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Research Article

Invasive records of *Eriocheir hepuensis* Dai, 1991 (Crustacea: Brachyura: Grapsoidea: Varunidae): Implications and taxonomic considerations

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Abstract

A non-ovigerous female mitten crab captured in Iraq on the 20th June 2005 was initially identified as *Eriocheir sinensis* H. Milne Edwards, 1853. More material has now been made available from Iraq and Kuwait and these specimens were compared with the extant type series of *E. sinensis* and *E. hepuensis* Dai, 1991. From this morphological study the Persian Gulf material was identified as the Hepu mitten crab, *E. hepuensis*. This is the first report of this mitten crab species outside its native range of southern China. However, because mitten crab taxonomy and systematics requires further clarification especially with respect to species of *Eriocheir* De Haan, 1835, the DNA of the Persian Gulf material was compared with a suite of GenBank COI sequences from various mitten crab taxa. The results of this extensive examination indicate that the Iraqi and Kuwaiti specimens are *E. hepuensis* and the DNA analysis indicates that mitten crabs can be assigned to three genera and six species; although the present study does discuss reports of hybridisation between three *Eriocheir* species associated with aquaculture. Also reported, and of concern, is that *E. hepuensis* may have been first collected about thirty years ago in Iraq and has now become well established within that country. As a consequence the Hepu mitten crab could, given its catadromy, disperse widely throughout the extensive riverine systems of the region.

Key words: *Eriocheir hepuensis*; invasive species; Persian Gulf; molecular and morphological analyses; mitten crab taxonomy

Introduction

Four species of “hairy” (colloquially Southeast and East Asia) or “mitten” (vernacular Europe) crabs are currently assigned to *Eriocheir* De Haan, 1835 (Brachyura: Grapsoidea: Varunidae), initially, *E. japonica* (De Haan, 1835), and *E. sinensis* H.Milne Edwards, 1853, and recently, *E. hepuensis* Dai, 1991, and *E. ogasawaraensis* Komai, Yamasaki, Kobayashi, Yamamoto and Watanabe, 2006 (Ng et al. 2008). *Eriocheir* species are indigenous to China, the Korean Peninsula and Japan (Figure 1), and are all catadromous, spending most of their lives in fresh water, but returning to higher salinity water for mating, spawning and larval development. The seaward migration is annual, occurring during the autumnal months, and after mating the adults die (semelparous). The life cycle is completed by the upstream migration of early crab stages into freshwater.

Eriocheir sinensis has become arguably the most notorious brachyuran species on the planet. It is one of only two crabs on the world’s list of 100 most invasive aquatic invertebrates (Lowe et al. 2004). Incidentally, the other brachyuran species listed is the portunid *Carcinus maenas* (Linnaeus, 1758). The Chinese mitten crab is also cited in the handbook of alien species in Europe (DAISIE 2009: 312). This crab has successfully invaded NE Europe and has been reported from the east (Ruiz et al. 2006; Dittel and Epifanio 2009; Schmidt et al. 2009) and west coast (Cohen and Carlton 1997) of the United States and eastern Canada (Nepszy and Leach 1973; de Lafontaine 2005) with records from southern Iraq (Clark et al. 2006; Hashim 2010), and Tokyo Bay, Japan in 2004 (S. Kobayashi,

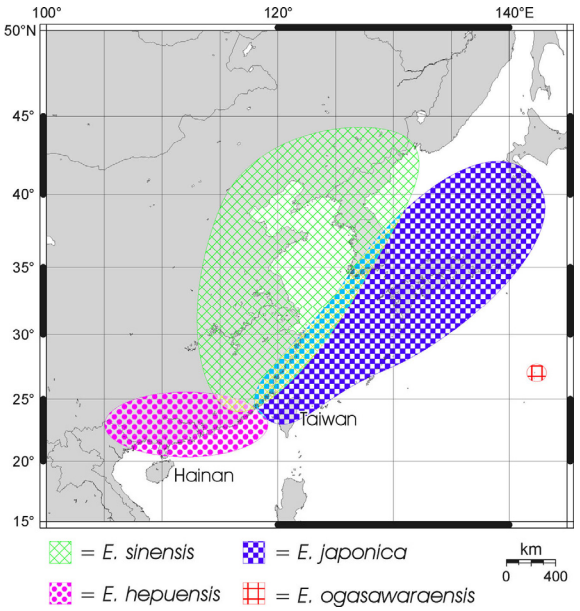

Figure 1. Known distribution of native *Eriocheir* De Haan, 1835 species.

Figure 2. Advertisement in a local newspaper promoting mitten crab on the menu of the Hôtel Plaza Athénée, Bangkok for the whole of November, 2001.

(pers. comm.; Doi et al. 2011). Furthermore, *E. sinensis* is one of the most commercially valuable crabs in East and Southeast Asia (e.g., see Peng 1986; Zhao 1988; Ng 1998; Lai et al. 1992), where the gonads, which develop during the annual downstream migration, are regarded as a delicacy (Figure 2).

Eriocheir japonica is also commercially exploited, but not to the same extent as *E. sinensis*, being consumed predominantly in Japan. The Japanese mitten crab too has been reported from outside its home range when a solitary male crab was captured in the Columbia River approximately 3km west of the Astoria-Megler Bridge, Astoria, Oregon, USA (see Jansen and Armstrong 2004). No further exotic reports have been recorded.

Recently, new mitten crab specimens from a number of localities in Iraq and Kuwait have been reported (Figure 3). Some of this material, which included several large males, has become available for detailed examination. The specimens were identified as *Eriocheir hepuensis*, the Hepu mitten crab. This is the first report of the crab outside its native range of southern China. However, because mitten crab taxonomy and systematics requires further clarification especially with respect to species of *Eriocheir* De Haan, 1835, a more extensive investigation was undertaken. This included a thorough examination of available *E. sinensis* and *E. hepuensis* material, a morphometric analysis, and the DNA barcode (*sensu* Costa et al. 2007; the mitochondrial COI gene) of an Iraqi mitten crab was compared against a suite of GenBank sequences for *Eriocheir*.

The present study reports upon the results of the morphological and genetic investigations of the mitten crabs from the Persian Gulf, and the implications for mitten crab taxonomy and systematics. Also discussed is the capacity of *E. hepuensis* for further dispersal throughout the extensive watersheds of the region.

