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Taxonomic re-examination of the two camptandriid crab species *Deiratonotus japonicus* (Sakai,) and *D. tondensis* Sakai, , and genetic differentiation among their local populations

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Taxonomic re-examination of the two camptandriid crab species *Deiratonotus japonicus* (Sakai, 1934) and *D. tondensis* Sakai, 1983, and genetic differentiation among their local populations

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Abstract

Morphological and genetic characteristics were investigated for two allied brachyuran species, *Deiratonotus japonicus* (Sakai, 1934) and *D. tondensis* Sakai, 1983 (family Camptandriidae), occurring in upstream brackish water areas of western Japan. Our observations on over 400 crab specimens from 13 localities, including the types, revealed that there were many specimens that could not be identified morphologically as either species by their diagnostic characters indicated in original descriptions. We grouped specimens into three morphological types of “*D. japonicus*”, “*D. tondensis*” and species-unknown. Specimens of three morphological types were often found even in the same localities. Moreover, there was no correspondence between the morphological types and mitochondrial DNA haplotypes (COI, 540 bp), which was revealed by phylogenetic analyses using maximum likelihood methods and a statistical-parsimony haplotype network constructed with the TCS computer program. In addition, genetic distance between “*D. japonicus*” type and “*D. tondensis*” type (0–1.7%) were in the intraspecific range of Crustacea. Thus, *D. japonicus* and *D. tondensis* ought to be treated as the same species and the latter nominal species as a junior synonym of the former. Significant genetic differentiation was recognized among populations with a positive correlation between geographic and genetic distances, suggesting isolation by distance. In consequence, a redescription of the newly defined *Deiratonotus japonicus* is presented, taking into account the newly recognized morphological variability in this species.

Keywords: *Brackish waters*, *Deiratonotus*, *genetic differentiation*, *mtDNA COI region*, *synonym*

Introduction

Two allied brachyuran species of the family Camptandriidae, *Deiratonotus japonicus* (Sakai, 1934) and *D. tondensis* Sakai, 1983, have been known to be endemic to Japan,

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occurring in upstream brackish-water areas (Sakai 1976, 1983). Records of the two species have been limited to several localities of western Japan: *D. japonicus* from eight localities from Kanagawa Prefecture to Okinawa Prefecture (Sakai 1934, 1976; Terada 1995; Yamanishi et al. 2001; Nakasone and Irei 2003), *D. tondensis* from three localities of Wakayama Prefecture and Okinawa Prefecture (Sakai 1983; Nakasone and Irei 2003). Regardless of these records, it is doubtful whether the two species should be treated as different species, because there are many *Deiratonotus* individuals with a combination of morphological characters of *D. japonicus* and *D. tondensis*. Hence, the validity of morphological characteristics distinguishing between the two species should be reconsidered.

Limited distribution of the two species in upstream brackish-water areas, on the other hand, is expected to enhance genetic differentiation among the local populations, even though they have a planktonic larval stage (Terada 1995). Recently, genetic differentiation has been explored for littoral decapod crustaceans even having a planktonic larval stage. Some studies using mitochondrial DNA (mtDNA) revealed clear genetic differentiation among populations on a geographic mesoscale (tens to thousands of kilometres), e.g. the white shrimp *Litopenaeus schmitti* (Burkenroad) on the Cuban coast (Borrell et al. 2004), the European lobster *Homarus gammarus* (Linnaeus) along the European coast (Triantafyllidis et al. 2005), the hermit crab *Pagurus longicarpus* Say in the eastern USA (Young et al. 2002), and the mud crab *Scylla serrata* (Forsk.) in the Indian Ocean (Fratini and Vannini 2002), whereas no genetic differentiation among populations has been found in others, e.g. the blue crab *Callinectes sapidus* Rathbun in the eastern USA (McMillen-Jackson and Bert 2004), and the swimming crab *Callinectes bellicosus* (Stimpson) in the Gulf of California (Pfeiler et al. 2005).

