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A reliable DNA barcode reference library for the identification of benthic invertebrates: essential for biomonitoring of the North Sea





EUROPEAN UNION

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GEANS: <u>Genetic tools for Ecosystem health Assessment in the</u> <u>North Sea region (https://northsearegion.eu/geans/;</u>@GEANS\_Interreg)

- Funding: EU InterregNorth Sea region
- Duration: 1 March 2019 –1 March 2022
- Budget: € 2.5 million
- Consortium: 9 partners
- Project coordinator: ILVO, Belgium

Objective: GEANS strives to implement DNA-based tools in routine monitoring programs in support of policy and decision making concerning ecosystem health.



#### **GEANS – Overview**



Benthic macroinvertebrates are key components in environmental impact assessments but their use as bioindicators can be constrained by the time- and cost-consuming processes needed for their morphological identification.

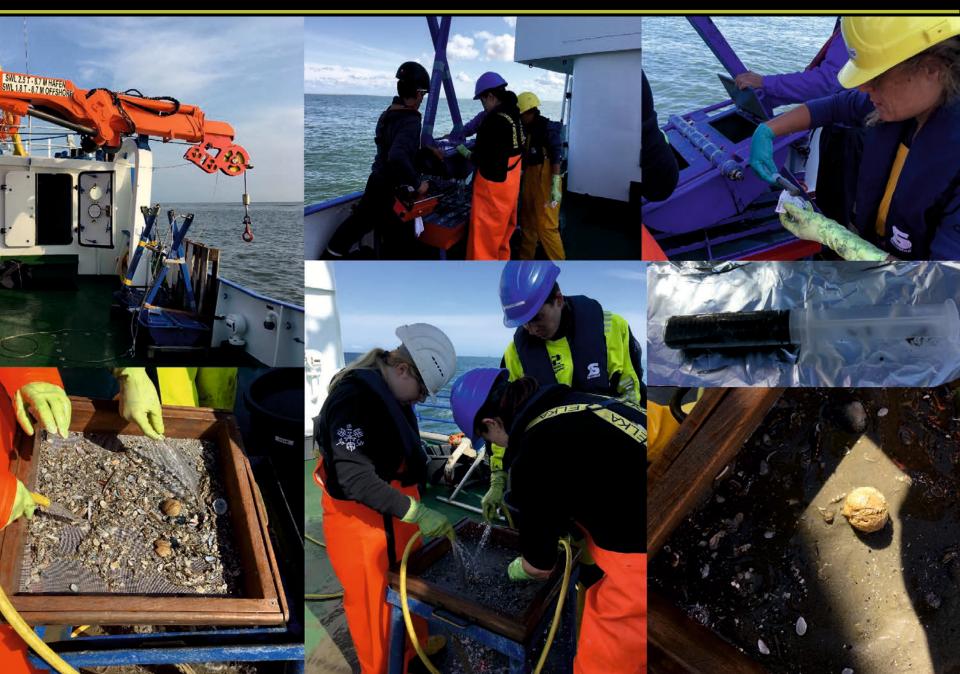


The recent advances in high-throughput sequencing (i.e. DNA metabarcoding) can provide an alternative to morphology-based approaches. The main limitation is the considerable investment needed to build high-quality and curated taxonomic reference DNA sequence libraries for species identification.

