Sommersemester 2025 Vertiefte Bestimmungsübungen an Tieren (MEES003/C3)

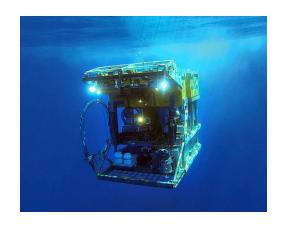
# **Environmental DNA (eDNA) Metabarcoding Analysis**

Day 3



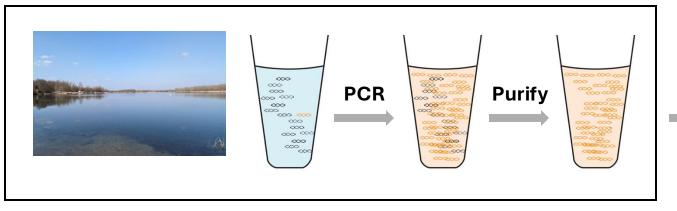
Day1, 2: eDNA Metabarcoding for identifying animals living in a pond near Halle

Day3: Metabarcoding analysis in different samples and a public dataset



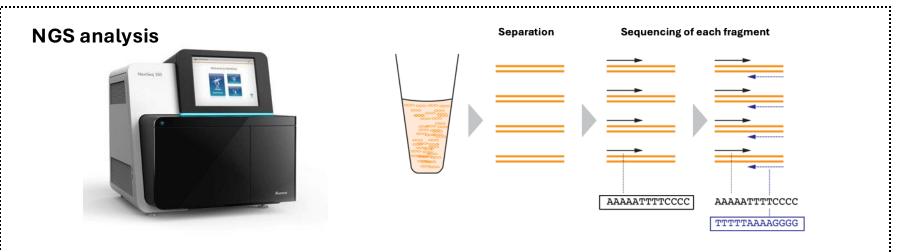






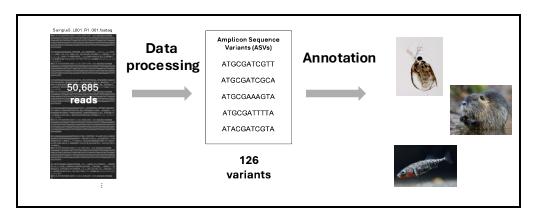
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Receive the sequencing data



## **Analysis/Annotation**







#### **Publication**

# MOLECULAR ECOLOGY RESOURCES

RESOURCE ARTICLE 🙃 Full Access

Comparing iDNA from mosquitoes and flies to survey mammals in a semi-controlled Neotropical area

Bruno H. Saranholi 🔀 Karen G. Rodriguez-Castro, Carolina S. Carvalho, Samira Chahad-Ehlers, Carla C. Gestich, Sónia C. S. Andrade, Patrícia D. Freitas, Pedro M. Galetti Jr

First published: 03 August 2023 | https://doi.org/10.1111/1755-0998.13851 | Citations: 4

Handling Editor: Sebastien Calvignac-Spencer

Abstract

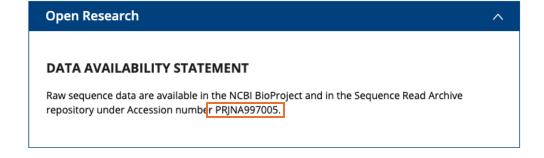
**:**■ SECTIONS

Ingested-derived DNA (iDNA) from insects represents a powerful tool for assessing vertebrate diversity because insects are easy to sample, have a diverse diet and are

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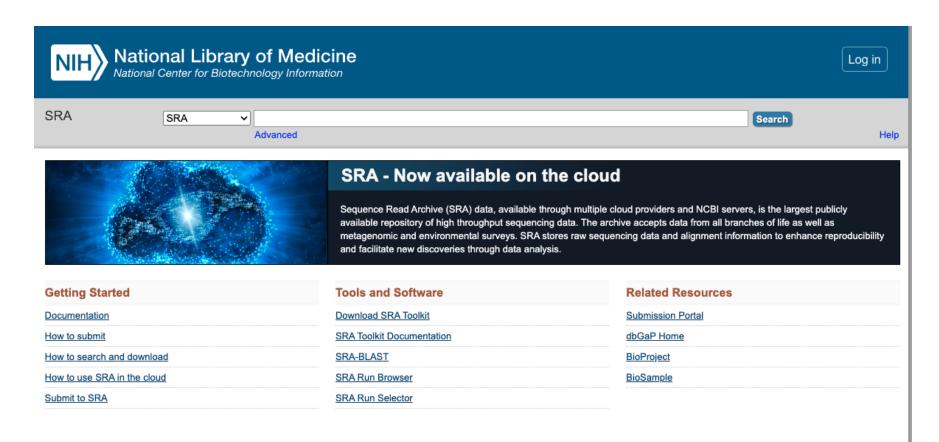


