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Research article

The invasion of *Macrobrachium nipponense* (De Haan, 1849) (Caridea: Palaemonidae) into the Southern Iraqi Marshes

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Abstract

Forty-two specimens of *Macrobrachium nipponense* (de Haan, 1849) were collected from Abu-Zirig Marsh in the south of Iraq, in July 2005. DNA sequences confirmed the morphological identification by 99 % similarity to published 16S sequences. The introduction vector for this non-native species into the wild is considered to be unintentional escapes from Iranian aquaculture.

Key words: *Macrobrachium nipponense*, escapes, Iranian aquaculture, Al-Hammar Marshes, Iraq, DNA sequences

Introduction

Before 2002, *Macrobrachium* Bate, 1868 had not been reported from Iraq. However, early in that year, specimens started to appear frequently in benthic samples from the Garmat-Ali River, near the confluence of the Shatt Al-Arab River with the branch of the River Euphrates emerging out of the Al-Hammar Marshes (Figure 1). The water in this area is oligohaline, with a salinity of 1.299 - 2.690 % (Ali et al. 1995). Since 2002, specimens have been collected from the Al-Hammar Marshes (Hour Al-Hammar), the central (Al-Chibayish) Marshes and occasionally from the Al-Huwaizah Marshes (Figure 1, Annex 1). Studies of the life history and population dynamics of *Macrobrachium* in Garmat-Ali River are in progress (Dr. K. D. Saoud, pers. comm.).

The identification process was initiated in early 2003. Although the figures of the appendages and the whole animals are still extant, the original material based on specimens from Garmat-Ali River were lost during the looting in April 2003. Last year Dr. Ali Douable of the Iraq Foundation loaned some well-preserved specimens. Identification followed Holthuis (1950 and 1980) and Holthuis and Miquel (1984), with confirmation by DNA analysis.

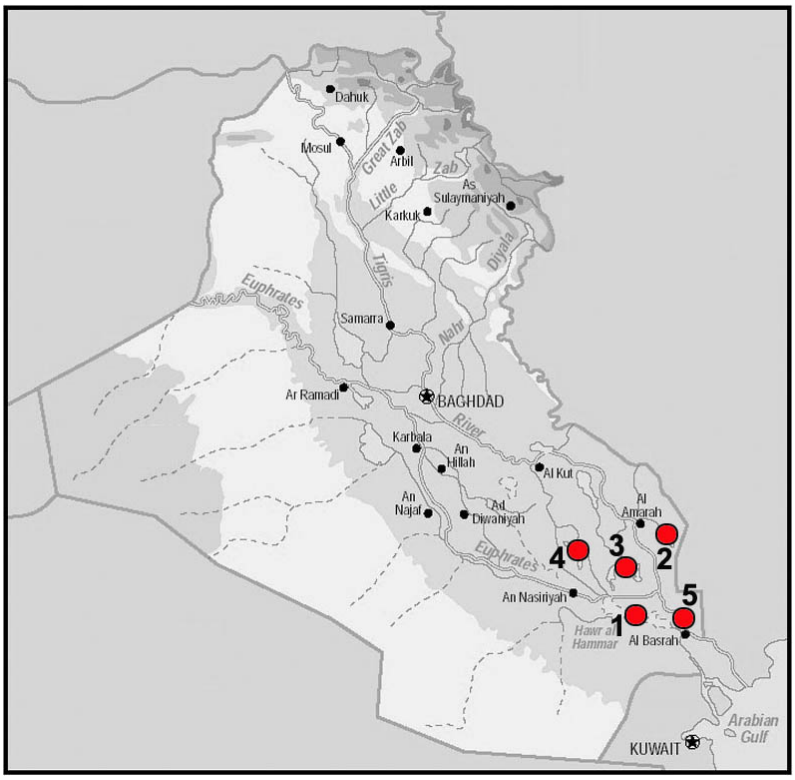

Figure 1. Map of Iraq with locations of *Macrobrachium nipponense* records (geographic coordinates of specific locations are provided in Annex 1)

Morphology

Material examined: Forty-two specimens were collected from Abu-Zirig Marsh in the south of Iraq (Figure 1) during July 2005. Of these 42 specimens, 25 were males, most of them fully grown, and the rest females. All the females were ovigerous.

Males: total length 71, 91-99.80 mm; carapace length (from posterior margin of carapace to tip of rostrum) 34, 49-49.62 mm; dorsal rostral teeth 11 (3 specimens), 12 (9 specimens), 13 (11 specimens), 14 (2 specimens); ventral rostral teeth 1 (2 specimens), 2 (8 specimens), 3 (15 specimens), 4 (none).

