



Pole to pole: the deep-sea king crab *Lithodes couesi* (Decapoda: Lithodidae) in the Burdwood Bank, Southwestern Atlantic Ocean

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Abstract

In recent years, remote exploration around the Scotia Arc and waters off the Antarctic Peninsula yielded new records of Lithodidae suggesting our knowledge on their distribution is biased by our ability to sample areas with difficult access. In the present study, we used molecular methods to identify and report the occurrence of the deep-sea king crab *Lithodes couesi*, so far reported as a North Pacific lithodid, in the marine protected area (MPA) Namuncurá/Burdwood Bank II, Southwestern Atlantic Ocean; more than 10,000 km away from its known distribution. Our finding suggests that the distributions of deep-water lithodid species are more extensive than they have been previously thought. We emphasize the need for a worldwide key to identify lithodid species, and prompt to barcoding specimens, at least those from poorly known species or remote locations, in order to corroborate their specific status. Finally, our study stresses the importance of MPAs in the conservation of biodiversity, as well as the processes involved in its evolution.

Keywords MPA Namuncurá · Marine biogeography · Antipodal distribution · Lithodid

Introduction

Around the southern tip of South America (south of -40°), Scotia Arc and waters off the Antarctic Peninsula, lithodid species are represented by 3 genera (*Lithodes*, *Neolithodes* and *Paralomis*) and 13 species (Stevens and Lovrich 2014; Anosov et al. 2015). During recent years, remote exploration in this area has yielded new records of Lithodidae (e.g., García Raso et al. 2005; Thatje et al. 2008; Smith et al. 2012; Anosov et al. 2015), suggesting our knowledge on their distribution is biased by our ability to sample areas with difficult access (García Raso et al. 2005).

In 2013, Argentina created its first oceanic MPA, named “Namuncurá,” which is located over the plateau of the Burdwood Bank (National Law No 26,875 <https://www.argentina.gob.ar/areamarinanamuncura>, Fig. 1). The Burdwood Bank is a Subantarctic submerged plateau located in the northwestern Scotia Arc, 200 km south of Islas Malvinas/Falkland Islands and 50 km east of Isla de los Estados (Tierra del Fuego, Argentina). It comprises nearly 34,000 km², circumscribed to a plateau above the 200 m isobath (Law 26,875) surrounded by steep slopes that reach down to 3,500 m. The Burdwood Bank is featured by a high vulnerability due to its high biomass of sessile species, very sensitive to bottom trawling (Schejter et al. 2016). The aim of this MPA is to conserve an area with high environmental sensitivity, to promote the sustainable management of marine benthic ecosystems, and to facilitate scientific investigations oriented to the application of the ecosystem approach to fisheries, and the mitigation of the effects of global change (Law 26,875).

In this context, several research expeditions were conducted in the area, the results of which evidenced a benthic specific richness higher than in other productive areas of the Mar Argentino (e.g., Schejter et al. 2016). The intensive surveying of this area yielded the description of new species of several phyla, and new occurrences of known species. Studies also revealed that the benthic community changes

