

Encounter of lithodid crab *Paralomis birsteini* on the continental slope off Antarctica, sampled by ROV

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Abstract A population of stone crab (Lithodidae) was encountered on the continental slope off Antarctica in the Bellingshausen Sea between 1,123 and 1,304 m water depths using the ROV-*I*sis during leg 166 of the RV James Clark Ross, in January 2007. Specimens were video recorded and one specimen was retrieved by ROV for morphological and molecular identification. Based on morphology and molecular data from the mitochondrial COI gene, this specimen identified as *P. birsteini*, Macpherson, 1988a. The significance of the molecular data and their implications for biogeography and evolution of lithodids in the Southern Ocean are briefly discussed.

Keywords Southern Ocean · Stone crab · Molecular phylogeny · Biogeography

Introduction

The shallow waters of the Antarctic continental shelf are virtually free of benthic top predators, such as shark, rays, teleost fish, and crabs (Aronson et al. 2007). The absence of such predators results from harsh physiological constraints, mainly low temperature that has prevailed in this environment for tens of millions of years. The process of Antarctic cooling was

initiated by the breakup of Gondwana in the early Eocene, with a last cooling step until about 14 million years ago that resulted in conditions similar to those as seen today (see Aronson et al. 2007, and references therein). Since then, and in the absence of top predators structuring the faunal community, the Antarctic benthos of the shallow continental shelf evolved and maintained a rather ancient structure that today is not found anywhere else on Earth (Aronson et al. 2007).

The increased records of lithodid crabs in deeper waters and on seamounts surrounding the Antarctic continent in recent years raised the question of established lithodid crab populations in the Southern Ocean (López Abellan and Balguerías 1993; Klages et al. 1995; Arana and Retamal 1999; Thatje and Arntz 2004; Thatje and Lörz 2005). Although the origin and especially the timescale of lithodid radiation in the Southern Ocean remains obscure (Thatje et al. 2005), there is consensus that these largest arthropods currently inhabiting the oceans are the most likely candidates to invade the shallow waters of the Antarctic continental shelf under conditions of climate change (Meredith and King 2005; Thatje et al. 2005). Warming is likely to remove physiological barriers on lithodid crabs that currently place a limit on the invasion of shallow waters of the high Antarctic; a scenario that is especially likely for waters off the Antarctic Peninsula (Aronson et al. 2007, and references therein).

First records of lithodid crabs of the species *Neolithodes capensis* and *Paralomis birsteini* from 1,408 to 1,947 m, respectively, were made on the continental rise of Antarctica in the Bellingshausen Sea (García Raso et al. 2005). *P. birsteini* now appears to be widespread in the Bellingshausen Sea and so far remains the most commonly recorded Antarctic lithodid species south of 60°S (Arana and Retamal 1999; Thatje and Arntz 2004; Ahyong and Dawson 2006).

In the present work, we present new records of *P. birsteini* Macpherson, 1988a (= *P. spectabilis* Birstein and

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Vinogradov 1967, not Hansen 1908) from 1,100 to 1,400 m water depths in the Bellingshausen Sea, which constitute the shallowest records of lithodids on the continental slope/rise of Antarctica. Comparative analysis of a fragment of the mitochondrial cytochrome oxidase I gene from the sampled specimen of *P. birsteini* and sequences obtained from related species are discussed from a biogeographical and evolutionary point of view.

Materials and methods

Sampling—thirteen specimens of the lithodid genus *Paralomis* were video-recorded on the continental slope/rise off

Antarctica in the Bellingshausen Sea between 1,123 and 1,394 m water depth using the ROV-*Isis* during leg 166 of the RV James Clark Ross in January 2007 (Fig. 1, dive stations 5 and 6, Tyler et al. 2007). One male specimen (Fig. 2a–d) was sampled using the ROV's manipulator arm (66°24'81 S; 71°30'79 W; 1,394 m). The specimen was surfaced and died shortly thereafter.

