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NOTES ON GEOGRAPHIC DISTRIBUTION

First record of the freshwater leech *Hemiclepsis schrencki* Bolotov, Klass, Bespalaya, Konopleva, Kondakov & Vikhrev, 2019 (Hirudinea, Glossiphoniidae) in Hokkaido, Japan

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Abstract. Freshwater leeches of the genus *Hemiclepsis* Vejdovský, 1884 were collected in Hokkaido, Japan, and identified as *H. schrencki* Bolotov, Klass, Bespalaya, Konopleva, Kondakov & Vikhrev, 2019. This marks the first recorded occurrence of the species beyond the Eurasian continent. The *H. schrencki* specimen from Hokkaido displays a distinct mitochondrial cytochrome *c* oxidase subunit I (COI) sequence variant, represented by a single terminal in the phylogeny, suggesting that the local population is indigenous to this region.

Key words. Hirudinea, molecular phylogeny, DNA barcoding, COI, Far East Asia

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INTRODUCTION

Hemiclepsis Vejdovský, 1884 is a genus of freshwater leeches, primarily composed of blood-feeding species, with some members known to associate with mussels (Bolotov et al. 2019). The type species, *H. marginata* (Müller, 1774), was initially described briefly without reference to a type locality (Müller 1774) and later revisited by Blanchard (1892) based on specimens from various European localities. Subsequently, the specimens of *H. marginata* collected mainly from Kashmir, India, were described by Moore (1924), who established a subspecies, *H. m. asiatica* Moore, 1924, mainly based on eye characteristics. Several additional species have been described under *Hemiclepsis* using morphological traits; however, consensus on their taxonomic validity is lacking, and some have been reassigned to other genera (Soós 1967). However, using molecular data, recent studies have described five new species classified within *Hemiclepsis*, suggesting that East and Southeast Asia may represent the center of distribution for this genus (Bolotov et al. 2019; Xu et al. 2021).

In Japan, five species, *H. marginata*, *H. smaragdina* (Oka, 1910), *H. kasmiana* (Oka, 1910), *H. okadai* Oka, 1925, and *H. japonica* (Oka, 1932), are known to have previously been classified under the genus *Hemiclepsis* (Oka 1935; Soós 1967). In this study, free-living *Hemiclepsis* specimens were collected from Hokkaido, Japan, and identified as *H. schrencki* Bolotov, Klass, Bespalaya, Konopleva, Kondakov & Vikhrev, 2019 based on mitochondrial sequence data. This species has only been reported from the Russian Far East (Bolotov et al. 2019, 2022), indicating that the present specimens represent the first record of *H. schrencki* outside the Eurasian continent.

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METHODS

Leech specimens were collected from submerged fallen branch or stones in the Bibi River and Tomakomai River, Hokkaido, Japan (Figure 1). The collected leeches were kept individually at 13 °C in the laboratory to digest blood meals. Subsequently, the specimens were relaxed by the gradual addition of 99% ethanol to freshwater and fixed in 70% ethanol. The following four 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 to the posterior margin of the sucker (CL), and caudal sucker width from the right to the left margin of the sucker (CW). Examination, dissection and drawing of the

