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Shedding light on an East-Mediterranean mesophotic sponge ground community and the regional sponge fauna

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Abstract

Sponges are a diverse and abundant phylum, globally occupying many hard-bottom habitats. However, data on East-Mediterranean sponge communities are scarce, outdated, and mostly concern the shallow waters. This study aimed to expand the knowledge on poriferan fauna along Israel's Mediterranean coast. A newly-discovered mesophotic sponge ground at ~100 m depth was studied using a Remotely-Operated Vehicle, while shallow-water surveys were conducted by scuba diving. In this mesophotic ecosystem, sponges serve as environmental engineers, creating complex 3D structures that attract invertebrates and fish. Quantitative surveys of the mesophotic sponge ground revealed a rich and diverse sponge community with a high percentage cover (~35%). Several mesophotic species are reported for the first time from the Levantine Sea, while others might be novel species. Here we report 111 sponge species along the Mediterranean coast of Israel (from our current surveys and previous studies), 36 of which were collected from the mesophotic habitat. The updated sponge list supports the hypothesis that the sponge diversity in the Levantine Sea is not as species-poor as previously considered. A comparison of the sponge community composition between the shallow waters and the mesophotic depth revealed only partial overlap, with merely a few species thriving along the entire depth range. The mesophotic habitat was found to harbor some species that seem to have disappeared from the shallow habitats decades ago, suggesting that the former may serve as refugia for species stressed by rising temperatures in shallow waters, and that this deeper habitat thus require protection from negative anthropogenic influences.

Keywords: Levantine Sea, sponge grounds, mesophotic, community structure, quantitative survey, Mediterranean, ROV, diversity.

Introduction

Porifera constitute one of the dominant invertebrate phyla in the Mediterranean benthos, displaying very high endemism (48% according to Coll *et al.*, 2010). Sponges are important habitat builders in the Mediterranean, creating complex three-dimensional structures, attracting fish and invertebrates by increasing the number and complexity of available microhabitats, providing refuge from predators, and serving as spawning and nursery grounds (Hogg *et al.*, 2010; Beazley *et al.*, 2013; Bo *et al.*, 2012). They support local species richness and diversity and provide diverse ecosystem services, thus serving as environmental engineers (Kenchington *et al.*, 2013; Gerovasileiou *et al.*, 2016).

Whereas sponge fauna of the Western Mediterranean has been studied quite thoroughly, data on the Levant fauna are scarce (see Carteron, 2002; Perez *et al.*, 2004; Voultsiadou, 2005a, 2009; Vacelet *et al.*, 2007; Vacelet *et al.*, 2008; Evcen & Cinar., 2012; Van Soest *et al.*, 2012; Topaloglu *et al.*, 2014). Specifically, current knowledge on the sponge fauna of the Mediterranean coast of Israel is outdated, limited to the shallow waters, and derived from several old publications, including surveys of the benthic fauna in the bay of Haifa conducted in the 1950s (Levi, 1957; Gottlieb, 1959). Levi (1957) identified 31 sponge species, of which only three were found deeper than 42 m. Another more comprehensive study was carried out in the mid-1960s, listing 61 demosponge and two homoscleromorph species from shallow (<7 m) coastal habitats (Tsurnamal, 1968). Although a few studies reported some of the latter findings (Tsurnamal 1967, 1969a, 1969b), a complete checklist was published only in Hebrew (Tsurnamal, 1968). Moreover, data on sponges dwelling in deeper habitats (below 7 m) along the Israeli coast are scarce (Levi, 1957; Ilan *et al.*, 1994; Ilan *et*

al., 2003). Generally, the data from deep and mesophotic habitats are often biased towards soft-bottom environments, which are easier to sample by means of trawling or grab than the less accessible hard substrata (Cerrano *et al.*, 2010; Hogg *et al.*, 2010; Bo *et al.*, 2012).

The mesophotic zone of the continental shelf occupies the deeper half of the photic zone, and in tropical and subtropical regions it starts at 30-40 m and extends to below 150 m depth (Lesser *et al.*, 2009). Due to its inaccessibility by scuba diving, until recently it was therefore one of the less-studied habitats in the ocean (Lesser *et al.*, 2009). Thus, basic information on the mesophotic sponge communities, including community composition, species depth range, habitat preferences, and species abundance and distribution, are scarce worldwide (Bo *et al.*, 2011; Schönberg *et al.*, 2012; Olson *et al.*, 2013; Slattery *et al.*, 2015). Furthermore, the processes that structure these communities are virtually unknown.

The term "sponge grounds" refers to the habitat in which sponges are the dominant phylum in species abundance, diversity, coverage, and size of the individuals (Hogg *et al.*, 2010). Mesophotic sponge grounds are often situated on patchy hard substrata surrounded by unstable soft bottom (Hogg *et al.*, 2010) and, as such, they constitute an oasis of local richness and diversity (Bo *et al.*, 2012). In such habitats sponges frequently act as environmental engineers by increasing the structural complexity and creating niches for both invertebrates and fish (Kenchington *et al.*, 2013; Gerovasileiou *et al.*, 2016). Hard-bottom substrate has an ecological, scientific, and economic importance due to its complex structure, which is able to support rich communities (Maldonado *et al.*, 1996). Along the Israeli coast of the Mediterranean Sea there are several submerged sandstone ridges at depths ranging from 10 to 130 m. These submerged ridges are mostly covered by sediment, with their exposed parts constituting less than 10% of the sea floor at that depth (Yahel *et al.*, 2012; Israel marine plan 2015).

The development of new tools, such as the Remotely Operated Vehicle (ROV), has enabled a more thorough study of these habitats, revealing these Mediterranean sponge grounds. However, no data on them have been published to date. These Levant mesophotic sponge grounds are under constant anthropogenic threat due to bottom trawling and oil and gas exploration. Consequently, it is vital to study these unique habitats in order to better understand their role in the local ecosystem and determine how best to protect them.

Voultsiadou (2009) analyzed the spatial distribution of the Mediterranean sponge fauna and revealed a north-north-west to south-south-east gradient in sponge diversity, rather than the commonly accepted west-east gradient (Voultsiadou, 2005a; Coll *et al.*, 2010; Mouillot *et al.*, 2011; Coll *et al.*, 2012). Nonetheless, we hypothesized that this gradient might not be as steep as described, and that the Levant's low sponge biodiversity could be the result of lower research efforts in the Eastern-Mediterranean region (Van Soest *et al.*, 2012). We therefore assumed that additional research in the Levant region would lead to an increase in the number of species known to reside there. While the temperature of shallow waters in the Levantine Sea exceeds 30˚C in summer, below the seasonal thermocline the temperature is stable and does not exceed 18˚C (Kress *et al.* 2014). We therefore also hypothesized that the environment below the thermocline would be suitable for a community that differs from that of the shallow waters.

To test these hypotheses, we examined sponge biodiversity along the Israeli Mediterranean coast by conducting field surveys in shallow and mesophotic habitats and incorporating the available data from the literature. The goals of this study were:

1. To examine the sponge fauna (Demospongiae and Homoscleromorpha) along the Israeli Mediterranean coast.
2. To characterize the sponge community structure in the recently discovered East-Mediterranean mesophotic sponge ground.
3. To compare the sponge assemblages in the Israeli Mediterranean upper photic zone with the mesophotic sponge ground.

Materials and Methods

Study site and sampling

Sponges (classes Demospongiae and Homoscleromorpha) were collected along the Israeli coast of the Mediterranean Sea from north to south at 13 sites (Fig. 1, Table 1). Calcarean species were not studied since they are usually small and as such are not visible in the quantitative surveys. Sampling down to 30 m was done by scuba diving (with permits from the Israel Nature and Parks Authority). At deeper locations (90-130 m) samples were collected by ROV (Remotely Operated Vehicle).

The 12 shallow sites were sampled qualitatively during 33 dives, while at the mesophotic site, during six expeditions, both quantitative and qualitative surveys were conducted. Most of these sites are either nature reserves or are planned to be declared as such in the future. Thus, the current work provides a basis for the future study and monitoring of these areas. In the shallow water 172 sponge specimens were collected (Fig.1). An effort was made to collect as many different species as possible, focusing on the most conspicuous sponges, in order to obtain a better picture of the species composition. The mesophotic sponges (98 specimens) were collected at the Herzliya deep site, which is a part of the deepest ridge of the continental shelf, approximately 15 km off

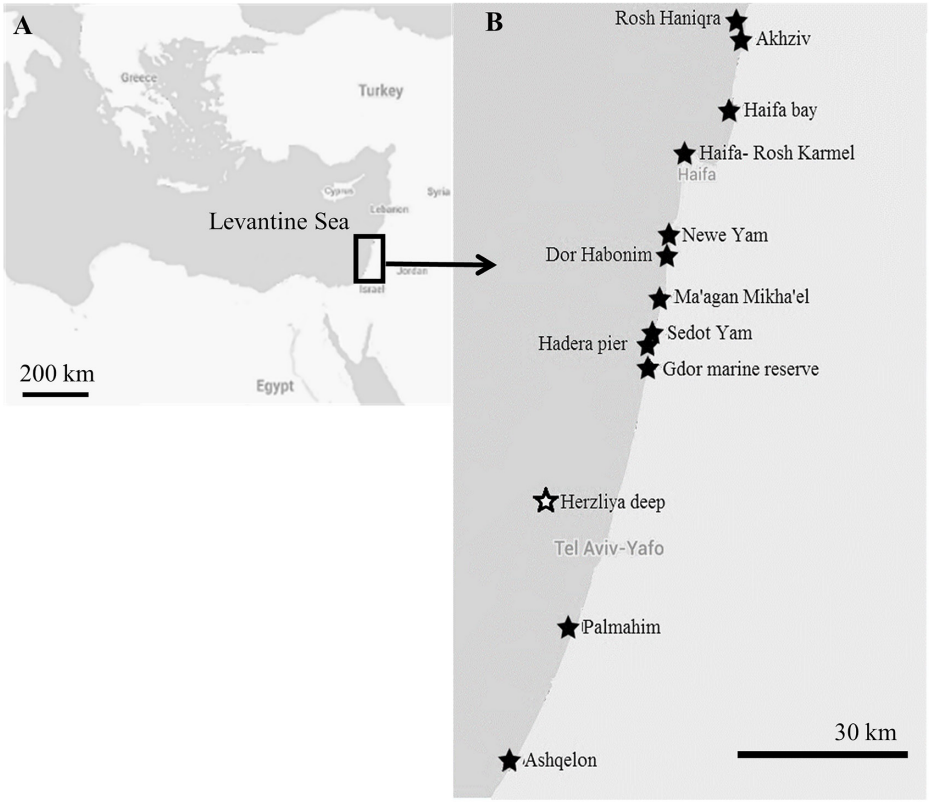

Fig. 1: The study area: A. Map of the Levantine Sea. B. Map of the sampling sites along the Israeli coast. The mesophotic site is marked with a white star.

