

Original article

# Major harmful shell-boring species of polydorids from cultured Akoya oysters in Ago Bay, Japan

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## Introduction

Polydorids are among the most common shell-boring worms in molluscan aquaculture. In 1964, Mizumoto reported that *Polydora ciliata* was the most harmful species affecting the Akoya oyster *Pinctada fucata martensii*, on pearl culturing farms in Ago Bay, Japan, and described control technique such as immersing oysters in saturated brine [1]. The effect of this treatment can be maximized if the peak settlement period for polydorid larvae is known, so it is important to gather the information on the larval morphology and planktonic period of harmful polydorid species. However, several species are morphologically indistinguishable. Because of their wide range of morphologic variations [2], leading to confusion in their classification [3]. This study was therefore designed to identify the major polydorid species in Ago Bay and their larval ecology.

## Materials and methods

From May 2015 to September 2016, we collected polydorid worms from 1- to 3-year-old hatchery-bred Akoya oysters reared on the MIKIMOTO Tatoku Pearl Farm, Ago Bay, Mie. After removing oyster soft tissue from shells, we dissected the shells to collect inhabiting worms. Representative worms were photographed. Under a stereomicroscope, we noted the morphologic characteristics of both live specimens and those preserved in 99.5% ethanol [4].

For molecular sequence analysis, we chose three individuals from the most abundant species, which appeared to be *P. haswelli* from the morphological characteristics. Total DNA was extracted from whole specimen with Phenol-Chloroform (1:1, vol:vol). A fragment of nuclear 18s rRNA gene was amplified by PCR using the following primer set: forward, 5'-TACCTGGTTGATCCTGCCAGTAG-3'; reverse, 5'-GATCCTCCGCAGGTTACCTAC-3' [5]. The amplified products were sequenced by the Fasmac sequencing service (Atsugi, Japan). Partial sequences of

the nuclear 18S rRNA gene were aligned with the sequences of related species obtained from GenBank (<https://www.ncbi.nlm.nih.gov/nucleotide/>). The phylogenetic relationships of polydorid species were constructed using the maximum likelihood method based on the Kimura 2-parameter model in MEGA6 software [6].

Any polydorid egg capsules found were placed in 6-well microplates in 8 mL of filtered seawater. About 50 planktonic larvae per well were cultured at room temperature, and the water was changed every 2 or 3 days. Cultured algae—*Chaetoceros neogracile*, *Chaetoceros calcitrans* and *Pavlova lutheri*—were fed daily to the larvae at  $0.4\text{--}1.5 \times 10^4$  cells mL<sup>-1</sup>.

## Results

From 549 oysters sampled, we collected 147 worms. Of these, 120 were classified into 4 *Polydora* species, 11 were identified to genus only, and 16 could not be identified to species or genus (Table 1). *Polydora haswelli* was the most abundant at 96 worms (65.3%). The morphologic identification was supported by the results of molecular sequence analysis (Fig. 1).

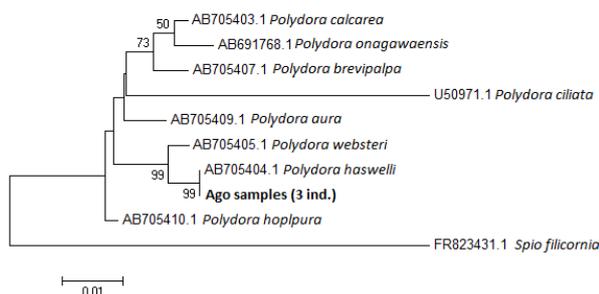
**Table 1.** Polydorid species collected from cultured pearl oysters in Ago bay, Japan

Species	Number of individuals	% of total
<i>Polydora aura</i>	17	11.6
<i>Polydora haswelli</i>	96	65.3
<i>Polydora uncinata</i>	1	0.7
<i>Polydora websteri</i>	6	4.1
<i>Polydora</i> spp.	3	2.0
<i>Dipolydora</i> spp.	7	4.8
<i>Pseudopolydora</i> sp.	1	0.7
Polydorids spp.	16	10.9
Total	147	100.0

We found nine mature *P. haswelli* females. Those collected on 4 June 2015 and 14 September 2016 had many planktonic 3-setiger larvae in egg capsules. These larvae were reared for more than a month. Those reared in 2015 developed only to the 5–7-setiger stage. But in

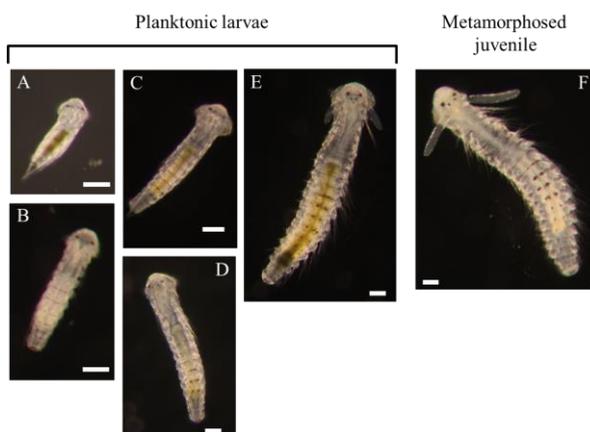
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**Fig. 1.** Maximum likelihood tree inferred from the nuclear 18S rRNA gene sequences of polydorids. The sequence of Ago samples is in bold. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

2016, we observed 5-setiger larvae at 2 weeks, 11-setiger larvae at 3 weeks, and 18-setiger larvae at 1 month (Fig. 2A–E). The 18-setiger larvae were placed in wells containing seawater with pieces of Akoya oyster shell. Four days later, they metamorphosed into juveniles with long palps (Fig. 2F).



**Fig. 2.** Planktonic development and metamorphosed juvenile of *P. haswelli*. A: 5-setiger (body length: 300  $\mu$ m). B: 9-setiger (550  $\mu$ m). C: 11-setiger (750  $\mu$ m). D: 13-setiger (100  $\mu$ m). E: 18-setiger (1450  $\mu$ m) F: metamorphosed juvenile (1600  $\mu$ m). Scale bar, 100  $\mu$ m.

## Discussion

The major harmful polydorid species on cultured Akoya oysters in Ago Bay was *P. haswelli*. This species is well known as one of the most virulent pests of cultured mollusks [7]. Collected worms ranged in size from 1 cm to 3 cm, and in maturity, and egg capsules were found in some burrows. These results confirm that *P. haswelli* reproduced in burrows excavated in shells of cultured Akoya oysters.

*Polydora ciliata* has long been known as a major pest [1], but was not found in this study. Polydorid species associated with mollusk shells have spread globally through commercial transport of host mollusk

shells [4]. *P. haswelli* also may have been transferred from other areas to Ago Bay after the 1960s.

The planktonic period of *P. haswelli* is about a month. Hatched larvae developed from the 3-setiger stage to the 18-setiger stage during rearing in this study. The metamorphosis of 18-setiger larvae into benthic juveniles was induced in the presence of oyster shell but not in the absence. This result suggests that metamorphosis is induced by calcareous substrate such as shells or by mud or biofilm.

Among the three species identified by morphology only (*P. aura*, *P. websteri*, and *P. uncinata*) the morphology of planktonic larvae of except the *P. aura* has been reported [8, 9]. Our results add to these earlier findings. Weekly monitoring of harmful species larvae in culturing area in Ago Bay to reveal seasonal fluctuations and to predict peak settlement times will support better control of polydorids.

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