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# A new brackish tanaidacean, *Sinelobus kisui* sp. nov. (Crustacea, Peracarida, Tanaidacea), from Japan, with a key to *Sinelobus* species and barcode information from two loci

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#### Abstract

We describe the new brackish tanaidid species *Sinelobus kisui* **sp. nov.** from Hagi, Yamaguchi, Japan. *Sinelobus kisui* is similar to *S. barretti* and *S. vanhaareni* in having antennal article 2 with one outer distal seta, the dorsodistal crotchet on percopods 2 and 3 carpi shorter than half propodus length, and percopodal carpi 2–6 with five distal crotchets, but differs from them in having (1) the inner of two ventro-subdistal circumplumose setae on the maxillipedal endite longer than the outer; (2) the maxillipedal endite with one mid-inner spiniform seta; (3) the percopod-1 propodus with one middle setulate seta; and (4) the pleopod-1 protopod lacking inner plumose setae. Our study confirmed that character states of the chelipeds in strongly dimorphic males are useful in *Sinelobus* taxonomy. We determined partial sequences for the cytochrome *c* oxidase subunit I (COI; cox1) and 18S rRNA (18S) genes in *S. kisui* for future DNA barcoding and phylogenetic analyses. Morphological and/or molecular data reveal that *S. kisui* also occurs in Kagawa and Osaka, Japan. A key to species in *Sinelobus* is provided.

#### Key Words

Integrative taxonomy, intraspecific variation, non-marine, Tanaididae, tube dweller

### Introduction

The brackish genus *Sinelobus* Sieg, 1980 is one of 20 genera in Tanaididae Nobili, 1906. After Sieg's (1980) revision of Tanaididae, the genus was regarded as monotypic, containing only the single cosmopolitan species *Sinelobus stanfordi* (Richardson, 1901), originally described from Clipperton Island in the eastern Pacific Ocean. Since 2008, however, five additional species have been described: *Sinelobus barretti* Edgar, 2008 (Tasmania), *Sinelobus bathykolpos* Bamber, 2014 (Hong Kong), *Sinelobus pinkenba* Bamber, 2008 (Australia), *Sinelobus stromatoliticus* Rishworth, Perissinotto & Błażewicz, 2018 (South Africa), and *Sinelobus vanhaareni* Bamber, 2014 (Netherlands) (Fig. 1a).

*Sinelobus* tanaidaceans are benthic tube-dwellers, lack a planktonic larval stage, and inhabit brackish water (note:

there are some records from freshwater environments; e.g., Stephensen 1936), suggesting that they have low dispersibility, especially across oceans. It is thus likely that this genus contains many locally endemic species that have not yet been detected. They have been reported from various localities in Japan, either identified as *S. stanfordi* or not identified to species level (e.g., Stephensen 1936, Ariyama and Ohtani 1990, Kakui 2022) (Fig. 1b).

We investigated *Sinelobus* tanaidaceans collected from Hagi, Yamaguchi, Japan and concluded they represent an undescribed species. Here we describe this species as new, present partial sequences for the mitochondrial cytochrome c oxidase subunit I (COI) and nuclear 18S rRNA (18S) genes for future DNA barcoding and phylogenetic analyses, and provide a key to the species in *Sinelobus*.

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#### Materials and methods

Sinelobus specimens were collected among tubular Ulva algae in a brackish stream (no salinity data) at Hagi, Yamaguchi, Japan on 22 May 2014 (Fig. 1). Some individuals were maintained in a small aquarium (20 °C; 14 h light/10 h dark; 3‰ salinity; fed porphyrized dry feed for crayfish [JAN code 4971618829092; Kyorin, Japan] every 3 days), and one female and one male hatched in the aquarium were photographed live to document their body pigmentation pattern. All other individuals were fixed and preserved in 70–99% ethanol. We also obtained *Sinelobus* specimens from two other Japanese localities, Kagawa (collected by Yoshihiro Hayashi) and Osaka (collected by Michio Ohtani). The Kagawa individuals (= *Sinelobus* sp. 1 sensu Kakui et al. [2011]) came from the mouth of the Aya River on 2 June 2008 and were fixed in 99% ethanol. The Osaka individuals were collected at Station 4 in the Shin Yodo River on 19 February 1989 and fixed in 10% formalin (see Ariyama and Ohtani [1990] for additional details). The methods used for dissection, preparation of slides, light microscopy, 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, and the Osaka Museum of Natural History (**OMNH**), Osaka, 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)," and the term "protopod" is used instead of "basal article" for pleopods



