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

Introducing GEANS



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



Cefas



AARHUS UNIVERSITY

SEANALYTICS AB

Species and Ecosystem Analyses



NORD
University

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VLIZ



WAGENINGEN
UNIVERSITY & RESEARCH



Naturalis
Biodiversity
Center

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.

GEANS Reference Species Library

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

| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y | Z | AA | AB | AC | AD | AE | | |
|----|---------------|--------------|---------------|--------------|--------|------------------------|------------|------------|------------|------------|------------|------------|------------|------------|-----|-----|-----|-----|-----|-------|-----|-----------|-----|------|-----|---------|-----|----------|-----|-----|-----|-----|----|
| 1 | Taxonomy | | | | | Material and Sequences | | | | | | | | | | | | | | GEANS | | part-BOLD | | BOLD | | Genbank | | Invasive | | | | | |
| 2 | Genus | Species | Phylum | Class/Order | ApbiID | SGN | ILVO | Naturalis | AU | Cefas | VLIZ | SeAnalytic | s AB | NU | COI | 18S | COI | 18S | COI | 18S | COI | 18S | COI | 18S | COI | 18S | COI | 18S | | | | | |
| 3 | | | | | | Spec. Seq. | Spec. Seq. | Spec. Seq. | Spec. Seq. | Spec. Seq. | Spec. Seq. | Spec. Seq. | Spec. Seq. | Spec. Seq. | | | | | | | | | | | | | | | | | | | |
| 4 | Abietinaria | abietina | Cnidaria | Hydrozoa | 117870 | no | no | no | no | yes | no | no | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | no | no | no | no | yes | no | yes | no | yes | yes | no |
| 5 | Abludomelita | obtusata | Arthropoda | Amphipoda | 102788 | no | no | yes | yes | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | no | no | yes | no | yes | no | yes | no | no | |
| 6 | Abra | alba | Mollusca | Bivalvia | 141433 | yes | yes | yes | yes | no | yes | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | SGN | no | no | no | yes | yes | yes | no | no | |
| 7 | Abra | nitida | Mollusca | Bivalvia | 141435 | yes | yes | -- | -- | -- | yes | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | SGN | no | no | no | yes | yes | no | no | no | |
| 8 | Abra | prismatica | Mollusca | Bivalvia | 141436 | yes | yes | yes | no | -- | -- | -- | -- | -- | no | no | -- | -- | -- | -- | -- | yes | no | SGN | no | yes | no | yes | yes | no | no | no | |
| 9 | Acanthocardia | echinata | Mollusca | Bivalvia | 138992 | yes | yes | -- | -- | yes | no | yes | no | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | SGN | no | yes | no | yes | no | yes | no | no | |
| 10 | Acanthodoris | pilosa | Mollusca | Gastropoda | 140627 | -- | -- | -- | -- | yes | yes | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | SGN | no | yes | no | yes | yes | no | no | no | |
| 11 | Achelia | echinata | Arthropoda | Pycnogonida | 134599 | -- | -- | -- | -- | yes | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | no | no | no | no | yes | yes | no | no | no | |
| 12 | Acidostoma | obesum | Arthropoda | Amphipoda | 102497 | -- | -- | no | no | -- | -- | yes | no | -- | -- | -- | -- | -- | -- | -- | -- | no | no | no | no | yes | no | no | no | no | no | no | |
| 13 | Acrocnida | brachiata | Echinodermata | Ophiuroidea | 236130 | -- | -- | yes | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | SGN | no | no | no | yes | yes | no | no | no | |
| 14 | Acteon | tornatis | Mollusca | Gastropoda | 138691 | yes | yes | -- | -- | -- | yes | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | SGN | no | no | no | yes | yes | no | no | no | |
| 15 | Admete | viridula | Mollusca | Gastropoda | 138969 | -- | -- | -- | -- | -- | -- | -- | -- | -- | no | no | -- | -- | -- | -- | -- | no | no | no | no | no | no | yes | no | no | no | no | |
| 16 | Aeolidia | papillosa | Mollusca | Gastropoda | 138709 | -- | -- | -- | -- | yes | yes | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | SGN | no | yes | no | yes | yes | no | no | no | |
| 17 | Aequipecten | opercularis | Mollusca | Bivalvia | 140687 | -- | -- | yes | no | yes | yes | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | SGN | no | no | no | yes | yes | no | no | no | |
| 18 | Aglantha | digitale | Cnidaria | Hydrozoa | 117849 | -- | -- | -- | -- | yes | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | SGN | no | yes | no | yes | yes | no | no | no | |
| 19 | Alcyonidium | diaphanum | Bryozoa | Gymnolaemata | 111597 | -- | -- | -- | -- | yes | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | no | no | no | no | no | no | no | no | no | |
| 20 | Alcyonidium | gelatinosum | Bryozoa | Gymnolaemata | 111600 | -- | -- | -- | -- | yes | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | no | no | no | no | no | no | no | yes | no | no | no | |
