

# UNESCO environmental DNA (eDNA) Expeditions in marine World Heritage sites

Biodiversity survey for Everglades National Park

Interim Results Based on 50% of Samples Analyzed  
Not for public distribution



Credit: NPS Photos by Federico Acevedo.

## About eDNA Expeditions

UNESCO's eDNA Expeditions is a global, citizen science initiative that is conducted with the intention to measure marine biodiversity and predict the impact climate change on marine community composition across UNESCO World Heritage marine sites.

eDNA 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 extracting this DNA from water samples, and subsequently multiplying and sequencing specific regions within these DNA strands, a wide variety of species can be detected without removing any organisms from their environment. The regions in the DNA targeted for sequencing 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 to get high resolution insight for a more specific taxonomic group such as fish. The UNESCO eDNA Expeditions initiative combines a number of markers tailored for marine vertebrates (fish, mammals, and turtles). A general marker was added to also allow detection of species from other groups such as invertebrates, which can give a broader insight in the marine species diversity in a given area.

Between September 2022 and May 2023, UNESCO organized eDNA sampling campaigns in 21 marine World Heritage sites across 17 countries around the world. At every World Heritage site, about 20 samples were collected from different habitats. Over 250 young people participated in the local sampling expeditions. They were guided by local experts and park management staff, using protocols and eDNA sampling equipment provided by UNESCO.

Biodiversity inventories generated from the samples are being combined with existing species distribution data from public biodiversity databases such as the Ocean Biodiversity Information System (OBIS) in view of obtaining a comprehensive overview of marine life across marine World Heritage sites. Through the use of climate scenarios and species distribution models, an estimate will be made of the impact of climate change, in particular rising temperatures, on local biodiversity and its potential future distribution patterns.

More information about the initiative and a press kit are available on the UNESCO website: <https://www.unesco.org/en/edna-expeditions>.

## Results

### Sampling and sample processing

eDNA sampling was conducted in Everglades National Park in April 2023. A total of 20 samples were collected at 5 locations in the park covering different habitats: Captain, Crocodile Dragover, North Eagle Key, Trout Creek, and Whip Ray (Figure 1). Up to 1,800 mL of seawater was filtered through filter cartridges containing a filter with a 0.8  $\mu\text{m}$  pore size. After sampling, the filter cartridges were flushed with preservation liquid and shipped to UNESCO. From the 20 samples, 11 have now (December 2023) been processed. A second batch of samples will be processed by January 2024. DNA from these samples was extracted and amplified, and subsequently sent to the sequencing facility at KU Leuven in Belgium, a specialized university eDNA lab contracted by UNESCO for this work. 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. The results in this preliminary report are based on a first batch of 11 samples that were analyzed covering all locations that were sampled in the World Heritage site.

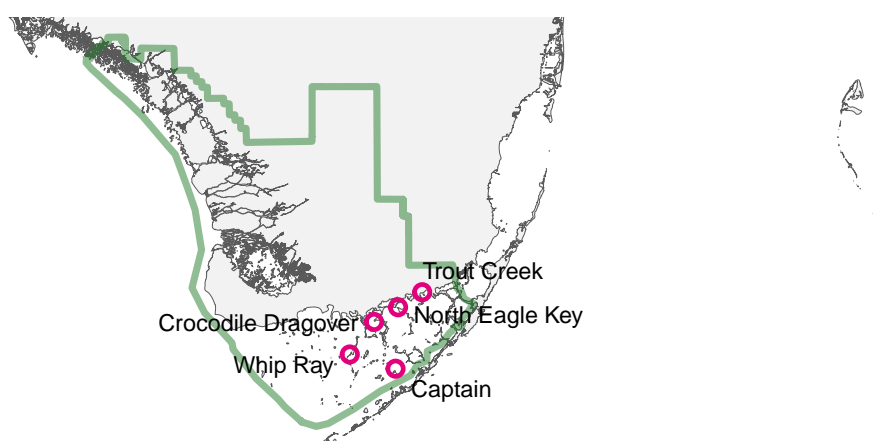


Figure 1: Map of the sampling locations.

