Short original article

**The mouth morphology of diving beetle *Hyphydrus japonicus* (Dytiscidae:
Hydroporinae) is specialized for predation on seed shrimps**

Short running title: Diving beetles mouth morphology

MASAKAZU HAYASHI^{1,*} AND SHIN-YA OHBA²

¹*Hoshizaki Green Foundation, Sono, Izumo, Shimane 691-0076, Japan*

²*Biological Laboratory, Faculty of Education, Nagasaki University, Bunkyo 1-14,
Nagasaki 852-8521, Japan*

*Corresponding author. E-mail: hgf-haya@green-f.or.jp

14 Abstract

15 Two-shelled organisms, such as bivalves and seed shrimps, protect themselves from
16 external stresses by firmly closing both shells. We found that the diving beetle
17 *Hyphydrus japonicus* Sharp, 1873 (Dytiscidae: Hydroporinae) larvae frequently prey on
18 seed shrimps. Other predatory aquatic insects have difficulty in capturing seed shrimps
19 owing to the general form of their mandibles. Larvae of *H. japonicus* bear a projection
20 on the head (the nasale), which is used in conjunction with vertical movements of the
21 mandibles to fixate the valves of their prey and gain access to the soft body within them.
22 This capture method does not destroy shells and is extremely efficient. The present
23 study provides the first report on the use of a unique morphological trait for attacking
24 prey animals without destroying their shells.

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26 ADDITIONAL KEYWORDS: diversity – food selection – freshwater crustacean –
27 insect – predator/prey interactions – lab experiment

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INTRODUCTION

Many insects have exaggerated traits on their heads. For example, male-specific elongated horns in scarab beetles Scarabaeinae and rhinoceros beetles Dynastinae are used for competition among males over females and have evolved by sexual selection (Emlen, Lavine & Ewen-Campen, 2007). Such horns are also effective for defence against predators and for attacking other species. The larvae of several Nymphalidae butterflies have long horns that are used to drive off other larvae of the same species (Teshirogi, 1990). In addition to defence, horns are used for feeding. Larvae of the pipevine swallowtail *Battus philenor* L. (Papilionidae) have long fleshy frontal projections that help them find host plants, although these projections develop on the prothorax (Kandori *et al.*, 2015). As predatory insect larvae and nymphs generally develop large mandibles, labium or claws (e.g. Inoda *et al.*, 2003; Ohba & Tatsuta, 2016), examples of head projections specialized for capturing specific prey items are not known.

Hard tissues that cover invertebrate body surfaces such as molluscan shells typically serve as protection against predators. Bivalves, for example, can tightly close both hard shells to protect their organs. Predators have therefore developed a range of physical (and chemical) means for attacking bivalves, such as breaking shells (mantis shrimp and crab), breaking the adductor muscle and prying shells open (starfish), and drilling (octopus, dog whelk, and bladder moon shell) (Chrystensen, 1957; Wells, 1978; Taylor, 1998; Gosling, 2003; Lawrence, 2013). Certain crustaceans also possess two shells, possibly used for defensive purposes (Tsukagoshi, 2017). Due to their shells, seed shrimps (ostracods) are avoided by small predators including aquatic insects (Smith & Delorme, 2010; Klecka & Boukal, 2012; Smith *et al.*, 2015). These shells also increase the probability of ostracods surviving through the digestive tract of large

predators (Lopez *et al.*, 2002).

The larvae of diving beetle family Dytiscidae prey on living organisms using their large mandibles. They usually eat soft-bodied animals, such as small fishes, amphibian nymphs, and insect nymphs and larvae, but there is a tendency to choose specific prey (e.g., Ohba, 2009a, b). Larvae of Dytiscidae species within the subfamily Hydroporinae are characterized by a strong anterior projection of the anterior margin of the head (front clypeus; Miller & Berrgsten, 2016). This projection is accompanied with mandibles moving vertically that is known as hold the prey (Friis, Bauer & Betz, 2003). Species within the North American genus *Uvarus* (Hydroporinae) show a triangular nasale bearing five subequal denticles, which larvae use to capture ostracods but not copepods (Matta, 1983). Under laboratory conditions, we noticed that larvae of *Hyphydrus japonicus* Sharp 1873 (Fig 1A, B) prey on living Podocopa seed shrimps. *Hyphydrus* species are known to have unique mouth morphology (Böving & Craighead, 1931), comprising a sensory organ and a dentate character at the tip of the front clypeus, the nasale (Friis, Bauer & Betz, 2003). They capture prey by protruding the front clypeus and holding the prey in their curved mandibles, although the functional role of these mouthparts remains unclear.

