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Left-right Reversal Arrangement of Glochidial Threads in *Sinanodonta lauta* (Bivalvia: Unionidae)

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Abstract: This is the first report on the left-right reversal arrangement of organs in *Sinanodonta lauta* glochidia. A glochidial larva possesses a pair of subtriangular-shaped shells, a pair of claw-like hooks with spines at the ventral margin, internal and external glochidial threads, four pairs of sensory hair tufts and one adductor muscle. The arrangement of the internal thread and the origin of the external thread is asymmetric between the pair of valves. As a result of examination of approximately 50 glochidia by light microscopy, one was observed with the internal thread and the origin of the external thread positioned on the left valve. These morphological features are positioned on the right valve in normal glochidia. The relative position of these threads between abnormal and normal glochidia represents a left-right reversal.

Keywords: situs inversus viscerum, internal thread, external thread, larva, freshwater mussel

Introduction

Left-right reversal of organs, shell, internal and external skeletons is observed in many metazoan taxa, such as mammals (Oki *et al*., 2009), amphibians (Toyoizumi *et al*., 1997), fishes (Follett *et al*., 1960), insects (Hayashi & Murakami, 2001), crustaceans (Duguid, 2010), molluscs (Nakadera *et al*., 2010) and echinoderms (Emlet, 2009). However, examples of situs inversus viscerum are rarely found in nature (Toyoizumi *et al*., 1997). One of causes of left-right reversal in the animal body is genetical control (Hozumi *et al*., 2006) and it is also induced by physical stimulation (Kuroda *et al*., 2009), associated with adaptation to the environment. However, little is known about the controlling mechanism in Bivalvia.

Glochidia are the larvae in three families of Unionida: Unionidae, Hyriidae and Margaritiferidae (Graf & Cummings, 2006). Most of these glochidia parasitise suitable fish or amphibians to facilitate metamorphosis into mussels, although some unionid glochidia can grow into juveniles without parasitism (Lefevre & Curtis, 1911; Howard, 1914). Glochidia are small (70–392 μm) with a calcified or amorphous bivalved glochidial shell and a single adductor muscle (Kondo, 1997; Graf & Cummings, 2006; Kondo, 2008). Some glochidia have other morphological features such as hooks, spines, glochidial threads and sensory hair tufts (McMahon & Bogan, 2001), and left-right organ asymmetry is another morphological feature. In the present study, to clarify the presence of left-right reversal arrangement in soft part of freshwater mussels, glochidia of *Sinanodonta lauta* were examined in detail by light microscopy.

Materials and Methods

Two mussel species, *Sinanodonta lauta* and *S. japonica*, were caught from the bottom of an

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agricultural ditch through a paddy field in Kinomoto-cho, Shiga Prefecture, central Japan in June 2011. These two species are widely distributed in lotic and lentic habitats in Japan (Kondo, 2008). Identification based on shell morphology is difficult because of shell plasticity, which is dependent on environments. However, Kondo *et al*. (2011) reported that species can be identified in specific areas, including Kinomoto-cho, using the following discriminant function: Y = –1.045 × shell length (mm) + 1.092 × shell height (mm) +1.383 × shell width (mm) – 13.165. When Y > 5, the specimen is always regarded as being *S. lauta*, at least in Kinomoto-cho, and we employed this measure in species identification. Shell dimension was measured using a digital vernier calliper (Mitsutoyo Corporation) with an accuracy of 0.01 mm. To determine the presence of glochidia and eggs in the brood pouch, the valves were slightly opened using a shell opener and the outer demibranch was examined by the naked eye. Only the outer demibranch functions as a brood pouch in *S. lauta*. Two large mussels with an inflated demibranch were selected and transported to the laboratory. The mussels were reared in aquaria containing 1000 ml dechlorinated tap water with aeration (water temperature, 20°C). Enforced release of glochidia from the female mussel was induced under these conditions (Aldridge & McIvor, 2003). Live glochidia (n = approximately 50) settling at the bottom of the aquaria were collected using a graduated pipette, and the morphology of their exterior and visceral organs was carefully examined by light microscopy. The glochidia of *S.lauta* are subtriangular, hooked and buff in color. The number of teeth larger than 10 μm on each hook is more than 12, and shell height (284–329 μm) is greater than shell length (263–329 μm), as revealed by previous studies (Kondo *et al*., 2006; Kondo, 2008). Anterior-posterior orientation of glochidia was determined based on the positional relationships between the shell margin and adductor muscle. The anterior margin of a glochidial larva is characterized as the margin closest to the single adductor muscle (Hoggarth, 1987).

