

Description of a New *Hamatipeda* Species, with an 18S Molecular Phylogeny (Crustacea: Tanaidacea: Typhlotanaidae)

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We describe a new typhlotanaid species, *Hamatipeda kohtsukai* sp. nov., collected from between 167 and 488 m depth in the Sagami Sea, Japan. This is the first record of *Hamatipeda* from the northern hemisphere. *Hamatipeda kohtsukai* resembles *Hamatipeda trapezoida* from the Subantarctic region in having pereonites 1–3 widest anteriorly (not rectangular), but differs from it in the length ratio of antennal articles 4/5; the number of setae on the dactyli of pereopods 1–3, ischia of pereopods 4–6, and carpi of pereopods 4–6; the shape of the unguis of pereopods 4–6; and the shape of the uropodal endopod. We determined partial sequences for the cytochrome *c* oxidase subunit I (COI; cox1) and 18S rRNA (18S) genes in *H. kohtsukai*. A phylogenetic reconstruction based on the 18S sequences recovered a highly supported Typhlotanaidae clade containing *H. kohtsukai* and *Typhlotanais mixtus*, with *Paranarthrura* sp. (Agathotanaidae) as the sister taxon. A key to species of *Hamatipeda* is presented.

Key words: DNA barcode, Malacostraca, new species, North Pacific, Peracarida

INTRODUCTION

Hamatipeda Błażewicz-Paszkowycz, 2007, one of 14 genera in the tanaidacean family Typhlotanaidae, contained four species at the onset of our study: *Hamatipeda longa* (Kudinova-Pasternak, 1975) from the Subantarctic region; *Hamatipeda prolata* Segadilha and Błażewicz in Segadilha et al. (2019) from the Southwestern Atlantic; *Hamatipeda sima* Błażewicz-Paszkowycz and Bamber, 2012 from the South Pacific; and the type species, *Hamatipeda trapezoida* Błażewicz-Paszkowycz, 2007, from the Subantarctic (Kudinova-Pasternak, 1975; Błażewicz-Paszkowycz, 2007; Błażewicz-Paszkowycz and Bamber, 2012; Segadilha et al., 2019). Unlike most typhlotanaids, species in *Hamatipeda* lack "prickly tubercles" sensu Błażewicz-Paszkowycz (2007) on the carpi of pereopods 4–6, but instead bear specialized hooks (= spiniform setae in Larsen's [2003] terminology) on these carpi. Five other typhlotanaid genera (*Aremus* Segadilha et al., 2018, *Meromonakantha* Sieg, 1986, *Obesutanais* Larsen et al., 2006, *Paratyphlotanais* Kudinova-Pasternak and Pasternak, 1978, and *Targaryenella* Błażewicz and Segadilha in Segadilha et al., 2019) also lack the tubercles, but *Hamatipeda* differs from these genera in that (1) the body is elongate, with the length/width ratio reaching at least 10 (in *Obesutanais*, the body is short, with the length/width ratio less than 6), (2) pereonite 1 is longer than wide and lacks a hyposphenium (in *Paratyphlotanais*, the segment is wider than long and has a hyposphenium), (3) pereonites 4 and 5 are rectangular in dorsal view, with parallel lateral margins (in *Meromonakantha*, these segments are hexagonal or rounded in dorsal view, and the segmental boundaries between pereonites are well constricted), (4) the carpi of pereopods 1–3 lack dorsodistal spiniform setae (in *Targaryenella*, each of these carpi has one large dorsodistal spiniform seta), and (5) pleopods are present (in *Aremus*, pleopods are absent).

During the 16th Japanese Association for Marine Biology (JAMBIO) Coastal Organism Joint Survey, we collected an undescribed species of *Hamatipeda* from between 167 and 488 m depth in the Sagami Sea, Japan. This is the first record for this genus from the northern hemisphere. Here we describe this species as new, provide partial sequences for its cytochrome *c* oxidase subunit I (COI) and 18S rRNA (18S) genes, and infer its phylogenetic position in the superfamily Paratanaoidea based on 18S data.

MATERIALS AND METHODS

An individual of *Hamatipeda* was collected by RV *Rinkai-maru* (Misaki Marine Biological Station, The University of Tokyo, Japan) by means of a biological dredge, photographed, and fixed in 80% ethanol. Ethanol-fixed specimens of *Nototanoides ohtsukai* Kakui and Yamasaki, 2013, *Paranesotanais longicephalus* Larsen and Shimomura, 2008, and *Akanthophoreus* sp., collected during cruises of the RV *Soyo-maru* (National Research Institute of Fisheries Science, Japan) or TRV *Toyoshio-maru* (Hiroshima University, Japan) were also used to determine their 18S sequences. The methods used for dissection, preparation of slides, light microscopy, and drawing were as described by Kakui and Angsupanich (2012). Our specimens were deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo.

