Geothelphusa makatao sp. nov. (Crustacea: Brachyura: Potamidae), a new freshwater crab from an uplifted Pleistocene reef in Taiwan

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Abstract

A new species of potamid freshwater crab, Geothelphusa makatao, is described from southwestern Taiwan, based on morphological characters and mitochondrial DNA evidence. The new species differs from close congeners, G. pingtung Tan & Liu, 1998, G. shernshan Chen, Cheng & Shy, 2005, and G. ancylophallus Shy, Ng & Yu, 1994 and a superficially similar species, G. albogilva Shy, Ng & Yu, 1994, in the structure of its ambulatory legs, thoracic sternum, male abdomen, and male first pleopods. The unique gene sequences of 16S rRNA and cytochrome oxidase I also support the recognition of this coastal population, which is isolated from other closely related species inhabiting montane areas. Except for G. makatao, the phylogenetic analysis showed that there are three additional hill subclades within the G. pingtung clade, G. shernshan, G. pingtung and the Liangshan subclade, situated in different watersheds of rivers or streams near the Central Range in the southwestern Taiwan.

Key words: 16S rRNA, cytochrome oxidase I, Geothelphusa makatao, G. pingtung, Brachyura, Potamidae, Taiwan, taxonomy, new species

Introduction

The genus Geothelphusa Stimpson, 1858, are endemic and dominant, either in species diversity or abundance, freshwater crabs on islands of East Asia (Taiwan, the Ryukyus, and mainland Japan) (Shih et al. 2009). More than 50 Geothelphusa species have been described, making it the second largest genus of the family Potamidae after Sinopotamon Bott, 1967, which is endemic to China and has around 80 species (see Dai 1999; Ng et al. 2008). The highest diversity (36 species) of Geothelphusa is found in Taiwan (Shy et al. 1994; Shy & Ng 1998; Shy & Yu 1999; Ng et al. 2008; Shih et al. 2008), though some species have been suggested to be synonyms (Ng et al. 2001, 2008; Shih et al. 2004, 2007b). Recent molecular phylogenetic and phylogeographic studies of Taiwanese freshwater crabs (Shih et al. 2004, 2005, 2006, 2007a, b, 2008) have helped clarify the taxonomy and systematics of some problematic and cryptic species.

A population of Geothelphusa at Chaishan (= Shoushan), Kaohsiung City, southwestern Taiwan, an area isolated from the Central Range of Taiwan, was previously misidentified as G. albogilva Shy, Ng & Yu, 1994, based on morphology and coloration (Chen et al. 2001, 2003). However, based on a molecular study using two mitochondrial gene markers (haplotypes of 16S rRNA (Gp-1 and Gp-2) and cytochrome oxidase I (COI; Gp-C1 and Gp-C2)) (see Shih et al. 2007b: table 1, fig. 2), this population should instead belong to the G. pingtung clade, which includes G. pingtung Tan & Liu, 1998 (considered a senior synonym of G. neipu Chen, Cheng & Shy, 1998) and G. shernshan Chen, Cheng & Shy, 2005. As to its monophyly, basepair (bp) differences, and the disjunct geographic distributions between Chaishan and the Central Range populations led Shih et al. (2007b) to suggest that the latter is at least a geographic subspecies of G. pingtung.
We compared a series of specimens from Chaishan with type specimens of *G. albogilva*, *G. ancylophallus* Shy, Ng & Yu, 1994, *G. pingtung*, and *G. shernshan*. In addition, we also examined sequences of 16S rRNA and COI from the Chaishan population and from the holotypes of the *G. pingtung* clade and *G. ancylophallus*. These combined studies show that the population of *Geothelphusa* in Chaishan, Kaohsiung, southwestern Taiwan is in fact a new species, which we describe in the present paper.

Materials and methods

Specimens from Chaishan (including the campus of the National Sun Yat-sen University) were preserved in 70%~95% ethanol after collection, illustrated with the help of a drawing tube attached to a stereomicroscope, and deposited in the National Museum of Natural Science, Taichung, Taiwan (NMNS); the Zoological Collections of the Department of Life Science, National Chung Hsing University, Taichung, Taiwan (NCHUZOOL); and the Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Keelung, Taiwan (NTOU). The following abbreviations are used: G1 for the male first pleopod and G2 for the male second pleopod (Ng 1988; Shy *et al*. 1994). Type specimens of *G. albogilva*, *G. ancylophallus*, *G. pingtung*, and *G. shernshan* deposited in NTOU and the National Taiwan Museum (TMCD) were also used for morphological comparisons.

