1	(verso) H. Ueda and H. Nagai
2	(recto) Three new species of Halicyclops
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4	RESEARCH ARTICLE
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6	Three new species of the brackish-water copepod Halicyclops
7	(Crustacea, Cyclopoida) from Ariake Bay, Japan
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14	(Received)
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1 Abstract 2 Three new species of the cyclopoid copepod Halicyclops are described from river estuaries of Ariake 3 Bay, Japan. H. continentalis sp. nov. is most similar to H. laminifer, but differs by the shape of a 4 angular protuberance on the genital double-somite and a serrate hyaline frill of the fourth urosomite. H. 5 sinensis described by Tai and Chen is widely distributed in China and is identifiable to H. continentalis, 6 indicating the population in Ariake Bay is likely a continental relict. H. uncus sp. nov., belonging to the 7 thermophilus group, is distinguishable by the shape of the lateral process on the genital double-somite, a 8 frill of the fourth urosomite, and caudal rami; it is probably endemic to Japan. H. ariakensis sp. nov. 9 differs from the other congeners by a combination of the shape of the prosome, urosomal hyaline frills, 10 and caudal ramus length; it is considered endemic to Ariake Bay. 11 12 **Keywords:** Copepoda, *Halicyclops*, brackish-water, continental relict, Ariake Bay 13 14 *Corresponding author. Email: hueda@kochi-u.ac.jp 15

Introduction

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The cyclopoid copepod genus *Halicyclops* is predominatly found in brackish waters but 2 members have also been found in a wide range of habitats from coastal marine to ground 3 4 waters (Pesce et al. 1996). Globally, Halicyclops presently consists of 80 species and 5 subspecies, 72 of which were listed by Pesce et al. (1996) with the remaining having been 6 subsequently described (Rocha et al. 1998; De Laurentiis 1999, 2001; Baribwegure and 7 Dumont 2000; Karanovic 2004, 2006; Menu-Marque and Sorarrain 2007). Many of the 8 species have been recorded from very restricted or narrow geographical ranges, probably 9 due in-part from limitations of brackish-water species to disperse across marine and 10 freshwater environments. The following five species of *Halicyclops* have been recorded 11 from the Japanese Archipelago: H. fosteri Wilson, 1958, H. higoensis Ito, 1957, H. 12 japonicus Ito, 1956, H. ryukyuensis Ito, 1962 and H. sinensis Kiefer, 1928 (Ito 1957, 1962; 13 Ishida 2002). Among them, H. higoensis and H. japonicus are endemic to the Japanese 14 Archipelago, whereas *H. ryukyuensis* and *H. sinensis* have also been described from 15 continental waters of China (Tai and Chen 1979). 16 This paper describes three new *Halicyclops* species from river estuaries of Ariake 17 Bay, Japan, based on female specimens. Special attention was given to ornamentation of 18 middle caudal setae, which have been used in the taxonomic distinction of Halicyclops 19 species (Rocha, 1984). Zoogeographies of the new species described here are also briefly 20 discussed since among Japanese estuarine waters the Ariake Bay estuary has been known

- 1 to have specific habitats associated with continental relicts of various brackish-water
- 2 species, including planktonic copepods (Hiromi and Ueda 1987; Ohtsuka et al. 1995; Ueda
- and Bucklin 2006).

5

Materials and methods

6 Halicyclops specimens were sorted from the following plankton samples: Honmyo-gawa 7 River mouth in a detention pond of Isahaya Bay, a branch of Ariake Bay, collected in 8 August 1997; Rokkaku-gawa River mouth collected on 22 August 2002; and eleven other 9 river estuaries collected on 14-16 March 2002 (Figure 1). Samples were taken by surface 10 tow using a 0.1-mm mesh plankton net from the shore or from a bridge and immediately 11 fixed in 5% formalin. Salinity at each sampling site was measured using a water quality 12 meter (Alec Electronics, ADR-1000) in the Honmyo-gawa River mouth and a portable 13 electric salinometer (Merbabu Trading, Sinar NS-3P) at the other locations. Halicyclops 14 was found in eight of the 12 river estuaries, shown as closed circles in Figure 1. 15 Microscopic examination and dissection of specimens were made in lactophenol. Length 16 measurements and drawings were made with the aid of an optic micrometer and a camera lucida attached to a differential interference microscope. Ornamentation of the middle 17 caudal setae was illustrated with computer software (Adobe Illustrator®) using 18 19 microscopic photographs as a background picture. The type specimens were deposited in 20 the National Science Museum in Tokyo. The terminology of oral appendages follows Huys

- and Boxshall (1991). Abbreviations used are P1-P5 for first to fifth legs, Exp1-Exp3 for
- 2 first to third exopod segments, Enp1-Enp3 for first to third endopod segments, and L/W for
- 3 length/width ratio.

- 5 Systematic account
- 6 Order Cyclopoida Burmeister, 1834
- 7 Family Cyclopidae Dana, 1846
- 8 Subfamily Halicyclopinae Kiefer, 1927
- 9 Genus Halicyclops A. M. Norman, 1903
- 10 Halicyclops continentalis sp. nov.
- 11 (Figures 2-3)

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13 Synonym. Halicyclops sinensis, Tai and Chen (1979): 313-315, figs. 176, 177.

