HOW to PROCEED with SCIENTIFIC RESEARCH & HOW to MAKE SCIENTIFIC REPORTS

By Nagasaki West High School, Super Science Program
In cooperation with *Division of Invertebrate Zoology, American Museum of Natural History, NY, USA*

21 JULY 2022

PREFACE

The SCIENTIFIC METHOD is a process for experimentation that is used to explore observations and answer questions. All scientists must not follow this process exactly; some areas of science can be more easily tested than others. When direct experimentation is not possible, scientists may modify the scientific method. There are probably as many versions of the scientific method as number of scientists. Even when modified, the goal remains the same — to discover cause and effect relationships by asking questions, carefully gathering and examining the evidence and results, and seeing if all the available information can be combined into a logical, reasonable and reliable answer.

Even though we show the scientific method as a series of steps, keep in mind that new information or thinking might cause a scientist to back-up and repeat steps at any point during the process. A process like the scientific method that involves such backing up and repeating is called an iterative (repeatable) process. Whether you are doing a classroom science activity, independent (personal) research, or any other expert examination, understanding the steps of the scientific method will help you to answer the question as well as possible.

Scientific Process — *The Five Obligatory Steps*

The scientific method is a dynamic and open-ended process that scientists use when they investigate a question they have. It is not a series of prescribed steps that scientists follow to prove a hypothesis. Rather, it is a general plan that helps guide their investigation. And while all scientists use the Scientific Method, they might not use all the steps, or they may complete the steps in a different order. For example, a scientist might make observations and collect data about a subject that interests him or her for years before formulating a hypothesis.

Defining a question to investigate

As scientists conduct their research, they make observations and collect data. The observations and data often lead them to ask why something is the way it is. Scientists

pursue answers to these questions in order to continue with their research. Once scientists have a good question to investigate, they begin to think of ways (or methods) to answer it.

Making predictions

Based on their research and observations, scientists will often come up with a hypothesis. A hypothesis is a possible answer to a question. It is based on: their own observations, existing theories, and information they gather from other sources. Scientists use their hypothesis to make a prediction, a testable statement that describes what they think the outcome of an investigation will be.

Gathering data

Evidence is needed to test the prediction. There are several strategies for collecting evidence, or data. Scientists can gather their data by observing the natural world, performing an experiment in a laboratory, or by running a model. Scientists decide what strategy to use, often combining strategies. Then they plan a procedure and gather their data. They make sure the procedure can be repeated, so that other scientists can evaluate their findings.

Analyzing the data

Scientists organize their data in tables, graphs, or diagrams. If possible, they include relevant data from other sources. They look for patterns that show connections between important variables in the hypothesis they are testing.

Drawing conclusions

Based on whether or not their prediction came true, scientists can then decide whether the evidence clearly supports or does not support the hypothesis. If the results are not clear, they must rethink their procedure. If the results are clear, scientists write up their findings and results to share with others. The conclusions they draw usually lead to new questions to pursue.

§1 HOW TO PLAN AN EXPERIMENT

Variables

Scientists ask questions to find out more about the world, like "how can we get more energy from the sun?" and "how can we cure diseases or protect ourselves from infection?" To answer these questions, every scientist must do experiments. During experiments, factors that can change are called variables.

A variable is anything that can change and be measured. Two important types of variables are:
Independent Variables – the variable that is being changed during the experiment
Dependent Variables – the variable being tested or measured during the experiment
In an experiment, the effect of changing just one variable on another is tested; testing how the independent variable affects the dependent variable.
For this reason, other variables must be properly controlled, so that they do not affect the independent variable. These variables are *Control Variables*.

Making predictions (or hypothesizing)

Making a scientific prediction (hypotheses) involves statement what might happen.
Knowing which variables to control is important when designing experiments to find out if a prediction is right or wrong.
Identifying control variables makes sure that only the independent variable affects the dependent variable. This will ensure that the results from the experiment are valid.

Variables in experiments

A variable is a factor that can be changed or measured. It is important to be able to identify each variable in an experiment. Below are several examples.

Examples of experiments

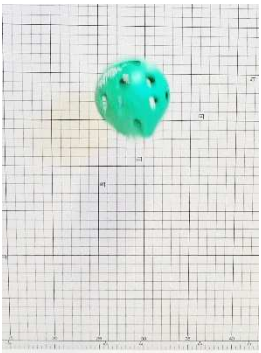
1. Plant growth and water

Adding different amounts of water to a plant could affect its growth. To investigate this, each planted seed is supplied with different amounts of water.

- *Independent variable* is the volume of water given to each plant.
- *Dependent variable* is how high the plant grows.
- *Control variables* include the size of the pots, the type of soil and the condition of environment (temperature, humidity, illuminance, etc.).

2. Dropping a ball from different heights

Dropping a ball from different heights could affect how high it bounces.



- ✧ *Independent variable* is the height of the drop.
- ✧ *Dependent variable* is how high the ball bounces.
- ✧ *Control variables* include the type of the ball, the surface (condition or quality) that it is dropped onto and the size of the ball.

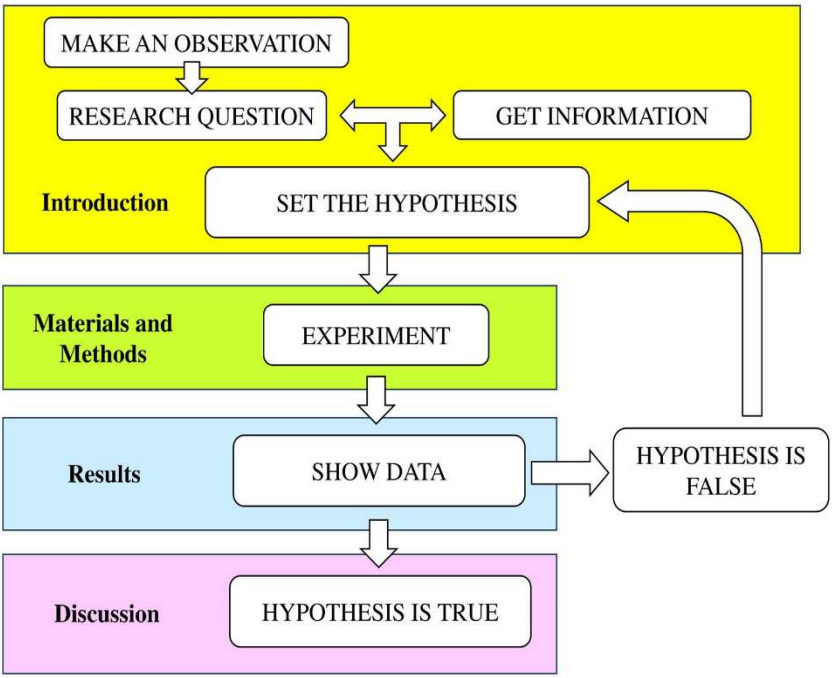
3. Testing reaction times

Reaction times can be investigated by dropping a ruler and seeing how quickly someone can grab it.

- ➢ *Independent variable* is the person who is trying to catch the ruler.
- ➢ *Dependent variable* is how far the ruler dropped.
- ➢ *Control variables* include the position of the ruler above the hand and the distance between the finger and thumb.

The above mentioned examples contain variables that are all measurable. Nonetheless, in some experiences, the variables are not always measured easily. In the following examples, the variables sometimes are difficult or impossible to be evaluated quantitatively.

- Verifying a recently discovered organism as a new species or which genus group accommodates the taxon.
- Which vaccine is the most effective against Corona-virus.
- Which color effects the rapid development of a fish species.



Schematic chart — How to proceed with scientific research

§2 PRINCIPAL STRUCTURE OF SCIENTIFIC REPORT

In scientific reports, IMRAD (composed of Introduction, Methods, Results and Discussion) is a common and standard structure. Almost of all scientific articles follow the IMRAD style. In addition, (References and Acknowledgements if any) are also necessary to be included (usually at the end). Paragraph writing should be used throughout the text for papers and articles.

TITLE

A scientific report and presentation should commence with a ***TITLE*** that succinctly explains the content, new finding and/or topic of the research. Using descriptive words that associate strongly with the content of the research is necessary.

ABSTRACT or SUMMARY

Usually, this section is forwarded to the ***INTRODUCTION*** (see below), and the author(s) should briefly provide the essence, or the most valuable, noteworthy highlights of the research (within 200–300 words). The statements must be concise and informative and should not include ambiguous assumptions or suppositions. An attractive ***ABSTRACT*** stands on its own and can be understood fully even when made available without reading the full contents.

INTRODUCTION

This section principally state: 1) background of research, 2) research process, 3) novel or significant result of new finding, 4) topic or highlight, etc. The ***INTRODUCTION*** not only clarifies the motivation for the work presented and previous information as well as research question but also prepares readers or audience for the structure of the paper or presentation.