Results

The morphology of a normal *S. lauta* glochidium is shown in Figs. 1 and 2. The glochidium is composed of hard shells and a soft body. The shells are subtriangular in outline with a straight hinge line (Fig. 1), and a pair of claw-like hooks is present at the ventral margin (Fig. 2). Long spines are present on the hook and small spines are situated close to it (Fig. 2). One adductor muscle (Fig. 1A, C) and four pairs of sensory hair tufts are present in the soft parts. A coiled, thick thread (internal thread) is embedded in the soft body, and a thin thread (external thread) protrudes from the centre of the hinge line (Fig. 1C, D). A connection between the internal and external threads could not be ascertained. The internal thread and the origin of the external thread appear to be arranged asymmetrically (Fig. 1), but the shell outline, hooks, sensory hair tufts and adductor muscle show a bilaterally symmetrical arrangement. In other words, the internal thread and the origin of the external thread are normally positioned only on the right valve of the glochidium (Fig. 1).

As a result of our investigation, one glochidium with the internal thread and the origin of the external thread positioned on the left valve was identified (Fig. 3). The presence of both threads was easy to confirm by light microscopy at ×150 magnification or higher. This arrangement is clearly different from that observed in normal glochidia (Figs. 1, 3). Irrespective of the difference in the position of glochidial threads between normal and abnormal glochidia, an external thread protruded through the near anterior margin of shells in all specimens with the thread examined (Figs. 1C, D and 3). The difference in positional relationship between normal and abnormal glochidia is thus a left-right reversal arrangement. This asymmetric relationship could not be confirmed for other organs because of their bilaterally symmetric arrangement.

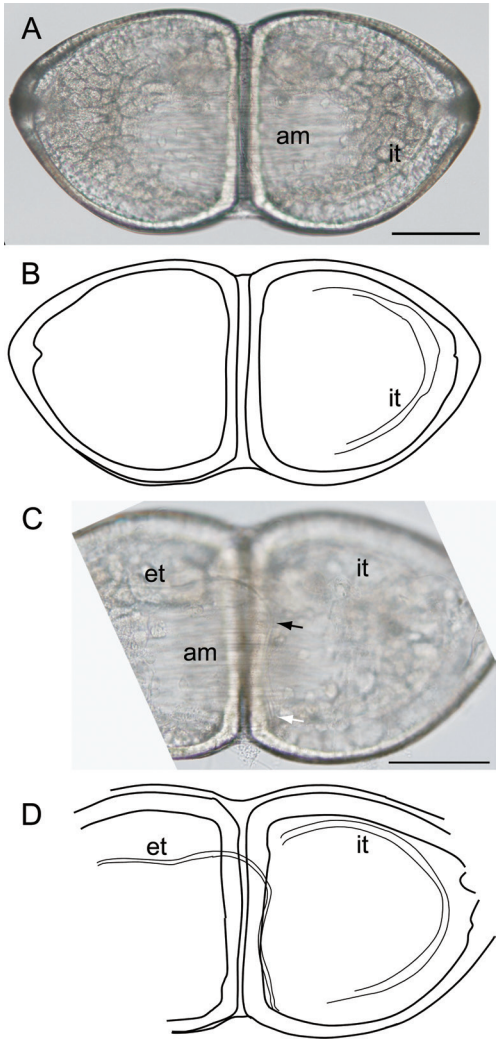

Fig. 1. Normal glochidia of *Sinanodonta lauta*. Upper and lower images represent posterior and anterior part of glochidia, respectively. **A**, **B**. Normal glochidium without external thread because of dissolve. **C**, **D**. Normal glochidium with external thread but the image is partly lacked. Black and white arrows in C indicate the protrusion site of the external thread and the origin of the external thread, respectively. Abbreviations: am, adductor muscle; et, external thread; it, internal thread. Scale bars = 100 μm.

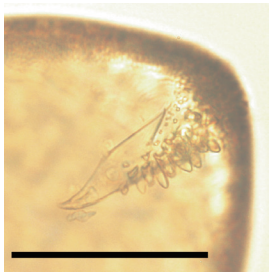

Fig. 2. A claw-like hook with spines on the ventral margin of *Sinanodonta lauta* glochidium. Scale bar = 50 μm.

