

A New Species of *Deiratonotus* (Crustacea: Brachyura: Camptandriidae) Found in the Kumanoe River Estuary, Kyushu, Japan

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Camptandriid crabs collected in the Kumanoe River Estuary, Kyushu, Japan were studied on the basis of morphological characters and molecular analysis. As a result, a new species, *Deiratonotus kaoriae*, was recognized. These crabs were found mainly in a creek of the sandy tidal flat within the Kumanoe River Estuary. The new species shares a very diagnostic character, the presence of a transverse ridge on the carapace, with *D. cristatus* (de Man, 1895) and differs markedly from the other congeners that lack this feature. The new species, however, differs from *D. cristatus* in the absence of harpoon-shaped setae on the subdistal end of the first gonopod and the presence of an extremely reduced second abdominal segment. According to a molecular analysis based on 12S+16S mitochondrial rRNA gene sequences, with *Cleistostoma dilatatum* (de Haan, 1833) and *Camptandrium sexdentatum* Stimpson, 1858 as outgroups, *Deiratonotus kaoriae* is more closely related to *D. cristatus* than to *D. japonicus* (Sakai, 1934).

Key words: new species, camptandriid crab, river estuary, morphological characters, molecular analysis, Miyazaki

INTRODUCTION

Along the Pacific coast of Miyazaki Prefecture on Kyushu Island, Japan, 67% of the coastline remains in its natural state, and recent studies have added numerous crab and mollusc species to the coastal fauna (Sato, 1997; Miura *et al.*, 2005, 2006, 2007). An undescribed species of the genus *Deiratonotus* Manning and Holthuis, 1981 obtained in the Kumanoe River Estuary on this coast in May 2004 was found to be morphologically different from previously known species (Miura *et al.*, 2004). This study describes this new species and illustrates its phylogenetic relationships with congeneric species on the basis of a mitochondrial DNA analysis.

Manning and Holthuis (1981) established the genus *Deiratonotus* in the family Camptandriidae, with *Paracleistostoma cristatus* de Man, 1895, as the type species. This genus differs from other genera of the family in that the gonopod lacks the subapical lobe or is not bilobed. Besides the type species, which has been recorded from Japan (Sakai, 1976) to Korea and China (Tan and Ng, 1999), the genus includes two other species, *D. japonicus* (Sakai, 1934) and *D. tondensis* Sakai, 1983, which are endemic to Japan (Sakai, 1976; 1983). Kawane *et al.* (2005), however, recently synonymized *Deiratonotus tondensis* with *D. japonicus*. All these species inhabit estuaries or inland seas

(Ono, 1965; Sakai, 1976; Fukui and Wada, 1986). Our study adds another species from Japan to the genus *Deiratonotus*.

MATERIALS AND METHODS

Specimens used for morphological observations were collected from the Kumanoe River Estuary, Miyazaki Prefecture, Kyushu Island, Japan (32°40'38"N, 131°46'53"E). The first specimens including the holotype were obtained on 7 May 2004, and additional samples including fresh material for molecular analysis were collected on 24 April 2005. To collect these crabs, bottom sediment was sieved by a hand net with an approximately 2-mm mesh size. Most crabs were found on the sandy bottom of tidal-flat creeks and on sandy bottoms along the river. Crabs were transferred into 99% ethyl alcohol and preserved in the same medium or transferred to 70% ethanol. Specimen drawings and photographs were made with the aid of an Olympus SZX-DA camera lucida and a Nikon Coolpix 950 digital camera system attached to an Olympus SZX12 binocular dissection microscope. Additional morphological observations were made with an Olympus BH2 light microscope. Measurements of maximum carapace length and width and other structures are given in millimeters in the text. The type specimens and the materials for molecular analysis were deposited in the National Science Museum (NSMT), Tokyo.