Methods and material

Abbreviations used: ASIZ = National Zoological Museum of China, The Chinese Academy of Sciences, Beijing, People's Republic of China (previously known as Institute of Zoology, The Chinese Academy of Sciences, Beijing, People's Republic of China); coll. = collected; det. = determined by; MNHN = Muséum national d'Histoire naturelle; NHM = Natural History Museum, London; ZRC = Zoological Reference Collection, Raffles Museum of Biodiversity Research, Singapore; ZSM = Zoologische Staatssammlung München; ovig. = ovigerous; reg. = registration number.

Measurements: Carapace measurements = width × length in millimetres.

Figure 3. *Eriocheir hepuensis* specimens collected from Iraq and Kuwait.

Collected in Jan - Feb 2004 from Kuwaiti waters.

Collected in Jun 2006 from Shatt Al-Basrah Canal, Iraq (Clark et al. 2006).

Collected in Mar 2009 - Jan 2010 from Iraq (Hashim 2010).

Collected in Nov 2010 - Aug 2011 from Iraq (M.D. Naser, pers. comm.).

? Reports in Aug 2011 by fishermen from Iraq (M.D. Naser, pers. comm.).

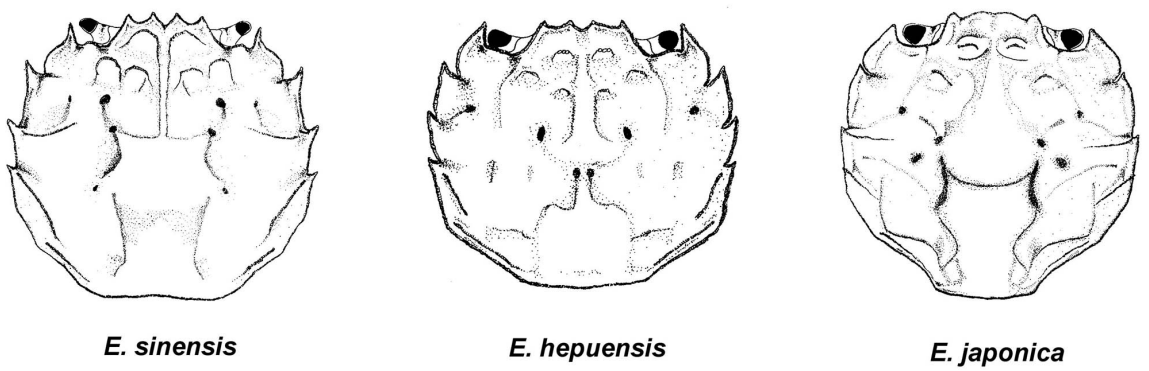

Figure 4. Dorsal carapace morphology of three mitten crab species (from Guo et al. 1997).

Literature: Only a selected synonymy for *E. sinensis* and *E. hepuensis* is provided here, but see Guo et al. (1997) for additional references.

Identification and Taxonomy

***Eriocheir sinensis* H. Milne Edwards, 1853** (Figures 4, 5a, 6a, 7a, 8)
Eriochirus sinensis H. Milne Edwards 1853: 177.
Eriocheir sinensis – H. Milne Edwards, 1854: 146-148, Pl. 9; Figures 1, 1a-c; Peters and Panning 1933: 1–180; Shen and Dai 1964: 127; Kim 1973: 465, text-fig. 202, Pl. 40, Figure. 154; Hwang and Mizue 1985: 12; Gu 1986: 268; Dai et al. 1986: 523; Yu and Ho 1986: 116; Adema 1991: 201, Fig. 79; Dai and Yang 1991: 523; Lai and Lu 1992: 23; Hong et al. 1993: 10, Pls. A and F; Chan et al. 1995: 301, Figure 3B; N.K. Ng et al. 1998: 493, 1999: 154; Sun et al. 2003: 592; Chu et al. 2003: 738; Tang et al. 2003: 309, 2004: 255; Chan et al. 2005: 457; Robbins et al. 2006: 33, Figures 1-2; Ng et al. 2008: 228.

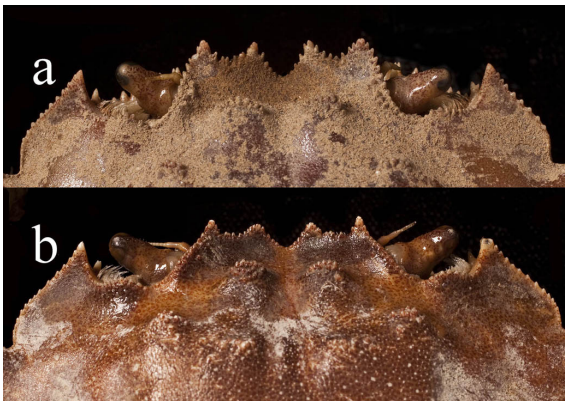

Figure 5. Frontal margin of a. *Eriocheir sinensis*, 1♂ (74.0×68.5mm), NHM reg. 1993.1, River Cray, Hall Place, near Crayford, Kent, England, coll. 20 Aug. 1992; b. *E. hepuensis*, 1♂ (70.6×66.4 mm), Shatt Al-Basrah canal near the dam at 30°24'33.75"N 47°46'32.32"E, Iraq, coll. M. Naser, 30 Nov. 2010, NHM reg. 2011. 8035. Photograph taken by Phil Hurst, NHM Photo Unit.

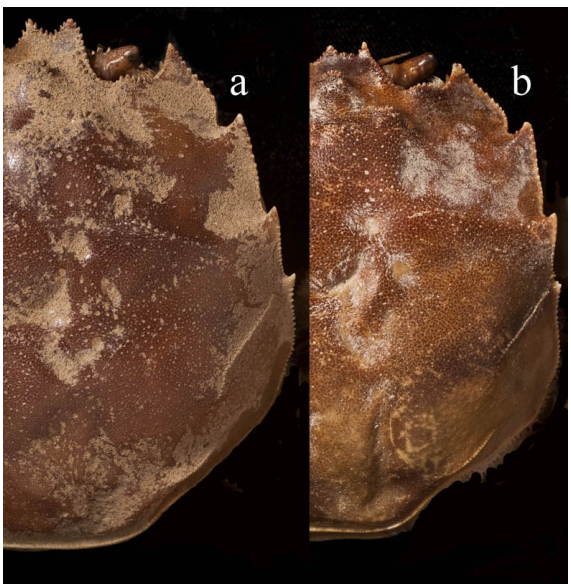

Figure 6. Anterolateral carapace margin of a. *Eriocheir sinensis*, 1♂ (74.0×68.5mm), NHM reg. 1993.1, River Cray, Hall Place, near Crayford, Kent, England, coll. 20 Aug. 1992; b. *E. hepuensis* 1♂ (70.6×66.4 mm), Shatt Al-Basrah canal near the dam at 30°24'33.75"N 47°46'32.32"E, Iraq, coll. M. Naser, 30 Nov. 2010, NHM reg. 2011. 8035. Photograph taken by Phil Hurst, NHM Photo Unit.

