eDNA Expeditions biodiversity survey of Everglades National Park

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In collaboration with World Heritage Marine Programme

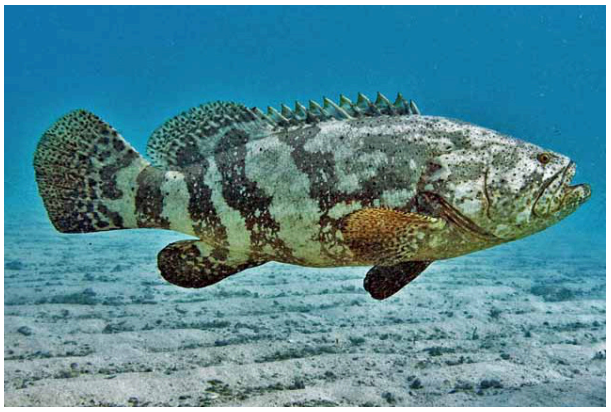
Credit: NPS Photos by Federico Acevedo.

Highlights

With 50% of the samples collected at Everglades National Park now analyzed, we can share the following highlights: 17.5 l of water, roughly two buckets, contained:

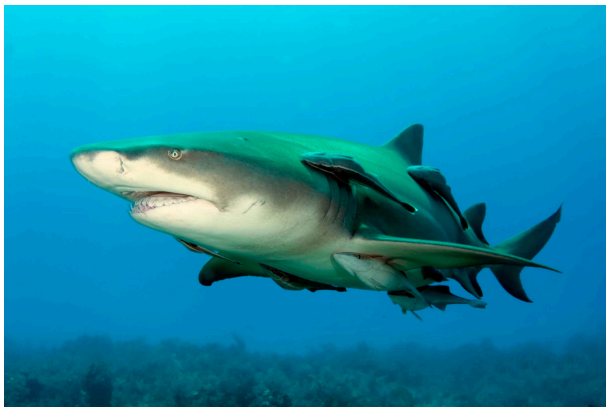
- DNA from **163 identifiable species** of which **45 species not previously reported in the Everglades**. The Everglades is home to over 2,500 marine species (source UNESCO/Ocean Biodiversity Information System OBIS).
- DNA of the following **7 IUCN Red List threatened species**: Sphyrna tiburo (bonnethead shark, EN), Limulus polyphemus (horseshoe crab, VU), Negaprion brevirostris (lemon shark, VU), Trichechus manatus (West Indian manatee, VU), Megalops atlanticus (tarpon, VU), Epinephelus itajara (Atlantic goliath grouper, VU), Caretta caretta (loggerhead sea turtle, VU).

(a) Sphyrna tiburo (bonnethead shark)



(b) Epinephelus itajara (Atlantic goliath grouper)

(c) Limulus polyphemus (horseshoe crab)



(d) Negaprion brevirostris (lemon shark)

(e) Trichechus manatus (West Indian manatee)

(f) Caretta caretta (loggerhead sea turtle)

Figure 1: Some of the detected species. Credits: Robin Riggs (a), Smithsonian Tropical Research Institute (b), Albert Kok (d), U.S. Fish and Wildlife Service Headquarters (e), Roberto Pillon (f).

What is the eDNA Expeditions project?

eDNA Expeditions is a global, citizen science initiative that will measure marine biodiversity and predict the impact climate change will have on marine community composition across UNESCO World Heritage marine sites.

eDNA or environmental DNA is a cost effective and minimally invasive method to measure biodiversity in any given area. Marine species continuously shed DNA into the water around them in the form of waste, mucus, or cells. By collecting this DNA from water samples, and subsequently amplifying and sequencing specific regions within these DNA strands, we can detect a wide variety of species without removing any organisms from their environment. The amplified regions are selected depending on the scope of the study: while some markers are suitable to obtain a broad overview of biodiversity across many groups of species, other markers are particularly suited get high resolution insight for a more specific taxonomic group. The eDNA Expeditions project combines a number of markers tailored for marine vertebrates (fish, mammals, and turtles), and a marker which allows us to cast a wider net and also detect some species from other groups such as invertebrates.