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Orientation and terminology here follow Larsen (2003), except that the term 'plumose sensory seta' (PSS; Bird, 2011) is used instead of 'broom seta' and the term 'protopod' is used instead of 'basal article' for pleopods and uropods; the term 'vestigial proximal article' (Bird, 2012) was also used. Body length (BL) was measured from the base of the antennules to the tip of the pleotelson, and body width at the widest portion of the cephalothorax (CW, cephalothorax width). Measurements were made axially from digital images by using ImageJ (Rasband, 2021): dorsally on the body, antennules, antennae, and uropods; laterally on the chelipeds, pereopods, and pleopods.

Total DNA was extracted by using a NucleoSpin Tissue XS Kit (TaKaRa Bio, Japan). Primers used for PCR and sequencing are listed in Supplementary Table S1. PCR amplification conditions for COI and 18S with Ex Taq DNA polymerase (TaKaRa Bio) were 94°C for 1 min; 35 cycles of 98°C for 10 s, 42°C (COI) or 50°C (18S) for 30 s, and 72°C for 50 s (COI) or 3 min (18S); and 72°C for 2 min (COI) or 7 min (18S). Conditions for 18S amplification with KOD FX Neo (Toyobo Life Science, Japan) were 94°C for 2 min; 45 cycles of 98°C for 10 s, 52°C for 30 s, and 68°C for 90 s; and 68°C for 3 min. The 18S amplicons of *N. ohtsukai* and *P. longicephalus* were cloned with the pGEM-T Easy vector system (Promega, USA), and insert sequences were determined from colony PCR products; other sequences were determined by direct sequencing. All nucleotide sequences were determined with a BigDye Terminator Kit ver. 3.1 and a 3730 DNA Analyzer (Life Technologies, USA). Fragments were concatenated by using MEGA7 (Kumar et al., 2016). The sequences we determined were deposited in the International Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan (DDBJ). BLAST searches (Altschul et al., 1990) were used to detect the nucleotide sequences in INSD most similar to our COI and 18S sequences.

The 18S dataset for a phylogenetic analysis included the four sequences we determined and 16 sequences from 15 paratanaoidean species and one outgroup taxon (*Arctotanais alascensis* [Richardson, 1899], Tanaidoidea) taken from public databases (see Supplementary Table S2). Among four typhlotanaid species with sufficiently long (greater than 1600 bp) 18S sequences available in public databases, *Typhlotanais finmarchicus* Sars, 1882 (accession number MN337129), *Typhlotanais variabilis* Hansen, 1913 (MK804186), and *Typhlotanais trispinosus* Hansen, 1913 (MK804184) were not included in our dataset because they contained long gaps not present in the other sequences, as shown by a preliminary alignment. Sequences were aligned (3475 positions in the aligned 18S dataset) and the optimal substitution model (GTR + G + I) was selected in the manner described by Homma et al. (2020). A maximum likelihood (ML) analysis was conducted in RAxML v8.2.10 (Stamatakis, 2014), with nodal support values obtained by analysis of 1000 bootstrap pseudoreplicates. The ML tree was drawn with FigTree v1.4.4 (Rambaut, 2021). A maximum parsimony (MP) tree with nodal support values obtained by analysis of 1000 bootstrap pseudoreplicates (see Supplementary Figure S1) was obtained with MEGA7 under the "complete deletion" option.

RESULTS

Taxonomy

Family Typhlotanaidae Sieg, 1984
[New Japanese name: Kurayami-tanaisu-ka]
Genus ***Hamatipeda*** Błażewicz-Paszkowycz, 2007

Amended diagnosis. Typhlotanaid genus with BL more than 10 times CW; pereonite 1 longer than wide, without hyposphenium; pereonites 4 and 5 rectangular in dorsal view, with parallel lateral margins; carpus of pereopods 1–3 without dorsodistal spiniform setae; carpus of pereopods 4–6 without prickly tubercles; unguis of pereopods 4–6 bifurcate or trifurcate; pleopods present.

Remarks. The unguis of pereopods 4–6 is bifurcate in the species newly described here (see below), not trifurcate like the other congeners (Błażewicz-Paszkowycz, 2007; Segadilha et al., 2019); we therefore amended the diagnosis to include the condition.

