Corresponding author

Noriko AZUMA

Division of Marine Bioscience, Graduate School of Fisheries Sciences, Hokkaido University,

3-1-1 Minato, Hakodate, 041-0821, JAPAN

e-mail; anoriko@fish.hokudai.ac.jp

Tel & FAX; 81-138-40-8864

Genetic variation and population structure of hair crab (*Erimacrus isenbeckii*) in Japan inferred from mitochondrial DNA sequence analysis

Noriko Azuma[1], Yasushi Kunihiro[2], Jun Sasaki[2], Eiji Mihara[3], Yukio Mihara[4], Tomoaki Yasunaga[5], Deuk-Hee Jin[6], Syuiti Abe[1],

[1] Division of Marine Bioscience, Graduate School of Fisheries Sciences, Hokkaido University, Japan.

[2] Hokkaido Mariculture Fisheries Experiment Station, Japan.

[3] Hokkaido Wakkanai Fisheries Experiment Station, Japan.

[4] Hokkaido Central Fisheries Experiment Station, Japan.

[5] Hokkaido Kushiro Fisheries Experiment Station, Japan.

[6] Kangnung National University, Korea.

ABSTRACT

Genetic variation and population structure of hair crab (*Erimacrus isenbeckii*) were examined using nucleotide sequence analysis of 580bp in the 3' portion of the mitochondrial cytochrome *c* oxidase subunit I gene (COI) of 20 samples collected from 16 locales in Japan (the Hokkaido and Honshu Islands) and one in Korea. A total of 27 haplotypes was defined by 23 variable nucleotide sites in the examined COI region. Pairwise population F_{ST} estimates and neighbor-joining tree inferred distinct genetic differentiation between the representative samples from the Pacific Ocean off the Eastern Hokkaido Island and the Sea of Japan, while others intermediated these two groups. AMOVA also showed a weak but significant differentiation among these three groups. The present results suggest a moderate population structure of hair crab, probably influenced by high gene flow between regional populations due to sea current dependent larval dispersal of this species.

INTRODUCTION

In many species of sea crab, the ecology and population biology are still poorly understood, mainly due to the difficulty in direct observation of their life history and larval dispersal. Population genetic study with molecular markers can be considered as one of the effective approaches to provide significant information of population structure and genetic diversity in such case. Following the allozyme analysis in the 1990s (McMillen-Jackson et al., 1994; Creasey et al., 1997), population genetic analyses in some crab species were performed with TGGE (Gopurenko et al., 1999) and PCR-PFLP analyses (Yamasaki et al., 2006) of mitochondrial (mt) DNA and microsatellite DNA analysis (Herborg et al., 2007). The sequence analysis of mtDNA has been considered as a useful tool for phylogeny and systematics among closely related crab species (Geller et al., 1997; Schubart et al., 2001; Tang et al., 2003; Imai et al., 2004), and recently used in intraspecific population genetics (Roman and Pulmbi, 2004; Pfeiler et al., 2005; Cassone and Boulding, 2006).

Population genetics of sea crabs in the North Pacific Ocean has so far been limited to tanner crab and king crab for their considerable commercial values as fishery resources around Alaska, using allozyme (Seeb et al., 1989; Seeb et al., 2001), microsatellite DNA (Seeb et al., 2001) and mtDNA markers (Bunch et al., 1998). However, in the Far East, only a preliminary allozyme (Chow et al., 1987) and mtDNA analyses (Azuma et al., 2006) have been attempted for hair crab around the Hokkaido Island, Japan.

Hair crab or horsehair crab (*Erimacrus isenbeckii*) is one of the most important fishery resources around Hokkaido, widely distributed in the North Pacific Ocean and

its adjacent waters, from east coast of the Bering Sea to the Aleutian Islands, westward to the Kuril Islands and southern part of the Sakhalin Islands, and then southward to northern Japan and east coast of the Korean Peninsula. Adults are found on the sand seafloor with the water temperature about 15 ^{o}C below. Offspring hatch in March to May, and the larvae appear as zooplankton in March to July, until they reach to the bottom of the sea in the shape of small crabs (Mihara, 2003). Such a species with long planktonic larval stage for more than a month may be expected to have high dispersal potential causing a decrease in the genetic or demographic differentiations among local populations (Palumbi, 1994), meanwhile the coastal fisheries resource statistics has suggested several distinguishable local groups of hair crab populations around Hokkaido showing different demographic fluctuation (Mihara, 2003).

We recently revealed a certain genetic differentiation among the populations of hair crab in this area using sequence variation in the 3' half of the COI as a molecular marker, discriminating northern samples of Rishiri/Rebun in the Soya coast and Monbetsu and Abashiri in the Sea of Okhotsk coast from southern samples of Kiritappu, Tokachi, Shiraoi, and Oshamanbe in the Pacific Ocean coasts (Azuma et al., 2006). The aim of the present study is to estimate detailed genetic structure of hair crab population around Hokkaido and other northern Japan to provide genetic information available for conservation and stock management of this species, using the mtDNA COI and samples including those newly collected from the distant locales and the previously analyzed ones (Azuma et al., 2006).

MATERIALS AND METHODS

Crab samples and DNA extraction

The samples of Rishiri/Rebun, Monbetsu, Abashiri, Kiritappu, Tokachi, Shiraoi, and Oshamanbe in the previous work (Azuma et al. 2006) were designated RR, MN, AB, KI, TK, SR and OS, respectively, for the present analysis. Newly collected samples were from seven locales including Soya (SY), Nemuro (NE), Kushiro-west (KW), Erimo-west (EW), Erimo-east (EE), Shizunai (SZ), and Otoshibe (OT) from Hokkaido, two including Ishikawa (IS) and Iwate (IW) from Honshu, and one from Korea (KR) (Fig. 1). Muscle samples of the leg were collected from 564 adult individuals of the above 10 newly collected samples of hair crab from Hokkaido and Honshu, Japan, and Korea from 2004 to 2006 (Table 1 and Fig. 1). Two individuals of related species, *Telmessus acutidens* were also sampled as out group in the analysis of haplotype genealogy. Collected samples were stored at -30 ^{o}C, or kept in 99% ethanol at room temperature until DNA extraction.

