

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* (Parisi, 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 *Nanhaiopotamon* 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	Locality ^a	DDBJ accession no.
<i>N. dongyinense</i> sp. nov.	NHDY	2	Dongyin, Matsu, Taiwan [1]	AB212863
<i>N. formosanum</i> (Parisi, 1916)	NHTW1	1	Jiji, 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
<i>N. nanriense</i> Dai, 1997	NHNR	1	Nanri, Putian City, Fujian, China [6]	AB212868
<i>N. hongkongense</i> (Shen, 1940)	NHHK	1	Hong Kong	AB212869
<i>N. huaanense</i> Dai, 1997	NHHA	1	Gao-an, Hua-an, Zhangzhou City, Fujian, China [7]	AB212870
<i>Geothelphusa albogilva</i> Shy, Ng and Yu, 1994		9 ^b	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 female (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 19.15–29.56 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.

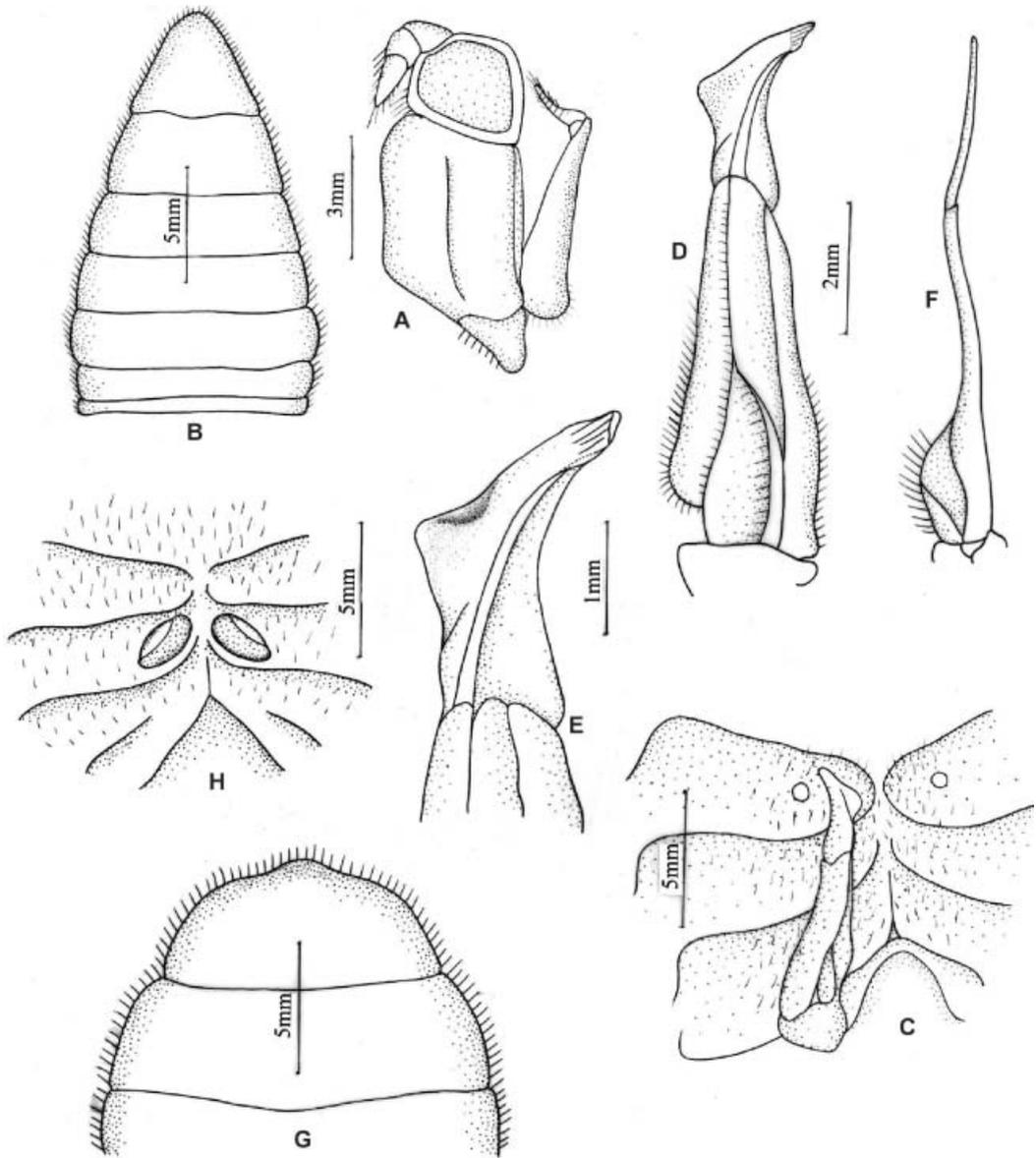


Figure 2. *Nanhaiopotamon dongyinense* sp. nov.: (A–F) holotype male (31.3 × 25.8 mm) (NMNS 4557-001); (G, H) allotype female (26.5 × 22.2 mm) (NMNS 4557-002). (A) Third maxilliped; (B) male abdomen; (C) left G1, in situ; (D) left G1; (E) left G1, distal segment; (F) left G2; (G) female abdomen; (H)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

