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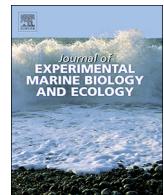
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The host-specific whale louse (*Cyamus boopis*) as a potential tool for interpreting humpback whale (*Megaptera novaeangliae*) migratory routes



Tammy Iwasa-Arai^{a,b,*}, Cristiana S. Serejo^b, Salvatore Siciliano^c, Paulo H. Ott^{d,e},
Andrea S. Freire^f, Simon Elwen^g, Enrique A. Crespo^h, Adriana C. Colosioⁱ, Vitor L. Carvalho^j,
Ghennie T. Rodríguez-Rey^{k,1}

^a Programa de Pós-Graduação em Zoologia, Museu Nacional/Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 20940-040, Brazil

^b Laboratório de Carcinologia, Departamento de Invertebrados, Museu Nacional/UFRJ, Rio de Janeiro, RJ 20940-040, Brazil

^c Instituto Oswaldo Cruz/Fiocruz and Grupo de Estudos de Mamíferos Marinhos da Região dos Lagos, Pavilhão Mourisco – sala 122, Av. Brasil, 4365, Manguinhos, Rio de Janeiro, RJ 21040-900, Brazil

^d Laboratório de Biodiversidade e Conservação, Universidade Estadual do Rio Grande do Sul (UERGS), Unidade do Litoral Norte, Osório, RS 95520-000, Brazil

^e Grupo de Estudos de Mamíferos Aquáticos do Rio Grande do Sul (GEMARS), Torres, RS 95560-000, Brazil

^f Laboratório de Crustáceos e Plâncton, Departamento de Ecologia e Zoologia, Universidade Federal de Santa Catarina, Florianópolis, SC 88040-900, Brazil

^g Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, C/o Sea Search Research and Conservation NPC, 4 Bath Rd, Muizenberg, Cape Town 7945, South Africa

^h Marine Mammal Laboratory, Centro Nacional Patagónico, CONICET, University of Patagonia, Puerto Madryn, Chubut 9120, Argentina

ⁱ Instituto Baleia Jubarte, Rua Barão do Rio Branco, 125, Centro, Caravelas, BA 45900-000, Brazil

^j Centro de Reabilitação de Mamíferos Marinhos, Associação de Pesquisa e Preservação de Ecossistemas Aquáticos (AQUASIS), Av. Pintor João Figueiredo, s/n, Sesc Ipirana, Praia de Ipirana, Caucaia, CE 61627-250, Brazil

^k Laboratório de Biodiversidade Molecular, Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 21941-590, Brazil

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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 oxidase 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

* Corresponding author at: Programa de Pós-Graduação em Zoologia, Museu Nacional/Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 20940-040, Brazil.

E-mail addresses: tammy@grad.ufsc.br (T. Iwasa-Arai), andrea.freire@ufsc.br (A.S. Freire), kike@cenpat-conicet.gob.ar (E.A. Crespo), adriana.colosio@baleijubarte.org.br (A.C. Colosio), vitorluz@yahoo.com.br (V.L. Carvalho), gtrodriguezr@gmail.com (G.T. Rodríguez-Rey).

¹ Present address: Departamento de Ciencias Biológicas, Universidad de Caldas, Manizales, Cl. 65 #26-10, Colombia.

Indian Ocean, stock E of the western south Pacific, stock F of Oceania and stock G of the eastern 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 migration 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 sea 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 oxidase 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 MgCl₂, 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

Table 1

Cyamus boopis samples data. Sequences from the North Pacific were obtained from GenBank (*).