In the present study, we verified the validity of morphological characteristics distinguishing *D. japonicus* and *D. tondensis*, based on type specimens, their original descriptions and many specimens collected from wide areas in distributional ranges of these species. We also examined the genetic characteristics of the two nominal species using mtDNA sequence data, from which the genetic difference between the two species and genetic structure of them were investigated.

Materials and methods

Specimens

Type specimens were examined for *Deiratonotus japonicus* (three of indeterminable number of syntypes: KPH-NH0107898; most disarticulated) deposited in Kanagawa Prefectural Museum, Japan and *D. tondensis* (holotype: Senckenberg-Mus. 28971; two of three paratypes: Senckenberg-Mus. 28972; another specimen is of *Ptychognathus ishii* Sakai) in Senckenberg Museum, Germany.

Specimens of the two species were obtained from 13 localities of western Japan for morphological observation and DNA analysis (Figure 1; Table I). Specimens of *Deiratonotus cristatus* (de Man, 1895), the only species in the same genus, were also collected in the Tonda River Estuary in Wakayama Prefecture (Osaka Museum of Natural History, Japan, OMNH-Ar 6877) and the Shimanto River Estuary in Kohchi Prefecture (OMNH-Ar 6878) to use as outgroups in DNA analysis. Parts of the specimens were deposited at Osaka Museum of Natural History, Japan, and others are stored by the authors.

Figure 1. Sampling localities of *Deiratonotus* specimens. 1, Isuzu R., Shizuoka; 2, Yukashi Lagoon, Wakayama; 3, Ohta R., Wakayama; 4, Tsun R., Wakayama; 5, Hiki R., Wakayama; 6, Hukuro R., Wakayama; 7, Tonda R., Wakayama; 8, Urauchi Bay, Kohchi; 9, Fukiage R., Kohchi; 10, Shimanto R., Kohchi; 11, Takahama R., Kumamoto; 12, Amikake R., Kagoshima; 13, Miyashi R., Kagoshima.

Morphological comparison

A total of 435 specimens were collected at 13 localities in 1981, 2001, and 2002, and their morphology studied to identify the species, including the measurement of carapace width (CW). Morphological characteristics in which *D. japonicus* and *D. tondensis* differ were

Table I. Data of specimens for morphological observation.

Locality		Morphological type ^b						
No. ^a	Name	Male			Female			Carapace width (mm)
		I	II	III	I	II	III	
1	Isuzu R., Shizuoka	6 (5)			9			5.5–10.7
2	Yukashi Lagoon, Wakayama	2		2	2		3	5.0–6.8
3	Ohta R., Wakayama	8		6 (5)	21		14	4.3–10.2
4	Tsuni R., Wakayama	5 (5)	1 (1)	14 (4)	13		19	3.7–10.7
5	Hiki R., Wakayama	6 (5)	2 (1)	23 (5)	20		10	4.8–10.5
6	Fukuro R., Wakayama	4		2	17		3	4.4–8.8
7	Tonda R., Wakayama	6 (5)	11 (5)	29 (5)	29		17	4.2–10.2
8	Urauchi Bay, Kohchi	6 (1)			7 (4)		2	4.9–10.0
9	Fukiage R., Kohchi				1		1	9.3
10	Shimanto R., Kohchi			3 (3)	7 (2)		1	5.8–7.1
11	Takahama R., Kumamoto	6		13 (5)	10		9	6.9–10.7
12	Amikake R., Kagoshima	3		10 (5)	17		3	4.3–10.9
13	Miyashi R., Kagoshima	9 (4)		3 (1)	12		8	4.5–11.4

Numbers in parentheses indicate the numbers of specimens used in DNA analysis. ^aLocality no. corresponds to that in Figure 1. ^bMorphological type I, *Deiratonotus japonicus*; II, *D. tondensis*; III, unknown.

enumerated from their original descriptions (Sakai 1934, 1983). Based on three such characteristics shown in Table II, we assigned each specimen to a species. The specimens which had all three characteristics of *D. japonicus* were classified into '*D. japonicus*'-morphological type, and those of *D. tondensis* into '*D. tondensis*'-morphological type. The other specimens with combinations of characteristics of both species were treated as species-unknown-morphological type.

