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Using Molecular Tools to Establish the Type Locality and Distribution of the Endemic Taiwanese Freshwater Crab *Geothelphusa chiui* Minei, 1974 (Crustacea: Brachyura: Potamidae), with Notes on the Genetic Diversity of *Geothelphusa* from Eastern Taiwan

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Peter K.L. Ng, Hsi-Te Shih, Tohru Naruse, and Jhy-Yun Shy (2010) Using molecular tools to establish the type locality and distribution of the endemic Taiwanese freshwater crab *Geothelphusa chiui* Minei, 1974 (Crustacea: Brachyura: Potamidae), with notes on the genetic diversity of *Geothelphusa* from eastern Taiwan. *Zoological Studies* **49**(4): 544-555. The potamid freshwater crab genus *Geothelphusa* reaches its highest diversity in Taiwan, and since the last major revision in 1994, substantial progress has been made in confirming the identities of the various species. Despite those efforts, the validity of the type locality of *G. chiui* Minei, 1974, from Nanpu, Hsinchu, northwestern Taiwan, has remained doubtful because repeated efforts to establish its presence at its type locality have been unsuccessful. DNA sequences of the holotype and paratype of *G. chiui* show that this taxon belongs to a subclade the members of which are found in southern Hualien County in eastern Taiwan, and that it is the sister species of *G. cinerea* Shy, Ng and Yu 1994, from central Hualien County. This study confirms that the stated type locality of *G. chiui* is incorrect, and it is accordingly revised herein. http://zoolstud.sinica.edu.tw/Journals/49.4/544.pdf

Key words: mtDNA sequences, 16S rRNA, Cytochrome *c* oxidase subunit I.

The potamid freshwater crab genus *Geothelphusa* Stimpson, 1858, is one of the largest genera in the Potamidae, with 51 species (Ng et al. 2008) from Taiwan and Japan. Taiwan has the majority of these taxa, with 37 known species (Shy et al. 1994, Shy and Ng 1998, Ng et al. 2001 2008, Shih et al. 2008, Shih and Shy 2009). In recent years, there has been an increase in studies of the biology, ecology, and phylogeny of *Geothelphusa* species (e.g., Liu and Li 2000, Shih et al. 2004 2007 2008 2009, Shih and Shy 2009), and this has resulted in the subsequent collection of most of the species that have been described from Taiwan. Despite this, a few Taiwanese species such as *G. yangmingshan* Shy, Ng and Yu 1994, and *G. wangi* Shy, Ng and Yu 1994, are known from very few specimens, and not much is known about their biology. Their identities are nevertheless reasonably well established as they are well described with precise locations. In fact, the only species in Taiwan the taxonomy of which is still shrouded in doubt is *G. chiui* Minei, 1974 (Shy et al. 1994).

Ever since the revision of the Taiwanese

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species of *Geothelphusa* by Shy et al. (1994), researchers have attempted to test the validity of *G. chiui* and ascertain its precise taxonomic identity. Repeated efforts to collect topotypic material of *G. chiui* from Hsinchu in northwestern Taiwan all failed, and specimens collected from that area were all referable to another taxon. The present work clarifies the taxonomy of this problematic species on the basis of DNA and morphological evidence derived from our examination of the type series and of fresh collections of *G. chiui* from Taiwan. The data confirm that the original provenance of the specimens is incorrect.

MATERIALS AND METHODS

A preliminary comparison of sequences of 16S ribosomal (r)RNA of the type series of *G. chiui* (see Table 1 and "Material examined" under "RESULTS"), with that of specimens collected from other parts of Taiwan, showed that *G. chiui*, the recorded type locality of which was in Hsinchu, belonged to a clade comprised of species of *Geothelphusa* from eastern Taiwan. Additional specimens from Hsinchu (the original type locality), Hualien, and Taitung (both in eastern Taiwan) were therefore sequenced and included in the island-wide study (Fig. 1, Table 1), with the Japanese *G. dehaani* (White, 1847) as the most distant outgroup.

Specimens used for the molecular study and morphological examination are deposited in the following collections: the Zoological Laboratory, Kyushu Univ., Fukuoka, Japan (ZLKU), but were transferred to the Kitakyushu Museum of Natural History and Human History in Fukuoka; the Zoological Reference Collection of the Raffles Museum of Biodiversity Research, National Univ. of Singapore (ZRC); the Department of Life Science, National Chung Hsing Univ. (NCHUZOOL), Taichung, Taiwan; and the Department of Environmental Biology and Fisheries Science, National Taiwan Ocean Univ. (NTOU), Keelung, Taiwan. The abbreviations, G1 and G2, are used for the male 1st and 2nd pleopods, respectively. Measurements (in millimeters) are of the maximum carapace widths and lengths, respectively.

Genomic DNA was isolated from the muscle tissue of legs using a GeneMark tissue and cell genomic DNA purification kit (Taichung, Taiwan). A region of approximately 550 basepairs (bp) of the 5'-end of the 16S rRNA gene was selected for amplification with a polymerase chain reaction (PCR) using the primers 1471 (5'-CCTGTTTANCA AAAACAT-3') and 1472 (5'-AGATAGAAACCAACC TGG-3') (Crandall and Fitzpatrick 1996). A portion of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified by a PCR using the primers LCO1490 (5'-GGTCAACAAATCATAAAGA TATTGG-3') and HCO2198 (5'-TAAACTTCAGGG TGACCAAAAAATCA-3') (Folmer et al. 1994). An internal primer designed by Roman and Palumbi (2004) for *Carcinus maenas* (5'-GCTTGAGCT GGCATAGTAGG-3') was also used. The PCR conditions for the above primers were denaturation for 50 s at 94°C, annealing for 70 s at 45-47°C, and extension for 60 s at 72°C (40 cycles), followed by extension for 10 min at 72°C. Sequences were obtained by automated sequencing (Applied Biosystems 3730, Foster City, CA, USA) and were aligned with the aid of Clustal W (vers. 1.4, Thompson et al. 1994), after verification with the complimentary strand. Sequences of the different haplotypes were deposited in the DNA Data Bank of Japan (DDBJ) databases (accession nos. are given in Table 1).

