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Systematics of the Genus *Geothelphusa* (Crustacea, Decapoda, Brachyura, Potamidae) from Southern Taiwan: A Molecular Appraisal

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Hsi-Te Shih, Peter K. L. Ng and Hsueh-Wen Chang (2004) Systematics of the genus *Geothelphusa* (Crustacea: Decapoda, Brachyura, Potamidae) from southern Taiwan: a molecular appraisal. *Zoological Studies* **43**(3) : 561-570. There are 5 freshwater crab species of the genus *Geothelphusa* Stimpson, 1858 in southernmost Taiwan, *G. albogilva* Shy, Ng and Yu, 1994; *G. ferruginea* Shy, Ng and Yu, 1994; *G. tawu* Shy, Ng and Yu, 1994; *G. lanyu* Shy, Ng and Yu, 1994; and *G. lutao* Shy, Ng and Yu, 1994. Among these, *G. lanyu* and *G. lutao*, are only known from 2 offshore islands, Lanyu (Orchid I.) and Lyudao (Green I.), respectively. Comparisons of the DNA sequences encoding part of the mitochondrial large subunit 16S rRNA gene showed that all 5 species constitute a single monophyletic clade distinct from species in nearby areas. The genetic and morphological differences among *G. tawu*, *G. lanyu*, and *G. lutao* are very small, and further studies may show that all 3 are conspecific. This is supported by the geological history of the area, with both islands having a violent volcanic history, and the presence of freshwater crabs on the island must have been a relatively recent event (~500 000 years), and may even have been the result of human introduction. The phylogeographical patterns of the genus in southernmost Taiwan and adjacent areas were examined at the molecular level, and the isolating effects of mountains on freshwater crab dispersal are discussed. The genetic data also suggest that the 2 species from southwestern Taiwan, *G. pingtung* Tan and Liu, 1998 and *G. neipu* Chen, Jeng and Shy, 1998, are synonymous, with the former name having priority. http://www.sinica.edu.tw/zool/zoolstud/43.3/561.pdf

Key words: Phylogeography, mtDNA sequence, 16S rRNA.

The East Asian freshwater crabs of the genus *Geothelphusa* Stimpson, 1858, are distributed from Taiwan to Japan (Shy and Yu 1999, Yoshigou 1999) but are absent from continental Asia. Currently, 31 species are known from Taiwan (Shy et al. 1994, Chen et al. 1998, Tan and Liu 1998). In the southern part of Taiwan, 9 species are present: *G. albogilva* Shy, Ng and Yu, 1994; *G. ferruginea* Shy, Ng and Yu, 1994; *G. tawu* Shy, Ng and Yu, 1994; *G. lanyu* Shy, Ng and Yu, 1994; *G. lutao* Shy, Ng and Yu, 1994; *G. cinerea* Shy, Ng and Yu, 1994; *G. bicolor* Shy, Ng and Yu, 1994; *G. pingtung* Tan and Liu, 1998; and *G. neipu* Chen, Jeng and Shy, 1998. Of these, *G. albogilva*, *G. ferruginea*, and *G. tawu* occur on the Hengchun Peninsula (south of the Fenggang River). *Geothelphusa cinerea* and *G. bicolor* are present on the northeastern portion of the Hengchun Peninsula but are also distributed along much of the east coast of Taiwan (Shy et al. 1994). On the northern Hengchun Peninsula to central Pingtung County, 2 other species, *G. pingtung* and *G. neipu*, have been reported. Two species, *G. lanyu* and *G. lutao*, are known only from the offshore islands of Lanyu (Orchid I.) and Lyudao (Lutao or Green I.), respectively.

Geothelphusa albogilva is a large species (adult carapace width ~35 mm) and can be separated from sympatric species in southernmost Taiwan by morphology and coloration. However,

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the smaller-sized *G. ferruginea*, *G. tawu*, *G. lanyu*, and *G. lutao* (adult carapace width ~20 mm) closely resemble each other externally, with the main differences between them being the shape and proportions of the male 1st gonopods (Shy et al. 1994).

The entire life cycle of freshwater crabs is spent in the freshwater environments. The fertilized eggs develop directly into juvenile crabs, and females brood the early juveniles for periods of different lengths (Ng 1988). From a biogeographic perspective, this reproductive biology, together with their intolerance of brackish or sea water, means that oceans are effective barriers to the dispersal of freshwater crabs, except during periods of extreme glaciation when land masses and islands are connected by land bridges (Ng and Rodriguez 1995). In the case of Lanyu and Lyudao I. in southern Taiwan, there are 2 deep troughs (the 2500 m deep Taitung Trough and 3000 m deep Lyudao-Lanyu Trough) that separate them from Taiwan (Huang et al. 1995) (Fig. 1). Even during the last major glaciation period (at which time the maximum sea level was as low as ~120 m) (Fairbanks 1989, Voris 2000), there is no evidence that there was a land bridge connecting these islands to each other or to the main island.