Table 1. List of sampling sites with GPS coordinates.

	Site name	Depth	GPS coordinates	
			Latitude	Longitude
A	Rosh Haniqra	<5 m	33.08463° N,	035.08346° E
B	Akhziv	<5 m, 15-27 m	33.04867° N,	035.09410° E
C	Haifa bay	15-25 m	32.91562° N,	035.06595° E
D	Haifa- Rosh Karmel	14 m	32.83474° N,	034.96055° E
E	Newe-Yam	<5 m	32.68099° N,	034.92330° E
F	Dor Habonim	<5 m	32.64092° N,	034.91879° E
G	Ma'agan-Mikha'el	2-7 m	32.56012° N,	034.90184° E
H	Sedot-Yam	<5 m, 25-30 m	32.49528° N,	034.88399° E
I	Hadera pier	<5 m , 15-25 m	32.47211° N,	034.87232° E
J	Gdor marine reserve	<5 m	32.42854° N,	034.87313° E
K	Herzliya deep	95-120 m	32.17710° N,	034.63306° E
L	Palmahim	2-6 m	31.93526° N,	034.68452° E
M	Ashqelon	2-6 m	31.69553° N,	032.55095° E

the coast of Herzliya (32°10.62' N 034°37.98' E, indicated as Herzliya deep in Fig. 1). This site was selected based on bathymetric data from the National Bathymetric Survey project of the Israeli sea bed (received from the Israel Oceanographic and Limnological Research institution). The bathymetric map of this site showed that eight pinnacles of the ridge were elevated well above the sediment and were about 300 m apart from each other. The pinnacles rise from 120-130 m depth at the base to about 93-97 m below the surface. Sponges were sampled from four of these pinnacles, while an extensive quantitative survey was conducted on three of the four (named here South, Middle, and North). Each of the pinnacles features a different structure (Fig. 2): the South pinnacle has the most moderate slopes of the three, with its top at 92 m and bottom at 118 m, the Middle pinnacle's slopes are steeper; with the top at 95 m having a saddle shape while the bottom is at 124 m, and the North pinnacle is shaped like a horseshoe, with a plateau top at 94 m and very steep slopes leading to the bottom at 121 m.

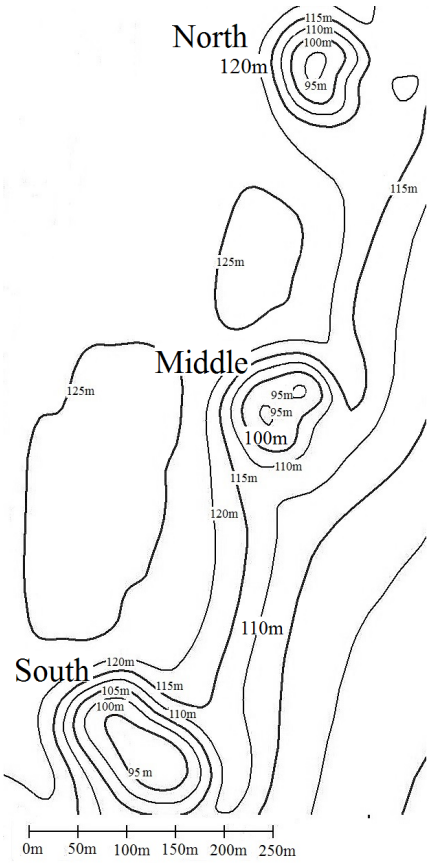

Fig. 2: Contour map of the mesophotic pinnacles studied (North, Middle, and South). Depth is noted on the contour lines.

Sample collection

In the upper-photic zone the most abundant and conspicuous sponges were sampled by scuba diving. The specimens were photographed *in situ* (underwater) prior to collection, and again in the laboratory, followed by preservation in 85% and 100% ethanol for morphological and molecular taxonomic evaluation, respectively.

Collection at the mesophotic site was conducted from the R/V Mediterranean Explorer (EcoOcean) using a Falcon Seaeye ROV equipped with a five-function manipulator and a storing basket, allowing collection of up to 20 samples per dive (depending on sample size). The ROV was also fitted with a sonar, HD (high definition) camera (GoPro Hero3+), an acoustic positioning system USBL (ultra-short baseline), and navigation software that enabled determination of the absolute position of the ROV when sampling. Collected specimens were documented and preserved as described above. Vouchers of the collected material have been deposited in the Steinhardt Museum of Natural History and National Research Center at Tel Aviv University (museum numbers are presented in Table S1 in the Supplement).

Sponge identification

Preparation of spicules and tissue sections followed standard methods (Hooper, 2003). In some cases, spicules and tissue were examined using scanning electron microscopy (SEM). The spicule composition was analyzed for each sample according to Rützler (1978). For spicule type, the length and width were measured and the size range, mean and standard deviation were calculated (n = 30 spicules per sample). The diameter of sponge fiber and skeletal arrangement were examined using a light microscope on hand-cut sections of the ectosome and choanosome. We used Hooper and van Soest (2002b), Morrow and Cárdenas (2015), and the World Porifera Database (Van Soest *et al.*, 2016) for classification and identification to genus level.

Following species identification, we compiled a list of species recorded in the current surveys, together with data acquired from previous studies along the Israeli coast (Levi 1957; Tsurnamal 1967, 1968, 1969a; Ilan *et al.,* 1994, 2003).

Molecular Identification

Molecular identification of the samples was performed in order to support the morphological identification and to create a molecular database for the local fauna. In cases of uncertain identification, all collected samples were sequenced. Small tissue sections were carefully cleaned of epi-fauna in order to avoid contamination from foreign DNA. DNA was extracted with DNeasy (Qiagen #69504)

following the manufacturer's protocol (for the elution of the DNA we used only 40 µl of elution buffer). For the molecular identification we amplified and sequenced fragments from either the 18S rDNA or the mitochondrial COI (the barcoding region) commonly used for sponge phylogeny (Cárdenas *et al.,* 2012). The choice of molecular marker to amplify for species identification was based on data available for comparison in GenBank, or on PCR success. Additionally, when only a few sequence data were available for comparison in the GenBank database, the 28S rDNA (the C1 D2 domain) was amplified to complement the inference based on COI or 18S sequences. Primers used for the PCR amplifications are listed in Table S2 (in the Supplement). The 18S rDNA gene was amplified using the primer sets 18S1/18S2 (Borchiellini *et al.*, 2001). If nothing was obtained in the first (external) PCR, re-amplification was performed in two overlapping fragments using the primer-pairs 18S1/18S6 and 18S3/18S2. For irciniid sponges the primer-pairs 18S1/18S_R1425_Irc and 18S_D1000b_Irc/18S2 were also used for re-amplification (Table S2 in the Supplement). The 28S rDNA gene was amplified using the primer sets C1' modified/D2 (Chombard *et al.*, 1998) or C1' modified/28S_R1t. For irciniid sponges, the primer set 28S_IrcD1/28S_IrcR1 was designed instead. Since the variability among irciniid sequences was low, the set of primers SP58bF/SP28cR from Thacker & Starnes (2003) was used to amplify a fragment of the ITS2, upstream to the 28S gene. A specific set of primers ITS2_G1_D1/ITS2_G1_R1 was also designed for dictyoceratid sponges (Table S2 in the Supplement). The COI gene was amplified with the primers LCO1490 (Folmer *et al.*, 1994) and COX1R1 (Rot *et al.*, 2006), followed by a re-amplification of the initial PCR product using LCO1490 and COX820R_G1 when needed. Some irciniid COI were amplified as described in Belinky *et al.* (2012). *Aaptos aaptos* (Po.25875) was amplified with LCO1490/HC02198 (Folmer *et al.*, 1994).

To support the identification based on sequence similarity, phylogenetic reconstructions were performed. To reduce computation time, only sequences closely related to the specimens collected were included in the phylogenetic analyses. Specifically, each sequence was submitted to a Blastn search (BLASTN 2.5.0+ Zhang *et al.,* 2000) against the nucleotide database of the National Center for Biotechnology Information (NCBI). The five highest sequence results, with a percentage of identity over 93%, were downloaded. For each dataset, sequences were aligned with MAFFT 7.304 (Katoh & Standley 2013) using L-INS-i parameters. In cases in which several sequences were very long compared to the rest of the sequences, 5' and 3' ends of the alignment were trimmed. In order to avoid regions of poor alignment, separate 18S rDNA and 28S rDNA datasets were considered for each Demospongiae clade (i.e., Keratosa = *G1*, *Myxospongiae* = G2, marine Haplosclerida = G3, the rest of demosponges = G4; Borchiellini *et al.,* 2001). Following Belinky *et al.,* (2012), phylogenetic trees were reconstructed under the maximum likelihood criterion with PhyML 3.0 (Dereeper *et al.,* 2008) using the GTR+G4+I model of sequence evolution (the model parameters were estimated). Other parameters were set to default. Bootstrap percentages (BPs) were computed for each dataset based on 100 replicates. The results are presented in the supplemental Figs. S1 - S4. The sequences have been submitted to Genbank under accession numbers (KX622143- KX622163; KX866734- KX866812) (See Supplementary Table S1).