Although it is considered difficult for aquatic insects to destroy the shells of Podocopa using these specialized mouthparts, *Hyphydrus* sp. larvae appear to feed efficiently on these organisms without destroying their shells. However, *Hyphydrus* sp. and other Hydroporinae might occupy a unique niche in freshwater ecosystems by preying on Podocopa. The present study aimed to examine the predation of Podocopa by aquatic Coleoptera larvae, and compared the predation rates of *Hyphydrus japonicus* larvae with intact and surgically treated front clypei to examine the role of this projection on predation.

MATERIALS AND METHODS

First experiment: interspecific comparison

For this experiment, we selected predatory aquatic insects that appeared in the same season as seed shrimp and lived in the same type of shallow water as *Hyphydrus japonicus* larvae. Larvae/nymphs of the following six aquatic insect species were used in the present study (Fig. 1C–I): two species of diving beetle, *Hyphydrus japonicus* (3rd instar, $n = 16$) and *Platambus optatus* (Sharp, 1884) (3rd instar, $n = 12$); three species of water scavenger beetle, *Enochrus simulans* (Sharp, 1873) (3rd instar, $n = 15$), *Amphiops mater* Sharp, 1873 (3rd instar, $n = 14$), and *Sternolophus rufipes* (Fabricius, 1792) (2nd instar, $n = 15$); and one species of damselfly, *Copera annulata* (Selys, 1863) (unknown instar, head width: 1.8–3.2 mm, $n = 15$). All insects were collected from ponds and/or rice fields in east Shimane Prefecture (35°19–26′N, 132°43–51′E), Honshu Island, Japan. The larvae of *Hyphydrus japonicus* used in the present study originated from eggs laid by reared adults collected in March 2017.

Heterocypris incongruens (Ramdohr, 1808) was selected as the seed shrimp species. Individuals of *H. incongruens* hatched from dormant eggs contained in a ‘water lily bowl’ placed in the field, were used when the diameter of their shells was 1.4 to 1.6 mm. *Heterocypris incongruens* shells (valves) are elliptical, with a smooth surface bearing some granular projections (Supporting Information Fig. S1). This nektobenthic species is widely distributed in shallow freshwater habitats, such as rice fields and puddles, in which it can be observed alternately swimming or resting (Tanaka *et al.*, 2015).

The first experiment was conducted for 3 h, using the six predator species. Five seed shrimps were provided to each individual predator at the beginning of the

experiment, and the number of prey individuals eaten per hour was recorded. This experiment was conducted in 100-mL beakers containing 20 mL dechlorinated tap water and a bamboo skewer (length: 120 mm; diameter: 2.5 mm), which was diagonally placed as a scaffold for resting and breathing above the water surface (Supporting Information Fig. S2). The experiment was set in a room at 20 °C, under continuous lighting, as all replicate experiments were performed during the day (between 0900 and 1700 h, Japan Standard Time). To standardize the degree of hunger among predatory larvae, adequate quantities of chironomid larvae, cladocerans, and seed shrimps were provided before fasting for approximately 24 h. Chironomid larvae and cladocerans are commonly used in aquatic insects rearing (e.g., Tsuzuki, Taniwaki & Inoda, 1999; Watanabe, Hayashi & Kato, 2017; Watanabe, Suda & Fukutomi, 2017b).

Second experiment: comparison between intact and treated *Hyphydrus japonicus*

In the second experiment, we only used *Hyphydrus japonicus* larvae, which were divided into two experimental groups: “intact” ($n = 10$) and “surgically treated” ($n = 11$) front clypeus. Surgically treated *H. japonicus* larvae were adequately fed, the tip of their nasale (Fig. 1C, D) was excised using tweezers, and the experiment was conducted approximately 24 h later (Supporting Information Fig. S3). The number of individuals that successfully preyed upon one seed shrimp during 1 h was recorded. After this initial phase, crushed seed shrimp were provided to *Hyphydrus japonicus* larvae before fasting for approximately 24 h. This experiment was repeated for “intact” larvae.

