Molecular Phylogenetics and Evolution 55 (2010) 202–209

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Incongruence between molecular phylogeny and morphological classification in amphipod crustaceans: A case study of Antarctic lysianassoids

Charlotte Havermans [a,b,*], Zoltán T. Nagy [a], Gontran Sonet [a], Claude De Broyer [a], Patrick Martin [a]

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

ARTICLE INFO

Article history:
Received 6 July 2009
Revised 19 October 2009
Accepted 20 October 2009
Available online 24 October 2009

Keywords:
Molecular phylogeny
Amphipoda
Lysianassoidea
Southern Ocean
COI
28S rRNA

ABSTRACT

In Antarctic waters, the superfamily Lysianassoidea is one of the most important amphipod groups both in terms of species number and abundance. Dominant members of this superfamily are species of the orchomenid complex, found throughout the Southern Ocean. This study presents the first molecular phylogenetic analysis based on a representative subset of the Antarctic species belonging to different orchomenid genera and hence provides a framework for a systematic revision of these taxa. The current classification of the orchomenid genera is mainly based on mouthpart morphology. The validity of these morphological characters was assessed by resolving phylogenetic relationships using nuclear 28S rRNA and mitochondrial cytochrome oxidase subunit I sequences. The molecular data rejected most of the previously proposed taxonomic subdivisions within this complex. The genera *Abyssorchomene* and *Orchomenella* as well as the subgenus *Orchomenopsis* appeared to be non-monophyletic. This implies that the supposed diagnostic characters are likely a result of convergent evolution. Further, our results indicated the necessity of a revision of the family-level systematics.

1. Introduction

For a long time, the concept of a global-scale increase in species richness from the poles to the equator (Clarke and Johnston, 2003) let assume that the Southern Ocean was depressed in species richness. Recently, the number of studies on Antarctic biodiversity and biogeography increased and challenged this view for several higher taxa. These studies suggested, in contrast, that the species richness in certain animal groups in the Southern Ocean might be comparable to this from temperate and tropical continental slopes in the Southern Hemisphere (Brandt et al., 2007), with species endemism rates of around 50% (Griffiths et al., 2009).

With more than 1500 strictly Antarctic species, the Southern Ocean is nowadays considered as a hotspot of biodiversity and that of endemisms for several orders of peracarid crustaceans (Malacostraca) like isopods and amphipods. Moreover, peracarids have undergone spectacular adaptive radiations in the Southern Ocean (Watling and Thurston, 1989; Brandt, 1999; Lörz and Brandt, 2004; Lörz and Held, 2004; Brandt, 2005). Among them, amphipods are the most diverse with more than 815 gammaridean and corophiidean species in the Southern Ocean *sensu lato* and more than 500 species from the Antarctic region only (De Broyer et al., 1999, 2003a, 2007).

The superfamily Lysianassoidea is one of the dominant gammaridean amphipod groups in Antarctic waters, both in number of species and abundance (Arnaud et al., 1986; De Broyer et al., 2001). Members of the group are common in deep oceanic basins as well as in shallow waters in high latitudes. Many species are scavengers and play a key role in deep-sea benthic communities by consuming and dispersing food falls of all sizes (Slattery and Oliver, 1986; De Broyer et al., 2004). In the Southern Ocean, dominant members of this superfamily are species of the orchomenid genus complex comprising the genera *Abyssorchomene* De Broyer, 1984, *Falklandia* De Broyer, 1985, *Orchomenella* G.O. Sars, 1895 (including the subgenera *Orchomenella* and *Orchomenopsis*), *Orchomenyx* De Broyer, 1984 and *Pseudorchomene* Schellenberg, 1926. The genera *Falklandia*, *Orchomenyx* and *Pseudorchomene* are endemic to the Southern Ocean *sensu lato*. Although the other two genera, *Orchomenella* and *Abyssorchomene*, may be considered as cosmopolitan (Barnard and Karaman, 1991), they also harbour some species endemic to the Southern Ocean.