MATERIALS and/or METHODS

In the majority of cases, this section follows the *INTRODUCTION* and can be separated into two respective subdivision, ***MATERIALS*** and ***METHODS***. The section will explain

the choices that the researchers made in the experimental procedure, such as — What justifies using a given compound, concentration, or dimension? —What is special, unexpected, or different in the approach? In this section the authors or presenters also should explain clearly how they carried out their own investigations in the following general structure and organization, for example:

- The organism(s) studied (plant, animal, human, etc., shown by Scientific Names) and, when relevant, their pre-experiment handling and care, as well as when (date and/or study period) and where (research sites) the study was carried out.
- In the case of a field investigation, a description of the study site, including the significant physical and biological features should be provided, (if necessary) with the punctual location (e.g. latitude and longitude, detailed map).
- The experimental or sampling design (i.e., how the experiment or study was structured. For example, controls, treatments, what variable(s) were measured, how many samples were tested, what kind of chemical reagents were used, etc.).
- How the data was analyzed (e.g. qualitative analyses and/or statistical procedures employed to determine significance, data transformations used, what probability was used to decide a proper conclusion).

RESULTS

The *RESULTS* section should objectively present genuine results, without any interpretation, modification nor supposition, in an orderly and logical sequence using both text and visual materials (Tables and Figures including Graphs). The *RESULTS* section always begins with text, reporting the key results and properly referring to the figures and tables shown. Summaries of the statistical analyses may appear either in the text (usually parenthetically) or in the relevant Tables or Figures (with the explanation or the legend along with every Table or Figure).

The *RESULTS* section is best organized around or nearby Tables and/or Figures that should be sequenced to present novel findings in a logical order. The text of the *RESULTS* section should be crafted to follow this sequence and highlight the evidence needed to answer the questions/hypotheses investigated. Important negative results also should be reported. Authors usually write the text of this section based upon the

sequence of Tables and Figures.

The *RESULTS* and *DISCUSSION* (see below) sections are frequently combined, because results make little sense to most readers without interpretation. In this case the combined section is shown as '***RESULTS AND DISCUSSION***'.

DISCUSSION

This is the most important part of any scientific report, which evaluates the level or quality of the researches. The function of the ***DISCUSSION*** is to interpret the results in light of what was already known about the subject of the investigation, and to explain a new understanding of the problem after taking the results into consideration. The ***DISCUSSION*** will always connect to the ***INTRODUCTION*** by way of the questions or hypotheses posited and the references cited, but it does not simply repeat or rearrange the ***INTRODUCTION***.

Fundamental research questions to answer would include:

- Do the results provide answers to the testable hypotheses? If so, how can the findings can be interpreted?
- Do the findings agree with what others have shown? If not, do the statements suggest an alternative explanation or perhaps an unexpected design flaw in the experiment?
- Given to conclusions, what novel understandings can be acquired from the problem investigated and outlined in the ***INTRODUCTION***?
- If warranted, what would be the next step of the study (further investigation), e.g. what experiments are encouraged in the future?

ACKNOWLEDGEMENT(S)

Most research works are supported by some or many people, namely colleagues, mentors, teachers, assistants, institutes… The author(s) politely expresses gratitude to such supporters in this section.

REFERENCES [*or LITERATURE CITED*]

There are no scientific papers or reports without any references. All genuine academic

reports must refer to previous relevant work(s) published in journals, books, and/or reliable websites provided by public institutions or universities (individual blogs or websites are often subjective and unreliable for scientific purposes).

Text Citations

In the text (from Introduction to Discussion sections), citations are, in spite of some different styles depending on books or journals, usually given as follows: Yasunaga (2021), (Yasunaga, 2010) or (Yasunaga & Duwal, 2019); more than one references are separated by a comma or semicolon (Brown & White, 1975; Schuh, 1988; Yasunaga, 2005) [as a rule, ordered by year]. The references with more than three authors are better cited as Asanabe et. al. (2019), Tamada et al. (2020) [only the first author shown]. All cited papers should be listed alphabetically at the end of the paper under the heading 'References', 'Literature Cited' or 'Bibliography'; papers not cited in the text should be omitted from the list of references. Order references in the text by date and number of the authors.

Reference List

Titles of journals are best not abbreviated. When available, adding DOI* numbers or URL citations are necessary and recommendable. *DOI — Digital Object Identifier: a unique series of numbers attached to a piece of digital information such as a website, file, or online article that is globally accessible.

Journal Article

Author(s) + Published year + Journal + Volume (number) + page(s)

<One author>

Aukema, B., 2018. Catalogue of the Palaearctic Heteroptera (searchable database). https://catpalhet.linnaeus.naturalis.nl/ (Accessed 7 Dec 2021)

Yasunaga, T., 1998. Revision of the mirine genus *Castanopsides* Yasunaga from the eastern Asia (Heteroptera: Miridae). Entomologica Scandinavica 29: 99–119. DOI: https://doi.org/10.1163/187631298X00221

<Two authors>

Yasunaga T. & M. D. Schwartz, 2007. Revision of the mirine plant bug genus *Philostephanus* Distant and allies (Heteroptera: Miridae: Mirinae: Mirini). Tijdschrift voor Entomologie 150: 101–180.
DOI:10.1163/22119434-900000216

<Three or more authors>

Yasunaga, T., H. Asanabe, A. Hirano, H. Momosaka, T. Nagashima & M. Hayashi, 2018. A unique new species of halophilous water strider of the genus *Aquarius* Schellenberg (Hemiptera: Gerridae: Gerrinae) endemic to Omura Bay, Nagasaki, Japan. The Canadian Entomologist 150: 413–439.
DOI: https://doi.org/10.4039/tce.2018.22

Book

Author(s) + Published year + Book title + Publisher & location + page(s)

Wheeler, A. G., 2001. Biology of the Plant Bugs (Hemiptera: Miridae), Pests, Predators, Opportunists. Cornell University Press, Ithaca & London, xv+507 pp.

Yasunaga, T., S. Maehara, T. Ishikawa & M. Takai, 2018. Guidebook to the heteropteran world — Basic ecology, morphology, classification and research methodology. Zenkoku Noson Kyoiku Kyokai, Tokyo, 212 pp.

Book Chapter

Yasunaga, T., 2001. Family Miridae, plant bugs. In: Yasunaga, T., M. Takai & T. Kawasawa (eds.), A Field Guide to Japanese Bugs II, pp. 112–276, figs. 2–331. Zenkoku Noson Kyoiku Kyokai, Tokyo.

Elucidation of cryptic ecology of 'Runner Plant Bugs' (Miridae: Phylinae: Hallodapini), with emphasis on stridulatory mechanism

Yui Tamada, Haruka Hinami, Ayana Miyazaki, Tomohide Yasunaga,
Tetsuya Nagashima & Serrien Serrano Leon

Published in Rostria (Hemipterological Society of Japan), No 65, 1–14 p. (2020)

Introduction

Runner plant bugs are ground-inhabiting members of the genus *Hallodapus* Fieber, 1858 and its allied genera (e.g., *Alloeomimella* Yasunaga & Duwal, *Wygomiris* Schuh) belonging to the tribe Hallodapini (Miridae: Phylinae). They are small-sized insects with a total body length 2.2–3.5 mm (cf. Fig. 2). All members of *Hallodapus* are known to uniquely prefer epigeic habitats and are presumed to prey upon other tiny arthropods on the ground under the cover of thick shrubs, dominated by graminoid weeds (Yasunaga 2001, Yasunaga et al. 2013a, 2013b, Duwal et al. 2017, Yasunaga et al. 2019). However, little is known about their ecology, due to the fundamental difficulty of sampling sufficient numbers of these bugs in their natural habitat, a complex undercover of deep meadows.

Schuh (1974, 1984) hypothesized that the minutely serrated or notched edge of the forewing (exocorium or embolium) and minute bumps or plectra on the dorsal surface of metafemur found in some members of Hallodapini represent a stridulatory device (e.g., *Hallodapus albofasciatus* (Motschulsky) known widely from the Old World tropics and subtropics). However, the practical function of these structures and the fundamental question of whether these tiny bugs actually emit sound remains to be verified (Yasunaga et al. 2019), although several previous works have documented acoustic communication in large-sized stinkbugs (Goula 2008, Schuh & Slater 1995). The present work was therefore initiated to confirm stridulation and to demonstrate that the structures (the forewing edge – dorsal metafemur) undoubtedly perform

stridulatory function as well as to clarify the cryptic ecology of these bugs, particularly for the Japanese species of *Hallodapus* Fieber. In addition, novel methodology to collect, rear and observe the targets was developed and applied.

This paper represents the second part of recent attempt to document the novel taxonomic, morphological and ecological findings for the taxa of Asian Hallodapini, subsequent to Yasunaga et al. (2019). The present part particularly reveals the enigmatic ecology of the hallodapine plant bugs.

SUGGESTION & HINT

INTRODUCTION is where you describe briefly and clearly why you are writing the paper. The section must supply sufficient background information for the reader to understand and evaluate the experiment you did. It also supplies a rationale for the study.

- Present the problem and the proposed solution
- Present the nature and scope of the problem investigated
- Review the relevant literature to orient the reader
- State the method of the experiment, the principle results and highlights of the work

Your own checklist

□ Indicate the field of the work, why this field is important, and what has already been done (with proper citations of references).

□ Indicate a gap, raise a research question, or challenge prior work in a similar field.

□ Outline the purpose and announce the present research, clearly indicating what is novel and why it is significant.

□ Avoid simply repeating the abstract; providing unnecessary background information; exaggerating the importance of the work; claiming novelty without a proper literature search.

