



# **Mediterranean Marine Science**

Vol 19, No 3 (2018)



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doi: 10.12681/mms.14146

# To cite this article:

PAZ, G., YUDKOVSKY, Y., SHAISH, L., STERN, N., LUBINEVSKI, H., MIENIS, H. K., DOUEK, J., GALIL, B. S., GOREN, M., & RINKEVICH, B. (2019). Initiating DNA barcoding of Eastern Mediterranean deep-sea biota. *Mediterranean Marine Science*, *19*(3), 416–429. https://doi.org/10.12681/mms.14146

# Initiating the DNA barcoding of Eastern Mediterranean deep-sea biota

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Received: 27 June 2017; Accepted: 23 March 2018; Published on line: 29 August 2018

#### Abstract

This study presents the results obtained from a DNA barcoding survey of the deep-sea macro- and mega-fauna off the Israeli coast (SE Mediterranean, depths of 700 to 1500 meters; during the years 2012-2013). Using trawling and gillnetting we collected 846 organisms, assigned to 37 species, consisting of fish (18), crustaceans (10), mollusks (5), echinoderms (3) and a single brachiopod. The most abundant species were the blackmouth catshark *Galeus melastomus*, the cosmopolitan decapod *Polycheles typhlops* and the bivalve *Abra longicallus*. Two species were sampled for the first time in the southern Levant: the long armed chiroteuthid squid, *Chiroteuthis veranyi*, and the common mora, *Mora moro*. Four of the 18 fish species represented 78% of the total fish collected, and two of the 10 crustacean species represented 61% of the total crustaceans collected were abundant. Most other species were represented by fewer than 10 individuals. PCR products for the cytochrome *c* oxidase sub unit I (COI) gene were successfully sequenced for the 37 species (1-3 vouchers/species). The vouchered individuals were stored at the Steinhardt Museum of Natural History (Tel Aviv University, Israel) and their COI sequences were uploaded to the BoLD universal data center as part of the Israeli national marine barcoding project. The COI sequences of *Acanthephyra eximia, Gryphus vitreus, Galeodea echinophora, Mesothuria intestinalis* and *Astropecten irregularis,* are the first records of these species in the BoLD. When compared to the COI sequences in BoLD, the present results reveal some inconsistencies in species identification. This outcome should be taken into consideration, calling for further meticulous taxonomical work on some BoLD vouchered specimens. This study is the first step in the DNA barcoding of the little-known Levant's benthic deep-sea fauna.

Keywords: Deep sea; Levantine basin; Israel; BoLD; national barcoding.

### Introduction

While there is a scientific interest in exploring the Mediterranean deep-sea ecosystem, the accumulated knowledge on benthic deep-sea biodiversity is still meager, though it is believed to be a biodiversity hot spot, with a high percentage of endemic species (Fredj & Laubier, 1985; Coll et al., 2010; Danovaro et al., 2010; Myers *et al.*, 2000). This knowledge gap is greatest in the easternmost basin of the Mediterranean Sea. The Levantine bathyal populations have a lower biomass than populations elsewhere in the Mediterranean, are markedly eurybathy, with an upper bathymetric boundary that permits the penetration of the shallow Gibraltar and Siculo-Tunisian sills and a much lower bathymetric boundary than recorded for conspecifics elsewhere (Fishelson & Galil, 2001; Galil & Goren, 1994; Goren & Galil, 1997, 2015; Galil, 2004). The study of the bathyal fauna in the Israeli Mediterranean Sea, has intensified since the early

1990s, and has already revealed new species, previously unknown to science, as well as other species recorded for the first time in the region (Ben-Tuvia, 1971; Golani, 1986; Galil & Clark, 1993; Ilan *et al.*, 1994, 2003; Galil & Goren, 1994; Goren & Galil, 1997, 2002, 2015; Galil & Zibrowius, 1998; Corbera & Galil, 2001; Sorbe & Galil, 2002; Bogi & Galil, 2004; Galil, 2004; Goren *et al.*, 2006; Campani *et al.*, 2011; Mienis *et al.*, 2013). Yet it is far from completion and there is no doubt that further exploration, use of additional sampling methodologies, and examination of small sized taxa, will further enhance our knowledge base (Coll *et al.*, 2010) regarding the DNA barcoding of the deep sea biota.

Limitations on morphological identifications, and the lack of expert taxonomists, have highlighted the need for DNA barcoding, and the employment of the ca. 650 bp fragment of the mitochondrial cytochrome c oxidase sub-unit I (COI) gene for species identification. This tool is also used for spatial mapping of species boundaries and

discover cryptic speciation, and may flag the existence of new species (Stern *et al.*, 2016). When this molecular tool is linked to authoritatively identified voucher specimens (Hebert *et al.*, 2003a, b; Ratnasingham & Hebert, 2013), it ensures results of the highest quality currently available.

In this study we present the first list of DNA barcoding results for 37 deep-water Levantine macro- and mega-faunal taxa. An extension to the barcoding study of the biota in the continental shelf of Israel. This study provides a validated data set of COI sequences in the Levant, and demonstrates the application of DNA barcoding for this benthic deep-sea biodiversity, albeit highlighting some limitations and obstacles.