Females: total length 60, 64-88.69 mm; carapace length 27, 22-42.45 mm; dorsal rostral teeth 11 (1 specimen), 12 (3 specimens), 13 (6 specimens), 14 (3 specimens); ventral rostral teeth 1 (2 specimens), 2(8 specimens), 3 (15 specimens); 4 (1 specimen).

The remaining specimens had broken rostrums.

Diagnosis: Rostrum strait, broad in the middle (width about ¼ length, from orbit to tip of rostrum), extending well beyond antennal peduncle, and beyond tip of scaphocerite (excluding setae). About 2 dorsal rostral teeth behind orbit. Hepatic spine at a level lower than antennal spine. Second pair of pereiopods of adult male equal in size, long and all segments covered with a short and dense pubescence; carpus shorter than propodus and longer than merus; chela with stiff or velvety hairs on entire surface; cutting edge of finger of propodus with 1 proximal tooth; cutting edge of dactylus without tubercules (Figure 2).

Identification: The maximum size of *M. nipponense* (de Haan 1849) recorded in the literature coincides with the measurements reported here. Holthuis (1950) reported a size limit of 61-99 mm from 12 Japanese specimens, four of which were ovigerous females. For three specimens from Takao, South Formosa, he reported lengths of 44-61 mm, and 92 mm for a

dried specimen from an unknown locality. However, Holthuis (1980) has stated that the maximum length of the species is 86 mm for males and 75 mm for the females. A photograph of *Macrobrachium nipponense* from Garmat-Ali River is found in Figure 3.

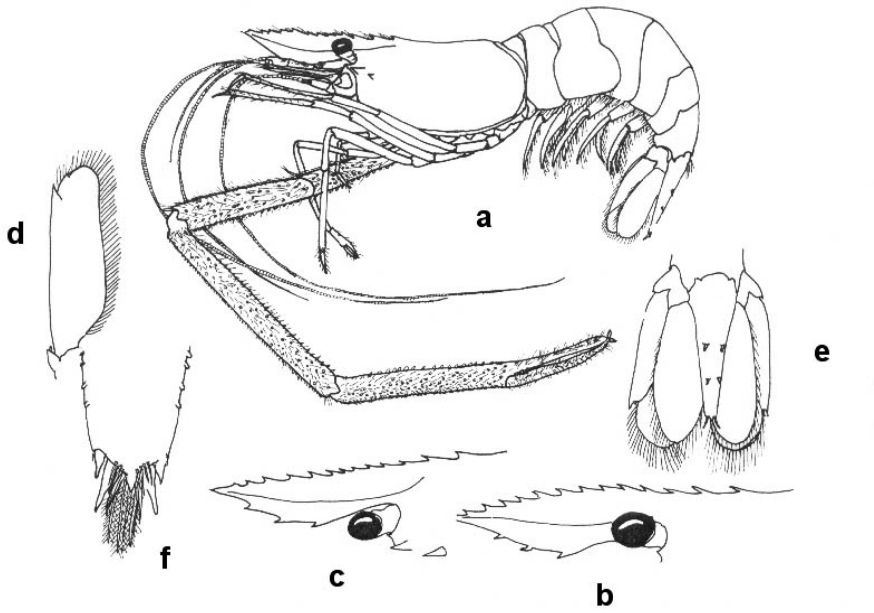

Figure 2. Macrobrachium nipponense male (9.4 cm) a. lateral view; b. rostrum of another specimen (9.0 cm); c. rostrum of a third specimen; d. Scaphocerite; e. Tail fan; f. Telson.

Figure 3. Photographs of *Macrobrachium nipponense* from Garmat-Ali River

Molecular analysis

Three specimens were collected from Garmat-Ali River. They were preserved in 70% EtOH. Genomic DNA was extracted using a modified version of a CTAB-phenol/chloroform extraction (Doyle and Doyle 1987). Three fragments of mitochondrial DNA (mtDNA) were amplified using the polymerase chain reaction (PCR). These were two fragments (5' and 3') of cytochrome oxidase subunit I (COI), and one fragment of 16S ribosomal DNA (rDNA). The 5' COI fragment (popularly known as the "DNA Barcode" sensu Hebert et al. 2003) 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) and the 3' fragment of COI was amplified using CDC0.La (5'-CCN GGG TTY GGR ATA ATT TCT C-3'; Page et al. 2005) and COIa.H (5'-AAG CAT CTG GGT ART C-3') (Palumbi et al. 1991). Primers for the 16S PCR were 16S-F-Car (5'-TGC CTG TTT ATC AAA

AAC ATG TC-3') and 16S-R-Car (5'-AGA TAG AAA CCA ACC TGG CTC-3') (Zitzler et al. in review).

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 ddH20. The following cycling conditions were used for the COI primers: 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. The 16S primers had the following cycling conditions: 40 cycles of 30 s at 94°C, 30 s at 50°C, 30 s at 72°C.

