

SYSTEMATICS OF THE INDO-WEST PACIFIC BROAD-FRONTED FIDDLER CRABS (CRUSTACEA: OCYPODIDAE: GENUS *UCA*)

Hsi-Te Shih

Department of Life Science, National Chung Hsing University, Taichung 40227, Taiwan
Email: htshih@dragon.nchu.edu.tw

Peter K. L. Ng

Department of Biological Sciences, National University of Singapore, Kent Ridge, Singapore 119260, Republic of Singapore
Email: peterng@nus.edu.sg

Min-Yun Liu

Taiwan Ocean Research Institute, National Applied Research Laboratories, Qiding, Kaohsiung City 85243, Taiwan
Email: mylalex@tori.narl.org.tw (Corresponding author)

Abstract. — Fiddler crabs (genus *Uca*) with broad-fronts (BF) belong to a group of small-sized species with complex behaviors and have been suggested to be more “advanced” compared to the narrow-fronted species groups. Three Indo-West Pacific subgenera, *Austruca* Bott, 1973, *Cranuca* Beinlich & von Hagen, 2006, and *Paraleptuca* Bott, 1973, are reappraised using two mitochondrial (16S rRNA and cytochrome oxidase I) and one nuclear (28S rRNA) markers. The phylogenetic analyses show that the three clades agree relatively well with the three subgenera as currently defined. Our study confirms that the Indo-West Pacific BF species that had been placed with the American *Celuca sensu* Crane, 1975, are genetically unsupported, and should be classified in *Austruca*, together with *U. sindensis* (Alcock, 1900) (currently in *Paraleptuca*). *Austruca* now contains 11 species. *Cranuca*, a subgenus established with only *U. inversa* (Hoffmann, 1874), is supported by its monophyly and its significant distance from other subgenera. In addition, *Paraleptuca* (= *Amphiuca* Crane, 1975) is restricted for *U. chlorophthalmus* (H. Milne Edwards, 1837), *U. crassipes* (White, 1847) and *U. splendida* (Stimpson, 1858). The two American BF subgenera, *Minuca* Bott, 1954 and *Leptuca* Bott, 1973, form a mixed clade and further studies will be needed to clarify their validities.

KEY WORDS. — *Uca*, *Austruca*, *Cranuca*, *Paraleptuca*, fiddler crab, 16S rRNA, cytochrome oxidase I, 28S rRNA, systematics

INTRODUCTION

Fiddler crabs (genus *Uca* Leach, 1814) are a common group of crabs on most tropical and subtropical coastal areas and are one of best-studied brachyuran groups (Crane, 1975; von Hagen, 1976; Rosenberg, 2001). Crane (1975) revised the genus and divided it into nine subgenera. However, most of her subgeneric names have to be replaced by taxa briefly diagnosed by Bott (1973) but have nomenclatural priority (von Hagen, 1976; Rosenberg, 2001; Beinlich & von Hagen, 2006).

The members of the nine subgenera can be categorised into two groups - narrow-fronted (NF) and broad-fronted (BF). According to Crane (1975), the BF species (including *Uca tangeri* (Eydoux, 1835)) and American NF species, have advanced social behavior with complex waving displays. Indo-West Pacific (=IWP) NF species, however, only have

simple displays and were considered as primitive (i.e., ancestral) forms.

Five BF subgenera were recognised by Crane (1975), viz. *Afruca* Crane, 1975 (type species *Gelasimus tangeri* Eydoux, 1835, eastern Atlantic), *Amphiuca* (type species *Gelasimus chlorophthalmus* H. Milne Edwards, 1837, IWP), *Boboruca* Crane, 1975 (type species *Uca thayeri* Rathbun, 1900, America), *Celuca* Crane, 1975 (type species *Uca deichmanni* Rathbun, 1935, IWP and America) and *Minuca* Bott, 1954 (type species *Gelasimus mordax* Smith, 1870, America). *Afruca* is only for *U. tangeri*, although the subgenus was treated as a synonym of the subgenus *Uca* Leach, 1814 (see Rosenberg, 2001; Beinlich & von Hagen, 2006; Ng et al., 2008). However, Spivak & Cuesta (2009) made a good case to keep *U. tangeri* in its own subgenus *Afruca*, with which we agree. Crane (1975) proposed *Amphiuca* to include *U. chlorophthalmus* (H. Milne Edwards, 1837), *U. crassipes*

(White, 1847), *U. inversa* (Hoffmann, 1874) and *U. sindensis* (Alcock, 1900), but her name has to be synonymised with *Paraleptuca* Bott, 1973, which has priority and the same type species (von Hagen, 1976; Rosenberg, 2001). Later, *U. inversa* was removed to a new subgenus *Cranuca* Beinlich & von Hagen, 2006. As for *Celuca*, although many authors agreed that it can in fact be separated into two taxa: *Leptuca* Bott, 1973 (type species *Gelasimus stenodactylus* H. Milne Edwards & Lucas, 1843, America) and *Austruca* Bott, 1973 (type species *Gelasimus annulipes* H. Milne Edwards, 1837, IWP) (see Rosenberg, 2001), Beinlich & von Hagen (2006) preferred to refer all the IWP “*Celuca*” species to *Paraleptuca* instead. Naderloo et al. (2010) disagreed and resurrected *Austruca* as a valid subgenus for members of the *Uca lactea* species-complex based on morphology and a genetic study by Shih et al. (2009) that showed that the group was monophyletic. *Boboruca* (= *Planuca* Bott, 1973, type species *Uca thayeri* Rathbun, 1900) contains only *U. thayeri* Rathbun, 1900, and *U. umbratila* Crane, 1941, but it is now regarded as a junior synonym of *Minuca* (see Rosenberg, 2001; Beinlich & von Hagen, 2006; Ng et al., 2008). *Minuca* is superficially close to the American *Celuca* (= *Leptuca* Bott, 1973), but Bott (1973) and Crane (1975) separated the two taxa on the basis of a suite of adult characters.

As the taxonomic treatments for the IWP BF fiddler crabs have been based on different characters (Crane, 1975; Beinlich & von Hagen, 2006; Naderloo et al., 2010; Shih et al., 2012), it is clearly necessary to clarify the phylogenetic relationships of the species involved using molecular tools. In this study, we revise the BF subgenera from IWP by using the mitochondrial 16S rRNA and cytochrome oxidase subunit I (COI), and the nuclear 28S rRNA.