DNA was extracted as previously (Azuma et al., 2006) from about 20 mg of the stored muscle specimens with a commercially available PureGene kit (Gentra). Extracted DNA was dissolved in 100-500 µl tris-EDTA buffer (TE; 10mM Tris-HCl, and 1 mM EDTA, pH 8.0).

Nucleotide sequence analysis

Following our previous work, the nucleotide sequence of 580 bp in the 3' region of the mtDNA COI was analyzed for the present study. PCR was performed as previously (Azuma et al. 2006) in the reaction mixture containing template DNA, dNTPs, *Taq* DNA polymerase (Sigma) and the hair crab specific primers KBCOImf and BeCOIr2, using the following cycling profile; pre-cycling denaturation at 94 ^{o}C for 3 min, followed by 30-35 cycles of denaturation at 94 ^{o}C for 30 sec, annealing at 49 ^{o}C for 30

sec and extension at 72 °C for 40 sec, with post-cycling extension at 72 °C for 4 min. Low annealing temperature at 46 - 48 °C was also attempted in case of insufficient amplification.

Direct nucleotide sequencing of the 3' portion of COI was performed on PCR products purified with magnetic beads, AMPure (Agencourt). The forward or reverse PCR primers were used for sequencing reaction with a BigDye® Terminator Cycle Sequencing kit version 3.1 (ABI), according to the manufacturer's instruction. The obtained sequence data was aligned by the DNASIS software (Hitachi) to determine the genotypes (haplotypes) of the COI. The haplotype genealogy was resolved with a parsimony network using the TCS Network Program (Clement et al., 2000).

Population genetic data analysis

Arlequin version 2.000 program package (Schneider et al., 2000) was used for estimation of the haplotype and nucleotide diversities within samples and pairwise F_{ST} values between samples (Reynolds et al., 1983) and for exact tests of population differentiation (Raymond and Rousset, 1995), on a total of 20 samples (902 crabs) from 16 Japanese and one Korean locales. Significance of the variance components and F_{ST} values was tested with a permutation method. After construction of genetic distance matrix based on nucleotide divergence between samples estimated according to Nei (1987) and Nei and Tajima (1981), a neighbor-joining (NJ) tree was constructed for each replicated distance matrix from bootstrap resampling of 1000 replication with a NEIGHBOR in PHYLIP version 3.5 software package (Felsenstein, 1993), and the consensus tree was generated following 50% majority rule using CONSENSUS in the same package of PHYLIP. Based on the F_{ST} estimates, the results of the exact tests,

and the topology of the NJ consensus tree, the samples was divided into subgroups, and the variance component was evaluated in each hierarchical level by AMOVA (Excoffier et al., 1992) using Arlequin ver. 2.000 for assessing genetic divergence within and among the groups.

RESULTS

Hair crab mtDNA COI haplotypes

As shown in Table 2, the observed 23 variable nucleotide sites of the COI contained 18 synonymous and 5 non-synonymous substitutions, among which transition and transversion were 21 and two, respectively (Table 2). Substitution at each site was biallelic, suggesting the occurrence of single base substitution between sequences and no saturation of substitutions. Total of 27 haplotypes were detected, including newly defined ten in the present study, *EI422*, *EI424*, *EI647*, *EI526*, *EI1014*, *EIK30*, *EIW42*, *EI2225*, *EIW14* and *EII35*, deposited to the DDBJ/EMBL/GeneBank with accession number AB300421 – AB300430, and the previously reported 17 (AB241420 to AB241437, Azuma et al., 2006).

The sequence of the same COI region was successfully determined in *Telmessus acutidens*, and the sequence data were deposited to the DDBJ/EMBL/GeneBank with accession number AB300431. *T. acutidens* is probably one of the closest species to hair crab among extant species in the different genus of the same family. However, nucleotide substitutions between *T. acutidens* and hair crab reached up to more than 60 nucleotides, so that the genetic distance between these species was too large to recruite *T. acutidens* as an outgroup in haplotype analysis. The rooted NJ tree actually showed very low bootstrap support for major branching

among haplotypes of hair crab COI (not shown). Therefore, unrooted network genealogy was much preferable and informative to investigate intraspecific analysis. The parsimony network of the observed 27 haplotypes is presented in Fig. 2, with haplotype abundance in circle size. In the network, focal haplotypes of *EIcom*, *EI26*, *EIR8* and *EI705* were apparent with relatively high abundance, from which other infrequent haplotypes were radiated with a single subsitution, suggesting that focal haplotypes were old enough to be considered as a tool of population genetics. The distribution of haplotypes among 20 samples of hair crab is presented in Table 3. The occurrence of haplotypes was different from one sample to another, although *EIcom* haplotypes commonly occurred in all the samples.

Population genetic analysis

Table 1 showed the haplotype and nucleotide diversities and the frequencies of focal haplotypes in each samples. Both diversity indices were highest in RR, followed by IS and MN. The frequencies of the *EIcom* were higher than 75% in NE, KI, KW, TK, EW and OS2 from the Pacific Ocean off Hokkaido. The frequencies of the *EIR8* were high in samples from the Sea of Japan and the Sea of Okhotsk, while the haplotype *EI26* is abundant in several samples from the Pacific Ocean off the eastern Hokkaido. Diversity parameters seems affected by the observed haplotype numbers and the frequency of *EIcom*, as RR, IS and MN contained relatively high number of haplotypes and low frequency of *EIcom* compared with other samples. In addition, the samples from the northern part of Hokkaido and the Sea of Japan tended to show increased values of both parameters. These results suggest the larger genetic variation of hair crab from the northern part of Hokkaido and the Sea of Japan than those from the Pacific Ocean off the eastern and other parts of Hokkaido and Honshu.