Hamatipeda kohtsukai sp. nov.
[New Japanese name: Kihoshi-kurayami-tanaisu]
(Figs. 1–3, 5)

Diagnosis. Body length 10 times CW. Pereonites 1–3 widest anteriorly in dorsal view, with lateral margins not parallel. Length ratio of antennular articles 3/2 greater than 2.0. Length ratio of antennal articles 4/5 ca. 2.0. Dactylus of pereopods 1–3 with proximal simple seta. In pereopods 4–6, ischium with ventral spiniform seta; carpus with four distal spiniform setae; unguis bifurcate. In uropod, exopod length ca. three-fourths endopod length; endopod uniarticulate but with suture.

Etymology. The specific name is a noun in the genitive case, honoring Hisanori Kohtsuka, a crinoid researcher and one of the organizers of the JAMBIO Coastal Organism Joint Surveys.

Material examined. Holotype: Female, ICHUM-6255 (BL 2.50 mm, CW 0.25 mm), dissected, eight slides and one vial; INSD accession numbers LC632703 (COI) and LC632702 (18S); Stn. 4, Sagami Sea, North Pacific, dredged between 35°7.3887′N 139°33.7933′E, 167 m depth and 35°7.0851′N 139°33.3836′E, 488 m depth, 22 February 2018, collected by K.K.

Description of female. Body length 10 times CW (Figs. 1, 2A–D, C'), translucent, slightly yellowish, paired yellow spots in cephalothorax and pereonites when alive (Fig. 1). Cephalothorax length 0.15 times BL and 1.40 times CW; eye lobe absent. Pereonites 1–6 with length ratio 1.00:1.31:1.25:1.04:0.93:0.63, length-to-width ratios 1.17, 1.51, 1.50, 1.36, 1.27, 0.82, respectively; pereonites 1–3 anteriorly widest in dorsal view, with lateral margins not parallel; pereonites 4–6 with lateral margins nearly parallel. Pleon 0.11 times BL; pleonites 1–5 wider than long; pleotelson pentangular, narrower than pleonites.

Fig. 1. *Hamatipeda kohtsukai* sp. nov., female, holotype, fresh specimen.

