Host Species for Glochidia of the Freshwater Unionid Mussel

Inversiunio jokohamensis

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Abstract: The host species for the glochidia of the freshwater unionid mussel Inversiunio jokohamensis were identified by determining whether these glochidia had infected the following fish taxa collected from a paddy field ditch in Tochigi Prefecture, Kanto area, central Japan: Nipponocypris spp., Gnathopogon elongatus, Carassius spp., Misgurnus anguillicaudatus, Cobitis biwae, Lefua echigonia, and Rhinogobius sp. OR (orange type). The fishes were kept in tanks for 10 or 11 days, and the numbers of glochidia and metamorphosed juveniles detached from the hosts were counted. Living juveniles detached from the bodies of only Nipponocypris spp., L. echigonia, and Rhinogobius sp. OR. The number of glochidia infecting the fishes, the infection site, and the rate of glochidial encystment were determined using formalin-fixed specimens of N. temminckii, N. sieboldii, G. elongatus, Ca. buergeri subsp.1, M. anguillicaudatus, Co. biwae, L. echigonia, Rhinogobius sp. OR, and the frog Hyla japonica (tadpole). Glochidia had infected 3 host species i.e. L. echigonia, Rhinogobius sp. OR, and N. temminckii. Most glochidia were attached to the gills and fins of the fishes. More glochidia were attached to and encysted on L. echigonia and Rhinogobius sp. OR than on N. temminckii. L. echigonia was identified as a new host species for the glochidia of I. jokohamensis in addition to Rhinogobius sp. OR and N. temminckii.

Keywords: glochidia, host, Inversiunio jokohamensis, juveniles, Lefua echigonia

Introduction

The freshwater unionid mussel Inversiunio jokohamensis inhabits ponds and rivers in Japan. Like other unionid mussels, it is used by bitterlings as a spawning site and is important for the conservation of bitterling species (Kihira et al., 2003; Masuda & Uchiyama, 2004; Kondo, 2008). In recent years, however, the number of mussels has decreased, and bitterlings have almost become extinct, particularly at several sites in Kanto area in central Japan (Kondo, 1994; Negishi et al., 2008). The larvae of unionid mussels (glochidia) parasitize living fish (Dudgeon & Morton, 1984; Itoh et al., 2003; Itoh et al., 2008; Kondo, 1989), and metamorphose into juveniles at the end of their parasitic stage and then settle on the riverbed (Fukuhara et al., 1990). The breeding season and host specificity of I. jokohamensis have already been examined. Rhinogobius spp., Nipponocypris temminckii, and Tridentiger brevispinis are known as the hosts of I. jokohamensis glochidia (Kondo, 2008). In this study, we report a new host species, Lefua echigonia, for the

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glochidia of *I. jokohamensis* in a paddy field ditch in Tochigi Prefecture, Kanto area, central Japan.

**Materials and Methods**

**Proportions of glochidia and juveniles detached from host fishes**

On July 26, 2008 and March 21, April 11, and May 30, 2009, fishes were collected from a branch of the Tonegawa River (a paddy field ditch, approximately 500 m long) inhabited by mussels and located in the east of Tochigi Prefecture (Kakino *et al.*, 2006a; Kakino *et al.*, 2006b; Kakino *et al.*, 2007). *I. jokohamensis* was the only unionid mussel inhabiting the ditch.