All individuals were sequenced in both directions for all three primer sets. BigDye v.1.1 Terminator (Applied Biosystems) was used for the sequencing reaction and all sequences were 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, 2000).

From the molecular analyses, the final sequences produced were 602 base pairs (5' COI), 557 base pairs (3' COI) and 534 base pairs long (16S rDNA) (lodged in Genbank under the accession numbers DQ656414 - DQ656416; Annex 2). The haplotypes for each specimen were identical for each respective gene fragment. A BLASTN search of Genbank (www.ncbi.nih.gov) failed to find *Macrobrachium* sequences that were closely related (i.e. within 10%) to either of the COI fragments. There are currently no *M. nipponense* COI sequences on Genbank. However, our 16S sequence closely matched (99% similarity) seven 16S sequences of *M. nipponense* from Japan (Genbank AY282771, Murphy and Austin 2004) and China (Genbank DQ462406-411, Sun, Li and Feng, unpublished).

Discussion

Macrobrachium nippponense inhabits fresh and brackish waters throughout much of East Asia (Holthuis 1980). This species has become important economically, especially in China and Japan, where it is fished commercially and used heavily in aquaculture (New 2005). While most *Macrobrachium* species are subtropical or tropical, *M. nipponense* survives well in colder temperate climates, thus making it a useful alternative to other popular aquaculture species such as *M. rosenbergii* (de Man, 1879) (Wong and McAndrew 1994a, New 2005). The use of *M. nipponense* in aquaculture has been growing since the 1990s and is spreading (New 2005) to Vietnam (Nguyen et al. 2002), Iran (Wong and McAndrew 1994b) and other locations. It is quite possible that the specimens found in southern Iraq may have escaped from aquaculture ponds in Iran recently and dispersed to Southern Iraq through the Al-Huwaizah marshes or the Karun River. However, recent sampling has revealed the presence of only a few specimens of *M. nipponense* in the Al-Huwaizah marshes, much fewer than in Al-Hammar, Al-Chibayish marshes and the Garmat-Ali River.

The wide ecological tolerance of *Macrobrachium nipponense* encompasses temperature and salinity. It can live at a range of salinities from brackish to fully freshwater, and can quickly adapt to a change to fully freshwater in three generations (Wong and McAndrew 1994a). The biological basis for this species being a competent coloniser of new areas, and therefore an invasive species threat, is completed by a significant intra-population and intra-individual variation in egg size (Mashiko and Numachi 2000) and larval characters (Alekhnovich and Kulesh 2001). All of these factors make this species effective in dispersing to and surviving in new environments, as it has naturally throughout its native range in East Asia (Wong and McAndrew 1994a).

Macrobrachium nipponense has been unintentionally introduced and flourished in many water bodies, and thus threatening native species, including in Lake Biwa, Japan (Hall and Mills 2000), Lake Dianchi, China (Yang 1996), Singapore (Chong et al. 1997) and the Caspian Sea, Iran (Abassi 2005). It is also present in Europe, as in 1960 it was inadvertently introduced to the cooling reservoir of a Moscow power plant (Alekhnovich and Kulesh 2001). Subsequently it has also appeared in power plants in Belarus, Moldova, Uzbekistan and Kazakhstan (Alekhnovich and Kulesh 2001). While this may be an opportunity for a developing aquaculture industry (New 2005), it also represents potential source populations for aquatic invasions elsewhere in Europe and the Middle East.

Another successful crustacean coloniser, the Chinese mitten crab *Eriocheir sinensis* H. Milne Edwards, 1853, has recently been reported in same area of Southern Iraq where *M. nipponense* has appeared, but the geographical source for these Chinese mitten crabs is unclear as DNA remains to be analysed (Clark et al. 2006). DNA

"barcoding" (Hebert et al. 2003) also provides a potentially effective method to supplement morphological analysis in species identification, as in the present study. A further factor that requires consideration when identifying *Macrobrachium* is that environmental rather than genetic factors can determine expressed morphological characters (Dimmock et al. 2004). Non-native species are unlikely to appear in locally appropriate morphological identification keys and because invading species may not be morphologically distinct, biosecurity can be significantly improved by a combined morphological and molecular approach (Armstrong and Ball 2005).

