



## ***Geothelphusa boreas*, a new montane freshwater crab (Crustacea: Potamidae: *Geothelphusa*) from northeastern Taiwan, and the identity of *G. hirsuta* Tan & Liu, 1998**

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### Abstract

A new freshwater crab is described from the montane area in northeastern Taiwan based on morphological and molecular evidence. *Geothelphusa boreas* **sp. nov.**, from the Fushan Botanical Garden situated around New Taipei City and Yilan County, is distinct from similar congeners by the structure of the male first gonopod and the proportions of the male thoracic sternites. In addition, after comparing the holotypes of *G. takuan* and *G. hirsuta* Tan & Liu, 1998, no substantial difference could be found. Molecular evidence from mitochondrial cytochrome oxidase subunit I also supports the recognition of the new species and the conspecificity of *G. hirsuta* with *G. takuan*.

**Key words:** Potamidae, freshwater crab, new species, *Geothelphusa boreas*, *G. hirsuta*, *G. takuan*, Taiwan, mitochondrial DNA, cytochrome oxidase subunit I

### Introduction

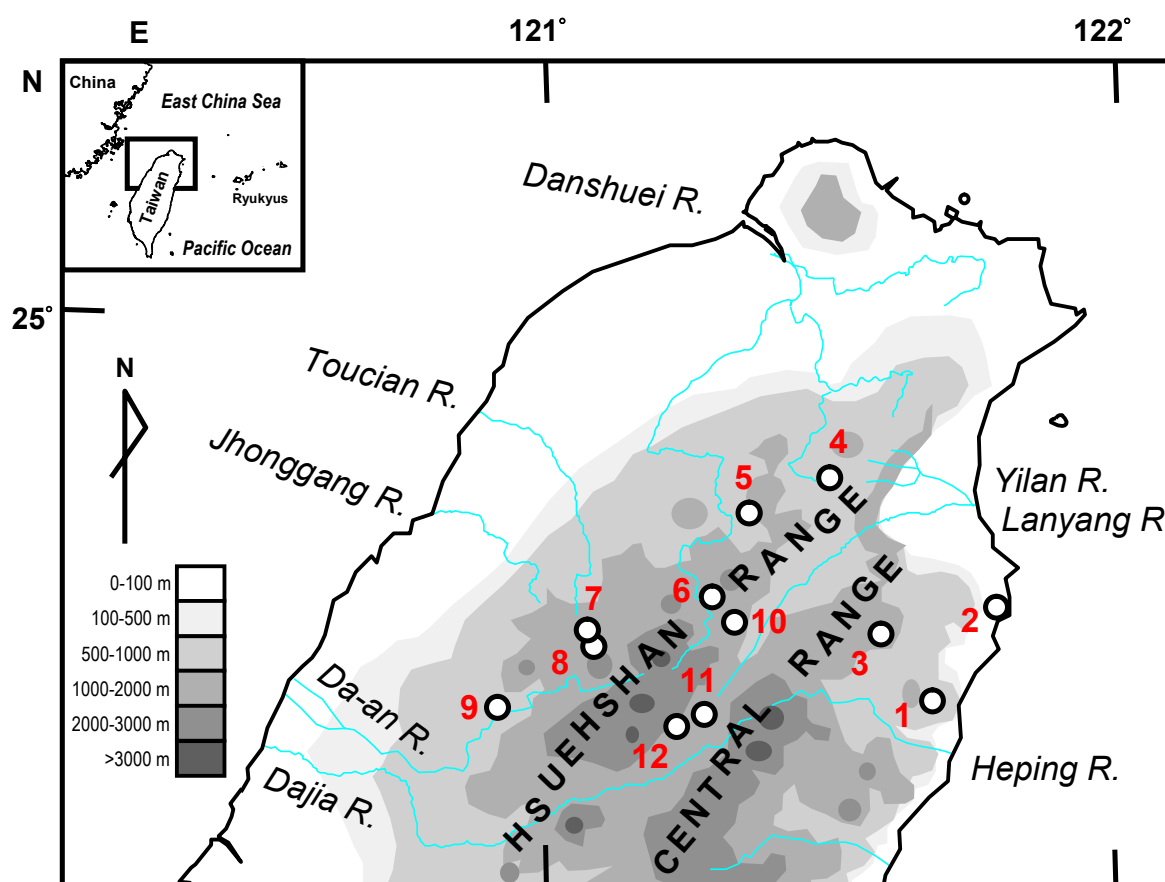
While many taxa of freshwater crabs have been described from China after the review in Shih & Ng (2011) (e.g. KL Chu *et al.* 2017, 2018a, b; Huang *et al.* 2014, 2016, 2017a, b, 2018, 2020a, b, c; Huang 2018; Wang *et al.* 2019; Mao & Huang 2020; PF Wang *et al.* 2020), fewer have been reported from the East Asian arc (Taiwan, the Ryukyus and main islands of Japan) (Suzuki & Kawai 2011; Shy *et al.* 2014, 2020; Ng *et al.* 2017), although some are still awaiting description (Shy *et al.* 2020: p. x). *Geothelphusa* Stimpson, 1858 is the second largest genus in the Potamidae (Ng *et al.* 2008; Shih & Ng 2011) and is endemic to the East Asian arc. There are 57 recognized species, with 39 species in Taiwan, 15 in the Ryukyus and three in the main islands of Japan (Shih & Ng 2011; Suzuki & Kawai 2011; Shy *et al.* 2014, 2020). While most Taiwanese *Geothelphusa* species live in low to middle altitudes, seven species, viz. *G. cilan* Shy, Shih & Mao, 2014; *G. eury soma* 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 the montane region at altitudes higher than 1000 m a.s.l. (Shy & Lee 2009; Shy *et al.* 2014). Molecular evidence has supported most of these montane species having a close phylogenetic relationship (Ng *et al.* 2010; Shih *et al.* 2010, 2011; Shy *et al.* 2014).

