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A new species of *Zeuxo* (Crustacea: Peracarida: Tanaidacea) from Japan, with remarks on carapace pigmentation as a potentially useful taxonomic character

Nobuya Okamoto[a], Yuki Oya[a], and Keiichi Kakui[b]

[a]*Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan*

[b]*Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan*

Corresponding author: Nobuya Okamoto, nobu.okmt@gmail.com; Graduate School of Science, Hokkaido University, N10 W8, Kita-ku, Sapporo 060-0810, Japan

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Abstract

We describe *Zeuxo ezoensis* sp. nov. from Hokkaido, Japan. This species closely resembles *Z. phytalensis*, *Z. shitipingensis*, and *Z. turkensis* in having the pleopodal endopod with one inner plumose seta, maxillipedal palp article 4 with one outer simple seta, the left mandible with a wide, denticulate lacinia mobilis and one bifurcate accessory seta, and the right mandible with a peg-like lacinia mobilis and two accessory setae. It differs from them in having (1) antennal article 6 longer than wide, (2) the distal region of maxillipedal endite with four spiniform setae and two circumplumose setae, (3) maxillipedal palp article 2 with one outer simple seta, (4) the chelipedal carpus with three or four dorsodistal simple setae, (5) the chelipedal dactylus with one inner simple seta, (6) the pereopod-1 basis with one or two ventrodistal simple setae, (7) the pereopod-1 carpus with one ventrodistal simple seta, and (8) the uropod with four or five articles (basal article plus three or four ramus articles). Partial nucleotide sequences for the cytochrome *c* oxidase subunit I (COI) gene (655 nt) from *Z. ezoensis* specimens from four localities in Hokkaido showed Kimura 2-parameter (K2P) divergences of 0–0.5% and 0.6–1.5% within and between populations, respectively. Based on the COI data, we examined the phylogenetic position of *Z. ezoensis* within *Zeuxo*. Six specimens used for morphological observations and 10 specimens used for COI sequencing showed a similar dorsal pigmentation pattern on carapace. We briefly discuss the validity of using this pattern as a diagnostic

character in *Zeuxo* taxonomy.

KEYWORDS: Malacostraca, Tanaididae, North Pacific, Hokkaido, Oshoro, DNA barcoding

Introduction

The genus *Zeuxo* Templeton, 1840, with 37 species (Bird 2019; but see the next paragraph), is the most speciose genus in the family Tanaididae. *Zeuxo* differs from the other 19 confamilial genera in having the following combination of characters: (1) five free pleonites, (2) the length/width ratio of antennular article 1 more than 2.5, (3) the antenna with seven articles, (4) no distal cluster of setae on antennal article 2, and (5) the uropod with the terminal article not reduced (Sieg 1980; Bamber 2005; Bamber and Boxshall 2006; Chim and Tong 2019).

Species of *Zeuxo* have been found from tropical to subpolar regions worldwide. They are tube-dwellers, making a self-woven tube in bottom sediments, on seagrasses, or on seaweeds (Kakui 2016) and have been reported from depths of 0–30 m (García-Herrero et al. 2019). Three species have been reported from Japan: *Zeuxo maledivensis* Sieg, 1980, *Zeuxo coralensis* Sieg, 1980, and *Zeuxo normani* (Richardson, 1905). *Zeuxo maledivensis* was synonymized with *Z. kurilensis* (Kussakin and Tzareva, 1974) by Sieg (1983), but here we reinstate *Z. maledivensis* as a valid species because it morphologically differs from *Z. kurilensis* (e.g., the number of inner plumose setae on the pleopodal endopod was one in the former but three in the latter; Kussakin and Tzareva 1974; Sieg 1980). In Japan, *Zeuxo maledivensis* and *Z. coralensis* were reported from Nagasaki Prefecture (Sieg 1980), and *Z. normani* from Wakayama (Shiino 1951), Mie (Shiino 1951), and Hokkaido prefectures (Takashima et al. 2002). However, their type localities are far away (Addu Atoll, Maldives for

Z. coralensis; Fadiffolu Atoll, Maldives for *Z. maledivensis*; Monterey Bay, USA for *Z. normani*; Sieg 1980), so the occurrence of these species in Japan are suspect and need to be checked.

Around Hokkaido, northern Japan, unidentified *Zeuxo* individuals all having a similar pigmentation pattern on the carapace have been reported from Rishiri Island, Okushiri Island, and Oshoro Bay (Kakui et al. 2014, 2017; Kakui 2015); specimens from Rebun Island reported in Kakui et al. (2011) also show a similar pigmentation pattern (KK unpublished data). Through morphological observations and molecular phylogenetic analyses, we found these tanaidaceans to be conspecific and to represent an undescribed taxon. Here we describe it as new and report partial sequences for the mitochondrial cytochrome *c* oxidase subunit I (COI) to access its phylogenetic position within *Zeuxo*. Additionally, we briefly discuss the dorsal pigmentation pattern on the carapace as a diagnostic character in *Zeuxo* taxonomy.

Materials and methods

Tanaidaceans were collected among brown algae in the intertidal zone at Oshoro and on Rebun, Rishiri, and Okushiri islands. Some individuals from Oshoro were maintained for several months in a small aquarium (20°C; 14h L/10h D; fed every 3 days), and one female and one male hatched in the aquarium were photographed live to document their body pigmentation pattern. All specimens were fixed and preserved in 70–99% ethanol. The methods used for dissection, preparation of slides, light microscopy, scanning electron microscopy (SEM), and drawing were as described by Kakui and Angsupanich (2012).

Orientation and terminology here follow Larsen (2003), except that the term “plumose sensory seta(e)” (PSS; Bird 2011) is used instead of “broom seta(e)”, and two additional setal terms are used: 'flattened denticulate seta' (Edgar 2008) and 'step-tipped plumose seta' (Kakui et al. 2010). 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). Appendages were measured in holotype and allotype specimens. Measurements were made axially with ImageJ (Rasband 2020) from digital images: dorsally on the body, antennules, antennae, and uropods; laterally on the pereopods and pleopods.

Total DNA was extracted from the cheliped or whole body of 2, 2, 3, and 3 specimens from Oshoro and Rebun, Rishiri, and Okushiri islands, respectively, by using a NucleoSpin Tissue XS Kit (TaKaRa Bio, Japan); the carapace pigmentation patterns on these specimens were photographed before extraction. After extraction, exoskeletons were recovered and preserved in 99% ethanol. Part of the COI gene was amplified by PCR with primers LCO-1490 and HCO-2198 (Folmer et al. 1994). PCR amplification conditions with TaKaRa Ex Taq DNA polymerase (TaKaRa Bio) were 94°C for 1 min; 35 cycles of 98°C for 10 s, 50°C for 30 s, and 72°C for 50 s; and 72°C for 2 min. Nucleotide sequences were determined by direct sequencing with a BigDye Terminator Kit ver. 3.1 and a 3730 Genetic Analyzer (Life Technologies, USA).