| 21 | Alcyonium | digitatum | Cnidaria | Anthozoa | 125333 | -- | -- | no | no | yes | yes | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | no | no | yes | no | yes | yes | no | no | no | |
| 22 | Alitta | succinea | Annelida | Polychaeta | 234850 | yes | yes | -- | -- | yes | yes | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | no | no | yes | no | yes | yes | no | no | no | |
| 23 | Alitta | virens | Annelida | Polychaeta | 234851 | yes | no | -- | -- | yes | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | no | no | no | no | yes | no | yes | yes | yes | no | no | |
| 24 | Alloteuthis | subulata | Mollusca | Cephalopoda | 153131 | -- | -- | -- | -- | yes | yes | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | no | no | no | no | yes | no | no | no | no | |
| 25 | Alvania | cimicoides | Mollusca | Gastropoda | 141168 | -- | -- | -- | -- | -- | -- | -- | -- | -- | no | no | -- | -- | -- | -- | -- | no | no | no | no | no | no | no | no | no | no | no | |
| 26 | Alvania | lactea | Mollusca | Gastropoda | 141203 | -- | -- | no | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | no | no | no | no | no | no | no | no | no | no | no | no |
| 27 | Amacea | trilobata | Annelida | Polychaeta | 131471 | -- | -- | -- | -- | -- | -- | -- | -- | -- | no | no | -- | -- | -- | -- | -- | no | no | no | no | no | no | yes | no | no | no | no | |
| 28 | Amage | auricula | Annelida | Polychaeta | 129771 | -- | -- | -- | -- | -- | -- | -- | -- | -- | no | no | -- | -- | -- | -- | -- | no | no | no | no | no | no | yes | no | no | no | no | |
| 29 | Ammonothea | hilgendorfi | Arthropoda | Pycnogonida | 134607 | -- | -- | -- | -- | yes | yes | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | no | no | yes | no | yes | yes | yes | yes | no | |
| 30 | Ampelisca | brevicornis | Arthropoda | Amphipoda | 101891 | yes | yes | yes | yes | -- | yes | no | -- | -- | no | no | -- | -- | -- | -- | -- | yes | yes | no | no | yes | no | yes | yes | no | no | no | |
| 31 | Ampelisca | diadema | Arthropoda | Amphipoda | 101896 | -- | -- | -- | -- | -- | yes | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | SGN | no | yes | no | yes | no | no | no | no | |
| 32 | Ampelisca | macrocephala | Arthropoda | Amphipoda | 101908 | -- | -- | -- | -- | -- | yes | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | no | no | no | no | yes | no | yes | no | no | no | no | |
| 33 | Ampelisca | typica | Arthropoda | Amphipoda | 101933 | -- | -- | no | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | no | no | no | no | yes | no | yes | no | no | no | no | |
| 34 | Ampelisca | tenuicornis | Arthropoda | Amphipoda | 101930 | yes | yes | -- | -- | -- | yes | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | yes | no | SGN | no | yes | no | yes | no | no | no | no | |
| 35 | Ampharete | lindstroemi | Annelida | Polychaeta | 129781 | -- | -- | -- | -- | -- | -- | -- | -- | -- | no | no | -- | -- | -- | -- | -- | no | no | no | no | no | no | no | no | no | no | no | |
| 36 | Ampharete | acutifrons | Annelida | Polychaeta | 129775 | -- | -- | -- | -- | -- | yes | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | no | no | no | no | no | no | no | no | no | no | no | |
| 37 | Ampharete | baltica | Annelida | Polychaeta | 129776 | no | no | -- | -- | -- | yes | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | no | no | no | no | no | no | no | no | no | no | no | |
| 38 | Ampharete | falcata | Annelida | Polychaeta | 129777 | no | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | no | no | no | no | yes | no | yes | yes | no | no | no | |
| 39 | Ampharete | finmarchica | Annelida | Polychaeta | 129778 | no | no | -- | -- | -- | yes | no | -- | -- | -- | -- | -- | -- | -- | -- | -- | no | no | no | no | no | no | no | yes | yes | no | no | no |
| 40 | Amphibalanus | amphitrite | Arthropoda | Cirripedia | 421137 | -- | -- | -- | -- | -- | -- | no | no | no | no | -- | -- | -- | -- | -- | -- | no | no | no | no | yes | no | yes | yes | yes | no | no | |
| 41 | Amphibalanus | improvisus | Arthropoda | Cirripedia | 421139 | yes | yes | -- | -- | yes | yes | -- | -- | -- | no | no | -- | -- | -- | -- | -- | yes | no | yes | no | yes | no | yes | no | yes | yes | no | |
| 42 | Amphibalanus | reticulatus | Arthropoda | Cirripedia | 421140 | -- | -- | -- | -- | -- | -- | no | no | -- | -- | -- | -- | -- | -- | -- | -- | no | no | no | no | no | no | yes | yes | yes | yes | no | |
| 43 | Amphictene | auricoma | Annelida | Polychaeta | 152448 | no | no | -- | -- | -- | yes | no | -- | -- | no | no | -- | -- | -- | -- | -- | no | no | no | no | no | no | no | no | no | no | no | |
| 44 | Amphipolis | norvegica | Echinodermata | Onchiuroidea | 125057 | -- | -- | -- | -- | -- | -- | -- | -- | -- | no | no | -- | -- | -- | -- | -- | no | no | no | no | yes | no | yes | yes | no | no | no | |