DNA analysis

Five to 15 specimens preserved in 100% ethanol were chosen from each locality (except for localities 2, 6, and 9) and used for DNA analysis. Each sample was assigned to a morphological type as described above (Table I). The specimens used for the analysis are deposited at Osaka Museum of Natural History, Japan (OMNH-Ar 6867–6891).

Total genomic DNA was extracted from musculature of each crab using SIGMA GenElute[™] Mammalian Genomic DNA Kit. The target DNA segment of a portion of the mitochondrial cytochrome oxidase subunit I (COI) was amplified by the polymerase chain reaction (PCR), with primers mtd10 5'-TTGATTTTTTGGTCATCCAGAAGT-3' (Roehrdanz 1993) and C/N2769 5'-TTAAGTCCTAGAAAATGTTGRGGGA-3' (Gopurenko et al. 1999). PCR amplification was conducted in a total volume of 25 µl

Table II. Morphological characteristics that are different in the original descriptions between *Deiratonotus japonicus* and *D. tondensis*.

Character	<i>D. japonicus</i>	<i>D. tondensis</i>
Ridges on carapace surface	Clear	Not clear
Granules on carapace surface	Present	Absent
Hairiness of ambulatory legs	Sparse	Thick

containing 0.125 µl of TaKaRa Ex Taq[™] (5 units/µl), 2.5 µl of 10 × Ex Taq[™] buffer, 2.0 µl of dNTP mixture (2.5 mM each), 0.5 mM of each primer, and 1.0 µl of template. PCR conditions comprised 35 cycles of denaturation (94°C, 30 s), annealing (52–53°C, 30 s), and extension (72°C, 1 min) on a GeneAmp 2400 thermal cycler (Applied Biosystems). Amplification products were checked for size by loading 5 µl on a 1% agarose gel (TaKaRa) with 0.5 µg/ml ethidium bromide. The remaining product was purified using ExoSAP-IT (usb), and sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (ABI) with an automated DNA sequencer (Genetic Analyzer 310, ABI). Sequencing reactions followed the protocol suggested by the manufacturer. All final sequences were obtained from both strands for verification and a sequence of ingroup and that of outgroup were deposited at DDBJ (accession numbers AB188112 and AB188859, respectively).

Genetic differentiation and phylogenetic analysis

To determine which model of DNA substitution best fitted our COI sequence data, we used the program Modeltest 3.0 (Posada and Crandall 1998), which uses Akaike Information Criterion. The selected model was TrN (Tamura and Nei 1993)+I, a special case of the general time reversible model with the following substitution rates: A-C=1.0000, A-G=35.8810, A-T=1.0000, C-G=1.0000, C-T=14.3334, G-T=1.0000; and base frequencies: A=0.2858, C=0.1702, G=0.1750, T=0.3691. The proportion of invariable sites was found to be 0.8388.

Phylogenetic relationships among haplotypes were analysed by maximum likelihood (ML) methods with the TrN+I model using PAUP* version 4.0b10 (Swofford 2002), with 1000 bootstrap replications. A statistical-parsimony haplotype network was constructed with the TCS computer program (Clement et al. 2000).

Genetic distances between specimens classified as '*D. japonicus*'-type and those classified as '*D. tondensis*'-type were calculated using TrN+I distances with PAUP*. Pairwise F_{ST} values based on sequence difference (Wright 1951; Excoffier et al. 1992), computed with Arlequin version 2.001 (Schneider et al. 2001), were used as the index of genetic differentiation between populations, with the significance calculated by means of 100,000 permutations with Arlequin version 2.001 and the significance was corrected by sequential Bonferroni methods (Rice 1989). A Mantel (1966) test (10,000 permutations) was carried out to test whether genetic distances [$F_{ST}/(1-F_{ST})$] (Rousset 1997) were correlated with geographic distances (the shortest distance between sites in sea area).