We collected seven fish taxa from this ditch by using a dip net. The following taxa were identified (Table 1): *Nipponocypris* spp. (*N. temminckii* and *N. sieboldii*), *Gnathopogon elongatus*, *Carassius* spp. (*Ca. buergeri* subsp.1 and *Ca. langsdorfii*), *Misgurnus anguillicaudatus*, *Cobitis biwae*, *Lefua echigonia*, and *Rhinogobius* sp. OR (orange type; see Akihito *et al.*, 2000, for the identification of this undescribed species). We conducted a total of 11 tests in this experiment, with each test lasting 10 or 11 days. The fishes were moved to 11 discrete tanks respectively for the 11 tests. Each tank was filled with 6 L of aerated water at 28°C, and half the water was changed every 1–3 days. The light-dark regime was not controlled. The fishes were not fed during the tests. The water in the tanks was filtered through a plankton net (mesh opening, 0.113 mm), and the glochidia and juveniles on the net were counted under a stereoscopic microscope at magnifications of ×10 to ×40 every 1–3 days (average, 36.8 h) for 10 or 11 days. The proportions of the glochidia and juveniles that detached from the fishes in each tank were calculated.

**Number of glochidia infecting the hosts’ bodies and infection sites**

The following eight fish species and one amphibian were collected on May 30, 2009, and fixed in 10% formalin: *N. temminckii*, *N. sieboldii*, *G. elongatus*, *Ca. langsdorfii*, *M. anguillicaudatus*, *Co. biwae*, *L. echigonia*, *Rhinogobius* sp. OR, and the frog *Hyla japonica* (tadpoles) (Table 2). We examined these specimens for the number of infecting glochidia, the infection sites, and the development of glochidial encystment. We examined gill filaments, gill rakers, the inner side of the opercula, pectoral fins, pelvic fins, anal fin, caudal fin, dorsal fin, buccal cavity, and other surfaces for the glochidia, under a stereoscopic microscope of 10 or 40 times power.

**Results**

**Proportion of glochidia and juveniles after detachment from host fishes**

The proportions of glochidia and juveniles are shown in Fig. 1 and Table 1. Glochidia (75.0–93.7%) were observed in the tank of *Nipponocypris* spp. on days 1–3 of all tests (1st–4th), and juveniles (6.3–25.0%) were observed on days 2–4; neither were observed on days 5–11. In the case of *L. echigonia*, glochidia (16.8%) were observed on days 1–4 of the 10th test, and juveniles (83.2%) were observed on days 1–5; neither glochidia nor juveniles were observed on days 6–11. In the case of *Rhinogobius* sp. OR, both glochidia (10.7%) and juveniles (89.3%) were observed on days 1–5 of the 11th test, and neither were observed on days 6–10. For the remaining 4 species, glochidia were observed on the first day of the tests, and no juveniles were seen.

All glochidia that detached from the bodies of fishes during the tests were dead (Fig. 2A); the shells of the glochidia were light brown and half-open. Most of the juveniles detached from the hosts were alive (Fig. 2B); they opened and closed their shells, and moved by stretching and contracting their foot. Their body was light brown and equal in size to that of the glochidia, and they retained their glochidial shells.
### Table 1. Details of the fish species for the experiment 1, collected in a paddy field ditch in Tochigi Prefecture, Kanto Area, central Japan.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Nipponocypris spp.</td>
<td>1st Jul 26–Aug 5, 2008</td>
<td>5</td>
<td>41.4</td>
<td>5</td>
<td>38</td>
<td>7</td>
</tr>
<tr>
<td>(N. temminckii and N. sieboldii)</td>
<td>2nd Mar 21–Apr 2, 2009</td>
<td>13</td>
<td>48.7</td>
<td>2</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>3rd Apr 11–21, 2009</td>
<td>7</td>
<td>61.6</td>
<td>0</td>
<td>56</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>4th May 30–Jun 10, 2009</td>
<td>15</td>
<td>33.7</td>
<td>15</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Gnathopogon elongatus</td>
<td>5th Mar 21–Apr 2, 2009</td>
<td>6</td>
<td>49.4</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Carassius spp.</td>
<td>6th Apr 11–21, 2009</td>
<td>13</td>
<td>42.3</td>
<td>11</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>(Ca. buergeri subsp.1 and Ca. langsdorfii)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Misgurnus anguillicaudatus</td>
<td>7th Mar 21–Apr 2, 2009</td>
<td>10</td>
<td>65.4</td>
<td>3</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>Cobitis biwae</td>
<td>8th Jul 26–Aug 5, 2008</td>
<td>10</td>
<td>43.4</td>
<td>10</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9th Apr 11–21, 2009</td>
<td>17</td>
<td>43.9</td>
<td>14</td>
<td>249</td>
<td>0</td>
</tr>
<tr>
<td>Lefua echigonia</td>
<td>10th Mar 21–Apr 2, 2009</td>
<td>11</td>
<td>35.5</td>
<td>11</td>
<td>19</td>
<td>96</td>
</tr>
<tr>
<td>Rhinogobius sp. OR</td>
<td>11th Jul 26–Aug 5, 2008</td>
<td>2</td>
<td>26.3</td>
<td>1</td>
<td>6</td>
<td>42</td>
</tr>
</tbody>
</table>

