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First record of *Aequorea macrodactyla* (Cnidaria, Hydrozoa) from the Israeli coast of the eastern Mediterranean Sea, an alien species indicating invasive pathways

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Abstract

The species of *Aequorea* attract much scientific interest as they contain the unique Green Fluorescent Protein (GFP). In this work we describe for the first time the discovery of a hydrozoan jellyfish belonging to the genus *Aequorea* from the Israeli eastern Mediterranean that contains and exhibits fluorescent protein. Finding *Aequorea macrodactyla* (Brandt, 1835) in the eastern Mediterranean indicates that changes are occurring in the gelatinous fauna of this area. This hydromedusa is known in the seas adjoining the Mediterranean though most of its records are more than four decades old. We examined and identified the newly discovered Israeli *Aequorea* species by combining two phylogenetic systems, traditional morphological phylogeny and molecular phylogenetics. The molecular identification determined that the species is *A. macrodactyla* but with minor genetic differences in the mtDNA 16S gene marker. A 1% difference between the Israeli and the Japanese *A. macrodactyla* was demonstrated, which suggests that the genetic difference between the Israeli and the Japanese population is small but existent. Invasive pathways for this jellyfish were examined by phylogenetic and taxonomic relationships with similar Cnidaria. The results indicate introduction from the Indo-Pacific as invasive pathway, probably by human transportation, and the discovery of *A. macrodactyla* in the eastern Mediterranean Sea could be interpreted as part of the changes in marine biota as a result of cumulative effects of anthropogenic and global changes that affect the eastern Mediterranean basin.

Keywords
Aequorea macrodactyla, hydromedusa, gelatinous fauna, phylogenetic markers, migration of jellyfish, Lessepsian migration, anthropogenic changes

Introduction

The class Hydrozoa, or the superclass Hydrozoa as suggested by Bouillon and Boero (2000), consists of typical carnivores which are considered to be among the most important planktonic and benthic predators (Bouillon et al. 2006). They prey on fish eggs, fish larvae, some marine invertebrates, and on different sizes of planktonic animals (Frost et al. 2012; Laakmann and Holst 2014). The class contains approximately 3800 species worldwide (Schuchert 2014). *Aequorea macrodactyla* (Brandt, 1835) (Fig. 1) belongs to the family Aequoreidae Eschscholtz, 1829 and is one of the 27 accepted species of the genus *Aequorea* Péron & Lesueur, 1810 (Schuchert 2014). *Aequorea victoria* (Murbach & Shearer, 1902) is the most studied species from this genus. For the first time bioluminescence and biofluorescence were described in a jellyfish and green fluorescent protein (GFP) was discovered (Shimomura et al. 1962) in this species. This discovery enabled the utilization of fluorescent protein (FP) in science and medicine and attracted scientific attention to the unique ability of this genus. *Aequorea macrodactyla* is the second species of jellyfish that has been studied for its bioluminescence and GFP, and is used for commercial products (Xia et al. 2002). In this work we present for the first time a hydrozoan jellyfish belonging to the genus *Aequorea* (Fig. 1A) that contains and exhibits fluorescent protein (Figs 1B, 2) in the Israeli eastern Mediterranean basin. Since the discovery of GFP more than fifty years ago (Shimomura et al. 1962) nearly two hundred natural GFP-like fluorescent proteins (FPs) have been described and the FP became an important tool in biological research (Chudakov et al. 2010; Hunt et al. 2012). By using the term "jellyfish" we include the medusa stage of the scyphomedusae from the class Scyphozoa and the hydromedusae from the class Hydrozoa.

Aequorea macrodactyla is a common hydroidomedusa found in the warmer waters of the coastal region of the East China Sea (Xia et al. 2002). Kramp (1956) claimed that *A. macrodactyla* is widely distributed in the Indian and Pacific Ocean from Africa to America and was recorded also at the southern part of the west coast of Africa. Reports of *A. macrodactyla* from the seas adjoining the Mediterranean began with fishery investigations in the years 1937 and 1938, conducted by Dr. H. Blegvad in the Persian Gulf. He collected one specimen of *A. macrodactyla* in this survey. "No previous records of medusa exist from the Iranian Gulf" (Kramp 1956). From the Arabian Sea, *Aequorea macrodactyla* was collected from great depths and also near the shore (Kramp 1965). Schmidt (1973) refers to *A. macrodactyla* existing in the Red Sea, the Gulf of Eilat and the Gulf of Aqaba. Russell reports in 1939 and 1953 finding *A. macrodactyla* in the English Channel (Kramp 1956). Goy et al. (1991) reviewed jellyfish proliferation based on a survey (1969–1989) from the neritic Lebanese waters and suggested that hydro-

