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Recent occurrence of *Olindias muelleri* Haeckel, 1879 (Cnidaria, Hydrozoa, Limnomedusae, Olindiidae) in the Aegean Sea

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Abstract: A new occurrence of the hydrozoan *Olindias muelleri* and the first case of an associated envenomation were recorded from the eastern Aegean Sea. A total of eight adult medusae of *O. muelleri* were collected near the surface on 22 and 23 June 2018 in Boyalık Bay, Turkey following a sting report by a bather. Medusae were identified as *O. muelleri* using morphological keys and confirmed through COI-based DNA barcoding. The presence of *O. muelleri* may have not been noticed in the past in the region or it could stem from a recent range expansion from other parts of the Mediterranean Sea. Monitoring is needed to better understand the spread of *O. muelleri* and the threat it presents for human health and coastal tourism in Çeşme.

Key words: Aegean Sea, COI, DNA barcoding, Hydrozoa, Stinger

Introduction

Olindias muelleri Haeckel, 1879 is a shallow water hydrozoan (Cnidaria) that occurs in the Mediterranean Sea and Tropical-Atlantic Ocean (van der Land et al. 2001, Bouillon et al. 2004, Schuchert 2018). It has a biphasic life cycle with a benthic polyp and a free-swimming medusa (Weill 1936). The medusa has been reported to reside among seagrass and algae with its contracted tentacles during daytime, and after sunset it becomes active and swims into the water column (Larson 1986). *Olindias muelleri* feeds on other zooplankton; its diet mainly consists of copepods, chaetognaths, polychaetes and crustacean larvae, fish eggs and larvae (Breder 1956, Larson 1986). Thus, it can play an important role in the pelagic ecosystem as a predator and a competitor of fish.

Olindias muelleri has been reported as *O. phosphorica* Delle Chiaje, 1841, however, the status of *O. phosphorica* has recently changed. According to the World Register of Marine Species (http://www.marinespecies.org/aphia.php?p=taxdetails&id=117774), *O. phosphorica* is not a valid name for the Mediterranean species and the correct taxon name is *O. muelleri* Haeckel, 1879.

Although reports on *Olindias muelleri* medusae originate over a wide geographical area in the Mediterranean Sea (Fig. 1), there are only a limited number of reports (Barangè & Gili 1987, Daly Yahia et al. 2003, Vukanic 2006, Deidun 2010, Eleftheriou et al. 2011, Soler et al. 2016) most likely due to it is nocturnal habit. The first occurrence record in the Mediterranean Sea dates to 1824 according to Global Biodiversity Information Facility (GBIF) (Fig. 1). There is only one long-term data record between 1898–1917 based on human observations (214 occurrences), published in GBIF by the Hellenic Centre for Marine Research using data from Observatoire Océanologique de Villefranche-sur-Mer (GBIF 2018). In the following years, reports in the Mediterranean are limited. *Olindias muelleri* was found in September 1985 and in March 1986 in a coastal lagoon on Mallorca Island (NW Mediterranean) as a part of a Cnidarian survey (Barangè & Gili 1987). Regular appearances of *O. muelleri* were recorded during August and September in the Bay of Tunis (SW Mediterranean) between December 1993 and November 1995 (Daly Yahia et al. 2003). It was also found in 2002 during a zooplankton survey in the Bay of Boka Kotorska (Southern Adriatic) (Vukanić 2006). The occurrence of *O. muelleri*, through the collection of voucher species was reported in

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Maltese coastal waters in July and August 2010 (Deidun 2010). According to the citizen science campaign METEOMEDUSE, *O. muelleri* was the third most abundant jellyfish between 2009 to 2011 along the Salento coasts (Southern Italy) (De Donno et al. 2014). A monitoring programme of jellyfish presence in the Valencian community (NW Mediterranean) showed that it lives permanently in certain locations along the east and southeast coast of Spain (Soler et al. 2016). It has been reported only once from the NE Aegean Sea in 2011 (Eleftheriou et al. 2011). To the best of our knowledge, there are no other published records available from the Aegean Sea before that date. Thus, the Aegean Sea is the eastern limit of the geographical distribution of this species in the Mediterranean Sea. Recently, *O. muelleri (*as *O. phosphorica)* was listed as a harmful species in the poster of "Watch out for the gelatinous organisms of the Eastern Mediterranean" from a Citizen Science program (http://www.cmas.org/science/watch-out-for-the-gelatinous-organisms-of-the-eastern-mediterranean).