The best-fitting model for sequence evolution of the combined 16S rRNA and COI dataset was determined by MrModeltest (vers. 2.2, Nylander 2005), selected by the Akaike information criterion (AIC), and was subsequently applied to the maximum likelihood (ML) analysis. The best-fitting models for sequence evolution of the 16S rRNA and COI datasets, respectively, were also determined by MrModeltest and were subsequently used for the partitioned Bayesian inference (BI) analysis. The BI analysis was performed with MrBayes (vers. 3.1.1, Ronquist and Huelsenbeck 2003), with the parameters estimated from MrModeltest. The search was run with 4 chains for 10×10^6 generations and 4 independent runs with trees sampled every 1000 generations (the 1st 5000 trees were later discarded as the burn-in). A consensus maximum parsimony (MP) tree was constructed using PAUP* (vers. 4.0b10, Swofford 2003) with 2000 bootstrap replications 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. All characters were equally weighted. The ML analysis was also carried out using PAUP* with 200 bootstrap replications and 20 random-addition sequence replications. The other parameters were the same as in the MP analysis. In order to avoid an excessive computation time, the total number of rearrangements for each search was limited to

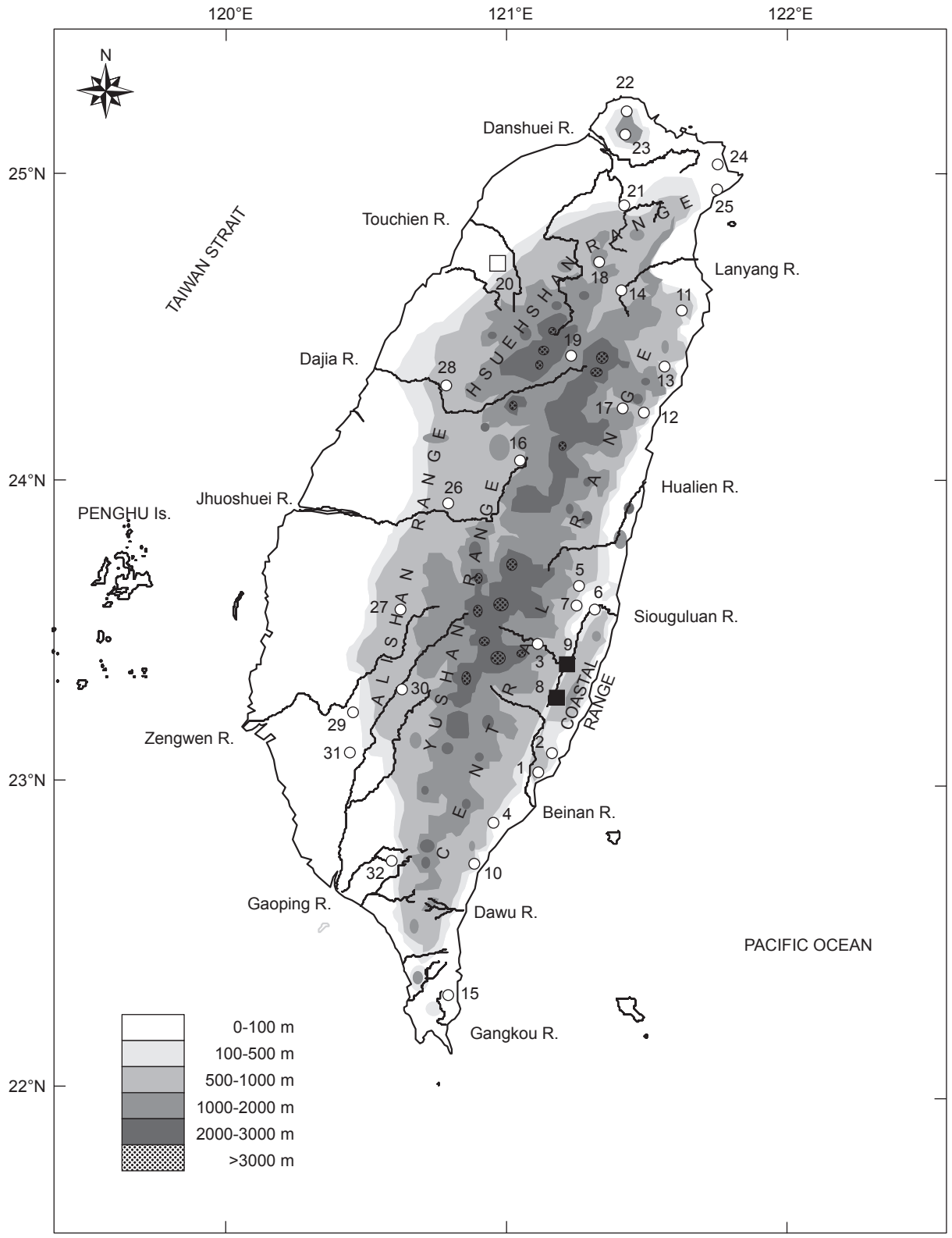

Fig. 1. Collection sites for species of *Geothelphusa* in Taiwan. For locality names and haplotypes, see table 1. The original type locality of *G. chiui* Minei, 1974 was "Nanpu, Beipu, Hsinchu" in northwestern Taiwan (no. 20, indicated by an empty square), and the revised type locality is near Fuli (no. 8) and Nantong (no. 9) (both indicated by black squares), southern Hualien in eastern Taiwan. Is., Islands; R, river.

Table 1. Thirty-five haplotypes of the 16S ribosomal RNA gene and 37 haplotypes of the cytochrome *c* oxidase subunit I (COI) gene of *Geothelphusa* species, corresponding to specimens collected from various places of Taiwan, and outgroups. Most species were identified based on Shy et al. (1994) and Shy and Yu (1999). Numbers within brackets correspond to localities in figure 1. NCHUZOOL, Department of Life Science, National Chung Hsing Univ.; NTOU, Department of Environmental Biology and Fisheries Science, National Taiwan Ocean Univ.; ZLKU: Zoological Laboratory, Kyushu Univ.

