

# eDNA Expeditions biodiversity survey of Everglades National Park

IOC-UNESCO and 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 to 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.

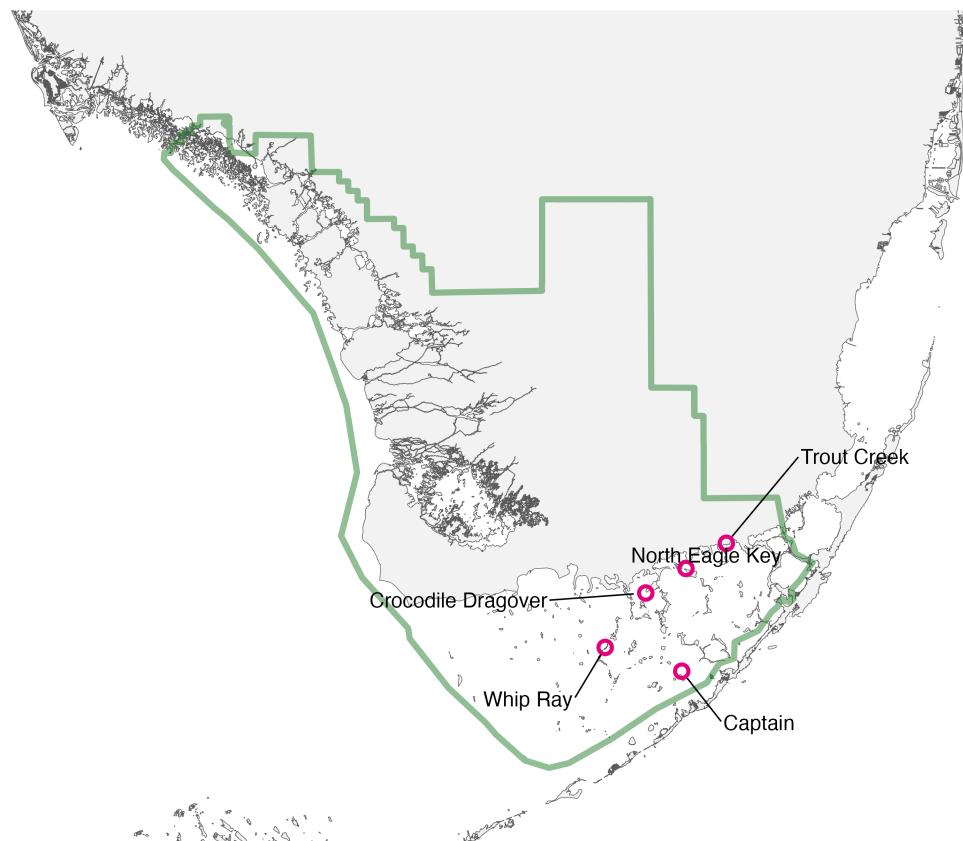


Figure 2: Map of eDNA sampling locations.

# 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 de-

tected 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.

