

INTRODUCTION OF AN INDOCHINESE FRESHWATER CRAB *SAYAMIA GERMAINI* (CRUSTACEA: BRACHYURA: GECARCINUCIDAE) TO TAIWAN: MORPHOLOGICAL AND MOLECULAR EVIDENCE

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ABSTRACT. – Freshwater crabs of the genus *Sayamia* (family Gecarcinucidae) are native to Indochina and northern Peninsular Malaysia. Recently, individuals of *Sayamia* were collected from the coastal areas of Kaohsiung, southwestern Taiwan. The species is identified as *S. germaini* (Rathbun, 1902) [from southern Vietnam and Cambodia] by morphological characters as well as 16S rRNA and cytochrome oxidase I DNA sequences. Individuals shared identical haplotypes, which suggest that they are derived from a small founder population. The possible source and pathways of introduction of this alien species and its potential impacts on Taiwanese aquatic biodiversity, human health, and agriculture are discussed.

KEY WORDS. – *Sayamia germaini*, freshwater crab, alien species, Vietnam, Cambodia, Taiwan, 16S rRNA, cytochrome oxidase I.

INTRODUCTION

True (or primary) freshwater crabs are land-locked organisms with direct development of fertilized eggs and maternal care (Ng, 1988; Cumberlidge & Ng, 2009). Owing to their relatively poor dispersal abilities, they are easily isolated by geographical barriers such as mountains, seas and even rivers, with their biogeography often reflecting past geological events (see Shih et al., 2009). Not surprisingly, many species of freshwater crabs have very restricted

distributions. The richness of freshwater crabs on Taiwan island (and islets) is the highest among the East Asian islands (Taiwan, the Ryukyus and mainland Japan), with 42 species belonging to four genera: *Geothelphusa*, *Candidiopotamon*, *Nanhaipotamon* (family Potamidae) and *Somanniathelphusa* (family Gecarcinucidae) (Shy et al., 1994; Ng et al., 2008; Shih et al., 2008, 2010, in press; Shih & Shy, 2009; Shih & Ng, in press). While *Geothelphusa* and *Candidiopotamon* are endemic to the East Asian islands, most species of *Nanhaipotamon* and *Somanniathelphusa* are more widely

distributed on the Asian mainland (Ng & Naiyanetr, 1993; Dai, 1999; Shy & Yu, 1999; Ng et al., 2001; Shih & Ng, in press; Shih et al., in press).

Recently, specimens of *Sayamia* Naiyanetr, 1994 (family Gecarcinucidae) were collected from two canal systems in Kaohsiung, southwestern Taiwan. *Sayamia* species are native to southern Indochina (Thailand, Cambodia and southern Vietnam) and northern Peninsular Malaysia (Rathbun, 1902, 1905; Ng, 1988, 1997; Ng & Naiyanetr, 1993; Naiyanetr, 1994; Yeo & Ng, 1999; Ng et al., 2008), and have never been reported from Taiwan, despite the island being extensively surveyed and the fauna very well documented (Shy et al., 1994; Shy & Yu, 1999; Ng et al., 2001). Thus, the presence of this genus in Taiwan is certainly the result of anthropogenic introduction. In this paper, we report the presence of the Indochinese species, *Sayamia germaini* Rathbun, 1902, in Taiwan. The species is identified using morphological and molecular (16S rRNA and cytochrome oxidase I, COI) characters. Possible pathways of introduction into Taiwan and potential impacts of the species on native fauna, human health and agriculture are discussed.

MATERIALS AND METHODS

Fresh specimens of *Sayamia* from the introduced areas of Kaohsiung in southwestern Taiwan, and markets of southern Vietnam and Cambodia, within the native range of the genus, were preserved in 70% to 95% ethanol after collection, illustrated with the help of a drawing tube attached to a stereomicroscope, and deposited in the Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Taiwan (NTOU) and the Zoological Collections of the Department of Life Science, National Chung Hsing University, Taiwan (NCHUZOOL). The following abbreviations are used: G1 for the male first pleopod and CW for the carapace width. Comparative specimens are deposited in the Muséum national d'Histoire naturelle, Paris (MNHN), and Zoological Reference Collection of the Raffles Museum of Biodiversity Research, National University of Singapore (ZRC) (Table 1).

