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The actinopterygian diversity of the CEAMARC cruises: Barcoding and molecular taxonomy as a multi-level tool for new findings

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ABSTRACT

In the winter 2007–2008, the CAML-CEAMARC cruises prospected in the Eastern part of the Antarctic continental shelf (Dumont d'Urville Sea, off Terre Adélie). The Australian R/V "Aurora Australis" and the Japanese R/V "Umitaka Maru" sampled in locations and at depths previously uninvestigated in this region. In total, 538 teleost specimens collected during these cruises were sequenced for the mitochondrial cytochrome oxidase I gene (COI), with the goal of barcoding a representative sampling from the campaign. The efficiency of barcoding for identification has been questioned for some taxonomic groups, thus we compared the COI results for a few of the families and genera included here (genus *Trematomus*, Artedidraconidae, Liparidae) to results for other markers for the same specimens. To better explore intra- and interspecific variability, sequences from previous campaigns and public databases were added to the analysis for these groups. The congruence among the results for different genes (COI, cytochrome b, D-loop and the nuclear rhodopsin retrogene) and morphological identification was used to assess the efficiency of the COI dataset at recovering species delimited using other data. Where discrepancies were present among the different data sources, a morphological re-identification was performed.

The partial COI sequence yields reliable identification in most Antarctic teleost families when using their position in the clusters on a NJ tree. However, for several groups of species neither COI nor the other molecular markers investigated nor morphology recover unambiguously the currently accepted species. The taxonomy of these groups needs to be reconsidered. Identification through sequence similarity using the Barcode of Life Data System (BOLD) works for some groups, but is hampered by the incompleteness of the taxonomic coverage for antarctic teleosts. For four families (Artedidraconidae, Zoarcidae, Liparidae and Channichthyidae), several interspecific divergences were very small, and of the same magnitude as intraspecific divergences for other antarctic species. Despite these small divergences, almost all the species investigated in artedidraconids have molecular synapomorphies in the COI sequences, and a barcoding gap from the closest species. In the genus *Trematomus*, almost all species are well separated except for two pairs of closely related species that could not be distinguished by the other molecular markers either. For the typically hard to identify zoarcids and liparids, the results of barcoding are in agreement with in-depth morphological study. Once a reasonably complete reference dataset is available, barcoding will be invaluable to discriminate species from one another in these families. A careful comparison of the morphological and molecular results for our specimens allowed us to add numerous well-identified specimens (including some rare species) and sequences to BOLD. It helped to pinpoint the specimens that needed to be re-identified morphologically, and highlighted groups where barcoding is most helpful for specimen identification (*Chionodraco* species). This large-scale project underlines the need for further taxonomic work in antarctic actinopterygians.

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1. Introduction

With over 30 000 valid species, and more than 300 described each year (Froese and Pauly, 2009), actinopterygian fish remain the last great challenge in our discovery of vertebrate species diversity. They hold a crucial place in marine ecosystems and possess great economic importance. Precise and reliable identification is needed as a basis for scientific studies (Bely and Weisblat, 2006; Bortolus, 2008) as well as for fraud detection (Lockley and Bardsley, 2000; Wong and Hanner, 2008). Moreover, identification must be based on a sound knowledge of the taxonomy, as a faulty delineation of the species limits often precludes identification altogether. Our knowledge of this group comes principally from morphological studies, but in recent years, molecular taxonomy studies have begun to prove their worth, especially when combined with morphology. For instance, they have helped to detect cryptic species (Kon et al., 2007; Zemlak et al., 2009; Steinke et al., 2009) and, conversely, to relate morphologically different life stages and sexes to a single species (Johnson et al., 2009). However, identification remains primarily based on morphology. It can be limited in the case of incomplete specimens (i.e. stomach contents), for determining eggs, larvae or juveniles (Koubbi et al., 2009), or simply because of the sheer diversity of species. Some specimens can be very hard to identify even for specialists, and there is a dire lack of experts on many groups.

Molecular identification based on mitochondrial DNA has been around for several decades (see Ward et al., 2009), but has recently taken a new dimension through larger scale projects with a standardised approach and high quality control (FishTrace www.fishtrace.org, and especially the Barcode of Life (BOL) http://www.barcodinglife.org/). These rely on the sequencing of standardized gene regions (cytochrome b and rhodopsin for FishTrace, cytochrome oxidase I for BOL). Identification is then performed through a comparison to publicly accessible reference datasets, in which sequences are linked to voucher specimens. More stringent control, as well as the link with vouchers, add a reliability and an *a posteriori* controllability that is absent (Harris, 2003) from sequences deposited in other databases. The link between a sequence and its voucher specimen allows to recheck the specimen, should the systematics of a group or an identification been questioned.

