

***Atyaephyra tuerkayi* Christodoulou et al., 2012 and *Palaemon turcorum* (Holthuis, 1961) (*Decapoda*, *Caridea*) in the Middle Eastern Region of the Mediterranean**

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Abstract

The real distribution range of a species is mostly unknown due to the lack of sampling in certain regions in the world. This may be especially problematic for freshwater species in the Mediterranean basin since its freshwater inhabitants are taxa highly sensitive to anthropogenic disturbances. The cytochrome oxidase subunit 1 (COI) gene in *Atyaephyra* recognized seven *Atyaephyra* species in the Mediterranean basin in recent years. However, the lack of sampling in the Middle East does not help to clarify what *Atyaephyra* species are present in this region and their distributions. The most extended species in the area is *A. orientalis*, while *A. tuerkayi* has been found only in the River Nahr Al-Kabir, which is located along the borders of Lebanon with Syria. DNA barcodes of *Atyaephyra* specimens collected in this study confirm the first record of *A. tuerkayi* in Turkey. In addition, *Palaemon turcorum*, an endemic species to the Sakarya River Basin which flows into the Black sea was collected outside this basin, in rivers of the Mediterranean coast. The fact that the Middle East has not been well surveyed for shrimp could explain the unknown distribution of these species.

Introduction

Most biodiversity is unknowable because of: the regions in the world that have not been surveyed well, the relative neglect of taxonomy, the growing evidence of multiple cryptic species, and the high predicted rate of extinction has not allowed the scientific communities to know the former fauna (e.g.: Barnosky *et al.*, 2011; Macpherson & Machordom, 2005). Regional ecosystems such as estuaries and coastal communities are rapidly losing populations, species, or entire functional groups (Lotze *et al.*, 2006; Jackson *et al.*, 2001). Habitat loss and alteration, excessive nutrient and sewage inputs, chemical contaminations, and overfishing are traditional anthropogenic activities which have a high impact on inland and transitional waters (Kennish, 2002). However, freshwater transfers between basins are turning into a problem, especially in the ariddest Mediterranean regions, where extraordinary

droughts periodically occur (Baptista *et al.*, 2010; Cyrus *et al.*, 2011). Man-made reservoirs modify natural river discharges, affecting physicochemical conditions of the estuaries and the dynamic population of the species (González-Ortegón *et al.*, 2016), which is critical to survival and meta-population dynamics (Fahrig & Merriam, 1985; Levins, 1970).

Atyaephyra de Brito Capello, 1867 is the most widespread atyid taxon in the Mediterranean region with its native range spanning from the Middle East to North Africa, a large part of Southern Europe, and to some Mediterranean islands (d'Udekem d'Acoz, 1999). Currently, this genus is represented by 7 valid species, 8 according to WoRMS (<http://www.marinespecies.org/aphia.php?p=taxdetails&id=234114>, August 24, 2020), because they consider *A. acheronensis* Christodoulou, Antoniou, Magoulas & Koukouras (2012) as a valid species, although García Muñoz *et al.* (2014) showed that it is a synonym of *A. desmarestii* (Millet, 1831). The

other 6 valid species are *A. stankoi* Karaman, 1972; *A. orientalis* Bouvier, 1913; *A. thyamisensis* Christodoulou, Antoniou, Magoulas & Koukouras, 2012; *A. strymonensis* Christodoulou, Antoniou, Magoulas & Koukouras, 2012; *A. tuerkayi* Christodoulou, Antoniou, Magoulas & Koukouras, 2012; and *A. vladoi* Jabłońska, Mamos, Zawal & Grabowski, 2018.