Sequences of 16S and COI from the above specimens ($n = 24$), with *Somanniathelphusa taiwanensis* Bott, and *Siamthelphusa improvisa* (Lanchester) used as outgroups (Table 1), were obtained following the method described by Shih et al. (2007) and analysed. The best-fitting model for sequence evolution of the combined 16S and COI dataset was determined by MrModeltest (vers. 2.2, Nylander, 2005), and selected by Akaike Information Criterion (AIC), with subsequent Bayesian Inference (BI) 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 and the first 500 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). A consensus 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 random addition sequence replicates. All characters were equally weighted. Gaps in the MP tree construction were treated as missing data. The relationships of the combined 16S and COI haplotypes in *S. germaini* and its closest congener, *S. sexpunctata*, were examined by using the program TCS (vers. 1.20, Clement et al., 2000) with gaps treated as a missing data.

RESULTS

Taxonomy

Five adult male specimens (NTOU F10301, 10302, 10303, 10306, 10307; CW 46.12–54.58 mm) and two adult females (NTOU F10304, CW 48.14 mm, ovigerous; NTOU F10305, 48.69 mm) were collected from a canal near the coast in Siaogang, Kaohsiung City, southwestern Taiwan in October and November of 2005; and two females (NCHUZOOL 13386, 13387; CW 50.72, 42.41 mm) from a depression

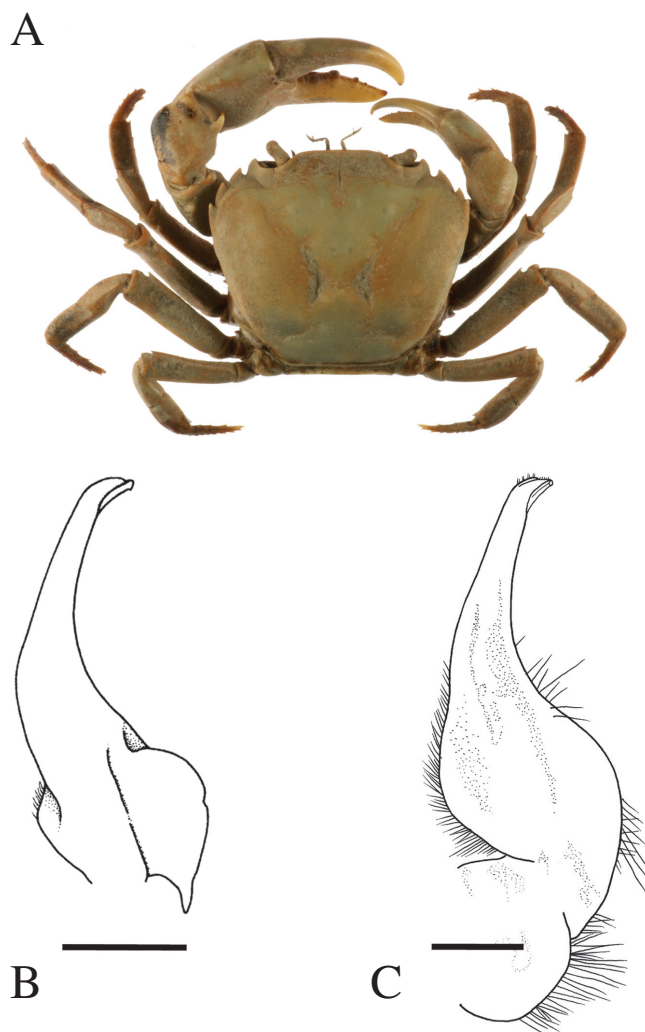


Fig. 1. *Sayamia germaini* (Rathbun, 1902). A, dorsal view of a male (NTOU F10307, CW 46.1 mm) from the coastal region of Kaohsiung, southwestern Taiwan; B, C, G1s of holotype (MNHN-B5162, CW 44.2 mm) and NTOU F10301 (CW 50.4 mm)

