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DNA barcoding reveals new insights into the diversity of Antarctic species of *Orchomene sensu lato* (Crustacea: Amphipoda: Lysianassoidea)

C. Havermans [a,b,*], Z.T. Nagy [a], G. Sonet [a], C. De Broyer [a], P. Martin [a]

[a] Royal Belgian Institute of Natural Sciences, Rue Vautier 29, B-1000 Brussels, Belgium
[b] Laboratory of Marine Biology, Place Croix du Sud 3, Catholic University of Louvain, B-1348 Louvain-la-Neuve, Belgium

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ABSTRACT

Recent molecular analyses revealed that several so-called "circum-Antarctic" benthic crustacean species appeared to be complexes of cryptic species with restricted distributions. In this study we used a DNA barcoding approach based on mitochondrial cytochrome oxidase I gene sequences in order to detect possible cryptic diversity and to test the circumpolarity of some lysianassoid species. The orchomenid genus complex consists of the genera *Abyssorchomene*, *Falklandia*, *Orchomenella*, *Orchomenyx* and *Pseudorchomene*. Species of this genus complex are found throughout the Southern Ocean and show a high species richness and level of endemism. In the majority of the studied species, a genetic homogeneity was found even among specimens from remote sampling sites, which indicates a possible circum-Antarctic and eurybathic distribution. In four investigated species (*Orchomenella* (*Orchomenopsis*) *acanthurus*, *Orchomenella* (*Orchomenopsis*) *cavimanus*, *Orchomenella* (*Orchomenella*) *franklini* and *Orchomenella* (*Orchomenella*) *pinguides*), genetically divergent lineages and possible cryptic taxa were revealed. After a detailed morphological analysis, *O.* (*O.*) *pinguides* appeared to be composed of two distinct species, formerly synonymized under *O.* (*O.*) *pinguides*. The different genetic patterns observed in these orchomenid species might be explained by the evolutionary histories undergone by these species and by their different dispersal and gene flow capacities.

1. Introduction

According to most estimations on global biodiversity, the majority of species living on this planet are currently undescribed (Novotny et al., 2002; Blaxter, 2003, 2004; Bouchet, 2006). Aiming to have a "complete" account of all living organisms would require more work than the present manpower and technology can handle. Moreover, in the context of the current biodiversity crisis and the declining number of taxonomists, several authors suggest the use of DNA barcoding to accelerate and simplify species identification (Hebert et al., 2003a,b; Blaxter, 2004; Janzen et al., 2005; Schander and Willassen, 2005; Schindel and Miller, 2005). DNA barcoding uses a short DNA sequence as the standard genetic marker for species identification (a ca. 648 bp segment near the 5′ end of the mitochondrial cytochrome oxidase I gene, COI, for animals). The barcode sequence from each unknown specimen is compared with a reference library of sequences derived from specimens of known identity (Hajibabaei et al., 2007). This sequence library is currently being established. This approach speeds up species identification and also facilitates the discovery of undescribed species (Witt et al., 2003). The efficiency of a barcoding marker in species delimitation depends on the separation between intra- and interspecific divergences (Hebert et al., 2003a,b; Meyer and Paulay, 2005; Waugh, 2007). In accordance with the biological species definition, intraspecific genetic distances have to be generally smaller (mostly by an order of magnitude) than interspecific genetic distances. This provides the basis for species delimitation (Waugh, 2007; Meier et al., 2008). In several animal taxa, the effectiveness of this approach has been confirmed, such as in birds (Hebert et al., 2004b), fish (Ward et al., 2005), molluscs (Meyer and Paulay, 2005), spiders (Barrett and Hebert, 2005) and several groups of butterflies (Hebert et al., 2004a; Janzen et al., 2005; Hajibabaei et al., 2006). In poorly studied groups, DNA barcoding can be performed prior to "conventional", morphology-based taxonomic studies in order to quickly sort specimens into genetically divergent groups (Hajibabaei et al., 2007). However, the DNA barcoding approach is not without controversy when it is considered as a tool for classification and identification (e.g., Lipscomb et al., 2003; Moritz and Cicero, 2004; Will and Rubinoff, 2004). It has raised some debates about traditional