Between September 2022 and May 2023, the eDNA Expeditions project organized eDNA sampling campaigns in 21 marine World Heritage sites around the world. At every site, around 20 samples are collected from different habitats by teams of citizen scientists and local staff. Biodiversity inventories generated from these samples will be combined with existing species distribution data from public biodiversity databases such as the Ocean Biodiversity Information System (OBIS) to get a comprehensive overview of marine life at the sites. Using climate scenarios and species distribution models, we will estimate the impact of rising temperatures on local biodiversity.

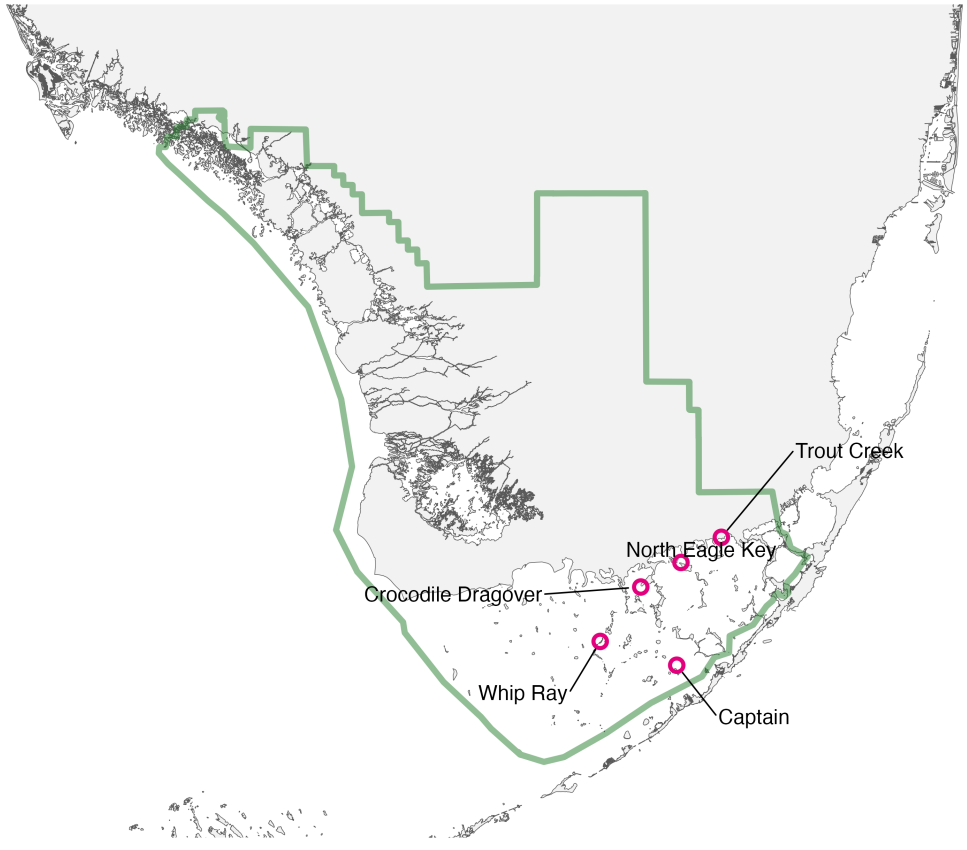


Figure 2: Map of eDNA sampling locations.