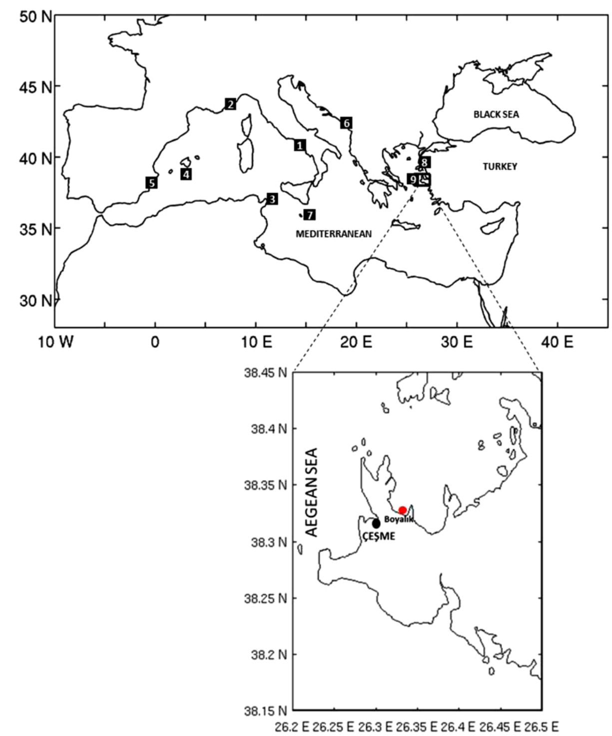

Fig. 1. First records of *Olindias muelleri* from Mediterranean Sea given in chronological order (1- Italy: 1824; 2- France: 1898, 3- Tunisia: 1972, 4-Mallorca Island: 1984, 5- Spain: 1985, 6- Southern Adriatic: 2002, 7- Maltese: 2010, 8- NE Aegean Sea: 2011, 9- Eastern Aegean Sea: 2018 (new occurrence of *Olindias muelleri* (red circle) in Çeşme Peninsula).

Although it occurs in low numbers and does not form large populations, *Olindias muelleri* is a serious stinger and can cause serious human envenomation events near shore (De Donno et al. 2014). It was the major species responsible for stings along the Valencian coast between the summers of 2010 and 2015 (Soler et al. 2016), and the third most common stinger along the Catalan coast (Canepa et al. 2013). *O. muelleri* became a dreaded species for bathers and fishermen during the summers of 1994 and 1995 in Tunisian coastal waters (Daly Yahia et al. 2003).

DNA barcoding has been frequently used in molecular identification of living organisms. The mitochondrial cytochrome oxidase I (COI) gene is commonly used in DNA barcoding for invertebrates (Bucklin et al. 2011) and has aided species identification of hydrozoans (Moura et al. 2008, Ortman et al. 2010, Lee et al. 2011, Lindner et al. 2011, Karouzas et al. 2015, Lindsay et al. 2015, Gaynor et al. 2016, Maggioni et al. 2016, Schuchert 2016, Govindarajan et al. 2017).

In the present study, based on morphological and molecular identification, we document the presence of *Olindias muelleri* in the popular touristic area of Boyalık Bay in Çeşme (Turkey, Eastern Aegean). An *O. muelleri* sting is also reported for the first time from the Aegean Sea. This finding is discussed in relation to earlier *O. muelleri* records from the Mediterranean.