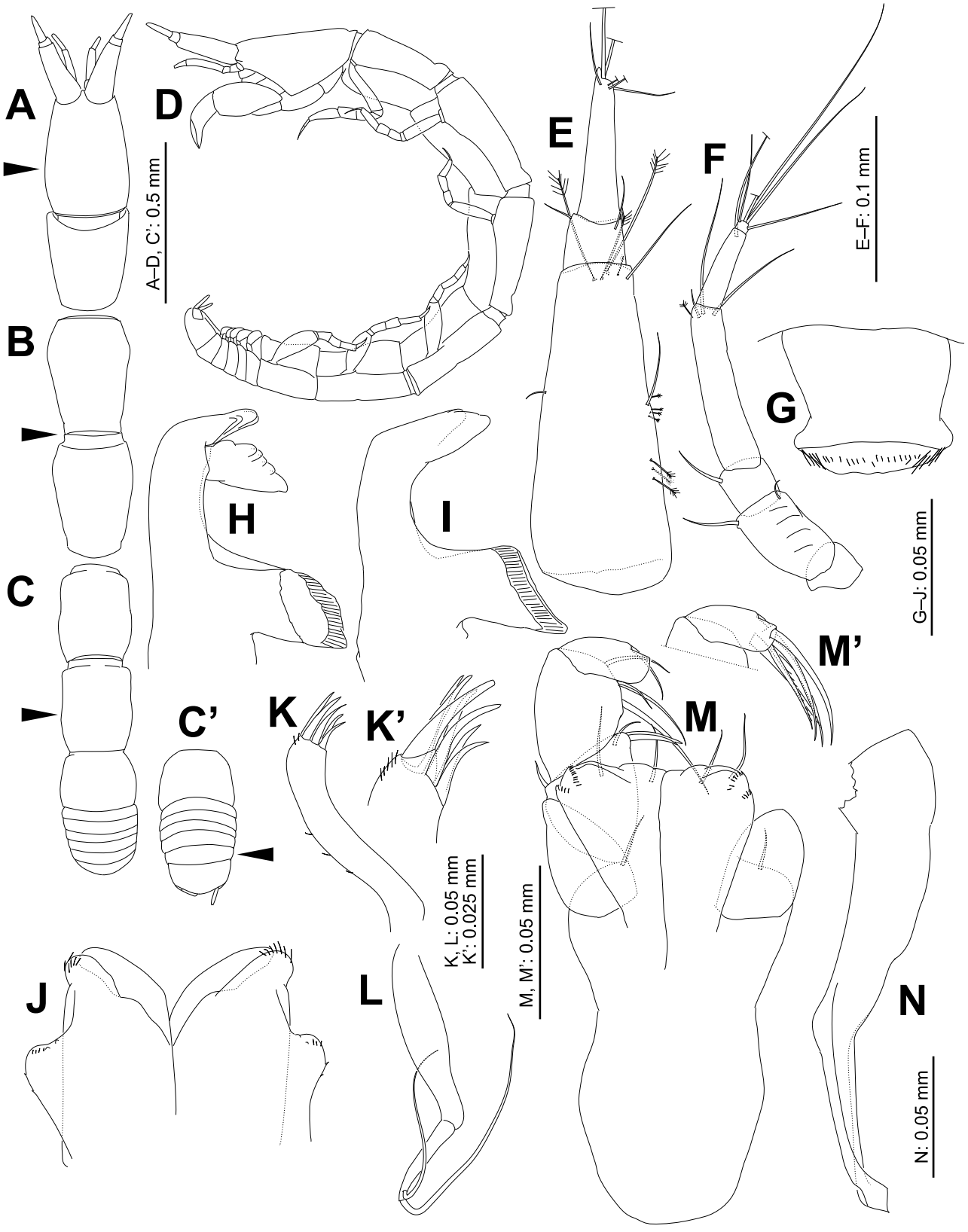

Fig. 2. *Hamatipeda kohtsukai* sp. nov., female, holotype. **(A–C)** body, dorsal view. **(C’)** pereonite 6, pleon, and pleotelson, dorsal view. **(D)** body, left view. **(E, F)** right antennule and antenna, dorsal view. **(G)** labrum. **(H, I)** left and right mandibles. **(J)** labium. **(K)** right maxillule. **(K’)** right maxillular endite. **(L)** right maxillular palp. **(M)** maxillipeds, dorsal view; articles 2–4 of right palp and distal setae on article 4 of left palp omitted. **(M’)** article 4 of left maxillipedal palp. **(N)** left epignath. Arrowheads indicate segments/points where the dorsal surface of the body lies parallel to the plane of the figure.

