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Two species of the genus Anchistrocheles (Bairdioidea: Ostracoda: Crustacea) from Japan and their developmental characteristics for adaptation to interstitial environments

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Abstract

A new species and an already known species of the genus Anchistrocheles Brady & Norman, 1889 from the Pacific coast of Japan, are described in detail using scanning electron microscopy of the carapaces and soft-part anatomy of the appendages. The new species, Anchistrocheles hayatotanakai sp. nov., is only the second to be described in the genus Anchistrocheles from Japan, and this study is also the first to describe the appendages of A. yamaguchii Yajima, 1987, previously only known as a fossil from Pleistocene strata of the Atsumi Formation in central Japan. The two species are considered to be closely related because they share many common characters in their appendages, but they are distinguishable from each other based on the male and female copulatory organs, furcal chaetotaxy, brush-shaped organs, number of lateral pore systems, and outlines of the carapaces in dorsal view. Ontogenetic changes of carapace sizes were compared between three interstitial species (Anchistrocheles hayatotanakai sp. nov., A. yamaguchii, and Neonesidea sp. I) and an epifaunal species (N, sp. S). The reduction in carapace width was found to be the primary reason for the reduction in the cross-sectional area and is probably related to the adaptation to interstitial environments. The brushshaped organs located between the 6th pair of limbs are also shown. This study is the first to report the anatomically precise position of these organs.

Key words: soft-part anatomy, new species, ontogeny, three-dimensional carapace size, Arthropoda, brush-shaped organ

Introduction

The genus Anchistrocheles (type species: A. fumata Brady, 1890), was established by Brady & Norman (1889), and 15 nominal species (including fossil species but not their synonyms) have been recognised until now (Brandão et al. 2022). The species of this genus are distributed throughout the globe in shallow marine environments of low to medium latitude zones. The oldest fossil record of this genus is from the Miocene, found in Australia (Warne 1990). Anchistrocheles species have been known since the end of the 19th century, but there were no reports of their habitat as interstitial environments before Maddocks (1976). There are 12 extant species in this genus (Brandão et al. 2022), of which only five have the male copulatory organs described in detail, which is an important character for taxonomy (A. fumata by Brady 1890; A. bensoni Maddocks, 1969, A. hartmani Maddocks, 1976, and A. mcquadei Maddocks, 1976 by Maddocks 1976; A. darwini Maddocks & Ilife, 1991 by Maddocks & Ilife 1991). There are a large number of reports on only the carapace morphology or non-nominal species of Anchistrocheles sp., indicating the difficulty in collecting individuals with soft parts that could clarify their taxonomy. Ontogenetic changes in ostracods provide important information on phylogeny and ecology (Kamiya 1988a; Tsukagoshi 1990; Smith & Kamiya 2002; Okada et al. 2008). However, information on the ontogeny of interstitial ostracods is limited because of their small and fragile bodies. The carapace size of most interstitial ostracods is approximately 0.3 mm in the adult stage, however, the carapace size of most Anchistrocheles species is approximately 0.8 mm at the adult stage. To the best of our knowledge, this is the first study on the ontogenetic changes in carapace sizes of this genus. In addition, the characteristics of the ontogeny of interstitial species can be clarified in comparison with those of epiphytic species.

Material and methods



FIGURE 1. Map showing the sampling locations of *Anchistrocheles* species. Motojima (A, Loc. 1), Miho-Uchihama (B, Loc. 2), and Arai-Hama (C, Loc. 3).

Specimens of *Anchistrocheles* species were collected from coarse sand to pebble beach sediments (sediment depth approximately 5–20 cm) around the strand line during the lowest spring tide at Motojima, Tanabe City, Wakayama Prefecture (33° 43' 54" N, 135° 20' 57" E; Loc. 1 in Fig. 1A) on 19 September 2017; Miho-Uchihama, Shizuoka City, Shizuoka Prefecture (35° 00' 54" N, 138° 31' 51" E; Loc. 2 in Fig. 1B) on 14 February, 5 October, and 6 December 2017; and Arai-Hama, Miura City, Kanagawa Prefecture (35° 09' 36" N, 139° 36' 42" E; Loc. 3 in Fig. 1C) on 27 March 2017. The sediments were washed five times in a bucket of freshwater, and then strained through 16-mesh (# 1 mm) and 250-mesh (# 0.063 mm) sieves. Interstitial *Neonesidea* sp. I was collected from coarse sand to pebble beach sediments (sediment depth of approximately 10–20 cm) around the strand line during the lowest low tide in the spring tide in Miho-Masaki Beach, Shizuoka City, Shizuoka Prefecture (35° 01' 13" N, 138° 31' 11" E) on 5 October 2017. Epiphytic *Neonesidea* sp. S were collected from algae on the breakwater wall by hand in Miho-Uchihana (35° 00' 54" N, 138° 31' 51" E) on 5 October 2017. All collected specimens were fixed in 5–10% formaldehyde solution neutralised with hexamethylenetetramine in the field, and then transferred to 70–80% alcohol

solution in the laboratory. The soft parts were dissected and mounted in Neo-Shigaral medium on glass slides and were observed under a transmitted light microscope (Olympus BX-50) with a camera lucida, and sketched. The position of brush-shaped organs was observed using a scanning electron microscope (JEOL JSM-5600LV) after freeze drying (Sun Technologies Aqua FD-6500) and coating with osmium ion (Filgen OPC40). The carapaces were air-dried, coated in gold with a quick auto coater (JEOL JFC-1500, ion sputtering device), and then observed and photographed under a scanning electron microscope (JEOL JSM-5600LV). The distribution of pore systems, muscle scars, and the marginal infoldment (calcified inner lamella) in some carapaces were observed under a transmitted light microscope (Olympus BX-50) with a camera lucida, and sketched. The sketches were digitized using an LCD tablet (Wacom DTK-1301/K0) and paint software (FireAlpaca). For measurements, an optical stereo-microscope with an ocular micrometre or a digital micrometre (Peacock Digital Gauge D-10S) was used.