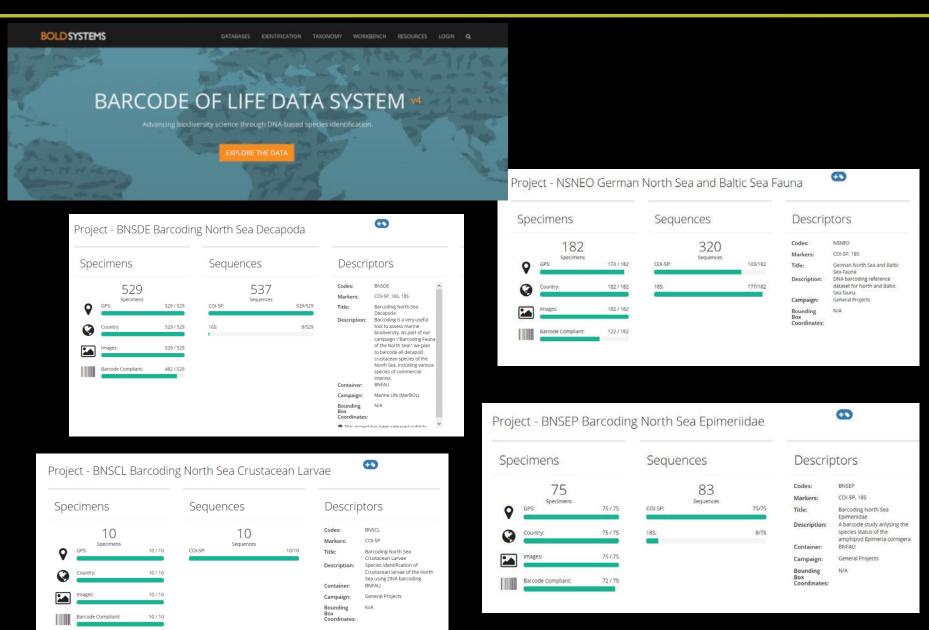
GEANS aims in developing a curated DNA reference library based on mitochondrial cytochrome c oxidase subunit I (COI) for the North Sea macrobenthos that will serve as the backbone of all the molecular protocols.

#### First Key list (V1): 800 species (96 NIS species)

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Abludomelita	obtusata	Arthropoda	Amphipoda	102788			ves	no	yes		no								1000		no ves	no	no	no	yes	no	yes	yes	no	+
Abra	alba	Mollusca	Bivalvia	141433			ves	yes	ves	no	ves	no				1					ves	no	SGN	no	yes		yes		no	+
Abra	nitida	Mollusca	Bivalvia	141435	12.00	ves	yes	yes	yes	10	ves		1.			12	1		1000		A.C.C.	no	SGN	no		no	yes	yes		+
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Acteon	tornatilis	Mollusca	Gastropoda	138691	yes	yes					yes	no	**				**	***			yes	no	SGN	no	no	no	yes	yes	no	+
Admete	viridula	Mollusca	Gastropoda	138969			**		-		**						no	no			no	no	no	no	no	no	yes	no	no	+
Acolidia	papillosa	Mollusca	Gastropoda	138709					yes	yes											yes	no	SGN	no	yes	no	yes	yes	no	+
Aequipecten	opercularis	Mollusca	Bivalvia	140687	17		yes	no	yes	yes	**						**				yes	no	SGN	no	no	no	yes	yes	no	+
Aglantha	digitale	Cnidaria	Hydrozoa	117849	**				yes	no				••			**		5.4	••	yes	no	SGN	no	yes	no	yes	yes	no	+
Alcyonidium	diaphanum	Bryozoa							yes	no									1.44		yes	no	no	no	no	no	no	no	no	+
