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Notes on the distribution and biology of northern brown shrimp *Farfantepenaeus aztecus* (Ives, 1891) in the eastern Mediterranean

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Abstract: From November 2013 to March 2014, 14 female specimens of the nonindigenous penaeid species northern brown shrimp *Farfantepenaeus aztecus* were caught in Thermaikos Gulf and Nestos estuaries (northern Aegean Sea), as well as one male in the Argolikos Gulf (central Aegean Sea) and 1 female in the Ionian Sea, further confirming their expansion range in the eastern Mediterranean Sea. Along with the external morphology of the specimens, sequencing analysis of the 16S rDNA gene of mtDNA confirmed the species identification. Recognition of large mature individuals strongly supports that the already established species in the Greek seas could be efficiently reproduced.

Key words: Northern brown shrimp, *Farfantepenaeus aztecus*, eastern Mediterranean, sequencing, 16S rDNA

1. Introduction

In the northwestern Atlantic, *Farfantepenaeus aztecus* (Ives, 1891), *F. brasiliensis* (Latreille, 1817), and *F. duorarum* are among the most commercially important shrimp catches (Diamont, 2004). Among them, the northern brown shrimp *F. aztecus* is the most abundant decapod along the eastern coast of the United States (Texas coast, the southwestern Bay of Campeche, and off North Carolina), with catches exceeding those of other penaeids taken in the United States (Tavares, 2002). The northern brown shrimp is an estuarine and oceanic littoral decapod, found from the coastline to depths of about 110 m (occasionally in deeper waters to 165 m). It is naturally distributed along the western Atlantic: along the Atlantic coast of the United States from Martha's Vineyard, Massachusetts, and around the Florida Peninsula to Texas, around the Gulf of Mexico to northwestern Yucatán (Tavares, 2002). Its maximum total length is 236 mm for females and 195 mm for males (Holthuis, 1980; Tavares, 2002).

Until 1939, *F. aztecus* was not distinguished from the other eastern American species of the genus *Farfantepenaeus*, all of which were indicated as *Penaeus brasiliensis* (Latreille, 1817). However, in 1967, *P. aztecus* was divided into 2 species (*P. aztecus* and *P. subtilis*) (Holthuis, 1980), which are now considered as valid *F. aztecus* and *F. subtilis*, respectively (Tavares, 2002).

The presence of the northern brown shrimp in the Mediterranean Sea was recorded for the first time by Deval et al. (2010) and afterwards by Gökoğlu and Özvarol (2013), based on specimens collected in the southeastern region from the gulfs of Finike, Antalya, İskenderun, Mersin, and Yumurtalık (Turkey). With the present report, *F. aztecus* is the second Atlantic decapod crustacean that has been reported in the Thermaikos Gulf (northern Aegean Sea), after the blue crab *Callinectes sapidus* Rathbun, 1896, and the new species increases to 28 the number of alien decapod crustaceans (21 Indo-Pacific, 7 Atlantic species) in the entire Aegean Sea (Kapiris et al., 2012). The increase of marine nonindigenous species in the eastern Mediterranean, and therefore in the Aegean Sea, is certainly attributable to an increase of human activities (shipping transport), but it is also a consequence of climate change.

The present study gives an updated report on the distribution and biology of the northern brown shrimp in the eastern and central Mediterranean Sea, together with some new data on its occurrence in the northern and central Aegean Sea. In addition to the above data, 16S rRNA sequencing analysis was also used for identity confirmation of the species.

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2. Materials and methods

From November 2013 to March 2014, 14 female individuals (Figure 1a) were collected through bottom trawling performed by professional fishing vessels at a depth of 45–50 m in the Thermaikos Gulf (40°21′N, 22°47′E) and 7 nautical miles from the Nestos River estuaries (40°50′N, 24°58′E), northern Aegean Sea, Greece. In addition, one male was collected by a bottom trawler in the Argolikos Gulf (Ermioni area, central Aegean Sea) (Y Kotzamanis, personal communication) and one female in the Ionian Sea (Kapiris and Apostolidis, 2014).

The specimens were identified according to Pérez Farfante (1988), Pérez Farfante and Kensley (1997), and Tavares (2002).

