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

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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

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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.

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Table 1
Data on specimens used for this study. Abbreviations: AP–Antarctic Peninsula, BB–Burdwood Bank, BI–Bouvet Island, JI– Joinville Island, KGI–King George Island, LB–Larsen B area, PAE–Polish Antarctic IPY Expedition 2007, SS–Scotia Sea, RS–Ross Sea, WS–Weddell Sea, n.d.–no data.

Species	Individual codes	Expedition	Station number	Locality	Longitude/latitude	Depth (m)	Accession no.
Uristidae							
Abyssorchomene charcoti (Chevreux, 1912)	AC-SS205	ANDEEP I&II	128	SS	62°43′S 55°30′ W	205	GU109230
Abyssorchomene charcoti (Chevreux, 1912)	AC-0510075	ANDEEP I&II	128	SS	62°43′S 55°30′ W	205	HM053979
Abyssorchomene charcoti (Chevreux, 1912)	AC-1110071	ANDEEP I&II	058	JI	60°59′S 55°43′ W	113	HM053980
Abyssorchomene charcoti (Chevreux, 1912)	AC-1110072	ANDEEP I&II	127	JI	62°42′S 55°22′ W	295	HM053981
Abyssorchomene charcoti (Chevreux, 1912)	AC-1403073	ANT XXI-2	103	WS	70°49′S 10°39′W	387	HM053982
Abyssorchomene chevreuxi (Stebbing, 1906)	AC-WS4700	ANDEEP III	110	WS	65°00′S 43°02′W	4700	GU109248
Abyssorchomene chevreuxi (Stebbing, 1906)	AC-P3076	ANDEEP I&II	131-1	AP	65°17′S 51°35′W	3076	GU109229
Abyssorchomene chevreuxi (Stebbing, 1906)	AC-2609074	ANDEEP I&II	131-1	AP	65°17′S 51°35′W	3076	HM053983
Abyssorchomene chevreuxi (Stebbing, 1906)	AC-0810074	ANDEEP III	81	WS	70°31′S 14°34′W	4409	HM053984
Abyssorchomene chevreuxi (Stebbing, 1906)	AC-26090710	ANDEEP I&II	131-1	AP	65°17′S 51°35′W	3076	HM053985
Abyssorchomene nodimanus (Walker, 1903)	AN-WS393	ANT XXI-2	167	WS	70°48′S 10°39′W	393	GU109241
Abyssorchomene nodimanus (Walker, 1903)	AN-WS387	ANT XXI-2	103	WS	70°49′S 10°39′W	387	GU109260
Abyssorchomene plebs (Hurley, 1965)	AP-0304076	ANDEEP III	150	SS	61°48′S 47°27′W	1943	HM053986
Abyssorchomene plebs (Hurley, 1965)	AP-SS1943	ANDEEP III	150	SS	61°48′S 47°27′W	1943	GU109255
Abyssorchomene plebs (Hurley, 1965)	AP-SS270	ANT XIX-5	191	SS	57°41′S 26°24′W	270	GU109258
Abyssorchomene plebs (Hurley, 1965)	AP-08100719	ANT XIX-5	191	SS	57°41′S 26°24′W	270	HM053987
Abyssorchomene plebs (Hurley, 1965)	AP-08100722	ANT XIX-5	191	SS	57°41′S 26°24′W	270	HM053988
Abyssorchomene plebs (Hurley, 1965)	AP-0506081	ANT XIX-5	191	SS	57°41′S 26°24′W	270	HM053989
Abyssorchomene plebs (Hurley, 1965)	AP-2409073	ANT XXI-2	14	BI	54°37′S 03°06′E	515	HM053990
Abyssorchomene plebs (Hurley, 1965)	AP-2109078	ANT XXI-2	14	BI	54°37′S 03°06′E	515	HM053991
Abyssorchomene plebs (Hurley, 1965)	AP-0510071	ANT XXI-2	14	BI	54°37′S 03°06′E	515	HM053992
Abyssorchomene plebs (Hurley, 1965)	AP-LB383	ANT XXIII-8	698-1	LB	65°59′S 60°24′W	383	GU109233
Abyssorchomene plebs (Hurley, 1965)	AP-J12	ANT XXIII-8	684-1	SS	62°57′S 57°57′W	822	HM053993
Abyssorchomene plebs (Hurley, 1965)	AP-G10	ANT XXIII-8	620	JI	60°56′S 55°49′W	334	HM053994
Abyssorchomene plebs (Hurley, 1965)	AP-1110076	ANDEEP I&II	114	SS	61°44′S 60°45′W	2889	HM053995
Abyssorchomene plebs (Hurley, 1965)	AP-08100721	ANT XIX-5	194	SS	57°40′S 26°25′W	308	HM053996
Abyssorchomene plebs (Hurley, 1965)	AP-2609072	ANDEEP I&II	083	SS	61°07′S 56°08′W	349	HM053997
Abyssorchomene plebs (Hurley, 1965)	AP-0810072	ANDEEP I&II	083	SS	61°07′S 56°08′W	349	HM053998
Abyssorchomene plebs (Hurley, 1965)	AP-A1	ANT XXIII-8	n.d.	n.d.	n.d.	n.d.	HM053999
Abyssorchomene plebs (Hurley, 1965)	AP-31100710	ANT XV-3	T13	WS	70°29′S 07°57′W	550	HM054000
Abyssorchomene rossi (Walker, 1903)	AR-1010076	ANT XXI-2	288	WS	72°47′S 19°29′W	847	HM054001
Abyssorchomene rossi (Walker, 1903)	AR-3110078	ANT XV-3	T13	WS	70°29′S 07°57′W	550	HM054002
Abyssorchomene rossi (Walker, 1903)	AR-I19	ANT XXIII-8	698-1	LB	65°59′S 60°24′W	383	HM054003
Abyssorchomene scotianensis (Andres, 1983)	AS-SS3408	ANDEEP III	142	SS	62°11′S 49°29′W	3408	GU109242
Abyssorchomene scotianensis (Andres, 1983)	AS-2210075	ANDEEP I&II	131-1	AP	65°17′S 51°35′W	3076	HM054004
Abyssorchomene scotianensis (Andres, 1983)	AS-2609079	ANDEEP I&II	131-1	AP	65°17′S 51°35′W	3076	HM054005
Abyssorchomene scotianensis (Andres, 1983)	AS-2210072	ANDEEP I&II	131-1	AP	65°17′S 51°35′W	3076	HM054006
Abyssorchomene scotianensis (Andres, 1983)	AS-P3076	ANDEEP I&II	131-1	AP	65°17′S 51°35′W	3076	GU109240
Abyssorchomene scotianensis (Andres, 1983)	AS-05100710	ANDEEP I&II	131-1	AP	65°17′S 51°35′W	3076	HM054007
Abyssorchomene scotianensis (Andres, 1983)	AS-2210071	ANDEEP I&II	131-1	AP	65°17′S 51°35′W	3076	HM054008
Abyssorchomene scotianensis (Andres, 1983)	AS-2210074	ANDEEP I&II	131-1	AP	65°17′S 51°35′W	3076	HM054009
Abyssorchomene scotianensis (Andres, 1983)	AS-22100919	ANDEEP III	78	WS	71°09′S 14°00′W	2166	HM054010
Abyssorchomene scotianensis (Andres, 1983)	AS-0810073	ANDEEP III	80	WS	70°39′S 14°43′W	3088	HM054011
Abyssorchomene scotianensis (Andres, 1983)	AS-1110077	ANDEEP I&II	114	SS	61°44′S 60°45′W	2889	HM054012
Abyssorchomene sp. 1	An-SS3406	ANDEEP III	142	SS	62°11′S 49°29′W	3406	GU109239
Abyssorchomene sp. 1	An-0810076	ANDEEP III	80	WS	70°39′S 14°43′W	3088	HM054013
Abyssorchomene sp. 1	An-WS3088	ANDEEP III	80	WS	70°39′S 14°43′W	3088	GU109236
Abyssorchomene sp. 1	An-0810078	ANDEEP III	81	WS	70°31′S 14°34′W	4409	HM054014
Abyssorchomene sp. 1	An-08100710	ANDEEP III	78	WS	71°09′S 14°00′W	2166	HM054015

