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Rapid Communication

An American in the Aegean: first record of the American lobster *Homarus americanus* H. Milne Edwards, 1837 from the eastern Mediterranean Sea

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Abstract

A male *Homarus americanus* individual, commonly known as the American lobster, was caught by artisanal fishermen at Chalkidiki Peninsula, Greece, north-west Aegean Sea on 26 August 2019. The individual weighted 628.1 g and measured 96.7 mm in carapace length (CL) and 31.44 cm in total length (TL). The specimen was identified by both morphological and molecular means. This is the species' first record from the eastern Mediterranean Sea and Greece, and only the second for the whole basin. However, several hypotheses for potential introduction vectors are discussed, as well as the potential implication to the regional lobster fishery.

Key words: Decapoda, biological diversity, non-indigenous species, Aegean Sea, Greece

Introduction

It is well acknowledged that non-indigenous and invasive species are negatively affecting biodiversity. The diet of several non-indigenous species consists of many different native species, altering the food webs. For instance, it is documented that the piscivorous cornetfish *Fistularia commersonii* Rüppell, 1838 affects the native fauna (e.g. Kalogirou et al. 2007). Similarly, the invasive pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) and the blue crab *Callinectes sapidus* Rathbun, 1896 are posing some threats at the regional food webs (Kalogirou 2013; Kampouris et al. 2019). Moreover, a recent study from Cyprus demonstrated that the alien goatfish *Parupeneus forsskali* (Fourmanoir & Guézé, 1976) is now the most abundant mullid species, competing for habitat with the native counterpart species (Evagelopoulos et al. 2020). The Mediterranean Sea is strongly impacted by Lessepsian migration and many Red Sea species are established in Mediterranean waters (Galil et al. 2019; Katsanevakis et al. 2020). Biological invasions in the Mediterranean Sea are also affected by other human-associated activities such as shipping, given that two-thirds of global marine traffic occurs in the region (Ulman et al. 2017). Additionally, some accidental and intentional introductions, either for farming purposes or associated with the live animal trade negatively contribute to regional biodiversity. The examples are numerous and include freshwater crayfish species (e.g., Snovsky and Galil 2011; Cilenti et al. 2017) and both marine and freshwater fish species (e.g., Emiroğlu et al. 2016; Zenetos et al. 2016; Tiralongo et al. 2018; Kampouris et al. 2020a).

Non-indigenous crustaceans are the second-most abundant group in the Mediterranean Sea, among which 91 species belong to the order Decapoda (Galil et al. 2015). Most recent records include species of Indo-Pacific and Red Sea origin such as the moon crab, *Matuta victor* (Fabricius, 1781) from Turkey and Greece (Rhodes Island) (Ateş et al. 2017; Kondylatos et al. 2018), and *Penaeus pulchricaudatus* Stebbing, 1914 and *P. hathor* (Burkenroad, 1959) from Crete Island (Kampouris et al. 2018) among others. The majority of reported occurrences are thermophilic species, mainly of Indo-Pacific and Red Sea origin, but there are also reports of decapod species of Atlantic origin that are established. Perhaps the species with the biggest negative effect is *C. sapidus*, with established populations across Mediterranean coasts (Mancinelli et al. 2017; Kampouris et al. 2019). In Greece, *C. sapidus* are posing serious threats to local biodiversity and are affecting regional societies and economy (Kampouris et al. 2019). The northern brown shrimp *Penaus aztecus* Ives, 1891 is also well established in Mediterranean Sea (Kampouris et al. 2018; Özcan et al. 2019). Moreover, the Atlantic *Xiphopenaeus kroyeri* (Heller, 1862) is established on the Mediterranean coast of Egypt (Khafage and Tasha 2019). In addition to the above, other species of Atlantic and Pacific origin were recorded sporadically in Mediterranean waters, characteristic examples are the record of *Cancer bellianus* Johnson, 1861 from the Alboran Sea (Garrido and Peña-Rivas 2016) and the record of *Paralithodes camtschaticus* (Tilesius, 1815) in the Ionian Sea (Faccia et al. 2009), both based on single specimens. Also, an unexpected finding of a *Panulirus longipes longipes* (A. Milne-Edwards, 1868) exuvia from Israel was reported recently (Spanier and Friedmann 2019). Relatedly, two non-indigenous lobster species occur on European and Mediterranean coasts, the Lessepsian *Panulirus ornatus* (Fabricius, 1798) (Galil et al. 1989) and the American lobster *Homarus americanus* H. Milne Edwards, 1837 (Stebbing et al. 2012a; Øresland et al. 2017; Pavičić et al. 2020).

