Proc. Arthropod. Embryol. Soc. Jpn. 57, 7–10 (2026)

ISSN print 1341-1527 online 2759-2065

[SHORT COMMUNICATION]

Eggs of *Aphelocheirus vittatus* Matsumura, 1905 (Hemiptera, Heteroptera, Nepomorpha, Aphelocheiridae)

Ko NISHIMOTO[1] and Shodo MTOW[2]

[1] Graduate School of Agriculture, Meijo University, Shiogamaguchi 1–501, Nagoya, Aichi 468–8502, Japan
[2] Department of Agrobiological Resources, Faculty of Agriculture, Meijo University, Shiogamaguchi 1–501, Nagoya, Aichi 468–8502, Japan
Corresponding author: KN (helsinki19181214@gmail.com)

https://doi.org/10.60372/paesj.57.0_7

Nepomorpha, commonly known as aquatic bugs, is an infraorder of Heteroptera, comprising 13 families with about 2,400 species distributed on all continents except Antarctica (Schuh and Slater 1995; Polhemus and Polhemus 2008; Ye et al. 2020). Problems persist in the higher-level phylogeny of Heteroptera, and the phylogenetic position of Nepomorpha remains uncertain. Accordingly, the following groups have been proposed as sister group candidates of Nepomorpha: the remaining heteropteran infraorders (e.g., Mahner 1993; Weirauch and Schuh 2011; Weirauch et al. 2018; De Moya 2019), Cimicomorpha (Li et al. 2012), Pentatomomorpha + Cimicomorpha (Song et al. 2024), Leptopodomorpha + (Pentatomomorpha + Cimicomorpha) (Wheeler et al. 1993; Johnson et al. 2018), Leptopodomorpha + ((Gerromorpha + Dipsocoromorpha) + (Pentatomomorpha + Cimicomorpha)) (Xie et al. 2008), and (Enicocephalomorpha + Dipsocoromorpha) + Gerromorpha (Kieran et al. 2019).

Aphelocheiridae is a family of Nepomorpha distributed worldwide except in the Americas and Antarctica, comprising one genus, two subgenera, and 106 species (Schuh and Slater 1995; Zettel and Rattanachan 2020). Aphelocheirid nymphs and adults possess a plastron respiratory system that allows them to complete their entire life underwater (Polhemus and Polhemus 1988). The phylogenetic position of Aphelocheiridae within Nepomorpha has been debated based on molecular and morphological data, but it remains to be unresolved. Proposed sister groups include Naucoridae (e.g., Popov 1971; Mahner 1993; Hua et al. 2009), Potamocoridae (e.g., Hebsgaard and Damgaard 2004; Ye et al. 2020; Xian et al. 2025), and Notonectidae + (Pleidae + Helotrephidae) (Rieger 1976). Comparative embryology represents a promising approach for reconstructing the groundplan of a group and discussing the phylogenetic issues (e.g., Machida 2006; Fujita and Machida 2017; Mtow and Machida 2018; Shimizu and Machida 2024). However, information on embryonic development in Aphelocheiridae is totally lacking, except for brief descriptions of the egg (Ussing 1910; Larsén 1927; Messner and Adis 1999). On this background, we have started an embryological study of Aphelocheiridae, using the Japanese species *Aphelocheirus vittatus* Matsumura, 1905. In the present note, we examine and describe the egg structure of *A. vittatus* with scanning electron microscopy and report new observations on its external morphology.