# **Materials and Methods**

# Sampling

The sampling was carried out by the R/V Shikmona at nine stations along the coast of Israel (SE Mediterranean Sea, at depths of 676m to 1610m), using an otter trawl with a semi balloon net (1.6m high, 14m wide and with a cod-end mesh of 10mm, 25mm at the top, and 20mm at its' middle section), and a bottom gill net (100 m wide, 1.5m long). Each trawl tow lasted 90 minutes and covered app. 7 km. A one-day cruise was completed on November 26, 2012 at Station 0 (average depth of 1546m; Suppl. Fig. 1, Suppl. Table 1). Three trawl transects were conducted, each 60 min long, covering app. 4.7 km. During the second cruise (July 28-31, 2013) trawl net transects were carried out at eight stations (altogether 20 hauls; the transects coordinates were recorded by GPS; Table 1), 2 or 3 were repeated back and forth hauls, app. 90 min each, at each station. At station 3 a baited gill net was deployed for 11h at a depth of 923-973m (Suppl. Table 1). The collected material was sorted onboard, counted, labeled, and 1-3 specimens of each taxon were photographed, measured and tissue samples were taken for DNA extraction. Each tissue sample was placed in a separate vial with 200µL of lysis buffer (1M Tris-borate pH 8.2, 0.5 M EDTA, 10% SDS, 5M NaCl) and 40µL of 5M NaClO<sub>4</sub> shaken, and filled with 240µL of phenol-chloroform-isoamyl alcohol (25:24:1, v:v:v). Most specimens were stored in 99% alcohol (few fish were preserved in 4% formalin). Alcohol-fixed specimens were brought to the Steinhardt museum of Natural History at the Tel Aviv University, examined onsite or sent to taxonomists for final identification. Voucher specimens were archived in the natural museum's collection.

# DNA extraction, amplification, sequencing

Genomic DNA was extracted according to Graham (1978), with the following modifications (Douek *et al.*, 2002, 2010; Israel *et al.*, 2010): the samples were centrifuged for 5 min at 14,000 rpm; the aqueous upper phase was transferred to a new tube, further mixed with 240

 $\mu$ l of chloroform-isoamyl alcohol (24:1, v/v) and centrifuged for 5min at 14,000rpm. The upper phase was then collected once more and the DNA was precipitated overnight at -20°C, with 500 $\mu$ l of 100% cold ethanol. The samples were centrifuged for 15 min at 4°C at 14,000 rpm, washed with 500  $\mu$ l of 70% ethanol and centrifuged for 5 min. The ethanol was then removed and the precipitated DNA was dissolved in 40 $\mu$ L of DDW.

DNA barcoding was performed with four sets of COI primers. For the fish samples two pairs of primers were used alternatively, as described in Ward *et al.* (2005):

# FishF1-5'TCAACCAACCACAAAGACATTGG-CAC3';

FishR1-5'TAGACTTCTGGGTGGCCAAAGAATCA3';

FishF2-5'TCGACTAATCATAAAGATATCGGCAC3';

# FishR2-5'ACTTCAGGGTGACCGAAGAAT-CAGAA3'.

For non-fish and non-crustaceans samples we used the primers designed according to Folmer *et al.* (1994):

# LCO1490-5'GGTCAACAAATCATAAAGATATTGG3' and HCO2198-5'TAAACTTCAGGGTGAC-CAAAAAATCA3'.

The crustaceans primers were designed according to Radulovici *et al.* (2009):

# CrustDF1-5 'GGTCWACAAAYCATAAAGAY-ATTGG3' and CrustDR1-5 'TAAACYTCAGGRTGAC-CRAARAAYCA3'.

The PCR protocols followed Folmer et al. (1994), Ward et al. (2005) and Radulovici et al. (2009), with minor modifications: Two microliters of diluted DNA (1:50) from each specimen were added to a PCR reaction mixture for a total solution volume of 50 µl, containing a PCR Master Mix (2×; kt201 Tiangen Biotech, China) and 5µM from each of the forward and reverse primers. PCR amplification included an initial denaturation step at 95°C for 2 min, followed by 35 cycles, each including 1 min at 95°C, 1 min for primer annealing at 45°C for the Folmer primers' samples, or 55°C for the crustacean or the fish specimens, ending with 1 min at 72°C for all samples. A final extension step was executed at 72°C for 10 min. The PCR products were screened on TAE 1.0% agarose gel. The PCR primers were then used for directly sequencing the PCR products (Macrogen Inc., South Korea).

### Data analysis

The DNA sequences were aligned and corrected with BioEdit version 7.2.3 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) (Hall, 1999), and compared to the

GenBank using the BLASTN algorithm from the NCBI website (ncbi.nlm.nih.gov). The criteria for the liable sequencing were a good match between the forward and the reverse sequences, and good quality of the sequence's chromatograms. A comparison between the taxonomical identification and the NCBI BLASTN algorithm results was made prior to uploading the data to the BoLD (Barcode of Life Data Systems) site under the project's name: "Marine Biota of the Israel-Mediterranean" [BIM].