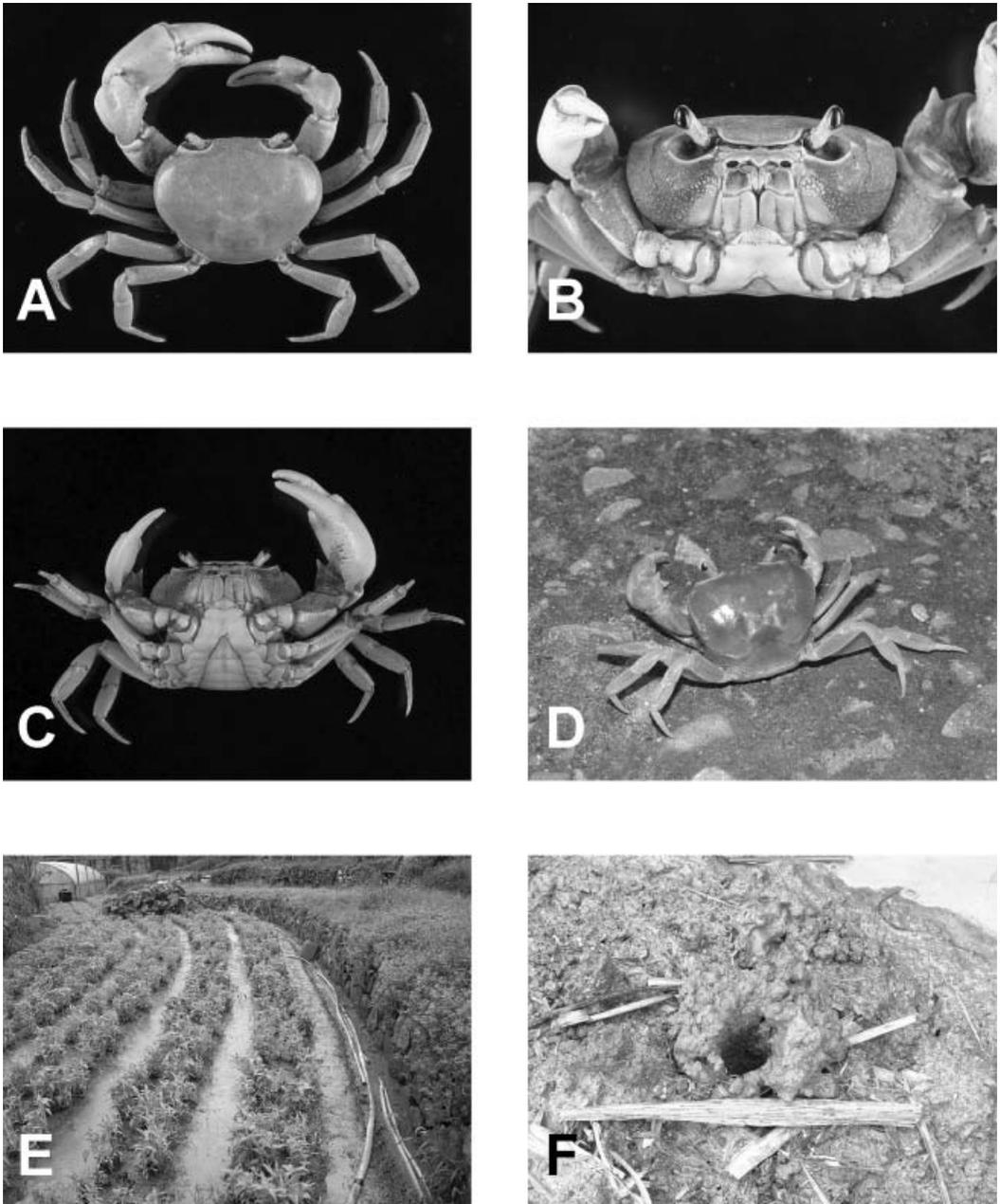


Figure 3. (A–C) Dorsal, frontal and ventral views of the fresh-preserved male paratype (CW 36.28 mm) (NMNS 4557-003) of *Nanhaiipotamon dongyinense* sp. nov., Dongyin Island, Matsu, Taiwan; (D) live coloration of the female paratype (CW 29.66 mm) (NMNS 4557-008); (E) habitat; (F) a chimney around the entrance of burrow.

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

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*.

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

Character	<i>N. dongyinense</i> sp. nov.	<i>N. nanriense</i> Dai, 1997	<i>N. formosanum</i> (Parisi, 1916)	<i>N. huaanense</i> 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, female: 1.19	Male: 1.21, female: 1.18	Male: 1.21, female: 1.20	Male: 1.24, female: 1.23
Larger cheliped: length of manus	Shorter than movable finger	Longer than movable finger	Shorter than movable finger	Shorter than movable finger
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

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 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*.

	<i>N. dongyinense</i>	<i>N. formosanum</i>				<i>N. nanriense</i>	<i>N. hongkongense</i>	<i>N. huaanense</i>
	NHDY	NHTW1	NHTW2	NHTW3	NHTW4	NHNR	NHHK	NHHA
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	14s,4v,2i	19s,6v,5i
NHTW4						5s,1i	15s,4v,2i	20s,6v,5i
NHNR							16s,4v,3i	21s,6v,6i
NHHK								20s,10v,3i
NHHA								

Types of difference are abbreviated as follows: s, transitions; v, transversions; i, indels.

cemented trenches, and pollution from a winery. Without appropriate conservation, the endemic species may well be exterminated in the future.