beside a canal in Renwu, Kaohsiung County (Table 1). The external morphology (Fig. 1A) and the structure of the G1 (Fig. 1B) of these specimens agree very well with *Sayamia germaini* (Rathbun, 1902) collected from southern Vietnam (Table 1), including the holotype of the species (MNHN-B5162, Fig. 1C), and we have little doubt they are conspecific. *Sayamia germaini* is naturally distributed in southern Vietnam (Rathbun, 1902, 1905) and Cambodia (this study) (Table 1).

DNA analysis

A ~550 basepair (bp) segment (excluding the primer regions) of the 16S from all 24 ingroup specimens was amplified and aligned; 29 positions were variable and 18 parsimoniously informative. Among the total number of sequences, 10 different haplotypes were distinguished (Table 1). The studied segment of 16S sequences was AT rich (69.9%) (T, 33.9%; A, 36.0%; G, 20.1%; and C, 10.0%). For the COI gene, a 616 bp segment was compared, resulting in 9 different haplotypes of the ingroups (Table 1). The studied segment of the COI sequence was also AT rich (63.5%) (T, 36.4%; A, 27.1%; G, 14.9%; and C, 21.5%). In this gene, 72 positions were variable and 61 parsimoniously informative.

The best model selected by MrModeltest was the GTR+I+G model (Rodríguez et al., 1990) for the combined 16S and COI segment of 1170 bp. For MP, a single tree was recovered with a tree length of 301 steps, a consistency index of 0.89, and a retention index of 0.87.

The phylogenetic tree constructed by BI and MP methods (Fig. 2) agrees with the morphological results, with the haplotypes from Vietnam, Cambodia and Taiwan forming a highly-supported clade of *Sayamia germaini*, with the closely related *S. sexpunctata* from southern Thailand and northern Peninsular Malaysia forming a sister clade. Another clade is composed of *S. bangkokensis* and *S. cf. maehongsonensis* from Thailand. The bp differences and nucleotide divergence with the Kimura (1980) 2-parameter distance (K2P distance) between those haplotypes of COI is shown in Table 2. For *S. germaini*, the bp difference is ≤ 7 (1.14%) and the nucleotide divergence is $\leq 1.15\%$; for *S. sexpunctata*, the bp difference is ≤ 4 (0.65%) and the nucleotide divergence is $\leq 0.65\%$. The minimum interspecific bp differences and divergence is 18 (2.92%) and 3.00% (between *S. germaini* and *S. sexpunctata*), respectively. Thus, the minimum COI divergence is 2.6 times higher between species than within. Only a single haplotype of 16S and COI was sequenced from the seven *S. germaini* specimens from Taiwan, compared

