

## Host Fish Species for the Glochidia of *Anodonta japonica* Inhabiting Drainage Ditches for Rice Cultivation in Hikone City

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The larvae of freshwater mussels in the family Unionidae are obligate parasites of fishes and amphibians prior to their metamorphosis into juveniles. Suitable host species vary among mussel species, and even where two mussel species in the same genus use fishes of a single genus as host, the host species is different in some cases (Jones *et al.*, 2006). Therefore, determining the hosts for individual mussel species represents a very important ecological study on Unionidae as well as other families in the Unionoida.

In Japan, three species of the genus *Anodonta* exist: *Anodonta japonica*, *A. lauta*, and *A. calipygos* (Kondo, 2008). *A. calipygos* lives in lentic habitats such as Lake Biwa and its effluent rivers (Kondo, 2008; Negishi *et al.*, 2008; Itoh *et al.*, 2010), whereas, the other two species live in lentic and lotic habitats (Fukuhara *et al.*, 1994; Fukuhara & Nagata, 1995). *A. japonica* and *A. lauta* were previously identified as a single species, *A. woodiana* (Habe, 1977), until the study of Tabe *et al.* (1994) in which they were identified as two distinct species. However, clear distinction between the two species based on morphology of the settled mussels and larvae could not be achieved, due to their adaptive plasticity to a broad range of environments (Kondo *et al.*, 2006; Kondo *et al.*, 2011). Thus, the host for *A. japonica* and *A. lauta* has been cited without discriminating between the two species.

The larval incubation period in the external gills of gravid females varies between *A. japonica* and *A. lauta*, *i.e.*, throughout the year and between March and July, respectively (Fukuhara *et al.*, 1994; Kondo, 2008), with no overlap between the two species for about half a year. The larval parasitic period for *Anodonta* species depends on the water temperature and is shortened in warmer water (Kondo, 2008). The glochidium of *A. lauta* can metamorphose within a month at water temperatures above 14°C, which was calculated using a linear regression equation (Kondo, 2008). This indicates that any *Anodonta* glochidia found

on a host between September and February at water temperatures above 14°C in August can be identified as *A. japonica*.

In the previous studies, the glochidia that were assumed to be *A. japonica* were found attached on the gills and fins of six species of fish: *Zacco platypus*, *Gnathopogon elongatus elongatus*, *Rhodeus ocellatus*, *Misgurnus anguillicaudatus*, *Tridentiger brevispinis* and *Rhinogobius brunneus* (Fukuhara *et al.*, 1986; Kondo, 1997). Furthermore, it was shown that glochidia like *A. japonica* do not include *Pseudorasbora parva* among their hosts (Fukuhara *et al.*, 1986). However, the glochidia of some species have been attached to non-host fish species by artificial manipulation *in vitro* (Watters & O'dee, 1998; Khym & Layzer, 2000). Thus, in identifying the host fish for mussel species, simply checking for the presence of glochidia attached to the host body surface is inadequate. In the present study, culture experiments were performed to determine with certainty the host fish species for *A. japonica*.

### Materials and Methods

Sampling was performed in agricultural drainage ditches in Hikone City, Shiga Prefecture, central Japan on October 25 and 27, 2010. The water through the drainage mainly comes from the Uso River. The river mouth of the drainage connects to Lake Biwa. Four mussel species were identified: *Anodonta* spp. (*A. japonica* and/or *A. lauta*), *Lanceolaria grayana*, *Unio douglasiae biwae*, and *Pronodularia japonensis* (Akiyama, unpublished data). Of these species, only *A. japonica* releases glochidia in October (Kondo, 1987; Fukuhara *et al.*, 1994; Kondo, 2008). Fishes and shrimps were collected using an electric shocker (LR-24 Electro fisher; Smith-Root, Inc.) in the mussel habitat. The precise sampling site is not given in this paper in order to protect the mussels. Collected organisms were taken to the laboratory and categorized according to species. Up to 30