Stranding location	Coll. no.	Whale ID	Collection year	Cyamids sequenced	GenBank access. no.
Western South Atlantic (WSA) – breeding stock A					
Ceará (CE), Brazil	MNRJ25986	AQUASIS349	2009	3	MG720497–MG720499
Bahia (BA), Brazil	MNRJ25982	IBJ226	2006	4	MG720490–MG720493
Bahia (BA), Brazil	IBJ668	IBJ668	2012	3	MG720494–MG720496
Rio de Janeiro (RJ), Brazil	MNRJ25993	GEMM115	2006	4	MG720519–MG720522
Santa Catarina (SC), Brazil	MNRJ25995	–	2014	10	MG720533–MG720542
Rio Grande do Sul (RS), Brazil	MNRJ25990	GEMARS1684	2015	5	MG720523–MG720527
Rio Grande do Sul (RS), Brazil	GEMARS1695	GEMARS1695	2016	5	MG720528–MG720532
Puerto Madryn, Argentina (AR)	MNRJ28869	Megnov003	2011	11	MG720479–MG720489
Eastern South Atlantic (ESA) – breeding stock B					
Walvis Bay, Namibia (NA)	MNRJ28870	–	2012	11	MG720500–MG720510
Western South Pacific (WSP) – breeding stock E					
Victoria (VIC), Australia	NMVJ20994	–	2009	2	MG720511; MG720512
New South Wales (NSW), Australia	AMP86650	–	2009	6	MG720513–MG720518
North Hemisphere (NH) – breeding stock North Pacific					
Isla Socorro, Mexico (MEX)	–	–	–	2	FJ751158*; FJ751159*
Alaska, USA (AL)	–	–	–	1	DQ095150*

sequenced in both directions using an ABI 3500 automated DNA sequencer (Applied Biosystems).

2.3. Intraspecific variability and population genetic differentiation

Three sequences of *C. boopis* from the North Pacific in the Northern Hemisphere were obtained from GenBank (National Center for Biotechnology Information, NCBI) and correspond to cyamids from two humpback whales found in Socorro Island, Mexico, and Alaska.

The sequences obtained were edited using SEQMAN II 4.0 (DNAstar Inc.), aligned with the Clustal W algorithm implemented in MEGA 6 (Tamura et al., 2013) and checked manually for misalignments. Nucleotide divergences between sequences were estimated in MEGA, using the K2P distance model. Number of haplotypes (N_H) and their frequencies, as well as haplotype (h) and nucleotide (π) diversities were estimated using ARLEQUIN 3.5.2 (Excoffier et al., 2005).

To evaluate the genetic population structure, samples by localities were grouped into breeding stocks delineated by the International Whaling Commission (IWC, 2015) (Table 1). Pairwise genetic divergences between breeding stocks were estimated using ϕ_{ST} statistics (which considers the nucleotide diversity) and F_{ST} statistics (which considers only the haplotype frequencies) using ARLEQUIN, and corrected with two-stage sharpened method of False Discovery Rate (FDR) (Benjamini and Hochberg, 1995). The population structure, considering the patterns of humpback whale migration, was examined through analyses of molecular variance (AMOVA) using the pairwise difference algorithm in ARLEQUIN and the statistical significance of estimates was assessed by 10,000 permutations. First, to assess how the genetic variability is distributed at a global scale, two alternative scenarios were examined: (Scn1) between hemispheres, South (WSA + ESA + WSP) and North (NH); and (Scn2) between ocean basins, Atlantic (WSA + ESA) and Pacific (WSP + NH). Second, to assess