graphic conditions and climate are the most determining factors in temporal distribution of jellyfish. They found some *Aequorea* spp. but not *Aequorea macrodactyla*. Turan et al. (2011) report the presence of *Aequorea globosa* Eschscholtz, 1829 for the first time near the coast of Iskenderun Bay in the northeastern Mediterranean and they suggest ship-mediated transport as probable vector. A year later in 2012, Mamish et al. (2012) observed *Aequorea globosa* near the coast of Syria and considered its appearance an indicator of climate change in the eastern Mediterranean marine environment. Gravili et al. (2013) published a comprehensive review to update the diversity poll of species of what is known as a non-Siphonophora Hydrozoa (NSH) in the Mediterranean, which are traditionally studied as a separate group as their polyp and/or medusa stage is distinctive in global distributions (Kramp 1961; Kramp 1965). In this recent work *A. macrodactyla* was absent from the lists of Mediterranean species but mentioned as being present in neighboring areas: the Gulf of Eilat, the Gulf of Aden and the Red Sea (Gravili et al. 2013). The online Encyclopedia of Life shows more up to date information for the locations of *A. macrodactyla* but the specimen closest to the Mediterranean comes from the Indian Ocean dated 23.7.2007 (Encyclopedia of Life 2014).

Boero and Bouillon (1993) state in their study that the fauna of Mediterranean hydromedusae contains 346 species and consists of 19.5% endemic species. The majority, as much as 72.5%, of the Mediterranean species is present in the Atlantic, as well as distributed worldwide, and it can be considered an Atlantic species that could have entered the Mediterranean Sea through the Strait of Gibraltar after the Messinian Salinity Crisis (MSC), also referred to as the Messinian Event. Only 8% are considered Indo-Pacific and these are mainly restricted to the eastern Mediterranean basin. Twenty years later Gravili et al. (2013) presented more or less the same picture for non-siphonophoran hydrozoan fauna distribution in the Mediterranean Sea. From the 400 species they recorded, as much as 68% originate from the Atlantic Ocean, 20% are endemic to the Mediterranean, 8% are Indo-Pacific and the other 4% are defined unclassifiable.

Morphological species identification in hydrozoan taxonomy is difficult for many species and can be misleading as a result of phenotypic variability (Erpenbeck et al. 2005; Laakmann and Holst 2014). Because of this we combined molecular phylogenetics with morphological species identification to achieve the most accurate results. Since molecular phylogenetics by DNA sequences became a main source of information for the clarification of evolutionary relationships (Wörheide and Erpenbeck 2007), we used this tool with three DNA markers. Defining a single general marker for the taxonomic classification of an organism on all taxonomic levels can also be limited and inaccurate (Lunt et al. 1996; Schultz et al. 2005). Thus, in order to achieve better accuracy, multiple genetic markers should be used for research instead of one single genetic marker (Gruenthal et al. 2007). In this study we used genetic methods that are used on the Hydrozoa by comparing DNA sequencing sites from the nuclear large 28S subunit ribosomal DNA (rDNA) genes (Cartwright et al. 2008; Collins et al. 2008; Cantero et al. 2010). We employed two mitochondrial DNA (mtDNA) markers, the cytochrome c oxidase subunit 1 (COI) gene and the large subunit ribosomal RNA (rRNA) gene 16S. The slow evolutionary rates of COI and 16S rDNA in some hydro-

zoans allow them to be useful markers (McFadden et al. 2011; Zheng et al. 2014). The COI is known as the main metazoan barcoding marker, although in hydrozoans the COI gen has scarcely been employed because of the difficulties involved in amplifying DNA fragments using standard primers (Cantero et al. 2010; Schuchert 2010).

The 16S rDNA has been widely accepted for hydrozoan barcoding purposes and is widely used to resolve phylogenetic questions within the Hydrozoa and has been shown as a better marker for barcoding. It is considered to be a gene that is much easier to amplify and is more available in the GenBank database (Cantero et al. 2010; Moura et al. 2011; Laakmann and Holst 2014; Zheng et al. 2014).

By using both methods in this work, molecular phylogenetics sequences as distance-based methods for the evaluation of genetic distances within and between species and morphological species identification, we anticipated improved accuracy in the taxonomic classification for this new discovery of the Israeli eastern Mediterranean *Aequorea* species.