The mitochondrial 16S ribosomal RNA (rRNA) encoding gene have been shown to be effective in various phylogenetic and population studies of crustaceans (see review by Schubart et al. 2000). They are also useful in recognizing questionable species (e.g., Sarver et al. 1998, Schubart et al. 1998, 2001a, b, c, Spivak and Schubart 2003). In the present study, we compared the DNA sequences of the mitochondrial 16S rRNA gene among *Geothelphusa* species from southern Taiwan and adjacent areas to estimate the extent of genetic diversity among these species. The taxonomy of Taiwanese species is discussed and reviewed in connection with these new genetic data and the geological history of this region.

MATERIALS AND METHODS

Specimens of the genus *Geothelphusa* were collected from streams around the Hengchun Peninsula, Pingtung County, and southern Taitung County, areas which belong to the southernmost part of the east side of the Central Range of Taiwan. Samplings were also carried out on the 2 offshore islands, Lanyu and Lyudao (Fig. 1). In total, 50 specimens were collected between 1993

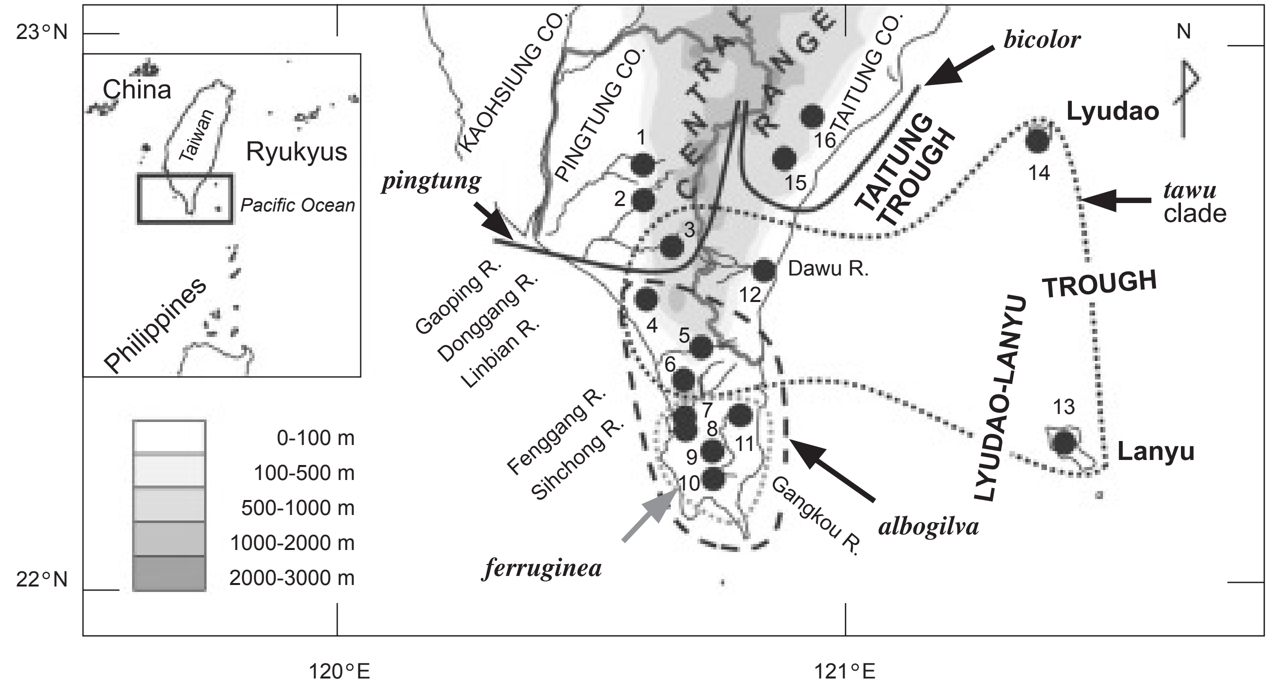

Fig. 1. Collection sites (black circles) for *Geothelphusa* species in southern Taiwan and adjacent areas. The black and dotted curves shown on the map show the possible distributional ranges indicated by species names based on the molecular results in this study. Numbers beside the collection sites correspond to those following the localities in Table 1.