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- 15 Type material. One female holotype (NSMT Cr-18237) dissected and mounted on two
- glass slides, and eight undissected female paratypes (NSMT Cr-18238) preserved in
- alcohol; collected from the Honmyo-gawa River mouth on 28 August 1997.

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- 9 Type locality. Honmyo-gawa River mouth (32°_53' 02" N, 130° 08' 10" E) in a detention
- 20 pond of Isahaya Bay, Kyushu, Japan.

- 1 Etymology. The specific epithet refers to the primary distribution of the species along the
- 2 coast of the East Asian continent.
- 3 Description
- 4 Female. Body (Figure 2A) length 0.55-0.59 mm (n = 5, holotype 0.59 mm). Prosome L/W
- 5 1.3-1.4, widest at end of cephalosome, and 1.8-1.9 times longer than urosome.
- 6 Cephalosome somewhat triangular in dorsal view, with round integumental window on
- 7 dorsal surface. Genital double-somite (Figure 2B) L/W 0.7-0.9, laterally expanding into
- 8 angular protuberance. Genital double-somite and subsequent two urosomites with coarsely
- 9 serrate hyaline frills; teeth of middorsal frill of fourth urosomite (Figure 2B, C) larger than
- those of lateral and ventral frills. Anal somite (Figure 2B, C) with row of spinules along
- distal margin except on dorsal and medial sides.
- 12 Caudal rami (Figure 2B) L/W 1.1-1.2, distally with rows of spinules near base of
- dorsal terminal seta and on lateral margin; proximal dorsolateral, lateralmost terminal, and
- medialmost terminal setae about as long as ramus; dorsodistal seta about 2.0 times longer
- than ramus; lateral median seta 0.9 time as long as urosome; median terminal seta 2.0
- times longer than urosome. Middle terminal setae (Figure 2D) almost homogenously
- setulose, but setules on medial seta distally longer and hair-like.
- Antennule (Figure 2E) 6-segmented, reaching midlength of cephalosome, with setal
- formula (ae indicates aesthetasc): I = 9, II = 12, III = 5 + spine, IV = 6 + ae, V = 2, VI = 10
- 20 + ae; first segment with 2 rows of spinules ventrally; spinules of distal row longer than

- 1 those of proximal one; fourth segment longest and L/W 1.3.
- 2 Antenna (Figure 2F) 3-segmented, consisting of coxobasis and 2-segmented endopod,
- 3 with setal formula 3, 1, 12; coxobasis proximally with tuft of long hairs on anterior surface
- 4 and 2 rows of short hairs medially; Enp2 slightly longer than Enp1, with proximal row of
- 5 spinules and 2 distal spinules on anterior surface and intermittent row of spinules along
- 6 lateral margin.
- 7 Mandible (Figure 2G) with 2 unequal setae on reduced palp, and 2 rows of spinules
- 8 near palp and base of segment, respectively.
- 9 Maxillule (Figure 3A) consisting of praecoxal arthrite and 1-segmented palp;
- praecoxal arthrite medially with strong spine at midlength, short seta distal to spine, and 5
- spinules around spine, and with 4 strong spines apically; palp with 1 proximal and 6 apical
- setae.
- Maxilla (Figure 3B) incompletely 4-segmented; praecoxa and coxa partly fused on
- posterior surface; praecoxa with 2 setae on endite; coxa with 2 setae on medial margin and
- on distal endite, respectively, strong spine fused to endite, and setule on spine; basis
- medially expanding into strong naked claw, with one-sidedly teethed strong spine and
- medial seta; endopod with seta, 2 one-sidedly teethed claw-like spines, and few spinules at
- 18 socket of spine.
- Maxilliped (Figure 3C) 2-segmented; syncoxa with 3 setae, 2 of them inserted at
- about midlength of medial margin and 1 at distal one-fourth on anterior surface, and row of

- 1 fine spinules on distolateral corner; endopod L/W 1.3, with 5 setae.
- 2 P1-P4 (Figure 3D-G) with 3-segmented rami. Spine (Roman numeral) and seta
- 3 (Arabic numeral) formula as follows (lateral/medial or lateral/apical/medial):

	Coxa	Basis	Exopod			Endopod		
			1	2	3	1	2	3
P1	0/1	1/I	I/1;	I/1;	III+1/1/3	0/1;	0/1;	II/1/3
P2	0/1	1/0	I/1;	I/1;	IV/1/4	0/1;	0/2;	II/I/3
P3	0/1	1/0	I/1;	I/1;	III/1/4	0/1;	0/2;	II/I/3
P4	0/1	1/0	I/1;	I/1;	III/1/4	0/1;	0/2;	I/II/2