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References

- Boggs SJ, Wang W-C, Lewis FS, Chen J-C. 1979. Sediment properties and water characteristics of the Taiwan Shelf and Slope. *Acta Oceanographica Taiwanica* 10:10–49.
- Bott R. 1967. Potamiden aus Ost-Asien (*Parapotamon* De Man, *Sinopotamon* n. gen., *Candidiopotamon* n. gen., *Geothelphusa* Stimpson) (Crustacea, Decapoda). *Senckenbergiana Biologica* 48:203–220.
- Bott R. 1968. Potamiden aus Sud-Asien (Crustacea, Decapoda). *Senckenbergiana Biologica* 49:119–130.
- Cheng Y-Z, Yang W-C, Zhong Y-H, Li L. 2003. A new species of the genus *Nanhaipotamon* (Decapoda: Potamidae). *Journal of Xiamen University (Natural Science)* 42:676–678. (Chi).
- Crandall KA, Fitzpatrick JFJ. 1996. Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. *Systematic Biology* 45:1–26.
- Dai A-Y. 1997. A revision of freshwater crabs of the genus *Nanhaipotamon* Bott, 1968 from China (Crustacea: Decapoda: Brachyura: Potamidae). *The Raffles Bulletin of Zoology* 45:209–235.
- Dai A-Y. 1999. Fauna Sinica. Arthropoda: Crustacea: Malacostraca: Decapoda: Parathelphusidae, Potamidae. Beijing: Science Press, 501 p, 30 plates. (Chi with Eng abstract).
- Hall TA. 2001. BioEdit: a user-friendly biological sequence alignment editor and analysis, version 5.09. Raleigh: North Carolina State University, Department of Microbiology.
- Kambhampati S. 1995. A phylogeny of cockroaches and related insects based on DNA sequence of mitochondrial ribosomal RNA genes. *Proceedings of the National Academy of Sciences of the U S A* 92:2017–2020.
- Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111–120.
- Miura T, Roisin Y, Matsumoto T. 2000. Molecular phylogeny and biogeography of the nasute termite genus *Nasutitermes* (Isoptera: Termitidae) in the Pacific Tropics. *Molecular Phylogenetics and Evolution* 17:1–10.
- Ng PKL. 2000. A.-Y. Dai, 1999: Fauna Sinica. Arthropoda: Crustacea: Malacostraca: Decapoda: Parathelphusidae, Potamidae [book review]. *Crustaceana* 73:249–251.
- Ng PKL, Wang C-H, Ho P-H, Shih H-T. 2001. An annotated checklist of brachyuran crabs from Taiwan (Crustacea: Decapoda). *National Taiwan Museum Special Publication Series* 11:1–86.
- Parisi B. 1916. I decapodi giapponesi del Museo di Milano. IV. Cyclometopa. *Atti della Società Italiana di Scienze Naturali* 55:153–190.
- Shen C-J. 1940. Four new species of Brachyura from Chinese seas. *Journal of the Hong Kong Fisheries Research Station* 1:255–262.
- Shih H-T, Ng PKL, Chang H-W. 2004. Systematics of the genus *Geothelphusa* (Crustacea: Decapoda, Brachyura, Potamidae) from southern Taiwan: a molecular appraisal. *Zoological Studies* 43:519–526.
- Shy J-Y, Ng PKL, Yu H-P. 1994. Crabs of the genus *Geothelphusa* Stimpson, 1858 (Crustacea: Decapoda: Brachyura: Potamidae) from Taiwan, with descriptions of 25 new species. *The Raffles Bulletin of Zoology* 42:781–846.
- Shy J-Y, Yu H-P. 1999. Freshwater crabs of Taiwan. *Kaohsiung (Taiwan): National Museum of Marine Biology/Aquarium*. (Chi).
- Stimpson W. 1858. *Prodromus descriptionis animalium everttebratorum, quae in Expeditione ad Oceanum Pacificum Septentrionalem, a Republica Federata missa, Cadwaladaro Ringgold et Johanne Rodgers Ducibus, observavit et descripsit W. Stimpson. Pars VII. Crustacea Anomoura*. *Proceedings of the Academy of Natural Sciences of Philadelphia* 10:225–252.

- Swofford DL. 2001. PAUP: phylogenetic analysis using parsimony (and other methods), version 4.0b8. Sunderland (MA): Sinauer Associates.
- Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22:4673–4680.
- Tong JG, Chan T-Y, Chu KH. 2000. A preliminary phylogenetic analysis of *Metapenaeopsis* (Decapoda: Penaeidae) based on mitochondrial DNA sequences of selected species from the Indo West Pacific. *Journal of Crustacean Biology* 20:541–549.
- Van de Peer Y, De Wachter R. 1997. Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. *Computer Applications in the Biosciences* 13:227–230.
- Yoshigou H. 1999. Potamidae (Crustacea: Decapoda: Brachyura) of Japan. *Hibakagaku* 191:17–26. (Jpn).