As shown in Table 4, F_{ST} values were generally low, suggesting small genetic differentiation between samples, probably due to high gene flow among regions. The higher F_{ST} estimates, e.g. exceeding 0.15, were found between the samples from the Pacific Ocean off the eastern and northern parts of Hokkaido or the Sea of Japan (SY2-NE, SY2-TK, RR-KI, IS-NE, IS-KI, IS-KW, IS-TK, IS-EW, IS-EE, IS-SR), between IS and AB, and between IS and OS2 (Table 4). Significantly higher differentiation was shown between IS and all other samples but SY, SY2, RR, MN and SZ, suggesting that IS from the Sea of Japan off the Noto Peninsula, central Honshu, is genetically distinctive from others except for the samples from northern Sea of Japan.

The unrooted consensus tree of hair crab samples also favored weak to moderate branching without distinctive cluster (Fig. 3). The IS, genetically distinctive as suggested by the above F_{ST} estimates, positioned at the end of the tree together with SY, SY2 and RR forming the cluster of the rim of the Sea of Japan, whereas the KI, KW, SR, OS and EW, from the Pacific Ocean off the Hokkaido, were at the other end, and others intermediated these two clusters (Fig. 3). Although the topology of this tree certainly reflected the regional population structure of hair crab, the discrepant geographic relationships among samples, such as the deep split among OS, OS2 and OT, from similar or very close sampling point, and exclusion of KR from the group of the samples from the Sea of Japan, might suggest again that the regional genetic structure is weak and unstable in this species, probably due to high gene flow among regions suggested by the aforementioned F_{ST} test.

Based on the results of F_{ST} estimates, topology of the population NJ tree and geographic positions of sampling sites, the present hair crab samples could tentatively be separated into three groups, A, B and C. The group A contained the samples from

the Pacific Ocean off the eastern parts of Hokkaido (NE, KI, KW, TK, TK2, EE and EW), while the group C included the samples from the Soya Strait (SY and SY2), the boundary of the Sea of Japan and the Sea of Okhotsk, and the Sea of Japan off the northwest Hokkaido, the Noto Peninsula and Korea (RR, IS and KR). The group B included other samples from the Sea of Okhotsk and the Pacific Ocean off the Hokkaido, and those from the Pacific Ocean off the Iwate Prefecture, northern Honshu (MN, AB, OS, OS2, OT, SR, SZ and IW). The clustering of these three groups was favored by AMOVA with low variance component among samples within each group (Table 5). Nevertheless, rather low variance component among three groups (6.05 %) and extremely high component within samples (93.73 %) might also suggest a weak and unstable regional genetic structure in this species, probably due to high gene flow among regions as described above.

Thus, the population genetic data described above suggested genetic differentiation at least between the groups A and C, favoring a structure separating the Rim of the Sea of Japan and the Pacific Ocean off the eastern Hokkaido**.**

DISCUSSION

The sequence data of mtDNA COI has often been used as a tool for population genetics of marine invertebrates, including sea cucumber (Uthicke and Benzie, 2003), sea urchin (Lessios et al., 2001 and 2003), marine shrimp *Penaeus* (Baldwin et al., 1998), green crab (Roman and Palumbi, 2004), swimming crab (Pfeiler et al., 2005), and lined shore crab (Cassone and Boulding, 2006), although similar molecular population genetic studies have been performed with microsatellite DNA and PCR-RFLP of mtDNA control region in Kuruma Prawn (Sugaya et al., 2002) and sequence variation of NCR2

(non cording region) in Japanese scallop mtDNA (Nagashima et al., 2005) and 12S–16SrRNA in sea-star (Waters and Roy, 2004). Our previous study using COI sequence variation inferred the genetic differentiation as predicted between the northern samples (RR and MN in the present study) and others (TK, KI, SR, OS and AB in the present study). Detailed population genetic profile obtained by recruit of additional samples in this study suggests that the differentiation lies not between north and south but basically between the rim of the Sea of Japan and The Pacific Ocean. Although the previous small scale allozyme study (Chow et al., 1987) showed only the probable geographical population structure of hair crab around Japan with excess homozygosity, the present sequence analysis of COI provided more detailed picture of genetic structure, proven its increased potential in population analysis of hair crab.

The parsimony network of the observed 27 haplotypes revealed four focal haplotypes of the mtDNA COI, *EIcom*, *EI26*, *EIR8* and *EI705*, from which other minor ones were radiated. The observed haplotype distribution suggested an association of high frequency of the *EIcom* and *EI26* with the Pacific Ocean and the *EIR8* with the Sea of Japan. In fact, the *EIR8* is more abundant than the *EI26* in the every sample from the Sea of Japan. However, the *EIR8* also occurred more often than the *EI26* in EE, EW and SZ from the Pacific Ocean, which did not favor regional association of haplotype lineages and determination of the exact origin of hair crab in the current species range. These situations may likely favor post-expansion shuffling rather than recent rapid expansion of the major maternal lineages of hair crab.

The nucleotide divergence was less than 1.1% (six substitutions in 580 bp of sequenced region) among the observed focal haplotypes of in the present hair crab samples (Table 2 and Fig. 2), suggesting a shallow haplotype genealogy. Although the

molecular clock estimation for the mtDNA COI in shrimps gave different rate of molecular evolution, 1.4% (Knowlton and Weight, 1998) to 3.0% (Baldwin et al, 1998) per one million year, the divergence among main haplotypes of hair crab was considered to occur in middle or upper Pleistocene, in any estimate.

It has been inferred that the main lineages seemed to be generated by population expansion and isolation with change of sea level and/or sea environment such as water temperature or currents during glacial periods in Pleistocene in many marine invertebrates (Palumbi, 1994; Lessios et al., 2001 and 2003; Uthicke and Benzie, 2003) including crabs (Pfeiler et al., 2005; Cassone and Boulding, 2006; Gopurenko et al., 1999). In such case, a strait, which was once closed as a land bridge or isthmus in glacial periods, likely considered to remain as a border between distinct lineages. However, population structure of hair crab did not show any of clear borders at straits, i.e. the Soya Strait and the Tsugaru Strait. Probably, after established main framework of haplotypes during Pleistocene, haplotypes in each population of hair crab were exchanged and mixed by high gene flow mainly occurring in the larval dispersal.