Carapace length (*CL*) was measured in all specimens to the nearest 0.01 mm, from the posterior margin of the orbit to the posterior hind edge of the cephalothorax, using electronic calipers. Some other morphometric characteristics such as total length, body length, and rostrum length were taken for the individuals caught in the northern Aegean Sea. Total length (*TL*) was measured as the linear distance from the tip of the rostrum to the posterior margin (end) of the telson, body length (*BL*) as the distance from the tip of the carapace (without rostrum) to the end of the telson, and rostrum length (*RL*) as the distance from the tip of the rostrum to the orbital margin of the carapace. Additionally, the total wet weight (*W*) was measured to the nearest 0.01 g. Maturity stage (MS) was determined by macroscopic observations of the ovaries as follows and was classified as one of the stages I–VII proposed by Renfro and Brusher (1963): Stage I: undeveloped ovaries; Stage II: early developed; Stage III: developed; Stage IV: late developed; Stage V: ripe; Stage VI: spent; Stage VII: resting.

Sequencing analysis of the mtDNA 16S rDNA gene from 2 specimens caught in the Thermaikos Gulf was used for the taxonomic identity confirmation of the studied decapods. Total DNA was extracted from muscle according to Hillis et al. (1996). A universal primer set (Palumbi, 1996) was used for the amplification of the 16S rDNA gene. The reaction mixture contained template DNA (approximately 100 ng), 1X PCR buffer, 2.2 mM $MgCl_2$, 20 pmol of each primer, 0.25 mM of each dNTP, 0.5 U of Taq DNA polymerase (New England Biolabs, Inc., UK), and 19.6 µL of ddH_2O. Amplification was started at 94 °C for 3 min, followed by 31 cycles at 94 °C for 50 s, 49 °C for 50 s, and 72 °C for 50 s, and a final extension at 72 °C for 5 min. Electrophoresis of 3 µL of the PCR product was performed in 1X TBE buffer for 1 h at 150 V, in 1.5% agarose gel (AppliChem GmbH, Darmstadt, Germany) containing 0.5 µg/mL ethidium bromide (Merck KGaA, Darmstadt, Germany). The resulting PCR products were visualized by UV transillumination and photographed. PCR products (without purification) were sent to VBC (Vienna, Austria) for bidirectional sequencing. The returned nucleotide sequences of the 2 individuals were aligned using Clustal X software (Thompson et al., 1997) and BioEdit software (Hall, 1999).

The final dataset included in total 13 sequences from different *Farfantepenaeus* species (Table 1). Species *Palaemon elegans* was used as an outgroup. Phylogenetic relationships were estimated with MEGA6 software (Tamura et al., 2013), using the maximum likelihood method. The best-fit substitution model (T92 + G)

Table 1. Sequences used for phylogenetic analysis in the present study.

Abbreviation	Species	GenBank accession no.	References
Fa1	*Farfantepenaeus aztecus*	AF192051.1	Maggioni et al. (2001)
Fa2	*Farfantepenaeus aztecus*	HQ214010.1	Unpublished
Fa3	*Farfantepenaeus aztecus*	HM014401.1	Alvarado Bremer et al. (2010)
Fa4	*Farfantepenaeus aztecus*	KF953962.1	Nikolopoulou et al. (2013)
Fd1	*Farfantepenaeus duorarum*	HQ214013.1	Unpublished
Fd2	*Farfantepenaeus duorarum*	HQ214011.1	Unpublished
Fd3	*Farfantepenaeus duorarum*	AF192055.1	Maggioni et al. (2001)
Fd4	*Farfantepenaeus duorarum*	AF279812.1	Lavery et al. (2004)
Fs1	*Farfantepenaeus subtilis*	AY344194.1	Unpublished
Fs2	*Farfantepenaeus subtilis*	AF192068.1	Maggioni et al. (2001)
Fs3	*Farfantepenaeus subtilis*	AF192062.1	Maggioni et al. (2001)
Fs4	*Farfantepenaeus subtilis*	AF192061.1	Maggioni et al. (2001)
Fa-Gr	*Farfantepenaeus aztecus*	KF983532.1	Present study
Pe	*Palaemon elegans*	HE573180.1	Reuschel et al. (2010)

was provided by the MEGA6 software (Hall, 2013). Nonuniformity of evolutionary rates among sites was modeled using a discrete Gamma distribution (+G) with 5 rate categories and by assuming that a certain fraction of sites were evolutionarily invariable (+*I*). The parameters of this model were: nucleotide frequencies A = 33.5%, C = 16.5%, G = 16.5%, T = 33.5%, +G = 0.24, R = 3.56. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. Branch support was assessed by 1000 replicates, and sites with missing data were removed only as the need arose.

