Zoological Studies **46**(6): 680-694 (2007)

Zoological Studies

Two New Species of the Land-Locked Freshwater Shrimps Genus, *Neocaridina* Kubo, 1938 (Decapoda: Caridea: Atyidae), from Taiwan, with Notes on Speciation on the Island

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(Accepted July 2, 2007)

Hsi-Te Shih and Yixiong Cai (2007) Two new species of the land-locked freshwater shrimps genus, *Neocaridina* Kubo, 1938 (Decapoda: Caridea: Atyidae), from Taiwan, with notes on speciation on the island. *Zoological Studies* **46**(6): 680-694. Two new species of land-locked shrimp, *Neocaridina saccam* and *N. ketagalan* (Decapoda: Caridea: Atyidae), are described from Tainan, southwestern Taiwan, and Taipei, northern Taiwan, respectively. Based on morphological and molecular (mitochondrial 16S rRNA and cytochrome oxidase I genes) evidence, both can be distinguished from their congeners. A speciation model for this genus on the island of Taiwan is also proposed based on geological events and molecular clock estimates.
http://zoolstud.sinica.edu.tw/Journals/46.6/680.pdf

Key words: *Neocaridina saccam*, *N. ketagalan*, 16S rRNA, Cytochrome oxidase I, Biogeography.

The freshwater shrimp genus, *Neocaridina* Kubo, 1938, comprises a group of land-locked organisms of the family Atyidae which is distributed throughout Russia, Korea, Japan, China, Taiwan, and Vietnam (Cai 1996, Liang 2004). Unlike the genera *Caridina* and *Macrobrachium* with both catadromous and land-locked species, all known species of *Neocaridina* are land-locked as evidenced by the abbreviated larval development and large-sized eggs (Shokita 1976 1979, Shy et al. 1987 1992, Hung et al. 1993, Xue et al. 1995, Shy and Yu 1998).

In his monograph of the family Atyidae of China, Liang (2004) revised this genus from China and Taiwan, and described 5 new species (and subspecies) from China. Recently, a new species was also described from Iriomote I., the Ryukyus, by Naruse et al. (2006). Although 26 species (and subspecies) are reported for this genus, the taxonomy of some taxa is currently controversial (Cai 1996, Liang 2004). Based on Hung et al. (1993) and Shy and Yu (1998), who reported on the Taiwanese atyid shrimp fauna, only 1 species, *N. denticulata* (de Haan, 1849), is distributed throughout the island of Taiwan, except for the southeastern part in Taitung County.

The phylogeny and biogeography of land-locked freshwater crabs of Taiwan and other areas has recently been studied in detail (Shih et al. 2004 2005 2006 2007a b, Yeo et al. 2007), but no molecular study on the genus *Neocaridina* with similar land-locked habits has been reported yet. In a recent survey of the Taiwanese atyid and potamid fauna, the senior author and his students collected several specimens around the main island of Taiwan. Specimens of the land-locked *Neocaridina* from southwestern Taiwan (Tainan Co.) and northern Taiwan (Taipei Co.) were found to possess different morphological characters and genetic structures, compared with their congeners. Hence, both are described as new in the present study. Based on molecular dating and geological

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events, the speciation of the genus within Taiwan is discussed.

MATERIALS AND METHODS

Specimens of the genus *Neocaridina* were collected from small creeks in Taiwan (including 6 localities in Taipei, Taichung, Yunlin, Tainan, and Kinmen Cos.) and Hawaii. Comparative specimens of *N. spinosa*, *Caridina formosae*, and *C. cantonensis*, were included as outgroups for construction of a phylogenetic tree (Fig. 1, Table 1). Specimens were preserved in 70%-95% ethanol after collection, were illustrated with the help of a drawing tube attached to an Olympus stereo microscope (model SZX7), and were deposited in the Zoological Collections of the Department of Life Science, National Chung Hsing University, Taichung, Taiwan (NCHUZOOL), the National Museum of Natural Science, Taichung, Taiwan (NMNS), and the Zoological Reference Collection, Raffles Museum, National University of Singapore, Singapore (ZRC). cl refers to the carapace length, and the mode means the number occurring most frequently. The rostral formula was counted based on all specimens available. Other measurements, including the ratio, were based on 5 males and 5 females for each species, and egg measurements were based on 8 eggs from 1 individual.

Genomic DNA was isolated from the muscle tissue of the abdomen using a Sigma mammalian genomic DNA miniprep kit. A region of approximately 510-550 base pairs (bp) of the 5'-end of the 16S ribosomal (r)RNA gene was selected for amplification with a polymerase chain reaction (PCR) using the primers 1471 (5'-CCTGTTTAN CAAAAACAT-3'), 1472 (5'-AGATAGAAAC CAACCTGG-3') (Crandall and Fitzpatrick 1996), 16Sar (5'-CGCCTGTTTATCAAAAACAT-3'), and

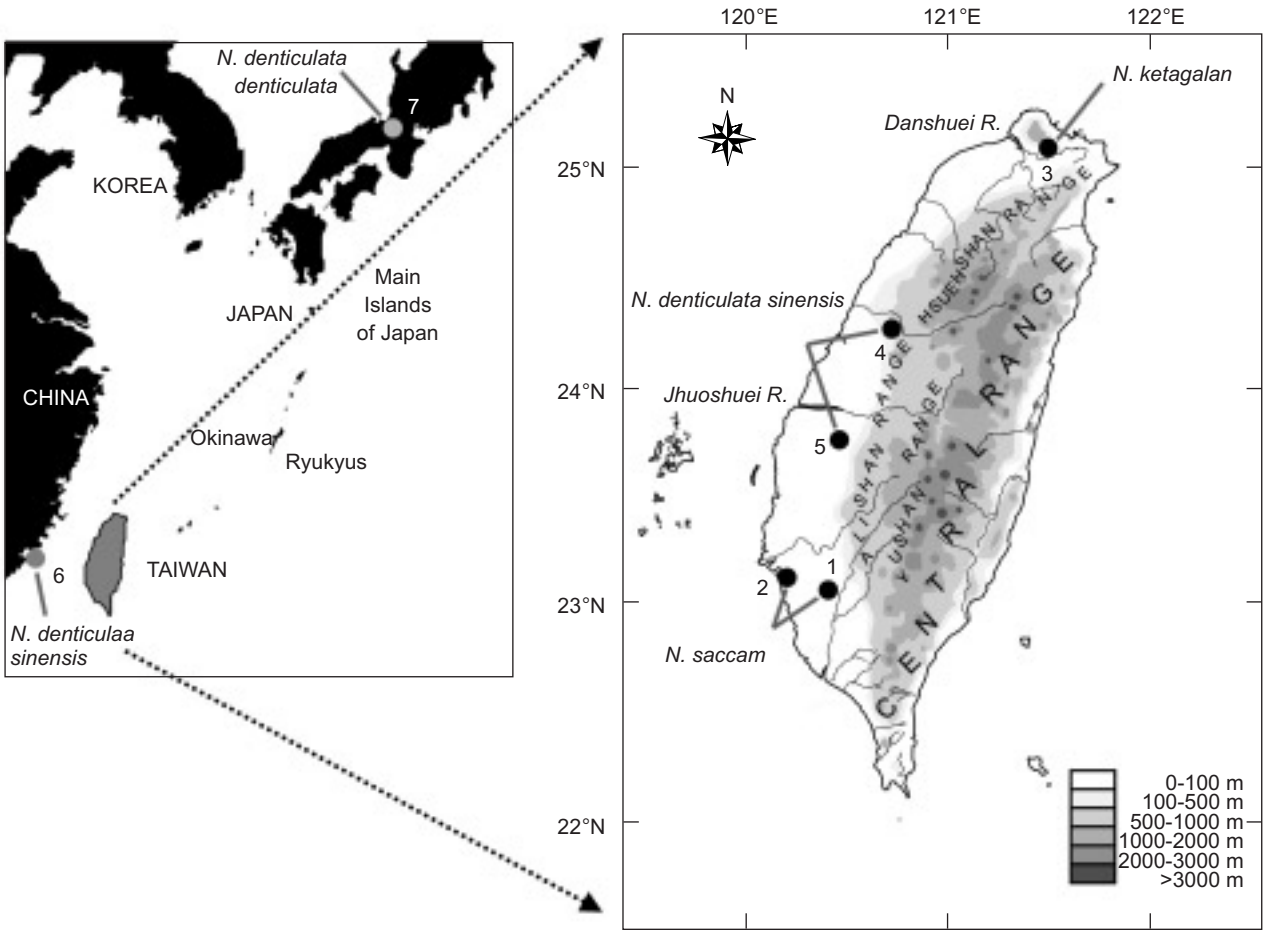

Fig. 1. Collection sites for *Neocaridina* in Taiwan and adjacent areas. The numbers beside the circles refer to localities described in table 1. R., river.