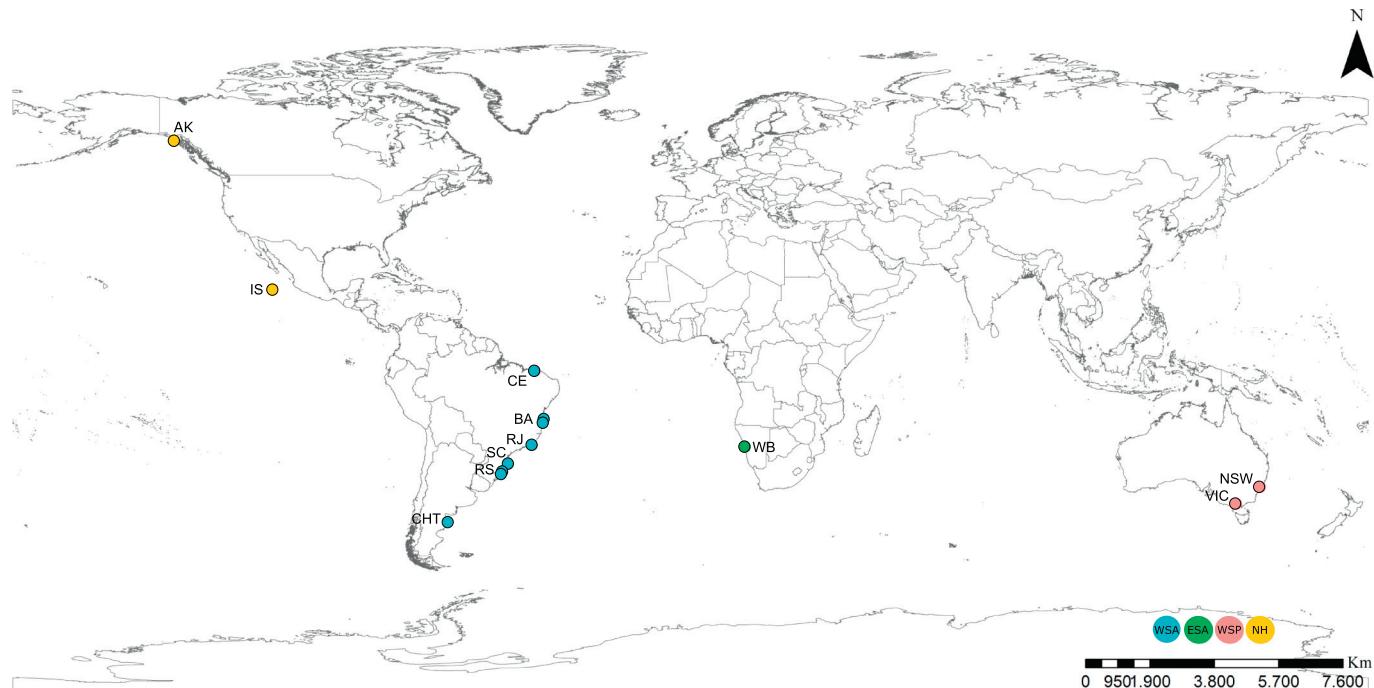


Fig. 1. Distribution map of collected samples and GenBank sequence localities. AK: Alaska, United States of America; BA: Bahia, Brazil; CE: Ceará, Brazil; CHT: Chubut, Argentina; ESA: Eastern South Atlantic; IS: Isla Socorro, Mexico; NH: Northern Hemisphere; NSW: New South Wales, Australia; RJ: Rio de Janeiro, Brazil; RS: Rio Grande do Sul, Brazil; SC: Santa Catarina, Brazil; VIC: Victoria, Australia; WB: Walvis Bay, Namibia; WSA: Western South Atlantic; WSP: Western South Pacific.

how the genetic variability is distributed at regional scale, across the Southern Hemisphere, four alternative structure scenarios between breeding stocks were considered: (Scn3) stocks A and B grouped (WSA + ESA); (Scn4) stocks A and E grouped (WSA + WSP); (Scn5) stocks B + E grouped (ESA + WSP); and (Scn6) all breeding stocks separated. Genealogical relationships among haplotypes were assessed through a parsimony haplotype network constructed using a median-joining algorithm as implemented in the software NETWORK 5.0.0.1 (Bandelt et al., 1999).

3. Results

3.1. Intraspecific variability and population genetic differentiation

An aligned segment of 740 base pairs (bp) of the COI gene was obtained from each of the 67 *C. boopis* specimens analysed. The value of divergence between the analysed sequences was 0–2.5%, suggesting that these individuals belong to a single species once the divergence is congruent with the intraspecific distances inferred for the genus *Cyamus* (0.9–9.0%) (Iwasa-Arai et al., 2017a,b). Sixty-seven sites were polymorphic (9.2%), of which 60 were transitions and only seven resulted in non-synonymous changes. Of the 42 haplotypes identified, 38 were private to single breeding stock, and four (H_27, H_31, H_32 and H_33) were shared by localities in WSA and WSP (breeding stocks A and E, respectively) (Fig. 2). The most common haplotype (H_27) was found in only seven individuals of *C. boopis* from two whales found in the WSA (southern Brazil) and the WSP (Australia). All haplotypes (H_22–H_26)

from localities in the ESA (Namibia - breeding stock B) and from localities in the Northern Hemisphere (H_1, H_20 and H_21) were exclusives, differed by one to six mutations from the closest one (Fig. 2). Haplotype (h) and nucleotide (π) diversities obtained are presented in Table 2. The nucleotide diversity of *C. boopis* within each whale (a maximum of 11 whale lice per whale was analysed) ranged from 0.005 to 0.012 and the haplotype diversity ranged from 0.7 to 1. A pattern of high haplotype diversity and high nucleotide diversity was found both within each humpback whale breeding stock as for the species (overall $\pi = 0.01$; overall $h = 0.974$).