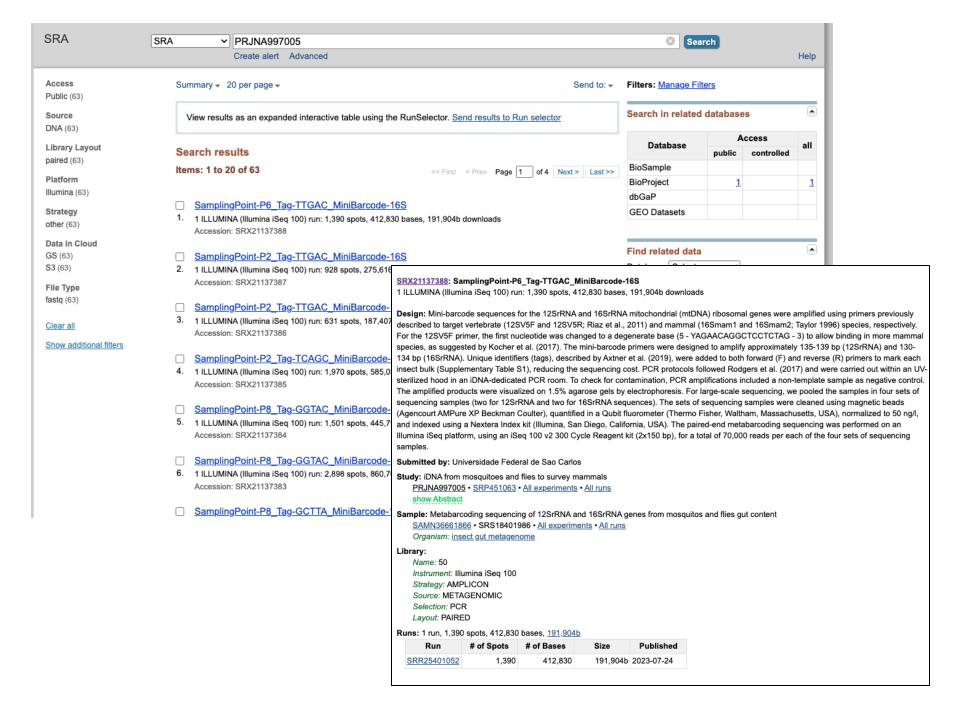
# **Data deposition**

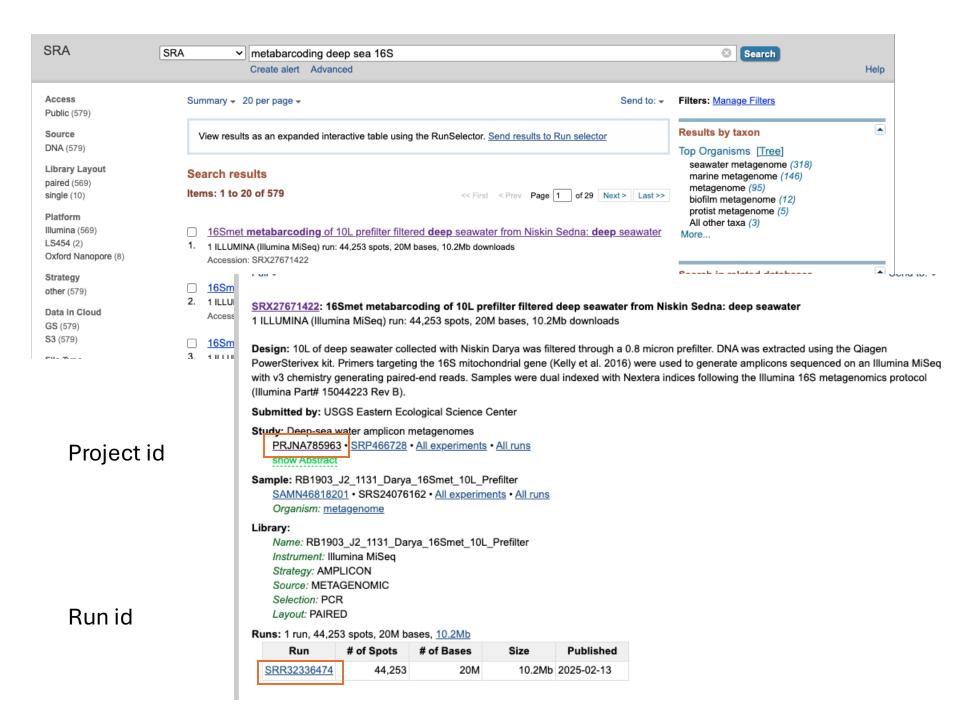


### SRA: Sequence Read Archive

https://www.ncbi.nlm.nih.gov/sra

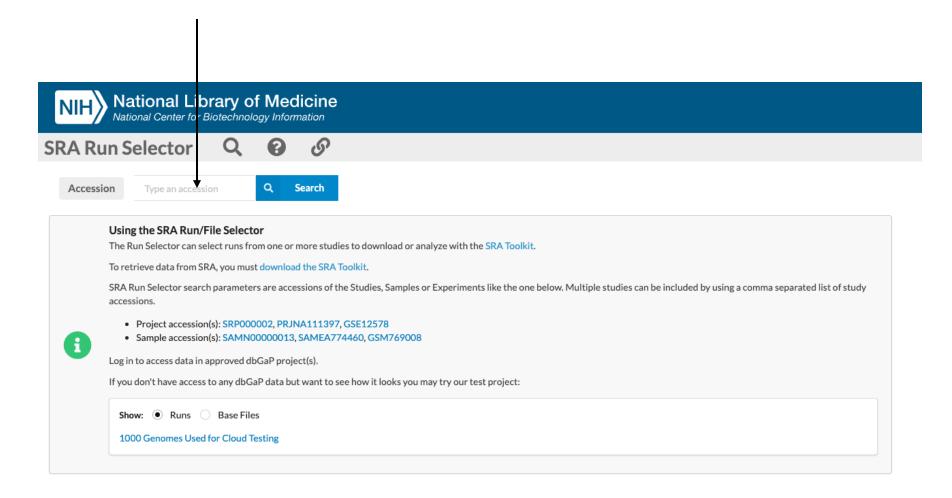






# Details of the analysis may be found in SRA Run selector https://www.ncbi.nlm.nih.gov/Traces/study/

#### PRJNA785963



ound 125	<b>Items</b> Sear	rch within results							Q	Clear				<	1 1 :	3 >
<b></b> ×	▲ Run	BioSample		Bases  4	Bytes	Collection_Date	Depth	env_broad_scale	env_local_scale	* \$ Experiment	filter_material	filter_pore_size	‡ lat_lon		Niskin_name	pucl_acid_ext
1	SRR26406384	SAMN37846936	330	177.21 M	64.81 Mb	2019-04-29	2571m	oceanic bathypelagic zone biome	oceanic bathypelagic zone biom	e SRX22112076	Sterivex	0.2	32.97398 N 75.91420 W	RB1903_J2_1137_Darya_185_1L_Sterivex_run2	Darya	Sterivex filters were extracted using the
2	SRR26406385	SAMN37846935	337	214.81 M	78.93 Mb	2019-04-25	948m	oceanic epipelagic zone biome	oceanic epipelagic zone biome	SRX22112075	Prefilter	0.8	33.91893 N 75.83331 W	RB1903_J2_1135_Sedna_18S_10L_Prefilter_run2	Sedna	Prefilters were extracted using the Qi
3	SRR26406386	SAMN37846934	337	148.81 M	52.73 Mb	2019-04-23	298m	oceanic epipelagic zone biome	oceanic epipelagic zone biome	SRX22112074	Sterivex	0.2	35.67351 N 74.79731 W	RB1903_J2_1133_Darya_18S_1L_Sterivex_run2	Darya	Sterivex filters were extracted using t
4	SRR26406387	SAMN37846933	337	208.12 M	82.07 Mb	2019-04-17	516m	oceanic epipelagic zone biome	oceanic epipelagic zone biome	SRX22112073	Prefilter	0.8	31.76121 N 79.19059 W	RB1903_J2_1130_Sedna_18S_10L_Prefilter_run2	Sedna	Prefilters were extracted using the Q
5	SRR26406388	SAMN37806937	325	75.21 M	31.91 Mb	2019-04-11	731m	oceanic epipelagic zone biome	oceanic epipelagic zone biome	SRX22112071	Sterivex	0.2	31.88915 N 77.36567 W	RB1903_J2_1128_Darya_18S_1L_Sterivex	Darya	Sterivex filters were extracted using t
6	SRR26406389	SAMN37807008	128	54.81 M	26.77 Mb	2019-10	Surface	laboratory	laboratory	SRX22112070	Prefilter	0.8	NA	Lab_extraction_ctl_cox1_10L_Prefilter	NA	Prefilters were extracted using the Q
7	SRR26406390	SAMN37807006	116	20.56 M	9.59 Mb	2019-09	Surface	laboratory	laboratory	SRX22112068	Sterivex	0.2	NA	Lab_extraction_ctl_cox1_1L_Sterivex	NA	Sterivex filters were extracted using t
8	SRR26406391	SAMN37807005	98	1.39 M	444.34 kb	2019-09	Surface	laboratory	laboratory	SRX22112067	Sterivex	0.2	NA	Lab_extraction_ctl_18S_1L_Sterivex	NA	Sterivex filters were extracted using t
9	SRR26406392	SAMN37807004	122	83.54 M	40.02 Mb	2019-04-30	Surface	laboratory	laboratory	SRX22112066	Prefilter	0.8	NA	RB1903_Postcruise_ctl_Sedna_cox1_10L_Prefilter	Sedna	Prefilters were extracted using the Q
10	SRR26406393	SAMN37807003	486	140.44 M	61.83 Mb	2019-04-30	Surface	laboratory	laboratory	SRX22112065	Prefilter	0.8	NA	RB1903_Postcruise_ctl_Darya_cox1_10L_Prefilter	Darya	Prefilters were extracted using the Q
11	SRR26406394	SAMN37807002	178	85.94 M	42.97 Mb	2019-04-30	Surface	laboratory	laboratory	SRX22112064	Sterivex	0.2	NA	RB1903_Postcruise_ctl_Sedna_cox1_1L_Sterivex	Sedna	Sterivex filters were extracted using t
