A new species of freshwater crab (Decapoda: Brachyura: Potamidae) from Dongyin Island, Matsu, Taiwan, defined by morphological and molecular characters, with notes on its biogeography

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Abstract
A new species of freshwater crab, Nanhaipotamon dongyinense sp. nov. (Decapoda: Brachyura: Potamidae), is described from Dongyin Island, Matsu, Taiwan. Based on morphological and molecular (mitochondrial 16S rRNA) evidence, it can be distinguished from its congeners. The biogeography of the species, found on an island 50 km from mainland China, is also discussed.

Keywords: Biogeography, freshwater crab, mtDNA sequence, Nanhaipotamon dongyinense, new species, 16S rRNA, Taiwan

Introduction
The freshwater crab fauna of China was recently revised by Dai (1999). Although there are several problems with the book, it is still the most comprehensive compilation yet for Chinese freshwater crabs (Ng 2000). While the freshwater crab fauna of East Asia (China, Japan, Ryukyus, and Taiwan) is reasonably well studied (e.g. Dai 1999; Shy and Yu 1999; Yoshigou 1999; Ng et al. 2001), it remains interesting that only the genera Nanhaipotamon Bott, 1968, Somanniathelphusa Bott, 1968, and Candidiopotamon Bott, 1967 are shared between mainland China and the East Asian islands. With regards to Nanhaipotamon, 12 species are currently known, with 11 species in the coastal provinces (Zhejiang, Fujian, and Guangdong) of south-east China (Dai 1999; Cheng et al. 2003) and one, Nanhaipotamon formosanum (Parisi, 1916), from Taiwan (Shy and Yu 1999; Ng et al. 2001).

Dongyin (26°22′42″N, 120°29′E, Matsu) is a small island about 50 km off Fujian, China. It is composed of two islands which are connected by an artificial causeway.
(Jhongjhu Bank) and the highest point is 174 m above sea level (Enaishan). Recently, several specimens of the genus *Nanhaipotamon* were collected in this island with the local name “shalulu” or “halulu” (“mountain crab”). Based on morphology and molecular data (mitochondrial 16S rRNA), these specimens are clearly distinct from other known species of *Nanhaipotamon*, and are therefore here described as a new species. The presence of this species on an isolated island 50 km from mainland China is also discussed in relation to past glaciation events.

**Materials and methods**

Specimens were collected from irrigation ditches beside vegetable gardens, Yansiouwo, Dongyin, Matsu, Taiwan (Figure 1). After collection, specimens were preserved in 75–95% ethanol. Specimens examined are deposited in the National Museum of Natural Science, Taichung, Taiwan (NMNS), Institute of Zoology, Chinese Academy of Sciences, Beijing, China (IZCAS), and the Zoological Reference Collection, Raffles Museum, National University of Singapore, Singapore (ZRC).

Related species of the genus *Nanhaipotamon* from adjacent areas were also collected for molecular comparison, including *N. formosanum* (Paris, 1916) (Nantou, Yunlin, Chiayi, and Tainan, Taiwan), *N. nanriense* Dai, 1997 (Nanri, Putian, Fujian, China), *N. hongkongense* (Shen, 1940) (Hong Kong), and *N. huaanense* Dai, 1997 (Gao-an, Hua-an, Fujian, China) (Figure 1; Table I). *Geothelphusa albogilva* Shy, Ng and Yu, 1994 (Kending, Pingtung, Taiwan) (accession no. AB127366) was used as an outgroup for the construction of molecular tree.

Genomic DNA was isolated from the muscle tissue of legs by a Sigma mammalian genomic DNA miniprep kit. A region of approximately 550 base pairs (bp) of the 5′-end of the 16S rRNA gene was selected for amplification with polymerase chain reaction (PCR) using the primers 1471 (5′-CCTGTTTANCAAAAACAT-3′) and 1472 (5′-AGATAGAAACCAACCTGG-3′) (Crandall and Fitzpatrick 1996). The PCR conditions were 35–40 cycles for primers 1471 and 1472 were 50 s at 94°C, 70 s at 45°C, and 60 s at 72°C (denaturation, annealing, and extension), followed by 72°C extension for 10 min. Sequences were obtained by automated sequencing (ABI PRISM 377 Sequencer and MegaBACE DNA Analysis System 500) and were aligned with the aid of CLUSTAL W (version 1.4; Thompson et al. 1994) and BioEdit (version 5.09; Hall 2001), after verification with the complimentary strand. Sequences of the different haplotypes have been deposited in the DDBJ nucleotide sequence databases (accession nos AB212863 to AB212870).