All sequences we determined were deposited in the International Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan (DDBJ). MEGA7 (Kumar et al. 2016) was used to align the 10 COI sequences we obtained (655 nt, no indels, encoding 218 amino acids) and to calculate Kimura (1980) 2-parameter (K2P) and *p* distances within and among populations.

The COI dataset for a phylogenetic analysis included the 10 COI sequences we determined and eight sequences from the following species, obtained from INSD: *Zeuxo exsargasso* Sieg, 1980 (three sequences, accession numbers KF928318–928320; Larsen et al. 2014); *Zeuxo holdichi* Bamber, 1990 (KF928322; Larsen et al. 2014); *Zeuxo koreaensis* Larsen, 2014 (KF928321; Larsen et al. 2014); *Zeuxo turkensis* Larsen, 2014 (KF928323; Larsen et al. 2014); *Zeuxo* cf. *normani* (Richardson, 1905) (HM016203; Drumm 2010); and *Arctotanais alascensis* (Richardson, 1899) (outgroup taxon; LC322249; Tanabe et al. 2017). The dataset was aligned by using MAFFT ver. 7 (Katoh and Standley 2013) with the “Auto” strategy (“L-INS-i”) selected (Katoh et al. 2005), after which the aligned sequences were trimmed in MEGA7 to the shortest length among the sequences (389 nt). The optimal substitution models for different partitions determined under the corrected AIC in PartitionFinder 2.1.1 (Lanfear et al. 2016) were TVM+I for the first codon position, K81u+G for second position, and HKY for the third position. A partitioned ML analysis was conducted in IQ-TREE ver. 1.6.8 (Nguyen et al. 2015; Chernomor et al. 2016), with nodal support values obtained by ultrafast bootstrap analysis of

10,000 pseudoreplicates (Hoang et al. 2018). The ML tree was drawn by using FigTree v1.4.4 (Rambaut 2020).

Systematics

Family Tanaididae Nobili, 1906

Genus *Zeuxo* Templeton, 1840

[Japanese name: *Nami-tanaisu-zoku*]

Zeuxo Templeton, 1840: 203. Type species: *Zeuxo westwoodiana* Templeton, 1840

Zeuxo ezoensis sp. nov.

[New Japanese name: *Ezo-nami-tanaisu*]

(Figures 1–5)

Anatanais normani (not of Richardson 1905): Kito 1975, p. 152.

Zeuxo sp. 1: Kakui et al. 2011, p. 751; Kakui et al. 2012, p. 128, figure 1F; Kakui et al. 2017, p. 130, figures 4, 5.

Zeuxo spp.: Kakui et al. 2014, p. 9, figure 3Z1, Z2 (in part).

Zeuxo sp.: Kakui 2015, p. 2, figures 1, 4B–D.

Diagnosis

Antennal article 2 with inner distal simple seta. Antennal article 6 longer than wide. Left mandible with wide denticulate lacinia mobilis and bifurcate accessory seta. Right mandible with peg-like lacinia mobilis and two accessory setae. Maxilliped with endite bearing four spiniform setae and two circumplumose setae in distal region; palp article 4 with outer simple seta. Chelipedal dactylus with inner simple seta. Male chelipedal fixed finger with nine outer dorsal simple setae but without triangular mid-dorsal process on cutting surface. Pereopod 1 with coxa bearing slight dorsal process; basis with one or two ventrodistal simple setae; carpus with ventrodistal simple seta. Pleopods 1–3 with endopod bearing inner plumose seta. Uropod with four or five articles (including basal article). Carapace pigmentation comprising dark background with V-shaped pattern of lighter, irregular spots.

Etymology

The specific name is an adjective referring to the old name for Hokkaido Island, where the type locality is located.

Material examined

See Table I.

Description of female, based primarily on holotype, with observation of maxillular palp from ICHUM6064

Body (Figures 1A, a1, 3A, B) 5.34 times as long as wide, with reddish brown pigmentation (retained in ethanol). Cephalothorax 0.22 times BL, with pair of mid-lateral simple setae and pair of simple setae posterior to eyes; dorsal pigmentation comprising dark background with V-shaped zone of lighter, irregular spots (Figures 1, 2). Pereonites 1–6 with length ratio 1.0:1.85:1.96:2.42:2.23:1.51; each pereonite with several pairs of simple setae. Pereonites 1–6 with width to length ratios 0.28, 0.46, 0.58, 0.79, 0.72, and 0.46, respectively. Pleonites 1–3 with one or two pairs of dorsolateral simple setae and two to five pairs of lateral plumose setae; pleonites 4 and 5 with one to three pairs of lateral simple setae. Pleotelson with three to five pairs of simple setae.

Antennule (Figure 3C) 0.59 times as long as cephalothorax; articles 1–4 with length ratio 1.00:0.42:0.40:0.05. Article 1 with one inner proximal, two inner distal, and three outer distal simple setae and several proximal and distal PSS. Article 2 with four distal simple setae and three distal PSS. Article 3 with three distal simple setae and distal PSS. Article 4 with three shorter and eight longer simple setae, and five aesthetascs.

Antenna (Figure 3D) 1.06 times as long as antennule; articles 1–7 with length ratio 1.00:1.30:0.72:1.51:1.36:0.41:0.01. Articles 1 and 3 naked. Article 2 with one inner distal, one dorsodistal, and two mid-ventral simple setae. Article 4 with five distal simple setae. Article 5 with three inner distal simple setae and three distal PSS. Article 6 longer than wide, with four distal simple setae. Article 7 with six distal simple setae.

Labrum (Figure 3G) setulate distally. Mandibles (Figures 3H–J, 5) with well-developed molar process bearing many small teeth on masticatory surface; left mandible (Figures 3H, 5A) with smooth incisor, wide, denticulate lacinia mobilis, and bifid serrate accessory seta; right mandible (Figures 3I, J, 5B) with smooth incisor, peg-like lacinia mobilis, and two serrate accessory setae. Labium (Figure 3K) with inner and outer lobes setulate on distal margin; labial palp setulate, articulated with outer lobe. Maxillule (Figure 3L, M) with endite bearing seven distal spiniform setae and outer subdistal setation; palp (from paratype female ICHUM6064) with six distal simple setae. Maxilla (Figure 3N) with finely serrate outer and distal margins.