Amphipods have a history of taxonomic instability concerning higher ranks: there has been much contention concerning their classification and phylogenetic relationships (Bousfield and Shih, 1994). In fact, higher-level relationships within Amphipoda are still so uncertain that several taxonomic treatments simply list families alphabetically (Barnard and Karaman, 1975; Barnard and

* Corresponding author. Address: Department of Freshwater Biology, Royal Belgian Institute of Natural Sciences, Rue Vautier 29, 1000 Brussels, Belgium. 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).

1055-7903/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2009.10.025

Barnard, 1983; Barnard and Ingram, 1990; Martin and Davis, 2001). The masking effects of convergent or homoplastic morphology was reasserted as a major issue in amphipod taxonomy (Englisch et al., 2003; Macdonald et al., 2005; Hou et al., 2007; Fišer et al., 2008; Ito et al., 2008).

Despite several studies intending to clarify the systematics of the orchomenid genus complex (Shulenberger and Barnard, 1976; De Broyer, 1983, 1984, 1985a; Barnard and Karaman, 1991), the relationships among these taxa remain obscure (Table 1). The taxonomic history of this orchomenid genus complex dates back to Barnard (1964) who put the genera *Orchomenella*, *Orchomenopsis* and *Allogaussia* Schellenberg, 1926, in synonymy with *Orchomene*. In his revision of this group, De Broyer (1983, 1984, 1985a,b) deemed that the morphology of mouthparts was of prime importance in the systematics of the Lysianassoidea. Taking this new set of morphological characters into account, he was able to identify a combination of characters that supported the revalidation of the genus *Orchomenella*, to combine *Orchomenopsis* to *Orchomenella* as a subgenus, together with *Orchomenyx* as a new subgenus, and to identify *Abyssorchomene* and *Falklandia* as new genera. Barnard and Karaman (1991) found these new generic characters very difficult to evaluate and considered, as a conservative measure, all these taxa but *Pseudorchomene* as a monophyletic assemblage within a supergenus *Orchomene* in the family Lysianassidae. Lowry and Stoddart (1997) raised *Orchomenyx* to the generic level and moved it together with *Orchomenella* and *Pseudorchomene* to the Tryphosinae. This subfamily was established within Lysianassidae, with the notable exception of *Abyssorchomene* and *Falklandia* which were omitted from that revision. The most recent systematic classification (De Broyer et al., 2007) followed Lowry and Stoddart (1997) and corrected those omissions in placing *Falklandia* in the Lysianassidae (Tryphosinae), and *Abyssorchomene* within the Uristidae.

Given this framework, the present study aims at resolving the phylogenetic relationships of the orchomenid complex of genera of the Southern Ocean and testing the validity of the morphological characters used for their taxonomy. This study is the first molecular phylogenetic analysis of a representative subset of this species assemblage, using DNA sequences of the mitochondrial cytochrome oxidase subunit I (COI) and the nuclear 28S rRNA genes. It could serve as a basis for further systematic studies of this dominant group in the Southern Ocean.

2. Material and methods

2.1. Sampling

Specimens were collected with the research vessel *Polarstern* during several Antarctic expeditions: EASIZ II (ANTARKTIS XV-3, De Broyer et al., 1999), LAMPOS (ANTARKTIS XIX-5, De Broyer et al., 2003b), ANDEEP I, II, III (De Broyer et al., 2003b, 2006), ANTARKTIS XXIII-8 (d'Udekem d'Acoz and Robert, 2008). These campaigns provided shelf and deep-sea samples from the Scotia Sea, the Scotia Arc, the eastern shelf of the Antarctic Peninsula, the Weddell Abyssal Plain, the eastern Weddell Sea and Bouvet Island. Additional samples from the Ross Sea and from King George Island (South Shetland Islands) were provided by the National Institute of Water and Atmospheric Research (New Zealand) and the Polish Antarctic IPY Expedition 2007, respectively. The following collecting gears were used: Agassiz and bottom trawls, dredges, epibenthic sledges, grabs and multi-box corers as well as baited traps. Specimens for DNA analysis were fixed in 96% or absolute ethanol, pre-chilled at −20 °C.

