Deeply divergent DNA barcode lineages within Dynamene edwardsi (Isopoda) from the Northeast Atlantic suggest between four to eight cryptic species

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Problem statement

Isopods have direct development and lack larval stages, therefore their dispersal ability and gene flow between populations may be potentially lower compared to other marine invertebrates with planktonic larvae. This may contribute to increase the genetic isolation of populations and eventually facilitate speciation events. A sound discrimination of species is crucial in biodiversity assessment, but the establishment of species boundaries requires a combination of multiple approaches (e.g. molecular, morphological and ecological data). However, numerous studies in marine invertebrates, including isopods (e.g. Hurtado et al. 2010), have been reporting the occurrence of cryptic species, which can only be detected through the use of molecular markers, since they can hardly be distinguished through morphological features. Standardized universal molecular markers such as DNA barcodes, the cytochrome c oxidase gene (COI-5P) (Hebert et al. 2003) have been highly successful in the detection of potential and actual cryptic species in a vast diversity of animals, including crustaceans (Costa et al. 2007). In fact, the existence of cryptic species are suspected within most of the large genera in the family Sphaeromatidae such as Cymodoce, Dynamenella and Dynamene (Poore and Bruce 2012). Dynamene is a species-poor genus of sphaeromatid isopods, characterized by the presence of the bidentate process in adult males, a structure unique to this genus. The shape of the bidentate process is necessary to identify and distinguish the different Dynamene species. This implies that adult males are required to accurately discriminate the different species and, in their absence, species identifications of females and juveniles are extremely difficult. Members of this genus are very common and abundant among the invertebrate fauna of intertidal and shallow subtidal rocky substrates in Northeast Atlantic Ocean, where they are represented by 3 species only: D. bidentata, D. edwardsi and D. magnitorata (Holdich 1968).
In this study, we examine the genetic diversity of Dynamene morphospecies in rigorously identified specimens collected along Macaronesian, European and Moroccan Atlantic rocky shores, using the DNA barcode region (COI-5P).

Methodology

Dynamene specimens' collection was carried out in continental coastal areas and Macaronesian archipelagos (except Cabo Verde) of the Northeast Atlantic Ocean between 2009 and 2015. Specimens were taken from rocky shore habitats by scraping of the algal cover. Morphology-based taxonomic identification was supported by specialized literature (Holdich 1968). The sampled specimens for genetic analyses by species and location were the following: D. bidentata specimens collected in Scotland, Spain, Portugal and Morocco, D. magnitorata specimens sampled in Spain, Portugal and Terceira and D. edwardsi individuals collected in Spain, Portugal, Morocco and São Miguel, Madeira, Porto Santo, Gran Canaria, Tenerife, El Hierro and La Palma.
The DNA extraction, amplification and sequencing of the standard 658 base pair (bp) barcode region (COI-5P) were performed according to published protocols (e.g. Costa et al. 2007; Lobo et al. 2013). The software MEGA 6.06 was used for sequence edition and subsequent analyses. After inspection and quality verification of the bidirectional trace files a total of sequences 155 sequences of 658 bp were aligned. No indels or spurious sequences with stop codons were detected upon translation. For phylogenetic reconstruction we used the Maximum Likehood method, applying Hasegawa-Kishino-Yano best fit substitution model and with 1000 boostrap for determine node support. Genetic distances were calculated using the Kimura 2-parameter model.

Results:

Cytochrome oxidase I (COI-5P) clearly discriminated between the three species, confirming their morphology-based identifications. Among Dynamene morphospecies the pair D. bidentata / D. magnitorata differed less (19%) when comparing with D. edwardsi (26 and 25% respectively). Both D. bidentata and D. magnitorata displayed intra-specific distances below 2%, in spite the specimens originated from relatively distant populations (e.g. Scotland and Morocco).
While in D. bidentata and D. magnitorata nearly none or little population structure was observed, D. edwardsi displayed extensive structure, where it is possible to discriminate up to eight geographically circumscribed lineages with complete sorting and exclusive haplotypes, and displaying over 4% of genetic distance between them. All lineages showed little within lineage diversity (less than 1%). Inspection of the deep phylogeny of D. edwardsi reveals four major deeply divergent lineages, displaying genetic distances between

16% and 22%, strongly suggesting the existence of a cryptic species complex. One group integrates specimens from São Miguel and Portugal and Spain, another from Porto Santo, the third one comprises specimens from Gran Canaria and Morocco and the last, specimens from Madeira, Tenerife, El Hierro and La Palma. Average divergence among D. edwardsi deep lineages surpasses the divergence among the pair D. bidentata / D. magnitorata.

The most salient finding, however, was that populations within a lineage were not grouped by geographic proximity. For example, populations as geographically close as Porto Santo and Madeira, split into two lineages displaying as much as 22% genetic distance, while populations geographically more distant as Madeira and Tenerife are grouped in the same lineage.

Detailed comparative morphological analyses of specimens from the various lineages revealed no patent fixed distinction, and all of them shared the diagnostic features of D. edwardsi.

Major conclusions:
DNA barcodes unravelled a complex of four to eight probable cryptic species within Dynamene edwardsi in Northeast Atlantic, highlighting the role of this molecular tool to detect the diversity of marine invertebrates, which should be used regularly to complement taxonomic research and species delimitation.
It is noteworthy that Dynamene is a genus with only six known species present in the Northern Hemisphere and just three in the Northeast Atlantic (Holdich 1968). If the putative cryptic species here reported are confirmed by further studies, it would represent a staggering increase of over 100 % in the known species diversity in the northern hemisphere for this species-poor genus.
These findings highlight the relevance of Macaronesia islands in isolation and genetic diversity of this passively dispersed marine invertebrate and can contribute in the investigation of comparative patterns of evolution and speciation of marine invertebrates in this region. Given the frequent occurrence and dominance of this isopod in the rocky shore communities, this information can be highly pertinent for coastal management and conservation strategies in Macaronesia region.

References

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