Materials and methods

Aequorea macrodactyla (Brandt, 1835), known also as *Mesonema macrodactyla* Brandt, 1835 was collected near the Israeli shore line at two different locations during the summer of 2013. The first group of specimens was collected in Sdot Yam, GPS position: 32°29'35"N, 34°51'48"E, on 16.6.2013, with an umbrella diameter of 65 mm to 75 mm. Three other groups of *A. macrodactyla* specimens were found and collected at different sites in the Bay of Haifa, GPS general position: 32°52'N, 35°01'E, on 19.12.2013, with an umbrella diameter ranging between 75 mm to 80 mm. A total of 23 specimens were taken to the laboratory for further identification. Finding *A. macrodactyla* concentrations in two different places at a distance of approximately 55 km from each other and half a year apart indicates that this hydromedusa is established in the region. For the species identification of the Israeli *Aequorea*, two phylogenetic systems were combined: traditional morphological phylogenetics and molecular phylogenetics. Morphological identification was executed according to Mayer (1910), Kramp (1956, 1961, 1965), Russell (1970), Bouillon et al. (2004) and Bouillon et al. (2006).

Samples from the collected jellyfish were processed immediately *in situ*, excising sample cuts from the animals and starting with DNA extraction. Total genomic DNA was extracted using the Wizard® SV Genomic DNA Purification System kit (Promega). Other samples were preserved *in situ* in 95% ethanol for further processing in the laboratory. Throughout the work, care was taken to use sterilized tools and containers, and gloves were worn. The genetic classification was done by matching DNA sequences to global and local data to achieve the most accurate species identification for the sampled specimens (Bayha et al. 2010; Pett et al. 2011).

We designed DNA primers (Table 1) for the locally found jellyfish. After some experimenting a suitable marker was determined for the specimens. The DNA concentration was measured with the NanoDrop, using absorption at 260 nm, and the DNA

itself was prepared using the Promega kit for Polymerase chain reaction and sequencing (PCR) amplification and diluted to a final concentration of 2 ng/μL. DNA quality was assessed by running samples on 1% agarose gels. All amplifications were carried out in a T100™ Thermal Cycler (BIO-RAD) using GoTaq® Green Master Mix (Promega). PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega). The sequencing of the specimens was performed by HyLabs sequencing service (Israel).

For the analyses, the sequences were processed and aligned with BioEdit version 7.2.5 (Hall 1999), ClustalX (Larkin et al. 2007), RAxML (Silvestro and Michalak 2012), Nucleotide composition and analyzed with MEGA6. Each phylogenetic tree was processed with MEGA 5.2 and MEGA 6 tools in order to select the best and most appropriate evolutionary phylogenetic model (Tamura et al. 2013).

Results

Systematics

Class HYDROZOA Owen, 1843
Order LEPTOTHECATA Cornelius, 1992
Family AEQUOREIDAE Eschscholtz, 1829

Genus *Aequorea* Péron & Lesueur, 1810
Fig. 1A

Aequorea macrodactyla (Brandt, 1835)

Morphological description. The freshly collected jellyfish were observed carefully in the lab by using: Nikon SMZ100 Binocular, Nikon AZ100 Binocular with DS-Ri1 camera, and Zeiss Imager microscope M2. All specimens have a biconvex lens umbrella (central disc is lens-shaped) of around 20 mm thick and 65 mm to 80 mm in diameter. The stomach is shallow with a diameter of a little less than half of the umbrella, around 30 mm to 38 mm (Fig. 1A). There is an average of 32 straight radial canals (Fig. 1B) and the gonads are linear on both sides of each radial canal (Fig. 1C). The jellyfish have between 10–20 broad marginal tentacle bulbs (Fig. 1A) each with abaxial keel (Fig. 1D). The identification of *Aequorea* spp. can be confusing mainly due to their considerable variability, and misidentification has occurred in the past (Kramp 1965). The amount of radial canals and tentacles, and the shape of the bases of the marginal tentacles are of great importance and the key to distinguish morphologically between other similar jellyfish belonging to the genus *Aequorea*, for example *Aequorea pensilis* and *Aequorea macrodactyla* that bear a close resemblance one to another. The marginal bulb should be determined in order to distinguish between similar species of *Aequorea* (Mayer 1910; Kramp 1956; Kramp 1965; Russell 1970; Bouillon et al. 2006; Gul and Gravili 2013). In our findings we can see clearly (Fig. 1C) the cross-shaped bases of