Provided: no. of fish provided to the test; Survived: no. of fish survived until the end of the test.
Table 2. Details of the specimens for the experiment 2, used to determine the number of *Inversinumio jokohamensis* glochidia attached to the host species, the sites of attachment, and occurrence of glochidial encystment. All specimens were collected from a paddy field ditch in Tochigi Prefecture, Kanto area, central Japan on May 30, 2009.

<table>
<thead>
<tr>
<th>Species</th>
<th>Fish</th>
<th>Standard length (mm)</th>
<th>I. Jokohamensis</th>
<th>Glochidian encystment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Median</td>
<td>Maximum</td>
<td>Minimum</td>
</tr>
<tr>
<td>Nipponocypris temminckii</td>
<td>6</td>
<td>43.5</td>
<td>52.0</td>
<td>32.3</td>
</tr>
<tr>
<td>Nipponocypris sieboldi</td>
<td>2</td>
<td>35.5</td>
<td>40.7</td>
<td>30.2</td>
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<tr>
<td>Gnathopogon elongatus</td>
<td>4</td>
<td>44.8</td>
<td>47.0</td>
<td>37.2</td>
</tr>
<tr>
<td>Carassius buergeri subsp.</td>
<td>4</td>
<td>46.4</td>
<td>53.1</td>
<td>40.1</td>
</tr>
<tr>
<td>Misgurnus anguillicaudatus</td>
<td>7</td>
<td>48.6</td>
<td>95.0</td>
<td>42.4</td>
</tr>
<tr>
<td>Cobitis biwaie</td>
<td>4</td>
<td>42.0</td>
<td>45.2</td>
<td>41.5</td>
</tr>
<tr>
<td>Lefua echigonia</td>
<td>5</td>
<td>34.6</td>
<td>47.1</td>
<td>29.5</td>
</tr>
<tr>
<td>Rhinogobius sp. OR</td>
<td>9</td>
<td>38.0</td>
<td>47.6</td>
<td>27.7</td>
</tr>
<tr>
<td>Hyla japonica (tadpole)</td>
<td>9</td>
<td>33.0</td>
<td>36.1</td>
<td>33.0</td>
</tr>
</tbody>
</table>

Fig. 1. Proportion of glochidia and juveniles after detachment from the host fishes (July 2008 to May 2009).

**Number of glochidia infecting the hosts’ bodies and glochidial encystment rate**

Glochidia were attached to only three host species: *L. echigonia*, *Rhinogobius* sp. OR, and *N. temminckii* (Table 2). The median standard lengths of the host, *L. echigonia* (median, 34.6 mm), *Rhinogobius* sp. OR (median, 38.0 mm), and *N. temminckii* (median, 43.5 mm) did not differ significantly (Kruskal-Wallis test, $n_1 = 5$, $n_2 = 6$, $n_3 = 9$, $H = 41.8$, $P > 0.05$). The prevalences of glochidia in both *L. echigonia* and *Rhinogobius* sp. OR were 100% and that in *N. temminckii* was 66.7%.