### DNA sequencing

Sequencing of the DNA from 11 samples resulted in over 19 million sequence reads, from which we collected 12,337 unique sequences or ASVs (Tables 1 and 2).

reads	asvs
19530371	12337

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

### 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 169 species, including 87 fish species, 2 mammalian species, and 2 turtle species (Table 3). Species from other groups, such as molluscs, worms, algae, cnidarians, and sponges, were also detected (Figures 2 and 3). This number of species detected represents about 14% of the 2,568 species known from Everglades National Park in the OBIS database. Of the 169 species detected, 50 are not among the species previously reported from Everglades National Park to the UNESCO OBIS database. 7 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.

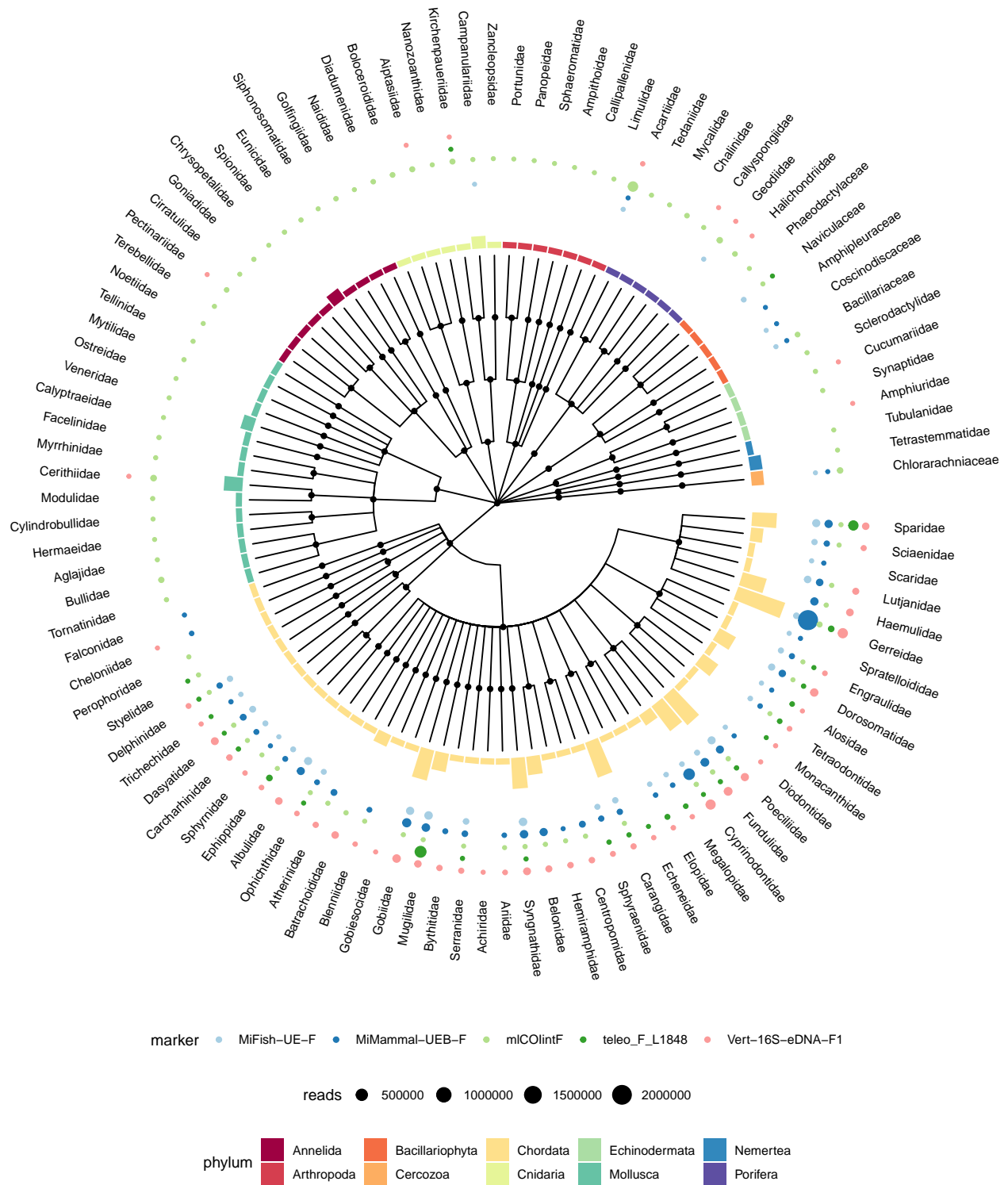


Figure 2: Number of DNA reads (bubbles) and species detected (bars) by family.

