

A systematic revision of the snail-leech genus *Alboglossiphonia* in Japan
(Hirudinea: Glossiphoniiformes: Glossiphoniidae)

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Running title: Systematic revision of Japanese *Alboglossiphonia*

Abstract

Freshwater leeches of the genus *Alboglossiphonia* Lukin, 1976 are predatory invertebrates with a global distribution. The genus' centre of diversity is in Africa, where 11 nominal species have been recorded. Several species also occur in Europe, North America, New Zealand, Tasmania and Asia, although taxonomic studies in these regions remain limited. *Alboglossiphonia lata* (Oka, 1910), long considered widespread in East Asia, has been studied in terms of morphology, ethology and developmental biology. However, its original description was based on only a few characteristics, leaving its taxonomic status uncertain. In this study, *A. lata* is redescribed based on newly collected specimens from its type localities. As the type specimens have been lost and multiple type localities exist, a neotype was designated for this species to resolve zoological and nomenclatural issues. Additionally, a new species, *A. reisui* sp. nov., is described from the Japanese Archipelago. This species shares many characteristics with *A. lata* but differs in its dorsal tubercle arrangement and colouration pattern. Phylogenetic analyses using nuclear and mitochondrial markers revealed that *A. reisui* sp. nov. forms a sister clade to a monophyletic group comprising *A. lata* and *A. pahariensis* Nesemann & Sharma, 2007. We also report the first records of *A. cf. hyalina* (O. F. Müller, 1774) and *A. sibirica* Bolotov, Eliseeva, Klass & Kondakov, 2022 in Japan.

Key words: Glossiphoniiformes, Hirudinea, molecular phylogeny, neotypification, redescription, 'Rhynchobdellida'

Introduction

Alboglossiphonia Lukin, 1976 is a freshwater leech genus in the family Glossiphoniidae (Sawyer 1986), comprising species that prey on invertebrates (Proctor and Young 1987). The genus is characterised by a unique eye arrangement of three pairs in a triangular shape, with some species also exhibiting united male and female gonopores. At least 25 species are recognised globally across all continents except Antarctica, with Africa serving as a diversity centre where 11 species have been recorded (Jueg 2023). However, Asia is also known for high *Alboglossiphonia* species richness. In this region, numerous species, including *Alboglossiphonia annandalei* (Oka, 1922), *A. bhamoensis* Bolotov, Eliseeva, Kondakov, Konopleva & Vikhrev, 2022, *A. heteroclita* (Linnaeus, 1761), *A. hyalina* (O. F. Müller, 1774), *A. kashiensis* Nesemann, 2007, *A. lata* (Oka, 1910), *A. pahariensis* Nesemann and Sharma, 2007, *A. sibirica* Bolotov, Eliseeva, Klass & Kondakov, 2022 and *A. weberi* (Blanchard, 1897), have been identified (Bolotov et al. 2022a, b; Jueg 2023). Among these, *A. weberi*, originally described from specimens collected in Lake Manindjau, Sumatra, was the first species to be described outside Europe, although the description was lacking internal morphological details (Blanchard 1897). The second oldest described species, *A. lata*, is the only one previously known from the Japanese Archipelago.

Alboglossiphonia lata is considered widespread in East Asia beyond Japan. Its ease of collection and rearing have made it the subject of study in morphology, ethology and developmental biology, among other fields (Jiménez et al. 2017; Kwak et al. 2021; Kwak et al. 2023). The species was originally described with limited morphological details in a simple key to Japanese leeches (Oka 1910a). Additionally, this description, published in Japanese in the journal *Doubutsugaku Zasshi* (= *Zoological Magazine*),

Volume 22, Issue 256, on 15 February 1910, lacked locality information. A redescription, seemingly intended as the original description by Oka, was published in German in *Annotationes Zoologicae Japonenses*, Volume 7, Issue 3, on March 27, 1910, specifying Honshu and Tokushima Prefecture as collection sites (Oka 1910b). As the limited features given were insufficient to distinguish *A. lata* from its congeners, the species has occasionally been treated as a subspecies or junior synonym of *A. weberi* (e.g. Autrum 1936; Soós 1969; Lukin 1976).

According to Oka (1910b), *A. lata* has multiple type localities, and its type specimens are presumed lost or destroyed, based on Nakano and Itoh (2011) and our own survey. Therefore, this study revisits the systematic accounts of *A. lata* with the topotypic specimens. Additionally, *Alboglossiphonia* specimens from various Japanese localities were newly collected, revealing individuals differing from *A. lata* in terms of their dorsal papillation pattern, colouration and body size. Using an integrative approach combining morphological examinations and phylogenetic analyses of nuclear and mitochondrial markers, we describe a new species and report two known species in Japan for the first time.

Materials and methods

Sampling. Leeches were collected by hands from underneath submerged rocks or debris at a total of 45 sites across various waterbodies in Japan (Fig. 1). Altitudes and coordinates for the localities were obtained using a Garmin eTrex GPS unit.

The collected leeches were kept at 13°C in the laboratory to digest meals. Subsequently, the specimens were relaxed by the carbonated water or gradual addition of 99% ethanol to freshwater. A quarter of the caudal sucker was taken from every

specimen for DNA extraction, and the remaining bodies were fixed in 10% formalin and preserved in 70% ethanol. The following four body measurements were taken: body length from the anterior margin of the oral sucker to the posterior margin of the caudal sucker (BL), maximum body width (BW), caudal sucker length from the anterior margin to the posterior margin of the sucker (CL) and caudal sucker width from the right to the left margins of the sucker (CW). Examination, dissection and drawing of the specimens were accomplished under stereoscopic microscopes with a drawing tube (Leica M125C and M165C). Some small specimens were compressed between two glass slides, stained with a mixture of Mayer's paracarmine and Ehrlich's haematoxylin, and then mounted on slides. Specimens used in this study have been deposited in the Zoological Collection of Kyoto University (KUZ).

The somite numbering convention is based on Moore (1927): body somites are denoted by Roman numerals and the annuli in each somite are given alphanumeric designations.