## Vouchers, Taxonomy and Molecular data

This study is part of the National Israeli Marine Barcoding project (BIM) at the Israel Oceanographic and Limnological research (IOLR) and the data produced was uploaded to the IOLR site at http://isramar.ocean.org.il/ IsraelBarcoding/BarcodingDef.aspx and to the BoLD - at boldsystems.org. The BIM project is the first barcoding project performed in Israel using the BoLD center. The DNA samples are kept at 4°C at the National Institute of Oceanography, Haifa, Israel (IOLR).

## Results

# General census

A total of 846 organisms were collected and identified (Tables 1, 2). Four of the 18 fish species were abundant, amounting to 75% (149/197) of the fish specimens collected. The most common fish species was the blackmouth catshark Galeus melastomus (Rafinesque, 1810) (n= 59; 30% of fish specimens: Table 2). This species was first recorded along the Israeli coast during the 1980's, at a depth of 1440m (Golani, 1986). The second most abundant species was the roughtip grenadier Nezumia sclerorhynchus (Valenciennes, 1838) (n= 42; 21% of fish specimens: Table 2), first recorded in the Levantine basin during the 1990's at a depth of 1440-1500m (Galil & Goren, 1994), and noted as one of the most common species in it (Galil, 2004). The third fish species in abundance was the velvet belly Etmopterus spinax (Linnaeus, 1758) (n=26; 13% of fish specimens: Table 2), first recorded in the Levant two decades ago at depths of 1335-1400m (Galil & Goren, 1994). The fourth fish species in abundance was the Mediterranean spiderfish Bathypterois dubius (Vaillant, 1888) (n=18; 9% of fish specimens: Table 2), trawled at depths of 1003-1585m. Galil (2004) noted that this species, also photographed at 2900m depth (as B. mediterraneus), comprised 27% of the deep-sea fish off the coast of Israel. From the fifth species on, the percentage dropped to <5% of the total fish specimens.

Two of the 10 crustacean species sampled were highly abundant, representing 61% (321/530) of all the crustacean specimens collected, with *Polycheles typhlops* (Heller, 1862) as the most abundant species (36% of all crustaceans; at depths of 726-1585m; Table 2), previously recorded at depths of 1000-1527m (Galil & Goren,

1994). The second most common crustacean species was *Aristeus antennatus* (Risso, 1816) (25%; at depths of 726-1546m; Table 2), recorded in the Levant by Galil & Goren (1994) at depths of 100-1527m. *Abra longicallus* (Scacchi, 1835) was the most abundant mollusk species found, comprising 89.5% of mollusks collected (77/86) (at depths of 726-1382 m; Table 2). This species was previously recorded at a depth of 1362m off the Israeli coast (Bogi & Galil, 2004). Koutsoubas *et al.* (2000) reported finding it at a wide bathymetric distribution (depths of 40-1570m).

The gill net yielded four fish species and one crustacean species (Table 2). Two of the fish species (G. *melastomus; E. spinax*) were collected both by trawling and gillnetting. The two other fish species were only found in the gill net: Centrophorus granulosus (Bloch & Schneider, 1801), recorded in Israel at depths of 1330-1440m (Golani, 1986) and Mora moro (Risso, 1810), a species abundant in the eastern Ionian Sea at depths of 700-1200m (Ben-Tuvia, 1971; D'Onghia et al., 2004; Mytilineou et al., 2005) as well as in other regions of the Mediterranean Sea (Ben-Tuvia, 1971; Galil, 2004). This is the first record of this species along the Israeli coast. The single crustacean caught in the gill-net (also collected in the trawl) was Gervon longipes (Milne-Edwards, 1882), first recorded in the Levant basin by Galil & Goren (1994) at depths of 1230-1400m.

The first sampling site (Station 0, Table 1, Fig. 1) yielded four fish species (11 specimens), four crustacean species (99 specimens) and one specimen each of two mollusk species (Table 2). The two mollusk species were *Heteroteuthis dispar* (Rüppell, 1844), first recorded at a depth of 1550m in the SE Mediterranean by Mienis *et al.* (2013), and *Rossia macrosoma* (Delle Chiaje, 1830), described in the eastern Mediterranean by Koutsoubas *et al.* (2000) at depths of 200-700m and first collected near the Israeli coast at a depth of 375m off Ashqelon (Mienis, 2005).

## Barcoding

A total of 35 taxa (18 fish species, nine crustaceans, one brachiopod, five mollusks species and two echinoderms; Table 2) were identified up to species level. Five species are first records in the BoLD, *Acanthephyra eximia* (Smith, 1884), *Gryphus vitreus* (Born, 1778), *Galeodea echinophora* (Linnaeus, 1758), *Mesothuria intestinalis* (Ascanius, 1805) and *Astropecten irregularis* (Pennant, 1777) (Table 2).