The American lobster, *H. americanus*, is native to the northwest Atlantic coast. It is a closely related species, with genetic differences being present, to the European lobster *H. gammarus* (Linnaeus, 1758), that have been geographically isolated since the Pleistocene (van der Meeren et al. 2008 and references therein). *Homarus americanus* is a species with high commercial value and it is imported worldwide. In the U.S.A. alone, the landings for 2019 were reported to be more than half a billion Euros (NOAA 2020). In Singapore, one of the biggest fishery related harbours in

Asia, wild caught *H. americanus* are imported live (Yeo et al. 2011). It is known that several individuals escaped in Japan (Watabe 1993; Doi et al. 2011). In the European Atlantic, the species occurs in Norway and Sweden (Jørstad et al. 2007; van der Meeren et al. 2008; Øresland et al. 2017), and the UK (Stebbing et al. 2012a). In the Mediterranean Sea there is only one confirmed record from the Adriatic Sea, from Croatian waters (Pavičić et al. 2020).

In Mediterranean waters the lobster fishery [*H. gammarus*, *Palinurus elephas* (Fabricius, 1787) and *Scyllarides latus* (Latreille, 1803)] is mainly small-scale. All three species are not targeted; however, they are highly esteemed. For instance, in Greece the wholesale price ranges from 30 to 90 €/kg^{-1} (Kampouris et al. 2020b). *Palinurus elephas* is found across the Mediterranean Sea and adjacent Atlantic Ocean, it occurs in shallow waters up to 200 m, commonly found in hard substrates, and it is the only spiny lobster species of the eastern Mediterranean basin (Goñi and Latrouite 2005). *Homarus gammarus* inhabits the eastern Atlantic coasts and the Mediterranean Sea, it prefers hard substrates and it is territorial (Pere et al. 2019 and references therein). In fact, the region of Chalkidiki Peninsula supports a lobster fishery (Kampouris et al. 2020b). For the above reasons, a potential establishment of *H. americanus* in the region can pose some risk to the native lobster species.

The present paper reports the first record of *H. americanus* in the eastern Mediterranean Sea and the second record for the whole Mediterranean.

Materials and methods

On 26 August 2019, a single male *H. americanus* individual, was caught on the east coast of Kassandra Peninsula, Chalkidiki (39°54′50.7″N; 23°41′10.7″E), in the northwest Aegean Sea, off Greece. A 10.5-m-long plastic artisanal fishing vessel was used. Trammel nets of 5 km in length and of 50 mm in mesh size (knot to knot) were used. Small weights were placed equidistantly along the net's whole length to keep it close to the bottom. Additionally, small floats were placed equidistantly along the net's whole length to prevent possible entanglement. The soak time ranged from 10 to 12 h. The fishing depth was from 45–55 meters, over hard bottom substrate. Measurements of the carapace length, total length and wet weight were obtained. The carapace length, from the posterior margin of the eye socket to the posterior end of the carapace and the total length, from the posterior margin of the eye socket to the end of the telson, of the specimen were measured (Hepper 1966). The wet weight was measured with a digital scale to the nearest gram (Kampouris et al. 2020b). Comparisons on the morphometric characters like the absence/presence of the ventral spines at the rostrum or the overall coloration were made with a *H. gammarus* individual that was caught in the region a couple days later. The *H. gammarus* was sold by the fishermen at the local market. The specimen of *H. americanus* was caught during scientific surveys for other lobster species (*H. gammarus* and *P. elephas*).

The *H. americanus* individual was covered with a wet cloth (soaked with seawater), and transferred live with a portable cooler, then was euthanized by chilling (Butler 2017). The dissection was conducted in four steps: (1) cephalothorax isolation, (2) carapace removal from around the stomach, (3) detach and remove the stomach and (4) open the stomach (Bierman and Tobin 2009).

DNA extraction and PCR amplification

White muscle tissue was placed in 15 ml falcon tube containing 90% EtOH and stored at −20ºC. Total genomic DNA was extracted according to a phenolechloroform based protocol (Sambrook et al. 1989). PCR fragments were amplified using two universal primer pairs targeting the cytochrome oxidase subunit I (COI) gene (Folmer et al. 1994).