All illustrated specimens have been deposited in the collection of the Shizuoka University Museum with the prefix SUM-CO.

Taxonomy

Superfamily Bairdioidea Sars, 1888

Family Bythocyprididae Maddocks, 1969

Genus Anchistrocheles Brady & Norman, 1889

Anchistrocheles hayatotanakai sp. nov. (Figs. 2, 3A, B, 4, 5A, B)

Type series. All examined specimens collected at intertidal coarse sand beach in Miho-Uchihana, Shizuoka City, Shizuoka Prefecture (35°00′54″N, 138°31′51″E; Fig. 1A) on 5 October 2017. Holotype: adult male (SUM-CO-2481), carapace length 0.80 mm, height 0.39 mm, appendages mounted on glass slide and valves preserved in cardboard cell slide. Paratypes: 6 adult males (SUM CO-2482–2484, 2486, 2487 and 2490) and 3 adult females (SUM-CO-2485, 2488 and 2489).

Etymology. Named after Dr Hayato Tanaka (Kasai Aquarium, Tokyo, Japan), in recognition of his significant contribution to our knowledge on interstitial ostracod species.

Description. *Carapace* (Figs. 2 and 3A, B) streamlined sub-reniform in lateral view, surface extremely smooth, thin, and translucent. Outline convex in dorsal view. Marginal infold, with wide vestibule and about 40 short radial (marginal) pore canals, well developed along antero-ventral and postero-ventral margins. Left valve slightly larger than right valve and overlapping on both anterior and posterior ends of hingement. Surface covered with around 100 simple type normal (lateral) pore systems. Central muscle scars consisting of 4 adductor scars, 2 small elongated mandibular scars, 1 small elongated frontal scar, 1 limb scar in both valves. Hingement very simple: right valve bearing small blunt brim-like teeth at anterior and posterior ends; left valve bearing anterior and posterior elongated sockets with dorsal weak shelf.

Eye. Absent.

Antennule (Fig. 4A). Seven articulated podomeres and coxa, length ratio among them from proximal to distal 31: 24: 6: 7: 4: 2: 1. First podomere with numerous setulae along anterior and anterior distal margins. Second podomere with 1 long stout seta at posterior distal corner. Third podomere with 1 medium length seta at postero-distal corner and row of setulae on anterior distal margin. Fourth podomere with 1 medium length apical seta at antero-distal corner. Fifth podomere with 1 long and 1 very long setae on both antero- and postero-distal ends, respectively. Sixth podomere with 2 very long setae on antero- and postero-distal ends, respectively. Seventh (terminal) podomere with 5 very long distal setae longer than twice of total length of all seven podomeres.

Antenna (Fig. 4B). Coxa and five articulated podomeres, length ratio among them from proximal to distal 9: 16: 9: 7: 13: 4. Coxa with 1 long and 1 medium length setae at postero-distal corner. First podomere (basis) bearing reduced exopodite consisting of 1 long setulous, 1 medium length, and 1 very short setae on antero-distal end, and 1 long setulous seta on postero-distal end. Second podomere with 2 medium length setae at middle of posterior



FIGURE 2. Carapace morphology of *Anchistrocheles hayatotanakai* **sp. nov.** A and B, male right and left valves, respectively (SUM-CO-2486, paratype). C and D, internal view of male left and right valves, respectively (SUM-CO-2487, paratype). E and F, external view of female right and left valves, respectively (SUM-CO-2488, paratype). G and H, internal view of female left and right valves, respectively (SUM-CO-2489, paratype). I, dorsal view of male carapace (SUM-CO-2490, paratype). J, central muscle scars on male right valve (SUM-CO-2487, paratype). Scale 1: 200 µm for A–I. Scale 2: 20 µm for J.

margin and 1 long seta on postero-distal end. Third podomere with 1 very short seta on posterior proximal margin, and 1 medium length and very1 short setae at posterior distal corner. Fourth podomere with 2 short setae on middle of anterior margin, 1 short seta on postero-proximal margin and 1 medium length stout setulous seta at posterior distal corner. Fifth (terminal) podomere with 1 long claw-like seta and 2 medium length setae on ledge on middle of posterior margin, and 1 very long stout terminal claw and 1 short seta on anterior distal end.



FIGURE 3. Internal lateral view of the male carapace in two *Anchistrocheles* species. A and B, left and right valves of *Anchistrocheles hayatotanakai* **sp. nov.** (male paratype, SUM CO-2482), respectively. C and D, left and right valves of *Anchistrocheles yamaguchii* Yajima, 1987 (male, SUM-CO-2492), respectively. Scale: 100 µm.

