

Short Article

Does the symbiotic scale-worm feed on the host mussel in deep-sea vent fields?

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Abstract

The scale-worm *Branchipolynoe pettiboneae* is frequently found in the mantle cavity of *Bathymodiolus* mussels living in hydrothermal vents and seeps around Japan. To investigate its feeding habits and symbiotic relationship with the host mussels, we determined the stable nitrogen isotopic composition of glutamic acid and phenylalanine for representative scale-worms including a symbiotic juvenile and a symbiotic adult, both with their host mussels, and a free-living adult, collected from hydrothermal vents of the Hatoma Knoll (~1532 m in depth) in the Okinawa Trough, Japan. The isotopic data suggested the feeding habits that *Bathymodiolus platifrons* is a major source of amino acids for symbiotic adult scale-worm but not for free-living adult and symbiotic juvenile scale-worms.

Key words: *Branchipolynoe pettiboneae*, *Bathymodiolus platifrons*, feeding habits, nitrogen isotopic composition, symbiosis

1. Introduction

Highly productive chemosynthetic communities are associated with deep-sea hydrothermal vents and seeps, which are characterized by symbiotic interactions including primary chemosynthetic bacteria-animal (e.g., bivalve and tubeworm) and secondary animal-animal (e.g., scale-worm) combinations (Desbruyères and Segonzac, 1997; Dubilier et al., 2008). As a representative of animal-animal symbiosis, three species of the scale-worm *Branchipolynoe* spp. (*Branchipolynoe symmytilida*, *Br. seepensis*, and *Br. pettiboneae*) that live symbiotically in the mantle cavity of *Bathymodiolus* mussels, with each species found in a different biogeographic area, were studied (Pettibone, 1984; Pettibone, 1986; Miura and Hashimoto, 1991). *Br. pettiboneae* is frequently found in *Bathymodiolus platifrons* and *Ba.*

japonicus on hydrothermal vents and seeps in Japanese waters (e.g., Miura and Hashimoto, 1991).

So far, however, few studies of *Branchipolynoe* scale-worms have examined their biological and ecological behaviors including feeding habits and symbiotic relationship with the host mussels (e.g., Jollivet et al., 2000). *Br. symmytilida* is parasitic because mussel debris was found in the stomach content (Desbruyères et al., 1985). *Br. seepensis* is a commensal or kleptoparasite because the stomach content of this scale-worm was mainly composed of suspended organic or inorganic materials but lacked mussel debris (Britayev et al., 2003, 2007). However, little is known of the feeding habits, trophic function, and symbiotic relationship with the host mussels of *Br. pettiboneae*.

The stable nitrogen isotopic composition of amino acids has recently been employed as a new tool for the

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estimation of diets and the trophic position of consumers in the food web (e.g., Chikaraishi et al., 2009). Since the isotopic composition shows large (+8.0‰ for glutamic acid) or small (+0.4‰ for phenylalanine) increases from nutrient sources to consumers (e.g., Chikaraishi et al., 2009), trophic ^{15}N -enrichment should be observed in the combination of scale-worm and its host mussel if the only nutrition source for the scale-worm is its host mussel.

In this study, we collected *Br. pettiboneae* and *Ba. platifrons* from hydrothermal vents and determined the stable nitrogen isotopic composition of glutamic acid and phenylalanine in these species to evaluate the feeding habits of *Br. pettiboneae*.

2. Samples and methods

Samples including the scale-worm *Branchipolynoe pettiboneae*, its host mussel *Bathymodiolus platifrons*, and the surface sediment were collected using a suction-sampler of the ROV *Hyper-Dolphin* within approximately 0.04 m² in hydrothermal vents of the Hatoma Knoll field (~1532 m depth) in the Okinawa Trough, Japan, in September 2011 (NT11-20). Free-living (i.e., non-symbiotic) and symbiotic scale-worms were collected in the sediments below host mussel aggregations and in the mantle cavity of the host mussels (Fig. 1), respectively. They were fixed with neutral formalin or ethanol and stored in 70% ethanol at room temperature. The number of individual scale-worms collected was counted and they were classified into two stages, juvenile (less than 21 segments) or adult, based on the number of body segments (Miura and Hashimoto, 1991; Jollivet et al., 2000).