Conditions were the same as in the first experiment (Supporting Information Fig. S2), including temperature and lightning conditions (between 0900 and 1700 h, Japan Standard Time). All adults derived from surgically treated larvae were normal (Supporting Information Fig. S4).

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Statistical analyses

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The statistical software R (R Development Core Team, 2015) was used for all analyses.

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To examine the number of seed shrimps eaten by the larvae of the six species used in

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the first experiment, and the predation rate between the two experimental groups of *H.*

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japonicus in the second experiment, we used a generalized linear mixed model (GLMM,

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glmmML package) with binomial and Poisson distributions, respectively. For the first

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experiment, “species”, “time (after 1, 2, and 3 h)”, and their interaction were

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incorporated into the GLMM as explanatory variables. For the second experiment,

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“treatment (intact or cut nasale)”, “test (first and second)”, and their interaction were

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incorporated into the GLMM as explanatory variables. Each individual was considered

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a random effect in both GLMMs. If the interaction was not significant ($P > 0.05$), it was

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removed from the final model.

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RESULTS

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The first experiment showed that the number of seed shrimps eaten by other predatory

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species was significantly lower than that eaten by *Hyphidrus japonicus* larvae (Fig. 2A;

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Supporting Information Table S1). In the case of *Hyphidrus japonicus* larvae, when

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seed shrimps opened their shells, one or both mandibles were inserted, and the contents

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were consumed without destroying the shells (Supporting Information Movie S1). The

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other diving beetle, *P. optatus*, and the water scavenger beetles had difficulty in

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capturing seed shrimps using their mandibles (Supporting Information Movie S2). Even

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when shrimps were captured, their shells could not be destroyed in the closed state;

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when one mandible was inserted between the shells these could be destroyed and the

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contents digested.

In the second experiment, statistical analysis showed that “treatment” was a significant factor, and the coefficient of “treatment” was significantly negative (Fig. 2B; Supporting Information Table S2), indicating the lower predation rate in “cut nasale” than in “intact”. The larvae did not die due to excise of nasale, and reacted to moving seed shrimp (Supporting Information Movie S3).

DISCUSSION

Generally, predators attack two-shelled organisms by exerting physical actions, such as breaking the shell, prying open the two valves, and drilling. To the best of our knowledge, this is the first report on the use of a unique morphological trait of *Hyphydrus japonicus*, the nasale, for attacking two-shelled prey organisms, such as bivalves, without destroying their shells (Supporting Information Movie S1). The mouth morphology of the European congener of *Hyphydrus japonicus*, *Hyphydrus ovatus* (L.), has been observed in detail (Friis, Bauer & Betz, 2003). Individuals of this species have different types of sensory organs near the tip of the nasale, their mandibles move upwards like the lower jaw of vertebrates, and the cuticular teeth on the underside of the nasale facilitate prey capture, pressing, and holding. A type of sensory organ is also found in *Hyphydrus japonicus* (Supporting Information Fig. S5), and the results obtained here suggest that the shapes of the nasale and mandibles in *Hyphydrus japonicus* are advantageous for capturing seed shrimps. Furthermore, sensory organs detect the movement of seed shrimps and appear to facilitate the rapid insertion of the mandible when seed shrimps open their shells and start to move (Supporting Information Movie S4). In our experiments, removal of the tip of the nasale led to a loss or reduction in sensory function. Although seed shrimps close their shells and cease body movement when threatened, they continue to move their legs internally. It appears

that the sensory organs of intact *Hyphydrus japonicus* larvae can detect leg movements within the shells; the larvae gradually approach seed shrimps and await the opening of the shells. In contrast, *Hyphydrus japonicus* larvae in which the tip of the nasale had been excised, as well as the larvae of other aquatic insects, did not respond to seed shrimps.