16Sbr (5'-CCGGTCTGAACTCAGATCACGT-3') (Palumbi et al. 1991). A portion of the mitochondrial cytochrome oxidase I (COI) gene was amplified with the primers LCO1490 (5'-GGTCAA CAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). The PCR conditions for the above primers were 40 cycles of denaturation for 50 s at 94°C, annealing for 70 s at 45°C, and extension for 60 s at 72°C, followed by a 72°C extension for 10 min. Sequences were obtained by automated sequencing (Applied Biosystems 3730) and were aligned with the aid of Clustal W (vers. 1.4, Thompson et al. 1994) and BioEdit (vers. 5.09, Hall 2001), after verification with the complimentary strand. Sequences of the different haplotypes were deposited in the DDBJ database (accession nos. given in Table 1). Additional sequences of 16S rRNA and COI of *Neocaridina* specimens from central Japan and Hawaii were provided by Timothy J. Page (Griffith University, Queensland, Australia) (see Table 1).

The best-fitting models for sequence evolution of 16S rRNA, COI, and the combined 16S rRNA and COI dataset were determined by MrModeltest (vers. 2.2, Nylander 2005), selected by the hierarchical Likelihood Ratio Test (hLRT), and subsequently applied to the analyses of neighbor-joining (NJ), Bayesian inference (BI), and inter- and intraspecific genetic diversities with the PAUP* program (vers. 4.0b10, Swofford 2003). A maximum parsimony (MP) tree was constructed using PAUP* with 2000 bootstrap reiterations of a simple heuristic search, tree bisection-reconnection (TBR) branch-swapping, and 100 random-addition sequence replications. Gaps in the MP tree construction were treated as missing data. The NJ tree was constructed using PAUP* with the model selected from MrModeltest. All characters were equally weighted. Bayesian analyses (BI) were performed with MrBayes (vers. 3.1.1, Ronquist and Huelsenbeck 2003) using the model selected by MrModeltest. The search was run with 4 chains for 10^6 generations, with trees being sampled every 100 generations. The 1st 500 trees were discarded as the burn-in for the calculation.

RESULTS

Taxonomy

Family Atyidae de Haan, 1849
***Neocaridina* Kubo, 1938**
***Neocaridina saccam*, sp. nov.**
(Figs. 2-4, 7A, B)

Table 1. Nine haplotypes of 16S ribosomal (r)RNA and 12 haplotypes of cytochrome c oxidase I (COI) genes of *Neocaridina* and the outgroups. Co., County; R., River; Prov., Province. The numbers within brackets following localities correspond to those in figure 1

Species	Localities	NCHUZOOL catalog no.	Sample size	Haplotypes of 16S	DDBJ accession no.	Haplotypes of COI	DDBJ accession no.
N. saccam	Longci, Tainan Co., Taiwan [1]	13103	1	Nsc-1	AB300164	Nsc-C1	AB300177
	Longci, Tainan Co., Taiwan [1]	13104	1	Nsc-2	AB300165	Nsc-C2	AB300178
	Houjha, Tainan City, Taiwan [2]	13105	1	Nsc-3	AB300166	Nsc-C1	AB300179
N. ketagalan	Sijhih, Taipei Co., Taiwan [3]	13106, 13115	2	Nkt	AB300167, AB300169	Nkt-C1	AB300180, AB300181
	Sijhih, Taipei Co., Taiwan [3]	13107	1	Nkt	AB300168	Nkt-C2	AB300182
N. denticulata sinensis	Sinshe, Taichung Co., [4]; Shihguei R., Dounan, Yunlin Co., Taiwan [5]	13108, 13109	2	Nsn-1	AB300170, AB300171	Nsn-C1	AB300183, AB300184
	Oahu, Hawaii	13110	1	Nsn-1	AB300172	Nsn-C2	AB300185
	Oahu, Hawaii	-[a]	1	Nsn-1	DQ681268	Nsn-C3	AB300186
	Kinmen Co., Taiwan [6]	13111	2	Nsn-2	AB300173	Nsn-C4	AB300187
N. d. denticulata	Lake Biwa, Shiga Prefecture, Honshu, Japan [7]	-[b]	1	Nsn-1	DQ681268	Ndt-C	AB300191
Outgroups							
N. spinosa	Tongan, Fujian Prov., China	13112	1	Nsp	AB300174	Nsp-C	AB300188
Caridina formosae	Datun R., Taipei Co., Taiwan	13113	1	Cfm	AB300175	Cfm-C	AB300189
Caridina cantonensis	Zhapo, Guangdong Prov., China	13114	1	Cct	AB300176	Cct-C	AB300190

[a]The catalog number is NMNH#258719 (Smithsonian Institution, National Museum of Natural History, Washington DC); [b]From the personal collection of Timothy J. Page.

Material examined: *Holotype*: ovigerous ♀, cl 5.2 mm, NMNS-5472-001, mountain stream at Longci, Tainan Co., Taiwan, coll. Hsi-Te Shih, Yu-Hsi Wang, and Jian-Rong Li, 21 July 2006. Paratypes: 9 ♂♂, cl 3.6-4.2 mm, 3 ♀♀, cl 3.0-3.6 mm, NCHUZOOL 13116, data same as for holotype; 9 ♂♂, cl 2.5-3.8 mm, 3 ♀♀, cl 2.5-3.2 mm, NMNS-5472-002, data same as for holotype; 10 ♂♂, cl 3.5-4.2 mm, 6 ♀♀, cl 3.9-5.2 mm, 1 ovigerous ♀, cl 5.1 mm, ZRC 2007.0097, data same as for holotype. Others: 1 ♂, cl 3.1 mm, 2 ♀♀, cl 3.6, 4.6 mm, 1 ovigerous ♀, cl 3.9 mm,

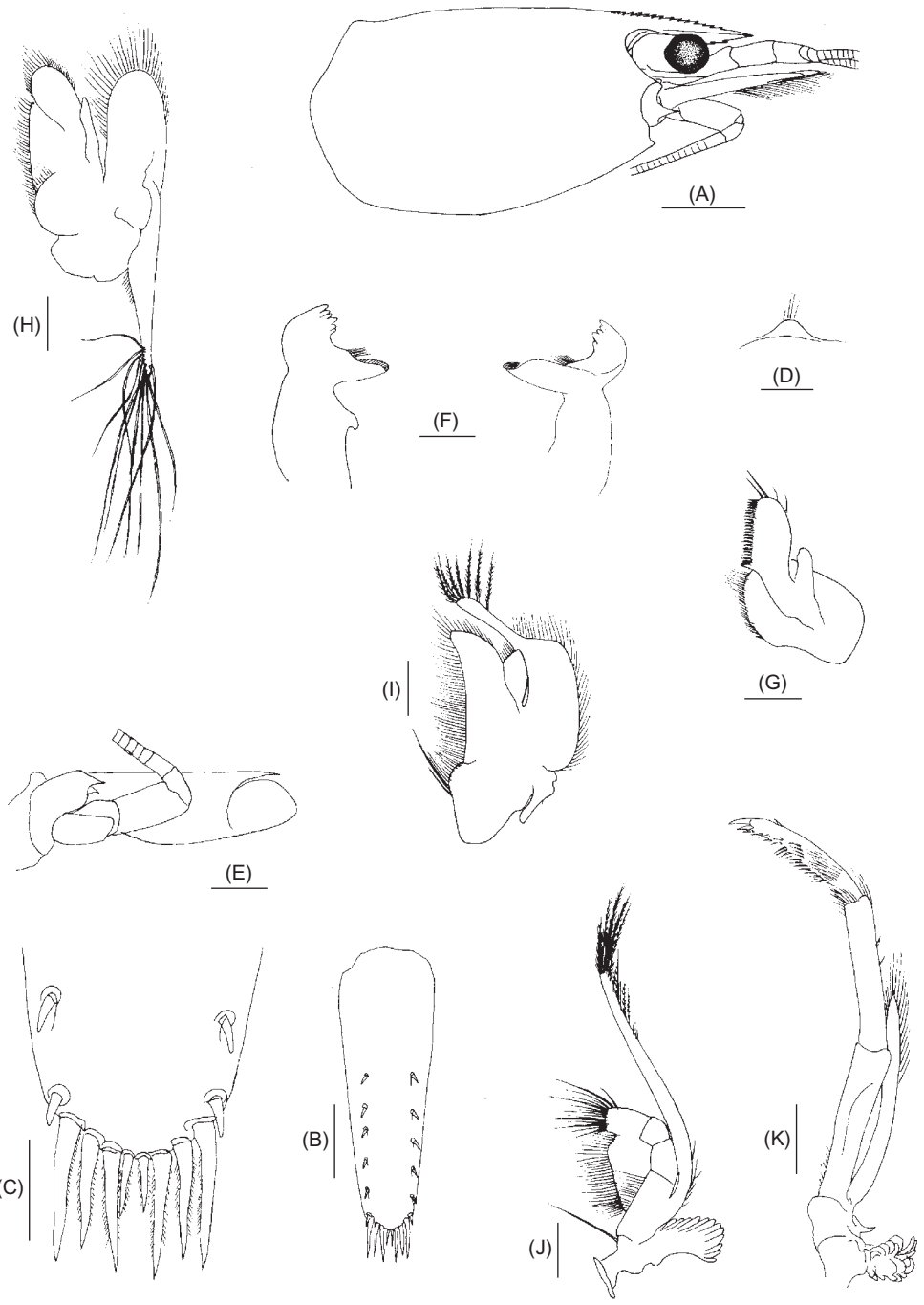

Fig. 2. *Neocaridina saccam* sp. nov. (A) Cephalothorax and cephalic appendages, lateral view; (B) telson; (C) distal portion of telson; (D) preanal carina; (E) scaphocerite; (F) mandible; (G) maxillula; (H) maxilla; (I) 1st maxilliped; (J) 2nd maxilliped; (K) 3rd maxilliped. Scales: A = 1 mm; B, K = 0.5 mm; C, E = 0.2 mm; D, F-J = 0.3 mm. (male, cl 3.5 mm, paratype, ZRC 2007.0097).

