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Description of a new montane freshwater crab (Crustacea: Potamidae: *Geothelphusa*) from northern Taiwan

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Abstract

A new freshwater crab is described from a montane area in northern Taiwan based on morphological characters and molecular evidence. *Geothelphusa cilan* **sp. nov.**, from the Cilan Forest, situated on the boundary of Hsinchu and Yilan (= Ilan) counties, is close to *G. monticola* Shy, Ng & Yu, 1994, and *G. takuan* Shy, Ng & Yu, 1994, but can be distinguished by its male first gonopod (G1) and the ratio of thoracic sternites. Molecular evidence from mitochondrial cytochrome oxidase subunit I (COI) also supports the identity of the new species.

Key words: Potamidae, freshwater crab, new species, Taiwan, DNA, COI

Introduction

Geothelphusa Stimpson, 1858, is the second largest genus of the family Potamidae (Ng *et al.* 2008; Shih & Ng 2011), which is distributed in East Asian islands (Taiwan, the Ryukyus and the main islands of Japan). Fifty-five species have been reported in the genus, with 38 species in Taiwan and the adjacent islets, 15 in the Ryukyus, and three in the main islands of Japan (Shih & Ng 2011; Suzuki & Kawai 2011). Whereas most Taiwanese *Geothelphusa* species live in low to middle altitudes, six montane species, viz. *G. eurysoma* Shy, Ng & Yu, 1994, *G. gracilipes* Shy, Ng & Yu, 1994, *G. haituan* Chen, Hsu & Cheng, 2007, *G. hirsuta* Tan & Liu, 1998, *G. monticola* Shy, Ng & Yu, 1994, and *G. takuan* Shy, Ng & Yu, 1994, inhabit in the mountain region with altitudes higher than 1000 m a.s.l. (Shy & Lee 2009).

Two additional montane species from northern Taiwan, as well as *G. takuan*, *G. hirsuta* and *G. monticola*, form a clade, sister to another clade composed of species from eastern Taiwan (Ng *et al.* 2010; Shih *et al.* 2010, 2011). Specimens of an undescribed species, *G.* sp. 2 in Shih *et al.* (2011), from the central region of northern Taiwan, were examined and compared with other related species. We confirm that it has distinct morphological characters different from congeners, a status that is also supported by the mitochondrial cytochrome oxidase subunit I (COI). We describe the specimens in question as a new species, *Geothelphusa cilan* **sp. nov.**

Material and methods

Specimens collected from the Cilan region (see no. 32 in fig. 1 of Shih *et al.* 2011) were preserved in 70%~95% ethanol after collection, illustrated with the help of a drawing tube attached to a stereomicroscope, and deposited in the Zoological Collections of the Department of Life Science, National Chung Hsing University, Taichung, Taiwan (NCHUZOOL). The following abbreviations are used: G1 for the male first gonopod and G2 for the second gonopod. Terminology used essentially follows Ng (1988) and Shy *et al.* (1994). Other specimens examined are deposited in the Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Keelung, Taiwan (NTOU) and the National Taiwan Museum (TMCD).

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Genomic DNA was isolated from the muscle tissue of legs by using the GeneMark tissue and cell genomic DNA purification kit (Taichung, Taiwan). A portion of the COI gene was amplified with PCR using the primers LCO1490, HCO2198 (Folmer *et al.* 1994), COL14 (Roman & Palumbi 2004), COH6 (Schubart & Huber 2006) and LCOA (Shih & Tu 2014). The PCR conditions for the above primers were 40 cycles of 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) and were aligned manually, after verification with the complementary strand. Sequences of different haplotypes were deposited in the DNA Data Bank of Japan (DDBJ) (accession numbers in Table 1), with other sequences published in earlier papers of HTS (Table 1).

TABLE 1. Haplotypes of *Geothelphusa cilan* **sp. nov.** and other related species collected from the montane areas in central and northern Taiwan. The numbers after haplotypes are the collecting localities in fig. 1 and table 1 in Shih *et al.* (2011).