Species identification—Morphological identification followed descriptions by Macpherson (1988a) in the form of the carapace and the antennal acicle, and additional comparison was made with other specimens of *P. birsteini* from the Musée National d'Histoire Naturelle (MNHN) in Paris. The specimen examined was 65 mm of Carapace Length (CL), measured from the orbit to the posterior carapace

Fig. 1 Biological dive stations with the ROV-*Isis* during leg 166 of RV James Clark Ross to the Antarctic Bellingshausen Sea in January/February 2007. Specimens of the lithodid crab *Paralomis birsteini* were encountered during dive Nos. 5 & 6 (starting points: 68°23'48 S; 71°32'95 W and 66°24'24 S; 71°81'82 W, respectively) on the continental slope/rise off the Western Antarctic Peninsula

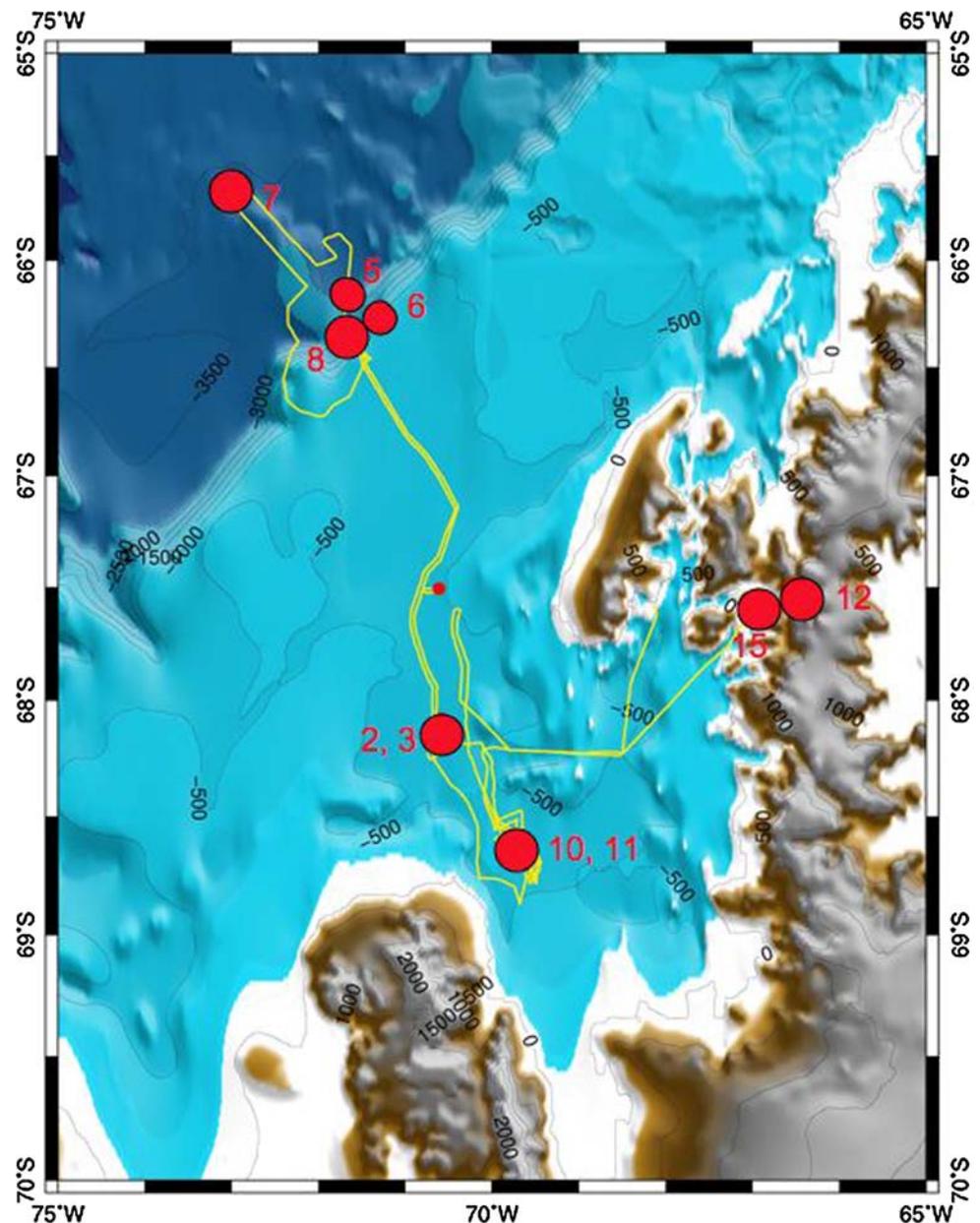
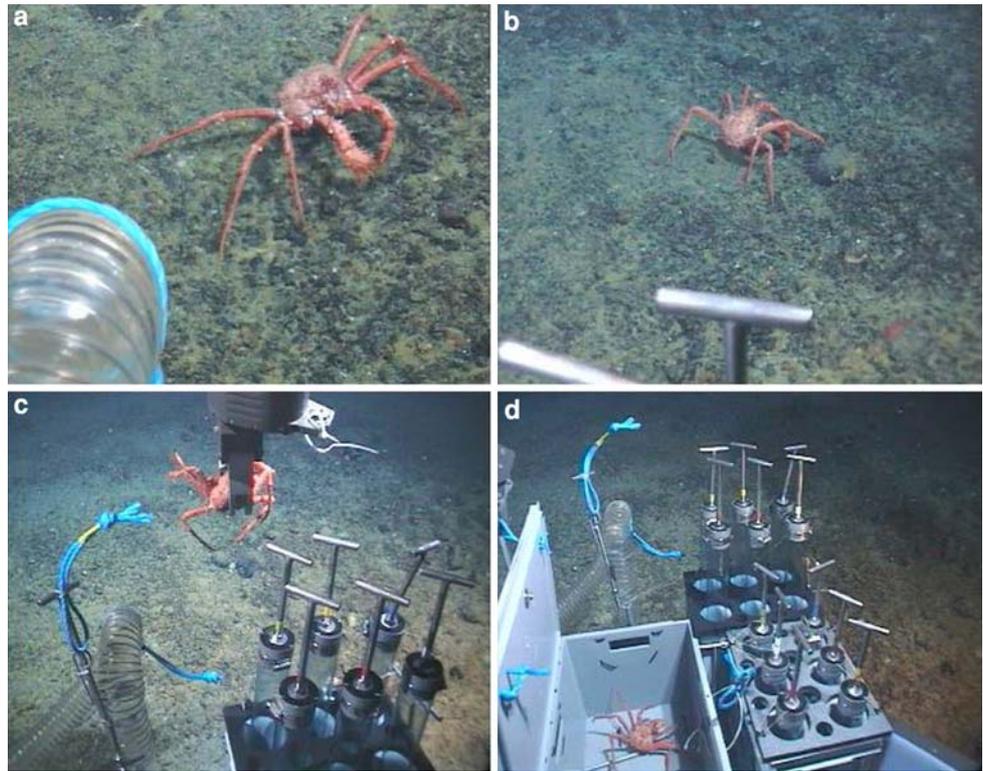