PCR reactions were performed in 25 ml reaction mixtures containing ~ 10 ng template DNA, 5 ml of 10X PCR buffer (Invitrogen), 2.5 mM $MgCl_2$ (Invitrogen), 0.2 μl of 10 mM each deoxyribonucleotide triphosphate (dNTPs) (Invitrogen), 0.3 μl of each 10 mM primer (Operon-Invitrogen) and 1 unit of Taq polymerase (Invitrogen). A PTC-200 thermocycler (MJ Research, Waltham, MA) was used and PCR amplification was applied under the following cycling conditions: an initial denaturation at 95 °C for 10 min followed by 35 cycles. Each cycle included the steps below: a denaturation at 95 °C for 30 s, an annealing at 53 °C for 30 s, and an extension at 72 °C for 1 min. A final extension at 72 °C for 10 min was applied. The PCR amplification products were separated in 1.5% (wt/vol) agarose gels using 1X Tris Borate EDTA (TBE) and photographed on a UV transilluminator. PCR amplification products were purified using the NucleoSpin Extract Kit (Macherey Nagel, Duren, Germany) to remove secondary metabolites prior to sequencing. All sequences were determined on an ABI PRISM® 3700 DNA Analyzer (Applied Biosystems). Each fragment used was sequenced in both directions to maximize the accuracy of the sequence, (Accession Number. No. MT551629).

Statistical analyses

Additional sequences from Nephropidae Family were derived from GenBank database, see Table 1, and the blue crab (*Callinectes sapidus* – MH235922) was inferred as outgroup. All data sets were aligned using the Clustal X v2.0 software (Larkin et al. 2007) confirmed by eye, edited and compiled using the Chromas Pro software and the extracted sequences were analysed through a BLAST search in GenBank in order to verify sequence orthology.

The kimura-2-parameter model was used through the MEGAX software (Kumar et al. 2018) to calculate nucleotide pairwise differences (bootstrap

Table 1. Taxa used for phylogenetic analyses.

Species	Genebank accession number	Reference
Eunephrops manningi	KF827998	Bracken-Grissom et al. 2014
Acanthacaris caeca	KF828007	Bracken-Grissom et al. 2014
Acanthacaris tenuimana	KF828006	Bracken-Grissom et al. 2014
Nephropides caribaeus	KF827999	Bracken-Grissom et al. 2014
Callinectes sapidus	MH235922	Aguilar et al. 2013
Nephropsis atlantica	JQ305976	Matzen da Silva et al. 2011
Nephropsis rosea	KF827997	Bracken-Grissom et al. 2014
Metanephrops boschmai	EU186141	Chan et al. 2009
Metanephrops armatus	KF828002	Bracken-Grissom et al. 2014
Nephrops norvegicus	JQ306237	Matzen da Silva et al. 2011
Homarus americanus	MH300656	Spielmann et al. 2019
Homarus americanus	**MT551629**	**This study**
Homarus americanus	MN654341	Pavicic et al. 2020
Homarus gammarus	KT208429	Raupach et al. 2015
Metanephrops challengeri	HQ944636	iBOL Data Release
Dinochelus ausubeli	KF278680	Chang et al. 2014
Thaumastocheles massonktenos	KF604906	Chang et al. 2014
Thaumastocheles bipristis	KF278685	Chang et al. 2014

replicates: 10000 with uniform rates). To infer phylogenetic relationships, a main Bayesian inference model-based method was used. MrBayes v.2.01 software was used for Bayesian inference (Huelsenbeck and Ronquist 2001). The data set was explored using four chains: one cold chain and subsequently three incrementally heated ones by temperature set at 0.20. A GTR model of sequence evolution was employed allowing a gamma shape of among-site rate variation. The GTR model usually fits real data better than the other (simpler) alternative models (Sumner et al. 2012). Through the evolution of speciation, nucleotide substitutions are not at equilibrium, thus the GTR model predicts better this complexity. Posterior probability distributions were obtained for the phylogenies and the parameters of the model of sequences' evolution were adjusted; random trees were used as seeds. Tree spaces were explored inferring 1,000,000 generations, with 100 generations sampled each time and the burn-in was set to 1000.

Results

The caught male lobster in view weighted 628.1 g and measured 96.7 mm in carapace length (CL), 314.4 mm in total length (TL). The overall coloration was dark-greenish to black, with some orange patches being present. The uropods and telson were mostly orange with some dark, almost black-coloured blotches (Figure 1). The rostrum had two ventral spines (Figure 2A). In contrast, the rostrum of *H. gammarus* usually has no ventral spine (Figure 2B). Furthermore, the dorsal spines on both rostrum and chelipeds were of orange-reddish coloration (Figure 2C) while those of *H. gammarus* are always white (Figure 2D) (Jørstad et al. 2007). The *H. americanus* individual was dissected, and its foregut was extracted to obtain the stomach content for further molecular analysis. The foregut was empty, and no material was obtained.