Results

Sampling and sample processing

eDNA sampling was conducted in Everglades National Park in April 2023, by filtering up to 1800 ml of seawater through filter cartridges with a 0.8 µm pore size. 20 samples were collected at five locations in the park: Trout Creek, North Eagle Creek, Crocodile Dragover, Whip Ray, and Captain. After sampling, the filter cartridges were flushed with preservation liquid and shipped to the OBIS secretariat in Belgium. From these 20 samples, a first batch of 11 samples covering all sites has now (November 2023) been processed. A second batch of samples will be processed before the end of the year. DNA from these samples was extracted and amplified, and then sent to the sequencing facility at KU Leuven in Belgium. PCR amplification was done using five primer pairs selected to capture as much biodiversity as possible in the target groups of the study: fish, mammals, and turtles.

DNA sequencing

Sequencing of the DNA from 11 samples resulted in over 20 million sequence reads. From these reads we were able to collect 12,458 unique sequences or ASVs. Matching those sequences with reference sequences in public databases, we were able to detect 163 species. Most of these species are vertebrates, but we also detected species from other groups including arthropods, cnidarians, echinoderms, molluscs, and sponges (Figure 3). Of the 163 species detected, 45 are not among the 2,566 species previously reported from Everglades National Park to the OBIS database.

reads	species	asvs
20543329	163	12458

Table 1: Reads, ASVs, and species across all samples.

locality	materialSampleID	reads	species	asvs	sampleSize
Captain	EE0386	1715700	25	1256	1500
Captain	EE0391	1504352	63	5470	1800
Captain	EE0402	1767896	56	5860	1800
Crocodile Dragover	EE0389	1539642	57	5163	1500
Crocodile Dragover	EE0390	2053533	55	5022	450
North Eagle Key	EE0388	1573556	39	2822	1500
Trout Creek	EE0403	1851194	77	3578	1500
Whip Ray	EE0387	2892694	69	3084	1800
Whip Ray	EE0404	2188565	88	5825	1800
Whip Ray	EE0405	1975938	85	4786	1800
Whip Ray	EE0406	1480259	90	4662	1800

Table 2: Reads, ASVs, and species by sample.

