

# A DNA barcode reference library for the superorder Peracarida (Crustacea) from the Southern European Atlantic coast

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## Problem statement

Peracarida is a Superorder of the subphylum Crustacea and one of the most diverse and widely distributed groups of crustaceans. In marine coasts, peracaridean species are among the most ecologically important invertebrates, with high relevance in trophic interactions and constituting one of the dominant groups and key components of marine benthic communities. The Superorder currently contains more than 18000 known species listed in the World Register of Marine Species (WORMS), but experts claim that numerous species still await formal description. Several orders are included in this group such as Amphipoda, Isopoda, Tanaidacea among others.

The peracaridean fauna of the southern Atlantic European coasts is particularly rich and unique, consisting on a mixture of species from adjacent biogeographic regions such as the Mediterranean Sea and the warm and cold temperate Northeast Atlantic. Being a biogeographic cross road region where many peracaridean species have their distribution limits, this region is particularly relevant for monitoring alterations in distributional ranges driven by different factors, such as climate change, the introduction of alien species or anthropogenic activities.

Although these organisms constitute one of the most diverse groups of crustaceans, they are often neglected in most ecological surveys and have been poorly studied in comparison to other taxa of similar ecological importance. A rigorous and extensive knowledge of the peracaridean biodiversity has not been reached yet, which may compromise the informed planning and management of the marine environment.

As with many other marine invertebrate taxa, rigorous species identifications can be rather challenging, with the knowledge of peracaridean diversity being constrained by the taxonomic impediment. In recent years, the use of molecular tools for specimen identification and classification has become quite widespread and it has been used to complement morphological identifications. The use of a standard universal system, like the DNA barcode, a standard region of mitochondrial cytochrome c oxidase gene (COI-5P) with 658 base pair (bp), has been shown to be successful in species classification and delimitation in several marine groups. Furthermore has been contributing to improve the detection of potential new species and to facilitate specimen identification, particularly of cryptic species and other organisms with complex or hardly-accessible morphology.

The availability of reliable, scrutinized and annotated reference libraries of DNA barcodes is a fundamental backbone of this DNA-based species identification system. Nevertheless, marine invertebrate species are still poorly represented in the published reference libraries, and only a small fraction of the species occurring in our European marine “backyard” are represented, namely the peracaridean crustaceans (Raupach and Radulovic 2015). In this study we report and examine a core DNA barcode library for the Peracarida from the Southern Atlantic European Coast, focusing on representatives of the orders Amphipoda, Isopoda and Tanaidacea.

## Methodology

Specimens were collected in various locations along the European coast and estuaries of the Northeast Atlantic Ocean, the vast majority of which was collected along the Portuguese and Spanish Atlantic coasts between 2008 and 2015. The DNA extraction, amplification and sequencing of a 658 bp barcode region (COI-5P) was carried out according to published protocols with minor adaptations (e.g. Lobo et al. 2013). DNA sequences of COI-5P were assigned to 34 morphospecies (140 specimens) of the orders Amphipoda, Isopoda and Tanaidacea. To this dataset we added publicly available COI-5P sequences of taxonomically close species (some genera) from Portuguese and Spanish Atlantic coasts, and also from other Northeast Atlantic locations, to inspect for taxonomic congruence among studies and collection location, hence producing a final dataset comprising 423 barcodes covering 30 isopod species, 4 tanaid species and 63 amphipod species. Only sequences with more than 500 bp were considered. All sequence edition and genetic analyses were performed using the software MEGA 6.06 and the Barcode of Life Data Systems (BOLD) analyses tools. A Neighbor-Joining phenogram was constructed applying the Kimura 2-parameter model (K2P) and bootstrap node support determined through 1000 iterations. Intra and interspecific genetic distances were calculated using K2P to facilitate comparison with other studies.

## Results:

The large majority of the morphospecies analyzed (79%) had low average intra-specific distances, (<2%), a result that matches well with the typical pattern of COI-5P variability observed in numerous crustaceans species (Costa et al. 2007; Hebert et al. 2003; Raupach and Radulovici 2015). Intraspecific variability data was not available for 18 singleton species (species represented by a single sequence). Two isopod species belonging to the genus *Lekanasphaera* were grouped in the same cluster, indicating some misidentification, where three previous species with non-defined identifications were attributed to clusters with identifications (*Ampelisca diadema* and two *Monocorophium* species). However, five non-defined species still continue to not match any cluster with identified taxa. Also, one *Dexamine spiniventris* record were attributed to the genus *Atylus*. As much as five morphospecies showed high intraspecific divergence (>2%) and were assigned to multiple lineages. But in three, *Aphersusa jurinei*, *Phistica marina* and *Gamarella fucicola*, the lineages were separated geographically (Iberian Peninsula and North Sea), suggesting that these populations are probably isolated.

## Major conclusions:

The percentage of concordant morphospecies and molecular lineages is almost 80% after revision, which shows a good reliability of our DNA barcode reference library. Nonetheless, the amount of discordance between morphological data and molecular lineages highlights the need for continuous taxonomic revision in marine invertebrates. These cases should be reviewed with more integrative studies to correctly understand the diversity of this group. Also, a more detailed analysis and comparison between Southern European and other Atlantic coasts, namely the North Sea should be performed to understand the hidden diversity of Peracarida species and the processes of speciation and their phylogeographic history in Northeast Atlantic.

Our study confirms the application of DNA barcodes as highly effective identification system for the study of marine peracaridean fauna of the Southern Atlantic European Coasts and represents an important tool for modern biodiversity assessment studies using barcode sequences. For poor-studied faunal groups as the superorder Peracarida, DNA barcodes offer great potential to assist taxonomic revisions and inventories, species discovery and identification and can be key tool for documentation of the diversity of this group.

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