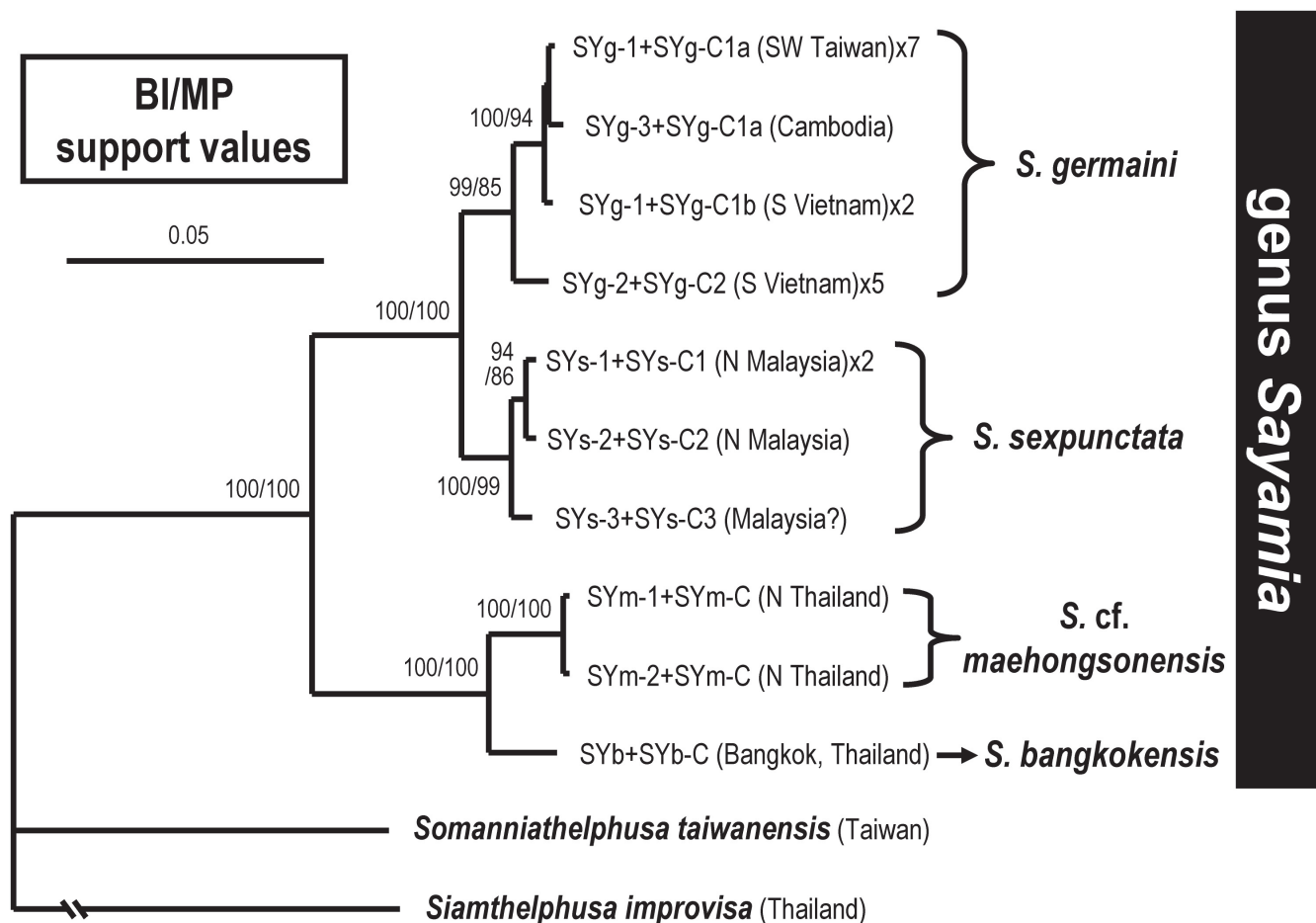


Fig. 2. A Bayesian inference (BI) tree of the *Sayamia* species from Taiwan, Vietnam, Cambodia, Malaysia and Thailand based on the combined 16S rRNA and cytochrome oxidase I genes. Probability values at the nodes represent support values for BI and maximum parsimony (MP). For haplotype abbreviations and detailed localities see Table 1.

Table 1. Haplotypes of 16S rRNA and cytochrome c oxidase I (COI) genes of *Sayamia* species, with the outgroups, used in this study. (CBM = Natural History Museum and Institute, Chiba, Japan; NCHUZOO = Zoological Collections of the Department of Life Science, National Chung Hsing University, Taiwan; NTOU = Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Taiwan; ZRC = Zoological Reference Collection of the Raffles Museum of Biodiversity Research, National University of Singapore, Singapore).