For the molecular analysis, specimens of camptandriid species, including *Deiratonotus cristatus* (de Man, 1895), *D. japonicus*, the new species, *Cleistostoma dilatatum* (de Haan, 1833), and *Camptandrium sexdentatum* Stimpson, 1858, were collected from some river estuaries in Japan. Specimens of the new species for molecular analysis were obtained from the Kumanoe River on 24 April 2005. A specimen of *D. cristatus* was collected from the Shimanto River, Kochi Prefecture, on 12 July 2002 and another specimen from the Tonda River, Wakayama Prefecture, on 14 March 2002. *Deiratonotus japonicus* was collected from the Takahama River, Kumamoto Prefecture on 8 August 2002.

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Cleistostoma dilatatum was collected from the Yorimo River, Ohita Prefecture, on 25 April 2005. *Camptandrium sexdentatum* was collected from Imazu Bay, Fukuoka Prefecture, on 5 June 2005. Two specimens of each species preserved in 99% ethyl alcohol were used in the DNA analysis.

Total genomic DNA was extracted from the leg musculature of each crab using a SIGMA GenElute™ Mammalian Genomic DNA Kit (SIGMA, St. Louis, MO). Portions of the 12S and 16S mitochondrial ribosomal RNA genes were amplified by the polymerase chain reaction (PCR; Saiki *et al.* 1988), with primer pairs L1496i (5'-GTA-CATATCGCCCGTCGCTT-3'; Kitaura *et al.*, 1998) and H2492i (5'-CAGACATGTTTTAATAAACAGGC-3'; Kitaura *et al.*, 2002) for 12S, and L2510i (5'-CGCCTGTTTAAACAAAACAT-3'; Kitaura and Nishida, pers. comm.) and H3059i (5'-CCGGTCTGAACTCAGAT-CATGT-3'; Kitaura and Nishida, pers. comm.) for 16S. Symmetric PCR amplifications were conducted on a GeneAmp 2400 thermal cycler (Applied Biosystems, Foster City, CA) in total volumes of 25 µl containing 0.125 µl of TaKaRa Ex Taq™ (Otsu, Shiga; 5 units/µl), 2.5 µl of 10X Ex Taq™ Buffer, 2.0 µl of dNTP Mixture (2.5 mM of each nucleotide), 0.5 mM of the appropriate forward and reverse primer, and 1.0 µl of template DNA. PCR conditions were 94°C for 2 min; 35 cycles of 94°C for 30 sec, 48–53°C for 30 sec (different temperatures were used for different species), and 72°C for 1 min; and a final extension step of 72°C for 7 min. Amplification products were checked for size by loading 5 µl on a 1% agarose gel (TaKaRa) with 0.5 µg/ml ethidium bromide in 1X TAE buffer. The remaining product was purified using ExoSAP-IT (USB, Ohio) and sequenced with an amplification primer using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (ABI) with an automated DNA sequencer (Genetic Analyzer 310, ABI). Sequences were verified with DNASIS software (HITACHI, Shinagawa, Tokyo) and aligned by Clustal W (Thompson *et al.*, 1994). All final sequences were obtained from both DNA strands for verification and those on the new species were deposited in the DNA Data Bank of Japan (DDBJ; <http://www.ddbj.nig.ac.jp/Welcome-j.html>; accession numbers AB332046 for male specimen NSMT-Cr 17833 and AB332047 for female specimen NSMT-Cr 17834).

To determine which model of DNA substitution best fit our combined 12S+16S data set (ILD test, $p=1.0000$), we used the program Modeltest 3.0 (Posada and Crandall, 1998), which uses hierarchical likelihood ratio tests. The selected model was HKY (Hasegawa *et al.* 1985)+G (-lnL=3379.6357), a special case of the general-time-reversible model with the following parameters: transition / transversion ratio=4.2278; base frequencies, A=0.3952, C=0.0784, G=0.1389, T=0.3875; among-site rate variation, gamma (G) distribution with shape parameter=0.1947.