Passive dispersal of planktonic larvae may be strongly affected by the sea current. The effect of sea current on the population structure was commonly suggested in marine invertebrate (Waters and Roy, 2004; Lessios et al., 2001 and 2003). The results of the present study indicated the breaking between the samples from the sea of Japan and from Pacific Ocean, and the higher diversity in the former than the latter, suggesting asymmetric gene flow probably caused by sea currents. In the Sea of Japan, the Tsushima current forms circulation current with the Liman current. The Tsushima current runs into the Sea of Japan along the Honshu Island, and reaches to the western coast of Hokkaido branching into substreams of the Tsugaru warm current and the Soya

warm current, flowing into west Pacific Ocean and the Sea of Okhotsk, respectively. As the water in main stream of the Tsushima current goes north, it becomes too cool to continue upwards and finally turns around to join to the Liman cold current, which proceeds south along the coasts of Asian continent (Naganuma, 1977; Senju, 1999). This movement of sea water may well explains the hair crab population structure, as samples from the rim of the Sea of Japan shared similar genetic character, and population in the Sea of Okhotsk seemed continuous to the Sea of Japan like the structure in Japanese scallop (Nagashima et al., 2005) and Japanese sandfish (Yanagimoto, 2004). In the Pacific Ocean, the structure seems influenced by both the Tsugaru current and Okhotsk cold current (Oyashio, Kuril current) flowing from northeast to southwest along the Kuril Islands until meet the Tsugaru current or Japan Current, Kuroshio (Watanabe, 1964; Kawai, 1972). While Takayanagi et al. (1999) reported the non random distribution of hair crab larvae affected by the Okhotsk current in the Funka Bay (the Volcano Bay, in western Pacific Ocean, close to the Tsugaru strait), it is also plausible that the Tsugaru current transports larvae to western part of the Pacific Ocean. The present study revealed that western samples in the Pacific Ocean showed lower differentiation to the rim of the Sea of Japan, while the eastern samples were remarkably different from the Sea of Japan samples, suggesting that eastern populations received the dominating effect from the Okhotsk current, and that the effect of the Okhotsk current was moderated by the Tsugaru current in the western populations. The similar population genetic profile among AB, IW and the samples from the western Pacific Ocean may be also explained the balanced effects of these two currents.

A slight genetic differentiation was detected between yearly samples collected from the same locales, namely SY vs. SY2, OS vs. OS2 and TK vs. TK2. Although

none of the pairs showed significant differentiation by F_{ST} and exact tests, but the F_{ST} values were not zero and the consensus tree allocated OS and OS2 on the distant branches, as well as TK and TK2 (Fig. 3). Such temporal genetic variations may reflect the year class structure in this species, because an adults female can spawn only once in every two years. More plausible explanation for such genetic fluctuations may be a possible occurrence of stochastic larval supply as described in sea urchin (Lessios et al., 2003).

The present study suggested not only a considerable extent of gene flow among local populations of hair crab, but also apparent limitation of gene flow to isolate more or less some local groups as previously mentioned by coastal fisheries resource statistics. Therefore, the occurrence of local extinction may not be easily recovered even by such a relatively high gene flow in hair crab, so that the fisheries management would be prerequisite to conservation and sustainable use of crab resources based on the demography in each local area, as well as the larger scale management across whole species range in hair crab.

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REFERENCES

1. Azuma N, Kunihiro Y, Sasaki J, Nozawa Y, Mihara E, Mihara M, Yasunaga, Abe S. (2006). Genetic variation of hair crab (*Erimacrus isenbeckii*) inferred from mitochondrial DNA sequence analysis. *Fish Genet Breed Sci* 35, 35-42
2. Baldwin JD, Bass AL, Bowen BW, Clark WC. (1998). Molecular phylogeny and biogeography of the marine shrimp *Penaeus. Mol Phylogenet Evol* 10, 399–407
3. Bunch T, Highsmith RC, Shields GF. (1998). Genetic evidence for dispersal of larvae of Tanner crabs *(Chionoecetes bairdi)* by the Alaskan Coastal Current. *Mol Mar Biol Biotechnol* 7, 153-159.
4. Cassone BJ, Boulding EG. (2006). Genetic structure and phylogeography of the lined shore crab, *Pachygrapsus crassipes*, along the northeastern and western Pacific coasts. *Mar Biol* 149, 213-226
5. Chow S, Abe S, Ueno Y, Torisawa M, Fujio Y. (1987). Homozygote excess observed in natural population of horsehair crab *Erimacrus isenbeckii*. *Nippon Suisan Gakkaishi* 53, 321
6. Clement M, Posada D, Crandall KA. (2000). TCS: a computer program to estimate gene genealogies. *Mol Ecol* 9, 1657–1659.
7. Creasey S, Rogers A, Tyler P, Young C, Gage J. (1997). The population biology and genetics of the deep-sea spider crab, *Encephaloides armstrongi* Wood-Mason 1891 (Decapoda:. Majidae). *Phil Trans Roy Soc Lond* 352, 356-376
8. Excoffier L, Smouse P, Quattro J. (1992). Analysis of molecular valiance inferred from metric distances among DNA haplotypes: Aplication to human mitochondrial DNA restriction data. *Genetics* 131, 479-491.