GEANS Reference Species Library



SGN Reference Species Libraries

BOLD SYSTEMS DATABASES IDENTIFICATION TAXONOMY WORKBENCH RESOURCES LOGIN

BARCODE OF LIFE DATA SYSTEM v4

Advancing biodiversity science through DNA-based species identification.

[EXPLORE THE DATA](#)

Project - BNSDE Barcoding North Sea Decapoda

Specimens

529
Specimens

GPS: 529 / 529

Country: 529 / 529

Images: 529 / 529

Barcode Compliant: 482 / 529

Sequences

537
Sequences

COI-SP: 529/529

16S: 8/529

Descriptors

Codes: BNSDE
Markers: COI-SP, 16S, 18S
Title: Barcoding North Sea Decapoda
Description: Barcoding is a very useful tool to assess marine biodiversity. As part of our campaign "Barcoding Fauna of the North Sea" we plan to barcode all decapod crustacean species of the North Sea, including various species of commercial interest.
Container: BNFAU
Campaign: Marine Life (MarBOL)
Bounding Box Coordinates: N/A

Project - NSNEO German North Sea and Baltic Sea Fauna

Specimens

182
Specimens

GPS: 174 / 182

Country: 182 / 182

Images: 182 / 182

Barcode Compliant: 122 / 182

Sequences

320
Sequences

COI-SP: 143/182

16S: 177/182

Descriptors

Codes: NSNEO
Markers: COI-SP, 18S
Title: German North Sea and Baltic Sea Fauna
Description: DNA barcoding reference dataset for North and Baltic Sea fauna
Campaign: General Projects
Bounding Box Coordinates: N/A

Project - BNSCL Barcoding North Sea Crustacean Larvae

Specimens

10
Specimens

GPS: 10 / 10

Country: 10 / 10

Images: 10 / 10

Barcode Compliant: 10 / 10

Sequences

10
Sequences

COI-SP: 10/10

Descriptors

Codes: BNSCL
Markers: COI-SP
Title: Barcoding North Sea Crustacean Larvae
Description: Species identification of Crustacean larvae of the North Sea using DNA barcoding
Container: BNFAU
Campaign: General Projects
Bounding Box Coordinates: N/A

Project - BNSEP Barcoding North Sea Epimeriidae

Specimens

75
Specimens

GPS: 75 / 75

Country: 75 / 75

Images: 75 / 75

Barcode Compliant: 72 / 75

Sequences

83
Sequences

COI-SP: 75/75

16S: 8/75

Descriptors

Codes: BNSEP
Markers: COI-SP, 18S
Title: Barcoding North Sea Epimeriidae
Description: A barcode study analysing the species status of the amphipod Epimeria corrigeria
Container: BNFAU
Campaign: General Projects
Bounding Box Coordinates: N/A