A moderate level of population structure (Hartl and Clark, 1997) was found among *C. boopis* samples (overall $\Phi_{ST} = 0.130$, $P < 0.005$), suggesting that the species does not behave as a single panmictic population. To evaluate the genetic heterogeneity, six scenarios (Scn) of population structure were tested through analyses of molecular variance (Table 3). Despite the overall population structure, no significant population clustering was detected through AMOVA ($\Phi_{CT} = -0.5445$ –0.2353, $P > 0.05$, Table 3). These high P values are expected when the number of sampled localities is small and thus, the AMOVA does not have the statistical power necessary to detect population differences (Fitzpatrick, 2009). The scenarios Scn1 and Scn4, which maximise the Φ_{CT} (0.2353 and 0.1878 respectively), separate the breeding stocks from the Northern and Southern Hemispheres in concordance with the patterns of humpback whale migration. Additionally, the Scn4 that presents the combination of a high Φ_{CT} and a non-significant low Φ_{SC} , separates the ESA breeding stock from the rest of the breeding stocks in the Southern Hemisphere.

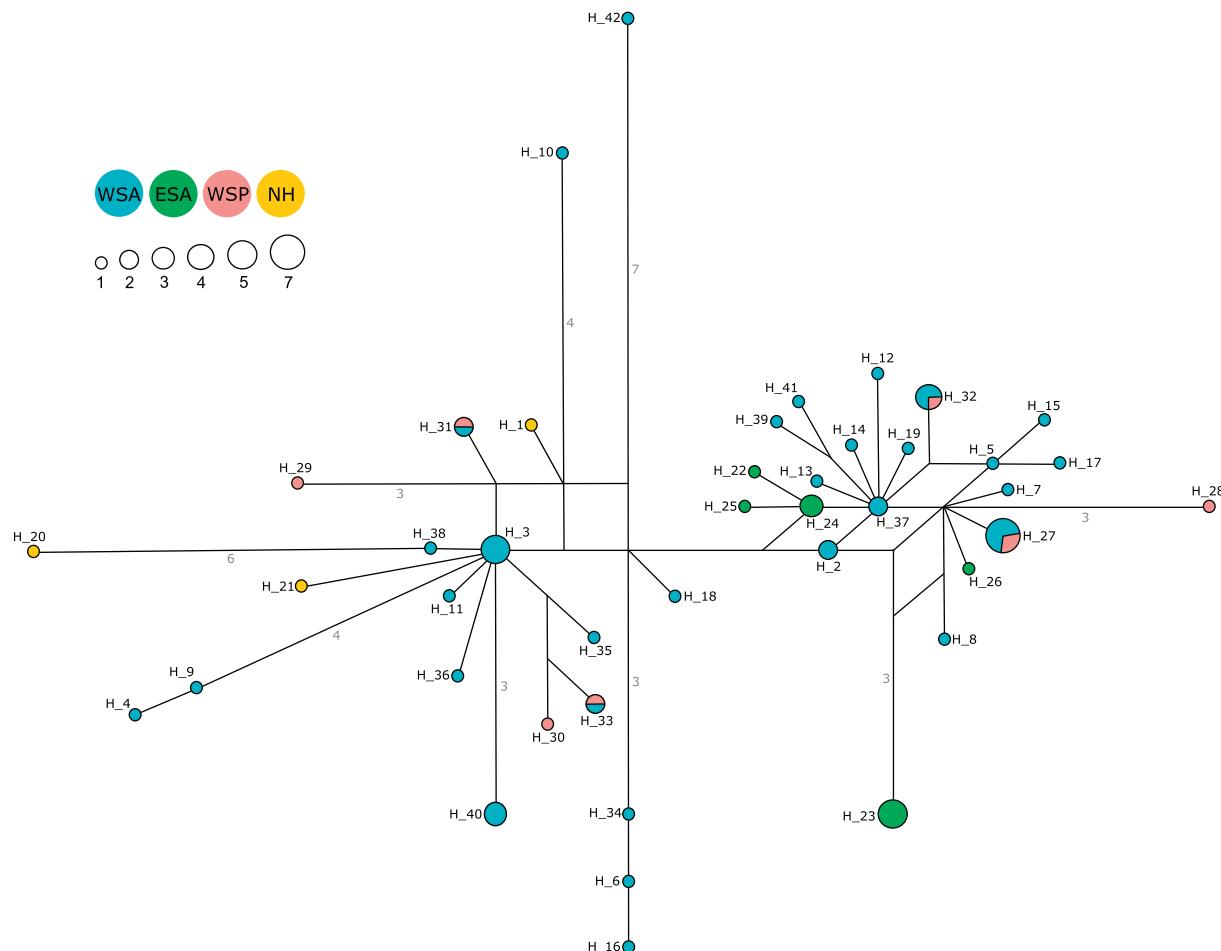


Fig. 2. Parsimony median-joining network of *Cyamus boopis*. Sizes of the circles are proportional to the frequency of each haplotype. Line lengths are relative to the number of mutations between haplotypes (shortest lines = 1 mutation).

ESA: Eastern South Atlantic; NH: Northern Hemisphere; WSA: Western South Atlantic; WSP: Western South Pacific.

Table 2

Genetic variability in *Cyamus boopis*. Number of specimens (N), number of polymorphic sites (S), number of observed haplotypes (K), haplotype diversity (H), nucleotide diversity (π), and standard deviation (SD).

Stranding location	N	S	K	H (SD)	π (SD)
Western South Atlantic (WSA) – breeding stock A	45	51	31	0.972 (0.013)	0.00936 (0.00065)
Whale CE – AQUASIS349	3	8	3	1.000 (0.272)	0.00721 (0.00233)