Fig. 2 Male specimen of *Paralomis birsteini* on the continental slope off Antarctica, Bellingshausen Sea, sampled by ROV-*Isis* during leg JCR166 on 25 January 2007. *P. birsteini* in its natural habitat (a, b), sampled by ROV-*Isis* (c, d), specimen CL = 64.9 mm



edge, and making it similar in size to the nominal *P. birsteini* (two paratypes studied: maximum CL = 67.78 mm; maximum CW = 67.74 mm).

DNA extraction, PCR, and sequencing—Tissue was sampled from the dactylus muscle of the retrieved sample and preserved in pre-cooled, 70% ethanol. Muscle samples from related species were also frozen very soon after death. DNA was extracted using Qiagen (Hilden, Germany) DNeasy Blood & Tissue kits following manufacturers' protocol. Using universal primers HCO2198 and LCO1490 (Folmer 1994) and Qiagen Taq polymerase approximately

850 bp of the mitochondrial COI gene were amplified (Saiki et al. 1988). The amplicons were cleaned using Qiagen QIAquick purification columns and sent to Macrogen Inc (Korea) for sequencing. Sequences can be retrieved from GenBank (Table 1).

Alignment and phylogenetic analyses—DNA sequences were aligned with no gaps or ambiguity using the Clustal W program (Thompson et al. 1994). Identical sequences were omitted from the analysis. Alignments were run through Modeltest 3.7 to obtain estimates of parameters for Maximum likelihood analysis. Phylogenetic trees were inferred

Table 1 Collection data for the lithodid specimens studied

Morphological ID	Lat	Long	Genbank accession	Identified by	Caught by
<i>Paralomis spinosissima</i>	53°36' S	36°38' W	EU493258	S. Hall	Long line off South Georgia
<i>Paralomis spinosissima</i>	53°36' S	36°38' W	EU493259	S. Hall	Long line off South Georgia
<i>Paralomis birsteini</i>	66°24'81 S	71°30'79 W	EU493260	S. Hall	JCR166 ROV- <i>Isis</i>
<i>Paralomis birsteini</i>	48°2' S	71°18' E	EU493261	S. Hall	Palangrier Aldbaran, Kerguelen St Pal. 60 6/12/1999
<i>Paralomis formosa</i>	53°36' S	36°38' W	EU493262	S. Hall	Long line off South Georgia
<i>Paralomis formosa</i>	53°36'4.42" S	36°38'43.86" W	EU493265	M. Belchier	Long line off South Georgia
<i>Paralomis granulosa</i>	54°47'59.14" S	65°15'0.15" W	EU493264	G.A. Lovrich	Artisanal trap fisheries, Beagle Channel
<i>Lithodes confundens</i>	54°47' S	65°15' W	EU493257	S. Hall	ICEFISH 04, st 10T1
<i>Neolithodes brodiei</i>	14°44'48 S	167°8'40 E	EU493263	Original, E. Macpherson; Reviewed S. Hall	BOAO Alis CP 2312 Vanuatu, 15/11/2004
<i>Pagurus bernhardus</i>	Unknown	Unknown	AF483157	Young et al. Genbank	Unknown

from DNA sequences using PAUP 4 beta version 10. Outgroups were taken from both within the family Lithodidae (*Neolithodes brodiei* and *Lithodes confundens*), and from a closely related group (*Pagurus bernhardus*). Inclusion of either lithodid outgroup did not change the outcome. In addition, an iterative Bayesian analysis was run with MrBayes 3.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The trees are presented for comparison (Fig. 3a, b).

Results

Thirteen specimens of *P. birsteini* were video recorded between about 1,123 and 1,394 m water depths on the Antarctic continental slope/rise in the Bellingshausen Sea (Figs. 1, 2). The present video footage included the record of one juvenile specimen of less than 2 cm CL in a gravel substratum that tried to escape the ROV's slurp gun and unfortunately was destroyed during hovering.

The genus *Paralomis* is well supported in the present molecular work, with species from the South Atlantic and

Indian oceans clustering very closely together. While the phylogeny doesn't resolve fully with molecular methods, the recognized morphospecies of *Paralomis spinosissima* and *P. formosa* are upheld (Fig. 3a, b). It may be of significance that the specimens of *P. birsteini* from the Crozet Islands appear in this analysis distinct from the specimen in question despite close morphological similarity. Comparative analysis of a fragment of the mitochondrial cytochrome oxidase I gene from the sampled specimen of *P. birsteini* and sequences obtained from related species indicates a close affinity of species of *Paralomis* from either side of the Scotia arc and the Bellingshausen Sea (Fig. 3a, b). Relationships within this group cannot be further resolved based on the present data.

Discussion

Paralomis birsteini is morphologically closely related to *P. spectabilis*, which so far has only been found off Iceland and eastern Greenland at depths ranging from 1,470 to 2,075 m (Macpherson 1988b) and *P. formosa* Henderson,