Table 1 (*continued*)

Species	Individual codes	Expedition	Station number	Locality	Longitude/latitude	Depth (m)	Accession no.
Abyssorchomene sp. 1	An-0707088	ANT XXIII-8	706-7	LB	65°26′S 61°26′W	828	HM054016
Abyssorchomene sp. 1	An-07070810	ANT XXIII-8	705-1	LB	65°33′S 61°37′W	310	HM054017
Abyssorchomene sp. 1	20814	Tangaroa (NIWA)	TAN0402-257	RS	66°12′S 162°26′E	1395	HM054018
Abyssorchomene sp. 2	An2-1010074	ANT XXI-2	14	BI	54°37′S 03°06′E	515	HM054019
Abyssorchomene sp. 2	An2-2202072	ANT XXI-2	14	BI	54°37′S 03°06′E	515	HM054020
Lysianassidae							
Falklandia reducta (Schellenberg, 1931)	FR-SS285	ANT XIX-5	252	SS	61°23′S 55°26′ W	285	GU109256
Orchomenella (Orchomenella) franklini (Walker, 1903)	OF-SS259-1	ANT XXIII-8	614-15	JI	60°52′S 55°27′W	259	GU109226
Orchomenella (Orchomenella) franklini (Walker, 1903)	OF-0707082	ANT XXIII-8	614-15	JI	60°52′S 55°27′W	259	HM054021
Orchomenella (Orchomenella) franklini (Walker, 1903)	OF-SS259-3	ANT XXIII-8	614-15	JI	60°52′S 55°27′W	259	GU109235
Orchomenella (Orchomenella) franklini (Walker, 1903)	OF-0707084	ANT XXIII-8	614-15	JI	60°52′S 55°27′W	259	HM054022
Orchomenella (Orchomenella) franklini (Walker, 1903)	OF-0707087	ANT XXIII-8	n.d.	n.d.	n.d.	n.d.	HM054023
Orchomenella (Orchomenella) franklini (Walker, 1903)	OF-D1	ANT XXIII-8	614-3	JI	60°52′S 55°27′W	259	HM054024
Orchomenella (Orchomenella) franklini (Walker, 1903)	OF-I16	ANT XXIII-8	654-3	JI	61°22′S 56°03′W	363	HM054025
Orchomenella (Orchomenella) franklini (Walker, 1903)	OF-2610071	ANDEEP I&II	133-3	AP	65°19′S 54°14′W	1120	HM054026
Orchomenella (Orchomenella) franklini (Walker, 1903)	OF-24100720	ANDEEP I&II	133-3	AP	65°19′S 54°14′W	1120	HM054027
Orchomenella (Orchomenella) franklini (Walker, 1903)	OF-1010075	ANT XXI-2	245	WS	70°56′S 10°32′W	337	HM054028
Orchomenella (Orchomenella) pinguides (Walker, 1903)	OP-WS387	ANT XXI-2	103	WS	70°49′S 10°39′W	387	GU109247
Orchomenella (Orchomenella) pinguides (Walker, 1903)	OP-WS395	ANT XXI-2	108	WS	70°48′S 10°39′W	395	GU109259
Orchomenella (Orchomenella) pinguides (Walker, 1903)	OP-WS175	ANT XXI-2	39	WS	71°06′S 11°32′W	175	GU109237
Orchomenella (Orchomenella) pinguides (Walker, 1903)	OP-24100714	ANDEEP I&II	133-3	AP	65°19′S 54°14′W	1120	HM054029
Orchomenella (Orchomenella) pinguides (Walker, 1903)	20810	Tangaroa (NIWA)	TAN0402-206	RS	71°09′S 171°02′E	975	HM054030
Orchomenella (Orchomenella) pinguides (Walker, 1903)	20807	Tangaroa (NIWA)	TAN0402-103	RS	71°14′S 170°42′E	555	HM054031
Orchomenella (Orchomenopsis) acanthurus (Schellenberg, 1931)	OA-RS252	Tangaroa (NIWA)	TAN0402-133	RS	71°38′S 170°13′E	252	GU109263
Orchomenella (Orchomenopsis) acanthurus (Schellenberg, 1931)	20845	Tangaroa (NIWA)	TAN0402-134	RS	71°38′S 170°09′E	65	HM054032
Orchomenella (Orchomenopsis) acanthurus (Schellenberg, 1931)	OA-P137	ANT XXIII-8	605-3	AP	61°20′S 55°31′W	137	GU109266
Orchomenella (Orchomenopsis) acanthurus (Schellenberg, 1931)	OA-WS284	ANT XXI-2	132	WS	70°56′S 10°31′W	284	GU109225
Orchomenella (Orchomenopsis) acanthurus (Schellenberg, 1931)	OA-23040818	ANT XXI-2	146	WS	70°56′S 10°47′W	404	HM054033
Orchomenella (Orchomenopsis) acanthurus (Schellenberg, 1931)	OA-0707085	ANT XXIII-8	605	AP	61°20′S 55°29′W	151	HM054034
Orchomenella (Orchomenopsis) acanthurus (Schellenberg, 1931)	OA-0707086	ANT XXIII-8	605	AP	61°20′S 55°29′W	151	HM054035
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-3110073	ANDEEP I&II	042-2	SS	59°40′S 57°35′W	3683	HM054036
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-SS349	ANDEEP I&II	083	SS	61°07′S 56°08′W	349	GU109250
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-SS349-1	ANDEEP I&II	083	SS	61°07′S 56°08′W	349	GU109251
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-SS349-4	ANDEEP I&II	083	SS	61°07′S 56°08′W	349	GU109252
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-1909073	ANT XIX-5	162	SS	53°25′S 42°40′W	293	HM054037
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-2109075	ANT XIX-5	162	SS	53°25′S 42°40′W	293	HM054038
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-SS293	ANT XIX-5	162	SS	53°25′S 42°40′W	293	GU109264
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-WS515	ANT XIX-5	289	WS	72°49′S 19°30′W	515	GU109257
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-SS413	ANT XIX-5	157	BB	54°32′S 55°55′W	413	GU109262
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-SS1943	ANDEEP III	150	SS	61°48′S 47°27′W	1943	GU109254
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-0603075	ANT XV-3	T13	WS	70°29′S 07°57′W	550	HM054039
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-WS387	ANT XXI-2	103	WS	70°49′S 10°39′W	387	GU109244
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-WS847	ANT XXI-2	288	WS	72°47′S 19°29′W	847	GU109261
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-WS1017-5	ANDEEP III	74	WS	71°18′S 13°56′W	1017	GU109243
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-WS1017-4	ANDEEP III	74	WS	71°18′S 13°56′W	1017	GU109249
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-I2	ANT XXIII-8	683-1	AP	62°57′S 57°57′W	839	HM054040
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-0304075	ANDEEP III	80	WS	70°39′S 14°43′W	3088	HM054041
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	20829	Tangaroa (NIWA)	TAN0402-14	RS	71°43′S 171°45′E	451	HM054042
Orchomenella (Orchomenopsis) cavimanus (Stebbing, 1888)	OC-0707089	ANT XXIII-8	635	SS	60°56′S 55°55′W	231	HM054043
Orchomenyx macronyx (Chevreux, 1905)	OM-JI151	ANT XXIII-8	605-1	JI	61°20′S 55°29′W	151	GU109231
Orchomenyx macronyx (Chevreux, 1905)	OM-JI161	ANT XXIII-8	685-1	JI	62°33′S 55°41′W	161	GU109228
Orchomenyx schellenbergi (Thurston, 1972)	OS-23040820	PAE	2-07	KGI	58°27′S 62°09′W	210	HM054044
Orchomenyx schellenbergi (Thurston, 1972)	OS-KGI210	PAE	2-07	KGI	58°27′S 62°09′W	210	GU109265
Orchomenyx schellenbergi (Thurston, 1972)	OS-2304083	PAE	2-07	KGI	58°27′S 62°09′W	210	HM054045
Orchomenyx tabarini (Thurston, 1972)	OT-P211	ANT XXIII-8	689-5	AP	62°27′S 55°25′W	211	GU109227