Mandibula (Fig. 4C). Consisting of 5 podomeres, length ratio among them from proximal to distal 47: 12: 4: 6: 5. First podomere (coxa) with masticatory part of several denticles and several very short thin setae on distal end. One short simple seta on anterior distal margin and numerous minute process on anterior ventral area. First podomere of palp (basis) with 1 setulous seta on anterior margin at one-third from distal end, 1 seta on distal end, and branchial plate (reduced exopodite) consisting of 1 very long and 2 medium-long plumose setae on dorsal margin. Second podomere of palp (1st podomere of endopodite) with 1 setulous and 1 simple setae on distal end. Third podomere of palp with 1 long simple and 2 medium length setulous setae around middle of dorsal margin, and 1 short setulous and 1 medium length simple setae on anterior distal end. Fourth (terminal) podomere with 2 short setae on middle of dorsal margin, 2 brush-like terminal claws and 1 short seta on distal end.

Maxillula (Fig. 4D). Thin branchial plate (exopodite) bearing 25 plumose setae and 7 long reflexed setae. Basal podomere bearing 1 palp (endopodite) and 3 masticatory endites. Palp with 2 short setae on ventral margin at three-fourths from proximal end, 5 medium length setae and 1 spatulate seta bearing fine setulae along distal end. First (dorsal most) endite with 2 short setae, 1 claw-like and 1 spatulate setae on distal end. Second endite with 3 short setae, 1 claw-like and 1 spatulate seta at middle of ventral margin, short claw-like and 7 short simple setae on distal end.

Fifth limb in male (Fig. 4E1). Consisting of 4 articulated podomeres, length ratio among them from proximal to distal 8: 8: 5: 1. First podomere with branchial plate bearing 9 plumose setae and 5 reflexed setae on ventro-distal margin, 1 very short and 1 long setae on anterior margin, and 2 long setae on antero-distal corner. Second podomere with 1 long and 1 medium length setae at middle of anterior margin. Third podomere with 1 very short setulous seta on anterior distal end. Fourth podomere with 1 very short thin seta and 1 long stout terminal claw on distal end.

Fifth limb in female (Fig. 4E2). Consisting of 5 articulated podomeres, length ratio among them from proximal to distal 40: 23: 7: 23: 4. First podomere with branchial plate bearing 9 plumose setae and 5 reflexed setae on ventro-distal margin, 1 very short and 1 long setae on anterior margin, 2 long setae on anterior-distal corner. Second



FIGURE 4. Appendages of *Anchistrocheles hayatotanakai* **sp. nov.** A, antennule; B, antenna; C, mandibula; D, maxillula; E, 5th limb (E1, male; E2, female); F, 6th limb; G, 7th limb. H, brush-shaped organ. A, B, D, E1, F, and G, male holotype, SUM-CO-2481; C, male paratype, SUM-CO-2483; E2, female paratype, SUM-CO-2485; H, male paratype, SUM-CO-2484. Scale: 100 μm.

podomere with 2 unequal-length long setae on antero-distal corner. Third podomere without setae. Fourth podomere with 1 short setulous seta on antero-distal corner. Fifth podomere with 1 very short thin seta and 1 long stout terminal claw on distal end.

Sixth limb (Fig. 4F). Consisting of 5 articulated podomeres, length ratio among them from proximal to distal 19: 14: 6: 12: 2. First podomere with 1 very short and 1 medium length setae on anterior margin, 1 short and 1 medium length setae on distal margin. Second podomere with 1 long setulous seta at anterior-distal corner. Third podomere with 0 very thin and short seta and 1 long curved terminal claw on distal end.

Seventh limb (Fig. 4G). Consisting of 5 articulated podomeres, length ratio among them from proximal to distal 20: 15: 6: 11: 2. First podomere with 1 very short and 1 long setae on anterior margin, 1 short and 1 medium setae on distal margin. Second podomere with 1 long setae at anterior-distal corner. Third podomere without seta. Fourth podomere with 1 short setulous seta at anterior-distal corner. Fifth podomere with 1 very thin short seta and 1 long curved terminal claw on distal end.

Brush-shaped organ (in male) (Fig. 4H). Consisting of paired symmetrical lobes, each bearing about 10 to 12 long thin setae.

Male copulatory organ (Fig. 5A). Basal capsule semicircular with beak-like shape in distal part. Two clasping apparatus: proximal one (*CA1*) with branched tip; distal one (*CA2*) curved. Copulatory duct arched.

Female copulatory organ (Fig. 5B). Outline blunt sub-triangular. Inside tube coiled 2 times and straight at proximal parts.

Furca (Fig. 5A, B). Bearing 1 very short seta at middle of ventral margin and 2 setulous setae at distal part, distal most one slightly longer than second most one.



FIGURE 5. Copulatory organs, furcae, and caudal process of *Anchistrocheles hayatotanakai* **sp. nov.** (A, male holotype, SUM-CO-2481; B, female paratype, SUM-CO-2485) and *Anchistrocheles yamaguchii* Yajima, 1987 (C, male SUM-CO-2491; D, female SUM-CO-2494). *CA1*, clasping apparatus 1; *CA2*, clasping apparatus 2; *CD*, copulatory duct; *CP*, caudal process; *DP*, distal part of capsule. Scale: 100 μm.