Species	Localities	Catalog no. of museum (NCHUZOOL)	Sample size	Haplotypes of COI	Access. no.
G. cilan	Cilan, Jianshih, Hsinchu County [32]	13617 (holotype), 13430	6	GcL1	AB625746
		13618	2	GcL2	LC002663
		13619	1	GcL3	LC002664
G. monticola	Siaoyako, Heping, Taichung City [27]	NTOU F10204 (holotype)	1	Gmt1	AB535481
	Cijiawan R., Heping, Taichung City [26]	13286	1	Gmt1	AB535481
		13433, 13621	2	Gmt2	AB625751
	Upper reach of Lanyang R., Yilan County [27]	13287	3	Gmt1	AB535481
	Siyuanyakou, Datong, Yilan County [27]	13288	3	Gmt1	AB535481
G. takuan	Daguanshan, Fusing, Taoyuan County [28]	13285	1	Gtk1	AB625747
		13285	1	Gtk2	AB535480
G. eurysoma	Dasyueshan Forest Road, Heping, Taichung City	13622	1		LC002665
G. gracilipes	Lyushuei, Shioulin, Hualien County	TMCD	1		AB535479

By using jModelTest (vers. 0.1.1, Posada 2008; Guindon & Gascuel 2003), the best-fitting models for sequence evolution were HKY+I and TIM1+I, under the Bayesian information criterion (BIC) and the Akaike information criterion (AIC), respectively, which were subsequently applied for the analyses of Bayesian inference (BI) and maximum likelihood (ML), respectively. The BI was performed with MrBayes (vers. 3.1.1, Ronquist & Huelsenbeck 2003) and the search was run with four chains for 10 million generations, with trees sampled every 1000 generations. The convergence of chains was determined by the effective sample size (ESS) (>200 as recommended) in Tracer (vers. 1.5, Rambaut & Drummond 2009) and the first 1100 trees were discarded as the burnin (determined by the average standard deviation of split frequency values below the recommended 0.01; Ronquist *et al.* 2005). The ML analysis was conducted in GARLI (vers. 2.0, Zwickl 2006), with 10 replicate searches (searchreps = 10) and 1000 bootstraps (bootstrapreps = 1000). The consensus tree from GARLI output was computed using PAUP* program (vers. 4.0b10, Swofford 2003) to assess node supports. Other analyses, including the nucleotide composition, variable and parsimony informative positions, and the pairwise estimates of Kimura (1980) two-parameter (K2P) model distance for inter- and intraspecific genetic diversities were calculated using MEGA (vers. 5.2, Tamura *et al.* 2011).

Results

Taxonomy

Family Potamidae Ortmann, 1896

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

Geothelphusa Stimpson, 1858

Geothelphusa cilan sp. nov.

(Figs. 1–2)

Geothelphusa sp. 2—Shih *et al.* 2011: 461.

Material examined. **Holotype**: 1 ♂ (18.8 x 14.4 mm) (NCHUZOOL 13617), Cilan, Takejin (= Takazinm) River, the headstream of Danshuei (= Tansui) River, Jianshih, Hsinchu County (near Yilan [= Ilan] County), Taiwan, 24°32'08.7"N; 121°23'09.7"E, elevation of 1950 m, coll. H.-T. Shih & J.-J. Mao, 28 March 2009.

Paratypes: 2 ♂♂ (CW 12.4–13.5 mm), 3 ♀♀ (13.8–15.5 mm) (NCHUZOOL 13430); 2 ♂♂ (10.4–20.6 mm) (NCHUZOOL 13618); 1 ♂ (13.4 mm) (NCHUZOOL 13619); 5 ♂♂ (15.5–18.9 mm), 13 ♀♀ (14.5–19.3 mm) (NCHUZOOL 13620), same data as holotype.

Comparative material. *Geothelphusa monticola* Shy, Ng & Yu, 1994: 1 holotype male (NTOU F10204), Siaoyako (= Sheauyeakow), Heping (= Hoping), Taichung City, Taiwan, coll. J.-Y. Shy & W.-L. Tsay, 6 Jun. 1992; *Geothelphusa takuan* Shy, Ng & Yu, 1994: 1 holotype male (NTOU F10205), Daguan (= Takuan), Fusing (= Fuhsing), Taoyuan County, Taiwan, coll. J.-Y. Shy & W.-L. Tsay, 1 Nov. 1992.