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Alvania	cimicoides	Mollusca	Gastropoda	141168													no	no			no	no	no	no	no	no	no	no	no	+
Alvania	lactea	Mollusca	Gastropoda	141203			no	no											++		no	no	no	no	no	no	no	no	no	+
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Ampelisca	brevicornis	Arthropoda	Amphipoda	101891	yes	yes	yes	yes			yes	no					no	no	773		yes	yes	no	no	yes	no	yes	yes	no	
Ampelisca	diadema	Arthropoda	Amphipoda	101896							yes	no									yes	no	SGN	no	yes	no	yes	no	no	
Ampelisca	macrocephala	Arthropoda	Amphipoda	101908							yes	no					**		**		no	no	no	no	yes	no	yes	no	no	1
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Ampharete	baltica	Annelida	Polychaeta	129776	no	no					yes	no						100 C			no	no	no	no	no	no	no	no	no	
Ampharete	falcata	Annelida	Polychaeta	129777	no	no															no	no	no	no	yes	no	yes	yes	no	ſ
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Amphibalanus	improvisus	Arthropoda	Cirripedia	421139	yes	yes	99 99		yes	yes	44.5	442			no	no			no	no	yes	no	yes	no	yes	no	yes	no	yes	Γ
Amphibalanus	reticulatus	Arthropoda	Cirripedia	421140				-			-	144))	no	no			**				no	no	no	no	no	no	yes	yes	yes	
Amphictene	auricoma	Annelida	Polychaeta	152448	no	no					yes	no					no	no			no	no	no	no	no	no	no	no	no	
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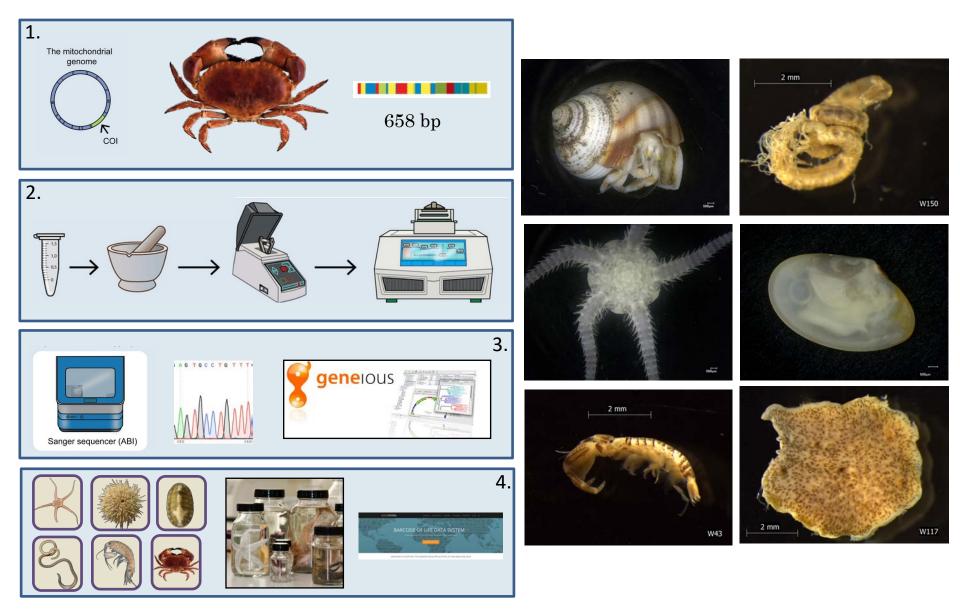