locality	materialSampleID	sampleSize	reads	asvs	species
Captain	EE0386	1500	1707430	1214	27
Captain	EE0391	1800	1318355	5357	66
Captain	EE0402	1800	1526511	5723	62
Crocodile Dragover	EE0389	1500	1323395	5092	62
Crocodile Dragover	EE0390	450	1877459	4965	60
North Eagle Key	EE0388	1500	1563335	2788	44
Trout Creek	EE0403	1500	1833731	3521	79
Whip Ray	EE0387	1800	2884264	3052	75
Whip Ray	EE0404	1800	2033657	5745	93
Whip Ray	EE0405	1800	1978944	4768	92
Whip Ray	EE0406	1800	1466428	4637	95

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

group	eDNA species	total species	fraction
fish	87	646	0.13
mammals	2	14	0.14
turtles	1	6	0.17

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

category	eDNA species	total species	fraction
CR	0	15	
EN	1	12	0.08
VU	6	35	0.17

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

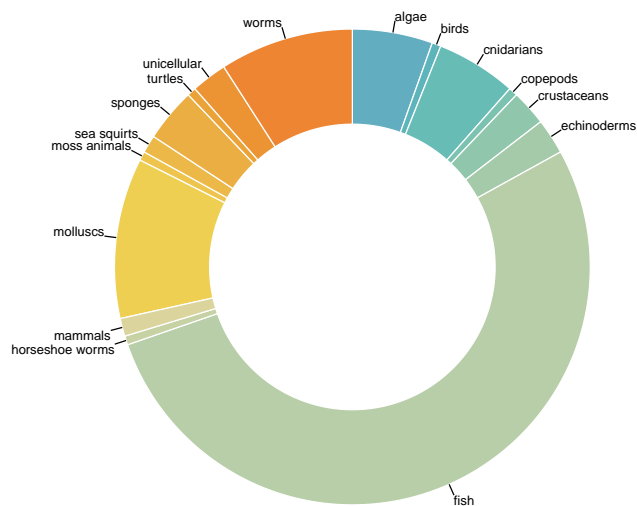


Figure 3: Distribution of detected species across groups.