These species typically inhabit headwaters and the middle stretch of rivers, but seasonal river discharges typical of Mediterranean basins can displace some populations towards the oligohaline zones of estuaries (González-Ortegón *et al.*, 2015; Cuesta *et al.*, 2006) and they could be coexisting with freshwater and/or brackish water species of the genus *Palaemon*. These freshwater shrimps are sensitive to the water oxygenation level (González-Ortegón *et al.*, 2012) and the human impacts on their habitat, especially in the Mediterranean region, making these species highly susceptible to the risk of extinction. However, the extent of this species wider distribution in the Mediterranean region is unknown despite the recent effort to recognize and delimit species within *Atyaephyra* due to the high number of cryptic species (García Muñoz *et al.*, 2009, 2014; Christodoulou *et al.*, 2012; Jablonska *et al.*, 2018). The Middle Eastern region is one example where the lack of surveys does not allow accurate knowledge concerning the distribution of the *Atyaephyra* species (De Grave, 2013). For example, until now only *A. orientalis* Bouvier, 1913 had been recorded in Turkey (Özbek *et al.*, 2004, 2009; Özbek & Ustaoglu, 2005) and

thus detailed studies of the *Atyaephyra* populations in the Middle East are necessary to clarify the number of *Atyaephyra* species in this region, considering the new advances in the composition of the species of this genus (García Muñoz *et al.*, 2009, 2014; Christodoulou *et al.*, 2012; Jablonska *et al.*, 2018).

The aim of this study is to provide knowledge on the current geographic distribution of the *Atyaephyra* species in the Middle Eastern region. We hypothesized that the restricted geographical distributions where *A. thyamisensis*, *A. strymonensis*, and *A. tuerkayi* had already been described, did not reflect the real distribution of these species, and surely some of them inhabited nearby regions, as already proved for *A. thyamisensis* reported in Albania and Macedonia (Jablonska *et al.*, 2018).

Materials and Methods

Specimens of the genus *Atyaephyra* were collected in several estuaries and lakes from the coastal waters of Antalya (Turkey) using nets. In November 2008, 46 specimens of *Palaemon turcorum* (Holthuis, 1961) and 20 specimens of the genus *Atyaephyra* were collected from the Yamansaz coastal lake (Kemeragzi, Turkey) and 20 specimens of *P. turcorum* and 48 of *Atyaephyra* sp. were collected from Kirkgöz spring lake (Döşemealti, Antalya, Turkey). These specimens were preserved in 5% buffered formalin (Figure 1; Table 1).