**Figure 1.** Sampling sites and type localities for *Sinelobus* species. **a.** Map showing the type localities for seven *Sinelobus* species, including *Sinelobus kisui* sp. nov.; **b.** Map showing the sampling site at Hagi, Yamaguchi and previously reported sites for *Sinelobus* individuals in Japan (\*, Kochi, where *Sinelobus* sp. 2 sensu Kakui et al. [2011] was collected); **c.** Location of the sampling site for *Sinelobus kisui* in Hagi; **d.** Photograph showing the point of sampling in a brackish stream in Hagi. Maps were generated by using GMT6 (**a**, **b**. Wessel et al. 2019) or were based on GSI Maps (**c.** Geospatial Information Authority of Japan 2022). Sources and raw data for the plots are available in the Figshare repository (Kakui 2022).

and uropods. Three other setal terms used are: "crotchet(s)" (Bird 2019), "flattened denticulate seta(e)" (Edgar 2008), and "step-tipped plumose seta(e)" (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 2022) from digital images: dorsally on the body, antennules, antennae, and uropods; laterally on the pereopods and pleopods. Unless otherwise noted, all measurements in the text are in millimeters.

DNA was extracted from three Yamaguchi specimens by using a NucleoSpin Tissue XS Kit (Macherey-Nagel, Germany). For PCR amplification, primers used for COI were LCO1490 (Folmer et al. 1994) and HCOoutout (Prendini et al. 2005); these two and HCO2198 (Folmer et al. 1994) were used in cycle sequencing. Amplification primers for 18S were 18S-a1F and 18S-a9R (Kakui et al. 2011); seven primers (18S-b3F, 18S-b4F, 18S-b4R, 18S-b6F, 18S-a6R, 18S-b8R, and 18S-b8F; Kakui et al. 2011, Kakui and Shimada 2017) were used in cycle 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 1 min; and 72 °C for 2 min. Amplification conditions for 18S with KOD FX Neo (Toyobo, Japan) were 94 °C for 2 min; 45 cycles of 98 °C for 10 s, 52 °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). Two previously determined, unpublished COI sequences from two Kagawa *Sinelobus* individuals were included in this study; the method used to determine these sequences was described by Okamoto et al. (2020). The sequences we determined were deposited in the International Nucleotide Sequence Database (**INSD**) through the DNA Data Bank of Japan. Kimura (1980) 2-parameter (K2P) distances among *Sinelobus* COI sequences were calculated with MEGA7.

#### Results

Taxonomy

Family Tanaididae Nobili, 1906

Genus *Sinelobus* Sieg, 1980 [New Japanese name: *Kisui-tanaisu-zoku*]

**Remarks.** *Sinelobus* differs from confamilial genera in the following combination of characters: pereonite 1 not extended anterolaterally; four free pleonites; dorso-transverse plumose setal row on pleonites 1 and 2 present but incomplete (absent in mid-dorsal region); antennal article 4 without distal setal tuft; antennal article 5 at least 0.6 times length of article 4; labial outer lobe without terminal process; and prominent sexual dimorphism (Sieg 1980, Bamber 2005, Bamber and Boxshall 2006, Chim and Tong 2019).

#### Sinelobus kisui sp. nov.

https://zoobank.org/FA0362B0-1A05-41B3-AC84-DA36C622D3BA [Japanese name: *Kisui-tanaisu*] Figs 2–6

Sinelobus stanfordi: Ariyama and Ohtani 1990, 25–26, figs 2–5; Kakui 2016, 610 (not Sinelobus stanfordi Richardson, 1901).

Sinelobus sp. 1: Kakui et al. 2011, 751; Kakui et al. 2012, 128–129, fig. 1D.

Sinelobus sp.: Kaji et al. 2016, 3-8, figs 1-5; Kakui et al. 2021, 3.