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Pseudorchomene coatsi (Chilton, 1912)	PC-1809076	ANDEEP I&II	083	SS	31°07′S 56°08′W	349	HM054046
Pseudorchomene coatsi (Chilton, 1912)	PC-05100712	ANDEEP I&II	083	SS	31°07′S 56°08′W	349	HM054047
Pseudorchomene coatsi (Chilton, 1912)	PC-SS349	ANDEEP I&II	083	SS	31°07′S 56°08′W	349	GU109245
Pseudorchomene coatsi (Chilton, 1912)	PC-2609071	ANDEEP I&II	083	SS	31°07′S 56°08′W	349	HM054048
Pseudorchomene coatsi (Chilton, 1912)	PC-SS2889-4	ANDEEP I&II	114	SS	61°44′S 60°45′W	2889	GU109232
Pseudorchomene coatsi (Chilton, 1912)	PC-SS2889-5	ANDEEP I&II	114	SS	61°44′S 60°45′W	2889	GU109234
Pseudorchomene coatsi (Chilton, 1912)	PC-2210079	ANT XV-3	T13	WS	70°29′S 07°57′W	550	HM054049
Pseudorchomene coatsi (Chilton, 1912)	PC-22100710	ANT XV-3	T13	WS	70°29′S 07°57′W	550	HM054050
Pseudorchomene coatsi (Chilton, 1912)	PC-2609076	ANT XXI-2	240	WS	70°48′S 10°39′W	406	HM054051
Pseudorchomene coatsi (Chilton, 1912)	PC-08100715	ANT XXI-2	103	WS	70°49′S 10°39′W	387	HM054052
Pseudorchomene coatsi (Chilton, 1912)	PC-1909075	ANT XIX-5	261	SS	62°16′S 58°15′W	723	HM054053
Pseudorchomene sp.	Pn-WS847	ANT XXI-2	288	WS	72°47′S 19°29′W	847	GU109238
Pseudorchomene sp.	Pn-0304072	ANDEEP III	150	SS	61°48′S 47°27′W	1943	HM054054
Pseudorchomene sp.	Pn-0510077	ANDEEP III	150	SS	61°48′S 47°27′W	1943	HM054055
Pseudorchomene sp.	Pn-SS1943	ANDEEP III	150	SS	61°48′S 47°27′W	1943	GU109253
Adelheilid group							
Ambasiopsis sp.	As-08100711	ANT XXI-2	19	BI	54°31′S 03°14′E	260	HM054056
Ambasiopsis sp.	As-08100712	ANT XXI-2	19	BI	54°31′S 03°14′E	260	HM054057
Ambasiopsis sp.	As-BI260	ANT XXI-2	19	BI	54°31′S 03°14′E	260	GU109246

3. Results

The alignment of COI sequences included 658 positions, comprising 272 variable sites with the outgroup included, 247 variable sites without considering the outgroup. The amino acid translation. The mean base frequencies were A, 0.24; C, 0.13; G, 0.21; T, 0.42. The transition/transversion ratio was 1.566.