In the study of Shih *et al.* (2011), the “*G. sp. 2*” has been published as *G. cilan*, while the “*G. sp. 1*”, collected from the Fushan Botanical Garden, located in the mountainous area of New Taipei City and Yilan County, northeastern Taiwan has remained undescribed. Additional surveys around this region confirmed a wider distribution of this species. The structure of the male first gonopod and the proportions of the male thoracic sternites are different from other similar species, and it is herein described as new.

In addition, there is a subclade composed of *G. takuan*, *G. hirsuta* and *G. cf. hirsuta* in Shih *et al.* (2011). After reexamining the type specimens of *G. takuan* and *G. hirsuta*, we now believe that *G. hirsuta* is a junior synonym of *G. takuan*. No substantial morphological difference could be found and they are also genetically almost identical.

## Material and methods

Specimens collected by hands, traps (with bait) or pitfalls (without bait) from northeastern Taiwan (Table 1; Fig. 1) were preserved in 70%–95% ethanol and deposited in the Zoological Collections of the Department of Life Science, National Chung Hsing University, Taichung, Taiwan (NCHUZOO). Other specimens deposited in the Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Keelung, Taiwan (NTOU), the National Taiwan Museum (TMCD), and the Zoological Reference Collection of the Lee Kong Chian Natural History Museum, National University of Singapore, Singapore (ZRC), were examined. Specimens were illustrated with the help of a drawing tube attached to a stereomicroscope. The terminology used primarily follows that in Ng (1988), Shy *et al.* (1994) and Davie *et al.* (2015). Measurements provided are of the maximum carapace width (CW) and carapace length (CL) in millimeters. The following abbreviations are used: G1 for the male first gonopod and G2 for the male second gonopod.



**FIGURE 1.** Collection sites (empty circles) for the *Geothelphusa* species from northern Taiwan. For locality names, see Table 1. R. = river.

Genomic DNA was isolated from the leg muscle tissue 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), and COL14 (Roman & Palumbi 2004; Schubart 2009). 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) and were aligned manually, after verification with the complementary strand. Sequences of different haplotypes were deposited in NCBI GenBank (accession numbers in Table 1), with other sequences of closely related species (Shih *et al.* 2010, 2011; Shy *et al.* 2014) (Table 1).

**TABLE 1.** Specimens and COI haplotypes of *Geothelphusa* collected from the montane areas of northern Taiwan. The numbers within brackets following localities correspond to those in Fig. 1. <sup>a</sup> Fushan Botanical Garden is located between Wulai, New Taipei City and Yuanshan, Yilan County; <sup>b</sup> Sequences are shorter and not included for further analyses (see “DNA analyses and discussion”).

species	locality in Taiwan	catalogue no. of NCHUZOO (unless indicated)	sample size	haplotype of COI	accession no.
<i>G. boreas</i>	Fushan Botanical Garden [4] <sup>a</sup>	13429	2	Gbr1	AB625744
<b>sp. nov.</b>		13429	2	Gbr2	AB625745
	Yilan: Dakeng Bridge, Suao [2]	13616	3	Gbr3	MZ673391
		13616	1	Gbr4	MZ673392
	Yilan: Shenmihu, Nan-ao [1]	16436	2	Gbr5	MZ673393
	Yilan: Tongshan, Nan-ao [3]	14998	2	Gbr6	MZ673394
<i>G. takuan</i>	Taoyuan: Daguanshan (= Lalashan), Fusing [5]	13285	1	Gtk1	AB625747
		13285	1	Gtk2	MZ673395
		13285	1	Gtk3	AB535480
		16435	1	Gtk3	AB535480
		16435	1	Gtk4	MZ673396
	Hsinchu County: Smangus, Jianshih [6]	17041	4	Gtk5, Gtk6, Gtk7	MZ854144, MZ854145, MZ854146
	Hsinchu County: Syueba Farm, Wufeng [7]	13432	2	Gtk8	MZ673397
		13432	1	— <sup>b</sup>	AB625750
	Miaoli: Sihmasian Forest Road, Tai-an [9]	13431	1	Gtk9	AB625749
( <i>G. hirsuta</i> )	Miaoli: Guanwu, Tai-an [8]	TMCD 3285 (holotype), 3292 (paratype), ZRC (paratype)	3	Gtk10	AB625748
<i>G. cilan</i>	Hsinchu County: Cilanshan, Jianshih [10]	13617 (holotype)	1	GeL1	AB625746
		13430	4	GcL1	AB625746
		13430	1	— <sup>b</sup>	MZ673398
		13618	2	GcL2	LC002663
		13619	1	GcL3	LC002664
<i>G. monticola</i>	Yilan: upper reach of Lanyang R. [11]	13287	2	Gmt1	MZ673399
		13287	1	— <sup>b</sup>	AB535481
	Yilan: Siyuanakou, Datong [11]	13288	2	Gmt1	MZ673400
		13288	1	— <sup>b</sup>	AB535481
	Taichung: Siaoayako, Heping [12]	NTOU F10204 (holotype)	1	— <sup>b</sup>	AB535481
	Taichung: Cijiawan R., Heping [12]	13286	1	— <sup>b</sup>	AB535481
		13621	1	Gmt2	MZ673401
		13433	1	— <sup>b</sup>	AB625751
outgroups					
<i>G. eurysoma</i>	Taichung: Dasyueshan Forest Road, Heping	13622	1		LC002665
<i>G. gracilipes</i>	Hualien: Lyushuei, Shioulin	TMCD	1		AB535479
<i>G. nanao</i>	Yilan: Jinyang, Nan-ao	NTOU F10195 (holotype)	1		AB535472
<i>G. tali</i>	Yilan: Dali	NTOU F10187 (holotype)	1		MZ673402