**Diagnosis.** Antennal article 2 with outer distal simple seta. Maxillipedal endite with one mid-inner and two dorsodistal spiniform setae; and two ventro-subdistal circumplumose setae, inner one longer than outer. Pereopod 1 with carpus bearing two dorsal and two ventral simple setae; propodus with middle setulate seta. Dorsodistal crotchet on carpi of pereopods 2 and 3 shorter than half propodus length. Pereopod 3 basis with ventral PSS. Pereopods 2–6 with carpus bearing five distal crotchets. Pleopod 1 protopod without inner plumose setae. In strongly sexually dimorphic males, chelipedal merus with three ventral processes; angle of dorsal margin of fixed finger to distal margin of palm about 90°.

**Etymology.** The specific name is derived from the Japanese noun *kisui* (brackish water), referring to the habitat of this species, and also referable to the Japanese name *Kisui-tanaisu*, proposed by Ariyama and Ohtani (1990) for this species in the Shin Yodo River, Osaka.

Material examined. Holotype: Female, ICHUM8301 (BL 4.00, CW 0.67), Hagi, Yamaguchi, Japan (34°26.168'N, 131°25.140'E) (type locality), among tubular Ulva algae, brackish stream, 22 May 2014, coll. by Keiichi Kakui. Allotype: Male, ICHUM8302 (BL 3.17, CW 0.62), same collection data as for holotype. Paratypes: Five females (ICHUM8303, BL 3.06, CW 0.56; ICHUM8304, BL 2.91, CW 0.52; ICHUM8305, CW0.51, INSD accession number LC705431 [COI]; ICHUM8306, BL 3.18, CW 0.52; ICHUM8312, BL 2.10, CW0.42, INSD accession numbers LC705430 [18S], LC705432 [COI]). Six males (ICHUM8307, BL 3.04, CW0.56; ICHUM8308, BL 2.85, CW0.56, INSD accession number LC705433 [COI]; ICHUM8309, BL 2.89, CW 0.52; ICHUM8310, BL 3.36, CW 0.61; ICHUM8311, BL 2.42, CW 0.46; ICHUM8313, BL 2.46, CW 0.53). Collection data for ICHUM8303-8311 same as for holotype. ICHUM8312 and 8313 hatched in an aquarium in Kakui laboratory, Sapporo, descendants of individuals collected from type locality on 22 May 2014.

*Other material*: One female, ICHUM8314 (BL 2.79, CW 0.51; INSD accession number LC664100 [COI]), Aya River, Sakaide, Kagawa, Japan (approximate coordinates



Figure 2. *Sinelobus kisui* sp. nov., paratype female (a. ICHUM8312) and male (b. ICHUM8313), dorsal views, fresh specimens. Scale bars: 0.5 mm.

34°19.693'N, 133°52.499'E), 2 June 2008, coll. by Yoshihiro Hayashi; one male, ICHUM4031 (INSD accession numbers AB618192 [18S], LC664101 [COI]), same collection data as for ICHUM8314; one male, OMNH-Ar12459 (BL 2.97, CW 0.67), Station 4, Shin Yodo River, Osaka, Osaka, Japan

(approximate coordinates: 34°41.447'N, 135°26.613'E), under rocks, 19 February 1989, coll. by Michio Ohtani.

**Description of females (based on holotype unless noted otherwise).** Body (Figs 2a, 3a, b) 5.96 times as long as wide, with brown pigmentation (retained in eth-



Figure 3. *Sinelobus kisui* sp. nov. **a**, **b**. Body, female holotype, dorsal (**a**) and left (**b**) views; **c**, **d**. Body, male allotype, dorsal (**c**) and left (**d**) views. Scale bars: 1 mm.

anol). Cephalothorax 0.19 times BL, tapering anteriorly, with mid-lateral pair of simple setae and pair of simple setae posterior to eyes. Dorsal pigmentation pattern on carapace: anterior region dark; subanterior region without dark pigmentation; posterolateral region with two dark diagonal bands; reticulate pattern of pigmentation elsewhere (often faint). Pereonites 1–6 with length ratio 1.00:1.71:2.07:2.50:2.58:2.20; all with one or two pairs of simple setae. Pereonites 1–6 width-to-length ratio 0.30, 0.51, 0.63, 0.82, 0.86, and 0.80, respectively. Pleo-

nites 1–3 with pair of dorsal simple seta; pleonites 1 and 2 with dorso-transverse plumose setal row (but incomplete, absent in dorsal region); pleonites 2 and 3 with lateral plumose setal row. Pleonite 4 with several pairs of simple setae. Pleotelson with seven pairs of simple setae.