3.1. Intraspecific divergence

The mean K2P divergence in the intraspecific pairwise comparisons is 1.86% for all orchomenid species. Distinct intraspecific divergence patterns could be observed within the different species. Most of the species showed intraspecific pairwise distances lower than 2.4%, except for four species: *Orchomenella* (*Orchomenella*) *pinguides*, *Orchomenella* (*Orchomenella*) *franklini*, *O.* (*O.*) *cavimanus* and *Orchomenella* (*Orchomenopsis*) *acanthurus*. These higher divergence values may be due to unrecognized cryptic species and may thus not represent intraspecific divergences. In *O.* (*O.*) *cavimanus*, a gradient of intraspecific divergences could be observed, varying from 0% to 10.6%. When these four putative species complexes are not included, the mean K2P intraspecific divergence becomes 0.4%. In several species (e.g. *Abyssorchomene plebs*, *Abyssorchomene* sp. 1, *Pseudorchomene coatsi*), very low genetic divergences could be observed. In *Abyssorchomene* sp. 1, a mean intraspecific variation of 0.7% exists between specimens from the Scotia Sea (3406 m depth), the Antarctic Peninsula (Larsen B, 828 and 310 m), the eastern Weddell Sea (4409 m) and the Ross Sea (1395 m) (Fig. 1). *A. plebs* showed a mean K2P distance of 0.2% between specimens of the Antarctic Peninsula, the Scotia Sea, the eastern Weddell Sea, the Atlantic sector with Bouvet Island, as well as between specimens from shelf (270 m) and abyssal depths (2889 m) in the Scotia Sea (Fig. 1). Specimens of *P. coatsi* from the continental shelf (350 m) and from abyssal depths (2889 m) also show low genetic distances with a mean K2P distance of 0.2%.

3.2. Interspecific divergence

The mean interspecific K2P divergence between species (except the four potential species complexes) is 14.5%, ranging from 6.3% (between *P. coatsi* and *Pseudorchomene* sp.) to 20.1% (between *Abyssorchomene chevreuxi* and *O.* (*O.*) *acanthurus*). The frequency distribution of pairwise K2P distances within and between well-defined orchomenid species (without the putative species complexes) is shown in Fig. 2. Interspecific divergence exceeds intraspecific divergence to such an extent that a "gap" can be observed. This gap range is the interval between the highest intraspecific and the lowest interspecific distances (Astrin et al., 2006; Meier et al., 2008). In our case, the gap size is about 3.9%.

3.3. Species delimitation based on the neighbour-joining tree

The neighbour-joining analysis (Fig. 1) shows that conspecifics based on morphological identification always group together and thus confirms the monophyly of all species investigated by multiple specimens. This analysis also revealed clusters corresponding to undescribed species. These appeared to be distinct from known species by a detailed morphological analysis. The species *Abyssorchomene* sp. 1 and *Abyssorchomene* sp. 2 are separated from their related species by genetic distances in the range of the formerly defined interspecific distances. The mean divergence between *A.* sp. 1 and *A. chevreuxi* is of 10.2% and between *A.* sp. 2 and *A. chevreuxi* of 14.0%. Another undescribed species, *Pseudorchomene* sp., can be distinguished as a sister species of *P. coatsi* from both genetic and morphological points of view.

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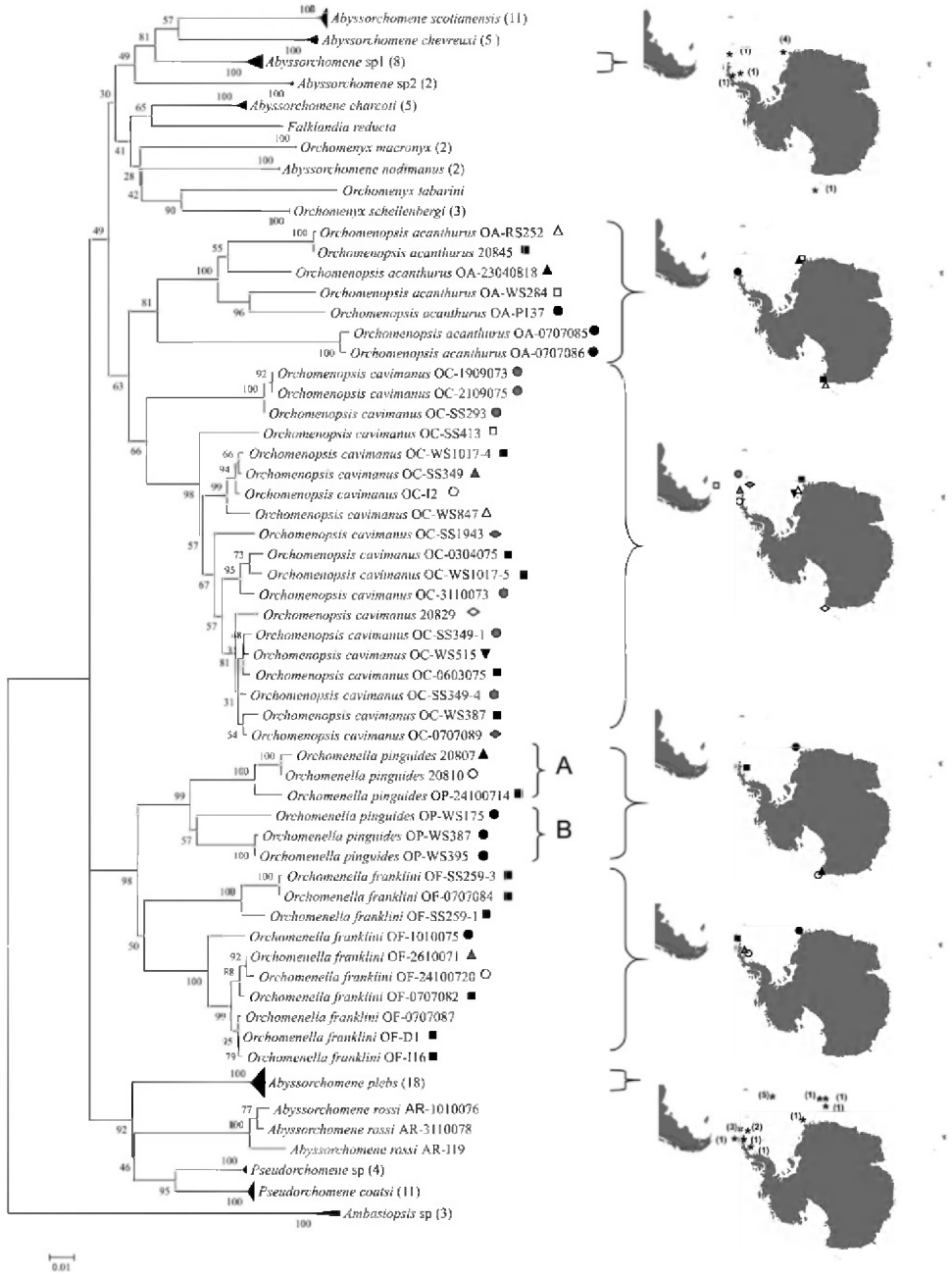