The median abundances of the glochidia infected to the 3 host species, *N. temminckii*, *L. echigonia*, and *Rhinogobius* sp. OR are shown in Table 2. More glochidia were attached to *L. echigonia* (median, 15.0 individuals) than to *N. temminckii* (median, 1.0 individuals) (Kruskal-Wallis test, $n_1 = 5$, $n_2 = 6$, $n_3 = 9$, $H = 9.3$, $P < 0.05$, Mann-Whitney U-test with Bonferroni
Host Species for *Inversianio jokohamensis*

Correction, \( n_1 = 5, n_3 = 9, U = 42.7, P < 0.0167 \). The number of glochidia attached to *L. echigonia* and *Rhinogobius* sp. OR (median: 6.0 individuals) did not differ significantly (Mann-Whitney U-test with Bonferroni correction, \( n_1 = 5, n_2 = 6, U = 48, P > 0.0167 \)). The number of glochidia attached to *Rhinogobius* sp. OR was slightly higher than that for *N. temminckii* (Mann-Whitney U-test with Bonferroni correction, \( n_3 = 9, n_2 = 6, U = 29, P < 0.0167 \).

The encystment rates for *Rhinogobius* sp. OR (100%) and *L. echigonia* (92.2%) were slightly higher than the rate for *N. temminckii* (75.0%) (Table 2).

The sites of glochidial infection on the hosts’ bodies are shown in Fig. 3. Glochidia were attached only to the gill filaments in *Rhinogobius* sp. OR and *N. temminckii*. In the case of *L. echigonia*, glochidia were attached to the gill filaments (60.9%), gill rakers (4.7%), the inner side of the operculum (23.4%), pectoral fins (3.1%), pelvic fins (1.6%), caudal fins (1.6%), and dorsal fins (4.7%). No glochidia were attached to the anal fins, buccal cavity, or other body surfaces in any of the three host species.

**Discussion**

We identified *L. echigonia* as a new major host for the glochidia of *I. jokohamensis*; in addition, we reconfirmed that *Rhinogobius* sp. OR and *N. temminckii* are hosts for these glochidia (Kondo, 2008). Many parasitic glochidia metamorphosed normally into juveniles, and the rate of metamorphosis exceeded 80% in the case of *L. echigonia*.

Our study has some limitations. A limited number of fishes were used. All glochidia could not be counted because some of the host fishes died during experiment 1. With some taxa (*G.
elongatus, Ca. spp., M. anguillicaudatus, Co. biwae), no juveniles detached from the fishes in Experiment 1 even though glochidia that detached from the bodies of the fishes were observed. In experiment 2, no glochidia were seen attached to these fishes. Formalin fixation, which was performed in experiment 2, leads to the opening of glochidial shells and may be responsible for the absence of glochidia in this experiment (Itoh, unpublished data). Thus, the calculated proportions of glochidia and juveniles may not be entirely accurate. Nonetheless, our findings are promising, and this topic should be studied in greater detail in the future.

The rate of metamorphosis from glochidia to juveniles in the case of Nipponocypris spp. (<25%) was lower than the rates previously reported (for example, Kondo, 2008). Although many glochidia had infected Nipponocypris spp., only a few of the glochidia attached to N. temminckii metamorphosed into juveniles. To determine a suitable host species for glochidia, the rate of metamorphosis should be calculated accurately (Itoh et al., 2003; Itoh et al., 2008).

We reconfirmed the previously listed host species for the glochidia of I. jokohamensis (for example, Kondo, 2008). The host species identified by Kondo (2008) were collected from the Kansai area, whereas those examined in this study were collected from the Kanto area. The difference in the study sites may explain the difference in the results of the two studies.