Region	Species	Locality	Catalogue no. of museum (NCHUZOOL)	Sample size	Haplotype of 16S	DDBJ accession no.	Haplotype of COI	DDBJ accession no.
Eastern region								
	G. sp. 1	Taitung Co. (Yanping) [1]	13270	1	Gsp-1	AB535433	Gsp-C1	AB535461
		Taitung Co. (Taiyuan) [2]	13271	1	Gsp-1a	AB535434	Gsp-C1a	AB535462
	G. sp. 2	Hualien Co. (Walami, Jhuosi) [3]	13272	1	Gsp-2	AB535435	Gsp-C2	AB535463
	G. bicolor	Taitung Co. (Jhihben) (holotype) [4]	NTOU F10191	1	Gb-1	AB535436	Gb-C1	AB535464
		Taitung Co. (Jhihben) [4]	13064	1	Gb-2	AB127384	Gb-C2	AB266306
	G. cinerea	Hualien Co. (Fuyuan R.) [5]	13273	1	Gcn-1	AB535437	Gcn-C1	AB535465
		Hualien Co. (Jinpu) [6]	13274	1	Gcn-2	AB535438	Gcn-C2	AB535466
		Rueishuei, Hualien Co. (holotype) [7]	NTOU F10052	1	Gcn-3	AB535439	Gcn-C3	AB535467
	G. chiui	Hualien Co. (Fuli) (holotype and paratype)	ZLKU 10151	2	Gch	AB535440	-	-
		Hualien Co. (Fuli) [8]	13275, 13276	2	Gch	AB535440	-	-
		Hualien Co. (Fuli) [8]	13267, 13268	3	Gch	AB535440	Gch-C1	AB535468
		Hualien Co. (Antong) [9]	13269	2	Gch	AB535440	Gch-C2	AB535469
		Hualien Co. (Luoshan) [8]	NTOU F10225	1	Gch	AB535440	Gch-C3	AB535470
	G. sp. 3	Taitung Co. (Taimali) [10]	13277	1	Gsp-3	AB266171	Gsp-C3	AB535471
	G. nanao	Ilan Co. (Nan-ao) (holotype) [11]	13278	1	Gna	AB535442	Gna-C	AB535472
	G. taroko	Hualien Co. (Taroko) [12]	13279	1	Gtr	AB535443	Gtr-C	AB535473
	G. dolichopodes	Hualien Co. (Heping R.) [13]	13280	1	Gdl	AB535444	Gdl-C	AB535474
	G. ilan	Ilan Co. (Lanyang R.) [14]	13281	1	Gi	AB535445	Gi-C	AB535475
	G. ferruginea	Pingtung Co. (Nanrenshan, Kending) [15]	13282	1	Gf	AB127383	Gf-C	AB535476
	G. sp. 4	Taitung Co. (Taimali) [10]	13283	1	Gsp-4	AB535446	Gsp-C4	AB535477
	G. albogilva	Pingtung Co. (Nanrenshan, Kending) [15]	13052	1	Ga	AB127366	Ga-C	AB266294
Montane region								
	G. eurysoma	Nantou Co. (Wushe) [16]	13284	1	Ger	AB535447	Ger-C	AB535478
	G. gracilipes	Hualien Co. (Lyushuei) [17]	TMCD	1	Gg	AB535448	Gg-C	AB535479
	G. takuan	Taoyuan Co. (Daguanshan) [18]	13285	1	Gtk	AB535449	Gtk-C	AB535480
	G. monticola	Taichung Co. (Siyuayakou; Wuling Farm, Heping) [19]	13286, 13287, 13288	3	Gmt	AB535450	Gmt-C	AB535481
Western region								
	G. eucrinodonta complex	Hsinchu Co. (Beipu) [20]	13289	1	Gec-1	AB127386	Gec-C1	AB535482
		Taipei Co. (Wulai) [21]	13290	1	Gec-2	AB535452	Gec-C2	AB535483
		Taipei Co. (Shihmen) [22]; Taipei City Yangmingshan) [23]	13291, 13292	2	Gec-3	AB535453	Gec-C3	AB535484
		Taipei Co. (Hemei) (holotype) [24]	NTOU F10199	1	Gec-4	AB535454	Gec-C4	AB535485
	G. tali	Ilan Co. (Dali) [25]	13164	2	Gtl	AB453217	Gtl-C	AB453226
	G. olea complex	Nantou Co. (Shueili, beside Sun Moon Lake) [26]	13293, 13294	2	Go-1	AB535455	Go-C1	AB535486
		Chiayi Co. (Jhuci) [27]	13295	1	Go-2	AB535456	Go-C2	AB535487
		Taichung Co. (Dongshih) [28]	13162	1	Go-3	AB453215	Go-C3	AB453224
		Tainan Co. (Nansi) (holotype) [29]	NTOU F10026	1	Go-4	AB535457	Go-C4	AB535488
		Kaohsiung Co. (Sanmin) (holotype of *G. tsayae*) [30]	NTOU F10082	1	Go-5	AB535458	Go-C5	AB535489
	G. ancylophallus	Kaohsiung Co. (Mucha) (holotype) [31]	NTOU F10117	1	Gan	AB266164	Gan-C	AB474180
	G. pingtung	Pingtung Co. (Taiwu; Dahou; Lili) (holotype) [32]	TMCD 3282; 13036; 13037	3	Gp	AB127365	Gp-C	AB266286
Others	*G. miyazakii*	Taipei City (Yangmingshan) [23]	13296	1	Gmy	AB535459	Gmy-C	AB535490
	G. dehaani	Japan (Ohsumi Peninsula, Kagoshima)	13200	1	Gd	AB535460	Gd-C	AB535491

5 × 10^5 for the MP analysis, and 2000 for the ML analysis.

RESULTS

Taxonomy

Potamidae Ortmann, 1896

Geothelphusa Stimpson, 1858

Geothelphusa chiui Minei, 1974

(Figs. 2-4)

Geothelphusa chiui Minei 1974: 243 (part), figs. 4, 5; 6E, F; Shy and Yu 1999: 38; Shy et al. 1994: 792, fig. 3; Ng et al. 2001: 50; Ng et al. 2008: 162.

Geothelphusa cinerea – Shy and Lee 2009: 115-118 (part).

Not *Geothelphusa chiui* – Hwang and Mizue 1985: 13, text fig. 8, pl. IIB; Dai 1999: 389, fig. 208A, pl. 26 (2); Shy and Lee 2009: 112-114.