Quantitative survey

Three of the four sampled pinnacles were analyzed for species richness, diversity, and percentage live cover (Fig. 2). Due to the high cost of each mesophotic expedition we could only conduct an extensive survey on three pinnacles. The quantitative survey was carried out using the Falcon Seaeye ROV equipped with two parallel laser beams (used as a scale). For the purpose of this survey, still images were taken with an HD camera (one photo per second per frame), creating a collection of quadrats of known sizes. The manipulator and collection basket were removed in order to provide a clear camera view. Each non-blurred, non-overlapping photo with a clear scale (obtained by the lasers) was defined as a quadrat suitable for analysis. A database of 187 quadrats was uploaded to CoralNet (Beijbom *et al.*, 2012; Beijbom *et al.*, 2015) in order to determine the sponge and total live coverage (including all sessile invertebrates). Since the GoPro Hero3+ camera has a wide-angle lens, it creates a distortion of the image edges. To avoid any bias in the analysis, only a 50X70 cm quadrat, from the non-distorted center of each image, was analyzed. To determine the sponge and total live coverage we employed a point count method in which points are scattered in a stratified random manner on the image, followed by manual annotation of each point (Shihavuddin *et al.*, 2013). Overall, 180 points were annotated in each image using CoralNet.

The percentage cover for each of the three surveyed pinnacles at the mesophotic site was calculated according to the proportion of number of times a point was placed on a specific object. For the North, Middle, and South pinnacles, 52, 87, and 48 photo quadrats were analyzed respectively. To calculate the percentage of sponge cover as well as the percentage of total live cover, three annotation categories were used: sponge, other invertebrates, and substrate. Other invertebrates included cnidarians, molluscs, annelids, bryozoans, and chordates (Class: Ascidiacea).

Species richness and diversity were calculated by counting the number of sponge species in each image. Some of the examined sponge species could later be eas-

ily identified in the pictures. Other individuals that were not sampled but were identified from the pictures based on distinctive morphology are generally referred to as morphospecies. It is therefore likely that some of the morphospecies consist in more than one species, but this could not be determined from the images alone (Bell *et al.*, 2001). Richness and diversity analyses were performed based on the 187 quadrats previously used for the CoralNet database, and encompassed both the identified sponge species and morphospecies. Since quantitative surveys were not conducted at the shallow sites, species were referred to as common based on their presence, spatial distribution, and size at the various sites. In the mesophotic sponge ground, in order to better describe the community we measured the abundance of each species.

To estimate the differences between the pinnacles, all statistical analyses were performed using the R 3.3.0 software and R studio 3.2 (R Development Core Team, 2014). A species-by-site matrix was created from the raw data and used for the analyses. Diversity, richness, evenness, rarefaction, and ordination were calculated with Vegan 2.3 (Oksanen *et al.*, 2011) and Rich 0.3 packages (Rossi, 2011) employing the above-noted matrix (raw data). Kruskal-Wallis rank sum test was used as a post-hoc test on the results of each photoquadrat for diversity (Shannon index), richness, and evenness (Pielou index).

To visualize the level of similarity of sub-samples in the mesophotic sponge community among the different pinnacles, Non-metric Multidimensional Scaling (NMDS) ordination was performed (Field *et al.*, 1982). NMDS was calculated using Bray-Curtis dissimilarity (Bray *et al.*, 1957) for sub-samples of eight sequential quadrats, resulting in six, ten, and five sub-samples for the North, Middle, and South pinnacles, respectively. These quadrats were regarded as a transect 5.6 m long by 50 cm wide (this step was required due to a larger number of samples than species (Field *et al.*, 1982)). Rarefaction analysis compared the richness among the pinnacles, considering the different sampling efforts and community structure.

Analysis of similarity (ANOSIM) was employed to examine the variation in species abundance and community composition by comparing distances between the pinnacles with distances within pinnacles, as well as a pairwise comparison in a post-hoc test (Clarke, 1993; Warton *et al.*, 2012). The ANOSIM analysis was performed using the rank order of dissimilarity values created for the raw data. These analyses provided a better understanding of the NMDS results by confounding the differences between groups and dispersion within groups.

SIMPER analysis was applied in order to identify the percentage contribution of each species to the overall similarity within pinnacles, and the dissimilarity among areas (Clarke *et al.*, 1994). To calculate the size range (length for branching sponges and diameter for massive species) of dominant sponge species, ImageJ 1.49p software was used utilizing the ROV's laser scale (Sheffield, 2007). The size of ball-shaped and rod-shaped sponge species was measured based on their diameter or length, respectively.

Results

During this study, 270 sponge specimens were collected and classified to the lowest possible taxonomic level (Table S1 in the Supplement). From these specimens (including 98 from the mesophotic depth), 60 sponge species were identified (Table S1 in the Supplement). The morphological identification was supported by molecular sequences, of which 44 are novel to the GenBank (COI, 18S and 28S new sequences, Table S1). Our data, together with the literature records, revealed that 111 sponge species belonging to the classes Demospongiae and Homoscleromorpha (Table S1 in the supplement) are currently known from along the coast of Israel. These species belong to 16 orders and 42 families of Demospongiae and one order of Homoscleromorpha (TableS1, Figs. S1- S4). We recorded 84 sponge species from the shallow waters (Table S1) and 36 species from the mesophotic sponge ground (Table 2). Of the latter, 27 species are absent from the upper-photic zone of the Israeli coast and at least 12 of these are new to the Levantine Sea (Table 2).

The five most common and conspicuous species found at nearly all shallow-water (0-7 m) sites were *Sarcotragus spinosulus, Chondrosia reniformis, Crambe crambe, Dysidea* sp. 1, and *Cinachyrella levantinensis*. At 20-30 m depth, *Axinella polypoides, Axinella verrucosa,* and *Petrosia ficiformis* were found at all sites. The most abundant species found in the mesophotic depth are presented in

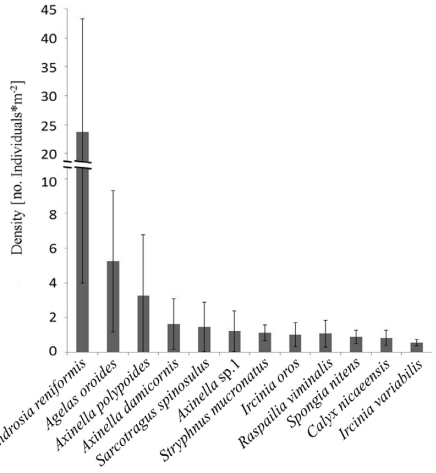

Fig. 3: Density (number of individuals m^{-2}) of the most prominent sponge species in the mesophotic sponge grounds (mean ± SD). Only species that were collected and could be identified in the photographed survey are presented in this figure (N = 184 quadrats, total area sampled 64.4m^2).

Figure 3. The most prominent sponges in the mesophotic zone were *C. reniformis,* followed by *Agelas oroides* and *A. polypoides* (Figs. 3 & 4). *C. reniformis* was by far the most abundant sponge (Fig. 3), and despite its individuals having a relatively small size (Table 3), they dominated the terrain, with up to 50 individuals recorded in a quadrat (50 X 70 cm). Other sponges, in contrast, such as *A. oroides, Stryphnus mucronatus*, *Ircinia oros*, and *Calyx nicaeensis* (Figs. 3 & 4), were less abundant but could reach a considerable size (Table 3) and therefore occupy a large area, thereby both contributing to the sponge coverage and creating more niches for other taxa.

Table 2. Sponge species collected from the mesophotic sponge ground and their distribution in the Levantine Sea. *- reported in Tsurnamal (1968). ?- unknown distribution of species that are not yet identified. ** Perez T. personal communication.

	Sponge taxa	Upper-photic zone Israel	Levantine Sea
Demospongiae			
1	*Agelas oroides**	-	+
2	*Axinella verrucosa*	+	+
3	*Axinella damicornis*	-	-
4	*Axinella* sp.1	-	?
5	*Axinella* sp.2	-	?
6	*Axinella polypoides*	+	+
7	*Raspailia viminalis**	-	+
8	*Dictyonella* sp.	-	?
9	*Dictyonella incisa*	-	-
10	*Thymosiopsis conglomerans*	-	-
11	*Chondrosia reniformis**	+	+
12	*Ircinia dendroides*	+	+
13	*Coscinoderma sporadense*	-	-
14	*Dictyoceratida* sp1.	-	?
15	*Lamellodysidea* sp.	-	?
16	*Fasciospongia cavernosa*	+	+
17	*Ircinia oros*	-	+
18	*Ircinia variabilis**	+	+
19	*Sarcotragus foetidus*	-	+
20	*Sarcotragus spinosulus*	+	+
21	*Spongia lamella*	-	-
22	*Spongia nitens*	-	-
23	*Spongia zimocca*	-	-
24	*Chalinidae* sp.	-	?
25	*Chalinula* sp.	-	?
26	*Haliclona* sp.	-	?
27	*Calyx nicaeensis*	-	+
28	*Phorbas topsenti*	+	+
29	*Phorbas tenacior*	-	-
30	*Jaspis* sp.	-	?
31	*Stryphnus mucronatus*	-	-
32	*Aplysina cavernicola*	-	-
Homoscleromorpha			
33	*Oscarella lobularis***	+	+
34	*Oscarella tuberculata***	-	-
35	*Oscarella* sp.	-	?
36	*Plakortis* sp.	-	?