NCHUZOOL 13118, Houjha, Tainan City, Taiwan, coll. Rong-Hsiang Li, 5 Apr. 2004.

Description: Rostrum straight, reaching middle of 2nd segment of antennular peduncle, nearly reaching or beyond its end. Rostral formula 1-3 (mode 2)+9-16 (mode 12-14)/1-5(mode 2-4), inferior orbital angle of carapace fused with antennal spine; ptergygostomial angle rectangular with a tiny spine.

Third abdominal somite with moderately con-

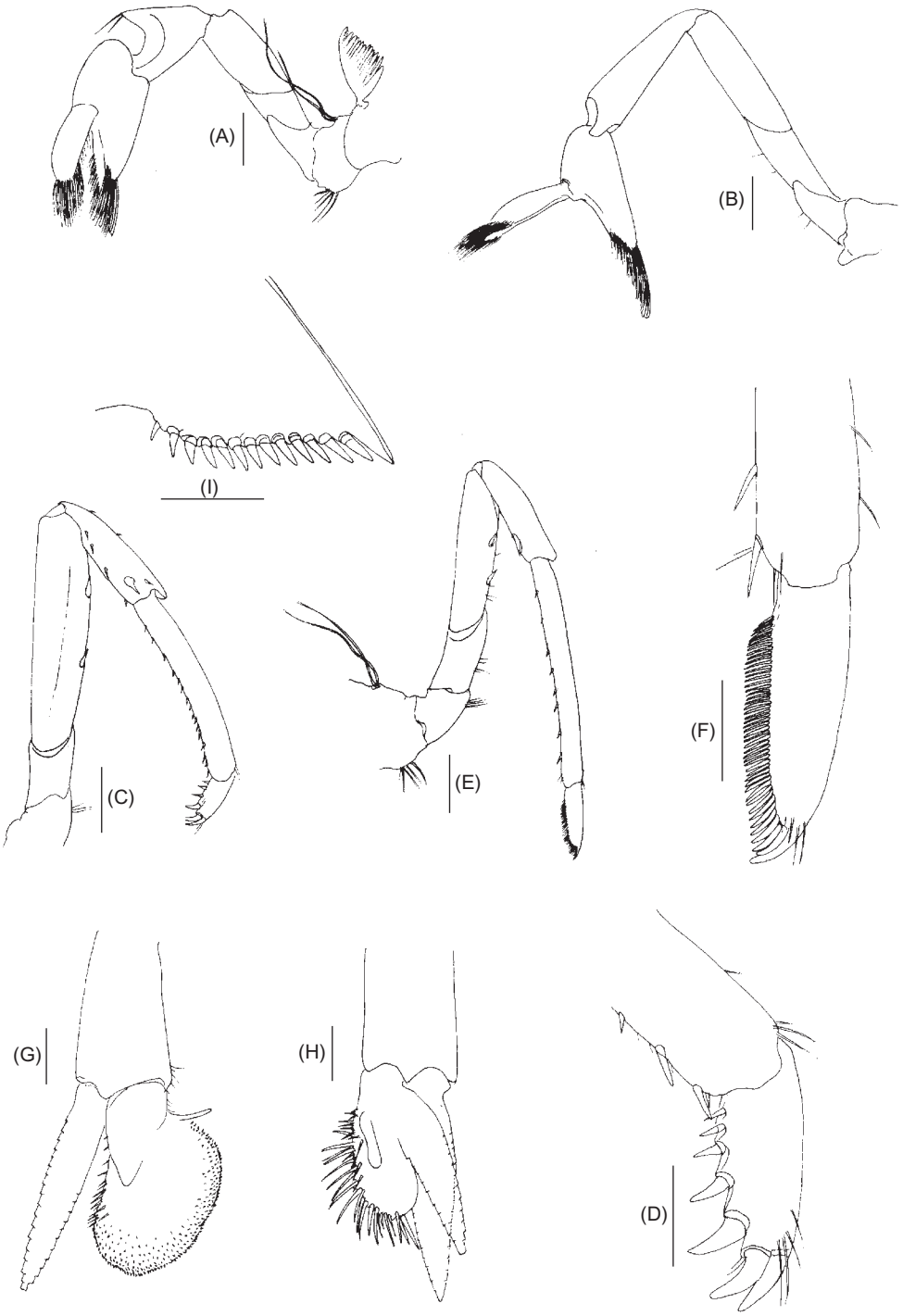

Fig. 3. *Neocaridina saccam* sp. nov. (A) 1st pereiopod; (B) 2nd pereiopod; (C) 3rd pereiopod; (D) the same, dactylus; (E) 5th pereiopod; (F) the same, dactylus; (G) male 1st pleopod; (H) male 2nd pleopod; (I) uropodal diaeresis. Scales: A, B, G, H = 0.3 mm; C, E = 0.5 mm; D, F, I = 0.2 mm. (male, cl 3.5 mm, paratype, ZRC 2007.0097).

vex dorsal profile. Sixth abdominal somite about 1/2 length of carapace, as long as telson, 1.8 times as long as 5th abdominal somite. Telson terminating or not terminating in a projection; 4 or 5 pairs of dorsal spinules, 1 pair of dorsolateral spines near distal end, 3 or 4 pairs of spines on distal margin, lateral pair longer than sublateral pair, slightly longer than intermediate pairs; preanal carina rounded, no spine present.

Eyes well developed. Antennular peduncle stout, 0.55-0.65 times as long as carapace; basal segment of antennular peduncle longer than sum