Material examined: Holotype: ♂, 33.9 × 26.8 mm, ZLKU 10151, "Nanpu, Hsinchu", locality incorrect, material most probably from southern Hualien County (Co.), coll. J.-K. Chiu, 3 Dec. 1960. Paratype: 1 ♀, 36.9 × 29.2 mm, same data as for holotype. Other material: 2 ♂♂, 22.1 × 17.6 mm, 36.0 × 29.0 mm, 1 ♀, 33.1 × 26.6 mm, NCHUZOOL 13267, Fuli, Hualien Co., 23°10'20.6"N, 121°15'13.2"E, 257 m elev., coll. Yu-Hsi Wang et al., 7 Mar. 2009; 1 ♂, 35.1 × 27.6 mm, 1 ♀, 27.8 × 22.3 mm, ZRC 2009.0909, Fuli, Hualien Co., 23°10'20.6"N, 121°15'13.2"E, coll. Yu-Hsi Wang et al., 7 Mar. 2009; 1 ♂, 17.8 × 14.1 mm, NCHUZOOL 13268, Fuli, Hualien Co., coll. Jung-Hsiang Lee, 20 July 2003; 2 ♂♂, 24.0 × 18.6 mm, 25.1 × 19.7 mm, NCHUZOOL 13269,

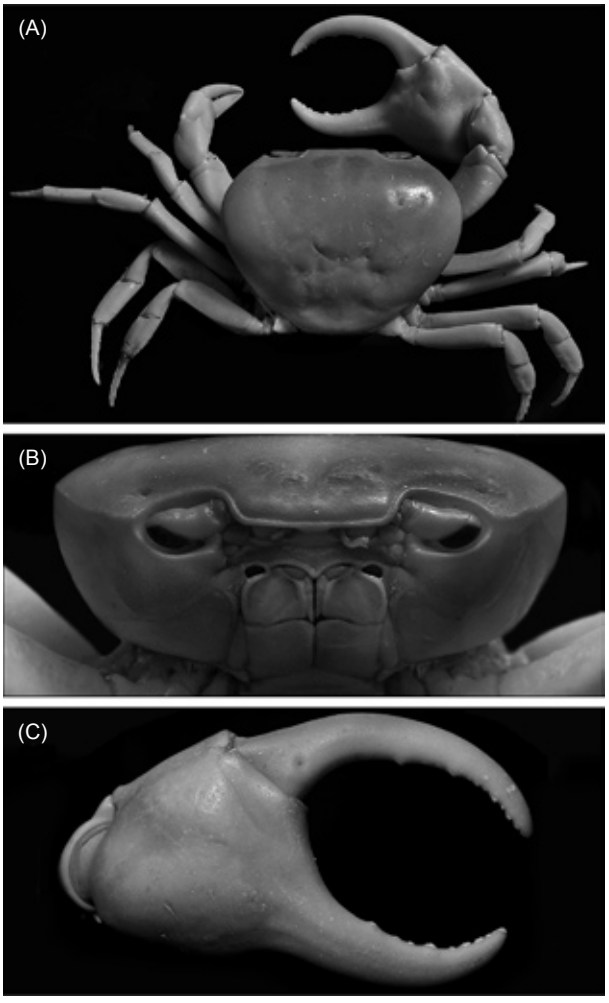

Fig. 2. Holotype male *Geothelphusa chiui* Minei, 1974 (ZLKU 10151). (A) Overall dorsal view; (B) anterior view of carapace; (C) major chela.

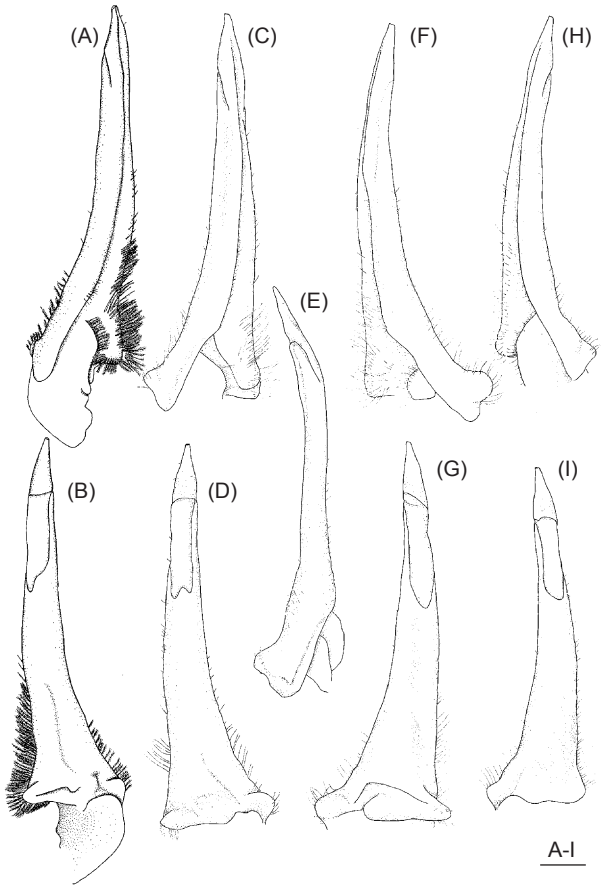

Fig. 3. *Geothelphusa chiui* Minei, 1974. G1 structures. (A, B) Holotype male, 33.9 × 26.8 mm, ZLKU 10151; (C-E), male, ZRC, 35.1 × 27.6 mm; (F, G) NCHUZOOL, 36.0 × 29.0 mm; (H, I) NTOU F10225, 29.6 × 23.6 mm. (A, C, F, H) Ventral view; (B, D, G, I) dorsal view; (E) lateral view. Scale = 1.0 mm.

Antong, Hualien Co., 15 May 2006; 1 ♂, 29.6 × 23.6 mm, 1 ♀, 32.8 × 26.9 mm, NTOU F10225, Luoshan, Hualien Co., coll. J.-Y. Shy and P.-W. Lee, 27 Aug. 2004.