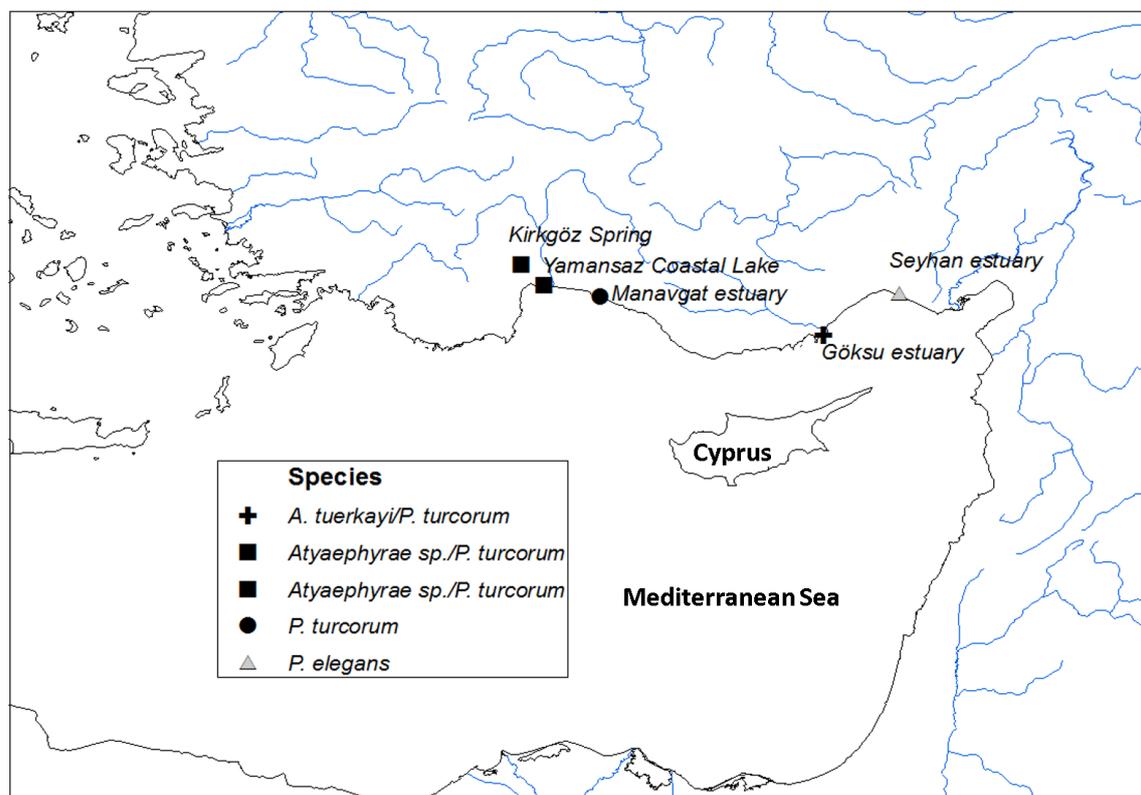


Figure 1. Sampling sites and caridean shrimp species found in the middle eastern region. Cross, square, circle, and triangle points indicate sites where, respectively, the species *A. tuerkayi/Palaemon turcorum*, *Atyaephyra* sp./*P. turcorum*, *P. turcorum*, and *Palaemon elegans* have been detected in this study.

Table 1. Locations, year, depth, salinity, temperature, and environmental conditions of shrimp sampling surveys conducted along the Southern coast in Turkey between 2008 and 2015

Locality	Latitude	Longitude	Year(s)	Depth Range (m)	Salinity	Water Temperature (°C)	Habitat Type
Göksu estuary	36°17'46.01"N	34° 2'41.30"E	2014, 2015	<1	1-26	9-20	mud and long dense eelgrass
Kirkgöz spring	37° 6'30.67"N	30°34'58.54"E	2008	<1	<1	12-20	Gravel and riparian vegetation
Yamansaz coastal lake	36°52'17.39"N	30°50'30.26"E	2008	<1	<1	14-20	Gravel with green algae
Manavgat estuary	36° 44'19.34"N	31°29'23.95"E	2015	<1	1-26	9-20	mud and long dense eelgrass
Seyhan estuary	36° 46'43.18"N	34°54'39.91"E	2015	<1.5	1-26	9-20	mud and long dense eelgrass

Between 2014 and 2015, 6 and 7 specimens of *P. turcorum* were collected from the Manavgat and Göksu river estuaries, respectively, 6 specimens of *Palaemon elegans* Rathke, 1837 from the Seyhan River and 2 specimens of *Atyaephyra* sp. from the Göksu River, which were preserved in analytical grade 96% ethanol.

The specimens of *Atyaephyra* sp. from Turkey used in the present study were morphologically identified according to Christodoulou *et al.* (2012) and photographed (Figure 2). Two specimens were deposited in the Decapoda and Stomatopoda Crustacean Collection (Spanish Institute of Oceanography in Cadiz, Spain) under the catalog numbers IEO-CD-CCDE15/1475 and 1476.

Total genomic DNA was extracted from pleon muscle tissue of one male specimen of *Atyaephyra* sp., collected from the Göksu estuary (2015), following a modified Chelex 10% protocol by Estoup *et al.* (1996). Partial sequences of the mitochondrial 16S and COI genes were amplified. Cycling conditions of the polymerase chain reaction (PCR), length of the sequences obtained, and primers used for each gene are the same as those in previous works (see Perez-Miguel *et al.*, 2019). PCR products were sent to Stab-Vida laboratories to be purified and then bi-directionally sequenced. The sequences were edited using the software Chromas v. 2.0, and assembled in Bioedit (Hall, 1999). The final DNA sequences obtained were compared with those of the *Atyaephyra* species retrieved from the GenBank database.

New sequences of 16S and COI are deposited in GenBank under the accession numbers MW020260 and MW020377, respectively.

Results

Taking into account the descriptions of the *Atyaephyra* species (García Muñoz *et al.*, 2014; Christodoulou *et al.*, 2012; Jablonska *et al.*, 2018) from the Mediterranean region and comparing them with the specimens of *Atyaephyra* collected from the Yamansaz coastal lake and Kirgöz Spring, these look morphologically more similar to *A. orientalis*, and those from the Göksu estuary to *A. tuerkayi* (Figure 2). Although, it is worth bearing in mind, and given the morphological variability within the genus *Atyaephyra*, that other species of this genus should not be discarded (Figure 2). The main differences were observed in the rostrum, 20 specimens (mainly females and some indeterminate individuals) from the Yamansaz coastal