- 4 P1 (Figure 3D) intercoxal sclerite with hairs along edge of round protuberance on each
- 5 side; coxa with row of long spinules along distolateral margin on anterior side; basis with
- 6 medial spine reaching beyond tip of Enp3 and long spinules near base of endopod on
- 7 anterior surface; spine on Exp2 naked; proximal 2 spines of Exp3 naked, 1.2-1.3 times
- 8 longer than segment and about 2 times longer than distal spine. P2-P4 intercoxal sclerite
- 9 without hairs; bases with row of long spinules near base of endopod on anterior surface;
- proximalmost medial seta of Enp3 spiniform on distal one-third. P4 Enp3 (Figure 3G) L/W
- 1.2; medial apical spine 2.0 times longer than segment and lateral apical spine; distormedial
- seta spiniform as in medial apical spine but with few short setules proximally.
- P5 exopod (Figure 3H) L/W about 1.1, with 2 lateral and 1 medial spines, apical seta,
- oblique rows of long spinules on lateral and medial sides, and smaller spinules around
- sockets of spines; length ratio of three spines (from lateral to medial) to segment
- 16 1.1:1.0:1.2; seta about 1.5 times longer than segment.

- 1 Remarks
- 2 Halicyclops continentalis sp. nov. is identical to H. sinensis described by Tai and Chen
- 3 (1979) in all diagnostic features they described. The species is distinguishable from most
- 4 other congeners by having angular protuberances on the genital double-somite, the 3.4.3.3
- 5 spine formula on the P1-P4 Exp3, and characteristic spines on the P1 Exp3, of which the
- 6 proximal two are much longer than the distal one. The hitherto known species and
- 7 subspecies with the same formula are *H. brevispinosus meridionalis* Herbst, 1953, *H. b.*
- 8 psammophilus Plesa, 1959, H. laminifer Herbst, 1982, H. exiguus Kiefer, 1934 described
- 9 by Defaye and Dussart (1988), H. tetracanthus Rocha, 1995, and H. eberhardi De
- Laurentiis et al., 2001. Among them, H. laminifer is the most similar to H. continentalis in
- having similar spines on the P1 Exp3, of which the proximal two are about twice as long as
- the distal one (Herbst 1982). However, H. laminifer differs from H. continentalis by a
- squarely produced hyaline frill along posterodorsal margin of the fourth urosomite and a
- round lateral protuberance of the genital double-somite. The other species with the 3.4.3.3
- spine formula are also distinguishable by longer female antennules, of which the L/W of
- the fourth segment is at least 2.0 (De Laurentiis et al., 2001; Defaye and Dussart 1988;
- Herbst 1953; Pleşa 1959; Rocha, 1995), in contrast to 1.3-1.6 in *H. continentalis*, and by
- the absence of a triangular protuberance on the lateral surface of the genital double-somite.
- 19 Halicyclops sinensis was described by Kiefer (1928) as a freshwater form from China
- which was previously described but not definitively identified by Burckhardt (1913) as "H.

- 1 aequoreus (Fischer, 1860) or a closely related new species." According to Burckhardt's
- description and illustration, the spine formula of the P1-P4 of the specimen is 3.4.4.3 and
- 3 the medial spine on the P1 basis reaches only to the midlength of the Enp3, indicating that
- 4 *H. sinensis* described by Tai and Chen (1979) from Chinese brackish waters differs from
- 5 Kiefer's (1928) original description.
- 6 Ishida (1993, 2002) also described *H. sinensis* from Japan. He noted that there were
- 7 no morphological differences among his specimens from Hokkaido and Malaysia, and Tai
- 8 and Chen's (1979) description provided no information on the spine number of P3 Exp3.
- 9 We reexamined Ishida's (1993) H. sinensis female specimens from Hokkaido, which were
- deposited in the National Science Museum, Tokyo (NSMT Cr-11269, 11270). Results
- showed Ishida's (1993) specimen had a 3.4.4.3 spine formula and that posterior margins of
- the urosomites lacked coarsely serrate frills, which are present in Burckhardt's specimen.
- This indicated that *H. sinensis* described by Ishida (1993, 2002) are neither Kiefer's (1928)
- original nor the present species.
- The detention pond of Isahaya Bay, in which the type locality is located, was closed
- off by a 7-km long dike in April 1997. Ueda et al. (2003) made plankton surveys in the
- pond seven times from May 1997 to July 2000 to know changes in plankton in relation to
- desalination. Mean surface water salinity in the pond steeply declined from 27 psu in May
- 19 1997 to 3.8 psu on 28 August 1997, and was almost constant around 1 psu after April 1999.
- 20 Halicyclops continentalis was first observed on 28 August 1997, when it was found