### **SGN Reference Species Libraries**



## **GEANS: DNA Sequence Reference Library**

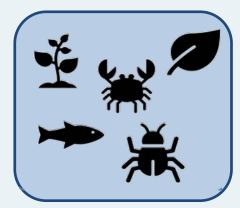
### Lab-Workflow



## **Reference Species Library – Curation process**

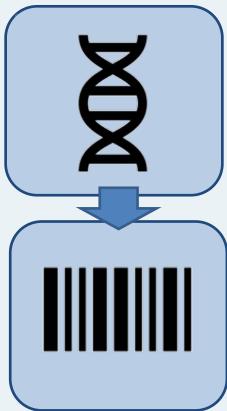
#### **Curation-Workflow**

- Specimens identified/checked by expert taxonomists
- Voucher deposition
- Accompanied specimen metadata deposition
- Barcode standards Sequence quality control





- Constant updating taxonomy (WORMS)
- Cross-referencing of data among institutes
- Tracked library versions
- Taq markers for sequences
- Curating new entries and control of old entries



### **Reference Species Library – Species page**

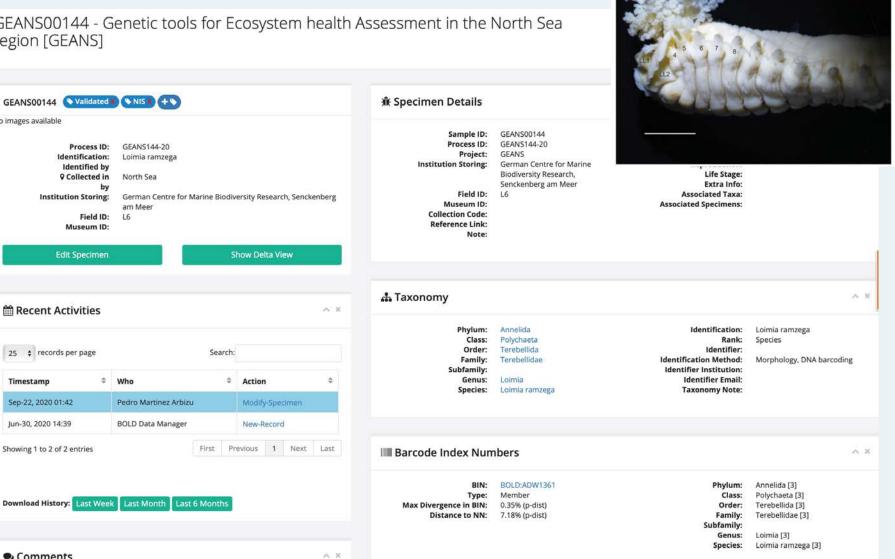
### Example: Pagurus bernhardus (Linnaeus, 1758)

GEANS00200 - Genetic tools for Ecosystem health region [GEANS]	Assessment in the North Sea	÷ ×	O Upload Traces	load Traces					Activity Report	to Show Delta View							
			Specimen Details Current			Marker Summar	y										
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## **Reference Species Library – Species page**

#### Example: Loimia ramzega Lavesque et al., 2017

GEANS00144 - Genetic tools for Ecosystem health Assessment in the North Sea region [GEANS]



No images available

Process ID:

Identification:

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Institution Storing:

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Museum ID:

Edit Specimen

Recent Activities

25 \$ records per page

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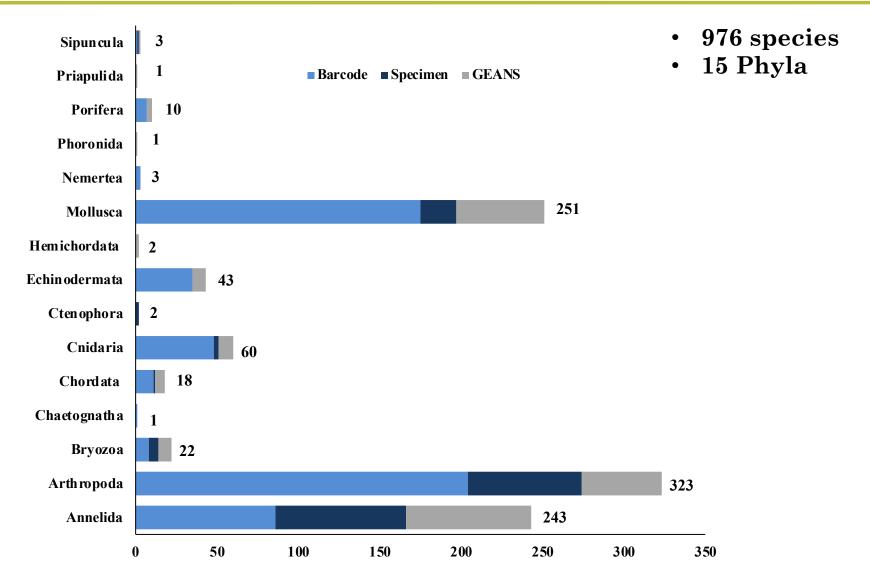
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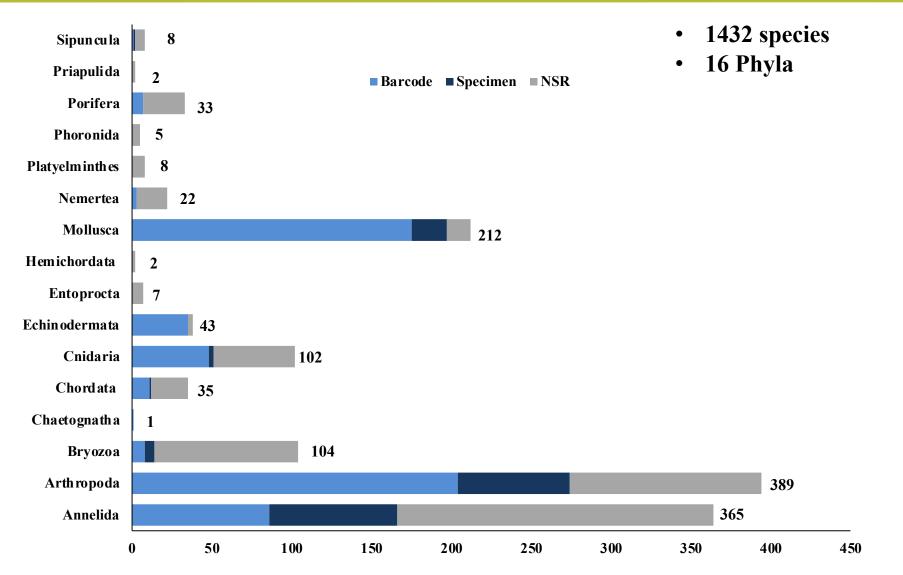
Showing 1 to 2 of 2 entries