Description. Carapace (Fig. 2A–B) swollen longitudinally, transversely; dorsal surface smooth, glabrous, with fine pits. Carapace length, width 1.7, 2.2 carapace height, respectively. Frontal margin slightly dived into 2 lobes, without tooth. Postorbital cristae distinct, supraorbital margin smooth, without granules; infraorbital margin smooth to almost smooth, lined with inconspicuous granules. External orbital angle stout, external orbital region concave. Anterolateral margin distinct, lined with inconspicuous granules, without epibranchial tooth. Postorbital crista faint, smooth. Gastric, cardiac, intestinal regions smooth. H-shaped groove distinct. Tip of medium lobe of epistome stout. Distance between tip of closed male abdomen, anterior margin of thoracic sternite 3 about 1.1 length of thoracic sternites 1, 2 (Fig. 2C).

Chelipeds of adult males unequal, fingers of larger chela forming obliquely triangular gape when closed. Ambulatory legs smooth, dorsal, ventral margins of dactyli with 2 rows of small spines, respectively. Second leg about 1.8 carapace length. Telson of male abdomen bell-shaped, moderately short, width about 1.4 length (Fig. 2C).

Subterminal segment of G1 (Fig. 1A–C) curving inwards, outer proximal margin with small tubercle, inner proximal margin clearly dilated; terminal segment slightly curving inwards to almost straight (length /width = 2.4); total length of G1 5.5 terminal segment; length of synovial membrane about 3.3 maximum width. Outer proximal margin of basal segment of G2 (Fig. 1D) dilated, showing a single lobe; distal segment short, about 0.12 total length.

Etymology. The species is named for the type locality, the Cilan Forest, in northern Taiwan. The name is used as a noun in apposition.

Coloration. Carapace and ambulatory legs grayish brown, mottled with dark brown spots; chelae orange-red, with dark brown spots; tip and inner edge of fingers white (Fig. 2D–G).

Ecological notes. The specimens were collected from the headstream of Danshuei River (Fig. 2H) near the boundary of Hsinchu and Yilan counties, near Yuanyang Lake Nature Reserve, with an altitude about 2000 m. The mean monthly water temperatures were 10.3–14.6°C during April to December, 2012 (mean 12.6°C) for the adjacent Yuanyang Lake, with the same drainage.

Remarks. *Geothelphusa cilan* **sp. nov.** is similar to *G. monticola* and *G. takuan* both of which are present in the adjacent regions. The subterminal segment of the G1 of *G. monticola* is slightly S-shaped, and those of *G. takuan* and *G. cilan* **sp. nov.** are slightly curved inwards, but *G. monticola* and *G. takuan* have a conspicuous tubercle and *G. cilan* **sp. nov.** a small tubercle at the outer proximal margin. The terminal segment of the G1 of *G. monticola* is slender (length/width = 3.3), and those of *G. takuan* and *G. cilan* **sp. nov.** are stouter (length/width = 1.8 and 2.4, respectively). *Geothelphusa takuan* and *G. cilan* **sp. nov.** have shorter distance between the tip of the closed male abdomen and anterior margin of thoracic sternite 3 (ratio = 1.0 and 1.1, respectively) than that in *G. monticola* (ratio = 1.6).

DNA analyses and discussion. A 616-658 basepair (bp) segment of the COI was amplified from 9 specimens of *G. cilan* and 10 specimens of the closely related *G. monticola*, resulting in 5 different haplotypes (Table 1). The studied segment was AT rich (63.2%) (T, 35.4%; A, 27.8%; G, 16.5%; and C, 20.3%). In this gene, 18 positions were variable and 13 parsimoniously informative.

The phylogenetic tree constructed by BI and ML methods (Fig. 3) supports the clade of *G. cilan* **sp. nov.,** which is a sister species to another montane clade, *G. monticola*. The pairwise nucleotide divergences for COI with K2P distance is shown in Table 2. The mean interspecific K2P distance of *G. cilan* is 2.26% with *G. monticola*, which is 38 or 17 times greater than the mean intraspecific distance of *G. cilan* (0.06%) or *G. monticola* (0.13%), respectively (Table 2). The lowest interspecific K2P distance of *G. cilan* is 2.15% with *G. monticola*, which is 13 or 4.7 times greater than the largest intraspecific distance of *G. cilan* (0.16%) or *G. monticola* (0.46%). The interspecific K2P distance of 2.15% – 2.65% between *G. cilan* **sp. nov.** and *G. monticola* is not high, but still higher than the distance between *G. marginata* Naruse, Shokita & Shy, 2004 and *G. fulva* Naruse, Shokita & Shy, 2004 (1.48%–1.99%, Shih *et al.* 2011); and the distance among *G. makatao* Shih & Shy, 2009, *G. shernshan* Chen, Cheng & Shy, 2005, and *G. pingtung* Tan & Liu, 1998 (1.65%–1.98%, recalculated from Shih & Shy 2009).

