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DNA Barcoding of Antarctic Freshwater Copepod *Boeckella poppei* (Crustacea: Copepoda: Calanoida: Centropagidae) Inhabiting King George Island, South Shetland Islands, Antarctica

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ABSTRACT

The Antarctic freshwater copepod, *Boeckella poppei* (Mrazek, 1901), has the widest range of distribution extending from southern South America to Antarctic continent, among all *Boeckella* species. *Boeckella poppei* is the only freshwater copepod known to be inhabiting the Antarctic continent. In present study, we analyzed the DNA barcodes of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene of *B. poppei* from King George Island, Antarctica. The intraspecific genetic distances varied from 0% to 13% and interspecific genetic distances ranged from 11% to 14%. The overlap of DNA barcode gap suggests careful threshold-based delimitation of species boundaries.

Keywords: *Boeckella poppei*, Antarctica, DNA barcode, *COI*, copepod

INTRODUCTION

Boeckella de Guerne & Richard, 1889, is a freshwater calanoid copepod belonging to the family Centropagidae, which is mostly restricted to the Southern Hemisphere (Bayly, 1992). A total of 14 species of *Boeckella* have been reported from the southern latitudes of South (Bayly et al., 2003; Adamowicz et al., 2007; Walter and Boxshall, 2018; Maturana et al., 2019).

Boeckella poppei (Mrázek, 1901) is the only freshwater copepod known to be distributed in Antarctica and extending widely from southern South America to Antarctica (Heywood, 1977; Bayly and Burton, 1993; Menu-Marque et al., 2000; Bayly et al., 2003; Gibson and Bayly, 2007; Nedbalová et al., 2009; Maturana et al., 2018, 2019).

A 650 base pair (bp) section of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) DNA barcode has been used to distinguish species belonging to the subphylum Crustacea (Costa et al., 2007) including copepods (Machida and Tsuda, 2010; Blanco-Bercial et al., 2011, 2014; Cepeda et al., 2012; Aarbakke et al., 2014; Sharma et al., 2014). Despite the widespread distribution of *B. poppei* in Southern Hemi-

sphere, the DNA barcode information of the species has been reported from two geographical regions: Argentina (Cabo Virgenes and Tierra del Fuego) (Adamowicz et al., 2007), and Signy Island (Maturana et al., 2020). Here, we report the DNA barcodes of *B. poppei* from King George Island and verify the usefulness of DNA barcode for this species compared with other populations and closely related species. The specimens of *B. poppei* were collected from Barton Peninsula (62°14'24"S, 58°44'36"W) of King George Island, South Shetland Islands (Fig. 1) in January 2020. Genomic DNA was extracted from the whole body using a QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The *Boeckella* specific primers (Maturana et al., 2019) were used under the following polymerase chain reaction conditions: initial denaturation at 94°C for 1 min; 10 cycles of 94°C for 1 min, 40°C for 90 s and 72°C for 1 min; followed by 30 cycles of 94°C for 1 min, 46°C for 90 s and 72°C for 90 s; and a final extension at 72°C for 10 min. We have retrieved GenBank sequences of *B. poppei* from Signy Island (MN87317–MN087400) and Argentina (DQ356568–DQ356570, DQ356576) and *Boeckella brasiliensis* (DQ356544–DQ356546) as an outgroup. The sequence

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Table 1. Pairwise genetic distances based on Kimura 2-parameter of *COI* DNA barcodes between *Boeckella poppei* populations and outgroup species

Species name	Location	N	No.	1	2	3	4	5	6	7	8	9	10	
<i>Boeckella poppei</i>	King George Island	Barton	20	1										
			12	2	0.003									
	Signy Island	Changing	8	3	0.003	0.001								
			14	4	0.003	0.007	0.007							
			14	5	0.003	0.003	0.003	0.005						
	Argentina	Cabo Vigenes	7	6	0.005	0.003	0.003	0.009	0.005					
			15	7	0.003	0.007	0.007	0.003	0.005	0.009				
			14	8	0.002	0.006	0.006	0.002	0.004	0.008	0.002			
			1	9	0.123	0.122	0.121	0.121	0.122	0.122	0.122	0.121		
	Argentina	Tierra del Fuego	3	10	0.126	0.123	0.123	0.123	0.123	0.124	0.123	0.123	0.123	0.012
			3	11	0.110	0.118	0.119	0.113	0.116	0.118	0.113	0.112	0.126	0.133

N, number of individuals.



Fig. 1. Location of *Boeckella poppei* (Mrázek, 1901) field sampling site (62°14'24\"S, 58°44'36\"W) at Barton Peninsula, King George Island (Google Earth V, 2020). This map from Google, Maxar Technologies.

Table 2. Within group mean distance based on Kimura 2-parameter of *Boeckella poppei* populations and outgroup species

Species name	Location	Mean distance	
<i>Boeckella poppei</i>	King George Island	Barton	0
		Signy Island	Changing
	Heywood		0.002
	Light		0.003
	Pumphouse		0.004
	Sombre		0.005
	Tranquil		0.003
	Twisted		0.001
	Argentina		Cabo Vigenes
		Tierra del Fuego	0.006
	<i>Boeckella brasiliensis</i>	Argentina	0.021

alignment, genetic distance calculation using the Kimura 2-parameter (K2P) model and neighbor-joining (NJ) phylogenetic tree construction based on K2P genetic distance and the bootstrap with 1000 iteration have been performed by using MEGA X (ver. 10.1.8) (Kumar et al., 2018).

RESULTS AND DISCUSSION

The length of three *COI* sequences obtained was 403 bp and these new sequences were registered to GenBank (accession No. MT590771–MT590773 and MW008651–MW008667).



Fig. 2. Neighbor joining tree based on Kimura 2-parameter distances of *COI* DNA barcodes *Boeckella poppei* individuals and out-group species.

Within the species, the pairwise genetic distances of *B. poppei* based on K2P model ranged from 0% to 13% and inter-specific genetic distances ranged from 11% to 14% (Table 1). Within population mean distance based on K2P model showed the lowest genetic distance value observed from Barton population (King George Island) (Table 2) and the Barton populations showed low level of pairwise genetic distance with Signy Island populations ranged from 2% to 5% even though 700 km geographic distance apart. The NJ phylogenetic tree successfully delimited *B. poppei* from the out-group species (Fig. 2). The previous molecular systematics of Centropagidae in Argentina based on *COI* gene showed that the average K2P genetic distance of the genus *Boeckella* varied from 10.9% to 25% (Adamowicz et al., 2007). Consequently, we observed an overlap between intraspecific and interspecific variations in *B. poppei*. Usually, the DNA barcode overlap does not affect the identification of unknown species in a thoroughly sampled tree but does have an impact when the sampled group is incomplete (Meyer and Paulay, 2005). In conclusion, the results suggest a threshold-based delimitation of species boundary is not promising for identification of *B. poppei* and closely related species, suggesting the need for careful consideration and large sample sizes.

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CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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