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Integrative taxonomy of *Zeuxo* (Crustacea: Peracarida: Tanaidacea) from Japan, with the description of a new species

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Abstract

We describe a new tanaidid species, *Zeuxo molybi* sp. n., from Kominato, Chiba, Japan. *Zeuxo molybi* closely resembles the Japanese congener *Zeuxo ezoensis*, but differs in having (1) the pereopod-1 carpus without dorsodistal spiniform setae; (2) two or three ventrodistal simple setae on the pereopod-1 propodus in females, and three or four in males; and (3) the pereopod-6 propodus with four or five flattened denticulate setae. The two species also differ in the pattern of dorsal pigmentation on the carapace. Kimura 2-parameter distances between the two species were 15.6–16.9% for the cytochrome *c* oxidase subunit I (COI) gene and 2.8% for the 18S rRNA gene; the former value is much greater than intraspecific distances previously reported for *Z. ezoensis* (0–1.5%), confirming our conclusion from morphology that *Z. molybi* and *Z. ezoensis* are not conspecific. In a COI-based maximum-likelihood phylogenetic tree, the Japanese taxa *Z. molybi*, *Z. ezoensis*, and *Z.* cf. *normani* did not form a clade.

Keywords cox1 · DNA barcode · intertidal · Malacostraca · phylogeny · Tanaididae

Statements and Declarations

Conflicts of interests/competing interests. The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Introduction

With 39 species, the genus *Zeuxo* Templeton, 1840 is the most species-rich genus in the family Tanaididae Nobili, 1906 (Okamoto et al. 2020). *Zeuxo* tanaidaceans have been reported at shallow depths (0–52 m; Kakui and Shimada 2022) from tropical to polar regions worldwide. They occasionally occur at high density (e.g., more than 320,000 individuals/m^2 in seaweed beds; Hayashi and Kanda 2007) and play an important role in food webs as omnivorous (KK unpublished data) or herbivorous (e.g., Nakaoka 2002; Karlson et al. 2021) consumers and as prey for various animals such as fishes, isopods, and staurozoan cnidarians (Kakui 2015, 2022; Shiraki and Kakui 2022).

The taxonomy of *Zeuxo* is challenging (Bird 2019), and identification can be difficult even at the genus level when the strongly sexually dimorphic males are absent from a sample (cf. Tait et al. 2021). Molecular data have indicated that one species, *Zeuxo turkensis* Larsen, 2014, may belong in *Hexapleomera* Dudich, 1931 (Tait et al. 2021), suggesting that a revision of *Zeuxo* and allied genera such as *Hexapleomera* based on both morphological and molecular data is necessary, an integrative approach taken in several recent studies (e.g., Larsen 2014; Larsen et al. 2014; Okamoto et al. 2020). Okamoto et al. (2020) proposed that the dorsal pigmentation pattern on the carapace may be useful in *Zeuxo* taxonomy, but its validity has not yet been sufficiently tested. Kakui (2016) suggested the possibility that each *Zeuxo* species has some microhabitat preference.

In this study, we describe a species of *Zeuxo* collected from Japan; present partial nucleotide sequences for its cytochrome *c* oxidase subunit I (COI) and 18S rRNA (18S) genes; and infer its phylogenetic position based on COI.

Material and methods

Tanaidaceans were obtained from muddy sediment covering an intertidal bedrock at Kominato, Chiba, Japan on 17 May 2017 and 17 May 2018. 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). The specimens studied were deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan.

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)"; two additional setal terms are used, "flattened denticulate setae" (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 the holotype and allotype specimens. Measurements were made axially with ImageJ (Rasband 2021) from digital images: dorsally on the body, antennules, antennae, and uropods; laterally on the pereopods and pleopods. All measurements in the text are in millimeters, unless noted otherwise.

DNA was extracted from a freshly killed individual hatched in an aquarium in the Kakui laboratory in Sapporo, Japan; a reared individual was used because amplification of target sequences from ethanol-fixed wild individuals was unsuccessful. This individual was a descendant of individuals collected from the type locality on 17 May 2018. Total DNA was extracted from the whole body of the specimen by using a NucleoSpin Tissue XS Kit (Macherey-Nagel, Germany). Table 1 lists the primers used for PCR and sequencing. PCR amplification conditions for COI with TaKaRa Ex Taq DNA polymerase (TaKaRa Bio, Japan) were 94 °C for 1 min; 35 cycles of 98 °C for 10 s, 42 °C for 30 s, and 72 °C for 50 s; and 72 °C for 2 min. Conditions for 18S amplification with KOD FX Neo (Toyobo, Japan) were 94 °C for 2 min; 45 cycles of 98 °C for 10 s, 65 °C for 30 s, and 68 °C for 75 s; and 68 °C for

3 min. Nucleotide sequences were determined by direct sequencing with a Big Dye 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. BLAST searches (Altschul et al. 1990) of the INSD were conducted to find the known sequences most similar to those we obtained.

The COI dataset for a phylogenetic analysis included the sequence we determined and those in Okamoto et al. (2020). The methods used for sequence alignment (389 positions in the aligned dataset), selection of the optimal substitution model (HKY+I, K81U+G, and HKY+G for the first, second, and third codon positions, respectively), a maximum likelihood (ML) analysis, drawing of the tree, and calculation of Kimura (1980) 2-parameter (K2P) distances among species were as described in Okamoto et al. (2020).