The leech collection at the National Museum of Nature and Science, Tsukuba, Japan (NSMT), including most of Oka's leech collection (Nakano 2010), was surveyed to determine whether the type series of *Alboglossiphonia* species still exists. Although a portion of Oka's collection is kept at The University Museum, The University of Tokyo (UMUT), the type series was not discovered in the UMUT zoological collection (Nakano and Itoh 2011).

Molecular analyses. Total DNA was extracted from the caudal sucker using phenol and chloroform extraction. The partial sequence of mitochondrial cytochrome *c* oxidase subunit I (COI), tRNA^{Leu} and NADH dehydrogenase subunit 1 (tRNA^{Leu}-ND1) and

nuclear 18S ribosomal RNA (18S) of representative specimens were PCR-amplified. The PCR and cycle sequencing reactions were performed according to Nakano (2016) except for the use of the PCR reaction kit, EmeraldAmp PCR Master Mix (Takara Bio). The newly determined sequences were deposited in the International Nucleotide Sequence Databases (INSD) through the DNA Data Bank of Japan.

Based on Siddall et al. (2005) and Bolotov et al. (2022b), we retrieved COI, ND1 and 18S sequences of the *Alboglossiphonia* species from INSD, three species of *Glossiphonia* Johnson, 1816 that has been shown to be most closely related to *Alboglossiphonia*, and 12 representative species of the family Glossiphoniidae (Table 1). The sequences were aligned with MAFFT v. 7.427 with the L-INS-i option. Phylogenetic trees were inferred with maximum likelihood (ML) and Bayesian inference (BI) methods. The best-fit partition scheme and substitution models were identified with AICc using PartitionFinder algorithm implemented in IQ-TREE v. 1.6.12 (Nguyen et al. 2015) as follows: for COI, GTR + I + G; for tRNA^{Leu}, F81; for ND1, GTR + G4; and for 18S, GTR + I. The ML phylogeny was inferred using IQ-TREE and nonparametric bootstrapping (BS) was subsequently conducted with 1000 replicates. BI tree and Bayesian posterior probabilities (PP) were obtained using MrBayes v. 3.2.6 (Ronquist et al. 2012). Two independent runs of four Markov chains were conducted for two million generations and a tree was sampled every 1000 generations. Parameter estimates and convergence were checked using Tracer v. 1.7.1 (Rambaut et al. 2018) and the first 10% of trees were discarded based on the result. Pairwise comparisons of the uncorrected *p*-distance for COI between the representative specimens of *Alboglossiphonia* obtained in this study were calculated using MEGA X (Kumar et al. 2018).

Two different methods were used for species delimitation analyses to assess the genetic differentiation within our dataset: Assemble Species by Automatic Partitioning (ASAP) (Puillandre et al. 2021) and Bayesian implementation of Poisson Tree Processes (bPTP) (Zhang et al. 2013). The ASAP analysis was conducted with the partial COI sequences, using the online ASAP version (<https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html>) with default settings and the K2P distance model. The bPTP analysis was performed using the BI tree based on COI, ND1 and 18S markers at the bPTP web server (<https://species.h-its.org>) with the following settings: No. MCMC generations, 100 000; Thinning, 100; Burn-in, 0.1; and Seed, 123.

Results

Molecular analyses. The BI tree ($\ln L = -9597.668$; Fig. 2) had a topology nearly identical to that of the ML tree ($\ln L = -9531.792$). Our analyses revealed that Japanese *Alboglossiphonia* comprise four lineages: *A. lata*, one undescribed species and two newly recorded species from the Japanese Archipelago (Fig. 2). The *A. lata* clade, including specimens from Japan, the Russian Far East, Taiwan and Hawaii, was moderately supported (BS = 56%, PP = 0.90) and formed a strongly supported clade with *A. pahariensis* and an undetermined Australian species (BS = 98%, PP = 1.0). *Alboglossiphonia reisui* sp. nov. formed a well-supported clade of Japanese specimens and one Korean sequence (BS = 92%, PP = 1.0), which was a sister to the *A. lata* + *A. pahariensis* + *A. sp.* clade (BS = 89%, PP = 1.0). *Alboglossiphonia* cf. *hyalina* was suggested to be a sister to *A. bhamoensis* (BS = 87, PP = 1.0). One *Alboglossiphonia* specimen collected from Hokkaido formed a clade with *A. sibirica* from Russia (BS = 100%, PP = 1.0). For *Alboglossiphonia* species, the ASAP analysis assigned the best

score to the hypothesis of 11 groups, and mPTP resulted in 12 groups. In both analyses, *A. lata*, *A. cf. hyalina* and *A. sibirica* were each classified as a single species, whereas *A. reisui* sp. nov. was split into three candidate species.

Systematics

Genus *Alboglossiphonia* Lukin, 1976

Alboglossiphonia lata (Oka, 1910)

Figs 3, 4 and 5A

Glossiphonia lata Oka, 1910a: 58; Oka 1910b: 168; Kaburaki 1921: 699–700; Moore 1924: 352–353; Oka 1928: 545–546, fig. 2; Moore 1930: 175; Chen 1959: 562–563; Chen 1960: 41; Yang 1996: 41–44, fig. 33.

Glossiphonia weberi lata Autrum 1936: 14; Moore 1946: 172–173; Lukin 1960: 41–42; Soós 1969: 414.

Glossiphonia (Alboglossiphonia) weberi Lukin 1976: 261–266, figs 116–119.

Alboglossiphonia lata Sawyer 1986: 659; Nesemann et al. 2007: 201, pl. 54, figs 10–12; Lai et al. 2009: 41; Lai & Chen 2010: 6–10, fig. 1; Jueg 2023: 15.

Amended diagnosis. Body greyish with a number of dark narrow stripes. Caudal sucker ventral, oval. Somites V–XXIV triannulate. Anus between somites XXVI and XXVII. Male and female gonopores united; common gonopore opened in posterior margin of XII a1. Eyes in three pairs; 1st pair closest together. Dorsal tubercles developed on every mid-body annulus forming one median row, one pair paramedian tubercles on a2, one pair outer paramedian tubercles on a3. Mouth pore on centre of oral

sucker. Salivary gland cells diffuse. Oesophagus simple, oesophageal gland absent. Bacteriosomes absent. Crop giving rise to six pairs of crop caeca, 1st pair diverticulated into two sections, 2–5th pairs simple, 6th pair (post-crop caeca) diverticulated into four or five sections. Intersomital testisacs in six pairs. Paired sperm ducts thick, tubular. Atrial cornua directed anteriorly, developed. Paired ovisacs tubular.