Although the nearest distance between the localities of *G. cilan* **sp. nov.** and *G. monticola* (see nos. 32 and 27 in fig. 1 of Shih *et al.* 2011) is only about 13 km, both areas belong to different drainages, the Danshuei and Daja rivers, respectively. Furthermore, several mountains more than 2000 m a.s.l. separated the two areas. Based on the substitution rates of 2.33% per 10^6 yr for COI of terrestrial crabs (see Schubart *et al.* 1998), the two sister species diverged at 1.0±0.2 million years ago (mya) (with uncorrected p-distance divergences of 2.35%±0.56%), which is quite young allopatric speciation, considering the geological history of Taiwan is about 5 mya (see Shih *et al.* 2006).

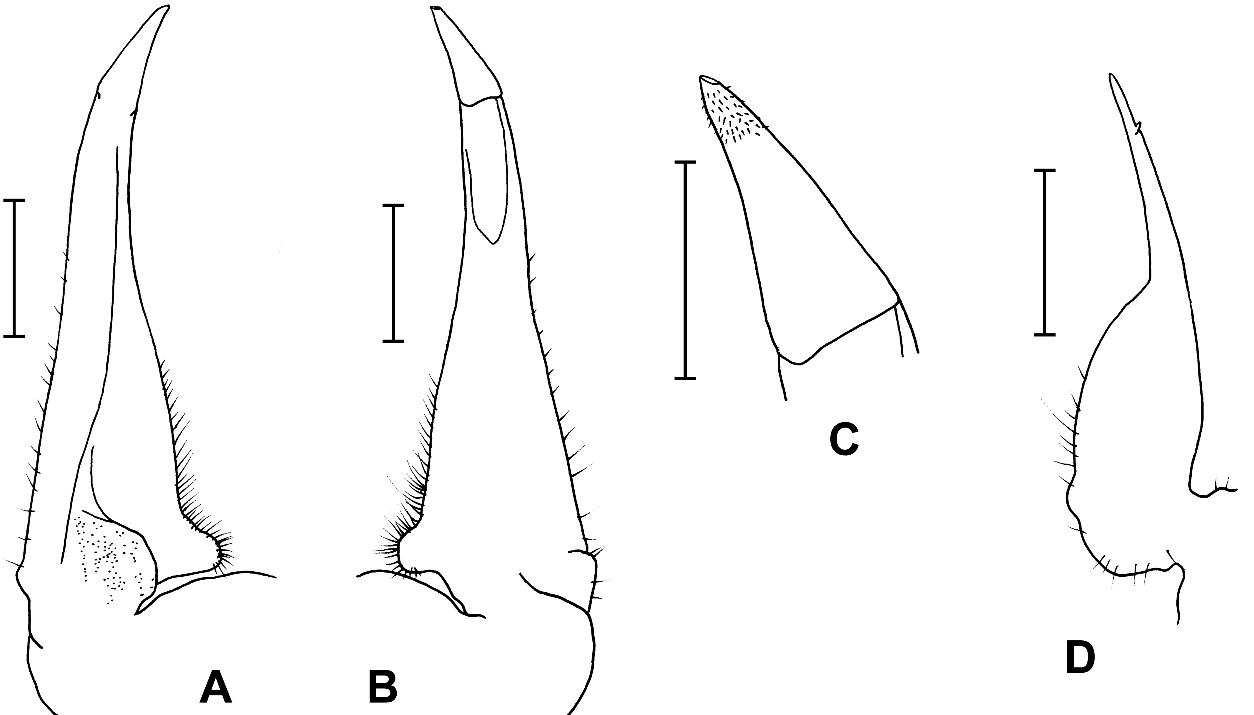

FIGURE 1. Male right first and second gonopods of *Geothelphusa cilan* **sp. nov.** (holotype, NCHUZOOL 13617). A, ventral view of right G1; B, dorsal view of right G1; C, terminal segment of G1; D, ventral view of right G2. Scale = 1 mm for A, B, D; scale = 0.5 mm for C.