Eriocheir sinensis form *rotundifrons* Panning, 1938: 109, Figure 5; Adema 1991: 201, Figure 79.
Eriocheir sinensis form *acutifrons* Panning, 1938: 109, Figure 6; Adema 1991: 201, Figure 79.

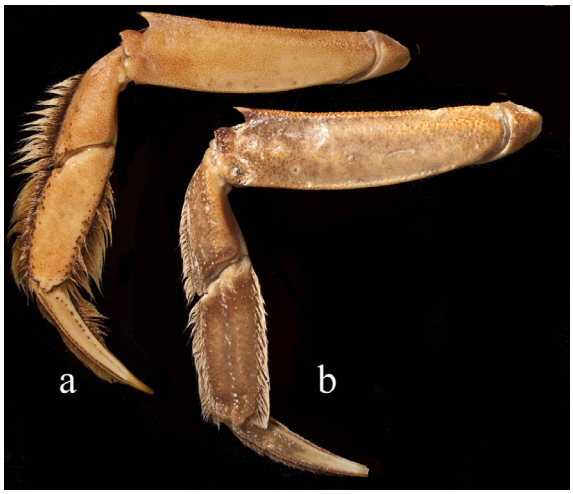

Figure 7. Fifth pereiopod, fourth ambulatory leg of a. *Eriocheir sinensis*, 1♂ (74.0×68.5mm), NHM reg. 1993.1, River Cray, Hall Place, near Crayford, Kent, England, coll. 20 Aug. 1992; b. *E. hepuensis*, 1♂ (70.6×66.4 mm), Shatt Al-Basrah canal near the dam at 30°24'33.75"N 47°46'32.32"E, Iraq, coll. M. Naser, 30 Nov. 2010, NHM reg. 2011. 8035. Photograph taken by Phil Hurst, NHM Photo Unit.

Eriocheir sinensis form *trilobata* Panning, 1938: 110, Figure 7; Adema 1991: 201, Figure 79.
Eriocheir sinensis form *rostrata* Panning, 1933: 53, Figure 22B; 1938: 110, Figure 8; Adema 1991: 201, Figure 79.

Material examined**.** Type material: lectotype. China. 1♀ (66.0×57.0mm), MNHN reg. B3383S, Macao, coll. M. Callery, no date. Non-type material: England. 1♂ (74.0×68.5mm), NHM reg. 1993.1, River Cray, Hall Place, near Crayford, Kent, coll. 20 Aug. 1992; 1♂ (69.0×62.0mm), NHM reg. 2003.315. China. Hong Kong Market, tied with rice grass, coll. P. Clark, Oct. 2002; 2♂ (70.9-72.8×62.5-63.3mm), 1♀ (61.6×57.0mm), NHM reg. 2011.8006-8008, Hong Kong, tied with rice grass, coll. B. Morton, Nov. 2010. In addition, see specimens listed in Guo et al. (1997).

Diagnosis. Carapace squarish, overall dorsal surface convex, regions well-defined (Figure 4). Frontal margin with four strong, sharp teeth with a narrow v-shaped median cleft (Figures 4, 5a). Anterolateral margins with four teeth including exorbital tooth (Figures 4, 6a). Third maxilliped broad; ischium, merus broad, exopod narrow. Ambulatory legs slender, long, long thick setae on anterior, posterior surfaces of carpi, propodi (Figure 7a). G1 long, slender, distal margin narrowly rounded, slopping shoulder shaped

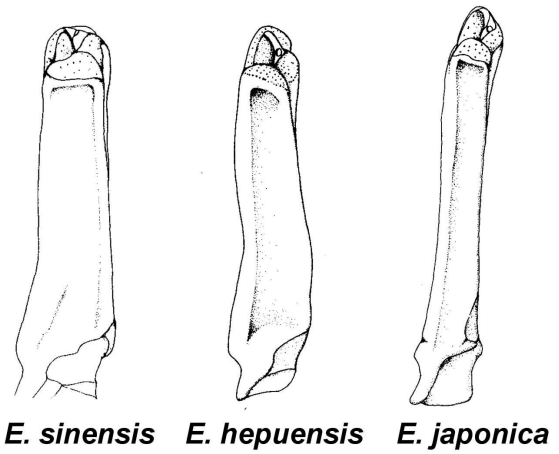

Figure 8. Morphology of the first male gonopod of three mitten crab species (from Guo et al. 1997).

Figure 9. *Eriocheir hepuensis*: distal tip of first gonopod, specimen from Kuwait (ZSM A20110200). Photograph taken by Michael Apel.

Figure 10. *Eriocheir hepuensis*: 1♂ (68.7×63.6mm) Khor Al-Gayed, East of Ras Al-Gayed, Bubiyan Isl., 29°52'N 48°21'E, Kuwait, coll. 26 January 2004, otter trawl, 4-6 m. Photograph taken by Michael Apel.

when viewed laterally, short, chitinuous prominence, slightly curved dorsally outwards with subdistal lobe (Figure 8), gonopore close to distal end. Female gonopore operculate, semicircular in shape, prominent, concave dorsally.

***Eriocheir hepuensis* Dai, 1991**

(Figures 4, 5b, 6b, 7b, 8, 9, 10)

Eriocheir japonica hepuensis Dai, 1991: 61, Figures 1-11; Dai 1993: 17.

Eriocheir hepuensis – Guo et al. 1997: 460; N.K. Ng et al. 1998: 493; N.K. Ng et al. 1999: 154; Sun et al. 2003: 592; Chu et al. 2003: 738; Tang et al. 2003: 309; Tang et al. 2004: 255; Chan et al. 2005: 457; Apel and Bishop 2006; Ng et al. 2008: 228.

Eriocheir sinensis – Chan et al. 1995: 301 (part), Figure 3D; Clark et al. 2006: 51, Figs. 2-3; Hashim 2010: 32-33, Figure 2.

Material examined. Type material: holotype. China. 1♂ (70.2×63.0mm), ASIZ reg. GX899024A), Hepu, Guangxi Province, southern China; Paratype, 1♀ (68.1×62.7mm), ASIZ reg. GX899024B, Hepu, Guangxi Province, southern China, coll. 18 Nov. 1989. Non-type material: Kuwait. 1♂ (68.7×63.6mm), ZSM reg.