On March 31 and May 12, 2024, and April 18, 2025, adults of *A. vittatus* were collected from the Akazu River, Wakamiya, Seto, Aichi, Japan. They were reared in plastic containers (22.5 cm × 14.5 cm × 12.5 cm) filled with tap water. The container bottoms were lined with gravel and stones, on which filtration (Roka Boy S, GEX, Osaka, Japan) and aeration (e–AIR 1000SB, GEX, Osaka, Japan) equipments were installed. Insects were fed on frozen bloodworms. Eggs deposited by females were cleaned with an ultrasonic cleaner (USC–2, Iuchi Seieido, Osaka, Japan) for 5 min, punctured with a fine tungsten needle in Ephrussi–Beadle solution (0.75% NaCl, 0.035% KCl, 0.021% $CaCl_2$), fixed with Kahle's fixative (ethyl alcohol : formalin : acetic acid : distilled water = 15 : 6 : 2 : 30) for 24 h, and stored in 70% ethyl alcohol at room temperature. Fixed eggs were then postfixed with 1% OsO_4 for 1 h, dehydrated through a graded ethyl alcohol series and 1,1,1,3,3,3-hexamethyldisilazane (HMDS), air-dried, mounted on stubs, coated with platinum using an ion sputter

(JEC–3000PC, JEOL, Tokyo, Japan), and observed under a scanning electron microscope (SEM; JSM–IT100, JEOL, Tokyo, Japan) at an accelerating voltage of 10 kV. To prevent shrinkage or deformation during dehydration, some eggs were processed with the nano-suit method following Fujita et al. (2016) and Nomura (2018): fixed eggs were immersed in a nano-suit solution for fixed specimens [ethyl alcohol : glycerin : polyoxyethylenesorbitan monolaurate (Tween 20) = 74 : 25 : 1] for 1 h, briefly blotted on dry filter paper, mounted on stubs, and observed under the SEM.

Females of *A. vittatus* lay eggs on stones in rearing containers. In the present study, egg orientation in *A. vittatus* is defined as follows: 1) the anterior is where the micropyle exists, and the posterior is its opposite; 2) the ventral is where the egg faces the substratum (stones), and the dorsal is the opposite.

Eggs of *A. vittatus* are ellipsoidal, with long and short diameters of about 1.2 mm and about 0.5 mm, respectively (Fig. 1A, B), and pale yellow immediately after oviposition. The egg surface shows a honeycomb pattern formed by chorionic ridges (Fig. 1A–D). Each honeycomb unit contains about 20–30 aeropyles (Fig. 1C). A micropylar process approximately 25 μm in diameter and about 5 μm height is present at the anterior pole of the egg, and a micropyle opens at its center; the honeycomb pattern is poorly developed around this region (Fig. 1D). A translucent, jelly-like adhesive substance covers the ventral surface of the egg (Fig. 1A, B).

The egg structure of Aphelocheiridae has previously been described only for *Aphelocheirus aestivalis* (Ussing 1910, described as *Aphelocheirus montandoni*; Larsén 1927; Messner and Adis 1999). Available information, which is based on light and/or scanning electron microscopy, remains fragmentary. In the present study, we examined the external egg morphology of *A. vittatus* for the first time and found no substantial differences between the *A. vittatus* eggs and *A. aestivalis* eggs. The egg structure of Aphelocheiridae can be characterized as (the feature newly found this time indicated in italics): 1) ellipsoidal in shape and pale yellow in color; 2) honeycomb surface pattern; 3) numerous aeropyles on the chorion; 4) a micropylar process at the anterior pole of the egg with a single micropyle; 5) *a jelly-like adhesive substance on the*