Antennule (Fig. 4a) 0.65 times as long as cephalothorax; articles 1–4 with length ratio 1.00:0.37:0.33:0.03. Article 1 with two outer distal and one inner distal simple setae, and several proximal and distal PSS. Article 2 with three outer distal and two inner distal simple setae, and



Figure 4. *Sinelobus kisui* sp. nov. **a**, **c**, **e**–**k**, **m**, **n**. Female holotype; **b**, **d**. Male allotype; **l**. Female paratype (ICHUM8304); **a**, **b**. Right (**a**) and left (**b**) antennules, dorsal views; **c**, **d**. Left antenna, dorsal (**c**) and outer (**d**) views; **e**. Labrum, lateral view; **f**, **g**. Left mandible, posterior (**f**) and inner (**g**) views; **h**. Right mandible, inner view; **i**. Right molar; **j**. Labium; **k**. Right maxillule, palp broken; **l**. Left maxilla; **m**. Maxillipeds, dorsal view (right palp omitted; outer portion of right endite bent inward); **n**. Left epignath. Scale bars: 0.1 mm.

several distal PSS. Article 3 with two distal simple setae and several distal PSS. Article 4 with tuft of distal simple setae, distal PSS, and two aesthetascs.

Antenna (Fig. 4c) 0.84 times as long as antennule; articles 1–6 with length ratio 1.00:3.00:1.08:3.29:2.37:0.34. Articles 1 and 3 naked. Article 2 with one outer distal, one ventrodistal, and one inner distal simple setae. Article 4 with distal simple seta and distal PSS. Article 5 with three distal simple setae and three distal PSS. Article 6 with tuft of distal simple setae. Labrum (Fig. 4e) setulate distally. Mandibles (Fig. 4f–i) with well-developed molar process bearing many small teeth on masticatory surface; left mandible (Fig. 4f, g) with weakly denticulate incisor, wide denticulate lacinia mobilis, and bifurcate serrate accessory seta; right mandible (Fig. 4h, i) with seven-toothed incisor, peg-like lacinia mobilis, and bifurcate serrate accessory seta. Labium (Fig. 4j) with distally setulate inner lobe; outer lobes smaller. Maxillule (Fig. 4k) endite bearing eight distal spiniform setae and outer subdistal setation; palp broken. Maxilla (ICHUM8304; Fig. 41) with small serrations on outer distal margins.

Maxilliped (Fig. 4m) with coxa bearing simple seta (not illustrated). Basis with ventrodistal simple seta. Endite with outer distal tufts of fine setae, one mid-inner and two dorsodistal spiniform setae; and two ventro-subdistal circumplumose setae, inner one longer than outer; distal region setulate. Palp article 1 with outer distal serrations; article 2 with one outer distal and four inner simple setae, and inner distal serrate spiniform seta; article 3 with four inner distal simple setae and six inner plumose setae; article 4 with one mid-outer and two outer-subdistal simple setae, two inner plumose setae, and five distal serrate setae. Epignath (Fig. 4n) with kidney-shaped lobe, margins finely setulate; terminal seta setulate.

Cheliped (Fig. 5a, b) with triangular articulation to cephalothorax via sclerite (Fig. 3b). Basis slightly longer than wide, with one outer dorsal and one ventrodistal simple setae. Merus with one outer dorsal and one ventral simple setae. Carpus 1.46 times as long as wide, with one dorsal, two dorsodistal and two ventral simple setae. Propodus 0.77 times as long as carpus; palm with two outer and one inner simple setae at insertion of dactylus; fixed finger with one ventral, four outer subdistal, and two inner subdistal simple setae, and dorso-subdistal broad lamellar expansion and triangular claw. Dactylus as long as fixed finger, with inner simple seta, row of ventral spiniform setae, and bifurcate serrate seta; unguis triangular, curved ventrally.

Pereopod 1 (Fig. 5e, f) length 0.25 times BL, with length ratio of basis, merus, carpus, propodus, and dactylus-unguis 1.00:0.28:0.36:0.51:0.40. Coxa with dorsal simple seta. Basis cylindrical, narrow, 4.61 times as long as wide, with dorso-subproximal simple seta and dorso-subproximal PSS. Merus with one ventrodistal simple seta. Carpus with two dorsal and two ventrodistal simple setae. Propodus with one dorsodistal and two ventrodistal simple setae, middle PSS, and middle setulate seta. Dactylus naked; unguis shorter than dactylus, naked.

