

The East Asian shore crab *Hemigrapsus sanguineus* (Brachyura: Varunidae) in the Mediterranean Sea: an independent human-mediated introduction*

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SUMMARY. A single adult male specimen of the East Asian crab *Hemigrapsus sanguineus* (de Haan, 1835) was collected in August 2001 in the northern Adriatic Sea along the northwest coast of the peninsula of Istra. This is the first record of this genus and species from the Mediterranean Sea. Previously, *Hemigrapsus sanguineus* had been reported to invade the Atlantic coasts of North America and of western France, while the congeneric East Asian species, *Hemigrapsus penicillatus* (de Haan, 1835), has established breeding populations along the Atlantic coast of Europe. The current absence of *Hemigrapsus sanguineus* in southern Europe and the western Mediterranean suggests an independent human-mediated introduction of the Croatian specimen. Preliminary genetic analyses reveal that specimens from Istra, North America, and Japan have identical DNA haplotypes corresponding to the mitochondrial large subunit rRNA gene (16S mtDNA), while the homologous sequence from a specimen of Taiwan differs in two out of 525 nucleotides.

Key words: Crustacea, human-mediated introduction, invasion, Adriatic Sea, Istra.

RESUMEN: EL CANGREJO LITORAL DEL ESTE ASIÁTICO *HEMIGRAPSPUS SANGUINEUS* (BRACHYURA: VARUNIDAE) EN EL MAR MEDITERRÁNEO: UNA INTRODUCCIÓN HUMANA INDEPENDIENTE. – Un ejemplar macho adulto del cangrejo del este Asiático *Hemigrapsus sanguineus* (de Haan, 1835) fue recolectado en agosto de 2001 en el norte del Mar Adriático en la costa noroeste de la península de Istria. Este hallazgo constituye la primera cita de *H. sanguineus* (y del género *Hemigrapsus*) en el Mar Mediterráneo. Previamente, *H. sanguineus* ha invadido las costas atlánticas de Norteamérica y de Francia occidental, mientras que la especie congénica del este Asiático, *Hemigrapsus penicillatus* (de Haan, 1835) ha establecido poblaciones reproductoras a lo largo de la costa atlántica europea. La presente ausencia de *H. sanguineus* en el sur de Europa y en el Mediterráneo occidental sugiere una introducción humana independiente del espécimen hallado en las costas croatas. Análisis genéticos preliminares revelan que los especímenes de Istria, Norteamérica y Japón presentan idénticos haplotipos de DNA mitocondrial correspondientes al gen subunidad mayor rRNA (16S mtDNA), mientras que la secuencia homóloga de un espécimen de Taiwan difiere en dos de 525 nucleótidos.

Palabras clave: Crustáceos, introducción humana, invasión, Mar Adriático, Istria.

INTRODUCTION

During the past twelve years, two East Asian crab species of the genus *Hemigrapsus* (family Varunidae, see Martin and Davis, 2001; Schubart *et*

al., 2002) have invaded almost simultaneously the temperate coasts of the eastern (*H. penicillatus*) and western (*H. sanguineus*) North Atlantic (Williams and McDermott, 1990; Noël *et al.*, 1997). Previously, this genus was unknown in the Atlantic Ocean. Within a few years, both species were found to reproduce in their new environments (McDermott,

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1991; 1998a; Noël *et al.*, 1997). They have rapidly extended their distributional ranges and are now successfully established over several hundred kilometres of the respective coastlines (McDermott, 1999; Nijland and Beekman, 2000).