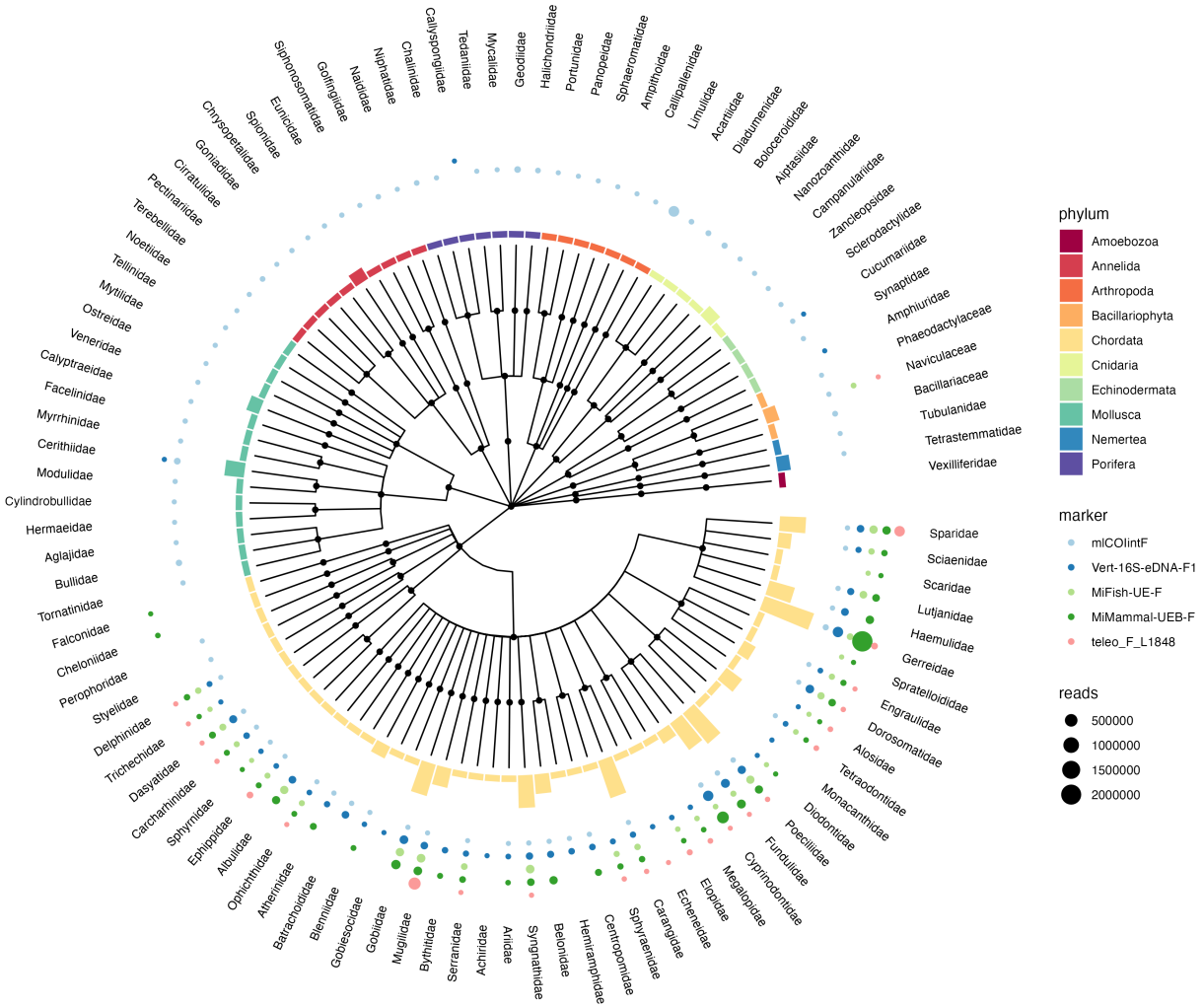


Figure 3: Number of DNA reads (bubbles) and species detected (bars) by family.

Species identification

The marker sequences obtained from sequencing were matched with sequence reference databases built using public data available from the National Center for Biotechnology Information (NCBI). This resulted in the identification of 163 species, of which 86 fish species, two mammalian species, and one turtle species (Table 3). This represents about one sixth of the species known from Everglades National Park in the OBIS database. Seven of the detected species are listed as threatened on the IUCN Red List (Table 4). A full list of species is added at the end of this report.

group	obis_species	edna_species	fraction
fish	643	86	0.13
mammal	13	2	0.15
turtle	6	1	0.17

Table 3: Number of species in the three target groups, from the OBIS database and from eDNA sampling.

category	obis_species	edna_species	fraction
CR	15		
EN	12	1	0.08
VU	35	6	0.17

Table 4: Number of species by IUCN Red List category, from the OBIS database and from eDNA sampling.