Caudal process (Fig. 5A, B). One simple seta. **Dimensions.** See Table 1A.

Occurrence. Miho-Uchihana, Shizuoka City, Shizuoka Prefecture (type locality, Loc. 2 in Fig. 1B) and Motojima, Tanabe City, Wakayama Pref., Japan (Loc. 1 in Fig. 1A).

Remarks. Anchistrocheles hayatotanakai **sp. nov.** and A. yamaguchii Yajima, 1987 are similar except for the soft-parts characters: the male and female copulatory organs, furcae, and brush-shaped organs. In the new species the proximal clasping apparatus is branched at the tip, but that of A. yamaguchii has a sharp tip. The inner tube of the female copulatory organ is coiled two times in A. hayatotanakai **sp. nov.**, but four times in A. yamaguchii. The middle seta on the furca is as long as the width of the furca in A. hayatotanakai **sp. nov.**, but is shorter than that in A. yamaguchii. Furthermore, the distal most seta of the furca is slightly longer than the second distal most seta in A. hayatotanakai **sp. nov.**, but both setae are of similar lengths in A. yamaguchii.

Yajima (1987) reported two *Anchistrocheles* species, *A. yamaguchii* and *A. hondai*, as new species from the Pleistocene Atsumi Formation in central Japan. However, the latter species could belong to the genus *Orlovibairdia* because of the numerous pits on the carapace surface and remarkable spines along the anterior and postero-ventral margins. Although there is no information on copulatory organs in the type specimen of *A. yamaguchii*, because they are fossil, the new species described herein can be distinguished from *A. yamaguchii* by the wider marginal infoldment and more convex carapace outline in dorsal view.

Anchistrocheles yamaguchii Yajima, 1987

(Figs. 3C, D, 5C, D, 6, 7, 11)

Anchistrocheles yamaguchii Yajima, 1987: 62, Figs 5–3, 4, 11-3a, b, 4a, b.

Description. *Carapace* (Figs. 3C, D, 6) characters almost corresponding to *Anchistrocheles hayatotanakai* **sp. nov.**

Outline in dorsal view flat around middle. Surface covered with around 120 simple type pore systems.

Eye. Absent.

Antennule (Fig. 7A). Seven articulated podomeres, length ratio among them from proximal to distal 27: 23: 5: 9: 5: 3: 2. Chaetotaxy almost corresponding to *Anchistrocheles hayatotanakai* **sp. nov.**

Antenna (Fig. 7B). Number and length ratio of podomeres and chaetotaxy almost corresponding to *Anchistrocheles hayatotanakai* **sp. nov.**

Mandibula (Fig. 7C). Number and length ratio of podomeres and chaetotaxy almost corresponding to *Anchistrocheles hayatotanakai* **sp. nov.**

Maxillula (Fig. 7D). Palp with 2 medium length setae on ventral margin of three-fourths from proximal end. Other chaetotaxy almost corresponding to *A. hayatotanakai* **sp. nov.**

Fifth limb in male (Fig. 7E1). Consisting of 4 articulated podomeres, length ratio among them from proximal to distal 9: 7: 5: 1. Second podomere with 2 long setae at middle of anterior margin. Third podomere with 1 short setulous seta on anterior distal end. Other chaetotaxy almost corresponding to *Anchistrocheles hayatotanakai* **sp. nov.**

Fifth limb in female (Fig. 7E2). Second podomere with 2 equal-length long setae on antero-distal corner. Number and length ratio of podomeres and other chaetotaxy almost corresponding to *Anchistrocheles hayatotanakai* **sp. nov.**

Sixth limb (Fig. 7F). First podomere with 1 very short and 1 medium length setae on anterior margin, 2 short setae on distal margin. Number and length ratio of podomeres and other chaetotaxy almost corresponding to *Anchistrocheles hayatotanakai* **sp. nov.**

Seventh limb (Fig. 7G). First podomere with 1 very short and 1 long setae on anterior margin, 2 short setae on distal margin. Number and length ratio of podomeres and other chaetotaxy almost corresponding to *Anchistrocheles hayatotanakai* **sp. nov.**

Brush-shaped organ (in male) (Fig. 7H). Consisting of symmetrical paired lobes without seta. Located between 6th legs.

Male copulatory organ (Fig. 5C). Basal capsule semicircular with beak-like shape in distal part. Two clasping apparatus: proximal one (*CA1*) short and claw-like shape; distal one (*CA2*) almost straight. Copulatory duct arched.

Female copulatory organ (Fig. 5D). Outline blunt sub-triangular. Inside tube coiled 4 times, proximal part with appearance of twisted, wrung-out cloth.



FIGURE 6. Carapace morphology of *Anchistrocheles yamaguchii* Yajima, 1987. A and B, external view of male right and left valves, respectively (SUM-CO-2495). C and D, internal view of male left and right valves, respectively (SUM-CO-2496). E and F, external view of female right and left valves, respectively (SUM-CO-2497). G and H, internal view of female left and right valves, respectively (SUM-CO-2498). I, dorsal view of male carapace (SUM-CO-2499). J, central muscle scars on female right valve (SUM-CO-2498). Scale 1: 200 µm for A–I. Scale 2: 20 µm for J.