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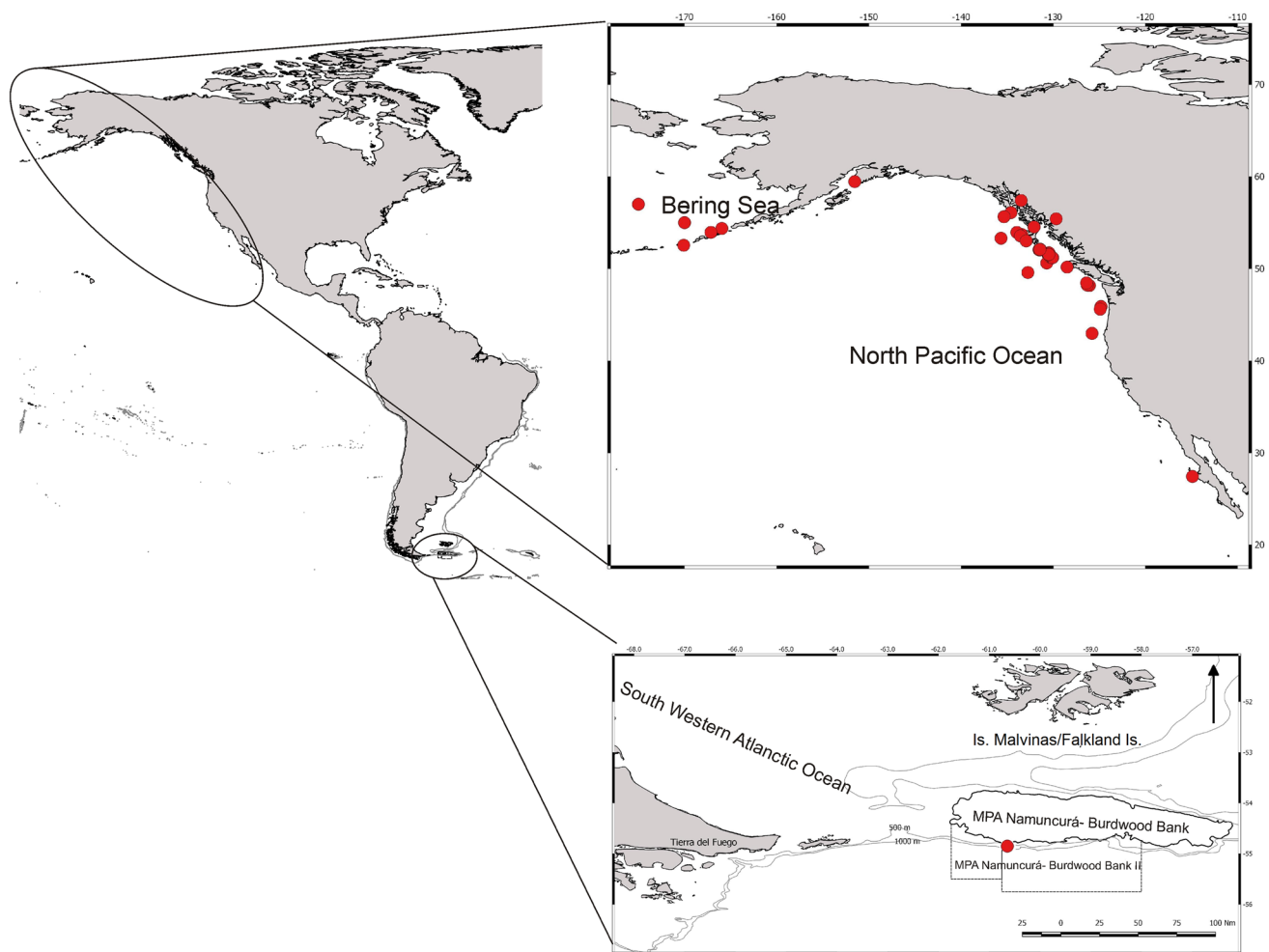


Fig. 1 Distribution of *Lithodes couesi* based on GBIF records and capture location in the MPA Namuncurá/Burdwood Bank II

in the slopes of the Burdwood Bank, which are dominated by cold water corals (Schejter et al. in prep.). Therefore, the MPA was recently enlarged to include its slope through the enactment of National Law No. 27,490, which created "Namuncurá-Burdwood Bank II" (<https://servicios.infoleg.gob.ar/infolegInternet/anexos/315000-319999/317651/norma.htm>) (Fig. 1).

In this study, we report the occurrence of the deep-sea king crab *Lithodes couesi*, so far reported as a North Pacific lithodid (Stevens and Lovrich 2014), in the Southwestern Atlantic ocean, more than 10,000 km away from its known distribution.

Materials and methods

Sampling

An individual of an unidentified lithodid species was collected on board the RV Puerto Deseado during April 2016

using a bottom otter trawl of 9.1 m mouth opening and 4 cm mesh size within the recently created MPA Namuncurá/Burdwood Bank II. The specimen was sorted from the benthic catch and fixed in ethanol 96°.

Molecular identification

DNA extraction, amplification, and sequencing

DNA was extracted from muscle of the ethanol preserved specimen using a salting-out protocol (Reiss et al. 1995). The COI gene was amplified using primers LCO1490/HCO2198 (Folmer et al. 1994). Polymerase chain reaction (PCR) was performed using a MJ Research thermal cycler in a 10 µl reaction consisting of 20 ng of DNA, 0.2 mM of each dNTP, 2 mM MgCl₂, 0.15 µM of each primer, 0.25 U of Taq, the corresponding buffer, and ddH₂O. Thermal cycling conditions consisted of an initial denaturation step of 94 °C for 3 min followed by 35 cycles at 94 °C for 30 s, 40 °C for 50 s, 72 °C for 1 min, and a final extension at

72 °C for 7 min. The amplification product was sequenced in Macrogen. The COI sequence obtained was deposited in GenBank (GB, accession no. MN047311).