Neotypification. The specimens examined in *A. lata*'s original description (Oka 1910a) were automatically designated as syntypes under Article 73.2 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999; hereafter, 'the Code'). However, no type series were found at NSMT or UMUT and the syntypes are believed to have been lost or destroyed, according to Nakano and Itoh (2011) and our own survey.

The original description (Oka 1910a) did not specify *A. lata*'s type locality, but the redescription, which is believed to have used the same collections, listed multiple localities (Oka 1910b). The present study describes *A. reisui* sp. nov., which shares a similar morphology with *A. lata* and can occur syntopically with this species. As the original description (Oka 1910a) lacks sufficient morphological details to distinguish the two, both species may have been mixed in Oka's collection, further obscuring *A. lata*'s taxonomic status. Under Article 75.1 of the Code, a neotype should be designated when no name-bearing type specimen is believed to be extant and such a type is needed to objectively define the nominal taxon. Therefore, to resolve zoological and nomenclatural issues, we designate a neotype for *A. lata*, which has been deposited at the KUZ, a repository for Japanese leech collections.

Oka (1910b) reported *Alboglossiphonia* specimens collected from Honshu

(Hondō in his paper) and Tokushima Prefecture (Shikoku [Tokushima]). Being employed at Tokyo Higher Normal School since 1897 (Fukui 1937), he may have collected specimens near Tokyo. Therefore, we selected one specimen (KUZ Z5764) newly collected from the Tokyo area as the *A. lata* neotype. This specimen exhibits dorsal papillae arrangement consistent with that of *A. lata*, as described by Oka (1928) and possesses the characteristics provided in the amended diagnosis.

Material examined. Neotype: KUZ Z5764, a mature specimen, dissected, collected from Hiki-gun, Saitama Prefecture, Japan (35°57'21"N, 139°31'11"E; elev. 27 m) on 1 June 2024. Additional material: In total, six mature specimens were examined and dissected: KUZ Z5766 collected from Seiro-machi, Niigata Prefecture, Japan (37°57'56"N, 139°15'36"E; elev. 5 m); KUZ Z5768 collected from Azumino City, Nagano Prefecture, Japan (36°20'45"N, 137°54'58"E; elev. 519 m); KUZ Z5769 collected from Shizuoka City, Shizuoka Prefecture, Japan (35°00'17"N, 138°23'37"E; elev. 8 m); KUZ Z5771 collected from Kusatsu City, Shiga Prefecture, Japan (35°03'11"N, 135°55'22"E; elev. 85 m); KUZ Z5772 and Z5773 (two specimens) collected from Otsu City, Shiga Prefecture, Japan (35°02'53"N, 135°52'27"E; elev. 85 m).

Description of the neotype. Body lanceolate (Fig. 3A–B). Caudal sucker ventral, oval; BL 21.2 mm, BW 5.90 mm, CL 2.06 mm, CW 1.84 mm.

Somites I and II comprising four annuli altogether. Somite III uniannulate. Somite IV biannulate ($a_1 + a_2 = a_3$). Somites V–XXIV triannulate $a_1 = a_2 = a_3$. Somite XXV biannulate $a_1 = (a_2 + a_3)$. Somites XXVI and XXVII uniannulate. Anus between

231 XXVI and XXVII. Total annuli 71.
 232 Clitellum unobservable.
 233 Male and female gonopore joined and common gonopore at posterior margin of
 234 XII a1.
 235 Eyes in three pairs on IV a1 to V a1; 1st pair closest together; following two
 236 pairs spaced from 1st pair. Dorsal tubercles developed medially on every mid-body
 237 annulus forming one conspicuous row; one pair paramedian tubercles on a2; one pair
 238 outer paramedian tubercles on a3 (Fig. 5A). Each ring more or less papillated.
 239 Nephridiopores undetectable.
 240 Mouth pore on centre of oral sucker. Proboscis in membranous sheath reaching
 241 to between XII and XIII, without forming loop (Fig. 4A). Salivary gland cells diffuse.
 242 Oesophagus simple (Fig. 4A); Oesophageal gland absent. Bacteriosomes absent. Crop
 243 reaching to between XIX a2 and a3, giving rise to six pairs of crop caeca (Fig. 4A); 1st
 244 pair of crop caeca diverticulated in two sections; 2–5th pairs simple; 5th pair, right
 245 caeca diverticulated into two sections: 1st pair XIII a3–XIV a2; 2nd pair XV a1–a3; 3rd
 246 pair XVI a1–a3; 4th pair XVII a1–a3; 5th pair XVIII a1–a3; and 6th pair (post-crop
 247 caeca) diverticulated into four to five sections, in XIX a1–XXII a3. Intestinal caeca in
 248 four pairs (Fig. 4A): 1st pair in XIX a2–a3; 2nd pair in XX a1–a2; 3rd pair in XX a3–
 249 XXI a1; 4th pair in XXI a2–a3. Rectum simple tubular (Fig. 4A). Testisacs in six pairs,
 250 intersomital (Fig. 4A): 1st pair in XIII a2–a3; 2nd pair in XIV a3–XV a1; 3rd pair in
 251 XV a3–XVI a1; 4th pair in XVI a3–XVII a1; 5th pair in XVII a3–XVIII a1; 6th pair in
 252 XVIII a3–XIX a1. Paired sperm ducts thick, tubular, in XI a1–XV a3 (Fig. 4B). Pair of
 253 muscular atrial cornua directed anteriorly, in XI a1–XII a1 (Fig. 4B). One pair of
 254 ovisacs tubular, thin-walled, slightly folded, running alongside ventral nerve cord, in

XII a1–XVII a1 (Fig. 4C).

