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The variations of internal and external morphology and body shape between and within three populations of *Helobdella stagnalis* (Linnaeus, 1758) in northern Honshu, Japan, were examined. The results suggest that (1) the location of internal organs relative to the segmentation is not informative for *Helobdella* taxonomy, (2) body shape may be useful as a species-level taxonomic character for properly fixed specimens, and (3) a purportedly cosmopolitan species, *H. stagnalis* may need to be separated into more than one species with restricted distributional ranges.

**Key Words:** Rhynchobdellida, Glossiphoniidae, Haementeriinae, *Helobdella stagnalis*, internal morphology, body shape, external morphology.

**Introduction**

The leech genus *Helobdella* Blanchard, 1896 is in the subfamily Haementeriinae of the family Glossiphoniidae. With approximately 39 currently recognized species, the genus shows a cosmopolitan distribution and is diagnosed by the following characters: 1 pair of eyes; diffuse salivary gland cells; crop with maximum of 6 pairs of caeca; male and female gonopores separated by 1 or 2 annuli; and mid-body somite 3-annulate, not subdivided (but subdivided in *H. diploides* Ringuelet, 1948) (Ringuelet 1948; Soos 1969; Sawyer 1972, 1986). Sawyer (1986) divided this genus into two species-groups based on presence/absence of the nuchal gland and scute, that is, the *H. stagnalis*-group possessing both structures and the *H. triseri- alis*-group lacking either of them.

In recent taxonomic studies of *Helobdella* with a dorsal scute, the species have been distinguished mainly by internal morphological characters (Kutschera 1988; Govedich and Davies 1998); however, internal morphological characters, especially the positions of internal organs in terms of segmentation, are not always stable within a given species (Miller 1929). Intra-specific variation in the position of internal organs may be due to the contraction or extension of muscles (Castle 1900; Miller 1929). External characters, such as pigmentation, body shape, position of eyes, and position and shape of the scute, had been often used to distinguish species until it was pointed out that they were often too variable within a given species, and even within a given population, to be diagnostic (Harding and Moore 1927; Miller 1929; Sawyer 1972; Klemm 1976). Sawyer (1972) showed that the body shape of freshwater leech specimens could vary due to fixation artifacts. Neverthe-
less, detailed analyses of variations in either internal or external characters between and within populations have not been made for any species of Helobdella. Helobdella stagnalis (Linnaeus, 1758) is the type species of the genus and the only species of Helobdella that has been recorded from all continents with the exception of Australia and Antarctica (Sawyer 1986). Such a cosmopolitan distribution is very unusual for freshwater animals with poor mobility, and some distributional records have referred to character variation between local populations (Castle 1900), yet no detailed taxonomic reconsideration of this species has been done. The Japanese fauna of the genus Helobdella is no exception. Oka (1910, 1917) recognized only H. stagnalis in this country, recording it from several localities. His identification was based only on the presence of the scute and the external appearance of the head; both characters are, in current practice, insufficient to identify species of Helobdella. Since Oka’s time, no one has studied the Helobdella fauna in Japan.

The present study aims to examine the variation in internal and external morphology between and within three populations of H. stagnalis in northern Honshu, Japan.

Materials and Methods

The leeches were collected from three lakes in the northern part of Honshu, the main island of Japan (Fig. 1): Lake Kayaharanoike (one of the Tsugaru-Juniko Lakes, Aomori Prefecture), April, 1997; Lake Suganuma (Tsuta-onsen, Aomori Prefecture), April, 1997; Lake Yunoko (Oku-nikko, Tochigi Prefecture), July, 1997, and February, April, and November, 1998.

Nearly all of the leeches collected in April had a membranous cocoon or young attached to their ventral side. At Lakes Kayaharanoike and Suganuma, the leeches were found underneath dead branches and leaves accumulated along shorelines at a depth of 10 to 50 cm, where the bottom substrate was silt. At Lake Yunoko, the leeches were collected from the undersides of stones of 20 to 30 cm diameter.

The leeches were brought to the laboratory alive, relaxed in 20% ethanol for 10–15 minutes, and then fixed with 10% formalin. External characters, such as body color, number of eyes, position of eyes, position of scutes, and positions of gonopores, were observed on the fixed specimens under a stereoscopic dissecting microscope. Some of the fixed specimens were embedded in paraffin, sectioned at 8–12 μm, and stained with haematoxylin and eosin. Observations of these preparations were made under an optical compound microscope.

In order to examine the deformation of body shape as a result of the fixation process, the body shapes of the specimens from Lake Yunoko were measured before (under narcotization with 20% ethanol) and after fixation. The length and maximum width of the body were used as the indices of body shape. These two measurements were traced on paper using a stereoscopic dissecting microscope with a drawing apparatus. Then, the traced lines were measured with a digital-curvimeter to the nearest 1 mm and converted to the actual lengths. For the specimens from the other two lakes, measurements were made only after fixation.