ZSMA20110200, Khor Al-Gayed, East of Ras Al-Gayed, Bubiyan Isl., 29°52'N 48°21'E, coll. 26 Jan. 2004, otter trawl, 4-6 m, det. M. Apel; 1♂ (52.7×49.3mm), ZSM reg. ZSMA20110201, Khor Abdullah off Ras Al-Gayed (between Bubiyan Island and Fao Peninsula, Iraq), coll. 24 Feb. 2004, otter trawl, 4-10 m, det. M. Apel. Iraq. 1♀ (47.8×45.9mm), NHM reg. 2006.98, Shatt-Al, Basrah canal, 30º15'41.25"N, 47º48'

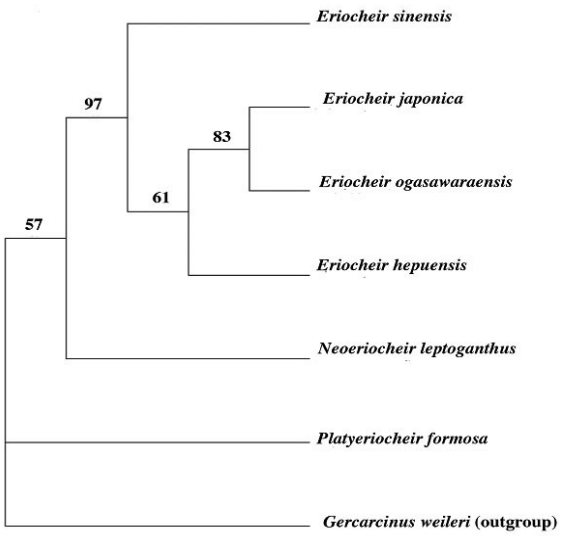

Figure 11. *Cladogram* of the four species of *Eriocheir* viz. *E. sinensis*, *E. hepuensis, E. japonica* and *E. ogasawaraensis*; *Neoeriocheir leptognathus; Platyeriocheir formosa* and *Gercarcinus weileri* (outgroup), based on 46 adult morphological characters (Ng, N.K. unpublished data).

56.91"E, coll. Ibtsam Abdul-Sahib, 20 Jun. 2005, det. P. Clark as *E. sinensis*; 2♂ (71.6×68.8-73.0×67.7mm), 1♀ ovi. (75.2×72.0 mm), ZRC reg. ZRC 2011.0732, Shatt Al-Basrah canal near the dam at 30°24'33.75"N 47°46'32.32"E, coll. M. Naser, 30 Nov. 2010; 2♂ (66.6×62.6-70.6×66.4 mm), 1♀ ovi. (68.6× 65.9mm), NHM reg. 2011.8035-8037, Shatt Al-Basrah canal near the dam at 30°24'33.75"N 47°46'32.32"E, coll. M. Naser, 30 Nov. 2010. In addition, see specimens listed in Guo et al. (1997).

Diagnosis. Carapace squarish, overall dorsal surface convex, regions well-defined (Figures 4, 10). Frontal margin with four sharp, broad teeth with a broad v-shaped median cleft (Figures 4, 6b). Anterolateral margins with four teeth including exorbital tooth (Figures 4, 6b, 10). Third maxilliped broad; ischium, merus broad, exopod narrow. Ambulatory legs broad, stout, long thick setae on anterior, posterior surfaces of carpi, propodi (Figure 7b). G1 long, slender, distal margin narrowly rounded, sloping shoulder shaped when viewed laterally, short, chitinuous prominence, slightly curved outwards with subdistal lobe, gonopore at ½ length of distal end (Figures 8, 9). Female gonopore operculate, bluntly triangular in shape, prominent, slightly concave dorsally.

Molecular

Genomic DNA was extracted from the leg of one *E. hepuensis* specimen collected at Shatt Al-Basrah, Iraq, using a modified version of a CTAB-phenol/chloroform extraction (Doyle and Doyle 1987). The 5' portion of the mitochondrial cytochrome oxidase subunit I (COI) was amplified using universal COI primers LCO-1490 (5'-TGA TTT TTT GGT CAC CCT GAA GTT CA-3') and HCO-2198 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') (Folmer *et al*., 1994).

PCR amplifications were 12.5µl reactions on a Geneamp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) of 0.5µl template DNA, 0.4µM primers, 0.1µM dNTPs, 2µM MgCl2, 2.5µl 10X PCR Buffer, 0.5 units of Taq polymerase (Bioline Pty Ltd, Alexandria, NSW, Australia) and the rest ddH_20. The following cycling conditions were used: 15 cycles of 30 s at 94°C, 30 s at 40°C, 60 s at 72°C; 25 cycles of 30 s at 94°C, 30 s at 55°C, 60 s at 72°C.

BigDye v.3.1 Terminator mix (Applied Biosystems) was used for the sequencing reaction in both directions and the sequences produced on an Applied Biosystems 3130xl Genetic Analyser at the DNA Sequencing Facility at Griffith University. Sequences were edited using Sequencher 4.1.2 (Gene Codes Corporation).

The COI sequence was compared against all GenBank sequences (17 Apr. 2011) using the BLASTN search at blast.ncbi.nlm.nih.gov, and also compared against the Barcoding of Life (BOLD) online database at http://www.barcodinglife.org. All GenBank *Eriocheir* COI sequences were downloaded and aligned with our sequence and a minimum evolution tree created using PAUP* version 4.0 b10 (Swofford 2002) with a Kimura 2-Parameter model (K2P), and bootstrapped 1000 times. A haplotype network was constructed with TCS version 1.21 (Clement et al. 2000) using sequences closely related to the Iraqi specimen (>99%) to explore within-species phylogeographic patterns. Molecular distances (Kimura 2-Parameter) between and within groups were calculated using Mega version 2.1 (Kumar et al. 2001).