Comparative material: *Geothelphusa olea* Shy, Ng and Yu 1994: 1 holotype ♂, 21.9 × 16.5 mm, NTOU F10190, Nansi (= Nanhsi), Tainan Co., coll. J.-Y. Shy and W.-L. Tsay; 2 ♂♂, 1 ♀ (paratypes), NTOU F10026, Nansi, Tainan Co., coll. J.-Y. Shy and W.-L. Tsay; 5 ♂♂, 19.7 × 15.3-27.5 × 21.6 mm, 6 ♀♀, 20.8 × 16.0-29.5 × 23.5 mm), ZLKU 13751 (paratypes of *G. chiui*), Sinyi (= Hsin-I), Nantou Co., coll. Hsiang-Ping Yu, 29 June 1972; *Geothelphusa* sp. (unidentified): 1 ♂, 32.6 × 26.0 mm, 1 ♀, 31.6 × 24.6 mm, Taiwan, ZLKU 10081 (paratypes of *G. chiui*), coll. Prof. Hwang, received 25 Dec. 1964; 1 ♂, 24.6 × 20.5 mm, ZLKU 1130 (paratype of *G. chiui*), Guansi (= Kuanhsi), Hsinchu Co., coll. H.-P. Yu, 29 Dec. 1972.

Fig. 4. Life color of *Geothelphusa chiui* Minei, 1974. (A) Overall dorsal view; (B) anterior view of carapace; (C) major chela. (A, C) Male, 35.1 × 27.6 mm, ZRC 2009.0909; (B) male, 36.0 × 29.0 mm, NCHUZOOL 13267.

Diagnosis: Carapace strongly convex longitudinally and transversely; postorbital and postfrontal cristate not discernible, pit present on postero-inner part of external orbital angle. Front deflexed; frontal, supraorbital, and infraorbital margins cristate, not granulated; external orbital angle acute, directed anteriorly. Subhepatic region swollen laterally. Anterolateral margin weakly cristate in small individuals, smooth in large individuals, barely separating subhepatic and epibranchial regions; epibranchial tooth absent. Male chelae unequal, major chela with high palm, fingers strongly curved, forming ovoid gape when closed. G1 almost straight to gently curving outwards, distal segment directed dorsodistally; proximolateral and proximomesial angles produced, with knob-like lobe.

Color: In adult specimens, dorsal surface of carapace and legs ashy-gray; other surfaces white. Male major chela white (Fig. 4).

Ecology: *Geothelphusa chiui* lives in burrows under forest cover. It prefers habitats some distance from streams where the ground water is still relatively high. Burrow depth often exceeds 50 cm with the bottom usually flooded. This species is semi-terrestrial and usually forages on land at night.

Distribution: Originally the type locality of *G. chiui* was recorded from Nanpu, Beipu, Hsinchu Co. (indicated by an empty square in figure 1 [no. 20]), northwestern Taiwan. The updated type locality is southern Hualien Co. (around Fuli, Antong, and Luoshan) (indicated by black squares in figure 1 [nos. 8, 9]), eastern Taiwan.

DNA analysis

A 553 bp segment (excluding the primer regions) of the 16S rRNA gene was amplified and aligned from all 50 specimens of the ingroups (excluding the distant *G. miyazakii* and *G. dehaani*); 68 positions were variable and 44 were parsimoniously informative. Among the total number of sequences, 33 different haplotypes were distinguished (Table 1). The studied segment of the 16S sequences was AT rich (72.6%) (T, 36.8%; A, 35.8%; G, 17.3%; C, 10.1%). For the COI gene, a 616-bp segment was compared, resulting in 35 different haplotypes, from 46 ingroup specimens. The studied segment of the COI sequences was also AT rich (65.6%) (T, 37.0%; A, 28.6%; G, 15.7%; C, 18.7%). In this gene, 163 positions were variable and 113 parsimoniously informative.

The COI sequence could not be obtained for the holotype and 1 paratype of *G. chiui* collected from the same locality even after several attempts (including a different combination of primers, internal primers, and a lower annealing temperature), and this was probably due to the poorly preserved condition of these older specimens that were collected on 3 Dec. 1960. However, the type specimens of *G. chiui* and those from Fuli and the adjacent area shared the identical haplotype of 16S rRNA (Table 1).

The best model for ML selected by MrModeltest was the GTR+I+G model (Rodríguez et al. 1990) for the combined 16S rRNA and COI segment of 1172 bp (with a proportion of invariable sites of 0.6856 and a gamma distribution shape parameter of 1.1897). The best models of the 16S rRNA and COI datasets were the HKY+I+G and GTR+I+G models, respectively. The phylogenetic tree constructed by the partitioned BI analysis, with the respective values of nodal support from the ML and MP analyses, is shown in figure 5. Only values larger than 50% are shown.

The recovered phylogenetic tree (Fig. 5) shows that *G. miyazakii*, from northern Taiwan, is the sister group to the remaining Taiwanese *Geothelphusa*. In addition, 3 highly supported clades could be distinguished, “eastern”, “montane”, and “western”, with the former 2 being more closely related. The “eastern” clade included material from Ilan, Hualien, Taitung, and Pingtung Cos., all of which are located to the east of the Central Range, and 6 could be identified within this. *Geothelphusa chiui* was situated within in the “eastern” clade and formed a sister group with *G. cinerea*. The “western” clade was comprised of haplotypes from Hsinchu together with material from Taipei City and Taipei Co. (including the holotype of *G. eucrinodonta*) that formed a complex within this clade (termed the “*G. eucrinodonta*” complex).

Nucleotide bp differences and percent nucleotide divergences of COI and 16S rRNA between species of *Geothelphusa* from Hualien and Taitung in eastern Taiwan (excluding the southern subclade) are shown in table 2. The COI of *G. chiui* differed from that of *G. cinerea* by 13.2 bp (2.14%), and 2.18% of the Kimura (1980) 2-parameter (K2P) distance; and 16S rRNA of *G. chiui* differed from that of *G. cinerea* by 3.7 bp (0.67%) and 0.67% (K2P distance).

DISCUSSION

The identity and taxonomy of *Geothelphusa chiui* Minei, 1974, has been especially frustrating for carcinologists in East Asia. In their revision of the Taiwanese *Geothelphusa*, Shy et al. (1994: 794) commented "The identity of this species causes some problems as we have not been able to find specimens in Taiwan exactly referable to it. Minei (1974) described the holotype from a village called Nanpu in Hsinchu Co. (= Hsien) (northwestern Taiwan) and provided good descriptions and illustrations of the species, including its G1. Fresh collections made in and around Nanpu have not uncovered this but another species instead, *G. olea*, new species. We have not been able to find any species in the Hsinchu area which has the inflated physiognomy characteristic of *G. chiui* s. str. The holotype male and one paratype female were supposedly collected by a parasitologist, Dr. J.K. Chiu in Dec. 1960 (Minei 1974: 243) but no other data are available. It is interesting to note that in Chiu's (1964) paper detailing his crab collections and their association with *Paragonimus*, there were no records from Nanpu in Hsinchu Hsien. It is possible that the specimens were actually collected from other areas and had been incorrectly labeled.