phylum	class	species	group	category	new	vernacular
Amoebozoa	Discosea	Neoparamoeba aestuarina	unicellular		yes	
Annelida	Clitellata	Thalassodrilides gurwitschi	worms		yes	
Annelida	Polychaeta	Bhawania goodei	worms		yes	
Annelida	Polychaeta	Glycinde multident	worms			
Annelida	Polychaeta	Loimia medusa	worms			medusa worm
Annelida	Polychaeta	Marphysa sanguinea	worms			red rock worm
Annelida	Polychaeta	Pectinaria gouldii	worms			ice cream cone worm
Annelida	Polychaeta	Polydora websteri	worms		yes	
Annelida	Polychaeta	Prionospio steenstrupi	worms			
Annelida	Polychaeta	Timarete caribous	worms		yes	
Annelida		Golfingia (Golfingia) elongata	worms			
Annelida		Siphonosoma cumanense	worms		yes	
Arthropoda	Copepoda	Acartia (Acanthacartia) tonsa	copepods			
Arthropoda	Malacostraca	Callinectes sapidus	crustaceans			blue crab, crabe bleu
Arthropoda	Malacostraca	Cymadusa compta	crustaceans			
Arthropoda	Malacostraca	Neopanope packardii	crustaceans			Florida grassflat crab
Arthropoda	Malacostraca	Paracerceis caudata	crustaceans			
Arthropoda	Merostomata	Limulus polyphemus		VU		horseshoe crab
Arthropoda	Pycnogonida	Callipallene brevirostris				
Bacillariophyta	Bacillariophyceae	Coscinodiscus granii	algae		yes	
Bacillariophyta	Bacillariophyceae	Cylindrotheca closterium	algae		yes	
Bacillariophyta	Bacillariophyceae	Halamphora calidilacuna	algae		yes	
Bacillariophyta	Bacillariophyceae	Navicula minima	algae		yes	
Bacillariophyta	Bacillariophyceae	Phaeodactylum tricornutum	algae		yes	
Bryozoa	Gymnolaemata	Amathia evelinae	moss animals		yes	
Cercozoa	Chlorarachnea	Bigelowiella natans	unicellular		yes	
Cercozoa	Chlorarachnea	Chlorarachnion reptans	unicellular		yes	
Chlorophyta	Mamiellophyceae	Micromonas pusilla	algae			
Chordata	Ascidiacea	Botrylloides niger	sea squirts			Black synascidia , Synascidie noire
Chordata	Ascidiacea	Ecteinascidia styeloides	sea squirts		yes	
Chordata	Aves	Falco sparverius	birds	LC	yes	
Chordata	Elasmobranchii	Hypanus americanus	fish	NT		
Chordata	Elasmobranchii	Negaprion brevirostris	fish	VU		lemon shark
Chordata	Elasmobranchii	Sphyrna tiburo	fish	EN		bonnethead
Chordata	Mammalia	Trichechus manatus	mammals	VU		West Indian manatee
Chordata	Mammalia	Tursiops truncatus	mammals	LC		bottlenose dolphin, grand dauphin
Chordata	Teleostei	Albula vulpes	fish	NT		bonefish
Chordata	Teleostei	Anarchopterus criniger	fish	LC		fringed pipefish
Chordata	Teleostei	Anchoa mitchilli	fish	LC		bay anchovy
Chordata	Teleostei	Archosargus probatocephalus	fish	LC		sheepshead
Chordata	Teleostei	Archosargus rhomboidalis	fish	LC		Western Atlantic seabream
Chordata	Teleostei	Ariopsis felis	fish	LC		hardhead catfish
Chordata	Teleostei	Atherinomorus stipes	fish	LC		hardhead silverside
Chordata	Teleostei	Belonesox belizanus	fish	LC		pike killifish
Chordata	Teleostei	Brevoortia patronus	fish	LC		Gulf menhaden
Chordata	Teleostei	Caranx crysos	fish	LC		blue runner, carangue coubali
Chordata	Teleostei	Caranx hippos	fish	LC		common jack, carangue crevalle
Chordata	Teleostei	Caranx latus	fish	LC		horse-eye jack
Chordata	Teleostei	Centropomus undecimalis	fish	LC		common snook
Chordata	Teleostei	Chaetodipterus faber	fish	LC		Atlantic spadefish