Results

Morphological observations

Male specimens of '*D. japonicus*'-morphological type were found at all localities except for two localities (locality nos 9 and 10) of Kohchi Prefecture (the Fukiage R. and the Shimanto R.), whereas specimens of '*D. tondensis*'-type were only found at three localities (locality nos 4, 5, and 7) of Wakayama Prefecture (the Tsuni R., the Hiki R., and the Tonda R.) (Table I). Many male specimens of the species-unknown-type were collected at all localities except for three localities of the Isuzu River (Shizuoka Pref.; locality no. 1), Urauchi Bay (Kohchi Pref.; locality no. 8), and the Fukiage River (Kohchi Pref.; locality no. 9). Female specimens of '*D. japonicus*'-type were found at all localities, but no female specimens of '*D. tondensis*'-type were

found at any locality. Female specimens of the species-unknown-type were caught at all localities except for the Isuzu River (Shizuoka Pref.; locality no. 1).

DNA analysis

DNA segments of 540 base pairs (bp) of the mitochondrial COI gene were sequenced and used for DNA analyses. Out of all sequenced specimens ($n=71$), we identified 34 different mtDNA haplotypes (Appendix 1) with a total of 36 variable sites, with no found insertions or deletions. Among the variable sites, there were 20 informative sites for phylogenetic analysis.

Haplotype Dj1, 2, 10, 11, 15, 19–25, and 33 were obtained from specimens of '*D. japonicus*'-morphological type and haplotype Dj9, 14, 16, and 18 were from those of '*D. tondensis*'-type. In contrast, haplotype Dj3 contained all three morphological types ('*D. japonicus*', '*D. tondensis*', and species-unknown) and haplotype Dj4, 13, and 34 contained two morphological types of '*D. japonicus*' and species-unknown, and haplotype Dj17 contained two morphological types of '*D. tondensis*' and species-unknown. Additional haplotypes were obtained from specimens of the species-unknown type. Among the 34 haplotypes from all localities, haplotype Dj3, 4, 13, 25, and 27 were recorded from two or more localities, but others were limited to one locality (Appendix 2).

A parsimonious haplotype network coincided with the ML tree (Figure 4). The phylogenetic relationship revealed that haplotypes from each morphological type showed no close relationship with one another (Figure 4). Genetic distances between specimens of '*D. japonicus*'-type and those of '*D. tondensis*'-type were $0\text{--}1.68\%$ ($\text{mean} \pm \text{SD} = 0.84 \pm 0.31\%$). Genetic distances within specimens of '*D. japonicus*'-type and those of '*D. tondensis*'-type were $0\text{--}1.91\%$ ($0.98 \pm 0.43\%$) and $0\text{--}1.10\%$ ($0.77 \pm 0.18\%$), respectively. Haplotypes from the Miyashi River (haplotype Dj33, 34; locality no. 13) formed a clade. Specimens within each locality of the Isuzu River (haplotype Dj1–3; locality no. 1), the Ohta River (haplotype Dj3–6; locality no. 3), Urauchi Bay (haplotype Dj21–25; locality no. 8), and the Amikake River (haplotype Dj27, 32; locality no. 12) revealed close genetic relationship with each other, respectively.

Pairwise F_{ST} values (Table III) showed a tendency for genetic differentiations between populations ($P < 0.05$ in comparisons of nine out of all 45 pairs). The population pairs showing the significant differentiation were the Tsuni River (locality no. 4) versus the Urauchi Bay (locality no. 8), the Amikake River (locality no. 12) and the Miyashi River (locality no. 13), the Hiki River versus the Urauchi Bay, the Takahama River (locality no. 11), the Amikake River and the Miyashi River, and the Tonda River (locality no. 7) versus the Urauchi Bay and the Miyashi River ($F_{ST} = 0.331\text{--}0.763$), with geographic distances of 180–800 km. There was significantly positive correlation between geographic distance and $F_{ST}/(1 - F_{ST})$ (Mantel test, $P < 0.01$; Figure 5).

Discussion

Deiratonotus japonicus and *D. tondensis* have been described as having different morphological characteristics from each other for the texture of the carapace surface and hairiness of ambulatory legs (Sakai 1934, 1983). However, many specimens that we collected from various localities could not be identified as either species, because they had morphological characteristics of *D. japonicus* and *D. tondensis* combined. In addition, the morphological types *D. japonicus* and *D. tondensis* were recorded sympatrically: the former

Table III. Pairwise F_{ST} value between populations in *Deiratonotus japonicus*.