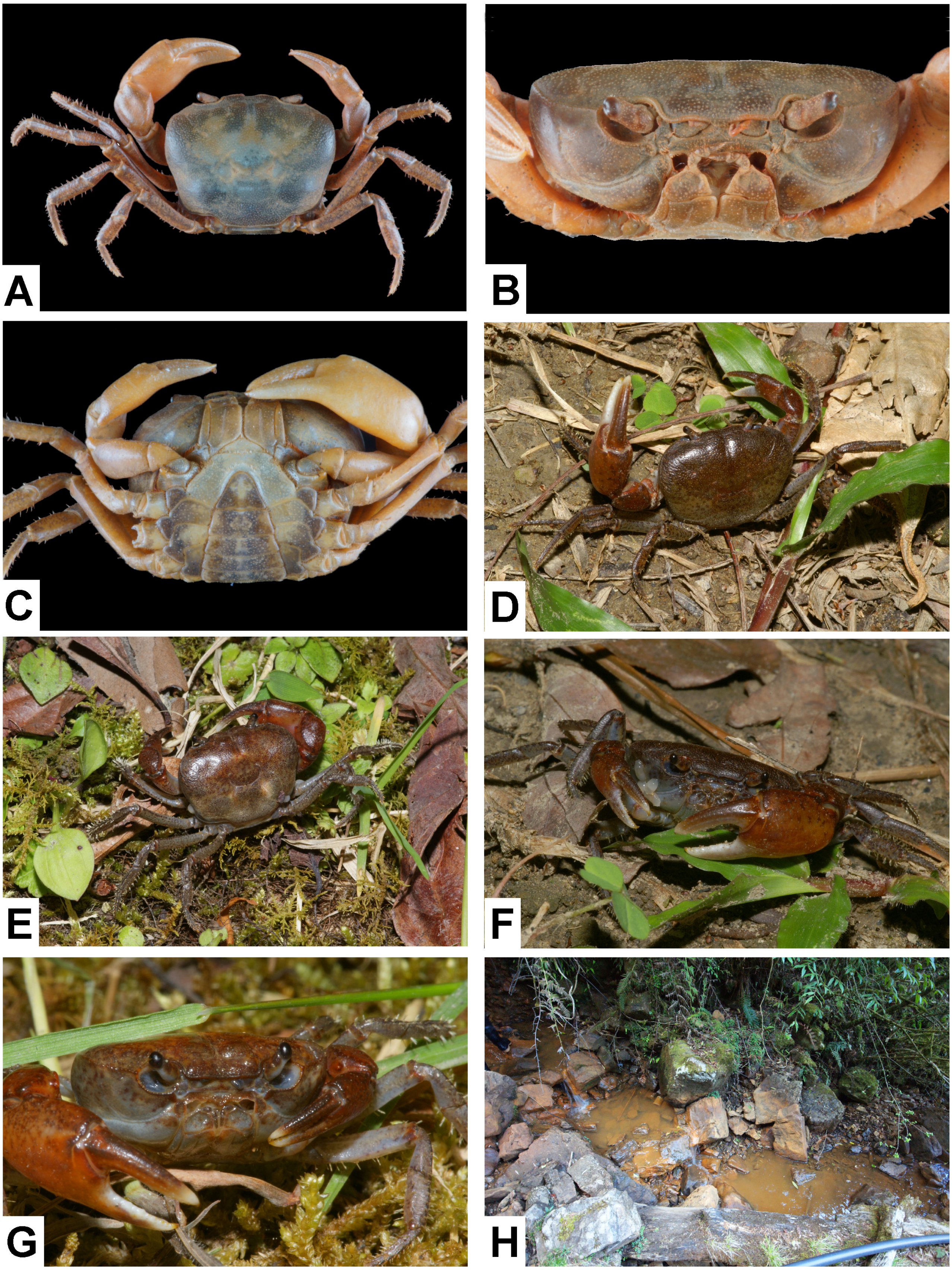

FIGURE 2. *Geothelphusa cilan* **sp. nov.** A, B, C, dorsal, frontal and ventral views of the holotype (NCHUZOOL 13617); D–G, live coloration of the male, dorsal view (D, E) and frontal view (F, G); D, F, NCHUZOOL 13618 (CW 20.6 mm); E, G, NCHUZOOL 13620 (CW 15.5 mm); H, habitat in a montane creek in the headstream of Danshuei River, Jianshih, Hsinchu County.