12	SRR26406395	SAMN37807001	453	126.22 M	56.91 Mb	2019-04-30	Surface	laboratory	laboratory	SRX22112063	Sterivex	0.2	NA	RB1903_Postcruise_ctl_Darya_cox1_1L_Sterivex	Darya	Sterivex filters were extracted using
13	SRR26406396	SAMN37807000	328	7.93 M	3.75 Mb	2019-04-30	Surface	laboratory	laboratory	SRX22112062	Prefilter	0.8	NA	RB1903_Postcruise_ctl_Sedna_18S_10L_Prefilter	Sedna	Prefilters were extracted using the Q
14	SRR26406397	SAMN37806999	344	59.25 M	23.99 Mb	2019-04-30	Surface	laboratory	laboratory	SRX22112061	Prefilter	0.8	NA	RB1903_Postcruise_ctl_Darya_18S_10L_Prefilter	Darya	Prefilters were extracted using the C
15	SRR26406398	SAMN37806936	527	88.92 M	39.52 Mb	2019-04-09	Surface	laboratory	laboratory	SRX22112060	Prefilter	0.8	NA	RB1903_Precruise_ctl_Sedna_cox1_10L_Prefilter	Sedna	Prefilters were extracted using the
16	SRR26406399	SAMN37806998	256	2.24 M	1.21 Mb	2019-04-30	Surface	laboratory	laboratory	SRX22112059	Sterivex	0.2	NA	RB1903_Postcruise_ctl_Sedna_18S_1L_Sterivex	Sedna	Sterivex filters were extracted using
17	SRR26406400	SAMN37806997	334	123.26 M	52.10 Mb	2019-04-30	Surface	laboratory	laboratory	SRX22112058	Sterivex	0.2	NA	RB1903_Postcruise_ctl_Darya_18S_1L_Sterivex	Darya	Sterivex filters were extracted using
18	SRR26406401	SAMN37807007	106	13.05 k	71.31 kb	2019-10	Surface	laboratory	laboratory	SRX22112069	Prefilter	0.8	NA	Lab_extraction_ctl_18S_10L_Prefilter	NA	Prefilters were extracted using the C
19	SRR26406402	SAMN37806996	557	83.35 M	38.04 Mb	2019-04-29	2571m	oceanic bathypelagic zone biome	oceanic bathypelagic zone biom	e SRX22112057	Prefilter	0.8	32.97398 N 75.91420 W	RB1903_J2_1137_Sedna_cox1_10L_Prefilter	Sedna	Prefilters were extracted using the C
20	SRR26406403	SAMN37806995	560	87.98 M	39.08 Mb	2019-04-29	2571m	oceanic bathypelagic zone biome	oceanic bathypelagic zone biom	e SRX22112056	Prefilter	0.8	32.97398 N 75.91420 W	RB1903_J2_1137_Darya_cox1_10L_Prefilter	Darya	Prefilters were extracted using the C
21	SRR26406404	SAMN37806994	551	128.02 M	54.97 Mb	2019-04-29	2571m	oceanic bathypelagic zone biome	oceanic bathypelagic zone biom	e SRX22112055	Sterivex	0.2	32.97398 N 75.91420 W	RB1903_J2_1137_Sedna_cox1_1L_Sterivex	Sedna	Sterivex filters were extracted using
22	SRR26406405	SAMN37806993	572	140.78 M	60.97 Mb	2019-04-29	2571m	oceanic bathypelagic zone biome	oceanic bathypelagic zone biom	e SRX22112054	Sterivex	0.2	32.97398 N 75.91420 W	RB1903_J2_1137_Darya_cox1_1L_Sterivex	Darya	Sterivex filters were extracted using
23	SRR26406406	SAMN37806992	339	105.31 M	46.46 Mb	2019-04-29	2571m	oceanic bathypelagic zone biome	oceanic bathypelagic zone biom	e SRX22112053	Prefilter	0.8	32.97398 N 75.91420 W	RB1903_J2_1137_Sedna_18S_10L_Prefilter	Sedna	Prefilters were extracted using the Q
24	SRR26406407	SAMN37806991	336	142.59 M	62.05 Mb	2019-04-29	2571m	oceanic bathypelagic zone biome	oceanic bathypelagic zone biom	e SRX22112052	Prefilter	0.8	32.97398 N 75.91420 W	RB1903_J2_1137_Darya_18S_10L_Prefilter	Darya	Prefilters were extracted using the C