Species	Countries	Localities	Voucher number	N	Haplotypes of 16S	DDBJ Acc. no.	Haplotypes of COI	DDBJ Acc. no.
<i>S. germaini</i>	TAIWAN	Yanhai 2nd Road, Siaogang, Kaohsiung City (22°33'25.96"N, 120°22'7.24"E)	NTOU F10301, 10302, 10304, 10306, 10307	5	SYg-1	AB601842	SYg-C1a	AB601853
		Renwu, Kaohsiung County (22°41'29.58"N, 120°21'29.19"E)	NZHUZOO 13386, 13387	2	SYg-1	AB601842	SYg-C1a	AB601853
	VIETNAM	market in Di Linh, Lam Dong	NZHUZOO 13319	2	SYg-1	AB601842	SY-C1b	AB601854
		market in Di Linh, Lam Dong	NZHUZOO 13319	4	SYg-2	AB601843	SY-C2	AB601855
<i>S. sexpunctata</i>	CAMBODIA	market in Duong Minh Chau, Tay Ninh	NZHUZOO 13320	1	SYg-2	AB601843	SY-C2	AB601855
		Trapeang Thom Khang Cheung, Tramkak, Takeo	CBM	1	SYg-3	AB601844	SYg-C1a	AB601853
	MALAYSIA	Paddy fields along Ulu Melaka, Langkawi Island	ZRC 1989.2026-38	1	SYs-1	AB601845	SYs-C1	AB601856
		Chuping, Perlis	ZRC 1984.7352-9, 7689-94	1	SYs-1	AB601845	SYs-C1	AB601856
<i>S. bangkokensis</i>	THAILAND	Northern Peninsular Malaysia	ZRC 2001.1101	1	SYs-2	AB601846	SYs-C2	AB601857
		Malaysia (aquarium purchase in Singapore)	ZRC	1	SYs-3	AB601847	SYs-C3	AB601858
	THAILAND	Khanom (in northern Peninsular Malaysia) (neotype)	ZRC1985.1956	1	SYs-4	AB601848	—	—
		Bangkok	ZRC 1989.2159-2165	1	SYb	AB601849	SYb-C	AB601859
<i>S. cf. maehongsonensis</i>	THAILAND	Bangkok	ZRC 1989.2151-2155	1	SYb	AB601849	—	—
		Phitsanulok (in lower northern Thailand)	ZRC 1997.108	1	SYm-1	AB601850	SYm-C	AB601860
	THAILAND	Mae Taeng (Taeng River) (in northern Thailand)	CBM	1	SYm-2	AB601851	SYm-C	AB601860
OUTGROUPS								
<i>Somaniathelphusa taiwanensis</i>	TAIWAN	Shihguei, Dounan, Yunlin	NCHUZOO 13001b	1		AB265228		AB265240
<i>Siamithelphusa improvisa</i>	THAILAND	Surat Thani	ZRC 1999.0024	1		AB601852		AB601861

Table 2. Matrix of percentage pairwise nucleotide divergences of K2P distance (lower left) and mean number of differences (including gaps) (upper right) based on 616 bp of cytochrome c oxidase I (COI) between *Sayamia* species from Taiwan, Vietnam, Cambodia, Malaysia and Thailand. For haplotype abbreviations and detailed localities see Table 1.

	<i>S. germaini</i>			<i>S. seipunctata</i>			<i>S. bangkokensis</i>	<i>S. cf. maehongsonensis</i>
	SYg-C1a	SYg-C1b	SYg-C2	SYs-C1	SYs-C2	SYs-C3	SYb-C	SYm-C
SYg-C1a		1	6	22	21	20	51	49
SYg-C1b	0.16		7	23	22	21	52	50
SYg-C2	0.98	1.15		20	19	18	49	51
SYs-C1	3.69	3.86	3.35		1	4	54	53
SYs-C2	3.51	3.69	3.17	0.16		3	53	52
SYs-C3	3.34	3.51	3.00	0.65	0.49		53	52
SYb-C	8.94	9.13	8.57	9.50	9.31	9.31		20
SYm-C	8.56	8.75	8.95	9.31	9.12	9.12	3.36	

with the higher diversity of haplotypes from Vietnam and Cambodia (Fig. 2, Tables 1, 2).