- densely aggregated at a single site with a salinity of 1.05 psu. At this time, copepod
- 2 densities of *H. continentalis* were at their highest level (6.9 individuals L⁻¹), and they
- 3 continued to occur at lower densities until the end of the survey (Ueda, unpublished data).
- 4 This species was also collected from the Kashima-gawa, Rokkaku-gawa, Kase-gawa,
- 5 Okinohata-gawa, and Yabe-gawa Rivers in the innermost area of the bay. The salinity
- 6 range of these waters was 1-10 psu. There were somewhat wide variations in body length
- between the localities: 0.63 mm from the Rokkaku-gawa River, 0.79 mm from the
- 8 Kashima-gawa River, and 0.96 mm from the Yabe-gawa River. The head of the specimen
- 9 from the Yabe-gawa River was rounded rather triangular.
- The Chinese population of the present species, described as *H. sinensis*, is
- distributed widely from Guanzhou to Tianjin (Tai and Chen 1979), whereas it is restricted
- 12 to Ariake Bay in Japan. This geographical pattern indicates that the Ariake Bay population
- is likely a continental relict of the East Asian continent as previously discussed for the
- brackish-water copepods, *Sinocalanus sinensis* (Poppe, 1889) (Hiromi and Ueda 1987),
- 15 Tortanus derjugini Smirnov, 1935 (Ohtsuka et al. 1995), and Acartia ohtsukai Ueda and
- Bucklin, 2006 (Ueda and Bucklin 2006) in this bay.
- 18 Halicyclops uncus sp. nov.
- 19 (Figures 4-5)

20 Synonym. Halicyclops japonicus, Ishida (2002): 41-42, figs. 1a-e.

- 2 Type material. One female holotype (NSMT Cr-18239), dissected and mounted on three
- 3 glass slides, collected from Kashima-gawa River estuary on 14 March 2002, and 8
- 4 undissected female paratypes (NSMT Cr-18240) preserved in alcohol, collected from
- 5 Midori-kawa River estuary on 15 March 2002.

- 7 | Type locality. Kashima-gawa River estuary (33° 06' 37"_N, 130° 04' 13"_E) in Ariake Bay,
- 8 Kyushu, Japan. The salinity was 2 psu.

9

- 10 Etymology. The specific epithet uncus, meaning "hook" in Latin, refers to the shape of
- acute protuberance on the genital double-somite.

- 13 Description
- 14 Female. Body (Figure 4A) length 0.66-0.80 mm (holotype 0.69 mm). Prosome L/W
- 1.7-1.9 and 1.5-1.9 times longer than urosome. Forehead round in dorsal view. Genital
- double-somite (Figure 4B) L/W 0.8-1.0, with backwardly directed hook-shaped acute
- process on each side in dorsal view; tip of process not chitinized. Genital double-somite
- and subsequent two urosomites (Figure 4B, C) with serrate distal frill; mid-dorsal part of
- 19 frill of fourth urosomite (Figure 4D) extending beyond anal operculum.
- Caudal rami (Figure 4D) L/W 1.5-1.7; proximal dorsolateral seta slightly shorter than

- 1 ramus; medialmost terminal seta 0.4 times as long as ramus; medial median terminal seta
- 2 1.4 times longer than urosome and about 2.0 times longer than lateral median terminal seta.
- 3 Middle terminal setae heterogeneously ornamented as follows: proximal half of lateral seta
- 4 sparsely spinulose on its distal half of lateral margin; distal half of lateral seta spinulose on
- 5 lateral margin and plumose on medial margin; proximal half of medial seta with 2-3
- 6 spinules on distal part of medial margin; distal half of medial seta spinulose proximally and
- 7 plumose distally.
- 8 Antennule (Figure 4F) with setal formula: I = 8, II = 12, III = 6 + spine, IV = 6 + ae,
- 9 V = 2, VI = 9 + ae; first segment with row of spinules ventrally; fourth segment L/W about
- 10 2.0.
- Antenna (Figure 4G) coxobasis 2 rows of spinules on medial and anterior surfaces,
- respectively; Enp2 L/W about 3.0, 1.6 times longer than Enp1, with several rows of short
- spinules scattered except on medial side.
- Mandible (Figure 4H) with 2 rows of spinules near palp and row of larger spinules
- 15 proximally.
- Maxillule (Figure 5A) praecoxal arthrite medially with 2 strong spines, 2 thick setae
- and 3 spinules around midlength, and with 4 strong spines apically; palp (Figure 5B) with
- 18 1 proximal and 3 apical setae on segment 1 (proximal and 1 apical setae missing in Figure
- 19 5B), and 3 setae on segment 2.
- 20 Maxilla (Figure 5C) basis and endopod with spine armed with long teeth on both

- 1 sides; claw of basis naked.
- 2 Maxilliped (Figure 5D) with distolateral row of long spinules on syncoxa; endopod
- 3 L/W 1.1.
- 4 P1-P4 (Figure 5E-H) spine and seta formula of Exp3 as follows: III+1/1/3, III/I+1/4,
- 5 III/I+1/4, II/I+1/4; spinules on lateral margins of coxa, distal margins of basis and lateral
- 6 margins of rami stronger than those of *H. continentalis*. P1 basis with medial spine
- 7 reaching midlength of Enp2; spines on Exp1 and Exp2 naked; spines on Exp3 subequal
- 8 and slightly shorter than segment. Proximalmost medial setae of P2-P3 Enp3 spiniform but
- 9 more slender than apical spine at distal half. P4 Enp3 (Figure 5H) L/W 1.4; medial apical
- spine 1.2 times longer than segment and 1.5 times longer than lateral apical spine; medial
- setae spiniform at distal half.
- P5 exopod (Figure 5I) L/W 1.3; length ratio of three spines to segment 0.7: seta about
- as long as segment; lateral and medial spinules thicker than those of *H. continentalis*.
- Other diagnostic features as in *H. continentalis*.
- 15
- 16 Remarks
- 17 Halicyclops uncus sp. nov. belongs to the thermophilus species group, which is
- characterized by a well-developed process on each side of the genital double-somite in
- dorsal view and a 3.4.4.3 spine formula of the P1-4 Exp3 (Herbst 1983). Other members
- are H. thermophilus Kiefer, 1929, H. spinifer Kiefer, 1935, H. venezuelaensis Lindberg,