individuals of each species were reared in a 1-L container filled with approximately 800 mL of tap water containing neutralized chlorine for 17 days, and the remaining individuals were released at the sampling site. The lack of a partition in the containers meant that glochidia and juveniles could possibly be eaten by fish after detachment from the host. Mean water temperature in the containers was 19°C. The light-dark regime was not controlled. The fishes and shrimps were not fed during the experiment. The containers were gently aerated with an airstone and air pump. The water in the container was changed every 1 to 4 days. Water removed from the container was filtered through a nylon net (mesh size, 25 µm), and the debris retained in the net was examined for glochidia with a stereomicroscope (× 15 power). The total number of live juvenile mussels and the number of empty glochidial shells, live glochidia, and dead juvenile mussels at the bottom of each container were counted and separately recorded. Settled juveniles were judged to be alive or dead depending on the movement of the foot (Itoh *et al.*, 2008). Glochidia on the fishes that died during the experiment were counted and evaluated as dead larvae.

## Results and Discussion

Six species of fish and one species of shrimp were collected from the study site and used in the rearing experiment (Table 1). *G. elongatus elongatus* and *Rhinogobius* sp. OR (orange type; see Akihito *et al.*, 2002, for the identification of this undescribed species) individuals were found dead during the rearing experiment. All examples of the former died on the 10th day after initiation of the

experiment. Perhaps a change of water condition in the container caused by the death of one individual brought on the death of the remainder. In the latter species, six, four and two individuals died 3, 4 and 7 days after initiation of the experiment respectively. Attacks among gobies observed sometimes were considered a possible cause of their death. Table 1 demonstrates the results of the culture experiment. Although all fishes and shrimps were collected from the main mussel habitat in the drainage ditches, only *A. japonica* juveniles appeared in the containers with *Rhinogobius* sp. OR. Glochidial shells were confirmed in containers with *Rhinogobius* sp. OR and *G. elongatus elongatus*. Although the presence of many empty shells was ascertained in the former, only a couple of empty shells were found in the latter. The rate of larvae metamorphosing into juvenile mussels was estimated at 30.5% in the container with *Rhinogobius* sp. OR but the actual rate might be high because all larvae on the 12 dead gobies were regarded as dead. The other species of fish and shrimp involved might be entirely non-functional. These results suggest that *Rhinogobius* sp. OR is the host species for *A. japonica*, at least at this study site. Fukuhara *et al.* (1986) cited *R. brunneus* as a valid host species for *A. woodiana*. However, following publication of their paper, Akihito *et al.* (2002) revealed that at least 11 forms included in the “yoshinobori” species complex, including *R. brunneus*, should be considered as distinct species. Unfortunately, the correct scientific name of the host goby species described by Fukuhara *et al.* (1986) is unknown. *Rhinogobius* sp. OR was the most dominant species and found to be widely distributed around the sampling site in the present

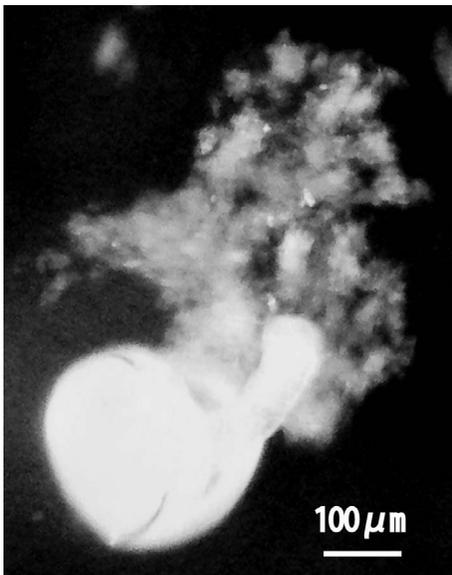
**Table 1.** Number of fish or shrimp individuals for the culture experiment, total numbers of live juveniles, and empty glochidial shells, dead glochidia, and dead juveniles obtained on the bottom of containers and dead host bodies. Abundance of dead individuals of each species during the experiment shown in parentheses.