Maxilliped (Figure 3O, P) with coxa bearing two simple setae. Basis with ventrodistal simple seta. Endite with outer serration, two tiny dorso-subdistal and two distal spiniform setae, and two ventro-subdistal circumplumose setae; ventrodistal region setulate. Palp article 1 with outer subdistal simple seta; article 2 with three inner ventral and one outer simple setae, and three inner plumose setae; article 3 with 13 inner plumose setae; article 4 with outer simple seta and 10 inner plumose setae. Epignath (Figure 3Q) with kidney-shaped lobe, margins finely setulate; terminal seta setulate.

Cheliped (Figures 3B, 4A) with triangular articulation to cephalothorax via sclerite (Figure 3B). Basis nearly as long as wide, with one outer dorsal and one ventrodistal simple setae. Merus with two dorsal and two ventral simple setae. Carpus 1.46 times as long as wide, with one dorsal, three dorsodistal, and three ventral simple setae. Propodal palm with three outer simple setae and inner plumose seta at insertion of dactylus; fixed finger with three ventral, five outer dorsal, and two inner subdistal simple setae, dorsal lamellar expansion showing straight dorsal margin, and triangular claw. Dactylus as long as fixed finger, with inner simple seta and row of ventral spiniform setae; unguis triangular.

Pereopods cylindrical, with length ratio 1.00:0.81:0.80:0.89:0.93:0.90 (unguis of pereopod 3 broken, not measured). Pereopod 1 (Figure 4C) 0.29 times as long as BL, with length ratio of basis, merus, carpus, propodus, and dactylus-unguis 1.00:0.26:0.34:0.60:0.30. Coxa with slight dorsal process bearing dorsal simple seta. Basis cylindrical, narrow, 2.67 times as long as wide, with one dorso-subproximal and one ventrodistal simple setae and dorso-subproximal PSS. Merus with ventrodistal simple seta. Carpus with one dorsodistal and one ventrodistal simple setae, and dorsodistal spiniform seta. Propodus with one mid-inner, one dorsodistal, and four ventrodistal simple setae, and mid-dorsal PSS. Dactylus with dorsal simple seta; unguis length half dactylus length, naked. Pereopod 2 (Figure 4D) with length ratio of articles from basis to dactylus-unguis 1.00:0.42:0.30:0.45:0.17. Coxa with dorsal simple seta. Basis cylindrical, narrow, 3.50 times as long as wide, with one dorso-subproximal and one ventrodistal simple setae, and dorso-subproximal PSS. Merus with one dorsodistal and two ventrodistal simple setae and ventrodistal spiniform seta. Carpus with one dorsodistal and one ventrodistal simple setae, and four distal spiniform setae. Propodus with two dorsodistal, one ventro-subdistal, and one ventrodistal simple setae, and mid-dorsal PSS; dactylus with dorsal simple seta; unguis as long as dactylus, naked. Pereopod 3 (Figure 4E) with length ratio of articles from basis to dactylus 1.00:0.52:0.35:0.51:0.15; similar to pereopod 2, except basis with two dorso-subproximal PSS, and carpus with five distal spiniform setae but without ventrodistal simple seta. Pereopod 4 (Figure 4F) with length ratio of articles from basis to dactylus-unguis 1.00:0.44:0.37:0.49:0.39. Coxa naked. Basis thicker than in pereopods 1–3, 1.98 times as long as wide, with two ventrodistal simple setae, three dorso-subproximal PSS, and two ventro-subdistal PSS. Merus with two dorsodistal and one ventrodistal simple setae, and two ventrodistal spiniform setae. Carpus with two dorsodistal simple setae and five distal spiniform setae. Propodus with two dorsodistal, one outer distal, and one mid-ventral simple setae, and dorsodistal PSS. Dactylus-unguis fused to form claw, strongly arched, with inner and outer rows of ventral spines. Pereopod 5 (Figure 4G) with length ratio of articles from basis to dactylus-unguis 1.00:0.48:0.44:0.56:0.37; similar to pereopod 4 except basis with one ventro-subdistal PSS but without dorso-subproximal PSS. Pereopod 6 (Figure 4H, I) with length ratio of articles from basis to dactylus-unguis 1.00:0.43:0.40:0.64:0.29. Basis, merus, carpus, and dactylus-unguis similar to those of pereopod 4 except basis with two dorso-subproximal PSS but without ventro-subdistal PSS. Propodus with two dorsodistal, one outer distal, and one mid-ventral simple setae, dorsodistal PSS, and seven inner distal flattened denticulate setae.

Pleopod 1 (Figure 4J) with basal article bearing one inner and five outer plumose setae; endopod with one inner and nine outer plumose setae, and outer distal step-tipped plumose seta; exopod 1.45 times as long as endopod, with 21 outer plumose setae. Pleopod 2 (Figure 4K) similar to pleopod 1 except exopod with 20 outer plumose setae (inner plumose seta on basal article broken in holotype). Pleopod 3 (Figure 4L) with basal article bearing two outer plumose setae; endopod with one inner and seven outer plumose setae, and outer distal step-tipped plumose seta; exopod 1.38 times as long as endopod, with 17 outer plumose setae.

Uropod (Figure 4M) with four articles (basal article and triarticulate ramus). Basal article with five distal simple setae. Ramus article 1 with distal simple seta and distal PSS; article 2 with four distal simple setae and two distal PSS; article 3 with six distal simple setae and two PSS.

Description of male, based on allotype

Body (Figure 1B, b1) similar to female.

Antennule (Figure 3E) 0.92 times as long as cephalothorax; articles 1–4 with length ratio 1.00:0.34:0.25:0.05. Article 1 with setation similar to that of female. Article 2 with eight distal simple setae and four PSS. Article 3 with four distal simple setae and two distal PSS. Article 4 with five shorter and 11 longer simple setae, and seven aesthetascs.

Antenna (Figure 3F) 0.88 times as long as antennule; articles 1–7 with length ratio 1.00:2.29:1.01:2.59:1.77:0.54:0.12. Articles 1 and 3 naked. Article 2 with one inner distal, one dorsodistal, and one mid-ventral simple setae. Article 4 with four distal simple setae and distal PSS. Article 5 with three distal simple and four distal PSS. Article 6 longer than wide, with seven distal simple setae. Article 7 with eight distal simple setae.

Labrum, mandibles, labium, maxillule, and maxilla similar to those of female.

Maxilliped with coxa bearing three simple setae. Basis with two ventrodistal simple setae. Endite similar to female. Palp article 1 with three (left) or two (right) outer subdistal simple setae; article 2 with five inner ventral and one outer simple setae and three inner plumose setae; article 3 with 11 inner plumose setae; article 4 with outer simple seta and 14 inner plumose setae. Epignath similar to female.