In their native East Asia, *H. penicillatus* and *H. sanguineus* are known to thrive in quite different ecological niches. The first species is more common along protected shores with low wave action and soft sediments (e.g. lagoons and estuaries), while *H. sanguineus* is typical on more exposed coasts with rocks and boulders (Fukui, 1988; Lohrer *et al.*, 2000). The initial distribution of *H. penicillatus* and *H. sanguineus* in the Atlantic brought up the question whether the restriction of *H. penicillatus* to European waters and of *H. sanguineus* to American ones could be a consequence of possible differences in ecological suitability of North American versus European habitats. However, the recent findings of *H. sanguineus* along the European Atlantic coasts of France (Le Havre) and the Netherlands (Oosterschelde) with accounts of breeding animals in Le Havre (Breton *et al.*, 2002) suggests that the second East Asian species of *Hemigrapsus* will also establish successfully along the French Atlantic and that both species of *Hemigrapsus* can potentially coexist in Europe. Continuing observations on the establishment and subsequent distribution of *H. sanguineus* in European waters, as well as on the number of independent introductions, are of crucial importance for understanding and predicting ecological scenarios that will determine successful invasion of alien species in the marine environment.

MATERIAL AND METHODS

The shallow subtidal and intertidal crustacean fauna of Uvala Slanik was studied along the rocky shore of the Ladin Gaj Campground in August 2001. U. Slanik is a small and shallow bay surrounded by flat rock terraces. It is located south of the small village of Lovrećica (13°32'26"E 45°22'54"N), on the northwestern coast of Istra, between Umag and Novigrad in Croatia.

The collected crustaceans were killed on ice (to prevent loss of appendages) or transported alive to the University of Regensburg (Germany) for colour photography previous to preservation in 95% ethanol. Samples of the collected crab species were archived at the Forschungsinstitut und Museum Senckenberg, in Frankfurt a. M. (Germany).

Genomic DNA of *Hemigrapsus sanguineus* was isolated from the muscle tissue of one walking leg. Selective amplification of a fragment from the mitochondrial large subunit rRNA gene (16S mtDNA) was carried out by polymerase-chain-reaction (PCR) with the primer combination 16L2 (5'-TGC-CTGTTTATCAAAAACAT-3') and 1472 (5'-AGATAGAAACCAACCTGG-3') (see Schubart *et al.*, 2000: Table 3). PCR products were purified and sequenced in an ABI Prism 310 Genetic Analyzer®. Sequences were aligned manually and compared with the multisequence editing program ESEE (Cabot and Beckenbach, 1989).

In addition to the specimen from Uvala Slanik, DNA of two other individuals of *H. sanguineus* from geographically distant populations was examined, one from Sippewissett (Massachusetts, USA) collected by Megan Tyrell in 1997 and deposited at the University of Louisiana at Lafayette (ULLZ 3793), and one from Taipei county (Taiwan) collected by Hung-Chang Liu on 7 June 1995 and deposited at the Senckenberg Museum, Frankfurt a. M. (SMF 27592). In addition, a third sequence corresponding to *H. sanguineus* from Japan (Kitaura *et al.*, 2002) was obtained from Genbank under the accession number AB058630. New DNA-sequences were submitted to the European Molecular Biology Laboratory (EMBL) genomic library (AJ493053-AJ493054). The genetic comparisons were carried out to confirm identification of the specimen from Istra as well as to establish the intraspecific variation of this gene. Genetic variation at the mtDNA level can be useful for future population genetic studies to determine possible founder populations.

RESULTS

The following species of brachyuran crabs were commonly encountered in shallow subtidal and intertidal habitats of Uvala Slanik (Istra, Croatia): *Pachygrapsus marmoratus* (Fabricius, 1787), *Eriphia verrucosa* (Forskål, 1775), *Carcinus aestuarii* Nardo, 1847, *Maja crispata* Risso, 1827, and *Xantho poressa* (Olivi, 1792). On 27 August 2001, one male specimen of *Hemigrapsus sanguineus* (de Haan, 1835) was collected from under a large boulder on a rocky platform at about sea level in the inner part of the bay. The crab was identified based on carapace and chelar morphology as well as on the characteristic punctuation pattern of the chelae (Fig. 1, colour picture in Galil *et al.*, 2002). This speci-