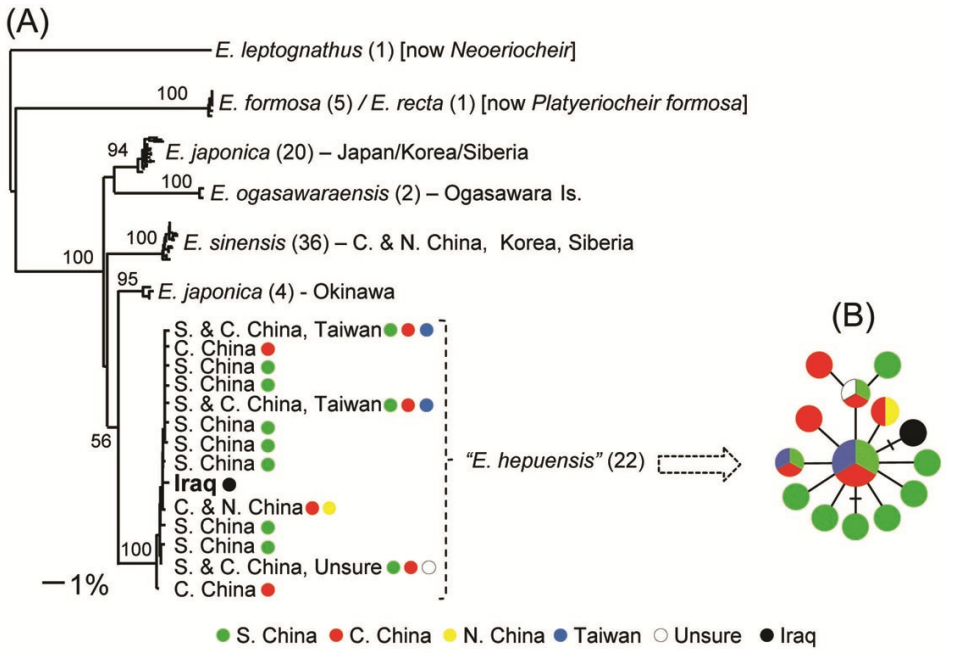

Figure 12. A) Minimum evolution tree of *Eriocheir* COI sequences with bootstrap support values (number of sequences in parentheses); B) Haplotype network of "*E. hepuensis*" clade showing geographic origin of relevant specimen.

Table 1. List of characters that can be used to distinguish between *Eriocheir sinensis* and *E. hepuensis*.

Characters/Species	*E. sinensis*	*E. hepuensis*
Teeth on frontal margin	Acutely triangular, sharp and high (Figure 5a)	Broadly triangular and sometimes not as sharp, and not as high (Figure 5b)
Median cleft on frontal margin	Narrow v-shaped (Figure 5a)	Broad v-shaped (Figure 5b)
Carapace physiognomy	High and convex	High but not convex
Anterolateral teeth of carapace	Usually sharp and strong (Figure 6a)	Weaker and not as sharp (Figure 6b)
4th leg merus	Long and slender (Figure 7a)	Short and broad (Figure 7b)
4th leg propodus	Long and slender (Figure 7a)	Shorter and broader (Figure 7b)
Male G1	Gonopore close to distal end	Gonopore about ½ length from distal end
Female gonopore	Semicircular in shape, concave dorsally	Triangular in shape, slightly concave dorsally

Mitten Crab taxonomy and systematics

Morphology

According to Ng et al. (2008), mitten crabs comprise three genera and six species namely *Eriocheir japonica* (De Haan, 1835), *E. sinensis*, *E. hepuensis*, and *E. ogasawaraensis* Komai, Yamasaki, Kobayashi, Yamamoto and Watanabe, 2006, *Neoeriocheir leptognathus* (Rathbun, 1913) and *Platyeriocheir formosa* (Chan, Hung and Yu, 1995). However, this classification and the present identification of the Hepu mitten crab specimens from the Persian Gulf may be considered as controversial to some researchers (Tang et al. 2003, 2004; Sun et al. 2003, 2005) who consider *E. hepuensis* as an invalid species. They consider the Hepu mitten crab to be a junior synonym of *E. sinensis*. Furthermore, Chu et al. (2003) have argued for maintaining five species (*E. ogasawaraensis* was described later) within *Eriocheir*, while Tang et al. (2003) suggested that *E. japonica*, *E. sinensis* and *E. hepuensis* were conspecific and should be considered as subspecies of *E. japonica*. In contrast, studies on morphology (Guo et al. 1997; Li and Zheng 2000a), reproductive biology (Li and Zheng 2000b), geography and habitats (Li and Zheng 2001), as well as a cladistic analysis based on 46 adult morphological characters (Ng, N.K. unpublished data; Figure 11) show that the Hepu mitten crab should be recognized as a separate taxon from *E. sinensis*.

Table 2. *Eriocheir* COI sequences downloaded from GenBank that are 99% similar to Iraqi specimen.

Area	Site	Species in original publication	GenBank accession number	Reference
Northern China				
	Changjiang R.	*E. sinensis*	DQ438944	Hu and Wang unpub.
Central China				
	Feiyunjiang R.	*E. japonica*	AY640088	Xu et al. 2009
	Feiyunjiang R.	*E. japonica*	AY640089	Xu et al. 2009
	Minjiang	*E. sinensis*	AF317336	Tang et al. 2003
	Oujiang R.	*E. japonica*	AY640088	Xu et al. 2009
	Oujiang R.	*E. japonica*	AY640089	Xu et al. 2009
	Oujiang R.	*E. japonica*	FJ750320	Xu et al. 2009
	Oujiang R.	*E. japonica*	FJ750321	Xu et al. 2009
	Tongan	*E. japonica*	AY640089	Xu et al. 2009
	Tongan	*E. japonica*	AY640090	Xu et al. 2009
	Tongan	*E. japonica*	FJ750322	Xu et al. 2009
Southern China				
	Aotou	*E. japonica*	AY640088	Xu et al. 2009
	Aotou	*E. japonica*	AY640089	Xu et al. 2009
	Aotou	*E. japonica*	FJ750323	Xu et al. 2009
	Aotou	*E. japonica*	FJ750324	Xu et al. 2009
	Hepu	*E. hepuensis*	AF317327	Tang et al. 2003
	Hepu	*E. hepuensis*	AF317328	Tang et al. 2003
	Hepu	*E. hepuensis*	AF516699	Chu et al. 2003
	Hepu	*E. japonica*	AY640088	Xu et al. 2009
	Hepu	*E. japonica*	AY640089	Xu et al. 2009
	Hepu	*E. japonica*	FJ750326	Xu et al. 2009
	Hepu	*E. japonica*	FJ750327	Xu et al. 2009
	Hepu	*E. japonica*	FJ750328	Xu et al. 2009
	Zhujiang	*E. japonica*	AY640091	Xu et al. 2009
	Zhujiang	*E. japonica*	FJ750324	Xu et al. 2009
	Zhujiang	*E. japonica*	FJ750325	Xu et al. 2009
	Zhujiang	*E. japonica*	FJ750326	Xu et al. 2009
Taiwan				
	Tamshui	*E. japonica*	AY640088	Xu et al. 2009
	Tamshui	*E. japonica*	FJ750321	Xu et al. 2009
Unsure				
	Unsure	*E. hepuensis*	FJ455506	Wang, Huang and Li unpub.
	Unsure	*E. japonica*	AF105245	Cheng, Chen and Chen unpub.
	Unsure	*E. japonica*	AF105246	Cheng, Chen and Chen unpub.