Materials and methods

Field sampling: Both adults and immature forms of four *Hallodapus* spp. were captured using an engine-vacuum-netting method (Fig. 1C–E) from July 2018 to May 2019 at the following sites in Nagasaki Prefecture, Kyushu, Japan — Nagasaki City: Taira-machi (32°48'52.5"N, 129°46'42.6"E) (Fig. 1A), Yotsue-machi (32°48'28.9"N, 129°47'51.2"E) (Fig. 1B), Azekari New Fishery Port (32°48'56.1"N, 129°46'31.2"E), Mieda (32°49'09.9"N, 129°44'03.2"E), Kabashima (32°33'19.2"N, 129°46'37.3"E); Omura City: Kushima (32°89'64.6"N, 129°95'45.1"E). A net was placed in the vacuum tube of a handheld blower/vacuum (EBVK-2650; Ryobi, Fuchu, Japan) (Fig. 1C–D). Vacuumed samples, along with fallen leaves and soil, were transferred to a plastic tray, and runner plant bugs were collected using an insect aspirator (Fig. 1E).

Fig. 1. Habitats of epigeic hallodapine plant bugs (A–B) and sampling method (C–E). A–B, Sampling sites, Taira-machi (A) and Yotsue-machi (B).

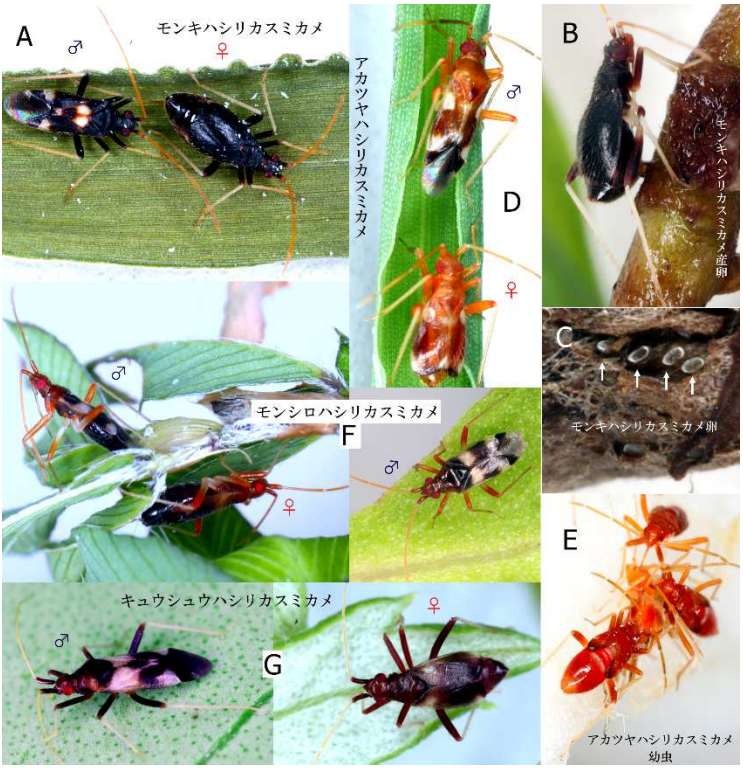


Fig. 2. *Hallodapus* species from Nagasaki. A–C, *H. centrimaculatus*; D–E, *H. ravenar*; F, *H. linnavuorii*; G, *H. kyushuensis*. A, D, F, G, Adults; B, oviposition; C, eggs laid near root of *Artemisia* weed; E, final instar nymphs feeding on a cadaver of conspecific nymph.

Long-term rearing method: Seventeen plastic cages [including 5 large (20 cm H x 30 cm W x 20 cm D) and 12 small (12 cm H x 20 cm W x 13 cm D) (Fig. 3B)] and more than 40 transparent plastic containers with a diameter of 12 cm (Fig. 3A) were used for breeding. The interior bottom surfaces of all plastic cages were sanded using sandpaper to prevent the samples from slipping and exhausting too much energy (as hallodapine pretarsal structures are simple, see Yasunaga et al., 2019). We used immature graminoid grasses and *Artemisia* weeds pulled up by their roots as egg-

laying sites. Water was constantly supplied via tissue paper. Breeding temperature and illuminance were 25℃ and 100–500 lx, respectively (equivalent to the illuminance near the roots of weeds at the field survey sites during daylight). A small folded tissue paper was immersed in a diluted fermented milk beverage and several dried red-worms (chironomid larva), both commercially available, were used as diet (Fig. 3A).

Species reared and tested in this work: *Hallodapus centrimaculatus* (Poppius, 1909) (Fig. 2A), *H. kyushuensis* Miyamoto, 1965 (Fig. 2F), *H. linnavuorii* Miyamoto, 1965 (Fig. 2E) and *H. ravenar* (Kirkaldy, 1902) (Fig. 2B).

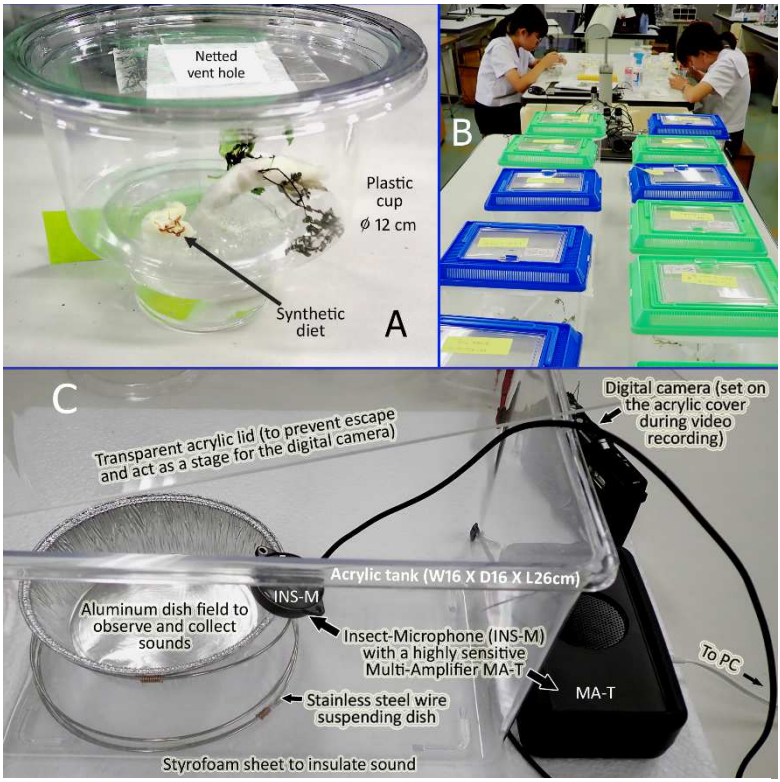


Fig. 3. A–B, Rearing and breeding containers; C, a stridulation recording unit used to detect the subtle vibrations made by runner plant bugs in motion.

Table 1. A–B, Rearing and breeding containers; C, a stridulation recording unit used to detect the subtle vibrations made by runner plant bugs in motion.

Species	Sex or stage	7/29	8/4	8/18	9/2	9/11	9/17	9/23	9/27	10/8	10/20	10/27	11/24	12/15	12/22	12/23	1/13	2/10	3/17	4/13	5/28
H. ravenar	♂	-	+	+	+	+	-	+	+	+	+	-	+	+	+	-	-	-	-	-	+
	♀	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	-	-	-	+
	nymph	-	+	+	+	+	-	+	+	-	-	-	-	-	+	-	-	-	-	-	+
H. centrimaculatus	♂	-	+	-	-	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-
	♀	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
	nymph	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
H. kyushuensis	♂	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	♀	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	nymph	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
H. linnavuori	♂	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-
	♀	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-
	nymph	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Laboratory observation and experiments: We maintained a group of individuals in the laboratory and observed each developmental stage. Video recording (by Olympus TG-5 digital camera) of the behavior of each individual and interactions between individuals in small breeding cages used for observation were made.

Examination of microstructures (stridulatory mechanism, phonoreceptor and the scent efferent system) of Hallodapine bugs: Using specimens collected from our fieldwork and other borrowed samples, we compared the morphology of femoral trichobothria. This structure is thought to function like a phonoreceptor and a scent efferent system. For our observations, we used a Hitachi Tabletop Scanning Electron Microscope ® TM 3030 (Hitachi High Technologies, Tokyo, Japan). Dry-preserved specimens of 33 Asian species (including 9 Japanese ones) in 9 genera of the Hallodapini were examined (see Yasunaga et al. 2019).

Development of the sound amplifying-recording system: The Insect-Microphone INS-M (Narika Corporation, Tokyo) was principally used to detect subtle sounds induced by *Hallodapus* spp. When a sensor is in contact with an insect needle attached to a branch of a tree with a bug walking on it, the vibration transmitted from the needle is converted into a signal of voltage change. Using this system, we can detect the subtle vibrations of insects in motion.

Runner plant bugs' bodies are too small to pick up the sounds they make as they move along solid surfaces. Therefore, we ultimately used a system that transfers the small

vibrations that the runner plant bugs make as they move. This system needed to be large enough to accommodate the bugs' mating and fighting behaviors, while at the same time, being able to amplify their sounds and transfer them into the needle. By testing various materials, we found that an aluminum dish suspended in the air was the best way to observe and collect the sounds (Fig. 3C).