The specimens of *F. aztecus* from the northern Aegean Sea were deposited in the ichthyological collection of the Alexander Technological Educational Institute of Thessaloniki, Department of Fisheries and Aquaculture Technology (catalogue numbers 2013-010 to 2013-023).

3. Results

3.1. Morphological description

Carapace smooth. Rostrum armed with 8 to 10 dorsal teeth, the epigastric tooth included, and 2 ventral teeth (Figure 1b); the ratio of rostrum length to carapace length (*RL/CL*) = 0.44–0.61 (0.51 ± 0.012). Gastrofrontal carina present and not turning anterodorsally (Figure 1b). The sixth abdominal somite bearing on each side 3 cicatrices (Figure 1a). Last (sixth) abdominal somite with

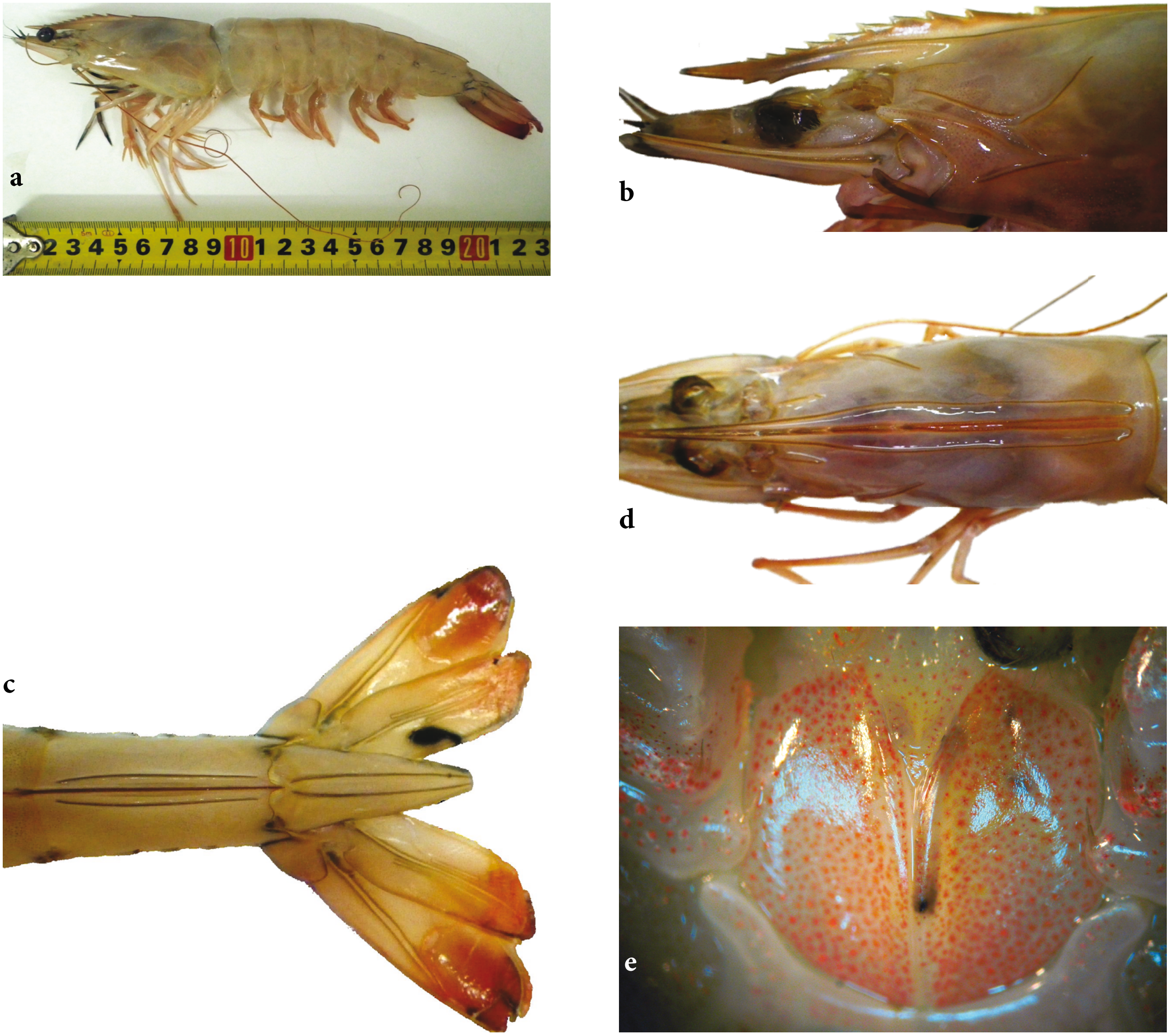

Figure 1. a) Northern brown shrimp *Farfantepenaeus aztecus* (Ives, 1891); **b)** lateral view of armed rostrum; **c)** dorsal view of telson; **d)** dorsal view of grooved carapace; **e)** thelycum.