The neighbour-joining (NJ) tree was established by the pairwise distance of nucleotide divergence constructed by the program TREECON for Windows (version 1.3b; Van de Peer and De Wachter 1997) with 2000 bootstrap replications. The distance estimation was based on Kimura’s (1980) model with the transition/transversion ratio estimated from the data. A maximum parsimony (MP) tree was constructed using the program PAUP* (version 4.0b8; Swofford 2001), with 2000 bootstrap reiterations of a simple heuristic search, random sequence addition, branch-swapping=TBR (tree bisection-reconnection) and random-addition sequence replications=100. All characters were equally weighted. Gaps in the 16S rRNA alignment were treated as a fifth character state in NJ and MP tree construction (Kambhampati 1995; Miura et al. 2000; Tong et al. 2000).
Figure 1. Collection sites for *Nanhaipotamon* species used in this study. For locality names see Table I.
Table I. Eight haplotypes of *Nanhaipotamon* species and one outgroup used in this study.

| Species                        | Haplotype | Sample size | Localitya | DDBJ accession no. |
|-------------------------------|-----------|-------------|------------|--------------------|
| *N. dongyinense* sp. nov.     | NHDY      | 2           | Dongyin, Matsu, Taiwan [1] | AB212863 |
| *N. formosanum* (Parisi, 1916)| NHTW₁     | 1           | Jiī, Nantou County, Taiwan [2] | AB212864 |
|                               | NHTW₂     | 1           | Dounan, Yunlin County, Taiwan [3] | AB212865 |
|                               | NHTW₃     | 2           | Botanical Garden, Chiayi City, Taiwan [4] | AB212866 |
|                               | NHTW₄     | 2           | Yungkan, Tainan City, Taiwan [5] | AB212867 |
| *N. nanriense* Dai, 1997      | NHNR      | 1           | Nanri, Putian City, Fujian, China [6] | AB212868 |
| *N. hongkongense* (Shen, 1940)| NHHK      | 1           | Hong Kong | AB212869 |
| *N. huaanense* Dai, 1997      | NHHA      | 1           | Gao-an, Hua-an, Zhangzhou City, Fujian, China [7] | AB212870 |
| *Geothelphusa albogilva*     | 9b        |             | Kending, Pingtung County, Taiwan [8] | AB127366 |

aThe numbers within square brackets correspond to the localities shown in Figure 1; bbased on Shih et al. (2004).

**Results**

**Taxonomy**

**Family POTAMIDAE** Ortmann, 1896

*Nanhaipotamon* Bott, 1968

*Nanhaipotamon dongyinense* sp. nov.

(Figures 2A–H, 3A–D)

Holotype: one male (31.3 × 25.8 mm) (NMNS 4557-001), Yansiouwo, Dongyin (26°21′91″N, 120°28′96″E), Matsu, Taiwan, coll. L.-M. Wang, June 2004. Paratypes: one male (allotype) (26.5 × 22.2 mm) (NMNS 4557-002), same data as holotype; five males (CW 29.47–36.28 mm) (NMNS 4557-003 to 4557-007), five females (CW 29.64–34.76 mm) (NMNS 4557-008 to 4557-012), same locality as holotype, coll. L.-M. Wang, H.-T. Shih, H.-T. Hung, and N.-H. Jang-Liaw, 15 July 2004; one male (CW 22.4 mm) (IZCAS 0401), same data as holotype; one male (CW 28.27 mm), one female (CW 27.84 mm) (IZCAS 0402), same locality as holotype, coll. L.-M. Wang, H.-T. Shih, H.-T. Hung, and N.-H. Jang-Liaw, 15 July 2004; two males (CW 32.05, 32.51 mm), two females (CW 26.62, 29.03 mm) (ZRC 2004.0694), same locality as holotype, coll. L.-M. Wang, H.-T. Shih, H.-T. Hung, and N.-H. Jang-Liaw, 15 July 2004. Others: five males (CW 19.15–26.56 mm), two females (CW 26.40–26.91 mm) (NMNS 4557-013), same locality as holotype, coll. L.-M. Wang, H.-T. Shih, H.-T. Hung, and N.-H. Jang-Liaw, 15 July 2004.