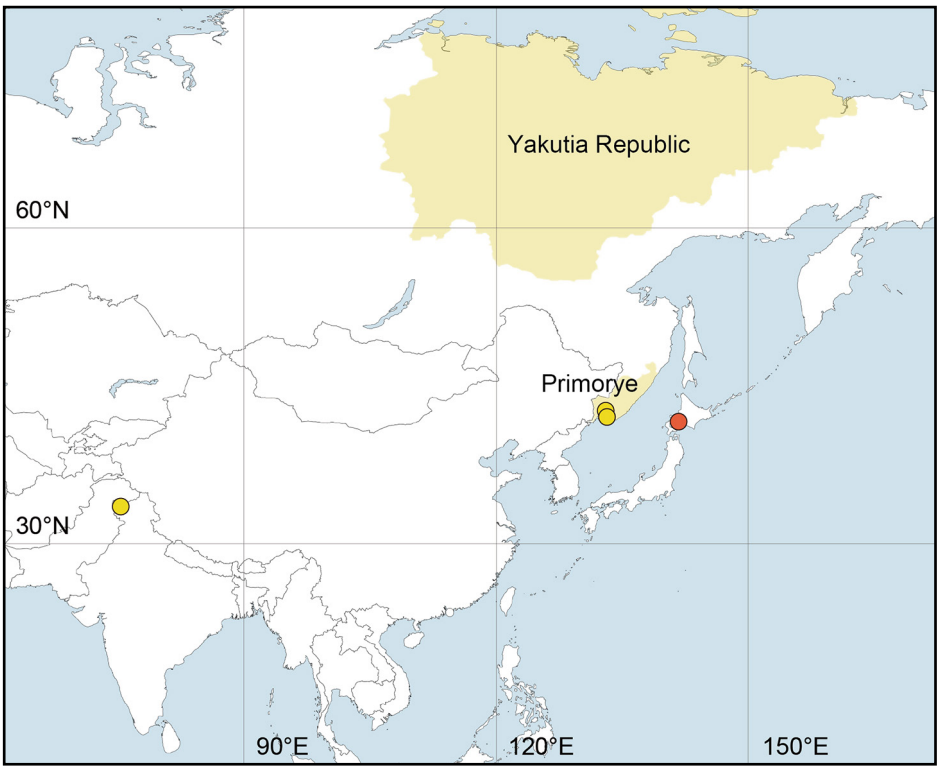

Figure 1. Map showing the collection localities of *Hemiclepsis schrencki*. The red circle indicates the newly recorded locality, while the yellow circles and shaded areas represent previously known sites from Bolotov et al. (2019, 2022). This map was created with mapchart.net.

specimens were accomplished under stereoscopic microscopes with a drawing tube (Leica M165C). Two specimens were compressed between two glass slides, stained with a mixture of Mayer's paracarmine and Ehrlich's hematoxylin, and then mounted on slides. These specimens were 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.

Total DNA of a representative specimen (KUZ Z6289) was extracted from the part of the caudal sucker using phenol/chloroform extraction. The partial sequence of cytochrome *c* oxidase subunit I (COI) was polymerase chain reaction (PCR) amplified using the primers LCO1490 and HCO2198 (Folmer et al. 1994). The PCR and cycle sequencing reactions were performed according to Nakano (2012), except for the use of PCR reaction kit, EmeraldAmp PCR Master Mix (Takara Bio). The newly determined COI sequence was deposited in the International Nucleotide Sequence Databases (INSD) through the DNA Data Bank of Japan (DDBJ). Based on Bolotov et al. (2019), we retrieved eight COI sequences of *H. schrencki* and six of other *Hemiclepsis* species, in which *H. tumniniana* Bolotov, Klass, Bespalaya, Konopleva, Kondakov & Vikhrev, 2019 and *H.* sp. were set as outgroup. Also, one COI sequence of *H. schrencki* collected from Dal Lake in Kashmir, India (INSD accession number PV291865) was obtained from INSD. The sequences were aligned using MAFFT v. 7.427 with the L-INS-i option (Katoh and Standley 2013). Phylogenetic analysis was conducted using IQ-TREE v. 1.6.12 (Nguyen et al. 2015), and nonparametric bootstrapping (BS) was subsequently conducted with 1000 replicates. The best-fit model was identified as GTR + F + G4 based on AICc using PartitionFinder algorithm implemented in IQ-TREE. Pairwise comparisons of the uncorrected *p*-distance for COI between the *H. schrencki* specimens obtained in this study and other populations were calculated using MEGA 12 (Kumar et al. 2024).

RESULTS

Family Glossiphoniidae Vaillant, 1890
Genus *Hemiclepsis* Vejdovský, 1884

***Hemiclepsis schrencki* Bolotov, Klass, Bespalaya, Konopleva, Kondakov & Vikhrev, 2019**
Figures 2, 3

New records. JAPAN — Hokkaido • Tomakomai, Bibi River; 42°44′26.1″N, 141°42′51.1″E; 27.II.2023; C. Kambayashi leg.; 4 specimens, KUZ Z6288–Z6291; DDBJ LC884667 • Tomakomai, Tomakomai River; 42°38′21.4″N, 141°33′44.9″E; 28.IX.2024; C. Kambayashi leg.; 4 specimens, KUZ Z6292–Z6295 • same locality; 29.IX.2024; 1 specimen, KUZ Z6296.