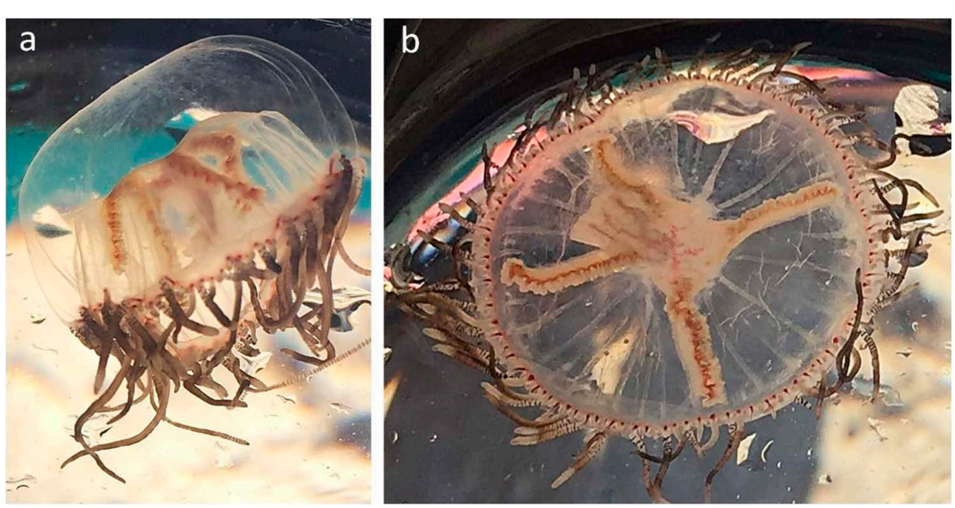

Fig. 2. Live specimen of *O. muelleri* caught on Boyalık Beach at 22 June 2018 (a-lateral and b-oral view). Photo credit Ü. Aytan.

Materials and Methods

Study sites and sample collection

The Çeşme peninsula (İzmir/Turkey) is surrounded by the Aegean Sea, with a coastline of 90 km characterized mostly by sandy beaches. It is the most important tourist resort in İzmir and has a resident population of 41,278 inhabitants (TUIK 2017). According to local authorities, the population increases up to nearly 600000 during the summer period due to tourism. Boyalık Bay is a sheltered sandy bay located in the north of Çeşme Peninsula (Fig. 1). Boyalık beach is a popular beach, about 2 km long, between Ilıca and Çeşme.

On 22th June 2018, just after a bather was stung, an adult medusa of *Olindias muelleri* was noticed in the near shore area in Boyalık Bay (38.3244722 N, 26.335315 E) (Fig. 1). *O. muelleri* was collected with a hand net near the surface and live colour photos were taken immediately (Fig. 2). On 23th June 2018, again in daytime, seven specimens were collected near the surface in the same area and preserved in 99.5% ethanol for taxonomic observation and DNA extraction. In this study, only a representative subsample of specimens were collected for morphological and molecular analyses. However, the number of specimens in the water was high. Sea surface temperature, wind speed and direction data for the sampling dates were obtained from the Turkish Meteorology Service.

Morphological identification

Taxonomic observations and measurements were made on preserved specimens under a stereomicroscope (Leica Sapo) according to Bouillon et al. (2004). Measurements were made to the nearest 0.1 mm using an ocular micrometer attached to the stereomicroscope.

Molecular identification and data analyses

Purification of DNA from tissue samples was automated on a QIAcube robot using the DNeasy Blood & Tissue Kit (Qiagen, Germany). DNA was stored at −20°C for PCR applications. The DNA was visualized on a 0.8% agarose-TBE gel containing 0.5 μg/mL of ethidium bromide by a UV Quantum-Capt ST4 system (Vilber Lourmat, France) and the quantity of the isolated DNA was measured using a NanoDrop 2000c Spectrophotometer (Thermo Scientific, USA). Mitochondrial COI sequences were amplified by PCR using the universal forward and reverse primers; HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) and LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) (Folmer et al. 1994). The PCR was performed with 50 μL of reaction mixture containing 30 μL sterile distilled water, 5 μL 10x PCR buffer (20 mM Tris-HCl pH 8.0), 5 μL $MgCl_2$ (25 mM), 6 μl dNTP mixture (25 mM), 1 μl of each primer (10 pmol), 1 U *Taq* DNA polymerase (Thermo Fisher Scientific Inc., USA) and 2 μL DNA template (60 ng). PCR programs were run with initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, annealing 49°C for 30 s and 72°C for 1 min, and a final extension at 72°C for 10 min using the gradient thermal cycler Biorad T100TM (Bio-Rad, Hercules, CA, USA). The PCR products were confirmed by 1% agarose gel electrophoresis. The concentrations of purified PCR products were estimated using a NanoDrop 2000C UV-Vis spectrophotometer (Thermo Fisher Scientific, USA). PCR products were sequenced by direct bidirectional sequencing or cloned into the PMD 18-T vector (TaKaRa, Japan). Double stranded PCR products were purified using the BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystem, Foster City, CA), and then were bi-directionally sequenced on an ABI Prism 3730x1 automatic DNA sequencer (Applied Biosystems, USA) using PCR primers the Macrogen Inc. (Amsterdam, Netherlands).

The COI-barcode sequences were manually checked and corrected with the BioEdit 7.2.5 software (Hall 1999). The number of haplotypes were calculated using DnaSP 5.0 (Librado & Rozas 2009). Using uncorrected p-distance, which yields higher or similar identification success rates for neighbor-joining tree and distance-based identification techniques (Srivatsan & Meier 2012), the neighbor-joining tree was constructed in MEGA 6.0 (Tamura et al. 2013) with 1000 iterations.