phylum	class	species	group	category	new	vernacular
Amoebozoa	Discosea	Neoparamoeba aestuarina			yes	
Annelida	Clitellata	Thalassodrilides gurwitschi			yes	
Annelida	Polychaeta	Marphysa sanguinea				red rock worm
Annelida	Polychaeta	Bhawania goodei			yes	
Annelida	Polychaeta	Glycinde multidens				
Annelida	Polychaeta	Polydora websteri			yes	
Annelida	Polychaeta	Prionospio steenstrupi				
Annelida	Polychaeta	Timarete caribous			yes	
Annelida	Polychaeta	Pectinaria gouldii				ice cream cone worm
Annelida	Polychaeta	Loimia medusa				medusa worm
Annelida		Golfingia (Golfingia) elongata				
Annelida		Siphonosoma cumanense			yes	
Arthropoda	Copepoda	Acartia (Acanthacartia) tonsa				
Arthropoda	Malacostraca	Cymadusa compta				
Arthropoda	Malacostraca	Neopanope packardii				Florida grassflat crab
Arthropoda	Malacostraca	Callinectes sapidus				blue crab, crabe bleu
Arthropoda	Malacostraca	Paracerceis caudata				
Arthropoda	Merostomata	Limulus polyphemus		VU		horseshoe crab
Arthropoda	Pycnogonida	Callipallene brevirostris				
Bacillariophyta	Bacillariophyceae	Cylindrotheca closterium			yes	
Bacillariophyta	Bacillariophyceae	Haslea crucigera			yes	
Bacillariophyta	Bacillariophyceae	Navicula minima			yes	
Bacillariophyta	Bacillariophyceae	Phaeodactylum tricornutum			yes	
Bryozoa	Gymnolaemata	Amathia evelinae			yes	
Cercozoa	Chlorarachnea	Chlorarachnion reptans			yes	
Chlorophyta	Mamiellophyceae	Micromonas pusilla				
Chordata	Ascidiacea	Ecteinascidia styeloides			yes	
Chordata	Ascidiacea	Botrylloides niger				Black synascidia , Synascidie noire
Chordata	Aves	Falco sparverius			yes	
Chordata	Elasmobranchii	Negaprion brevirostris	fish	VU		lemon shark
Chordata	Elasmobranchii	Sphyrna tiburo	fish	EN		bonnethead
Chordata	Elasmobranchii	Hypanus americanus	fish			
Chordata	Mammalia	Tursiops truncatus	mammal			bottlenose dolphin, grand dauphin
Chordata	Mammalia	Trichechus manatus	mammal	VU		West Indian manatee
Chordata	Teleostei	Chaetodipterus faber	fish			Atlantic spadefish
Chordata	Teleostei	Albula vulpes	fish			bonefish
Chordata	Teleostei	Myrophis punctatus	fish			speckled worm eel
Chordata	Teleostei	Atherinomorus stipes	fish			hardhead silverside
Chordata	Teleostei	Opsanus beta	fish			Gulf toadfish
Chordata	Teleostei	Opsanus tau	fish			oyster toadfish
Chordata	Teleostei	Strongylura notata	fish			redfin needlefish
Chordata	Teleostei	Strongylura timucu	fish			timucu
Chordata	Teleostei	Tylosurus crocodilus	fish			houndfish
Chordata	Teleostei	Chriodorus atherinoides	fish			hardhead halfbeak
Chordata	Teleostei	Chasmodes bosquianus	fish			striped blenny