Forty-one ingroup specimens representing 17 orchomenid species were used for the genetic analyses (Table 2). This sampling included 13 known Antarctic species (Table 1), and an undescribed species closely related to *Abyssorchomene scotianensis*, an undescribed species belonging to *Pseudorchomene* and a species closely related to *Orchomenella pinguides* (*Orchomenella* aff. *pinguides*). Moreover, specimens of *Abyssorchomene chevreuxi*, so far only recorded in Atlantic deep-sea, were found in the Southern Ocean and were included in this study.

Further, we added five specimens assigned to other lysianassoid genera (*Tryphosella*, *Kergueleni*a, *Eurythenes*, *Ambasiopsis*) as outgroup taxa, of which only the most closely related taxon (*Ambasiopsis* sp.) was eventually used in phylogenetic analyses. Detailed collection data and GenBank accession numbers are listed in Table 2.

2.2. Laboratory techniques

Total genomic DNA was extracted from the sixth pereiopod using the QIAamp DNA Mini Kit (Qiagen). Amplification of the COI marker was carried out in polymerase chain reactions using the universal primers LCOI1490 and HCOI2198 (Folmer et al., 1994). For 28S rRNA, the primers 28F and 28R (Hou et al., 2007) were used. The reaction mix contained 2.5 μl dNTPs (2 mM), 2.5 μl 10× PCR buffer including $MgCl_2$ (Sigma), 2.5 μl of each primer (2 μM), 0.25 μl of Taq DNA polymerase (5 units/μl), DNA template (around 40–80 ng) and water (depending on initial DNA concentration) in an end volume of 25 μl. PCR settings for amplifying COI sequences consisted of an initial denaturation of 180 s at 94 °C, followed by 10 cycles of 40 s at 94 °C, 40 s at 45 °C, 60 s at 72 °C, then 30 cycles of 40 s at 94 °C, 40 s at 50 °C and 60 s at 72 °C and a final elongation for 10 min at 72 °C. PCR settings for 28S sequences consisted of an initial denaturation of 180 s at 94 °C, followed by 40 cycles of 40 s at 94 °C, 40 s at 50 °C and 90 s at 72 °C and a final elongation for 10 min at 72 °C. Purified PCR products were sequenced bidirectionally using an ABI 3130xl DNA sequencer.

2.3. Phylogenetic analyses

Alignments were made manually and by MAFFT 6 web server (using the G-INS-i option) (Katoh et al., 2002; Katoh and Toh, 2008) for COI and 28S, respectively. In order to prevent the inclusion of pseudogenes in the analyses, COI sequences were translated into amino acids and checked for stop codons.

Phylogenetic analyses were conducted both on the separate and combined (COI + 28S) data sets. Parsimony analyses were carried out using Paup* 4.0b10 (Swofford, 2002). All characters were equally weighted and unordered. Alignment gaps were treated as a new state ("fifth character") or as missing data. Heuristic searches were carried out with random sequence addition (10 replicates) and using tree-bisection-reconnection (TBR) branch swapping. Branch support was evaluated using non-parametric bootstrapping (number of replicates was 2000).

Furthermore, Bayesian analyses were performed on the combined data set (COI + 28S) with four data partitions (three partitions for each codon position of COI and one partition for 28S). The best-fit model was selected using jModeltest 0.1.1 (Posada, 2008) by estimating and comparing maximum likelihood scores for different nucleotide substitution models. This has been carried out for each of the four data partitions. Different selection criteria (Akaike Information Criterion and Bayesian Information Criterion) identified the same best-fit substitution model for each data partition: the general time-reversible substitution model with a discrete γ correction for among site variation (GTR + G model). Hence, this model was used for each partition to conduct the Bayesian analyses using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Two parallel runs with four chains each were run for 2 mil-