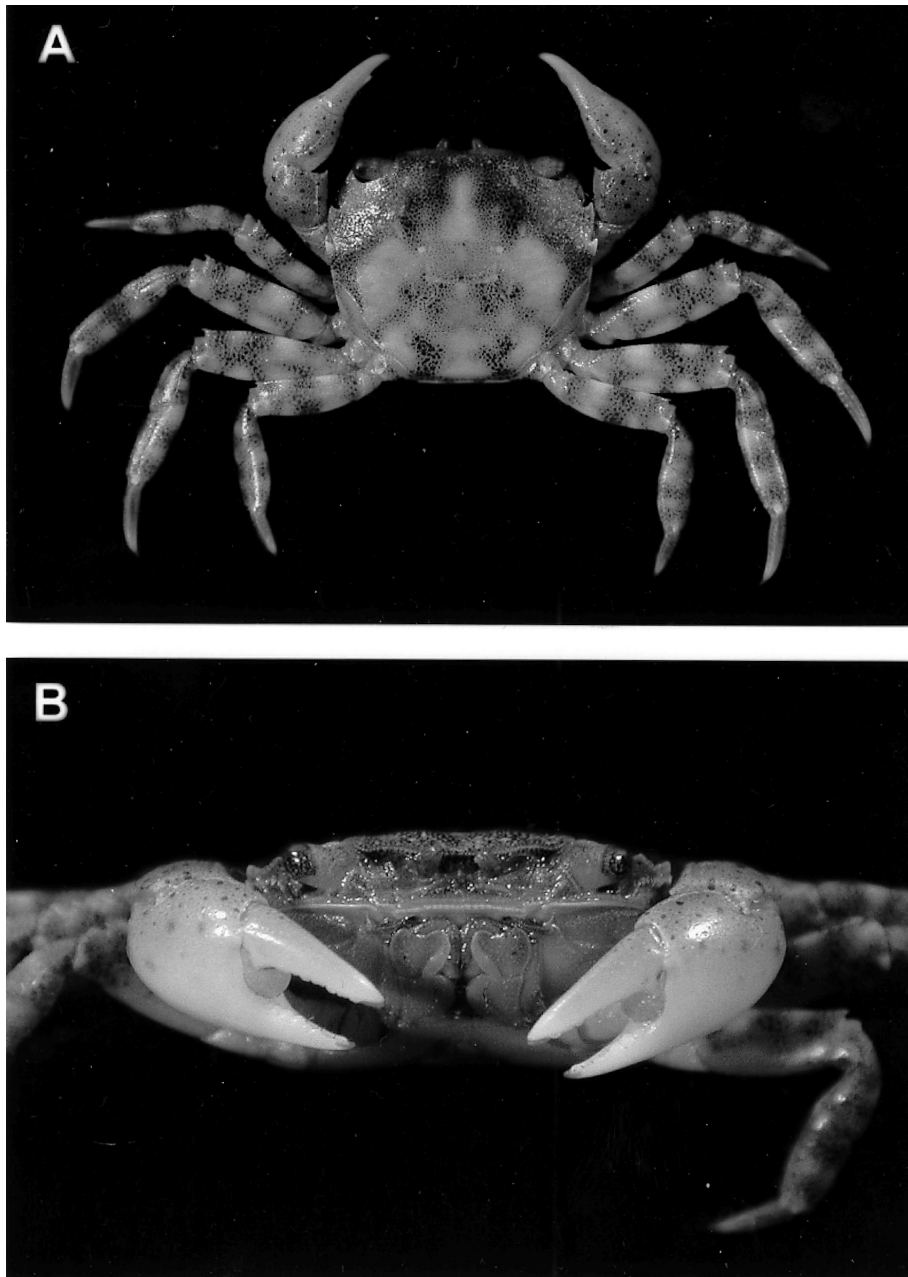


FIG. 1. – *Hemigrapsus sanguineus* (de Haan, 1835) from Istra (SMF 27591). A, dorsal view; B, frontal view.

men is the first record of this species and genus from the Adriatic Sea as well as from the entire Mediterranean Sea. The specimen is a small but adult male with a maximum carapace width (cw) of 17.2 mm, a carapace length (cl) of 15.4 mm and a chelar propodus height (prh) of 5.9 mm. It is deposited at the Senckenberg Museum, Frankfurt a. M. (SMF 27591). For comparison, the Taiwanese male crab (SMF 27592) measured 27.0 mm cw, 23.78 mm cl, 10.6 mm prh and the crabs reported from Le Havre by Breton *et al.* (2002) between 19 mm (ovigerous female) and 31.5 mm cw. According to Fukui

(1988), this species can reach ~40 mm cw. Subsequent efforts to find additional specimens of *Hemigrapsus sanguineus* at the same locality in Istra were unsuccessful.