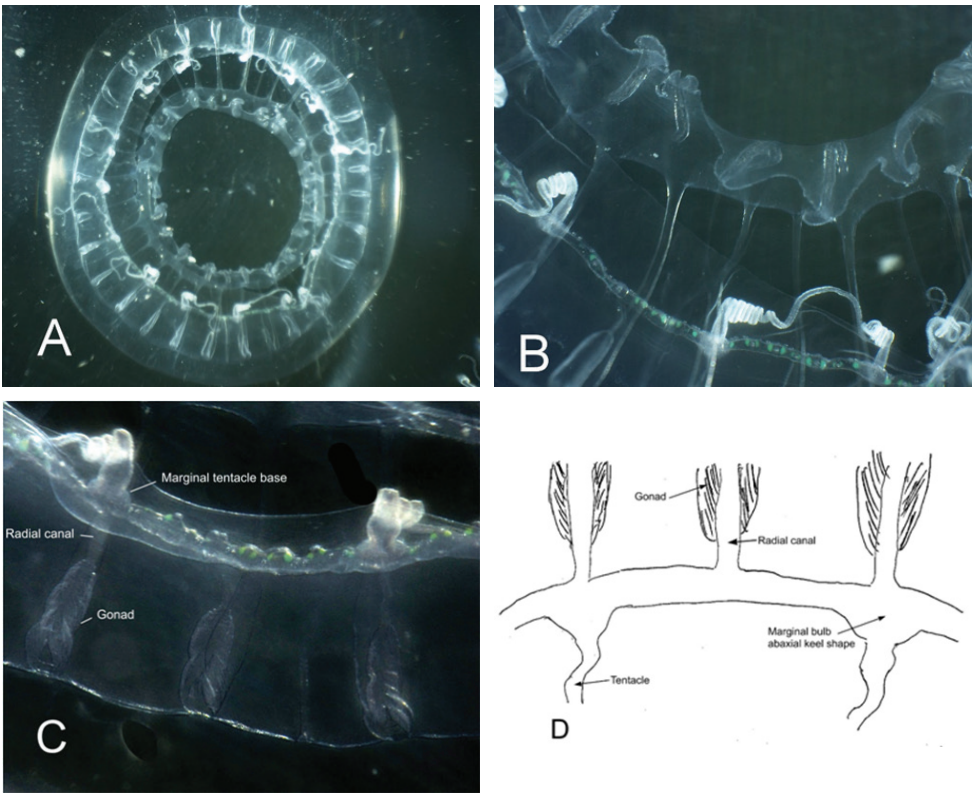

Figure 1. A Oral view of the first *Aequorea macrodactyla* collected at the Israeli shoreline in Sdot Yam, on 16.6.2013, size 8 cm **B** Magnification of the rudimentary marginal bulb and the GFP granules **C** The gonads around the radial canal and the shape of the marginal tentacle base **D** A schematic drawing of the marginal bulb tentacle base of the Israeli *A. macrodactyla*.

the marginal tentacles that continue the radial canal. Also it is evident (Fig. 1C) that some radial canals end as marginal bulbs but did not develop tentacles and should be considered as non-tentacular marginal bulbs or rudimentary bulbs (Fig. 1D), which is a characteristic phenomenon of the *Aequorea macrodactyla*. In Figure 1C we can see the position of the gonads surrounding the radial canal. The color of the radial canal, the endoderm of the lips and the tentacle bulbs present a milky color as other parts are mostly transparent.

Genetic identification

The DNA sequences were submitted (Table 1) to the European Nucleotide Archive (ENA).

Our results from the sequencing and processing of different genes through DNA alignment within the population of the Israeli *Aequorea macrodactyla* specimens show very minor differences. These differences are suggested to be negligible and the results

Table 1. Data of *Aequorea macrodactyla* nucleotide Sequence Database, submission to the European Nucleotide Archive.

Eukaryota; Metazoa; Cnidaria; Hydrozoa; Hydroida; Leptomedusae; Aequoreidae; *Aequorea*			
Accession#: HG964642.	16-JUN-13	*Aequorea macrodactyla*, partial 16S rRNA gene (Fig. 3)	
Latitude = 32°29'N, Longitude = 34°52'E	Israel. East Mediterranean coast. Sdot Yam		
Primer forward name	16S F756	Forward sequence	CCGTGATAAAGTAGCATAATCAC
Primer reverse name	16S R755	Reverse sequence	AATATTACCCTGTTATCCCTACGG
Accession#: HG964638.	16-JUN-13	*Aequorea macrodactyla* partial 28S rRNA gene (Fig. 4)	
Latitude = 32°29'N, Longitude = 34°52'E	Israel. East Mediterranean coast. Sdot Yam		
Primer forward name	28S F834	Forward sequence	GAGACCGATAGCGAACAAGTACCGTG
Primer reverse name	28S R833	Reverse sequence	AGAGTTTCCTCTGGCTTCACCCTACTC
Latitude = 32°52'N, longitude = 35°01'E	Israel. East Mediterranean coast. Haifa bay		
Primer forward name	COI F754	Forward sequence	TATGATTATAMGAYTGGAACTATCAGG
Primer reverse name	COI R755	Reverse sequence	GTYAACAACATGGTWATYGCCCCAGC