Table 1. Haplotypes of *Geothelphusa collected* from Hengchun Peninsula and adjacent areas of southern Taiwan (Fig. 1). Most species were identified following Shy et al. (1994) and Shy and Yu (1999). If the 16S rRNA of the species identified differed from that of the true species, the species name is enclosed in quotation marks (“ “). Numbers within brackets following localities correspond to those in Fig. 1. R., river; Co., county

Regions and haplotypes	Sample size (n)	Species identified	Localities	DDBJ accession no.
Southwestern region (SW)	9			
PTC1	2	“*albogilva*”	Pingtung Co. (Cijia, Chunrih [3])	AB127363
PTC2	5	*pingtung*, *neipu*, “*albogilva*”	Pingtung Co. (Taiwu [1]; Dahou, Laiyi [2]; Lili, Chunrih [3])	AB127365
PTC3	2	*neipu*, “*albogilva*”	Pingtung Co. (Taiwu [1])	AB127364
Southern region (S)	39			
PTHC1	1	*albogilva*	Pingtung Co. (Shihniu R., Hengchun [4])	AB127368
PTHC2	1	*albogilva*	Pingtung Co. (Sihchong R., Checheng [7])	AB127367
PTHC3	9	*albogilva*	Pingtung Co. (Fenggang R., Shihzih [5]; Sihchong R. [7], Baoli R., Checheng [8]; Maozaikengnei, Hengchun [9]; Nanrenshan, Manjhou [11])	AB127366
PTSW1, 2, 3	3	*albogilva*	Pingtung Co. (Shihwen, Chunrih [4])	AB127373, 127371, 127372
PTSW4	2	*albogilva*	Pingtung Co. (Shihwen, Chunrih [4])	AB127369
PTSW5	1	*albogilva*	Pingtung Co. (Shihwen, Chunrih [4])	AB127370
PTSW6	1	“*ferruginea*”	Pingtung Co. (Shihwen, Chunrih [4])	AB127375
PTCJ1	1	“*ferruginea*”	Pingtung Co. (Cijia, Chunrih [3])	AB127377
PTCJ2	2	“*ferruginea*”	Pingtung Co. (Cijia, Chunrih [3])	AB127376
TTS1, 2	2	*tawu*	Taitung Co. (Dawu R., Dawu [12])	AB127379, 127381
TTS3	4	“*ferruginea*”, *tawu*, *lanyu*	Pingtung Co. (Fenggang R., Shihzih [5]), Taitung Co. (Dawu R., Dawu [12]; Lanyu [13])	AB127380
TTS4	4	*lanyu*	Taitung Co. (Lanyu [13])	AB127378
TTS5	3	*lutao*	Taitung Co. (Lyudao [14])	AB127382
PTHC4	1	“*ferruginea*”	Pingtung Co. (Damei R., Mudan [6])	AB127374
PTHC5	4	*ferruginea*	Pingtung Co. (Shihniu R. [10], Maozaikengnei, Hengchun [9]; Nanrenshan, Manjhou [11])	AB127383
Eastern region (E)	2			
TT1	1	*bicolor*	Taitung Co. (Taimali [15])	AB127385
TT2	1	*bicolor*	Taitung Co. (Jhihben, Jinfeng [16])	AB127384
All localities	50			

and 2002, and these identified were as *G. albogilva*, *G. ferruginea*, *G. tawu*, *G. lanyu*, *G. lutao*, *G. pingtung*, *G. neipu*, and *G. bicolor*, mainly on the basis of Shy et al. (1994) and Shy and Yu (1999) (Table 1). After collection, specimens were preserved in 75~95% ethanol. Specimens of the morphologically distinct *G. olea*, collected from Nangang, Taipei City; Beipu, Hsinchu County; Touwu, Miaoli County; Shetou, Changhua County; Cingshuei River, Chiayi County; and Dongshan, Tainan County, were sequenced as outgroups.

Genomic DNA was isolated from the muscle tissue of legs by a phenol-chloroform extraction (Kocher et al. 1989) or a Sigma mammalian genomic DNA miniprep kit. A region of approximately 550 base pairs (bp) of the 5'-end of the 16S rRNA gene was selected for amplification with the polymerase chain reaction (PCR) using the primers 1471 (5'-CCTGTTTANCAAAAACAT-3') and 1472 (5'-AGATAGAAACCAACCTGG-3') (Crandall and Fitzpatrick 1996). The PCR conditions for primers 1471 and 1472 were denaturation for 50 s at 94°C, annealing for 70 s at 45°C, and extension for 60 s at 72°C, followed by extension for 10 min at 72°C. Sequences were obtained by automated sequencing (ABI PRISM 377 Sequencer and MegaBACE DNA Analysis System 500) and were aligned with the aid of Clustal W (vers. 1.4, Thompson et al. 1994) and BioEdit (vers. 5.09, Hall 2001), after verification with the complimentary strand. Sequences of the different haplotypes were deposited in the DDBJ nucleotide sequence databases (accession nos. AB127363 to 127386).