The COI sequences of 15 fish species from this study revealed a >99% match with the validated public BoLD records (Table 2). Seven fish species showed a similarity of <99% to at least some of the COI sequences in the BoLD's records (Table 2): *Nezumia sclerorhynchus* BIN AAD8590, showed a <98% similarity to our voucher specimen (93.53-93.86% similarity; museum IDs MB85-006072, MB85-006070, MB85-006073 and MB85-006071, all from Portugal);

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					~	Number	of spe	cimens ]	per stat	ion [ave	rage de	pth (m)]					
			Ē	Station:	0	1	2	3	4	ŝ	9	٢	œ	Gill net	Total no.	Depth	Voucher
	Urder	Family	laxonomic ID	Depth (m)	1546	1079	726	1003	1381	1585	978	1104	1381	948m	individuals (trawling)	range	id*
Fist																	
-	Anguilliformes	Congridae	Conger conger (Linnaeus, 1758)				-								1	726	P. 15182
7	Anguilliformes	Nettastomatidae	<i>Nettastoma melanu</i> (Rafinesque, 1810)	rum	1		1				7		7		9	726- 1546	P. 15171; P. 15166
ŝ	Aulopiformes	Ipnopidae	Bathypterois dubiu: (Vaillant, 1888)	X	×			7	1	4		-	7		18	1003- 1585	P. 15184; P. 15185
4	Beryciformes	Trachichthyidae	Hoplostethus medit (Cuvier, 1829)	erraneus			9								9	726	P. 15167; P. 15169
S	Carcharhini- formes	Scyliorhinidae	Galeus melastomus (Rafinesque, 1810)			б	1	9			б	3		43	16	726- 1104	P. 15163; P. 15190
9	Chimaeriformes	Chimaeridae	Chimera monstrosa (Linnaeus, 1758)	~				7							7	1003	P. 15172
Г	Gadiformes	Gadidae	<i>Mora moro</i> (Risso, 1810)											2		948	P. 15181; P. 15181
×	Gadiformes	Macrouridae	<i>Coryphaenoides me</i> (Giglioli, 1893)	editerraneus					1						1	1381	P. 15189
6	Gadiformes	Macrouridae	<i>Hymenocephalus it</i> (Giglioli, 1884)	alicus			7								7	726	P. 15168
10	Gadiformes	Macrouridae	<i>Nezumia sclerorhyr</i> (Valenciennes 1838	ıchus ()		6	12	б	7		9	8	7		42	726- 1381	P. 15176; P. 15178
11	Gadiformes	Phycidae	Phycis blennoides (Brünnich, 1768)			7	1								б	726- 1079	P. 15192; P. 15193
12	Myctophiformes	Myctophidae	Lampanyctus croco (Risso, 1810)	dilus					1		1				7	978- 1381	P. 15165; P. 15177; P. 15170
13	Notacanthiformes	Notacanthidea	Polyacanthonotus r (De Filippi & Vera	rissoanus ny 1857)	1										1	1546	P. 15393
4	Scorpaeniformes	Sebastidae	<i>Helicolenus dactylc</i> (Delaroche, 1809)	pterus			2								2	726	P. 15183; P. 15183
																0	continued)

						Number	· of spec	imens p	er statio	n [aver:	tge dept	h (m)					
	-	:	Ē	Station:	0	1	2	e	4	S	9	٢	00	Gill net	Total no.	Depth	Voucher
	Order	Family	laxonomic ID	Depth (m)	1546	1079	726	1003 1	1381	1585	978	1104	1381	948m	individuals (trawling)	range	id*
15	Squaliformes	Centrophoridae	Centrophorus grai (Bloch & Schneid	nulosus ler, 1801)										~		948	P. 15187
16	Squaliformes	Etmopteridae	Etmopterus spinax (Linnaeus, 1758)	64		5	Г	7			5	1		6	17	726- 1104	P. 15174; P. 15173
17	Stomiiformes	Sternoptychidae	Argyropelecus hen (Cocco, 1829)	nigymnus									1		1	1381	P. 15180
18	Stomiiformes	Stomiidae	Chauliodus sloani (Bloch & Schneide	er, 1801)	1	1							$\mathfrak{c}$		S	1079- 1546	P. 15188; P. 15164; P. 15175
Cri	ıstacean																
19	Decapoda	Aristeidae	Aristeomorpha fol. (Risso, 1827)	iacea		1	9								٢	726- 1079	AR29296; AR29305
20	Decapoda	Geryonidae	Geryon longipes (Milne-Edwards, 1	(882)		4	7	7			4		-	1	14	726- 1381	AR29297
21	Decapoda	Oplophoridae	Acanthephyra exin (Smith, 1884)	nia	-	ŝ	31		4	15	٢	5	16		79	726- 1585	AR29300
22	Decapoda	Paguridae	Pagurus alatus (Fabricius, 1775)				1		1		1				ς	726- 1381	AR29303
23	Decapoda	Palemonidi	Aristeus antennatu (Risso, 1816)	S1	٢	18	82		8		11	4			130	726- 1546	AR29299; AR29301; AR29294
24	Decapoda	Pandalidae	Plesionika edward (Brandt, 1851)	łsü			36								36	726	AR29295
25	Decapoda	Paguridae	Anapagurus laevis (Bell, 1846)	×			-								1	726	AR29306
					57										57	1546	* *
26	Decapoda	Polybiidae	Bathynectes maran standrea, 1839)	vigna (Pre-		1		7							ę	1003- 1079	AR29298
27	Decapoda	Polychelidae	Polycheles typhlop (Heller, 1862)	SC	34	15	10	8	20	7	17	19	61		191	726- 1585	AR29302
28	Decapoda	Nematocarcinidae	Nematocarcinus sț	p.						8					8	1585	AR29304
																U	continued)