MATERIAL AND METHODS

Specimens of all known species of the IWP BF *Uca* (except *U. cryptica* Naderloo, Turkey & Chen, 2010, but including an undescribed taxon [*U. aff. annulipes*] from Madagascar) from various localities were collected and preserved in 75–95% ethanol, or obtained from museums (Table 1). Other BF subgenera of *Minuca* and *Leptuca* from America, *Afruca* from eastern Atlantic, and IWP NF subgenera of *Tubuca*, *Australuca* and *Gelasimus*, were included as comparative taxa (Table 1). Based on the results of Levinton et al. (1996) and Sturmbauer et al. (1996), we select *Afruca* as the outgroup. While the mitochondrial 16S and COI markers are commonly used for brachyuran phylogenetic studies (e.g., Schubart, 2000; Yeo et al., 2007; Shih et al., 2011a–c), the nuclear 28S gene is also useful for phylogenetic studies of species as well as genera (e.g., Ragionieri et al., 2009; Shih et al., 2011c). In this study, the three markers were used for reconstructing the phylogeny of these fiddler crabs.

Genomic DNA was isolated from the muscle tissue of legs by using the GeneMark tissue and cell genomic DNA purification kit (Taichung, Taiwan). A region of ~550 basepairs (= bp) of the 5'-end of the 16S gene was selected for amplification with polymerase chain reaction (PCR) using the primers 1471, 1472

(Crandall & Fitzpatrick, 1996), 16Sar and 16Sbr (Palumbi et al., 1991). A portion of the COI gene was amplified with PCR using the primers LCO1490 and HCO2198 (Folmer et al., 1994). An internal primer from Roman & Palumbi (2004) was also used. 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. The primers for 28S were 28L4 and 28H4 (Ragionieri et al., 2009), and the new designed 28L4F (5'-TCGTGATGTAGGTCGCCGCGACCCG-3') and 28H4F (5'-GGACAGAGCAGGATCGGAAGGC-3'), with the annealing temperature 47–50°C in PCR condition. Sequences were obtained by automated sequencing (Applied Biosystems 3730) and were aligned with the aid of ClustalW (vers. 1.4, Thompson et al., 1994), after verification with the complimentary strand. The missing data of the COI haplotype of *U. umbratila* with shorter sequence were designated as a “?” in the alignment. Sequences of the different haplotypes have been deposited in the DNA Data Bank of Japan (DDBJ) (accession numbers in Table 1).

Several 28S sequences were found to be ambiguous so their PCR products were cloned. The products were purified by using the QIAquick Gel Extraction kit (Qiagen) first and were cloned using the pGEM-T Easy Vector System (Promega). Three colonies from each sample were selected, and used for insert verification. Verified colonies were used for additional PCR amplification using the original 28S primers. All products were visualised under ultraviolet light stained with ethidium bromide, with a comigrating 100-bp ladder molecular-weight marker to confirm the correct amplification. Amplification products were cycle-sequenced and the sequences were obtained by automated sequencing (see above). Hillis & Dixon (1991) and Colgan et al. (2000) have reported multiple copies in the ribosomal DNA, including 28S rRNA. In our cloning, the three sequences selected from the samples of *U. tangeri* and *U. splendida* (Stimpson, 1858) (#1) (Table 1) only differ in 0.3% and 1.6%, respectively. Therefore we randomly selected one sequence from each sample for the analyses.

For a combined analysis of mitochondrial (16S and COI) and nuclear (28S) markers, phylogenetic congruence among the three dataset partitions was tested under the maximum parsimony criterion using the incongruence length-difference (ILD) test (Farris et al., 1994) implemented in the PAUP* program (vers. 4.0b10, Swofford, 2003) as the partition homogeneity test. The parameters included 1000 reiterations of a heuristic search with 100 randomly added sequence replications, TBR branch-swapping, using Steepest Descent and the MULTREES option enabled. The topologies of the three data sets were congruent ($P = 0.17$) and as such, the sequences were combined.

For the combined 16S, COI and 28S dataset, the best-fitting models for sequence evolution of individual datasets were determined by jModelTest (vers. 0.1.1, Posada, 2008; Guindon & Gascuel, 2003), selected by the Bayesian information criterion (BIC). The obtained best models were TrN+I+G, TPM1uf+I+G and TPM2uf+I+G, respectively,

Table 1. Haplotypes of 16S rRNA, COI and 28S rRNA genes for the Indo-West Pacific (IWP) broad-fronted (BF) fiddler crabs, including the subgenera *Austruca*, *Paraleptuca* and *Cranuca*, as well as the comparative taxa, including the American BF *Minuca* and *Leptuca*, the eastern Atlantic *Afruca*, and IWP narrow-fronted *Tubuca*, *Australuca* and *Gelasimus*.