Inter- and intrapopulation genetic diversities were established by calculating pairwise distances of nucleotide divergence under a Kimura (1980) 2-parameter model with the pairwise deletion option using the program MEGA2 (vers. 2.1, Kumar et al. 2001). The neighbor-joining (NJ) tree was established using pairwise distances of nucleotide divergence and was constructed by the program TREECON for Windows (vers. 1.3b, Van de Peer and De Wachter 1997). The distance estimation was based on Kimura's (1980) model with the transition/transversion ratio estimated from the data. Two thousand bootstrap replications were performed for bootstrap analysis. The maximum parsimony (MP) tree was constructed using the program PAUP* (vers. 4.0b8, Swofford 2001), with 2000 replications of a heuristic search and random sequence addition. All characters were equally

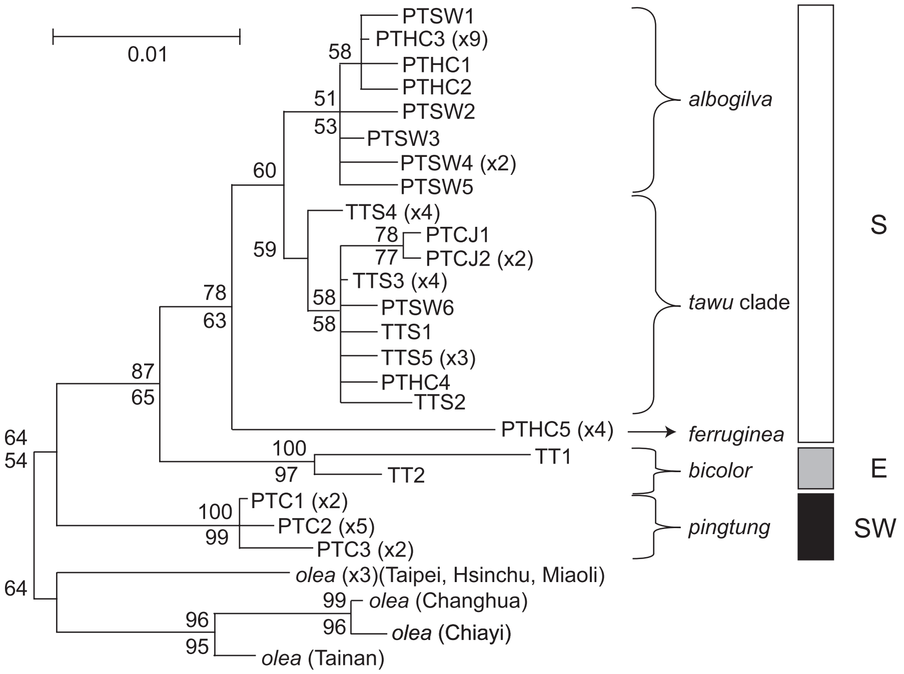

Fig. 2. Neighbor-joining tree from Kimura's 2-paramater distance matrix for *Geothelphusa* species of southern Taiwan and adjacent areas. Sequence data for the 550-bp domain of the mitochondrial 16S rRNA gene were used. Specimens of *G. olea* from other areas of Taiwan were included as outgroups. Values at a given node represent the percent support (> 50%) of the 2000 bootstrap replications in the neighbor-joining analysis (above the node) and in the maximum parsimony analysis (below the node). See Table 1 for abbreviations of haplotypes. S, southern Taiwan; E, eastern Taiwan; SW, southwestern Taiwan.

weighted. Gaps in both the NJ and MP tree construction were treated as a 5th character state (Kambhampati 1995, Miura et al. 2000, Tong et al. 2000). Phylogenetic reconstructions identified a *G. tawu* clade composed of very closely related haplotypes, which included individuals all sampled from Lanyu and Lyudao. To examine the relationships of these haplotypes in detail, a gene genealogy was constructed using the program TCS (Clement et al. 2000). Gaps were treated as a 5th state when the network was constructed.

RESULTS

A 550-bp segment (excluding the primer regions) of the 16S rRNA mtDNA gene was amplified and aligned for all 50 specimens. Out of those, 41 positions were variable and 21 were parsimoniously informative. Among the total number of sequences, 23 different haplotypes were found (Table 1). The amplified segment of 16S sequences was AT rich (72.4%) (T, 36.4%; A, 36.0%; G, 17.2%; and C, 10.4%). The phylogenetic tree constructed by Kimura's 2-parameter model and neighbor-joining analysis is shown in Fig. 2, with bootstrap values (> 50%) obtained from the NJ and MP analyses (above and below the nodes, respectively).