Phylogenetic relationships among species were analyzed by the maximum parsimony (MP: Camin and Sokai, 1965) and maximum likelihood (ML: Felsenstein, 1981) methods, using PAUP* version 4.0b10 (Swofford, 2002). The MP tree was found by a heuristic search using the tree-bisection-reconnection (TBR) branch-swapping algorithm with starting trees obtained by random stepwise addition with 100 replications. Gaps were treated as missing data. The ML analyses were performed by heuristic searches with the HKY+G model of substitution. Support for nodes was evaluated using non-parametric bootstrapping (Felsenstein, 1985) with the heuristic search option in PAUP* for parsimony, distance, and likelihood methods with 1,000 pseudoreplicates. Genetic distance between specimens for the combined 12S+16S data were calculated using the HKY+G model.

TAXONOMIC ACCOUNT

Genus *Deiratonotus* Manning and Holthuis, 1981

Deiratonotus kaoriae, new species

[Japanese name: Kumano-e-miosujigani]

Figs. 1–3

Type material

Holotype, NSMT-Cr 17830: male (6.7 by 7.3), tidal-flat creek, about 300 m upstream from mouth of the Kumano River, Miyazaki Prefecture, Japan; sieved from sandy sediment by using a hand net; collected by K. Shin and K. Miura on 7 May 2004. Paratypes, NSMT-Cr 17831: one male (7.0 by 7.6), two females (8.5 by 10.1, 7.7 by 9.3), and one juvenile (2.6 by 2.9), same site, collectors, and date as holotype. Paratypes, NSMT-Cr 17832: five males (6.3 by 8.7, 6.3 by 7.0, 7.0 by 8.1, 6.1 by 7.0, 6.6 by 7.6), two females (7.6 by 9.4, 7.3 by 8.7), and one juvenile (2.6 by 2.8), same site as holotype, collected by T. Miura on 24 April 2005.

Description

Carapace dorso-ventrally depressed, nearly quadrangular in outline; 1.1 times broader than long in holotype (Figs. 1, 3A, B); lateral margins moderately convex, without tooth behind outer orbital tooth; regions slightly demarcated; dorsal surface covered coarsely with fine granules, marginal rim and post-lateral ridges covered with dense granules and stiff, brown, short setae; epigastric crests present; hepatic region weakly concave; protogastric and mesogastric regions convex, with small cluster of granules; protobranchial and mesogastric regions forming clear transverse ridges accentuated by dense granules with short brown setae; metabranchial and intestinal regions weakly concave (Fig. 1). Marginal rim and ridges of carapace and ambulatory legs covered by granules with very short dark brown setae in holotype and some paratypes, but only granules present and most of short setae lacking in some paratypes.

Frontal margin weakly bilobed, slightly broader than orbit, thickened on either side. Inner dorsal orbital angle bluntly squarish; supraorbital margin flat without notch or projection; outer orbital tooth blunt (Fig. 1).

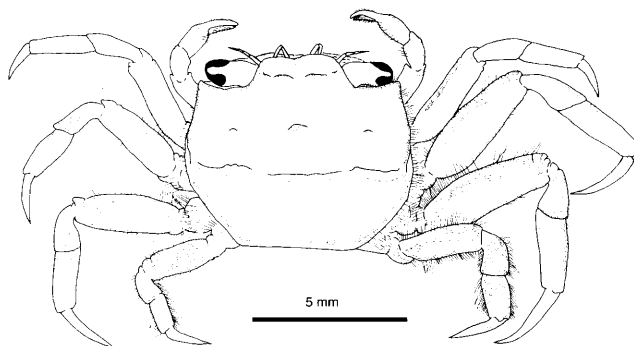


Fig. 1. *Deiratonotus kaoriae*, new species. Holotype, male, dorsal view.

Third maxilliped covered with sparse granules, with thickened inner lateral edges; palp articulated; ischium and merus not fused with complete suture; very broad, completely closing buccal orifice; merus rounded distally, with rounded notch on inner distal edge for placement of palp; merus slightly shorter than ischium (Fig. 2A).