Subgenus	Species	Locality	Catalogue no. of NCHUZOO (unless indicated)	DDBJ Access. no. of 16S	DDBJ Access. no. of COI	DDBJ Access. no. of 28S
<i>Austruca</i>	<i>U. annulipes</i> (H. Milne Edwards, 1837)	Thailand: Phuket	13258	AB471894	AB491161	AB813686
	<i>U. aff. annulipes</i>	Madagascar	ZRC	AB813648	AB813669	AB813687
	<i>U. iranica</i> Pretzmann, 1971	Iran: Gavbandi	13245	AB471896	AB471908	AB813688
	<i>U. albinana</i> (Kossmann, 1877)	Egypt: Sinai	13242	AB471893	AB471906	AB813689
	<i>U. mijobergi</i> Rathbun, 1924	Australia: Bedford I.	QM-W20253	AB471900	AB471914	AB813690
	<i>U. perplexa</i> (H. Milne Edwards, 1852) (#1)	Taiwan: Dulanwan, Taitung	NTOU	AB471901	AB471915	AB813691
	<i>U. perplexa</i> (#2)	New Caledonia	13573	AB813649	AB813670	AB813692
	<i>U. lactea</i> (De Haan, 1835)	Hong Kong	13250	AB471898	AB471912	AB813693
	<i>U. triangularis</i> (A. Milne-Edwards, 1873)	Philippines: Cebu	13574	AB813650	AB813671	AB813694
	<i>U. bengali</i> Crane, 1975	Malaysia: Selangor	13575	AB813651	AB813672	AB813695
	<i>U. sindensis</i> (Alcock, 1900)	Iran: Qeshm	13576	AB813652	AB813673	AB813696
	<i>U. splendida</i> (Stimpson, 1858) (#1)	Taiwan: Cingluo, Penghu	13457	AB813653	AB734641	AB813697
	<i>U. splendida</i> (#2)	Vietnam: Nha Trang	13448	AB813654	AB734654	AB813698
<i>U. splendida</i> (#3)	Hong Kong: Tai Tam	13368	AB813655	AB734648	AB813699	
<i>U. crassipes</i> (White, 1847) (#1)	Ryukyus: Hiyane, Okinawa	13467	AB813656	AB734656	AB813700	
<i>U. crassipes</i> (#2)	Moorea, French Polynesia: Haapiti	13478	AB813656	AB734656	AB813701	
<i>U. chlorophthalmus</i> (H. Milne Edwards, 1837) (#1)	East Africa: Mayotte	MNHN	AB813657	JX050999	AB813702	
<i>U. chlorophthalmus</i> (#2)	East Africa: Mayotte	MNHN	AB813657	(MDECA791-12)	AB813702	
<i>Cranuca</i>	<i>U. inversa</i> (Hoffmann, 1874) (#1)	Kenya: Gazi	MZUF 1024	AB813658	AB813674	AB813703
	<i>U. inversa</i> (#2)	Tanzania: Dar es Salaam	13255	AB471904	AB471917	AB813703
<i>Leptuca</i>	<i>U. uruguayensis</i> Nobili, 1901	Argentina: Samborombón	13577	AB813659	AB813675	AB813704
	<i>U. deichmanni</i> Rathbun, 1935	Panama: Culebra I.	13583	AB813660	AB813676	AB813705
	<i>U. terpsichores</i> Crane, 1941	Panama: Culebra I.	13582	AB813661	AB813677	AB813706
	<i>U. pugillator</i> (Bosc, 1802)	USA: Georgetown, South Carolina	13586	AB813662	AB813678	AB813707
	<i>U. umbratila</i> Crane, 1941	Panama: Diablo Heights	13579	AB813663	AB813679	AB813708
<i>U. herradurensis</i> Bott, 1954	Panama: Diablo Heights	13580	AB813664	AB813680	AB813709	
<i>U. rapax</i> (Smith, 1870)	USA: Corpus Christi Bay, Texas	13584	AB813665	AB813681	AB813710	
<i>U. tangeri</i> (Eyedoux, 1835)	Spain: Puerto de Santa Maria, Cadiz	13585	AB813666	AB813682	AB813711	
<i>U. arcuata</i> (De Haan, 1835)	China: Hainan I.	13363	AB813667	AB813684	AB813713	
<i>Aufruca</i>	<i>U. seismella</i> Crane, 1975	Indonesia: Irian Jaya	ZRC 2000.2059	AB813668	AB813685	AB813714
<i>Gelasimus</i>	<i>U. vocans</i> (Linnaeus, 1758)	Philippines: Bohol I.	13205	AB555399	AB813683	AB813712

MNHN, Museum National d'Histoire Naturelle, Paris, France; MZUF, Museo Zoologico de la Universitat de Firenze, Italy; NCHUZOO, the Zoological Collections of the Department of Life Science, National Chung Hsing University, Taichung, Taiwan; NTOU, Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Keelung, Taiwan; QM, Queensland Museum, Queensland, Australia; ZRC, Zoological Reference Collection of the Raffles Museum of Biodiversity Research, National University of Singapore, Singapore

and were subsequently applied for the partitioned Bayesian inference (BI) analysis. The BI was performed with MrBayes (vers. 3.2.1, Ronquist et al., 2012) 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 500 trees were discarded as the burn-in (determined by the average standard deviation of split frequency values below the recommended 0.01; Ronquist et al., 2005). Maximum likelihood (ML) analysis was conducted in RAxML (vers. 7.2.6, Stamatakis, 2006) for the combined dataset. The model GTR + G (i.e. GTRGAMMA) was used for all subsets with 100 runs, and found the best ML tree by comparing the likelihood scores. The robustness of the ML tree was evaluated by 1000 bootstrap pseudoreplicates under the model GTRGAMMA.

Other analyses, including the nucleotide composition, variable and parsimony informative positions, were calculated using MEGA (vers. 5.10, Tamura et al., 2011).

RESULTS

Sequence diversity. — For the 20 specimens of IWP BF *Uca*, a 543 bp segment of the 16S was amplified and aligned; of which 163 positions were variable and 120 were parsimony informative. Among the total number of sequences, 18 different haplotypes were distinguished (Table 1). The studied segment of 16S was AT rich (70.6%) (T: 36.6%, A: 34.0%, G: 18.7%, C: 10.7%). For COI, a 658 bp segment was compared, resulting in 19 different haplotypes. The COI segment was AT rich (61.7%) (T: 33.5%, A: 28.2%, G: 17.5%, C: 20.8%). In this gene, 221 positions were variable and 194 were parsimony informative. A 605 bp segment of the 28S was compared and 17 different haplotypes were obtained. The segment of 28S was GC rich (66.2%) (T: 19.5%, A: 14.3%, G: 35.1%, C: 31.1%), with 70 positions variable and 40 were parsimony informative.

Phylogenetic analyses. — The phylogenetic tree, based on 1815 bp of the combined 16S, COI and 28S, was constructed using BI, with the support values from BI and ML analyses (Fig. 1). With regard to the IWP BF *Uca*, there are three clades corresponding well to *Austruca*, *Paraleptuca*, and *Cranuca*, although some members under *Austruca* and *Paraleptuca* have to be transferred. The analysis indicates the IWP BF and NF subgenera form a major clade (only highly supported by BI). However, the *Paraleptuca* and *Cranuca* clades, as well as the three IWP NF subgenera, are closer.

It is clear that the *Austruca* clade is highly supported by BI, although weakly supported under ML. This clade includes three subclades. The first subclade contains three species groups – (1) *U. annulipes* group: *U. albimana* (Kossmann, 1877), *U. annulipes* (H. Milne Edwards, 1837), *U. aff. annulipes* and *U. iranica* Pretzmann, 1971; (2) *U. lactea* group: *U. lactea* (De Haan, 1835) and *U. perplexa* (H. Milne Edwards, 1852); and (3) *U. mjoebergi* Rathbun, 1924. The

second subclade is the *U. triangularis* complex with *U. bengali* Crane, 1975, and *U. triangularis* (A. Milne-Edwards, 1873). The last subclade only includes *U. sindensis*.

The *Paraleptuca* clade is highly supported and includes three species (*U. chlorophthalmus*, *U. crassipes*, and *U. splendida*). The distinct *Cranuca* clade contains only the East African *U. inversa* and forms a large clade with the NF *Gelasimus*. Both the NF *Tubuca* and *Australuca* are closely related.