Results

Taxonomy

Family Tanaididae Nobili, 1906

Genus *Zeuxo* Templeton, 1840

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

***Zeuxo molybi* sp. n.**

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

(Figs. 1–4)

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 bifurcate accessory seta. Maxilliped with endite bearing two dorso-subdistal and two distal spiniform setae; palp article 4 with outer simple seta. Chelipedal dactylus with inner simple seta. Male chelipedal fixed finger without triangular mid-dorsal process on cutting surface. Pereopod 1 with basis bearing one or two ventrodistal simple setae; carpus with ventrodistal simple seta but without dorsodistal spiniform setae; propodus with two or three (females) or three or four (male) ventrodistal simple setae. Pereopod-6 propodus with four or five flattened denticulate setae. Pleopods 1–3 with endopod bearing inner plumose seta. Pleopod-3 basal article lacking inner plumose setae. Uropod with five articles (including basal article).

Carapace pigmentation pattern: anterior region dark; subanterior region without dark pigmentation; posterolateral region with two dark diagonal bands; posteromedial region with irregular dark mesh pattern (often faint).

Description of females, based primarily on holotype

Body (Figs. 1a, c, 2a, b) 5.47 times as long as wide, with brown pigmentation (retained in ethanol). Cephalothorax 0.18 times BL, with mid-lateral pair of simple setae and two pairs of simple setae posterior to eyes. Dorsal pigmentation pattern: anterior region dark; subanterior region without dark pigmentation; posterolateral region with two dark diagonal bands; posteromedial region with irregular dark mesh pattern (often faint). Pereonites 1–6 with length ratio 1.00:1.40:1.76:2.30:2.20:1.70; all with several pairs of simple setae. Pereonites 1–6 width-to-length ratio 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 seven

pairs of simple setae.

Antennule (Fig. 2d) 0.62 times as long as cephalothorax; articles 1–4 with length ratio 1.00:0.42:0.27:0.03. Article 1 with one inner proximal and three distal simple setae, and three proximal and four distal PSS. Article 2 with six distal simple setae and four distal PSS. Article 3 with three distal simple setae and distal PSS. Article 4 with four simple setae, three aesthetascs, and distal PSS.

Antenna (Fig. 2f) 1.20 times as long as antennule; articles 1–7 with length ratio 1.00:1.80:1.03:1.87:1.76:0.61:0.23. Articles 1 and 3 naked. Article 2 with one inner distal, one dorsodistal, and two mid-ventral simple setae. Article 4 with four distal simple setae and distal PSS. Article 5 with three distal simple setae and four distal PSS. Article 6 longer than wide, with five distal simple setae. Article 7 with five distal simple setae.

Labrum (Fig. 3a) setulate distally. Mandibles (Figs. 3b, c, 4) with well-developed molar process bearing many small teeth on masticatory surface; left mandible (Figs. 3b, 4a) with smooth incisor, wide, denticulate lacinia mobilis, and bifurcate serrate accessory seta; right mandible (Figs. 3c, 4b) with smooth incisor, peg-like lacinia mobilis, and bifurcate serrate accessory seta. Labium (Fig. 3d) with inner and outer lobes setulate on distal margin; labial palp setulate, articulated with outer lobe. Maxillule (Fig. 3e, f) with endite bearing seven distal spiniform setae and outer subdistal setation; palp (from paratype ICHUM8257) with five distal simple setae. Maxilla (Fig. 3g) with small serrations on outer and distal margins.

Maxilliped (Fig. 3h) coxa bearing simple seta. 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 distal simple seta; article 2 with four inner ventral and one outer simple setae, and three inner distal plumose setae; article 3 with eight inner plumose setae; article 4 with outer simple seta

and six distal plumose setae. Epignath (Fig. 3i) with kidney-shaped lobe, margins finely setulate; terminal seta setulate.

Cheliped (Fig. 2h) with triangular articulation to cephalothorax via sclerite (Fig. 2a). Basis nearly as long as wide, with one outer dorsal and one ventrodistal simple setae. Merus with one dorsal and two ventral simple setae. Carpus 1.77 times as long as wide, with one dorsal, three dorsodistal and four ventral simple setae. Propodus 0.95 times as long as carpus length; palm with two 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, and dorsal lamellar expansion (dorsal margin straight) and triangular claw. Dactylus as long as fixed finger, with inner simple seta and row of ventral spiniform setae; unguis triangular.

Pereopods 1–6 cylindrical, with length ratio of 1.00:0.83:1.11:0.87:0.84:0.88.

Pereopod 1 (Fig. 3j) 0.28 times as long as BL, with length ratio of basis, merus, carpus, propodus, and dactylus-unguis 1.00:0.26:0.36:0.65:0.47. Coxa with slight dorsal process bearing two dorsal simple setae. Basis cylindrical, narrow, 3.72 times as long as wide, with one dorso-subproximal and one ventrodistal simple setae, one ventrodistal tiny simple seta, and one dorso-subproximal PSS. Merus with one ventrodistal and one dorsodistal simple setae. Carpus with two dorsodistal and one ventrodistal simple setae. Propodus with one mid-inner, one dorsodistal, and three ventrodistal simple setae, and mid-dorsal PSS. Dactylus with dorsal simple seta; unguis as long as dactylus, naked.

Pereopod 2 (Fig. 3k) with length ratio of articles from basis to dactylus-unguis 1.00:0.43:0.31:0.43:0.27. Coxa with dorsal simple seta. Basis cylindrical, narrow, 2.94 times as long as wide, with one dorso-subproximal and one ventrodistal simple setae, one ventrodistal tiny simple seta, and two dorso-subproximal PSS. Merus with one dorsodistal and two ventrodistal simple setae and ventrodistal spiniform seta. Carpus with two 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 (Fig. 3l) with length ratio of articles from basis to dactylus-unguis 1.00:0.44:0.34:0.49:0.33; similar to pereopod 2, except coxa without simple seta, basis without ventrodistal tiny simple seta, merus with one ventrodistal simple seta, and carpus with one dorsodistal simple seta.