25	SRR26406408	SAMN37806990	331	90.97 M	39.27 Mb	2019-04-29	2571m	oceanic bathypelagic zone biome	oceanic bathypelagic zone biom	e SRX22112051	Sterivex	0.2	32.97398 N 75.91420 W	RB1903_J2_1137_Sedna_185_1L_Sterivex	Sedna	Sterivex filters were extracted using
26	SRR26406409	SAMN37806989	417	314.85 k	212.07 kb	2019-04-29	2571m	oceanic bathypelagic zone biome	oceanic bathypelagic zone biom	e SRX22112050	Sterivex	0.2	32.97398 N 75.91420 W	RB1903_J2_1137_Darya_18S_1L_Sterivex	Darya	Sterivex filters were extracted using
27	SRR26406410	SAMN37806935	573	140.50 M	58.58 Mb	2019-04-09	Surface	laboratory	laboratory	SRX22112049	Prefilter	0.8	NA	RB1903_Precruise_ctl_Darya_cox1_10L_Prefilter	Darya	Prefilters were extracted using the C
28	SRR26406411	SAMN37806988	515	118.75 M	50.08 Mb	2019-04-28	2164m	oceanic bathypelagic zone biome	oceanic bathypelagic zone biom	e SRX22112048	Prefilter	0.8	32.49361 N 76.19091 W	RB1903_J2_1136_Sedna_cox1_10L_Prefilter	Sedna	Prefilters were extracted using the C
29	SRR26406412	SAMN37806987	542	111.85 M	48.85 Mb	2019-04-28	2164m	oceanic bathypelagic zone biome	oceanic bathypelagic zone biom	e SRX22112047	Prefilter	0.8	32.49361 N 76.19091 W	RB1903_J2_1136_Darya_cox1_10L_Prefilter	Darya	Prefilters were extracted using the C
30	SRR26406413	SAMN37806986	506	124.07 M	53.07 Mb	2019-04-28	2164m	oceanic bathypelagic zone biome	oceanic bathypelagic zone biom	e SRX22112046	Sterivex	0.2	32.49361 N 76.19091 W	RB1903_J2_1136_Sedna_cox1_1L_Sterivex	Sedna	Sterivex filters were extracted using
31	SRR26406414	SAMN37806985	516	114.24 M	48.19 Mb	2019-04-28	2164m	oceanic bathypelagic zone biome	oceanic bathypelagic zone biom	e SRX22112045	Sterivex	0.2	32.49361 N 76.19091 W	RB1903_J2_1136_Darya_cox1_1L_Sterivex	Darya	Sterivex filters were extracted using
32	SRR26406415	SAMN37806984	338	112.99 M	49.98 Mb	2019-04-28	2164m	oceanic bathypelagic zone biome			Prefilter	0.8	32.49361 N 76.19091 W	RB1903_J2_1136_Sedna_18S_10L_Prefilter	Sedna	Prefilters were extracted using the
33	SRR26406416	SAMN37806983	334	82.92 M	35.20 Mb	2019-04-28	2164m	oceanic bathypelagic zone biome	oceanic bathypelagic zone biom	e SRX22112043	Prefilter	0.8	32.49361 N 76.19091 W	RB1903_J2_1136_Darya_18S_10L_Prefilter	Darya	Prefilters were extracted using the
34	SRR26406417	SAMN37806982	332	106.40 M	46.41 Mb	2019-04-28	2164m		oceanic bathypelagic zone biom		Sterivex	0.2	32.49361 N 76.19091 W	RB1903_J2_1136_Sedna_18S_1L_Sterivex	Sedna	Sterivex filters were extracted using
35		SAMN37806981	326	91.25 M	39.24 Mb		2164m		oceanic bathypelagic zone biom		Sterivex	0.2		RB1903_J2_1136_Darya_185_1L_Sterivex	Darya	Sterivex filters were extracted using
36		SAMN37806980		124.43 M		2019-04-25	948m	oceanic epipelagic zone biome	oceanic epipelagic zone biome	SRX22112040		0.8		RB1903 J2 1135 Sedna cox1 10L Prefilter	Sedna	Prefilters were extracted using the C
			540	221.101.11	20.701.70		,	epiperagre zone sittife	beperage zone biome	31012222010			22207011733030117			