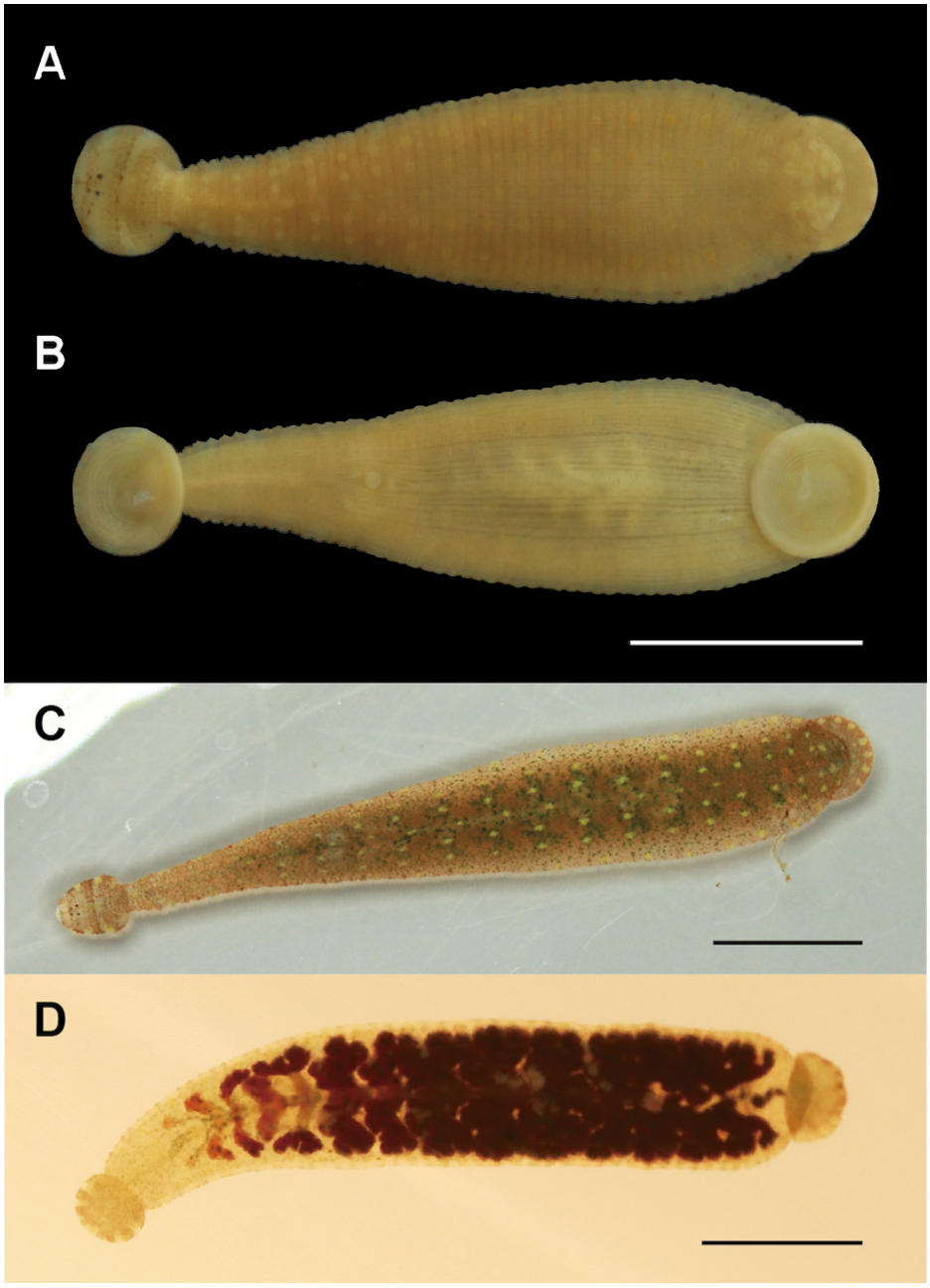

Figure 2. Specimens of *Hemiclepsis schrencki* examined in this study. **A.** Dorsal view. **B.** Ventral view. **C.** Dorsal view of a live individual (KUZ Z6289). **D.** Ventral view of an engorged live individual (KUZ Z6296). Scale bars: A–D = 5 mm (identical scale for A and B).