For the American BF *Uca*, three *Minuca* species and four *Leptuca* species form a highly supported, but mixed, clade without a clear division between *Minuca* and *Leptuca*.

DISCUSSION

Based on the classification of Crane (1975), the IWP BF *Uca* species belong to two subgenera, *Celuca* (= *Austruca* Bott, 1973) and *Amphiuca* (= *Paraleptuca* Bott, 1973). Beinlich & von Hagen (2006) subsequently established *Cranuca* for *U. inversa* because of some unusual characters (see below). In our results, the subgenera *Austruca*, *Paraleptuca* and *Cranuca* are strongly supported by two mitochondrial and one nuclear markers (Fig. 1), although the subgeneric assignments of some species need to be changed.

In Crane's monograph (1975), *Celuca* is the largest subgenus, with 27 species and subspecies from America and six from IWP. As noted by von Hagen (1976), *Celuca* Crane, 1975, has to be synonymised under *Leptuca* Bott, 1973. Their respective type species, *U. stenodactylus* (H. Milne Edwards & Lucas, 1843) and *Uca deichmanni* Rathbun, 1935, are American and closely related. Rosenberg (2001) suggested if the IWP species of *Celuca* form a different clade from the American one, then they would have to be called *Austruca* Bott, 1973, as its type species is the IWP *U. annulipes*. Beinlich & von Hagen (2006), however, regarded the subgenus *Paraleptuca* as including all of Crane's IWP species of *Celuca* (except *U. inversa*) and *Amphiuca*.

The subgenus *Austruca* (with the type species *U. annulipes*) is supported by its monophyly (Fig. 1), different from the American BF clade (including two subgenera, see below). Excluding *U. inversa*, the species in Bott's (1973) *Austruca* and Crane's (1975) IWP *Celuca* are largely retained, although *U. triangularis* was never treated by Bott (1973) and *U. sindensis* was placed in another subgenus (*Paraleptuca*) by Crane (1975). Our revised *Austruca* includes nine described species and one undescribed species. The *U. lactea* complex, with seven species, forms a highly supported clade (Fig. 1), with three subclades composed of the *U. annulipes*, *U. lactea* and *U. mjoebergi* species groups. These agree well with the results in Shih et al. (2009) which used only 16S and COI.

The undescribed species from the East African region has been identified as *U. annulipes* by Crane (1975) and followed by subsequent authors (e.g., Tanzania: Skov & Hartnoll, 2001; Zanzibar: Ólafsson & Ndaró, 1997; Mozambique: Litulo, 2005; South Africa: Backwell & Passmore, 1996; Jennions

& Backwell, 1996, 1998; Backwell et al., 1999). The identity of the East African “*U. annulipes*” has been questioned by Shih et al. (2009: fig. 1) (as a dotted line, different from the solid line of *U. annulipes* in Asia), because of its disjunct distribution and genetic distinctiveness. However, we have not been able to discern reliable and consistent morphological characters to characterise them. An extensive collection from various areas of East Africa and the detailed examination for stable distinguishing characters will be necessary to ascertain the identity of the East African taxon.

Another species within the *U. lactea* complex, *Uca cryptica* Naderloo, Türkay & Chen, 2010, was not included in our study. Based on morphology, Naderloo et al. (2010) have suggested it should be included in the Clade W (including *U. albimana*, *U. annulipes* and *U. iranica*) proposed by Shih et al. (2009). If so, it should be placed in our *U. annulipes* species group (Fig. 1). Further collections of this species for molecular study are necessary to confirm its phylogenetic position.

Crane (1975) treated the small-sized *U. triangularis* and *U. bengali* as two subspecies due to their morphological similarity. However, their genetic distance is relatively large according to the branch length (Fig. 1.) Their distribution seems to be isolated geographically by Malay Peninsula, i.e., *U. triangularis* is widely distributed in West Pacific, whereas *U. bengali* is limited to the eastern Indian Ocean (Andaman Sea and Bay of Bengal) (Crane, 1975). *Uca sindensis* is distributed along the northern coastal area of the Arabian Sea, including Pakistan, Iran, Iraq and Kuwait (Alcock, 1900; Crane, 1975; Collins et al., 1984; Naser et al., 2010; Mokhlesi et al., 2011). This species is sister to the remaining *Austruca* species (Fig. 1), suggesting it may represent an older lineage, and the Arabian Sea may be associated with the cladogenesis of this subgenus.

In the context of the present study, 11 species of *Austruca* are now identified - eight from the *U. lactea* complex, as well as *U. bengali*, *U. sindensis* and *U. triangularis*. In addition, some cryptic species based on molecular evidence within *Austruca*