Figure 1. The male American lobster *H. americanus* caught in the Aegean Sea. Photograph by the first author.

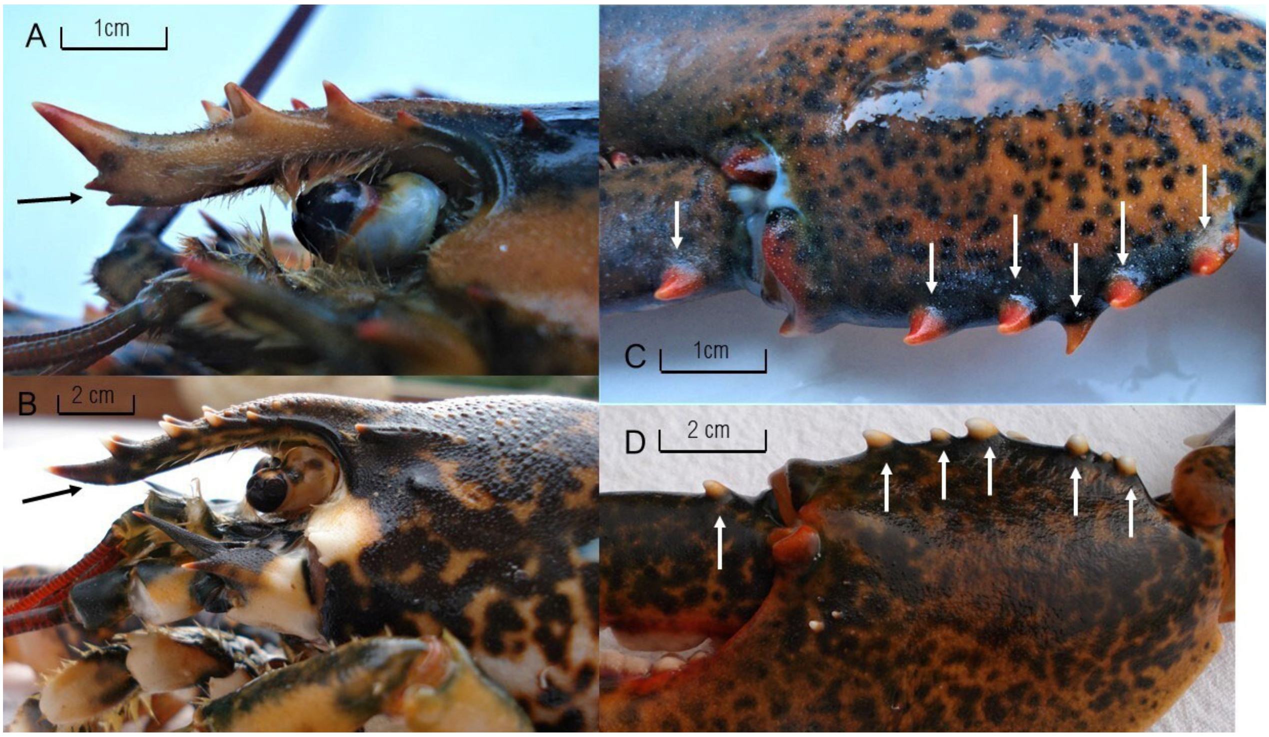

Figure 2. A) ventral spines, marked with pointing arrow, at the rostrum of the caught American lobster; B) in European lobster, ventral spines at the rostrum are usually absent marked with pointing arrow, this specimen was caught at the same region; C) reddish spines of the chelipeds, marked with pointing arrows, of the caught American lobster; D) white spines of the chelipeds, marked with pointing arrows of the European lobster that was caught at the same region. Photographs by the first author.

The aligned cytochrome oxidase subunit I sequence revealed a length of 595 bp (Genbank accession number: No. MT551629). The kimura 2-parameter model (Table 1) and phylogenetic analyses (Figure 3) revealed that our specimen is 100% identical with *Homarus americanus*.