Interestingly, Figure 11 does not support a single taxon as suggested by Tang et al. (2003, 2004). Instead this cladogram confirms the recognition of three genera as proposed by Chan et al. (2005), Komai et al. (2006) and P.K.L. Ng et al. (2008), i.e., *Eriocheir*, *Neoeriocheir* Sakai, 1983, and *Platyeriocheir* N.K. Ng, Guo and P.K.L. Ng, 1999. Figure 11 also confirms that *Eriocheir* s. str. comprises four species, *E. sinensis*, *E. japonica*, *E. hepuensis* and *E. ogasawaraensis*. Moreover, *E. hepuensis* can be distinguished from *E. sinensis* based on the characters listed in Table 1, which include the shape of the frontal (Figures 4, 5) and anterolateral (Figures 4, 6) teeth, the shape and position of the carapace ridges (Figure 4, 5, 6), the meral and propodal proportions of the fourth ambulatory leg (Figure 7) and the position of the genital pore on the male gonopod (Figures 8, 9).

Molecular

As the validity of *E. hepuensis* appears to be divisive, a DNA analysis was undertaken to further clarify mitten crab systematics. For the present study, a 659 base pair COI sequence was produced (GenBank accession number JF81 0991) which corresponds to positions 5343-6001

Table 3. Molecular COI average distances (Kimura 2-parameter) between *Eriocheir* groups from Figure 15a (numbers in parentheses are within group averages).

	E. japonica	*E. formosa/ E. recta*	*E. sinensis*	*E. hepuensis*	*E. leptognathus*	*E. japonica (Okinawa)*	*E. ogasawaraensis*
E. japonica	(0.5%)						
E. formosa/recta	13.6%	(0.1%)					
E. sinensis	4.3%	14.4%	(0.4%)				
E. hepuensis	4.2%	14.2%	4.7%	(0.3%)			
E. leptognathus	14.0%	16.8%	15.3%	14.5%	(N/A)		
E. japonica (Okinawa)	3.1%	14.8%	4.2%	3.0%	13.6%	(0.4%)	
E. ogasawaraensis	4.8%	16.1%	6.8%	6.3%	14.9%	4.9%	(0.4%)

of the *E. hepuensis* mitochondrial genome (GenBank genome reference NC011598; Wang, Huang and Li, unpublished data). BLAST searches of GenBank and BOLD identified 21 sequences that were 99% similar (Table 2 for sequence information; Table 3 for molecular distances; Appendix 1 for other *Eriocheir* sequences), all belonging to *Eriocheir*. Most of these were derived from Xu et al. (2009), but also included sequences from Tang et al. (2003) and Chu et al. (2003) and some unpublished sequences. The final alignment of all *Eriocheir* sequences was 554 base pairs. The Iraqi specimen formed part of a strong clade (bootstrap 100%) in a minimum evolution tree (Figure 12a) that includes all 21 closely related sequences (average 0.3% K2P molecular distance within the clade). This clade contains specimens listed on GenBank as being *E. hepuensis*, *E. japonica* and *E. sinensis*. Since different authors consider these *Eriocheir* taxa in many different ways (as separate species, separate subspecies or all conspecific), these sequences are labelled on the tree as the "*E. hepuensis*" clade on the basis of their geographic location rather than as a formal taxon.

Essentially, there were seven groups in the tree (Figure 12a). *Neoeriocheir leptognathus* and *Platyeriocheir formosa* were distinct from each other and all other taxa. The remaining five groups form a strong clade, but the relationship between them is uncertain. These groups consist of 1) *E. japonica* from mainland East Asia which corresponds to the species in the strict sense as this includes the type locality of mainland Japan; 2) *E. ogasawaraensis* from the Ogasawara Islands; 3) *E. sinensis* which is the species in the strict sense as it includes the type locality of mainland northern China; 4) "*E. japonica*" from Okinawa, which may represent a cryptic species (or a geographic lineage of one of the other species); and 5) a mix of "*E. sinensis*", "*E. japonica*" and "*E. hepuensis*", including the specimen from Iraq, here referred to as the "*E. hepuensis*" clade.

The mixed nature of the last group can be explained by a number of factors (which are not mutually exclusive). Firstly, the GenBank specimens may have simply been identified incorrectly. This is more likely if only juveniles were used, purchased mixed from markets and collectors, and/or the worker was inexperienced with *Eriocheir* taxonomy. Some of the taxonomic characters used to separate the different species may also be less reliable due to possible variation. Consequently, new characters may need to be considered. Table 1 presents morphological characters currently used to distinguish between *E. sinensis* from *E. hepuensis*. In any case, juveniles and immature specimens can be difficult to separate (see Guo et al. 1997). In addition, the two species are genetically close enough to hybridise and this would make definitive species level identification difficult. If sorted by DNA barcoding then the groupings would be determined by the species of the mother (because COI is a mitochondrial gene so only inherited maternally). A recent paper by Sui et al. (2009) covering six river systems using six microsatellite loci nevertheless support the species distinction of *E. japonica*, *E. sinensis* and *E. hepuensis* as recognised by Dai (1991), but does hint at possible hybridisation.

The records of *E. hepuensis* and *E. japonica* from coastal southern and central China (~21°40'N to ~32°N) and Taiwan (see cited localities in Xu et al. 2009) need to be re-examined using freshly collected wild specimens. Interestingly, *Eriocheir hepuensis* haplotypes were the only species identified from

Figure 13. Chinese art depicting cooked *Eriocheir sinensis* waiting to be eaten. By Meishuaihaozhe Zhiyou, Courtesy Jiangsu Fine Arts Publishing House.

Figure 14. Chinese mitten crabs for sale at the Turf City Seafood Restaurant, Bukit Timah Road, Singapore. Note usual price for 250grm (or above) male is $65SD = ca. $33US. Photograph taken by Peter Ng.

coastal southern China. In coastal central China, both *E. hepuensis* and *E. sinensis* were present. *Eriocheir sinensis* dominates northern China, with only a single *E. hepuensis* mitochondrial haplotype found in the Chiagjiang River (Hu and Wang, unpublished data). Sui et al. (2009) found nuclear DNA evidence of a split between northern and southern coastal China (Figure 1).