Locality	Isuzu R.	Ohta R.	Tsuni R.	Hiki R.	Tonda R.	Urauchi B.	Shimanto R.	Takahama R.	Amikake R.
Ohta R.	0.452								
Tsuni R.	0.254	−0.015							
Hiki R.	0.326	0.234	0.199						
Tonda R.	0.204	0.128	0.088	−0.001					
Urauchi B.	0.620	0.596	0.560*	0.383*	0.415*				
Shimanto R.	0.215	0.245	0.201	0.084	0.056	0.167			
Takahama R.	0.309	0.369	0.287	0.331*	0.255	0.479	0.097		
Amikake R.	0.409	0.462	0.333*	0.381*	0.274	0.587	0.188	0.290	
Miyashi R.	0.861	0.853	0.763*	0.757*	0.704*	0.821	0.696	0.734	0.822

* $P < 0.05$. Probability was calculated from 100,000 replications and corrected with sequential Bonferroni methods for multiple tests.

from all localities and the latter from localities in Wakayama Prefecture (locality nos 4, 5, and 7). These findings suggest that morphological characteristics characterizing the two species are insufficient to distinguish them into different taxonomic groups. On the other hand, mtDNA analysis showed that haplotype Dj3 was present in all morphological types of '*D. japonicus*', '*D. tondensis*', and species-unknown, haplotype Dj17 from morphological types '*D. tondensis*' and species-unknown, and haplotype Dj4, 13, and 34 from those of '*D. japonicus*' and species-unknown. Moreover, the haplotype network revealed no two distinctive groups between the morphological types '*D. japonicus*' and '*D. tondensis*', and the genetic distance between the two different types (0–1.68%) was equivalent to those within the morphological types. These values are inferior to the limit of intraspecific differentiation in several crustacean taxa (intraspecific, 0.0–3.47%; interspecific, 5.2–31.6%) (Meyran et al. 1997; Baldwin et al. 1998; Chu et al. 1999; Harrison and Crespi 1999).

Thus, our data on geographic distribution, morphological characteristics, and genetic relationship in *D. japonicus* and *D. tondensis* provide no clear basis for distinctness of the two species, suggesting that the morphological features having characterized the two species are intraspecific variation. Therefore, we conclude that *D. tondensis* is a junior synonym of *D. japonicus* and the former name becomes invalid for taxonomy. Recently, it has been reported in brachyuran crabs that morphologically similar pairs of species are genetically identical (Schubart 2001; Spivak and Schubart 2003), as in the case of *D. japonicus* and *D. tondensis*.

Though *D. japonicus* has been known to have a marine planktonic larval development (Terada 1995), high F_{ST} values (0.331–0.763) between populations indicate that local populations in this species, including '*D. tondensis*', have genetically differentiated. There have been some studies addressing genetic differentiation using mtDNA data among populations for brackish-water animals having a planktonic phase. The batillariid snail *Batillaria multiformis* (Lischke) occurring on the Japanese coast has been found to show no genetic differentiation among populations (Kojima et al. 2003). On the other hand, in the mud crab *Scylla serrata* living in estuarine and mangrove waters of the Indian Ocean, genetic differentiations were exhibited between populations separated by about 1000 km (Fratini and Vannini 2002). In the case of the gobiid fish *Tridentiger obscurus* (Temminck and Schlegel) and *T. brevispinis* Katsuyama, Arai and Nakamura inhabiting brackish water and fresh water throughout the Japanese coastline, there were clearly intraspecific genetic differentiations among three areas along the Kuroshio current, along the Tsushima current,

and along the Oyashio current (Mukai et al. 1997). For the tidewater goby *Eucyclogobius newberryi* (Girard) inhabiting discrete, seasonally closed estuaries and lagoons of the California coastline, genetic differentiations were shown in the scale of tens to hundreds of kilometres (Dawson et al. 2001). Our study has found genetic differentiations between *D. japonicus* populations separated on relatively small geographic scale such as 180 km, at least. Thus, genetic differentiation among populations of *D. japonicus* seems to be relatively large in comparison with other brackish-water animals previously investigated for genetic differentiation.