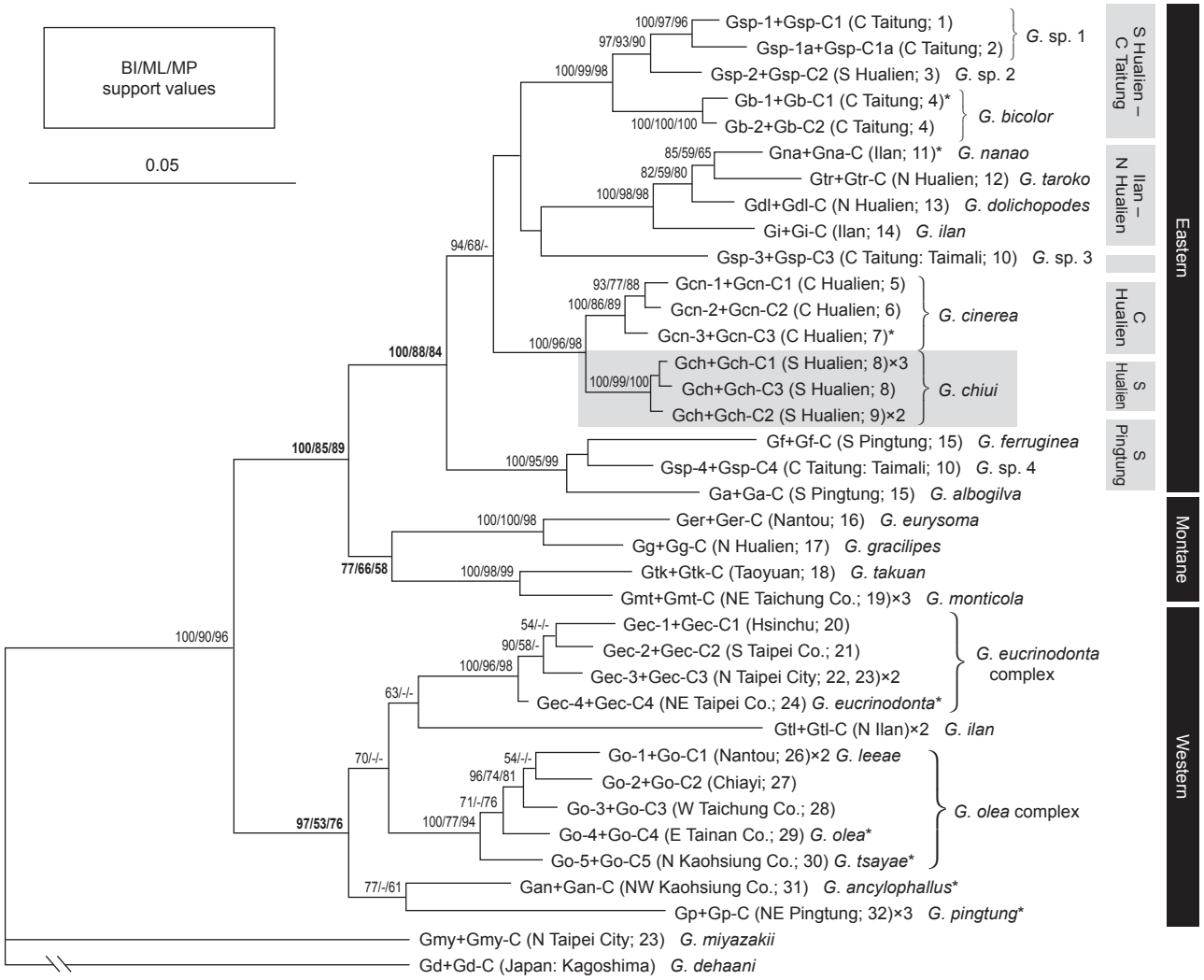

Fig. 5. Bayesian inference (BI) tree of *Geothelphusa* species from Taiwan based on 1172 base pairs of the combined 16S ribosomal RNA and cytochrome *c* oxidase subunit I genes. Probability values at the nodes represent bootstrap values for the maximum likelihood (ML) and maximum parsimony (MP) analyses. Locality names and corresponding numbers in figure 1 are placed in parentheses after the species names. For haplotype abbreviations see table 1. *DNA taken from the holotype; C, central; E, eastern; N, northern; NE, northeastern; NW, northwestern; S, southern; W, western.

We have obtained three other new species (*G. albogilva*, *G. ancylophallus* and *G. wangi*) which also have a similar physiognomy to *G. chiui*, but their G1s all differ from that of *G. chiui* substantially (see Minei 1974: Figs. 6E, F). The identities of the other specimens reported by Minei (1974: 243) from Kuanhsi (Hsinchu Hsien), Hsin-I (Nantow Hsien) and "Taiwan" as "*G. chiui*" will have to be checked to ascertain their identities. It is possible that these specimens contain more than one species."

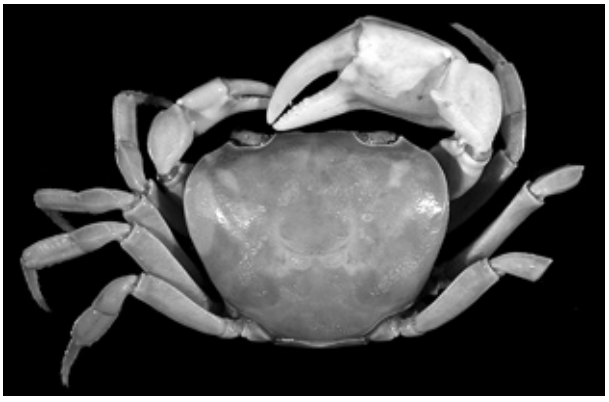

Fig. 6. *Geothelphusa olea* Shy, Ng and Yu 1994. Paratype male of *G. chiui* collected from Sinyi (as Hsin-I), Nantou Co. (33.9 × 26.8 mm, ZLKU 10151).