The haplotype network constructed to further depict the relationships among the combined 16S and COI haplotypes of clades of *S. germaini* and *S. seipunctata* are shown in Fig. 3. The combined haplotype SYg-1+SYg-C1a from Taiwan is more central relative to other haplotypes and is therefore assumed to represent the ancestral haplotype (cf. Clement et al., 2000) (but see Discussion).

DISCUSSION

Possible pathways and sources of introduction

Sayamia germaini was first observed in Kaohsiung City, Taiwan, in 2005, although the species may have arrived earlier. One ovigerous female was obtained (NTOU F10304) in 2005, and juveniles were also observed at the water's

edge that year (C.-H. Ho, pers. comm.), which indicate that the species was already established in Taiwan by 2005. Specimens were subsequently also observed in 2008 (J.-F. Huang, pers. comm.). Most recently, we also collected specimens from a second site about 15 km north of the first observed site at the end of 2010 (Table 1).

The source(s) of the introduced Taiwanese populations of *S. germaini* could not be confirmed. Interestingly though, Kaohsiung City also has a large population of Vietnamese migrant workers, the country from which *S. germaini* is naturally found. We do not believe this is a coincidence. Freshwater crabs, especially, gecarcinucid ricefield crabs like *Sayamia*, are commonly eaten in Vietnam (Ng, 1988; Ng & Kosuge, 1995; Yeo & Nguyen, 1999; Yeo et al., 2008) and it is possible *Sayamia* may have been introduced to Taiwan for this purpose. Grocery shops in the area of Kaohsiung City also specialise in importing fresh Vietnamese cuisine to cater for the large Vietnamese labour force in Kaohsiung. While it is illegal to do so, it is not inconceivable that businesses may have smuggled in live *Sayamia* for sale. In markets in Vietnam and Cambodia, *Sayamia* is regularly sold live for food (e.g., Table 1). The anthropogenic spread of live gecarcinucid crabs for food has precedence. Ng & Naiyanetr (1993) reported that *S. bangkokensis*, a species native to central Thailand, was introduced to Chiangmai in the northwestern part of the country by migrant workers as a protein source. *Sayamia* as well as allied genera like *Somanniathelphusa* and *Esanthelephusa* are always sold live to keep them fresh (Ng, 1988; Ng & Naiyanetr, 1993). Yet another possibility may be that the crab was brought in by Vietnamese restaurants in Kaohsiung City to serve to the migrant community there. This has happened elsewhere, for example, in the United States, where individuals of the Japanese potamid, *Geothelphusa dehaani*, were found in a lake at Las Vegas, Nevada, which almost certainly escaped from an adjacent hotel sushi bar (Carlton, 2001). In recent years, freshwater crabs have also become popular aquarium subjects, and we have observed specimens of *Sayamia seipunctata* and *S. bangkokensis* on sale in Singapore (e.g., Table 1), and some have even been exported to Europe and Japan (unpubl. data). As aquarium fish and plants are regularly exported from Singapore to Taiwan, the specimens