Cheliped (Figure 4B) with triangular articulation to cephalothorax via sclerite. Basis and merus similar to those of female. Carpus 0.90 times as long as wide, with one dorsal, four dorsodistal, and five ventral simple setae. Propodal palm with six outer simple setae and inner plumose seta at insertion of dactylus; fixed finger with five ventral, nine outer dorsal, and two inner subdistal simple setae, dorsal lamellar expansion showing straight dorsal margin, and triangular claw. Dactylus strongly arched ventrally, with inner simple seta and row of ventral spiniform setae; unguis triangular.

Pereopods 1–6 with length ratio 1.00:0.82:0.77:0.83:0.84:0.88; pereopod 1 length 0.35

times BL. Pereopods similar to those of female, with following exceptions. Pereopod 1: basis with two simple dorso-subproximal and one or two ventrodistal simple setae; merus with dorsodistal simple seta; carpus with one or two dorsodistal simple setae, propodus with six or seven ventrodistal simple setae. Pereopod 2: basis with two dorso-subproximal and three ventrodistal simple setae, and two dorso-subproximal PSS; merus with four ventrodistal simple setae; carpus with two dorsodistal simple setae; propodus with one dorsodistal and four ventrodistal simple setae. Pereopod 3: basis with two dorso-subproximal and two ventrodistal simple setae; merus with two dorsodistal and three ventrodistal simple setae; carpus with two dorsodistal simple setae; propodus with one dorsodistal and two mid-ventral simple setae. Pereopod 4: basis with three ventrodistal simple setae; merus with three dorsodistal and two ventrodistal simple setae; carpus with three dorsodistal simple setae and six distal spiniform setae. Pereopod 5: basis with three ventrodistal simple setae and dorso-subproximal PSS; merus with three dorsodistal and two ventrodistal simple setae; propodus with two mid-ventral simple setae. Pereopod 6: basis with three ventrodistal simple setae; merus with three dorsodistal and three ventrodistal simple setae; carpus with three dorsodistal simple setae; propodus with two mid-ventral simple setae and 10 inner distal flattened denticulate setae.

Pleopods similar to those of female, with following exceptions: basal articles of pleopods 1–3 with six, six, and three outer plumose setae, respectively; endopods of pleopods 1–3 with 12, 13, and 11 outer plumose setae, respectively; exopods of pleopods 1–3 with 30, 32, and 25 outer plumose setae, respectively.

Uropod with five articles (basal article and 4-articulate ramus). Basal article with eight distal simple setae. Ramus article 1 with two distal simple setae and distal PSS; article 2 with six distal simple setae and distal PSS; article 3 with five distal simple setae and two distal PSS; article 4 with six distal simple setae and two distal PSS.

Variation and stability

In addition to the holotype and allotype, two female (ICHUM6061, 6064) and two male (ICHUM6062, 6063) paratypes of *Zeuxo ezoensis* sp. nov. were dissected and all appendages were observed. The morphological data we obtained are in Table SI. All specimens shared the same character state for the following selected characters (for all characters, see Table SI): (1) dorsal pigmentation pattern on carapace comprising dark background with V-shaped zone of lighter, irregular spots; (2) antennal article 2 with one inner distal and one dorsodistal simple setae; (3) left mandible with wide, denticulate lacinia mobilis and one bifurcate accessory seta; (4) right mandible with peg-like lacinia mobilis and two accessory setae; (5) maxillipedal endite with two tiny dorso-subdistal and two distal spiniform setae; (6) maxillipedal palp articles 2 and 4 with one outer simple seta; (7) chelipedal basis with one outer dorsal simple seta; (8) chelipedal dactylus with one inner simple seta and row of ventral spiniform setae; (9) pereopod-1 carpus with one ventrodistal simple seta; (10) endopod of pleopods 1–3 with one inner plumose seta; and (11) pleopod-3 basal article without inner plumose setae.

The following selected setae or articles varied in number among specimens (for all characters, see Table SI) (ranges in parentheses): dorsodistal simple setae on chelipedal carpus (3–4), ventrodistal simple setae on pereopod-1 basis (1–2), and articles of uropod (4–5).

Sexually dimorphic characters found in this species were typical for this genus: male antennules and antennae were longer than those of females; male cheliped had a wider carpus and a larger chela than that of females.

Phylogenetic analysis and genetic divergence

Partial COI sequences (655 nt) were determined from 2, 2, 3, and 3 individuals collected from populations at Oshoro and Rebun, Rishiri, and Okushiri islands, respectively. In the ML tree (Figure 6) based on COI, the 10 sequences from Hokkaido form a clade (*Zeuxo ezoensis* sp. nov.), with K2P distances of 0–0.5% within populations and 0.6–1.5% among populations (Table II; the *p* distances were identical to the K2P distances). The *Z. ezoensis* clade is the sister group to a clade comprising *Z. holdichi*, *Z. koreaensis*, and *Z.* cf. *normani*, with 80% ultrafast bootstrap support (uBS), which in turn forms a clade with *Z. turkensis* (uBS, 93%), with *Z. exsargasso* comprising the sister group to the other *Zeuxo* species. Genetic distances among the six *Zeuxo* species were 12–36% (K2P) or 11–29% (*p* distance), much greater than the intraspecific distances for *Z. ezoensis*.

Discussion

The results that all our Hokkaido sequences form a single clade, with much lower genetic distances within the clade than between it and other, previously described *Zeuxo* species, indicate that our 10 specimens from four localities are conspecific. While there is relatively little information in the literature on ranges of intraspecific variation for COI in Tanaidacea, the K2P distances among populations we found were similar to those reported for the tanaidid *Hexapleomera ulsana* Wi et al., 2018 (up to 1.1%; Wi et al. 2018), and distances within populations were lower than within a single population of the kalliapseudid *Mesokalliapseudes macsweenyi* (Drumm, 2003) (up to 3%; Drumm and Kreiser 2012). In the tree, our species from Hokkaido was not in sister relation to another Japanese species, *Z.* cf. *normani*. This implies that there may be multiple species groups in *Zeuxo*.

Among 38 congeners [37 in Bird (2019) plus *Z. maledivensis*], *Zeuxo ezoensis* sp. nov. closely resembles *Z. phytalensis* Sieg, 1980 from the Kerguelen Islands, *Z. shitipingensis* Tzeng and Hsueh, 2015 from Taiwan, and *Z. turkensis* from Turkey in having the following combination of characters: (1) left mandible with wide denticulate lacinia mobilis and one bifurcate accessory seta, (2) right mandible with peg-like lacinia mobilis and two accessory setae, (3) maxillipedal palp article 4 with one outer simple seta, and (4) pleopodal endopod with one inner plumose seta.