The Barcode of Life project is the largest in scale. It uses a database with an associated data analysis system, the Barcode of Life Data System (BOLD, Ratnasingham and Hebert, 2007). The project has received much attention, and has been presented as a powerful tool for molecular taxonomy (Hebert et al., 2003 and others), not without generating heated debates about limits and advantages of the approach itself (see for instance DeSalle et al., 2005; Rubinoff et al., 2006; DeSalle, 2006; Buhay, 2009) and about the use of a cut-off value to differentiate inter- and intraspecific divergence levels (Meyer and Paulay, 2005; Hickerson et al., 2006). Both the evaluation of the approach and the development of ameliorations are still underway; however, it looks promising for numerous taxa. The part of the project devoted to fish diversity (www.fishbol.org) is very active (Ward et al., 2009) and the number of included species from all over the world rises steadily.

The Southern Ocean ecosystem is one of the places that will be most impacted by global warming (Clarke et al., 2005; Thatje, 2005; Aronson et al., 2009). While the changes are less noticeable in the Eastern Antarctic region, they are already visible in the Antarctic Peninsula (Steig et al., 2009; Naish et al., 2009). Monitoring of these changes requires a biodiversity baseline inventory as soon as possible, and a large amount of taxonomic work is still needed for the region, including for fish. An additional and reliable tool for identification would therefore be truly plutot que very welcome.

We explore here the efficiency of identification through barcoding with COI for Antarctic actinopterygian fish, as well as the use of this marker for preliminary studies in molecular taxonomy. For these purposes, we sequenced a large number of specimens from two of the Collaborative East Antarctic Marine Census (CEAMARC) cruises. These cruises were carried out by the Australian R/V "Aurora Autralis" and the Japanese R/V "Umitaka Maru" during the Antarctic summer 2007/2008. The project is part of the CAML initiative in the framework of the International Polar Year. Before CEAMARC, the coastal (0–200 m) fish fauna of this area had been investigated starting in the sixties (morphological studies, Hureau, 1966), and most recently for both morphological and molecular studies, by the IPEV French programme ICOTA (Ichtyologie Côtière en Terre Adélie). Only 21 teleost species had been recorded, mainly notothenioids. During CEAMARC, demersal fish were collected on board the R/V "Aurora Australis" (1172 actinopterygian specimens, 65 species) and pelagic fish and ichthyoplankton on board the R/V "Umitaka Maru" (totalling more than 350 000 actinopterygian specimens and 49 species), down to 2400 m deep. This brings the number of morphologically identified teleost species recorded in the area to at least 91, including one new and several rare species.

Whether for enriching the reference database or for biodiversity exploration, collecting cruises are highly efficient in gathering high quality material suitable for both morphological and molecular works. Yet, very often, the diversity collected is such that finding competent taxonomists for all fish groups is a very long and arduous process. Cruises in the Southern Ocean are an ideal case to explore the relevance of barcoding all specimens from a campaign because the number of actinopterygian groups (Eastman, 1993; Eastman and Clarke, 1998) is relatively low and therefore precise identification can be more easily obtained. On the CEAMARC cruises, specialists for almost all the sampled groups were involved, making reliable and fast identifications possible.

We present here the results of the barcoding of almost all specimens sampled for molecular study on the R/V "Aurora Australis", as well as some of those from the R/V "Umitaka Maru". This last cruise collected a high proportion of larvae, not all of which can be identified morphologically to the species level by the only taxonomic key available (North and Kellermann, 1990). This key describes only 58 of the 322 fish species currently known for the Southern Ocean (Koubbi et al., 2009). Therefore, misidentifications of larvae are possible and a re-examination of the specimens will need to be performed for this cruise after integrating the results of our molecular identification. While starting with molecular identification might have yielded a species list faster, it is also more destructive due to the very small size of many of the larvae and juveniles. It was therefore decided to first perform a morphological study, and to wait for a full dataset and a test of the methodology based on the sampling from the R/V "Aurora Australis" before barcoding collections from the R/V "Umitaka Maru".

2. Material and methods

2.1. Collection

Fish specimens were collected during the CEAMARC campaigns off Adélie and King George V lands (Dumont d'Urville Sea). The R/V "Aurora Australis" (AA) surveyed the benthic fauna using beam trawls on 89 stations between 139.3 and 145.53°E and between 65.44 and 67.05°S at depths ranging from 138 to 1260 m,