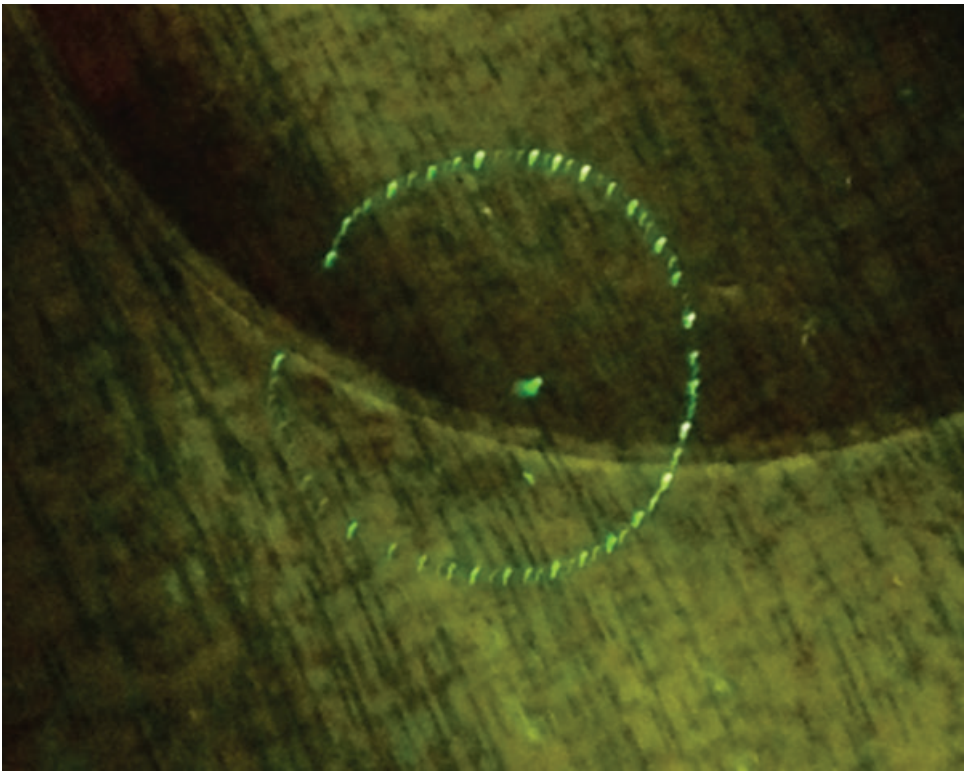

Figure 2. Localization of the Green fluorescent protein (GFP) in the margin of the umbrella in the Israeli *Aequorea macrodactyla*, size 9 cm. Photograph taken inside the boat in Haifa Bay with specimen in sea water using UV illumination with UV flashlight in a darkened room.

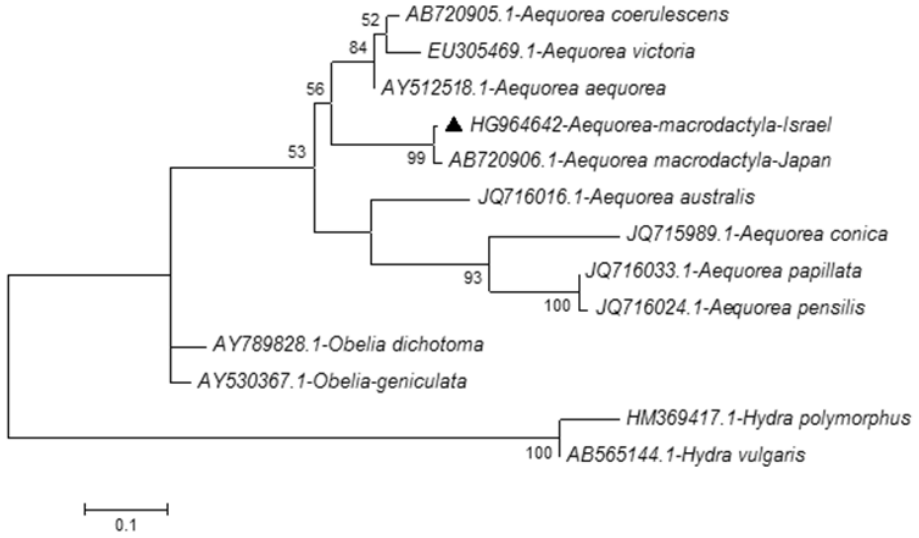

Figure 3. Evolutionary tree for Israeli *Aequorea macrodactyla* by mtDNA 16S marker compared with other members of *Aequorea*. Molecular phylogenetic analysis by Maximum Likelihood Method, an evolutionary tree based on partial sequences of 354 base pairs (bp) mitochondrial DNA (mtDNA) marker 16S. The black triangle represents the local *A. macrodactyla* jellyfish of the Mediterranean according to the DNA molecular sequence acquired in this work. The outgroup for this tree is represented by *Hydra polymorphus* and *H. vulgaris*. The test of phylogeny is by Bootstrap method and the number of bootstrap replications is 1000. For the taxon name we used the NCBI GenBank reference number.