In the descriptions, body segment numbers are given by Roman numerals; for example, “II” designates the second body segment, and “III/IV” means the intersegment between the third and fourth body segments.
Morphological variation of *Helobdella stagnalis*  

Vouchers are deposited in the Division of Biological Sciences, Graduate School of Science, Hokkaido University Sapporo, Japan.

**Results**

**External morphology**

The numbers of specimens examined for external characters were 12, 10, and 11 for Lake Kayaharanoike, Lake Suganuma, and Lake Yunoko, respectively. The external characters other than body shape did not show any significant differences separating the populations, but the states of some characters varied within a given population as summarized below.

Body coloration uniformly light yellow to light pink; 1 pair of eyes located between 2nd and 3rd annuli of head, but sometimes shifted frontward by 1 annulus (1 specimen each from Lake Kayaharanoike and Lake Yunoko); neither papillae nor sensory annuli corresponding to segmentation present; scute present at mid-dorsum of neck (between 11th and 13th body annuli), with egg-shaped to rounded triangular outline, but sometimes divided into 2 small scutes (2 specimens from Lake...
Kayaharanoike); male and female gonopores opening on ventral side of XI–XII, former anterior to latter by 1 annulus.

**Internal morphology**

The internal structures of 16 specimens from each population were examined. The following characters did not differ among the populations but showed intra-populational variations as given in parentheses (see also Table 1): proboscis located medially, with smooth surface; esophagus usually S-shaped; salivary gland cells arranged diffusely on each side of body from basis of proboscis; gonopores separated by 1 annulus among XI to XIII; typically 6 pairs of testisacs (5 pairs in 6 specimens from Lake Kayaharanoike; 4 and 5 pairs respectively in 2 and 7 specimens from Lake Suganuma; 5 pairs in 2 specimens from Lake Yunoko), located between crop caeca. Crop located near mid-length of body, with 6 pairs of crop caeca; anterior 5 pairs simple lobes, but most anterior one often much smaller than others; posterior pairs of caeca extending posteriorly and laterally to intestinal caeca (only 5 pairs of crop caeca in 1 specimen each from Lake Kayaharanoike, Lake Suganuma, and Lake Yunoko; in these specimens, anterior 4 pairs of caeca simple lobes of same size, and posteriormost pair of caeca as in ordinary specimens, thus small first pair apparently absent). Intestine with 4 pairs of caeca of nearly same size. Rectum S-shaped in longitudinal section, occupying 1 or 2 body segments.

The characters of the digestive system showing inter-populational variations are described below for each population.

**Lake Kayaharanoike:** Proboscis occupying 2 to 8 segments in VI–XV; salivary gland cells spreading over 1 to 4 segments in X–XV; crop occupying 3.5 to 7 segments in XII–XX; last pair of crop caeca arising between XIX and XXII/XXIII, extending posteriorly, ending between XXI and XXII/XXIII; intestine occupying 3 to 5 segments in XIX–XXV; rectum located in XXII–XXV.

**Lake Suganuma:** Proboscis occupying 6 to 8 segments in VI–XIV/XV; salivary gland cells spreading over 1 to 4 segments in XI–XV/XVI; crop occupying 3.5 to 8 segments in XII–XXI; last pair of crop caeca arising between XVIII and XX, and terminating between XX and XXIII; intestine occupying 1.5 to 4 segments in XVIII–XXIV; rectum located in XX/XXI–XXV.

**Lake Yunoko:** Proboscis occupying 4 to 8 segments in VI–XVII; salivary gland cells spreading over 1 to 3.5 segments in X–XV; crop occupying 4.5 to 6 segments in XIII–XX/XXI, with last pair of caeca arising in XVIII–XX and terminating in XXI–XXIII; intestine occupying 1.5 to 4.5 segments in XIX–XXIII/XXIV; rectum located in XXIII–XXIV.

Next, the characters of reproductive organs showing inter-populational variations are described below for each population.

**Lake Kayaharanoike:** Testisacs located among XII/XIII–XX, arranged in 5 or 6 pairs, and each pair occupying 1/3 to 1 body segment.

**Lake Suganuma:** Testisacs located among XIII–XIX, arranged in 4 to 6 pairs, and each pair occupying 1/3 to 1 body segment.

**Lake Yunoko:** Testisacs located in XII/XIII–XX, composed of 5 to 6 pairs, and each pair occupying 1/3 to 1 body segment.

**Deformation of body shape through fixation**

There was a significant correlation between values before and after fixation.
Table 1. Variations in internal characters of *Helobdella stagnalis* from three lakes in northern Honshu, Japan. Roman numerals designate body segment numbers. The range of the number of body segments that bear any respective internal structure is given in parentheses.