Both phylogenetic trees (Fig. 2) provide evidence that *Geothelphusa* species in southern Taiwan are monophyletic with 3 clades, i.e., *G. albogilva*, *G. ferruginea*, and *G. tawu* clades; however, the *G. tawu* clade was not separated by the MP analysis (with a bootstrap value of 42%). The resulting phylogenetic groupings agree well with most species identifications, except for the *G. tawu* clade (including *G. tawu*, *G. lanyu*, *G. lutao*, and "*G. ferruginea*") and for the *G. pingtung* clade (including *G. pingtung*, *G. neipu*, and "*G. albogilva*") (Table 1). The double quotation marks (" ") indicate that the species was identified based on Shy et al. (1994) and Shy and Yu (1999), but its 16S rRNA differed from that of the actual species.

The average nucleotide divergence (Kimura 1980) between haplotypes belonging to the *G. tawu* clade was 0.0043 ± 0.0014 (0~0.0074), and the number of nucleotide differences was less than 6 bp (Table 2). One haplotype (TTS5) was found only in the Lyudao population. Two haplotypes (TTS3 and TTS4) were present in the Lanyu population, of which 1 haplotype (TTS3) was further shared by specimens from the Fenggang River, Pingtung County and Tawu River, Taitung County (Table 1). The results suggest that *G. tawu*, *G. lanyu*, and *G. lutao* are genetically difficult to separate. The haplotype network depicting the relationship among the haplotypes of the *G. tawu* clade is shown in Fig. 3. Haplotype TTS3 is most closely related to other haplotypes and is the predicted ancestral haplotype (Clement et al. 2000). For the *G. pingtung* clade, several "species" share the same haplotypes, and the 3 haplotypes (PTC1, PTC2, and PTC3) only differ by less than 3 bp. In

Table 2. Pairwise 2-parameter distance (Kimura, 1980) matrix (lower-left) from the 550-bp portion of the mitochondrial 16S rRNA gene among the haplotypes of the *Geothelphusa tawu* clade from southern Taiwan. The numbers and types of differences are given in the upper right. Among them, TTS3 was found in the Fenggang and Dawu Rivers, and on Lanyu. TTS4 and TTS5 were only seen on Lanyu and Lyudao, respectively. Types of differences include transitions (s), transversions (v), and indels (i). See Table 1 for haplotype abbreviations

	PTSW6	PTCJ1	PTCJ2	TTS1	TTS2	TTS3	TTS4	TTS5	PTHC4
PTSW6		4s	3s	2s	1s,1v,1i	1s	3s	1s,1v	1s,1i
PTCJ1	0.0074		1s	4s	3s,1v,1i	3s	5s	3s,1v	3s,1i
PTCJ2	0.0055	0.0018		3s	2s,1v,1i	2s	4s	2s,1v	2s,1i
TTS1	0.0037	0.0074	0.0055		1s,1v,1i	1s	3s	1s,1v	1s,1i
TTS2	0.0037	0.0074	0.0055	0.0037		1v,1i	2s,1v,1i	2v,1i	1v,2i
TTS3	0.0018	0.0055	0.0037	0.0018	0.0018		2s	1v	1i
TTS4	0.0055	0.0092	0.0074	0.0055	0.0055	0.0037		2s,1v	2s,1i
TTS5	0.0037	0.0074	0.0055	0.0037	0.0037	0.0018	0.0055		1v,1i
PTHC4	0.0018	0.0055	0.0037	0.0018	0.0018	0	0.0037	0.0018	

the following, we use the *G*. *tawu* clade and *G*. *pingtung* to represent the 2 clades respectively in this study (also see the "Discussion"). Only 1 haplotype (PTHC5) was found in the *G*. *ferruginea* clade (Table 1), but specimens identified as "*G*. *ferruginea*" were also found in the *G*. *tawu* clade.

The nucleotide divergence (Kimura 1980) within each clade (0~0.0148; average, 0.0051) was significantly lower in comparison with the divergence between clades (0.0093~0.0319; average, 0.0241, see Table 3) ($p = 0.0013$, by the Mann-Whitney test).

DISCUSSION

The present genetic analyses show that there are 3 distinct clades of southern Taiwanese *Geothelphusa*. Southernmost Taiwan is represented by 1 large- (*G*. *albogilva*) and 4 small-sized species (*G*. *ferruginea*, *G*. *tawu*, *G*. *lanyu*, and *G*. *lutao*) (Shy et al. 1994, Shy and Yu 1999), all of which emerged as a single monophyletic clade (Fig. 2). This is herein referred to as the S clade. The species found in the southwestern area, *G*. *pingtung*, forms its own clade (herein referred to as the SW clade), and *G*. *bicolor* from the eastern part of Taiwan is in its own clade (referred to as the E clade) (Fig. 2). The data suggest that the southwestern extension of the Central Range, which separates the S and SW clades, has played a major role in isolating these freshwater crabs.