The best-fitting model for sequence evolution was determined by PartitionFinder (vers. 2.1.1, Lanfear *et al.* 2017) and was selected by the Bayesian information criterion (BIC). The obtained best model (HKY+G) was subsequently used for a Bayesian inference (BI) analysis. The BI analysis was performed with MrBayes (vers. 3.2.3, Ronquist *et al.* 2012). Phylogenetic analyses were run with four chains for 10 million generations and four independent runs, with trees sampled every 1000 generations. The convergence of chains was determined by the average standard deviation of split frequency values below the recommended 0.01 (Ronquist *et al.* 2020), and the first 1000 trees were accordingly discarded as burnin. The minimum evolution (ME) tree was constructed on MEGA (vers. 10.0.5; Kumar *et al.* 2018) with the gamma correction (= 0.934) obtained from jModeltest (vers. 2.1.4; Darriba *et al.* 2012; selected by the Akaike information criterion (AIC) and the Kimura (1980) two-parameter model (CNI level = 2, initial tree = NJ, and maximum number of trees to retain = 1) and 2,000 replicates. The pairwise estimates of Kimura (1980) two-parameter (K2P) model distance for inter- and intraspecific genetic diversities were calculated using MEGA.

## Results

### Systematic account

#### Family Potamidae Ortman, 1896

#### Subfamily Potamiscinae Ortman, 1896 (sensu Yeo & Ng 2003)

#### Genus *Geothelphusa* Stimpson, 1858

#### *Geothelphusa boreas* sp. nov.

(Figs. 4–5)

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*Geothelphusa* sp. 1. — Shih *et al.* 2011: 461.

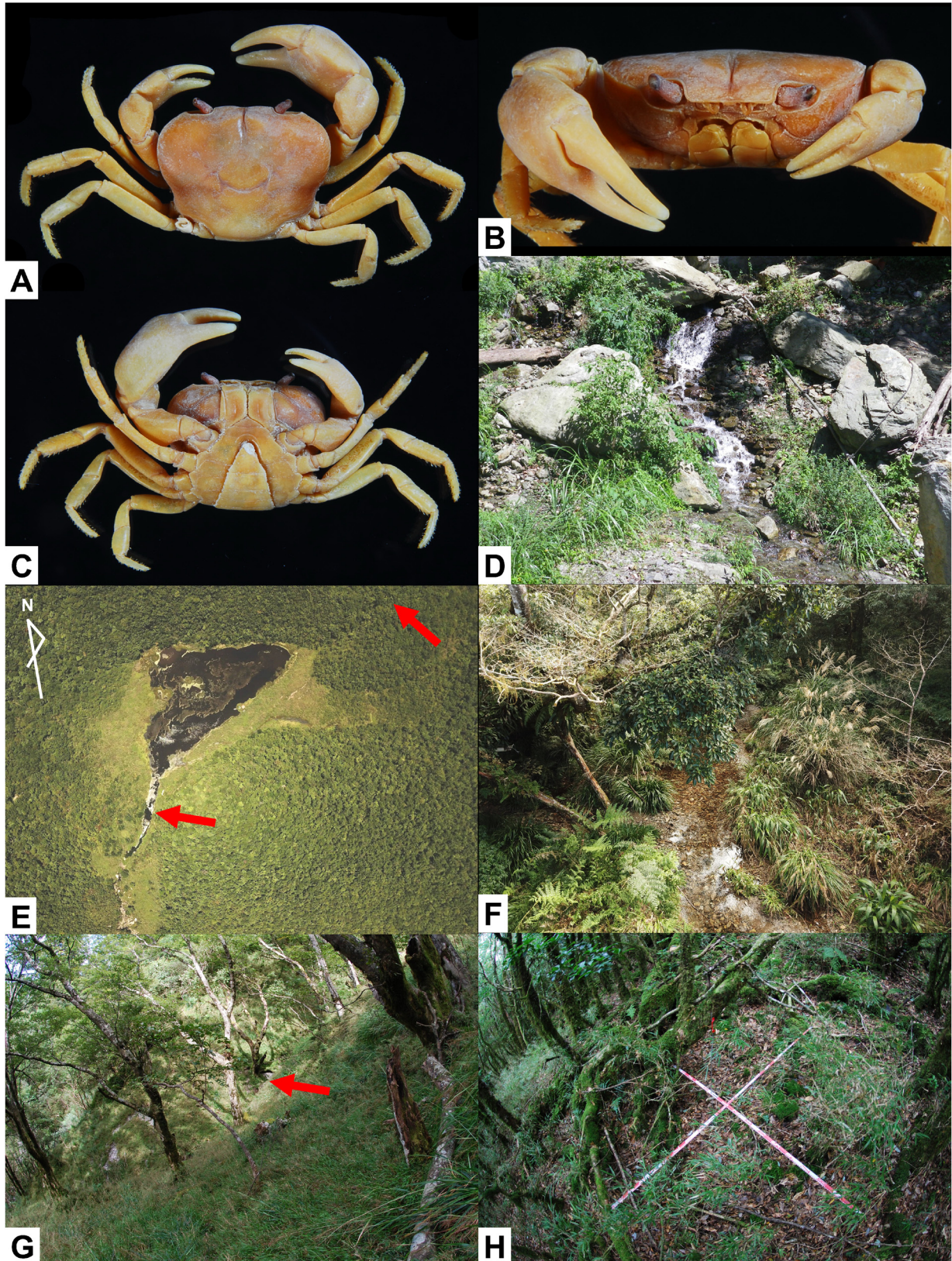
**Material examined. Holotype:** 1 ♂ (21.2 × 16.4 mm) (NCHUZOOL 13615), Hapen River, head branch of Danshuei (= Tansui) River, the Fushan Botanical Garden area, New Taipei City (24°45'30"N; 121°34'35"E), elevation of 740 m, coll. H.-T. Shih, 16 Feb. 1994. **Paratypes:** 3 ♂♂ (15.7 × 12.4–19.0 × 14.7 mm), 2 ♀♀ (15.3 × 12.1, 17.1 × 13.1 mm) (NCHUZOOL 13429), 1 ♂ (16.2 × 13.2 mm) (ZRC 2021.0648); same data as holotype. **Others:** 7 ♂♂ (11.4 × 9.2–15.3 × 12.2 mm), 1 ♀ (16.6 × 12.9 mm) (NCHUZOOL 13616), Dakeng Bridge, Suao, Yilan (24°31'48.3"N; 121°51'13.0"E), elevation of 280 m, coll. Y.-H. Wang *et al.*, 8 Sep. 2010; 2 ♀♀ (15.1 × 10.9, 16.7 × 12.8 mm) (NCHUZOOL 16436), around the lake of Shenmihu, Nan-ao, Yilan (24°22'43.9"N; 121°44'58.7"E), elevation of 1100 m, coll. J.-J. Mao, 10 Mar. 2018; 1 ♂ (12.7 × 9.7 mm), 1 ♀ (13.9 × 10.5 mm) (NCHUZOOL 14998), 1 ♀ (13.5 × 10.3 mm) (ZRC 2021.0649), Tongshan, Nan-ao, Yilan (24°30'15.5"N; 121°37'41.8"E – 24°30'03.5"N; 121°38'10.2"E), elevation of 1735–1880 m, coll. J.-J. Mao, 26 Sep. 2009.