Pereopod 4 (Fig. 3m) with length ratio of articles from basis to dactylus-unguis 1.00:0.36:0.42:0.48:0.36. Coxa naked. Basis 2.63 times as long as wide, with two ventrodistal simple setae, and two dorso-subproximal 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 dorso-subdistal PSS. Dactylus-unguis fused to form claw, strongly arched, with inner and outer rows of ventral spines.

Pereopod 5 (Fig. 3n) with length ratio of articles from basis to dactylus-unguis 1.00:0.43:0.34:0.53:0.38; similar to pereopod 4.

Pereopod 6 (Fig. 3o) with length ratio of articles from basis to dactylus-unguis 1.00:0.37:0.38:0.51:0.46; similar to pereopod 4 except basis without ventro-subdistal PSS and propodus with four inner distal flattened denticulate setae.

Pleopod 1 (Fig. 3p) with basal article bearing one inner and four outer plumose setae; endopod with one inner and ten outer plumose setae, and outer distal step-tipped plumose seta; exopod 1.37 times as long as endopod, with 21 outer plumose setae. Pleopod 2 (Fig. 3q) similar to pleopod 1. Pleopod 3 (Fig. 3r) with basal article bearing outer plumose seta; endopod with one inner and nine outer plumose setae, and outer distal step-tipped plumose seta; exopod 1.47 times as long as endopod, with 18 outer plumose setae.

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

Description of male, based on allotype

Body (Figs. 1b, d, 2c) similar to female.

Antennule (Fig. 2E) 0.75 times as long as cephalothorax; articles 1–4 with length ratio of 1.00:0.43:0.28:0.03. Article 1 with three distal and one inner proximal simple setae, and three proximal and one distal PSS. Article 2 with six distal simple setae and two distal PSS. Article 3 with three distal simple setae and one distal PSS. Article 4 with four simple setae and five aesthetascs.

Antenna (Fig. 2G) as long as antennule; articles 2–7 with length ratio 1.00:0.40:0.85:0.70:0.23:0.05 (article 1 broken during dissection, not examined). Article 2 with one inner distal, one dorsodistal, and two mid-ventral simple setae. Article 3 naked. Article 4 with four distal simple setae. Article 5 with three distal simple setae and two distal PSS. Article 6 longer than wide, with three distal simple setae. Article 7 with eight distal simple setae.

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

Maxilliped similar to that in female, except inner margin of palp article 3 with 12 setae and distal margin of palp article 4 with nine plumose setae. Epignath similar to that in female.

Cheliped (Fig. 2i) with triangular articulation to cephalothorax via sclerite. Basis and merus similar to those of female. Carpus 1.28 times as long as wide, with one dorsal and three dorsodistal and three ventral simple setae. Propodus 0.83 times as long as carpus length,

Propodus and dactylus setation similar to that in female. Fixed finger with dorsodistal large lamellar expansion on cutting surface. Dactylus strongly arched ventrally; unguis triangular.

Pereopods 1–6 with length ratio of 1.00:0.91:0.81:0.88:0.88:0.94; pereopod 1 length 0.33 times as long as BL. Pereopods similar to those of female, with following exceptions. Pereopod 1: coxa without dorsal simple seta; basis without ventrodistal simple seta; carpus with dorsodistal simple seta; propodus with four ventrodistal simple setae. Pereopod 2: coxa with two dorsal simple setae; carpus with dorsodistal simple seta. Pereopod 3: coxa with two dorsal simple setae; basis with one dorso-subproximal and one ventro-subproximal PSS; merus with two ventrodistal simple setae; carpus without dorsodistal simple seta. Pereopod 6: merus with dorsodistal simple seta.

Pleopods similar to those of female, with following exceptions: basal articles of pleopods 1–3 with five, five, and three outer plumose setae, respectively; exopods of pleopods 1–3 with 22, 24, and 19 outer plumose setae, respectively.

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

Variation and stability

In addition to the holotype and allotype, three female (ICHUM8255–8257) and two male (ICHUM8258, 8259) paratypes of *Zeuxo molybi* sp. n. were dissected and all appendages were observed. The raw morphological data are in Online Resource 1. All specimens shared the same state for the following characters (selected characters only are presented; for all characters, see Online Resource 1): (1) dorsal pigmentation pattern on carapace (anterior region dark; subanterior region without dark pigmentation; posterolateral region with two

dark diagonal bands; posteromedial region with irregular dark mesh pattern (often faint); cf. Fig. 1); (2) antennal article 2 with one inner distal and two mid-ventral simple setae; (3) antennal article 6 longer than wide; (4) left mandible with wide, denticulate lacinia mobilis and one bifurcate accessory seta; (5) right mandible with peg-like lacinia mobilis and one bifurcate accessory seta; (6) maxillipedal endite with two dorso-subdistal and two distal spiniform setae; (7) maxillipedal palp articles 2 and 4 each with one outer simple seta; (8) chelipedal merus with two ventral simple setae; (9) chelipedal dactylus with one inner simple seta and row of ventral spiniform setae; (10) pereopod-1 carpus with one ventrodistal simple seta but lacking spiniform setae; (11) pereopod-2 basis, merus, carpus, and propodus each with one ventrodistal simple seta; (12) endopod of pleopods 1–3 each with one inner plumose seta; (13) pleopod-3 basal article without inner plumose setae; and (14) uropod with five articles (including basal article).