9. Felsenstein J. (1993). *PHYLIP (Phylogeny Interference Package) version 3.5c*. Department of Genetics, Washington State University Seattle.
10. Geller JB, Walton ED, Grosholz ED, Ruiz GM. (1997). Cryptic invasions of the crab *Carcinus* detected by molecular phylogeography. *Mol Ecol* 6, 901-906
11. Gopurenko D, Hughes JM, Keenan CP. (1999). Mitochondrial DNA evidence for rapid colonisation of the Indo-West Pacific by the. mud crab *Scylla serrata*. *Mar Biol* 134, 227-233.
12. Herborg LM, Weetman DW, Oosterhout C, Hänfling B (2007). Genetic population structure and contemporary dispersal patterns of a recent European invader, the Chinese mitten crab, *Eriocheir sinensis*. *Mol Ecol* 16, 231–242
13. Imai H, Cheng JH, Hamasaki K, Numachi K. (2004). Identification of four mud crab species (genus Scylla) using ITS-1 and 16S rDNA markers. *Aquat Liv Resourc* 17, 31-34
14. Kawai H. (1972). Hydrography of the Kuroshio Extension. In: Stommel H, Yoshida K (eds). *Kuroshio* University of Tokyo Press, Tokyo. pp 235-352
15. Kimura M. (1980). A simple method for estimating evolutionary rates of base substitutions that compare studies of nucleotidesequences. *J Mol Evol* 16, 111-120.
16. Knowlton N, Weight LA. (1998). New dates and new rates for divergence across the Isthmus of Panama. *Proc R Soc Lond B* 265, 2257-2263
17. Lessios HA, Kessing BD, Pearse JS. (2001). Population structure and speciation in tropical seas: Global phylogeography of the sea urchin *diadema*. *Evolution* 55, 955-975

18. Lessios HA, Kane J, Robertson DR. (2003). Phylogeography of the pantropical sea urchin *Tripneustes*: contrasting patterns of population structure between oceans. *Evolution* 57, 2026–2036.
19. McMillen-Jackson AM, Bert TM, Steele P. (1994). Population genetics of the blue crab *Callinectes sapidus*: modest population structuring in a background of high gene flow. *Mar Biol* 118, 53–65
20. Mihara E. (2003). Kegani *Erimacrus isenbecckii* (Brandt). In Mizushima T, Torisawa M (eds) *Gyogyou Seibutu Zukan, Shin Kita no Sakanatachi*. Hokkaido Shinbun sha, Sapporo, Japan. pp 380-385 (in Japanese)
21. Naganuma K. (1977). The oceanographic fluctuations in the Japan Sea. *Mar Sci* (*Kaiyo Kagaku*) 9, 137–141 (in Japanese with English abstract).
22. Nagashima K, Sato M, Kawamata K, Nakamura A, Ohta T. (2005). Genetic structure of Japanese scallops population in Hokkaido, analyzed by mitochondrial haplotype distribution. *Mar Biotechnol* 7, 1–10.
23. Nei M. (1987). *Molecular Evolutionary Genetics*. Columbia University Press, New York 1987
24. Nei M, Tajima F. (1981). DNA polymorphism detectable by restriction endonucleases. *Genetics* 97, 145-163.
25. Palumbi SR. (1994). Genetic divergence, reproductive isolation, and marine speciation. *A Rev Ecol Syst* 25, 547-572.
26. Pfeiler E, Hurtado LA, Knowles LL, Torre-Cosío J, Bourillón-Moreno L, Márquez-Farías JF, Montemayor-López G. (2005). Population genetics of the swimming crab *Callinectes bellicosus* (Brachyura: Portunidae) from the eastern Pacific Ocean. *Mar Biol* 146, 559–569

27. Raymond M, Rousset F. (1995). An exact test for population differentiation. *Evolution* 49, 1280-1283.

28. Reynolds J, Weir BS, Cockerham CC. (1983). Estimation for the coancestry coefficient: basis for a short-term genetic distance. *Genetics* 105, 767-779.

29. Roman J, Palumbi SR (2004). A global invader at home: population structure of the green crab, *Carcinus maenas*, in Europe. *Mol Ecol* 13, 2891–2898.

30. Schneider S, Roessli D, Excoffier L. (2000). *ARLEQUIN: a software for population genetics data analysis. Version 2.000*. University of Geneva, Geneva, Switzerland.

31. Schubart CD, Cuesta JA, Rodrıguez A. (2001). Molecular. phylogeny of the crab genus *Brachynotus* (Brachyura: Varunidae) based on the 16S rRNA gene. *Hydrobiologia* 449, 41–46

32. Seeb JE, Kruse GH, Seeb LW, Weck RG. (1989). Genetic structure of red king crab stocks in Alaska facilitates enforcement of fishing regulations. *Proceedings of the International Symposium on King and Tanner Crabs, Anchorage, Alaska, USA, November 28-30, 1989. Alaska Sea Grant College Program, Fairbanks*. pp 491-502

33. Seeb LW, Grant S, Templin B, Kretschmer E, Olsen J. (2001). Crab Genetics. In: *King and Tanner Crab research in Alaska; Final Report.* Alaska Department of Fish and Game. Division of Commercial Fisheries. pp14-28

34. Senju T. (1999). The Japan Sea Intermediate Water; Its Characteristics and Circulation. *J Oceanograph* 55, 111-122.

35. Sugaya T, Ikeda M, Taniguchi N. (2002). Relatedness structure estimated by microsatellites DNA and mitochondrial DNA polymerase chain reaction -

restriction fragment length polymorphisms analyses in the wild population of kuruma prawn *Penaeus japonicus*. *Fish Sci* 68, 793-802