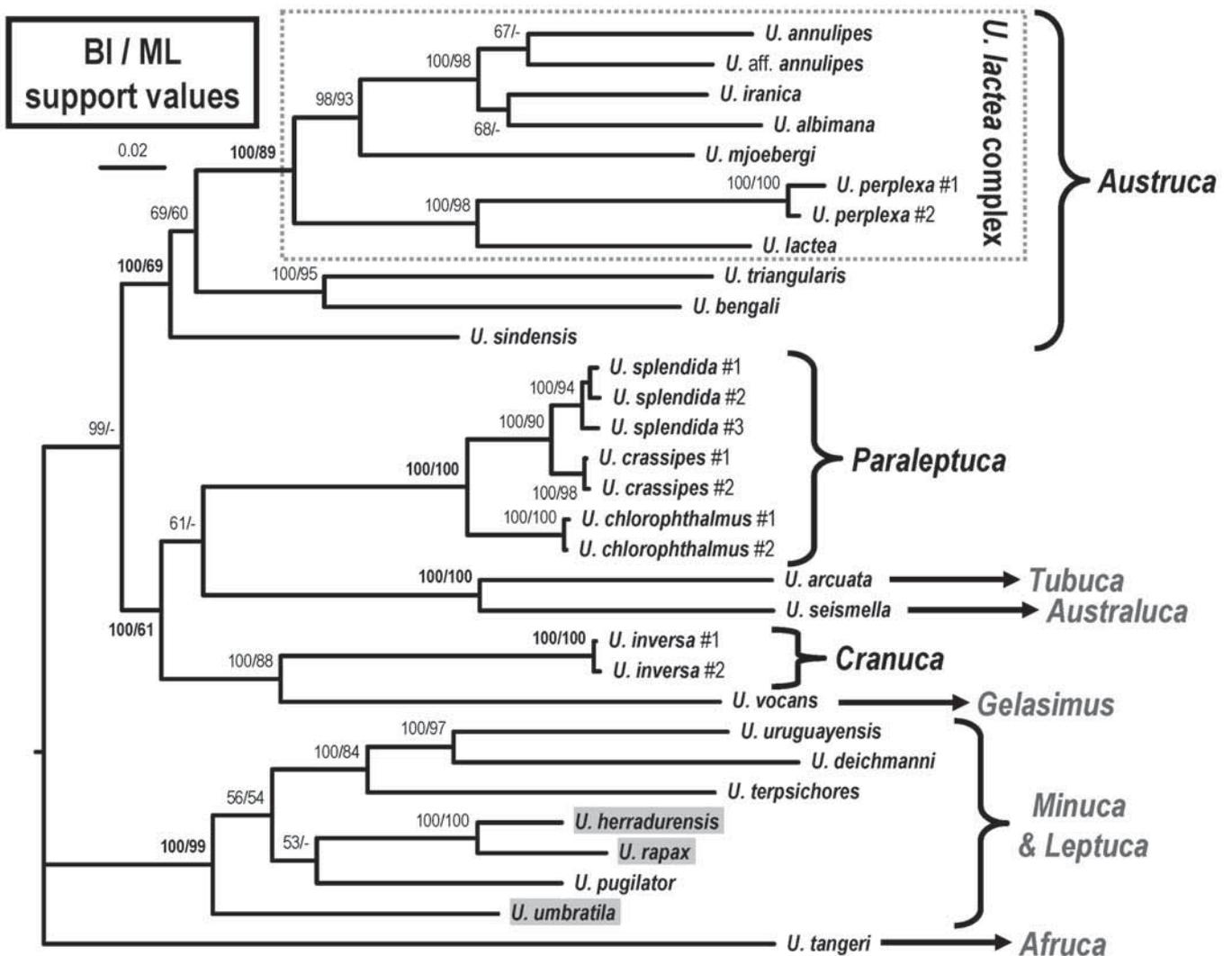


Fig. 1. A Bayesian inference (BI) tree of the Indo-West Pacific (IWP) broad-fronted (BF) fiddler crabs (subgenera *Austruca*, *Paraleptuca* and *Cranuca*) and the comparative taxa (the American *Minuca* and *Leptuca*, the eastern Atlantic *Afruca*, and IWP narrow-fronted *Tubuca*, *Australuca* and *Gelasimus*), based on the combined 16S rRNA, cytochrome oxidase subunit I genes (COI) and 28S rRNA. For the details of specimens see Table 1. Probability values at the nodes represent support values for BI and maximum likelihood (ML). The dotted lined block means the *Uca lactea* complex. For the clade of “*Minuca & Leptuca*”, the species names with gray block belong to the subgenus *Minuca*, and the remaining species belong to the subgenus *Leptuca*.

are still under study (unpublished data), and initial results suggest that this subgenus is even more diverse. Our results do not support the redefinition of *Paraleptuca* by Beinlich & von Hagen (2006), who transferred all the IWP BF *Uca* into this subgenus (except *U. inversa*), although they did highlight the possible confusion of the names between *Austruca* and *Australuca* Crane, 1975 (type species *Gelasimus bellator* White, 1847). Naderloo et al. (2010) proposed that members of the *U. lactea* complex be taken out of *Paraleptuca* and assigned to *Austruca* instead. Our study adds even more species into *Austruca*.

Some of Crane's (1975) conclusions, including the relationship between *Celuca* species from IWP and America, have been criticized by Salmon & Zucker (1988). They proposed the morphological similarity was due to parallel evolution and not a shared phylogenetic history (see also Rosenberg, 2001). This hypothesis was supported by Levinton et al. (1996) and Sturmbauer et al. (1996) based on a single mitochondrial 16S marker. Our study corroborates this hypothesis using three mitochondrial and nuclear markers (Fig. 1).

Bott (1973) only included *U. chlorophthalmus* (type species) and *U. gaimardi* (H. Milne Edwards, 1852) (= *U. crassipes*) (Crane, 1975; Shih et al., 2012) in *Paraleptuca* (= *Amphiuca* Crane, 1975). Although Crane (1975) included *U. inversa* and *U. sindensis* in her *Amphiuca*, this is not supported in our study. Instead, it indicates that *U. inversa* should be moved to *Cranuca* as suggested by Beinlich & von Hagen (2006), and *U. sindensis* be transferred to *Austruca* (Fig. 1). As *U. splendida* was recently resurrected from the synonymy of *U. crassipes* (Shih et al., 2012), three species can now be included in our redefined *Paraleptuca*. While *U. chlorophthalmus* occurs in the western Indian Ocean, *U. crassipes* is widely distributed from eastern Indian Ocean to central and southern Pacific Ocean (Crane, 1975). *Uca splendida*, however, is limited to continental East Asia and Vietnam (Shih et al., 2010, 2012). *Uca crassipes* and *U. splendida* are sympatric in Penghu (islands in the middle of Taiwan Strait), western Taiwan and Dongsha Island (= Pratas Island, in the northeastern South China Sea) (Shih et al., 2012).

The subgenus *Cranuca* was established for *U. inversa* based on some characters, like the absence of a pleonal clasping apparatus, presence of a large triangular subdistal tooth on the dactylus of the major cheliped, and lacking a tuberculate ridge on the inner surface of the manus (Beinlich & von Hagen, 2006). It is supported by our study as a distinct clade (Fig. 1). However, because all BF from IWP and America have a pleonal clasping apparatus (Beinlich & von Hagen, 2006), the absence of this character in *U. inversa* may suggest a close relationship with NF subgenera, which is supported by the monophyly between *Cranuca* and *Gelasimus* (Fig. 1). The mix of BF and NF in the IWP fiddler crabs has already been shown in Levinton et al. (1996) and Sturmbauer et al. (1996). Future studies with more taxa of IWP NF subgenera will be necessary to clarify their relationships.