Chelipeds with finger slightly longer than palm; movable finger with a single small, median tooth on cutting edge; immovable finger smooth on cutting edge; distal end of mov-

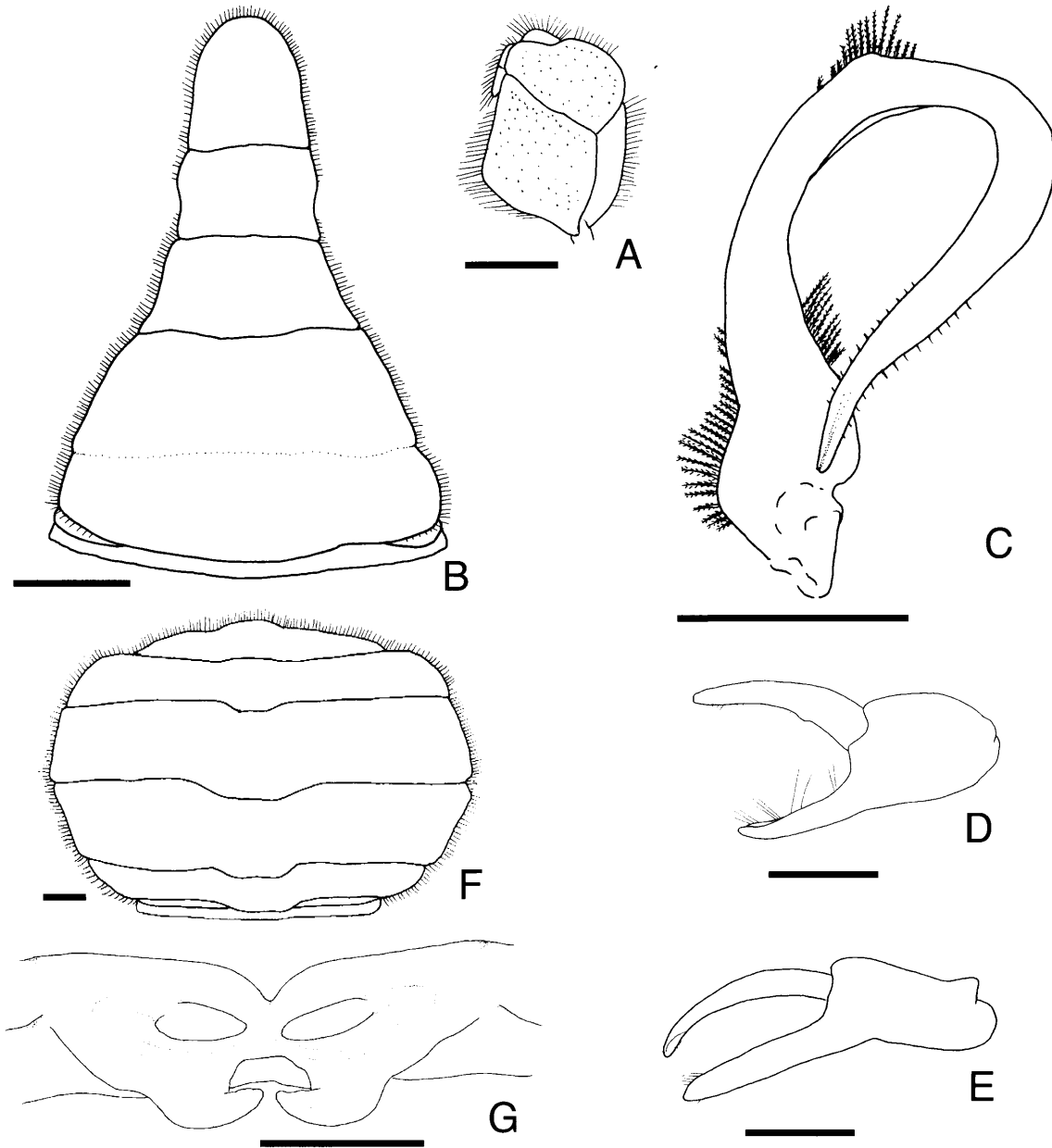


Fig. 2. *Deiratonotus kaoriae*, new species. **(A)** Left maxilliped 3, ventral view (outer face), holotype, male. **(B)** Abdomen, ventral view, holotype, male. **(C)** Left gonopod, outer face (ventral view), holotype, male. **(D)** Left cheliped, ventral view (outer face), holotype, male. **(E)** Left cheliped, ventral view (outer face), paratype, female. **(F)** Abdomen, ventral view (outer face), paratype, female. **(G)** Female genital organ on thoracic sternites 6 and 5 with hook-like projection, venter-anterior view, paratype. Scale=1 mm.

able and immovable fingers with spatulate tips (Figs. 2D, 3B). Female paratypes with slender chelipeds; fingers longer than palm (Figs. 2F, 3C).