The habitat of *D. japonicus* has been known to be limited to the upper region of brackish areas (Fukui and Wada 1986; Yamanishi et al. 2001). Because of such habitat characteristics, planktonic larval dispersal may be strongly restricted in each estuary. As a similar example, the brackish-water snail *Hydrobia ventrosa* (Montagu) revealed clearer genetic differentiations between local populations than the related marine snail *H. ulvae* (Pennant), though *H. ventrosa* has no planktonic larval stage (Wilke and Davis 2000). It is necessary in studying genetic differentiation to consider more closely the effect of the difference of habitat or life-cycle characteristics.

Redescription of *Deiratonotus japonicus* (Sakai, 1934)

Deiratonotus japonicus (Sakai, 1934)

(Figures 2, 3, 6)

Paracleistostoma japonicum Sakai 1934, p 321–322, Figure 26; Sakai 1935, p 219–221, Figure 117, Plate 60 Figure 3; Sakai 1939, p 634, Figure 104, Plate 68 Figure 3.

Camptandrium japonicum: Sakai 1976, p 618–619, Plate 212 Figure 1.

Deiratonotus japonicus: Miyake 1983, p 164, Plate 55–56; Wada 1995, p 417, Plate 118–10; Nakasone and Irei 2003, p 271, Figure 37.

Figure 2. A male specimen having morphological characteristics of *Deiratonotus japonicus*, collected in the Isuzu River, Shizuoka (OMNH-Ar 6879). Scale bar: 5 mm.

Figure 3. A male specimen having morphological characteristics of *Deiratonotus tondensis*, collected in the Tonda River, Wakayama (OMNH-Ar 6885). Scale bar: 5 mm.

Deiratonotus tondensis Sakai 1983, p 41–42, Plate 8-D; Nakasone and Irei 2003, p 271, Figure 37.

Material examined

Syntypes: three males, Shimoda, Shizuoka Prefecture, January 1933, collected by T. Sakai (KPH-NH0107898); one male (10.36 mm CW), Tonda R., Wakayama Pref., November 1977, coll. T. Sakai (Senckenberg-Mus. 28971); one male (6.12 mm CW), one female (10.10 mm CW), Isuzu River, Shizuoka Pref., 8 August 2002, coll. K. Wada (OMNH-Ar 6879); one male (5.84 mm CW), Yukashi Lagoon, Wakayama Pref., 26 April 2002, coll. K. Wada and M. Kawane (OMNH-Ar 6880); one male (9.80 mm CW), Ohta R., Wakayama Pref., 27 April 2002, coll. K. Wada and M. Kawane (OMNH-Ar 6881); one male (9.43 mm CW), Tsuni R., Wakayama Pref., 27 April 2002, coll. K. Wada and M. Kawane (OMNH-Ar 6882); one male (9.14 mm CW), one female (9.55 mm CW), Hiki R., Wakayama Pref., 27 April 2002, coll. K. Wada and M. Kawane (OMNH-Ar 6883); one male (7.81 mm CW), one female (7.68 mm CW), Fukuro R., Wakayama Pref., 27 April 2002, coll. K. Wada and M. Kawane (OMNH-Ar 6884); two males (7.41–8.94 mm CW), one female (7.23 mm CW), Tonda R., Wakayama Pref., 14 March 2002, coll. K. Wada and M. Kawane (OMNH-Ar 6885); one male (5.91 mm CW), one female (8.30 mm CW), Urauchi Bay, Kohchi Pref., 10 July 2002, coll. K. Wada (OMNH-Ar 6886); one female (9.30 mm CW), Fukiage R., Kohchi Pref., 13 July 2002, coll. K. Wada (OMNH-Ar 6887); one female (6.28 mm CW), Shimanto R., Kohchi Pref., 12 July 2002, coll. K. Wada (OMNH-Ar 6888); one male (7.52 mm CW), one female (8.96 mm CW), Takahama R., Kumamoto Pref., 8 August 2001, coll. S. Yodo (OMNH-Ar 6889); one male (7.13 mm