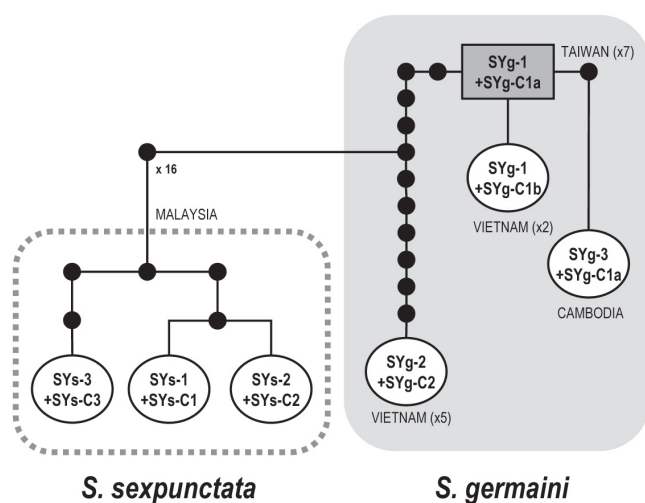


Fig. 3. Genealogical network for the combined 16S rRNA and COI haplotypes observed within the clades of *Sayamia germaini* (collected from Taiwan, Vietnam and Cambodia) and *S. seipunctata* (Malaysia). The ancestral haplotype, or root of the network, is indicated by a square. Unlabelled nodes indicate inferred haplotypes not found in the sampled populations

in Kaohsiung could also have arrived there via this pathway. Regardless of how *S. germaini* entered southern Taiwan, the original introduced populations were fortunate to have man-modified, earth-lined canals in Kaohsiung that were ecologically similar to their natural habitats in Indochina (Ng, 1988; Ng & Naiyanetr, 1993). The climate in Kaohsiung is also tropical and even in winter, is relatively warm, allowing this tropical species to survive.

Based on the molecular data, individuals of the populations of *S. germaini* in Kaohsiung City and Kaohsiung County share identical haplotypes of 16S rRNA and COI (Fig. 1, Tables 1, 2), suggesting a founder effect (Mayr, 1963). This indicates that the current two Kaohsiung populations probably originated from a single introduction event, where only a few individuals (perhaps even a single ovigerous female) were originally released (or escaped) into the wild. However, it is not clear which of the two localities, which are 15 km apart and likely to be connected by waterways, might be the original site of introduction. Although the Kaohsiung haplotypes appear to be more ancient compared to other haplotypes obtained from southern Vietnam and Cambodia (Fig. 3), there is only 1 bp difference (without gap) between the Kaohsiung haplotype and the nearest haplotype SYg-1+SYg-C1b from Lam Dong, southern Vietnam (Fig. 3), a very minor difference that suggests these haplotypes originate from one population. Firmer conclusions can therefore only be made after more extensive surveys of the range of species.

Potential impacts and management

It is important to note that known *Sayamia* species have a tropical distribution ranging from southern Indochina to northern Peninsular Malaysia (Rathbun, 1902, 1905; Ng, 1988, 1997; Ng & Naiyanetr, 1993; Yeo & Ng, 1999; Ng et al., 2008). That the alien population of *S. germaini* in Taiwan has been established for at least five years in the tropical climate of southern Taiwan is interesting in itself. How long the species will persist in southern Taiwan and whether it is able to spread north and survive in the cooler latitudes of central and northern Taiwan is not clear. In any case, if *S. germaini* was to spread to other parts of Taiwan, potential ecological, human health and agricultural problems can be anticipated. The present ranges of Taiwanese *S. germaini* are restricted to two canals located in the highly urbanized industrial park of Kaohsiung City and the suburban Kaohsiung County, respectively. No other native freshwater crab species has been recorded in these areas. The nearest location where a native freshwater species is known in Chaishan, a coastal hill in Kaohsiung City, where the potamid *Geothelphusa makatao* Shih & Shy, 2009, occurs. However, as the biology and habitat of potamids and gecarcinucids are very different (Ng, 1988), there is unlikely to be significant competition between them. For the endemic Taiwanese gecarcinucid, *Somanniathelphusa taiwanensis*, however, the presence of *S. germaini* presents a different problem. *Somanniathelphusa taiwanensis* is distributed in lowland habitats from central-western to southwestern Taiwan and is the only gecarcinucid

species native to the island (Shih et al., 2007). Over the past two decades, *So. taiwanensis* has become increasingly rare due to habitat destruction and the extensive use of insecticides and herbicides (Shih et al., 2007), and is currently regarded as an endangered species internationally (Cumberlidge et al., 2009). A serious concern would arise if the range of the alien *S. germaini* population expands northwards to overlap with that of *So. taiwanensis*, which would result in competition between the two species. As adult *S. germaini* grow to twice the size of *So. taiwanensis* and probably share similar habitat and food preferences, the survival of the latter species would be even more threatened. Not only could *S. germaini* directly interfere with and outcompete *So. taiwanensis* for common limited resources such food and habitat, but it could also have the added advantage of relative size refuge from predation.

