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The host-specific whale louse (*Cyamus boopis*) as a potential tool for interpreting humpback whale (*Megaptera novaeangliae*) migratory routes

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ABSTRACT

The whale louse *Cyamus boopis* is a host-specific amphipod that parasitizes humpback whales (*Megaptera novaeangliae*) across the world. Humpback whales from the Southern Hemisphere are currently separated into seven breeding stocks, each with its own migration route to/from Antarctic waters. The aim of this study was to determine the population structure of *C. boopis* from the Southern Hemisphere using cytochrome oxydase I sequences, and compare it to that of its host species found in previous studies. High haplotype and nucleotide diversities in *C. boopis* were observed, and the populations from western south Atlantic (WSA: Brazil + Argentina − Breeding stock A) and western south Pacific (WSP: Australia - Breeding stock E) did not show any significant difference but were differentiated from populations of eastern south Atlantic (ESA: Namibia - Breeding stock B) and the north Pacific. The genetic homogeneity between WSA and WSP populations, might reveal a higher genetic transfer within the Southern Hemisphere, since the feeding grounds of whales which are distributed throughout the circumpolar Southern Ocean could allow inter-mixing of individuals from different breeding populations during the feeding season. The present data reinforces that population dynamics of humpback whales seem more complex than stable migration routes, which could have implications for both management of the species and cultural transmissions of behaviours.

1. Introduction

Migration patterns of humpback whales *Megaptera novaeangliae* (Borowski, 1781) in the Southern Hemisphere have been extensively studied over the last few decades (Stevick et al., 2004, 2010; Rosenbaum et al., 2009; Felix et al., 2012; Jackson et al., 2014). Humpback whales are cosmopolitan, and for management and conservation purposes, breeding areas of the Southern Hemisphere were historically divided into seven stocks, according with their migration patterns and breeding areas. Breeding stock A includes the humpback whales of the western south Atlantic, stock B of the eastern south Atlantic, stock C of the western Indian Ocean, stock D of the eastern

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Indian Ocean, stock E of the western south Pacific, stock F of Oceania and stock G of theeastern south Pacific (IWC, 2015).

Although these divisions suggest typical routes for each one of the seven breeding stocks, occasional exchanges of individual humpback whales between oceans have been recorded within the Southern Hemisphere (e.g. Pomilla and Rosembaum, 2005; Stevick et al., 2010). Individual identification of humpback whales by photo identification of fluke and genetic markers largely corroborate the breeding stocks proposed by the International Whaling Commission (IWC) and the low gene flow between hemispheres (Jackson et al., 2014). According to Jackson et al. (2014), gene flow has been more restricted between inter-hemispheric oceans than across the Southern Hemisphere oceans. Therefore, resightings of individual humpbacks in different stocks of the same hemisphere might corroborate the potential for gene flow between southern breeding grounds (Stevick et al., 2010). Since humpback whales from both hemispheres are geographically and genetically differentiated, reflecting low organismal gene flow, three subspecies of *M. novaeangliae* were recently proposed, *M. novaeangliae australis* (Lesson, 1828) from the Southern Hemisphere, *M. novaeangliae novaeangliae* (Borowski, 1781) from the North Atlantic Ocean and *M. novaeangliae kuzira* (Gray, 1850) from the North Pacific Ocean (Jackson et al., 2014).

Rosenbaum et al. (2009) inferred the population structure of southern Atlantic humpback whales and found that although rare transoceanic migration events had been recorded (Pomilla and Rosembaum, 2005), there were different demographic aggregations with low genetic divergence and expected migration rates between populations of the two southern Atlantic stocks A (western south Atlantic stock) and B (eastern south Atlantic stock), corroborated by identical song structure among these stocks in a single breeding season (Darling and Sousa-Lima, 2005). Thus, male-mediated gene flow of these two populations may occur during migration or in feeding areas, as pointed out by Rosenbaum et al. (2009). Jackson et al. (2014) also compared the population of the southern Atlantic Ocean with those from the southern Pacific and Indian Ocean, and observed a low but significant differentiation with high migrations rates between the Southern Hemisphere oceans.

Dozens of *M. novaeangliae* carcasses wash up on the Brazilian coast every year (Groch et al., 2012) and a few of them strand along the Patagonian coast especially from July to November, when humpback whales migrate from temperate and polar feeding grounds to the tropics for breeding and nesting. These carcasses represent an important source of information on a wide range of questions from skeleton abnormalities to reproductive endocrinology (Groch et al., 2012; Mello et al., 2017), as well as diseases (e.g. Ott et al., 2016) and parasitic data (Moura et al., 2013). Among the ectoparasites, crustacean amphipods called “whale lice” are commonly found on *M. novaeangliae*. These whale lice have no free-swimming stage, so their transmission can only occur during contact between whales (Rowntree, 1996; Kaliszewska et al., 2005). The whale lice constitute the entire family Cyamidae Rafinesque, 1815, that comprise 28 species within eight genera, where *Cyamus* Latreille, 1796 is the most speciose genus, and the majority of species within *Cyamus* are parasites of see whales (Iwasa-Arai and Serejo, 2018). *Cyamus boopis*Lütken, 1870 is the only species found living on humpback whales, and it has been recorded from *M. novaeangliae* all over the world (Lütken, 1870; Hurley, 1952; Margolis, 1955; Gruner, 1975; Fransen and Smeenk, 1991; Rowntree, 1996; Abollo et al., 1998; De Pina and Giuffra, 2003; Iwasa-Arai et al., 2017a,b).