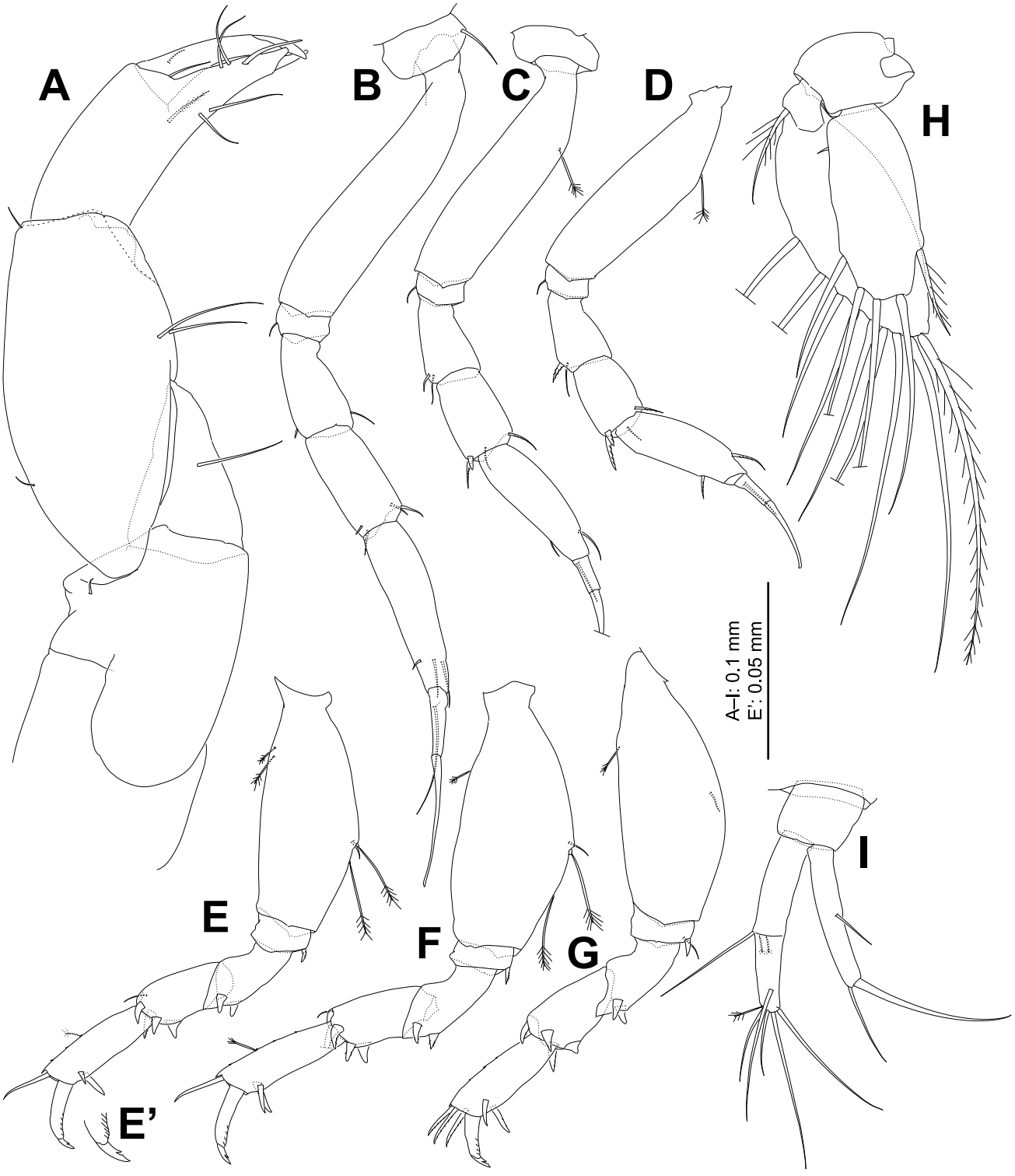

Fig. 3. *Hamatipeda kohtsukai* sp. nov., female, holotype. **(A)** cheliped, outer view. **(B–G)** right pereopods 1–6, outer view. **(E')** unguis and distal part of dactylus of pereopod 4. **(H)** left pleopod 1 (most setal ornamentation omitted). **(I)** right uropod.

Antennule length (Fig. 2E) 0.82 times cephalothorax length; articles 1–3 with length ratio 1.00:0.12:0.46. Article 1 with one mid-outer, one outer distal, and one mid-inner simple setae and several subproximal and distal PSS. Article 2 with one outer distal and one inner distal simple setae. Article 3 with distal spur and six distal simple setae.

Antenna length (Fig. 2F) 0.84 times antennule length; articles 1–6 with length ratio 0.50:1.00:0.55:2.04:1.05:0.11. Article 1 naked. Article 2 with one outer distal and one inner distal simple setae and dorsal series of slight ridges. Article

3 with inner distal simple seta. Article 4 with three distal simple setae and distal PSS. Article 5 with distal simple seta. Article 6 with two subdistal and three distal simple setae.

Labrum (Fig. 2G) setulate distally. Mandibles (Fig. 2H, I) with well-developed molar process bearing broad masticatory surface fringed by lamella; left mandible (Fig. 2H) with 4-denticulate incisor and wide, multidenticulate lacinia mobilis; right mandible (Fig. 2I) with incisor almost smooth (medial region slightly concaved). Labium (Fig. 2J) with outer and inner lobes setulate on outer distal margin. Maxillule (Fig. 2K, K', L) with endite bearing seven distal spiniform setae and outer subdistal setation; palp with two distal simple setae. Maxilla lost during dissection.

Maxillipeds (Fig. 2M, M') with medially fused bases, each base bearing ventrodistal simple seta. Endites not fused medially, each with outer distal rows of short setae and two ventro-subdistal simple setae; distal margin with slight, round process. Palp article 1 naked; article 2 with one outer distal and one inner distal simple setae and two inner distal spiniform setae; article 3 with four inner simple setae; article 4 with outer subdistal simple seta, four distal spiniform setae, and distal pinnate seta. Epignath (Fig. 2N) falciform.

Cheliped (Fig. 3A) with basis longer than wide, bearing large, free posterior portion and outer dorsal simple seta. Merus triangular, with ventral simple seta. Carpus slender, ca. twice as long as wide, with two ventral, one dorso-subproximal, and one dorsodistal simple setae; outer ventrodistal margin slightly extending as cuff. Propodus slender, ca. three times as long as wide; palm with outer simple seta and inner bipinnate seta at insertion of dactylus; fixed finger with two ventral and two outer dorsal simple setae, outer dorsal spiniform seta, dorsodistal digitiform process, and distal triangular claw. Dactylus-unguis as long as fixed finger, with inner subproximal simple seta; unguis triangular.