The following setae, articles, and ratios varied in number or value among specimens (selected characters only are presented; for all characters, see Online Resource 1) (ranges in parentheses): dorsodistal simple setae on chelipedal carpus (3–4), carpus-length/propodus-length ratio in male cheliped (0.79–0.95); ventrodistal simple setae on pereopod-1 basis (1–2), and flattened denticulate setae on pereopod-6 propodus (4–5).

Material examined

Holotype. Female, ICHUM8253 (BL 2.12, CW 0.38), Kominato, Chiba, Japan (35°07'10.3"N, 140°11'02.3"E) (type locality), muddy sediment covering bedrock, intertidal zone, 17 May 2018, coll. by Keiichi Kakui.

Allotype. Male, ICHUM8254 (BL 1.97, CW 0.39), same collection data as for holotype.

Paratypes. Seven females (ICHUM8255, BL 2.56, CW 0.46; ICHUM8256, BL 1.99,

CW 0.49; ICHUM8257, BL 2.24, CW 0.44; ICHUM8264; ICHUM8265, BL 2.34, CW 0.43, INSD accession numbers LC655993 [18S] and LC655994 [COI]; ICHUM8266, BL 2.31, CW 0.45; ICHUM8268) and seven males (ICHUM8258, BL 1.95, CW 0.44; ICHUM8259, BL 1.67, CW 0.38; ICHUM8260, BL 1.80, CW 0.36; ICHUM8261, BL 1.60, CW 0.37; ICHUM8262, BL 2.11, CW 0.38; ICHUM8263, BL1.86, CW 0.34; ICHUM8267, BL 2.13, CW 0.45). Collection data for ICHUM8255–8259, 8262, 8263 same as for holotype. ICHUM8260 and 8261 collected at type locality on 17 May 2017. ICHUM8264–8268 hatched in an aquarium in Kakui laboratory, Sapporo; descendants of individuals collected from type locality on 17 May 2018.

Etymology

The specific name is a noun in apposition derived from the Greek noun μολύβι (*molybi*; pencil), referring to the dorsal pigmentation pattern on the carapace resembling the tip of a pencil.

Phylogenetic analysis

The partial 18S (INSD accession number LC655993; 2043 bp long) and COI (LC655994; 605 bp long, encoding 201 amino acids) sequences were determined from one individual (ICHUM8265; Fig. 1i). The sequence in the INSD detected by a BLAST search to be most similar to our 18S sequence was from *Zeuxo* sp. KK-2011-2 (identity score 97.77%, query cover 92%) collected from Misaki, Kanagawa, Japan (Kakui et al. 2011). The K2P distances between the 18S sequence from *Z. molybi* and two *Zeuxo* listings in the INSD (*Zeuxo* sp. KK-2011-2 and *Z. ezoensis* [AB618194]) were 1.9% and 2.8%, respectively.

In the ML tree based on COI sequences (Fig. 5), *Z. molybi* was the sister group (with moderate support; ultrafast bootstrap value [uBS] = 88%) to a well-supported (97% uBS)

clade comprising *Z. koreaensis* Larsen, 2014, *Z. holdichi* Bamber, 1990, and *Z.* cf. *normani* (Richardson, 1905). K2P distances among COI sequences from the seven *Zeuxo* species were 12.4–36.2% (Table 2).

Discussion

Zeuxo molybi sp. n. differs from all congeners except *Z. ezoensis* Okamoto et al., 2020 from Japan 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 one bifurcate accessory seta (this condition has often been described as "two accessory setae"; e.g., Okamoto et al. 2020), (3) maxillipedal endite with two pairs of tiny dorso-subdistal spiniform setae, (4) maxillipedal palp article 4 with one outer simple seta, (5) basal article of pleopod-3 without inner plumose setae, and (6) uropod with five articles.

Zeuxo molybi differs from *Z. ezoensis* in the following combination of characters (character states of *Z. ezoensis* in parentheses): pereopod-1 carpus without dorsodistal spiniform setae (with one seta); two or three ventrodistal simple setae on pereopod-1 propodus in female (four or five), three or four in male (five to seven); and pereopod-6 propodus with four or five flattened denticulate setae (seven to ten). During this comparison, we realized that there was an error in the number of accessory setae on the right mandible and of ventrodistal setae on the pereopod 2 propodus in supplementary table S1 of Okamoto et al. (2020), which listed raw morphological data for type specimens of *Z. ezoensis*. We present a corrected version of this table as Online Resource 2.

Zeuxo molybi closely resembles *Z. ezoensis* in morphology but clearly differs in the pigmentation pattern on the dorsal region of carapace. In *Z. molybi* (Fig. 1), the anterior region of the carapace is dark; the subanterior region lacks dark pigmentation; the

posterolateral region has two dark diagonal bands; and the posteromedial region shows an irregular, dark mesh pattern (often faint). In *Z. ezoensis*, the carapace has a dark background, with a V-shaped zone of lighter, irregular spots (Okamoto et al. 2020: figs. 1, 2) (also see Online Resource 3). The K2P distance between these two species was 15.6–16.9% for COI and 2.8% for 18S; the former value is much greater than intraspecific distances previously reported for *Z. ezoensis* (0–1.5%; Okamoto et al. 2020). The molecular data support our conclusion from morphology that *Z. molybi* and *Z. ezoensis* are different species, and confirm that carapace pigmentation patterns are valid as a diagnostic character in *Zeuxo* taxonomy, as proposed by Okamoto et al. (2020).