17	ReleaseDate	create_date		Target_Gene
ATCAYGT	2025-02-13	2025-02-13 17:03:00Z	RB1903_J2_1128_Darya_16Smet_10L_Prefilter	mitochondrial 16S
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ATCAYGT	2025-02-13	2025-02-13 17:02:00Z	RB1903_J2_1137_Cedna_16Smet_1L_Sterivex	mitochondrial 16S
ATCAYGT	2025-02-13	2025-02-13 17:02:00Z	RB1903_J2_1136_Darya_16Smet_10L_Prefilter	mitochondrial 16S
ATCAYGT	2025-02-13	2025-02-13 17:03:00Z	RB1903_J2_1136_Darya_16Smet_1L_Sterivex	mitochondrial 16S
ATCAYGT	2025-02-13	2025-02-13 17:02:00Z	RB1903_J2_1136_Cedna_16Smet_10L_Prefilter	mitochondrial 16S
ATCAYGT	2025-02-13	2025-02-13 17:04:00Z	RB1903_J2_1136_Cedna_16Smet_1L_Sterivex	mitochondrial 16S
ATCAYGT	2025-02-13	2025-02-13 17:02:00Z	RB1903_J2_1135_Darya_16Smet_10L_Prefilter	mitochondrial 16S
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ATCAYGT	2025-02-13	2025-02-13 17:02:00Z	RB1903_J2_1135_Cedna_16Smet_10L_Prefilter	mitochondrial 169
ATCAYGT	2025-02-13	2025-02-13 17:03:00Z	RB1903_J2_1135_Cedna_16Smet_1L_Sterivex	mitochondrial 16S
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2025-02-13 17:03:00Z RB1903\_J2\_1128\_Cedna\_16Smet\_1L\_Sterivex mitochondrial 16S