Although we can confirm that there is no close genetic relationship between IWP and American *Celuca sensu* Crane, 1975, there remains a problem. Crane (1975) recognised three BF subgenera as present in America, viz. *Celuca* (= *Leptuca* Bott, 1973), *Minuca* and *Boboruca* (= *Planuca* Bott, 1973). *Boboruca* was established for *U. thayeri* Rathbun, 1900, and *U. umbratila* Crane, 1941, but it has been treated as a synonym of *Minuca* by several authors (Albrecht & von Hagen, 1981; Rosenberg, 2001; Beinlich & von Hagen, 2006). In our study, we do not find any support for the separation between the two American subgenera, because the clade composed of *Leptuca* and *Minuca* is mixed (Fig. 1). The mixed relationship between the two subgenera was reported by Levinton et al. (1996) and Sturmbauer et al. (1996) using the 16S marker. In fact, because of the mix of characters, it has proven difficult to assign some species to its subgenus, including *U. argillicola* Crane, 1941, *U. panamensis* (Stimpson, 1859), *U. pygmaea* Crane, 1941, and *U. subcylindrica* (Stimpson, 1859) (see Crane, 1975; Barnwell & Thurman, 1984; Levinton et al., 1996; Beinlich & von Hagen, 2006). To ascertain if the subgenera are monophyletic, more American species will need to be included and further morphological studies undertaken.

On the basis of the structure at the base of gastric mills, Beinlich & von Hagen (2006) proposed the American *Minuca* and *Leptuca* were derived from the IWP BF *U. sindensis* and *U. inversa*, perhaps via the ancient Tethys Sea. The hypothesis is not supported by the present study (Fig. 1) as we could detect no direct phylogenetic relationship between the American and IWP BF *Uca*.

In conclusion, our study supports the hypothesis that the IWP BF fiddler crabs can be separated into three distinct and monophyletic subgenera: *Austruca*, *Cranuca* and *Paraleptuca*. *Cranuca* contains only *U. inversa*; *Paraleptuca* includes *U. chlorophthalmus*, *U. crassipes* and *U. splendida*; and all the remaining IWP BF species belong in *Austruca*. There was no observable phylogenetic relationship between the BF subgenera from IWP and America, although the American BF subgenera do form a mixed clade.

ACKNOWLEDGEMENTS

This study was supported by grants from the National Science Council (NSC 98-2621-B-005-001-MY3, 101-2621-B-005-001-MY3), Executive Yuan, Taiwan, to HTS. Thanks are also due to John Christy, Carl Thurman, Pablo D. Ribeiro, Ehsan Kamrani, Christoph Schubart, Sara Fratini, Stefano Cannicci, Heok Hui Tan, A. Sasekumar, Joseph Poupin, Bertrand Richer de Forges, Laure Corbari and Benny K. K. Chan for helping specimen collection, and the members of the first author's laboratory for helping in molecular work. We acknowledge the kind comments of two anonymous referees who greatly improved the manuscript.

LITERATURE CITED

- Albrecht, H. & H. O. von Hagen, 1981. Differential weighting of electrophoretic data in crayfish and fiddler crabs (Decapoda: Astacidae and Ocypodidae). *Comparative Biochemistry and Physiology*, **70B**: 393–399.
- Alcock, A., 1900. Materials for a carcinological fauna of India. No. 6. The Brachyura Catometopa or Grapsoidea. *Journal of the Asiatic Society of Bengal*, **69**: 279–456.
- Backwell, P. R. Y., M. D. Jennions, J. H. Christy & N. I. Passmore, 1999. Female choice in the synchronously waving fiddler crab *Uca annulipes*. *Ethology*, **105**: 415–421.
- Backwell, P. R. Y. & N. I. Passmore, 1996. Time constraints and multiple choice criteria in the sampling behaviour and mate choice of the fiddler crab, *Uca annulipes*. *Behavioral Ecology and Sociobiology*, **38**: 407–416.
- Barnwell, F. H. & C. L. Thurman II, 1984. Taxonomy and biogeography of the fiddler crabs (Ocypodidae: genus *Uca*) of the Atlantic and Gulf coasts of eastern North America. *Zoological Journal of the Linnean Society*, **81**: 23–87.
- Beinlich, B. & H. O. von Hagen, 2006. Materials for a more stable subdivision of the genus *Uca* Leach. *Zoologische Mededelingen*, **80**: 9–32.
- Bosc, L. A. G., 1802. *Manuel de l'histoire naturelle des crustacés, contenant leur description et leurs moeurs; avec figures dessinées d'après nature. Volume 1*. Deterville, Paris. 258 pp.
- Bott, R., 1954. Dekapoden (Crustacea) aus El Salvador. 1. Winkerkrabben (*Uca*). *Senckenbergiana Biologica*, **35**: 155–180.
- Bott, R., 1973. Die verwandtschaftlichen Beziehungen der *Uca* Arten. *Senckenbergiana Biologica*, **54**: 315–325.
- Colgan, D. J., W. F. Ponder & P. E. Eggler, 2000. Gastropod evolutionary rates and phylogenetic relationships assessed using partial 28S rDNA and histone H3 sequences. *Zoologica Scripta*, **29**: 29–63.