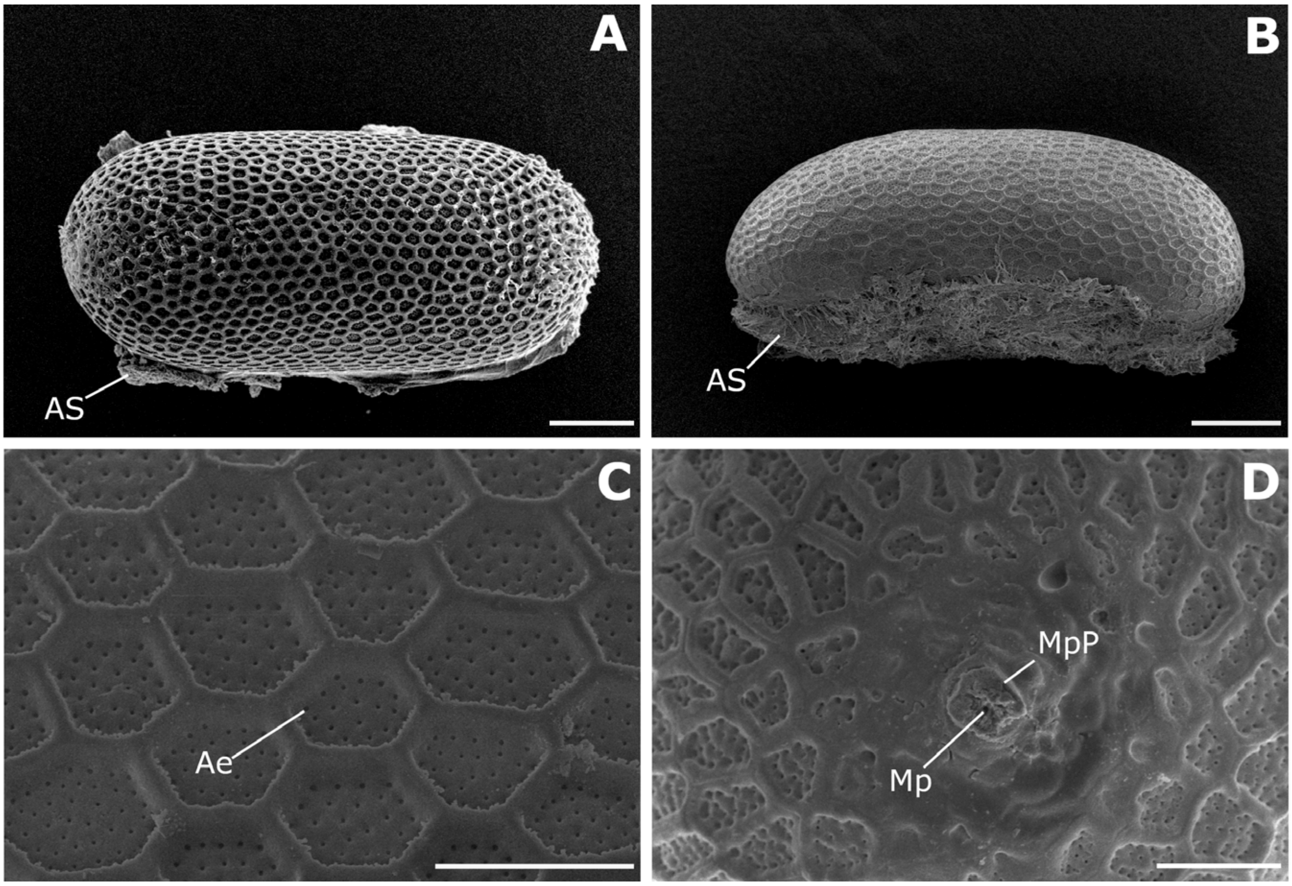

Fig. 1 SEMs of eggs of *Aphelocheirus vittatus*. A. Egg, dorsal view, anterior to the left, nano-suit method. B. Egg, lateral view, anterior to the left, conventional method. C. Enlargement of the ventral surface of the egg, conventional method. D. Anterior pole, conventional method. Ae: aeropyle, AS: adhesive substance, Mp: micropyle, MpP: micropylar process. Bars = A, B: 200 μm; C, D: 50 μm.

ventral surface of the egg.

While currently premature to infer the phylogenetic affinity of Aphelocheiridae within Nepomorpha, the information on the micropyles would be interesting. First, the eggs of Naucoridae possess multiple micropyles near the anterior pole, e.g., at least five in *Ambrysus* sp. (Cobben 1968), four in *Naucoris maculatus* (Poisson 1933), and three or four in *Ilyocoris cimicoides* (Poisson 1933; Cobben 1968), while lacking a micropylar process: these features differ from those observed in Aphelocheiridae. Second, the egg of *Potamocoris kleerekoperi* (Potamocoridae) bears a single micropyle at the anterior pole of the egg with mucous projections nearby (Cobben 1968, described as *Coleopterocoris kleerekoperi*), which may suggest a similarity to Aphelocheiridae in terms of the micropyle. To improve our understanding of the egg structures of Aphelocheiridae and Nepomorpha and to reconstruct their groundplan, additional data on egg structure, especially ultrastructural data from various lineages, are needed.

