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Initiating the DNA barcoding of Eastern Mediterranean deep-sea biota

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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 $NaClO_4$, 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 µl 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 500µl of 100% cold ethanol. The samples were centrifuged for 15 min at 4°C at 14,000 rpm, washed with 500 µl of 70% ethanol and centrifuged for 5 min. The ethanol was then removed and the precipitated DNA was dissolved in 40µL 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 *Geryon 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);

Table 1. Inventory of collected species, voucher identifications, depth ranges and numbers of collected specimens at the different sampling stations.

	Order	Family	Taxonomic ID	Number of specimens per station [average depth (m)]										Gill net 948m	Total no. individuals (trawling)	Depth range	Voucher id*
				Station:	0	1	2	3	4	5	6	7	8				
				Depth (m)	1546	1079	726	1003	1381	1585	978	1104	1381				
Fish																	
1	Anguilliformes	Congridae	*Conger conger* (Linnaeus, 1758)				1								1	726	P. 15182
2	Anguilliformes	Nettastomatidae	*Nettastoma melanurum* (Rafinesque, 1810)		1		1				2		2		6	726-1546	P. 15171; P. 15166
3	Aulopiformes	Ipnopidae	*Bathypterois dubius* (Vaillant, 1888)		8			2	1	4		1	2		18	1003-1585	P. 15184; P. 15185
4	Beryciformes	Trachichthyidae	*Hoplostethus mediterraneus* (Cuvier, 1829)				6								6	726	P. 15167; P. 15169
5	Carcharhiniformes	Scyliorhinidae	*Galeus melastomus* (Rafinesque, 1810)			3	1	6			3	3		43	16	726-1104	P. 15163; P. 15190
6	Chimaeriformes	Chimaeridae	*Chimera monstrosa* (Linnaeus, 1758)					2							2	1003	P. 15172
7	Gadiformes	Gadidae	*Mora moro* (Risso, 1810)											2		948	P. 15181; P. 15181
8	Gadiformes	Macrouridae	*Coryphaenoides mediterraneus* (Giglioli, 1893)						1						1	1381	P. 15189
9	Gadiformes	Macrouridae	*Hymenocephalus italicus* (Giglioli, 1884)				2								2	726	P. 15168
10	Gadiformes	Macrouridae	*Nezumia sclerorhynchus* (Valenciennes 1838)			9	12	3	2		6	8	2		42	726-1381	P. 15176; P. 15178
11	Gadiformes	Phycidae	*Phycis blennoides* (Brünnich, 1768)			2	1								3	726-1079	P. 15192; P. 15193
12	Myctophiformes	Myctophidae	*Lampanyctus crocodilus* (Risso, 1810)						1		1				2	978-1381	P. 15165; P. 15177; P. 15170
13	Notacanthiformes	Notacanthidea	*Polyacanthonotus rissoanus* (De Filippi & Verany1857)		1										1	1546	P. 15393
14	Scorpaeniformes	Sebastidae	*Helicolenus dactylopterus* (Delaroche, 1809)				2								2	726	P. 15183; P. 15183

(continued)

Table 1 Continued

				Number of specimens per station [average depth (m)]												
	Order	**Family**	**Taxonomic ID**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Gill net 948m**	**Total no. individuals (trawling)**	**Depth range**	**Voucher id***
			Station: / **Depth (m)**	**1546**	**1079**	**726**	**1003**	**1381**	**1585**	**978**	**1104**	**1381**				
15	Squaliformes	Centrophoridae	*Centrophorus granulosus* (Bloch & Schneider, 1801)										8		948	P. 15187
16	Squaliformes	Etmopteridae	*Etmopterus spinax* (Linnaeus, 1758)		5	7	2			2	1		9	17	726-1104	P. 15174; P. 15173
17	Stomiiformes	Sternoptychidae	*Argyropelecus hemigymnus* (Cocco, 1829)									1		1	1381	P. 15180
18	Stomiiformes	Stomiidae	*Chauliodus sloani* (Bloch & Schneider, 1801)	1	1							3		5	1079-1546	P. 15188; P. 15164; P. 15175
Crustacean																
19	Decapoda	Aristeidae	*Aristeomorpha foliacea* (Risso, 1827)		1	6								7	726-1079	AR29296; AR29305
20	Decapoda	Geryonidae	*Geryon longipes* (Milne-Edwards, 1882)		4	2	2			4	1	1	1	14	726-1381	AR29297
21	Decapoda	Oplophoridae	*Acanthephyra eximia* (Smith, 1884)	1	3	31		4	15	7	2	16		79	726-1585	AR29300
22	Decapoda	Paguridae	*Pagurus alatus* (Fabricius, 1775)			1		1		1				3	726-1381	AR29303
23	Decapoda	Palemonidi	*Aristeus antennatus* (Risso, 1816)	7	18	82		8		11	4			130	726-1546	AR29299; AR29301; AR29294
24	Decapoda	Pandalidae	*Plesionika edwardsii* (Brandt, 1851)			36								36	726	AR29295
25	Decapoda	Paguridae	*Anapagurus laevis* (Bell, 1846)			1								1	726	AR29306
				57										57	1546	**
26	Decapoda	Polybiidae	*Bathynectes maravigna* (Prestandrea, 1839)		1		2							3	1003-1079	AR29298
27	Decapoda	Polychelidae	*Polycheles typhlops* (Heller, 1862)	34	15	10	8	20	7	17	19	61		191	726-1585	AR29302
28	Decapoda	Nematocarcinidae	*Nematocarcinus* sp.						8					8	1585	AR29304