The analysis of the three pinnacles for species richness (Fig. 5, Table 4), diversity (Table 4), evenness (Table 4), and percentage cover (Fig. 6) revealed significant differences in all parameters (Table 5); hence, pairwise comparisons were made. We found that the richest areas in all pinnacles were the slopes, while the least rich was the plateau at the top. The North pinnacle was the richest, with 57 sponge species and morphospecies (Fig. 5, Table 4 & 5), the highest cover of other invertebrates, and the highest total live coverage (Fig. 6, Table 5). The Middle pinnacle, though the least rich and diverse (Fig. 5, Table 4), presented a sponge coverage as high as that of the North pinnacle (Fig. 6, no significant difference - Table 5). The rarefaction curve of the Middle pinnacle reached a plateau, indicating that it had been sufficiently sampled and no additional species were expected to be found by increasing the sampling effort, unlike the North and South pinnacles whose curves were not asymptotic (Fig. 5). The steep slope of the rarefaction curves (Fig. 5), as well as the Pielou index (Table 4), showed that all three sponge communities were relatively even. The pooled species richness of all three sites together (63) was higher than that of the richest pinnacle alone (57) (Fig. 5, Table 4).

The percentage of total live cover (Fig. 6) revealed a greater similarity between the Middle and North pinnacles. The South pinnacle was characterized by a relatively low total live cover, and had both the lowest sponge and other invertebrate coverage. Kruskal-Wallis rank sum test revealed a significant connection between site and percentage cover (Table 5), indicating that the sites significantly differed in this respect. Pairwise analysis of Kruskal-Wallis rank sum test revealed that sponge coverage was significantly lower in the South pinnacle (Table 5).

The differences between the three pinnacles in percentage cover and species composition were further supported by the NMDS and ANOSIM analyses (Figs. 7 & 8). Both analyses revealed that the North and South pinnacles shared more sponge species with similar abundances, while the Middle pinnacle differed significantly (Fig. 8 B - D). Furthermore, it was clear from the NMDS ordination that the North and Middle pinnacles differed in their community compositions from that of the South pinnacle, as their subsamples clustered closely together, while the South pinnacle's subsamples were spread apart. SIMPER analysis revealed that *C. reniformis* and *A. oroides* were the two species that contributed most to the difference among the three pinnacles.

Fig. 4: Some of the mesophotic sponge species (as well as morphospecies) identified. Species marked by an asterisk were also found in our survey of the shallow waters in Israel. (A) *Agelas oroides,* (B) *Lamellodysidea* sp., (C) *Axinella damicornis*, (D) *Axinella polypoides**, (E) *Axinella* sp. 1, (F) *Dictyonella incisa*, (G) *Raspailia (Raspailia) viminalis* (H) *Sarcotragus spinosulus**, (I) *Chondrosia reniformis**, (J) *Aplysina cavernicola* (red arrow) and *S. spinosulus** (blue arrow),(K) *Calyx nicaeensis*, (L) *Ircinia oros* (red arrow), *Spongia nitens* (blue arrow) and *S. spinosulus** (green arrow), (M) *Stryphnus mucronatus* with occupying fauna (N) morphospecies, identified as *Haliclona* sp., (O) *Phorbas topsenti**, (P) *Oscarella lobularis.* On pictures J and H the two red/green dots are the marks of laser rays that are 7 cm apart.

Table 3. Mesophotic sponge sizes (diameter or length depending on sponge shape, in cm). Size was measured only for collected sponges that could be easily identified in the photographic survey.

Sponge species	Size range (cm)	Average (±SE)	n
Agelas oroides	5.5-25.3	13.5± 4.7	35
Axinella polypoides	10.24-51.4	26.4±9	30
Calyx nicaeensis	4.6-41.8	18.2±10.4	35
Chondrosia reniformis	3.1-9.7	5.4±1.7	41
Ircinia variabilis	6.2-15.6	10.5±3.8	8
Ircinia oros	7.4-22.0	14±4.3	25
Sarcotragus spinosulus	6.8-26.6	13±4.7	33
Spongia nitens	6.5-21.7	13.7±5.2	8
Stryphnus mucronatus	7.4-47.4	27.6±8	23
Dictyonella incisa	3.2-9.6	6.7±1.9	10

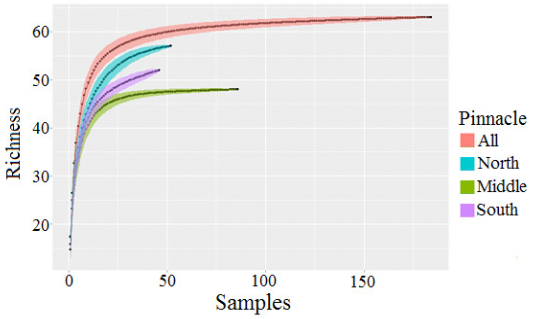

Fig. 5: Rarefaction curves of species richness (for both species and morphospecies) in the mesophotic sponge grounds. This is a species-by-sample analysis with 1000 permutations for each curve. The top curve sums all samples from the three pinnacles. The confidence interval is within the colored area of each curve.

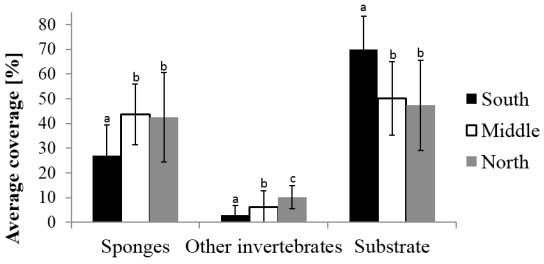

Fig. 6: Percentage cover at the mesophotic sponge ground (mean ±SE; n=187). Pairwise Kruskal-Wallis rank sum test revealed significant differences between pinnacles (Table 5). Pinnacles significantly differing in each category are marked with a different letter above the bar.

Table 4: Sponge diversity, richness, and evenness values (species and morphospecies) for three mesophotic pinnacles

Pinnacle	Diversity (Shannon index)	Richness	Evenness (Pielou index)
South	3.37	52	0.85
Middle	3.01	48	0.78
North	3.28	57	0.81
All	3.37	63	0.81

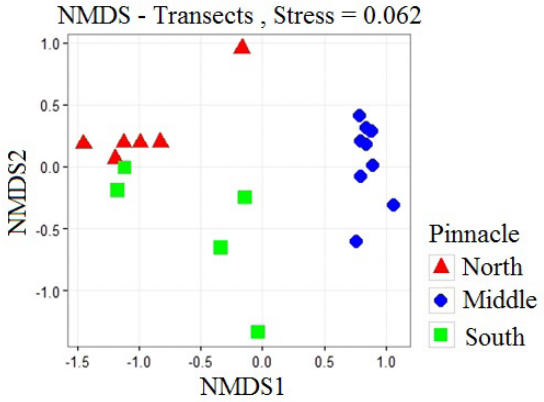

Fig. 7: NMDS ordination of mesophotic sites by sponge species, calculated with Bray Curtis dissimilarity matrix. The figure depicts the two dimensions that explain most of the dissimilarity.

Table 5: Summary of results of pairwise Kruskal-Wallis rank sum test done to test for significance of differences in measures of richness, diversity, evenness and percentage cover among the pinnacles at the mesophotic site.

Pinnacles compared	Richness	Diversity	Evenness	Percentage cover		
				Sponges	Other invertebrates	Substrate
All	χ^2 = 7.044, df = 2, p = 0.0295*	χ^2 = 10.79, df = 2, p = 0.0045**	χ^2 = 12.36, df = 2, p = 0.0021**	χ^2 = 46.032, df = 2, p = 1.01e-10**	χ^2 = 63.856, df = 2, p = 1.361e-14**	χ^2 = 62.035, df = 2, p = 3.382e-14**
North & Middle	χ^2 = 4.695, df = 1, p = 0.0303*	χ^2 = 11.96, df = 1, p = 0.0005**	χ^2 = 6.097, df = 1, p = 0.0135*	χ^2 = 0.4092, df = 1, p = 0.522	χ^2 = 19.473, df = 1, p = 1.02e-05**	χ^2 = 0.64, df = 1, p = 0.423
Middle & South	χ^2 = 0.686, df = 1, p = 0.4074	χ^2 = 0.551, df = 1, p = 0.4577	χ^2 = 9.562, df = 1, p = 0.0019**	χ^2 = 37.095, df = 1, p = 1.125e-09 **	χ^2 = 31.459, df = 1, p = 2.037e-08**	χ^2 = 44.726, df = 1, p = 2.266e-11**
South & North	χ^2 = 5.684, df = 1, p = 0.0171*	χ^2 = 2.834, df = 1, p = 0.0923	χ^2 = 1.957, df = 1, p = 0.162	χ^2 = 36.14, df = 1, p = 1.417e-08 **	χ^2 = 52.739, df = 1, p = 3.531e-12**	χ^2 = 55.901, df = 1, p = 7.264e-13**