					Ţ	Number	of spec	imens p	er statio	ın [aver£	ıge dept	h (m)]					
	-		Ē	Station:	0	1	7	3	4	S	9	7	8	ill net .	Total no.	Depth	Voucher
	Urder	Family	Taxonomic ID	Depth (m)	1546	1079	726	1003	1381	1585	1 978	104 1	381 9	48m	ndividuals (trawling)	range	id*
Moll	usca																
29	Littorinimorpha	Cassidae	<i>Galeodea echinopl</i> (Linnaeus, 1758)	hora			1	4			1				9	726- 1585	MO 78599
30	Sepiida	Sepiolidae	Rossia macrosoma (Delle Chiaje, 183(	, (C	1										1	1546	* *
31	Sepiolida	Sepiolidae	Heteroteuthis disp. (Rüppell, 1844)	ar	1										1	1546	* *
32	Veneroida	Semelidae	Abra longicallus (Scacchi, 1835)				63	7			12				ΤŢ	726- 1381	MO 78600
33	Teuthida	Chiroteuthidae	Chiroteuthis veran <sub>.</sub> 1835)	ıyi (Férussac,											1	978	MO 78622
Echi	nodermata																
34	Aspidochirotida	Mesothuriidae	Mesothuria intestiı (Ascanius, 1805)	nalis			8	٢	1		5				21	726- 1585	* *
36	Paxillosida	Astropectinidae	Astropecten irregu. (Pennant, 1777)	laris			1								1	726	* *
35	Valvatida	Goniasteridae	Ceramaster sp.				8	8			1				17	726- 1003	* *
Brac	hiopoda																
37	Terebratulida	Terebratulidae	Gryphus vitreus (Born, 1778)				5								5	726	BR 00001

\* voucher id as received from the Steinhardt Museum of Natural History, Tel Aviv university \*\* In archiving process.

Table 1 Continued

Sampling sites		eenland; Israel (Ashdodn 400m); Atlan- Ocean; unspecified (Genbank).	ited States; Mexico; South Africa; Ar- ntina; Indian Ocean; Northwest Atlantic	ean (Sargasso Sea).	rth Pacific Ocean; Atlantic Ocean	lantic Ocean; unspecified (GenBank)	geria; Israel (Ashdod, 400m); Italy; rtugal;	stralia (Tasmania, Bass Strait; South stralia); India	stralia; Cape Verde; United States ulf of Mexico); Norway	stralia (New South Wales; Tasmania; storia; South Australia); Italy; Austra- Gulf of Mexico.	xrico; United States; India; Japan; Indi- Ocean; Taiwan; Portugal.		lian Ocean (South Indian Sea); Italy cily); Greenland; South Africa; Atlantic ean; Malta; United States; Canada, xico; Tasmanian Sea.	stralia (Tasmania, Macquarie Island); lantic Ocean; New Zealand.	eden; Ireland; France; Italy; Portugal; specified (GenBank)	(continued)
Other IDs from same BIN (n)		Gr. Stomiiformes tic	Argyropelecus ac- Un uleatu ger	Stomiiformes Oc	Stomiiformes No	B. mediterraneus Atl (=dubius)	Al	<i>Centrophorus zee-</i> Au haani Au	Centrophorus uyato (G	Au Squaliformes Vic lia.	Centrophorus gran- Me ulosus Centrophorus ni- an	<i>aukang</i> Squaliformes	Inc Stomiiformes Oc Me	Stomiiformes Au Atl	Chimaeriformes Sw	
Number of vouchers related sequences		6	5	17	2		(89)	17	5	67	(33) 3	30	14	7	4	
Number of vouchers in BoLD		8	1		1	S	11				19		18	1	28	
Similarity (%)		99.52 - 99.84 (100)	83.9		87.02	99.28 – 99.84 (100)	99.02 - 100				95.5		99.35 - 100	87.16 - 87.74	98.71- 99.19 (100)	
BIN (cluster) in BoLD		AAC6584	AAB6595		AAC6585	AAF0023	AAB4327				ABZ3018		AAB1178	AAB1177	AAB3588	
Accession number in BoLD		BIM 325-13				BIM 323-13; BIM 335-13	BIM 326-13 BIM 401-15						BIM 319-13 BIM 324-13 BIM 337-13		BIM 389-14	
Taxonomic ID	FISH	Argyropelecus hemigymnus (Cocco, 1829)				Bathypterois dubius (Vaillant, 1888)	Centrophorus granulosus (Bloch & Schneider, 1801)						Chauliodus sloani (Bloch & Schneider, 1801)		Chimaera monstrosa (Linnaeus, 1758)	
#		1				7	б						4		5	

Table 2. Summary of all species sampled and their accession numbers, BIN affiliations and worldwide sampling sites from BoLD URL.

