Note

Morphological divergence of *Pseudodiaptomus inopinus* Burckhardt, 1913 (Copepoda: Calanoida) between the Japan Sea and Pacific coasts of western Japan

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Abstract: Two morphological groups of *Pseudodiaptomus inopinus* were detected in western Japan. One was from the Japan Sea and Tsushima Strait coasts and the other from the Pacific coast including the west and east coasts of Kyushu Island. Differences in females were seen in the posterior processes of the genital operculum, shape of the genital flap, and medial spinules on the first exopodal segment of the fifth leg, and those in males were in the shape of the first exopodal segment of the fifth left leg, and position of the medial process on the third exopodal segment of the fifth right leg. Proportional length of the caudal rami also differed in both sexes. Among these morphologies, the length of the genital operculum process discretely separated each group, but variations in the other morphometric characters were overlapping. Geographic distributions of the two groups were separated with a boundary between two rivers on the northwest coast of Kyushu Island. These results indicate that *P. inopinus* in Japan is a species complex and specimens from the Japan Sea and Pacific coasts belong to different species.

Key words: distribution, morphological differentiation, *Pseudodiaptomus inopinus*, species complex, western Japan

The brackish-water species *Pseudodiaptomus inopinus* Burckhardt, 1913 has been recorded widely from subtropical through cold-temperate waters in East Asia (e.g. Shen & Tai 1962, Mizuno & Miura 1984). It is a very common and often the most abundant copepod in brackish-waters of the mainland of Japan (Sakaguchi & Ueda 2010, Sakaguchi et al. 2011). Because of morphological differences among the literature from different localities, we (Sakaguchi & Ueda 2010) suggested that *P. inopinus* is a species complex. To confirm this suggestion, we examined *P. inopinus* specimens from various localities in western Japan, and discovered two morphologically distinguishable groups. This paper describes their morphological differences and geographical distribution in western Japan.

Adult specimens of *P. inopinus* used for examination were collected from 31 river mouths and a brackish lake in western Japan (Fig. 1) between August 2006 and October 2010. Collections were made by towing a 0.1, 0.2 or 0.3-mm mesh net and immediately fixing the contents in 2% buffered formalin or 99.5% ethanol. Specimens were dissected and examined in lactophenol. Morphological examinations, measurements and drawings were made under a differential interference microscope (Nikon Eclipse E600) with a drawing tube and ocular micrometer. Final illustrations were prepared with computer software (Adobe Illustrator®).

Morphologies we paid attention to were spinulation of the fifth pediger and the urosome, shape of the genital area, length of the caudal rami, and shape and spinulation of the fifth legs. Among these, significant variations related to sampling localities were found in the following morphologies: (1) length of posterior processes of the genital operculum (Fig. 2A, B; arrowhead *a*), (2) shape of the central part of the genital flap surrounding gonopores (Fig. 2A, B; arrowhead *b*), (3) medial spinules on the first exopodal segment of the female fifth leg (Fig. 2C, D); (4) shape of the first exopodal segment of the male fifth left leg (Fig. 2E, F), (5) position of a medial process on the third exopodal segment of the male fifth right leg (Fig. 2G, H), (6) proportional length of the caudal ramus in both sexes (Fig. 2I, J). Locality-related variations were not observed in the other morphologies.

To compare the morphometric characters statistically, the following measurements were made (the numbers in parentheses correspond to those above): (1) width of the genital double somite (GSW) and mean length of left and right posterior processes of the genital operculum (GOPL) (Fig. 3A) based on 79 females from sites #1–14, 16–28, 30–32 in Fig. 1; (4) width of the first exopodal segment of the fifth left leg at the narrowest point of the midlength (LSW) and depth of medial concavity of the segment (MCD) (Fig. 3B) on 78 males from sites #1, 3, 5–10, 12, 14–24, 27, 29; (5) length of the curved third exopodal segment of the male fifth right leg from the tip to proxi-

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Fig. 1. Map of western Japan with the 32 sampling sites where the present *Pseudodiaptomus inopinus* specimens were collected. Specimens from sites #15–27 and the others are referred to as the Japan Sea and Pacific groups, respectively.

mal end (RSL) and from the tip to the medial process (RPL) (Fig. 3C) on 74 males from sites #1, 3–12, 14, 18–24, 26, 32; (6) length/width of the left caudal ramus (CL/CW) and length of the anal somite (ASL) (Fig. 3D) based on 69 females from sites #1–14, 16–27, 30–32 and 70 males from sites #1, 3, 5–10, 12, 14–24, 27, 29, 30. For measurements of the curved third exopodal segment of the male fifth right leg, dissected legs were photographed with a digital camera by being leveled on the glass slide with a cover slip and the length of a curved line was measured on a computer display by using the free computer software “Magaru-monosashi” (<http://www.vector.co.jp/soft/winnt/util/se305305.html>). The other measurements were made directly under a microscope with an ocular micrometer. The male specimens included two morphological types of the second exopodal segment of the fifth left leg, i.e. thumb- and paddle-types (Burckhardt 1913, Shen & Song 1979).