Method for recordings of tiny bugs' stridulation: *Hallodapus centrimaculatus* and *H. ravenar* were tested, as sufficient numbers of individuals were available for the two species. Five to ten individuals (including both sexes) of the same species were released onto the dish field and observations were made of their behaviors. Simultaneously, videos were taken using the super-macro function of the digital camera. The recorded sounds were analyzed using a Wave-Pad Audio Editor (NCH Software, Colorado, USA) and the detected wave shapes for each species were constructed.

Verification of the usage of pheromones in the tribe Hallodapini: First, we put filter papers (9 mm in diameter and folded in four) in the cages with runner plant bugs for 120 hours. Then, we took out the filter papers from the cages and put them into new transparent plastic cases with different runner plant bugs. In the new cases, we also added new folded filter papers. We then observed which paper the runner plant bugs aggregated onto at regular time intervals.

SUGGESTION & HINT

Provide enough detail that any worker could repeat the experiment. Many of your readers will ignore this section because they already know from the Introduction or the general methods you used. However, careful writing of this section is important for your results to be of scientific merit. They must be reproducible. Otherwise your paper does not represent good science.

- Describe technical specifications, quantities of samples, source or method, equipment used, etc. as well as provide illustrations where relevant.
- Discuss detailed methodology if unusual, novel or advanced
- Prepare figure and tables for better recognition of the reader
- All examined organisms must be shown by 'Scientific Names'
- Show name and location of company if particular equipment is used

Results and discussion

Sampling and revealed life cycle of hallodapine bugs: Our continuing field investigation using the engine-vacuum-netting method successfully collected both adults and immature forms of four *Hallodapus* species (cf. Fig. 2, Table 1). Of these, *H. ravenar* (cf. Yasunaga et al., 2013a, b) was originally derived from tropical regions of Asia. The Amakusa area of central Kyushu, Japan was previously the northernmost extent of their distribution. However, we collected this species from Nagasaki Prefecture, extending their distribution further north as reported in our recent work (Ikeda et al. 2019). The beating or sweep-netting method did not yield any runner plant bugs, however ample individuals were collected using the engine-vacuum-netting method in a short amount of time. Therefore, we concluded that the latter method is best to collect runner plant bugs. Based on the collection records (2018–2019, Table 1), the four species occurring in Nagasaki likely overwinter in the egg stage, since no individuals were collected between late January and mid May, and both adults and nymphs of *H. centrimaculatus* and *H. ravenar* appeared from late May. These two species have at least two generations per year.

Synthetic diet applicable to the mass proliferation of biocontrol agents: A new synthetic diet was applied to four species of *Hallodapus* (see Table 1), and was used to rear all developmental stages of these bugs. For example, *Pilophorus typicus* (Distant) (a biocontrol agent for which mass proliferation is attempted, cf. Yasunaga et al. 2014) can be maintained by using this artificial diet. Significant cost reduction is expected with the new synthetic diet. For example, Mediterranean flour moth eggs (approx. 500 JPY/g) and brine shrimp eggs (approx. 200 JPY/g) are more expensive than our new synthetic diet (approx. 10 JPY/g). This diet can be put to practical use for low cost mass proliferation of biocontrol agents (natural enemies of pests).

Oviposition and food-preference: We could observe the oviposition of *H. centrimaculatus* and *H. ravenar*. The females laid eggs into the robust tissue of plants near their roots (Fig. 2B–C), which possibly affords protection of the eggs from natural enemies. *Hallodapus ravenar* required approximately 2 months to develop from the egg to the adult stage. Prior reports (cf. Yasunaga 2001) supposed that most hallodapines are predaceous. Our observation nonetheless suggested that the four

Japanese *Hallodapus* species we reared are not predators, but zoophagous. They did not attack any tiny 'living' arthropod, but instead fed primarily on arthropod cadavers (sometimes conspecific individual, as in Fig. 2E) and sometimes bird droppings. The runner plant bugs are therefore assumed to be 'scavengers'.

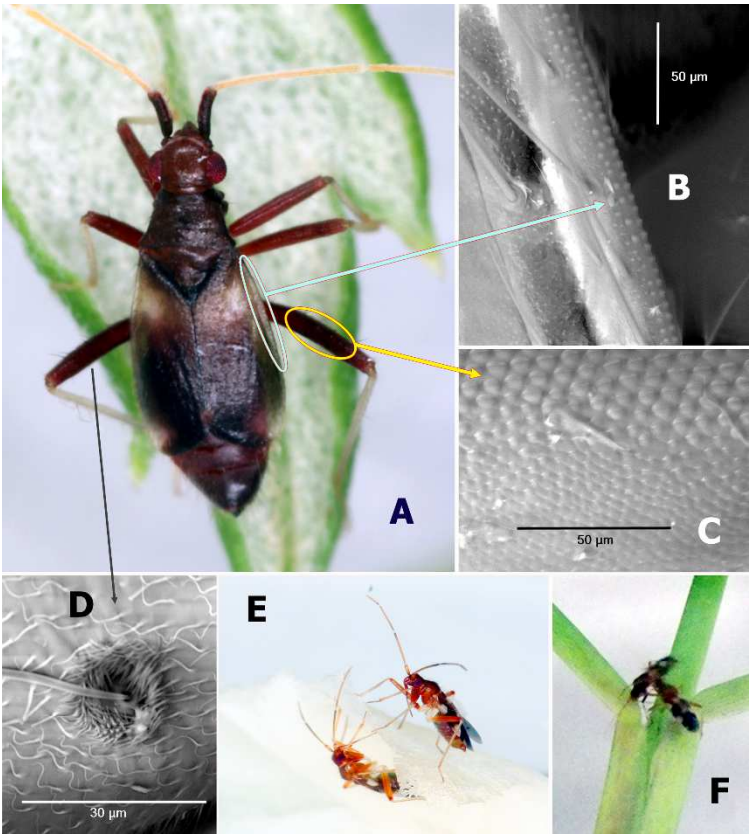


Fig. 4. *Hallodapus kyushuensis*, female (A–D) and *H. ravenar*, male (E–F). A, Dorsal habitus; B, edge of forewing (exocorium); C, metafemoral plectra; D, metafemoral trichobothrium; E, two males about to fight on synthetic diet; F, conflict between two males on a graminoid stem.

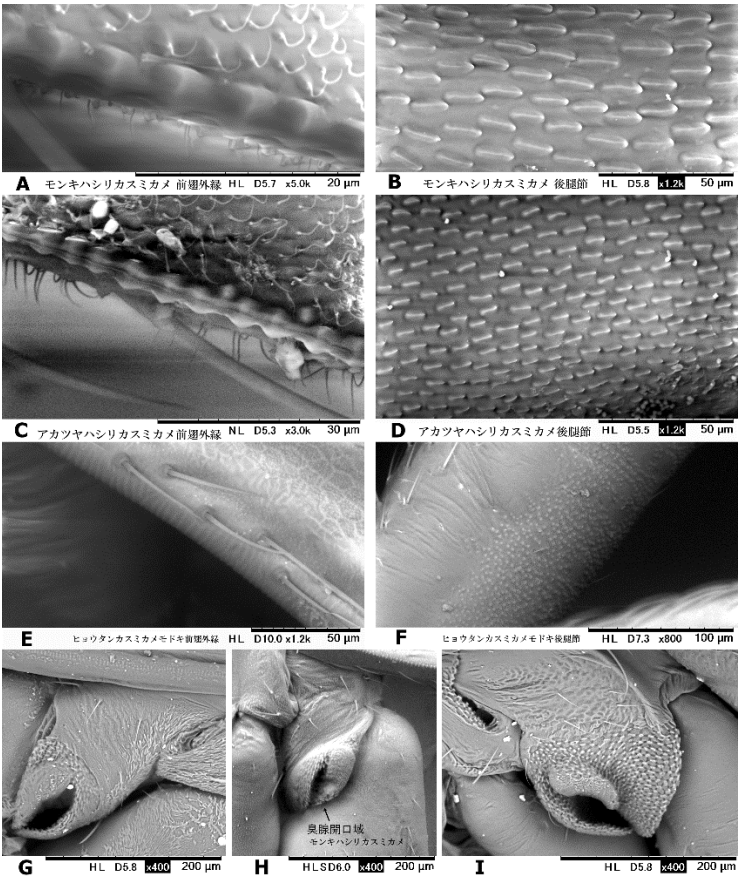


Fig. 5. Scanning electron micrographs showing stridulatory device (A–F) and metathoracic scent efferent system (G–I) of *Hallodapus centrimaculatus* (A–B, H), *H. ravenar* (C–D) and *Cleotomiris miyamotoi* (E–F), *Alloeomimella muiri* (Schuh) from Java, Indonesia (G) and *Wygomiris kaliyahae* Yasunaga from Nakhon Ratchasima, Thailand (I). A, C, E, Edge of forewing (exocorium); B, D, F, metafemoral plectra.

Aggressive conflict between male adults of the same species: Conspecific male individuals of *H. centrimaculatus* and *H. ravenar* occasionally fought for more than 1 minute when they encountered each other (e.g. Fig. 4E–F). Nothing was previously known about such intraspecific fighting behavior in the family Miridae. The significance of the intense fighting behavior found in runner plant bugs is yet to be confirmed. We presume that the fighting behavior may be territorial, concern courtship and/or food resources. Some of our sample movies are available from the following websites (You tube): Fighting between males of *H. centrimaculatus* (https://youtu.be/QjM1Q_24m9I); behavior between females of *H. centrimaculatus* (https://youtu.be/CWyv8plHHTw); and conflict between males of *H. ravenar* (https://youtu.be/RW0nHYj9Bw8).