(continued)

Table 1 Continued

	Order	Family	Taxonomic ID	Number of specimens per station [average depth (m)]													
				Station:	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Gill net 948m**	**Total no. individuals (trawling)**	**Depth range**	**Voucher id***
				Depth (m)	**1546**	**1079**	**726**	**1003**	**1381**	**1585**	**978**	**1104**	**1381**				
Mollusca																	
29	Littorinimorpha	Cassidae	*Galeodea echinophora* (Linnaeus, 1758)				1	4			1				6	726-1585	MO 78599
30	Sepiida	Sepiolidae	*Rossia macrosoma* (Delle Chiaje, 1830)		1										1	1546	**
31	Sepiolida	Sepiolidae	*Heteroteuthis dispar* (Rüppell, 1844)		1										1	1546	**
32	Veneroida	Semelidae	*Abra longicallus* (Scacchi, 1835)				63	2			12				77	726-1381	MO 78600
33	Teuthida	Chiroteuthidae	*Chiroteuthis veranyi* (Férussac, 1835)								1				1	978	MO 78622
Echinodermata																	
34	Aspidochirotida	Mesothuriidae	*Mesothuria intestinalis* (Ascanius, 1805)				8	7	1		5				21	726-1585	**
36	Paxillosida	Astropectinidae	*Astropecten irregularis* (Pennant, 1777)				1								1	726	**
35	Valvatida	Goniasteridae	*Ceramaster* sp.				8	8			1				17	726-1003	**
Brachiopoda																	
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 2. Summary of all species sampled and their accession numbers, BIN affiliations and worldwide sampling sites from BoLD URL.

#	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
	FISH							
1	*Argyropelecus hemigymnus* (Cocco, 1829)	BIM 325-13	AAC6584	99.52 - 99.84 (100)	8	9	Stomiiformes	Greenland; Israel (Ashdodn 400m); Atlantic Ocean; unspecified (Genbank).
			AAB6595	83.9	1	5	*Argyropelecus aculeatu*	United States; Mexico; South Africa; Argentina; Indian Ocean; Northwest Atlantic Ocean (Sargasso Sea).
						17	Stomiiformes	
			AAC6585	87.02	1	2	Stomiiformes	North Pacific Ocean; Atlantic Ocean
2	*Bathypterois dubius* (Vaillant, 1888)	BIM 323-13; BIM 335-13	AAF0023	99.28 – 99.84 (100)	5	1	*B. mediterraneus (=dubius)*	Atlantic Ocean; unspecified (GenBank)
3	*Centrophorus granulosus* (Bloch & Schneider, 1801)	BIM 326-13 BIM 401-15	AAB4327	99.02 - 100	11	(89)		Algeria; Israel (Ashdod, 400m); Italy; Portugal;
						17	*Centrophorus zeehaani*	Australia (Tasmania, Bass Strait; South Australia); India
						5	*Centrophorus uyato*	Australia; Cape Verde; United States (Gulf of Mexico); Norway
						67	Squaliformes	Australia (New South Wales; Tasmania; Victoria; South Australia); Italy; Australia; Gulf of Mexico.
			ABZ3018	95.5	19	(33)	*Centrophorus granulosus*	Mexico; United States; India; Japan; Indian Ocean; Taiwan; Portugal.
						3	*Centrophorus niaukang*	
						30	Squaliformes	
4	*Chauliodus sloani* (Bloch & Schneider, 1801)	BIM 319-13 BIM 324-13 BIM 337-13	AAB1178	99.35 – 100	18	14	Stomiiformes	Indian Ocean (South Indian Sea); Italy (Sicily); Greenland; South Africa; Atlantic Ocean; Malta; United States; Canada, Mexico; Tasmanian Sea.
			AAB1177	87.16 - 87.74	1	2	Stomiiformes	Australia (Tasmania, Macquarie Island); Atlantic Ocean; New Zealand.
5	*Chimaera monstrosa* (Linnaeus, 1758)	BIM 389-14	AAB3588	98.71- 99.19 (100)	28	4	Chimaeriformes	Sweden; Ireland; France; Italy; Portugal; unspecified (GenBank)