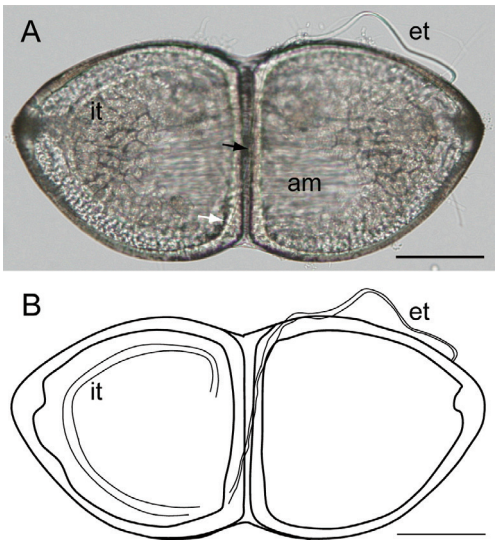

Fig. 3. Abnormal glochidia of *Sinanodonta lauta*. Upper (**A**) and lower (**B**) images represent posterior and anterior part of glochidia, respectively. Black and white arrows in A indicate the protrusion site of the external thread and the origin of the external thread, respectively. Abbreviations: am, adductor muscle; et, external thread; it, internal thread. Scale bars = 100 μm.

Discussion

The findings of the comparison between normal and abnormal *S. lauta* glochidia clarifies the fact that the latter exhibits situs inversus viscerum, which has also been reported in some invertebrates such as snails (Kuroda *et al*., 2009) and sea urchins (Emlet, 2009). In the present

study, the authors could not clarify if situs inversus viscerum of *S. lauta* larva happened to part of the internal organs (situs inversus partialis) or all organs (situs inversus totalis) because we did not investigate the embryonic development of the specimen. Furthermore, all organs except for the larval threads are symmetrically arranged in *S. lauta* glochidia.

The presence of an internal thread has been established in unionid glochidia of many genera, including *Anodonta*, *Unio*, *Acticosta*, *Lanceolaria*, *Cuneopsis*, *Lamprotula*, *Aculamprotula*, *Hyriopsis* and *Cristaria* (Wood, 1974; Pekkarinen & Englund, 1995; Wu *et al*., 1999). Glochidia of *Nodularia douglasiae biwae* and *Pronodularia japanensis* have the internal thread on the right valve (Y. Akiyama, unpublished data). However, mature glochidia of *Margaritifera margaritifera* and *Pseudanodonta camplanata* do not have the internal thread (Pekkarinen & Englund, 1995; Pekkarinen & Valovirta, 1996). Thus, discrimination among glochidia showing left-right reversal arrangement of larval thread organs can be examined in some unionid species.

An external thread protruding by approximately half of the hinge length from the anterior end has been observed in *Anodonta anatina*, *Anodonta cygnea* and *Anemina arcaeformis*, whereas this organ is at the anterior end in *Unio pictorum* and *Unio tumidus* (Wood, 1974; Pekkarinen & Englund, 1995; Lee *et al*., 2007). In *S. lauta*, the protrusion site of the external thread corresponds with that in the former cases, and thus the protrusion position site of this thread may be a determinant at the tribe level. The origin of the external thread in *A. cygnea* is positioned on the right valve according to Wood (1974), which is consistent with the observation made in *S. lauta*, but its position is unknown in other species. The position of the origin of the external thread is an indicator of situs inversus viscerum, although this thread dissolves immediately after release from gravid mussels in *Anodonta* and *Unio* (Arey, 1921). The external thread is generally thinner than the internal thread (Wu *et al*., 1999), while the former is absent in *Lampsilis*, *Obliquaria*, *Obovaria*, *Plagiola*, *Pleurobema*, *Quadrula*, *Tritogonia* and *Symphynota* (Lefevre & Curtis, 1910).

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ヌマガイにおける幼生糸の左右逆転配置

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要　約

本論文はイシガイ科貝類で初めて発見されたグロキディウム幼生の幼生糸の逆転配置について報告するものである。2011 年 6 月に滋賀県木之本町の農業用水路で成熟したグロキディウム幼生を有するヌマガイ 2 個体を採集し，母貝から放出された幼生の観察を行なった。ヌマガイの幼生は 1 対の亜三角形の殻が 1 辺で接合した対称的な形をしていた。軟体部を観察した結果，外部幼生糸の根元と内部幼生糸の配置が左右非対称であり，両者はほとんどの幼生で身体の右側に位置した。約 50 個体の幼生を観察した結果，身体の左側に外部幼生糸の根元と内部幼生糸を有する幼生が 1 個体のみ確認されたことから，この個体では少なくとも内臓が部分的に左右逆転していたと考えられる。