Pereopods 1–6 cylindrical, with length ratio 1.00:0.73:0.71:0.66:0.71:0.67 (unguis of pereopod 2 partly broken). Pereopod 1 (Fig. 3B) 0.20 times as long as BL, 13.77 times as long as pereopod-basis width, with length ratio of basis, ischium, merus, carpus, propodus, and dactylus-unguis 1.00:0.08:0.35:0.37:0.69:0.66. Coxa with dorsal simple seta; dorsal projection absent. Basis narrow, 4.50 times as long as wide, naked. Ischium with ventral simple seta. Merus with one dorsodistal and one ventrodistal simple setae. Carpus with four distal simple setae. Propodus with two inner subdistal and one ventral simple setae. Dactylus with proximal simple seta; unguis length ca. twice dactylus length, naked. Pereopod 2 (Fig. 3C) with length ratio of articles from basis to dactylus-unguis 1.00:0.08:0.27:0.33:0.55:0.31 (unguis partly broken). Coxa with dorsal simple seta (detached in right pereopod). Basis narrow, 4.18 times as long as wide, with dorso-subproximal PSS. Ischium with ventral simple seta. Merus with ventrodistal simple seta and ventrodistal spiniform seta. Carpus with dorsodistal serrate seta, inner distal simple seta, and two ventrodistal spiniform setae (one with serration). Propodus with one dorso-subdistal and one ventro-subdistal simple setae. Dactylus with proximal simple seta; unguis naked. Pereopod 3 (Fig. 3D) with length ratio of articles from basis to dactylus-unguis 1.00:0.08:0.28:0.28:0.53:0.47. Similar to pereopod 2, except several setae with serration and unguis length 1.92 times dactylus length (unavailable in pereopod 2). Pereopod 4 (Fig. 3E, E') with length ratio of articles from basis to dactylus-unguis 1.00:0.13:0.31:0.30:0.48:0.32. Coxa absent. Basis thicker than in pereopods 1–3, 2.31 times as long as wide, with mid-ventral simple seta and two dorso-subproximal and two mid-ventral PSS. Ischium with ventral serrate spiniform seta. Merus with two ventrodistal spiniform setae. Carpus with dorsodistal simple seta and four distal spiniform setae (one with serration). Propodus with one dorsodistal and two ventro-subdistal spiniform setae, mid-dorsal PSS, and dorsal serration. Dactylus with ventral setation; unguis not fused to dactylus, length 0.31 times dactylus length, bifurcate (Fig. 3E'). Pereopod 5 (Fig. 3F) with length ratio of articles from basis to dactylus-unguis 1.00:0.10:0.33:0.30:0.45:0.31; similar to pereopod 4, except several setae with serration and basis with one dorso-subproximal PSS. Pereopod 6 (Fig. 3G) with length ratio of articles from basis to dactylus-unguis 1.00:0.08:0.28:0.28:0.53:0.47; basis to carpus and dactylus-unguis similar to those of pereopod 5, except basis without mid-ventral PSS, ischium additionally with ventral simple seta, and carpus without dorsodistal simple seta. Propodus with three dorsodistal serrate spiniform setae, two ventro-subdistal spiniform setae (one with serration), and dorsal serration.

Pleopod 1 (Fig. 3H) with naked protopod. Endopod with one inner-subdistal, four distal, and one ventro-subproximal plumose setae. Exopod 1.45 times as long as endopod; ves-