To date, six genera and 12 species (except unidentified species) of Tanaididae have been reported around Japan (Kakui 2016; Tanabe et al. 2017; Tanabe and Kakui 2019; Okamoto et al. 2020; this study): *Arctotanais alascensis* (Richardson, 1899), *Hexapleomera sasuke* Tanabe and Kakui, 2019, *Hexapleomera urashima* Tanabe et al., 2017, *Protanais birsteini* (Kudinova-Pasternak, 1970), *Sinelobus stanfordi* (Richardson, 1901), *Tanais tinhauae* Bamber and Bird, 1997, *Tanais vanis* Miller, 1940, *Zeuxo coralensis* Sieg, 1980, *Zeuxo ezoensis*, *Zeuxo maledivensis* Sieg, 1980, *Zeuxo molybi* sp. n., and *Zeuxo normani*. As suggested by previous works (e.g., Okamoto et al. 2020), they contain species whose distribution around Japan is questionable. On the other hand, our ongoing projects have discovered several additional tanaidid species from Japan (KK unpublished). Current knowledge of Japanese tanaidids is based on samples from narrow areas. Further investigations will discover more tanaidid species from Japan.

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Table 1. List of PCR and cycle sequencing (CS) primers used in this study.

Gene	Primer	Sequence	Reaction	Source
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	PCR, CS	Folmer et al. (1994)
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	PCR, CS	Folmer et al. (1994)
18S	SR1	TACCTGGTTGATCCTGCCAG	PCR	Nakayama et al. (1996)
	18S-b3F	CCTGAGAAACGGCTACCACAT	CS	Kakui and Shimada (2017)
	18S-b3R	ATGTGGTAGCCGTTTCTCAGG	CS	This study
	18S-b4F	TGCGGTTAAAAAGCTCGTAGTTG	CS	Kakui et al. (2011)
	18S-b4R	TCCAACTACGAGCTTTTTAACC	CS	Kakui et al. (2011)
	18S-b5F	GATCGAAGGCGATYAGATACC	CS	Kakui et al. (2021)
	18S-b6F	CCTGCGGCTTAATTTGACTC	CS	Kakui et al. (2011)
	18S-a6R	AACGGCCATGCACCAC	CS	Kakui et al. (2011)
	18S-b8F	GGTCTGTGATGCCCTTAGATG	CS	Kakui et al. (2011)
	SR12	CCTTCCGCAGGTTCACCTAC	PCR	Nakayama et al. (1996)

Table 2. K2P distances (in percent) among COI sequences from six *Zeuxo* species (389 positions).

	Z. exsargasso	Zt	Ze	Zm	Zk	Zh
Z. turkensis (Zt)	35.3–35.7					
Z. ezoensis (Ze)	30.8–32.0	28.9–30.5				
Z. molybi sp. n. (Zm)	30.8–31.2	30.1	15.6–16.9			
Z. koreaensis (Zk)	31.2–31.6	32.5	16.0–17.4	17.3		
Z. holdichi (Zh)	32.8–33.1	35.7	18.3–19.3	22.8	13.7	
Z. cf. *normani* (Zn)	35.3–36.2	32.5	20.4–21.5	20.1	12.4	15.3

Figure legends

Fig. 1 *Zeuxo molybi* sp. n. a, c, paratype female (ICHUM8266); b, d, paratype male (ICHUM8267); e–h, paratype males (ICHUM8260–8263); i, paratype male (ICHUM8265; accession numbers LC655993 [18S] and LC655994 [COI]). **a, b** living individuals, dorsal view; **c–i** anterior portion of body, ethanol-fixed specimens. Scale bars: 0.5 mm (a, b), 0.2 mm (c, d)

Fig. 2 *Zeuxo molybi* sp. n. a, b, d, f, h, holotype, female; c, e, g, i, allotype, male. **a, b** body, lateral (a) and dorsal (b) views; **c** carapace and antennule, setae omitted; **d, e** right antennule, dorsal views; **f, g** right antenna, inner views; **h, i** cheliped, outer view. Scale bars: 0.5 mm (a, b); 0.1 mm (c–i)

Fig. 3 *Zeuxo molybi* sp. n. a–e, g–s, holotype, female; f, paratype (ICHUM8257), female. **a** labrum; **b** left mandible; **c** right mandible; **d** right labium; **e** maxillule; **f** maxillular palp; **g** maxilla; **h** maxilliped, all setae on left endite and inner and distal setae on left palp omitted; **i** epignath; **j–o** pereopods 1–6, inner views; **p–r** pleopods 1–3, setal ornamentation omitted; **s** uropod. Scale bars: 0.05 mm.

Fig. 4 *Zeuxo molybi* sp. n., distal region of mandibles, SEM images. **a** left mandible, female paratype (ICHUM8264); **b** right mandible, female paratype (ICHUM8268); lacinia mobilis was broken at the point with an arrowhead. Scale bars: 0.01 mm.

Fig. 5 ML tree based on COI sequences (389 positions). Numbers near nodes are ultrafast bootstrap values in percent. The scale indicates branch length in number of substitutions per site. Clades containing more than two conspecific sequences were collapsed (terminal