#### Second Key list (V2): 976 species (112 NIS species)

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17	Abra	alba	Mollusca	Bivalvia	141433			yes	yes	yes	no	yes	no			22		**			: 44	yes	no	SGN		no	no	yes	yes	no	
	Abra	nitida		Bivalvia	141435		yes					yes	no	44	**			1440)			-	yes	no	SGN		no	no	yes	yes	no	
	Abra	prismatica		Bivalvia	141436	yes	yes	yes	no					1.55				no	no			yes	no	SGN		ycs	no	yes	yes	no	
	Acanthocardia	echinata	and the second se	Bivalvia	138992	yes	yes			yes	no	yes	no					no	no			yes	no	SGN	no	yes	no	yes	no	no	
10	Acanthocardia	paucicostata	Mollusca	Bivalvia	138993																									no	
11	Acanthodoris	pilosa	Mollusca	Gastropoda	140627		442 I	<u>92</u>	÷4 .	yes	yes	++		**	144		++	++		-	44	yes	no	SGN	no	yes	no	yes	yes	no	
	Achelia	echinata	Arthropoda	Pycnogonida	134599				**	yes	no			-	-		122					yes	no	no	no	no	no	yes	yes	no	
13	Acidostoma	obesum	Arthropoda	Amphipoda	102497		÷ (	no	no		-	yes	no	-	-	-	-		-	-		no	no	no	no	yes	no	no	no	no	
14	Acrocnida	brachiata	Echinodermata	Ophiuroidea	236130			yes	no		++		447					no	no			yes	no	SGN	no	no	no	yes	yes	no	
15	Acteon	tornatilis	Mollusca	Gastropoda	138691	yes	yes				++ 1	yes	no									yes	no	SGN	no	no	no	yes	yes	no	
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	Aglantha	digitale			117849					ves	no											ves	no	SGN	no	yes	no	yes	yes	no	
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31	Alvania	cimicoides	Mollusca	Gastronoda	141168	8				103	yes											708	10	10	no	no	по	no	10	no	
32	Alvania		Mollusca	Gastropoda	141203				-														20				10	110			
33	Amacana	tricipata	Annelida	Polychaeta	131471													100	-				10	no	no	110	10				
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	Ampelisca	brevicornis	Arthropoda	Amphipoda	101891		A	yes	yes		**	yes	no					no	no			yes	yes	no	no	yes	no	yes	yes	no	
	Ampelisca	diadema		Amphipoda	101896	1.					**	yes	no	**			() and	***			194	yes	no	SGN	no	yes	no	yes	no	no	
	Ampelisca	macrocephala		Amphipoda	101908	1.T	1992 - S				182	yes	no	100	199	100	1411	10.	199	1993	**	no	no	no	no	yes	no	yes	no	no	
39	Ampelisca	typica	Arthropoda	Amphipuda	101933		-	110	110					-	- 10	- 10		-		- 10		no	10	110	10	3.62	10	1.4.8	.00	.00	
a state of the sta	Ampelisca	spinipes	Arthropoda	Amphipoda	101928																									no	
	Ampelisca	tenuicornis		Amphipoda		yes	yes					yes	no	**				no	no			yes	no	SGN	no	yes	no	yes	no	no	
42	Ampharete	indstroomi	Annelida	Polychaeta	129781	and a	-	-	and the second s	and a second		-	-	-	144		-	80	.110	- 112		no	80	no.	no.	no.	80	1200	no	Teo	
43	Ampharete	acutifrons	Annelida	Polychaeta	129775	100	<b>22</b> 2		22			yes	по		-	100	100		100	100	-	no	no	no	no	no	no	no	no	no	



Cumulative barcode and voucher specimen coverage of marine macrobenthic species in the GEANS DNA reference library. Numbers on bars refer to total number of species in GEANS checklist.