lake exhibited a dorsal margin that is slightly curved in the middle; from 13-17 preorbital teeth on the dorsal margin of the rostrum, 0-2 postorbital teeth and 4-8 teeth on the ventral margin of the rostrum. From the Kirkgöz spring lake, 31 specimens (mainly females, including 2 ovigerous females, and the rest indeterminate) showed a dorsal that is margin slightly curved in the middle or strongly curved in the middle and pointed upwards, from 17-20 preorbital teeth on the dorsal margin of the rostrum, 0-2 postorbital teeth and 5-10, mostly 10 teeth, on the ventral margin of the rostrum. The two males from the Göksu river estuary showed a straight dorsal margin, from 21-22 pre-orbital teeth on the dorsal margin of the rostrum, 1-2 postorbital teeth, and 8 teeth on the ventral margin of the rostrum. But, according to the rest of the features they could be identified as *A. tuerkayi*. The first pleopod in the male has a well-developed endopod, straight or bent outwards, bearing 7 to 8 spines on its edge. The distal part of the endopod, either curved inside or straight, ended with numerous cincinnuli (Figure 3A). The second pleopod in male has an appendix interna of endopod 5 times as long as wide, about 0.5 times the length of the appendix masculina, with numerous subterminal cincinnuli. The appendix masculina is 8 times as long as wide, bearing 8 spines on its proximal two-thirds; the endopod is shorter than the exopod and 7.1 times as long as its maximal width; the exopod is about 3.2 times as long as its maximal width (Figure 3B). The habitat conditions at these collection sites were consistent with the known habitats of *Atyaephyra* spp. (Table 1).

The preservation of the Göksu estuary specimens in ethanol in 2015 has allowed molecular analysis to confirm identification by DNA barcoding.

Specimens of *Palaemon turcorum* were collected from Göksu estuary, Kirkgöz spring, the Yamansaz coastal lake and Manavgat estuary, all in the Mediterranean basin (Figure 1). These are the first records for this species from outside the Sakarya Basin, where this species is known as an endemism (Holthuis, 1961). The most relevant features for assessing the identity of *P. turcorum* compared to *P. antennarius* H. Milne Edwards, 1837, as well as from *P. varians* (Leach, 1813), *P. zariquieyi* (Sollaud, 1938) and *P. mesopotamicus* (Pesta, 1913) are the endopod of the first male pleopod almost as large as the exopod and in the appendix masculina of the second male pleopod which is far longer than the endopod of that pleopod (González-Ortegón & Cuesta, 2006; Holthuis, 1961).