**Description.** Carapace distinctly convex longitudinally, surface smooth, finely pitted. Branchial region very swollen, cervical groove wide and deep. H-shaped groove between gastric and cardiac regions deep. Postfrontal lobe prominent, with large pits. Postorbital crest sharp, connected with epibranchial tooth. Front deflexed, anterior border emarginated medially, dorsal orbital border ridged. Exorbital angle triangular, outer border arched. Epibranchial teeth squarish, antero-lateral border carinated, anterior part with indistinct granules, posterior part smooth. Third maxilliped (Figure 2A) with merus about 1.2 times as broad as long, with ischium about 1.5 times as long as broad, exopod reaching to proximal third of merus, with a short flagellum.
Chelipeds strongly unequal, carpus with longitudinal depression on dorsal surface, with rugae on inner border, inner-distal angle with acute spine and spinule; larger manus about 1.3 times as long as high, slightly shorter than movable finger, with large gape when closed.

Ambulatory legs slender, last leg with propodus about 2.4 times as long as broad, shorter than dactylus.

Male abdomen (Figure 2B) triangular, sixth segment about 2.3 times as broad as long, telson about 1.2 times as broad as long. Median longitudinal groove of thoracic sternum

Figure 2. *Nanhaipotamon dongyinense* sp. nov.: (A–F) holotype male (31.3 × 25.8 mm) (NMNS 4557-001); (G, H) allotype female (26.5 × 22.2 mm) (NMNS 4557-002). (A) Third maxilliped; (B) male abdomen; (C) left G1, in situ; (D) left G1; (E) left G1, distal segment; (F) left G2; (G) female abdomen; (H) female gonopore.

Chelipeds strongly unequal, carpus with longitudinal depression on dorsal surface, with rugae on inner border, inner-distal angle with acute spine and spinule; larger manus about 1.3 times as long as high, slightly shorter than movable finger, with large gape when closed. Ambulatory legs slender, last leg with propodus about 2.4 times as long as broad, shorter than dactylus.

Male abdomen (Figure 2B) triangular, sixth segment about 2.3 times as broad as long, telson about 1.2 times as broad as long. Median longitudinal groove of thoracic sternum...
deep, interruption between sutures of sternites narrow, median longitudinal suture of sternites 7 and 8 moderately long.

Male G1 (first pleopod) (Figure 2D, E) reaching beyond tubercle of abdominal lock (Figure 2C), subterminal segment about 2.7 times as long as terminal segment, terminal

Figure 3. (A–C) Dorsal, frontal and ventral views of the fresh-preserved male paratype (CW 36.28 mm) (NMNS 4557-003) of *Nanhaipotamon dongyinense* sp. nov., Dongyin Island, Matsu, Taiwan; (D) live coloration of the female paratype (CW 29.66 mm) (NMNS 4557-008); (E) habitat; (F) a chimney around the entrance of burrow.
segment resembling an upside down boot in shape, its median line about 2.3 times as broad as long. Inner distal angle blunt triangular, distal margin arched inwards, outer distal angle produced, horn-like, distal end with pore. Male G2 (second pleopod) (Figure 2F) with subdistal segment about 1.9 times as long as distal segment.

Female abdomen (Figure 2G) oval, sixth segment about 3.1 times as broad as long, telson about 2.1 times as broad as long. Gonopore (Figure 2H) with upper part widening gradually, like watermelon seed, opening inwards and downwards.