FIGURE 7. Appendages of *Anchistrocheles yamaguchii* Yajima, 1987. A, antennule; B, antenna; C, mandibula; D, maxillula; E, 5th limb (E1, male, E2, female); F, 6th limb; G, 7th limb. H, brush-shaped organ. A–C and E1, male, SUM-CO-2491; D, F and G, male, SUM-CO-2493; E2, female, SUM-CO-2494. Scale: 100 µm.

Furca (Fig. 5C, D). Almost corresponding to *Anchistrocheles hayatotanakai* **sp. nov.** *Caudal process* (Fig. 5C, D). Almost corresponding to *Anchistrocheles hayatotanakai* **sp. nov. Dimensions**. See Table 1B.

Occurrence. Type locality and at coarse sand beach at Arai-hama, Miura City, Kanagawa Pref., east-central Japan (Loc. 3 in Fig. 1C).

Remarks. Anchistrocheles yamaguchii is similar to Anchistrocheles hayatotanakai **sp. nov.** (see Remarks section of the latter). There is an equal number of clasping apparatus in the copulatory organs of both species, and there is no significant difference in the external shape of the distal lobe. The differences between the two species are relatively minor, suggesting that they are closely related, mainly based on the similarity of their other appendages. Many cytherocopine species have setulae on the male brush-shaped organs, but the organs in *A. yamaguchii* have no setulae. Other Anchistrocheles species have a convex carapace outline in dorsal view, but the outline of *A. yamaguchii* is flat in the middle. The carapace size of the holotype of *A. yamaguchii* is 0.743 mm in length and 0.376 mm in height (Yajima 1987). These values were smaller than those of the specimens examined in this study (Table 1B). Previous studies have reported that carapace size depends on water temperature. For example, a study by Kamiya (1988b) showed seasonal changes in carapace size in *Loxoconcha japonica* Ishizaki, 1968 by monthly sampling. The maximum difference was between March (largest) and August (smallest), which was approximately 20%. The difference between the holotype of *A. yamaguchii* and the specimens illustrated in this study was within this range. Malz & Ikeya (1982) pointed out that the smaller fossil Pleistocene species *Cythere simplex* Hu, 1977 from Taiwan was a junior synonym of the larger extant species *Cythere omotenipponica* Hanai, 1959 from Japan. The carapace size of ostracods is difficult to diagnose.

Carapace development

The length, height, and width of carapaces were measured in A-6 to adult instars to calculate the growth rate for the two interstitial species (described herein) of the genus *Anchistrocheles* and two undescribed species of the genus *Neonesidea* (an interstitial species *N*. sp. I and an epiphytic species *N*. sp. S). The four species belong to two genera of the superfamily Bairdioidea, and their microhabitats can be distinguished as interstitial and epiphytic environments. The results showed that the adult carapace length is $< 900 \,\mu\text{m}$ in the interstitial species and $> 900 \,\mu\text{m}$ in the epiphytic species, regardless of the genus. Similarly, the height of the former is $< 500 \,\mu\text{m}$, whereas that of the latter exceeds it. Furthermore, the difference of the width between both is $> 200 \,\mu\text{m}$. (Table 1).

As for the growth rates of the carapace through moulting, the difference between the interstitial (*Anchistrocheles hayatotanakai* **sp. nov.**, *A. yamaguchii*, and *Neonesidea* **sp.** I) and epiphytic (*Neonesidea* **sp.** S) species was apparent in terms of height and width. The growth rate of carapace height in the interstitial species was suppressed (below the average growth rate) in the later stages, whereas that of the epiphytic species was suppressed in the earlier stages. Regarding the carapace width of interstitial species, the growth rate was suppressed twice, in the earlier and later growth stages. In contrast, in the epiphytic species, the growth rate was suppressed only in the earlier half of the growth period, and it was high in the later half. The mean growth rate in the interstitial species was low (1.16-1.18), whereas that of the epiphytic species was high (1.26; Table 2).

To compare the constituent carapace length, height, and width, excluding the absolute value of carapace size, the proportions of these three dimensions were determined for each instar in the four bairdioid species (Table 3). To facilitate visual comparisons, the developmental processes were compared in a three-dimensional triangular (ternary) diagram (Fig. 8). We found that, beyond the genus constraint, the three interstitial species followed similar growth patterns, with vectors moving from bottom to top in the triangular diagram. In contrast, for *Neonesidea* sp. S, the epiphytic species, a similar vector was drawn up to A-2, but switched to a downward vector starting from the A-2 to the adult instar. These three-dimensional comparisons using triangular diagrams were also applied to three other species of podocopids, namely, *Keijcyoidea infralittoraris* Tsukagoshi *et al.*, 2006, *Bicornucythere bisanensis* (Okubo, 1975), and *Cavernocypris* sp. 1. However, no uniform trend was observed (Table 4 and Fig. 9).

