

eDNA Expeditions biodiversity survey of Everglades National Park



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.

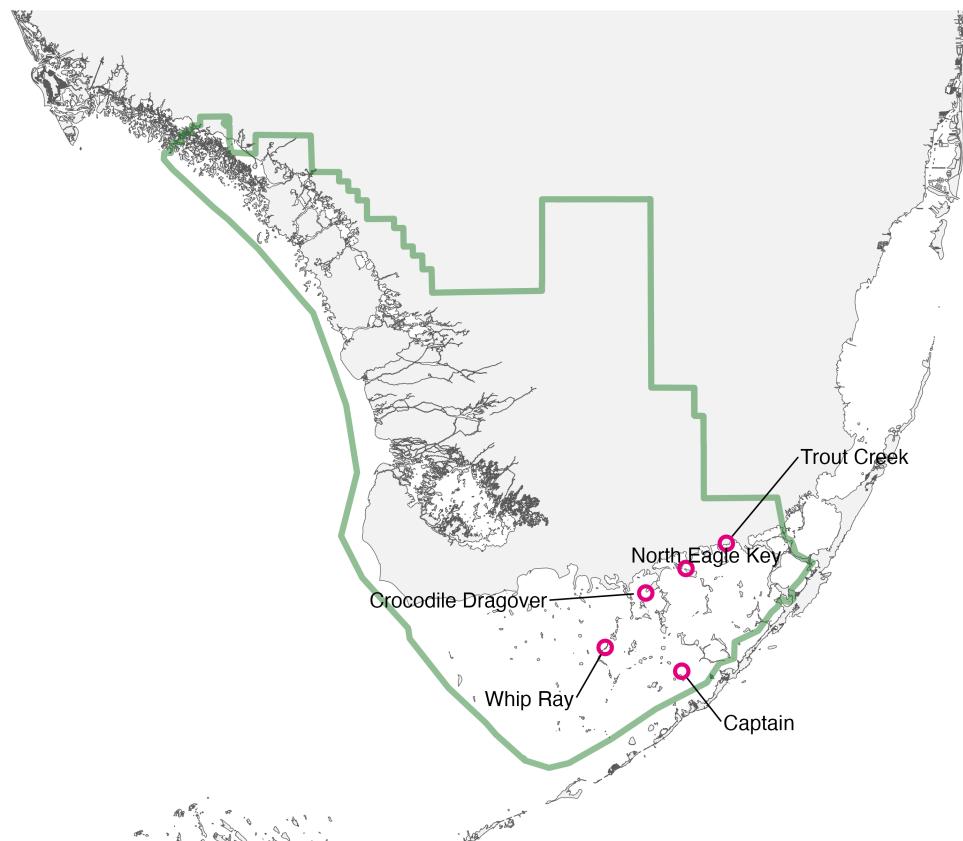


Figure 2: Map of eDNA sampling locations.

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.