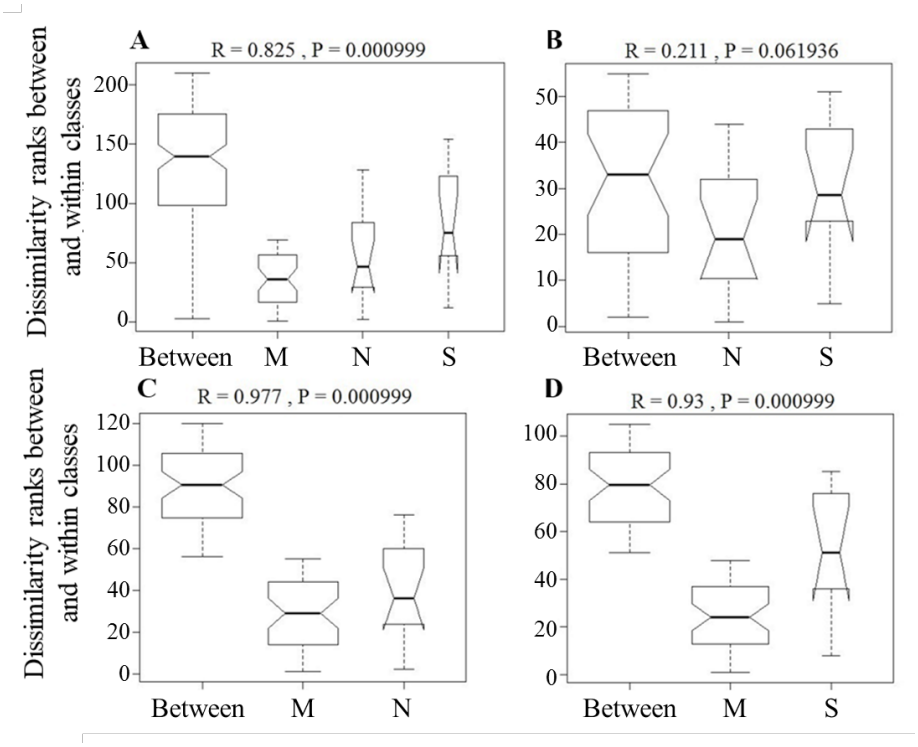

Fig. 8: Pairwise comparison of the species composition dissimilarity scores among the three mesophotic pinnacles, using ANOSIM analyses, (notches indicate the 95% CI). N-north pinnacle, M- middle pinnacle, S- south pinnacle. A. Comparison of all three pinnacles. B. Comparison of the North and South pinnacles. C. Comparison of Middle and North pinnacles. D. Comparison of Middle and South pinnacles (Clarke, 1993; Warton *et al*., 2012).

The ANOSIM analysis revealed that the dissimilarity among the three pinnacles was greater than the dissimilarity within sites (Fig. 8). Upon further examination of the pairwise comparison, the statistics presented in Fig. 8B imply that only a small part of the difference between the South and North pinnacles can be significantly explained by species composition. Figure 8C-D shows that the Middle pinnacle differed significantly from both the North and South pinnacles in species composition. Based on SIMPER analysis, *C. reniformis* explained 14% of the difference between the North and Middle pinnacles and 12.5% of the difference between the South and Middle pinnacles. *Agelas oroides* explained 5% of the difference between the North and Middle pinnacles but only 1.7% of the difference between the North and Middle pinnacles.

Discussion

The Levantine Sea sponge diversity had been regarded until now as species-poor compared to that of other parts of the Mediterranean Sea (Voultsiadou, 2009; Coll *et al.*, 2010; Van Soest *et al.*, 2012). This has been mainly attributed to the physicochemical conditions of the Levantine Sea, which are less suitable for the Atlantic species that inhabit the western and northern parts of the Mediterranean. However, it has also been suggested that the perceived lower sponge diversity of the south-east Mediterranean could be an artifact, due to insufficient study of the benthic fauna of this vast area (Voultsiadou, 2009). Our findings seem to support the latter hypothesis. Prior to this study, 44 sponge species (excluding Calcarea) had been reported from the Israeli coast in scientific publications (Levi, 1957; Tsurnamal, 1967; 1969a; Ilan *et al.*, 1994; Ilan *et al.*, 2003); while a few additional papers had studied or reviewed sponges of the Levantine Sea among those of other areas (Perez *et al.*, 2004; Voultsiadou., 2005a; Vacelet *et al.*, 2007; Vacelet *et al.*, 2008; Voultsiadou, 2009; Evcen & Cinar, 2012; Topaloglu & Evcen, 2014). Here, we have listed 111 species, 37 of which are new records for the Levantine Sea, thereby elevating this region's sponge richness to 143 species. Fourteen additional species have not yet been identified to species level, some of which could also probably be new records. Such a knowledge gap can be

attributed primarily to the outdated, less accessible, and incomplete published data of past studies (for example, 20 of the demosponge species were noted only in Tsurnamal's thesis, published in Hebrew and not available online (Tsurnamal, 1968)). The sponge species found in the present study in shallow waters (0-7 m) were also present in the area during the 1960s (Tsurnamal, 1968), except for *Liosina blastifera, Cinachyrella levantinensis,* and *Aplysina* sp. In addition, two species (*Agelas oroides* and *Raspailia (Raspailia) viminalis*) that were documented in the shallow waters by Levi (1957) and Tsurnamal (1968), and which for decades had been considered lost from the Israeli sponge fauna (Ilan unpubl.), were found in the mesophotic sponge ground, where they were abundant. *Raspailia (Raspailia) viminalis* was probably rare during the 1960's surveys (a single specimen was recorded), while *A. oroides* was at that time common in the shallow waters of Israel, inhabiting crevices and constituting only a small part of overall sponge coverage. Our current survey in the mesophotic zone revealed *A. oroides* to be the second most common sponge (Fig. 4), reaching up to 25 cm in width (Table 3). For 11 of the mesophotic sponges, *Axinella damicornis*, S*tryphnus mucronatus*, *Thymosiopsis conglomerances*, *Coscinoderma sporadense*, *Spongia lamella, Spongia nitens*, *Spongia zimocca, Oscarella tuberculata*, *Dictyonella incisa*, *Aplysina cavernicola,* and *Phorbas tenacior* (Fig. 3), this is the first record from the Levantine Sea (Table 2; see Levi, 1957; Tsurnamal, 1968; Voultsiadou, 2005a; Topaloglu *et al.*, 2014), while 15 species are known from the Levant but are new to the Israeli coast (see Levi, 1957; Tsurnamal, 1968; Vacelet *et al.*, 2007; Evcen & Cinar, 2012; Topaloglu & Evcen, 2014). These results are in accordance with Bo *et al.* (2012), Gerovasileiou & Voultsiadou (2012), and Pérez *et al.* (2004), demonstrating that studying a broader diversity of habitats can increase the known species richness of an area.

Some of the collected mesophotic sponges were not identified to species level and they may be new species. For example, *Jaspis* sp., which resembles *Jaspis johnstonii* in skeletal organization as well as in color and consistency, was found in the mesophotic sponge ground to have considerably longer oxeas. Unfortunately, no sequence was available for *J. johnstonii* in the databases.

As the mesophotic community described in the current study has proven to be rich and diverse, we expect that further research of other mesophotic communities in different localities along the Israeli coast will uncover additional species that have not been described previously from this area, some of which might be novel. Nearly all the species we identified have been found in other locations in the Mediterranean Sea. However, there is only a small overlap between the shallow and the mesophotic community along the Israeli coast, with merely nine species that thrive across this entire depth range (Table 2). A prominent phenomenon of those sponge species along the Israeli coast that were found solely at the mesophotic depth, is that in other parts of the Mediterranean they also grow at shallower depths. Thus, although most of them have a wide depth range, some were not previously known to occur at the mesophotic depth (see Voultsiadou, 2005a; 2005b; 2009; Topaloglu *et al.*, 2014).

The mesophotic sponge ground lies beneath the seasonal thermocline (Kress *et al.*, 2014). Temperatures at this depth are very stable and do not exceed 18˚C, whereas in recent years the local shallow-water temperature has often risen above 30˚C during the summer. Over the past 50 years, the average sea-surface temperature in the Levantine Sea has risen by over 2°C during summer (Shaltout *et al.*, 2014). It is suggested that *A. oroides* and *R. viminalis* may be sensitive to high temperatures and hence can now only occupy the mesophotic habitat and no longer the shallower habitats in Israel. Similar explanations for the observed trends in Mediterranean sponge richness were given by Voultsiadou (2005a; 2009) and Bianchi (2007). The stable environmental conditions of the mesophotic sponge ground, in addition to a lesser extent of anthropogenic disturbance (such as pollution and fishing), could explain the high richness and diversity of the sponge community in this habitat. Moreover, while in the shallow waters, exposed to high light intensity, the fast-growing algae have a competitive advantage (Easson *et al.*, 2014), in deeper waters, with diminishing light intensities, which limits photosynthesis, every part of the sandstone ridge that is sufficiently elevated becomes a sort of marine oasis for benthic fauna and is dominated by sponges (95% of the total live coverage). Sponges, as filter-feeders, have an inherent advantage at this depth (Gerovasileiou & Voultsiadou, 2012).

In addition to the differences between shallow and mesophotic communities, the communities on each of the mesophotic pinnacles differed in both species composition and total live coverage. A similar small-scale diversity in sponge assemblage was described for shallow-water coral reefs in Indonesia and Australia (Hooper *& van Soest 2002*; Bell *et al.*, 2004). We suggest that in our study the differences are probably due to the distinct morphology of the pinnacles, since the more complex pinnacles with vertical walls support a higher total live cover. Sponges were the most dominant phylum in this mesophotic community and were associated with a diverse epifauna and infauna. These sponge associates were usually invertebrates of various phyla (e.g. Cnidaria, Mollusca, Annelida, Bryozoa, Arthropoda, Echinodermata, as well as other Porifera; Goren unpublished data). Although the mesophotic sponge grounds are currently under less stress compared to the shallow-water communities, they might already be facing a serious threat. In recent years explorations for natural gas and oil have been carried out along the Israeli coast of the Mediterra-

nean (sometimes in proximity to these sponge grounds), with the aim of this area becoming a center for the petroleum industry. Thus, drilling lubricants and thin sediment suspended in the water column from such excavations could cover and clog the filtering apparatus of the sponges (Gerrodette *et al.*, 1979; Tompkins-MacDonald *et al.*, 2008; Tjensvoll *et al.*, 2013; Edge *et al.*, 2016). In addition, fishermen off the Israeli coast are deploying a new type of gear for deep bottom-trawling, which is specially designed to operate over rocky outcrops and can be destructive to this sensitive community.