Sampling sites	Portugal; Italy (Sicily); France (Brittany, Finistere); Spain (Balearic Islands).	Atlantic Ocean; Greenland; United States	Sweden; France (Northern Atlantic); Italy; Cyprus; Portugal (Faro, Algarve); Portu- gal; Malta; Italy.	Israel (Ashdod, 400m).	Italy; Portugal (Sagres, Faro, Algarve); Algeria; unspecified (GenBank); Malta; Portugal; Portugal (Faro);	South Africa (Western Cape); South Af- rica; Israel (Ashdod, 400m); Italy (Sic- ily); Portugal (Faro, Algarve); Portugal; Canada (Nova Scotia; New Brunswick,); Greece; Turkey; unspecified (Genbank); Malta; Greece; Spain.	Argentina.	Australia.	South Africa.	Australia (Western Australia).	South Africa.	Australia; North Sea; Argentina; New Zealand; Atlantic Ocean; Indonesia (Bali); Taiwan; Namibia; Indian Ocean; Atlantic Ocean	Italy (Sicily); Portugal (Faro, Algarve); Malta, Australia (Tasmania, East of Maria Island & West of Trial Harbour); Portu- gal; South Africa; unspecified (GenBank); Australia (New South
Other IDs from same BIN (n)	Anguilliformes	Gadiformes	Squaliformes	ı	Carcharhiniformes		Helicolenus la- hillei	Helicolenus per- coides	Helicolenus sp. B	Helicolenus barathri	Helicolenus sp. E	Scorpaeniformes	Trachichtyiformes
Number of vouchers related sequences	-	1	4		ų	(80)	13	S	4	Э	1	54	28
Number of vouchers in BoLD	27	9	34	1	42	60							34
Similarity (%)	99.82 - 100	99.52-99.84 (100)	98.62 - 99.14 (100)	96.45 - 97.24 (100)	99.65 - 100	99.02 - 100							99.42 - 100
BIN (cluster) in BoLD	AAB6795	AAC9963	AAB1404	ACJ2122	AA9550	AA9248							AAB3177
Accession number in BoLD	BIM 328-13	BIM 376-13	BIM 330-13	BIM 334-13	BIM 318-13 BIM 339-13	BIM 329-13 BIM 333-13							BIM 327-13 BIM 336-13
Taxonomic ID	Conger conger (Linnaeus, 1758)	Coryphaenoides mediter- raneus (Giglioli, 1893)	Etmopterus spinax (Linnaeus, 1758)		Galeus melastomus (Rafin- esque, 1810)	Helicolenus dactylopterus (Delaroche, 1809)							Hoplostethus mediterraneus (Cuvier, 1829)
#	9	7	8	ı	6	10	ı	ı	ı		ı	ı	11

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(continued)

#	Taxonomic ID	Accession number in BoLD	BIN (cluster) in BoLD	Similarity (%)	Number of vouchers in BoLD	Number of vouchers related sequences	Other IDs from same BIN (n)	Sampling sites
I								Wales); Mexico; New Zealand; Tasma- nian Sea; Italy.
12	<i>Hymenocephalus italicus</i> (Giglioli, 1884)	BIM 448-15	AAI8555	99.19 (100)	4	ı	ı	Malta; Israel (Ashdod, 400m).
13	Lampanyctus crocodilus (Risso, 1810)	BIM 332-13 BIM 322-13 BIM 321-13	AAD5280	98.21 - 99.84	14	ı		Malta; unspecified (GenBank);
			AAB3779		1	16	Nannobrachium atrum	Atlantic Ocean; Tasmainia Sea; Green- land; New Zealand, unspecified (Gen-
						5	Myctophiformes	Bank)
14	Mora moro (Risso, 1810)	BIM 380-13 BIM 381-13	AAB9786	99.19 - 99.84	4	14	Gadiformes	Australia (Tasmania); New Zealand.
15	Nettastoma melanurum (Rafinesque, 1810)	BIM 377-13 BIM 386-13	AAG0809	99.65 - 100	9	1	Anguilliformes	Italy; Malta; Portugal; Mexico.
16	Nezumia sclerorhynchus (Valenciennes, 1838)	BIM 331-13 BIM 385-13 BIM 388-14	AAD8591	99.83-100	Ś	1	Nezumia aequalis	Greenland; Malta.
			AAD8590	93.5-93.86	4	ı	ı	Portugal.
17	Phycis blennoides (Brünnich, 1768)	BIM 320-13 BIM 338-13	AAD4387	100	14	ı	ı	Portugal; Italy (Sicily); Greenland; Israel (Ashdod, 400m), Malta.
18	Polyacanthonotus rissoanus (De Filippi & Vérany, 1857)	BIM 390-14	AAC3511	99.16 - 99.83	٢	4	Notacantiformes	Greenland; Atlantic Ocean (Flemish Cap); unspecified (GenBank), Canada ,united states
ı	CRUSTACEA							
19	Acanthephyra eximia (Smith, 1884)	BIM 371-13 BIM 451-15	AAD7852	99.03(100)	2	First in BoLD		
·				97.92 - 99.83		7	Acanthephyra pur- purea	Portugal.
						1	Decapoda	Canada.
20	Anapagurus laevis (Bell, 1846)	BIM 449-15	ABX3753	99.24 - 99.85	3	1	Decapoda	North sea; Portugal.
								(continued)