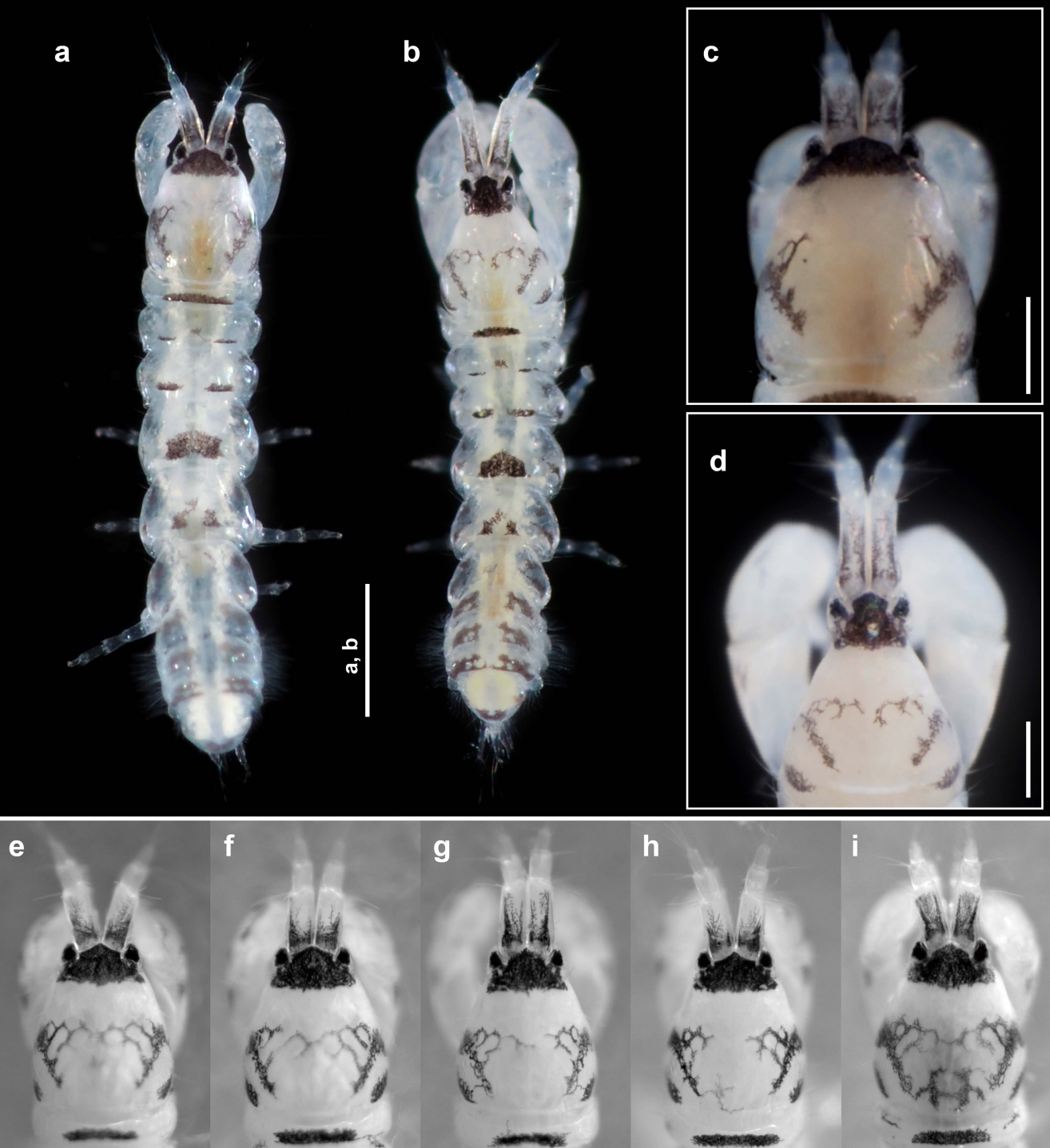
triangles).