Sequences of 16S rRNA and COI from the holotypes of the *G. pingtung* clade (*G. pingtung*, *G. neipu*, and *G. shernshan*) and *G. ancylophallus* (Table 1) were obtained following the method described by Shih *et al*. (2007b). The *G. pingtung* clade (Shih *et al*. 2007b) was re-analyzed to confirm the relationship within this clade, with the sister species, *G. ancylophallus*, as the outgroup (see Shih *et al*. 2007b). The best-fitting model for sequence evolution of the combined 16S and COI dataset was determined by MrModeltest (version 2.2, Nylander 2005), selected by the Akaike information criterion (AIC), and was subsequently applied for the analyses of minimum evolution (ME), maximum likelihood (ML), and Bayesian inference (BI). A maximum parsimony (MP) tree was constructed using the program PAUP* (vers. 4.0b10, Swofford 2003) with 2000 bootstrap reiterations of a simple heuristic search, tree bisection-reconnection (TBR) branch-swapping, and 100 randomly added sequence replications. All characters were equally weighted. Gaps in the MP tree construction were treated as missing data. The ME and ML analyses were also calculated using PAUP* with the same parameters as for the MP analysis. BI was performed with MrBayes (vers. 3.1.1, Ronquist & Huelsenbeck 2003). The search was run with four chains for 10 million generations and four independent runs, with trees sampled every 1000 generations (the first 5000 trees were later discarded as the burnin).

Taxonomy

Family Potamidae Ortmann, 1896

Subfamily Potamiscinae Ortmann, 1896 (*sensu* Yeo & Ng 2003)

*Geothelphusa* Stimpson, 1858

*Geothelphusa makatao* sp. nov.

(Figs. 1, 2)

Material examined. **Holotype**: 1 male (39.3 x 29.3 mm) (NMNS-5915-001), Longcyuan Temple, Chaishan, Kaohsiung City, Taiwan, coll. Jung-Hsiang Lee, 31 May 2002. **Paratypes**: 2 males, CW 32.5, 35.4 mm (NCHUZOOL 13033), same data as holotype; 1 male, CW 35.4 mm, 1 female, CW 43.0 mm (NCHUZOOL 13238), Chaishan, Kaohsiung City, Taiwan, coll. J.-H. Lee, 25 May, 2002; 4 ovigerous females, CW 37.1~42.6 mm (NCHUZOOL 13032, 13240, 13241), 22 May 2002, Longcyuan Temple, Chaishan, Kaohsiung
City, Taiwan, coll. Hsi-Te Shih; 1 male, CW 25.3 mm (NCHUZOOL 13239), Cueiheng Dormitory, National Sun Yat-sen University, Kaohsiung City, Taiwan, coll. H.-T. Shih, 9 Aug. 2000; 1 male, CW 36.6 mm (NTOU F10708), Cueiheng Dormitory, National Sun Yat-sen University, Kaohsiung City, Taiwan, coll. H.-T. Shih, 22 May 2002.

**TABLE 1.** Seven haplotypes of the 16S rRNA gene and ten haplotypes of the cytochrome oxidase I (COI) gene of specimens of the *Geothelphusa pingtung* clade (*G. makanao* sp. nov., *G. shernshan*, and *G. pingtung*) from southwestern Taiwan, with the outgroup *G. ancylophallus*, used in this study. The table is modified from Shih et al. (2007b) with additional sequences from the holotypes of the above species. DDBJ, DNA Data Bank of Japan; NCHUZOOL, Zoological Collections of the Department of Life Science, National Chung Hsing University; NMNS, National Museum of Natural Science; NTOU, Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University; TMCD, National Taiwan Museum.

<table>
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<th>Species</th>
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<th>Sample size</th>
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<th>DDBJ accession no. of 16S</th>
<th>Haplotype of COI</th>
<th>DDBJ accession no. of COI</th>
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<td>Gan-C2a</td>
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**Comparative material examined.** see Table 2 for the holotypes of *G. albogilva*, *G. ancylophallus*, *G. pingtung*, and *G. shernshan*.

**Diagnosis.** Carapace swollen longitudinally, transversely; dorsal surface smooth, glabrous, with fine pits. Carapace length, width 1.5-, 1.9-times carapace height, respectively. Frontal margin straight, without tooth. Postorbital cristae distinct, supraorbital margin smooth, without granules; infraorbital margin smooth to almost smooth, lined with very low granules. External orbital angle stout, external orbital region concave. Anterolateral margin faint, smooth, without epibranchial tooth. Postorbital cristae faint, smooth. Gastric, cardiac, intestinal regions smooth. H-shaped groove distinct. Tip of medium lobe of epistome slightly stout. Distance between tip of closed male abdomen, anterior margin of thoracic sternite 4 about 2.4 times length of thoracic sternites 1-3. Chelipeds of adult male unequal, fingers of larger chela forming large gape when closed. Ambulatory legs smooth, dorsal, ventral margins of dactyl with 2 rows of small spines. Second leg about 1.8 carapace length. Telson of male abdomen bell-shaped, moderately short, width about 1.4 carapace length. Subterminal segment of G1 (Fig. 1a-c) slightly curving outwards, outer proximal margin with blunt tooth, inner proximal margin moderately dilated; terminal segment straight or slightly curving inwards; total length of G1 4.9 terminal segment; length of synovial membrane about 6.1 maximum width. Outer proximal margin of basal segment of G2 (Fig. 1d) dilated, showing a single lobe; distal segment short, about 0.14 total length.