Data analysis

The COI sequence was compared against GB (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch) and the Barcode of Life database (BOLD) (http://v3.boldsystems.org/index.php/IDS_OpenIdEngine). Furthermore,

the sequence was aligned with sequences of all the species of *Neolithodes* and *Lithodes* available in GB (Table 1) and all the sequences of *L. couesi* available in the BOLD database (Table 2) using BioEdit v7.1.3 with default gap-opening and gap-extension penalties (Hall 1999). A phylogenetic reconstruction was conducted using Bayesian inference. A COI sequence of *Paralomis granulosa* obtained from GenBank (Table 1) was used as outgroup in the phylogenetic analysis. The bayesian analysis was performed using Mr. Bayes v3.1.2 (Ronquist and Huelsenbeck 2003). Model selection

Table 1 GenBank accession numbers of lithodid sequences used in the phylogenetic reconstruction

Species	GenBank accession numbers
<i>Neolithodes grimaldii</i>	JQ305971–JQ305973
<i>Neolithodes duhameli</i>	HM020892, HM020896
<i>Neolithodes brodiei</i>	HM020893–HM020894
<i>Neolithodes sp.</i>	HM020895
<i>Neolithodes asperrimus</i>	HM020890–HM020891
<i>Neolithodes diomedea</i>	KC196528
<i>Lithodes turkayi</i>	KC196529, KC196531, KC196539, KC196540
<i>Lithodes formosae</i>	GU289678
<i>Lithodes paulayi</i>	GU289677
<i>Lithodes aequispinus</i>	KC196523–KC196524
<i>Lithodes maja</i>	FJ581740–FJ581746, MG934982, MG935272, KT209132, KT209176, KT209197, KT209315, KT209524, KT209429, KT209538, KT209480
<i>Lithodes longispina</i>	AB476813–AB476817
<i>Lithodes nintokuuae</i>	AB375149–AB375158
<i>Lithodes santolla/confundens</i> ^a	HM020897–HM020898, HM020900–HM020901, KM887460, KM887467, KM887469–KM887471, KM887487, KM887489–KM887492, KM887494–KM887497, KY426275
<i>Lithodes ferox</i>	HM020903, KY426276
<i>Lithodes murrayi</i>	HM020899
<i>Lithodes aotearoa</i>	HQ944653–HQ944655, HQ944658–HQ944661, HQ944668, HQ944671–HQ944672
<i>Lithodes couesi</i>	Same as Table 2
<i>Paralomis granulosa</i>	HM020926

^aSpecies complex

Table 2 Results of the sequence comparison made by the BOLD identification engine

BOLD record	Collection location	% similarity	Latitude	Longitude	Depth (m)	GenBank accession number
DSALA010-06	Aleutian Islands, USA	99.83	52.33	– 170.47		
DSALA011-06	Aleutian Islands, USA	99.83	52.33	– 170.47		
FCDPA055-04	British Columbia, Canada	99.82	53.56	– 133.56	1075	DQ882085
DSALA013-06	Aleutian Islands, USA	99.66	52.33	– 170.48		
DSCRA073-06	British Columbia, Canada	99.66	52.03	– 131.36		
DSCRA074-06	British Columbia, Canada	99.66	52.03	– 131.36		
DSALA012-06	Aleutian Islands, USA	99.33	52.33	– 170.47		
FCDPA054-04	British Columbia, Canada	99.32	53.56	– 133.56	1075	DQ882086
DSALA009-06	Aleutian Islands, USA	99.16	52.33	– 170.47		

was made using jModelTest (Guindon and Gascuel 2003; Posada 2008) which suggested a HKY + I + G model. The analysis was run for 5,000,000 generations. Trees were sampled every 100 generations. To determine convergence, we examined the average standard deviation of split frequencies, and 12,500 trees were discarded as burn in. Genealogical relationships among specimens of *L. couesi* (Table 2) were reconstructed as median-joining networks using Network v4.6.1.1 (Bandelt et al. 1999).

Results

Specimen collected

The individual was collected on the southwest slope of the Burdwood Bank at 605 m depth, at -54.82° , -60.69° (Fig. 1). Bottom temperature and salinity were 4.54°C and 34.19, respectively. The individual was a non-ovigerous female of 40.3 mm of carapace length and 26 g wet mass approximately (after ethanol fixation) (Fig. 2).

Molecular identification

The sequence obtained in this study was assigned to the species *L. couesi* by the identification engine of BOLD systems ($>99\%$ similarity, Table 2), and formed a monophyletic group in the phylogenetic reconstruction performed with all *L. couesi* sequences downloaded (posterior probability = 1). Regarding its genealogical relationships, the specimen captured in the present study was more closely related to individuals from Alaska and British Columbia, than some specimens from Alaska and British Columbia between themselves (Fig. 3).

Morphology

The collected specimen fits the diagnosis of *L. couesi* (Benedict 1895), except for the fact that the carapace has rounded granules rather than “short, sharp, conical spines.” However, it also conforms with the description of *Lithodes turkayi* or *Lithodes murrayi* given by Macpherson (1988) and could be easily confounded with them.