t is in µm.			OR	145–153	120–142	102-113	83-88	72–75	63-65	55-60		lg)	OR	255–265	204–211	139–158	116–119	92–97	78-82	65–66
D). Each uni		terstitial)	Width	149	133	106	86	74	64	57		rface-dwellir	Width	261	206	150	118	95	79	99
and N. sp. S (uchii	(in	OR	118-432	362-382	303-313	262-272	217-228	183-187	145–157	S	(su	OR	578–593	159-468	342-350	264-272	206-207	167-172	143-145
ea sp. I (C),	A. yamag		Height	422	374	308	267	222	184	152	N. sp.		Height	588	462	346	266	207	169	144
8), Neonesid			OR	823-858	708-730	572-583	482-493	382–390	310-317	242-257			OR	974-1006	792-804	615-626	459-471	340–355	277–282	230–236
na, 1987 (E			Length	840	722	578	486	385	314	249			Length	966	797	622	464	350	280	233
<i>ii</i> Yajir			Ν	5	4	5	4	4	4	4			Ν	3	3	3	3	3	б	2
amaguch	В		Instar	Adult	A-1	A-2	A-3	A-4	A-5	A6	D		Instar	Adult	A-1	A-2	A-3	A-4	A-5	A-6
p. nov. (A), A.			OR	133-142	121 - 130	100 - 107	80-87	72–80	72–73	57-63			OR	185-190	162 - 170	162 - 170	136-143	116-119	92–94	71
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strocheles ha	<i>ıakai</i> sp. nov.	(interst	OR Wid	375–413 139	338–378 128	285–305 104	247–257 84	213–223 76	178–182 73	148–160 59	sp. I	(interstitial)	OR Width	449-454 188	394-401 167	335–337 140	267 117	214–223 93	175–179 83	145–148 71
s in Anchistrocheles ha	<i>hayatotanakai</i> sp. nov.	(interst	Height OR Widt	393 375–413 139	359 338–378 128	297 285–305 104	253 247–257 84	217 213–223 76	180 178–182 73	154 148–160 59	N. sp. I	(interstitial)	Height OR Width	451 449-454 188	398 394-401 167	336 335–337 140	267 267 117	219 214–223 93	177 175–179 83	147 145–148 71
sional valve in Anchistrocheles ha	A. hayatotanakai sp. nov.	(interst	OR Height OR Wid	753-843 393 375-413 139	675–723 359 338–378 128	<i>555–568</i> 297 285–305 104	437–463 253 247–257 84	372–382 217 213–223 76	277–292 180 178–182 73	148–160 154 148–160 59	N. sp. I	(interstitial)	OR Height OR Width	850–864 451 449–454 188	741–753 398 394–401 167	595-612 336 335-337 140	468–473 267 267 117	364–376 219 214–223 93	287–299 177 175–179 83	231–235 147 145–148 71
uree-dimensional valve in Anchistrocheles ha	A. hayatotanakai sp. nov.	(interst	Length OR Height OR Wid	795 753–843 393 375–413 139	696 675–723 359 338–378 128	563 555–568 297 285–305 104	452 437–463 253 247–257 84	376 372–382 217 213–223 76	287 277–292 180 178–182 73	243 148–160 154 148–160 59	N. sp. I	(interstitial)	Length OR Height OR Width	858 850–864 451 449–454 188	749 741–753 398 394–401 167	603 595-612 336 335-337 140	469 468–473 267 267 117	370 364–376 219 214–223 93	295 287–299 177 175–179 83	233 231–235 147 145–148 71
1. Three-dimensional valve in Anchistrocheles ha	A. hayatotanakai sp. nov.	(interst	N Length OR Height OR Wid	5 795 753-843 393 375-413 139	5 696 675–723 359 338–378 128	5 563 555–568 297 285–305 104	5 452 437–463 253 247–257 84	4 376 372–382 217 213–223 76	4 287 277–292 180 178–182 73	<u>5 243 148–160 154 148–160 59</u>	N. sp. I	(interstitial)	N Length OR Height OR Width	3 858 850–864 451 449–454 188	3 749 741–753 398 394–401 167	3 603 595-612 336 335-337 140	3 469 468-473 267 267 117	3 370 364-376 219 214-223 93	3 295 287–299 177 175–179 83	2 233 231–235 147 145–148 71



FIGURE 8. Three-dimensional triangular (ternary) diagram of the carapace in four bairdioid species. L, length; H, height; W, width. (a), full diagram; (b), close-up view; (c), movement of the average value for each instar.



FIGURE 9. Three-dimensional triangular (ternary) diagram of the carapace developing in three podocopid species. L, length; H, height; W, width. (a), full diagram; (b), close-up view; (c), movement of the average value for each instar.

Α	A. hayatotanakai	sp. nov.		В	A. yamaguc	hii	
Interval of Instan	r Length	Height	Width	Interval of Instar	Length	Height	Width
Adult/A-1	1.14	1.10	1.08	Adult/A-1	1.16	1.13	1.12
A-1/A-2	1.24	1.21	1.24	A-1/A-2	1.25	1.22	1.26
A-2/A-3	1.24	1.18	1.24	A-2/A-3	1.19	1.15	1.23
A-3/A-4	1.20	1.17	1.11	A-3/A-4	1.26	1.21	1.16
A-4/A-5	1.31	1.20	1.04	A-4/A-5	1.23	1.20	1.17
A-5/A-6	1.18	1.17	1.23	A-5/A-6	1.26	1.22	1.11
Average	1.22	1.17	1.16	Average	1.23	1.19	1.17
С	<i>N</i> . sp. I			D	<i>N</i> . sp. S		
Interval of Instan	r Length	Height	Width	Interval of Instar	Length	Height	Width
Adult/A-1	1.15	1.13	1.13	Adult/A-1	1.25	1.27	1.27
A-1/A-2	1.24	1.19	1.19	A-1/A-2	1.28	1.34	1.37
A-2/A-3	1.29	1.26	1.19	A-2/A-3	1.34	1.30	1.27
A-3/A-4	1.27	1.22	1.26	A-3/A-4	1.33	1.29	1.25
A-4/A-5	1.26	1.23	1.12	A-4/A-5	1.25	1.22	1.19
A-5/A-6	1.27	1.21	1.16	A-5/A-6	1.20	1.18	1.21
Average	1.24	1.21	1.18	Average	1.27	1.27	1.26