GEANS: DNA Sequence Reference Library

Lab-Workflow

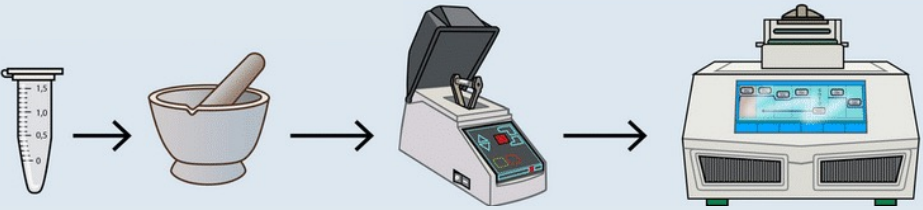
1.

The mitochondrial genome


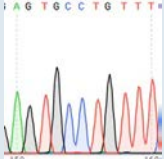



658 bp

2.

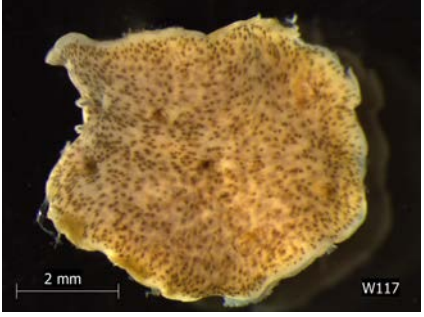


3.



Sanger sequencer (ABI)

4.



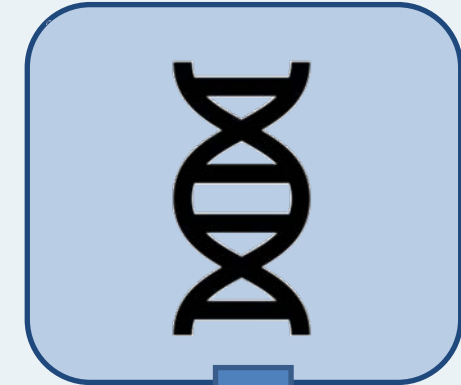
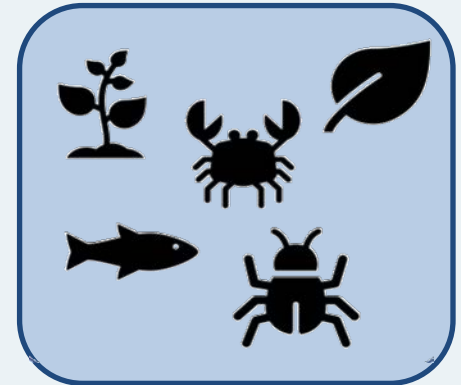
Reference Species Library – Curation process

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: *Loimia ramzega* Lavesque et al., 2017

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

GEANS00144 Validated NIS

No images available

Process ID: GEANS144-20
Identification: *Loimia ramzega*
Identified by
Collected in North Sea
by
Institution Storing: German Centre for Marine Biodiversity Research, Senckenberg am Meer
Field ID: L6
Museum ID:

Edit Specimen

Show Delta View

Specimen Details

Sample ID: GEANS00144
Process ID: GEANS144-20
Project: GEANS
Institution Storing: German Centre for Marine Biodiversity Research, Senckenberg am Meer
Field ID: L6
Museum ID:
Collection Code:
Reference Link:
Note:



Life Stage:
Extra Info:
Associated Taxa:
Associated Specimens:

Recent Activities

25 records per page

Search:

| Timestamp | Who | Action |
|--------------------|-----------------------|-----------------|
| Sep-22, 2020 01:42 | Pedro Martinez Arbizu | Modify-Specimen |
| Jun-30, 2020 14:39 | BOLD Data Manager | New-Record |

Showing 1 to 2 of 2 entries

First Previous 1 Next Last

Download History: Last Week Last Month Last 6 Months

Taxonomy

Phylum: Annelida
Class: Polychaeta
Order: Terebellida
Family: Terebellidae
Subfamily:
Genus: *Loimia*
Species: *Loimia ramzega*

Identification: *Loimia ramzega*
Rank: Species
Identifier:
Identification Method: Morphology, DNA barcoding
Identifier Institution:
Identifier Email:
Taxonomy Note:

Barcode Index Numbers

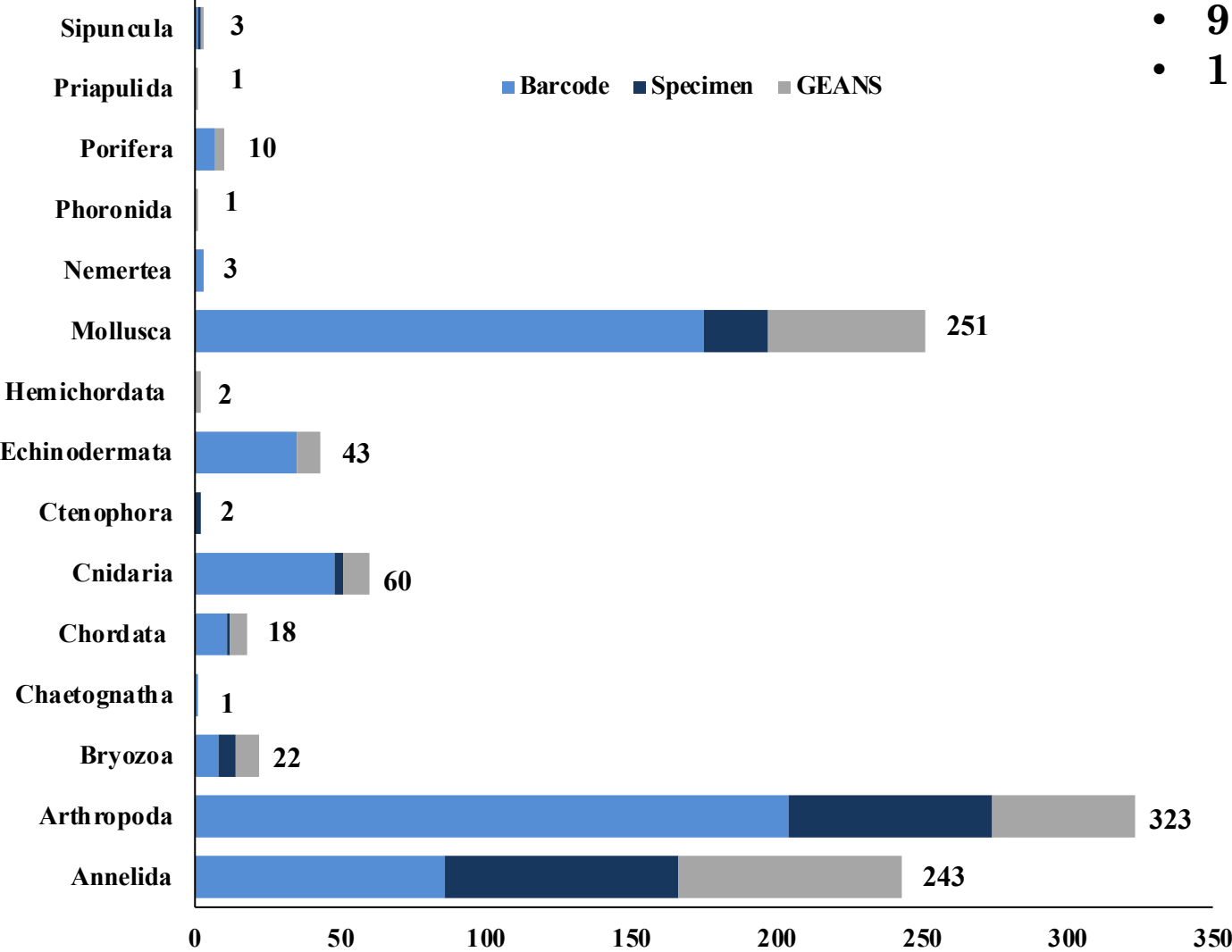
BIN: BOLD:ADW1361
Type: Member
Max Divergence in BIN: 0.35% (p-dist)
Distance to NN: 7.18% (p-dist)

Phylum: Annelida [3]
Class: Polychaeta [3]
Order: Terebellida [3]
Family: Terebellidae [3]
Subfamily:
Genus: *Loimia* [3]
Species: *Loimia ramzega* [3]