Host-parasite relationships provide a useful comparative framework for examining evolutionary processes, as rates of molecular evolution in parasites have been shown to be considerably faster than in their hosts (Page and Hafner, 1996; Kaliszewska et al., 2005). In the Cyamidae, synonymous sequence divergences can be 10 times faster than in their whale hosts for homologous markers (Kaliszewska et al., 2005), considering their short generation time (Callahan, 2008; Woolfit, 2009). Therefore, the genetic structure of cyamids could also reveal encounters between whales of different stocks. Hence, historical demographic patterns in cyamids should be more evident than in their hosts.

Populational studies of cyamids are still scarce, limited to analyses using the mitochondrial gene cytochrome *c* oxydase subunit I (COI) fragments of whale lice from right whales (*Eubalaena* spp.). (Kaliszewska et al., 2005) and from gray whales [*Eschrichtius robustus* (Lilljeborg, 1861)] (Callahan, 2008). Both studies showed high levels of genetic diversity for all cyamid species and no population structure was found. Moreover, Kaliszewska et al. (2005) observed a high haplotype diversity in right whale lice, and although the same species populations exhibited genetic homogeneity, cyamids from different species of *Eubalaena* have been geographically separated for several million years and therefore constitute three distinct lineages, one from each *Eubalaena* species.

To date, no studies have been performed on the population genetic structure of *C. boopis*, ectoparasite of one of the most studied and cosmopolitan whales in the world. The aim of our study was to estimate the population structure of *C. boopis* from three *M. novaeangliae* breeding stocks of the Southern Hemisphere (stocks A, B and E) based on COI gene sequences and compare with sequences of *C. boopis* from the Northern Hemisphere, to establish whether that population structure is correlated with humpback whales genetic structure found in previous studies.

2. Material and methods

2.1. Sampling locations

Samples of *C. boopis* were collected on 11 humpback whales carcasses from the western south Atlantic (eight whales from five locations in Brazil and one in Argentina), eastern south Atlantic (one whale from Namibia), and western south Pacific (two whales from two locations in eastern Australia) (Table 1). Localities refer to provinces where the whales were found stranded for Brazilian and Australian specimens, which have more than one location sampled. Abbreviations used for localities include: WSA for localities from breeding stock A in western south Atlantic; ESA for locality from breeding stock B in eastern south Atlantic; WSP for localities from breeding stock E in western south Pacific; and NH for localities from breeding stock north Pacific in Northern Hemisphere (Fig. 1; Table 1).

Three pereopods (P5–P7) from each cyamid were removed and preserved in absolute ethanol and the remaining body was deposited as hologenophores at the following museum collections: Museu Nacional, Rio de Janeiro, Brazil (MNRJ); Australian Museum, Sydney, Australia (AM); and Museum Victoria, Melbourne, Australia (NMV).

2.2. DNA extraction, amplification and sequencing

Sixty-seven specimens of *C. boopis* were subjected to genetic analyses (Table 1). Total genomic DNA was obtained by CTAB extraction (lysis buffer: CTAB 2%; RNAse 10 mg/mL; proteinase K 10 mg/mL), followed by precipitation in isopropanol (Gusmão and Solé-Cava, 2002). A fragment of the COI gene was amplified using the primers Jercy (5′ TAC CAA CAT TTA TTC TGR TTT TTY GG 3′) and Patcy (5′ ACT AGC ACA TTT ATC TGT CAC ATT A 3′) (Kaliszewska et al., 2005). Amplification reactions included approximately 10–50 ng of genomic DNA, 1 U of GoTaq Flexi DNA polymerase (Promega), 3 μL of Green GoTaq Flexi Buffer (5 ×), 0.2 mM of dNTPs, 2.5 mM of $MgCl_2$, 0.3 μM of each primer and 4 μg of BSA in a final volume of 15 μL.

Reactions were carried out with an initial denaturation step of 5 min at 95 °C, followed by 35 cycles consisting of a denaturation step of 20 s at 95 °C, an annealing step of 30 s at 50 °C, and an extension step of 50 s at 72 °C; and a final extension step of 2 min at 72 °C. PCR products amplified were purified using the Agencourt AMPure PCR purification kit in the epMotion 5075 Automated Pipetting System (Eppendorf) and