In the intervening years, the holotype male and paratype female of *G. chiui* were transferred from the Zoological Laboratory, Kyushu Univ. (ZLKU) to the Kitakyushu Museum of Natural History and Human History (Fukuoka, Japan). These specimens are refigured here (Figs. 2, 3A, B). The holotype male and 1 female paratype of *G. chiui* (as designated by Minei 1974) both came from the same type locality, "Nanpu, Hsinchu". As was discussed by Shy et al. (1994: 794), the other 10 males and 8 female paratype specimens listed by Minei (1974) from Kuanhsi (= present day Guansi), Hsin-I (= present day Sinyi), and an unspecified part of Taiwan as belonging to *G. chiui* do not belong to this species. The original type locality cited for *G. chiui* sensu stricto (s. str.) – "Nanpu, Hsinchu", which is in northwestern Taiwan, is problematic. In the years since 1994, several researchers from Taiwan, including the present authors, have surveyed the area, and all have failed to find this species. Only *G. olea* is known from the area, and as was discussed at length by Shy et al. (1994), the species looks very different from *G. chiui*. In fact, one of the authors (TN) visited the Kitakyushu Museum and re-identified Minei's (1974) specimens of "*G. chiui*" from Sinyi as *G. olea* (Fig. 6). This is to be expected from the distribution data given in Shy et al. (1994) for *G.*

Table 2. Matrix of percentage pairwise nucleotide divergences based on the Kimura 2 parameter model (lower left) and mean number of differences (including gaps) (upper right) based on 616 bp of the cytochrome *c* oxidase subunit I (COI) and 556 bp of 16S ribosomal (r)RNA (in parentheses) within and between species of *Geothelphusa* from Hualien and Taitung of eastern Taiwan (see Table 1; Fig. 5)

	Within species		Between species									
	Mean nucleotide difference	Nucleotide divergence	*G.* sp. 1	*G.* sp. 2	*G. bicolor*	*G. cinerea*	*G. chiui*	*G.* sp. 3	*G. nanao*	*G. taroko*	*G. dolichopodes*	*G. ilan*
G. sp. 1	7 (4)	1.15 (0.74)	-	13.5 (5)	22 (7.5)	29.5 (11.7)	33 (11)	37.5 (7)	39.5 (7)	42.5 (12)	34.5 (10)	41.5 (8)
G. sp. 2	-	-	2.24 (0.92)	-	19.5 (6.5)	30 (11.7)	28.5 (12)	37 (7)	40 (6)	41 (11)	35 (7)	41 (8)
G. bicolor	3 (1)	0.49 (0.18)	3.71 (1.39)	3.27 (1.2)	-	34.2 (10.2)	32 (10.5)	38.5 (5.5)	37.5 (6.5)	40.5 (11.5)	32.5 (9.5)	34.5 (8.5)
G. cinerea	5.3 (1.3)	0.87 (0.24)	5.01 (1.98)	5.09 (1.98)	5.85 (1.7)	-	13.2 (3.7)	34.3 (4.7)	42 (7.7)	41.7 (12.7)	35.7 (10)	37.7 (9.7)
G. chiui	1 (-)	0.02 (-)	5.64 (1.86)	4.83 (2.05)	5.46 (1.76)	2.18 (0.67)	-	37.5 (5)	44.5 (8)	43.5 (13)	37.5 (11)	35.5 (10)
G. sp. 3	-	-	6.46 (1.29)	6.37 (1.29)	6.64 (1.01)	5.85 (0.67)	6.43 (0.74)	-	39 (3)	36 (8)	34 (6)	39 (5)
G. nanao	-	-	6.79 (1.11)	6.88 (0.92)	6.42 (1.01)	7.22 (1.04)	7.69 (1.11)	6.67 (0.37)	-	13 (5)	9 (3)	23 (4)
G. taroko	-	-	7.36 (2.05)	7.08 (1.86)	6.98 (1.95)	7.17 (1.98)	7.51 (2.05)	6.14 (1.29)	2.14 (0.92)	-	11 (8)	20 (9)
G. dolichopodes	-	-	5.91 (1.67)	6 (1.11)	5.55 (1.57)	6.09 (1.48)	6.43 (1.67)	5.8 (0.92)	1.48 (0.55)	1.81 (1.48)	-	18 (7)
G. ilan	-	-	7.17 (1.3)	7.08 (1.29)	5.89 (1.39)	6.45 (1.42)	6.06 (1.48)	6.68 (0.74)	3.84 (0.37)	3.34 (1.29)	3 (0.92)	-

olea.

Apart from the structure of the G1, 2 external characters of *G. chiui* stand out: (1) its very ovate and swollen carapace in which the anterolateral cristae are very low, not sharp, and with no trace of any epibranchial tooth or angle in large specimens (Figs. 2A, B, 4A, B); and (2) the adult male with an enlarged major chela in which the fingers are very strongly curved, forming a very wide gape when closed (Figs. 2C, 4C). These characters are shared by only 2 other known species of Taiwanese *Geothelphusa* – *G. wangi* Shy, Ng and Yu 1994, and *G. ancylophallus* Shy, Ng and Yu 1994. In these 2 species, however, the forms of their G1s markedly differ from that of *G. chiui*. Compared to *G. chiui*, *G. ancylophallus* has a prominently hooked G1 with a prominently longer terminal segment (Shy et al., 1994: Fig. 2c, d); while in *G. wangi*, the G1 is more curved, with the inner margin of the basal part of the subterminal segment forming a relatively more-rounded lobe (Shy et al., 1994: Fig. 5c, d; present Fig. 3). In addition, *G. wangi* has a relatively strong postorbital crest, its anterolateral margin is weakly granulated (completely smooth in large *G. chiui*, present Figs. 2A, B, 4A, B), and the suborbital, subhepatic, and pterygostomial regions are all relatively more rugose (Shy et al. 1994: Fig. 5a, b; present Figs. 2B, 4B).