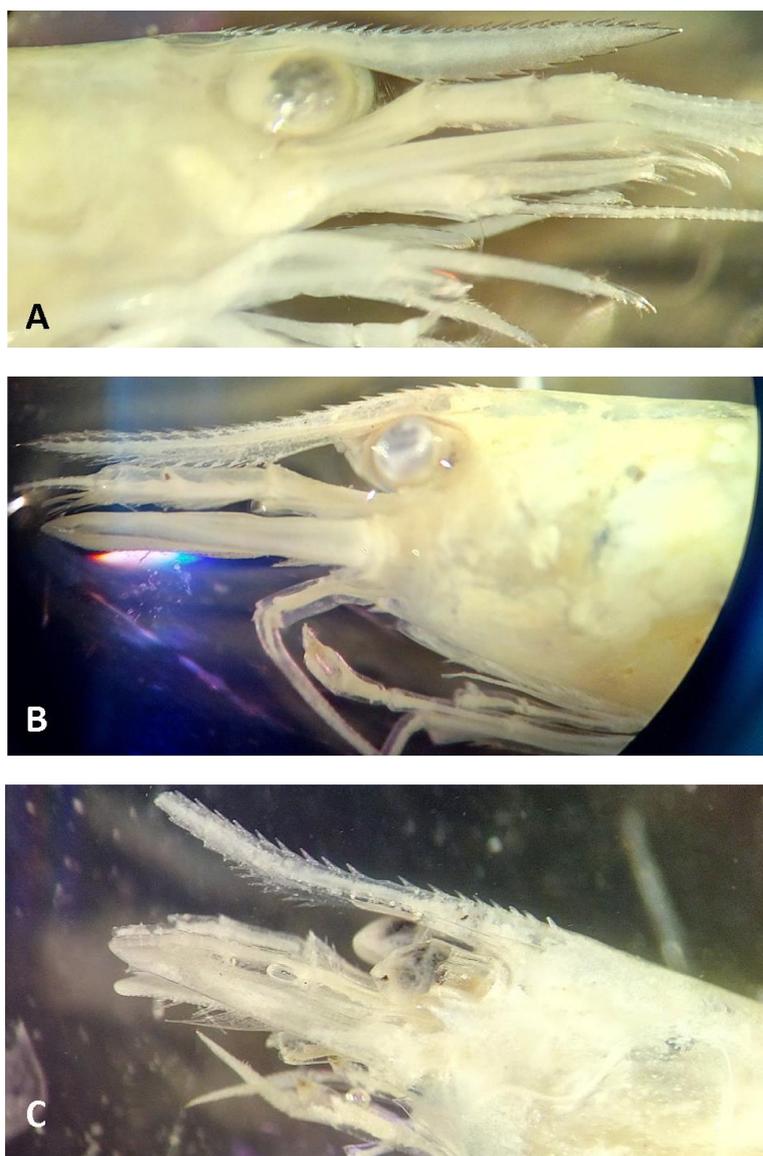


Figure 2. Photographs of *Atyaephyra* sp. species' cephalotorax collected in Turkey. A – *Atyaephyra tuerkayi* specimen from the Göksu river estuary; B – *Atyaephyra orientalis* from the Kirkgöz Spring; C - *Atyaephyra* sp. specimen from the Yamansaz coastal lake. Photographs by EGO.

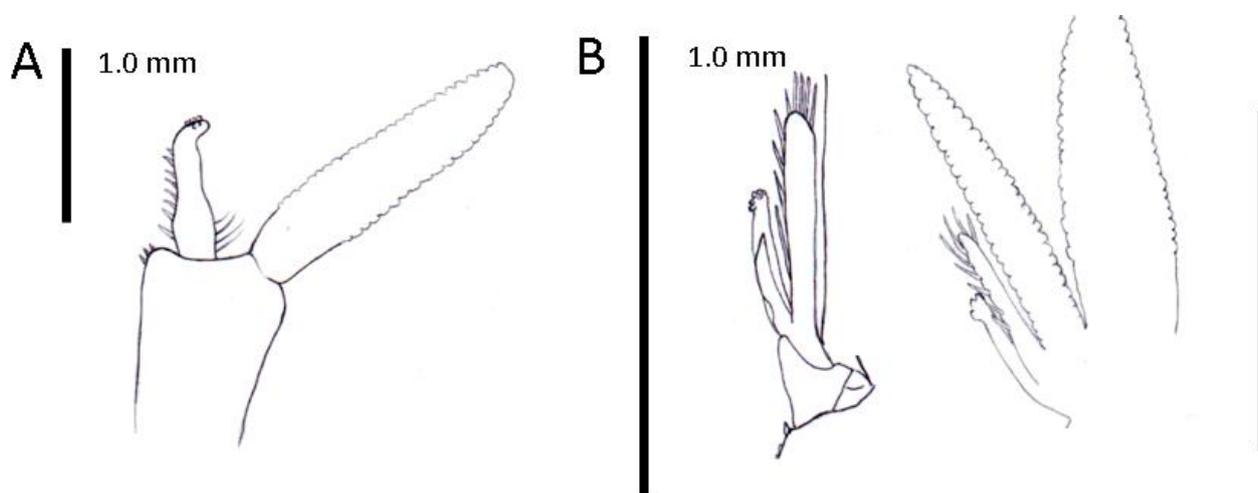


Figure 3. *Atyaephyra tuerkayi* Christodoulou, Antoniou, Magoulas & Koukouras, 2012, male (IEO-CD-CCDE15/1475), from Göksu river estuary: A, right endopod of the first pleopod; B, right second pleopod.

DNA barcoding identification of *Atyaephyra* sp.