- 1 1954, H. japonicus Ito, 1956, H. latus Shen and Tai, 1964, H. antiguanensis Herbst, 1983,
- 2 H. dedeckeri Brownell, 1983 and H. soqotranus Baribwegure and Dumont, 2000.
- 3 Karanovic (2008) considered H. spinifer, H. japonicus, H. latus, H. dedeckeri and H.
- 4 antiguanensis as junior synonyms of *H. thermophilus*. The reasoning behind this was noted
- 5 in his remarks and can be summarized as follows: according to the revision of the genus by
- 6 Kiefer (1936), who created *H. thermophilus*, the nominotypical species of the group, and
- 7 the second member *H. spinifer*, both provided in brief descriptions (Kiefer 1929, 1935), the
- 8 main differences between the two species seemed to be only their relative lengths of the
- 9 lateral protuberance on the genital double-somite and of the medial spine on the female P5,
- which were longer in *H. spinifer*. However, specimens subsequently described as *H*.
- 11 thermophilus or H. spinifer by others such as Lindberg (1941) had different combinations
- of these characters or different shapes (spiniform or plumose) of setae on the P4 Enp3. The
- subsequent species of the group were generally created only by a difference in a single
- character, that is, the shape of the setae on the P4 Enp3 in *H. japonicus* and *H. dedeckeri*,
- and the length of the medial spine on the P5 in *H. latus*. However, the shape of the setae on
- the P4 Enp3 "could not be accepted as only distinguishing character in the genus
- 17 Halicyclops, as this was also shown to be variable in H. venezuelaensis by D. Rocha
- 18 (1995)."
- We do not agree with Karanovic (2008) based on three principal conclusions. First,
- 20 Karanovic (2008) disregarded other important diagnostic characters of the genus, such as

- 1 the serrate hyaline frill of the fourth urosomite, the L/W of the caudal ramus, and that of
- 2 the P4 Enp3. The caudal ramus of *H. japnicus* described by Ito (1956) is about twice as
- 3 long as wide while that of *H. thermophilus* is almost as long as wide in Kiefer's (1929,
- 4 1936) figure. The posterior margin of the fourth urosomite of *H. latus* is smooth, which
- 5 was mentioned by Shen and Tai (1964) as a character distinguishing it from *H. japonica*.
- 6 Disregard of the urosomal frill, which is serrate but not so developed at the middorsal part
- 7 of the fourth urosomite in *H. thermophilus* (Kiefer 1936), is also seen in Karanovic's
- 8 (2008) synonym list, in which *H. spinifer* described by Pesce et al. (1996) bearing the
- 9 well-developed middorsal frill is synonymized. Second, it is unlikely for a difference in the
- shape of setae on the P4 Enp3 as a result of intraspecific variation. Karanovic (2008) cited
- 11 H. venezuelaensis described by Rocha (1995), of which the setae are different from those
- in the original description of the species (Lindberg 1954), but failed to cite that Rocha had
- suggested the possibility of interspecific variation. Although there is currently no way to
- determine whether the characteristics in question are intra- or interspecific in origin, it is
- reasonable to regard it as interspecific by considering the following: 1) as far as we are
- aware the degree of variation of these setae within a population has never been recorded,
- not only in *Halicyclops*, but also other cyclopoid genera; 2) specialized setae like spiniform
- ones on the swimming legs are hypothesized as apomorphies by Abiahy et al. (2006) and
- are thus employed as an important diagnosis of a species; 3) the extremely wide
- 20 geographic range of a brackish-water *Halicyclops* species is unlikely due to the extreme