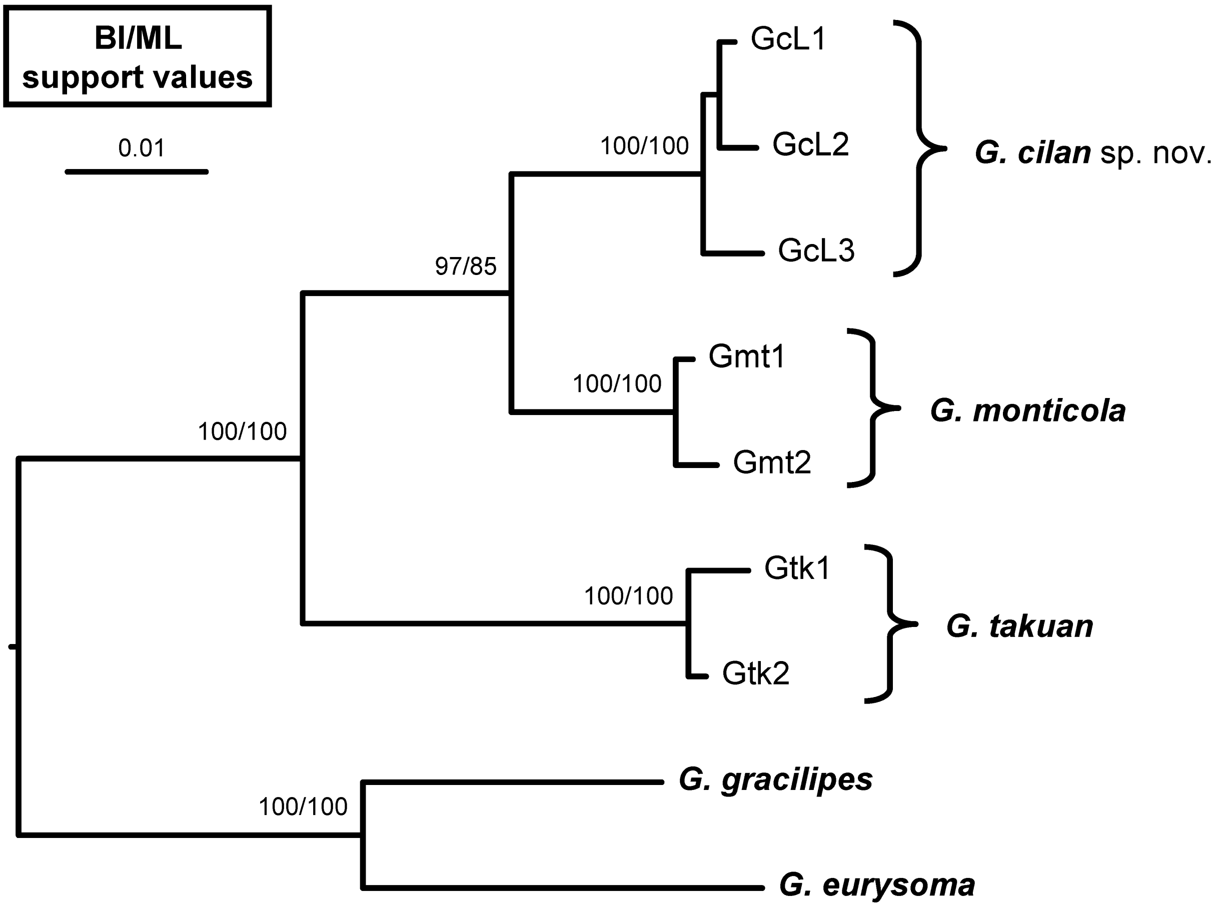

FIGURE 3. A Bayesian Inference (BI) tree of *Geothelphusa cilan* **sp. nov.** and related montane species from northern Taiwan based on cytochrome oxidase I (COI) marker. Probability values at the nodes represent confidence values for BI and maximum likelihood (ML). For haplotype abbreviations, see Table 1.

TABLE 2. Matrix of percentage pairwise nucleotide divergences with K2P distance based on COI within and between species of *Geothelphusa cilan* **sp. nov.**, *G. monticola*, and *G. takuan*. Values of range are shown in parentheses.

	Intraspecific	Interspecific		
	Nucleotide divergence	*G. cilan*	*G. monticola*	*G. takuan*
G. cilan	0.06 (0–0.16)	-		
G. monticola	0.13 (0–0.46)	2.26 (2.15–2.65)	-	
G. takuan	0.22 (0–0.33)	4.34 (4.20–4.58)	4.77 (4.72–5.08)	-

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