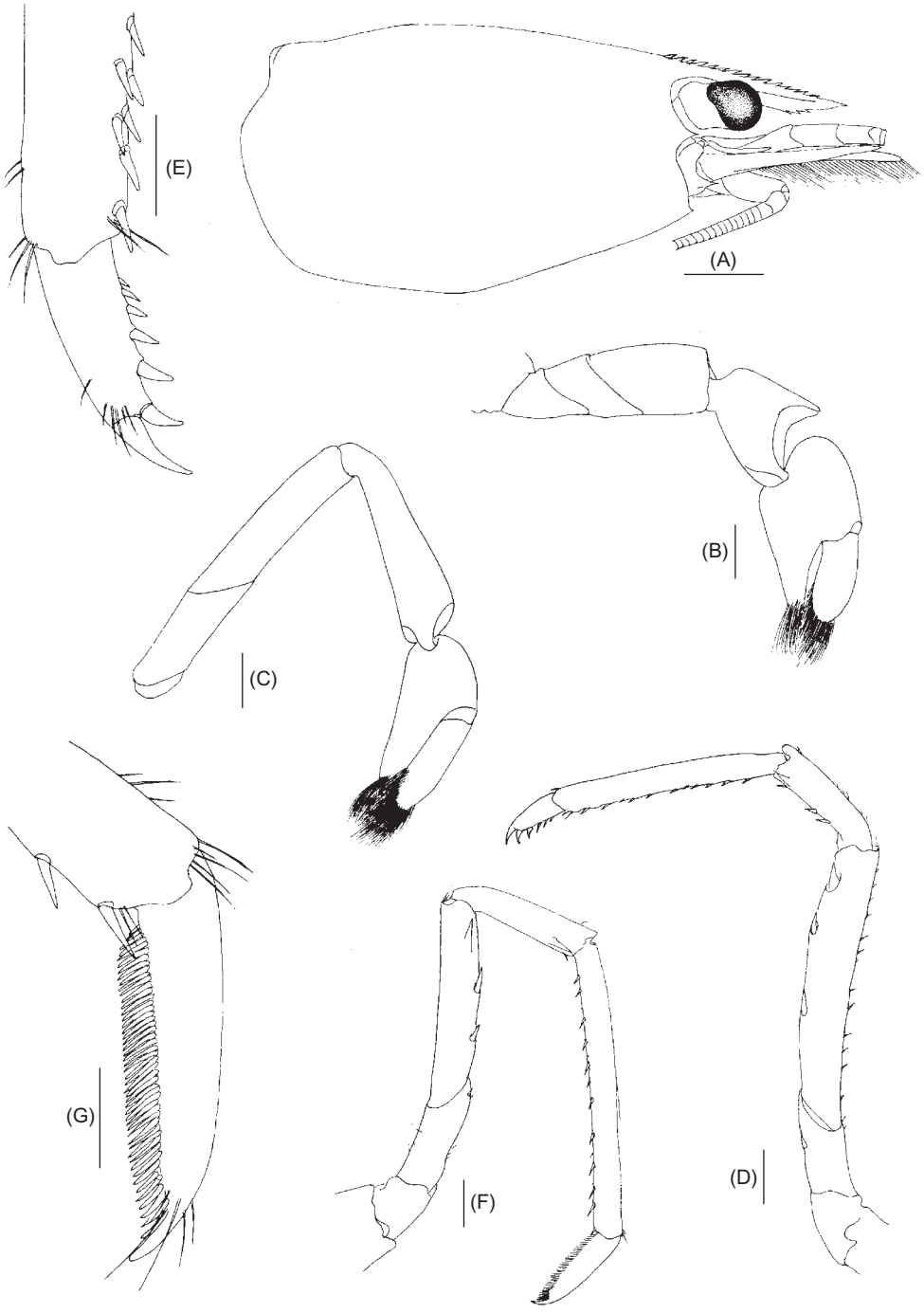

Fig. 4. *Neocaridina saccam* sp. nov. (A) Cephalothorax and cephalic appendages, lateral view; (B) 1st pereiopod; (C) 2nd pereiopod; (D) 3rd pereiopod; (E) the same, dactylus; (F) 5th pereiopod; (G) the same, dactylus. Scales: A = 1 mm, B-D, F = 0.3 mm; E, G = 0.2 mm. (female, cl 5.2 mm, paratype, ZRC 2007.0097).

of 2nd and 3rd segment lengths; 2nd segment distinctly longer than 3rd segment; stylocerite reaching 0.8-0.9 times length of basal segment of antennular peduncle, anterior angle of basal segment reaching 0.3 times length of 2nd segment. Scaphocerite 3.1 times as long as wide.

Incisor process of mandible ending in irregular teeth, molar process truncated. Lower lacinia of maxillula broadly rounded, upper lacinia elongate, with a number of distinct teeth on inner margin, palp slender. Upper endites of maxilla subdivided, palp short, scaphognathite tapering posteriorly with numerous long, curved setae at posterior end. End of palp of 1st maxilliped truncate. Podobranch of 2nd maxilliped well developed. Third maxilliped reaching beyond end of 2nd segment of antennular peduncle, with ultimate segment subequal to penultimate segment.

Epipods on 1st 4 pereiopods. First pereiopod stout, short, only reaching middle of basal segment of antennular peduncle; merus stout, as long as carpus, 1.4-1.7 times as long as wide; carpus short, as long as palm, 1.3 times (females) to 1.4 times (males), as long as high; chela 1.8-1.9 times as long as broad, fingers as long as palm. Second pereiopod slender, reaching slightly beyond end of 2nd segment of antennular peduncle; lower posterior margin of coxa of male specimens with a triangular plate, ending posteriorly in a hooked projection; merus slightly shorter than or as long as carpus, 3.3-4.4 times as long as wide; carpus 1.2 times as long as chela, 3.6-3.8 times as long as high; chela 2.2-2.3 times as long as broad; fingers 1.4-1.6 times as long as palm. Third pereiopod reaching end of antennular peduncle, merus stout, propodus straight in females, slightly incurved in males, 2.7-3.2 times as long as dactylus (terminal spine included), 7.5-8.7 times as long as broad, numerous spinules on posterior margin; dactylus terminating in 2 claws, 4-6 accessory spines on flexor margin, strongly incurved in males. Fifth pereiopod reaching end of basal segment of antennular peduncle; propodus 10-11 times as long as broad, 2.9-3.1 times as long as dactylus; and slender, dactylus 3.0-3.5 times as long as broad, terminating in 1 elongate claw, with 43-48 spinules on flexor margin.

Endopod of male 1st pleopod extending to 0.9 times exopod length, rounded, pear-shaped, 1.4 times as long as broad, numerous tiny spinules on dorsal surface. Appendix interna slender, elongate, at base of inflated part. Appendix masculina of male 2nd pleopod cylindrical, reaching 0.7 times endopod length, inner and distal surfaces densely lined with long, stout spines; appendix interna at basal 1/4 of appendix masculina, extending to base of distal 1/3 of appendix masculina.

Uropodal diaeresis with 12-14 spinules.

Eggs 1.10-1.15 x 0.65-0.70 mm in diameter.

Color: Body color varies from translucent, white, light green, to light brown; with or without brown stripes on abdomen (Fig. 7A, B).

Etymology: The new species is named after Saccam Village (inhabited by a subtribe of the Siraya Tribe which belongs to the Pingpu Tribes), a large aboriginal village formerly located on the plains and hills around the Tainan area of southwestern Taiwan. Saccam is also the name of a famous building, Chihkan Tower, built in 1653 by the Dutch, in the center of Tainan City. The name is used as a noun in apposition.

Habitat: The shrimp from the type locality, Longci, Tainan Co., live in a small pond, the water source of which is crevices in a small mountain (with an elevation of 250 m) (Fig. 7C), composed of mudstone and loosely structured material that are the result of strong river erosion giving it an appearance of badlands. *Geothelphusa ancylophallus* is sympatric with this species (Shih et al. 2007b).

Remarks: *Neocaridina saccam* sp. nov. is morphologically closest to *N. denticulata sinensis* (Kemp, 1918) from central and eastern China, and Taiwan (cf. Englund and Cai 1999). It can be distinguished from the latter by the short rostrum (reaching the middle of the 2nd segment of the antennular peduncle, rarely to or beyond the end of this segment vs. reaching the end of the 2nd segment to the end of the antennular peduncle in *N. d. sinensis*); the continuous row of rostral teeth almost throughout the upper margin vs. the anterior 1/4 unarmed in *N. d. sinensis*; the short antennular peduncle (0.55-0.65 times as long as the carapace vs. 0.75-0.85 in *N. d. sinensis*), and the stout 1st pereiopod (1.3 vs. 1.4 times as long as high in females, and 1.4 vs. 1.7 times in males). With respect to the short rostrum, the new species is most similar to *N. ishigakiensis* (Fujina and Shokita, 1975) from Ishigaki, the Ryukyus. However, it can be easily separated from *N. ishigakiensis* by its stout carpus of the 1st pereiopod (1.3-1.4 times as long as high vs. 1.6-1.8 in *N. ishigakiensis*) and the pear-shaped endopod of the male's 1st pleopod (vs. palm-shaped in *N. ishigakiensis*).

***Neocaridina ketagalan*, sp. nov.**
(Figs. 5, 6, 7D, E)

Material examined: *Holotype*: ovigerous ♀, cl 4.6 mm, NMNS-5472-003, Sijhih, Taipei Co., Taiwan, coll. H.T. Shih, Y.H. Wang, and Chiou-Ping Huang, 27 Oct. 2006. Paratypes: 5 ♂♂, cl 4.0-4.4 mm, 8 ♀♀, cl 3.8-5.2 mm, NCHUZOOL 13117, data same as for holotype; 5 ♂♂, cl 3.4-4.2 mm, 8 ♀♀, cl 3.3-4.7 mm, NMNS-5472-004,