Mesophotic sponge grounds are scarce along the Israeli coast. To date, we have located only four such habitats at ca. 100 m depth, none of which have previously been studied. These mesophotic communities might act as refugia for some sponge species as well as for other invertebrates and fish, as exemplified by the sponges *A. oroides* and *R. viminalis*, which can now only be found in the mesophotic habitat, where they are very common. Consequently, the exploration of this habitat that hosts a diverse sponge community, including new species records for this part of the Mediterranean, has revealed the necessity for the protection of such a unique community and ecosystem. The Barcelona Convention for Protecting the Mediterranean Sea (1995) calls for its signatories to create specially protected areas in the sea in order to safeguard, among others, sensitive benthic habitats. This could be achieved by applying the precautionary principle, calling for the protection of habitats that might be at risk. It is argued that the mesophotic habitat described here meets all the above criteria, and thus should be urgently designated as an endangered habitat that requires protection as such.

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Appendixes

Table S1. List of sponge species reported up to date from the Israeli coast, with finding places and depth range, and their distribution in the Mediterranean. For sponges collected in the present study museum voucher numbers and GenBank accession numbers are given.

Abbreviations: Adriatic Sea- AdS, Aegean Sea- AS, Alboran Sea- AlS, Egypt- Eg, Gulf of Sidra – GS, Ionian Sea- IS, Israel- Is, Levantine Sea- LS, Mediterranean Sea- MD, East Mediterranean Sea- ME, West Mediterranean Sea- MW, Syria- Sy, Turkey- Tu. Depth range (I: 0–10 m; II: 11–50 m; III: 51–100 m; IV: 101–200 m; VII: >600 m).

Species	Distribution in the Levant	Depth range in the Levant	References	Distribution in the Mediterranean[#]	Museum numbers	GenBank accession numbers		
						COX	18S	28S
Class: Demospongiae								
Order: Agelasida								
Family: Agelasidae								
Agelas oroides (Schmidt, 1864)	Is, Eg, Le	I- IV	2, 3, 4, 5, 8, 16	MD	Po.25598 Po.25569	LN868208[a]	KX866803 KX622155	KX688750 KX688753
Family: Hymerhabdiidae								
Hymerhabdia pori Tsurnamal, 1969	Is	I	5, 6	LS				
Hymerhabdia reichi Tsurnamal, 1969	Is	I	5, 6	LS				
Order: Axinellida								
Family: Axinellidae								
Axinella cannabina (Esper, 1794)	Is, Tu	II	3, 15, 12	AdS, AS, IS, MW, LS	Po.25604	KX866735[N]		KX688755
Axinella minuta Lévi, 1957	Is	II	3					
Axinella verrucosa (Esper, 1794)	Is, Tu	II- IV	3, 12, 15, 16	MD	Po.25674, Po.25600 Po.25627	LN868210[a]	KX622143 KX622144 KX866787	
Axinella damicornis (Esper, 1794)	Is	II- IV	16	AdS, AS, IS, MW	Po.25764, Po.25924		KX622156	KX688743 KX688749
Axinella polypoides Schmidt, 1862	Is, Tu	II- IV	3, 12 15, 16	MD	Po.25597 Po.25596	LN868209[a]	KX622145	KX688754
Axinela sp. 1		III-IV	16		Po.25779 Po.26035	KX866742 KX866777	KX866782 KX866812	KX688746
Axinella sp. 2		III-IV	16		Po.25781		KX866781	KX688747
Family: Heteroxyidae								
Didiscus stylifer Tsurnamal, 1969	Is, Eg	I	2, 5, 6	AS, IS, LS, ME, WM				
Family: Raspailiidae								
Raspaciona aculeata (Johnston, 1842)	Is	II	3	MD	Po.26042	KC869426		
Raspailia (Raspailia) viminalis Schmidt, 1862	Is	II- IV	3, 5, 16	MD	Po.25743	KX866741[N]	KX622146[N]	
Raspaillidae sp.*	Is	II	15		Po.25592 Po.25574	KX866747[N] KX866752[N]	KX622162[N] KX221559[N]	

(continued)

Table S1 continued

Order: Bubarida								
Family: Dictyonellidae								
Acanthella annulata Sarà, 1958	Is	I	5	MW				
Dictyonella sp.	Is	III- IV	16		Po.25926		KX866798	
Dictyonella incisa (Schmidt, 1880)	Is	III- IV	16	MW, AdS, AS, IS, AlS	Po.25741 Po.25919	KX866740 KX866771	KX866797	
Family: Bubaridae								
Bubaris sarai Ilan, Ben-Eliahu & Galil, 1994	Is	VII	7	LS				
Order: Chondrillida								
Family: Chondrillidae								
Chondrilla nucula Schmidt, 1862	Is, Le, Tu, Eg	I-II	2, 5 ,8, 14, 15	MD	Po.25854	FR819682[b]	FR819690[b]	
Thymosiopsis conglomernsconglomerns Vacelet, Borchiellini, Perez, Bultel-Poncé, Brouard & Guyot, *2000*	Is	III-IV	16	MW	Po.25914	KX866779	KX866796[N]	
Order: Chondrosiida								
Family: Chondrosiidae								
Chondrosia reniformis Nardo, 1847	Is, Le, Tu, Eg	I- IV	2, 3, 5, 8, 12, 15, 16	MD	Po.25193	AM076986[c]	FR819689[b]	
Order: Clionaida								
Family: Clionaidae								
Cliona celata Grant, 1826	Is, Tu	I	5, 13	MD				
Cliona schmidtii (Ridley, 1881)	Is, Le, Tu	I-II	3, 5, 8, 13	MD				
Cliona vermifera Hancock, 1867	Is	I	5	AdS, AS, IS, ME, MW				
Cliona viridis (Schmidt, 1862)	Eg, Is	I-II	2, 3, 5, 15	MD	Po.25908		KX866795	
Cliothosa hancocki (Topsent, 1888)	Is	I	5	AdS, AS, IS, MW				
Pione vastifica (Hancock, 1849)	Is	I	5	AdS, IS, MW,				
Family: Spirastrellidae								
Diplastrella bistellata (Schmidt, 1862)	Is, Tu	I	5, 13	AdS, AS, IS, MW				
Diplastrella ornata Rützler & Sarà, 1962	Is	I	5	AdS, AS, IS, ME, MW				
Spirastrella cunctatrix Schmidt, 1868	Eg, Is, Tu	I-II	2, 3, 13, 15	MD	Po.25510 Po. 25527	KX866753[N]	KX622153 KX866784	KX688739[N]
Family: Placospongiidae								
Placospongia decorticans (Hanitsch, 1895)	Is, Le	I	5, 8	AdS, AS,IS, LS, MW				
Order: Dendroceratida								
Family: Darwinellidae								
Aplysilla sulfurea Schulze, 1878	Is	I	5	AdS, MW				

(continued)

Table S1 continued

Chelonaplysilla erecta Tsurnamal, 1967	Is, Le	I	4, 5, 8, 15	LS	Po.25497 Po.25531	KX866758	 KX866811[N]	KX688734[N]
Order: Dictyoceratida								
Dictyceratida sp.		III-IV	16		Po.25766	KX866765[N]		KX688744[N]
Family: Dysideidae								
Dysidea sp.	Is	IV	15		Po.25602 Po.25509 Po.25611	KX866763[N] KX866750[N] KX866764[N]	KX866786[N] KX866808[N]	KX688723[N] KX688733[N]
Lamellodysidea sp.	Is	I, III-IV	15, 16		Po.25778	KX866767		KX688738[N]
Dysidea fragilis (Montagu, 18)	Eg, Tu, Is	I	2, 5, 13	MD				
Pleraplysilla spinifera (Schulze, 1879)	Is, Tu	I	5, 13	AS, MW				
Family: Thorectidae								
Fasciospongia cavernosa (Schmidt, 1862)	Eg, Is	II- IV	2, 3, 15,16	MD	Po.25963 Po.26087			
Family: Irciniidae								
Ircinia dendroides (Schmidt, 1862)	Is, Tu	I- IV	5, 13, 15, 16	MD	Po.25798 Po.25799 Po.25500 Po.25518 Po.25830	 KX866759 KX866768	KX866790[N] KX866791[N] KX866809[N] KX866810[N] KX866793[N]	 KX688735 KX688728
Ircinia oros (Schmidt, 1864)	Eg, Tu, Is	II- IV	2, 12, 16	MD	Po.25670			KX688731
Ircinia retiderma-ta Pulitzer-Finali & Pronzato, 1981	Is	VII	7	AS, LS, MW				
Ircinia variabilis (Schmidt, 1862)	Is, Eg, Tu, Le	I- IV	2, 3, 5, 8, 13, 15,16	MD	Po.25496 Po.25492		HE591466[b]	 KX688725
Sarcotragus foetidus Schmidt, 1862	Is, Tu	I-VII	5, 7, 13, 16	MD	Po.25921 Po.25069	KX866772 KX866743		
Sarcotragus spinosu-lus Schmidt, 1862	Is, Tu, Le	I-VII	5, 8, 13, 15, 16	MD	Po.25501 Po.25502 Po.25517 Po.25673	HE591460[b]	HE591467[b] KX866788	 KX688727 KX688737 KX688732
Family: Spongiidae								
Coscinoderma spo-radense Voultsia-dou-Koukouras, van Soest & Koukouras, 1991	Is	III-IV	16	AS	Po.25932	KX866774[N]	KX866800	
*Spongia (Spongia) la-mella (*Schulze*, 1879)*	Is	III-IV	16	AdS, MW	Po.26215			
Spongia (Spongia) zimocca Schmidt*, 1862*	Is	III-IV	16	AdS, MW, GS	Po.25742			
Spongia (Spongia) ni-tens (Schmidt, 1862)	Is	III-IV	16	AdS, AS, IS, MW,	Po.25493 Po.25665	KX866744[N]	KX866807[N]	KX688726[N] KX688730[N]
Spongia (Spongia) officinalis Linnaeus, 1759	Is, Eg, Sy, Tu	I- II	1, 2, 3, 5, 12	MD				
Spongia (Spongia) virgultosa (Schmidt, 1868)	Is	I	5	AdS, AS, MW				
Order: Haplosclerida								
Family: Phloeodictyidae								
Oceanapia deci-piens (Sarà, 1958)	Is	I	5	MW				
Calyx nicaeen-sis (Risso, 1826)	Tu, Is	II- IV	14, 16	AS, IS, LS, MW,	Po.25570	KX866755[N]	KX622154[N]	KX688751[N]
Family: Chalinidae								