TABLE 2. Rates of carapace growth through moulting in *Anchistrocheles hayatotanakai* **sp. nov.** (A), *A. yamaguchii* Yajima, 1987 (B), *Neonesidea* sp. I (C), and *N.* sp. S (D). Values in italics indicate values less than the average.

Discussion

Considering the three occurrence points of the two extant Anchistrocheles species in Japan (Fig. 1), the distribution of the two species appears to be split into two by the Izu Peninsula. A previous study of interstitial ostracods suggested that the Izu Peninsula is a barrier between populations at the genetic level. Higashi et al. (2011) examined the mt CO1 region of Microloxoconcha dimorpha Higashi et al., 2011 and showed differences between the west and east sides, across the Izu Peninsula. They also showed that the populations along the eastern side of the Izu Peninsula were more derived than those along the western side. However, the geographical differences were not necessarily reflected in the morphology, suggesting that genetic differences precede morphological differences. Anchistrocheles species in this study were distributed from east to west as two morphologically distinct species separated by the Izu Peninsula, but the evidence is not sufficient to determine whether the Izu Peninsula was a barrier causing speciation into two species. The species of the genus Anchistrocheles in Japan probably migrated from the south, based on the distribution of the entire genus. Considering the example of M. dimorpha by Higashi et al. (2011), A. yamaguchii may have speciated later than Anchistrocheles hayatotanakai sp. nov., as a newer (derived) species because of its distribution in the eastern region. A. yamaguchii was found during a warm (high sea-level) period in the Pleistocene strata of the Atsumi Formation, which is distributed in the Atsumi Peninsula (Yajima 1987), located approximately 200 km southwest of the Izu Peninsula, and its deposition age is approximately < 0.5 Ma (Nakashima *et al.* 2008). It has been suggested that the Izu Peninsula was established approximately 1 Ma (Amano et al. 2007).

Among the four species of the superfamily Bairdioidea compared here, the differences in carapace size and growth patterns were found to be habitat dependent. First, the three interstitial species have a small carapace (Table 1). A smaller carapace must be the first necessary condition for adaptation to the interstitial environment. In general, the epiphytic (epibenthic) bairdioid species are relatively large among marine benthic ostracods. The interstitial species derived from this superfamily should adapt their relatively large carapace, inherited from their common ancestor, to the new environment. The average length and height of podocopid interstitial species from Japan are less than one-half and one-third of those of the bairdioid species, respectively (Fig. 10). The carapace height of common interstitial podocopid species is particularly small. This can be explained by the fact that the cross-sectional area of the carapace is reduced to adapt to the narrow space of interstitial environments. Another parameter that reduces

the cross-sectional area of the carapace is its width. Data on the carapace width of known interstitial species are scarce, which does not allow extensive discussion, but the two *Neonesidea* species, N. sp. S and N. sp. I, which are epiphytic and interstitial species, respectively, of the same genus were compared. The increase in carapace width during development was particularly suppressed in the interstitial species (Table 2), suggesting that the suppression of carapace width is an important factor for the adaptation of species with a large carapace of the superfamily Bairdioidea to the interstitial environment.



FIGURE 10. Comparison of carapace sizes in interstitial ostracods from Japan. Size data were obtained from the holotype descriptions in the original papers.

The reason for the lack of a uniform trend in three-dimensional ratios of carapace sizes in other podocopid epiphytic species (Table 4 and Fig 9) must be their habitat diversity, especially microhabitats, even if they are combined as epiphytic. *Keijcyoidea infralittoralis* inhabits the coarse-grained sediments around the rhizoids of seaweeds thriving in the reef subtidal zone (Tsukagoshi *et al.* 2006), and *Bicornucythere bisanensis* is found in the sandy mud bottom of inner bays (Abe 1983). *Cavernocypris* sp. 1 was collected from sand and gravel bottoms in a very shallow terrestrial (spring) water environment. Some interstitial species with a small carapace height have a large carapace width relative to their carapace length (e.g., *Cobanocythere lata* Higashi & Tsukagoshi, 2011, *Semicytherura sagittiformis* Yamada & Tanaka, 2011). If the absolute carapace size is significantly smaller than that of *Anchistrocheles* species, it can be considered that the constraint of preferentially suppressing carapace width observed in the four bairdioid species in this study is negated, and other functional morphological aspects are emphasised. In the future, more data should be collected to examine how body size and three-dimensional proportions are affected by phylogenetic and various microenvironmental constraints.