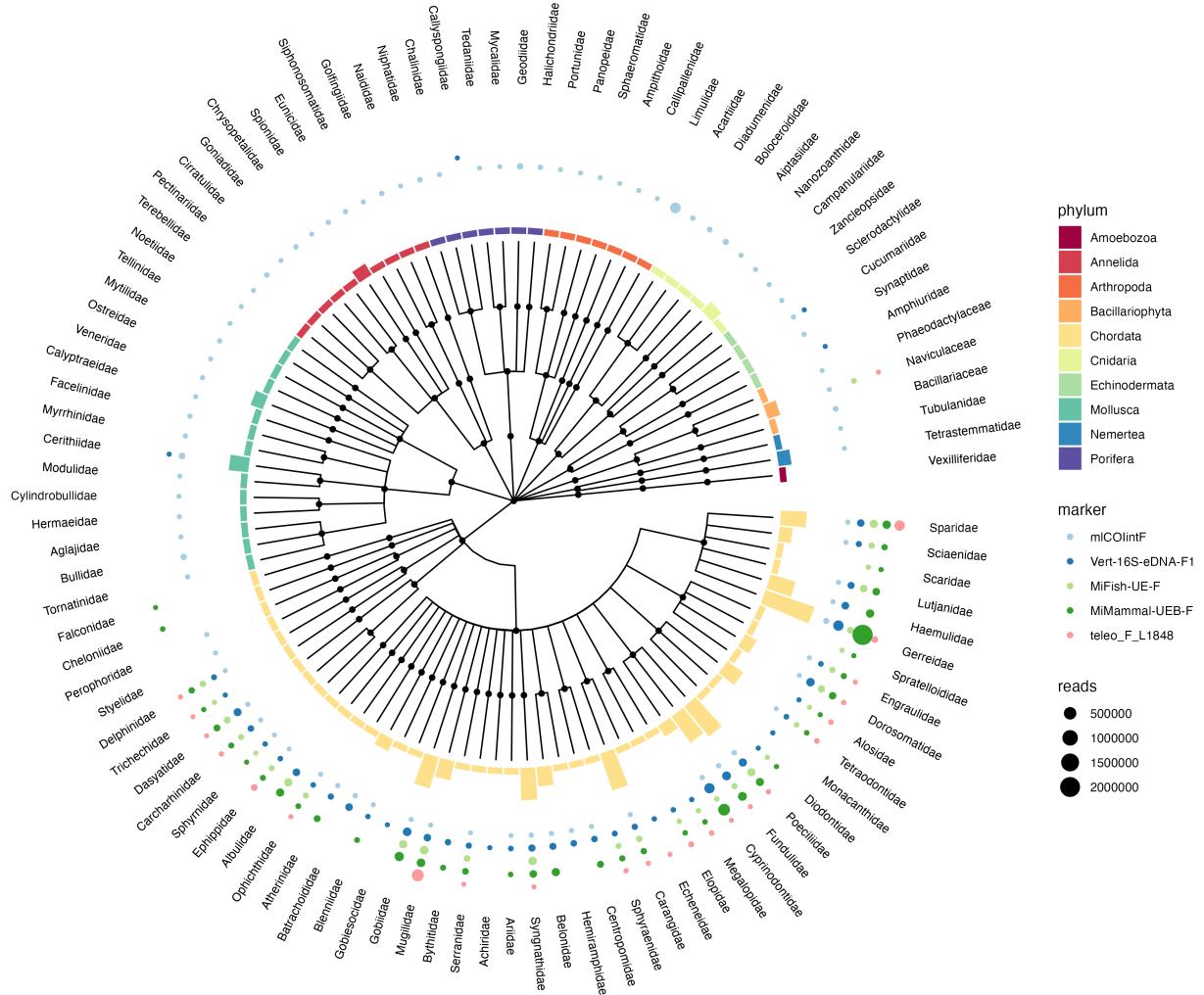


Figure 3: Number of DNA reads (bubbles) and species detected (bars) by family.

## 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 (Ta-

ble 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.

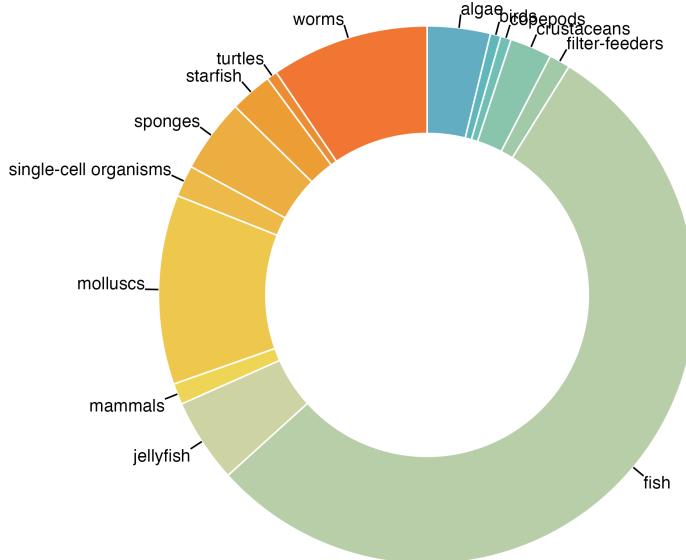


Figure 4: Distribution of species across groups.