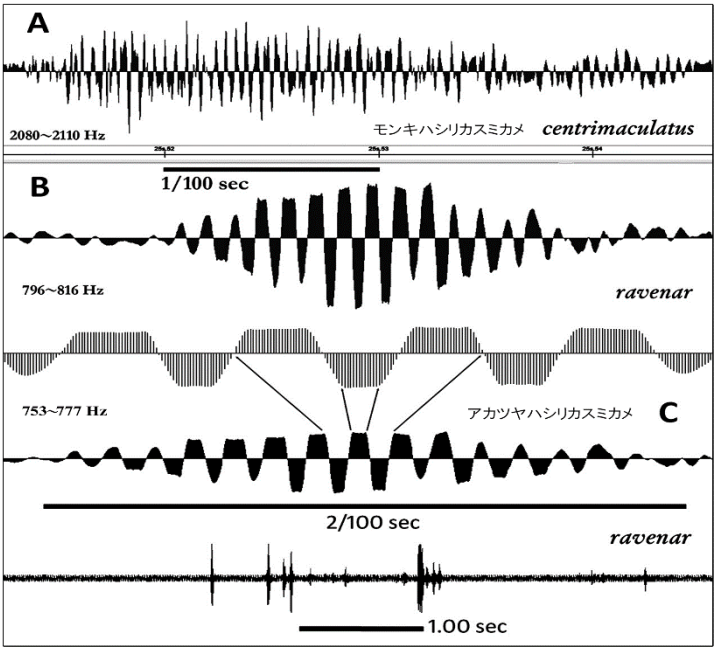


Fig. 6. Sound patterns emitted by *Hallodapus centrimaculatus* (A) and *H. ravenar* (B–C) species (detected with courtship behavior); y-axis showing sound in decibels (db)/ x-axis showing time in seconds (s)

Demonstration of hallodapines' acoustical communication: The wave shapes from the emitted sounds varied depending on the species. We observed their stridulation patterns during courtship behavior. We could successfully detect stridulation in two Japanese species, *H. centrimaculatus* (approx. 2,100 Hz) and *H. ravenar* (750–820 Hz) during laboratory observations (Fig. 6). Stridulatory patterns were found to be slightly variable even within the same species (Fig. 6B–C). Male and female hallodapine bugs exhibit stridulation only in the adult stage. It is reasonable to assume that they use stridulation during courtship behavior because the nymphs lack stridulatory structures. Since the stridulation patterns are different between species, these sounds may be used for distinguishing conspecific individuals.

Slightly different stridulatory patterns in the same species were recorded during our observation. It is possible that these sounds may represent differences in behavior. When the fighting behavior was observed between two males under the laboratory conditions (Fig. 4E), they seemed to rub their stridulatory structures against one another; however, these sounds were not recorded because the bugs did not fight on the dish field. Further observations regarding a stridulation pattern while in conflict are required.

Incidentally, we could not confirm the use of pheromones in runner plant bugs in the present experiment. Runner plant bugs showed no preference for which filter paper they selected. A close physiological examination, which is beyond the scope of the present study, is further required to verify the utilization of pheromones.

Phylogenetic discussion: The surface microstructures of 33 species in 9 Asian hallodapine genera were observed (see Yasunaga et al. 2019). We confirmed the presence of stridulatory structures in 4 genera (*Alloeomimella* Yasunaga & Duwal, *Cleotomiris* Schuh *, *Hallodapus* Fieber *and *Wygomiris* Schuh — *genera known from Japan). The species known to prefer epigeic habitats, without exception, have stridulatory structures (e.g., Fig. 4A–C) and no differences were found in these structures between male and female adults. The stridulatory structures are absent in immature forms. The size and shape of the stridulatory structures vary among species and can be used for identification (Fig. 5). Two Ethiopian genera, *Laemocoris* Reuter, 1879 and *Trichophthalmocapsus* Poppius, 1914 (Fig. 7), were also reported to have the

lateral hemelytral margins and dorsal surface of metafemora modified to form a stridulatory mechanism (Schuh 1974, 1984).

The species with stridulatory structures have a noticeably reduced scent efferent system (Fig. 5G–H). As evidenced by the results (cf. Fig. 6), the stridulatory structures are undoubtedly used for intraspecific communication (or possibly for courtship behavior). Unlike cicadas or other sound-emitting insects (e.g., orthopteran crickets), both males and females of hallodapine bugs can produce stridulation.

Schuh & Slater (1995) and Wheeler (2001) presumed that trichobothria (Fig. 4D) were used as phonoreceptors; however, further verification is required. Hallodapine bugs adapted to epigeic habitats are considered to have developed stridulation as a tool for communication and have reduced scent efferent systems (Fig. 5G–H). We confirmed the presence, shapes and function of the stridulatory structures, as well as the size of the scent efferent system (Fig. 5G–I). *Wygomiris kaliyahae* Yasunaga, 2012 known to inhabit aerial part of broadleaf trees in Thailand has a large scent efferent system (Fig. 5I).

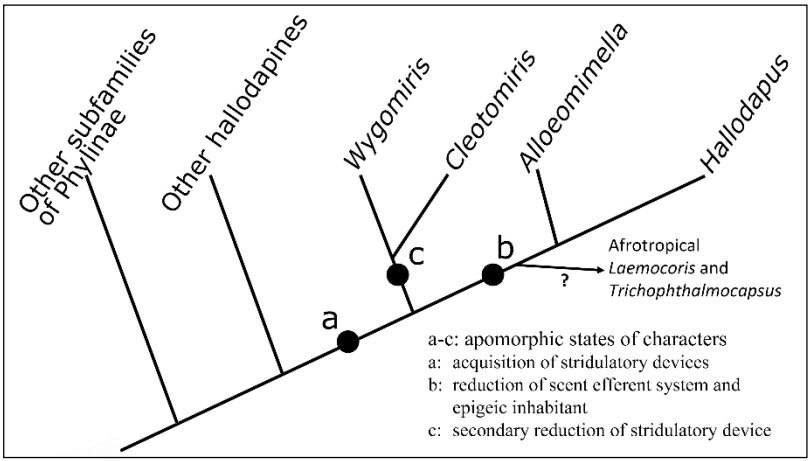


Fig. 7. Inferred phylogeny of (epigeic) hallodapines with stridulatory mechanism (modified from Schuh 1984, Yasunaga 2012).

Based on these observations, we propose a new hypothesis regarding the taxonomy and morphology of the plant bug tribe Hallodapini. The hallodapine bugs are presumed to have derived from an epigeic common ancestor that migrated from the aerial parts of plants to the ground and developed stridulation (Fig. 7). Many species in the subfamily Phylinae, including hallodapine bugs, are zoophagous; however, they depend on plants to propagate (Schuh 1984, Wheeler 2001, Yasunaga et al. 2001). Thus, we hypothesize that the common ancestor migrated from the aerial parts of plants, speciated into several taxa of the tribe Hallodapini, and acquired the stridulatory structures for intraspecific communication. Due to chaotic ground environments, sounds are most probably more effective than pheromones for the intraspecific communication of hallodapines. Stridulation is a significant mean of communication to differentiate between conspecific males and females, as well as to increase the chance of encounters on the messy ground surface.

SUGGESTION & HINT

RESULTS and DISCUSSION are often combined as this way is more rational and informative in some scientific reports. Results and discussion sections may be subdivided into subsections with short, informative headings.

'Results' should be the core of the paper. Do not initiate the section with methods you left out of the Materials and Methods section. You need to provide an essential description of the experiments and present the genuine data found.

- Factual statements supported by evidence, without excessive words
- Show representative data rather than endlessly repetitive data
- Discuss variables only if they had an effect (positive or negative)
- Use meaningful statistics, figures and/or tables
- Avoid redundancy. With good figures or tables, do not explain repeatedly, or re-state much of the data from a figure or table in the text. You are recommended to use the text to summarize what the reader will find in the table, or mention one or two of the most important, selected data. It is usually much easier to read data in a table or figure than in the text.

SUGGESTION & HINT

Discussion may be the most difficult section to write, although the section will evaluate the quality of your whole work. You are trying to bring out the true meaning of your data (results) without ambiguous words. Do not conceal your facts or reasoning and do not repeat your results; this is a discussion.

- Present principles, relationships and generalizations shown by the results
- Point out exceptions or lack of correlations. Define why you think this is so.
- Show how your results agree or disagree with previously published works
- Mention the theoretical implications of the work as well as practical applications
- State your conclusions clearly. Summarize good evidence for each conclusion.
- Discuss the significance, highlight and/or topic of the results
- Add future study or research plan if necessary

Academic reports usually have more than a few people who have helped in various way in the preparation of the written version or the research itself. These people need to be mentioned in the Acknowledgments section [as shown below].

Acknowledgements

Special thanks are due to the following individuals and/or institutions for offering or loaning materials, supporting fieldworks and/or sharing valuable information: Dr. R. T. Schuh (Curator Emeritus, American Museum of Natural History, New York); Dr. A. G. Wheeler (Clemson University, SC, USA); Drs. M. D. Schwartz & R. K. Duwal (Agriculture & Agri-Food Canada, Canadian National Collection of Insects, Ottawa, Ontario). We also thank CSR Division, Hitachi High-Technologies Corporation, Tokyo for generously allowing to use a tabletop scanning electron microscope (demo unit). This study was partly supported by Super Science High School (SSH) program promoted by the Japan Science & Technology Agency (JST).