Hydroporinae larvae are characterized by a strong projection on the anterior margin of the head (Miller & Berrgsten, 2016). This projection has diverse shapes, including a narrow and parallel-sided nasale, a triangular nasale, or a nasale with lateral branches (Michat, Alarie & Miller, 2017). The genus *Uvarus* having a triangular nasale capture ostracods (Matta, 1983). We observed that the three species of Hydroporinae, *Hydrovatus stridulus* Biström, 1997, *Hydroglyphus japonicus* (Sharp, 1873) and *Leiodytes frontalis* (Sharp, 1884) also captured ostracods without destroying their shells (Supporting Information Movie S5–7). However, the capture frequencies of the three species of Hydroporinae are unknown. Also, since the four species are smaller than *Hyphydrus*, it is difficult to compare under the same conditions. The evolution of the nasale in Hydroporinae might have enabled larvae of these species to capture seed shrimps. Ostracods, including seed shrimps, are generally considered minor prey species that are not an important component of the diet of most aquatic insect predators (Klecka & Boukal, 2012). It has been reported that one diving beetle species within genus *Agabus* (subfamily Agabinae) does not prey on ostracods (Culler & Lamp, 2009). In the present study, most predatory larvae other than *Hyphydrus japonicus* had difficulty in capturing and consuming ostracods but preyed predominantly upon chironomid larvae and cladocerans before the experiment. Seed shrimps are difficult to catch, and therefore not consumed by many predatory larvae. The Dytiscidae is one of the largest families of water beetles, comprising 4303 known species (Short, 2017).

Among these, the subfamily Hydroporinae is the largest group, comprising 51% of Dytiscidae species (Miller & Berrgsten, 2016), and it is estimated that it diverged from other subfamilies more than 100 million years ago (Desamore *et al.*, 2018). A specific foraging ecology might have enabled a unique niche in freshwater ecosystems for these species thereby increasing the diversity of this subfamily.

Insect mandibles move horizontally; therefore, vertical changes are dramatic and rarely occur. To use the mandible as a substitute for the lower jaw, a protrusion substituting the upper jaw is also necessary. Our research suggests that specific prey organisms are important for the evolution of the mouth morphology of predators. For example, Cretaceous fossil ants have special mouthparts (Perrichot, Wang & Engel, 2016; Barden, Herhold & Grimaldi, 2017) with large mandibles that move in a vertical direction, paired with a projection of the head similar to that of Hydroporinae. It is unknown how they use mandibles and protrusions, but it might indicate the presence of specific organisms to capture.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Scanning electron micrographs of the seed shrimp *Heterocypris incongruens*.

A, left valve. B, right valve. C, right valve, inner surface. D, left valve, inner surface.

Figure S2. The 100-mL beaker with a diagonal bamboo scaffold used in the predation experiment (A) and a *Hyphydrus japonicus* larva perching on the scaffold, preying upon a seed shrimp (B).

Figure S3. A nasale-excised larva attempting to prey on a living seed shrimp, *Heterocypris incongruens*.

Figure S4. Normal-morphology adult (A) derived from a nasale-excised larva (B). The arrow indicates the larval nasale in the exuvium of pupation.

Figure S5. Scanning electron micrographs of the mouthparts of *Hyphydrus japonicus*. A, lateral view of nasale. B, under side of the tip of the nasale. n, nasale; lm, left mandible; ps, plate-like sensilla; sb, sensilla basiconica; st, sensilla trichodea near the tip of the nasale; tlp, tooth-like protrusion; tn, tip of the nasale.

Table S1. Generalized linear mixed model results for the number of seed shrimps eaten by each predator species

Table S2. Generalized linear mixed model results for differences in predation rate between 'intact' and 'excised nasale' *Hyphydrus japonicus*

Movie S1. *Hyphydrus japonicus* capturing a swimming seed shrimp, *Heterocypris incongruens*.

Movie S2. *Platambus optatus* attempting to prey upon a live seed shrimp, *Heterocypris*

335 *incongruens*.

336 **Movie S3.** Nasale excised *Hyphydrus japonicus* reacted to moving seed shrimp,

337 *Heterocypris incongruens*.

338 **Movie S4.** *Hyphydrus japonicus* capturing a resting seed shrimp, *Heterocypris*

339 *incongruens*.

340 **Movie S5.** *Hydrovatus stridulus* Biström capturing a swimming seed shrimp,

341 *Stenocypris* sp.

342 **Movie S6.** *Hydroglyphus japonicus* capturing a swimming seed shrimp, *Stenocypris* sp.

343 **Movie S7.** *Leidytes frontalis* capturing a swimming seed shrimp, *Stenocypris* sp.