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phylum	class	species	group	category	new	vernacular
Chordata	Teleostei	Centropomus undecimalis	fish			common snook
Chordata	Teleostei	Sphyraena barracuda	fish			great barracuda
Chordata	Teleostei	Caranx crysos	fish			blue runner, carangue coubali
Chordata	Teleostei	Caranx hippos	fish			common jack, carangue crevalle
Chordata	Teleostei	Caranx latus	fish			horse-eye jack
Chordata	Teleostei	Oligoplites saurus	fish			leatherjack
Chordata	Teleostei	Selene vomer	fish			lookdown
Chordata	Teleostei	Trachinotus carolinus	fish			pompano
Chordata	Teleostei	Echeneis naucrates	fish			sharksucker
Chordata	Teleostei	Brevoortia patronus	fish			Gulf menhaden
Chordata	Teleostei	Harengula jaguana	fish			scaled sardine
Chordata	Teleostei	Opisthonema oglinum	fish			Atlantic thread herring
Chordata	Teleostei	Anchoa mitchilli	fish			bay anchovy
Chordata	Teleostei	Jenkinsia lamprotaenia	fish			dwarf herring
Chordata	Teleostei	Cyprinodon variegatus	fish			sheepshead minnow
Chordata	Teleostei	Floridichthys carpio	fish			goldspotted killifish
Chordata	Teleostei	Fundulus confluentus	fish			marsh killifish
Chordata	Teleostei	Fundulus grandis	fish			Gulf killifish
Chordata	Teleostei	Fundulus xenicus	fish			
Chordata	Teleostei	Lucania goodei	fish		yes	
Chordata	Teleostei	Lucania parva	fish			rainwater killifish
Chordata	Teleostei	Belonesox belizanus	fish			pike killifish
Chordata	Teleostei	Gambusia affinis	fish			mosquitofish
Chordata	Teleostei	Gambusia holbrooki	fish			eastern mosquitofish
Chordata	Teleostei	Gambusia rhizophorae	fish			mangrove gambusia
Chordata	Teleostei	Poecilia latipinna	fish			sailfin molly
Chordata	Teleostei	Poecilia sphenops	fish		yes	Mexican molly
Chordata	Teleostei	Elops saurus	fish			ladyfish
Chordata	Teleostei	Megalops atlanticus	fish	VU		tarpon
Chordata	Teleostei	Diapterus auratus	fish			Irish mojarra
Chordata	Teleostei	Eucinostomus argenteus	fish			silver mojarra
Chordata	Teleostei	Eucinostomus gula	fish			Jenny mojarra
Chordata	Teleostei	Eucinostomus jonesii	fish			slender mojarra
Chordata	Teleostei	Eucinostomus melanopterus	fish			flagfin mojarra
Chordata	Teleostei	Eugerres plumieri	fish			striped mojarra
Chordata	Teleostei	Gerres cinereus	fish			yellowfin mojarra
Chordata	Teleostei	Ulaema lefroyi	fish			
Chordata	Teleostei	Haemulon aurolineatum	fish			tomtate grunt
Chordata	Teleostei	Haemulon parra	fish			sailors choice
Chordata	Teleostei	Haemulon plumierii	fish			grunt
Chordata	Teleostei	Haemulon sciurus	fish			bluestriped grunt
Chordata	Teleostei	Lutjanus griseus	fish			grey snapper
Chordata	Teleostei	Nicholsina usta	fish			emerald parrotfish
Chordata	Teleostei	Cynoscion nebulosus	fish			spotted weakfish
Chordata	Teleostei	Pogonias cromis	fish			blackdrum

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phylum	class	species	group	category	new	vernacular
Chordata	Teleostei	Archosargus probatocephalus	fish			sheepshead
Chordata	Teleostei	Archosargus rhomboidalis	fish			Western Atlantic seabream
Chordata	Teleostei	Diplodus holbrookii	fish			spottail pinfish
Chordata	Teleostei	Lagodon rhomboides	fish			pinfish
Chordata	Teleostei	Gobiesox strumosus	fish			skilletfish
Chordata	Teleostei	Gobiosoma bosc	fish			naked goby
Chordata	Teleostei	Gobiosoma robustum	fish			code goby
Chordata	Teleostei	Lophogobius cyprinoides	fish			crested goby
Chordata	Teleostei	Microgobius gulosus	fish			clown goby
Chordata	Teleostei	Microgobius microlepis	fish			banner goby
Chordata	Teleostei	Mugil cephalus	fish			grey mullet, muge
Chordata	Teleostei	Mugil curema	fish			silverside mullet
Chordata	Teleostei	Mugil rubrioculus	fish			
Chordata	Teleostei	Ogilbia cayorum	fish			key brotula
Chordata	Teleostei	Epinephelus itajara	fish	VU		itajara
Chordata	Teleostei	Trinectes maculatus	fish			hogchoker
Chordata	Teleostei	Ariopsis felis	fish			hardhead catfish
Chordata	Teleostei	Anarchopterus criniger	fish			fringed pipefish
Chordata	Teleostei	Hippocampus zosterae	fish			dwarf seahorse
Chordata	Teleostei	Syngnathus floridae	fish			dusky pipefish
Chordata	Teleostei	Syngnathus fuscus	fish			northern pipefish
Chordata	Teleostei	Syngnathus schlegeli	fish		yes	Seaweed pipefish
Chordata	Teleostei	Chilomycterus schoepfii	fish			striped burrfish
Chordata	Teleostei	Stephanolepis hispida	fish			
Chordata	Teleostei	Sphoeroides maculatus	fish			puffer
Chordata	Teleostei	Sphoeroides parvus	fish		yes	least puffer
Chordata	Teleostei	Sphoeroides spengleri	fish			bandtail puffer
Chordata		Caretta caretta	turtle	VU		logerhead sea turtle, tortue Caouanne
Cnidaria	Anthozoa	Exaiptasia diaphana				
Cnidaria	Anthozoa	Boloceroides mcmurrichi			yes	
Cnidaria	Anthozoa	Diadumene leucolena			yes	white anemone
Cnidaria	Anthozoa	Nanozoanthus harenaceus			yes	
Cnidaria	Hydrozoa	Zancleopsis dichotoma			yes	
Cnidaria	Hydrozoa	Clytia hemisphaerica			yes	
Cnidaria	Hydrozoa	Obelia bidentata			yes	doubletoothed hydroid
Ctenophora	Tentaculata	Vallicula multiformis			yes	
Echinodermata	Holothuroidea	Leptosynapta tenuis				slender footless sea cucumber, holothurie grle
Echinodermata	Holothuroidea	Thyonella gemmata			yes	
Echinodermata	Holothuroidea	Sclerodactyla briareus				hard-fingered sea cucumber, holothurie de Briare
Echinodermata	Ophiuroidea	Amphipholis squamata				dwarf brittle star
Gnathostomulida		Gnathostomula mediterranea			yes	
Mollusca	Bivalvia	Arcopsis adamsi				
Mollusca	Bivalvia	Ameritella mitchelli			yes	
Mollusca	Bivalvia	Brachidontes exustus				
Mollusca	Bivalvia	Crassostrea virginica				eastern oyster, hutre creuse amricaine