With regards to the S clade, all the small-sized species are closely genetically related to each other and form a discrete cluster, except for *G*. *ferruginea*, which seems to belong to a separate subclade (Fig. 2). Although the *G*. *tawu* clade is not supported by maximum parsimony because of the TTS4 haplotype from Lanyu, it could be inferred by the neighbor-joining tree (Fig. 2), and there are only 2 transversions between TTS3 and TTS4 (Table 2), both of which were found in the

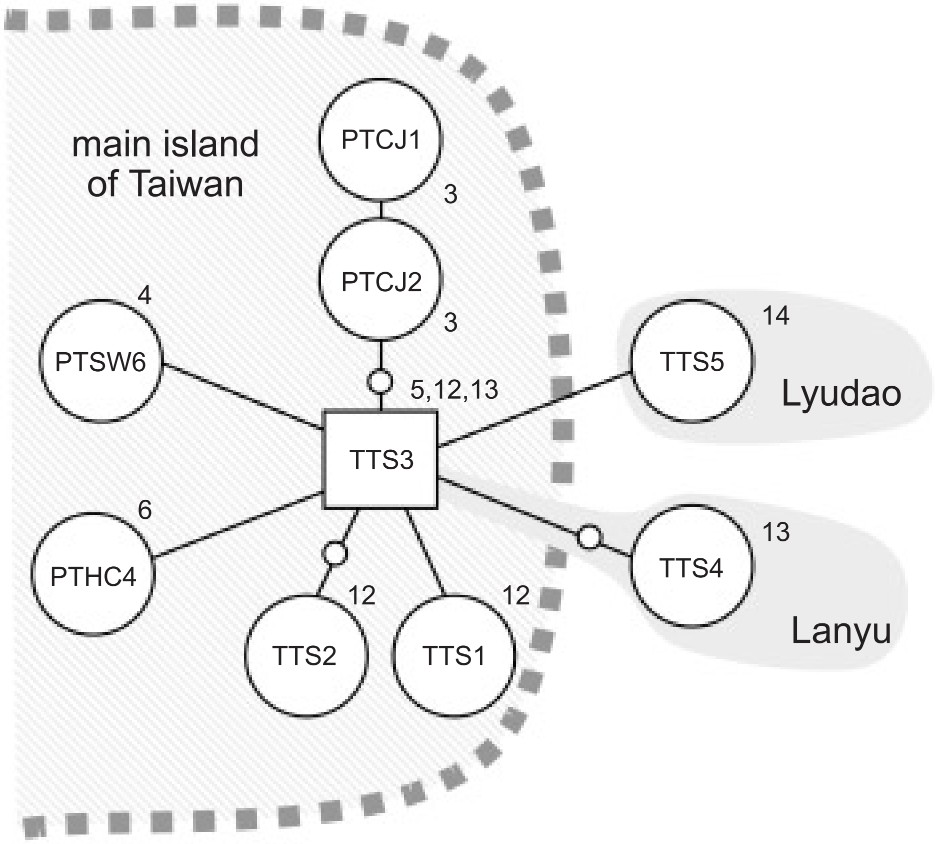

Fig. 3. A genealogical network for the haplotypes observed in the *Geothelphusa tawu* clade, including specimens collected from the northern part of the Hengchun Peninsula of the main island of Taiwan, and from 2 offshore islands (Fig. 1). The ancestral haplotype, or root of the network, is indicated by a square. Unlabelled nodes indicate inferred steps not found in the sampled populations. Numbers beside the nodes correspond to the collection sites in Table 1 and Fig. 1.

Lanyu population. Haplotype TTS3 is even shared by individuals from the Fenggang River (as "*G. ferruginea*"), Tawu River (as *G. tawu*), and Lanyu (as *G. lanyu*) (Table 1). The evidence suggests that these base-pair variations are intraspecific. However, we prefer to regard these taxa as separate species for the moment, at least until additional genetic and morphological studies can be conducted to conclusively establish that all 3 are conspecific. It is useful to point out, though, that the 3 species here were published as new in the same paper (Shy et al. 1994), and if the 3 are formally synonymized later, then one will have to have priority. It is, however, not necessary for the 1st name used in such a paper (in this case *G. lanyu*) to be the one selected. The current zoological code (ICZN 1999: Articles 24A and 50.6) makes it clear that since all 3 names were published in the same paper, the names are to be regarded as simultaneously published, and the senior name should then be selected by the 1st reviser.

