterns should be studied. Of the eight species confirmed during the survey in March, only a few large individuals of *P. onagawaensis* were found to have oocytes. Further, only *B. proboscidea* produced egg capsules with developed larvae and many nurse eggs. *Polydora onagawaensis*, *D. giardi*, and *B. hamata* have shown simultaneous development and produce planktotrophic larvae with a relatively long planktonic larval phase before settling (Sato-Okoshi 1999; 2000; Teramoto et al. 2013). In contrast, *P. websteri*, *P. hoplura*, and *B. proboscidea* are known to produce adelphophagic larvae, that usually emerge soon before settlement or as a mixture of these and planktotrophic larvae (reviewed by Simon and Sato-Okoshi 2015); *B. pseudonatrix* is known to produce adelphophagic larvae (Sato-Okoshi et al. 2008, as *B. knoxi*; Simon et al. 2010). Further confirmation of the developmental pattern of larvae in this water may lead to the elucidation of distribution expansion.

Effects of polydorids on cultured oysters in Normandy

A high occurrence rate of abnormal shell formation was observed in wild and cultured oysters, with particularly high rates in wild oysters caused by *P. hoplura* and in suspended cultured oysters caused by *P. websteri* and *P. onagawaensis*. Host oysters secrete excess organic and calcareous shell materials to cover and defend against polydorid excavation and repair part of the shell after its penetration. Although we did not confirm oyster death or apparent soft body shrinkage due to the infestation of the oyster shells by polydorids, based on the levels of shell infestation observed, negative impacts on oysters were a serious concern. The decrease in the commercial value of the host oysters, unpredictable environmental conditions, and other unfavorable factors could easily lead to growth inhibition and mortality. For these high-risk polydorid species groups, biological and ecological control measures, such as avoiding the period when the planktonic larvae settle on the host shells and avoiding water areas where larvae aggregate, to prevent invasion, are required to minimize damage to the culture system. At the same time, considering the high density and wide distribution of these species reported today, we need to recognize that invasive alien species have already established their populations in non-indigenous waters and continue to expand their distribution. It is necessary to discuss and evaluate global impacts on ecosystems.

Author contribution W. S.-O.: Research design, field sample collection, specimen examination, data analyses, and manuscript drafting. K. O.: Field sample collection, specimen examination, data analyses, review, and manuscript editing. H. A.: Specimen examination, molecular analyses, review, and editing of the manuscript, and J.-C. D. Research design, field sample collection, and review and editing of the manuscript. All the authors have read and approved the final manuscript.

Data availability Results from the experiments performed by the authors are available upon reasonable request.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Conflict of interest The authors declare no competing interests.

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