Comments

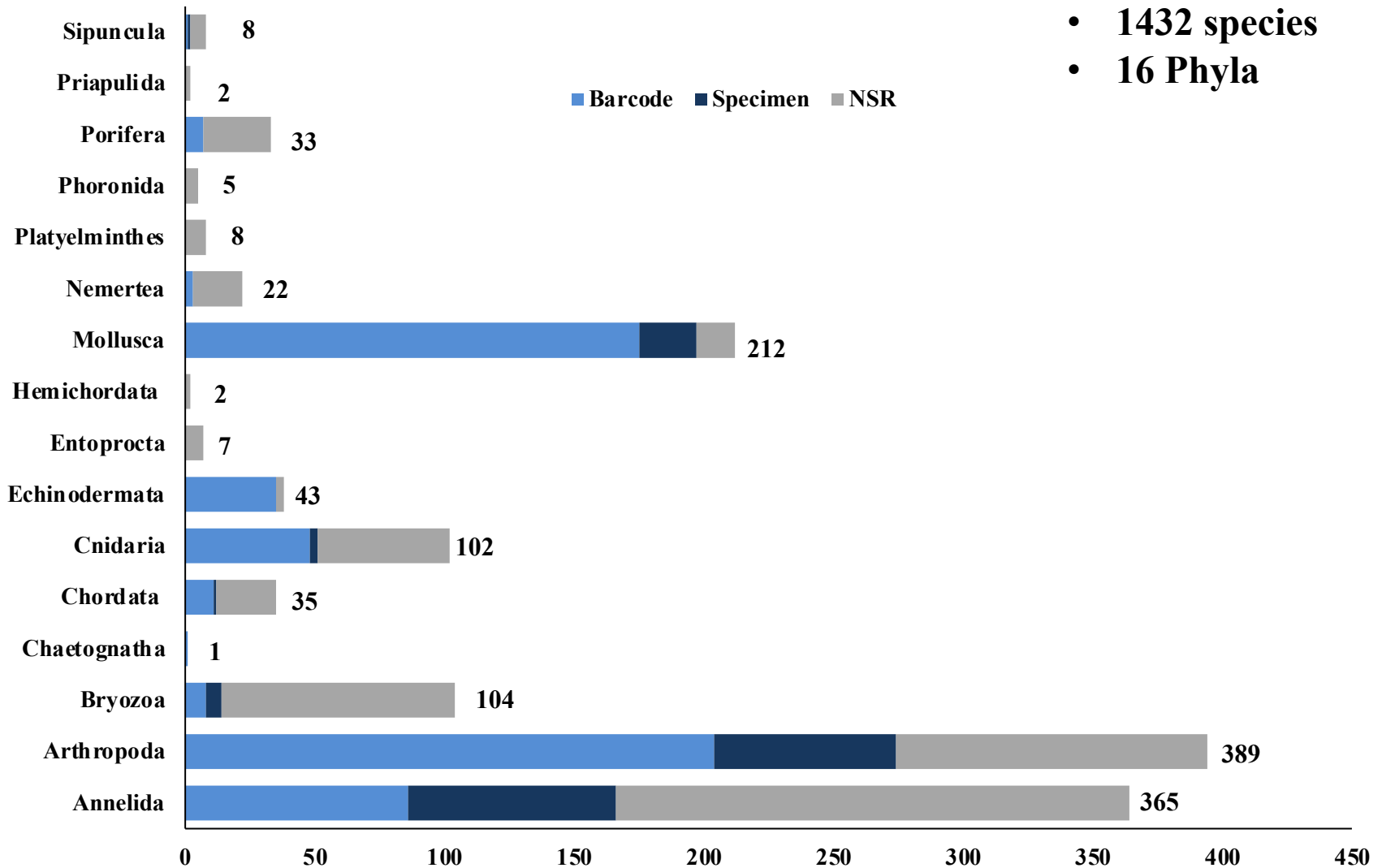
GEANS Reference Species Library

- 976 species
- 15 Phyla



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.

GEANS Reference Species Library



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.