Zeuxo ezoensis differs from *Z. phytalensis* in the following characters (character states of *Z. phytalensis* in parentheses; Sieg 1980): antennal article 6 longer than wide (shorter), chelipedal basis with one outer dorsal simple seta (without the seta), chelipedal carpus with three or four dorsodistal simple setae (one or two setae), chelipedal dactylus with one inner simple seta (without the seta), and pleopod-3 basal article without inner plumose setae (with one plumose seta). Additionally, male *Z. ezoensis* differs from male *Z. phytalensis* in having the chelipedal fixed-finger with nine outer dorsal simple setae (six in *Z. phytalensis*).

Zeuxo ezoensis differs from *Z. shitipingensis* in the following characters (character states of *Z. shitipingensis* in parentheses; Tzeng and Hseuh 2015): maxilipedal endite with two circumplumose setae (four); chelipedal dactylus with one inner simple seta (without the seta), dorsal process on pereopod-1 coxa slight (prominent), pereopod-1 carpus with one ventrodistal simple seta (without the seta), and uropod with four or five articles (six or seven). Additionally, male *Z. ezoensis* differs from male *Z. shitipingensis* in having maxillipedal palp article 2 with one outer simple seta (the seta is lacking in *Z. shitipingensis*), and the chelipedal fixed-finger with nine outer dorsal simple setae (five in *Z. shitipingensis*).

Zeuxo ezoensis differs from *Z. turkensis* in the following characters (character states of *Z. turkensis* in parentheses; Larsen 2014): antennal article 2 with one inner distal simple seta (without the seta), distal region of maxillipedal endite with four spiniform setae (two setae), chelipedal dactylus with one inner simple seta (without the seta), and pereopod-1 basis with one or two ventrodistal simple setae (without setae in this position). Males of the two species differ in the shape of the fixed finger: the dorsal margin is nearly straight in *Z. ezoensis* whereas it bears one triangular mid-dorsal process in *Z. turkensis*. *Zeuxo ezoensis* was 29–31% divergent (K2P) from *Z. turkensis* in the partial COI sequences (389 nt), and in our ML tree (Figure 6) the two species are separated by the clade comprising *Z. koreaensis*, *Z.* cf. *normani*, and *Z. holdichi*. These results support the conclusion from our morphological analysis, that *Z. ezoensis* and *Z. turkensis* are different species.

The pigmentation pattern on the carapace differs between *Z. ezoensis* and *Z. shitipingensis*. In *Z. ezoensis*, the carapace has a dark background, with a V-shaped zone of lighter, irregular spots (Figures 1, 2); this pattern was the same in the six specimens studied morphologically and the 10 specimens used for DNA sequencing (Figure 2). At least in one female and one male of *Z. shitipingensis*, the anterior and middle regions of the carapace are dark, while other regions lack dark pigmentation (Tzeng and Hsueh 2015: figures 6A, 9A). Larsen et al. (2014) concluded that the pigmentation pattern on carapaces in *Zeuxo* can show intraspecific variation, though his paper did not show the pigmentation patterns of specimens actually used for sequencing DNA. While we observed slight differences in the degree of dark pigmentation among *Z. ezoensis* specimens, and some specimens collected had strongly faded pigmentation on the carapace (data not shown), the overall pattern was the same. Our observation of multiple specimens suggests that carapace pigmentation patterns may be consistent within species. If this is the case, differences among species may be consistent and provide a useful character for *Zeuxo* taxonomy in situations where “species discrimination is extremely difficult” (Bird 2019: p. 47). The validity of carapace pigmentation patterns as a diagnostic character in *Zeuxo* taxonomy should be tested with additional studies involving multiple individuals from populations, combined with molecular confirmation of conspecificity.

Key to species of *Zeuxo* having the combination of characters referred in the Discussion section

1. Antennal article 6 shorter than wide ....*Z. phytalensis*

Antennal article 6 longer than wide 2

2. Maxillipedal endite with four plumose setae; dorsal process on pereopod-1 coxa prominent; uropod with six or seven articles ....*Z. shitipingensis*

Maxillipedal endite with two plumose setae; dorsal process on pereopod-1 coxa slight or not appreciable; uropod with four or five articles.. 3

3. Maxillipedal endite with two distal spiniform setae; chelipedal dactylus without simple setae; ventrodistal corner of pereopod-1 basis without simple setae; (in male) dorsal margin of fixed finger with one triangular mid-dorsal processs .... *Z. turkensis*

Maxillipedal endite with four distal spiniform setae; chelipedal dactylus with one inner simple seta; ventrodistal corner of pereopod-1 basis with one or two simple setae; (in male) dorsal margin of fixed finger nearly straight ....*Z. ezoensis* **sp. nov.**

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Figure legends

Figure 1. *Zeuxo ezoensis* sp. nov., paratype female (A, a1; ICHUM6068) and male (B, b1; ICHUM6069), dorsal views. A, B, living individuals; a1, b1, anterior portion of body, ethanol fixed specimens. Scales: 1 mm (A, B), 0.1 mm (a1, b1).

Figure 2. Carapaces of *Zeuxo ezoensis* sp. nov. specimens used for DNA extraction, ethanol fixed, dorsal view. A, B, Oshoro specimens (ICHUM6066, 6067); C, D, Rebun Island specimens (ICHUM6070, 6071); E–G, Rishiri Island specimens (ICHUM6072–6074); H–J, Okushiri Island specimens (ICHUM6075–6077).

Figure 3. *Zeuxo ezoensis* sp. nov. A–D, G–K, M–Q, holotype, female; E, F, allotype, male; L, paratype (ICHUM6064), female. A, B, body, dorsal (A) and lateral (B) views; C, E, antennule, outer view of left (C) and inner view of right (E); D, F, left (D) and right (F) antenna, inner view; G, labrum; H, left mandible (distal portion and molar); I, right mandible; J, distal portion of right mandible; K, labium, L, maxillule; M, maxillular palp; N, maxilla; O, maxillipeds (right palp, setal ornamentation and setation on right basis and left endite omitted), ventral view; P, distal portion of maxillipedal endites (circumplumose setae on left endite omitted), ventral view; Q, epignath. Scales: 1 mm (A, B); 0.1 mm (C–I, K–O, Q); 0.05 mm (J, P).

Figure 4. *Zeuxo ezoensis* sp. nov. A, C–M, holotype, female; B, allotype, male. A, B, right cheliped, inner view; C–H, pereopods 1–6, inner (C, H) and outer (D–G) views; I, distal portion of pereopod 6, inner view; J–L, right pleopods 1–3, setal ornamentation omitted; M, uropod. Scales: 0.1 mm.