Identification. Body lanceolate (Figure 2A, B). Oral and caudal sucker ventral, oval. Measurements (mean, followed by ranges of non-compressed specimens in parentheses; n = 7, KUZ Z6288–Z6291 and Z6294–Z6296): BL 12.7 mm (6.92–17.4), BW 3.60 mm (2.87–4.70), CL 2.07 mm (1.54–2.79), CW 2.21 mm (1.59–2.92). Somites I–III uniannulate. Somites IV and V biannulate, (a1 + a2) = a3. Somites VI–XXIII triannulate, a1 = a2 = a3. Somites XXIV and XXV biannulate, a1 < (a2 + a3). Somites XXVI and XXVII uniannulate. Anus on somite XXVII. Male gonopore in somite XI/XII. Female gonopore in somite XII a2/a3. Gonopores separated by 2 annuli. Eyes in 2 pairs; 1st pair on anterior margin of somite II; 2nd pair on anterior margin of somite III. Dorsal surface smooth. Mouth pore on center of oral sucker.

Proboscis in membranous sheath reaching to X a1/a2, without forming loop (Figure 3A). Crop caeca in 11 pairs; 1st–4th pairs simple; 2nd pair textured differently and uneven; 5th–10th pairs diverticulated; 11th pair (post-crop caeca) diverticulated into five or more sections (Figures 2D, 3A). Intestinal caeca in four pairs (Figure 3A). Intersomital testisacs in 6 pairs (Figure 3A). Paired sperm ducts thick, tubular, in XI a1–XX a1 (Figure 3B). Pair of muscular atrial cornua directed anteriorly, in XI a2–XII a1 (Figure 3B). One pair of ovisacs tubular, slightly folded, running alongside ventral nerve cord, in XII a2–XVI a3 (Figure 3B).

In life, dorsal surface generally orangish brown with seven rows of ovate yellow spots; two pairs paramedian spots on a2; median and one pair lateral spots on a3; caudal sucker with yellow spots (Figure 2C).