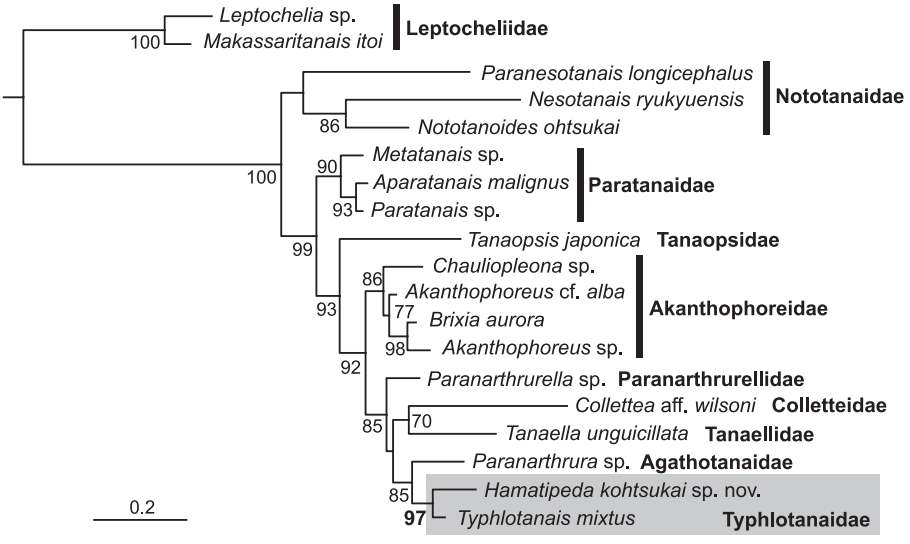

Fig. 4. Maximum-likelihood (ML) tree for 18S sequences (3475 positions) from tanaidaceans, including *Hamatipeda kohtsukai* sp. nov. Values near nodes are bootstrap values (< 60% not shown). Family names are indicated in bold font. The outgroup taxon (*Arctotanais alascensis*) is not shown. The scale bar indicates branch length in number of substitutions per site.

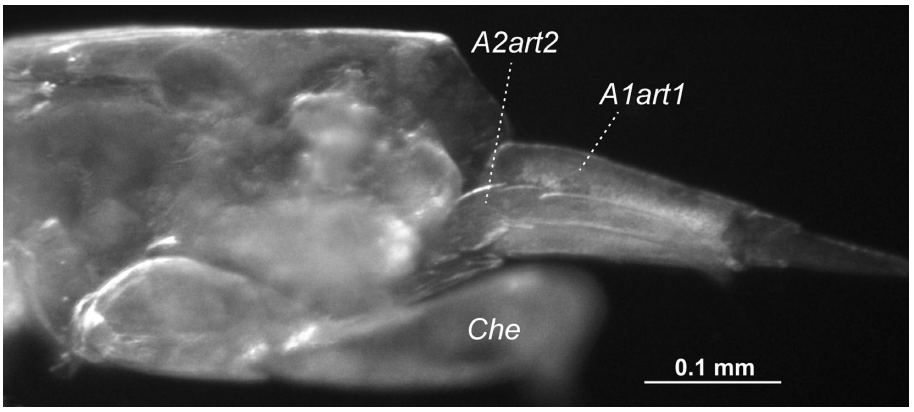

Fig. 5. Positional relationship between antennule and antenna in *Hamatipeda kohtsukai* sp. nov. in right ventral view; right antennule, antenna, and cheliped detached. *A1art1*, left antennular article 1; *A2art1*, left antennal article 2; *Che*, left cheliped.

tigial proximal article with ventral plumose seta; remaining portion with nine outer plumose setae. Other pleopods similar to pleopod 1, except exopod (excluding vestigial proximal article) with eight outer plumose setae.

Uropod (Fig. 3I) with protopod wider than long, naked. Endopod uniarticulate (but suture observed), with one middle, one subdistal, and four distal simple setae, and two middle and one distal PSS. Exopod 0.76 times as long as endopod, with one middle and two distal simple setae (one of which is robust).

Genetic information and phylogenetic analysis

A partial COI sequence (658 bp, encoding 219 amino acids; INSD accession number LC632703) and the nearly complete 18S sequence (2497 bp; LC632702) were determined from the holotype specimen. In BLAST searches, the COI sequence most similar to ours was from the insect *Lestrimelitta maracaia* Marchi and Melo, 2006 (accession number KC853378.1; identity score 72.32%, query cover 82%; Françoso and Arias, 2013); the most similar 18S sequence was from the confamilial species *Typhlotanais finmarchicus* (MN337129.1; identity score 89.36%, query cover 53%; unpublished). Our sequences were the first from any *Hamatipeda* species deposited in public databases (DDBJ, 2021).

In the ML tree based on 18S sequences (Fig. 4), *H. kohtsukai* was the sister taxon to the confamilial species *Typhlotanais mixtus* Hansen, 1913, with 97% bootstrap support. The sister taxon to the Typhlotanaidae clade was the agathotanaid *Paranarthrura* sp., with 85% bootstrap support.

DISCUSSION

Hamatipeda kohtsukai sp. nov. is the fifth species described in this genus. In having pereonites 1–3 widest anteriorly rather than rectangular, this species closely resembles *H. trapezoida*, reported from depths of 2375–3678 m in the Drake Passage, Subantarctic region (Błażewicz-Paszkowycz, 2007), but differs from the latter as follows (character state of *H. trapezoida* in parentheses): the length ratio of antennal articles 4/5 is ca. 2.0 (1.7); the dactylus on pereopods 1–3 bears one proximal simple seta (no seta); the ischium on pereopods 4–6 bears one ventral spiniform seta (no spiniform seta); the carpus on pereopods 4–6 bears four distal spiniform setae (two or three); the unguis on pereopods 4–6 is bifurcate (trifurcate); and the uropodal endopod has a suture (no suture).