Comparison of DNA-sequences corresponding to the 16S mtDNA revealed that among the studied 525 DNA-nucleotides, the Adriatic representative of *H. sanguineus* was genetically identical to the specimen from Massachusetts and to 474 nucleotides from a specimen of Japan. In contrast, the individual from Taiwan differed in two positions (two transitions) from the other three specimens (Fig. 2).

ish and Portuguese Atlantic coast as well as in the western Mediterranean strongly suggests that the single record from Istra is based on an independent introduction. If colonisation were carried out by a larval stage, our individual would have settled in Uvala Slanik approximately in autumn of 1999, based on Fukui's (1988) observations that *H. sanguineus* grows to 20 mm cw in about two years.

Although DNA sequences from American and European populations of *Hemigrapsus sanguineus* are identical to a Japanese sequence and two nucleotides differ from a Taiwanese sequence, it would be premature to interpret the molecular results obtained in this study with respect to the possible origin of the introduced populations. For this purpose, populations from the entire range of this species in East Asia should be sampled and more individuals should be studied to establish intra- and interpopulation genetic variation (see for example Fratini and Vannini, 2002). Only if the analysed molecular marker shows a complete lineage sorting among East Asian populations, it can be used to determine, or at least exclude, possible founder populations from the entire range of the invasive species. Geller *et al.* (1997) have shown that in some cases marine invasions may originate from more than one source population.

There have been several ecological studies monitoring the range extension and biotic interactions of the two species of *Hemigrapsus* in their new environments (Noël *et al.*, 1997; Lohrer and Whitlatch, 1997; McDermott, 1998b, 1999; Gerard *et al.*, 1999; Tyrrell and Harris, 2000). In western Europe as well as in the USA, the most important potential competitor is believed to be the green crab *Carcinus maenas* (Linnaeus, 1758) (introduced in the USA) and the most important prey, despite a predominantly algal diet, molluscs of the genera *Mytilus* and *Littorina* (see Gerard *et al.*, 1999; Tyrrell and Harris, 2000). To date, no measurable impact on the native (or previously introduced) fauna and flora has been reported (Breton *et al.*, 2002).

Noël *et al.* (1997) predicted that *H. penicillatus* may have a future range from Norway to North Africa, including the Mediterranean and Black Seas, based on its distribution across climatic regions in native East Asia. *H. sanguineus* has a similar native distribution and shows wide physiological tolerances (Watanabe, 1982; Gerard *et al.*, 1999). In the more southern regions of the potential European range, the grapsid crab *Pachygrapsus marmoratus* and the varunid crab *Brachynotus atlanticus* Forest,

1957 may be postulated as the main competitors of the two species of *Hemigrapsus* (see also d'Udekem d'Acoz, 1999). The first record of *Hemigrapsus sanguineus* from the Mediterranean Sea supports the assumption of potential competition with *Pachygrapsus marmoratus*, as the single specimen was collected under rocks, in a habitat shared with *P. marmoratus*. The two species did differ in their escape response, which was more sluggish in *Hemigrapsus sanguineus*. Feeding competition experiments as carried out between *H. sanguineus* and *Carcinus maenas* in North America might help to predict the outcome of a potential competitive exclusion. The dominant role of *P. marmoratus* as a herbivore and scavenger of the rocky intertidal in southern European waters casts doubt on the hypothesis that this species could be endangered by the invasive *H. sanguineus*. It is even possible that *P. marmoratus* will prevent the establishment of *Hemigrapsus* in southern Europe. Additional records of *H. sanguineus* from the Mediterranean Sea, especially of ovigerous females, will be needed to determine whether this species will become established and can be considered a new member of the Mediterranean fauna.

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