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phylum	class	species	group	category	new	vernacular
Chordata	Teleostei	Chasmodes bosquianus	fish	LC		striped blenny
Chordata	Teleostei	Chilomycterus schoepfii	fish	LC		striped burrfish
Chordata	Teleostei	Chriodorus atherinoides	fish	LC		hardhead halfbeak
Chordata	Teleostei	Cynoscion nebulosus	fish	LC		spotted weakfish
Chordata	Teleostei	Cyprinodon variegatus	fish	LC		sheepshead minnow
Chordata	Teleostei	Diapterus auratus	fish	LC		Irish mojarra
Chordata	Teleostei	Diplodus holbrookii	fish	LC		spottail pinfish
Chordata	Teleostei	Echeneis naucrates	fish	LC		sharksucker
Chordata	Teleostei	Elops saurus	fish	LC		ladyfish
Chordata	Teleostei	Epinephelus itajara	fish	VU		itajara
Chordata	Teleostei	Eucinostomus argenteus	fish	LC		silver mojarra
Chordata	Teleostei	Eucinostomus gula	fish	LC		Jenny mojarra
Chordata	Teleostei	Eucinostomus jonesii	fish	LC		slender mojarra
Chordata	Teleostei	Eucinostomus melanopterus	fish	LC		flagfin mojarra
Chordata	Teleostei	Eugerres plumieri	fish	LC		striped mojarra
Chordata	Teleostei	Floridichthys carpio	fish	LC		goldspotted killifish
Chordata	Teleostei	Fundulus confluentus	fish	LC		marsh killifish
Chordata	Teleostei	Fundulus grandis	fish	LC		Gulf killifish
Chordata	Teleostei	Fundulus xenicus	fish			
Chordata	Teleostei	Gambusia affinis	fish	LC		mosquitofish
Chordata	Teleostei	Gambusia holbrooki	fish	LC		eastern mosquitofish
Chordata	Teleostei	Gambusia rhizophorae	fish	LC		mangrove gambusia
Chordata	Teleostei	Gerres cinereus	fish	LC		yellowfin mojarra
Chordata	Teleostei	Gobiosox strumosus	fish	LC		skilletfish
Chordata	Teleostei	Gobiosoma bosc	fish	LC		naked goby
Chordata	Teleostei	Gobiosoma robustum	fish	LC		code goby
Chordata	Teleostei	Haemulon aurolineatum	fish	LC		tomtate grunt
Chordata	Teleostei	Haemulon parra	fish	LC		sailors choice
Chordata	Teleostei	Haemulon plumierii	fish	LC		grunt
Chordata	Teleostei	Haemulon sciurus	fish	LC		bluestriped grunt
Chordata	Teleostei	Harengula humeralis	fish	LC		pilchard
Chordata	Teleostei	Harengula jaguana	fish	LC		scaled sardine
Chordata	Teleostei	Hippocampus zosterae	fish	LC		dwarf seahorse
Chordata	Teleostei	Jenkinsia lamprotaenia	fish	LC		dwarf herring
Chordata	Teleostei	Lagodon rhomboides	fish	LC		pinfish
Chordata	Teleostei	Lophogobius cyprinoides	fish	LC		crested goby
Chordata	Teleostei	Lucania goodei	fish	LC	yes	
Chordata	Teleostei	Lucania parva	fish	LC		rainwater killifish
Chordata	Teleostei	Lutjanus griseus	fish	LC		grey snapper
Chordata	Teleostei	Megalops atlanticus	fish	VU		tarpon
Chordata	Teleostei	Microgobius gulosus	fish	LC		clown goby
Chordata	Teleostei	Microgobius microlepis	fish	LC		banner goby
Chordata	Teleostei	Mugil cephalus	fish	LC		grey mullet, muge
Chordata	Teleostei	Mugil curema	fish	LC		silverside mullet
Chordata	Teleostei	Mugil rubrioculus	fish	LC		
Chordata	Teleostei	Myrophis punctatus	fish	LC		speckled worm eel
Chordata	Teleostei	Nicholsina usta	fish	LC		emerald parrotfish
Chordata	Teleostei	Ogilbia cayorum	fish	LC		key brotula
Chordata	Teleostei	Oligoplites saurus	fish	LC		leatherjack
Chordata	Teleostei	Opisthonema oglinum	fish	LC		Atlantic thread herring