The posterior process of the genital operculum was clearly classifiable into two types. One type of process is short (Fig. 2A, arrowhead *a*) and was observed in specimens from the Japan Sea and Tsushima Strait coasts of Honshu and Kyushu Islands (sites #15–27); henceforth specimens from these sites are referred to as ‘the Japan Sea group’. The long type of the process (Fig. 2B; arrowhead *a*) was seen in specimens from the other sampling sites (#1–14, 28–32); henceforth referred to as ‘the Pacific group’. When GOPL/GSW was plotted against

GSW, the data of the Japan Sea and Pacific groups were discretely separated into those with short ($\text{GOPL/GSW} \leq 0.26$) and long (≥ 0.27) processes, respectively (Fig. 3A).

Differences in females between the two groups were also found in the shape of the central part of the genital flap surrounding the gonopores, which was generally triangular (Fig. 2A; arrowhead *b*) in the Japan Sea group and more round in the Pacific group (Fig. 2B; arrowhead *b*), and in the medial spinules on the first exopodal segment of the fifth legs, which were relatively thick in the Japan Sea group (Fig. 2C) and absent or fine in the Pacific group (Fig. 2D). As for the differences in the male fifth leg, the medial concavity of the first exopodal segment of the left leg was deeper (Fig. 2E) than in the Pacific group (Fig. 2F). The medial process on the third exopodal segment of the right leg was slightly proximal to the segment in the Japan Sea group (Fig. 2G) while it was generally almost located at the midlength in the Pacific group (Fig. 2H). The results of measurements on these male characters indicated that the values were significantly different between the groups ($p < 0.001$, *t*-test) with variations that overlapped with each other (Fig. 3B, C). The proportional length of the caudal rami of both sexes were significantly greater in the Japan Sea group ($p < 0.001$, *t*-test) although the variation greatly overlapped in the female specimens (Figs. 2I, J and 3D). The significant difference between the specimens with thumb- and paddle-type fifth legs in each group was seen in the medial

Fig. 2. Morphologies of *Pseudodiaptomus inopinus* specimens from the Japan Sea and Pacific coasts of western Japan. A, B: genital double somite; C, D: female fifth left leg; E, F: exopod of the male fifth left leg; G, H: third exopodal segment of the male fifth right leg; I, J: male anal somite and caudal rami. Left and right figures of each pair are of specimens from the Japan Sea (#20 for A, C, E; #22 for G; #24 for I) and Pacific groups (#1 for B, D, F, J; #12 for H), respectively. Morphologies to be compared are indicated by arrowheads.

concavity of the first exopodal segment of the left leg, which was deeper in the thumb-type ($p < 0.01$ for the Japan Sea group, and $p < 0.001$ for the Pacific group, t -test), but the differences in the other two morphologies were not significant. The paddle- and thumb-type fifth left legs are also known for *P. poplesia* Shen, 1955 (Shen & Song 1979). The present result that found no significant morphological difference other than this character in the fifth left leg confirms that these types are an example of intraspecific dimorphism.

The present study revealed that there are two morphological types of *P. inopinus* in western Japan, of which the distribution

ranges are discretely separated from each other with a boundary between two 30-km distant rivers (sites #10 and #15) on the northwest coast of Kyushu Island. Notable morphological clines were not observed within each range. These facts indicate that specimens of the two groups belong to different species and *P. inopinus* in Japan is a species complex. However, we do not decide their taxonomic statuses herein, because it is still uncertain whether one of the two types is identical to *P. inopinus* s. str. or not. Compared with Burckhardt's (1913) original figures of *P. inopinus* s. str. from Taifu Lake near the Yangtze River mouth, China, the Pacific group is more similar

Fig. 3. Comparisons of morphometric characters of *Pseudodiaptomus inopinus* specimens from the Japan Sea and Pacific coasts of western Japan. A: mean length of left and right posterior processes of the genital operculum; B: depth of medial concavity of the first exopodal segment of the male fifth left leg; C: positions of medial process of the third exopodal segment of the male fifth left leg; D: proportional length of the left caudal ramus. Filled and open symbols represent specimens from the Japan Sea and Pacific groups, respectively. Circles and triangles in males indicate specimens with thumb- and paddle-type fifth legs, respectively.

to *P. inopinus* s. str. in having longer posterior processes of the genital operculum. However, the morphology of the male fifth leg in the original description is somewhat different from that of the Pacific group; the ratios of RPL/RSL measured for the original figures (Burckhardt 1913: plate 12, figs. 7 and 8) are 0.62 for the thumb type and 0.58 for the paddle type, which are apparently the same as for the Japan Sea group (Fig. 3C). Besides, Lee & Frost (2002) suggested that morphological stasis of a species complex is common in free-living copepods. This means that morphological similarity is not a sufficient condition for specific identification within the *P. inopinus* complex. Specimen-based morphological comparisons with *P. inopinus* s. str. from China and genetic comparisons between Japanese and Chinese specimens as well as between the two types in Japan are necessary to make a final decision on their taxonomic status.

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