Whale BA – IBJ226	4	7	4	1.000 (0.177)	0.00473 (0.00106)
Whale BA – IBJ668	3	13	3	1.000 (0.272)	0.01171 (0.00353)
Whale RJ – GEMM115	4	8	3	0.833 (0.222)	0.00541 (0.00214)
Whale SC	10	19	4	0.711 (0.117)	0.01003 (0.00183)
Whale RS – GEMARS1684	5	11	4	0.900 (0.161)	0.00757 (0.00188)
Whale RS – GEMARS1695	5	13	3	0.700 (0.218)	0.00919 (0.00269)
Whale AR	11	25	10	0.982 (0.046)	0.01042 (0.00140)
Eastern South Atlantic (ESA) – breeding stock B	11	10	5	0.764 (0.107)	0.00590 (0.00065)
Western South Pacific (WSP) – breeding stock E	8	22	7	0.964 (0.077)	0.01134 (0.00144)
Whale VIC	2	5	2	1.000 (0.500)	0.00676 (0.00338)
Whale NSW	6	18	6	1.000 (0.096)	0.01063 (0.00168)
Northern Hemisphere (NH) – breeding stock North Pacific	3	9	3	1.000 (0.272)	0.00856 (0.003)
Whale MX	2	7	2	1.000 (0.500)	0.00946 (0.00473)
Whale AL	1	–	1	–	–
Total	67	67	42	0.974 (0.009)	0.00969 (0.001)

Table 3

Hierarchical analysis of molecular variance for *Cyamus boopis* based on COI sequences. Shades of blue indicate the compared groups (for more details see material and methods). ESA: Eastern South Atlantic; NH: Northern Hemisphere; WSA: Western South Atlantic; WSP: Western South Pacific.

Scenarios	Within localities				Among localities/within groups				Among groups			
	%	Φ_{ST}	P	%	Φ_{SC}	P	%	Φ_{CT}	P			
1 WSA	70.03	0.2997	0.0002	6.45	0.0843	0.0079	23.53	0.2353	0.2552			
2 WSA	89.36	0.1064	0.0004	16.46	0.1556	0.0013	-5.82	-0.0582	0.6670			
3 WSA	86.94	0.1307	0.0007	12.79	0.1282	0.0030	0.28	0.0028	0.3340			
4 WSA	81.86	0.1814	0.0006	-0.64	0.0079	0.4995	18.78	0.1878	0.1602			
5 WSA	87.31	0.1269	0.0004	16.68	0.1604	0.0159	-3.99	-0.0399	0.4959			
6 WSA	85.03	0.1497	0.0005	4.74	0.0528	0.1137	10.23	-0.1023	0.0759			

Table 4

Pairwise estimates of Φ_{ST} (below the diagonal) and F_{ST} (above diagonal) between localities. Numbers in parentheses are P-values. Values Φ_{ST} and F_{ST} significant after False Discovery Rate correction in bold.