Variation. Measurements (mean $\pm$ SD, followed by ranges in parentheses; $n = 7$, including neotype): BL 16.1 ± 2.52 mm (13.7–21.2 mm), BW 5.79 ± 1.01 mm (4.67–7.49 mm), CL 2.16 ± 0.20 mm (1.85–2.44 mm), CW 1.77 ± 0.22 mm (1.50–2.16 mm). Somite III uniannulate with secondary furrow dorsally (KUZ Z5771). The 1st crop caeca in XIV a1–XIV a2 (KUZ Z5766) and XIV a1–XIV a3 (KUZ Z5771 and Z5773). The post-crop caeca in XIX a1–XXIII a1 (KUZ Z5771 and Z5773) and XIX a1–XXIII a2 (KUZ Z5766 and Z5769). Pair of atrial cornua directed anteriorly reaching to XI a2 (KUZ Z5766, Z5772 and Z5773). Paired sperm ducts reaching to XV a1–XVIII a2. Ovisac reaching to XV a3–XIX a2. Somites I and II comprising three annuli; total annuli 70 (KUZ Z5768 and Z5769).

Colouration. In life, generally appears grey or yellowish-brown; ground-colour white, slightly transparent. Dorsum with several dark narrow stripes (Fig. 3C); dark pigmentation on lateral margin and dorsal tubercles in a3 of each mid-body somite pigmented; caudal sucker with dark radial patterns in larger individuals. Stripes absent on ventral surface; dark irregular markings present on anterior body in larger individuals. In preservative, colouration faded, but thin longitudinal stripes and markings remain visible.

Distribution. Although *A. lata* was previously confused with *A. weberi* (refer to the Discussion), its distribution, based on distinguishing features, such as longitudinal stripes, dorsal papillation and annuli count, includes Japan (Oka 1910a), the Russian Far

East (Lukin 1960), continental China (Yang 1996), Taiwan (Lai and Chen 2010) and Hawaii (Moore 1946). Although *A. lata*'s presence on the Japanese Archipelago was previously limited to Honshu and Shikoku (Oka 1910b), the present study expands its range across Japan, from Miyagi Prefecture southward to Yonaguni-jima Island.

Natural history. This species is common in the stagnant areas of rivers and ponds where freshwater snails are abundant. In captivity, it consumes freshwater snails, e.g. *Physa acuta* and *Semisulcospira* spp.

Remarks. Originally described with limited characteristics (Oka 1910a), *A. lata* was later redescribed with additional morphological details regarding the dorsal tubercles (Oka 1928). The four *Alboglossiphonia* species identified in the present study differ in their dorsal tubercle arrangement and the species matching the description of Oka (1928) was determined to be *A. lata*. Although Oka (1928) noted that *A. lata*'s common gonopore opens in the furrow between XII a1 and a2, our observations place it at the posterior margin of XII a1.

Autrum (1936) classified this taxon as a subspecies of *A. weberi*, originally described with 65 annuli (Blanchard 1897), as annuli counts can vary within glossiphoniids. Moore (1946) indicated that discrepancies in annuli counts can result from actual variation, preservation conditions or differences in how shallow furrows are interpreted. However, *A. weberi*'s original description depicted three biannulate somites at the posterior end, likely due to the fusion of two annuli (possibly a2 and a3; $a1 < [a2 + a3]$ according to the count of Moore [1927]) (Blanchard 1897). In contrast, *A. lata* specimens in the current study exhibited nearly uniform annuli size at the posterior end,

with no individuals showing fewer than 70 annuli, consistent with the description of Oka (1928). As many congeners also have 70 annuli (Table 2), annulation appears fixed within each lineage, suggesting that *A. weberi* may be characterised by reduced annulation. Furthermore, *A. weberi*'s original description (Blanchard 1897) did not mention dorsal longitudinal stripes, which are prominent in *A. lata*. Therefore, we consider these taxa distinct species but recommend a future re-evaluation of *A. weberi*.

***Alboglossiphonia reisui* sp. nov.**

(Figs 5B, 6 and 7)

Diagnosis. Body yellowish with several fuzzy dark stripes. Caudal sucker ventral, oval. Somites V–XXIV triannulate. Anus between somites XXVI and XXVII. Male and female gonopore united; common gonopore opened in posterior margin of XII a1. Eyes in three pairs; 1st pair are closest together. Dorsal tubercles developed on every mid-body annulus forming one median row, one pair on a2 faintly forming paramedian row. Mouth pore on centre of oral sucker. Salivary gland cells diffused. Oesophagus simple, oesophageal gland absent. Bacteriosomes absent. Crop giving rise to six pairs of crop caeca, 1st–6th pairs simple, 6th pair (post-crop caeca) diverticulated into four sections. Intersomital testisacs in six pairs. Paired sperm ducts thick, tubular. Atrial cornua directed anteriorly, developed. Paired ovisacs tubular.

Material examined. Holotype: KUZ Z5792, dissected, collected from Kamo City, Niigata Prefecture, Japan (37°39'45"N, 139°03'16"E; elev. 10 m) on 26 May 2024. Additional material: In total, five mature specimens were examined and dissected: KUZ

327 Z5784 collected from Hirosaki City, Aomori Prefecture, Japan (40°34'27"N,
328 140°26'13"E; elev. 94 m); KUZ Z5794 and Z5795 (two specimens) collected from
329 Tsuruga City, Fukui Prefecture, Japan (35°37'16"N, 136°02'52"E; elev. 24 m); KUZ
330 Z5798 collected from Gojo City, Nara Prefecture, Japan (34°20'57"N, 135°41'12"E;
331 elev. 109 m); KUZ Z5797 collected from Tamba City, Hyogo Prefecture, Japan
332 (35°08'58"N, 135°03'31"E; elev. 103 m).

333

334 **Description of the holotype.** Body lanceolate (Fig. 6A–B). Caudal sucker ventral, oval;
335 BL 17.1 mm, BW 5.09 mm, CL 1.82 mm, CW 1.60 mm.

336 Somites I and II comprising four annuli altogether. Somite III uniannulate, with
337 slight dorsal furrow. Somite IV biannulate ($a_1 + a_2 = a_3$). Somites V–XXIV triannulate
338 $a_1 = a_2 = a_3$. Somite XXV biannulate $a_1 = (a_2 + a_3)$. Somites XXVI and XXVII
339 uniannulate. Anus between XXVI and XXVII. Total annuli 71.