References

Goula, M. 2008. Acoustical communication in Heteroptera (Hemiptera: Heteroptera). In: Capinera, J. L. (Ed.) Encyclopedia of Entomology. Springer, Dordrecht (https://doi.org/10.1007/978-1-4020-6359-6).

Ikeda N., H. Asanabe, Y. Tamada, H. Hinami, A. Miyazaki , T. Yasunaga, T. Nagashima & K. Tanaka, 2019. New distributional records for ten heteropteran species recently found in Nagasaki Prefecture, southwestern Japan. *Rostria,* 63: 45–51.

Schuh, R. T., 1974. The Orthotylinae and Phylinae (Hemiptera: Miridae) of South Africa with a phylogenetic analysis of the ant-mimetic tribes of the two subfamilies for the world. *Entomologica Americana,* 47: 1–332.

Schuh, R.T., 1984. Revision of the Phylinae (Hemiptera, Miridae) of the Indo-Pacific. *Bulletin of the American Museum of Natural History*, 177: 1–476.

Schuh, R. T. & J. A. Slater, 1995. *True bugs of the world (Hemiptera: Heteroptera). Classification and natural history.* Ithaca and London: Cornell University Press, xii+337 pp.

Wheeler, A. G., 2001. *Biology of the Plant Bugs (Hemiptera: Miridae), Pests, Predators, Opportunists*. Cornell University Press, Ithaca and London, xv+507 pp.

Yasunaga, T., 2001. Family Miridae Hahn, plant bugs. In Yasunaga T., M. Takai & T. Kawasawa (Eds.). *A Field Guide to Japanese Bugs II*. Zenkoku Noson Kyoiku Kyokai Publ. Co. Ltd., Tokyo, pp. 2–96, 111–351 (in Japanese).

Yasunaga, T. 2012. Review of the phyline plant bug tribe Auricillocorini from Asia, with descriptions of a new genus and nine new species from Japan, Nepal and Thailand (Hemiptera: Heteroptera: Miridae: Phylinae). *Zootaxa,* 3530: 1–24.

Yasunaga T., K. Yamada & T. Artchawakom, 2013a. New or little known taxa of the plant bug tribe Hallodapini (Hemiptera: Heteroptera: Miridae: Phylinae) from Thailand, with descriptions of three new species of *Acrorrhinium* Noualhier. *Zootaxa,* 3647 (3): 429–442.

Yasunaga T., K. Yamada & T. Artchawakom, 2013b. Additional records of the plant bug genus *Hallodapus* Fieber from Thailand, with proposal of a new synonymy (Hemiptera: Heteroptera: Miridae: Phylinae). *Zootaxa,* 3701 (5): 596–599.

Yasunaga T., K. Yamada & T. Artchawakom, 2014. Additional records and descriptions of the ant-mimetic plant bug genus *Pilophorus* from Thailand (Hemiptera: Heteroptera: Miridae: Phylinae: Pilophorini). *Zootaxa*, 3795: 1–15.

Yasunaga, T., S. Maehara, T. Ishikawa & M. Takai, 2018. *Guidebook to the heteropteran world — Basic ecology, morphology, classification and research methodology*. Zenkoku Noson Kyoiku Kyokai Publ. Co. Ltd., Tokyo, 212 pp. (in Japanese).

Yasunaga, T., Y. Tamada, H. Hinami, A. Miyazaki, R. K. Duwal & T. Nagashima, 2019. Taxonomic review for the Asian taxa of plant bug tribe Hallodapini, with emphasis on stridulatory mechanism (Hemiptera: Heteroptera: Miridae). *Acta Entomologica Musei Nationalis Pragae*, 59: 71–99.

SUGGESTION & HINT

This is the final section of the report. Here you should provide an alphabetical listing of all the published work you cited in the text. You may only include the works you actually mentioned in the text of your manuscript.

Your own checklist

- □ Include citations that provide sufficient context to allow for critical analysis of this work by others.
- □ Include citations that give the reader sources of background and related material.
- □ Include citations that provide examples of alternate ideas, data, or conclusions to compare and contrast with this work, if they exist.
- □ Include citations that contain additional relevant data or research.
- □ Are the citations up to date, referencing that the latest work on this topic?
- □ Does the author(s) verify the accuracy of the references?
- □ Do not provide spurious citations (citations that are not needed but are included anyway); biased citations (any reference added or omitted without good reason); and excessive self-cites (citations to author's own papers).

2020	Heteropterus Revista de Entomología **Heteropterus Rev. Entomol.**	**20(2):** 181-191

ISSN: 1579-0681

A preliminary assessment of the nymphal characters for ant-mimetic plant bugs of the genus *Pilophorus* (Hemiptera: Heteroptera: Miridae: Phylinae)

K. FUKUDA[1], K. KATAOKA[1], M. OZAKI[1], H. SASANO[1], T. YASUNAGA[2,*], H. ASANABE[3]

[1]Nagasaki West High School; SSH Biology (Mission II); Takenokubo 12-9; Nagasaki 852-8014; Japan; E-mail: ssh@news.ed.jp
[2]Research Associate; Division of Invertebrate Zoology; American Museum of Natural History; New York, NY 10024; USA; E-mail: [redacted]
*Corresponding author
[3]College of Arts and Science; The University of Tokyo; Komaba 3-8-1; Meguro; Tokyo 153-8902; Japan; E-mail: [redacted]

Abstract

Nymphal characters of the ant-mimetic plant bug genus *Pilophorus* Hahn, 1826 are reported, based on examination of the immature forms of nine Asian congeners. All developmental stages were successfully reared and investigated for two Japanese species, *P. okamotoi* Miyamoto & Lee, 1966 and *P. typicus* (Distant, 1909). A unique structure which currently is considered a synapomorphy shared by *Pilophorus* congeners is recognized on the nymphal scutellum, or metanotal area, and is herein documented as the «metanotal ridge».

Key words: Hemiptera, Miridae, Phylinae, *Pilophorus*, immature forms, morphology, biology, Japan.

SUGGESTION & HINT

An abstract (or summary) concisely introduces the essence, topics and/or highlight of your whole research. This section is usually mentioned in one paragraph and should provide the readers with a clear description of your study and its results without reading the entire paper. Do not make it lengthy.

NOTE

This article was based on a students' research activity performed as a part of Super Science High School program '**Mission II**' (2019–2020) and contains novel findings on small insects commonly occurring in a school campus and its neighboring areas where the students could access easily.

Introduction

Pilophorus Hahn, 1826 is one of the most speciose genera within the plant bug subfamily Phylinae (Schuh, 2002-2013). The genus is composed of more than 130 species (Aukema, 2018; Schuh 1984, 1995, 2002-2013), most of which have an ant-mimetic body form and ecologically more or less obvious relationships with ants (Schuh, 1989, 1991; Yasunaga and Schuh, 2013; Yasunaga *et al.*, 2014; Yasunaga and Duwal, 2016). As argued by some works (*e.g.*, Yasunaga *et al.*, 2001; Yasunaga and Duwal, 2016), several potential biocontrol agents are considered to be utilizable for integrated pest management (IPM) programs, or nature friendly farming (*cf. P. typicus*; see Ito *et al.*, 2009, 2011).

We initiated the present observation while screening suitable candidates of biocontrol agents within Asian *Pilophorus* species. During breeding and subsequently observing the immature forms of some congeners, unique morphological structures were found on the thoracic scutellar area, which possibly represents a synapomorphy shared by some members of the genus.

Nymphal characters and the natural history of *Pilophorus* species have hitherto been documented only briefly in a few publications (*cf.* Yasunaga *et al.*, 2001; Yasunaga and Schuh, 2013). Because of their unique myrmecomorphy, the nymphs of *Pilophorus* species are characteristic in shape from those of other mirids (*cf.* Wheeler, 2001; Yasunaga *et al.*, 2001; Yasunaga and Schuh, 2013). The present paper reports a preliminary result of some observations on the immature forms, with emphasis on the unique unknown character exhibited on the nymphal metanotum, or scutellar area.

Material and methods

Immature forms of the following *Pilophorus* species (with localities and hosts) were examined:

P. erraticus Linnavuori, 1962 – Locality: Nagasaki West High School, Nagasaki City, Nagasaki Pref., Japan (32.765907, 129.859517) – Host association: *Ficus superba* (Miq.) Miq. (Moraceae).

P. lucidus Linnavuori, 1962 – Agri-Hills Park, Yotsue Town, Nagasaki City (32.810000, 129.796255) – *Castanea crenata* Sieb. & Zucc., *Quercus acutissima* Carruth. (Fagaceae).

P. okamotoi Miyamoto & Lee, 1966 – Hiyoshi, Izuhara Town, Tsushima Island, Nagasaki Pref. (34.209555, 129.291200) – *Artemisia* sp. (Asteraceae).

P. typicus (Distant, 1909) – Nagasaki West High School Garden, Nagasaki City (32.766222, 129.859222) – Herbaceous vegetation dominated by *Artemisia* sp.