<table>
<thead>
<tr>
<th>Character</th>
<th>Lake Kayaharanoike (N=16)</th>
<th>Lake Suganuma (N=16)</th>
<th>Lake Yunoko (N=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>male gonopore</td>
<td>XI-XII</td>
<td>X-XI/XII</td>
<td>XI-XII</td>
</tr>
<tr>
<td>female gonopore</td>
<td>XII-XIII</td>
<td>XI-XII/XIII</td>
<td>XI-XII</td>
</tr>
<tr>
<td>salivary gland</td>
<td>X-XV (1-4)</td>
<td>XI-XV/XVI (1-4)</td>
<td>X-XV (1-3.5)</td>
</tr>
<tr>
<td>crop</td>
<td>XII-XX (3.5-7)</td>
<td>XII-XXI (3.5-8)</td>
<td>XIII-XX/XXI (4.5-6)</td>
</tr>
<tr>
<td>crop caeca</td>
<td>5-6 pairs</td>
<td>5-6 pairs</td>
<td>5-6 pairs</td>
</tr>
<tr>
<td>1st crop caecum</td>
<td>XIV-XV/XVI</td>
<td>XIII-XVI</td>
<td>XIII-XIV-XVI</td>
</tr>
<tr>
<td>2nd crop caecum</td>
<td>XV-XVI/XVII</td>
<td>XIV-XVI/XVII</td>
<td>XIV-XVI</td>
</tr>
<tr>
<td>3rd crop caecum</td>
<td>XVI/XVII</td>
<td>XV-XVI/XVII</td>
<td>XV-XVI/XVII</td>
</tr>
<tr>
<td>4th crop caecum</td>
<td>XVI/XVII-XVIII</td>
<td>XVI-XVII/XVIII</td>
<td>XVI/XVII-XVIII</td>
</tr>
<tr>
<td>5th crop caecum</td>
<td>XVII/XVIII-XIX</td>
<td>XVI/XVII-XIX</td>
<td>XVI/XVII-XVIII</td>
</tr>
<tr>
<td>6th crop caecum</td>
<td>XIX-XI/XX</td>
<td>XVIII-XX</td>
<td>XVIII-XX</td>
</tr>
<tr>
<td>terminal point of last caecum</td>
<td>XXI-XXII/XXIII</td>
<td>XXI-XXIII</td>
<td>XXI-XXII/XXIV</td>
</tr>
<tr>
<td>intestine</td>
<td>XIX-XXV (2-5)</td>
<td>XVIII-XXIV (1.5-3.5)</td>
<td>XIX-XXII/XXIV (1.5-4)</td>
</tr>
<tr>
<td>intestinal caeca</td>
<td>4 pairs</td>
<td>4 pairs</td>
<td>4 pairs</td>
</tr>
<tr>
<td>1st intestinal caecum</td>
<td>XX-XX/XXI</td>
<td>XVIII-XXII/XXII</td>
<td>XX-XXI/XXII</td>
</tr>
<tr>
<td>2nd intestinal caecum</td>
<td>XXI-XXI/XXII</td>
<td>XVIII-XXII</td>
<td>XXI-XXII/XIII</td>
</tr>
<tr>
<td>3rd intestinal caecum</td>
<td>XXI-XXII/XXIII</td>
<td>XVIII-XXII/XXIII</td>
<td>XXI-XXII/XXIII</td>
</tr>
<tr>
<td>4th intestinal caecum</td>
<td>XIX-XIII</td>
<td>XIX-XIII/XXIV</td>
<td>XIX-XIII</td>
</tr>
<tr>
<td>proboscis*</td>
<td>-VI-XV (2-8)</td>
<td>-VI-XV (6-8.5)</td>
<td>-VI-XV (4-8)</td>
</tr>
<tr>
<td>rectum</td>
<td>XXII-XXV</td>
<td>XX/XXI-XXV</td>
<td>XXIII-XXIV</td>
</tr>
<tr>
<td>testes</td>
<td>5-6 pairs</td>
<td>4-6 pairs</td>
<td>6-5 pairs</td>
</tr>
<tr>
<td>1st testisac</td>
<td>XII-XIII-XVI/XV</td>
<td>XIII-XVII/XIV-XVII</td>
<td>XII-XIII-XV</td>
</tr>
<tr>
<td>2nd testisac</td>
<td>XIII/XIV-XVII</td>
<td>XIV-XVII/XV</td>
<td>XIII-XVI</td>
</tr>
<tr>
<td>3rd testisac</td>
<td>XV-XVIII</td>
<td>XV-XIX</td>
<td>XIV-XVII</td>
</tr>
<tr>
<td>4th testisac</td>
<td>XVI-XIX</td>
<td>XVI-XX</td>
<td>XV/XVI-XVIII</td>
</tr>
<tr>
<td>5th testisac</td>
<td>XVII-XX</td>
<td>XVI/XVII-XIX/XX</td>
<td>XVI/XVII-XIX</td>
</tr>
<tr>
<td>6th testisac</td>
<td>XVII/XVIII-XX</td>
<td>XVII/XVIII-XIX</td>
<td>XVIII-XX</td>
</tr>
<tr>
<td>ovary</td>
<td>XVI-XXI</td>
<td>XVIII-XXIII/XXIV</td>
<td>XIV-XX</td>
</tr>
</tbody>
</table>