(continued)

Table S1 continued

Chalinula limbata (Montagu, 1817)	Is	I	5	IS, MW				
Haliclona (Reniera) cinerea (Grant, 1826)	Eg, Is	I	2, 5	MD				
Haliclona (Reniera) cratera (Schmidt, 1862)	Is,Tu	I-II	3, 12	MD				
Haliclona (Gellius) fibulata (Schmidt, 1862)	Eg, Is	II	2, 3	MD				
Haliclona (Haliclona) simulans (Johnston, 1842)	Is	I-II	3, 15	MD	Po.25866			
Haliclona (Rhizoniera) rosea (Bowerbank, 1866)	Is	I	5, 15	MW, AdS	Po.25851 Po.25853			
Haliclona (Haliclona) varia (Sarà, 1958)	Is	I	5	AdS, MW				
Haliclona sp.	Is	III-IV	16		Po25929		KX866799	
Chalinidae sp.	Is	III-IV	16		Po.25928			
Chalinula sp.	Is	III-IV	16		Po.25782			KX688748[N]
Family: Niphatidae								
Niphates toxifera Vacelet, Bitar, Carteron, Zibrowius & Perez, 2007	Le, Is	I-II	10, 15	LS	Po.25723	KX866739[N]		
Family: Petrosiidae								
Petrosia (Petrosia) ficiformis (Poiret, 1789)	Is, Eg, Tu, Le	I-II	2, 3, 8, 13, 15	MD	Po.25572	KX866751	KX622161	KX688752
Order: Poecilosclerida								
Family: Chondropsidae								
Batzella inops (Topsent, 1891)	Is	I	5	AdS, IS, MW				
Family: Microcionidae								
Clathria (Microciona) toxitenuis Topsent, 1925	Is	I	5, 15	AdS, IS, MW,	Po.25881	KX866770[N]	KX866794[N]	
Family: Crambeidae								
Crambe crambe (Schmidt, 1862)	Eg, Is, Tu	I-II	2, 5, 13, 15	MD	Po.25545 Po.25519 Po.25800	KX866761 KX866760	KX622152 KX866783 KX866792	KX688742
Family: Hymedesmiidae								
Hymedesmia (Hymedesmia) pansa Bowerbank, 1882	Is	I	5	IS, MW				
Hymedesmia (Hymedesmia) rissoi Topsent, 1936	Is	I	5	MW				
Family: Coelosphaeridae								
Lissodendoryx (Lissodendoryx) isodictyalis (Carter, 1882)	Is, Tu	I	5, 13	AS				
Phorbas topsenti Vacelet & Perez, 2008	Le, Is	I-IV	11, 15, 16	MW, LS, AS	Po.25767 Po.25573 Po.25591	KX866766[N] KX866745[N]	KX622157[N] KX622163[N]	
Phorbas fictitius (Bowerbank, 1866)	Is, Tu	I	5, 13	MD				

(continued)

Table S1 continued

Phorbas tenacior (Topsent, 1925)	Is	III -IV	16	MD				
Family: Mycalidae								
Mycale (Aegogropila) contarenii (Lieberkühn, 1859)	Is, Tu	I	5, 13	AdS, AS, IS, MW				
Mycale (Carmia) macilenta (Bowerbank, 1866)	Is	I	5	AS, MW				
Mycale (Mycale) massa (Schmidt, 1862)	Eg, Is, Tu	I-II	2, 3, 13	MD				
Mycale (Carmia) sanguinea Tsurnamal, 1969	Is	I	5, 6, 15	LS			Po.25539[N]	
Family: Myxillidae								
Myxilla (Myxilla) rosacea (Lieberkühn, 1859)	Eg, Is	I-II	2, 3, 5, 15	MD	Po.25912 Po.25913			
Family: Tedaniidae								
Tedania (Tedania) anhelans (Vio in Olivi, 1792)	Eg, Is	I	2, 5, 15	MD				
Order: Polymastiida								
Family: Polymastiidae								
Tentorium levantinum Ilan, Gugel, Galil & Janussen, 2003	Is	VII	9	LS				
Order: Suberitida								
Family: Halichondriidae								
Ciocalypta carballoi Vacelet, Bitar, Carteron, Zibrowius & Perez, 2007	Le, Tu, Is	I-II	10, 12, 15	LS	Po.25633	KX866757[N]		KX688741[N]
Halichondria (Halichondria) genitrix (Schmidt, 1870)	Is	I-II	3, 5	MW				
Halichondria (Halichondria) panicea (Pallas, 1766)	Is	I	5	MW, IS, AS, AdS				
Halichondria (Halichondria) semitubulosa Lieberkühn, 1859	Is, Eg	I	2, 5	AdS, IS, LS, MW				
Topsentia lacazei (Schmidt, 1868)	Is	I	5, 6, 15	LS, MW	Po.25580			
Family: Suberitidae								
Aaptos aaptos (Schmidt, 1864)	Eg, Is	I-II	2, 3, 5, 15	MD	Po.25875	KX866769[N]	KX622158[N]	
Rhizaxinella shikmonae Ilan, Gugel, Galil & Janussen, 2003	Is	VII	9	LS				
Terpios gelatinosus (Bowerbank, 1866)	Is	I	5, 15	AS, MW	Po.25564	KX866738[N]	KX866789[N]	KX688722[N]
Order: Tethyida								
Family: Hemiasterellidae								
Liosina blastifera Vacelet, Bitar, Carteron, Zibrowius & Perez, 2007**	Le, Is	I-II	10, 15	LS	Po.25640 Po.25631 Po.25551 Po.25593	 KX866756[N] KX866762[N] KX866749[N]	KX622147[N] KX622148[N] KX866785[N]	KX688740[N]
Family:Tethyidae								

(continued)

Table S1 continued

Tethya aurantium (Pallas, 1766)	Eg, Is, Tu	I-II	2, 3, 5, 13, 15	MD	Po.25553	KX866754	KX622150	
Timea stellata (Bowerbank, 1866)	Is	I	5	AdS, AS, IS, MW				
Order: Tetractinellida								
Suborder: Astrophorina								
Family: Geodiidae								
Erylus discophorus (Schmidt, 1862)	Is, Tu, Le	I	5, 8, 13	MD				
Geodia conchilega Schmidt, 1862	Is, Eg	I-II	2,3	MD				
Penares helleri (Schmidt, 1864)	Is, Le	I-II	3, 8	MD				
Family: Ancorinidae								
Dercitus (Stoeba) plicatus (Schmidt, 1868)	Is	I	5	AdS, AS, IS, MW				
Jaspis johnstonii (Schmidt, 1862)	Is, Le	I-II	5, 8	MD				
Jaspis sp.	Is	III-IV	16		Po.25931		KX866780[N]	
Stelletta grubii Schmidt, 1862	Is	I	5	AdS, AS, MW				
Stryphnus mucronatus (Schmidt, 1868)	Is	III-IV	16	AdS, AS, IS, MW	Po.25733	KX866734[N]		
Suborder: Spirophorina								
Family: Tetillidae								
Cinachyrella levantinensis Vacelet, Bitar, Carteron, Zibrowius & Perez, 2007	Eg, Le, Is	I-II	2, 5, 10, 15	LS	Po.25456 Po.25568 Po.25529 Po.25618	AM076987[c] JX177903[c] JX177904[c] JX177906[c]	HM629802[d] JX177969[c] JX177970[c]	JX177938[c] JX177939[c] JX177940[c]
Family: Samidae								
Samus anonymus Gray, 1867	Is	I	5	AdS, AS, IS, MW				
Order: Trachycladida								
Family: Trachycladidae								
Trachycladus minax (Topsent, 1888)	Is	I	5	AdS, MW				
Order: Verongida								
Family: Aplysinidae								
Aplysina cavernicola (Vacelet, 1959)	Is	III-IV	16	AdS, AS, MW	Po.25922	KX866736		
Aplysina sp.	Is	I	15		Po.25526 Po.25953	KX866737 KX866776		
Family: Ianthellidae								
Hexadella racovitzai Topsent, 1896	Is, Le	I-II	3, 8	MD				
Class: Homoscleromorpha								
Order: Homosclerophorida								
Family: Plakinidae								
Plakortis simplex Schulze, 1880	Is, Le	I	5, 8	MD				
Plakortis sp. 1	Is	III-IV	16		Po.25927	KX866773		
Family: Oscarellidae								
Oscarella lobularis (Schmidt, 1862)	Is	I-IV	3, 5, 15, 16	MD	Po.25934 S157	KX866748	KX866801	

(continued)