Figure 1. Aquatic insect species used in the present study. A and B, Diving beetle *Hyphydrus japonicus* larva capturing the podocopan ostracod *Heterocypris incongruens* (A, Dorsal view; B, lateral view). C–I, Mouthparts of the aquatic insect larvae examined in this study: *H. japonicus* (3rd instar; C, D), arrows indicate the site at which the nasale was cut; *Platambus optatus* (3rd instar; E); *Enochrus simulans* (3rd instar; F); *Amphiops mater* (3rd instar; G); *Sternolophus rufipes* (2nd instar; H); Labium of *Copera annulata* (instar unknown; I). D, Lateral view. C, E–I, dorsal view.

Figure 2. Number of seed shrimps eaten by each of the six insect predator species (A) and predation rates of *Hyphydrus japonicus* with intact and surgically removed nasale (B).

Figure 1.

Figure 2.

SUPPORTING INFORMATION

Figure S1. Scanning electron micrographs of the seed shrimp *Heterocypris incongruens*.

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Table S1. Generalized linear mixed model results for the number of seed shrimps eaten by each predator species

	Source	Coefficient	SE	Z	P
Full model	Intercept	1.499	0.149	10.05	< 0.001
	Species (vs. <i>Amphiops mater</i>) ^a	-3.590	0.971	-3.70	0.000
	Species (vs. <i>Sternolophus rufipes</i>) ^a	-1.881	0.611	-3.08	0.002
	Species (vs. <i>Platambus optatus</i>) ^a	-2.303	0.622	-3.70	0.000
	Time	0.211	0.096	2.18	0.029
	Species (vs. <i>Amphiops mater</i>):time	0.556	0.376	1.48	0.139
	Species (vs. <i>Sternolophus rufipes</i>):time	0.061	0.255	0.24	0.812
	Species (vs. <i>Platambus optatus</i>):time	0.117	0.257	0.45	0.650
Final model	Intercept	0.643	0.218	2.953	0.003
	Species (vs. <i>Amphiops mater</i>) ^a	-2.305	0.314	-7.335	0.000
	Species (vs. <i>Sternolophus rufipes</i>) ^a	-1.750	0.258	-6.786	0.000
	Species (vs. <i>Platambus optatus</i>) ^a	-2.050	0.259	-7.915	0.000
	Time	0.264	0.081	3.250	0.001

^aThe coefficient indicates the relative effect of each predator species compared to that of

Hyphydrus japonicus during the first hour of the experiment.

Enochrus simulans and *Copera annulata* were excluded from the analysis because these

predators ate no seed shrimps.

Table S2. Generalized linear mixed model results for differences in predation rate between 'intact' and 'excised nasale' *Hyphydrus japonicus*

	Source	Coefficient	SE	<i>z</i>	<i>P</i>
Full model	Intercept	31.920	97.230	0.328	0.743
	Treatment (intact vs. cut) ^a	-62.360	107.010	-0.583	0.560
	Test (first vs. second) ^b	-19.090	97.100	-0.197	0.844
	Treatment:test	36.600	106.770	0.343	0.732
Final model	Intercept	7.361	3.242	2.271	0.023
	Treatment (intact vs. cut)^a	-14.820	5.201	-2.851	0.004
	Test (first vs. second) ^b	0.000	2.118	0.000	1.000

^aThe coefficient indicates the relative effect of the “excised” nasale compared with that of the “intact” nasale during the first test.

^bThe coefficient indicates the relative effect of the second test compared to the first test, for “intact” larvae.

26
27 **Figure S3.** A nasale-excised larva attempting to prey on a living seed shrimp.

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Figure S4. Normal-morphology adult (A) derived from a nasale-excised larva (B). The arrow indicates the larval nasale in the exuvium of pupation.

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- 42 **Movie S3.** Nasale excised *Hyphydrus japonicus* reacted to moving seed shrimp,
43 *Heterocypris incongruens*.
- 44 **Movie S4.** *Hyphydrus japonicus* capturing a resting seed shrimp, *Heterocypris*
45 *incongruens*.
- 46 **Movie S5.** *Hydrovatus stridulus* Biström capturing a swimming seed shrimp,
47 *Stenocypris* sp.
- 48 **Movie S6.** *Hydroglyphus japonicus* capturing a swimming seed shrimp, *Stenocypris* sp.
- 49 **Movie S7.** *Leiodytes frontalis* capturing a swimming seed shrimp, *Stenocypris* sp.