With regard to the *G. tawu* clade, there was only 1 haplotype (TTS5) found on Lyudao and 2 haplotypes (TTS3 and TTS4) on Lanyu (Table 1), indicating that invasions from the main island of Taiwan to these islands were rare if they occurred at all. The TTS3 haplotype has a wide distribution from the Fenggang River and Dawu River to Lanyu and is also the predicted ancestral haplotype (Fig. 3). There is only 1 transversion difference between TTS3 and TTS5, and 2 transversions between TTS3 and TTS4 (Table 2). The genetic structures of populations on Lanyu and Lyudao are thus believed to have resulted from the founder effect, with the TTS3 haplotype from the northern Hengchun Peninsula (e.g., the Fenggang and Tawu Rivers) invading the islands.

It is thought that the islands of Lanyu and Ludao have never been connected to the main island of Taiwan even during periods of glacial maxima as there are deep troughs between them and Taiwan proper (Fig. 1; Ota 1991, Toda et al. 1998). These offshore islands are believed to have gradually been pushed toward Taiwan from the southeast by an oblique collision between the Luzon volcanic arc and the Asian continent since the late Neogene (Huang et al. 1995). The latest periods of volcanic activity of Lanyu and Lyudao are estimated to have been some time between 0.54 and 0.8, and 1.4 and 2.3 mya, respectively (Chen 1990, Huang et al. 1995). All endemic terrestrial and freshwater organisms originally occurring on these small volcanic islands, if any, then were likely to have been exterminated during those periods by volcanic eruptions. The present fauna including the crabs must thus have colonized the islands after those periods of volcanic explosions. From the present data, the freshwater crabs on

Table 3. Pairwise 2-parameter distance (Kimura, 1980) matrix (lower-left) from the 550-bp portion of the mitochondrial 16S rRNA gene between *Geothelphusa* spp. from southern Taiwan and adjacent areas. Mean numbers of differences are given in the upper right. Standard errors of distances are shown in parentheses. The abbreviations for species are Ga, *G. albogilva*; Gf, *G. ferruginea*; Gtw, *G. tawu* clade; Gp, *G. pingtung*; and Gb, *G. bicolor*

	Within clades		Between clades				
	nucleotide divergence	nucleotide number difference	Ga	Gf	Gtw	Gp	Gb
Ga	0.0028 (0.0011)	1.5	-	10.0	5.0	13.3	13.6
Gf	0	0	0.0186 (0.0057)	-	9.0	16.0	16.0
Gtw	0.0043 (0.0014)	2.3	0.0093 (0.0031)	0.0167 (0.0051)	-	14.3	14.7
Gp	0.0037 (0.0019)	2.0	0.0248 (0.0061)	0.0300 (0.0072)	0.0268 (0.0066)	-	17.0
Gb	0.0148 (0.0049)	8.0	0.0255 (0.0060)	0.0300 (0.0070)	0.0275 (0.0064)	0.0319 (0.0066)	-

these islands are likely to have originated from the northern part of the Hengchun Peninsula as discussed above. However, potamid freshwater crabs are not capable of dispersing across the sea to islands, due to their intolerance of salt water (see Ng 1988). Their appearance in Lanyu and Lyudao is thus interesting as there has been no known land bridge connection with the main island of Taiwan. We propose 3 possible mechanisms to explain their presence there.

Typhoons are common in southern Taiwan, and occur during summer and autumn in the West Pacific. Taiwan, especially the eastern part, is annually impacted by violent typhoons, at which time rainfall is very heavy and rivers often flood. Freshwater crabs may easily be carried from mountains into estuaries as part of the flotsam and then to offshore islands. If the crabs were marooned on rafts of floating vegetation during this time, this would have minimized their contact with the seawater, and their semiterrestrial habits and the heavy rainfall would have increased their chances of surviving and arriving safely on distant islands. For example, Censky et al. (1998) reported on the dispersal of green iguanas onto islands by rafting during storms. Toda et al. (1998) also believed that the Indian rice frog populations in Lanyu and Lyudao originally came from eastern Taiwan via a similar mode of dispersal.

A 2nd explanation is that the dispersal may have been a result of the brooding behavior of freshwater crabs. Taiwanese freshwater crabs are prey for many kinds of wildlife, especially in winter when food is scarce (Shih 2000). When a brooding crab is attacked by a bird, some juveniles may evacuate the brood chamber and scatter, including onto the body and among the feathers of the predator. If these birds then migrate between Taiwan and the offshore islands, then some of the juveniles may have been carried across as well and inadvertently colonized the islands. However, this is a very chancy event, and cases must have been rare.