Fig. 1. Neighbour-joining tree of COI sequences based on K2P distances. Bootstrap supports (2000 replications) are shown on the branches. Clusters with low genetic divergences are collapsed (number of studied representatives are indicated in parentheses). In cases where species complexes of the genus *Orchomenella* were found, the locality is indicated for each specimen. Within *Orchomenella*, the subgeneric assignment was used in the figure. *Orchomenella* (*Orchomenella*) *pinguides* is divided into two clusters, A and B, which appeared to be distinct based on a morphological analysis. In addition, locality data of two *Abyssorchomene* species are also represented on maps, with the number of specimens for each location in parentheses.

Although distances between these two species (between 6.3% and 7.2%) are in the lower range of the interspecific distances, they are significantly higher than the highest intraspecific distance (2.4%).

3.4. Cryptic species

Within *O.* (*O.*) *pinguides*, *Orchomenella* (*O.*) *franklini*, *O.* (*O.*) *acanthurus* as well as *O.* (*O.*) *cavimanus*, we observed several clusters supported by high bootstrap values (Fig. 1). The frequency distributions of pairwise K2P distances in these species complexes are presented in Fig. 3. In *O.* (*O.*) *acanthurus*, distances range from 0% to 15.7%, which can be separated in three blocks ranging from 0% to 0.6%, from 5.8% to 8.4% and from 12.3% to 15.5%. In *O.* (*O.*) *pinguides*, distances vary from 0.2% to 7.9%. In *O.* (*O.*) *franklini* distances range from 0.2% to 3.5% and from 8.4% to 10.1%. These distances clearly indicate that some specimens are separated from each other by genetic distances in the range of interspecific distances. In the fourth case, *O.* (*O.*) *cavimanus*, genetic K2P distances vary from 0% to 10.6%. Without the most divergent cluster of *O.* (*O.*) *cavimanus* (i.e. the uppermost one in Fig. 1), K2P distances decrease to 0.3–5.6%.

In addition, representatives of these *Orchomenella* species occur in (partial) sympatry. For example, in *O.* (*O.*) *franklini*, specimens coming from the same sample locations at Joinville Island pop up in clusters separated by high genetic distances

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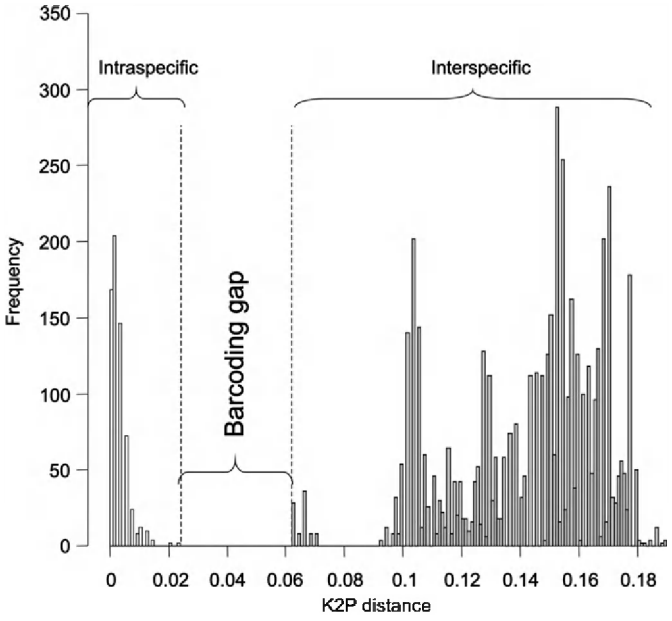

Fig. 2. Frequency distribution of pairwise K2P distances of "well-defined" Antarctic orchomenid species (fourteen ingroup species are included and putative species complexes are excluded).

(8.8–10.5%) (Fig. 1 and Table 1). In *O.* (*O.*) *acanthurus*, specimens coming from the same site at the Antarctic Peninsula appear in two clusters separated by distances of more than 15%. In *O.* (*O.*) *cavimanus*, a gradient of genetic divergences could be observed between one specimen of the Magellanic region (Burdwood Bank), specimens of the Scotia Sea, the Antarctic Peninsula, the Eastern Weddell Sea and a specimen of the Ross Sea. Specimens of the same sample locations were found scattered within the species cluster and no geographically related clusters could be observed (Fig. 1 and Table 1).

O. (*O.*) *pinguides* can be divided into at least two clusters (A and B, see Fig. 1), each comprising three specimens and separated by distances higher than 7%. Cluster A comprises specimens from the Ross Sea and the Antarctic Peninsula while the second includes specimens from the Eastern Weddell Sea. Within cluster B, one specimen is separated by a distance of more than 5%, while occurring in sympatry with the other two specimens. A detailed morphological investigation was conducted on the specimens belonging to the different clusters detected in *O.* (*O.*) *pinguides*. This required the revision of the type material of the species, as well as the type material of *Allogaussia lobata*, synonymized with *O.* (*O.*) *pinguides* by Hurley (1975; see complete taxonomic references and geographic records in De Broyer et al., 2007). Supported by the barcoding results, this revision permitted the detection of minor but consistent morphological differences between *O.* (*O.*) *pinguides* and *A. lobata*