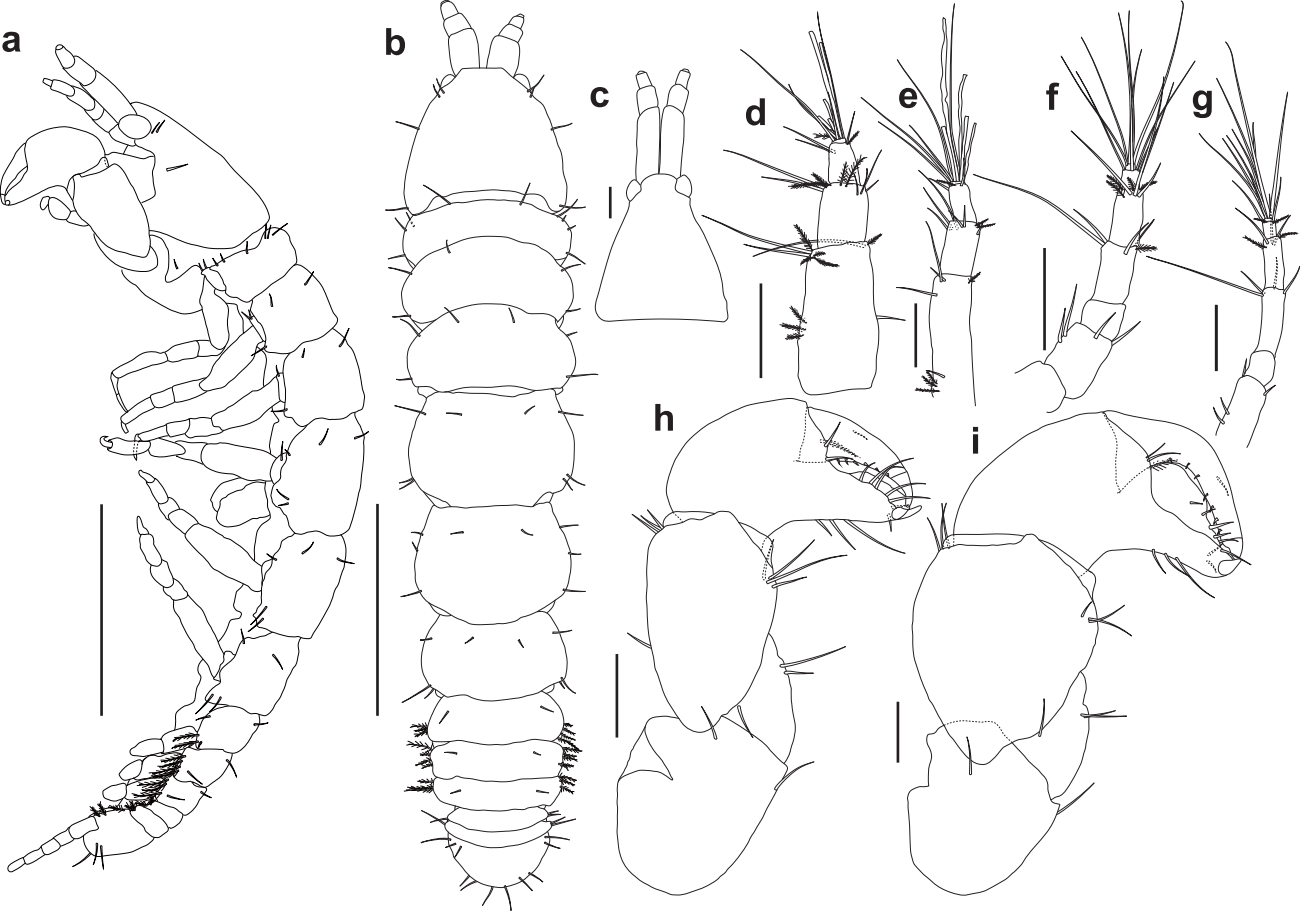
List of Online Resources

Online Resource 1 Variation in characters among dissected specimens of *Zeuxo molybi* sp. n.

Online Resource 2 Variation in characters among dissected specimens of *Zeuxo ezoensis* Okamoto et al., 2020.

Online Resource 3 Dorsal pigmentation pattern on two Japanese *Zeuxo* species, *Zeuxo molybi* sp. n. and *Zeuxo ezoensis* Okamoto et al., 2020.