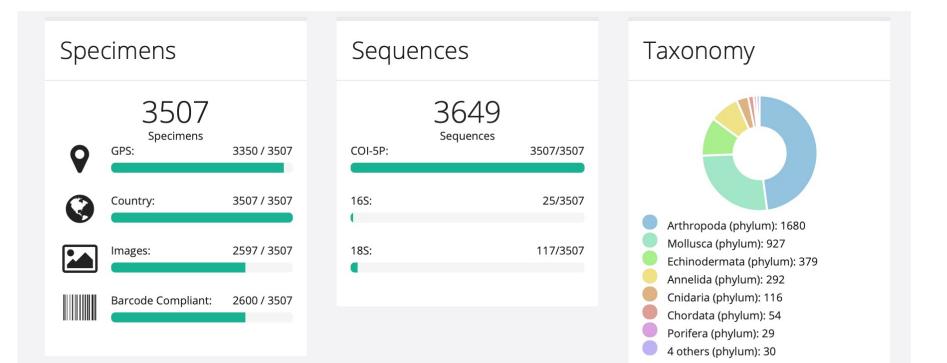


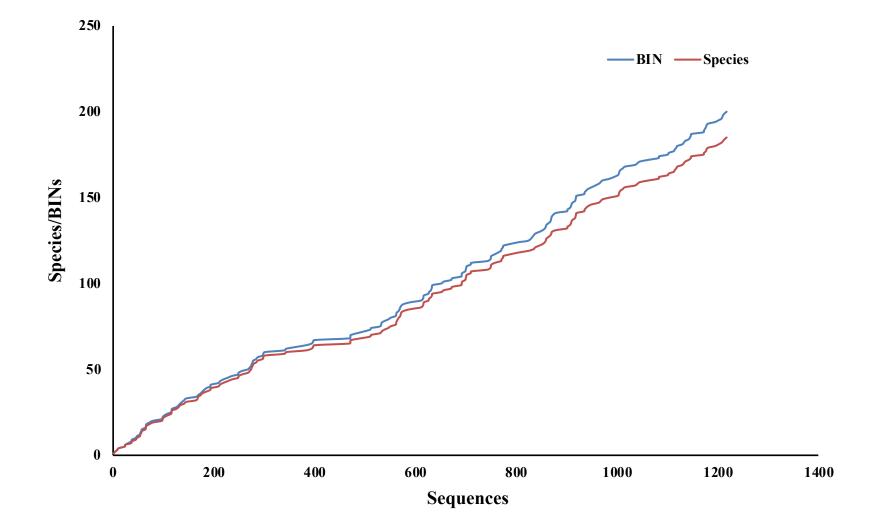
Cumulative barcode and voucher specimen coverage of marine macrobenthic species in the GEANS DNA reference library in relation to the North Sea Region. Numbers on bars refer to total number of species in DE-NSR checklist.

#### Dataset – DS-GEANS1 GEANS Reference Library

- 3507 specimens
- 592 species (613 BINs)
- 11 Phyla
- 3507 COI sequences







Accumulation curve for morphological species and barcode index numbers (BINs)

### In conclusive

- Currently GEANS Reference Library holds 3507 COI sequences belonging to 592 species (613 BINs).
- Once completed the GEANS reference library will be the first wellcurated DNA-library covering all the spectrum of macrobenthic animals in the North Sea.
  - Once available, this DNA-library will support the implementation of fast, cost-efficient and reliable DNA-based identifications and subsequently environmental health assessments in the North Sea.

Be a part of this effort: By offering voucher specimens!! (magdalini.christodoulou@senckenberg.de)





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Thank you!