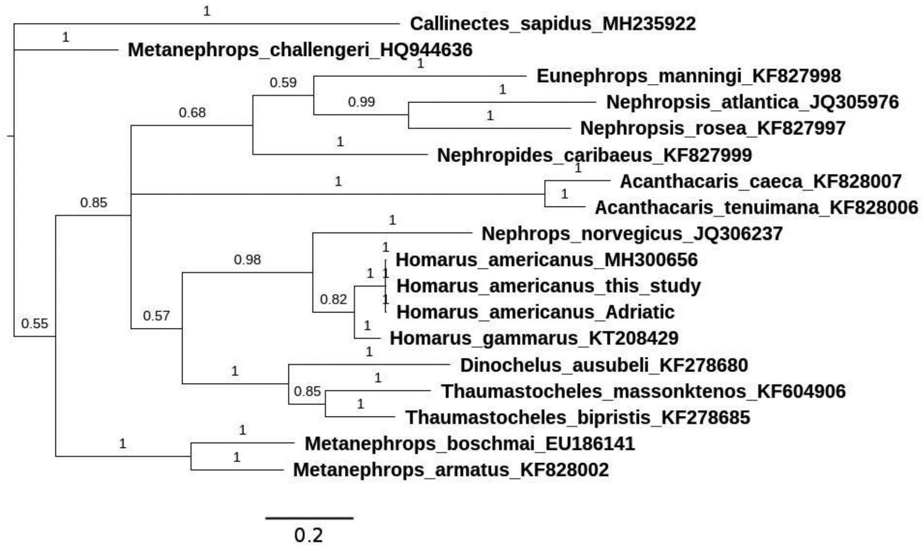

Figure 3. Topology tree for COI gene of different crustacean species within Nephropidae family. *Callinectes sapidus* – MH235922, was inferred as outgroup. Numbers shown next to the nodes indicate Bayesian Inference posterior probabilities.

Discussion

The collected specimen is the first record of the American lobster, *H. americanus*, from eastern Mediterranean waters. Lacking evidence, we cannot speculate on specific pathway of introduction. However, a likely vector is the seafood industry, as has occurred in Japan and Singapore, (Doi et al. 2011; Yeo et al. 2011). An intentional release from a private aquarium cannot be ruled out, but this species is not very popular among hobbyists, making this method unlikely. Lobster introductions through the aquarium industry are unusual, with an introduction of the spiny lobster *Panulirus versicolor* (Latreille, 1804) in Georgia, USA, being a rare example (Page 2013). Also, other human facilitated activities such as larvae transportation through ballast water cannot be excluded, especially since other decapod species of Atlantic origin are introduced in the Aegean Sea (Kampouris et al. 2018 and references therein).

If this is a persistent introduction, and not just an isolated incident, there are substantial ecological risks involved. *Homarus americanus* may negatively impact Mediterranean biodiversity and affect ecosystem services (Katsanevakis et al. 2014). The species is documented to be able to hybridize with the European lobster *H. gammarus*, (Jørstad et al. 2007). Furthermore, *H. americanus* can prey on the European species (Øresland et al. 2017), are more aggressive, more tolerant of varying physical conditions, and more flexible in habitat use (Stebbing et al. 2012a; Jørstad et al. 2007). However, while both species compete for habitat (Stebbing et al. 2012a; Øresland et al. 2017), *H. americanus* does more commonly select cobble reefs mixed with unvegetated sediment (Selgrath et al. 2007) and even though there are no confirmed effects on spiny lobster species, e.g., *P. elephas*, some potential negative agonistic impacts can occur, since it is known that American lobsters threatened and attacked the California spiny lobster, at least under laboratory conditions (Krekorian et al. 1974). Finally, there is a risk of introduction of associated microbes into the region, such as the bacterium *Aerococcus viridans* var. *homari* was introduced by American lobsters in east Atlantic waters, infected European lobster and caused mortality events (Stebbing et al. 2012b).

American lobsters are both nomadic and shelter-using (Scopel et al. 2009). Therefore, they are not easy to detect. The region off Chalkidiki Peninsula supports important lobster fishery (Kampouris et al. 2020b) and more recently, systematic monitoring was taking place. Perhaps the increased intensity of continuous sampling contributed positively in revealing the caught specimen. To a keen and trained eye, the morphological differences between the two homarids are evident. Nonetheless, those differences might not be profound to people not trained in morphology, increasing the probability of species misidentification. The current finding is alarming. For the time being, there is no evidence of the establishment of a population of American lobsters in the region. The regional fishermen at Chalkidiki Peninsula should be informed about the potential negative effects of the American lobster and they should understand – through dialogue, that the species should not act as a new fishery resource.

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