**Comparative material.** *Geothelphusa takuan* Shy, Ng & Yu, 1994: see “Material examined” under *Geothelphusa takuan*; *Geothelphusa tali* Shy, Ng & Yu, 1994: 1 holotype male (NTOU F10187), Dali (= Tali), Yilan Co., Taiwan, coll. J.-Y. Shy & W.-L. Tsay, 4 Apr. 1993.

**Diagnosis.** Carapace length, width 1.7-, 2.3-times carapace height, respectively. Anterolateral margins distinct, lined with inconspicuous granules, without epibranchial tooth. Subterminal segment of G1 (Fig. 3A–C) curving inwards, outer proximal margin without tooth; terminal segment slightly curving outwards to almost straight; total length of G1 5.8 times terminal segment.

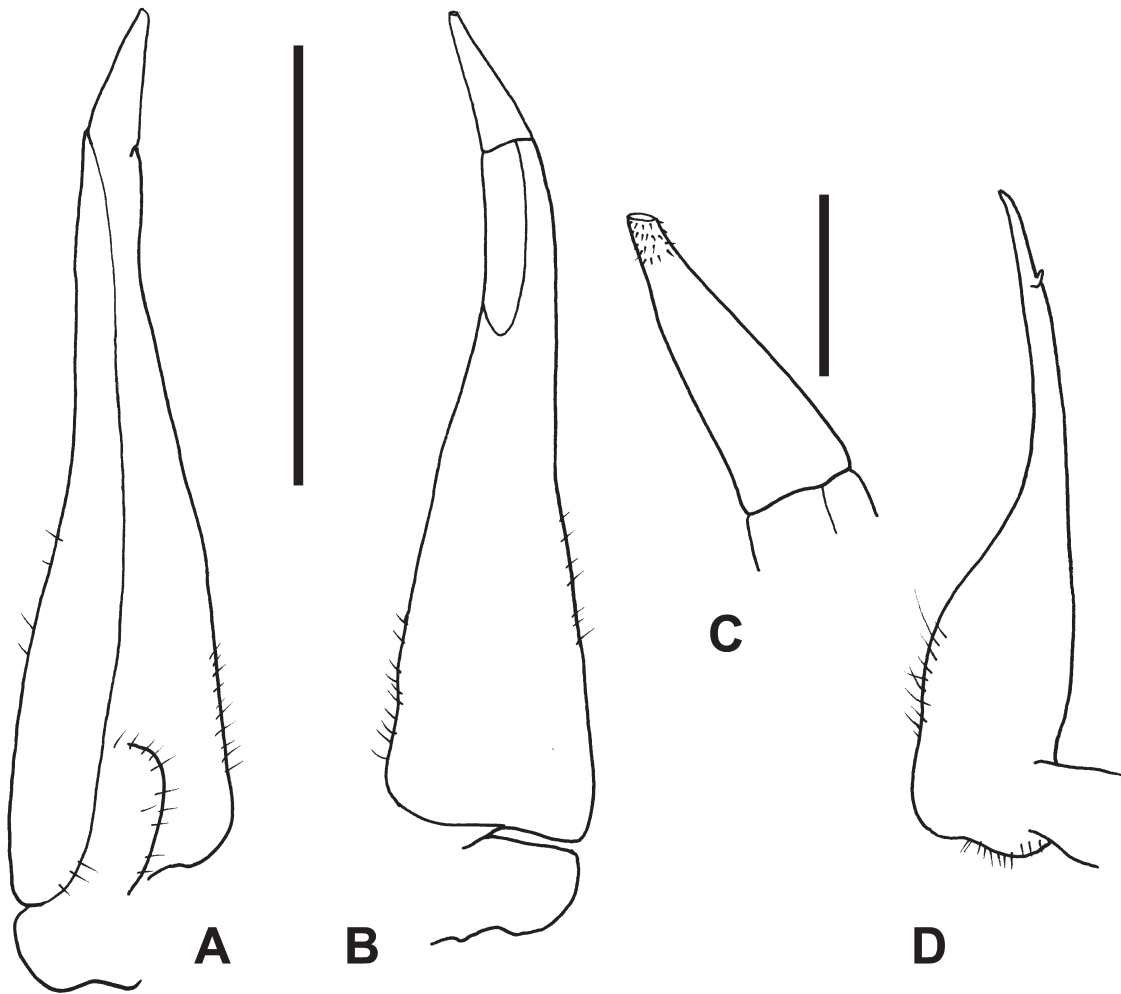
**Description.** Carapace (Fig. 2A, B) swollen longitudinally, transversely; dorsal surface smooth, glabrous, with fine pits. Carapace length, width 1.7-, 2.3-times carapace height, respectively. Frontal margin slightly dived into 2 lobes, without tooth. Postorbital cristae distinct, supraorbital margin smooth, without granules; infraorbital margin smooth to almost smooth, lined with inconspicuous granules. External orbital angles stout, external orbital regions concave. Anterolateral margins distinct, lined with inconspicuous granules, without epibranchial tooth. Postorbital cristae faint, smooth. Gastric, cardiac, intestinal regions smooth. H-shaped groove distinct. Tip of medium lobe of epistome stout. Distance between tip of sternopleonal cavity (Fig. 2C) and anterior margin of thoracic sternite 3 about 2.2 times length of thoracic sternites 1 and 2.