Species	Number of individuals	Number of live juveniles	Total number of empty shell, dead larvae and dead juveniles
<i>Gnathopogon elongatus elongatus</i>	30 (30)	0	1
<i>Misgurnus anguillicaudatus</i>	30 ( 0)	0	0
<i>Oryzias latipes latipes</i>	30 ( 0)	0	0
<i>Odontobutis obscura</i>	1 ( 0)	0	0
<i>Rhinogobius</i> sp. OR	30 (12)	26	59
<i>Neocaridina denticulate</i>	30 ( 0)	0	0

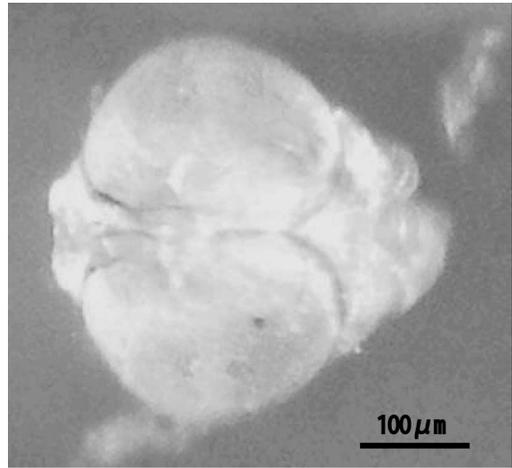
study (Kanao & Nishida 2007; Y. B. Akiyama, unpublished data), and thus, in terms of host abundance, the suitable habitat for *A. japonica* may have formed a wide part of the study area. *Rhinogobius* was noted in Kondo (2008) as the host species for approximately 94% of Japanese unionid species and subspecies. Accordingly, *Rhinogobius* species are a very important host group for unionids in Japan.

Kondo (1997) reported that four *A. japonica* glochidia were found after examining four individuals of *M. anguillicaudatus*, larvae were absent on eight individuals of *O. obscura* and two were associated with 31 individuals of *G. elongatus elongatus*, collected at two sites in the Shiga Prefecture. The evaluation of *M. anguillicaudatus* as a potentially functional host for *A. japonica* was made by Kondo (1997) but it is considered a non-functional host in the present study. To examine the reason for these differences, the effects of sampling method and sampling season on the abundance of glochidia on *M. anguillicaudatus* should be examined. Kondo's results regarding *O. obscura* and *G. elongatus elongatus* are comparable to those in the present study.

Several settled juveniles examined immediately after detachment from the host fish exhibited glochidial shells, and sticky mucus was noted exuding from the softer parts that caused detritus to



**Fig. 1.** Juvenile of *Anodonta japonica*, a day after commencement of the postparasitic stage. Detritus gathers around the soft parts.



**Fig. 2.** Juvenile of *Anodonta japonica*, 3 days after commencement of the postparasitic stage.

become attached around this area (Fig. 1). This behavior is possibly pedal-sweep feeding, which is known in the unionid species *Villosa iris* (Yeager *et al.*, 1994). The juveniles did not respond to tapping on the side of the container. Two to three days after detachment from the host surface, the soft parts of the juvenile *A. japonica* grew rapidly, their size exceeding that of the glochidial shell (Fig. 2). Adherence of mucus to juveniles was weak. The juveniles were very sensitive to tapping on the side of the container, and they responded by rapidly closing their valves. These results indicate that, after detachment from *Rhinogobius* sp. OR, juveniles of *A. japonica* grow and exhibit lively behavior.

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## 彦根市内の稲作用排水路における タガイのゲロキディウム幼生の宿主

秋山吉寛

### 要 約

スマガイとの識別が困難なタガイの宿主を明らかにするため、タガイの幼生が放出される10月下旬に、滋賀県彦根市内の稲作用排水路で採集した魚類および甲殻類の飼育を行ない、宿主から脱落したタガイ稚貝の有無を確認した。5種類の魚類と1種類の甲殻類のうち、稚貝の離脱が確認されたのはトウヨシノボリだけだった。離脱したばかりの稚貝は粘性の強い粘液を吐き出し、周囲のデトリタスを軟体部の周囲に吸着させる行動を見せた。さらに、2~3日後にも生存し、軟体部の急速な成長や、振動に対する敏感な反応が見られたことから、トウヨシノボリがタガイの宿主となることが確かめられた。