Results

All specimens of *Olindias muelleri* exhibited radial symmetry, with four radial canals and numerous centripetal canals (7–11) in each quadrant (Fig. 2b). The umbrella was hemispherical (Fig. 2a), and ranged between 2.2–4.4 cm (mean 3.36±0.74 cm) in diameter. The mesoglea was moderately thick. The orange gonads with papillliform processes were observed along the radial canals. Numerous primary tentacles (48–60) occurred above the bell margin and secondary tentacles (96–120) were observed on the bell margin.

The mitochondrial COI sequences of six Aegean *Olindias* samples were analyzed. The analysis showed four different haplotypes, determined by five transitions without insertion and/or deletion mutations. Haplotype sequences were deposited in GenBank under accession numbers MH700547-MH700550. To identify *Olindias*, the samples were aligned with the closest query sequences (Table 1) deposited in GenBank using the BLAST program. There is no available genetic data for *O. muelleri* from Aegean Sea for comparison. The medusae of *O. muelleri* from the Aegean Sea, Turkey have identical sequences to the *O. muelleri* medusae from Spain (GenBank number: JX121605) with a similarity ratio of over 99%. Phylogenetic tree topology revealed that the Aegean *Olindias* haplotypes clustered together with those identified from Spain (Fig. 3).

We also report that a young male was stung by *Olindias muelleri* while bathing at Boyalık beach on 22th June 2018.

Table 1. GenBank accession numbers and classifications of sequences used in the phylogenetic analysis.

Scientific name	GenBank accession no.	References
Gonionemus agilis Watson & Govindarajan, 2017	MF135184	Watson & Govindarajan 2017
Gonionemus vertens A. Agassiz, 1862	MF135185	Watson & Govindarajan 2017
Gonionemus sp. A. Agassiz, 1862	MF135186	Watson & Govindarajan 2017
Cubaia aphrodite Mayer, 1894	NC016467	Kayal et al. 2012
Maeotias marginata Modeer, 1791	AF383926	Vainola & Oulasvirta 2001
Craspedacusta sowerbii Lankester, 1880	KP231217	Karaouzas et al. 2015
	FJ423620	Fritz et al. 2009
Olindias muelleri Haeckel, 1879	JX121605	Nawrocki et al. 2013
	MH700547, MH700548 MH700549, MH700550	This study

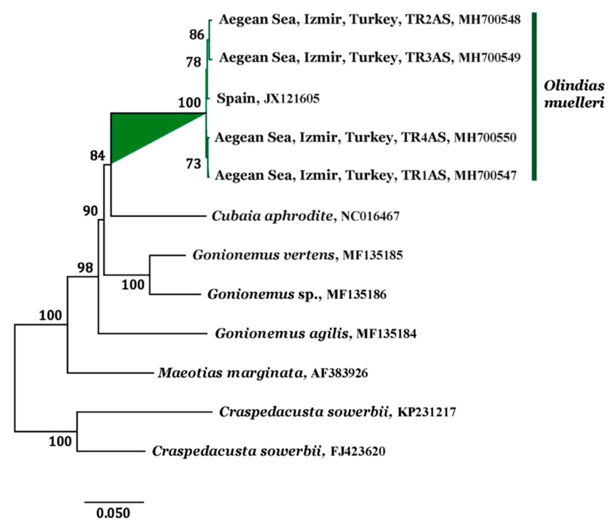

Fig. 3. Neighbor-joining phylogenetic trees based on mtDNA COI sequences. Scale bars indicate branch length in substitutions per site. Nodal support values are presented as the NJ bootstrap value.

A couple of minutes after the stung, urticaria were formed in the contacted area, accompanied by a burning and sore sensation (Fig. 4).