- barriers of marine, freshwater and land associated with brackish water habitats. If
- 2 Karanovic's (2008) synonymization of *H. thermophilus* is correct, it would be almost a
- 3 cosmopolitan species, recorded from Java (type locality), Japan (Ito 1956, as *H. japonicus*),
- 4 China (Shen and Tai 1964, as *H. latus*), Australia (Pesce et al. 1996, as *H. spinifer*),
- 5 Somalia (Dumont and Maas 1987), Uzbekistan (Mirabdullayaev and Getz 1996, as H.
- 6 spinifer), Madagascar (Lindberg 1952), South Africa (Brownell 1983, as H. dedeckeri),
- 7 North America (Wilson 1958), West Indies (Herbst 1983, as *H. antiguaensis*), South
- 8 America (Reid 1985), and so on. Karanovic noted passive dispersal in ship ballast water as
- 9 a partial cause for its current, very wide distribution. However, such a mode of introduction
- is very unlikely for the Japanese population (*H. japonicus*), of which the first record (the
- type locality of *H. japonicus*) occurred from a brackish pond on a small island of 1 km²,
- about 50 km north of the Noto Peninsula, the middle mainland of Japan. In conclusion, it
- seems more likely that populations hitherto reported as *H. thermophilus* consists of rather
- 14 distinct species.
- The most distinguishing characteristic of the new species is a hook-shaped acute
- process on each side of the genital double-somite, by which the following species are
- distinguished: *H. soqotranus* (Baribwegure and Dumont 2000) and *H. venezuelaensis*
- 18 (Lindberg 1954), which have blunt and short processes, and *H. antiguanensis* (Herbst
- 19 1983), H. japonicus (Ito 1956) and H. latus (Shen and Tai, 1964), of which the processes
- are short and/or produced laterally rather than posteriorly and thereby not hook-shaped.

- 1 The new species is also distinctive by the following: caudal rami of the new species [L/W
- 2 1.5] is longer than *H. antiguanensis* [1.25 (Herbst 1983)], *H. dedeckeri* [0.9 measured in
- 3 Brownell's (1983) figure], *H. soqotranus* [1.0 measured in Baribwegure and Dumont's
- 4 (2000) figure], and *H. venezuelaensis* [1.17 (Lindeberg 1954)]; posterior margins of the
- 5 urosomites of *H. latus* are smooth (Shen and Tai 1964); P2-P4 Enp3 of *H. japonicus* from
- 6 Hegurajima Island in the Japan Sea (Ito 1956) is obviously longer [e.g., P4 Enp3 with L/W
- 7 1.9 in contrast of 1.4 in the new species] and has a normal plumose proximomedial seta
- 8 [spiniform in the new species].

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Of the eight other members of the *thermophilus* group, the remaining two, *H. thermophilus* and *H. spinifer*, are difficult to compare because Kiefer's (1929, 1935) original descriptions were too brief and insufficient. However, subsequent descriptions from various collections around the world were not used for comparison due to the possibility of confusing with other species, because significant differences are apparent among these descriptions. For example, the L/W of the caudal ramus of *H. spinifer* is 1.22-1.27 by Kiefer (1936, as *H. thermophilus spinifer*) from India, whereas it is about 1.0 in Pesce et al. (1996) from Australia, and the lateral spines on the P5 of *H. thermophilus* are much shorter in descriptions from Madagascar (Lindberg 1952) and Somalia (Dumont and Maas 1987) compared to that from Java (Kiefer 1929). Therefore comparison of the new species should be made primarily with Kiefer's (1929, 1935) original descriptions and his subsequent redescription of his own specimen (Kiefer 1936) even though the available

- 1 information is limited. Their significant difference from the new species is seen again in
- 2 the short caudal ramus, of which the L/Ws are about 1.2 for *H. thermophilus* (measured in
- 3 Kiefer's (1929) figure) and 1.22-1.27 for *H. spinifer* (Kiefer 1936). The two species are
- 4 also distinguishable by the lateral process of the genital double-somite in dorsal view; the
- 5 processes of *H. thermophilus* are very short and produced laterally (Kiefer 1936) and those
- 6 of *H. spinifer* are strongly chitinized at the tip (Kiefer 1935).
- 7 The *Halicyclops* specimen described as *H. japonicus* by Ishida (2002) from a
- 8 brackish lake of northernmost Honshu is identifiable to the new species by hook-shaped
- 9 lateral processes on the genital double-somite, caudal rami with the L/W 1.5, serrate
- urosomal frills of which the middorsal part of the fourth urosomite partly covers the anal
- somite. However, it is uncertain whether Ishida's (2002) *H. japonicus* specimen from
- 12 Yakushima Island, south of Kyushu (Ishida 1993) is identical to the new species or Ito's
- 13 (1956) *H. japonicus*, because Ishida's illustration shows that the process on the genital
- double-somite is short and produced laterally as in Ito's.
- The new species was collected from estuaries of the Kashimagawa (type locality) and
- Midori-kawa Rivers, where the salinity ranged from 4-11 psu. This species is probably
- 17 endemic to the Japanese Archipelago since there have been no descriptions identical to the
- new species from East Asia (Tai and Chen 1979).

20 Halicyclops ariakensis sp. nov.

1 (Figures 6-7) 2 3 Type material. One female holotype (NSMT Cr-18241) dissected and mounted on three 4 glass slides, and 6 undissected female paratypes (NSMT Cr-18242) preserved in alcohol; 5 collected from the Kashima-gawa River estuary on 14 March 2002. 6 Type locality. Kashima-gawa River estuary (33° 06' 37"_N, 130° 04' 13" E) in Ariake Bay, 7 8 Kyushu, Japan. The salinity was 2 psu. 9 Etymology. The specific epithet refers to the locality, to which the species is considered 10 11 endemic. 12 13 Description 14 Female. Body (Figure 6A) length 0.50-0.66 mm (holotype 0.60 mm). Prosome L/W 15 1.4-1.5, widest at distal one-third of cephalosome, and 1.5-1.9 times longer than urosome. 16 Forehead round in dorsal view. Genital double-somite (Figure 6B) L/W 0.9-1.0, without 17 lateral protuberance. Genital double-somite and subsequent two urosomites (Figure 6B, C) 18 with finely serrate distal frill; middorsal part of frill of fourth urosomite more coarse and 19 extending nearly to end of anal somite on medium line.