a well-defined dorsolateral groove on either side (Figure 1a) of dorsal keel (Figure 1c). Dorsolateral sulcus on last abdominal segment well-defined and broad (Figure 1c). Telson unarmed, without lateral spines (Figure 1c). Postrostral carina was long, extending almost to the posterior margin of carapace framed by a deep adrostral sulcus and a long carina (Figure 1d). Thelycum with pair of lateral plates, their anteromedian angles divergent; posterior process armed with a median crest bifurcate anteriorly (Y-shaped) and exposed (Figure 1e). Orange to yellowish color; pereopods and tail fan darker; uropods with a purple edge. No dark lateral spot at junction of third and fourth abdominal segments, as in *F. brasiliensis* or *F. duorarum* (Tavares, 2002).

3.2. Measurements

Data on the measurements obtained from the 16 specimens of *F. aztecus* are shown in Table 2. *TL* ranged from 177 to 216 mm (204.4 ± 2.8) (mean ± standard error), *BL* ranged from 158 to 190 mm (179 ± 2.3), *CL* ranged from 41 to 54 mm (49.6 ± 0.9), *RL* ranged from 21 to 32 mm (25.6 ± 0.8), and total weight ranged between 47.27 and 138.42 g (76.85 ± 4.8).

3.3. Gonad maturity stage

Females caught during late November (n = 4, Thermaikos Gulf; n = 1, Ionian Sea) were in late developed (n = 3, Stage IV), resting (n = 1, Stage VII), or developed (n = 1, Stage III) stages (Kapiris and Apostolidis, 2014). The male caught in November (n = 1, Argolikos Gulf) was mature (Stage II), having hemipetasmata joined to each other by means of interlocking hooks. Females caught during early December (Nestos estuaries) had late developed (n = 1, Stage IV) or ripe (n = 2, Stage V) ovaries. Both females found in the Thermaikos Gulf in late December (No. 9) and early January (No. 10) were in the spent stage (n = 2, Stage VI). Females caught in late February and early March (Thermaikos Gulf) were in the early developed maturity stage (n = 5, Stage II) and had not yet mated.

3.4. Genetic analysis

The size of the PCR products was checked against a 100-bp DNA ladder and was approximately 540 bp. In total, 522 bp at the 5′ end of the mtDNA 16S rDNA gene for the 2 individuals were sequenced. The specimens revealed an identical haplotype, which was deposited in GenBank (accession number: KF983532.1).

Table 2. Morphometrical data of the specimens of *Farfantepenaeus aztecus* (Ives, 1891) caught in the Aegean Sea (northern and central) and Ionian Sea.

Sampling date	Sampling area	Sex	Total length (mm)	Carapace length (mm)	Body length (mm)	Rostrum length (mm)	Total weight (g)	Maturity stage
Nov 2013	Ionian Sea	F	-	53	-	-	138.42	III
24 Nov 2013	N Aegean Sea	F	203	51	177	26	77.70	IV
24 Nov 2013	N Aegean Sea	F	206	50	184	22	78.45	IV
24 Nov 2013	N Aegean Sea	F	211	52	185	26	81.44	IV
24 Nov 2013	N Aegean Sea	F	216	51	190	26	71.51	VII
1 Dec 2013	N Aegean Sea	F	191	41	168	23	62.08	IV
1 Dec 2013	N Aegean Sea	F	195	46	170	25	63.40	V
1 Dec 2013	N Aegean Sea	F	203	50	179	24	72.31	V
22 Dec 2013	N Aegean Sea	F	177	42	158	21	47.27	VI
6 Jan 2014	N Aegean Sea	F	207	51	183	24	75.06	VI
Nov 2013	C Aegean Sea	M	-	45	-	-	62.80	II
28 Feb 2014	N Aegean Sea	F	213	50	188	25	73.33	II
9 Mar 2014	N Aegean Sea	F	214	54	183	31	85.58	II
9 Mar 2014	N Aegean Sea	F	209	52	185	24	84.03	II
9 Mar 2014	N Aegean Sea	F	209	53	180	29	82.89	II
9 Mar 2014	N Aegean Sea	F	208	52.5	176	32	73.34	II
	Mean value		204.4	49.6	179	25.6	76.85	
	Standard deviation		10.49	3.95	8.72	3.18	19.20	
	Standard error		2.804	0.989	2.33	0.85	4.799	