*Third to sixth body segments are almost fused and this condition is expressed as "-VI".*
Fig. 2. Relationships between values of body length (a) and maximum width (b) before and after fixation in *Helobdella stagnalis* from Lake Yunoko.
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both for body length ($r=0.91$; Fig. 2a) and body maximum width ($r=0.88$; Fig. 2b) in the individuals from Lake Yunoko. When the values after fixation (Y) were analyzed as a linear function of the values before fixation (X), the relationships were expressed by the following equations:

Body length: $Y=0.79+0.96X$

Maximum body width: $Y=0.18+1.0X$

These linear regression analyses indicated that both body length and width increased slightly after fixation, and the proportional changes in both body length and width through fixation were size-dependent. On the other hand, the fact that there was a significant correlation in the arcsine-transformed width/length ratios before and after fixation (Fig. 3) showed that qualitative differences in body shape could be retained through the present method of fixation (fixation after narcotization).

**Body shape**

The body shape in dorsal view is spearhead-like or broad-leaf shaped in all three populations, and this characteristic was well retained after fixation. There was a significant allometric correlation between body length and maximum body width in all three populations. The correlation coefficient in the Lake Yunoko pop-
Fig. 4. Relationships between body length and maximum body width in three populations of *Helobdella stagnalis*. Both axes are represented by logarithmic scales.

ulation (*r*=0.41) was lower than those for the other two populations (Fig. 4: Lake Kayaharanoike, *r*=0.74; Lake Suganuma, *r*=0.66). When the log-transformed maximum body width is analyzed as a function of log-transformed body length, there is no significant difference in the regression coefficient between the Lake Suganuma and Lake Yunoko populations (ANCOVA, *P > 0.1*), while the regression coefficient for the population in Lake Kayaharanoike was significantly larger than those in the former two populations (ANCOVA, *P < 0.001* for both combinations); that is, individuals from Lake Kayaharanoike were proportionally wider than those from either Lake Suganuma or Lake Yunoko.

**Discussion**

The present study showed that the overall body shape of freshwater leeches is little modified if narcotization is properly conducted prior to fixation. On the other hand, contraction or relaxation of muscles and resultant positional changes of ventral nerves (positions of ganglia) were observed despite full narcotization. Such
changes of muscle condition have already been reported (Castle 1900; Miller 1929) and tend to reduce the value of positional data of internal organs in relation to segmentation as reliable characters in the taxonomy of freshwater leeches.

Aside from a few exceptional individuals as mentioned in the results, internal characters that should not be affected by muscular contraction (the numbers and shapes of crop caeca and intestinal caeca, the number of testisacs, and the number of annuli between gonopores) did not differ among the three populations examined in this study; the states of these internal characters in all three populations fell into the range so far reported for *H. stagnalis* (Moore 1901; Harding and Moore 1927; Miller 1929; Ringuelet 1944; Sawyer 1972; Kutschera 1985, 1988).

The position of the eyes and the condition (position and shape) of the scute varied within a given population, as has been pointed out for this species before (Sawyer 1972; Klemm 1976). These characters are no longer used in species-level taxonomy of the genus *Helobdella* (Kutschera 1985, 1988). The number of annuli between the male and female gonopores was constant in all specimens examined and agreed with the condition that diagnoses *H. stagnalis*. On the other hand, the fact that the body shape of the leeches from Lake Kayaharanoike was distinctly different from that of the leeches from Lake Suganuma or Lake Yunoko suggests that the two forms belong to different species.

In conclusion, the present study showed that (1) the location of internal organs relative to segmentation is not informative for *Helobdella* taxonomy, (2) body shape can possibly be used, in some cases, as a species-level taxonomic character if the specimens are fixed by a standard procedure (Madill 1983), and (3) a supposedly cosmopolitan species *H. stagnalis* may possibly be separated into more than one species with restricted distributional ranges. These findings indicate that a worldwide taxonomic revision of the species of *Helobdella* by introducing new characters or by experimental interbreeding (Shankland et al. 1992) is necessary to clarify even a local *Helobdella* fauna, such as that in Japan.

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