Fig. 2 **a** Dorsal, **b** posterior, **c** ventral, and **d** lateral views of the *Lithodes couesi* collected specimen. **e** Dorsal and **f** ventral views of the third walking leg. Scale bar: 25 mm



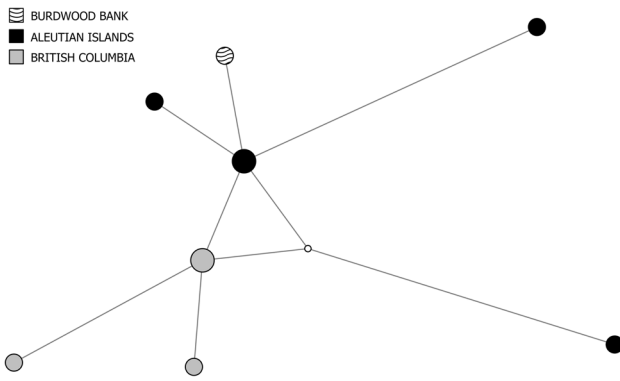


Fig. 3 Median-joining network of mitochondrial COI haplotypes of *Lithodes couesi*. Each circle represents a distinct haplotype; circle area and line length are proportional to haplotype frequency and number of mutational steps, respectively. The area of the smallest full circle corresponds to a frequency of one. The small empty circle is a median vector. The length of the shortest line corresponds to one mutational step. GenBank or BOLD accession numbers same as in Table 2

Discussion

This finding greatly puzzled us since the southernmost record of *L. couesi* before this study was off Bahía Tortugas, Baja California at 25.40°, -113.27°, and 1344 m depth (GBIF 2019). The specimen of *L. couesi* found in the Burdwood Bank slope represents the first case in the genus *Lithodes* of a species that inhabits the center of origin of the superfamily, i.e., the North Pacific Ocean (Zaklan 2002), and emerges in the Subantarctic (Hall and Thatje 2009). So far, species occurring in the Subantarctic were different to those of the subarctic/boreal region (Zaklan 2002). The only lithodid species with such ample distributional range, i.e., from 36.70° to -54.81° off South Georgia, always at depths >700 m, is *Neolithodes diomedea* which is sporadically reported along the East Pacific (Stevens and Lovrich 2014; GBIF 2019; Spivak et al. 2019). Furthermore, the specimen of *L. couesi* found in the Burdwood Bank is more closely related to some specimens of the North Pacific than some specimens of the North Pacific between themselves.

Regarding antipodal records of other marine organisms, Arkhipkin et al. (2010) reported the occurrence of three endemic North Pacific deep-water species, i.e., the zoarcid fish *Lycodapus endemoscotus*, the gonate squid *Gonatopsis octopedatus*, and the grenadier *Albatrossia pectoralis*, for the first time in the Southwestern Atlantic (i.e., on the northern side of Burdwood Bank, south of Islas Malvinas/Falkland Islands, and on the eastern side of Burdwood Bank, respectively). The authors suggest that these deep-water species might have dispersed from their common species ranges with Pacific Deep Water along the western slope of America and through the Drake Passage. More recently, the North

Pacific grenadier *Coryphaenoides acrolepis* was found on the northeast slope of the Burdwood Bank (Laptikhovskiy et al. 2013). *Lithodes couesi* is a deep-water species, with larvae probably associated with the bottom [Somerton 1981; c.f. *Lithodes aequispinus* (Jewett et al. 1985) and *Lithodes santolla* (Lovrich 1999; Tapella et al. 2012)], making it unlikely that this species could have been introduced via ship ballast waters, since ship water intakes are made close to the surface. We hypothesize that this species arrived at the Burdwood Bank via deep-sea circulation as in the case of those reported by Arkhipkin et al. (2010).

Morphologically, the specimen found in the slope of the Burdwood Bank could have been confounded for *L. turkayi*, especially since *L. turkayi* is known to inhabit this area (Stevens and Lovrich 2014; Anosov et al. 2015; Spivak et al. 2019). So far, lithodid species distributions have been thought to be more restricted than they actually are (García Raso et al. 2005; Olguín et al. 2015; this study). Therefore, we emphasize the need for a worldwide key to identify lithodid species (rather than locally restricted ones); and we prompt to barcoding specimens, at least those from poorly known species or remote locations, in order to corroborate their specific status.

Lithodes couesi lives at depths that are infrequently sampled and in rocky habitats that are hostile to sampling by trawls (Stevens and Lovrich 2014), it is probable that new records will be made in intermediate locations, i.e., between its previously known distribution and the Burdwood Bank, as sampling in these difficult to sample areas increases. Our finding highlights the need to intensify the sampling effort in the area, and to use molecular genetic methods to identify individuals. Furthermore, it stresses the importance of MPAs in the conservation of biodiversity as well as the processes involved in its evolution.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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