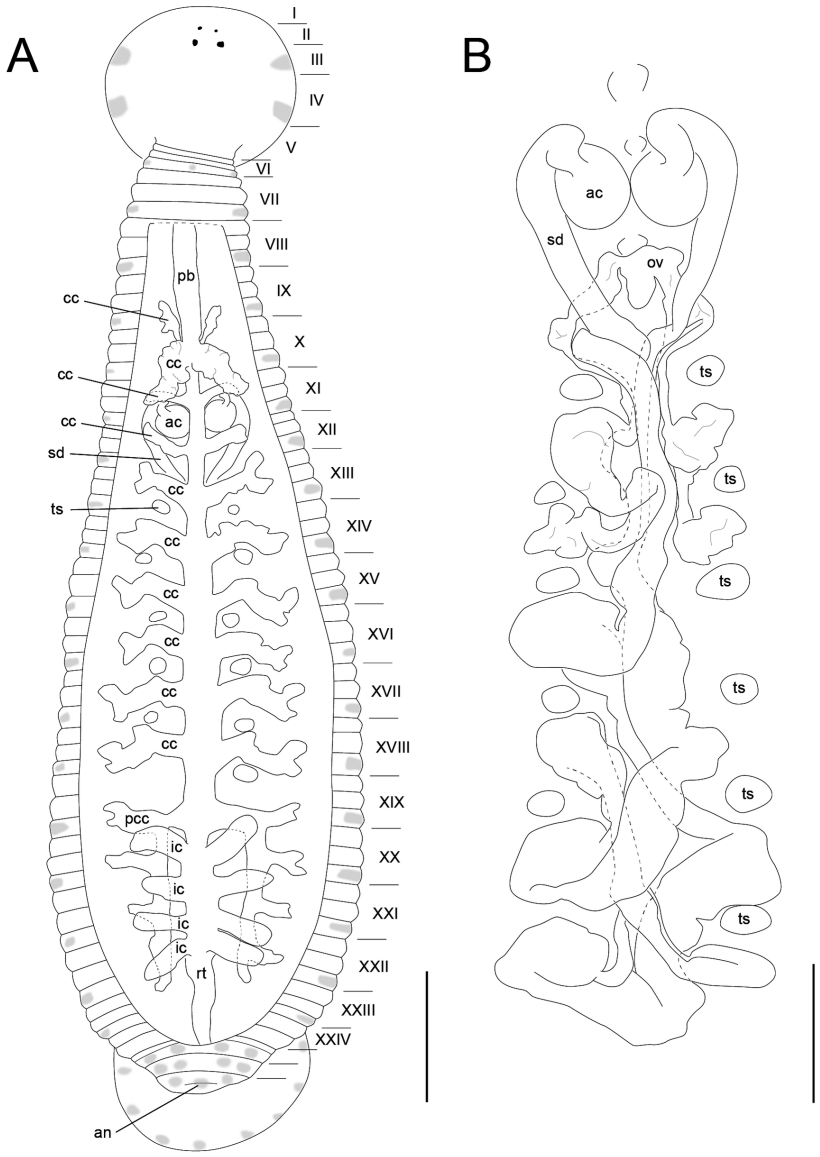

Figure 3. Anatomical features of *Hemiclepsis schrencki* from Japan (KUZ Z6289). **A.** Dorsal view showing the digestive tract and male reproductive organs. **B.** Dorsal view of the complete reproductive system. Abbreviations: ac, atrial cornu; cc, crop caecum; ic, intestinal caecum; ov, ovisac; pb, proboscis; pcc, post-crop caecum; rt, rectum; sd, sperm duct; ts, testisac. Scale bars: A = 2 mm; B = 1 mm.

The *H. schrencki* specimen from Hokkaido clustered in a monophyletic group with populations from Russia and Kashmir (BS = 100; Figure 4), showing minimal genetic variation (*p*-distance < 2.0%), which falls below the interspecific variation typically found among other glossiphoniid species (< 2.0%; de Carle et al. 2017; Kaygorodova et al. 2020; Jovanović et al. 2021).

DISCUSSION

Mitochondrial COI sequence analysis identified the *Hemiclepsis* specimens collected in this study as *H. schrencki*. Although the possibility that this population was introduced from the Eurasian continent cannot be ruled out, it is likely indigenous to Hokkaido, given the distinct COI sequence variant of the Hokkaido specimen, which is represented by a single terminal in the phylogeny. The slight genetic differentiation of the Hokkaido population from the Russian Far East population mirrors a pattern reported in *Alboglossiphonia sibirica* Bolotov, Eliseeva, Klass & Kondakov, 2022 (Kambayashi and Nakano 2025).

Although most of the morphological traits of the current specimens matched the diagnostic features of *H. schrencki*, a discrepancy was noted in the position of the gonopores. In the original description of the species, the male and female gonopores were reported at XI/XII and XII a1/a2, respectively (Bolotov et al. 2019). This arrangement was described as consistent across all *Hemiclepsis* species newly characterized by Bolotov et al. (2019). However, upon re-examining the annulation patterns in the original figures, the female gonopore of *H. tumniniana* appears to lie in the furrow between annuli a2 and a3, possibly at XII a2/a3,