There were a total of 404 positions in the final dataset. The maximum likelihood topology (Figure 2) revealed three different clusters: the first includes all the *F. duorarum* sequences, the second includes the *F. subtilis* individuals, and the third includes the *F. aztecus* sequences. The sequence of the Thermaikos Gulf individual (Fa-Gr) was grouped with the *F. aztecus* individuals in the last clade (Figure 2).

4. Discussion

A total of 10 nonindigenous crustacean species (3 Dendrobranchyata, 1 Caridea, and 6 Brachyura) have been reported only from the Greek coasts of the Aegean Sea. Almost half of them (*Synalpheus tumidomanus africanus*, *Percnon gibbesi*, *Calappa pelii*, and *Sirpus monodi*) have an Atlantic origin, while the rest (*Metapenaeopsis aegyptia*, *Metapenaeopsis mogiensis consobrina*, *Trachysalambria curvirostris*, *Coelusia signata*, *Myra subgranulata*, and *Gonioinfradens paucidentatus*) have an Indo-Pacific origin (Kapiris et al., 2012).

In the study areas, the identification of the individuals of *F. aztecus* is indubitable due to the combination of a) the use of the 16S rDNA gene, which is a very good species-specific marker, according to Perez et al. (2005); b) the study of the morphological characteristics; and c) the identical period of gonadal maturation of the studied specimens as others of the same species as those mentioned in the bibliographical resources could also confirm the correct identification of the species. Indeed, specimens captured from the Thermaikos Gulf (Fa-Gr) clustered with the *Farfantepenaeus aztecus* sequences (Figure 2). Thus, the genetic analysis confirmed the morphological identification of the species captured in the Thermaikos Gulf.

A significant increase of the population's abundance and an increasing expansion rate of the studied species are shown in the eastern part of the Mediterranean basin. Specifically, only 12 individuals of *F. aztecus* were caught in the Gulf of Antalya during 2010 (Deval et al., 2010), while its abundance has increased in southern Turkey within 3 years (Gulf of İskenderun to the east and Finike to the west), where around 1600 individuals were caught (Gökoğlu and Özvarol, 2013). In addition, the presence of *F. aztecus* has also been recorded recently elsewhere in the Mediterranean Sea—for example, in the Adriatic Sea, e.g., Montenegro (Markovic et al., 2014), the Ionian Sea, off Corfu Island (Kapiris and Apostolidis, 2014), and the northern Aegean Sea (Nikolopoulou et al., 2013).

Perhaps the presence of the northern brown shrimp in the northern Aegean could be dated back 4 to 5 years, since local professional fishermen and fish traders (V Stamidis, personal communication) have dated its presence in commercial shrimp catches since late 2010. In these last years, its abundance was very low and only a few large individuals were caught, according to the local fishermen, in comparison to the larger number of other decapods caught, like *Parapeneus longirostris* and