Second and third ambulatory legs longer than first and fourth legs; first ambulatory legs without long, soft plumose setae; second legs with a few plumose setae on anterior and posterior borders of meri; third and fourth legs with plumose setae on meri, carpi and propodi (Figs. 1, 3A, B).

Abdomen triangular in male; first segment very wide, reaching to coxae of fifth legs; second segment very narrow, extremely reduced, with visible right and left parts; third and fourth segments fused, narrowing distinctly in distal part; fifth

segment narrowing distally, with slightly constricted lateral margin; sixth segment quadrate; seventh segment longer than broad, with semicircular distal margin (Fig. 2B). Fifth and sixth segments with reddish color band on ventral surface in life (Fig. 3B). Part of gonopods evident on both sides of fourth to sixth segments (Fig. 3B). Female abdominal segmental sutures clearly visible. Distal margin of seventh segment slightly protruded (Fig. 2F). For female abdomen, only central parts of fifth and sixth segments reddish in life (Fig. 3C).

First gonopod very long, strongly curved, with distal part extending over proximal part; distal third gradually narrow-

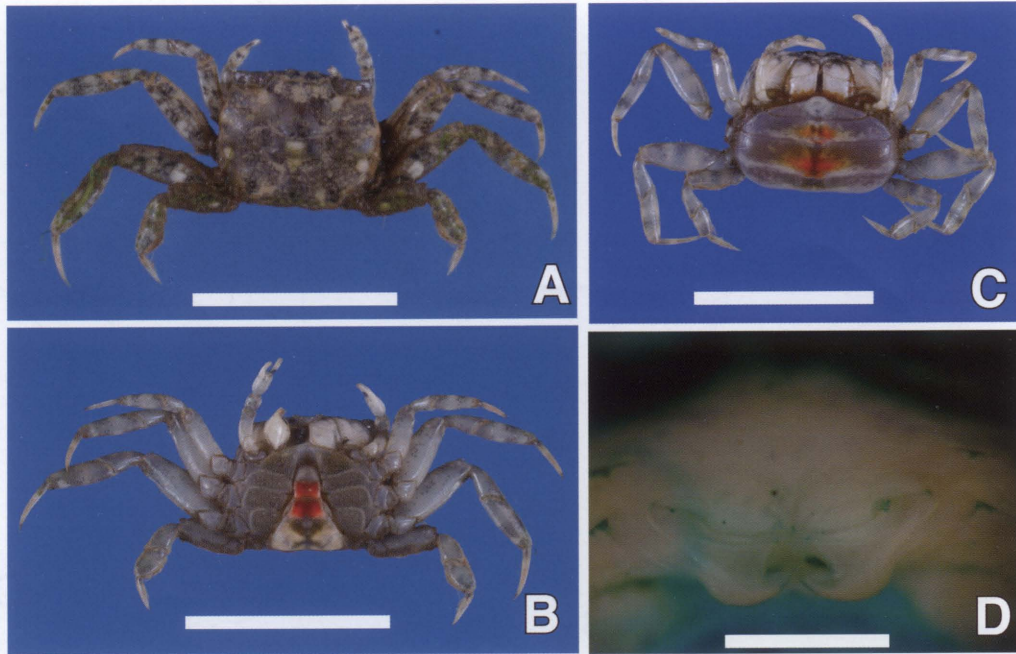


Fig. 3. *Deiratonotus kaoriae* new species. (A) Holotype, male, dorsal view, fresh sample. (B) Same, in ventral view. (C) Female paratype, ventral view, fresh sample. (D) Female genital organ on thoracic sternites 6 and 5 with hook-like projection of unknown function, vento-anterior view, paratype. Scales=10 mm in A–E and 1 mm in F.

ing; distal end slightly broadened, truncated with cutting edge oblique to axis; proximal part with numerous long plumose setae on both sides; median external swollen edge also with plumose setae; distal third with very short setae on both sides (Fig. 2C).