Brush-shaped organs are frequently found in male podocopids (Athersuch *et al.* 1989). However, their anatomical location has rarely been definitively demonstrated; for example, Athersuch *et al.* (1989) stated that brush-shaped organs are "situated variously in front of or between the fifth or sixth organs". In this study, the soft body of *Anchistrocheles yamaguchii* was freeze-dried and observed by SEM. It was found that the organs are not arranged as one of the rows of appendages, but located between the sixth pair of limbs (the second walking legs) (Fig. 11). In addition, the organs in both species of *Anchistrocheles* (family Bythocyprididae) are symmetrical (equal right and left). This fact supports the hypothesis that the asymmetrical brush-shaped organs in *Neonesidea* and closely related genera are a synapomorphic character of the family Bairdiidae (i.e., Horikoshi *et al.* 2019).

TABLE 3. Th	nree-dimens	ional carapac	ce proportions in	a Anchistroch	eles hayatotanakai	sp. nov. (A), <i>A</i> .	yamaguchii	Yajima, 198'	7 (B), Neonesid	lea sp. I (C), an	nd N. sp. S (D). L,
length; H, heig	ght; W, widt	h.									
V		A. hayat	totanakai sp. no	v.		В		А.	yamaguchii		
		(in	nterstitial))	interstitial)		
Instar	Ν	L(%)	H(%)	W(%)	L+H+W(%)	Instar	Ν	L(%)	H(%)	W(%)	L+H+W(%)
Adult	5	59.9	29.6	10.5	100	Adult	5	59.5	29.9	10.6	100
A-1	5	58.8	30.4	10.8	100	A-1	4	58.7	30.4	10.8	100
A-2	5	58.4	30.8	10.8	100	A-2	5	58.3	31.1	10.7	100
A-3	5	57.3	32.1	10.6	100	A-3	4	57.9	31.8	10.2	100
A-4	4	56.3	32.4	11.3	100	A-4	4	56.5	32.6	10.9	100
A-5	4	53.2	33.4	13.5	100	A-5	4	55.8	32.8	11.3	100
A-6	5	53.3	33.7	13.0	100	A-6	4	54.4	33.1	12.5	100
С		1	N. sp. I			D			N. sp. S		
		(in	nterstitial)					Ŭ	(epiphytic)		
Instar	Ν	L(%)	H(%)	W(%)	L+H+W(%)	Instar	Ν	L(%)	H(%)	W(%)	L+H+W(%)
Adult	3	57.3	30.1	12.5	100	Adult	3	54.0	31.9	14.2	100
A-1	3	57.0	30.3	12.7	100	A-1	3	54.4	31.5	14.1	100
A-2	З	55.9	31.1	13.0	100	A-2	3	55.7	30.9	13.4	100
A-3	б	55.0	31.3	13.7	100	A-3	3	54.7	31.4	13.9	100
A-4	б	54.3	32.1	13.6	100	A-4	3	53.7	31.8	14.5	100
A-5	Э	53.1	32.0	14.9	100	A-5	3	52.9	32.0	15.0	100
A-6	2	51.7	32.5	15.8	100	A-6	2	52.7	32.5	14.8	100

TABLE 4. Thr	ee-dimens	ional carap	ace proportion	ns in three non	-bairdioid species,	Keijcyoidea infraliti	<i>toralis</i> Tsuki	agoshi <i>et al.</i> ,	2006 (A), <i>Bi</i>	cornucythere l	visanensis (Okubo,
1975) (B), and (Cavernocy	pris sp. 1 (C	C). L, length;	H, height; W, v	vidth.						
V		K. in	ofralittoralis			В		B. bi	sanensis		
		(sub	-interstitial)					(epi	phytic)		
Moult stage	Ν	L(%)	H(%)	W(%)	L+H+W(%)	Moult stage	Ν	L(%)	(%)H	W(%)	L+H+W(%)
Adult	5	56.5	31.2	12.3	100	Adult	4	55.0	29.9	15.0	100
A-1	1	55.9	32.1	12.0	100	A-1	4	52.5	32.0	15.5	100
A-2	1	56.7	32.6	10.7	100	A-2	4	51.9	32.7	15.4	100
A-3	1	55.9	32.5	11.6	100	A-3	4	53.4	32.7	13.9	100
A-4	4	55.5	32.6	11.9	100	A-4	4	53.2	32.9	13.9	100
A-5	1	55.1	32.3	12.6	100	A-5	1	53.4	34.0	12.6	100
A-6	1	55.2	33.1	11.7	100						
A-7	3	53.0	33.2	13.7	100	C		C.	sp. 1		
A-8	4	51.3	34.6	14.1	100			(epi	phytic)		
						Moult stage	z	L(%)	(%)H	W(%)	L+H+W(%)
						Adult	4	56.8	29.1	14.1	100
						A-1	4	56.9	29.5	13.5	100
						A-2	3	56.5	30.2	13.3	100
						A-3	ю	55.7	30.8	13.5	100
						A-4	2	54.5	31.4	14.1	100

100 100

15.0 15.1

32.5 30.7

52.5 54.2

5 5

A-5 A-6



FIGURE 11. Anatomical location of brush-shaped organ in *Anchistrocheles yamaguchii* Yajima, 1987. A, anatomical location among appendages. B, high magnification. Male, SUM-CO-2500. *L5*, fifth limb; *L6*, sixth limb; *L7*, seventh limb. Scale: ca. 35µm for A, ca. 10µm for B.

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