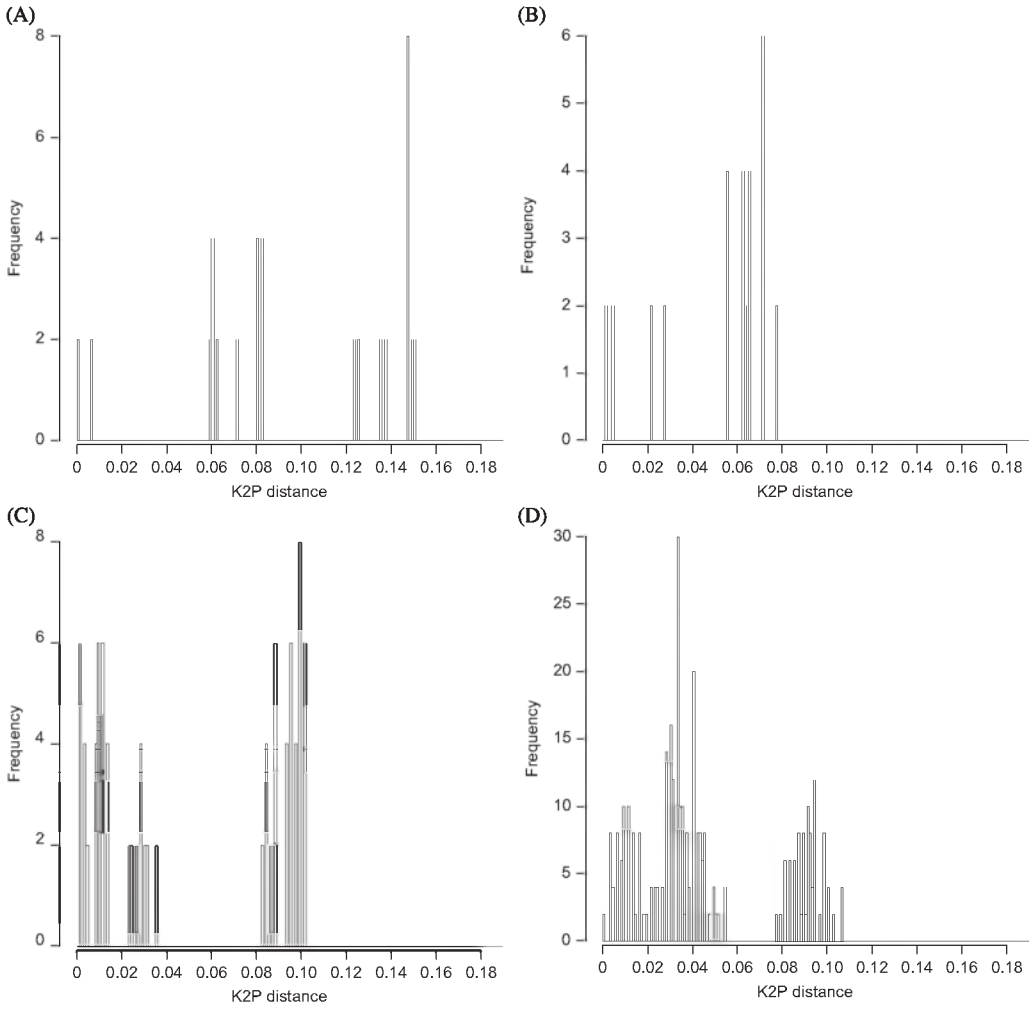

Fig. 3. Frequency distribution of pairwise K2P distances of (A) *Orchomenella (Orchomenopsis) acanthurus*, (B) *Orchomenella (Orchomenella) pinguides*, (C) *Orchomenella (Orchomenella) franklini* and (D) *Orchomenella (Orchomenopsis) cavimanus*.

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Table 2
Morphological differences between specimens of the two clusters observed within *Orchomenella* (*Orchomenella*) *pinguides*. Cluster A corresponds to *O.* (*O.*) *pinguides sensu stricto* and cluster B corresponds to *Orchomenella* (*Orchomenella*) *lobata*, a species previously synonymized with *O.* (*O.*) *pinguides*.

	***O.* (*O.*) *pinguides* s.s. (Cluster A)**	***O.* (*O.*) *lobata* (Cluster B)**
Epistome: front margin	Straight	Regularly convex
Epistome: proximal angle	Distinct, rounded	Absent
Gnathopod 1: length carpus *vs* propodus	±75% (75–80%)	±90% (81–100%)
Pereiopod 7: basis postero-distal margin	Regularly convex (or very weakly truncate)	Distinctly truncate
Pleosomite 3: dorso-distal angle	Moderately developed, regularly convex; weakly overvaulting urosomite 1	Moderately to strongly developed, subrectangular with angle rounded; strongly overvaulting urosomite 1
Urosomite 1: dorsal hump	Moderately to well developed, without weak mid-dorsal carina; strongly overvaulting urosomite 2	Well developed, with weak mid-dorsal carina; strongly overvaulting urosomite 2
Epimeral plate 3: proximal angle on hind margin	Distinct	Indistinct (margin nearly regularly rounded)
Epimeral plate 3: postero-distal angle	Indistinct, rounded	Distinct, well marked
Telson cleft	<50% (38–50%)	>50% (50–68%)

and thus re-establish the latter species as valid (as *Orchomenella* (*Orchomenella*) *lobata*). This morphological analysis will be presented in details elsewhere but is summarized in Table 2. The three specimens of cluster A initially identified as *O.* (*O.*) *pinguides* clearly belong to *O.* (*O.*) *lobata*. The specimens of cluster B were identified as *O.* (*O.*) *pinguides sensu stricto*. Based on the observation of morphological differences, which can be interpreted as interspecific variation and the high divergences separating these two clusters, this species complex seems to consist of two distinct species. Within cluster B, one specimen is separated from the other two by distances higher than 5% but it was not possible to separate them on a morphological basis. Both species, *O.* (*O.*) *lobata* and *O.* (*O.*) *pinguides*, have been recorded several times in sympatry (De Broyer, pers. comm.) and are characterized by a circumpolar distribution.