**FIGURE 2.** *Geothelphusa boreas* **sp. nov.** A, B, C, dorsal, frontal and ventral views of the holotype (NCHUZOO 13615); D, habitat in Dakeng Bridge, Suao, Yilan; E, F, habitat in Shenmihu, Nan-ao, Yilan; G, H, habitat in Tongshan, Nan-ao, Yilan. The arrows in E and G indicated the localities with crabs observed. The two rulers in H were used for a separate ecological study of the habitat.





**FIGURE 3.** Male right G1 and G2 of *Geothelphusa boreas* sp. nov. (holotype, NCHUZ00L 13615). A, D, ventral view of G1; B, dorsal view of G1; C, terminal segment of G1; D, ventral view of G2. Scale = 2.5 mm for A, B, D; scale = 0.5 mm for C.

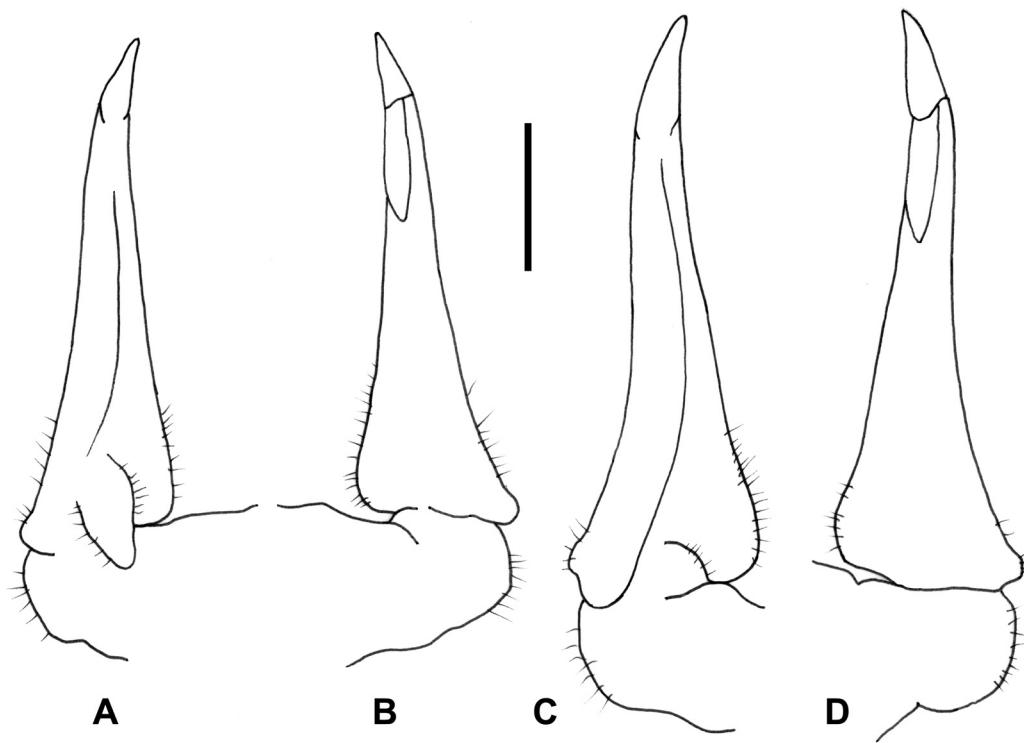
Chelipeds (Fig. 2A, B) of adult male unequal, fingers of larger chela forming obliquely triangular gape when closed. Ambulatory legs (Fig. 2A, C) smooth, dorsal, ventral margins of dactyli with 2 rows of small spines, respectively. Second leg about 1.8 carapace length. Telson bell-shaped, moderately short, width about 1.5 length.