ATCAYGT 2025-02-13

Runs: 1	run,	1,390 s	pots, 4	12,830	bases,	191,904b
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Run	# of Spots	# of Bases	Size	Published
SRR25401052	1,390	412,830	191,904b	2023-07-24

#### Download fastq files from SRA

Find the BioProject ID and search for it in the SRA Run Selector (<a href="https://www.ncbi.nlm.nih.gov/Traces/study/">https://www.ncbi.nlm.nih.gov/Traces/study/</a>) to find the run ID.

```
fastq-dump --gzip --split-files ***RUN ID***
```

Q

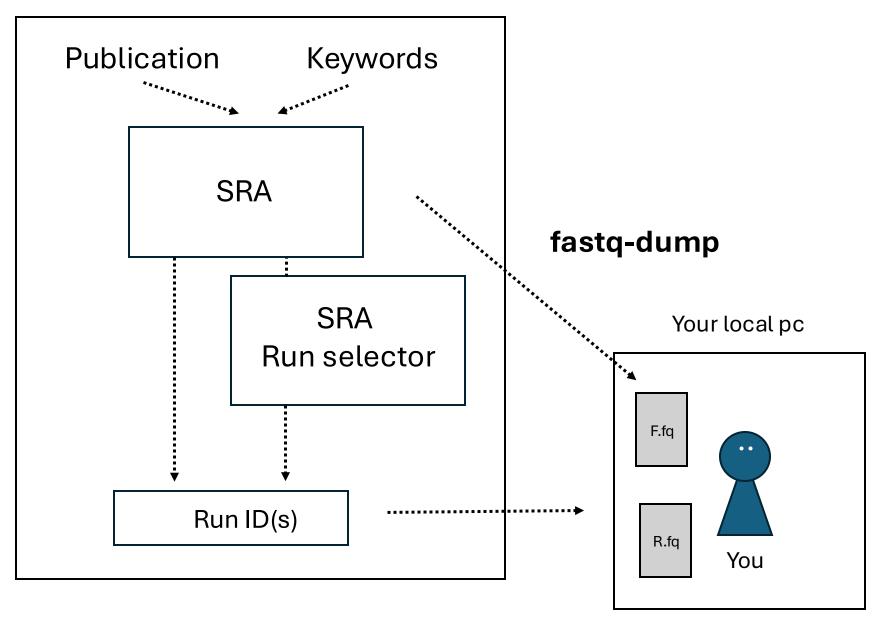
```
shumpei_yamakawa@cs-918058803005-default:~$ fastq-dump --gzip --split-files SRR3233649 0

Read 83944 spots for SRR32336490

Written 83944 spots for SRR32336490 shumpei_yamakawa@cs-918058803005-default:~$ ls a b c ncbi-blast-2.16.0+ New_directory1 R README-cloudshell.txt sratoolkit.3.2.

1-ubuntu64 SRR32336490_1.fastq.gz SRR32336490_2.fastq.gz test test2 test_meta
```

## Online



Tasks:

(Remaining Day2) Sample6 and Sample7

**Analysis of public datasets 1** 

- example 1: deep sea samples
- example 2: mosquito/fry samples

**Analysis of public datasets 2** 

find and analyze the datasets in SRA

### Example 1: Deep-sea water amplicon metagenomes. PRJNA785963

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DEEP SEARCH: DEEP Sea Exploration to Advance Research on Coral/Canyon/Cold seep Habitats

Science & Technology -

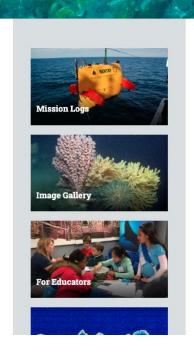
Home / Expeditions / DEEP SEARCH / Mission Logs / September 22

Expeditions -

# DEEP SEARCH 2017 Comes to an Early End, but the Project Is Just Beginning

By Caitlin Adams, NOAA Office of Ocean Exploration and Research, Web Coordinator September 22, 2017







The two Niskin bottles used to collect deep-sea water samples are mounted to the bottom of ROV Jason and can be triggered by the ROV's manipulator arms. Image courtesy of DEEP SEARCH 2019 - BOEM, USGS, NOAA. Download larger version (jpg, 704 KB).