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		<i>Discosphaera aestuaria</i>		yes	
Annelida	Clitellata	<i>Thalassodrilides gurwitschi</i>		yes	red rock worm
Annelida	Polychaeta	<i>Marphysa sanguinea</i>		yes	
Annelida	Polychaeta	<i>Bhawania goodei</i>		yes	
Annelida	Polychaeta	<i>Glycinde multidens</i>		yes	
Annelida	Polychaeta	<i>Polydora websteri</i>		yes	
Annelida	Polychaeta	<i>Prionospio steenstrupi</i>		yes	
Annelida	Polychaeta	<i>Timarete caribous</i>		yes	ice cream cone worm
Annelida	Polychaeta	<i>Pectinaria gouldii</i>		yes	medusa worm
Annelida	Polychaeta	<i>Loimia medusa</i>			
Annelida	Copepoda	<i>Golfingia (Golfingia) elongata</i>			
Arthropoda	Arthropoda	<i>Siphonosoma cumanense</i>			
Arthropoda	Arthropoda	<i>Acartia (Acanthacartia) tonsa</i>			
Arthropoda	Arthropoda	<i>Cymadusa compta</i>			
Arthropoda	Arthropoda	<i>Neopanope packardi</i>			
Arthropoda	Arthropoda	<i>Callinectes sapidus</i>			
Arthropoda	Arthropoda	<i>Paracerceis caudata</i>			
Arthropoda	Malacostraca	<i>Limulus polyphemus</i>		yes	
Arthropoda	Malacostraca	<i>Callipallenae brevirostris</i>		yes	
Arthropoda	Malacostraca	<i>Cylindrotheca closterium</i>		yes	
Arthropoda	Merostomata	<i>Haslea crucigera</i>		yes	
Arthropoda	Pycnogonida	<i>Navicula minima</i>		yes	
Arthropoda	Bacillariophyceae	<i>Phaeodactylum tricornutum</i>		yes	
Arthropoda	Bacillariophyceae	<i>Amathia evelinae</i>		yes	
Arthropoda	Bacillariophyceae	<i>Chlorarachnion reptans</i>		yes	
Arthropoda	Gymnolaemata	<i>Micromonas pusilla</i>		yes	
Arthropoda	Gymnolaemata	<i>Ecteinascidia styloides</i>		yes	
Arthropoda	Chlorophyta	<i>Botrylloides niger</i>			
Bryozoa	Asciacea	<i>Falco sparverius</i>			
Cercozoa	Chlorophyta	<i>Negaprion brevirostris</i>			
Chordata	Chordata	<i>Sphyraena tiburo</i>			
Chordata	Chordata	<i>Hypanus americanus</i>			
Chordata	Elasmobranchii	<i>Tursiops truncatus</i>			
Chordata	Elasmobranchii	<i>Trichechus manatus</i>			
Chordata	Mammalia	<i>Chaetodipterus faber</i>			
Chordata	Mammalia	<i>Albula vulpes</i>			
Chordata	Teleostei	<i>Myrophis punctatus</i>			
Chordata	Teleostei	<i>Atherinomorus stipes</i>			
Chordata	Teleostei	<i>Opsanus beta</i>			
Chordata	Teleostei	<i>Opsanus tau</i>			
Chordata	Teleostei	<i>Strongylura notata</i>			
Chordata	Teleostei	<i>Tylosurus crocodilus</i>			
Chordata	Teleostei	<i>Chridodus atherinoides</i>			
Chordata	Teleostei	<i>Chasmodes bosquianus</i>			