Caudal rami (Figure 6D) L/W 1.4-1.6; proximal dorsolateral seta as long as ramus;

- 1 medialmost terminal seta 0.4 times as long as ramus; dorsodistal seta about 2.5 times
- 2 longer than ramus; medial median terminal seta 1.6 times longer than urosome and about
- 3 2.3 times longer than lateral median terminal seta. Ornamentation of middle terminal setae
- 4 (Figure 6E) similar to *H. uncus*, but with denser ornamentation of certain features,
- 5 particularly more numerous spinules on proximal half of setae.
- Antennule (Figure 6F) with setal formula: I = 8, II = 13, III = 5 + spine, IV = 5 + ae,
- V = 2, VI = 10 + ae; first segment with row of spinules ventrally; fourth segment L/W 2.6.
- 8 Antenna (Figure 6G) coxobasis 2 rows of fine and large spinules on medial and
- 9 anterior surfaces, respectively; Enp2 L/W about 2.8, 1.6 times longer than Enp1.
- Mandible (Figure 6H) and maxillule (Figure 6I, J) as in *H. uncus* sp. nov.
- 11 Maxilla (Figure 6K) basis with claw and spine armed with long teeth on both sides;
- spines on endopod with similar spines.
- Maxilliped (Figure 7A) endopod L/W 1.8; distolateral 2 setae more slender than
- 14 others.
- 15 P1-P4 (Figure 7B-E) with spine and seta formula as follows: III+1/1/3, III/I+1/4,
- 16 III/I+1/4, II/I+1/4. P1 basis with medial spine reaching base of Enp3; exopod with no
- 17 naked spines; proximal spine on Exp3 slightly shorter than distal two and segment;
- Proximalmost medial setae of P2-P3 Enp3 spiniform at distal two-thirds. P4 Enp3 (Figure
- 7E) L/W 1.5; medial apical spine 1.5 times longer than segment and 1.7 times longer than
- 20 lateral apical spine; medial setae spiniform at distal three-fourths.

- P5 exopod (Figure 7F) L/W 1.5; length ratio of three spines (from lateral to medial)
- 2 to segment 1.1:0.9:1.0; seta 1.4 times longer than segment.
- 3 Other diagnostic features as in *H. continentalis*.

20

- 5 Remarks
- 6 Halicyclops ariakensis sp. nov. is similar to the following six species in having the 3.4.4.3 7 spine formula on the P1-P4 Exp3, serrate urosomal hyaline frills, of which middorsal part 8 of the fourth urosomite extends beyond the anal operculum, and no conspicuous lateral 9 protuberances on the genital double-somite: H. denticulatus Kiefer, 1960, H. gauldi Pleşa, 10 1961, H. reunionensis Bozic, 1964, H. clarkei Herbst, 1982, H. laminifer Herbst, 1982, H. 11 bowmani Rocha and Iliffe, 1993 and H. lindbergi Rocha, 1995. These species are 12 distinguishable from the new species first by the shape of the prosome, which is the widest 13 at about the end of the cephalosome as in most other congeners (Bozic 1964; Herbst 1982; 14 Kiefer 1960; Rocha 1995; Rocha and Iliffe 1993) whereas the new species is the widest at 15 the distal one-third of the cephalosome. Other features distinct from the new species are as 16 follows: the genital double-somite is longer in H. bowmani [L/W 1.2 (Rocha and Iliffe 17 1993)]; the middorsal hyaline frill of the fourth urosomite is shorter in *H. clarkei* [not 18 extending to end of the anal somite on the medium line (Herbst 1982)] and rectangular in 19 H. laminifer (Herbst 1982); the caudal ramus is shorter in H. laminifer [L/W 1.1 (Herbst

1982)] and longer in H. denticulatus [at least 2.0 (Kiefer 1960)] and H. reunionensis [2.0