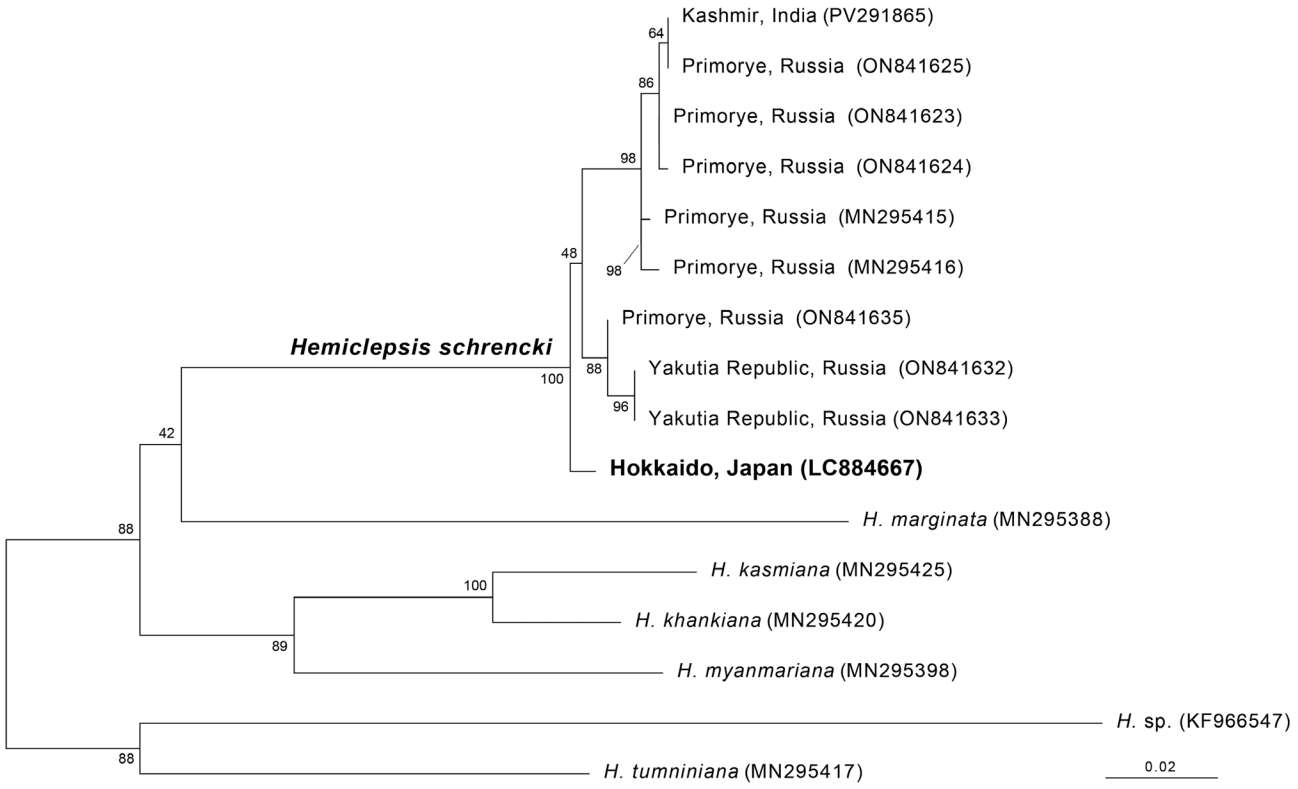

Figure 4. Maximum-likelihood phylogenetic tree based on cytochrome *c* oxidase subunit I (COI) gene sequences. International Nucleotide Sequence Database (INSD) accession numbers and collection localities are shown in parentheses. Bootstrap support values are indicated at the nodes.

which aligns with our observations of *H. schrencki* from Hokkaido. A further difference was observed in the number of crop caeca: as per the original description, the Russian specimens had seven pairs (Bolotov et al. 2019), whereas our specimens exhibited 11 pairs. These discrepancies may indicate morphological variation between populations; however, a future re-evaluation using the *H. schrencki* type specimens is warranted.

Hemiclepsis schrencki has been regarded as distinguishable from the morphologically similar *H. marginata* based on the shape, size, and arrangement of the dorsal spots (Bolotov et al. 2019). However, the specimens examined in this study closely resemble the redescription of *H. marginata* by Blanchard (1892). The presence of small anterior pairs of eyes in *H. schrencki* is more consistent with *H. m. asiatica*, originally described from Kashmir, India (Moore 1924). Moreover, this study revealed that the COI sequence of the specimen from Dal Lake in Kashmir is identical to one of the sequences from the Russian *H. schrencki*, suggesting that this species also occurs at the type locality of *H. m. asiatica*. It is known that the fish community of Dal Lake is currently dominated by non-native species (Bhat et al. 2024), and there is a possibility that *H. schrencki* was introduced along with its host fishes. In addition, *H. m. asiatica* has been described as possessing dorsal papillae absent in *H. schrencki*; nonetheless, the taxonomic validity of *H. schrencki* remains uncertain. Further studies based on type specimens or topotypes will be necessary to resolve the relationships among these *Hemiclepsis* species.

ADDITIONAL INFORMATION

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement

No ethical statement is reported.

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Author contributions

Conceptualization: CK. Data curation: CK, TN. Visualization: CK. Writing – original draft: CK. Supervision: TN. Validation: TN. Writing – review & editing: TN.

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Data availability
All data that support the findings of this study are available in the main text.

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