We managed to obtain tissues from the holotype male and 1 paratype female of *G. chiui* and compared the DNA sequences with other species from Taiwan. These methods were recently used to interpret species boundaries and biogeographic patterns of this genus in Taiwan (Shih et al. 2004 2007 2008, Shih and Shy 2009). Surprisingly, the DNA study placed *G. chiui* in a clade which included species known only from eastern Taiwan such as *G. bicolor* Shy, Ng and Yu 1994, *G. cinerea* Shy, Ng and Yu 1994, *G. dolichopodes* Shy, Ng and Yu 1994, and *G. taroko* Shy, Ng and Yu 1994 (Fig. 5). Species from the northwestern part of Taiwan (e.g., *G. olea*) were placed in a completely different clade (Fig. 5). Based on the present molecular study, there are also 2 populations of what is now called "*G. olea*." One belongs to the *G. eucrinodonta* complex (in the northern part of western Taiwan) while the other (from the type locality) belongs to the *G. olea* complex (southern part of western Taiwan) (Fig. 5). Clearly more studies are needed to clarify the identity of these cryptic taxa. In any case, the present specimens collected from Nanpu (Gec-1+Gec-C1) well fit within the *G. eucrinodonta* complex, and show no relationship to those from the type series of *G. chiui*. This is clear evidence that the type locality cited by Minei (1974) was wrong and supports the suggestion by Shy et al. (1994) that the original locality data was recorded incorrectly.

Between 2007 and 2009, 2 of the authors (HTS and JYS) independently obtained specimens from an area in southern Hualien Co. in southeastern Taiwan which bear a striking resemblance to *G. chiui*. Morphological comparisons showed them to be almost identical with the types of *G. chiui*, possessing the characteristic carapace physiognomy, enlarged male chela, and G1 structures. They agree very well with the holotype male and paratype female from the same locality; and confirm the differences observed with *G. wangi* and *G. ancylophallus* discussed earlier. There is a slight variation in the form of the G1, with that of one of the males been slightly less curved (Figs. 3C, D), and the terminal segment slightly longer (Figs. 3F-I), but these differences are unlikely to be significant. The 16S sequences of these specimens were identical to the types of *G. chiui*. The morphological and genetic evidence now strongly suggests that southern Hualien Co. is the actual type locality for *G. chiui*, not Hsinchu Co. in northwestern Taiwan. As the COI sequences of the types of *G. chiui* could not be obtained, comparisons for this gene could not be made.

Interestingly, one of the localities *G. chiui* found from Hualien was Fuli, which is exactly the same site as one of Chiu's (1964: Fig. 1) study sites, although he did not list any specimens of *Geothelphusa* from this location. However, since the study by Chiu (1964) was a parasitological one in which he dissected crabs to check for infection by the lung fluke *Paragonimus*, if he only managed to obtain 1 pair of specimens which was sent away for taxonomic study, then he would have had no reason to have included that material. Although the pereopods of the type specimens of *G. chiui* are still joined by wires, there is no sign of them having been dissected for parasitological studies. This circumstantial evidence supports our hypothesis that the original material which Chiu passed to Minei came from or around Fuli.

Since the original description of *G. chiui*, several authors have recorded what they thought were *G. chiui*. Dai (1999) identified specimens in the Senckenberg Museum (catalogue no. SMF 2859) collected from Takao (= Kaohsiung) as *G. chiui*. However, its cristate anterolateral margin

and less-curved dactylus of the major chela (Dai, 1999: pl. 26 (2)) indicate that it is not *G. chiui*, but more likely to be *G. ancylophallus*, *G. caesia* Shy, Ng and Yu 1994, or *G. tsayae* Shy, Ng and Yu 1994, which are known from Kaohsiung Co. (Shy et al. 1994). Similarly, Hwang and Mizue (1985) recorded *G. chiui* based on material from Pingtung, Miaoli, and Hsinchu Cos. On the basis of the figures (Hwang and Mizue, 1985: pl. IIB), their specimens have well-defined anterolateral margins and a markedly less-gaping male chela, indicating that they are also not *G. chiui* as presently defined. Similarly, the record of *G. chiui* by Shy and Lee (2009) is also mixed, and includes both *G. chiui* s. str. and *G. olea*.

Despite the close morphological similarities in carapace physiognomy and form, and the enlarged chela structure, the present molecular analysis suggests that *G. chiui* and *G. ancylophallus* are not closely related, with the 2 species appearing in 2 separate subclades (Fig. 5). Tissues of *G. wangi* could not be obtained as the preserved condition of both of the type specimens is very poor. The molecular study again demonstrates the tendency for these freshwater crabs to converge in body form and even chelar structure (Shih et al. 2004 2007).

In our study, the distribution of *G. chiui* is limited to the southern part of Hualien Co. which is very close to central Hualien inhabited its sister species, *G. cinerea*, based on genetic relationships (Table 2, Fig. 5). Regarding the K2P distance of COI, *G. chiui* differed from *G. cinerea* by an average of 2.18%, which is not high, but within the minimum difference for published Taiwanese *Geothelphusa* (e.g., *G. makatao* vs. *G. pingtung* s. str.: 1.65%, recalculated from Shih and Shy 2009; *G. siasiat* vs. *G. olea* (including *G. nanhsi* and *G. tsayae* from southwestern Taiwan): 3.18%, Shih et al. 2008). The average K2P distance of 16S rRNA of *G. chiui* and *G. cinerea* was 0.67%, which is also within the minimum range of Taiwanese *Geothelphusa* (e.g., *G. makatao* vs. *G. pingtung* s. str. (and *G. shenshan*): 0.55%, recalculated from Shih and Shy 2009; *G. siasiat* vs. *G. olea* from southern Taiwan: 0.87%, Shih et al. 2008). Both *G. chiui* and *G. cinerea* are separate species as supported by genetic evidence (Table 2, Fig. 5).

Previously, only *G. cinerea* and *G. bicolor* were reported from central Hualien to Taitung in eastern Taiwan. Although our study confirms that *G. chiui* is also in eastern Taiwan, there are clearly more unknown taxa in this area (e.g., at least 1 in Hualien and 3 in Taitung) (Fig. 5). The high diversity of freshwater crabs in eastern Taiwan can be explained by the isolation effects of the numerous mountains in this area. The Coastal Range in eastern Taiwan was formed by the collision of different plates (see Shih et al. 2006 2009), and this is believed to have led to the genetic differentiation of animals, including freshwater crabs (*Candidiopotamon*, Shih et al. 2006) and vipers (*Trimeresurus*, Creer et al. 2001). Additional molecular and morphological studies on the freshwater crabs in this region are necessary to further clarify the diversity and biogeography of these taxa.

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