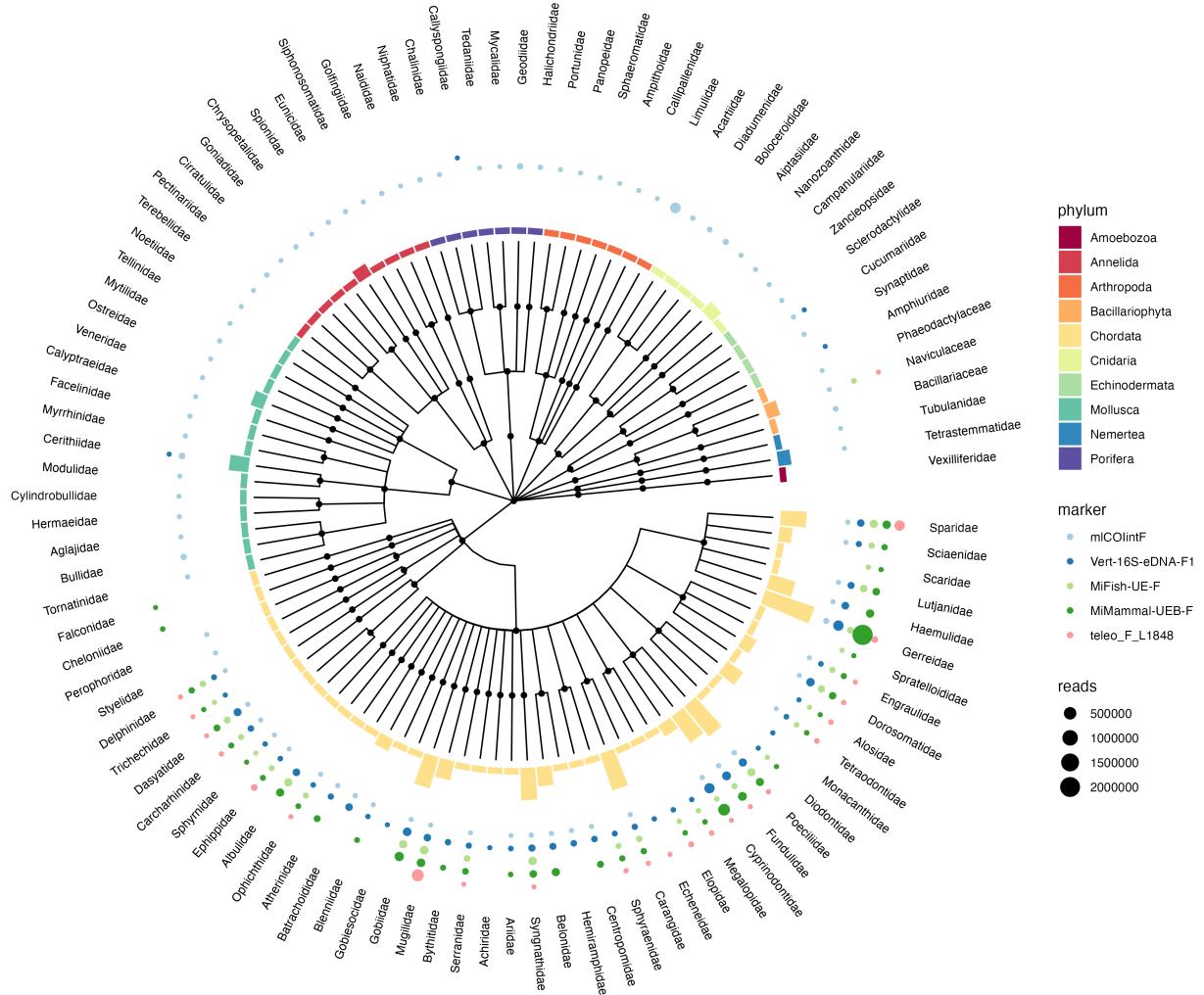


Figure 3: Number of DNA reads (bubbles) and species detected (bars) by family.

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.

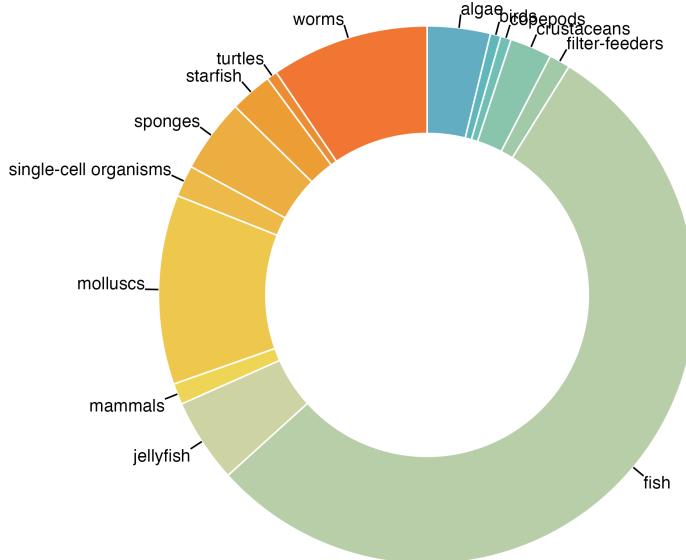


Figure 4: Distribution of species across groups.