* Corresponding author at: Department of Freshwater Biology, Royal Belgian Institute of Natural Sciences, Rue Vautier 29, B-1000 Brussels, Belgium. Tel.: +32 2 627 42 78; fax: +32 2 627 42 77.
E-mail addresses: chavermans@naturalsciences.be (C. Havermans), zoltan-tamas.nagy@naturalsciences.be (Z.T. Nagy), gontran.sonet@naturalsciences.be (G. Sonet), claude.debroyer@naturalsciences.be (C. De Broyer), patrick.martin@naturalsciences.be (P. Martin).

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taxonomy becoming extinct and being replaced by DNA sequencing. However, DNA barcoding should not be considered as a substitute for conventional taxonomy; its principal utility is as a searchable label, by linking barcodes to fully described voucher specimens (Waugh, 2007). The coupling of a detailed morphological and ecological investigation with the barcode results is critical for species descriptions. Nevertheless, DNA barcoding has its limitations: its accuracy seems to depend on the taxonomic knowledge and the sample coverage of the group (e.g., Meyer and Paulay, 2005). Additionally, the phenomena of incomplete lineage sorting, genetic introgression, pseudogenes (e.g., Buhay, 2009) or bacterial infections (*Wolbachia*, e.g., Whitworth et al., 2007) can make species identification inadequate with this tool.

The Southern Ocean is considered as a hotspot of biodiversity and endemism for several orders of peracarid crustaceans (Malacostraca), which have undergone spectacular adaptive radiations (Watling and Thurston, 1989; Brandt, 1999, 2005; Lörz and Brandt, 2004; Lörz and Held, 2004). Peracarids comprise about 1500 strictly Antarctic species and, among them, amphipods represent the most speciose group with more than 815 gammaridean and corophiidean species recorded in the Southern Ocean *sensu lato* (De Broyer et al., 1999, 2003, 2007). The superfamily Lysianassoidea is one of the most dominant gammaridean amphipod groups in Antarctic waters, both in number of species and in abundance (Arnaud et al., 1986; De Broyer et al., 2001).

Unlike Antarctic benthic communities living in shallow water, little is known about the biodiversity of the Antarctic deep-sea region where many collected invertebrate species are new to science (Brandt et al., 2007). Moreover, species counts for the fauna of the Southern Ocean are suspected to be underestimated. Indeed, many Antarctic marine benthic invertebrates are currently considered to have a circum-Antarctic and/or eurybathic distribution (Arntz et al., 1994). The circum-Antarctic distribution can be explained by similar environmental conditions in the sea around the continent, as well as by the circumpolar current systems (Arntz et al., 2005). The high degree of eurybathy is considered as an evolutionary adaptation to the oscillation of the ice cap extension during the Antarctic glacial and interglacial cycles. Ice extensions and retreats could have been followed by a migration of taxa up and down the Antarctic continental shelf and slope (Brey et al., 1996). However, recent molecular analyses revealed that several of these species represent in fact complexes of morphologically similar (cryptic) species showing restricted distribution ranges. This is the case for several Antarctic organisms: isopods (Held, 2003; Held and Wägele, 2005; Raupach and Wägele, 2006; Raupach et al., 2007; Brökeland and Raupach, 2008), molluscs (Beaumont and Wei, 1991; Page and Linse, 2002; Allcock et al., 2004; Strugnell et al., 2008), crinoids (Wilson et al., 2007), pycnogonids (Mahon et al., 2008) and fish (Bernardi and Goswami, 1997; Smith et al., 2008).

The lysianassoid genus *Orchomene sensu lato* represents a good model for biodiversity studies due to its (relative) species richness, high degree of endemism, its abundance and important role in the Southern Ocean, and the presence at both shallow and abyssal depths. Following the most recent systematic classification (De Broyer et al., 2007), this orchomenid genus complex includes the genera *Abyssorchomene* De Broyer, 1984, *Orchomenella* G.O. Sars, 1895 (including the subgenera *Orchomenella* and *Orchomenopsis*), *Orchomenyx* De Broyer, 1984 and *Pseudorchomene* Schellenberg, 1926. A recent molecular phylogenetic study also suggested the inclusion of the monotypic genus *Falklandia* De Broyer, 1985 within this genus complex (Havermans et al., 2010). The genera *Falklandia*, *Orchomenyx* and *Pseudorchomene* are endemic to the Southern Ocean. Although two genera, *Orchomenella* and *Abyssorchomene*, may be considered as cosmopolitan (Barnard and Karaman, 1991), they also comprise some species endemic to the Southern Ocean.