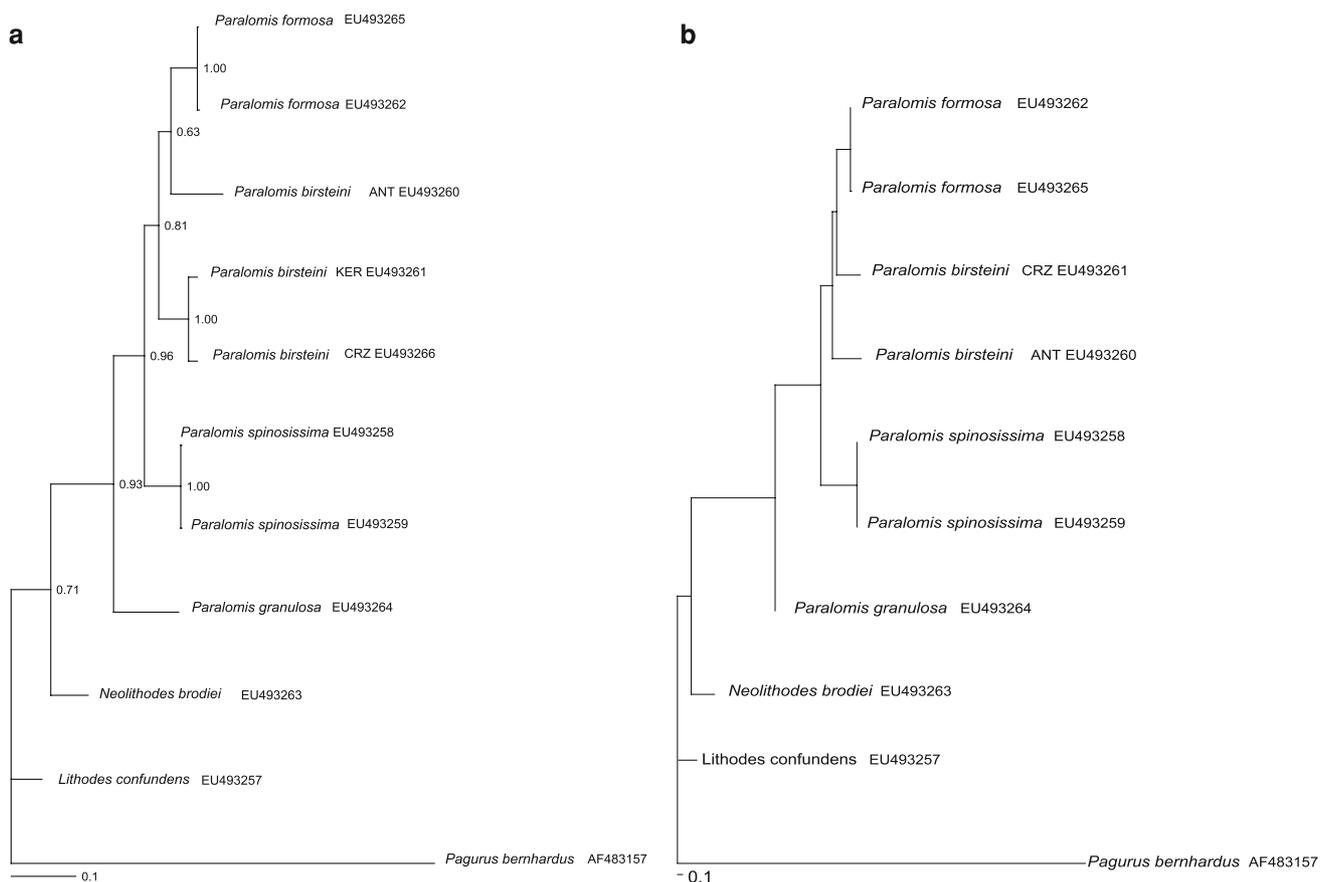


Fig. 3 a Phylogram produced using the MrBayes program, displaying Bayesian posterior probabilities at the internal nodes. Genbank accession numbers provided. *ANT* Bellingshausen Sea, *CRZ* Crozet, *KER*

Kerguelen Plateau. **b** Phylogram produced using PAUP 4 beta 10 using a Maximum Likelihood Method and a GTR_G_I model of substitution. *ANT* Bellingshausen Sea, *CRZ* Crozet, *KER* Kerguelen Plateau

which is common in waters off the island of South Georgia in the South Atlantic, at depths ranging from around 300 to 1,700 m (Thatje et al. 2005). *P. birsteini* is distinguished from *P. spectabilis* by shorter, stouter legs and the dactylus shorter than the propodus in *P. spectabilis*; the rostrum not pedunculate in *P. birsteini*, which is pedunculate in *P. spectabilis*; and the antennal acicle having short spines on its inner surface in *P. birsteini* (for details see Birstein and Vinogradov 1967; Macpherson 1988a). Personal observations of southern ocean *P. birsteini* specimens (Table 1) reveal a high degree of variability in these character states, and we recognize little substantial difference between the two species.