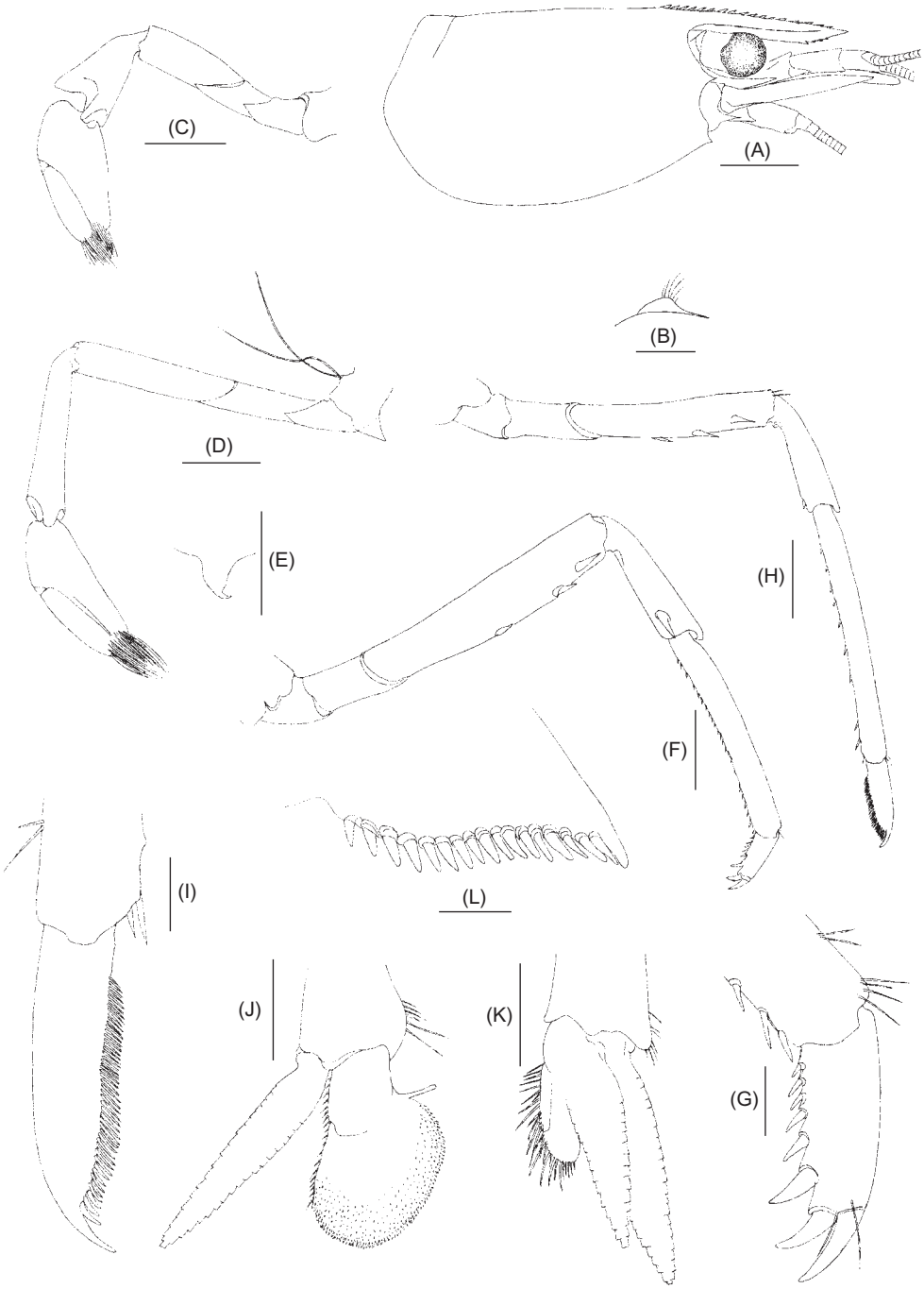

Fig. 5. *Neocaridina ketagalan* sp. nov. (A) Cephalothorax and cephalic appendages, lateral view; (B) preanal carina; (C) 1st pereiopod; (D) 2nd pereiopod; (E) hooked plate on lower posterior margin of coax; (F) 3rd pereiopod; (G) the same, dactylus; (H) 5th pereiopod; (I) the same, dactylus; (J) male 1st pleopod; (K) male 2nd pereiopod; (L) uropodal diaeresis. Scales: A = 1 mm, B-F, H, J, K = 0.5 mm; G, I, L = 0.2 mm. (male, cl 4.2 mm, paratype, ZRC 2007.0098).

data same as for holotype; 20 ♂♂, cl 3.5-5.1 mm, 27 ♀♀, cl 3.2-5.2 mm, ZRC 2007.0098, data same as for holotype. Others: 5 ♀♀, cl 3.9-4.5 mm, ZRC 2007.0494, Sijhih, Taipei Co, coll. H.T. Shih, Y.H. Wang, J.R. Li, and Min-Wan Chen, 31 Aug. 2006.

Description: Rostrum straight, reaching to middle or end of 2nd segment of antennular peduncle, rarely beyond it. Rostral formula 2-4 (mode 3)+9-14 (12 or 13)/2-5 (mode 4), inferior

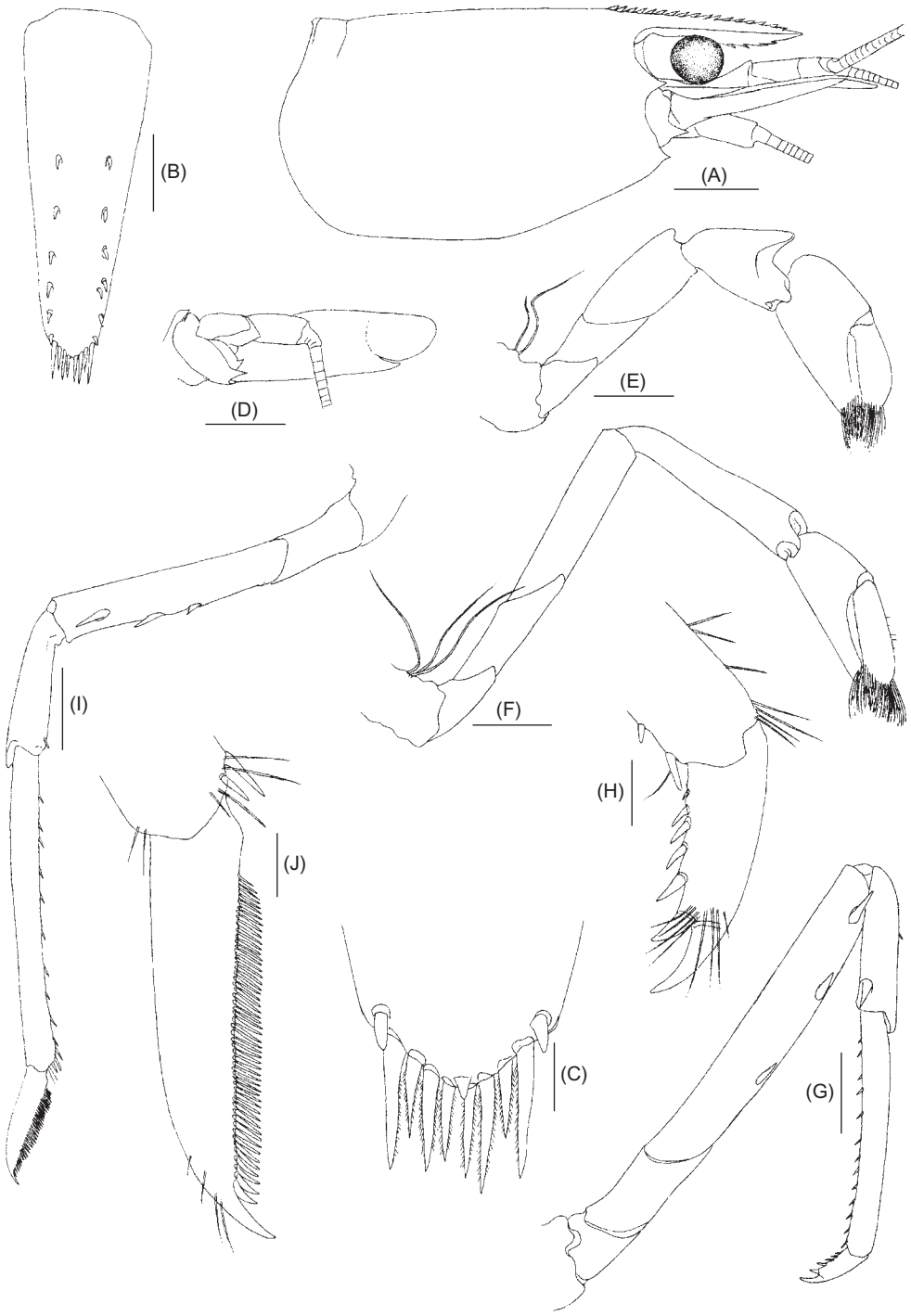

Fig. 6. *Neocaridina ketagalan* sp. nov. (A) Cephalothorax and cephalic appendages, lateral view; (B) telson; (C) distal portion of telson; (D) scalphocerite; (E) 1st pereiopod; (F) 2nd pereiopod; (G) 3rd pereiopod; (H) the same, dactylus; (I) 5th pereiopod; (J) the same, dactylus. Scales: A, D = 1 mm, B, E, F, G = 0.5 mm; C, H, J = 0.2 mm (male, cl 5.2 mm, paratype, ZRC 2007.0098).

orbital angle of carapace fused with antennal spine; ptergygostomial angle rectangular with a tiny spine.

Third abdominal somite with moderately convex dorsal profile. Sixth abdominal somite about 1/2 length of carapace, as long as telson, 1.6 times as long as 5th abdominal somite. Telson terminating or not terminating in a projection; 4 or 5 pairs of dorsal spinules, 1 pair of dorsolateral spines near distal end, 3 or 4 pairs of spines on distal margin, lateral pair longer than sublateral pair, slightly longer than intermediate pairs; preanal carina rounded, no spine present.

Eyes well developed. Antennular peduncle stout, 0.65-0.72 times as long as carapace; basal segment of antennular peduncle longer than sum of 2nd and 3rd segment lengths; 2nd segment distinctly longer than 3rd segment; stylocerite reaching 0.8-0.9 times length of basal segment of antennular peduncle, anterior angle of basal segment

Fig. 7. (A, B) Live coloration of *Neocaridina saccam* sp. nov. (A) Specimen with stripes; (B) ovigerous female with pale stripes; (C) habitat of *N. saccam*: a pond beside a road on a low-elevation mountain, Longci, Tainan Co., Taiwan. (D, E) Live coloration of *N. ketagalan* sp. nov. (D) White specimen with stripes; (E) deep-blue specimen with pale yellow longitudinal stripe on the mid-dorsal surface; (F) habitat of *N. ketagalan*: a small stream with slow velocity under dense vegetation.

reaching 0.3-0.5 times length of 2nd segment. Scaphocerite 3.4 times as long as wide.

