

A DNA barcode reference library for the superorder Peracarida (Crustacea) from European coasts

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ABSTRACT

Background: The superorder Peracarida is a highly diverse crustacean taxon, comprising numerous prominent members in European coastal areas' communities and ecosystems. Here we present a core DNA barcode reference library for marine European superorder Peracarida, comprising specimens from Black and Mediterranean Seas and from Northeast Atlantic coasts, ranging from Iberian Peninsula to Scandinavia, including Azores, Iceland and British Islands.

Results: A total of 953 DNA barcodes were compiled in a BOLD dataset, with 220 new DNA barcodes. The dataset included specimens of the orders Amphipoda (67.2%), Cumacea (1.6%), Isopoda (25.3%), Mysida (4.1%), and Tanaidacea (1.8%). In total, 176 peracaridean morphospecies were assigned to 205 BINs in BOLD, with 163 (92.6%) represented by single BINs, comprising specimens collected from geographically distant populations, up to approximately 3500 km in the most extreme cases (e.g. *Idotea granulosa* from Azores, Portugal, Iceland, Scotland, North Sea and Norway). The remaining thirteen morphospecies, belonging to Amphipoda and Isopoda, split between two to six BINs each, and had maximum intraspecific genetic distances between 3 to 25%. All multiple intraspecific BINs were allopatric, although the geographic distance between members of each BIN lineage ranged from 60 km up to 3000 km. Major splits were detected between upper north and south regions of the NE Atlantic, between Atlantic and the Mediterranean Sea, or sometimes even within countries. The most striking case was revealed for the isopod *Janira maculosa*, which split into six BINs (maximum intraspecific distance 25.16%).

Significance: The high percentage of morphospecies matching unique BINs (92.6%) shows the good reliability of our DNA barcode's reference library. However, the presence of deeply divergent intraspecific lineages suggests the presence of considerable overlooked taxonomic diversity. These findings indicate the need for a comprehensive revision and DNA barcode-based screening of the peracaridean fauna from the European coasts.

BACKGROUND

- Peracarida is a Superorder of the subphylum Crustacea¹.
- Among the most ecologically important marine benthic invertebrates¹.
- The main goal is to compile a core reference library for shallow water Peracarida from Europe.
- Orders Amphipoda, Isopoda, Tanaidacea, Mysida and Cumacea used.

METHODOLOGY

- DNA barcodes from 220 individuals obtained using publishing protocols².
- Peracaridean COI sequences available on BOLDsystems (accessed May 2017) added.
- Only barcodes with a minimum length of 500 base pairs, without stop codons, from marine or estuarine coasts, and with the indication of the respective source were used.
- Area: Black and Mediterranean Seas and NE Atlantic.
- NJ phenogram was constructed applying the K2P model.
- Mean and Maximum (Max) Intraspecific distances (ISD) were calculated using K2P distances.

RESULTS

- A total of 953 DNA barcodes were compiled, 67.2% of which from Amphipoda.
- 176 peracaridean morphospecies were assigned to 205 BINs.
- Most morphospecies (92.6%) represented by single BINs.
- Thirteen morphospecies split in several BINs each (total 33), and had maximum intraspecific genetic distances between 3 to 25% (Figure 1, Table 1).
- All multiple intraspecific BINs were allopatric.
- Major splits were detected between upper north and south regions of the NE Atlantic, between Atlantic and the Mediterranean Sea or within countries.
- The isopod *Janira maculosa* displayed the highest max ISD Ref(25.16%) and highest number of BINs (6, Table 1).

REFERENCES

- ¹Cunha MR et al. (1997) On the structure of the neritic suprabenthic communities from the Portuguese continental margin. *Marine Ecology Progress Series* 157: 119–137.
- ²Lobo J et al. (2013) Enhanced primers for amplification of DNA barcodes from a broad range of marine metazoans. *BMC Ecology* 13: 34.

Order	Species	Mean ISD (K2P)	Max ISD (K2P)	Number of BINs
Amphipoda	<i>Ampelisca brevicornis</i>	4.53	6.6	2
Amphipoda	<i>Ampelisca spinipes</i>	20.53	20.53	2
Amphipoda	<i>Ampelisca typica</i>	13.65	18.61	3
Amphipoda	<i>Amphithoe rubricata</i>	12.2	24.76	3
Amphipoda	<i>Corophium multisetosum</i>	9.77	17.8	2
Amphipoda	<i>Dexamine spinosa</i>	16.76	24.06	3
Amphipoda	<i>Gammarella fucicola</i>	9.41	18.65	2
Amphipoda	<i>Jassa pusilla</i>	14.74	22.35	2
Amphipoda	<i>Phtisica marina</i>	2.34	3.83	2
Isopoda	<i>Astacilla intermedia</i>	3.17	4.76	2
Isopoda	<i>Janira maculosa</i>	14.77	25.16	6
Isopoda	<i>Ligia oceanica</i>	2.58	4.84	2
Isopoda	<i>Sphaeroma serratum</i>	5.78	5.78	2

Table 1. Mean and maximum Intraspecific distance (K2P) in morphospecies with more than one BIN.

Max Intra-Specific vs Nearest Neighbour

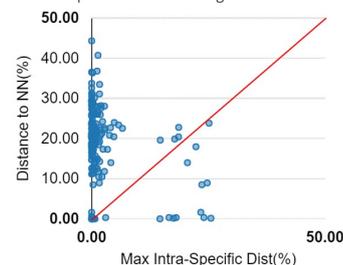


Figure 1. Scatterplot representing for species the minimum distance to Nearest Neighbour (NN) plotted against the maximum intra-specific distance.

SIGNIFICANCE

- The high percentage of morphospecies matching unique BINs confirms the typical pattern of COI variability observed in numerous crustaceans species³.
- Globally, the high proportion of congruent results indicate the reliability of DNA barcodes for Peracarid identification.
- As much as 20 putative new species detected in the well-studied peracaridean fauna of the NE Atlantic (increase of about 11 % in the number of species here examined).
- The reference library is still very incomplete; much more effort needed for its completion and to proceed with the revision and DNA barcode-based probation of the peracaridean fauna from the European coasts.
- A core reference library of marine shallow water peracarids available for metabarcoding-based biomonitoring in Europe⁴.

³Costa FO et al. (2007) Biological identifications through DNA barcodes: the case of Crustacea. *Can J Fish Aquat Sci* 64: 272–295.

⁴Leese et al. (2016) DNAqua-Net: Developing new genetic tools for bioassessment and monitoring of aquatic ecosystems in Europe. *RIO* 2: e11321.

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