Pereopod 2 (Fig. 5g–i) with length ratio of articles from basis to propodus 1.00:0.53:0.34:0.46. Coxa with dorsal simple seta (not illustrated). Basis cylindrical, narrow, 3.17 times as long as wide, with ventrodistal simple seta and dorso-subproximal PSS. Merus with ventrodistal simple seta and ventrodistal crotchet (Fig. 5h). Carpus with dorsodistal simple seta and six distal crotchets (dorsodistal one shorter than half propodus length). Propodus with two dorsodistal and one ventrodistal simple setae and mid-dorsal PSS. Dactylus (ICHUM8303; Fig. 5i) naked; unguis shorter than dactylus, naked.

Pereopod 3 (ICHUM8303; Fig. 5j) with length ratio of articles from basis to dactylus-unguis 1.00:0.39:0.34:0.36:0.37; similar to pereopod 2, except basis with ventral PSS, carpus with five distal crotchets, and propodus with dorsodistal simple seta.

Pereopod4(ICHUM8303;Fig.5k) with length ratio of articles from basis to dactylus-unguis 1.00:0.29:0.32:0.43:0.32. Coxa with simple seta. Basis 3.21 times as long as wide, with ventrodistal simple seta, and two dorso-subproximal and two ventro-subdistal PSS. Merus with outer distal simple seta and two ventrodistal crotchets. Carpus with inner dorsodistal simple seta and five distal crotchets. Propodus with outer distal simple seta, dorso-subdistal PSS, and one outer dorsodistal and one inner dorsodistal setulate setae. Dactylus-unguis fused to form claw, strongly arcuate, with inner and outer rows of ventral spines.

Pereopod 5 (Fig. 51) with length ratio of articles from basis to dactylus-unguis 1.00:0.43:0.30:0.51:0.35; similar to pereopod 4.

Pereopod 6 (Fig. 5m, n) with length ratio of articles from basis to dactylus-unguis 1.00:0.40:0.33:0.58:0.39; similar to pereopod 4 except basis without ventro-subdistal PSS and propodus with five inner distal flattened denticulate setae and inner distal biserrate seta.

Pleopod 1 (Fig. 6a) with protopod bearing six outer plumose setae; endopod with one inner and 14 outer plumose setae, and outer distal step-tipped plumose seta; exopod 1.42 times as long as endopod, with 29 outer plumose setae. Pleopod 2 (Fig. 6b) with protopod bearing six outer plumose setae; one inner plumose seta present on right protopod but absent on left; endopod with one inner and 13 outer plumose setae, and outer distal step-tipped plumose seta; exopod 1.37 times as long as endopod, with 30 outer plumose setae. Pleopod 3 (Fig. 6c) smaller than pleopods 1 and 2, with protopod bearing three outer plumose setae; endopod with one inner and 11 outer plumose setae, and outer distal step-tipped plumose seta; exopod 1.36 times as long as endopod, with 22 outer plumose setae.

Uropod (ICHUM8303; Fig. 6g) with four articles (protopod and triarticulate ramus). Protopod with four distal simple setae. Ramus article 1 naked; article 2 longest, with one middle and two distal simple setae and two distal PSS; article 3 with one middle and four distal simple setae.

**Description of males (based on allotype).** Body (Figs 2b, 3c, d) similar to female except for cephalothorax shape: cephalothorax strongly narrowed anteriorly.

Antennule (Fig. 4b) 0.93 times as long as cephalothorax; articles 1–4 with length ratio of 1.00:0.33:0.27:0.02. Article 1 with two outer distal and two inner distal simple setae, and several proximal and distal PSS. Article 2 with three outer distal and two inner distal simple setae, and several distal PSS. Article 3 with two distal simple setae and several distal PSS. Article 4 with tuft of distal simple setae, distal PSS, and three aesthetascs.

Antenna (Fig. 4d) 0.84 times as long as antennule; articles 1–6 with length ratio 1.00:2.47:1.59:3.43:1.93:0.19. Articles 1 and 3 naked. Article 2 with one outer distal, one ventrodistal, and one inner distal simple setae. Article 4 with distal simple seta and distal PSS. Article 5 with three distal simple setae and three distal PSS. Article 6 with tuft of distal simple setae and several distal PSS.