The mitochondrial DNA sequence of COI generated from one male from the Göksu estuary consists of 667 bp and a 99.78% fit with the *A. tuerkayi* holotype (SMF43020) sequence (JX289935, 474 bp), and 99.75% with *A. tuerkayi* paratype (SMF43021) sequence (JX289936, 406 bp). This new sequence increases in 193 bp the COI sequence for this species. This result strongly supports the idea that the specimens collected from the Göksu estuary belong to the species *A. tuerkayi*, with a low genetic variability concerning the Syrian specimens.

The DNA sequence of 16S (544 bp) generated from the same specimen of the Göksu estuary cannot be used to compare with the only two DNA vouchers of *A. tuerkayi*, because, unfortunately, the authors describing *A. tuerkayi* did not include any sequences of the 16S gene in their study. When this new sequence of 16S is blasted on the homepage of NCBI (<http://blast.ncbi.nlm.nih.gov>), the maximum homology obtained is with 21 sequences of *A. desmarestii* (93-95%) (i.e. EU123848, JX469086, FJ594305-FJ594355), and one of *A. orientalis* (94.02%)(JX469105).

Discussion

Our great ignorance of biodiversity probably allows many 'cryptic extinctions' to go undetected (Carlton, 1993). This work is one example of the diversity of cryptic species of the genus *Atyaephyra* and *Palaemon* that live in the Mediterranean basin without our knowing. The Mediterranean basin has a very complex geological history, with major events like the Messinian salinity crisis (5 Ma) and the Last Glacial Maximum (18,000 years ago) contributing to the high level of endemism found in peri-Mediterranean areas (Lejeune *et al.*, 2010; Reyjol *et al.*, 2007). Rapid advances in molecular genetics are key to understanding the genetic variation (Storfer *et al.*, 2010). In the present study, we are reporting a new occurrence of *A. tuerkayi* based mainly on data from a partial sequence of COI and the morphological features, and *P. turcorum* based on morphological features which match the previous descriptions. The fact that the Middle East has not been well surveyed for freshwater and estuarine shrimps could suggest that its potentially wider distribution is unknown (De Grave, 2013). This finding suggests that *P. turcorum* and *A. tuerkayi* are potentially distributed in the Antalya peninsula and could be coexisting with the species *A. orientalis*. In the case of *A. tuerkayi*, previous specimens were collected in 1979 from the Nahr Al-Kabir River (between Lebanon and Syria), close to the sea (Christodoulou *et al.*, 2012), they are considered on the IUCN Red List as Data Deficient. This new finding of a population of *A. tuerkayi* could lead to a reconsideration of this category on the IUCN Red List. Despite the high morphological variability found, specimens from the Yamansaz coastal lake and Kirkgöz Spring could be identified as *A. orientalis*. The results of

these two populations have limited conclusive significance, as the individuals could not be used for genetic analyses.

The Mediterranean basin is one of the regions most vulnerable to climate change (Ribas *et al.*, 2010), and several studies have shown that it is already facing the impacts of climate change on water yields (e.g. Ludwig *et al.*, 2011). The long-term unsustainable use of water resources leading to water scarcity is common in semi-arid regions, such as the Mediterranean region, where endemic freshwater species have been reported among the most threatened biota in the world (Hermoso & Clavero, 2011). Although data on freshwater invertebrates are limited, the latent effects of water scarcity should not pass unnoticed for small populations of caridean shrimps such as the species *A. tuerkayi*. The strong genetic differentiation at spatial scales of the species of *Atyaephyra* has been shown recently (García-Muñoz *et al.*, 2009, 2014; Christodoulou *et al.*, 2012; Jablonska *et al.*, 2018). In this arid region, with a dominance of small basins and regulated streams, the extended drought events lead to a loss of hydrologic connectivity between stream compartments (Sabater & Tockner, 2009). The geographic distribution of atyid species seems to be affected by freshwater availability and quality, especially in the smallest basins such as the coastal lake Yamansaz (Kemeragzi, Turkey) and may increase the risk of extinction of this population of the genus *Atyaephyra* with species pending confirmation (e.g. Holmquist *et al.*, 1998; Benstead *et al.*, 1999). If the environmental scenario of water scarcity under global warming remains unchanged, Mediterranean coastal species will be one of the most threatened biotas in the world.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

EGO conceived and designed the research. DY: performed the fieldwork. EGO and JAC: analyzed the species. EGO: wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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