![Figure 1](image_url)

**FIGURE 1.** Male right first and second pleopods of *Geothelphusa makatao* sp. nov. (holotype, NMNS-5915001). a, G1, ventral view of right G1; b, terminal segment of G1; c, dorsal view of right G1; d, ventral view of right G2. Scale = 0.5 mm.
FIGURE 2. Geothelphusa makatao sp. nov. a, dorsal view of the holotype (NMNS-5915-001); b, frontal view of the holotype; c, ventral view of the holotype; d, e, live coloration of a male, dorsal view; f an individual eating mud; g, a female with brood in a natural habitat (the campus of National Sun Yat-sen University); h, habitat in a dry creek bed where individuals were hidden under rocks (near Longcyuan Temple, Chaishan).
TABLE 2. Morphometric comparisons among the holotypes of Geothelphusa makataoa sp. nov. (NMNS-5915-001), G. albogilva Shy, Ng & Yu, 1994 (NTOU F10189), G. pingtung Tan & Liu, 1998 (TMCD 3282), G. shernshan Chen, Cheng & Shy, 2005 (NTOU F000101), and G. ancylophallus Shy, Ng & Yu, 1994 (NTOU F10117). CW, carapace width; CH, carapace height; CL, carapace length; DS4, distance between tip of closed male abdomen and anterior margin of thoracic sternite 4; LS1-3, length of thoracic sternites 1-3; P2L, 2nd ambulatory leg length; G1, male first pleopod; TL, total length; TSL, terminal segment length; SS, subterminal segment.

<table>
<thead>
<tr>
<th>Geothelphusa makataoa sp. nov.</th>
<th>Geothelphusa albogilva</th>
<th>Geothelphusa pingtung</th>
<th>Geothelphusa shernshan</th>
<th>Geothelphusa ancylophallus</th>
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<td>1.9</td>
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<tr>
<td>CL/CH</td>
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<td>1.5</td>
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<td>DS4/LS1-3</td>
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<td>1.7</td>
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<td>P2L/CL</td>
<td>1.8</td>
<td>1.7</td>
<td>1.6</td>
<td>1.8</td>
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<tr>
<td>G1 TL/TSL</td>
<td>4.9</td>
<td>6.0</td>
<td>6.1</td>
<td>5.4</td>
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<tr>
<td>G1 SS</td>
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<td>curving outward</td>
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</table>

Etymology. The species is named for the aboriginal Makatao Tribe, one of the Pingpu Tribes, which once lived in the Chaishan area. The name is used as a noun in apposition.

Coloration. Body, including legs, of most adults is yellow (Fig. 2d). Some young individuals range from pale yellow, orange (Fig. 2f, g), or greenish (Fig. 2e), to brown.

Ecological notes. The maximum elevation of type locality, Chaishan, is only 330 m and no permanent surface running water is present in this uplifted coral reef mountain system. Aquatic habitats rely on the presence of small ephemeral springs and periodic rainfall. Specimens were collected under rocks (Fig. 2h) or as they moved around on a road after a rain. Some individuals were observed near a burrow entrance. Ovigerous females were observed and collected in May (Fig. 2g).

Distribution. This species is found only in the Chaishan area, Kaohsiung City, southwestern Taiwan.

Remarks. Based on Shih et al. (2004, 2007b), G. makataoa sp. nov. (as “G. albogilva” in Shih et al. 2007b), G. shernshan, G. neipu, and G. pingtung belong to the G. pingtung clade, but G. neipu was formally synonymized under G. pingtung recently (Ng et al. 2008). Morphologically, this new species is close to G. pingtung and G. shernshan, but they can be separated by several characters including carapace height and G1 morphology (Table 2). In particular, the new species differs from G. shernshan by the relatively taller and higher carapace (Table 2; Fig 2A, B in Chen et al. 2005). The external morphology of G. makataoa sp. nov. and G. albogilva, both of which inhabit the uplifted coral reef mountain and are yellow in color, is also superficially similar. However, the distance between the tip of the closed male abdomen and the anterior margin of thoracic sternite 4, and the relative lengths of the second leg and terminal segment of the G1 of G. makataoa sp. nov. are clearly greater than those of G. albogilva (Table 2; Fig. 4 in Shy et al. 1994).