Ex.) SRR32336490 Sea water (depth 2,571 m) 16s rRNA amplicon / metazoa





# **MOLECULAR ECOLOGY** RESOURCES

### Comparing iDNA from mosquitoes and flies to survey mammals in a semi-controlled Neotropical area

Bruno H. Saranholi X, Karen G. Rodriguez-Castro, Carolina S. Carvalho, Samira Chahad-Ehlers, Carla C. Gestich, Sónia C. S. Andrade, Patrícia D. Freitas, Pedro M. Galetti Jr

First published: 03 August 2023 | https://doi.org/10.1111/1755-0998.13851 | Citations: 4

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:≡ SECTIONS





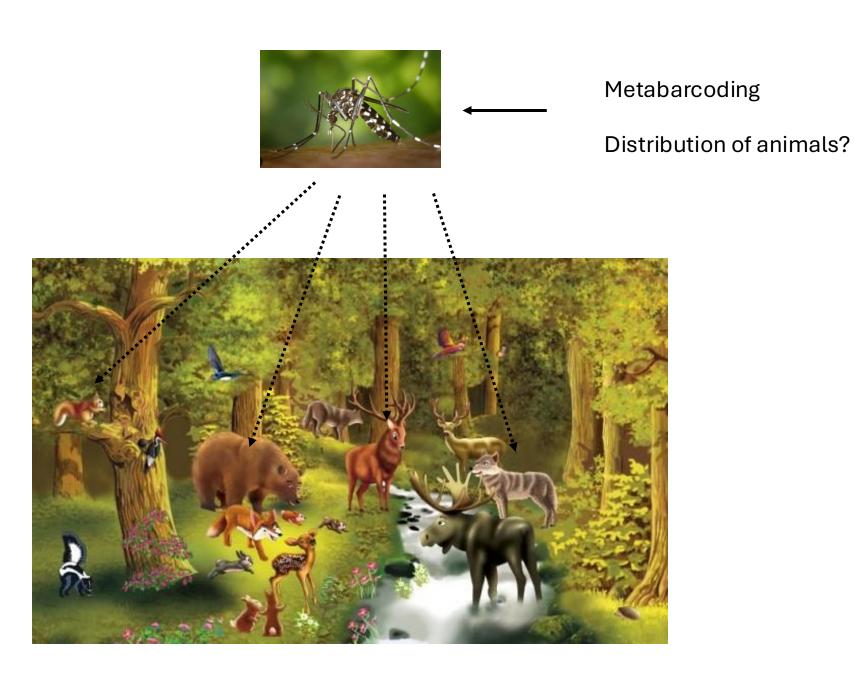


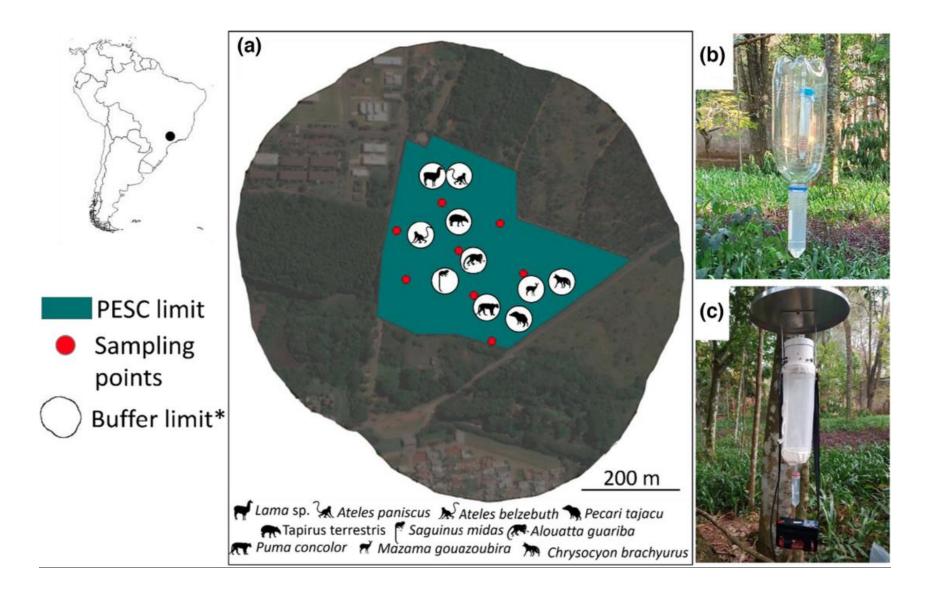


#### **Abstract**

Ingested-derived DNA (iDNA) from insects represents a powerful tool for assessing vertebrate diversity because insects are easy to sample, have a diverse diet and are Example 2







Parque Ecológico de São Carlos (Zoo and nature park)