GEANS Reference Species Library

Dataset – DS-GEANS1 GEANS Reference Library

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



Specimens

3507

Specimens



GPS: 3350 / 3507



Country: 3507 / 3507



Images: 2597 / 3507



Barcode Compliant: 2600 / 3507



Sequences

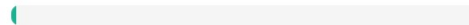
3649

Sequences

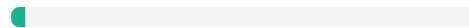
COI-5P: 3507/3507



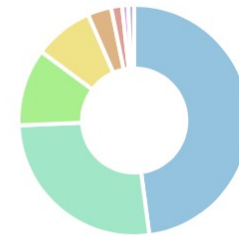
16S: 25/3507



18S: 117/3507

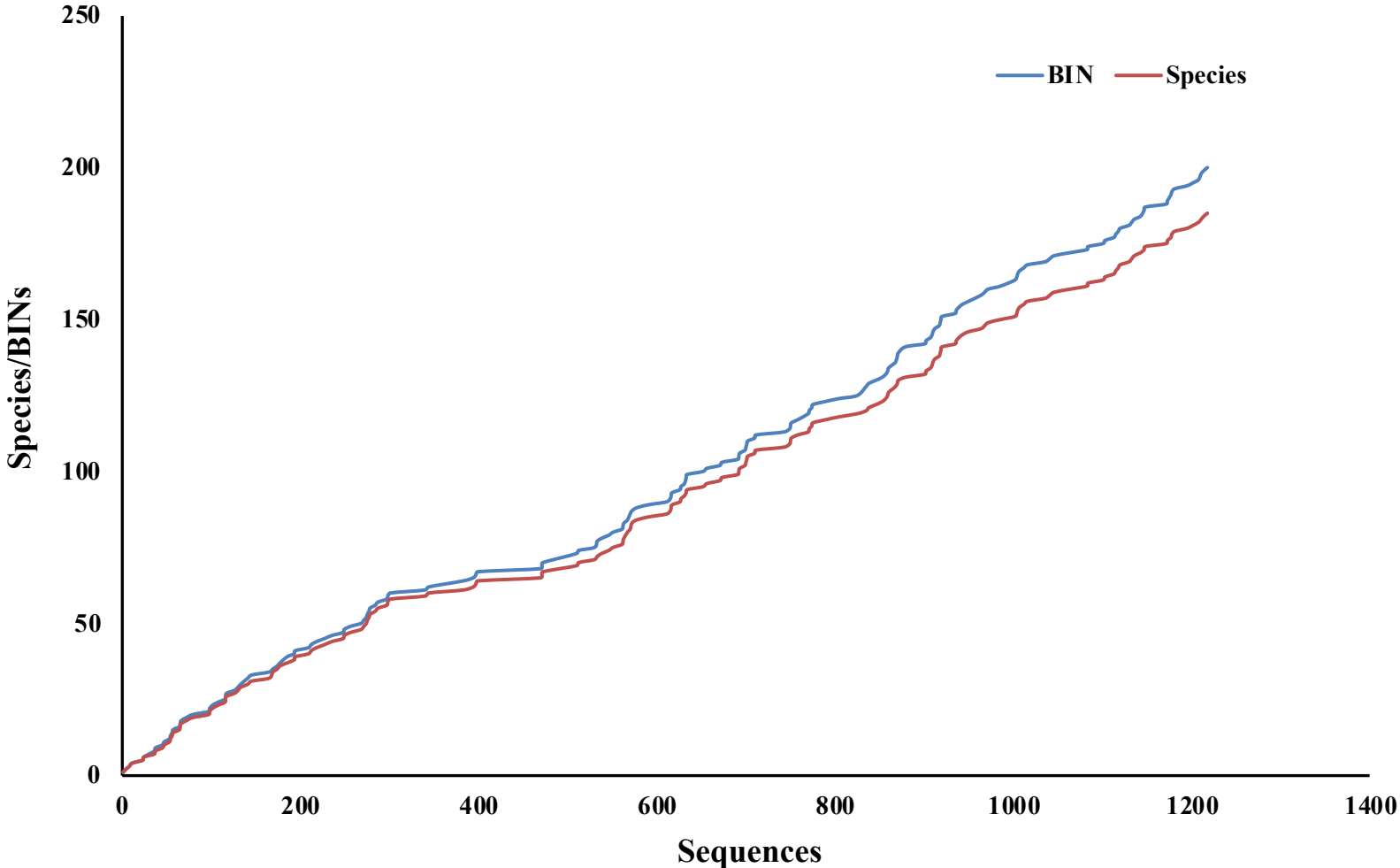


Taxonomy



- Arthropoda (phylum): 1680
- Mollusca (phylum): 927
- Echinodermata (phylum): 379
- Annelida (phylum): 292
- Cnidaria (phylum): 116
- Chordata (phylum): 54
- Porifera (phylum): 29
- 4 others (phylum): 30

GEANS Reference Species Library



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 well-curated 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)**



Thank you!