340 Clitellum unobservable.

341 Male and female gonopore joined and common gonopore at posterior margin of
342 XII a_1 .

343 Eyes in three pairs on IV a_1 to V a_1 . 1st pair closest together; following two
344 pairs spaced from 1st pair. Dorsal tubercles developed medially on every mid-body
345 annulus forming one conspicuous row; one pair on a_2 faintly forming two paramedian
346 rows; irregularly arranged on a_3 (Fig. 5B). Each ring more or less papillated.

347 Nephridiopores undetectable.

348 Mouth pore on centre of oral sucker. Proboscis in membranous sheath reaching
349 to between XII and XIII, without forming loop (Fig. 7A). Salivary gland cells diffuse.
350 Oesophagus simple (Fig. 7A); Oesophageal gland absent. Bacteriosomes absent. Crop

reaching to between XIX a2 and a3, giving rise to six pairs of crop caeca (Fig. 7A); 1–5th pairs of crop caeca simple; 3th pair on right side diverticulated into two sections; 5th pair diverticulated into two sections: 1st pair XIV a1–a3; 2nd pair XV a1–a3; 3rd pair XVI a1–a3; 4th pair XVII a1–a3; 5th pair XVIII a1–a3; and 6th pair (post-crop caeca) diverticulated into four sections, in XIX a1–XXIII a1. Intestinal caeca in four pairs (Fig. 7A): 1st pair in XIX a1–a3; 2nd pair in XX a1–a2; 3rd pair in XX a3–XXI a1; 4th pair in XXI a2–a3. Rectum simple tubular (Fig. 7A). Testisacs in six pairs, intersomital (Fig. 7A): 1st pair in XIII a3–XIV a1; 2nd pair in XIV a3–XV a1; 3rd pair in XV a3–XVI a1; 4th pair in XVI a3–XVII a1; 5th pair in XVII a3–XVIII a1; 6th pair in XVIII a3–XIX a1. Paired sperm ducts thick, tubular, in XI a1–XVIII a1 (Fig. 7B). Pair of muscular atrial cornua directed anteriorly, in XI a1–XII a1 (Fig. 7B). One pair of ovisacs tubular, thin-walled, slightly folded, running alongside ventral nerve cord, in XII a1–XVIII a1 (Fig. 7C).

Variation. Measurements (mean $\pm$ SD, followed by ranges in parentheses; n = 6, including neotype): BL 15.5 ± 1.59 mm (13.7–17.7 mm), BW 4.99 ± 0.69 mm (4.38–6.28 mm), CL 1.80 ± 0.26 mm (1.56–2.26 mm), CW 1.50 ± 0.08 mm (1.38–1.60 mm). Pair of atrial cornua directed anteriorly reaching to XI a2 (KUZ Z5794 and Z5797) and XI a3 (KUZ Z5795 and Z5798). Paired sperm ducts reaching to XII a1–XVIII a1. Ovisac reaching to XVI a1–XVIII a1. Somites I and II comprising three annuli; total annuli 70 (KUZ Z5784, Z5794 and Z5798).

Colouration. In life, generally appears whitish or yellowish; ground-colour white, slightly transparent. Several faint, dark stripes present on dorsum (Fig. 6C) but absent

on ventral surface. Dark irregular markings present in larger individuals. In preservative, colouration faded, but dark stripes and markings remain visible.

Etymology. The specific name *reisui* derives from the Japanese word *reisui* (= cool water), referring to this species' preference for cooler environments compared with *A. lata*. The specific name is treated as indeclinable.

Natural history. This species is common in streams and the stagnant areas of rivers where freshwater snails are abundant. In captivity, it consumes freshwater snails, e.g. *Physa acuta* and *Semisulcospira* spp.

Distribution. This species was collected across Japan, including Honshu (south of Aomori Prefecture), Shikoku and Kyushu. Its COI sequence has also been recorded from South Korea. *Alboglossiphonia reisui* sp. nov. is typically found at higher latitudes and in cooler environments compared with *A. lata*. Occasionally, the two species occur syntopically (Locality Nos. L3 and R07, as well as L14 and R08; Fig. 1 and Table 1).

Remarks. This species closely resembles *A. lata* but differs in its faint dark stripes on the dorsal surface and irregularly arranged dorsal tubercles on a3 (Fig. 5B). *Alboglossiphonia reisui* sp. nov. is also distinguishable from other congeners with a united common gonopore by its dorsal tubercle arrangement, colouration pattern and number of testisacs (Table 2).

Genetically, this species comprises three major clades: eastern Honshu, western Honshu and Kyushu + Korea. The *p*-distance between the eastern and western Honshu

populations is 6.3%–7.8% (Table 3), which is relatively high and comparable to the interspecific variation observed in other glossiphoniid species (de Carle et al. 2017; Kaygorodova et al. 2020; Jovanović et al. 2021). A greater genetic distance of 7.8%–10.2% separates the eastern + western Honshu populations from Kyushu + Korea (Table 3), and the delimitation analyses suggests that each of the three lineages represent a distinct species. However, no clear morphological differences were detected among these populations. Furthermore, no morphological data exist for Korean specimens and only one specimen has been examined from Kyushu. Consequently, we tentatively treat all of them as a single species in this study, but further evaluation is needed to clarify the taxonomy of this lineage.

***Alboglossiphonia cf. hyalina* (O. F. Müller, 1774)**

(Figs 8 and 9)

Hirudo hyalina O. F. Müller, 1774: 49–51.

Clepsine hyalina Moquin-Tandon 1826: 106.

Glossobdella hyalina De Blainville 1828: 565.

Glossiphonia heteroclita var. *hyalina* Pawlowski 1936: 130, fig. 89; Bennike 1943: 98, fig. 28E; Verriest 1950: 230, fig. 15; Mann 1953: 381, figs 5–6, 10; Autrum 1958: 7; Soós 1969: 413.

Alboglossiphonia hyalina Ben Ahmed et al. 2008: 137–138, figs. 8–10; Jueg 2013: 103–109; Kaygorodova et al. 2012: 2; Kaygorodova and Pronin 2013: 5; Grosser et al. 2015: 23; Ben Ahmed et al. 2015a: 5–6, fig. 2B, Ben Ahmed et al. 2015b: 78–79, fig. 1c; Kaygorodova 2015: 41; Mabrouki et al. 2019: 201–204; Fedorova and

Kaygorodova 2022: 78; Jueg 2023: 15, fig. 20.