NH	WSA	ESA	WSP
NH		0.0188 (0.2612–0.2742)	
WSA	0.2581 (0.0050–0.0035)		0.1567 (0.1388–0.1943)
ESA	0.5349 (0.0033–0.0034)	0.1333 (0.0030–0.0034)	0.1182 (0.0000–0.0000)
WSP	0.1965 (0.0507–0.0213)	0.0000 (0.5020–0.1757)	0.1417 (0.0095–0.0199)

ESA: Eastern South Atlantic; NH: Northern Hemisphere; WSA: Western South Atlantic; WSP: Western South Pacific.

Pairwise analyses, considering the nucleotide diversity, detected significant differences between all comparisons ($\Phi_{ST} = 0.133$ –0.535, $P < .05$) except between the breeding stock from the WSA and the WSP ($\Phi_{ST} = 0$, $P > .05$; Table 4). The values of higher Φ_{ST} were for the comparisons between the Northern Hemisphere and other breeding stocks ($\Phi_{ST} = 0.197$ –0.535, $P < .05$; Table 4). Considering the haplotype frequencies, significant differences were detected only between ESA and WSA breeding stocks ($F_{ST} = 0.118$, $P < .05$), and between the ESA and the WSP in the Southern Hemisphere ($F_{ST} = 0.142$, $P < .05$; Table 4). These results indicated that the most likely structuring scenario is of three populations: i) WSA + WSP (stocks A and E), ii) ESA (stock B) and iii) NH (stock North Pacific) which, indeed, was the grouping with the smallest intragroup heterogeneity in AMOVA

($\Phi_{SC} = 0.0079$, $P > .05$; Table 2).

4. Discussion

This study is the first to investigate the genetic structure and diversity of the whale louse *C. boopis*. Our results suggest that the populations from WSA and WSP were genetically homogenous but differentiated from populations from the ESA and the North Pacific (NH).

The high genetic variability observed in *C. boopis* ($h = 0.974$) seems to be typical of cyamids, since similar results have been reported using the COI gene for *C. scammoni* Dall, 1872, *C. ceti* (Linnaeus, 1758) and *C. kessleri* A. Brandt, 1873 of gray whales ($h = 0.986$, 0.975 and 0.901 respectively) (Callahan, 2008), as well as for *C. ovalis* Roussel de

Vauzème, 1834, *C. gracilis* Roussel de Vauzème, 1834 and *C. erraticus* Roussel de Vauzème, 1834 from right whales, in which most haplotypes were unique (Kaliszewska et al., 2005).

The genetic variability found among *C. boopis* is distributed in three populations that correspond to WSA + WSP, ESA and NH, which suggests that there is a partial correlation between the population structure of *C. boopis* and the humpback whale.

At a global scale, between ocean basins, the humpback whale genetic structure is driven by a combination of maternal fidelity to feeding areas and natal philopatry to breeding areas (Kershaw et al., 2017). Those natural trends result in minimal dispersion or interbreeding of whales from different ocean basins and therefore, they can result on some ancestral linkage between WSA and WSP. Although genetic exchange between these two humpback stocks appears to be very limited nowadays (Engel et al., 2008), the genetic structure of whale lice may reflect an ancestral genetic structure of the host. On the other hand, the African stocks (stocks B and C) are thus highly separated in the present day from stocks A and D in the Atlantic and Indian Ocean, respectively (Kershaw et al., 2017). Thus, the high differentiation and independent evolutionary trajectories observed between ocean basins, have resulted in the potential delimitation of three *M. novaeangliae* subspecies in the North Atlantic, North Pacific and Southern Hemisphere (Jackson et al., 2014). Given that the whale lice have no free-swimming stage and their transmission can only occur during contact between whales, it is expected that the population structure of the lice mimics that of their hosts. In fact, our results reflect that the greater differentiation was observed between *C. boopis* of humpback whales from the Southern Hemisphere and *C. boopis* from the Northern Hemisphere, representing two of the three *M. novaeangliae* subspecies proposed: *M. novaeangliae kuzira* in the north Pacific Ocean and *M. novaeangliae australis*, in the Southern Hemisphere.