ACKNOWLEDGMENTS

We would like to express our sincere gratitude to Dr. Mari Fujita of the University of Tsukuba, for her valuable assistance in obtaining the literature.

REFERENCES

Cobben RH (1968) Evolutionary Trends in Heteroptera. Part 1. Eggs, Architecture of the Shell. Gross Embryology, and Eclosion. Centre for Agricultural Publishing & Documentation, Wageningen.

De Moya RS, C Weirauch, AD Sweet, RK Skinner, KK Walden, DR Swanson, CH Dietrich, KP Johnson (2019) Deep instability in the phylogenetic backbone of Heteroptera is only partly overcome by transcriptome-based phylogenomics. Insect Systematics & Diversity, **3**, 1–14.

Fujita M, A Blanke, S Nomura, R Machida (2016) Simple, artifact-free SEM observations of insect embryos: Application of the nano-suit method to insect embryology. Proceedings of the Arthropodan Embryological Society of Japan, **50**, 7–10.

Fujita M, R Machida (2017) Embryonic development of *Eucorydia yasumatsui* Asahina, with special reference to external morphology (Insecta: Blattodea, Corydiidae). Journal of Morphology, **278**, 1469–1489.

Hebsgaard MB, J Damgaard (2004) Phylogeny of the true water bugs (Nepomorpha: Hemiptera–Heteroptera) based on 16S and 28S rDNA and morphology. Systematic Entomology, **29**, 488–508.

Hua J, M Li, P Dong, Y Cui, Q Xi, W Bu (2009) Phylogenetic analysis of the true water bugs (Insecta: Hemiptera: Heteroptera: Nepomorpha): Evidence from mitochondrial genomes. BMC Evolutionary Biology, **9**, 134.

Johnson KP, CH Dietrich, F Friedrich, RG Beutel, B Wipfler, RS Peters, JM Allen, M Petersen, A Donath, KK Walden, AM Kozlov, L Podsiadlowski, C Mayer, K Meusemann, A Vasilikopoulos, RM Waterhouse, SL Cameron, C Weirauch, DR Swanson, DM Percy, NB Hardy, I Terry, S Liu, X Zhou, B Misof, HM Robertson, K Yoshizawa (2018) Phylogenomics and the evolution of hemipteroid insects. Proceedings of the National Academy of Sciences, **115**, 12775–12780.

Kieran TJ, ERL Gordon, M Forthman, R Hoey-Chamberlain, RT Kimball, BC Faircloth, C Weirauch, TC Glenn (2019) Insight from an ultraconserved element bait set designed for hemipteran phylogenetics integrated with genomic resources. Molecular Phylogenetics & Evolution, **130**, 297–303.

Larsén O (1927) Über die Entwicklung und Biologie von *Aphelocheirus aestivalis* Fabr. Entomologisk Tidskrift, **48**, 181–206.

Li H, H Liu, A Shi, P Stys, X Zhou, W Cai (2012) The complete mitochondrial genome and novel gene arrangement of the unique-headed bug *Stenopirates* sp. (Hemiptera: Enicocephalidae). PLoS One, **7**, 1–13.

Machida R (2006) Evidence from embryology for reconstructing the relationships of hexapod basal clades. Arthropod Systematics & Phylogeny, **64,** 95–104.

Mahner M (1993) Systema cryptoceratorum phylogeneticum (Insecta, Heteroptera). Zoologica, **143**, 1–302.

Messner B, J Adis (1999) Zur Atmung der Eier von der Ständig Submers Lebenden Grundwanze (*Aphelocheirus aestivalis*) (Hydrocorisae, Heteroptera). Naturschutzarbeit in Mecklenburg Vorpommern, **42**, 66–67.