Fig. 4. A young male was stung by *O. muelleri* in the Boyalık beach

Discussion

Taxonomy

Medusae of *Olindias* are easily distinguished from other marine genera of Limnomedusae. Among the genera of Limnomedusae, only *Eperetmus* Bigelow, 1915, *Maeotias* Ostroumoff, 1896, and *Olindias* Müller, 1861 have centripetal canals and *Olindias* differ from the other two genera by having two types of tentacles (Boullion et al. 2006). The specimens analyzed in this study possessed the defining characteristics of medusae in the genus *Olindias*, including four radial canals, centripetal canals, primary tentacles that originate from above the bell margin, and secondary tentacles that originate at the bell margin (Bouillon et al. 2006). The genus *Olindias* has been divided into six species, each of them occurring within a separate warm-water area: *O. muelleri* Haeckel, 1879 in the Mediterranean and central Atlantic Ocean, *O. tenuis* Fewkes, 1882 in the west-central Atlantic Ocean, *O. malayensis* Maas, 1905 in the central Indo-Pacific Ocean, *O. formosus* Goto, 1903 in the North Pacific Ocean, *O. sambaquiensis* Müller, 1861 in South Africa and *O. singularis* Browne, 1905 in the Indo-Pacific Ocean and as an alien species in the Egyptian part of the Mediterranean Sea. There are insufficient morphological descriptions of *O. muelleri* occurring in the Mediterranean Sea. However, the characteristics of the present specimens of *O. muelleri* (number of centripetal canals, number of primary and secondary tentacles) agree with previous descriptions of this species (Haeckel 1879, Bouillon et al. 2004) with some minor differences. COI-based DNA barcoding also confirmed that medusae in this study were *O. muelleri*. The umbrella diameter of *O. muelleri* was slightly smaller than the umbrella diameter of *O. muelleri* (4–6 cm) according to Boullion et al. (2004). The specimens were preserved in ethanol and thus they might have become smaller than living ones due to dehydration.

Occurrence

In the Mediterranean, medusae of *Olindias muelleri* have been mostly observed during summer in the warm waters of sheltered bays with aquatic vegetation (Table 2). It has been found locally in low to moderate numbers and does not have population outbreaks, in contrast jellyfish species (e.g., Daly Yahia et al. 2003, Deidun 2010). Sporadic appearances of *O. muelleri* and its nocturnal habits makes it difficult to assess the real distribution of this species in the Mediterranean. The only report of *O. muelleri* from the Aegean Sea was in 2011. Only one specimen was found in Geyikli Harbour (NE Aegean Sea). Its presence was linked to transportation by currents and/or ballast waters (Eleftheriou et al. 2011). The present study provides further evidence that this species is present in the Aegean Sea. The high genetic similarity and phylogenetic match between our samples and those from Spain, which were isolated from two populations almost 1500 nautical miles distant from each other, suggests an eastward expansion of the western populations by currents or by ships during the benthic stage as a part of the fouling fauna. It has been speculated that warming might favor the increase in the number of warm-water species such as *O. muelleri* in the Mediterranean Sea (Boero et al. 2008). The occurrence of *O. muelleri* in the Aegean Sea may also be related to recent warming trends.

A jellyfish monitoring programme along the Valencian coast (NW Mediterranean) showed that *Olindias muelleri* was resident in certain locations (Soler et al. 2016). Their results suggested that *O. muelleri* completes its polyp-medusa cycle in sheltered waters, and thus its behavior is different compared to other jellyfish that are carried by winds and currents from distant areas (Soler et al. 2016). The *Olindias* medusae are typically associated with seagrass and attached themselves to the leaves during daytime (Breder 1956, Larson 1986). In a coastal lagoon of Mallorca Island (NW Mediterranean), *O. muelleri* was found at a station characterized by widgeon weeds (*Ruppia)* (Barangè & Gili 1987). Similarly, it was found in an area with dense meadows (*Posidonia*) in the Bay of Tunis (Daly Yahia et al. 2003). Regular appearance of this species in eastern and southeastern Spain was also linked to *Caulerpa prolifera* (Forsskål) J.V.Lamouroux, 1809 meadows (Soler et al. 2016). In agreement with these previous studies, *Posidonia* meadows are also common in the bay where *O. muelleri* was found in this study, and may have provided an attachment substrate for the medusae. According to frequent users of this bay during previous summers, the occurrence of stinging jellyfish is an unprecedented phenomenon. Nevertheless, it is possible that this species is distributed in warm sheltered bays with aquatic vegetation along the Turkish Aegean coast, as well as in the western Aegean, but they simply have not been sampled or encountered before.