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phylum	class	species	group	category	new	vernacular
Chordata	Teleostei	Opsanus beta	fish	LC		Gulf toadfish
Chordata	Teleostei	Opsanus tau	fish	LC		oyster toadfish
Chordata	Teleostei	Poecilia latipinna	fish	LC		sailfin molly
Chordata	Teleostei	Poecilia sphenops	fish	LC	yes	Mexican molly
Chordata	Teleostei	Pogonias cromis	fish	LC		blackdrum
Chordata	Teleostei	Selene vomer	fish	LC		lookdown
Chordata	Teleostei	Sphoeroides maculatus	fish	LC		puffer
Chordata	Teleostei	Sphoeroides parvus	fish	LC	yes	least puffer
Chordata	Teleostei	Sphoeroides spengleri	fish	LC		bandtail puffer
Chordata	Teleostei	Sphyræna barracuda	fish	LC		great barracuda
Chordata	Teleostei	Stephanolepis hispidus	fish			
Chordata	Teleostei	Strongylura notata	fish	LC		redfin needlefish
Chordata	Teleostei	Strongylura timucu	fish	LC		timucu
Chordata	Teleostei	Syngnathus floridae	fish	LC		dusky pipefish
Chordata	Teleostei	Syngnathus fuscus	fish	LC		northern pipefish
Chordata	Teleostei	Syngnathus scovelli	fish	LC		Gulf pipefish
Chordata	Teleostei	Trachinotus carolinus	fish	LC		pompano
Chordata	Teleostei	Trinectes maculatus	fish	LC		hogchoker
Chordata	Teleostei	Tylosurus crocodilus	fish	LC		houndfish
Chordata	Teleostei	Ulaema lefroyi	fish			
Chordata		Caretta caretta	turtles	VU		loggerhead sea turtle, tortue Caouanne
Cnidaria	Anthozoa	Boloceroïdes mcmurricchi	cnidarians		yes	
Cnidaria	Anthozoa	Diadumene leucolea	cnidarians		yes	white anemone
Cnidaria	Anthozoa	Exaïptasia diaphana	cnidarians			
Cnidaria	Anthozoa	Nanozoanthus harenaceus	cnidarians		yes	
Cnidaria	Hydrozoa	Clytia hemisphaerica	cnidarians		yes	
Cnidaria	Hydrozoa	Kirchenpaueria halecioides	cnidarians		yes	
Cnidaria	Hydrozoa	Obelia bidentata	cnidarians		yes	doubletoothed hydroid
Cnidaria	Hydrozoa	Zancleopsis dichotoma	cnidarians		yes	
Cryptophyta	Cryptophyceae	Hemiarma marina			yes	
Cryptophyta	Cryptophyta incertae sedis	Leucocryptos marina			yes	
Ctenophora	Tentaculata	Vallicula multiformis	cnidarians		yes	
Echinodermata	Holothuroidea	Leptosynapta tenuis	echinoderms			slender footless sea cucumber, holothurie grêle
Echinodermata	Holothuroidea	Sclerodactyla briareus	echinoderms			hard-fingered sea cucumber, holothurie de Briarée
Echinodermata	Holothuroidea	Thyonella gemmata	echinoderms		yes	
Echinodermata	Ophiuroidea	Amphipholis squamata	echinoderms			dwarf brittle star
Gnathostomulida		Gnathostomula mediterranea	worms		yes	
Mollusca	Bivalvia	Ameritella mitchelli	molluscs		yes	
Mollusca	Bivalvia	Anomalocardia puella	molluscs			
Mollusca	Bivalvia	Arcopsis adamsi	molluscs			
Mollusca	Bivalvia	Brachidontes exustus	molluscs			
Mollusca	Bivalvia	Chione cancellata	molluscs			cross-barred venus
Mollusca	Bivalvia	Crassostrea virginica	molluscs			eastern oyster, huître creuse américaine
Mollusca	Gastropoda	Acteocina canaliculata	molluscs			channeled barrel-bubble, bulle cannelée
Mollusca	Gastropoda	Bittium varium	molluscs			grass cerith
Mollusca	Gastropoda	Bulla arabica	molluscs		yes	
Mollusca	Gastropoda	Cerithium eburneum	molluscs			ivory cerith
Mollusca	Gastropoda	Cerithium muscarum	molluscs			flyspeck cerith
Mollusca	Gastropoda	Crepidula convexa	molluscs			convex slippersnail
Mollusca	Gastropoda	Cyerce antillensis	molluscs		yes	

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phylum	class	species	group	category	new	vernacular
Mollusca	Gastropoda	Cylindrobulla beauui	molluscs			
Mollusca	Gastropoda	Dondice occidentalis	molluscs		yes	
Mollusca	Gastropoda	Learchis poica	molluscs		yes	
Mollusca	Gastropoda	Modulus modulus	molluscs			Atlantic modulus
Mollusca	Gastropoda	Philinopsis pusa	molluscs		yes	
Myzozoa	Dinophyceae	Amphidinium massartii	unicellular		yes	
Nemertea	Hoplonemertea	Tetrastemma elegans	worms		yes	four-eyed nemertean
Nemertea	Hoplonemertea	Tetrastemma wilsoni	worms		yes	
Nemertea	Palaeonemertea	Tubulanus riceae	worms		yes	
Ochrophyta	Chrysoparadoxophyceae	Chrysoparadoxa australica	algae		yes	
Ochrophyta	Dictyochophyceae	Pseudopedinella elastica	algae		yes	
Phoronida		Phoronis psammophila	horseshoe worms		yes	
Porifera	Demospongiae	Callyspongia (Cladochalina) aculeata	sponges		yes	
Porifera	Demospongiae	Geodia neptuni	sponges		yes	
Porifera	Demospongiae	Halichondria (Halichondria) melanadocia	sponges		yes	
Porifera	Demospongiae	Haliclona (Halichoclona) vansoesti	sponges		yes	
Porifera	Demospongiae	Mycale (Carmia) fibrexilis	sponges		yes	yellow flabby-sponge
Porifera	Demospongiae	Tedania (Tedania) ignis	sponges			
Rhodophyta	Florideophyceae	Acanthosiphonia echinata	algae		yes	