In the dorsal region of antennal article 2 of *H. kohtsukai* (Fig. 2F), we found a series of ridges facing the ventral surface of the antennule in situ (Fig. 5). The location of these ridges greatly resembles that of the ridges on antennal article 2 in *Tanaopsis japonica* Kakui and Shimada, 2017, which possibly contribute to a stridulatory organ in that species (Kakui and Shimada, 2017, figs. 2C, 4B, 7). The ridges were slighter in *H. kohtsukai*, however, and we did not observe the slight ventral grooves on antennular article 1 that presumably comprise plectra in *T. japonica*. Hence, functions other than sound-production must be considered for *H. kohtsukai*.

The sequence from INSD most similar to our COI sequence from *H. kohtsukai* was from an insect species. A similar situation occurred in a BLAST search using a query COI sequence from the teleotanaid *Teleotanais madara* Tanabe and Kakui, 2019, the first teleotanaid sequence, where the most-similar sequence was likewise from an insect (Tanabe and Kakui, 2019). While there are no other *Hamatipeda* COI sequences in INSD, there are sequences from confamilial species. Our result suggests that COI sequences are quite divergent among typhlotanaid genera and have reached saturation, with multiple substitutions at many sites leading to extensive homoplasy, and consequently that BLAST searches will likely reliably detect only congeneric sequences.

In our ML tree (Fig. 4), *H. kohtsukai* and *T. mixtus* formed a highly supported clade (97% bootstrap value), confirming the familial affiliation of *Hamatipeda*. Except for Nototanaidae, other families with two or more species included in our analysis (Akanthophoreidae, Leptocheliidae, and Paratanaidae) were also retrieved as clades, with moderate to high (86–100%) bootstrap support. Interfamilial relationships in our tree differed from those in the 18S tree of Błażewicz et al. (2019). In our tree, for example, Colletteidae and Tanaellidae appear in a moderately supported clade along with Paranarthrurellidae, Agathotanaidae, and Typhlotanaidae, which is different from the topology in Błażewicz et al. (2019: fig. 2), though the differing topologies had high nodal support in both studies. There are many differences between the two analyses, however, including the method of analysis, ML in our study but MP in Błażewicz et al. (2019). In phylogeny reconstructions focusing on higher-level (e.g., interfamilial) relationships, reverse, parallel, or multiple substitutions are expected to be common among sequences, but MP does not take into account such substitutions. In the tree from a MP analysis of our dataset, most interfamilial relationships lost support (see Supplementary Figure S1). This and other differences may account for the incongruence between

our tree and that of Błażewicz et al. (2019).

Key to species of *Hamatipeda*

1. Pereonites 1–3 rectangular in dorsal view, with lateral margins parallel 2
- Pereonites 1–3 widest anteriorly in dorsal view, with lateral margins not parallel 4
2. Length ratio of antennular articles 3/2 around 1.0 .... *H. sima*
- Length ratio of antennular articles 3/2 greater than 2.0.... 3
3. Merus of pereopods 2 and 3 without ventrodistal spiniform seta; carpus with ventrodistal spiniform seta ..... *H. longa*
- Merus of pereopods 2 and 3 with ventrodistal spiniform seta; carpus with two ventrodistal spiniform setae .... *H. prolata*
4. Carpus of pereopods 4–6 with two or three distal spiniform setae; unguis trifurcate ..... *H. trapezoida*
- Carpus of pereopods 4–6 with four distal spiniform setae; unguis bifurcate ..... *H. kohtsukai* sp. nov.

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COMPETING INTERESTS

We declare no competing interests.

AUTHOR CONTRIBUTIONS

KK conceived and designed the study and made morphological observations; KK and CH conducted the molecular analysis, wrote the manuscript, and read and approved the final draft.

SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online. (URL: https://doi.org/10.2108/zs210065)

Supplementary Table S1. List of PCR and cycle sequencing (CS) primers used in this study.

Supplementary Table S2. Species included in the 18S phylogenetic analysis.

Supplementary Figure S1. Maximum parsimony (MP) tree for 18S sequences from tanaidaceans, including *Hamatipeda kohtsukai* sp. nov.

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