Table S1 continued

Oscarella tuberculata (Schmidt, 1868)	Is	III-IV	16	MW, AS, IS	Po.25935		KX866802
Oscarella sp.	Is	III-IV	16		Po.25936	KX866775	

* -These specimens were examined by Dr. B. Alvarez de Glasby who suggested it might be a new species of *Raspailia*. These specimens indeed clustered within the Raspailiidae family, both for 18S and COI (Figs. S1 & S3).
** -*Liosina blastifera* was related to the order Tethyida following Morrow and Cardenas (2015), supported by our molecular data (Figs. S1 & S3 in the Suplement).
# - Distribution according to the World Porifera Database.
[abcde]Sequences that were published in previous papers: [a]Huchon *et al.,* 2015, [b]Belinky *et al.,* 2012, [c]Rot *et al.*, 2006, [d]Szitenberg *et al.,* 2010, [e]Szitenberg *et al.,* 2013
[N]First sequence of this gene for this species

1- (Gruvel, 1931), 2- (Burton, 1936), 3- (Levi 1957), 4- (Tsurnamal, 1967), 5- (Tsurnamal, 1968), 6- (Tsurnamal, 1969a), 7- (Ilan *et al.,* 1994), 8- (Carteron, 2002), 9- (Ilan *et al.,* 2003), 10- (Vacelet *et al.,* 2007), 11- (Vacelet & Perez, 2008), 12- (Gözcelioğlu *et al.*, 2011), 13- (Evcen & Cinar, 2012), 14- (Topaloglu & Evcen, 2014), 15- present study shallow, 16- present study deep.

Table S2. Primers used to identify sponge samples.

primer source	primer sequence	Direction	Usage	Ref.
18S rRNA gene				
18S1	5'-AACCTGGTTGATCCTGCCA-3'	Forward	External	Borchiellini *et al.* (2001)
18S2	5'-TGCAGGTTCACCTACRGAA-3'	Reverse	External	Borchiellini *et al.* (2001)
18S3	5'-GCGTATATTAAAGTTGTTGCRGTT-3'	Forward	Re-amplification	
18S6	5'-CCTTCCGTCAATTCCTTTAAGT-3'	Reverse	Re-amplification	Belinky *et al.* (2012)
18S_D1000b_Irc	5'-GAATGACTCCGTTGGCACCTTAT-3'	Forward	Re-amplification	
18S_R1425_Irc	5'-GGCTCGCTGGCTCGATCA-3'	Reverse	Re-amplification	
C1' modified	5'-ACCCGCYGAAYTTAAGCAT-3'	Forward	External	Chombard *et al.* (1998)
28S rRNA gene				
D2	5'-TCCGTGTTTCAAGACGGG-3'	Reverse	External	Chombard *et al.* (1998)
28S_R1t	5'-CGGCAGGTGAGTTGTTACA-3'	Reverse	External	
SP58bF	5'-AATCATCGAGTCTTTGAACG-3'	Forward	External	Thacker & Starnes (2003)
SP28cR	5'-CTTTTCAYCTTTCCCTCA-3'	Reverse	External	Thacker & Starnes (2003)
28S_IrcD1	5'-GGGTTGTTTGGGAWTGCAGC-3'	Forward	External	
28S_IrcR1	5'-GGGATCTGATGAGCGTYG-3'	Reverse	External	
ITS2				
ITS2_G1_D1	5'-GCAAGCTGCGATACCTAGTGTGAA-3'	Forward	External	
ITS2_G1_R1	5'-GCTCTCACCCTCTYYGGCCCGCCT-3'	Reverse	External	
COI gene				
LCO1490	5'-GGTCAACAAATCATAAAGATATTGG-3'	Forward	External	Folmer *et al.* (1994)
COX1R1	5'-TGTTGRGGGAAAAARGTTAAATT-3'	Reverse	External	Rot *et al.* (2006)
HC02198	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	Reverse	External	Folmer *et al.* (1994)
COX820R_G1	5'-GCATAMACCATCCCCAAATA-3'	Reverse	Re-amplification	

Fig. S1: Phylogenetic tree used to support the morphological identification of Israeli sponges. The maximum likelihood tree was reconstructed with PhyML 3.0 (Dereeper *et al*. 2008) based on COI mtDNA sequences (1214 bp), under the GTR model of sequence evolution. Bootstrap supports higher than 50% are given near the corresponding nodes. Sequences from Mediterranean sponges obtained in the present study are marked by an asterisk*.

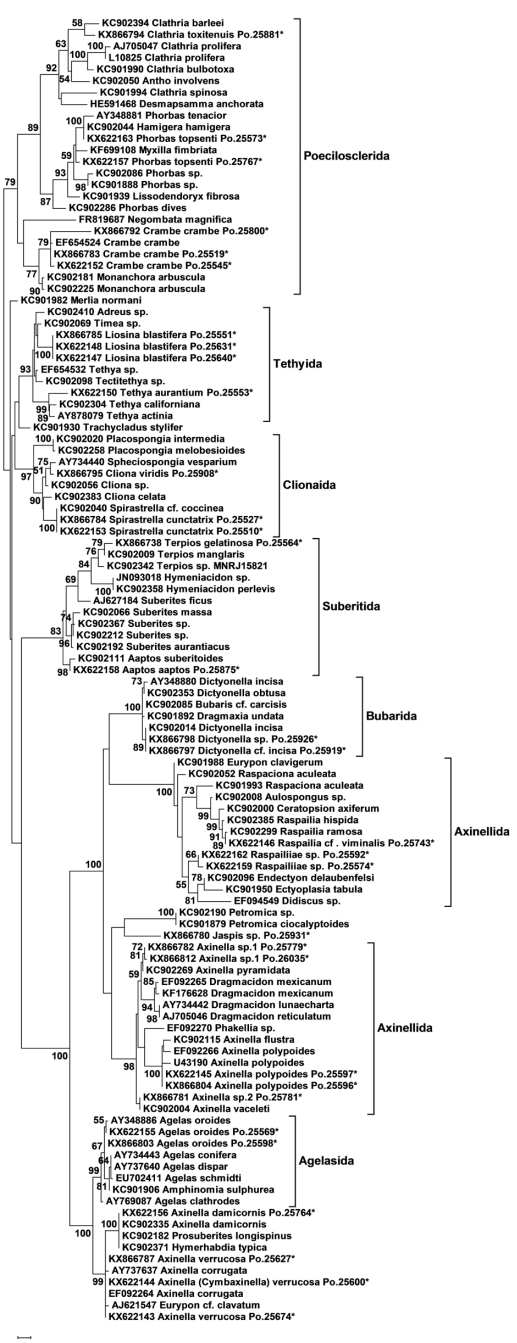

Fig. S2: Phylogenetic tree used to support the morphological identification of Israeli demosponges from clade G4 (base on the division by Borchiellini *et al*., 2004). The maximum likelihood tree was reconstructed with PhyML 3.0 (Dereeper *et al*., 2008) based on 18S rDNA sequences (1632 bp), using the GTR model of sequence evolution. Bootstrap supports higher than 50% are given near the corresponding nodes. Sequences from Mediterranean demosponges obtained in the present study are marked by an asterisk*.

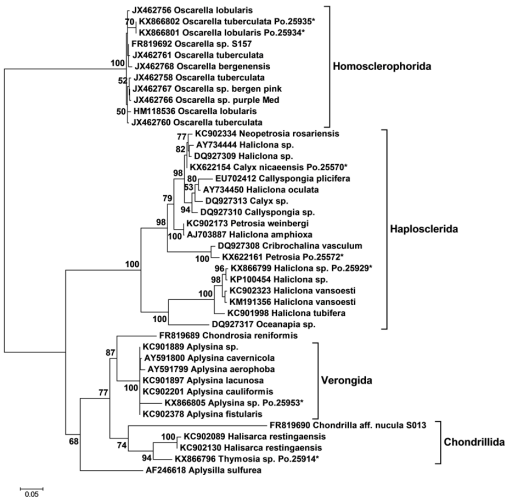

Fig. S3: Phylogenetic tree used to support the morphological identification of Israeli homoscleromorphs and demosponges from clades G2 and G3 (base on the division by Borchiellini *et al.,* 2004). The maximum likelihood tree was reconstructed with PhyML 3.0 (Dereeper *et al*., 2008) based on 18S rDNA sequences (1820 bp), using the GTR model of sequence evolution. Bootstrap supports higher than 50% are given near the corresponding nodes. Sequences from Mediterranean sponges (Demospongiae and Homoscleromorpha) obtained in the present study are marked by an asterisk*.

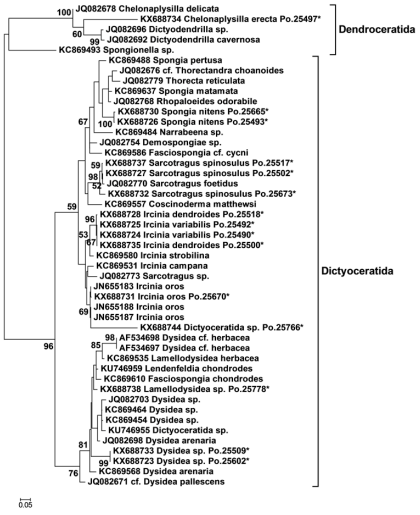

Fig. S4: Phylogenetic tree used to support the morphological identification of Israeli demosponges from clade G1 (base on the division by Borchiellini *et al.*, 2004). The maximum likelihood tree was reconstructed with PhyML 3.0 (Dereeper *et al.,* 2008) based on 28S rDNA sequences (1644 bp), using the GTR model of sequence evolution. Bootstrap supports higher than 50% are given near the corresponding nodes. Sequences from Mediterranean demosponges obtained in the present study are marked by an asterisk*.