Figure 5. *Zeuxo ezoensis* sp. nov., paratype (ICHUM6065), female, SEM images. A, B, distal portion of left (A) and right (B) mandibles, inner view. Scale: 10 μm.

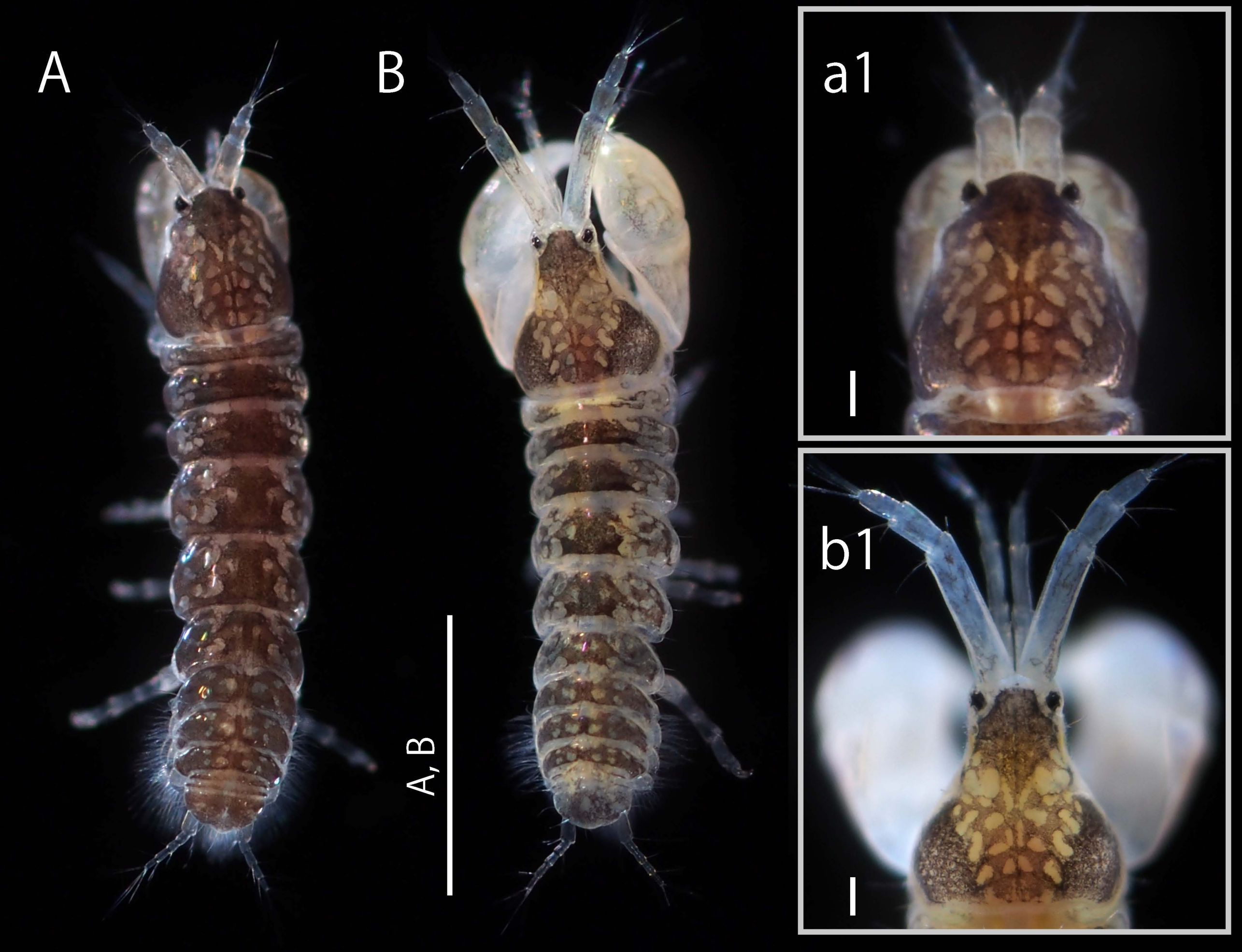
Figure 6. ML tree based on COI sequences (389 nt) from six *Zeuxo* species and an outgroup taxon, *Arctotanais alascensis*. Numbers near nodes are ultrafast bootstrap values in percent (80% or more). The scale indicates branch length in number of substitutions per site.

Table I. Collection information for specimens of *Zeuxo ezoensis* sp. nov. utilized in this study. –, COI not determined; nd, no data.

ICHUM/INSD	Status	Sex; BL/CW (mm)	Substratum; locality; date; collector
6059/–	Holotype	Female; 2.71/0.55	Brown algae; intertidal zone, west coast of Oshoro Bay, Oshoro (43°12'40.3"N 140°51'24.2"E); 11.v.2018; NO
6060/–	Allotype	Male; 3.70/0.81	
6061/–	Paratype	Female; 2.84/0.70	
6062/–	Paratype	Male; 2.41/0.60	
6063/–	Paratype	Male; 2.23/0.51	
6064/–	Paratype	Female; 2.90/0.53	
6065/–	Paratype	Female: 1.64/0.36	
6066/529716	Paratype	Female; nd/0.59	*Sargassum confusum*; intertidal zone, Uchikabuto, Oshoro (43°12'40.3"N 140°51'24.2"E); 2.v.2012; KK
6067/529717	Paratype	Female; nd/0.65	
6068/–	Paratype	Female; nd/0.51	(hatched in aquarium; fixed on 11.i.2019) *Neorhodomela aculeata*; intertidal zone, Uchikabuto, Oshoro, 3.ix.2018; KK and NO
6069/–	Paratype	Male; nd/0.57	
6070/529718	Nontype	Male; nd/0.47	*Sargassum* sp.; intertidal zone, Nishiuedomari, Rebun Island (45°24'27.2"N 140°59'29.3"E); 17.x.2007; KK
6071/529719	Nontype	Male; nd/0.39	
6072/529720	Nontype	Male; nd/0.47	*Sargassum* sp.; intertidal zone, Motodomari, Rishiri Island (45°15'09.3"N 141°11'15.1"E); 7.vii.2018; Shinri Tomioka
6073/529721	Nontype	Female; nd/0.48	
6074/529722	Nontype	Female; nd/0.40	
6075/529723	Nontype	Female; nd/0.77	*Sargassum thunbergii*; intertidal zone, Monai, Okushiri Island (42°06'53.05"N 139°25'04.98"E); 9.v.2010; KK
6076/529724	Nontype	Female; nd/0.61	
6077/529725	Nontype	Female; nd/0.56	

Table II. K2P distances (in percent) among COI sequences from four tanaidacean populations around Hokkaido (2, 2, 3, and 3 sequences from Oshoro and, Rebun, Rishiri, and Okushiri Islands, respectively; 655 nt). Average values are in parentheses.