Material examined. One specimen collected from Joetsu City, Niigata Prefecture, Japan: KUZ Z5807 (37°09'47"N, 138°14'20"E; elev. 4 m). Two specimens collected from Ena City, Gifu Prefecture, Japan: KUZ Z5808 and Z5809 (35°27'30"N, 137°24'35"E; elev. 264 m), the latter compressed. One specimen collected from Mima City, Tokushima Prefecture, Japan: KUZ Z5811 (34°02'41"N, 134°01'02"E; elev. 57 m). One specimen collected from Chikugo City, Fukuoka Prefecture, Japan: KUZ Z5812 (33°14'08"N, 130°29'05"E; elev. 17 m), compressed.

Description. Body lanceolate or ovate (Fig. 8A–B). Caudal sucker ventral, oval. Measurements (mean, followed by ranges of non-compressed specimens in parentheses; $n = 3$): BL 4.94 mm (4.40–5.40 mm), BW 1.37 mm (0.85–1.89 mm), CL 0.74 mm (0.64–0.84 mm), CW 0.66 mm (0.52–0.78 mm).

Somites I and II fused, comprising three annuli altogether. Somite III uniannulate, with slight dorsal furrow. Somite IV biannulate ($a1 + a2$) = $a3$. Somites V–XXIV triannulate $a1 = a2 = a3$. Somite XXV biannulate $a1 = (a2 + a3)$. Somites XXVI and XXVII uniannulate. Anus between XXVI and XXVII.

Clitellum unobservable.

Male and female gonopore joined and common gonopore opened at centre of XII $a1$.

Eyes in three pairs on IV $a2$ to VI $a1$. First pairs closest together. Dorsal papillae developed on every mid-body annulus; similar in size; mid-dorsal papillae arranged in a single keel-shaped row (Fig. 8A).

Nephridiopores undetectable.

Crop reaching to between XIX and XX, giving rise to five pairs of crop caeca

(Fig. 9A); 1–4th pairs of crop caeca simple: 1st pair XV a1–a2; 2nd pair XVI a1–a3; 3rd pair XVII a1–a3; 4th pair XVIII a1–a3; and 5th pair (post-crop caeca) XIX a1–XXII a3.

Intestinal caeca in four pairs (Fig. 9A): 1st pair in XIX a2–XX a1; 2nd pair in XX a2–a3; 3rd pair in XXI a1–XXI a2; 4th pair in XXI a3–XXII a1. Rectum simple tubular

(Fig. 9A). Testisacs in six pairs, intersomital (Fig. 9A): 1st pair in XIII a2–XIV a1; 2nd pair in XIV a2–XV a1; 3rd pair in XV a2–XVI a1; 4th pair in XVI a2–XVII a1; 5th pair in XVII a2–XVIII a1; 6th pair in XVIII a2–XIX a1. Paired sperm ducts tubular, in XII a1–XIII a2 (KUZ Z5809) or XI a3–XVIII a3 (KUZ Z5812; Fig. 9B). Pair of muscular atrial cornua directed anteriorly, in XI a3–XII a1 (Fig. 9B). One pair of ovisacs tubular, running alongside ventral nerve cord, in XII a3–XIII a1 (KUZ Z5809) or XII a3–XVII a1 (KUZ Z5812; Fig. 9C).

Colouration. In life, generally appears yellowish or greenish-white; ground-colour white, translucent. Dorsum with irregularly arranged greenish chromatophores (Fig. 8C). In preservative, colouration faded.

Distribution and natural history. This species inhabits streams and the stagnant areas of rivers where freshwater snails are abundant. It is widely distributed in Japan, from Fukushima Prefecture southward to Fukuoka Prefecture (Fig. 1). It can occur syntopically with *A. reisui* sp. nov. (Locality Nos. H1 and R05; Fig. 1 and Table 1). Originally described from Denmark (O. F. Müller 1774), *A. hyalina* has been recorded in Europe, Central Asia, Russia and Northwest Africa (e.g. Kaygorodova et al. 2012;

Jueg 2013; Ben Ahmed et al. 2015a, b; Jueg 2023). Although smaller than other congeners in Japan, some *A. hyalina* individuals were found brooding embryos, indicating reproductive maturity.

Remarks. *Alboglossiphonia hyalina* was originally described by O. F. Müller (1774) with limited morphological details. The specimens from the Japanese Archipelago were tentatively identified as this species owing to their shared characteristics of a translucent body and yellow pigmentation. However, the species' true taxonomic status remains uncertain and a redescription incorporating internal morphology and DNA data are needed. The species is notably smaller than other congeners but is easily distinguishable in Japan, as juveniles of other species exhibit dark dorsal pigmentation.

Alboglossiphonia sibirica Bolotov, Eliseeva, Klass & Kondakov, 2022

(Fig. 10)

Alboglossiphonia cf. *papillosa* Klass et al. 2018: 26.

Alboglossiphonia sibirica Bolotov et al. 2022b: 20630, figs 4a, 5a.

Material examined. One specimen collected from Tomakomai City, Hokkaido, Japan: KUZ Z5813 (42°44'26"N, 141°42'51"E; elev. 5 m).

Description. Body ovate (Fig. 10A–B). Caudal sucker ventral, oval; BL 9.08 mm, BW 3.26 mm, CL 1.36 mm, CW 1.19 mm.