P. birsteini distinguishes from *P. formosa* in having longer legs and less prominent spines than in *P. formosa*; *P. formosa* has a large spine in the centre of its gastric region drawn out anteriorly which makes this region very convex. This spine is present but less pronounced in *P. birsteini*. *P. formosa* is also distinguishable by having long slender spines variable in number on both sides of its antennal acicle, and in having its carapace and walking legs covered completely in granules (although this disappears in larger individuals; for details see Macpherson 1988b).

Because only one specimen was retrieved by ROV for morphological and molecular studies it remains unclear whether other species of *Paralomis* co-occur with *P. birsteini* in the same habitat, which is not uncommon in this genus (Thatje and Arntz 2004). So far, species from other lithodid genera, *Neolithodes* and *Lithodes*, and *Paralomis* have been recorded for the Bellingshausen Sea and the Scotia arc region (Thatje and Arntz 2004; García Raso et al. 2005).

This record of a juvenile specimen of *P. birsteini* may indicate a reproductively active population in the area under investigation, given that lithodid species in the Southern Ocean are assumed to possess a low potential for larval dispersal. This was frequently discussed to be due to demersally drifting, lecithotrophic larvae with limited swimming ability, as found in several species from southern high latitudes based on field and laboratory observations (Lovrich 1999; Thatje et al. 2003; Watts et al. 2006; Reid et al. 2007). Radiation in Southern Ocean lithodids is thus likely dependent on adult migration, although one may indeed discuss the potential of demersally drifting larvae in bottom currents to distribute over long distance, given that larval development in Southern Ocean lithodids is likely exceeding 4–5 months in duration (Thatje et al. 2005). The topic needs much closer future investigation.

The phylogenetic analysis of the COI gene of *Paralomis* species from either side of the Scotia arc indicates a close affinity of species from South Georgia (*P. formosa* and *P. spinosissima*) with morphologically similar groups from as far away as Crozet in the Indian Ocean, and the Bellings-

hausen Sea (Table 1, Fig. 3a, b). Although defined species do show constant morphological characters that aid identification of morphospecies (Macpherson 1988a, b) the molecular analysis provides an initial suggestion of an ongoing or very recent speciation process within this group in the Southern Atlantic/Indian Ocean.

Analysis of the COI gene in the Bellingshausen Sea specimen of *Paralomis birsteini* allows us to suggest that gene flow within this morphotype is limited over distance, possibly to the extent of a cryptic speciation. Cryptic speciation has been previously discovered in other Antarctic taxa with limited dispersal potential (Held and Wägele 2005; Raupach and Wägele 2006). Genetic differences between *P. birsteini* from three different localities presented in this work (Fig. 3a, b) could point at a species complex that consists of at least two cryptic species. This supports the necessity of comparative analyses among type locality specimens from around Antarctica and adjacent seas in order to unravel biogeography and radiation patterns of Antarctic invertebrates in general.

Relatively close phylogenetic relationships between south Atlantic and Bellingshausen species supports hypothesis of a biogeographic relationship between these two areas (Gorny 1999), and gives further insight into the potential colonization of Antarctica from lower latitudes. Given that dispersal of larvae and thus potential gene flow between populations of Southern Ocean is discussed to be very low (Thatje et al. 2003; Watts et al. 2006), the close-relatedness of species across the Scotia Arc could point at a relatively recent separation of species and possible radiation in the Southern Ocean. Evolutionary timescales and exact radiation patterns of lithodid species remain obscure (Zaklan 2002; Thatje et al. 2005) and such work is so far particularly biased by the lack of sufficient numbers of lithodid specimens from Antarctic waters available for phylogenetic and molecular studies.

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