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phylum	class	species	group	category	new	vernacular
Chordata	Teleostei	<i>Centropomus undecimalis</i>	fish			common snook
Chordata	Teleostei	<i>Sphyraena barracuda</i>	fish			great barracuda
Chordata	Teleostei	<i>Caranx cryos</i>	fish			blue runner, carangue couballi
Chordata	Teleostei	<i>Caranx hippos</i>	fish			common jack, carangue crevalle
Chordata	Teleostei	<i>Caranx latus</i>	fish			horse-eye jack
Chordata	Teleostei	<i>Oligoplites saurus</i>	fish			leatherjack
Chordata	Teleostei	<i>Selene vomer</i>	fish			lookdown
Chordata	Teleostei	<i>Trachinotus carolinus</i>	fish			pompano
Chordata	Teleostei	<i>Echeneis naucrates</i>	fish			sharksucker
Chordata	Teleostei	<i>Brevoortia patronus</i>	fish			Gulf menhaden
Chordata	Teleostei	<i>Harengula jaguana</i>	fish			scaled sardine
Chordata	Teleostei	<i>Opisthonema oglinum</i>	fish			Atlantic thread herring
Chordata	Teleostei	<i>Anchoa mitchilli</i>	fish			bay anchovy
Chordata	Teleostei	<i>Jenkinsia lamprotaenia</i>	fish			dwarf herring
Chordata	Teleostei	<i>Cyprinodon variegatus</i>	fish			sheepshead minnow
Chordata	Teleostei	<i>Floridichthys carpio</i>	fish			goldspotted killifish
Chordata	Teleostei	<i>Fundulus confluentus</i>	fish			marsh killifish
Chordata	Teleostei	<i>Fundulus grandis</i>	fish			Gulf killifish
Chordata	Teleostei	<i>Fundulus xenicus</i>	fish			
Chordata	Teleostei	<i>Lucania goodei</i>	fish			
Chordata	Teleostei	<i>Lucania parva</i>	fish			
Chordata	Teleostei	<i>Belonesox belizanus</i>	fish			
Chordata	Teleostei	<i>Gambusia affinis</i>	fish			
Chordata	Teleostei	<i>Gambusia holbrookii</i>	fish			
Chordata	Teleostei	<i>Gambusia rhizophorae</i>	fish			
Chordata	Teleostei	<i>Poecilia latipinna</i>	fish			
Chordata	Teleostei	<i>Poecilia sphenops</i>	fish			
Chordata	Teleostei	<i>Elops saurus</i>	fish			
Chordata	Teleostei	<i>Megalops atlanticus</i>	fish			
Chordata	Teleostei	<i>Diapterus auratus</i>	fish			
Chordata	Teleostei	<i>Eucinostomus argenteus</i>	fish			
Chordata	Teleostei	<i>Eucinostomus gula</i>	fish			
Chordata	Teleostei	<i>Eucinostomus Jonesii</i>	fish			
Chordata	Teleostei	<i>Eucinostomus melanopterus</i>	fish			
Chordata	Teleostei	<i>Eugerres plumieri</i>	fish			
Chordata	Teleostei	<i>Ulaema lefroyi</i>	fish			
Chordata	Teleostei	<i>Haemulon aurolineatum</i>	fish			
Chordata	Teleostei	<i>Haemulon parra</i>	fish			
Chordata	Teleostei	<i>Haemulon plumieri</i>	fish			
Chordata	Teleostei	<i>Lutjanus griseus</i>	fish			
Chordata	Teleostei	<i>Nicholsina ustata</i>	fish			
Chordata	Teleostei	<i>Cynoscion nebulosus</i>	fish			
Chordata	Teleostei	<i>Pogonias cromis</i>	fish			