Subterminal segment of G1 (Fig. 3A–C) curving inwards, outer proximal margin without tooth in most specimens, some smaller specimen (< 13.8 mm) with low tooth, inner proximal margin slightly dilated; terminal segment slightly curving outwards to almost straight; total length of G1 5.8 times terminal segment; length of synovial membrane about 4.6 times maximum width. Outer proximal margin of basal segment of G2 (Fig. 3D) dilated, showing a single lobe; distal segment short, about 0.16 times total length.

**Etymology.** The species is derived from the Greek “*boreas*” (for north), alluding to the northernmost distribution of this species in the Central Range.

**Ecological notes.** The specimens were collected from the montane area with an elevation of 740 m (the Fushan Botanical Garden area) to 1100 m (Shenmihu) and about 1800 m (Tonghsan), but also extended to the coastal cliff area of Suao (elevation of 280 m) (Fig. 1). The distributional range of this species covers an area of 45 km (from Fushan to Shenmihu; Fig. 1). There was running water in most habitats (Fig. 2D–F), but some crabs were more semiterrestrial, hiding under fallen leaves with only some seepage in Tongshan (Fig. 2G, H). Other species sympatric with this species include members of the *G. eucrinodonta* species complex, *G. tali* and *G. ilan* Shy, Ng & Yu, 1994 in Fushan area (Shih *et al.* 2010). In the higher altitude (Tongshan and Shenmihu), the habitats of the new species are near the *Fagus hayatae* (Taiwan beech) forest (Fig. 2E–H), with the mean (and standard deviation) water temperature  $17.66 \pm 2.12$  °C (April 2018) and  $23.25 \pm 1.78$  °C (May to September 2018), pH  $7.4 \pm 0.3$  (April to October 2018) and dissolved oxygen  $8.7 \pm 0.9$  mg/L (April to October 2018) in Shenmihu (Fig. 2F).

**Remarks.** *Geothelphusa boreas* **sp. nov.** is morphologically most similar to *G. takuan* and *G. tali* which occurs in adjacent regions in northern Taiwan. The G1 structures remain the best way to separate them. The subterminal segment of the G1 of *G. tali* is straight (Shy *et al.* 2020: fig. 124), while those of present new species and *G. takuan* are distinctly curved inwards (Figs. 3, 4A, B; Shy *et al.* 2020: fig. 121). In addition, the G1 subterminal segment in *G. takuan* has a distinct tooth on the outer proximal margin (Fig. 4A), while in *G. boreas*, there is usually no tooth present on the structure (or at most with a faint knob in some small individuals) (Fig. 3A). The distance between the tip of the sternopleonal cavity and the anterior margin of thoracic sternite 3 in *G. takuan* and *G. tali* being 1.0 times and 1.2 times the length of sternites 1 and 2, respectively, which are shorter than the ratio 2.2 times in *G. boreas*.



**FIGURE 4.** Male right G1s of *Geothelphusa takuan* Shy, Yu & Ng, 1994 (holotype, NTOU F10205; A, B) and *G. hirsuta* Tan & Liu, 1998 (holotype, TMCD 3285; C, D). A, C, ventral view; B, D, dorsal view. Scale = 1 mm.

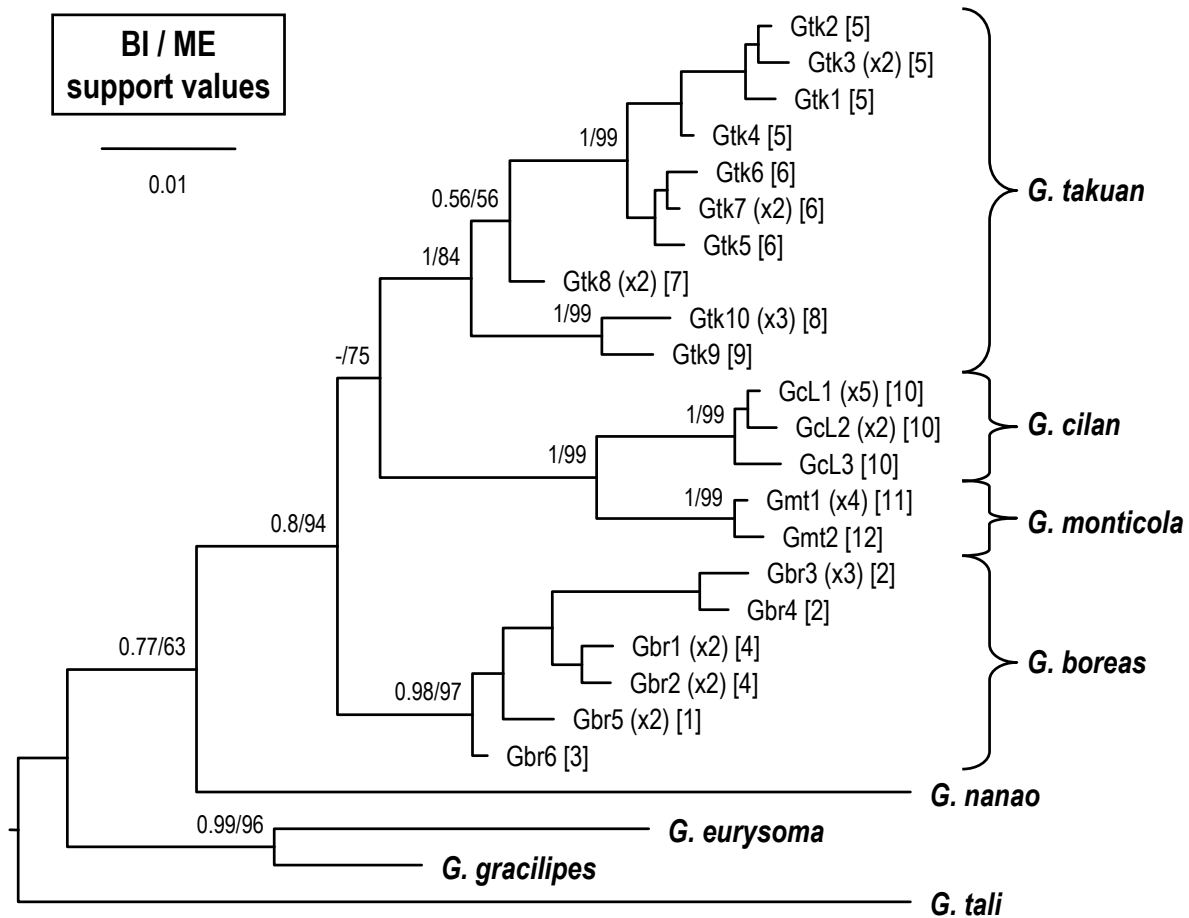
#### *Geothelphusa takuan* Shy, Ng & Yu, 1994