36. Takayanagi S, Utoh H, Yorita K, Ueda Y. (1999). Distribution and abundance of larvae of the atelecyclid crabs, *Erimacrus isenbeckii* (BRANDT) and *Telmessus cheiragonus* (TILESIUS) in Funka Bay and adjacent waters during April to early June 1989-1992. *Sci Rep Hokkaido Fish Exp Stn* 55, 79-87 (in Japanese with English abstract).
37. Tang BP, Zhou KY, Song DX, Yang G, Dai AY. (2003). Molecular systematics of the Asian mitten crabs, genus *Eriocheir* (Crustacea: Brachyura). *Mol Phylogenet Evol* 29, 309～316
38. Uthicke S, Benzie JAH. (2003). Gene flow and population. history in high dispersal marine invertebrates: mtDNA. analysis of *Holothuria nobilis* (Echinodermata:. Holothuroidea) populations from the Indo-Pacific. *Mol Ecol* 12, 2635-2648
39. Watanabe K. (1964). Hydrographic and meteorological conditions around Hokkaido. *Bull Coastal Oceanogr* 3, 23-30 (in Japanese).
40. Waters JM, Roy MS. (2004). Phylogeography of a high-dispersal New Zealand sea-star: does upwelling block gene-flow? *Mol Ecol* 13, 2797–2806
41. Yamasaki I, Yoshizaki G, Yokota M, Strüssmann CA, Watanabe S. (2006). Mitochondrial DNA variation and population structure of the Japanese mitten crab *Eriocheir japonica*. *Fish Sci* 72, 299–309.
42. Yanagimoto T. (2004). Geographic population subdivision of the Japanese sandfish, *Arctoscopus japonicus*, inferred from PCR-RFLP analysis on mtDNA. *Nippon Suisan Gakkaishi* 70, 583-591 (in Japanese with English abstract).

Figure legend

Fig. 1 Locations of sampling sites (see Table 1 for sample name). The Korean sample (KR) were bought in the market of Kangnung city, while all other samples were caught in the sea by fisheries research vessels. An arrow indicates the sampling locale of *Telmessus acutidens.*

Fig. 2 A single mininum spanning tree for the 27 haplotypes of mtDNA COI (580 bp) in hair crab presented in Table 2. Circle sizes reflect haplotype abundance.
Closed circle indicates a missing haplotype.

Fig. 3 Unrooted neighbor-joining tree showing genetic distance based on KP2 among 20 samples in hair crab. The inset shows the consensus phenogram with nodal bootstrap values of 1,000 replicates (%).

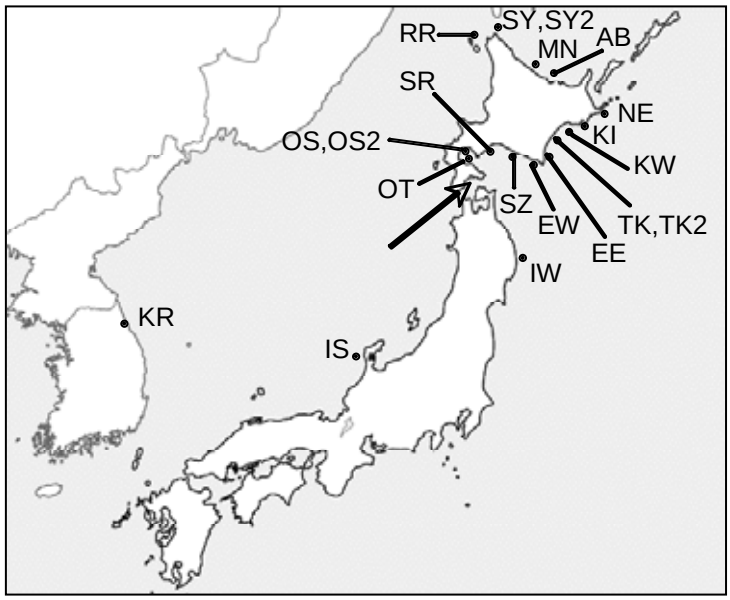

Fig. 1

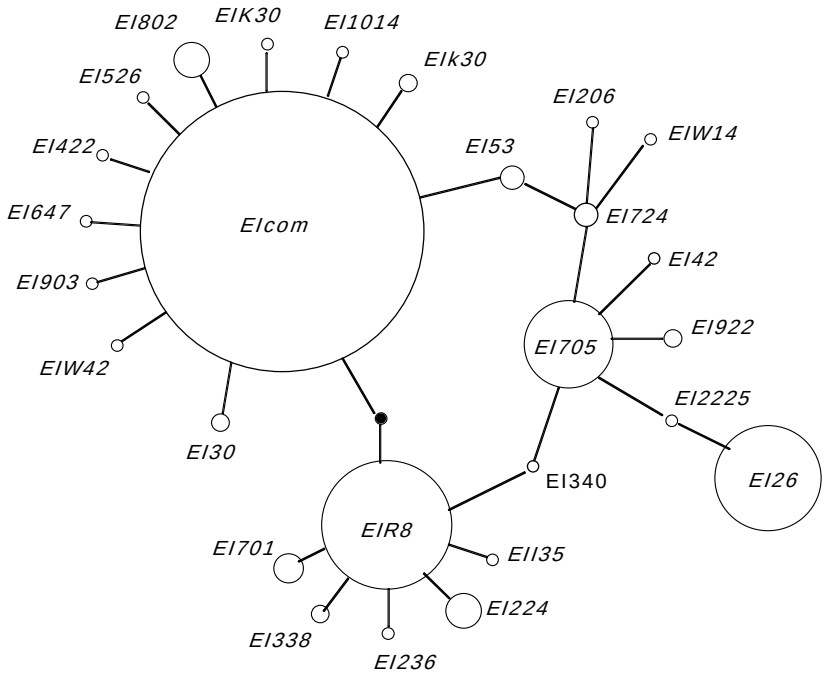
EI802
EIK30
EI1014
EIk30
EI526
EI206
EIW14
EI422
EI53
EI647
EIcom
EI724
EI903
EI42
EI922
EIW42
EI705
EI2225
EI30
EI340
EI26
EIR8
EI701
EII35
EI224
EI338
EI236