(continued)

Table 2 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
6	*Conger conger* (Linnaeus, 1758)	BIM 328-13	AAB6795	99.82 - 100	27	1	Anguilliformes	Portugal; Italy (Sicily); France (Brittany, Finistere); Spain (Balearic Islands).
7	*Coryphaenoides mediterraneus* (Giglioli, 1893)	BIM 376-13	AAC9963	99.52-99.84 (100)	6	1	Gadiformes	Atlantic Ocean; Greenland; United States
8	*Etmopterus spinax* (Linnaeus, 1758)	BIM 330-13	AAB1404	98.62 – 99.14 (100)	34	4	Squaliformes	Sweden; France (Northern Atlantic); Italy; Cyprus; Portugal (Faro, Algarve); Portugal; Malta; Italy.
-		BIM 334-13	ACJ2122	96.45 - 97.24 (100)	1		-	Israel (Ashdod, 400m).
9	*Galeus melastomus* (Rafinesque, 1810)	BIM 318-13 BIM 339-13	AAA9550	99.65 - 100	42	6	Carcharhiniformes	Italy; Portugal (Sagres, Faro, Algarve); Algeria; unspecified (GenBank); Malta; Portugal; Portugal (Faro);
10	*Helicolenus dactylopterus* (Delaroche, 1809)	BIM 329-13 BIM 333-13	AAA9248	99.02 - 100	60	(80)		South Africa (Western Cape); South Africa; Israel (Ashdod, 400m); Italy (Sicily); Portugal (Faro, Algarve); Portugal; Canada (Nova Scotia; New Brunswick,); Greece; Turkey; unspecified (Genbank); Malta; Greece; Spain.
-						13	*Helicolenus lahillei*	Argentina.
-						5	*Helicolenus percoides*	Australia.
-						4	*Helicolenus sp. B*	South Africa.
-						3	*Helicolenus barathri*	Australia (Western Australia).
-						1	*Helicolenus sp. E*	South Africa.
-						54	Scorpaeniformes	Australia; North Sea; Argentina; New Zealand; Atlantic Ocean; Indonesia (Bali); Taiwan; Namibia; Indian Ocean; Atlantic Ocean
11	*Hoplostethus mediterraneus* (Cuvier, 1829)	BIM 327-13 BIM 336-13	AAB3177	99.42 - 100	34	28	Trachichtyiformes	Italy (Sicily); Portugal (Faro, Algarve); Malta, Australia (Tasmania, East of Maria Island & West of Trial Harbour); Portugal; South Africa; unspecified (GenBank); Australia (New South

(continued)

Table 2 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
-								Wales); Mexico; New Zealand; Tasmanian Sea; Italy.
12	*Hymenocephalus italicus* (Giglioli, 1884)	BIM 448-15	AAI8555	99.19 (100)	4	-	-	Malta; Israel (Ashdod, 400m).
13	*Lampanyctus crocodilus* (Risso, 1810)	BIM 332-13 BIM 322-13 BIM 321-13	AAD5280	98.21 - 99.84	14	-		Malta; unspecified (GenBank);
-			AAB3779		1	16 5	*Nannobrachium atrum* Myctophiformes	Atlantic Ocean; Tasmainia Sea; Greenland; New Zealand, unspecified (GenBank)
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	6	1	Anguilliformes	Italy; Malta; Portugal; Mexico.
16	*Nezumia sclerorhynchus* (Valenciennes, 1838)	BIM 331-13 BIM 385-13 BIM 388-14	AAD8591	99.83-100	5	1	*Nezumia aequalis*	Greenland; Malta.
			AAD8590	93.5-93.86	4	-	-	Portugal.
17	*Phycis blennoides* (Brünnich, 1768)	BIM 320-13 BIM 338-13	AAD4387	100	14	-	-	Portugal; Italy (Sicily); Greenland; Israel (Ashdod, 400m), Malta.
18	*Polyacanthonotus rissoanus* (De Filippi & Vérany, 1857)	BIM 390-14	AAC3511	99.16 - 99.83	7	4	Notacantiformes	Greenland; Atlantic Ocean (Flemish Cap); unspecified (GenBank), Canada ,united states
-	**CRUSTACEA**							
19	*Acanthephyra eximia* (Smith, 1884)	BIM 371-13 BIM 451-15	AAD7852	99.03(100)	2	First in BoLD		
-				97.92 - 99.83	-	2	*Acanthephyra purpurea*	Portugal.
						1	Decapoda	Canada.
20	*Anapagurus laevis* (Bell, 1846)	BIM 449-15	ABX3753	99.24 - 99.85	3	1	Decapoda	North sea; Portugal.