- 1 (Bozic 1964)]; the spiniform distormedial seta of the P4 Enp3 is curved and has strong teeth
- 2 on only the medial margin in *H. lindbergi* (Rocha 1995); lateral two spines of P5 are much
- 3 shorter than the segment in H. gauldi and H. reunionensis according to Pleşa's (1961) and
- 4 Bozic's (1964) figures, respectively.
- 5 Among hitherto known species from Japan, *H. fosteri* illustrated by Ishida (2002)
- 6 from Miyazaki, Kyushu, is most similar to the new species in having the serrate hyaline
- 7 frills on posterior margins of the second to fourth urosomites, no lateral protuberance on
- 8 the genital double-somite, and the similar L/W of the caudal ramus. Ishida's (2002)
- 9 specimen, however, is neither identical to *H. fosteri* nor the new species. Wilson's (1958)
- 10 *H. fosteri* s. str. is characterized by the P4 having two spines and a slender seta between the
- spines on the Exp3 and with no medial setae on the Enp3 in females. *Halicyclops fosteri*
- described by Ishida (2002) has two medial setae on the P4 Enp3 as in most species of the
- genus. As for the Exp3, Ishida (2002) did not provide information of the specimen and
- noted that significant variations of spines and setae on the P4 Exp3 were observed in the
- population of the type locality. However, Ishida's (2002) statement is not a correct citation
- of Wilson's description, in which there was no description of variation in the P1-4 from the
- type locality (Louisiana), with the exception of differences in the spine numbers on the
- 18 P1-3 (not P4) Exp3 between two female specimens collected from Texas and between
- 19 Louisiana and Texas. Ishida (2002) synonymized *Halicyclops* sp. with the 3.4.4.3 spine
- formula on the P1-P4 Exp3 collected from Hokkaido (Ishida 1984) as *H. fosteri*. This

- 1 specimen again is neither *H. fosteri* s. str. nor the present new species because of its
- 2 obvious lateral protuberance on the genital double-somite. The differences between *H*.
- 3 fosteri described by Ishida (2002) and the new species are seen in the following
- 4 morphologies of the former, which are important in classification of *Halicyclops* species:
- 5 the middorsal hyaline frill on the fourth urosomite is not conspicuously extended, which he
- 6 pointed out as a distinctive feature of the species by an arrow in a figure, and the spines on
- 7 the P5 are much shorter than the segment.
- 8 The new species was collected from the Kashima-gawa, Kase-gawa, and Shiota-gawa
- 9 Rivers located in the innermost part of Ariake Bay. The salinity range in which the species
- occurred was between 2-10 psu. The three new species of the present study co-occurred in
- the 2-psu water of the Kashima-gawa River. It is considered *H. ariakensis* sp. nov. is
- 12 endemic to Ariake Bay, because there have been no records identifiable with this species in
- both Japan and the continental waters.

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- 1 Figure legends
- 2
- Figure 1. Map showing sampling points of river estuaries on the coast of Ariake Bay: (1)
- 4 Honmyo-gawa, (2) Kashima-gawa, (3) Shiota-gawa, (4) Rokkaku-gawa, (5)
- 5 Kase-gawa, (6) Chikugo-gawa, (7) Okinohata-gawa, (8) Yabe-gawa. (9)
- 6 Ohmuta-gawa, (10) Kikuchi-gawa, (11) Shira-kawa, (12) Midori-kawa, and (13)
- 7 Ohno-gawa Rivers. Closed and open circles indicate the sampling points where
- 8 *Halicyclops* was found and not collected, respectively.
- 9 Figure 2. Halicyclops continentalis new species, female. A. habitus, dorsal; B. urosome
- with P5 and caudal rami (lengths of dorsal setae are not properly shown due to
- oblique angles), dorsal; C. urosome, ventral; D. middle caudal setae; E. antennule,
- ventral; F. antenna, posterior; G. mandible, anterior. A-C, E-G, holotype; D, non-type
- from Kashima-gawa River.
- 14 Figure 3. *Halicyclops continentalis* new species, female (holotype). A. maxillule, posterior;
- B. maxilla, posterior; C. maxilliped, anterior; D. P1, anterior; E. P2, anterior; F. P3,
- anterior; G. P4, anterior; H. exopod of P5, anterior.
- 17 Figure 4. *Halicyclops uncus* new species, female. A. habitus, dorsal; B. urosome with P5,
- dorsal; C. urosome, ventral; D. fourth urosomite, anal somite and left caudal ramus,
- dorsal; E. middle caudal setae; F. antennule, ventral; G. antenna, posterior; H.

- 1 mandible, anterior. A, non-type from Midori-kawa River; B-D, F-H, holotype; E,
- 2 paratype.
- 3 Figure 5. Halicyclops uncus new species, female (holotype). A. maxillule, posterior; B.
- 4 palp of maxillule; C. maxilla, anterior; D. maxilliped, anterior; E. P1, anterior; F. P2,
- 5 anterior; G. P3, anterior; H. P4, anterior; I. exopod of P5, posterior.
- 6 Figure 6. Halicyclops ariakensis new species, female. A. habitus, dorsal; B. urosome,
- dorsal; C. urosome with right caudal ramus, ventral; D. right caudal ramus, dorsal; E.
- 8 middle caudal setae; F. antennule, ventral; G. antenna, anterior; H. mandible,
- 9 anterior; I. maxillule, medial; J. palp of maxillule; K. maxilla, anterior. A, non-type
- from Kashima-gawa River; B-D, F-K, holotype; E, non-type from Shiota-gawa
- 11 River.
- 12 Figure 7. Halicyclops ariakensis new species, female. A. maxilliped, posterior; B. P1,
- anterior; C. P2, anterior; D. P3, anterior; E. P4, anterior; F. exopod of P5, anterior. A,
- 14 C, F, holotype; B, D, E, non-type (single specimen) from Koshima-gawa River.