Mtow S, R Machida (2018) Egg structure and embryonic development of arctoperlarian stoneflies: A comparative embryological study (Plecoptera). Arthropod Systematics & Phylogeny, **76**, 65–86.

Nomura S (2018) Recent developments and future perspectives in entomology: Systematics and morphology. Japanese Journal of Entomology (New Series), **21**, 14–26. (in Japanese with English abstract).

Poisson R (1933) Quelques observations sur la structure de l'oeuf des Insectes Hémiptères–Hétéroptères. Bulletin de La Société Scientifique de Bretagne, **10**, 40–77.

Polhemus DA, JT Polhemus (1988) The Aphelocheirinae of tropical Asia (Heteroptera: Naucoridae). Raffles Bulletin Zoology, **36**, 167–300.

Polhemus JT, DA Polhemus (2008) Global diversity of true bugs (Heteroptera; Insecta) in freshwater. Hydrobiologia, **595**, 379–391.

Popov YA (1971) Historical development of the hemipterous infraorder Nepomorpha. Trudy Paleontological Institute Academy of Science, **129**, 1–228.

Rieger C (1976) Skelett und Muskulatur des Kopfes und Prothorax von *Ochterus marginatus* Latreille. Beitrag zur Klärung der Phylogenetischen Verwandtschaftsbeziehungen der Ochteridae (Insecta, Heteroptera). Zoomorphologie, **83**, 109–191.

Schuh RT, JA Slater (1995) True Bugs of the World (Hemiptera: Heteroptera) Classification and Natural History. Cornell University Press, New York.

Shimizu S, R Machida (2024) Development and reproductive biology of Dermaptera: A comparative study of thirteen species from eight families. Arthropod Systematics & Phylogeny, **82**, 35–75.

Song N, MM Wang, WC Huang, ZY Wu, R Shao, XM Yin (2024) Phylogeny and evolution of hemipteran insects based on expanded genomic and transcriptomic data. BMC Biology, **22**, 190.

Ussing H (1910) Beiträge zur Biologie der Wasserwanze: *Aphelocheirus Montandoni* Horvath. Internationale Revue der gesamten Hydrobiologie und Hydrographie, **3**, 115–121.

Weirauch C, RT Schuh (2011) Systematics and evolution of Heteroptera: 25 years of progress. Annual Review of Entomology, **56**, 487–510.

Weirauch C, RT Schuh, G Cassis, WC Wheeler (2018) Revisiting habitat and lifestyle transitions in Heteroptera (Insecta: Hemiptera): Insights from a combined morphological and molecular phylogeny. Cladistics, **35**, 67–105.

Wheeler WC, RT Schuh, R Bang (1993) Cladistic relationships among higher groups of Heteroptera: Congruence between morphological and molecular data sets. Entomological Scandinavica, **24**, 121–137.

Xian C, J Damgaard, JY Luo, P Chen, J Xie, Y Wu, Q Xie, Y Wang (2025) Serial amber fossils unveiling the innovation process of mouthparts in water boatmen (Hemiptera: Corixoidea) in the phylogenetic context of total evidence. Insect Systematics & Diversity, **9**, 1–19.

Xie Q, Y Tian, L Zheng, W Bu (2008) 18S rRNA hyper-elongation and the phylogeny of Euhemiptera (Insecta: Hemiptera). Molecular Phylogenetics & Evolution, **47**, 463–471.

Ye Z, J Damgaard, H Yang, MB Hebsgaard, T Weir, W Bu (2020) Phylogeny and diversification of the true water bugs (Insecta: Hemiptera: Heteroptera: Nepomorpha). Cladistics, **36**, 72–87.

Zettel H, K Rattanachan (2020) *Aphelocheirus narumonae* sp. n., second species of the subgenus *Micraphelocheirus* Hoberlandt & Štys, 1979 (Hemiptera: Aphelocheiridae) from Thailand. Zootaxa, **4853**, 109–116.