(Fig. 4)

*Geothelphusa takuan* Shy, Ng & Yu, 1994: 823, fig. 20a–f [type locality: Dagan, Fusing, Taoyuan]; Dai 1999: 387; Shy & Yu 1999: 75, 2 un-numbered figs.; Ng *et al.* 2001: 51; Chen *et al.* 2007a: 881, fig. 8A1–A3; Chen *et al.* 2007b: 906, fig. 4B; Shy & Lee 2009: 184, 8 un-numbered figs.; Shih *et al.* 2011: 461; Ng *et al.* 2017: 81; Shy *et al.* 2020: 154, figs. 119–121. *Geothelphusa hirsuta* Tan & Liu, 1998: 288, fig. 2A–E [type locality: Tai-an, Miaoli County, Taiwan]; Ng *et al.* 2001: 50; Shy & Lee 2009: 137, 7 un-numbered figs.; Shih *et al.* 2011: 461; Ng *et al.* 2017: 79; Shy *et al.* 2020: 82, figs. 66–68.

**Material examined. Types:** holotype male (NTOU F10205), Dagan (= Takuan), Fusing (= Fuhsing), Taoyuan Co., Taiwan, coll. J.-Y. Shy & W.-L. Tsay, 1 Nov. 1992. *Geothelphusa hirsuta* Tan & Liu, 1998: holotype male (TMCD 3285), 1 paratype male (TMCD 3292), Guanwu (= Kuanwu), Tai-an, Miaoli (24°30'17.5"N 121°07'09.9"E), elevation of 2060 m, coll. H.-C. Liu, 13 Nov. 1993. **Others:** 3 ♂♂ (12.0–16.2 mm), 3 ♀♀ (12.1–15.9 mm) (NCHUZOOL 13285), Daguanshan (= Lalashan), Fusing, Taoyuan (24°42'28.9"N, 121°26'09.2"E), elevation of 1650 m, coll. H.-T. Shih, 4 Dec. 2000; 3 ♂♂ (12.4 × 10.4–17.4 × 13.0 mm), 1 ♀ (17.3 × 12.8 mm) (NCHUZOOL 16435), Daguanshan (= Lalashan), Fusing, Taoyuan (24°42'28.9"N, 121°26'09.2"E), elevation of 1650 m, coll. Y.-H. Wang *et al.* 7

Sep. 2010; 4 ♂♂ (13.2 × 10.8–16.1 × 12.1 mm), 1 ♀ (16.2 × 13.4 mm) (NCHUZOO 17041), Smangus, Jianshih, Hsinchu County (24°34'41.6"N, 121°20'47.4"E), elevation of 1560 m, coll. J.-J. Li, 3 Apr. 2021; 3 ♂♂ (14.8 × 11.6–18.4 × 13.7 mm), 5 ♀♀ (16.5 × 13.3–20.2 × 14.9 mm) (NCHUZOO 13432), Syueba Farm, Wufeng, Hsinchu County, coll. 1 Dec. 2001; 1 ♂ (18.7 × 14.3 mm) (NCHUZOO 13431), Sihmasian Forest Road, Tai-an, Miaoli.



**FIGURE 5.** A Bayesian Inference (BI) tree of *Geothelphusa boreas* sp. nov. and related montane species from northern Taiwan based on cytochrome oxidase I (COI) marker. Probability values at the nodes represent the support values for BI and maximum evolution (ME). For haplotype abbreviations, see Table 1. Corresponding numbers of localities in Fig. 1 are in parenthesis behind haplotype abbreviations.

**Ecological notes:** In Daguanshan, *Geothelphusa takuan* is sympatric with *G. ilan*. In Smangus, *G. takuan* is sympatric with *G. ilan*, *G. cilan* and *G. sp.*

**Remarks:** Based on the figure in Tan & Liu (1998: fig. 2), the G1 of *G. hirsuta* with the subterminal segment curves outwards, which is different from the inward curved subterminal segment of *G. takuan* (Shy *et al.* 1994). However, after reexamining on the holotype of *G. hirsuta* (TMCD 3285), we found both the right and left G1s (and G2s) were dissected and stored in small tubes separately. By comparing the right G1 detached from the adult male paratype (TMCD 3292), the labels of left and right on the tubes of holotype are confirmed to be actually reversed. That is, the figure and description for the G1 and G2 in Tan & Liu (1998) are actually left ones instead. Our reexamination of the right G1s of the holotype (Fig. 4C, D) and paratype of *G. hirsuta*, found no difference from that of the holotype of *G. takuan* (NTOU F10205) (Fig. 4A, B). We also find that they differ only in the anterolateral margins, with *G. hirsuta* having lined with relatively clear granules (Shy *et al.* 2020: fig. 66) and *G. takuan* having lined with lower granules (Shy *et al.* 2020: fig. 119), too small a difference to warrant their recognition as separate taxa. With regard to the genetic similarity for the two species, see “DNA analyses and discussion”. Geographically, although the distance between the two type localities of *G. takuan* (Fig. 1: no. 5) and *G. hirsuta* (Fig. 1: no. 8) is about 40 km, they belong to the same mountain range, the Hsuehshan Range (Fig. 1), and a continuous distribution of a single species is expected.