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

Cheliped (Fig. 5c, d) with triangular articulation to cephalothorax via sclerite (Fig. 3d). Basis as long as wide, with one outer dorsal and one ventrodistal simple setae. Merus with one outer dorsal and one ventral simple



**Figure 5.** *Sinelobus kisui* sp. nov. **a, b, e–g, l–n.** Female holotype; **c, d.** Male allotype; **h–k.** Female paratype (ICHUM8303); **a, c.** Left (**a**) and right (**c**) chelipeds, outer views; **b.** Dactylus–unguis of cheliped and bifurcate serrate seta, inner view; **d.** Merus and carpus of cheliped, inner views; **e, g, j–m.** Left (**e, g, k–m**) and right (**j**) percopods 1–6, inner (**e, k**) and outer (**g, j, l, m**) views; **f, i, n.** Distal part of right (**f**) and left (**i, n**) percopods 1, 2, and 6, outer (**f, i**) and inner (**n**) views; **h.** Crotchet. White and black arrowheads, ventral processes on merus and carpus, respectively; gray arrowhead, inner broad thickening on merus; gray and black arrows, ventroproximal and mid-ventral processes on propodus, respectively. Scale bars: 0.1 mm (**a–g, i–m**); 0.05 mm (**h, n**).

setae, inner broad thickening (Fig. 5d, gray arrowhead), and three ventral processes (Fig. 5c, d, white arrowheads). Carpus 1.22 times as long as wide, with one dorsal, two dorsodistal, and two ventral simple setae, and three ventral processes (Fig. 5c, d, black arrowheads). Chela 1.15 times as long and 1.24 times as wide as carpus; ventral margin with middle simple seta and one proximal and one middle projection (Fig. 5c, gray and black arrows, respectively). Palm with two outer and one inner simple setae at insertion of dactylus. Fixed finger with four outer subdistal and two inner subdistal simple setae, dorso-subdistal triangular lamellar expansion, and triangular claw; base of fixed finger distant (greater than dactylus width) from base of dactylus; angle of dorsal margin of fixed finger to distal margin of palm about 90°. Dactylus strongly curved ventrally, with inner simple seta, row of ventral spiniform setae, and bifurcate serrate seta; unguis triangular.

Percopods 1–6 more slender than but similar to those of females, with differences in setal number (see Suppl. material 1: Table S1 for details).

Pleopods (Fig. 6d–f) similar to those of female, with differences in setal number (see Suppl. material 1: Table S1 for details).

Uropod similar to that of female.

**Variation.** In addition to the holotype and allotype, two female (ICHUM8303, ICHUM8304) and one male (ICHUM8307) paratypes of *Sinelobus kisui* sp. nov. were dissected, and selected characters were observed for the

antennule, antenna, maxilliped, cheliped, pereopods 1-6, and pleopods 1-3. The raw morphological data we obtained are in Suppl. material 1: Table S1. All specimens shared the same state for the following selected characters: (1) antennule with two (females) or three (males) aesthetascs; (2) antennal article 2 with one outer distal, one ventrodistal, and one inner distal simple setae; (3) maxillipedal endite with one mid-inner and two distal spiniform setae, and two ventro-subdistal circumplumose setae, inner one longer than outer; (4) percopod 1 with carpus bearing two dorsal and two ventral simple setae and propodus bearing one middle setulate seta; (5) dorsodistal crotchet on carpi of percopods 2 and 3 shorter than half propodus length; (6) percopod 3 basis with one ventral PSS; (7) pleopod 1 protopod lacking inner plumose setae. Two strongly sexually dimorphic males (allotype and paratype

The following setae varied in number among specimens (selected characters only are presented; ranges are in parentheses): ventrodistal simple setae on basis of pereopods 4–6 (1–2); inner distal flattened denticulate setae on pereopod 6 propodus (4–5); outer plumose setae on protopods of pleopods 1 and 2 (4–6) and on protopod of pleopod 3 (3–4); outer plumose setae on endopods of pleopods 1 and 2 (8–14) and on endopod of pleopod 3 (8–11); outer plumose setae on exopods of pleopods 1 and 2 (21–30) and on exopod of pleopod 3 (20–22). In the holotype individual, the right pleopod-2 protopod bears

ICHUM8307) shared all states observed in the chelipeds.