4. Discussion

4.1. "Barcoding gaps" and species delimitation

The clear barcoding gap observed in our COI dataset means that the assignment of a specimen to a particular species based on a "threshold" value of sequence divergence would mostly work for this group and would be also efficient to detect new and/or cryptic species (Hebert et al., 2003a, 2004b; Barrett and Hebert, 2005). Hebert et al. (2004a,b) proposed a standard sequence threshold of ten times the mean intraspecific divergence (K2P distance) to delimit animal species, which was also applied in studies on amphipods (e.g. Witt et al., 2006). In our case, this threshold would be 4.0%. However, the use of thresholds as an (exclusive) evidence ignores variation that may exist in different taxonomic groups. Meyer and Paulay (2005) assume that insufficient sampling on both intraspecific and interspecific levels can lead to false barcoding gaps. On the other hand, the main reason for an overlap between intra- and interspecific distances could be the poor taxonomic knowledge of a group, e.g. the presence of cryptic species that has been overlooked (Wiemers and Fiedler, 2007). This might be the case in the present study as well, where a barcoding gap apparently exists between well-defined species, but an overlap appears when considering the four putative species complexes. However, the species complex observed in *O.* (*O.*) *pinguides* appeared to be composed of two overlooked, distinct species. It is also possible that with a more extensive geographical sampling, which is the case in *O.* (*O.*) *cavimanus*, the intraspecific variation could increase further as individuals from more populations are sampled. By this, the barcoding gap range might decrease or become inexistent, which makes it impossible to designate a threshold value. Therefore, in such case, additional data from a morphological analysis or from nuclear markers are essential to verify the species status. In our previous study (Havermans et al., 2010), phylogenetic analyses were conducted on several of the specimens used for this barcoding study, based on COI and the nuclear gene 28S rRNA. The monophyletic clusters identified with the neighbour-joining tree corresponded to the clades revealed by the phylogenetic study, even in the case of the species complexes. In these complexes, specimens were also separated by higher divergences than within-species variations. Considering this, the phylogenetic species concept could also be applied, which defines a species as the smallest resolvable separately evolving lineage or the smallest diagnosable cluster (Vogler and Monaghan, 2007). The clusters within the species complexes identified in this study would then be recognized as different species. It also remains not less important to critically examine the morphology of the specimens belonging to the species complexes. A first examination of *O.* (*O.*) *pinguides* has been accomplished and revealed the presence of two distinct species. At first view, no morphological differences could be observed within the other species complexes, but this requires a detailed examination of all specimens and their types and this is clearly out of the scope of this paper.

4.2. Genetic structures of orchomenid species

Even within this group of closely related species, completely different genetic structures could be observed. The mitochondrial data revealed distinct, monophyletic clusters in *O.* (*O.*) *pinguides*, *O.* (*O.*) *franklini*, *O.* (*O.*) *cavimanus* and *O.* (*O.*) *acanthurus*. After a detailed morphological analysis some differences between specimens of *O.* (*O.*) *pinguides* suggested the presence of two morphologically similar species, which were formerly synonymized under *O.* (*O.*) *pinguides*. However, specimens of the three other species complexes seemed difficult to separate on a morphological basis. However, the genetic divergences between the clusters within *O.* (*O.*) *cavimanus*, *O.* (*O.*) *acanthurus* and *O.* (*O.*) *franklini* are congruent with species-level divergences in the orchomenid genus complex. Held (2003) developed a set of criteria to provide evidence for cryptic speciation of serolid isopods of the Antarctic waters: (1) a bimodal distribution of pairwise distance measures with no intermediate values, (2) a differentiation at a level known for this gene from other undisputed species pairs closely related to the studied species, and (3) the persistence of an expressed genetic divergence in sympatry. In our case, (1) a clear gap is observed in the distribution of intra- and interspecific distances, (2) the genetic distances between the different clusters of *O.* (*O.*) *cavimanus*, *O.* (*O.*) *acanthurus* and *O.* (*O.*) *franklini* are in the same range as interspecific distances of closely related orchomenid species, and

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(3) representatives of those genetic clusters occur in (partial) geographic and bathymetric sympatry. Different haplotypes of these *Orchomenella* species (complexes) occur in the same sampling site, or in a close geographical proximity, while still maintaining a high degree of genetic variation. Therefore, we suppose that these species consist of multiple lineages and include cryptic species.

There are increasing (molecular) evidences showing that several known species are in fact complexes of cryptic species with very similar morphological traits and restricted distributions. This raises a certain level of doubt about the circumpolarity of Antarctic invertebrates (Beaumont and Wei, 1991; Held, 2003; Held and Wägele, 2005; Page and Linse, 2002; Raupach and Wägele, 2006; Wilson et al., 2007). Raupach et al. (2007) even hypothesized that most peracarids with a benthic life style represent in fact groups of closely related but distinct species that can also appear in sympatry, which is called the "patchwork theory". This hypothesis is consistent with the statement of Knowlton (1993, 2000) arguing that since our knowledge of marine habitats is limited, the number of species is underestimated. Unfortunately, known facts on the influence of evolutionary events (e.g. formation of the Antarctic Circumpolar Current) on the endemism and distribution of faunal elements are still scarce (Thatje et al., 2005; Wilson et al., 2007).

On the other hand, a very low intraspecific diversity is observed in some other species, such as *A. plebs*, *Abyssorchomene* sp. 1 and *P. coatsi*, between specimens from geographically distant localities and between shelf and abyssal depths. In *A. plebs* we observed low genetic divergences between specimens of the Scotia Sea, the Antarctic Peninsula, the Eastern Weddell Sea and of the shelf of Bouvet Island. Bouvet Island is one of the most isolated places on Earth, situated on the mid-Atlantic Ridge between Africa and Antarctica, south of the Polar Front, and it has a young geological age of only 1 Ma (Gutt et al., 2006). Furthermore, bathymetric ranges extended from 270 to 2889 m. *Abyssorchomene* sp. 1 indicates evidence for a eurybathic and possible circum-Antarctic distribution, with low genetic variations between specimens from the Eastern Weddell Sea, the Antarctic Peninsula and Ross Sea, the latter situated on the opposite side of the continent. Furthermore, bathymetric ranges between 310 and 4409 m depth were observed. This observation supports the hypothesis of circum-Antarctic species' distributions in brooding amphipods, which was shown to be unlikely in the case of the epimeriids (Lörz et al., 2009).

4.3. Hypotheses on the speciation and the variation of genetic structures of orchomenids

Genetic distances between species (6.3–20.1%, mean of 14.5%) were much lower than those observed in studies using the same genetic marker on non-Antarctic amphipods, for example a mean interspecific divergence of 21.9% in *Gammarus* (Hou et al., 2009) and of 28% in Ponto-Caspian genera (Cristescu and Hebert, 2005). The genetic distances within and between orchomenid species were rather similar to those observed in *Hyalella* (Witt et al., 2006) and the Antarctic epimeriid species (Lörz et al., 2009). A lower interspecific divergence might indicate a more recent speciation, which was also observed in other Antarctic amphipod groups (Lörz et al., 2009). Precise dating would require more sophisticated analyses of additional data and fossil calibrations. Moreover, to our knowledge, there is no molecular clock specifically calibrated for amphipods. Quek et al. (2004) reported a rate of approximately 1.5% per million years, based on a literature survey for arthropod COI rates calibrated by fossils or biogeography. Considering this value, and the fact that the relative rate of nucleotide substitution does not differ in polar waters (Held, 2001), it seems that these Antarctic orchomenids might have speciated between 13.4 and 4.2 Myr ago, well after the isolation of the continent by the opening of the Drake Passage, ca. 34 Myr ago (Thomson, 2004).