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

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phylum	class	species	group	category	new	vernacular
Chordata	Teleostei	<i>Archosargus probatocephalus</i>	fish	LC		sheephead
Chordata	Teleostei	<i>Archosargus rhomboidalis</i>	fish	LC		Western Atlantic seabream
Chordata	Teleostei	<i>Diplodus holbrookii</i>	fish	LC		spottail pinfish
Chordata	Teleostei	<i>Lagodon rhomboides</i>	fish	LC		pinfish
Chordata	Teleostei	<i>Gobiesox strumosus</i>	fish	LC		skilletfish
Chordata	Teleostei	<i>Gobiosoma bosc</i>	fish	LC		naked goby
Chordata	Teleostei	<i>Gobiosoma robustum</i>	fish	LC		code goby
Chordata	Teleostei	<i>Lophogobius cyprinoides</i>	fish	LC		crested goby
Chordata	Teleostei	<i>Microgobius gulosis</i>	fish	LC		clown goby
Chordata	Teleostei	<i>Microgobius microlepis</i>	fish	LC		banner goby
Chordata	Teleostei	<i>Mugil cephalus</i>	fish	LC		grey mullet, mugé
Chordata	Teleostei	<i>Mugil curema</i>	fish	LC		silverside mullet
Chordata	Teleostei	<i>Mugil rubrioculus</i>	fish	LC		
Chordata	Teleostei	<i>Ogilbia cayorum</i>	fish	LC		key brotula
Chordata	Teleostei	<i>Epinephelus itajara</i>	fish	VU		itajara
Chordata	Teleostei	<i>Trinectes maculatus</i>	fish	LC		hogchoker
Chordata	Teleostei	<i>Ariopsis felis</i>	fish	LC		hardhead catfish
Chordata	Teleostei	<i>Anarchopterus criniger</i>	fish	LC		fringed pipefish
Chordata	Teleostei	<i>Hippocampus zosterae</i>	fish	LC		dwarf seahorse
Chordata	Teleostei	<i>Syngnathus floridae</i>	fish	LC		dusky pipefish
Chordata	Teleostei	<i>Syngnathus fuscus</i>	fish	LC		northern pipefish
Chordata	Teleostei	<i>Syngnathus schlegeli</i>	fish	LC		Seaweed pipefish
Chordata	Teleostei	<i>Chilomycterus schoepfii</i>	fish	LC		
Chordata	Teleostei	<i>Stephanolepis hispida</i>	fish	LC		puffer
Chordata	Teleostei	<i>Sphoeroides maculatus</i>	fish	LC		least puffer
Chordata	Teleostei	<i>Sphoeroides parvus</i>	fish	LC		bandtail puffer
Chordata	Teleostei	<i>Sphoeroides spengleri</i>	fish	VU		loggerhead sea turtle, tortue Caouanne
Chordata	Teleostei	<i>Caretta caretta</i>	turtle			
Cnidaria		<i>Exaiptasia diaphana</i>			yes	
Cnidaria		<i>Boloceroides mcmurrichi</i>			yes	
Cnidaria		<i>Diadumene leucolena</i>			yes	white anemone
Cnidaria		<i>Nanozoanthus harenaceus</i>			yes	
Cnidaria		<i>Zancleopsis dichotoma</i>			yes	
Cnidaria		<i>Clytia hemisphaerica</i>			yes	
Cnidaria		<i>Obelia bidentata</i>			yes	
Ctenophora		<i>Tentaculata</i>			yes	
Echinodermata		<i>Holothuroidea</i>			yes	
Echinodermata		<i>Holothuroidea</i>			yes	slender footless sea cucumber, holothurie grise
Echinodermata		<i>Holothuroidea</i>			yes	
Echinodermata		<i>Ophiuroidea</i>			yes	hard-fingered sea cucumber, holothurie de Briare
Gnathostomulida					yes	dwarf brittle star
Mollusca	Bivalvia	<i>Bivalvia</i>			yes	
Mollusca	Bivalvia	<i>Bivalvia</i>				
Mollusca	Bivalvia	<i>Bivalvia</i>				
		<i>Crassostrea virginica</i>				eastern oyster, hutre creuse américaine

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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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