#	Taxonomic ID	Accession number in BoLD	BIN (cluster) in BoLD	Similarity (%)	Number of vouchers in BoLD	Number of vouchers related sequences	Other IDs from same BIN (n)	Sampling sites
21	Aristaeomorpha foliacea (Risso, 1827)	BIM 368-13 BIM 383-13	AAD9249	99.61 - 100	12	I	ı	Italy (FAO zone 37; Sicily, Stretto di Sicilia); Portugal (south coast Portugal); Portugal, Israel (Ashdod, 400m).
ı			AA05639	87.88	1	ı	1	Solomon Islands.
22	Aristeus antennatus (Risso, 1816)	BIM 372-13 BIM 374-13	ABZ0544	99.82 - 100	4	I		Italy.
I		BIM 373-13	AAP0130	97.53 - 97.8	4	ı		Italy.
23	Bathynectes maravigna (Prestandrea, 1839)	BIM 375-13	AAC9108	99.68 - 100	10	4	Decapoda	Portugal (Gulf of Cadiz; south coast); Ire- land; Spain (Gulf of Cadiz),
24	<i>Geryon longipes</i> (A. Milne-Edwards, 1882)	BIM 369-13	AAC4001	98.85 - 100	13	9	Decapoda	Portugal (south coast); Portugal; Malta (Castelammare del Golfo); Malta; United Kingdom, Norway, Sweden; Israel (Ash- dod, 400m).
25	Pagurus alatus (Fabricius, 1775)	BIM378-13	AAD0298	96.69 - 100	14	2	Decapoda	North Sea; Portugal (south coast); unspec- ified (GenBank); Portugal
26	Plesionika edwardsii (Brandt, 1851)	BIM 384-13	ACF4004	99.21 - 99.47	5	I	ı	Italy; Guinea
ı			AAD4482	97.83 - 98.48	5	ı		Italy (Sicily); Italy
			ABZ8369	97.7	1	ı		Guinea
			ABZ8370	93.86	1	ı		Reunion
ı			AAY4332	86.99	1	1	Plesionika lophotes	French Polynesia; Philippines
27	Polycheles typhlops (Heller, 1862)	BIM 370-13	AAC2840	99.67 - 99.84(100)	17	ı	ı	Portugal (south coast); Portugal; United Kingdom (Scotland, SW St Kilda); Italy; genbank
ı			ADC9785	98.36 - 98.52	4	ı	ı	Fiji ; Indonesia (Molusques); Tonga; Van- uatu; unspecified (GenBank)
28	Nematocarcinus sp.	BIM 450-15	ACY8588	100	μ	ı		
	MOLLUSCA							
29	Abra longicallus (Scacchi, 1835)	BIM 379-13	ACJ9436	98.2	2	·		unspecified (GenBank)
30	Chiroteuthis veranyi (Férussac, 1835)	BIM 387-14	ACK5688	100	-	ı	·	·
								(continued)

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#	Taxonomic ID	Accession number in BoLD	BIN (cluster) in BoLD	Similarity (%)	Number of vouchers in BoLD	Number of vouchers related sequences	Other IDs from same BIN (n)	Sampling sites
ı			AAM4348	95.64	1	2	Chirotheutis calyx; Discotheutis discus	unspecified (GenBank)
ı			AAJ0136	88.27	1	·		unspecified (GenBank)
ı			AAY4431	85.82	1	1	Planctotheutis sp.	Atlantic Ocean
31	Galeodea echinophora (Linnaeus, 1758)	BIM 457-15	ACY8866	100	1	First in BoLD	ı	
32	Heteroteuthis dispar (Rüppell, 1844)	BIM 456-15	ABW9322	100	1	5	Sepiolida	Morocco
ı			AAH9798	85.38	1	S	Sepiola atlantica; S.intermedia; S.ro- busta; S.affinis; S.ligulata	unspecified (GenBank)
33	Rossia macrosoma (Delle Chiaje, 1830)	BIM 455-15	ACY7926	100	1			
ı	2		ACI9643	88.38	8	ı	ı	North Sea
	ECHINODERMATA							
34	<i>Mesothuria intestinalis</i> (Ascanius, 1805)	BIM 452-15	ACT2215	100	П	First in BoLD 2	Aspidochirotida	- Sweden
35	Astropecten irregularis (Pennant, 1777)	BIM 454-15	ACY7948	100	1	First in BoLD	ı	
36	Ceramaster sp.	BIM 453-15	ACY9227	100	1	not found in BoLD	ı	
	BRACHIOPODA							