a
b
c
d
a, b
e
f
g
h
i


a
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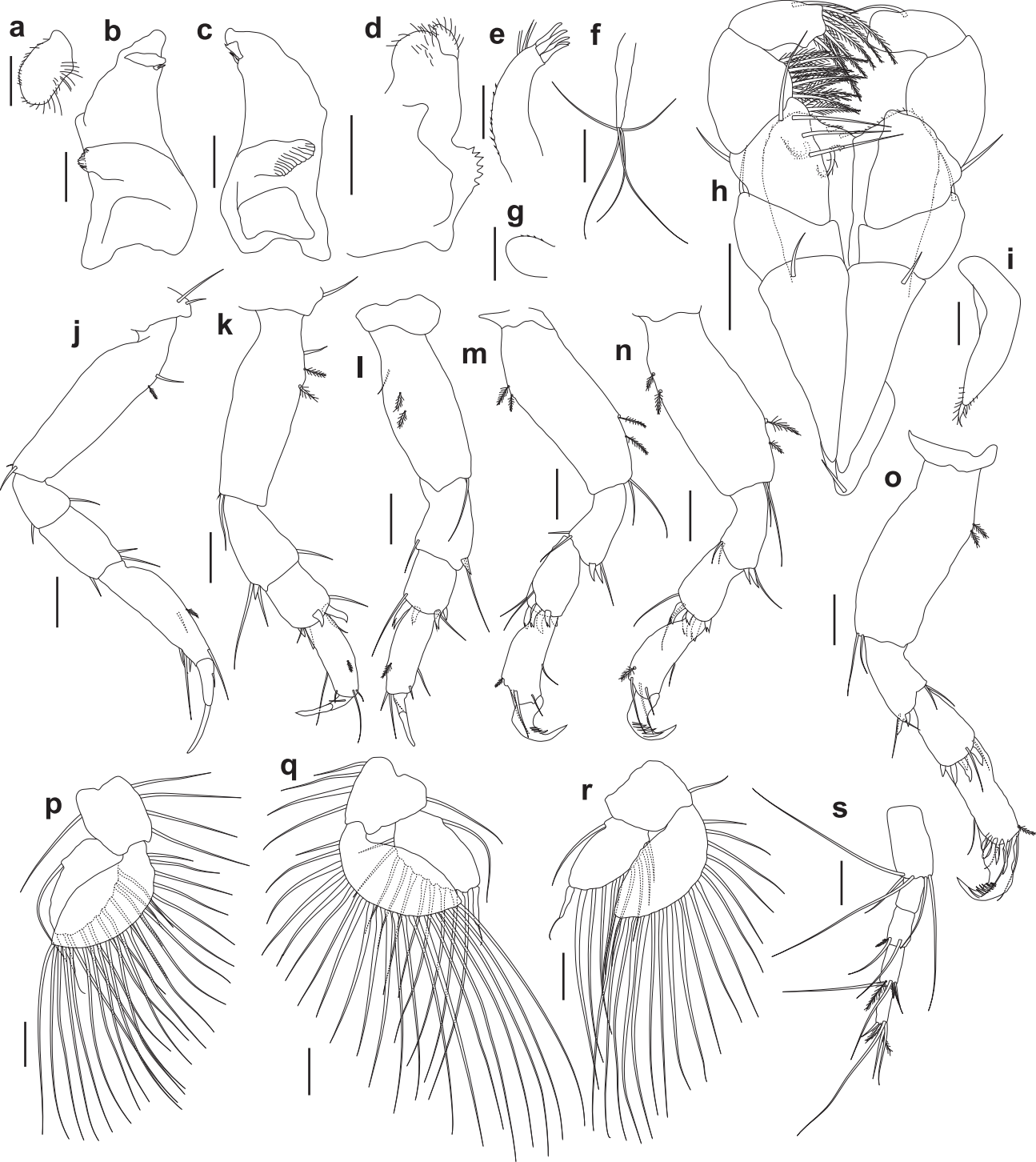
a
b
c
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e
f
g
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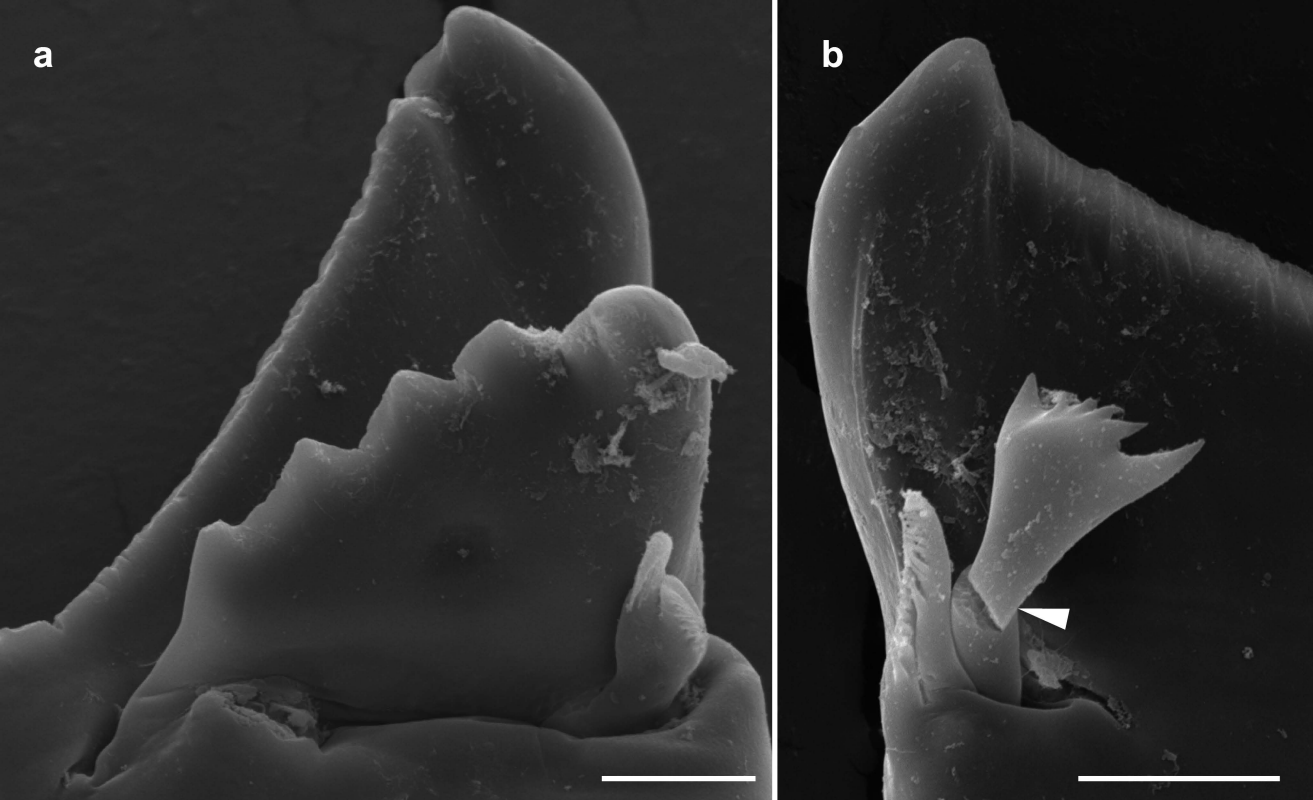
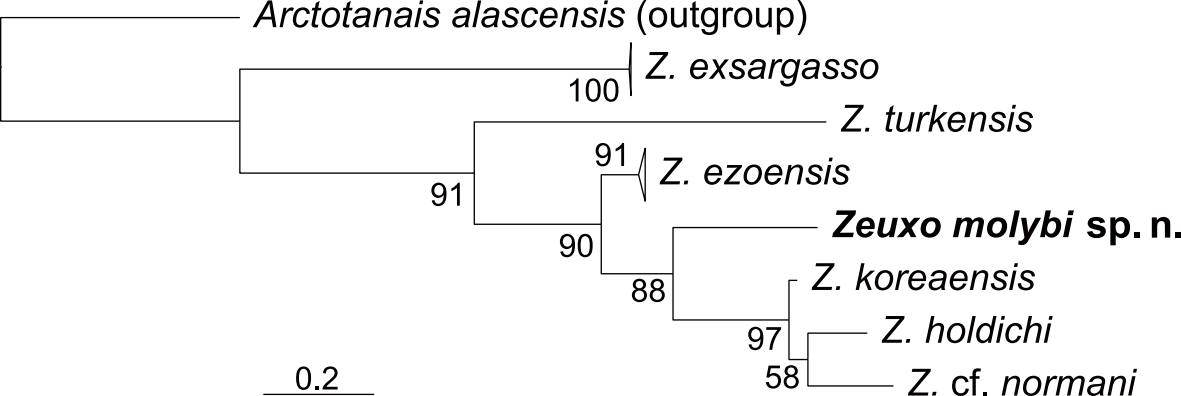
i
j
k
l
m
n
o
p
q
r
s


a
b


Arctotanais alascensis (outgroup)
Z. exsargasso
100
Z. turkensis
91
Z. ezoensis
91
***Zeuxo molybi* sp. n.**
90
Z. koreaensis
88
Z. holdichi
97
58
Z. cf. *normani*
0.2