The final possibility is that the crabs on these islands were introduced by humans. Both Lanyu and Lyudao have been inhabited by aborigines for several centuries, and they move between these islands and the main island via canoes or small boats. As *G. tawu* occurs in small streams near the estuaries of the Dawu River, it is possible that juveniles were accidentally collected with the drinking water on their boats and brought back to the islands. As freshwater crabs are also known to be eaten at times by the aborigines (Maa-Neu Dong, pers. comm.), the crabs could also have been brought to the islands by the locals as food, and then subsequently released accidentally or even intentionally. Human-introduced lizards among the Pacific islands have been recorded several times (see Austin 1999).

Near the boundary between southwestern and southernmost Taiwan (i.e., Cijia and Chunrih, Pingtung County), 1 large species, *G. pingtung*, and 1 small species, *G. tawu*, occur sympatrically. Based on the molecular data, *G. pingtung* belongs to the SW clade, but the sympatric *G. tawu* belongs to the S clade (Figs. 1, 2). Another large-sized species, *G. albogilva*, has been recorded in Cijia and even in Kaohsiung City (Mt. Chaishan) by Chen et al. (2001, 2003), but it is actually not found north of Cijia (Shih, unpubl. data). In the present study, several large-sized *Geothelphusa* specimens with different color patterns were also collected near Cijia. Those diverse colorations are considered to be important diagnostic characters and have been used as a key factor in distinguishing *G. neipu* from *G. pingtung* (Shy et al. 2000, Chen et al. 2001), and some “undescribed species” (Chen et al. 2003). In this study, individuals from the SW clade with the coloration of yellow and yellow-green (as “*G. albogilva*” in Table 1) should be included under these “undescribed species”. However, these “species” share the same haplotypes, and the 3 haplotypes (PTC1, PCT2, and PTC3; Table 1) differ by less than 3 bp, which we believe is not a significant difference. The haplotypes we have from the SW clade include material obtained from the type localities of *G. pingtung* and *G. neipu* and are thus likely to be reliable. Comparing the specimens of both species we have on hand (including the paratypes), we can discern no significant morphological differences between them, except for the slight difference in the form of the male 1st gonopod which is too subtle to be reliable. The different color patterns of the 2 species cited by Chen et al. (2001) to distinguish them are also not reliable characters, as we have found specimens with intermediate and alternative patterns (Shih, unpubl. data), suggesting they are too variable to be used for specific separation. As such, we consider that there is only 1 species near Cijia, and that it should be *G. pingtung* Tan and Liu, 1998. Although the original descriptions of both names, *G. pingtung* and *G. neipu*, bear 1998 as the year of publication, the accuracy in regard to the latter species was questioned by Ng (1999), who also discussed the problem of its authorship.

Geothelphusa pingtung was published in Oct. 1998, but the 1st valid use of *G*. *neipu* was in an abstract volume of the meeting of the Taiwan Fisheries Society in Dec. 1998. On the basis of this information, *G*. *pingtung* thus has priority and should be used over *G*. *neipu* if both names are regarded as subjective synonyms, as in the present case. Also, the small-sized populations in southwestern Taiwan clearly belong to the *G*. *tawu* clade, instead of being affiliated with *G*. *ferruginea* (see Chen et al. 2001, 2003). In Chen et al. (2003: 106), one undescribed species (called G. sp2) is mentioned which was collected from Lili (near Cijia and Shihwen) in Chunrih, Pingtung County. From its small size and coloration, this form is also likely to belong to the *G*. *tawu* clade.

On the main islands of Taiwan and Japan, large-sized *Geothelphusa* species invariably inhabit lower elevations compared to the montane species which are usually smaller (see Shy et al. 1994, Okano et al. 2000). As mountains play a key isolating role, the small-sized *G*. *tawu* most likely invaded Cijia in southern Taiwan from the southwestern tip of the Central Range. On the other hand, the large-sized lowland species (*G*. *albogilva* and *G*. *pingtung*) have not been able to do so. In a separate study of the molecular biogeography of another potamid freshwater crab, *Candidiopotamon rathbunae* (de Man), these 2 clades, which exactly correspond to the present S and SW clades, were found to have been isolated by the Central Range (Shih et al. submitted). The boundary between the E (eastern) and S clades is an area with several latitudinally oriented and high-elevation mountains extending eastwards to the coast (averaging ~500 m) (Fig. 1), which most likely were serious impediments to any gene flow northwards or southwards.

From the results of this study, it is obvious that the known morphological differences among some of the Taiwanese *Geothelphusa* species are not always reliable and do not necessarily reflect actual species boundaries. Preliminary studies show that there is still a lot to be done to elucidate the true taxonomic diversity of the Taiwanese *Geothelphusa*. Certainly, while it is clear that the freshwater crab diversity on this island is very rich, the validities of some species need to be reassessed while the identities of several supposedly wide-ranging ones will also need to be reexamined.

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