The sister clade to that including the Iraqi specimen contains only *E. japonica* from Okinawa (3.0% molecular distance from *E. hepuensis*). Other separate strong clades include: *E. sinensis* (coastal central and northern China from ~24°50'N to 41°10'N, and Korea, Siberia; 4.7% distance), *E. japonica* (Japan, Korea, Siberia; 4.2% distance), *E. ogasawaraensis* (Ogasawara Islands; 6.3% distance), and the more distant clades of *Platyeriocheir formosa* (14.2% distance) and *Neoeriocheir leptognathus* (14.5% distance).

A haplotype network was created using sequences from the *E. hepuensis* clade (Figure 12b). Five geographic areas were defined: Coastal China South (Hepu to Aotou), Coastal China Central (Tongan, Minjiang [see Tang et al. 2003], Feiyunjiang River, Ouijiang River), Coastal China North (Chiagjiang River, Hu and Wang unpublished) and Taiwan (Tamshui). There is little obvious geographic structuring between the areas, with haplotypes shared between many areas. The Iraqi haplotype was unique and separated from the common central haplotype by two base pairs. It is not possible to narrow down the native geographic origin of the Iraqi mitochondrial haplotype because there is little geographic structuring among the areas.

Commercial exploitation and hybridisation

Eating the developing mitten crab gonads has been part of Chinese culture for many centuries dating back to the Ming Dynasty (1368-1644), when skilled craftsmen made a set of gold utensils for eating a crab that included a mallet, scissors, a shell cracker, a round salver, scoop, spoon, a long fork and combined scraper and pricker (http://www.cultural-china.com/chinaWH/html/en/Kaleidoscope2789bye8009.html).

Mitten crabs have also been a popular subject for beautiful brush-stroke paintings depicting the consumption of this delicacy (Figure 13). Today, mitten crabs command a high price in SE Asian restaurants (e.g., ca. $40 for a single crab in the right condition, Figure 14) during the autumnal months when they are harvested during their

Figure 15. In season, Chinese mitten crabs tied-up in rice grass can be purchased from street markets in Hong Kong. Photograph taken by Phil Crabb, NHM Photo Unit.

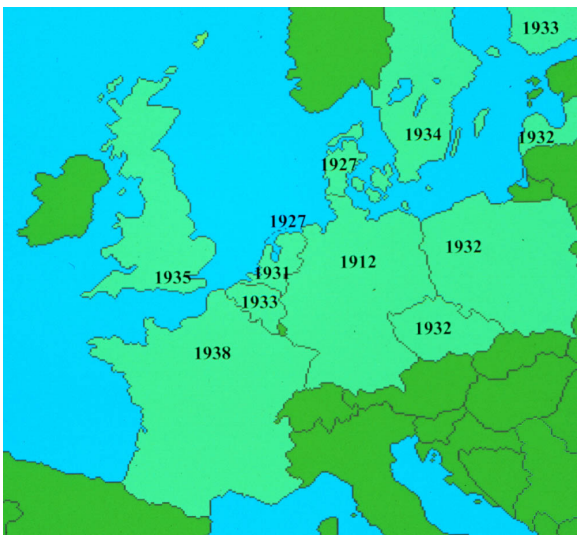

Figure 16. Map indicating rapid spread of *Eriocheir sinensis* throughout NE Europe, early 20th century.

annual migration from freshwater rivers to brackish estuarine water and marketed. Typically while in season, Chinese mitten crabs can be purchased from street markets as in Hong Kong (Figure 15). However, wild Chinese populations have dramatically declined due to over-exploitation, increased demand, river pollution and irrigation schemes that have disrupted the natural migration patterns of this species (Hymanson et al. 1999). But, local and international demands for *E. sinensis* have been met by an intensive aquaculture programme and this species has been farmed throughout China for the last 40 years (Sui et al. 2009) especially along the Yangtze valley (Jin et al. 2001; Wang et al. 2006). This industry is estimated to be worth ca. US$ 1.25 billion annually.

Studies on growth rates for the Hepu mitten crab, revealed maximum growth at water temperatures between 23 and 28°C (N.K. Ng, unpublished data). At this range of temperatures, *E. hepuensis* growth is better than that known for *E. sinensis*, and therefore in order to enhance the stock, farmers have cross-bred different genetic populations from various parts of China, including hybridising *E. sinensis* with *E. hepuensis* (see Zhao et al. 1988; Y. Cai, pers. comm., 1999). Although offspring have been produced in these *E. sinensis* and *E. hepuensis* crosses, there are no reports of their viability (Y. Cai, pers. comm. 1999; S. Yang, pers. comm. 2000). Furthermore, some farmers in northern China have also imported southern populations (including *E. hepuensis*) for breeding programmes (A.-Y. Dai, pers. comm. 1998). However, because farmers did not recognise *E. hepuensis* and *E. sinensis* as separate species but only as geographic populations, they did not keep records as to the sources of the breeding pairs. Moreover, the breeders did not check if the F1 generation were fertile. In addition, cross breeding between *E. sinensis* and *E. japonica* has been attempted in South Korea recently (S. H. Ko, pers. comm.), but again there are no reports on the outcome of this genetic experiment. One problem resulting from this practice is that according to S. H. Ko (pers. comm. 2002) there appears to be no 'pure' South Korean populations of *E. sinensis* left in the wild. In Japan, however, no interbreeding experiments of *E. sinensis* and *E. japonica* are known to date (T. Gao 2002; S. Kobayashi, pers. comm. 2010).

As a consequence of cross breeding, the taxonomy of mitten crabs native to East Asia has become even more problematic and controversial. This is why some taxonomists do not recognise *E. hepuensis* as a valid species. Even the validity of species like *E. japonica* and *E. sinensis* has been questioned because the genetic and morphological characters do not always agree. The presumed natural distributions (see Figure 1) of the four known *Eriocheir* species have been discussed at length by Guo et al. (1997) and Komai et al. (2006). Interestingly, *E. hepuensis* is currently considered locally to be an endangered species in China (Cheng et al. 2009).