The variation in the genetic structure of different orchomenid species (genetic homogeneity versus heterogeneity) might indicate that these species have undergone distinct evolutionary paths. Indeed, the use of different refugia during the Cenozoic glacial periods may give an explanation. For example, a recolonization from the deep-sea might have led to different genetic patterns than a recolonization from multiple shelf refugia (Thatje et al., 2005; Wilson et al., 2007). Furthermore, the maintenance of a low genetic divergence over a large geographic or bathymetric range can be explained by a high level of gene flow among populations and/or by a recent colonization and expansion event.

For the studied orchomenid species, little is known about the ecology and dispersal capacities. However, *A. plebs* is known to be a bentho-pelagic species, captured with baited bottom traps but also in the water column with pelagic nets, possibly forming suprabenthic swarms able to move rapidly (De Broyer, 1983). In a study on Antarctic lysianassoid amphipods, scavenging habits were compared between *O.* (*O.*) *pinguides* and *A. plebs* (Slattery and Oliver, 1986). *A. plebs* was more motile, swam actively, showed a swarming behaviour and occurred in vast numbers. On the contrary, *O.* (*O.*) *pinguides* did not show this swarming behaviour and was less motile and less abundant. *A. plebs* occurred over a wide range of depths and deep-water where sources of food are scarce, while *O.* (*O.*) *pinguides* occupied relatively constant and shallow depths with predictable high inputs of benthic and planktonic food. For *O.* (*O.*) *pinguides*, motility is thus not required to obtain food (Slattery and Oliver, 1986). These differences in motility could likely explain the genetic patterns observed in these species. A lower motility as in *O.* (*O.*) *pinguides* could reduce gene flow and lead to a geographical isolation of populations, particularly during glacial periods in which survival was only possible in the deep-sea or in shelters on the continental shelf (Thatje et al., 2005). During the isolation of different populations in these shelters, gene flow might have been interrupted and this could explain (cryptic) speciation events. On the contrary, the high motility of migrating species such as *A. plebs* could likely explain the high gene flow between geographically and bathymetrically remote populations and the recent colonization of the shelf of the geologically young (1 Ma) Bouvet Island.

In *O.* (*O.*) *pinguides*, at least two clusters, separated by high genetic distances, can be observed. After a detailed examination, these specimens appeared to belong to two distinct but morphologically very similar species. Cluster A, corresponding to the species *O.* (*O.*) *lobata*, comprises specimens from the Ross Sea and the Antarctic Peninsula (Fig. 1). The two specimens from the Ross Sea are separated from the specimen from the Antarctic Peninsula with a distance of more than 2%, which could be explained by a limited gene flow between these geographically distant populations. Cluster B included specimens belonging to *O.* (*O.*) *pinguides sensu stricto*, originating from the Eastern Weddell Sea (Fig. 1). Within this cluster, one specimen is separated from the other two by a distance of more than 5%, but all occur in sympatry. These distances are higher than the threshold value for species delimitation, set at 4% for the orchomenid species, and might thus represent a case of an ongoing sympatric speciation. Therefore, the circumpolar distribution of both species can be questioned but more samples with a larger geographical coverage are needed to analyze this.

In the case of *O.* (*O.*) *cavimanus*, two clusters are separated by distances in the same range as interspecific distances. When the most divergent cluster (comprising specimens from the Scotia Sea) is not included, K2P distances vary from 0.2% to 5.8%, which

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is less than the lowest interspecific variation (6.3%) but higher than the threshold value. No well-supported clusters could be observed, since there was an overlap of genetic divergences. This might suggest that *O.* (*O.*) *cavimanus* is in the process of speciation and had not yet the time to diverge genetically to the point where species distinction is possible. However, the specimens separated by intermediate genetic distances occur in sympatry and no clear geographical populations can be distinguished. Furthermore, a specimen from the Magellanic region (Burdwood Bank) clustered within species from the Scotia Sea and the Antarctic Peninsula, suggesting a possible dispersal across the Antarctic Polar Front, which normally presents a physical dispersal barrier for marine biota (Crame, 1999).

Nevertheless, we cannot assume that present distributions necessarily reflect ancient ones, knowing that the several cooling and warming episodes of the Antarctic geological history would have led to changes in the ranges of Antarctic marine taxa (Clarke and Crame, 1997; Page and Linse, 2002). This should be investigated with more fine-scaled molecular methods at the population level and an in-depth morphological examination.

5. Conclusion

A species identification by DNA barcoding was carried out for this group of Antarctic amphipods and revealed species new to science as well as the discovery of three likely species complexes and two genetically and morphologically distinct species formerly synonymized under one. Our barcoding study has been shown to be efficient for these amphipod taxa and will facilitate future taxonomic studies. The new and cryptic species will be submitted to a more accurate morphological analysis, since taxonomy should imply a holistic approach where morphological, ecological and genetic evidence is used together to delimitate species.

The application of DNA barcoding could be used in the future for species diversity studies in this group and other lysianassoid groups, as a way for non-specialists to discriminate taxa that are otherwise difficult to identify. It will thus make species identifications faster and more accessible at a lower cost at the same time.

In poorly known amphipod groups, high intraspecific genetic divergences could indicate overlooked species or species complexes. This barcode application can provide a preliminary signal of species richness. Moreover, the discovery of cryptic diversity could have profound implications for evolutionary theories and biogeography and may be a potentially important factor influencing future conservation decisions (Witt et al., 2006; Bickford et al., 2007). Furthermore, the Census of Antarctic Marine Life (CAML) states that there is an urgent need for more genetic barcode studies on Antarctic organisms, in view of the rate of climate-driven habitat changes which might lead to extinctions (Grant and Linse, 2009).

This study indicated that the species richness of Antarctic amphipods is underestimated, not only for the poorly known deep-sea but also for the better studied shelf fauna. Given the fact that our sampling mainly focused on the Atlantic sector and to a lesser extent on the Ross Sea, we expect that the entire diversity is even much higher. Therefore, additional samples from other areas in Antarctica are needed to assess the real diversity, and evaluate whether these identified clusters have a true circumpolar distribution.

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Appendix A. Supplementary Material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dsr2.2010.09.028.

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