Figure 4. Parsimony network of mtDNA COI haplotypes from *Deiratonotus* specimens. Haplotypes correspond to Table III. Solid lines show branches coincided with those of ML tree. Bootstrap probabilities (>50%) of 1000 replications in ML analysis are shown on internodes. Oval size indicates the number of each haplotype. Large oval, $n=14$; second, $n=8$; third, $n=4$; fourth, $n=2$; small oval, $n=1$. A haplotype of *Deiratonotus cristatus* from two localities was used as an outgroup.

CW), one female (9.39 mm CW), Amikake R., Kagoshima Pref., 2 October 2002, coll. T. Kishino (OMNH-Ar 6890); one male (6.98 mm CW), one female (7.36 mm CW), Miyashi R., Kagoshima Pref., 18 April 2001, coll. A. Nomoto (OMNH-Ar 6891).

Diagnosis

Carapace flat and anteriorly broadened. Anterolateral borders cut into three obtuse teeth, the foremost of which corresponds to outer orbital angle. Chelipeds equal and slender in both sexes but some male individuals have one larger. Tips of fingers spoon-shaped and somewhat hairy. Anterior pleopod strongly bent basally and pointed, with sparse hairs.

Description

Carapace flat and anteriorly broadened. Granulation on carapace visible under the microscope but some individuals have no granulation. A number of dull elevations on gastric, cardiac, hepatic, and branchial region of carapace. Anterolateral borders cut into three obtuse teeth, the foremost of which corresponds to outer orbital angle. Posterolateral

Figure 5. Relationship between geographic distance and $F_{ST}/(1-F_{ST})$ in *Deiratonotus* populations. Solid circles represent locality combinations showing significant F_{ST} values ($P < 0.05$) and open circles represent locality combinations showing insignificant F_{ST} values.

borders rapidly converge backwards. Posterior border straight and much wider than front. Front narrow, with anterior border about one-third of carapace width and divided into two lobules. Eyestalk thick, much shorter than front, and cornea does not reach outer orbital angle. Basal segment of antenna short and quadrate, placed in the orbital hiatus. Epistome short and pterygostomian regions furry. Ischium of outer maxilliped as long as its width, its antero-inner angle triangularly produced. Merus somewhat broader than its length and as long as ischium, its external angle rounded.

Chelipeds equal and slender in both sexes but some male individuals have one larger, with a large truncated tooth near base of movable finger. Tips of fingers spoon-shaped and somewhat hairy. Of ambulatory legs, the first and the fourth pairs shorter than the second and third pairs. The latter two longer than the former and about one and a half times as long as carapace. Each segment very sparsely hairy in both sexes but some males thickly hairy.

The first segment of the male abdomen very short, and the second to fourth segments fused together, and the fifth segment narrowed in the distal portion. Female abdomen broad and telson widely triangular. Anterior male pleopod strongly bent basally and pointed, with sparse hairs.

Distribution

Distributed in western Japan from Kanagawa Prefecture to Okinawa Prefecture: Hayama, Kanagawa Pref.; Izu Shimoda, Shizuoka Pref.; Isuzu R., Shizuoka Pref.; Owase, Mie Pref.; Yukashi Lagoon, Ohta R., Tsuni R., Hiki R., Fukuro R., Tonda R., Wakayama Pref.; Urauchi Bay, Fukiage R., Shimanto R., Kohchi Pref.; Kita R., Miyazaki Pref.; Takahama R., Kumamoto Pref.; Amikake R., Miyashi R., Kagoshima Pref.; Aha R., Fuku R.,

Figure 6. Male and female specimens of *Deiratonotus japonicus*. (A–C) Male from the Isuzu River, Shizuoka (OMNH-Ar 6879); (D) male from the Tonda River, Wakayama (OMNH-Ar 6885); (E) female from the Isuzu River, Shizuoka (OMNH-Ar 6879). (A, D) Cheliped; (B, E) abdomen; (C) first pleopod. Scale bar: 1 mm.