**Figure 6.** *Sinelobus kisui* sp. nov. **a–c.** Female holotype; **d–f.** Male allotype; **g.** Female paratype (ICHUM8303); **a–f.** Right (**a–c**) and left (**d–f**) pleopods 1 (**a**, **d**), 2 (**b**, **e**), and 3 (**e**, **f**) (all setal ornamentation omitted); **g.** Right uropod. Scale bars: 0.1 mm.

one inner plumose seta (Fig. 6b) but the left one lacks it. This seta was not found on the corresponding article in the other four individuals, suggesting that its presence in the one case observed may be abnormal. We observed six distal crotchets on carpi of left pereopods 2 and 3 in the holotype individual, but all other pereopods we observed bore five, indicating the former state may be abnormal.

Morphology of individuals from Kagawa and Osaka. One female from Kagawa (ICHUM8314) and one strongly sexually dimorphic male from Osaka (OMNH-Ar12459) were dissected and observed. In the former individual, all diagnostic character states were observed, except for those found only in strongly sexually dimorphic males. The latter individual was whitish, having lost its pigmentation (but see Ariyama and Ohtani 1990), and showed all diagnostic character states.

Genetic information. One partial 18S (INSD accession number LC705430; 1920 bp long) and three COI (LC705431–LC705433; 815 bp long, encoding 271 amino acids) sequences were determined from Yamaguchi individuals. The 18S sequence was identical to that from a *Sinelobus* sp. 1 sensu Kakui et al. (2011) individual from Kagawa (AB618192, Kakui et al. 2011) but was 4.6% divergent in K2P distance from an 18S sequence from *Sinelobus* sp. 2 sensu Kakui et al. (2011) (AB618193, Kakui et al. 2011). For COI, K2P distances among three individuals from Yamaguchi were 0.0–0.4%; those between three Yamaguchi individuals and two Kagawa individuals (LC664100, LC664101; 658 bp long, encoding 219 amino acids) were 0.3–1.5%; and that between two Kagawa sequences was 1.5%.

#### Discussion

*Sinelobus kisui* sp. nov. is similar to *S. barretti* and *S. vanhaareni* in having antennal article 2 with one outer distal seta, the carpi of pereopods 2 and 3 with a dorsodistal crotchet shorter than half the propodus length, and the carpi of pereopods 2–6 with five distal crotchets (Table 1).

Sinelobus kisui differs from S. barretti in having (1) the inner of the two ventro-subdistal circumplumose setae on the maxillipedal endite longer than the outer; (2) the pereopod-1 carpus with two dorsal and two ventral simple setae; (3) the pereopod-1 propodus with a middle Hirano, K. & Kakui, K.: A new Sinelobus species from Japan

setulate seta; and (4) the pleopod-1 protopod lacking inner plumose setae.

Sinelobus kisui differs from S. vanhaareni in having (1) the maxillipedal endite with one mid-inner seta; (2) the pereopod-1 carpus with two dorsal and two ventral simple setae; (3) the pereopod-3 basis with a ventral PSS; and (4) the pleopod-1 protopod lacking inner plumose setae. In addition, their strongly sexually dimorphic (SSD) males differ in the number of ventral processes on the chelipedal merus (three in S. kisui; two in S. vanhaareni). The two species share a state that is uncommon in Sinelobus, which is that the pleopod-3 protopod lacks inner setae, although S. vanhaareni bears a single inner plumose seta on the protopods of pleopods 1 and 2 (van Haaren and Soors 2009, Bamber 2014).

Our study confirms that character states of the chelipeds of SSD males (e.g., the number of ventral processes on the merus and carpus; the angle between the dorsal margin of the fixed finger and the distal margin of the palm; Table 1) are useful in *Sinelobus* taxonomy. Here we regard males having chelipeds in which the base of the fixed finger is distant (greater than dactylus width) from the base of the dactylus as SSD males. By this definition, *S. barretti* and *S. pinkenba* lack information on SSD males. Differences among *Sinelobus* species are not large, and we recommend that the chelipeds of SSD males be observed in future studies dealing with *Sinelobus* taxonomy.