The phylogeny of the group was recently investigated (Havermans et al., 2010) and it was shown that the molecular phylogeny does not correspond to the morphological classification at the genus level. Several (sub)genera (*Abyssorchomene*, *Orchomenella*, *Orchomenopsis*) appeared to be non-monophyletic and some diagnostic characters used in this complex of genera are likely a result of convergent evolution. The scope of the current paper does not focus on this issue but rather focuses on the issue of species delimitation within this group. Our aim is to test whether the COI gene is an appropriate barcoding marker for these taxa. Our previous study showed that previously proposed taxonomic subdivisions should be revised and these taxa remain difficult to identify for the non-expert. These taxa, with a confuse taxonomy, represent an interesting case to test the validity of the barcoding approach. Finally, the circumpolarity and species boundaries will be investigated using genetic and biogeographic data in several orchomenid species such as *Orchomenella* (*Orchomenopsis*) *cavimanus* (Stebbing, 1888) and *Abyssorchomene scotianensis* (Andres, 1983), which were characterized so far by a circum-Antarctic and eurybathic distribution (De Broyer et al., 2007).

2. Material and methods

During recent expeditions with the R/V "Polarstern", amphipod material was collected from the Magellanic region, the Scotia Sea, the eastern shelf of the Antarctic Peninsula, the Weddell Abyssal Plain, the Eastern Weddell Sea and Bouvet Island (ANTARKTIS XV-3, De Broyer et al., 1999; ANTARKTIS XIX-5, De Broyer et al., 2003; ANTARKTIS XXI-2, ANDEEP I, II, III, De Broyer et al., 2003, 2006; ANTARKTIS XXIII-8, d'Udekem d'Acoz and Robert, 2008). Additional samples from the Ross Sea (BIOROSS Cruise) and from King George Island (South Shetland Islands) were provided by the National Institute of Water and Atmospheric Research (NIWA, New Zealand) and the Polish Antarctic IPY Expedition 2007, respectively. Agassiz and bottom trawls, dredges, epibenthic sleds, grabs, multi-box corers and baited traps were used to collect amphipods. Samples were fixed in 96% or absolute ethanol.

The molecular analysis included 121 specimens belonging to ca. 19 species, identified by a preliminary morphological analysis. Specimens of the lysianassoid genus *Ambasiopsis* were used as outgroup. Genomic DNA was isolated from the sixth pereiopod using the QIAamp DNA Mini Kit (Qiagen). Amplification of the COI marker was carried out by polymerase chain reaction using the universal primers LCO1490 and HCO2198 (Folmer et al., 1994). Purified PCR products were sequenced bidirectionally using an ABI 3130xl capillary DNA sequencer (Applied Biosystems). Detailed information on specimens used in this study is given in Table 1 and sequences were deposited in GenBank.

Alignments were made manually (alignments are available from the first author upon request). A neighbour-joining tree (Saitou and Nei, 1987) was estimated using MEGA 4 (Tamura et al., 2007) and sequence divergences were calculated using the Kimura 2-parameter (K2P) distance model (Kimura, 1980), the best metric system when distances are low (Nei and Kumar, 2000) (see supplementary material available at doi:10.1016/j.dsr2.2010.09.028). Branch support was evaluated using non-parametric bootstrapping (number of replicates was 2000). Frequency distribution histograms of pairwise inter- and intraspecific distances were calculated with R (version 2.7.0) using the APE package (Paradis et al., 2004) and plotted using geneplotter, graphics related functions for Bioconductor (Gentleman et al., 2004). For further estimations on divergence, TaxonDNA v.1.5a12 (Meier et al., 2006) was used.