For sampling the material in Nagasaki, Japan, sweep-netting was mostly applied; an engine-vacuum-netting method (*cf.* Yasunaga *et al.*, 2019) was also effective to capture *Pilophorus typicus* (both adults and nymphs) in good numbers. The live samples were reared in acrylic petri dishes (9 × 2 cm) (Fig. 1a). Breeding methodology mainly followed Miyazaki *et al.* (2020) and Yasunaga *et al.* (2018); a folded tissue paper was immersed in a diluted fermented milk beverage (*e.g.*, Yakult), several dried red-worms (chironomid larva) and dried brine-shrimp eggs (all commercially available), were placed in each dish as diet (Fig. 1a-b). Young wormwood (*Artemisia* spp.) or *Kalanchoe daigremontiana* Raym.-Hamet & H. Perrier (Crassulaceae) were used as host plants to collect eggs (Fig. 1c-d).

Images of 5th instar nymphs, taken by one of the authors (Yasunaga), were also examined for *Pilophorus setulosus* Horváth, 1905 [from Aoyama, Tobetsu Town, Hokkaido, Japan (43.2977, 141.5611) – *Artemisia* sp. (Fig. 5b)]; *P. barbiger* Yasunaga & Schuh, 2013 and *P. maculatus* (Schuh, 1984) [Sakaerat Environmental Research Station (SERS), Wang Nam Khieo, Nakhon Ratchasima, Thailand (14.5099, 101.9300) – unidentified broadleaf tree (Fig. 5d)]; and *P. miyamotoi* Linnavuori, 1961 [Kibi-Hills, Okayama Pref., Honshu, Japan (34.8255, 133.7635) – *Pinus densiflora* Sieb. & Zucc. (Pinaceae) (Fig. 5a)]. Dried specimens of the 5th instar nymphs of two Oriental species were also examined for comparison: *Pilophorus barbiger* Yasunaga & Schuh, 2013 [Sakaerat Environmental Research Station, Nakhon Ratchasima, Thailand – *Gardenia sootepensis* Hutch. (Rubiaceae) (Fig. 5c)] and *P. palawanus* (Schuh, 1984) [MacRitchie Reservoir, Bukit Timah Hills, Singapore (1.3434, 103.8255) – flower of unidentified broadleaf tree (Fig. 7n)]. Fourth and 5th instar nymphs of *Pherolepis* sp. (possibly an undescribed taxon) were further examined for comparison [from Saga City, Kyushu, Japan (33.296777, 130.309085) – *Salix* sp. (Salicaceae) (Fig. 5e-f)]. Examined specimens are deposited in Nagasaki West High School or T. Yasunaga Collection, Nagasaki, Japan.

Scanning Electron Micrographs were taken with a Hitachi Tabletop Microscope® TM3030; some particular structures (Fig. 6a-b) were also observed using a Nikon Eclipse Ci upright microscope, with a photo-

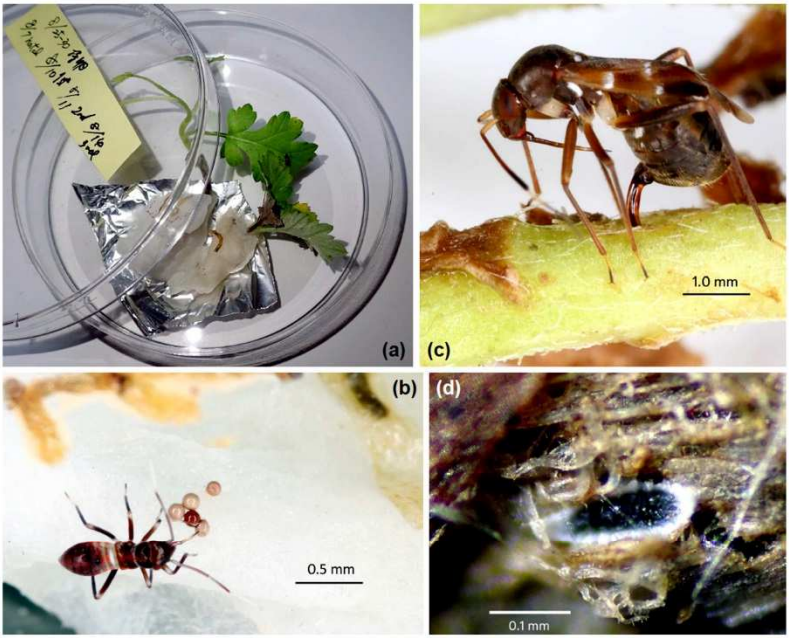


FIGURE 1. *Pilophorus okamotoi*, breeding samples: (a) Rearing unit; (b) 3rd instar immature feeding on brine-shrimp egg; (c) A female ovipositing into stem of *Artemisia* sp.; (d) Exposed operculum on the host stem.

phase unit. Measurements are given in millimeters; for some of the SEM images, scale bars are shown in micrometers (μm).

A new term «metanotal ridge (MR)» is provisionally given for the unique structure on the metanotum, or scutellar area. The following abbreviations are used to indicate some structures in the text and figures:

AS	abdominal segment
AW	mesonotal (anterior) wing-pad
MR	metanotal ridge
PW	metanotal (posterior) wing-pad
SG	abdominal scent gland opening

Results and discussion

During our recent attempts to clarify the life history and immature forms of *Pilophorus* species, we could confirm the nymphal characters for the following four species: *P. erraticus* Linnavuori, 1962 (3rd to 5th instar nymphs), *P. lucidus* Linnavuori, 1962 (3rd to 5th instar nymphs), *P. okamotoi* Miyamoto & Lee, 1966 (all immature stages, from egg to adult) and *P. typicus* (Distant, 1909) (all immature stages, from egg to adult). All of these were verified as predominantly carnivorous insects but sometimes sucked plant sap from the host leaves or stems during laboratory observation. As pointed out by some applied entomologists, *P. typicus* is regarded as an effective candidate for an indigenous natural enemy against various agricultural

Discussion section and Figures 2–5 are omitted. Complete version of PDF is available online: https://www.heteropterus.org/images/HRE/articulos/Heteropterus_Rev_Entomol_20(2)_181-191.pdf

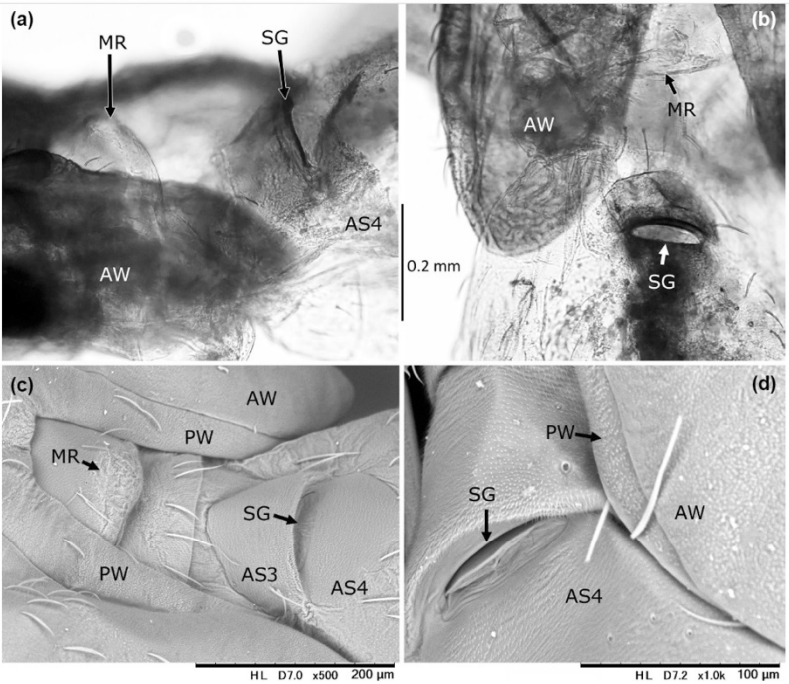


FIGURE 6. Detailed structures of posterior thorax and anterior abdomen of *Pilophorus typicus*, 5th instar: (a)-(b) Observed by compound microscope; (c)-(d) Scanning electron micrographs.

Acknowledgements

We are grateful to Mr. D. Terada and Mr. A. Hama (CSR Division, Hitachi High-Technologies Corporation, Tokyo) for generously allowing to use a tabletop scanning electron microscope (demo unit) as well as Mr. K. Tanaka, Mr. T. Nagashima, Mr. S. Kawashita, Mr. S. Serrano Leon, Ms. K. Maruno and Biology Club members (Nagasaki West High School) and Super Science High School (SSH) program (promoted by the Japan Science & Technology Agency) for supporting our research activities. The first author is indebted to Drs. T. Artchawakom and C. Phuvasa (Sakaerat Environmental Research Station, Thailand Ministry of Science and Technology, Nakhon Ratchasima) for supporting field investigation in Thailand. Thanks are extended to an anonymous reviewer and editors of Heteropterus Rev. Entomol. for improving the manuscript with useful comments and suggestions.

References

AUKEMA B. 2018. *Catalogue of the Palaearctic Heteroptera (searchable database)*. Available from: https://catpalhet.linnaeus.naturalis.nl/. Last accessed: 18 Sep 2020.