# Hematophagous mosquito



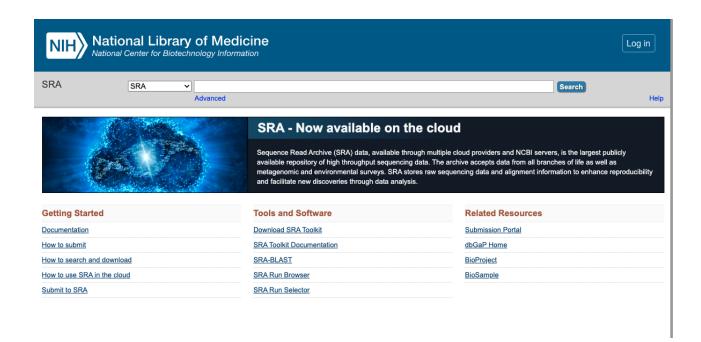
SRR25401090

Saprophagous fly



SRR25401082

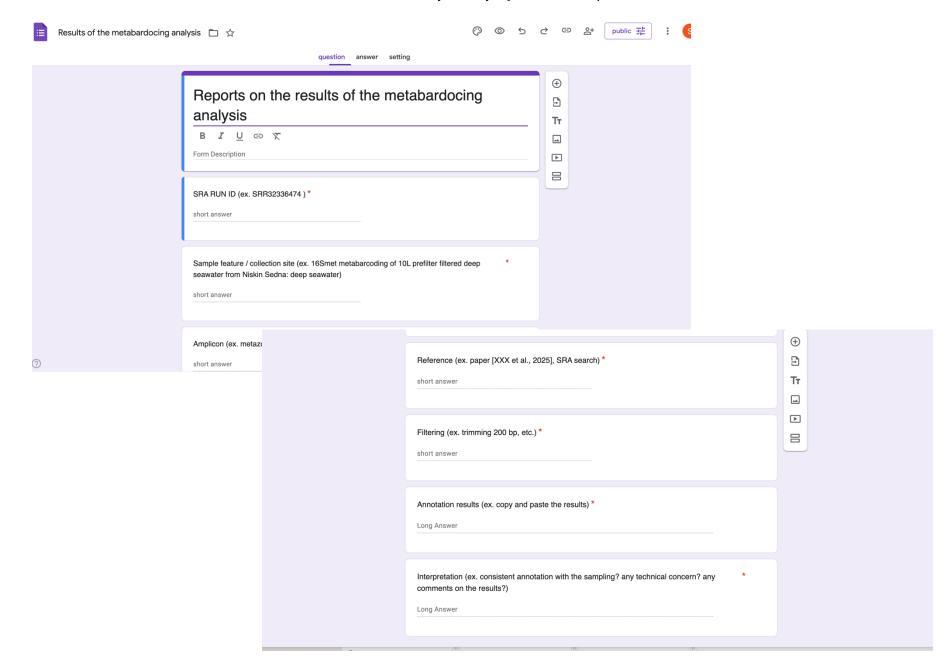
## **Analysis of public datasets**



Results							
Abandance: to 16 species wa	tal 31661 reads s identified						
Phylum	Class Speci	es blast_tophit	blast_ident	abunda	nce	seqs	
Arthropoda	Branchiopoda	Daphnia longispina	JN874595.1	97.521	128	seq63	
Arthropoda	Branchiopoda	Eubosmina cf. EU65068	5.1 98.3	347 67	seq93	12000000	
Arthropoda	Branchiopoda	Scapholeberis mucronata	EF189615.1	98.326	33	seq114	
Arthropoda	Branchiopoda	Simocephalus vetulus	LC382447.1	97.531	36	seq113	
Arthropoda	Insecta Cloeon	dipterum LC801945.1	96.680 43	seq109			
Bryozoa Phyla	ctolaemata Plumat	ella repens DQ30534	1.1 98.4	38 31	seq115		
Chordata	Actinopteri	Carassius auratus	DQ868870.1	99.674	59	seq101	
Chordata	Actinopteri	Gasterosteus aculeatus	DQ027919.1	99.340	135	seq59	
Chordata	Actinopteri	Leucaspius delineatus	NC_020357.1	99.342	68	seq92	
Chordata	Actinopteri	Pseudorasbora interrupt	a MN17	75390.1	99.342	5	seq125
Chordata	Amphibia	Bufo bufo JN64701	1.1 99.6	669 202	seq54,s	eq100	
Chordata	Amphibia	Pelophylax lessonae	MH105105.1	99.656	16	seq118	
Chordata	Amphibia				seq80		
Chordata	Aves Gallin	ula chloropus DQ48586	4.1 98.6	35 38	seq112		
Chordata	Mammalia	Myocastor coypus	AF422886.1	99.281	4	seq126	
Rotifera	Eurotatoria	Keratella quadrata	AF499046.1	99.010	4439	seq6,se	q8, seq18,

Amplicons need to be metazoan 16s rRNA for the customized scripts

# https://docs.google.com/forms/d/e/1FAlpQLSfTs3waEgm2DijM044nAUQhnMFUr2 luhOSFliwLmjz8MnwjGQ/viewform?usp=header



### Tasks:

(Remaining Day2) Sample6 and Sample7

# **Analysis of public datasets 1**

- example 1: deep sea samples
- example 2: mosquito/fry samples

### **Analysis of public datasets 2**

find and analyze the datasets in SRA (at least one)

-> Make a short report