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phylum	class	species	group	category	new	vernacular
Mollusca	Bivalvia	Anomalocardia puella				
Mollusca	Bivalvia	Chione cancellata				cross-barred venus
Mollusca	Gastropoda	Philinopsis pusa			yes	
Mollusca	Gastropoda	Bulla arabica			yes	
Mollusca	Gastropoda	Acteocina canaliculata				channeled barrel-bubble, bulle cannele
Mollusca	Gastropoda	Crepidula convexa				convex slippersnail
Mollusca	Gastropoda	Learchis poica			yes	
Mollusca	Gastropoda	Dondice occidentalis			yes	
Mollusca	Gastropoda	Bittiolum varium				grass cerith
Mollusca	Gastropoda	Cerithium eburneum				ivory cerith
Mollusca	Gastropoda	Cerithium muscarum				flyspeck cerith
Mollusca	Gastropoda	Modulus modulus				Atlantic modulus
Mollusca	Gastropoda	Cylindrobulla beauii				
Mollusca	Gastropoda	Cyerce antillensis			yes	
Myzozoa	Dinophyceae	Amphidinium massartii			yes	
Nemertea	Hoplonemertea	Tetrastemma elegans			yes	four-eyed nemertean
Nemertea	Hoplonemertea	Tetrastemma wilsoni			yes	
Nemertea	Palaeonemertea	Tubulanus riceae			yes	
Phoronida		Phoronis psammophila			yes	
Porifera	Demospongiae	Callyspongia (Cladochalina) aculeata			yes	
Porifera	Demospongiae	Haliclona (Halichoclona) vansoesti			yes	
Porifera	Demospongiae	Pachychalina tenera			yes	
Porifera	Demospongiae	Mycale (Carmia) fibrexilis			yes	yellow flabby-sponge
Porifera	Demospongiae	Tedania (Tedania) ignis				
Porifera	Demospongiae	Halichondria (Halichondria) melanadocia			yes	
Porifera	Demospongiae	Geodia neptuni			yes	
Rhodophyta	Florideophyceae	Acanthosiphonia echinata			yes	

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