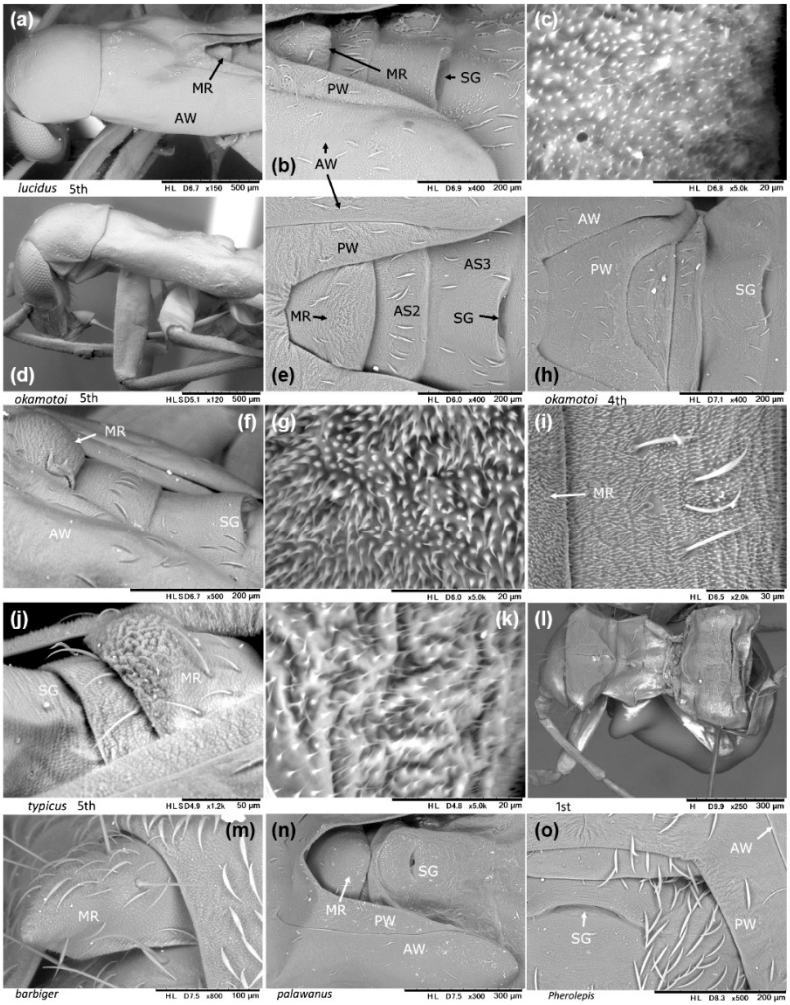


FIGURE 7. Scanning electron micrographs for immature forms of: (a)-(c) *Pilophorus lucidus*; (d)-(i) *P. okamotoi*; (j)-(l) *P. typicus*; (m) *P. barbiger*; (n) *P. palawanus*; (o) *Pherolepis* sp. [5th instars except for: (h)-(i) 4th; (l) 1st]: (a), (d) Anterior body; (b), (e), (f), (h), (i), (j), (n), (o) Dorsal parts of thoracic nota and abdominal sterna; (i), (m) Metanotum (scutellar area); (c), (g), (k) Metanotal ridge; (l) Dorsal habitus. Abbreviations as mentioned in Material and methods section.

ITO K, NISHIKAWA H, SHIMADA T, OGAWA K, MINAMIYA Y, HAYAKAWA H, FUKUDA T, ARAKAWA R. 2009. Molecular identification of genotypes of *Pilophorus typicus* (Heteroptera: Miridae) in Japan using PCR-RFLP analysis of mitochondrial DNA. *Environment Control in Biology* **47(4)**: 191-195.

ITO K, NISHIKAWA H, SHIMADA T, OGAWA K, MINAMIYA T, TOMODA M, NAKAHIRA K, KODAMA R, FUKUDA T, ARAKAWA R. 2011. Analysis of genetic variation and phylogeny of the predatory bug, *Pilophorus typicus*, in Japan using mitochondrial gene sequences. *Journal of Insect Science* **2**: 1-13.

MIYAZAKI A, NISHIDA M, UESUGI R, YAMADA U, YASUNAGA T, SERRANO LEON S, KAWASHITA S, NAGASHIMA T. 2020. Utilizing a synthetic diet containing a fermented milk beverage for rearing terrestrial heteropterans, with new distributional records of four species in Nagasaki Prefecture, Japan. *Rostria* **64**: 63-69. [in Japanese with English summary]

SCHUH RT. 1984. Revision of the Phylinae (Hemiptera, Miridae) of the Indo-Pacific. *Bulletin of the American Museum of Natural History* **177**: 1-476.

SCHUH RT. 1989. Old World Pilophorini: Descriptions of nine new species with additional synonymic and taxonomic changes (Heteroptera: Miridae: Phylinae). *American Museum Novitates* **2945**: 1-16.

SCHUH RT. 1991. Phylogenetic, host and biogeographic analysis of the Pilophorini (Heteroptera: Miridae: Phylinae). *Cladistics* **7**: 157-189.

SCHUH RT. 1995. *Plant bugs of the world (Insecta: Heteroptera: Miridae). Systematic catalog, distributions, host list and bibliography*. The New York Entomological Society. New York.

SCHUH RT. 2002-2013. *On-line systematic catalog of plant bugs* (*Insecta: Heteroptera: Miridae*). Available from: http://research.amnh.org/pbi/catalog/. Last accessed: 18 Sep 2020.

SCHUH RT, MENARD KL. 2011. Santatalean-feeding plant bugs: ten new species in the genus *Hypseloecus* Reuter from Australia and South Africa (Heteroptera: Miridae: Phylinae): their hosts and placement in the Pilophorini. *Australian Journal of Entomology* **50**: 365-392.

SCHUH RT, MENARD KL. 2013. A revised classification of the Phylinae (Insecta: Heteroptera: Miridae): Arguments for the placement of genera. *American Museum Novitates* **3785**: 1-72.

URAYAMA S, MATSUMOTO K, YAMAMICHI H, KAWASHITA S, NAGASHIMA T, YASUNAGA T. 2019. Heteropteran bugs found to inhabit *Zelkova* tree planted for landscaping at urbanized zones of Nagasaki City, Japan, close to «Hypocenter». *Rostria* **64**: 77-84. [in Japanese with English summary]

WHEELER AGJR. 2001. *Biology of the plant bugs (Hemiptera: Miridae). Pests, predators, opportunists*. Cornell University Press. Ithaca, NY.

YASUNAGA T, DUWAL RK. 2016. Three new species of the ant-mimetic plant bug genus *Pilophorus* from Japan (Hemiptera: Heteroptera: Miridae: Phylinae: Pilophorini). *Zootaxa* **4117**: 172-182.

YASUNAGA T, DUWAL RK. 2019. Reassessment of taxonomic characters for the plant bug genus *Hypseloecus* Reuter, with descriptions of new species of *Hypseloecus* and the related genus *Lasiolabops* Poppius (Heteroptera: Miridae: Phylinae). *Tijdschrift voor Entomologie* **159**: 41-65.

YASUNAGA T, SCHUH RT. 2013. Morphologically novel members of the ant-mimetic plant bug genus *Pilophorus* Hahn found in Thailand, with descriptions of three new species (Heteroptera: Miridae: Phylinae: Pilophorini). *American Museum Novitates* **3768**: 1-18.

YASUNAGA T, TAKAI M, KAWASAWA T (Eds.). 2001. *A field guide to Japanese bugs II*. Zenkoku Noson Kyoiku Kyokai Publ. Co. Ltd. Tokyo. [in Japanese]

YASUNAGA T, YAMADA K, ARTCHAWAKOM T. 2014. Additional records and descriptions of the ant-mimetic plant bug genus *Pilophorus* from Thailand (Hemiptera: Heteroptera: Miridae: Phylinae: Pilophorini). *Zootaxa* **3795**: 1-15.

YASUNAGA T, SCHUH RT, POORANI J, CASSIS G. 2015. A remarkable new genus and new species of the plant bug (Heteroptera, Miridae, Phylinae), inhabiting psyllid leaf margin roll gall on Indian banyan, *Ficus benghalensis*. *American Museum Novitates* **3839**: 1-15.

YASUNAGA T, MAEHARA S, ISHIKAWA T, TAKAI M. 2018. *Guidebook to the heteropteran world – Basic ecology, morphology, classification and research methodology*. Zenkoku Noson Kyoiku Kyokai Publ. Co. Ltd. Tokyo. [in Japanese]

YASUNAGA T, TAMADA Y, HINAMI H, MIYAZAKI A, DUWAL RK, NAGASHIMA T. 2019. Taxonomic review for the Asian taxa of plant bug tribe Hallodapini, with emphasis on stridulatory mechanism (Hemiptera: Heteroptera: Miridae). *Acta Entomologica Musei Nationalis Pragae* **59**: 71-99.

YESHWANTH HM. 2014. Five new species of *Hypseloecus* Reuter (Hemiptera: Miridae) on hemiparasitic Santalales from India. *Zootaxa* **3878**: 75-88.

Received / Recibido / Hartua: 19/09/2020
Accepted / Aceptado / Onartua: 22/09/2020
Published / Publicado / Argitaratua: 31/12/2020

Special thanks are due to Hitachi High-Tech Corporation, Toranomon, Minato-ku, Tokyo (Messrs. D. Terada and A. Hama), for allowing to use a Tabletop SEM TM3030®

How to proceed with scientific research & how to make scientific reports