Somites I and II fused, comprising three annuli altogether. Somite III uniannulate. Somite IV biannulate ($a_1 + a_2$) = a_3 . Somites V–XXIV triannulate $a_1 = a_2$

495 = a3. Somite XXV biannulate a1 = (a2 + a3). Somites XXVI and XXVII uniannulate.
 496 Anus between XXVI and XXVII.
 497 Clitellum unobservable.
 498 Common gonopore at posterior margin of XII a1.
 499 Eyes in three pairs on IV a1 to V a1. First pairs closest together. Dorsum with
 500 numerous small papillae on every mid-body annulus; similar in size (Fig. 10A).
 501 Nephridiopores undetectable.
 502
 503 **Colouration.** In life, generally appears whitish or yellowish; ground-colour white,
 504 slightly transparent. Irregularly arranged dark spots present on dorsum (Fig. 10C) but
 505 absent on ventral surface. In preservative, colouration faded, but dark pigmentation
 506 remains visible.
 507
 508 **Distribution and natural history.** In addition to Hokkaido, this species has been
 509 recorded in Western Siberia, Eastern Siberia, the Russian Far East and Mongolia
 510 (Bolotov et al. 2022b). The Japanese specimen was found attached to a branch in a
 511 river, although this species is known to associate with bryozoans in Russia (Klass et al.
 512 2018).
 513
 514 **Remarks.** The Hokkaido specimen of *Alboglossiphonia* was identified as *A. sibirica*
 515 based on its numerous small papillae and dark spots on the dorsal surface. The *A.*
 516 *sibirica* specimens from Hokkaido and Russia form a fully supported monophyletic
 517 group (BS = 100, PP = 1.0; Fig. 2), with a COI sequence divergence of only 2.1%,
 518 which is smaller than the interspecific variation observed in other glossiphoniid species

(de Carle et al. 2017; Kaygorodova et al. 2020; Jovanović et al. 2021). Although the male and female gonopores in the Russian specimens are fused and open in the furrow between XII a1 and a2 (Bolotov et al. 2022b), the Hokkaido specimen has a common gonopore at the posterior margin of XII a1. Additionally, the Russian specimens feature dark dashes arranged in longitudinal rows on the dorsal surface (Bolotov et al. 2022b), which are absent in the Hokkaido specimen.

Discussion

Confusion regarding the identity of *A. lata*. Owing to limited morphological data, *A. lata* has sometimes been treated as a subspecies or synonym of *A. weberi*. However, the original description of *A. weberi* indicates a reduced annulation pattern and fewer total rings compared with *A. lata* (Blanchard 1897), suggesting that the two species are distinct. Following Blanchard's original description of *A. weberi*, Kaburaki (1921) identified specimens from India as the same species based on the common feature of numerous dorsal papillae. However, total ring numbers in these specimens differed from those of true *A. weberi* and aligned more closely with those of *A. lata*. The arrangement of dorsal papillae also resembled that of *A. lata*. Nevertheless, Kaburaki (1921) distinguished the Indian species from *A. lata* by their lack of the prominent longitudinal stripes typically observed in this species. This suggests that the species identified by Kaburaki (1921) is not *A. weberi* but rather a distinct species closely related to *A. lata*. Moore (1924) later recorded *A. weberi* from Kashmir, using the characteristics described by Kaburaki (1921). Harding (1927) also documented this species from various South Asian localities, again relying on the description of Kaburaki (1921). Additionally, Autrum (1936) and Lukin (1967) discussed the taxonomic status of *A. lata*, primary referring to Kaburaki (1921)

and Harding (1927) for the characteristics of *A. weberi*. Therefore, it is likely that the identity of *A. lata* has been confused with a South Asian *Alboglossiphonia* species closely related to *A. lata*, which was mistakenly regarded as *A. weberi*. Although the present study concludes that *A. lata* and *A. weberi* are distinct species, a detailed redescription of *A. weberi* based on newly collected specimens remains necessary to clarify the taxonomic relationship between these two species.

Phylogenetic relationships and morphology. Japanese *Alboglossiphonia* has traditionally been thought to consist solely of *A. lata*; however, the present study identified four species that were clearly distinguished both morphologically and genetically. The closest relative to *A. lata* was *A. reisui* sp. nov., which forms a monophyletic group with *A. pahariensis* and *A. sp.* from Australia. Excluding the undetermined Australian species, all identified species have prominent tubercles on the dorsal surface of the mid-body somites. In particular, the tubercles are arranged paramedian on a2, a characteristic not observed in other lineages, which may represent a synapomorphy of the clade containing *A. lata* and *A. reisui* sp. nov. Given that syntopic occurrences of these two species were identified in the present study, as previously mentioned by Sawyer (1986), it is likely that the tubercle arrangement contributes to species recognition during mating, prior to spermatophore implantation between *A. lata* and *A. reisui* sp. nov.

Some *Alboglossiphonia* species possess a common gonopore, where the male and female gonopores are united. In the present phylogenetic analyses, these species formed a monophyletic group (Fig. 2). Considering the presence of two separate openings in the closely related genus *Glossiphonia* and the species *A. quadrata* (Moore,

1939), which branches early within *Alboglossiphonia*, two gonopores likely represent the ancestral state for this genus. The species with separated gonopores, *A. iberica* Jueg, 2008, has previously been suggested to form a clade with *A. quadrata* (Moser et al. 2022). However, as DNA sequence data are currently only available for *A. quadrata* with two gonopores, further analysis, incorporating additional species and molecular data, is required to fully understand the evolution of this unique characteristic.

Biogeographical implications. The present study demonstrated that *A. lata* is widely distributed across the Japanese Archipelago (from Honshu to the Ryukyu Islands), the Russian Far East, Taiwan and Hawaii. Its genetic diversity in Japan is relatively low (p -distance $< 3.1\%$), suggesting that *A. lata*'s expansion in this region is a recent event. Although eastern and western lineages are recognised in *Torix tagoi* (Oka, 1925), another glossiphoniid species inhabiting the Japanese Archipelago (Kambayashi and Nakano 2023), no clear geographic structure was observed in Japanese *A. lata*. This lack of correlation between geographic proximity and genetic similarity may be due to natural genetic exchange facilitated by the dispersal of freshwater insects, as observed in other glossiphoniids (Oosthuizen 1979). However, as *A. lata* inhabits stagnant waters, such as rice paddies, it is more plausible that individuals have been introduced to various regions through the transport of rice and other crops. In particular, the close genetic relationship between individuals from Hawaii and Okinawa (p -distance = 0.17%), despite being geographically separated by the Pacific Ocean, strongly suggests an artificial introduction. Nonetheless, the presence of various haplotypes in Japan and the historical abundance of *A. lata* in the early 20th century (Oka 1910b) indicate that this species is likely native to Japan.