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Annex 1

Records *of Macrobrachium nipponense* in the southern marshes of Iraq in 2005-2006*

Map Ref.	Location	Geographic coordinates		Record date	Collector
		Latitude, °N	Longitude, °E		
1	Al-Hammar Marshes	30.4130	47.3529	17.04.2006	S.A.Noor
2	Al-Huwaizah Marshes	31.3412	47.3011	04.01.2006	S.D.Salman
3	Al-Chibayish Marshes	31.0124	47.0220	12.11.2005	S.D.Salman
4	Abu-Zirig Marsh	31.1013	46.3530	15.07.2005	A. Douable
5	Garmat-Ali River	30.3501	47.4501	13.09.2005	M.D.Naser

*Full reference to the data: Salman SD, Page TJ, Naser MD and Yasser AG. (2006) The invasion of *Macrobrachium nipponense* (de Haan, 1849) (Caridea: Palaemonidae) into the Southern Iraqi Marshes. Aquatic Invasions 1(3): 109-115

Annex 2

Nucleotide sequences of cytochrome oxidase subunit 1 (COI) and rDNA from three specimens of *Macrobrachium nipponense* from Garmat-Ali River

5' COI fragment of *Macrobrachium* from Garmat-Ali River (**Genbank Accession Number DQ656415**)

TAGTAGGCACATCCTTAAGACTACTAATTCGAGCAGAATTAGGTCAACCAGGAAGATTGATTGG
AAACGATCAGATCTATAATGTTATTGTTACCGCCCACGCTTTCGTAATAATTTTCTTTATAGTAA
TACCTATTATAATTGGAGGCTTCGGTAATTGATTAGTTCCTCTAATACTAGGAGCGCCAGATATA
GCATTCCCACGAATAAATAACATAAGATTTTGACTTCTACCCCCATCACTTACCTTATTACTATC
AAGAGGGATAGTAGAAAGAGGAGTAGGCACAGGATGAACTGTCTACCCCCCACTAGCTGCAGG
AACTGCCCACGCAGGAGCCTCAGTAGACTTAGGAATCTTCTCCTTACACTTAGCAGGGGTATCT
TCAATCTTAGGAGCCGTTAATTTTATTACTACCGTAATTAACATACGATCACCAGGAATGACCAT
AGACCGACTACCTCTATTCGTATGGGCCGTGTTCTTAACGGCAATTCTTCTTCTTCTATCACTTCC
AGTGTTAGCAGGAGCTATTACTATACTTTTAACAGACCGAAATCTAAATACATCATTCTTTGACC
CGGCCGGAGGTGGTGATCCA

3' COI fragment *Macrobrachium* from Garmat-Ali River (**Genbank Accession Number DQ656414**)

ATATTGTAAGACAAGAATCAGGTAAAAAAGAATCATTTGGCACCCTAGGTATAGTTTATGCCAT
AATAGCAATTGGAGTTTTAGGCTTCGTAGTATGAGCTCACCACATATTTACAGTAGGAATAGAC
GTAGACACACGAGCTTACTTCACATCAGCCACAATAATTATTGCTGTTCCAACAGGGATTAAAA
TCTTCAGGTGATTAGCTACTCTTCACGGCACACAATTTACCTATAGACCATCACTGATTTGAGCA
TTAGGATTTATTTTCTTATTTACCATAGGAGGATTAACAGGAGTAGTCCTAGCTAATTCATCTAT
CGACATTATTCTCCACGATACTTACTATGTAGTAGCACACTTCCACTACGTATTATCTATAGGAG
CCGTATTTGGTATTTTTGCAGGAATTGCTCACTGATTCCCCCTATTTACCGGCCTATCACTCAACC
CTAAATGATTAAAAATTCACTTTACTACAATGTTTATTGGAGTAAATTTAACCTTCTTTCCACAA
CACTTCTTAGGATTAAACGGAATACCCCGACGATATTCT

16S rDNA *Macrobrachium* from Garmat-Ali River (**Genbank Accession Number DQ656416**)

GTCTGTGTGTATAAGTTATAAAGTCTAGCCTGCCCACTGATTGATTTAAAGGGCCGCGGTAATTT
GACCGTGCGAAGGTAGCATAGTCAGTAGTCTTTTAATTGAAGGCTTGAATGAAAGGTTGGACGA
GGGATGAGCTGTCTCCTTTACGTAATTTGAATTTAACTTTTGAGTGAAAAGGCTCAAATTATTTA
GTGGGACGATAAGACCCTATAAAACTTTATATAAGTTTAATTTGGCTTACAATTTGAGTGAAAA
GTAGTTTTATTAGGCTTATATTTCGTTGGGGAGATGTAGATATAATGTGTAACTGTCTGTTTAAT
TTAATAATTATAATTAGTGTGTGATCCTTCTTTGTGGATTAAAAGTATAAGTTACTTTAGGGATA
ACAGCGTGATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACCTCGATGTTGAATTAAA
ATTTCAGTTAGGTGTAGCCGTCTAGCTGGTAGGTCTGTTCGACCTTTAAAATTTTACATGATTTG
AGTTCAAACCGGTGTG