Kondo (2008) has reported that glochidia mainly attach to the gills and fins of host fishes, and also that the glochidial infection site varies with the species of unionid mussel (Itoh et al., 2003; Kondo, 2008). For example, the glochidia of Anodonta “woodiana” (a species group comprising A. japonica and A. lata) are parasitic on gills and fins (Fukuhara et al., 1990); and glochidia of Pronodularia japonensis are parasitic on various sites (e.g. all fins, gills, the inner side of the operculum, the buccal cavity) (Itoh et al., 2003). In contrast, most glochidia of I. jokohamensis were parasitic on the gill filaments of the three host species. I. jokohamensis glochidia may be more likely to come into contact with the gill filaments, and these surfaces may be suitable for parasitism by them (Atkins, 1979; D’Eliscu, 1972). The glochidia of I. jokohamensis do come into contact with the fins or buccal cavity of the hosts but may not be able to parasitize these sites. Therefore, when identifying host species for the glochidia of I. jokohamensis, it is insufficient to examine only the fins and body surface of the hosts.

Encystment of glochidia on the host’s body prevents them from detaching and protects them from external damage. We found that many glochidia on suitable host species were encysted. The determination of the encystment rates may be therefore useful in studies on the host species for the glochidia of unionid mussels (D’Eliscu, 1972).

Rhinogobius sp. OR widely inhabits the study site. N. temminckii also widely inhabits the study site but is a nonindigenous species in the Kanto area. L. echigonia inhabits a limited area of the study site, for example, a branch in the upper stream and a thicket of emergent plants in the stream (Kakino et al., 2006a; Kakino et al., 2006b; Kakino et al., 2007; Kakino et al., unpublished data). The loaches Co. biwae and M. anguillicaudatus and the minnow G. elongatus and the crucian carp Carassius spp. also widely inhabit the study site but are not host species for I. jokohamensis glochidia. Therefore, in this study site, Rhinogobius sp. OR was the dominant host for these glochidia, and L. echigonia was the most suitable host.

Adult mussels can move against the stream current, but only very slowly (Kondo & Kano, 1993). Glochidia cannot swim, and only float downstream (Howard, 1951; Kondo, 1984; Kondo, 2008). Thus, to disperse over a wide area, mussels require hosts that can spread glochidia over large distances (Itoh et al., 2003). The detection of new host species will help clarify some aspects of the ecology of unionid mussels.

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References


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ヨコハマシジラガイのグロキディウム幼生の寄生生態

伊藤寿茂・柿野亘・吉田豊

要約

栃木県東部の水田用水路に生息するヨコハマシジラガイのグロキディウム幼生の宿主となる生物を調べた。

生息地においてその寄生を受けていると想定される7種類の魚類（カワムツ類、タモロコ、フナ類、ドジョウ、シマドジョウ、ホトケドジョウ、トウヨシノポリ）を採集し、実験水槽内で継続飼育して、魚体から離脱してきた幼生を観察、計数したところ、ホトケドジョウ、トウヨシノポリ、カワムツ類の3種より、変態を終了させた稚貝が得られた。全離脱数に占める稚貝の出現率はホトケドジョウで83.3%，トウヨシノポリで89.3%，カワムツ類で6.3～25.0%であった。

次に、同生息地において8種類の魚類（カワムツ、ヌマムツ、タモロコ、キンブナ、ドジョウ、シマドジョウ、ホトケドジョウ、トウヨシノポリ）とニホンアマガエル幼生を採集し、10%ホルマリン水溶液で固定して魚体に寄生した幼生の状態を観察、計数したところ、上述の3魚種の体に寄生が見られた。寄生部位は大部分が鰓弁であり、ホトケドジョウのみ各鰭や鰓蓋の内側、鰓耙にも寄生が見られた。寄生幼生の被嚢率はトウヨシノポリとホトケドジョウでは92.2%以上と高かったのに対して、カワムツではやや低く75.0%であった。

これらの結果から、カワムツとトウヨシノポリに加えて、新たにホトケドジョウがヨコハマシジラガイの宿主として有用であることが確かめられ、稚貝の出現率が高い種では、寄生数、被嚢率が高くなる傾向が認められた。