(continued)

Table 2 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	-	-	Italy (FAO zone 37; Sicily, Stretto di Sicilia); Portugal (south coast Portugal); Portugal, Israel (Ashdod, 400m).
-			AAO5639	87.88	1	-	-	Solomon Islands.
22	*Aristeus antennatus* (Risso, 1816)	BIM 372-13 BIM 374-13	ABZ0544	99.82 - 100	4	-	-	Italy.
-		BIM 373-13	AAP0130	97.53 - 97.8	4	-	-	Italy.
23	*Bathynectes maravigna* (Prestandrea, 1839)	BIM 375-13	AAC9108	99.68 - 100	10	4	Decapoda	Portugal (Gulf of Cadiz; south coast); Ireland; Spain (Gulf of Cadiz),
24	*Geryon longipes* (A. Milne-Edwards, 1882)	BIM 369-13	AAC4001	98.85 - 100	13	6	Decapoda	Portugal (south coast); Portugal; Malta (Castelammare del Golfo); Malta; United Kingdom, Norway, Sweden; Israel (Ashdod, 400m).
25	*Pagurus alatus* (Fabricius, 1775)	BIM378-13	AAD0298	96.69 - 100	14	2	Decapoda	North Sea; Portugal (south coast); unspecified (GenBank); Portugal
26	*Plesionika edwardsii* (Brandt, 1851)	BIM 384-13	ACF4004	99.21 - 99.47	5	-	-	Italy; Guinea
-			AAD4482	97.83 - 98.48	5	-	-	Italy (Sicily); Italy
-			ABZ8369	97.7	1	-	-	Guinea
-			ABZ8370	93.86	1	-	-	Reunion
-			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	-	-	Portugal (south coast); Portugal; United Kingdom (Scotland, SW St Kilda); Italy; genbank
-			ADC9785	98.36 – 98.52	4	-	-	Fiji ; Indonesia (Molusques); Tonga; Vanuatu; unspecified (GenBank)
28	*Nematocarcinus* sp.	BIM 450-15	ACY8588	100	-	-	-	-
	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	1	-	-	-

(continued)

Table 2 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
-			AAM4348	95.64	1	2	*Chirotheutis calyx; Discotheutis discus*	unspecified (GenBank)
-			AAJ0136	88.27	1	-		unspecified (GenBank)
-			AAY4431	85.82	1	1	*Planctotheutis sp.*	Atlantic Ocean
31	*Galeodea echinophora* (Linnaeus, 1758)	BIM 457-15	ACY8866	100	1	First in BoLD	-	-
32	*Heteroteuthis dispar* (Rüppell, 1844)	BIM 456-15	ABW9322	100	1	2	Sepiolida	Morocco
-			AAH9798	85.38	1	5	*Sepiola atlantica; S.intermedia; S.robusta; S.affinis; S.ligulata*	unspecified (GenBank)
33	*Rossia macrosoma* (Delle Chiaje, 1830)	BIM 455-15	ACY7926	100	1	-	-	
-			ACI9643	88.38	8	-	-	North Sea
	ECHINODERMATA							
34	*Mesothuria intestinalis* (Ascanius, 1805)	BIM 452-15	ACT2215	100	1	First in BoLD 2	 Aspidochirotida	- Sweden
35	*Astropecten irregularis* (Pennant, 1777)	BIM 454-15	ACY7948	100	1	First in BoLD	-	-
36	*Ceramaster* sp.	BIM 453-15	ACY9227	100	1	not found in BoLD	-	-
	BRACHIOPODA							
37	*Gryphus vitreus* (Born, 1778)	BIM 382-13	ACK0176	100	1	First in BoLD	-	-

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 veranyi*, 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 veranyi* (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.

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