Mouthpart structure same as that described for previous species. Epipods on 1st 4 pereiopods. First pereiopod reaching end of basal segment of antennular peduncle; merus stout, as long as carpus, 1.8-2.2 times as long as wide; carpus short, as long as palm, 1.4 times as long as high in female, 1.6 times in male; chela 2.1-2.2 times as long as broad, fingers slightly longer than palm. Second pereiopod slender, reaching slightly beyond end of 2nd segment of antennular peduncle; lower posterior margin of coxa of male specimens with a triangular plate, ending posteriorly in a hooked projection; merus slightly shorter than carpus, 3.6-4.6 times as long as wide; carpus 1.1-1.2 times as long as chela, 4.0-4.2 times as long as high; chela 2.5-2.7 times as long as broad; fingers 1.5 times as long as palm. Third pereiopod reaching end of 2nd segment of antennular peduncle, propodus straight in females, slightly incurved in males, 3.5-3.9 times as long as dactylus (terminal spine included), 8.0-8.3 times as long as broad, numerous spinules on posterior margin; dactylus terminating in 2 claws, 4-6 accessory spines on flexor margin, strongly incurved in males. Fifth pereiopod reaching middle of 2nd segment of antennular peduncle; propodus 11 times as long as broad, 2.6-3.0 times as long as dactylus; and slender, dactylus 3.7-4.0 times as long as broad, terminating in 1 elongate claw, with 48-61 spinules on flexor margin.

Endopod of male 1st pleopod extending to 0.7 times exopod length, rounded, pear-shaped, 1.4 times as long as broad, numerous tiny spinules on dorsal surface. Appendix interna slender, elongate, at base of inflated part. Appendix masculina of male 2nd pleopod cylindrical, reaching 0.7 times endopod length, inner and distal surfaces densely lined with long, stout spines; appendix interna at basal 1/4 of appendix masculina, extending to base of distal 1/3 of appendix masculina.

Uropodal diaeresis with 14-17 spinules.

Eggs 1.10-1.20 x 0.75-0.80 mm in diameter.

Color: Body color varies from translucent, white, light green, deep green, deep blue, brown, to black; always with brown stripes on abdomen; some individuals with a pale yellow longitudinal stripe on mid-dorsal surface (Fig. 7D, E).

Etymology: The new species is named after

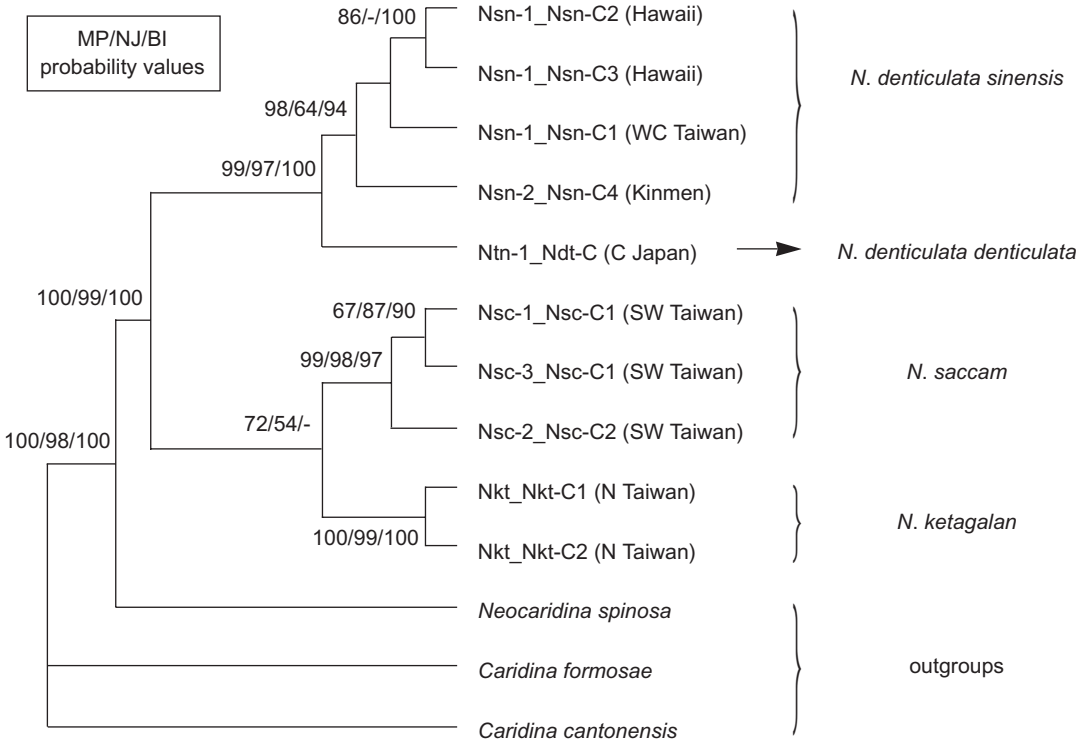

Fig. 8. Maximum parsimony (MP) tree of *Neocaridina* spp. from Taiwan and adjacent regions, and outgroups based on 1173 bp of the combined 16S ribosomal (r)RNA and cytochrome c oxidase I (COI) genes. Probability values at the nodes represent bootstrap values for neighbor-joining (NJ) and posterior probability for Bayesian inference (BI). For abbreviations of haplotypes see table 1. WC, west-central; C, central; N, northern; SW, southwestern.

the Ketagalan Tribe (which belongs to the Pingpu Tribes) which was formerly distributed on the plains and hills around the Taipei area of northern Taiwan. The name is used as a noun in apposition.

Habitat: The habitat is a small stream in an upstream area near the peak of a hill (with an elevation of 75 m), with heavy vegetation and slow water velocity (Fig. 7F).

Remarks: Morphologically, *N. ketagalan*, sp. nov. is closest to *N. denticulata sinensis* and *N. saccam*. In many characters, it falls into a range between these 2 species, but can be separated from both species as follows: its rostrum length reaches nearly to the end of the 2nd segment of the antennular peduncle (vs. mostly to the middle of the 2nd segment of the antennular peduncle in *N. saccam*, and between the end of the 2nd segment and the end of the antennular peduncle in *N. d. sinensis*); the length of the antennular peduncle is 0.65-0.72 times as long as the carapace (vs. 0.55-0.65 times in *N. saccam* and 0.75-0.80 times in *N. d. sinensis*); the merus of the 1st pereiopod is 1.8-2.2 times as long as wide (vs. 1.4-1.7 times in *N. saccam* and 2.5 times in *N. d. sinensis*; the carpus of the 1st pereiopod in the male is 1.6 times as long as high (vs. 1.4 times in *N. saccam* and 1.7 times in *N. d. sinensis*). *Neocaridina ketagalan* also shares several characters with *N. saccam* which can be used to separate both from *N. d. sinensis*, i.e., the short rostrum and rostral teeth placement on the upper margin; the lower number of accessory spines on the dactylus of the 3rd pereiopod (4-6 in both *N. saccam* and *N. ketagalan* vs. 5-8 in *N. d. sinensis*), and the relatively elongate endopod of the male 1st pleopod (1.4 times as long as wide in both *N. saccam* and *N. ketagalan* vs. 1.2 times in *N. d. denticulata*). *Neocaridina ketagalan* can be distinguished from *N. saccam* by its longer propodus of the 3rd pereiopod (3.5-3.9 times as long as dactylus vs. 2.7-3.2 times in *N. saccam*), the elongated dactylus of the 5th pereiopod (3.7-4.0 times as long as broad vs. 3.0-3.5 times in *N. saccam*), and the larger number of spinules on the uropodal diaeresis (14-17 vs. 12-14 in *N. saccam*).

DNA analysis

A 513-bp segment (excluding the primer regions) of 16S rRNA from 13 specimens of the ingroups (*N. saccam*, *N. ketagalan*, *N. denticulata sinensis*, and *N. d. denticulata*) was amplified and aligned. Of these, 18 positions were variable and 14 parsimoniously informative, and 6 different haplotypes were distinguished (Table 1). The studied segment of the 16S rRNA sequences was AT rich (67.4%) (36.1% T, 31.3% A, 20.8% G, and 11.8% C). For the COI gene of the ingroups, a 658-bp segment was compared, resulting in 9 different haplotypes. The studied segment of the COI sequences was also AT rich (59.8%) (32.7% T, 27.1% A, 20.8% G, and 19.4% C). In this gene fragment, 73 positions were variable and 64 were parsimoniously informative.

The best model selected by MrModeltest for 16S rRNA was the GTR+G model (gamma distribution shape parameter (shape) = 0.0578), for COI was the GTR+G model (shape = 0.1235), and for the combined 16S rRNA and COI segment was the GTR+G model with 1173 bp (shape = 0.1017). The phylogenetic tree constructed from the MP analysis, with the respective confidence values from the NJ and BI analyses, is shown in figure 8. Only confidence values > 50% are shown. For the MP analysis, a single tree was recovered with a tree length of 426 steps, a consistency index of 0.80, and a retention index of 0.78.