Takazato R., Okinawa Pref. (Sakai 1934, 1976, 1983; Terada 1995; Yamanishi et al. 2001; Nakasone and Irei 2003; this study).

Remarks

Deiratonotus tondensis Sakai, 1983 has been assigned to junior synonymy with *D. japonicus*, as shown in the present study. This species differs in features of the carapace, chelipeds, and the first pleopod of the male from a congeneric species, *Deiratonotus cristatus* (De Man,

1895), which is the type-species of the genus. The carapace of *D. cristatus* has a transverse ridge on cardiac and mesobranchial regions and the lateral margins are closely pubescent without teeth, while *D. japonicus* has no ridge on carapace, lateral margins are not pubescent and have three teeth. Chelipeds are hairy at the tip in *D. japonicus*, but not in *D. cristatus*. The male pleopod has four stout subdistal spines on the tip in *D. cristatus*, while there are no spines in *D. japonicus*.

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Appendix 1. Variable nucleotide sites from 540 bp sequences of mtDNA COI region in *Deiratonotus* specimens

	Nucleotide no. ^a																																					
Haplotype	13	19	28	35	46	52	58	61	67	130	136	199	235	244	247	250	253	256	271	277	298	325	349	406	409	430	433	442	448	450	478	482	487	505	526	529		
Dj1 ^b	G	G	A	C	A	G	C	A	G	C	T	A	G	G	C	C	T	T	T	T	G	A	C	C	A	T	G	C	G	C	T	T	C	A	A	T		
Dj2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	
Dj3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	
Dj4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.
Dj5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	G	G	.	
Dj6	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	
Dj7	.	.	.	.	.	.	.	G	.	A	.	G	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	
Dj8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	
Dj9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	
Dj10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	
Dj11	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	
Dj12	A	.	.	.	T	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	
Dj13	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	
Dj14	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	
Dj15	.	A	.	.	.	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	
Dj16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	
Dj17	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	
Dj18	.	.	G	.	.	A	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	
Dj19	.	.	.	A	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	
Dj20	.	.	.	.	.	T	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	G	.	.		
Dj21	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	C		
Dj22	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	G	.	.		
Dj23	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	C	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	G	.	.		
Dj24	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.		
Dj25	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.		
Dj26	.	A	.	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.		
Dj27	.	.	G	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	G	.	.		
Dj28	.	.	.	.	.	.	.	.	A	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.		
Dj29	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.		
Dj30	.	.	.	.	.	.	.	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	A	.	.	G	.	.		
Dj31	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.		
Dj32	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	G	.	.		
Dj33	.	.	.	.	.	A	.	.	A	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	G	.	.		
Dj34	.	.	.	.	.	A	.	.	A	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	G	.	.	.		

Dots indicate the same state as in first haplotype. ^aNucleotide no. refers to position beginning from 5' end; ^bDDBJ accession number of Dj1 is AB188112.

Appendix 2. Haplotype composition of each population in 10 localities

Locality no. ^a	Haplotype																																		Total		
	Dj1	Dj2	Dj3	Dj4	Dj5	Dj6	Dj7	Dj8	Dj9	Dj10	Dj11	Dj12	Dj13	Dj14	Dj15	Dj16	Dj17	Dj18	Dj19	Dj20	Dj21	Dj22	Dj23	Dj24	Dj25	Dj26	Dj27	Dj28	Dj29	Dj30	Dj31	Dj32	Dj33	Dj34			
1	2	1	2																																	5	
3			1	2	1	1																														5	
4			3	2			1	1	1	1	1																									10	
5			1	1								1	6	1	1																					11	
7			4	3									2			1	2	1	1	1																15	
8																					1	1	1	1	1											5	
10			2																							1	1	1									5
11			1																										1	1	1	1					5
12																												1					4				5
13																																		1	4		5
Number of localities	1	1	7	4	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	2	1	2	1	1	1	1	1	1	1	1		

^aLocality no. corresponds to that in Figure 1.