The sea surface temperature (SST) was around 26°C along the coast when the specimens were collected. Mean daily wind direction was offshore (166°, 1.6 m/s) on 22 June 2018 and (200°, 2.1 m/s) on 23 June 2018. Nevertheless, at the end of the afternoon when samples were collected there was a light onshore wind. This might have caused accumulation of this species near the shore. According to Bouillon et al. (2004), the medusa occurs between July and November in the Atlantic and Mediterranean. However, most reports on the occurrence of *O. muelleri* in the Mediterranean Sea are in July and August (Table 2). Records of SST from the Turkish Meteorology Service show significant increases during June of 2018 (data not shown). In the first ten days of June, the temperature increased by 2.4°C, reaching 25°C, and then stayed stable for nine days. From 19 June to 22 June, another increase of 1°C occurred. Thus, local warming might have been crucial in facilitating the proliferation of this warm water species in Boyalık Bay. Numerous hot water subma-

Table 2. Some previous reports on *Olindias muelleri* in the Mediterranean Sea.

Location	Period	Environment	Number	References
Coastal Lagoon of Mallorca, NW Mediterranean	September 1985 March 1986	*Ruppia*	2 adults 2 juveniles	Barangè & Gili 1987
Tunis Gulf, SW Mediterranean	August–September 1994–1995	*Posidonia*	1–2 ind. m^{-3}	Daly Yahia et al. 2003
Boka Kotorska Bay, SouthernAdriatic	Occasionally during 2002			Vukanic 2006
Ghadira, St. Thomas and St. Paul'sBay, Malta, Mediterranean	18–19 July 2010 13,22,31 August 2010		1 specimen	Deidun 2010
Geyikli Harbour, NE Aegean	24 July 2010		1 specimen	Eleftheriou et al. 2011
Salento coast, Southern Italy	Summer 2009–2011			De Donno et al. 2014
Santa Pola, Valencian coast, East and Southeast Spain	Summer 2009–2015	*Caulerpa*		Soler et al. 2016
Boyalık Bay, Eastern Aegean	22–23 June 2018	*Posidonia*	8 adults	This study

rine springs along the Northern Çeşme coast might also sustain a stable warm environment throughout the year, enabling the colonization of *O. muelleri* in the region.

Impacts of *O. muelleri*

Here we report the first case of an *Olindias muelleri* sting in the Aegean Sea. Jellyfish stings are a severe threat for human health, for coastal tourism and for sea-based economies (Marsh & Smith 2010, Boero 2013, De Donno et al. 2014). *O. muelleri* has been frequently associated with human envenomation in populated tourist areas in the Mediterranean (Daly Yahia et al. 2003, Deidun 2010, De Donno et al. 2014, Soler et al. 2016). The sting of this species can cause severe pain and other localized symptoms (Marsh & Smith 2010). According to Daly Yahia et al. (2003), periodic invasion of *O. muelleri* during summer in the coastal waters of the Tunis Gulf (SW Mediterranean) makes it a fearsome species for swimmers/fishermen and negatively affects tourism. Along the South Italian Coasts, 897 bathers required medical assistance following contact with jellyfish in summer (July and August) between 2009–2011 and *O. muelleri* was the third most abundant stinger (6.48%) in the same period (De Donno et al. 2014). In a monitoring programme of jellyfish presence along the Valencian coast of Spain, *O. muelleri* was found to be the most abundant species (66%–75% of all jellyfish) during the summer between 2010 and 2015. It was responsible for the high number of stings registered in this location (Soler et al. 2016). It is also reported as the third most abundant species responsible for stings that require first aid along the Catalan coast (Canepa et al. 2013). Jellyfish stings are a common reason for people to seek health assistance in summer in the Mediterranean. However, it is difficult to determine which species is responsible for stings. Citizen science is a key tool for detection of these species, reduction of health hazards, and determining the social cost of this emerging phenomenon.

Conclusion

A new occurrence of the hydrozoan *Olindias muelleri* and the first case of associated envenomation is reported for the eastern Aegean Sea. A pronounced increase in the frequency and extent of jellyfish outbreaks in the Mediterranean Sea has been observed during the last decade, associated with overfishing, global warming and pollution (Boero et al. 2013). New occurrences of O. *muelleri* in the eastern Aegean Sea might be a warming-related, eastward range expansion of this species in the Mediterranean. Çeşme is an important holiday spot in the Aegean and Mediterranean. Along the Çeşme coast, the most important economic activities are beach-associated tourism. More than half a million tourists visit Çeşme during summer and use the beaches for bathing, windsurfing, kite-surfing and sailing. Jellyfish stings might cause serious threats for human health and for coastal tourism in the region. The presence of adult medusae of *O. muelleri* in Boyalık Bay might lead to the development of numerous polyps, which in turn might form numerous medusae, with serious ecological and socio-economic impacts in the region. Monitoring surveys are needed to understand the population dynamics, life cycle and proliferation of *O. muelleri* throughout the Aegean Sea.

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