In contrast, our molecular analyses revealed that *A. reisui* sp. nov. can be divided into three clades reflecting geographic area (eastern Honshu, western Honshu and Kyushu + Korea), suggesting a speciation process influenced by geohistorical events. The boundary between the eastern and western Honshu populations aligns with the Fossa Magna, a major rift valley formed during the separation of the Japanese Archipelago from the continent. This rift may have acted as a barrier to the dispersal of *A. reisui* sp. nov. (Watanabe 1998). The Kyushu + Korea population diverged more basally; thus, they likely represent a lineage that dispersed before the formation of the Tsushima Strait, which prevents exchange between the continent and Japan. To better understand the speciation process of this species, further analysis, including divergence time estimation, is required. Currently, *A. reisui* sp. nov. is known only from Japan (Honshu, Shikoku and Kyushu) and South Korea; however, it is possible that this species has been confused with the similar *A. lata* in other East Asian regions.

Although the phylogenetic relationships within *A. cf. hyalina* were not fully resolved, the intraspecific variation was relatively low (p -distance < 2.7%), indicating a dispersal pattern similar to that of *A. lata*. Among the species with available sequences, *A. bhamoensis* from Myanmar was identified as the most closely related species. To elucidate the evolutionary history of the Japanese population, further phylogenetic analysis with *A. hyalina* specimens from other known localities is necessary.

The *A. sibirica* specimen from Hokkaido formed a monophyletic group with the Russian population, exhibiting minimal genetic variation (p -distance < 2.1%). However, as the Hokkaido specimen exhibited a unique COI haplotype (Fig. 2), the Hokkaido population is likely endemic to the region rather than artificially introduced from Russia. During the last glacial period, the sea level dropped and Hokkaido was connected to the

continent through Sakhalin (e.g. Kinoshita et al. 2024), potentially allowing *A. sibirica* to expand its distribution at that time.

In recent years, molecular studies have revealed the hidden species richness of *Alboglossiphonia* in Asia (Bolotov et al. 2022a, b). The present study revisits the taxonomic classification of an old *Alboglossiphonia* taxon, integrating molecular characterisation. Our findings indicate that the species richness of this genus in East Asia is greater than previously recognised, highlighting its true diversity. Specifically, our molecular phylogenetic analyses showed that these Palearctic species do not form a monophyletic group, with *A. pallida* (Verrill, 1872) from North America and an undetermined *Alboglossiphonia* species from Australia deriving from this lineage. The widespread distribution of *Alboglossiphonia* across all continents except Antarctica further implies that the origin of this genus is ancient and complex, as noted by Sket and Trontelj (2008). Despite only limited sequence data being available, future comprehensive taxon sampling, combined with dated molecular phylogeny and morphological information, will help clarify the dispersal history and true species diversity of *Alboglossiphonia*.

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641

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Figure legends

Figure 1. Map showing the sampling localities of the *Alboglossiphonia* specimens used in this study (filled circles, *A. lata*; open circles, *A. reisui* sp. nov.; filled triangles, *A. sibirica*; open triangles, *A. cf. hyalina*). Locality numbers are provided in Table 1.

Figure 2. Bayesian inference tree based on 3,160 bp of nuclear 18S rRNA, mitochondrial COI, tRNA^{Leu} and ND1 markers. Real branch length is shown on the upper left. Numbers on nodes represent bootstrap values for maximum likelihood and Bayesian posterior probabilities. Bars on the right show the species delimitation results of ASAP and bPTP.

Figure 3. *Alboglossiphonia lata*, neotype (KUZ Z5764). A. Dorsal view. B. Ventral view. C. Dorsal view of live animal. Scale bars: 5 mm (same scale for A and B).

Figure 4. *Alboglossiphonia lata*, neotype (KUZ Z5764). A. Dorsal view of the digestive tract and male genital organs. B. Dorsal view of the male reproductive system. C. Dorsal view of the female reproductive system. Abbreviations: ac, atrial cornu; cc, crop cecum; cg, common gonopore; ic, intestinal cecum; oes, oesophagus; ov, ovisac; pb, proboscis; pcc, post-crop cecum; rm, retractor muscle; rt, rectum; sd, sperm duct; sg, salivary gland; ts, testisac. Scale bars: 5 mm for A; 2 mm for B and C.

Figure 5. Arrangement of mid-dorsal tubercles in *A. lata* (A) and *A. reisui* sp. nov. (B).

869 Figure 6. *Alboglossiphonia reisui* sp. nov., holotype (KUZ Z5792). A. Dorsal view. B.
870 Ventral view. C. Dorsal view of live animal. Scale bars: 5 mm (same scale for A and B).

871

872 Figure 7. *Alboglossiphonia reisui* sp. nov., holotype (KUZ Z5792). A. Dorsal view of
873 the digestive tract and male genital organs. B. Dorsal view of the male reproductive
874 system. C. Dorsal view of the female reproductive system. Abbreviations: ac, atrial
875 cornu; cc, crop cecum; cg, common gonopore; ic, intestinal cecum; oes, oesophagus; ov,
876 ovisac; pb, proboscis; pcc, post-crop cecum; rm, retractor muscle; rt, rectum; sd, sperm
877 duct; sg, salivary gland; ts, testisac. Scale bars: 5 mm for A; 2 mm for B and C.

878

879 Figure 8. *Alboglossiphonia* cf. *hyalina* (KUZ Z5812). A. Dorsal view. B. Ventral view.
880 C. Dorsal view of live animal. Scale bars: 4 mm (same scale for A and B).

881

882 Figure 9. *Alboglossiphonia* cf. *hyalina* (KUZ Z5812). A. Ventral view of the digestive
883 tract and male genital organs. B. Ventral view of the male reproductive system. C.
884 Ventral view of the female reproductive system. Abbreviations: ac, atrial cornu; cc, crop
885 cecum; cg, common gonopore; ic, intestinal cecum; ov, ovisac; pcc, post-crop cecum; rt,
886 rectum; sd, sperm duct; ts, testisac. Scale bars: 2 mm for A; 1 mm for B and C.

887

888 Figure 10. *Alboglossiphonia sibirica* (KUZ Z5813). A. Dorsal view. B. Ventral view. C.
889 Dorsal view of live animal. Scale bars: 5 mm (same scale for A and B).