	Oshoro	Rebun	Rishiri	Okushiri
Oshoro	0			
Rebun	1.2 (1.2)	0.5		
Rishiri	0.8 (0.8)	0.8–1.1 (0.9)	0–0.2 (0.1)	
Okushiri	1.1–1.4 (1.2)	1.1–1.5 (1.3)	0.6–1.1 (0.8)	0–0.3 (0.2)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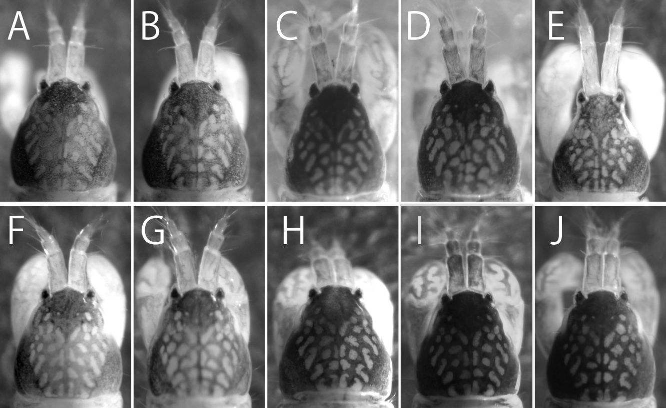
b1
A, B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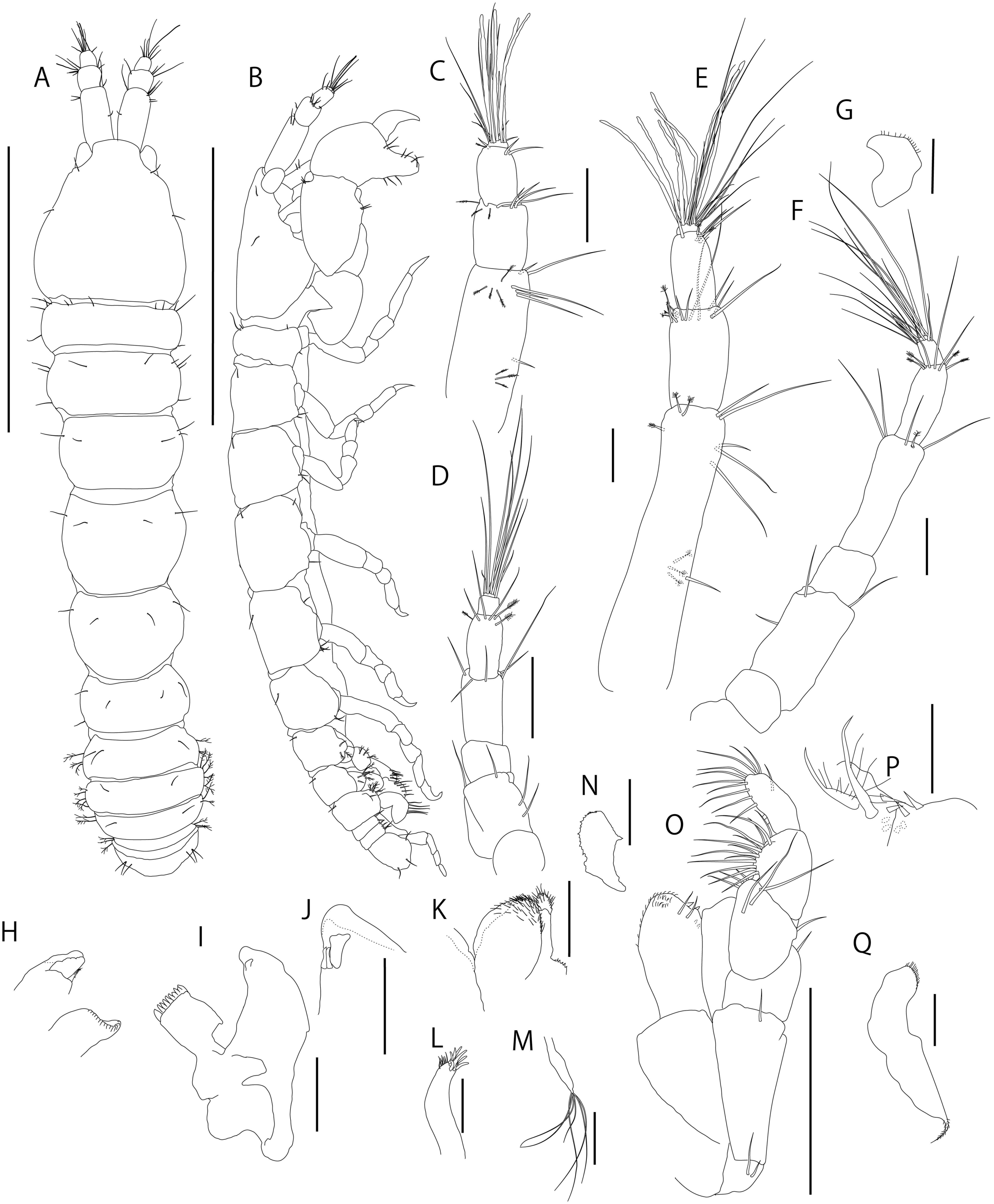
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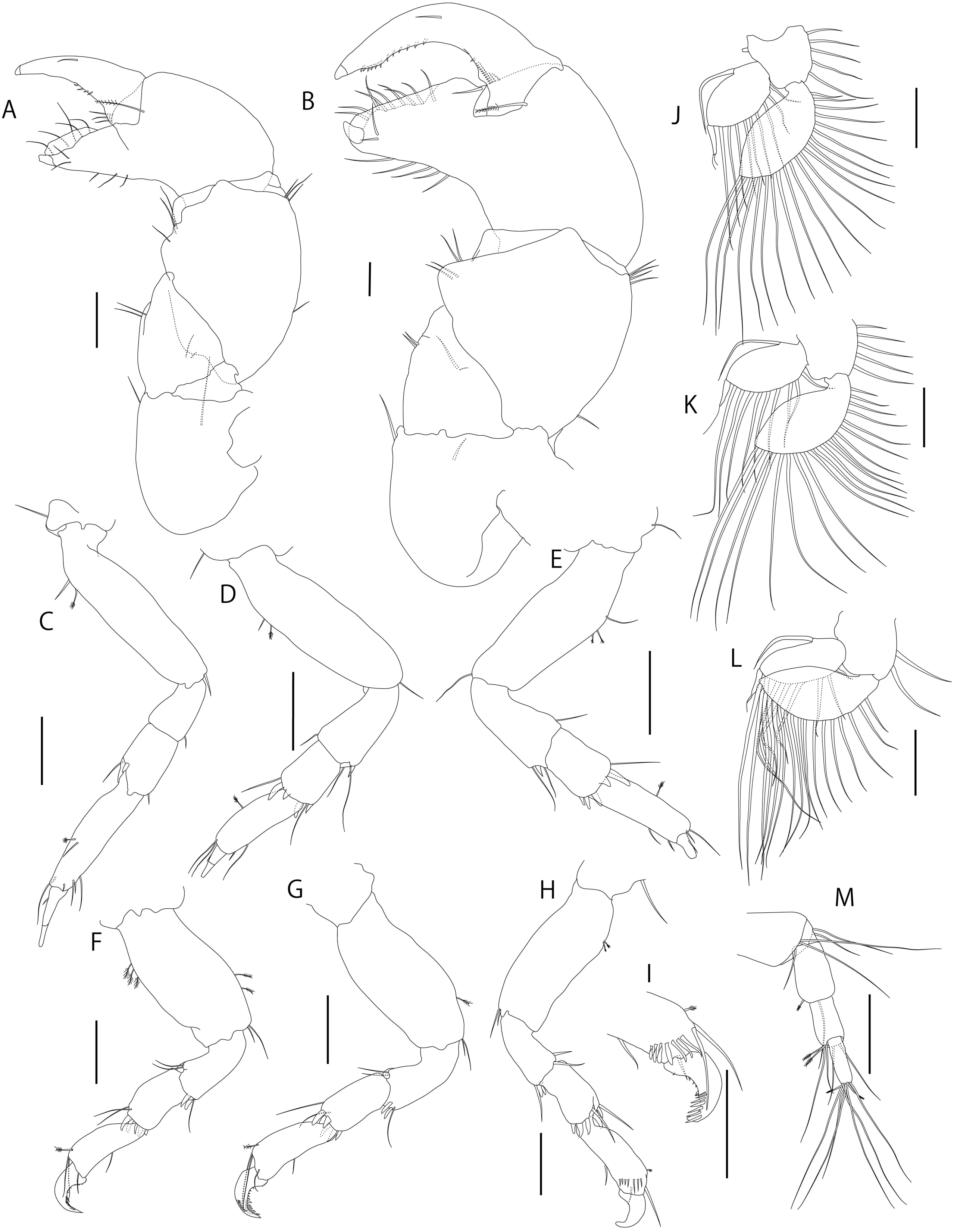