For the isotopic analysis, we used five representative samples including one free-living adult (21 segments), one symbiotic juvenile scale-worm (16 segments) and its host mussel (79 mm in shell length), and one symbiotic adult scale-worm (21 segments) and its host mussel (97 mm in shell length). The entire samples of scale-worms and a small portion of the labial palps of the host mussels were used for the isotopic analysis.

The stable nitrogen isotopic compositions ($\delta^{15}\text{N}$, ‰

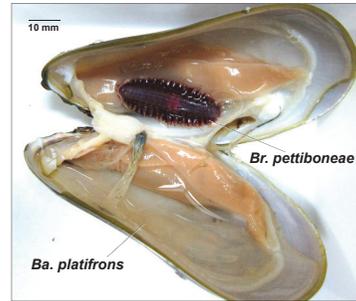


Fig. 1. Photograph of the symbiotic scale-worm *Branchipolynoe pettiboneae* and its host mussel *Bathymodiolus platifrons*.

relative to AIR) of glutamic acid and phenylalanine were determined with a gas chromatograph/isotope ratio mass spectrometer (GC/IRMS) after HCl hydrolysis and *N*-pivaloyl/isopropyl derivatization, according to the method of Chikaraishi et al. (2009). Analytical error in the $\delta^{15}\text{N}$ measurement was less than 0.5‰.

The trophic position (TP) was calculated using an equation with the observed $\delta^{15}\text{N}$ values of glutamic acid (Glu) and phenylalanine (Phe), according to Chikaraishi et al. (2009):

$$\text{TP}_{\text{Glu/Phe}} = [(\delta^{15}\text{N}_{\text{Glu}} - \delta^{15}\text{N}_{\text{Phe}} - 3.4) / 7.6] + 1$$

The standard deviation (1σ) of accuracy in the $\text{TP}_{\text{Glu/Phe}}$ (= [actual TP] - $\text{TP}_{\text{Glu/Phe}}$) was estimated to be 0.12 units for aquatic organisms by Chikaraishi et al. (2009).

3. Results and discussion

3.1. Sample composition and symbiotic ratios

Fifty-seven (11–21 segments) and 20 individuals (13–127 mm in shell length) of *Branchipolynoe pettiboneae* and *Bathymodiolus platifrons* were collected, respectively. Fifty-six *Br. pettiboneae* individuals were found as symbionts in all *Ba. platifrons*. Thirty-six (<21) and 20 (21 segments) individuals were juveniles and adults, respectively. Each single host mussel was infested with single adult scale-worm and 0–21 juvenile scale-worms. The 100% infestation rate was greater than those of two other *Branchipolynoe* species (<~80%) (e.g., Britayev et al., 2007). Only 1 (21 segments) individual was found as a free-living

adult scale-worm.

3.2. Nitrogen isotopic composition of amino acids and estimated trophic position

The $\delta^{15}\text{N}$ values of glutamic acid and phenylalanine and the estimated $\text{TP}_{\text{Glu/Phe}}$ value were +18.1‰, +0.9‰, and 2.8 for the free-living adult; +12.8‰, -1.0‰, and 2.4 for the symbiotic juvenile; and +12.8‰, +0.3‰, and 2.2 for the symbiotic adult scale-worms, respectively (Fig. 2). The $\delta^{15}\text{N}$ values of glutamic acid and phenylalanine were -0.7‰ and -4.6‰ for the host mussel of the juvenile scale-worm and +4.3‰ and +0.1‰ for the host mussel of the adult scale-worm, respectively (Fig. 2). The estimated $\text{TP}_{\text{Glu/Phe}}$ value was 1.1 for both host mussels (Fig. 2), suggesting that they are primarily amino acid producers in this ecosystem.