- Collins, M. J., D. A. Jones & D. A. Clayton, 1984. Redescription of *Uca sindensis* (Alcock, 1900) (Brachyura: Ocypodidae) with notes on the ecology of a population from Kuwait. *Journal of Crustacean Biology*, **4**: 318–328.
- Crandall, K. A. & J. F. J. Fitzpatrick, 1996. Crayfish molecular systematics: Using a combination of procedures to estimate phylogeny. *Systematic Biology*, **45**: 1–26.
- Crane, J., 1941. Crabs of the genus *Uca* from the west coast of Central America. Eastern Pacific Expeditions of the New York Zoological Society. XXVI. *Zoologica*, **26**: 145–208.
- Crane, J., 1975. *Fiddler Crabs of the World (Ocypodidae: Genus Uca)*. Princeton University Press, Princeton, New Jersey. xxiii + 736 pp.
- De Haan, W., 1833–1850. Crustacea. In: von Siebold, P. F. (ed.), *Fauna Japonica sive Descriptio Animalium, Quae in Itinere per Japoniam, Jussu et Auspiciis Superiorum, qui Summum in India Batava Imperium Tenent, Suscepto, Annis 1823–1830 Collegit, Noitis, Observationibus et Adumbrationibus Illustravit*. Lugduni-Batavorum, Leiden. Pp. i–xvii, i–xxxii, ix–xvi, 1–243, pls. 1–55, A–Q, (2).
- Eydoux, F., 1835. Gélasime. *Gelasimus* Latr. G. de Tanger. G. *Tangeri*. F. Eydoux. *Magasin de Zoologie*, **5** (Cl. VII): 4 unnumbered pages, pl. 17.
- Farris, J. S., M. Källersjö, A. G. Kluge & C. Bult, 1994. Testing significance of incongruence. *Cladistics*, **10**: 315–319.
- Folmer, O., M. Black, W. Hoeh, R. Lutz & R. Vrijenhoek, 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**: 294–299.
- Guindon, S. & O. Gascuel, 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, **52**: 696–704.
- Hillis, D. M. & M. T. Dixon, 1991. Ribosomal DNA: Molecular evolution and phylogenetic inference. *Quarterly Review of Biology*, **66**: 411–453.
- Hoffmann, C. K., 1874. Crustacés et échinodermes de Madagascar et de l'île de la Réunion. In: Pollen, F. P. L. & D. C. Van Dam (eds.), *Recherches sur la Faune de Madagascar et de ses dépendances, part 5*. E. J. Brill, Leiden. Pp. i–xvii, i–xxxii, ix–xvi, 1–58, pls. 1–10.
- Jennions, M. D. & P. R. Y. Backwell, 1996. Residency and size affect fight duration and outcome in the fiddler crab, *Uca annulipes*. *Biological Journal of the Linnean Society*, **57**: 293–306.
- Jennions, M. D. & P. R. Y. Backwell, 1998. Variation in courtship rate in the fiddler crab, *Uca annulipes*: Is it related to male attractiveness? *Behavioral Ecology*, **9**: 605–611.
- Kossmann, R., 1877. Malacostraca (1. Theil: Brachyura). Chapter Erste Hälfte, III. In: Kossmann, R. (ed.), *Zoologische Ergebnisse einer im Auftrage der Königlichen Academie der Wissenschaften zu Berlin ausgeführten Reise in die Küstengebiete des Rothen Meeres*. W. Engelmann, Leipzig. Pp. 1–66, pls. 1–3.
- Leach, W. E., 1814. Crustaceology. In: Brewster, D. (ed.), *The Edinburgh Encyclopaedia*, vol. 7. A. Balfour, Edinburgh. Pp. 383–437, pl. 221.
- Levinton, J. S., C. Sturmbauer & J. Christy, 1996. Molecular data and biogeography: Resolution of a controversy over evolutionary history of a pan-tropical group of invertebrates. *Journal of Experimental Marine Biology and Ecology*, **203**: 117–131.
- Linnaeus, C., 1758. *Systema Naturae per Regna Tria Naturae, Secundum Classes, Ordines, Genera, Species, cum Characteribus, Differentiis, Synonymis, Locis, Edition 10, vol. 1*. Laurentii Salvii, Holmiae. Pp. i–iii+1–824.
- Litulo, C., 2005. Population biology of the fiddler crab *Uca annulipes* (Brachyura: Ocypodidae) in a tropical East African mangrove (Mozambique). *Estuarine, Coastal and Shelf Science*, **62**: 283–290.
- Milne-Edwards, A., 1873. Recherches sur la faune carcinologique de la Nouvelle Calédonie, II. *Nouvelles Archives du Muséum d'Histoire Naturelle, Paris*, **9**: 155–332, pls. 4–18.
- Milne Edwards, H., 1837. *Histoire naturelle des Crustacés comprenant l'anatomie, la physiologie et la classification de ces animaux*. Librairie Encyclopedique de Roret, Paris. Vol. II: 531 pp.; + separate atlas to Vol. II: 32 pp.
- Milne Edwards, H., 1852. Observations sur les affinités zoologiques et la classification naturelle des Crustacés. *Annales des Sciences Naturelles, Zoology*, **3**(18): 109–166.
- Milne Edwards, H. & H. Lucas, 1842–1844. Crustacés. In: d'Orbigny, A. D. (ed.), *Voyage dans l'Amerique meridionale (le Brésil, la république orientale de l'Uruguay, la république Argentine, la Patagonie, la république du Chili, la république de Bolivia, la république du Pérou), exécuté pendant les années 1826, 1827, 1828, 1829, 1830, 1831, 1832 et 1833*. Vol. 6. P. Bertrand and Vve Levrault, Paris and Strassbourg. Pp. 1–37, 1–17.