In the Southern Hemisphere, the feeding grounds of humpback whales are mainly distributed throughout a broad inter-connected circumpolar area, providing the potential for longitudinal movements of individuals from different breeding populations during the feeding season (Cypriano-Souza et al., 2017). Although rare inter-oceanic events had been recorded in the Southern Hemisphere, several authors have inferred low genetic divergence and higher migration rates between populations of humpback whales in the Southern Hemisphere supporting the current designation of breeding stocks A, B, C, D, E, F and G (Rosenbaum et al., 2009; Jackson et al., 2014; Cypriano-Souza et al., 2017; Kershaw et al., 2017). Thus, despite the absence of geographic barriers, the population structure of *M. novaeangliae* at regional scales, across the Southern Hemisphere, is probably the result of long-term maternal transmission of migration routes and fidelity to breeding grounds, with a degree of gene flow that likely erodes the strong differentiation (Rosenbaum et al., 2009; Kershaw et al., 2017). The genetic structure obtained for *C. boopis* in the Southern Hemisphere partially agrees with that observed in their hosts. Differentiation was observed between the breeding stock from ESA (stock B) and other breeding stocks in the Southern Hemisphere, while genetic homogeneity between the WSA (stock A) and WSP (stock E). Considering the maternal fidelity to feeding areas of humpback whales, the homogeneity observed between the WSA and the WSP in *C. boopis* could be result of contact among male whales of these two breeding stocks somewhere in their annual migratory cycle or on the feeding areas. Furthermore, the high genetic variability observed in *C. boopis*, even within a single host (Table 2), suggests that a single humpback whale can host several maternal lineages of whale lice, thereby the transmission by contact between whales may occur regularly. However, the differentiation found between *C. boopis* populations from WSA (stock A) and ESA (stock B) was unexpected to some extent given the close proximity between these two areas and the similarity of the song structure amongst humpback whales of breeding stocks A and B (Darling and Sousa-Lima, 2005).

The genetic population structure found in *C. boopis* within the

Southern Hemisphere contrasts with the results obtained for others *Cyamus* species. Kaliszewska et al. (2005) analysed the genetic structure of *C. ovalis* and *C. erraticus*, including populations from southern right whales from Argentina, South Africa and Australia, and no variation was estimated to occur among groups, suggesting a single panmictic population in the Southern Hemisphere. Interestingly, population genetic analysis of mitochondrial DNA in southern right whales revealed significant differentiation between most of calving grounds (Argentina, South Africa, New Zealand, Southwest Australia), except for western south Atlantic (Argentina) and South Africa (Patenaude et al., 2007).

Thus, although both humpback and southern right whales exhibit the typical north-south seasonal migration in each hemisphere and some degree of maternal site fidelity to calving grounds, apparently higher in the last species (e.g. Best, 2000; Wedekin et al., 2010). The genetic structure of cyamids seems to be stronger in *C. boopis* than in any other *Cyamus* species. However, in both hosts the cyamid populations on opposite sides of the equator are clearly more divergent, given support for the existence of distinct taxonomic units (i.e. species for right whales and subspecies for humpback whales). To conclude, even though more studies including new sample locations such as the Chilean and Peruvian coast in the eastern south Pacific, as well as more sampling from the current sampled locations in Namibia and Australia, are needed to accurately determine the population structure of *C. boopis* within the Southern Hemisphere, our preliminary discovery has important implications for the conservation and management of the humpback whales of the Southern Hemisphere as a whole, and indicates that population dynamics of humpbacks seem more complex than stable migratory routes, as also previously revealed by some inter-oceanic migratory events (e.g. Pomilla and Rosebaum, 2005; Stevick et al., 2010). It should also be considered that the recovery process of the humpback whale populations is under a dynamic change and it has not been completed yet.

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Declarations of interest

None.

Contributors

TIA conceived the project, performed the molecular experiments and led the writing. GTRR designed and supervised the molecular experiments, and interpreted the results. CSS supervised the project and interpreted the results. SS, PHO, ASF, SE, EAC, CSS, VLC collected the material and field data and interpreted the results. All authors read, contributed to the intellectual discussion and approved the final manuscript.

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