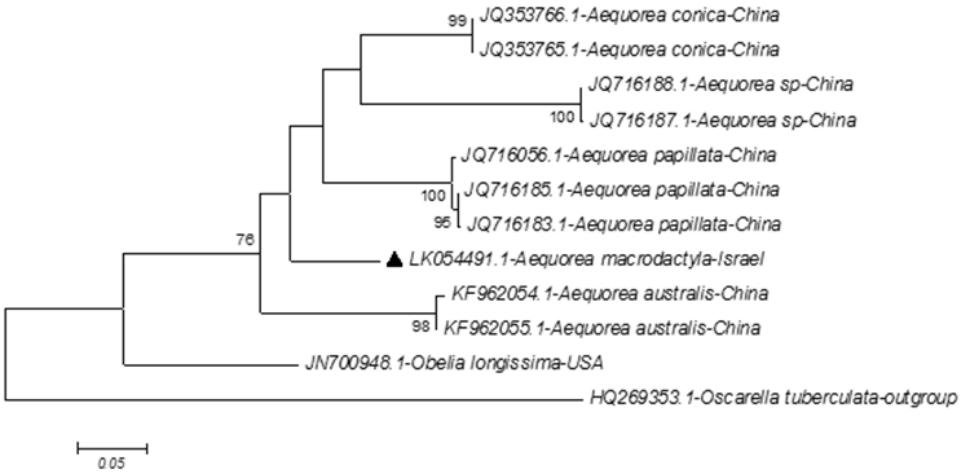

Figure 4. Evolutionary tree by COI marker for Israeli *Aequorea macrodactyla* (black triangle).

show that the Israeli *A. macrodactyla* specimens belong to one group as presented in the following phylogenetic trees (Figs 3, 4, 5) under the reference number HG964642,

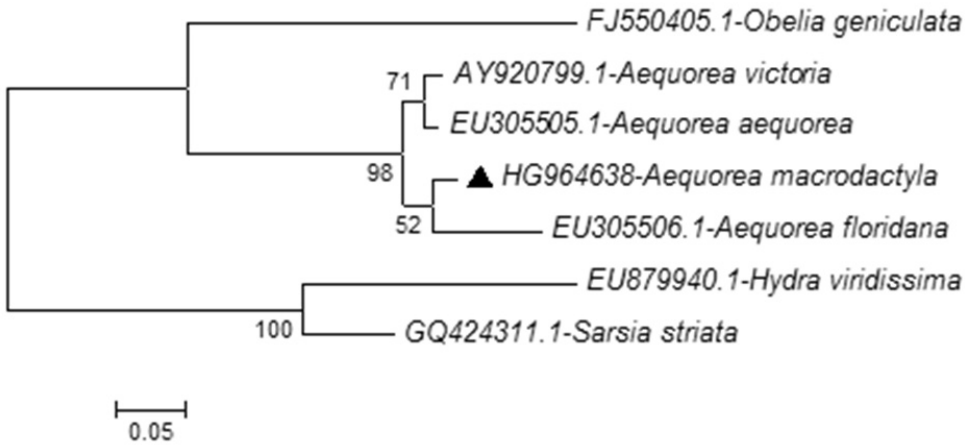

Figure 5. An evolutionary tree of Israeli *Aequorea macrodactyla* by 28S rDNA marker based on specimens from Sdot Yam and Haifa bay (marked by black triangle). The tree is based on partial sequences of 679 base pairs (bp) of the nuclear Ribosomal DNA 28S (rDNA) gene. The outgroup is based on *Hydra viridissima* Pallas, 1766. The reference number used in this figure represents the NCBI GenBank reference number and is followed by the taxon name. The data in this phylogenetic tree include all published data from the species of *Aequorea* existing in NCBI GenBank. The test of phylogeny is by Bootstrap method and the number of bootstrap replications is 500.

which is the National Center for Biotechnology Information (NCBI) GenBank reference number as received from the European Nucleotide Archive (ENA).

The evolutionary history was inferred by using the Maximum Likelihood Method based on the Tamura 3-parameter model (Tamura 1992). The tree with the highest log likelihood (-1506.2803) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The initial tree for the heuristic search was obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.3008)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 13 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 350 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

The tree is based on partial sequences of 691 base pairs (bp) mitochondrial cytochrome c oxidase subunit I (COI) with other family members from the same class. The outgroup is the Porifera, *Oscarella tuberculata*. The test of phylogeny is by Bootstrap method and the number of bootstrap replications is 1000. The reference number used in this figure represents the NCBI GenBank reference number followed by the taxon name.

The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model (Tamura 1992). The tree with the highest log likelihood (-2473.0463) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4266)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 12 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$+Noncoding. All positions containing gaps and missing data were eliminated; there was a total of 618 positions in the final dataset (Tamura et al. 2013).