- Mokhlesi, A., E. Kamrani, P. Backwell & M. Sajjadi, 2011. Study on the behaviour of two fiddler crabs, *Uca sindensis* and *Uca annulipes* (Decapoda: Ocypodidae), in Bandar Abbas, Iran. *Journal of the Marine Biological Association of the United Kingdom*, **91**: 245–249.
- Naderloo, R., M. Türkay & H.-L. Chen, 2010. Taxonomic revision of the wide front fiddler crabs of the *Uca lactea* group (Crustacea: Decapoda: Brachyura: Ocypodidae) in the Indo West Pacific. *Zootaxa*, **2500**: 1–38.
- Naser, M. D., M. H. Ali & A. G. Yasser, 2010. New record of the fiddler crab *Uca (Paraleptuca) sindensis* (Crustacea: Brachyura: Ocypodidae) from Khor Al Zubair, Basrah, Iraq. *Marine Biodiversity Records*, **3**: e87.
- Ng, P. K. L., D. Guinot & P. J. F. Davie, 2008. Systema Brachyurorum: Part I. An annotated checklist of extant brachyuran crabs of the world. *Raffles Bulletin of Zoology, Supplement*, **17**: 1–296.
- Nobili, G., 1901. Decapodi raccolti dal Dr. Filippo Silvestri nell'America meridionale. *Bollettino dei Musei di Zoologia ed Anatomia comparata della R. Università di Torino*, **16**(402): 1–16.
- Ólafsson, E. & S. G. M. Ndaró, 1997. Impact of the mangrove crabs *Uca annulipes* and *Dotilla fenestrata* on meiobenthos. *Marine Ecology Progress Series*, **158**: 225–231.
- Palumbi, S. R., A. Martin, S. Romano, W. O. McMillan, L. Stice & G. Grabowski, 1991. *The Simple Fool's Guide to PCR. A Collection of PCR Protocols, Version 2*. University of Hawaii, Honolulu, Hawaii.
- Posada, D., 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**: 1253–1256.
- Pretzmann, G., 1971. Ergebnisse einiger Sammelreisen nach Vorderasien 2. Teil: Marine Brachyura. *Annalen des Naturhistorischen Museums in Wien*, **75**: 477–487.
- Ragionieri, L., S. Fratini, M. Vannini & C. D. Schubart, 2009. Phylogenetic and morphometric differentiation reveal geographic radiation and pseudo-cryptic speciation in a mangrove crab from the Indo-West Pacific. *Molecular Phylogenetics and Evolution*, **52**: 825–834.
- Rambaut, A. & A. J. Drummond, 2009. *Tracer, Version 1.5*. <http://beast.bio.ed.ac.uk/Tracer>.
- Rathbun, M. J., 1900. Results of the Branner Agassiz expedition to Brazil. I. The decapod and stomatopod Crustacea. *Proceedings of the Washington Academy of Sciences*, **2**: 133–156.
- Rathbun, M. J., 1924. Results of Dr. E. Mjöberg's Swedish scientific expeditions to Australia 1910–1913. 37. Brachyura, Albuneidae and Porcellanidae. *Arkiv för Zoologi*, **16**(23): 1–33.
- Rathbun, M. J., 1935. Preliminary descriptions of six new species of crabs from the Pacific coast of America. *Proceedings of the Biological Society of Washington*, **48**: 49–52.
- Roman, J. & S. R. Palumbi, 2004. A global invader at home: Population structure of the green crab, *Carcinus maenas*, in Europe. *Molecular Ecology*, **13**: 2891–2898.
- Ronquist, F., J. P. Huelsenbeck & P. van der Mark, 2005. *MrBayes, Version 3.1 Manual*. <http://mrbayes.csit.fsu.edu/manual.php>.
- Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard & J. P. Huelsenbeck, 2012. MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**: 539–542.
- Rosenberg, M. S., 2001. The systematics and taxonomy of fiddler crabs: A phylogeny of the genus *Uca*. *Journal of Crustacean Biology*, **21**: 839–869.
- Salmon, M. & N. Zucker, 1988. Interpreting differences in the reproductive behaviour of fiddler crabs (genus *Uca*). In: Chelazzi, G. & M. Vannini (eds.), *Behavioral Adaptation to Intertidal Life*. Plenum Press, New York. Pp. 387–407.
- Schubart, C. D., J. E. Neigel & D. L. Felder, 2000. Use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. *Crustacean Issues*, **12**: 817–830.
- Shih, H.-T., E. Kamrani, P. J. F. Davie & M.-Y. Liu, 2009. Genetic evidence for the recognition of two fiddler crabs, *Uca iranica* and *U. albimana* (Crustacea: Brachyura: Ocypodidae), from the northwestern Indian Ocean, with notes on the *U. lactea* species complex. *Hydrobiologia*, **635**: 373–382.
- Shih, H.-T., P. K. L. Ng, S.-H. Fang, B. K. K. Chan & K. J. H. Wong, 2010. Diversity and distribution of fiddler crabs (Brachyura: Ocypodidae: *Uca*) from China, with new records from Hainan Island in the South China Sea. *Zootaxa*, **2640**: 1–19.
- Shih, H.-T., P. K. L. Ng, T. Naruse, S. Shokita & M.-Y. Liu, 2011a. Pleistocene speciation of freshwater crabs (Crustacea: Potamidae: *Geothelphusa*) from northern Taiwan and southern Ryukyus, as revealed by phylogenetic relationships. *Zoologischer Anzeiger*, **250**: 457–471.
- Shih, H.-T., P. K. L. Ng, K. J. H. Wong & B. K. K. Chan, 2012. *Gelasimus splendidus* Stimpson, 1858 (Crustacea: Brachyura: Ocypodidae), a valid species of fiddler crab from the northern South China Sea and Taiwan Strait. *Zootaxa*, **3490**: 30–47.
- Shih, H.-T., J.-Y. Shy, T. Naruse, D. C. J. Yeo & P. K. L. Ng, 2011b. Introduction of an Indochinese freshwater crab *Sayamia germaini* (Crustacea: Brachyura: Gecarcinucidae) to Taiwan: Morphological and molecular evidence. *Raffles Bulletin of Zoology*, **59**: 83–90.
- Shih, H.-T., X.-M. Zhou, G.-X. Chen, I.-C. Chien & P. K. L. Ng, 2011c. Recent vicariant and dispersal events affecting the phylogeny and biogeography of East Asian freshwater crab genus *Nanhaipotamon* (Decapoda: Potamidae). *Molecular Phylogenetics and Evolution*, **58**: 427–438.
- Skov, M. W. & R. G. Hartnoll, 2001. Comparative suitability of binocular observation, burrow counting and excavation for the quantification of the mangrove fiddler crab *Uca annulipes* (H. Milne Edwards). *Hydrobiologia*, **449**: 201–212.
- Smith, S. I., 1870. Notes on American Crustacea. No. 1. Ocypodoidea. *Transactions of the Connecticut Academy of Arts and Science*, **2**: 113–176.
- Spivak, E. D. & J. A. Cuesta, 2009. The effect of salinity on larval development of *Uca tangeri* (Eydoux, 1835) (Brachyura: Ocypodidae) and new findings of the zoeal morphology. *Scientia Marina*, **73**: 297–305.
- Stamatakis, A., 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**: 2688–2690.
- Stimpson, W., 1858. Prodrómus descriptionis animalium everttebratorum, quae in Expeditione ad Oceanum Pacificum Septentrionalem, a Republica Federata missa, Cadwaladaro Ringgold et Johanne Rodgers Ducibus, observavit et descripsit. Pars V. Crustacea Ocypodoidea. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **10**: 93–110.
- Stimpson, W., 1859. Notes on North American Crustacea, No. 1. *Annals of the Lyceum of Natural History of New York*, **7**: 49–93.
- Sturmbauer, C., J. S. Levinton & J. Christy, 1996. Molecular phylogeny analysis of fiddler crabs: Test of the hypothesis of increasing behavioral complexity in evolution. *Proceedings of the National Academy of Sciences of the United States of America*, **93**: 10855–10857.

- Swofford, D. L., 2003. *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4*. Sinauer Associates, Sunderland, Massachusetts.
- Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei & S. Kumar, 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, **28**: 2731–2739.
- Thompson, J. D., D. G. Higgins & T. J. Gibson, 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**: 4673–4680.
- von Hagen, H. O., 1976. Review of Jocelyn Crane: Fiddler crabs of the world. Ocypodidae: genus *Uca*. *Crustaceana*, **31**: 221–224.
- White, A., 1847. Short descriptions of some new species of Crustacea in the collection of the British Museum. *Proceedings of the Zoological Society of London*, **1847**(15): 84–86.
- Yeo, D. C. J., H.-T. Shih, R. Meier & P. K. L. Ng, 2007. Phylogeny and biogeography of the freshwater crab genus *Johora* (Crustacea: Brachyura: Potamidae) from the Malay Peninsula, and the origins of its insular fauna. *Zoologica Scripta*, **36**: 255–269.