Female genital opening on suture between thoracic sternites 6 and 5, with hook-like projection directed vento-posteriorly (Figs. 2G, 3D). All observed female specimens incubating eggs, with hook-like projections.

Etymology

The new species is named for Mrs. Kaori Shin, Ohita City, who helped us to collect the specimens in the field.

Molecular analysis

The multiple-sequence alignment of the combined 12S+16S data for the three species of *Deiratonotus* and outgroup taxa was 1,259 basepairs (bp) in length, including gaps (*i.e.*, insertion-deletion character states, or indels) and ambiguously aligned regions. Deletion of the latter regions resulted in an alignment 1,236 bp in length. Out of 1,236 total characters, 249 characters were parsimony-informative.

The MP and ML trees from the phylogenetic analysis were congruent. Therefore, only the ML tree is presented here, with both ML and MP bootstrap values (Fig. 4). The tree shows all species of the genus *Deiratonotus* to be included in the same clade, separate from the outgroups *Cleistostoma dilatatum* and *Camptandrium sexdentatum* also within the family Camptandriidae, and the new species to be more closely related to *D. cristatus*, even though the bootstrap values were not high. Genetic distance between specimens of *D. kaoriae* and *D. cristatus*, *D. kaoriae* and *D. japonicus*, and *D. cristatus* and *D. japonicus* for the combined 12S+16S data were 18.58 ± 0.12 (mean \pm SD), 21.38 ± 0.05 , and 19.87 ± 0.45 , respectively (Table 1).

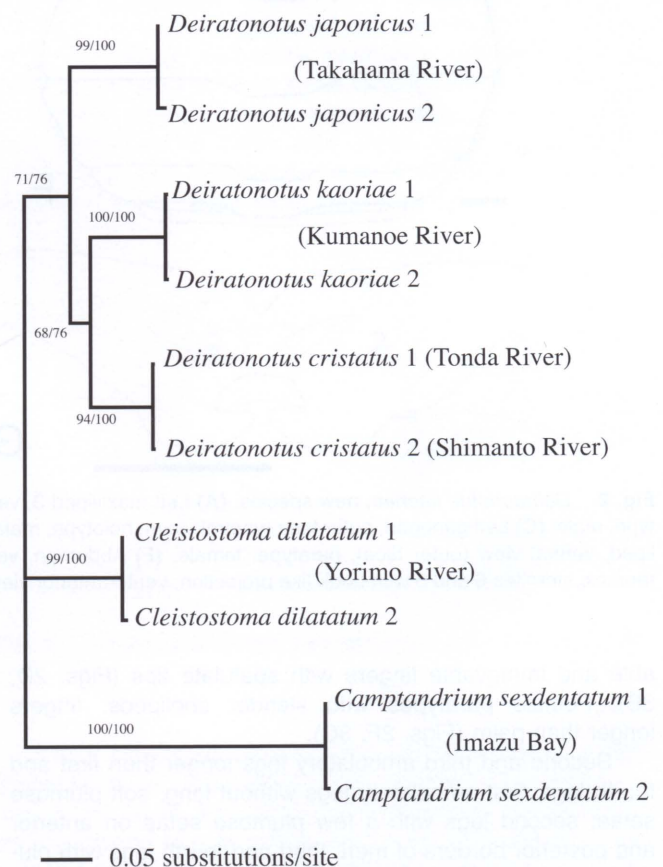


Fig. 4. Maximum-likelihood tree of the combined 12S+16S mitochondrial rRNA gene sequences under the HKY+G model of nucleotide substitution for three *Deiratonotus* species and two outgroup taxa (two individuals for each taxon). Bootstrap probabilities (> 50%) based on 1,000 replicates for each analysis (ML/ MP) are shown on internodes.