ı

ı

First in BoLD

-

100

ACK0176

BIM 382-13

Gryphus vitreus (Born, 1778)

37

*Argyropelecus hemigymnus* BIN's AAB6595 and AAC6585 showed <98% similarity with 2/5 different BoLD sequences (83.9% similarity, museum ID Ve37.1 from the Atlantic Ocean and 87.02% similarity, museum ID SIO 05-4 from Scripps Institution of Oceanography, California); *Chauliodus sloani*, BIN AAB1177 exhibiting a single BoLD sequence with 87.16% similarity to our voucher (ID AL275748, Australian Antarctic Division; Table 2).

Eight crustacean species in this study matched >98% to existing BoLD COI sequences (Table 2). The other species were: Aristeomorpha foliacea with 13 BoLD records, of which one sample had 87.88% similarity to our voucher's COI (museum ID MNHN IU20081449, Museum National d'Histoire Naturelle, Paris, from the Solomon Islands); Acanthephyra eximia, 97.22-99.83% similarity to our voucher (museum ID MNHN IU200814604, from Mozambique); Aristeus antennatus with 4 BoLD records, revealing 97.53-97.80% similarities with our voucher's COI (museum ID MIVSA from Italy, and ID MB89000673 from Italy); Plesionika edwardsii with eight BoLD sequences, of which only one record had 97.83-98.48% similarity to our voucher (ID MB89000672, Malta). In addition, one mollusk species found at a depth of 978m, Chiroteuthis veranvi, showed 95.64% similarity with a COI sequence in BoLD that had no museum ID, nor a sampling site record (Table 2). The three echinoderms are first records in the BoLD.

Our specimens were authoritatively identified, the COI sequences showed a full match between the forward and the reverse, in addition to the high quality of the sequencing chromatograms. We thus advise on validating BoLD records with sequences below the threshold accepted for the natural variation within a species (>2%).

# Discussion

This study is the first to utilize DNA barcoding on deep-sea macro- and mega fauna collected along the Israeli Mediterranean coast, combining the expertise of local taxonomists with molecular identification. The collecting survey resulted in 846 organisms (37 taxa), with 18 fish species (four of which amounted to 78% of all fish collected), 10 crustacean species (two comprising 61% of all crustacean collected), five molluscan species, three echinoderms and a single brachiopod. The blackmouth catshark G. melastomus (n=59 specimens) was the most common fish species, the cosmopolitan decapod crustacean Polycheles typhlops (n=191) was the most common deep-sea decapod species and Abra longicallus (n=77; 89.5% of all mollusk specimens) was the most common mollusk species. Two new species from the southern Levantine Sea were sampled for the first time, the long armed chiroteuthid squid Chiroteuthis veranvi (Férussac, 1835) (Shaish et al., 2014) and the worldwide temperate deep-sea fish the common mora - Mora moro (Risso, 1810). Most (65%; 24/37), of the species sampled were presented by >10 individuals/species. In this study,

35 taxa were identified to the species level, with a single echinoderm and crustacean identified to the genus level.

This study emphasizes the need for morphological taxonomy validation as part of the DNA barcoding protocol. More notable is the emerging need for comparisons with the existing and publicly available BoLD COI sequences. The similarities of 19 out of the 56 (33.9%) COI BIN sequences of our Levantine species and existing COI sequences in BoLD were < 98% (as low as 83.9%) for the same given scientific name (Table 3). This high incongruence for COI sequences, that are below the accepted threshold for defining natural variation within a species (set up at 2-3%; Hebert et al., 2003a), further indicates the urgent needs for taxonomic validations of COI sequences, for quality checks of the chromatograms, for possible mitochondrial introgression between closely related species, the appearance of cryptic species and for possible contaminations. The above conclusion is further supported by studies on fish from the eastern Mediterranean Sea (Karahan et al., 2017), on fish from the central Mediterranean basin (Landi et al., 2014), on deep water sharks off Portugal (Moura et al., 2015), macroinvertebrates (Shackleton & Rees, 2016) and various other groups (e.g., Shen et al., 2013; Sonet et al., 2013).

The Levantine deep water biota is barely known and poorly understood. The meso- and bathypelagic assemblages, and their relation to the deep seabed communities, are yet unexplored. The immensely important meio- and microfauna are virtually unknown. Molecular tools are uniquely suitable for establishing the full inventory of the biodiversity and characteristics of the Levant.

# Acknowledgements

This study was supported by a grant from the Israeli Ministry of Energy and partly by the EC-COST-CA15219. It is within the National Israeli Marine Barcoding project (BIM) at IOLR. We thank E. Hagai, the team of the R/V Shikmona and members of the Steinhardt Museum of Natural History for field and technical assistance.

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