Based on figure 8 of the combined dataset, 4 clades, *N. d. sinensis*, *N. d. denticulata*, *N. saccam*, and *N. ketagalan* formed a monophyletic group, and each of the taxa was separated with high support. The *N. d. sinensis* specimens collected from west-central Taiwan, Kinmen I. off the coast of China, and Hawaii, formed a monophyletic clade, but the confidence value of NJ was not very high. Specimens of *N. d. denticulata* from Japan formed a sister clade to *N. d. sinensis*. However, the 16S rRNA of *N. d. denticulata* was the same as that of *N. d. sinensis* specimens from Taiwan and Hawaii. The Taiwanese clade (*N. saccam* and *N. ketagalan*) was weakly supported, and only the MP bootstrap value was higher.

In table 2, the pairwise nucleotide divergences for 16S rRNA and COI (in parentheses) with models selected by MrModeltest and differences in the total base pair numbers (gaps considered) are shown. For 16S rRNA, the genetic distances within the 4 main ingroup clades (*N. d. denticulata*, *N. d. sinensis*, *N. saccam*, and *N. ketagalan*) (ranging from 0% to 1.29%, mean, 0.39%) did not significantly differ from those between species (from 0% to 3.80%, mean, 1.47%) (p = 0.52, Mann-Whitney U-test). The mean number of differences within species (from 0 to 1.3, mean, 0.58) also did not significantly differ from those between species (from 0 to 14.2, mean, 5.89) (p = 0.52, Mann-Whitney U-test). For the COI gene,

the genetic distance within each clade (from 0% to 0.46%, mean, 0.26%) was significantly lower than that between species (from 3.03% to 12.74%, mean, 10.32%) (p = 0.01, Mann-Whitney U-test); and the mean number of differences within species (from 0 to 3, mean, 1.7) was also significantly lower than that between species (from 16.8 to 47.5, mean, 40.1) (p = 0.01, Mann-Whitney U-test).

DISCUSSION

Hung et al. (1993) found that most specimens of the genus *Neocaridina* from Taiwan should be attributed to *N. denticulata sinensis*, but some had characters of *N. denticulata denticulata*. However, they used the name *N. denticulata* for all of their materials. In his revision on the genus *Neocaridina*, Cai (1996) redescribed *N. d. sinensis* (Kemp, 1918), based on specimens from Taihu Lake, Jiangsu Province, China, the type locality of that subspecies, and considered that the real *N. d. denticulata* is probably endemic to the main islands of Japan while *N. d. sinensis* is distributed in East and Central China, and possibly Taiwan. The introduced species of *Neocaridina* in Hawaii was also identified as *N. d. sinensis*, and populations from Taiwan and China were projected as being its native counterparts for comparison (Englund and Cai 1999).

Liang (2002) described a new species, *N. heteropoda*, from Zhejiang Province, China, and referred some of Cai's (1996) *N. d. sinensis* to his new species. Liang (2002 2004) also considered that the characters separating *N. d. denticulata* and *N. d. sinensis* are not reliable, and treated *N. d. sinensis* from China and Taiwan as a synonym of *N. d. denticulata*, and referred to the form introduced to Hawaii as *N. heteropoda heteropoda*. As neither Cai (1996) nor Liang (2002 2004) had examined the type material of *Caridina denticulata sinensis* Kemp, 1918, the definition of that subspecies is rather uncertain for the time being. The validity of *N. heteropoda* Liang, 2002 is also doubtful as Liang (2004: 91) listed *Caridina davidi* Bouvier, 1904 as a synonym of *N. heteropoda*, which in turn, should make *H. heteropoda* a junior synonym, if Liang's (2004) conclusion is correct. However, it cannot be ascertained as Liang (2004) did not examine the types of *C. davidi* and did not explain this synonymization in the text. With all these taxonomic and nomenclatural uncertainties regarding the Chinese subspecies, and to prevent further confusion, we hereby follow Englund and Cai (1999) and retain the name *N. denticulata*

Table 2. Matrix of percentage pairwise nucleotide divergences (lower left) and mean number of differences (including gaps) (upper right) based on 515 bp of 16S ribosomal (r)RNA and 658 bp of cytochrome c oxidase I (COI) (in parentheses) within and between clades of *Neocaridina* and the outgroups. Nucleotide divergence was estimated from pairwise distances of nucleotides based on the estimated GTR+I model for both 16S rRNA and COI

	Within clades		Between clades						
	Nucleotide divergence	Mean nucleotide difference	*N. saccam*	*N. ketagalan*	*N. denticulata sinensis*	*N. d. denticulata*	*N. spinosa*	*Caridina formosae*	*Caridina cantonensis*
N. saccam	0.27 (0.46)	1.3 (3)	-	12.7 (38.0)	14.2 (46.3)	13.7 (47.5)	31.7 (83.5)	59.3 (113.5)	59.0 (105.5)
N. ketagalan	0 (0.15)	0 (1)	3.17 (9.03)	-	8.5 (45.8)	9.0 (46.5)	36.0 (85.5)	59.0 (107.5)	61.0 (102.5)
N. denticulata sinensis	1.29 (0.44)	1 (2.8)	3.80 (12.49)	1.83 (12.07)	-	0.5 (16.8)	33.5 (83.8)	57.5 (108.5)	60.5 (104.8)
N. d. denticulata	0 (0)	0 (0)	3.64 (12.74)	1.97 (12.54)	0.10 (3.03)	-	33.0 (85.0)	57.0 (111.0)	60.0 (107.0)
N. spinosa	0 (0)	0 (0)	14.87 (40.72)	17.82 (41.47)	15.40 (41.71)	15.05 (42.9)	-	53.0 (119.0)	58.0 (102.0)
Caridina formosae	0 (0)	0 (0)	70.38 (93.01)	71.87 (85.57)	65.82 (86.53)	64.62 (91)	43.33 (113.79)	-	59.0 (108.0)
Caridina cantonensis	0 (0)	0 (0)	65.95 (80)	79.27 (74.66)	70.00 (83.86)	68.72 (88.35)	61.27 (75.77)	71.57 (93.94)	-

sinensis for populations of *Neocaridina* from East and Central China, Taiwan, and the Hawaiian Is. Further work is required to unravel these inconsistencies.

Neocaridina denticulata sinensis was distinguished from *N. d. denticulata* by Kemp (1918) mainly on the basis of the rostral formula: 14-22/3-8 (vs. 10-15/2-5), and the anterior carpal margin of the 1st pereiopod, which is deeply excavated compared to being slightly excavated. The difference in the rostral formula of the 2 species is not significant (8-19/1-9 in *N. d. sinensis* vs. 10-20/0-7 in *N. d. denticulata*), but the rostral length is more useful in separating them as suggested by Kubo (1938). Normally, the rostrum of *N. d. sinensis* does not reach beyond the end of the antennular peduncle, whereas it reaches well beyond it in *N. d. denticulata*. Sexual dimorphism of the last 3 pereiopods appears in *N. d. sinensis* but not in *N. d. denticulata*. This is a very reliable character which easily separates the 2 taxa.

The analysis of 16S rRNA failed to distinguish *N. d. denticulata* (from Japan) and *N. d. sinensis* (from Kinmen (Taiwan), Taiwan, and Hawaii) because they are identical or only 1 bp apart (Table 2), and they might be the same species. However, the more-variable COI gene marker distinguished them. There are 15-18 bp difference in COI between the 2 subspecies, and the 2 clades are well supported, which can be considered a significant difference, as in the case of Taiwanese potamid crabs (Shih et al. 2007b). More studies on 16S rRNA and COI and morphometric calculations of specimens from various localities in East Asia may confirm their relationships and species boundary.

From table 2, there was an average 12.7 bp difference in 16S rRNA (38 bp of COI) between *N. saccam* and *N. ketagalan*, and an 8.5 bp difference in 16S rRNA (45.8 bp of COI) between *N. ketagalan* and *N. d. sinensis*. Those differences are beyond or close to the critical species boundaries of potamids (Shih et al. 2004 2005 2007b). If we accept the assumption that freshwater and terrestrial decapods have similar evolution rates, then both new species *N. saccam* and *N. ketagalan* could be well supported genetically.

If the substitution rates of 0.88% for 16S rRNA and 2.33% per 10^6 yr for COI for terrestrial *Sesarma* (see Schubart et al. 1998) is applied, the Taiwanese clades (*N. saccam* and *N. ketagalan*) diverged at a similar time, about 2.8 ± 0.8 million yr ago (mya) for 16S rRNA and 3.0 ± 0.4 mya for COI (with uncorrected p-distance divergences of 2.44% ± 0.59% and 7.02% ± 0.85%, respectively). Global sea levels were 20 m lower around 3.5 mya and 100 m lower around 2.75 mya (see Woodruff 2003), possibly providing a landbridge for the dispersal of atyids from continental China into the island of Taiwan through the Taiwan Strait. Based on the postulated paleo-drainage system on the Taiwanese continental shelf during glaciations when the sea level fell to 140 m, the ancestral Minjiang R. from Fujian Province, China was connected to the Jhuoshuei R. of west-central Taiwan (Boggs et al. 1979). The ancestral Taiwanese *Neocaridina* is proposed to have invaded west-central Taiwan through this river system and then dispersed northward and southward due to the barrier of the Central Range which restricted easterly migration since around 3 mya.