Table 1. Mean genetic distances with standard deviations among 5 species of Camptandriidae. The values calculated with the model HKY+G are shown in percentage

	<i>D. kaoriae</i>	<i>D. cristatus</i>	<i>D. japonicus</i>	<i>C. dilatatum</i>
<i>Deiratonotus cristatus</i>	18.58±0.12	–		
<i>D. japonicus</i>	21.38±0.05	19.87±0.45	–	
<i>Cleistostoma dilatatum</i>	38.07±0.16	28.77±0.96	29.88±1.24	–
<i>Camptandrium sexdentatum</i>	85.84±0.52	47.69±1.12	62.96±0.80	54.90±1.56

DISCUSSION

The genus *Deiratonotus* contains four nominal species: *D. japonicus*, *D. tondensis*, *D. cristatus*, and *D. kaoriae*. Kawane *et al.* (2005), however, recently synonymized *D. tondensis* with *D. japonicus*. *Deiratonotus kaoriae* and *D. cristatus* are easily distinguished from *D. japonicus* in having on the carapace a very prominent submedian transverse ridge that is absent in the other. In *D. kaoriae*, the distal end of the first gonopod is blunt or truncated with a cutting edge oblique to the axis and lacks subdistal appendages. In *D. cristatus*, however, the distal end is acute with subdistal harpoon-shaped setae (Manning and Holthuis, 1981). These two species are also distinguished by the shape of the second segment of male abdomen. *Deiratonotus cristatus* has a reduced but well-defined second abdominal segment, whereas this segment is separated into two lateral parts in *D. kaoriae*.

The ML tree based on the combined 12S+16S data reveals that *Deiratonotus kaoriae* is more closely related to *D. cristatus* than to *D. japonicus*. Genetic distances between the two species (18.58±0.12) exceed the limit of intraspecific differentiation in various crustacean taxa (intraspecific, 0.00–1.47; interspecific, 5.2–31.6; Meyran *et al.*, 1997; Baldwin *et al.*, 1998; Chu *et al.*, 1999; Harrison and Crespi, 1999), and suggest that *D. kaoriae* is an independent species from its congeners.

In the Kumanoe River, *D. kaoriae* was found mainly on sandy bottoms, and *D. japonicus* under cobbles or among pebbles (Miura, unpublished). Both species were sometimes obtained in a same hand-net catch in the Kumanoe River. In other river estuary systems, *D. japonicus* also inhabits intertidal cobble shores (Fukui and Wada, 1996 as *D. tondensis*), while *D. cristatus* lives on muddy sand bottoms (tentatively more muddy than the habitat of the new species) and often co-occurs with *Cleistostoma dilatatum* (Ono, 1965; Miyake, 1983; Miura *et al.* 2004).

Female specimens of *D. kaoriae* have very interesting hook-like projections just behind the genital opening. These may aid copulation in grasping the very long male gonopods. The anterior parts of female sternite 5 are also swollen as knob-like projections in *D. japonicus*, but not elongated as are those in *D. kaoriae*. These external hook-like projections associated with the female genital organ and their function remain unknown and need further study.

ACKNOWLEDGMENTS

We are grateful to Mrs. Kaori Shin, Ohita City, and Mr. Kaname Miura, Ohmiya Junior High School, for their help in collecting the type material. The first author expresses his sincere gratitude to Dr. Masanori Sato, Kagoshima University, who introduced him to the

type locality (a well-protected and important river estuary in the Kyushu area) of the new species, and to Dr. Hiroshi Suzuki, Kagoshima University, who supplied some important references cited in this study. We acknowledge the helpful comments of Dr. Peter K. L. Ng in improving earlier drafts of this paper. This work was supported in part by funds to KW from the Foundation of River and Watershed Environment Management (FOREM), Japan, and by funds to TM from the Nippon Life Insurance Foundation.

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(Received January 23, 2007 / Accepted July 6, 2007)