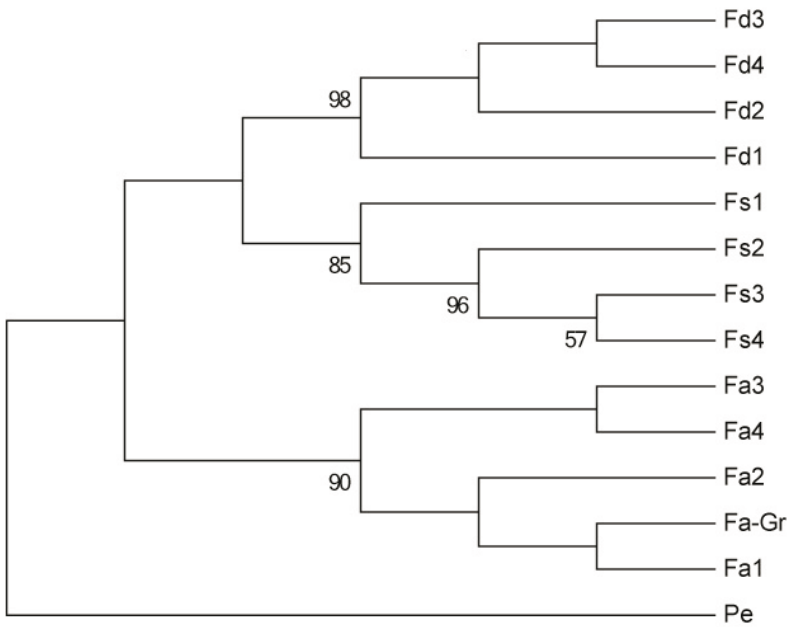

Figure 2. Maximum likelihood tree from 16S rRNA data under the best-fitting model T92 + G. Numbers above branches indicate bootstrap values among 1000 replicates. Branches without bootstrap numbers mean that the bootstrap values are below 50%.

Melicertus kerathurus. In addition to this, the presence of the northern brown shrimp in the Ionian Sea has been almost contemporaneously reported, according to the local fishermen, as in the Aegean Sea. According to fishermen, the highest catches of *F. aztecus* occurred during October and November each year; it is worth noting that adult northern brown shrimp have been caught by bottom trawling at depths of 40–45 m in the Thermaikos Gulf and by net in shallow waters at depths of 10–15 m in the Gulf of Thessaloniki (inner part of the Thermaikos Gulf).

Based on our results, it seems that the reproductive period of the species *F. aztecus* in the northern part of the Aegean Sea is during late autumn–early winter, a fact that agrees with previously reported data in other areas (Brown and Patlan, 1974; Renfro and Brusher, 1982). Indeed, this species is known to have an extended spawning season that is likely to vary in different geographic areas of its range. Gonads mature in August and become opaque white, yellow, or tan in color when they are fully ripe (Brown and Patlan, 1974). Its spawning occurs offshore at depths that generally exceed 18 m (Larson et al., 1989). The same reproductive period of this species is also reported in the northwestern part of the Gulf of Mexico, where Renfro and Brusher (1982) reported that at a sampling depth of 46 m the spawning peak was found to occur in October through December. In the above area, males were distributed over a broader area and further offshore than were females, though differences in spatial distribution between the sexes were not large (approximately 10%–15%) (Craig et al., 2005). This partially different distribution could be responsible for the predominance of females in our samples.

According to Deval et al. (2010), ballast waters are the most likely vector for the species' introduction to the eastern Mediterranean. Since the species has not been reported previously in the western Mediterranean or the eastern Atlantic, range expansion through the Gibraltar Strait cannot be considered as a possible vector. The scenario of ballast water transportation could also be accepted for the study area, since two large, busy ports with dense maritime traffic are located in the Thermaikos Gulf, and Indo-Pacific Ocean fish species have also been reported from this area (Minos et al., 2012).

The conditions surrounding the natural spread of *F. aztecus* and the initial indications of its establishment in the eastern Mediterranean suggest that favorable conditions in the area may facilitate the permanent settlement of this invasive species, whose presence could possibly be explained as a result of ballast water transportation. Moreover, the identification of mature individuals in both regions of the northern Aegean Sea is a strong indication that local conditions actually permit oogenesis and production of mature oocytes. However, if its establishment in the eastern Mediterranean from an accidental transfer is considered the most likely scenario, combined with the lack of reports from the eastern Atlantic, then the above would indicate lack of the ability of the species to expand its distribution range naturally over long distances. The above deduction could partially explain the discontinuous presence of the species.

In Antalya Bay, the continuous catches of *F. aztecus* through the years (Gökoğlu and Özvarol, 2013) following the first report in 2010 (Deval et al., 2010) could suggest the existence of an established population. In the present study, the large size (CL ≈ 50 cm) and the small number of *F. aztecus* individuals appearing in catches over the last 3 years (V Stamidis, personal communication) lead also to the hypothesis of an established population of the species in the northern Aegean Sea. Since *F. aztecus* supports an important fishery in the western Atlantic area (Tavares, 2002), perhaps following its successful adaptation in the Mediterranean Sea, an inshore sustainable shrimp fishery could be developed in the future. In this case, scientific knowledge on the biology of this species could support management measures of the stock in the coming years to ensure the sustainable exploitation of its stocks. Further studies are necessary in order to clarify aspects of its life span and the relationships between this nonindigenous decapod species and native species.

Finally, the present report of the new species reinforces the continuous entry of invasive alien species into the Mediterranean basin, influencing its biodiversity and relationships with indigenous species. The development of alien species distribution models under present and future climatic conditions is of crucial importance.

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