Carapace length of holotype male 25.8 mm, breadth 31.3 mm; of allotype female, length 22.2 mm, breadth 26.5 mm.

**Etymology.** *Nanhaipotamon dongyinense* is named for the type locality, Dongyin Island, Matsu.

**Coloration** (Figure 3A–D). Carapace is greenish gray and ambulatory legs are yellowish green. Female individuals tend to be more yellowish.

**Habitat.** The specimens were found in the irrigation ditches next to vegetable gardens (Figure 3E). The water source is from the springs of this small island. No freshwater shrimps or freshwater snails were found. Brooding females could be found from April to June. A mating pair was found near the entrance within the burrow during July. Sometimes a wall of mud (hood or chimney) around the entrance can be observed (Figure 3F), especially in habitats far from surface water.

**Distribution.** The largest population is located in Yansiouwo, eastern part of Dongyin. Some burrows could also be found in Bei-ao, north of Yansiouwo. In the western part of Dongyin (Siyin), local people stated that some crabs were seen in Hou-ao and Rendingshengtian (Figure 1), but none were obtained in the present study.

**Remarks.** *Nanhaipotamon dongyinense* sp. nov. can be distinguished from the other congeners—*N. nanriense* Dai, 1997, *N. formosanum* (Parisi, 1916), and *N. huaanense* Dai, 1997. The morphological differences among them are shown in Table II.

**DNA analysis**

A 548 base pair segment (excluding the primer regions) of the 16S mtDNA gene from five species of *Nanhaipotamon* was amplified and aligned. Out of those, 45 positions were variable and 10 were parsimony informative. Among the total number of sequences, eight different haplotypes were found (Table I). The segment of 16S sequences is AT-rich (72%) (T: 36.4%, A: 35.6%, G: 17.8%, C: 10.3%).

Among the populations of *Nanhaipotamon formosanum*, the difference in nucleotide number is within 2 bp. *N. dongyinense* differs from *N. formosanum* by 6–8 bp, and differs from *N. nanriense* by 10 bp. There is a 5–6 bp difference between *N. nanriense* and *N. formosanum* (Table III). The phylogenetic tree constructed by the Kimura two-parameter model of the NJ analysis, with the bootstrap values larger than 50% from MP analysis, is shown in Figure 4. Both NJ and MP methods support that *N. formosanum* from the populations of Taiwan form one clade, with *N. nanriense* forming the sister groups of *N. formosanum*. 
Discussion

Most species of the genus *Nanhaipotamon* inhabit deep holes in muddy areas near rice paddies or vegetable gardens below 500 m above sea level in the coastal provinces of China. Only four species are recorded on small islands near mainland China, i.e. *N. dongyinense* sp.

Table II. Morphological differences among *Nanhaipotamon dongyinense* sp. nov. and the congeners from adjacent areas.

| Character | *N. dongyinense* sp. nov. | *N. nanriense* Dai, 1997 | *N. formosanum* (Parisi, 1916) | *N. huaanense* Dai, 1997 |
|-----------|---------------------------|--------------------------|-------------------------------|--------------------------|
| Size      | Large (the largest male is CW 36.28 mm) | Small (the largest male is CW 22.5 mm) | Large (the largest male is CW 36.8 mm) | Medium (the largest male is CW 26.7 mm) |
| Carapace: proportion of breadth to length | Male: 1.21, | Male: 1.21, | Male: 1.21, | Male: 1.24, |
| Larger cheliped: length of manus | female: 1.19 | female: 1.18 | female: 1.20 | female: 1.23 |
| Last leg: proportion of breadth to length of propodus | 2.4 | 1.9 | 2.1 | 2.2 |
| Third maxilliped: proportion of breadth to length of merus | 1.2 | 1.1 | 1.1 | 1 |
| Male abdomen: proportion of breadth to length of sixth segment | 2.3 | 2.1 | 2.1 | 2.2 |
| Male G1: proportion of subterminal to terminal segment | 2.7 | 2.6 | 3.7 | 2.2 |
| Male G1: outer-lateral margin of distal segment | Arched | Straight | Straight | Straight |
| Male G1: inner-distal and outer-distal angles of distal segment | Angular | Blunt rounded | Blunt rounded | Blunt rounded |
| Female gonopore: upper part | Widening gradually | Narrowing gradually | Narrowing gradually | Narrowing gradually |