Fig. 2

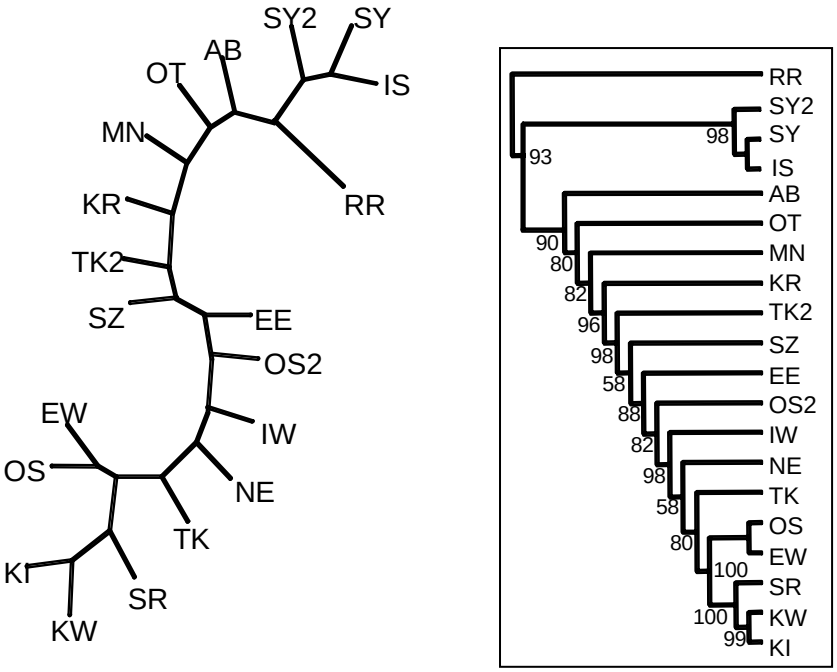
SY2
SY
AB
OT
IS
MN
RR
KR
TK2
SZ
EE
OS2
EW
IW
OS
NE
TK
KI
SR
KW
RR
SY2
98
SY
IS
93
AB
OT
90
MN
80
KR
82
TK2
96
SZ
98
EE
58
OS2
88
IW
82
NE
98
TK
58
OS
80
EW
100
SR
100
KW
99
KI

Fig. 3

Table 1. Sample name, date of collection, number of examined individuals, haplotype and nucleotide diversities and the frequency of focal haplotypes in each sample. Collection locales are shown in Figure 1.

Sample Name	Date of Collection	No. of Examined Individuals	Haplotype diversity (±SD)	Nucleotide diversity (±SD)	No. of haplotypes	Frequency of *EIcom (%)*	Frequency of *EIR8 (%)*	Frequency of *EI26 (%)*	Frequency of *EI705 (%)*
SY	Mar., 2005	20	0.6421+/-0.0871	0.0019+/-0.0014	4	55.0	15.0	0.0	25.0
SY2	May, 2006	44	0.6374+/-0.0441	0.0025+/-0.0017	4	50.0	31.8	2.3	15.9
RR	Mar., 2005	37	0.6997+/-0.0675	0.0031+/-0.0020	6	51.4	13.5	10.8	13.5
MN	Jun., 2005	51	0.6659+/-0.0569	0.0029+/-0.0019	7	62.7	21.6	9.8	0.0
AB	Jun., 2005	51	0.6478+/-0.0665	0.0023+/-0.0016	7	56.9	9.8	7.8	13.7
NE	Apr., 2006	45	0.4172+/-0.0865	0.0017+/-0.0013	6	75.6	2.2	13.3	4.4
KI	Dec., 2004	52	0.2707+/-0.0731	0.0014+/-0.0011	3	84.6	0.0	13.5	0.0
KW	Oct., 2006	46	0.3449+/-0.0851	0.0018+/-0.0013	4	80.4	8.7	8.7	0.0
TK	Oct., 2004	49	0.3636+/-0.0863	0.0013+/-0.0011	7	79.6	2.0	8.2	4.1
TK2	Dec., 2006	45	0.5525+/-0.0751	0.0024+/-0.0016	4	64.4	11.1	15.6	8.9
EW	Jun., 2005	47	0.3395+/-0.0848	0.0015+/-0.0012	5	80.9	10.6	4.3	0.0
EE	Jun., 2005	29	0.4778+/-0.1124	0.0015+/-0.0012	7	72.4	6.9	3.4	6.9
SZ	Jun., 2005	47	0.6078+/-0.0690	0.0027+/-0.0018	6	59.6	17.0	12.8	6.4
SR	Mar., 2005	35	0.4840+/-0.0995	0.0023+/-0.0016	7	71.4	5.7	11.4	0.0
OS	Feb., 2005	60	0.4712+/-0.0744	0.0027+/-0.0018	6	71.7	6.7	11.7	0.0
OS2	Jun., 2006	46	0.3797+/-0.0866	0.0015+/-0.0012	4	78.3	6.5	8.7	6.5
OT	Jun., 2006	50	0.5984+/-0.0655	0.0025+/-0.0017	4	60.0	14.0	14.0	12.0
IS	Oct., 2006	45	0.6758+/-0.0394	0.0028+/-0.0018	6	40.0	40.0	0.0	13.3
IW	Dec., 2006	52	0.5475+/-0.0738	0.0025+/-0.0017	7	65.4	15.4	9.6	3.8
KR	Jul., 2006	51	0.5945+/-0.0542	0.0027+/-0.0018	5	56.9	29.4	5.9	5.9

Table 2. Variable nucleotide sites in the 3' half of the mitocondrial DNA CO1 gene (580 bp) and defined haplotypes in hair crab. Dots indicate the nucleotide identical to that in the EIcom sequence. Non-synonymous and synonymous substitutions are shown in bold and plain letters, respectively.