The presence of *S. germaini* also raises potential human health concerns with regards to lung fluke disease or paragonimiasis. Fortunately, the lung fluke (*Paragonimus* spp.) infection rates of Taiwanese and Chinese gecarcinucid species is relatively low compared to that of potamids (see Chiu, 1964; Chung et al., 1975; Li & Lin, 1994); with *So. taiwanensis* from Taiwan reported to be infected with *Paragonimus westermanii* only in 1915 (locality questioned, Nakagawa, 1917) and *So. sinensis* sensu lato from Fujian, China, infected with *P. fukienensis* in 1936 (Li et al., 1999). The high level of public hygiene in Taiwan also helps to keep the infection rates low (Li & Huang, 2002). However, Indochinese *Sayamia* species have been reported as secondary intermediate hosts of lung flukes, e.g., *Paragonimus siamensis* in “*S. germaini*” from Thailand (Kawashima et al., 1989; Sugiyama et al., 2006; Binchai et al., 2007). Hence, the proliferation of *Sayamia* and their possible associated alien parasites may present problems in controlling this disease in Taiwan.

Sayamia germaini may also present economic problems. Crabs of the genus *Somanniathelphusa* and *Sayamia* inhabit lentic water bodies and are especially common in ricefields in China, Taiwan and Indochina. They dig deep burrows into the bunds of the ricefields, and these have been known to compromise the structural integrity of the bunds (Ng, 1988; Dai, 1999; Shy & Yu, 1999). In Taiwan, ricefield bunds are usually relatively narrow (ca. 30 cm in width) and will probably not be able to cope with extensive excavations by the much larger *Sayamia*. Any damage to ricefield bunds by *Sayamia* burrowing activities may in turn translate into economic damages in the form of reduction or losses in rice production and increased repair and maintenance of these structures.

Although *S. germaini* in Taiwan has yet to cause any obvious or detectable impacts, if the alien population was to expand, it may affect not only the survival of the endemic and endangered *So. taiwanensis*, but is also likely to raise public health issues, and cause serious problems for rice cultivators through their burrowing activities. If this alien species persists, an attempt at eradication to remove or control the species (e.g., see Myers et al., 2000; Lodge et al., 2006; Roche et al., 2009) should be seriously considered. Although

complete eradication is often difficult or impossible (Lodge et al., 2006), there have been successful instances, mostly attributed to early detection, or to limited and localized distribution of the invading species (Myers et al., 2000; Roche et al., 2009), of which, the latter scenario appears to be the case with the *S. germaini* invasion in Taiwan. Recent surveys suggest that the species seems to be slightly more widely distributed now than when first detected. There is nevertheless an opportunity to eradicate it by removing reproductively mature individuals or at least lowering the population to a non-viable level (see Myers et al., 2000). Such a program should therefore be done as soon as is practical, and could include, among other methods, biological control by fish predation through stocking of predatory fishes native to the area (targeted at reducing the population size) and trapping (targeted at reducing the population growth rate) (Hein et al., 2006). These could be supplemented by physical removal by hand and net collection. These methods must be accompanied by monitoring of the invaded habitat and surrounding unaffected areas to evaluate the effectiveness of the program. To further complement eradication efforts, the invaded stretches of the canals should be isolated to slow any spread of the species (Myers et al., 2000; Lodge et al., 2006), while education, prevention and enforcement measures directed at likely or potential sources of introduction (see earlier) probably need to be carried out at the same time.

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