Table III. Pairwise differences based on the 548 base pairs of the 16S rRNA gene among the haplotypes of *Nanhaipotamon dongyinense*, *N. formosanum*, *N. nanriense*, *N. hongkongense*, and *N. huaanense*.

| N. dongyinense | N. formosanum | N. nanriense | N. hongkongense | N. huaanense |
|----------------|--------------|--------------|----------------|--------------|
| NHDY | 4s,2v,2i | 3s,1v,2i | 4s,1v,2i | 5s,1v,2i | 6s,1v,3i | 16s,3v,2i | 20s,7v,5i |
| NHTW1 | 1s,1v | 1v | 1s,1v | 4s,1v,1i | 14s,5v,2i | 19s,7v,5i |
| NHTW2 | 1s | 2s | 5s,1i | 15s,4v,2i | 20s,6v,5i |
| NHTW3 | 1s | 4s,1i | 5s,1i | 14s,4v,2i | 19s,6v,5i |
| NHTW4 | 5s,1i | 15s,4v,2i | 20s,6v,5i |
| NHNR | 5s,1i | 15s,4v,2i | 20s,6v,5i |
| NHHK | 16s,4v,3i | 21s,6v,6i |
| NHHA | 20s,10v,3i |
nov. (Dongyin, Matsu), *N. nanriense* (Nanri, Fujian), *N. hongkongense* (Hong Kong) and *N. aculatum* (Hong Kong) (Dai 1999; this study). In comparison, there are two species of the genus *Geothelphusa* Stimpson, 1858 on Lanyu and Lyudao, two offshore islands east of Taiwan (Shy et al. 1994). Because deep oceanic trenches separate these two islands and Taiwan, a land bridge theory cannot explain their distribution. Considering the genetic similarities between *G. tawu* Shy, Ng and Yu, 1994 (from main island of Taiwan), *G. lanyu* Shy, Ng and Yu, 1994 (Lanyu) and *G. lutao* Shy, Ng and Yu, 1994 (Lyudao), the three species are very close, and are considered as the same species. Their presence on the two islands may be explained by rafting, or dispersal by birds, or even humans from Taiwan Island (Shih et al. 2004). The colonization of *N. dongyinense* sp. nov. is believed to be different from that of *Geothelphusa* spp. in Lanyu and Lyudao. Although Dongyin is more than 50 km from mainland Fujian, it nevertheless sits on the continental shelf. During periods of glaciation and low sea levels, Taiwan was probably connected to mainland China (Boggs et al. 1979). The isolation and evolution of *N. dongyinense* sp. nov. in Dongyin is thus believed to be a recent vicariant event.

In addition to the morphological difference (Table II), *N. dongyinense* sp. nov. also differs from congeners at the molecular level. Based on the tree topology (Figure 4), *N. dongyinense* sp. nov. forms a distinct clade with *N. formosanum* and *N. nanriense*. From Table III, there is at least 6 bp difference between *N. dongyinense* and *N. formosanum*. In comparison, Shih et al. (2004) have reported that there is no more than 5 bp nucleotide difference within the *Geothelphusa tawu* clade in southern Taiwan.

It is believed that *N. dongyinense* was originally distributed throughout Dongyin Island, but anthropogenic factors have affected several populations. The main reasons include the development of military building operations, overuse of pesticides and herbicides,

Figure 4. Neighbour-joining tree (Kimura two-parameter model) of *Nanhaipotamon* spp. and the outgroup *Geothelphusa albogilva* (GA) in this study based on 551 base pairs of the 16S rRNA gene. The bootstrap values (>50%) at the nodes represent confidence based on the percentage of 2000 bootstrap replications. For abbreviations of haplotypes see Table I.
cemented trenches, and pollution from a winery. Without appropriate conservation, the endemic species may well be exterminated in the future.

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