	Variable nucleotide site																						
haplotyp	3	21	36	42	**52**	63	105	**136**	138	177	180	198	213	237	**244**	**327**	351	354	384	**403**	534	546	564
EIcom	T	C	T	G	**G**	T	G	**G**	T	T	C	C	A	A	**G**	**T**	T	G	G	**A**	G	A	G
EI422	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•
EI424	•	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•
EI647	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•
EI526	•	•	•	•	•	•	•	•	•	•	•	•	•	•	**C**	•	•	•	•	•	•	•	•
EI1014	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•
EI30	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
EI53	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•
EI802	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•
EI903	•	•	•	•	**A**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
EIK30	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•
EIW42	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•
EIR8	•	•	•	A	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A
EI224	•	•	•	A	•	•	A	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	A
EI338	•	T	•	A	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A
EI701	•	•	•	A	•	•	A	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•	A
EI236	•	•	•	A	•	C	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A
EI340	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A
EI705	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
EI42	•	•	•	•	•	•	A	**A**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
EI206	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•
EI724	•	•	•	•	•	•	A	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•
EI922	A	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
EI2225	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	**G**	•	•	•
EII35	•	•	•	A	•	•	A	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	A
EI26	•	•	•	•	•	•	A	•	•	•	T	•	•	•	•	**C**	•	•	•	**G**	•	•	•
EIW14	•	•	C	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

Table 3. Distribution of mtDNA CO1 haplotypes among 20 samples of hair crab examined

Sample	*EIcom*	*EIR8*	*EI26*	*EI705*	*EI30*	*EI42*	*EI53*	*EI206*	*EI224*	*EI236*	*EI338*	*EI340*	*EI422*	*EI424*	*EI526*	*EI647*	*EI701*	*EI724*	*EI802*	*EI903*	*EI922*	*EI1014*	*EI2225*	*EIK30*	*EII35*	*EIW14*	*EIW42*	Total
SY	11	3	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	20
SY2	22	14	1	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	44
RR	19	5	4	5	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	37
MN	32	11	5	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	51
AB	29	5	4	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1	1	0	0	0	0	0	0	51
NE	34	1	6	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	45
KI	44	0	7	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	52
KW	37	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	46
TK	39	1	4	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	49
TK2	29	5	7	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	45
EW	38	5	2	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	47
EE	21	2	1	2	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	29
SZ	28	8	6	3	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	47
SR	25	2	4	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	35
OS	43	4	7	0	0	0	0	1	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	60
OS2	36	3	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	46
OT	30	7	7	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50
IS	18	18	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	45
IW	34	8	5	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	52
KR	29	15	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	51
Total	598	121	81	57	2	1	3	1	5	1	2	1	1	1	1	1	4	3	8	1	2	1	1	2	1	1	1	902

Table 4. F_{ST} values between samples (below diagonal) and probability of differentiation with p value in F_{ST} estimate and Fisher's exact test (above diagonal), where "-" indicates not significant in both estimates, "+", significant in both, "- +", nonsignificant in F_{ST} but significant in exact test, "+ -", significant in F_{ST} but nonsignificant in exact test. Significance was tested at the 5% level with a Bonferroni-corrected p for multiple tests. F_{ST} estimation followed to Raynolds et al., 1983.

Sample	SY	SY2	RR	MN	AB	NE	KI	KW	TK	TK2	EW	EE	SZ	SR	OS	OS2	OT	IS	IW	KR
SY	0.000	-	-	-	-	-	−+	-	-	-	-	-	-	-	−+	-	-	-	-	-
SY2	0.014	0.000	-	-	-	+	+	−+	+	-	+	-	-	−+	−+	-	-	-	-	-
RR	0.000	0.001	0.000	-	-	−+	−+	-	+−	-	-	-	-	-	-	-	-	-	-	-
MN	0.000	0.000	0.000	0.000	-	-	−+	-	+−	-	-	-	-	-	-	-	-	-	-	-
AB	0.000	0.074	0.020	0.018	0.000	-	−+	-	-	-	-	-	-	-	−+	-	-	+−	-	-
NE	0.061	0.183	0.084	0.081	0.008	0.000	-	-	-	-	-	-	-	-	-	-	-	+	-	+
KI	0.103	0.103	0.232	0.126	0.118	0.033	0.000	-	-	-	-	-	-	-	-	-	-	+	-	+
KW	0.021	0.118	0.054	0.048	0.001	0.000	0.004	0.000	-	-	-	-	-	-	-	-	-	−+	-	-
TK	0.076	0.211	0.117	0.108	0.023	0.000	0.000	0.000	0.000	-	-	-	-	-	-	-	-	+	-	+
TK2	0.006	0.073	0.007	0.009	0.000	0.007	0.032	0.006	0.035	0.000	-	-	-	-	-	-	-	+	-	-
EW	0.040	0.148	0.091	0.077	0.019	0.018	0.018	0.000	0.000	0.038	0.000	-	-	-	-	-	-	+	-	-
EE	0.031	0.147	0.080	0.071	0.004	0.001	0.007	0.000	0.000	0.029	0.000	0.000	-	-	-	-	-	+−	-	+
SZ	0.000	0.030	0.000	0.000	0.000	0.032	0.060	0.014	0.056	0.000	0.041	0.036	0.000	-	-	-	-	-	-	-
SR	0.013	0.102	0.036	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	-	-	-	+	-	-
OS	0.000	0.061	0.015	0.012	0.000	0.012	0.026	0.000	0.023	0.000	0.011	0.009	0.000	0.000	0.000	-	-	+	-	−+
OS2	0.030	0.147	0.069	0.062	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.021	0.000	0.001	0.000	-	+	-	-
OT	0.000	0.046	0.000	0.000	0.000	0.027	0.057	0.018	0.056	0.000	0.049	0.041	0.000	0.002	0.000	0.020	0.000	−+	-	-
IS	0.084	0.000	0.044	0.039	0.160	0.293	0.351	0.216	0.329	0.153	0.252	0.248	0.094	0.192	0.135	0.255	0.117	0.000	−+	-
IW	0.000	0.044	0.005	0.001	0.000	0.020	0.040	0.000	0.031	0.000	0.013	0.010	0.000	0.000	0.000	0.003	0.000	0.117	0.000	-
KR	0.000	0.000	0.000	0.000	0.041	0.120	0.155	0.064	0.138	0.037	0.087	0.090	0.006	0.054	0.026	0.088	0.020	0.020	0.013	0.000

Table 5. Results of the hierarchical analysis of molecular variance based on mtDNA CO1 sequence data of hair crab. The percentage of variance (%), probability estimated from permutation (P), and the fixersion indice (φ) are given at each level. See the text for the sample groups.

Source of variation	%	P	φ
Among the group A, B and	6.05	<0.001	0.0023
Among samples within groups	0.22	>0.1	0.062
Within samples	93.73	<0.001	0.06