Geothelphusa ancylophallus is also close to this new species, with the body height of both species being greater than most other species of Geothelphusa. The G1 of the former, however, is strongly curved outwards, while that of the latter is only slightly curved (Table 2; Fig. 2 in Shy et al. 1994).

DNA analysis and discussion

The best model selected by MrModeltest was GTR+I for the combined 16S and COI segments of 1164 bp (with a proportion of invariable sites of 0.7954). The phylogenetic tree constructed by the ME analysis, with the respective confidence values from the MP, BI, and ML analyses, is shown in Fig. 3. Within the G. pingtung clade, there are four subclades supported by high confidence values: G. pingtung, G. makataoa, G. shernshan, and Liangshan subclades (Fig. 3). The relationship among the subclades is not clear, however, except that the
G. shernshan and Liangshan subclades are quite close.

Geographically, the three hill subclades located in different watersheds of rivers or streams near the Central Range. The G. pingtung subclade, situated in the southern tributary, Jiaping River, of Donggang River, has a more-southerly distribution than other members of the clade. Sequences from holotypes of both G. pingtung and G. neipu were included in our study, and the results showed that they shared the same 16S rRNA and COI, confirming the conspecificity of the two species “(Tan & Liu 1998; Shih et al. 2007b; Ng et al. 2008)”. The minor morphological differences between the two holotypes should be considered to be variation within a single species (Shy et al. 2000; Shih et al. 2007b). G. neipu has been formally synonymized under G. pingtung recently (Ng et al. 2008) and our study supports this action. In addition, the type localities of G. pingtung and G. neipu are very close to one another (Ng et al. 2001) and in the lowlands (< 200 m in elevation) with no mountains or hills between them; thus there are no obvious geographic barriers between them.

FIGURE 3. A minimum evolution (ME) tree of the Geothelphusa pingtung clade, with the outgroup, G. ancylophallus, in southwestern Taiwan based on 1164 bp of the combined 16S rRNA and cytochrome oxidase I (COI) genes. Probability values at the nodes represent confidence values for ME, maximum parsimony (MP), Bayesian inference (BI), and maximum likelihood (ML). Only confidence values of ≥ 50% are shown. An asterisk (*) with a haplotype indicates that the holotype was included.

The Liangshan subclade is situated in the northern tributary, Nioujiaowan River, of Donggang River, between the G. pingtung and G. shernshan subclades, at elevation of < 100 m, but it possesses the highest genetic divergence. Shih et al. (2007b: fig. 3) showed that the Liangshan subclade has the supposed ancestral haplotypes of the combined 16S rRNA and COI sequences. This subclade is close to the G. shernshan subclade. Genetically, it may either belong to G. shernshan, as a geographic subspecies or be a distinct
closely-related species, but additional morphological comparisons are required to confirm this. The *G. shernshan* subclade, although close to the locality of the Liangshan subclade, situated in a remote tributary, Ailiao River, of Gaoping River, occupies the northernmost range among members of this clade, but is near a highland area (> 500 m in elevation). This species has a unique genetic structure compared to the other species (Fig. 3). Noteworthy, the holotype and other specimens collected from the type locality share identical 16S rRNA and COI sequences (Table 1), compared with the nucleotide divergence of 16S rRNA (and COI) of others in this study (*G. mакataо* sp. nov.: 0.18% (0.22%); *G. pingtung*: 0 (0.33%); Liangshan subclade: 0.24% (0.35%); *G. angolopallus*: 0.37% (0.27%)), indicating the narrow genetic structure of this species.

Compared to the coastal *G. mакataо* sp. nov. in Chaishan near Taiwan Strait, the geographic distances separating the three subclades in the hill area near the Central Range are quite short—the largest distance between them is only about 20 km. There are no mountains or hills on the 40 km-wide plain between Chaishan and the Central Range. Although the Chaishan reefs were uplifted above sea level during the Pleistocene (Gong *et al*., 1996, 1998), other parts of the Kaohsiung area were covered by seawater during two interglaciations and glaciations since the late Pleistocene (Sun 1964). The most recent transgression-regression cycle occurred during the Holocene (Chen *et al*., 1994), and the Kaohsiung area was reexposed as dry land between 8000 and 1000 years b.p. (Su 1999).

The genetic data of the hill subclades (Fig. 3) support the hypothesis that large-sized species of freshwater crabs are more easily isolated by mountains (Okano *et al*., 2000; Shih *et al*., 2004, 2007b). The unique genetic structure composed of only two haplotypes in *G. mакataо* sp. nov. (Fig. 3) might be the result of a founder effect from a few ancestors that colonized the area from peripheral hills of the Central Range during the early Pleistocene, and which were subsequently isolated (Shih *et al*., 2007b).

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