Dispersal beyond Iraq

The "invasion" and subsequent dispersal of the Chinese mitten crab in Europe has been well documented. *Eriocheir sinensis* was accidentally introduced into Germany during 1912 when a large male was captured from the River Aller, a tributary of the River Weser (Peters and Panning 1933; Panning 1939). During the 1920s and 1930s, the Chinese mitten crab spread rapidly throughout northeast Europe (Figure 16) and beyond (e.g., Harold 1935; Petit 1960; Haahtela 1963; Petit and Mizoule 1974; Ingle and Andrews 1976; Christiansen 1977; Wall and Limbert 1983; Clark 1984; Adema 1991; Zibrowius 1991; Vigneux et al. 1993; Gomoiu and Skolka 1998; Cabral and Costa 1999; Murina and Antonovsky 2001; Zaitsev and Öztürk 2001; Slynko et al. 2002; Herborg et al. 2003; Paunovic et al. 2004; Herborg et al. 2005; Mizzan, 2005; Robbins et al. 2006; Shakirova et al. 2007; Bentley 2011; Berezina et al. 2011; Clark 2011; Jażdżewski and Grabowski 2011). In addition, Chinese mitten crabs have been recorded in various river catchments at considerable distances from the coast: ~1000 km, Yangtze River, China (Schellenberg 1928); ~700-750 km, Elbe River as far as Prague, Czech Republic (Peters 1938); ~460 km, Oder River, Breslau, Poland (Herborg et al. 2003); ~512 km River Rhine Netherlands (Herborg et al. 2003) and ~2000 km, Kuibyshev Reservoir, Volga River, Russia (Shakirova et al. 2007). These data and observations are of concern because if its behaviour is comparable to *E. sinensis*, the Hepu mitten crab could now spread rapidly throughout the extensive watersheds and catchments flowing through Iraq and nearby countries.

The first actual record of a "mitten crab" in Iraq may date back to 1980 when S.D. Salman (pers. comm. in letter to JM Bishop dated 17.04.06) reported possible collection of mitten crabs from the Shatt Al-Arab and later from the "Marshes", south of Al Fao, Shatt Al-Arab and Shatt Al-Basrah. This suggests that there has already been a long period (over 30 years) of establishment which includes ovigerous material. A rapid expansion of range could be expected as three rivers feed the Shatt Al-Arab waterway in Iraq - the Euphrates, Tigris, and Karun (see Clark et al. 2006). The origins of the Euphrates and the Tigris are in eastern Turkey and both flow to the Persian Gulf via Syria and Iraq. The Euphrates River is approximately 2800 km in length compared with the Tigris River, ca. 1800 km. Rising in the Zagros Mountains, West Iran, the Karun River flows south for about 720 km to meet the Shatt Al-Arab waterway on the Iraqi border (see Figure 3). Due to the catadromy of mitten crab species, all three watersheds would be vulnerable and these distances are within the range of those already reported for *E. sinensis*.

Origins and vectors

The *Eriocheir hepuensis* specimens reported from Kuwait (Figure 3) may just be Iraqi mitten crabs that have moved westward along the coastline or down the Shatt Al-Basrah Canal. As Kuwait has no riverine system, this species it not expected to become established in the sheikhdom. However, with regard to the origin of *E. hepuensis* from Khor Abdullah (Figure 3), it is possible that the species was introduced to the Gulf region by ship traffic. Either adult or subadult specimens may have been transported on ship hulls or larvae with ballast water. The latter mechanism has been identified as the main pathway for dispersal of *Eriocheir* species as well as for other marine invasive species (Cohen and Carlton 1997; Minchin and Gollasch 2002).

Commercial vessel traffic in the Persian Gulf is heavy and increasing. During the 365-day period from May 2006 through April 2007, 44,845 commercial vessels entered the Gulf for a daily average of 123. This represents a 40% increase over the previous year (Capt. A.M. Al-Janahi, Marine Emergency Mutual Aid Centre, Bahrain, pers. comm., 9 Oct. 2007). Many of these vessels were tankers, which are empty and discharge ballast water as they load crude oil. Although records of mitten crabs from the Gulf have only recently been published (Apel and Bishop 2006; Clark et al. 2006), their presence in the upper Gulf's Shatt Al-Arab area has been known since 1980 (S. Salman, Basrah University, pers. comm., 17 Apr. 2006), and a "very large sample" of mitten crabs was captured by trawl some miles south of Iraq's Fao Peninsula. Salman also reports capturing mitten crabs in the Iraqi marshes and the Shatt Al-Arab (pers. comm., 17 Apr. 2006). As the world's demand for crude oil increases, so will tanker traffic, and re-introductions of this species or introductions of other exotic species is likely.

Conclusion

The identification of *Eriocheir hepuensis* from Kuwait and Iraq based on morphological

characters was confirmed by DNA analysis. Furthermore, these molecular data supports the view that mitten crabs comprise three genera and six species i.e., *Eriocheir japonica*, *E. sinensis*, *E. hepuensis* and *E. ogasawaraensis*, *Neoeriocheir leptognathus* and *Platyeriocheir formosa*. However, hybridisation between *E. sinensis*/*E. hepuensis* and *E. japonica*/*E. sinensis* are reported from aquaculture experiments. Furthermore, evidence presented here suggests that *E. hepuensis* may have been present for over thirty years and has now become well established in Iraq. Considering the natural distribution of *E. hepuensis* and its congeners, it appears that the Hepu mitten crab inhabits subtropical and tropical regions, while *E. sinensis* is restricted to more temperate climates (Figure 1). Therefore *E. sinensis* has successfully invaded mainly temperate regions in central and northern Europe and North America and *E. hepuensis* might be much better adapted for the subtropical climate in the Gulf region, thus implying that an even larger area of the world may be at risk of *Eriocheir* invasions.

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Appendix 1. Other *Eriocheir* COI sequences downloaded from GenBank (<99% similar to Iraqi specimen).

Species in original publication	GenBank accession number	Species in original publication	GenBank accession number
E. formosa	AF105249	*E. sinensis*	AF105247
	AF317326		AF105248
	AF516698		AF279269
	F105250		AF317333
	FJ750332		AF317335
E. japonica	AF317330		AF435113
	AF317331		AF435114
	AF516700		AF435115
	AY640095		AF435116
	AY640096		AF435117
	AY640097		AF435118
	AY640098		AF435119
	AY640099		AF516702
	AY640100		AY640082
	AY640101		AY640083
	AY640102		AY640084
	FJ455505		AY640085
	FJ750312		AY640086
	FJ750313		AY640087
	FJ750314		DQ438943
	FJ750315		DQ882062
	FJ750316		FJ750306
	FJ750317		FJ750307
	FJ750318		FJ750308
	FJ750319		FJ750309
E. japonica (Okinawa)	AY640092		FJ750310
	AY640093		FJ750311
	AY640094		HM640253
	FJ750329		HM640254
E. leptognathus	AF516701		HM640255
E. ogasawaraensis	FJ750330		HM640256
	FJ750331		HM640257
E. recta	AF317332		HQ534046
			HQ534047
			HQ534048
			HQ534049