Neocaridina saccam and *N. ketagalan* separated at about the same time, 2.6 ± 0.7 mya for 16S rRNA and 2.5 ± 0.4 mya for COI (with p-distance divergences of 2.28% ± 0.61% and 5.78% ± 0.90%, respectively). The orogeny of northern Taiwan from 2.8 to 2.5 mya (Wang and Chen 1990, Juang 1992) was suggested to have isolated the population in Taipei from others, and *N. ketagalan* speciated in northern Taiwan.

Based on the molecular tree in this study (Fig. 8), the Hawaiian *Neocaridina* is very close to *N. d. sinensis* from west-central Taiwan in terms of both 16S rRNA and COI. Their 16S rRNA is the same, and there is only a 3-bp difference in COI between them. The molecular evidence suggests that *N. d. sinensis* in Hawaii was probably introduced from populations native to west-central Taiwan. However, more sampling of different localities around Taiwan and China in the future is necessary to clarify this issue.

Acknowledgments: This study was supported by a grant from the National Science Council of Taiwan (NSC95-2621-B-005-004-) to the senior author. We wish to thank Timothy J. Page for sequencing the specimens from Japan and Hawaii, and Yu-Hsi Wang, Rong-Hsiang Li, Jian-Rong Li, Min-Wan Chen, Hsiao-Wei Kao, Min-Yun Liu, Tohru Naruse, Nian-Hong Jiang-Liaw, Chiou-Ping Huang, Rafael Lemaitre, and Koji Nishi for specimen collection. Thanks are also due to Peter K. L. Ng and Lena Chan for providing laboratory facilities and comparative materials for the morphological study. Two anonymous reviewers, who helped to improve this manuscript, are also acknowledged.

REFERENCES

Boggs SJ, WC Wang, FS Lewis, JC Chen. 1979. Sediment properties and water characteristics of the Taiwan Shelf and Slope. Acta Oceanogr. Taiwan. **10:** 10-49.

Cai Y. 1996. A revision of the genus *Neocaridina* (Crustacea: Decapoda: Atyidae). Acta Zootaxon. Sin. **21:** 129-160. (in Chinese)

Crandall KA, JFJ Fitzpatrick. 1996. Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. Syst. Biol. **45:** 1-26.

de Haan W. 1849. Crustacea. *In* von Siebold PF, ed. Fauna Japonoca sive descriptio animalium, quae in itinere per Japoniam, jussu et auspiciis superiorum, qui summum in India Batavia Imperium tenent, suscepto annis 1823-1830 collegit, notis, observationibus et adumbrationibus illustravit, (Crustacea). Leiden, the Netherlands: Lugduni-Batavorum.

Englund RA, Y Cai. 1999. The occurrence and description of *Neocaridina denticulata sinensis* (Kemp, 1918) (Crustacea: Decapoda: Atyidae), a new introduction to the Hawaiian Islands. Bishop Mus. Occas. Pap. **58:** 58-65.

Folmer O, M Black, W Hoeh, R Lutz, R Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. **3:** 294-299.

Fujino T, S Shokita. 1975. Report on some atyid shrimps (Crustacea, Decapoda, Caridea) from the Ryukyus Islands. Bull. Sci. Engin. Div. Univ. Ryukyus (Math. Nat. Sci.) **18:** 93-113.

Hall TA. 2001. BioEdit: a user-friendly biological sequence alignment editor and analysis, vers. 5.09. Raleigh, NC: Department of Microbiology, North Carolina State Univ.

Hung MS, TY Chan, HP Yu. 1993. Atyid shrimps (Decapoda: Caridea) of Taiwan, with descriptions of three new species. J. Crustacean Biol. **13:** 481-503.

Juang WS. 1992. Volcanic activity and igneous rocks of Taiwan. Taichung, Taiwan: National Museum of Natural Science. (in Chinese)

Kemp S. 1918. Zoological results of a tour in the Far East, Crustacea Decapoda Stomatopoda. Mem. Asiat. Soc. Bengal **6:** 219-297.

Kubo I. 1938. On the Japanese atyid shrimps. J. Imp. Fish. Inst. Tokyo **33:** 67-100.

Liang XQ. 2002. On new species of atyid shrimps (Decapoda, Caridea) from China. Oceanol. Limnol. Sin. **33:** 167-173. (in Chinese)

Liang XQ. 2004. Fauna Sinica. Invertebrata: Crustacea: Decapoda: Atyidae. Beijing, China: Science Press. (in Chinese)

Naruse T, S Shokita, Y Cai. 2006. *Neocaridina iriomotensis*, a new species of land-locked freshwater shrimp (Crustacea: Decapoda: Atyidae) from Iriomote island, southern Ryukyus, Japan. Proc. Biol. Soc. Wash. **119:** 25-31.

Nylander JAA. 2005. MrModeltest vers. 2.2. Uppsala, Sweden: Evolutionary Biology Centre, Uppsala Univ.

Palumbi SR, A Martin, S Romano, WO Mcmillan, L Stice, G Grabowski. 1991. The simple fool's guide to PCR. A collection of PCR protocols, version 2. Honolulu, HI: Univ. of Hawaii.

Ronquist F, JP Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19:** 1572-1574.

Schubart CD, R Diesel, SB Hedges. 1998. Rapid evolution to terrestrial life in Jamaican crabs. Nature **393:** 363-365.

Shih HT, GX Chen, LM Wang. 2005. 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. J. Nat. Hist. **39:** 2901-2911.

Shih HT, SH Fang, PKL Ng. 2007a. Phylogeny of the freshwater crabs genus *Somanniathelphusa* Bott (Decapoda: Parathelphusidae) from Taiwan and the coastal regions of China, with notes on their biogeography. Invertebr. Syst. **21:** 29-37.

Shih HT, HC Hung, CD Schubart, CA Chen, HW Chang. 2006. Intraspecific genetic diversity of the endemic freshwater crab *Candidiopotamon rathbunae* (Decapoda, Brachyura, Potamidae) reflects five million years of geological history of Taiwan. J. Biogeogr. **33:** 980-989.

Shih HT, PKL Ng, HW Chang. 2004. Systematics of the genus *Geothelphusa* (Crustacea: Decapoda, Brachyura, Potamidae) from southern Taiwan: a molecular appraisal. Zool. Stud. **43:** 519-526.

Shih HT, PKL Ng, CD Schubart, HW Chang. 2007b. Phylogeny and phylogeography of the genus *Geothelphusa* (Crustacea: Decapoda, Brachyura, Potamidae) in southwestern Taiwan based on two mitochondrial genes. Zool. Sci. **24:** 57-66.

Shokita S. 1976. Early life-history of the land-locked atyid shrimp, *Caridina denticulata ishigakiensis* Fujino et Shokita, from the Ryukyu Islands. Res. Crust. **7:** 1-10.

Shokita S. 1979. The distribution and speciation of the inland water shrimps and prawns from the Ryukyu Islands. II. Bull. Coll. Sci. Univ. Ryukyus **28:** 193-278. (in Japanese)

Shy JY, PH Ho, HP Yu. 1992. Complete larval development of *Neocaridina denticulata* (De Haan, 1884) (Crustacean, Decapoda, Caridea) reared in the laboratory. Ann. Taiwan Mus. **35:** 79-89. (in Chinese)

Shy JY, WH Liou, HP Yu. 1987. Morphological observation on the development of larval *Neocaridina brevirostris* Stimpson, 1860 (Crustacea: Decapoda: Atyidae) reared in the laboratory. J. Fish. Soc. Taiwan **14:** 15-24. (in Chinese)

Shy JY, HP Yu. 1998. Freshwater shrimps of Taiwan. Kaohsiung, Taiwan: National Museum of Marine Biology/Aquarium. (in Chinese)

Swofford DL. 2003. PAUP*: phylogenetic analysis using parsimony (*and other methods), vers. 4. Sunderland, MA: Sinauer Associates.

Thompson JD, DG Higgins, TJ Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:** 4673-4680.

Wang WH, CH Chen. 1990. The volcanology and fission track age dating of pyroclastic deposits in Tatun volcano group, northern Taiwan. Acta Geol. Taiwan. **28:** 1-30.

Woodruff DS. 2003. Neogene marine transgressions, palaeogeography and biogeographic transitions on the Thai-Malay Peninsula. J. Biogeogr. **30:** 551-567.

Xue JZ, L Zheng, ZF Cheng. 1995. Primary study of the embryo development of *Neocaridina denticulata sinensis*. J. Hangzhou Teach. Coll. **6:** 79-83. (in Chinese)

Yeo DCJ, HT Shih, R Meier, PKL Ng. 2007. Phylogeny and biogeography of the freshwater crab genus *Johora* (Crustacea: Brachyura: Potamidae) from the Malay Peninsula, and the origins of its insular fauna. Zool. Scr. **36:** 255-269.