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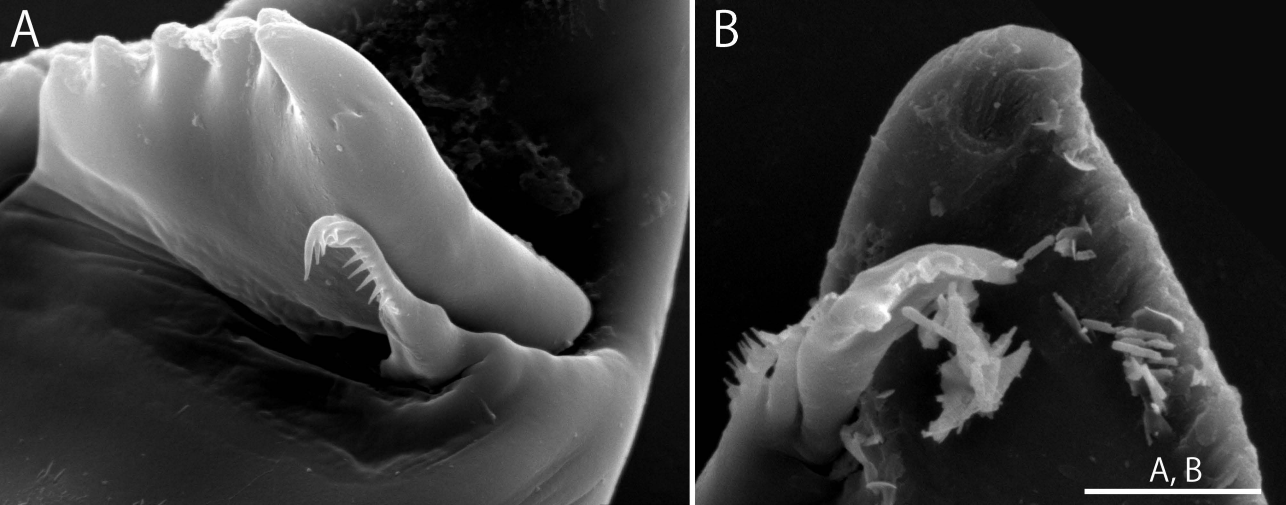
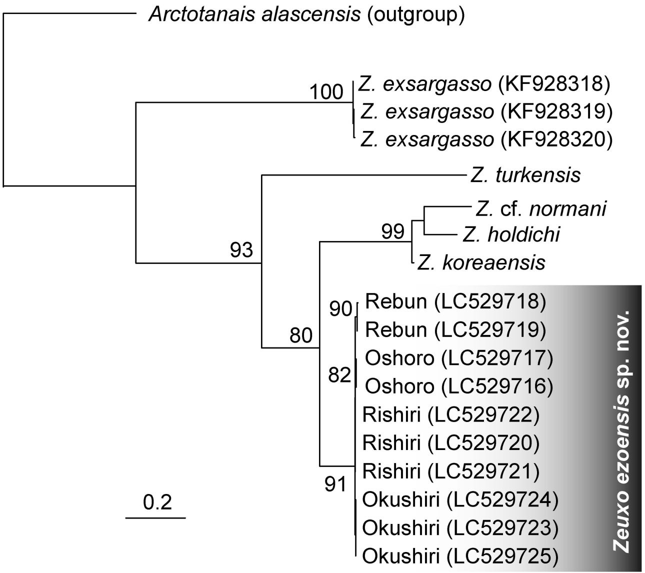

A
B
A, B



Arctotanais alascensis (outgroup)
100
Z. exsargasso (KF928318)
Z. exsargasso (KF928319)
Z. exsargasso (KF928320)
Z. turkensis
93
Z. cf. *normani*
99
Z. holdichi
Z. koreaensis
90
Rebun (LC529718)
Rebun (LC529719)
80
Oshoro (LC529717)
82
Oshoro (LC529716)
Rishiri (LC529722)
Rishiri (LC529720)
Rishiri (LC529721)
91
Okushiri (LC529724)
Okushiri (LC529723)
Okushiri (LC529725)
Zeuxo ezoensis sp. nov.
0.2