All tree model parameters were estimated by RAxML GAMMA model of rate heterogeneity, ML estimates of alpha-parameter GAMMA Model parameters were estimated up to an accuracy of 0.1 log Likelihood units, Alignment Patterns = 188, DataType = DNA, Substitution Matrix = GTR, and alpha = 0.300374 (Silvestro and Michalak 2012).

Discussion

The Mediterranean Sea is undergoing a dramatic change in biota as a result of biogeographical alterations and ecological changes (Monegatti and Raffi 2001; Monegatti and Raffi 2010; Zenetos et al. 2010; Vermeij 2012), and the East Mediterranean experiences even more changes in biota since the opening of the Suez Canal and fast urbanization along the Israeli Mediterranean coast (Galil et al. 2013). To better understand this ongoing process, it is essential to be familiar with the existing gelatinous fauna in the Mediterranean Sea and to document newly discovered jellyfish and existing ones, so that this important information can be utilized and provide us with the possibility to improve the management of our contributions to the changes in the sea. In this work we utilize phylogenetic and taxonomic relationships with similar Cnidaria to examine if the newly recorded species of *Aequorea* is an immigrant and if so, what constitutes the direction of its invasive pathways and speed of migration (migration vector), or if this species is local (Zenetos et al. 2012; Katsanevakis et al. 2014).

For the species identification of the Israeli *Aequorea*, two phylogenetic systems were combined: traditional morphological phylogenetics and molecular phylogenetic tools. Morphology: shape of the umbrella, position of the gonads, number of radial canals, and the morphology of the marginal bulbs show that the newly found Israeli hydrozoan belongs to the genus of *Aequorea* and we were able to identify this species as *Aequorea macrodactyla* according to the above described characteristics with some minor differences. The identification was made according to the description of this species from other sources (Mayer 1910; Kramp 1956; Kramp 1965; Russell 1970; Bouillon et al. 2006; Gul and Gravili 2013) and the genetic results, which will be discussed further on, support our identification (Fig. 3).

In order to establish the evolutionary history and molecular phylogenetics for the Israeli *Aequorea* widely used DNA markers were employed as a method to identify the species and to construct an evolutionary tree. We used the mitochondrial DNA

(mtDNA) marker 16S (Fig. 3), which is a widely used DNA marker for Hydrozoa; this marker has the most widely available data in the GenBank (Laakmann and Holst 2014). The result, based on partial sequences of 354 base pairs, presented in Fig. 3, supports the morphological results that *Aequorea* of the East Mediterranean Sea appears to be *Aequorea macrodactyla*. The evolutionary tree (Fig. 3) presents all *Aequorea* species by 16S gen available in the NCBI GenBank (Geer et al. 2010) and it is clear that the Israeli and Japanese *Aequorea macrodactyla* are related and different from all other *Aequorea* species presented in this tree (Fig. 3). Furthermore there is a difference in identity of 1% between the two *Aequorea macrodactyla* as shown by the results of 16S nucleotide, using basic local alignment search tool (blast). The Israeli *Aequorea macrodactyla* population shows negligible differences inside the four locally collected groups. 16S mtDNA gene exhibits a very low rate of mitochondrial nucleotide substitution, especially in Cnidaria, where it exhibits a considerably lower rate than in other invertebrates. The rate of substitution for 16S mtDNA in Cnidaria is 10–20 times lower than in vertebrates (Govindarajan et al. 2005). The 1% identity difference between the Israeli and the Japanese *A. macrodactyla* can be an indication of a significant difference between these two *A. macrodactyla* groups, but more worldwide data is required to resolve this issue.

The evolutionary tree resulting from mitochondrial cytochrome c oxidase subunit I (COI) for the Israeli *Aequorea macrodactyla* based on partial sequences of 691 base pairs (Fig. 4) and the evolutionary tree resulting from nuclear Ribosomal DNA 28S (rDNA) gene based on partial sequences of 679 base pairs (Fig. 5) strengthen the previous results from the 16S gene (Fig. 3) for the evolutionary locations of *Aequorea macrodactyla* among other existing *Aequorea* species. In this publication, we provide available sequences for COI and 28S genes and supply a basis for future reference regarding the use of COI and 28S gene markers.

This work emphasizes the importance of using a combination of the two phylogenetic systems especially when one tries to identify fragile and delicate creatures like members of the *Aequorea* spp. Our results show an average of 32 radial canals in the Israeli *A. macrodactyla*. Mayer (1910) wrote that there are 60 to 100 or more radial canals, Kramp (1965) stated that the "numbers of radial canals and tentacles are of great importance" but he presents average numbers of radial canals from 31 to 102, which is closest to our findings. However Kramp (1956) determines that the *A. macrodactyla* contains 153 radial canals. Russell (1970) presents numbers of 60 to 100, even up to 150 radial canals. This example of different and confusing morphological data demonstrates the importance of using both a phylogenetic approach and a genetic approach.