**TABLE 2.** Matrix of percentage pairwise nucleotide divergences with K2P distance and number of bp differences based on COI within and between species of *Geothelphusa* from northern Taiwan (see Table 1). In the right half, lower-left values are K2P distance and upper-right ones are bp differences. Values of range are given in parentheses.

	Intraspecific		Interspecific							
	Nucleotide divergence	bp difference	<i>G. boreas</i> <b>sp. nov.</b>	<i>G. takuan</i>	<i>G. cilan</i>	<i>G. monticola</i>	<i>G. eurysona</i>	<i>G. gracilipes</i>	<i>G. nanao</i>	<i>G. tali</i>
<i>G. boreas</i> <b>sp. nov.</b>	1.15 (0–2.01)	7.49 (0–13)		23.12 (14–31)	28.43 (25–33)	27.84 (25–30)	36.82 (34–41)	30.18 (26–35)	46.91 (43–52)	50 (49–51)
<i>G. takuan</i>	1.62 (0–3.33)	9.97 (0–20)	3.73 (2.17–4.91)		26.45 (23–32)	25.07 (22–29)	42.33 (40–46)	35.4 (31–39)	43.8 (40–47)	52.33 (48–55)
<i>G. cilan</i>	0.14 (0–0.46)	0.93 (0–3)	4.49 (3.93–5.26)	4.29 (3.6–5.26)		14.7 (14–17)	42.5 (42–44)	39.25 (39–41)	52.25 (52–54)	54.25 (54–56)
<i>G. monticola</i>	0.06 (0–0.15)	0.4 (0–1)	4.4 (3.94–4.77)	4.06 (3.45–4.73)	2.28 (2.17–2.65)		43.2 (43–44)	41.2 (41–42)	48.2 (48–49)	52.2 (52–53)
<i>G. eurysona</i>			5.88 (5.4–6.6)	6.99 (6.4–7.59)	6.83 (6.74–7.09)	6.96 (6.92–7.1)		24	49	59
<i>G. gracilipes</i>			5.11 (4.38–5.97)	6.04 (5.26–6.68)	6.73 (6.68–7.06)	7.1 (7.07–7.25)	4.03		41	49
<i>G. nanao</i>			8.13 (7.41–9.08)	7.55 (6.85–8.14)	9.12 (9.06–9.45)	8.36 (8.32–8.51)	8.48	7.02		56
<i>G. tali</i>			8.68 (8.48–8.87)	9.11 (8.3–9.6)	9.48 (9.42–9.82)	9.1 (9.06–9.25)	10.42	8.5	9.75	

## DNA analyses and discussion

A 616–658 basepair (=bp) segment of COI were amplified and aligned (Table 1). Some paratypes were a bit shorter (616 bp), but otherwise identical to other sequences with 658 bp, which omitted from further analyses.

The phylogenetic tree was reconstructed from BI analysis, with the support values from the ME analysis (Fig. 5). It is clear the haplotypes of *G. boreas* **sp. nov.** forms an independent clade with strong support, separate from other morphologically similar species. With regard to the clade composed of *G. takuan* and *G. hirsuta* (from type specimens; Table 1), specimens from northern area (“5” and “6” in Figs. 1, 5) seem different from those from southern area (“8” and “9” in Figs. 1, 5) on the phylogenetic tree (Fig. 5), but another subclade (“GtK8”) from southern area (“7” in Figs. 1, 5) situated in the intermediate position within this clade, which make this clade difficult to be subdivided. The *G. monticola* clade is sister to the *G. cilan* clade which form a main clade with strong support.

The pairwise nucleotide divergences for COI with K2P distance and bp differences are shown in Table 2. The mean (and minimum) interspecific K2P distance and bp differences of *G. boreas* are 3.73% (and 2.17%) and 23.12 bp (and 14 bp), with its closest relative, *G. takuan*. The above minimum interspecific distance is considered small, compared with other freshwater crabs (reviewed by Chu *et al.* 2015), but is still the same with or larger than some potamids, e.g. 2.17% between *G. cilan* and *G. monticola* (Shy *et al.* 2014; Table 2); 1.81% between *G. pingtung* and *G. makatao* (Chu *et al.* 2015). The mean (and highest) intraspecific distance and bp differences of *G. boreas* are as large as 1.15% (and 2.01%) and 7.49 bp (and 13 bp), and *G. takuan* also has larger intraspecific distance, 1.62% (and 3.33%) and 9.97 bp (and 20 bp), which could be cause by the wide distributions of the two species (Fig. 1), as in *Tiwaripotamon pluviosum* Do, Shih & Huang, 2016, 3.18% (and 5.25%) (Do *et al.* 2016). As a result, we treat *G. hirsuta* Tan & Liu, 1998 is a junior subjective synonym of *G. takuan* Shy, Ng & Yu, 1994, based on the morphological similarity, molecular evidence and the continued distribution.

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