3.3. Feeding habits of scale-worms

The $\delta^{15}\text{N}$ value of phenylalanine of the adult symbiotic scale-worm (0.3‰) was close to that of the host mussel (0.1‰), and the $\delta^{15}\text{N}$ values of glutamic acid increased by 8.5‰ from the host mussel (4.3‰) to the adult scale-worm (12.8‰) (Fig. 2). These results are consistent with the change in the $\delta^{15}\text{N}$ value generally found in the consumer-nutrient relationship in the

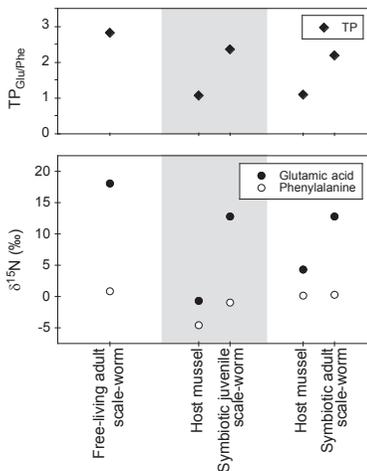


Fig. 2. Stable nitrogen isotopic composition ($\delta^{15}\text{N}$) of glutamic acid and phenylalanine and the estimated trophic position ($\text{TP}_{\text{Glu/Phe}}$) of *Branchipolynoe pettiboneae* and *Bathymodiolus platifrons*.

grazing food web (i.e., +0.4‰ for Phe and +8.0‰ for Glu from nutrient source to consumer, Chikaraishi et al., 2009), and therefore clearly show that the host mussel is a major source of amino acids for the symbiotic adult scale-worm (Fig. 3).

In contrast, the difference in $\delta^{15}\text{N}$ value between the host mussel and juvenile symbiotic scale-worm was 3.6‰ for phenylalanine and 13.5‰ for glutamic acid (Fig. 2), which is inconsistent with the general shift in the $\delta^{15}\text{N}$ value in the grazing food web. These results suggest that the host mussel may not function as a major source of amino acids for the juvenile scale-worms, even though the scale-worm was found in the mantle cavity of the mussel (Fig. 3). Moreover, the difference in the $\text{TP}_{\text{Glu/Phe}}$ value between the host mussel (1.1) and the juvenile scale-worm (2.4) was 1.3 units (i.e., did not fall in the range of 1.0 ± 0.1). This difference cannot be simply explained if the juvenile scale-worm specifically feeds on the host mussel.

The $\delta^{15}\text{N}$ value of phenylalanine in the free-living adult scale-worm (0.9‰) was close to the range of $\delta^{15}\text{N}$ values found in other samples (from -4.6‰ to 0.3‰) (Fig. 2). Its $\text{TP}_{\text{Glu/Phe}}$ value (2.8) indicates that mussel tissues are relatively minor source of amino acids for free-living scale-worms (Fig. 3) because the $\text{TP}_{\text{Glu/Phe}}$ value of the mussels was as small as 1.1. Thus, free-living scale-worms may be among the secondary consumers in the hydrothermal vent ecosystem.

The isotopic data suggest the feeding habits of *Br. pettiboneae* depend on whether it is free-living or

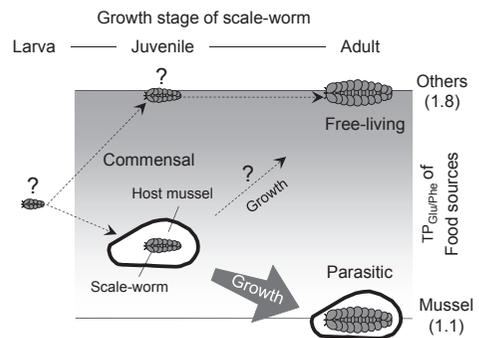


Fig. 3. Model of the life cycle and changes in feeding habits of the scale-worm *Branchipolynoe pettiboneae*.

symbiotic, and the life stage (i.e., juvenile or adult). *Ba. platifrons* is a major source of amino acids for symbiotic adult scale-worms, but not for free-living adult or symbiotic juvenile scale-worms.

In previous studies, the form of symbiosis of other scale-worm species was suggested to be parasitism for *Br. symmyitilida* and commensalism (or klepto-parasitism) for *Br. seepensis* (Desbruyères et al., 1985; Britayev et al., 2003, 2007). However, this study suggests a change in the form of symbiosis for *Br. pettiboneae* during the life stages, and we speculate that commensalism occurs in the juvenile stage (e.g., juvenile scale-worms may use mussels as shelter to increase their survival rate in this stage) and parasitism occurs in the adult stage (Fig. 3). We also discovered a free-living adult scale-worm and suggest that it feeds on other organisms as an omnivore and a carnivore.

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