The dynamic changes in gelatinous fauna in the Mediterranean and worldwide are of great interest, as jellyfish play an important role in the stability of marine ecology and the marine food web. In spite of the fact that they are considered simple creatures they rank high in the food web (Mills 2001). Jellyfish can affect the structure of pelagic ecosystems by competing for the same food resource or by predation (Richardson et al. 2009). The current trend, related to global change, reveals a considerable change in the marine biodiversity and marine habitats, reaching the point where marine habitats are experiencing hazardous pressure (Plan 2010). The discovery of *Aequorea macrodactyla*

in the eastern Mediterranean Sea could be interpreted as part of the change in marine biota resulting from the cumulative effects of anthropogenic and global changes that affect the eastern Mediterranean basin. Finding a new species could be explained by the sudden proliferation of a rare indigenous species that up until that point was evasive and stayed out of sight. It is possible that this species flourished as a consequence of changes in the environment that created more favorable conditions. The other possibility, which is more widely accepted, is that they are new immigrants introduced from the Atlantic or the Indo-Pacific, or are Indo-Pacific immigrants that arrived through the Suez Canal as Lessepsian immigrants (Plan 2010).

To deal with these important questions, whether *Aequorea macrodactyla* has been an evasive hydromedusa that kept a low profile or whether this is a new invasion by a non-indigenous species, we examined three DNA markers (Table 1): mitochondrial DNA (mtDNA) marker 16S (Fig. 3), mitochondrial cytochrome c oxidase subunit I (COI) marker (Fig. 4), and Ribosomal DNA 28S (rDNA) genes (Fig. 5). The results from marker 16S (Fig. 3) suggest that the genetic differences between the Israeli and the Japanese population are small but existent, and can be interpreted as a normal divergence within a population. As the Israeli *A. macrodactyla* population was collected from different places and at different times and demonstrated very little genetic diversion without any distinctive variance at all, we suggest that the local *A. macrodactyla* are not native to the East Mediterranean and should be considered a new immigrant jellyfish; we propose that the jellyfish from the Far East is the source of the Israeli East Mediterranean *Aequorea macrodactyla*.

The arrival of *A. macrodactyla* to the East Mediterranean could be explained by one of two possibilities, either Lessepsian migration or human transportation. Lessepsian migration is not a well-defined term and we propose to define it as successful migration of marine creatures from one side of the Suez Canal to the other, coming through the Suez Canal either step by step, swimming or even using local transportation (Por 1971) as long as the Canal enabled this immigration. For example, a hydromedusa arriving from the Arabian Sea to the Mediterranean by ballast water cannot be considered a Lessepsian migrant but should be defined as an anthropogenic migrant by means of human transportation. Human transportation is a means for the marine creature to immigrate to a new location, regardless of the transportation route: at present this appears to be the main path of introduction of a species (Gravili et al. 2013). As we compare our *A. macrodactyla* with the existing data one can assume that either one of the immigration options presented here is possible.

For *A. macrodactyla* in the seas adjoining the Mediterranean, records exist from the Bay of Eilat, the Gulf of Aden, the Red Sea, and the English Channel. All these published records are old i.e. from more than four decades ago, and the nearest and newest reports come from the Indian Ocean (23 July 2007) and from the North Atlantic in Central America on 15 September 1977 (Encyclopedia of Life 2014). The report of *A. macrodactyla* in the Red Sea (Schmidt 1973) is old, more than 40 years ago, and there have been no further records of *A. macrodactyla*. Moreover, Gravili et al. (2013) proposed to remove species from the list of taxonomic records that have not been found for a reasonable time (several decades), and we concur.

To summarize this discussion, we suggest that this jellyfish is an immigrant that used ship transportation from the Indo-Pacific to the Mediterranean, which is supported by the high DNA similarity with the Japanese (Xia et al. 2002) *Aequorea macrodactyla* (Fig. 3) and by the lack of other evidence or reliable information for this jellyfish existing in the Red Sea in recent years.

In this paper we emphasize the dynamic process in the East Mediterranean marine fauna as a significant part of the ecosystem's change in natural balance and as a result the introduction of a new species and change in the biodiversity. Moreover, genetic tools were utilized as a means for distinguishing and examining newly recorded species to determine whether this is a native species or an immigrant. As we pointed out, global information is limited, more work should be carried out, especially in the East Mediterranean as the marine habitat and its fauna are changing rapidly. The results indicating Indo-Pacific migration could be interpreted as part of the change in marine biota as a result from the cumulative effects of anthropogenic and global changes that affect the eastern Mediterranean basin (Duarte 2014; Tsikliras and Stergiou 2014).

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