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phylum	class	species	group	category	new	vernacular
Chordata	Teleostei	<i>Archosargus probatocephalus</i>	fish			sheephead
Chordata	Teleostei	<i>Archosargus rhomboidalis</i>	fish			Western Atlantic seabream
Chordata	Teleostei	<i>Diplodus holbrookii</i>	fish			spottail pinfish
Chordata	Teleostei	<i>Lagodon rhomboides</i>	fish			pinfish
Chordata	Teleostei	<i>Gobiosox strumosus</i>	fish			skilletfish
Chordata	Teleostei	<i>Gobiosoma bosc</i>	fish			naked goby
Chordata	Teleostei	<i>Lophogobius cyprinoides</i>	fish			code goby
Chordata	Teleostei	<i>Microgobius gulosis</i>	fish			crested goby
Chordata	Teleostei	<i>Microgobius microlepis</i>	fish			clown goby
Chordata	Teleostei	<i>Mugil cephalus</i>	fish			banner goby
Chordata	Teleostei	<i>Mugil curema</i>	fish			grey mullet, mugé
Chordata	Teleostei	<i>Mugil rubrioculus</i>	fish			silverside mullet
Chordata	Teleostei	<i>Ogilbia cayorum</i>	fish			key brotula
Chordata	Teleostei	<i>Epinephelus itajara</i>	fish			itajara
Chordata	Teleostei	<i>Trinectes maculatus</i>	fish			hogchoker
Chordata	Teleostei	<i>Ariopsis felis</i>	fish			hardhead catfish
Chordata	Teleostei	<i>Anarchopterus criniger</i>	fish			fringed pipefish
Chordata	Teleostei	<i>Hippocampus zosterae</i>	fish			dwarf seahorse
Chordata	Teleostei	<i>Syngnathus floridae</i>	fish			dusky pipefish
Chordata	Teleostei	<i>Syngnathus fuscus</i>	fish			northern pipefish
Chordata	Teleostei	<i>Syngnathus schlegeli</i>	fish			Seaweed pipefish
Chordata	Teleostei	<i>Chilomycterus schoepfii</i>	fish			striped burrfish
Chordata	Teleostei	<i>Stephanolepis hispida</i>	fish			puffer
Chordata	Teleostei	<i>Sphoeroides maculatus</i>	fish			least puffer
Chordata	Teleostei	<i>Sphoeroides parvus</i>	fish			bandtail puffer
Chordata	Teleostei	<i>Sphoeroides spengleri</i>	fish			loggerhead sea turtle, tortue Caouanne
Chordata	Teleostei	<i>Caretta caretta</i>	turtle	VU		
Cnidaria	Anthozoa	<i>Exaiptasia diaphana</i>			yes	
Cnidaria	Anthozoa	<i>Boloceroides mcmurrichi</i>			yes	
Cnidaria	Anthozoa	<i>Diadumene leucolena</i>			yes	
Cnidaria	Anthozoa	<i>Nanozoanthus harenaceus</i>			yes	
Cnidaria	Hydrozoa	<i>Zancleopsis dichotoma</i>			yes	
Cnidaria	Hydrozoa	<i>Clytia hemisphaerica</i>			yes	
Cnidaria	Hydrozoa	<i>Obelia bidentata</i>			yes	
Cnidaria	Tentaculata	<i>Valicula multiformis</i>			yes	
Ctenophora	Holothuroidea	<i>Leptosynapta tenuis</i>			yes	
Echinodermata	Holothuroidea	<i>Thyonella gemmata</i>			yes	
Echinodermata	Holothuroidea	<i>Sclerodactyla briareus</i>			yes	
Echinodermata	Ophiuroidea	<i>Amphipholis squamata</i>			yes	
Gnathostomulida		<i>Gnathostomula mediterranea</i>			yes	
Mollusca	Bivalvia	<i>Arcopsis adamsi</i>				
Mollusca	Bivalvia	<i>Ameritella mitchelli</i>				
Mollusca	Bivalvia	<i>Brachidontes exustus</i>				
Mollusca	Bivalvia	<i>Crassostrea virginica</i>				

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phylum	class	species	group	category	new	vernacular
Mollusca	Bivalvia	<i>Anomalocardia pulella</i>				cross-barred venus
Mollusca	Bivalvia	<i>Chione cancellata</i>			yes	
Mollusca	Gastropoda	<i>Philinopsis pusa</i>			yes	channeled barrel-bubble, bulle cannelé
Mollusca	Gastropoda	<i>Bulla arabica</i>				
Mollusca	Gastropoda	<i>Acteocina canaliculata</i>				
Mollusca	Gastropoda	<i>Crepidula convexa</i>				
Mollusca	Gastropoda	<i>Learchis poica</i>				
Mollusca	Gastropoda	<i>Dondice occidentalis</i>				
Mollusca	Gastropoda	<i>Bittium varium</i>				
Mollusca	Gastropoda	<i>Cerithium eburneum</i>				
Mollusca	Gastropoda	<i>Cerithium muscarum</i>				
Mollusca	Gastropoda	<i>Modulus modulus</i>				
Mollusca	Gastropoda	<i>Cylindrobulla beauii</i>				
Mollusca	Gastropoda	<i>Cyerce antillensis</i>				
Mollusca	Dinophyceae	<i>Amphidinium massartii</i>			yes	
Myzoza	Hoploneurtea	<i>Tetrasistema elegans</i>			yes	
Nemertea	Hoploneurtea	<i>Tetrasistema wilsoni</i>			yes	
Nemertea	Palaemonermetea	<i>Tubulanus riceae</i>			yes	
Nemertea	Phoronida	<i>Phoronis psammophila</i>			yes	
Porifera	Demospongiae	<i>Callyspongia (Cladodochalina) aculeata</i>			yes	
Porifera	Demospongiae	<i>Haliclona (Halichoclona) vansoestii</i>			yes	
Porifera	Demospongiae	<i>Pachychalina tenera</i>			yes	
Porifera	Demospongiae	<i>Mycale (Carmia) fibrexilis</i>			yes	
Porifera	Demospongiae	<i>Tedania (Tedania) ignis</i>				
Porifera	Demospongiae	<i>Halichondria (Halichondria) melanadocia</i>				
Rhodophyta	Florideophyceae	<i>Geodia neptuni</i>			yes	
		<i>Acanthosiphonia echinata</i>			yes	



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