In addition to individuals from the type locality (Yamaguchi), we observed Sinelobus individuals collected from Kagawa and Osaka and conclude that they are conspecific with Yamaguchi individuals, i.e., they are S. kisui. The K2P distances between the Yamaguchi and Kagawa COI sequences (0.3-1.5%) were within the range previously attributed to intraspecific variation in confamilial tanaidacean species: up to 1.1% in Hexapleomera ulsana Wi, Jeong & Kang, 2018 (Wi et al. 2018); up to 1.5% in Zeuxo ezoensis Okamoto, Oya & Kakui, 2020 (Okamoto et al. 2020). The fact that the 18S sequences were identical between the two populations supports the conclusion of conspecificity from our morphological observations. The species identity of Sinelobus tanaidaceans reported from other Japanese localities (cf. Fig. 1b) must be carefully investigated. Kakui et al. (2011) detected at least one other species molecularly, Sinelobus sp. 2, from Kochi, which shares a coastline with Kagawa (Fig. 1b).

#### Key to the seven species currently placed in Sinelobus

1	Pleopod 1 protopod with inner plumose seta	2
_	Pleopod 1 protopod without inner plumose seta	sp. nov.
2	Carpus of pereopods 2-6 with four distal crotchets	r, 2014
_	Carpus of pereopods 2–6 with five distal crotchets	3
3	Dorsodistal crotchet on carpi of pereopods 2 and 3 longer than about half propodus length	
	S. stromatoliticus Rishworth, Perissinotto & Błażewicz	z, 2018
_	Dorsodistal crotchet on carpi of pereopods 2 and 3 shorter than half propodus length	4
4	Antennal article 2 with three or more ventrodistal simple setae	5
_	Antennal article 2 with one ventrodistal simple seta	6

- 6 Maxillipedal endite with mid-inner spiniform seta; pereopod 1 propodus without middle seta.....S. barretti Edgar, 2008
- Maxillipedal endite without mid-inner spiniform seta; pereopod 1 propodus with middle seta..... S. vanhaareni Bamber, 2014

**Table 1.** Comparison of selected characters for seven *Sinelobus* species. L, length; Mxp, maxilliped; nd, no data; Pr, pereopod; PSS, plumose sensory seta; SSD, strongly sexually dimorphic.

	S. kisui	S. barretti	S. bathykolpos	S. pink-	S. stanfordi	S. stromatolit-	S. van-
	sp. nov.			enba		icus	haareni
Antennal article 2: number of outer distal setae	1	1	1	3	4	1	1
Mxp endite: mid-inner spiniform seta	Present	Present	Absent	Absent	Absent	Absent	Absent
Mxp endite: L of two circumplumose setae	lnner > outer	Outer > inner	Inner > outer	Inner > outer	Inner > outer	Inner > outer	Inner > outer
Chelipedal merus (SSD male): number of ventral processes	3	nd	1	nd	0	2	2
Chela (SSD male): angle of dorsal margin of fixed finger to distal margin of palm	≈ 90°	nd	≈ 50°	nd	≈ 90°	$\approx 70^{\circ}$	≈ 90°
Pr1 carpus: number of simple setae (dorsal, ventral)	2, 2	1,1	1,1	1,1	1,1	1, 1	2, 1
Pr1 propodus: middle seta	Present	Absent	Present	Present	Absent	Present	Present
$\ensuremath{Pr2-3}$ carpi: relative L of dorso distal crotchet to propodus L	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	≈ 0.5	< 0.5
Pr3 basis: ventral PSS	Present	Present	Absent	Absent	Absent	Absent	Absent
Pr2–6 carpi: number of distal crotchets	5 (rarely 6)	5	4	5	5	5	5
Pleopod 1 protopod: number of inner plumose seta	0	1	1	1	1	1	1
Pleopod 3 protopod: number of inner plumose seta	0	1	1	1?	1	1	0
Source	This study	Edgar (2008)	Bamber (2014)	Bamber (2008)	Sieg (1980)	Rishworth et al. (2018)	Bamber (2014)

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#### Supplementary material 1

#### Table S1

Authors: Kyoko Hirano, Keiichi Kakui

Data type: Xlsx file.

- Explanation note: Variation in characters among dissected specimens of *Sinelobus kisui* sp. nov.
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Artikel/Article: <u>A new brackish tanaidacean, Sinelobus kisui sp. nov.</u> (Crustacea, Peracarida, Tanaidacea), from Japan, with a key to Sinelobus species and barcode information from two loci 245-256