Immunogenetics (2020) 72:393–398
https://doi.org/10.1007/s00251-020-01171-9

SHORT COMMUNICATION

Latitudinal diversity gradient and cetaceans from the perspective of MHC genes

Maria Luiza Andreani[1] · Lucas Freitas[1] · Elisa K. S. Ramos[1] · Mariana F. Nery[1]

Received: 17 December 2019 / Accepted: 5 June 2020 / Published online: 20 June 2020

Abstract

Pathogen diversity is a key source of selective pressure on immune system genes, shaping molecular evolution mainly on widely distributed or migratory organisms such as cetaceans. Here, we investigated the effects of latitudinal span migration, different biomes occupation, and pathogen-mediated selection on MHC DQB locus divergence on cetaceans. We applied some evolutionary genetics methods using a dataset of 15 species and 121 sequences, and we found a trend on greater MHC divergence on tropical species when compared with either temperate or migratory species. In addition, oceanic cetaceans exhibit greater MHC divergence. Here, we show that, despite there was a correlation between the diversity of MHC DQB alleles with the distribution of organisms, the pattern of diversity found is not completely explained by pathogenic pressure, suggesting that other factors must be investigated for a better understanding of the processes related to the diversity of MHC in cetaceans.

Keywords evolution · MHC · cetaceans · migration · latitudinal gradient

Species diversity tends to diminish as latitude rises (Gaston 2000) and this pattern has been suggested to apply also to pathogens as previous studies described latitudinal gradients on diseases and parasites, both in continental and marine habitats (Rohde 2002; Guernier et al. 2004; Stearns and Koella 2008). This raises questions about the molecular dynamics of immune genes and the evolutionary processes behind ecological processes, more specifically, whether and how genetic variability of immune genes is related to the species' habitat.

Among immune genes, major histocompatibility complex (MHC) molecules act as a bridge between adaptive and innate immune responses, and they are subdivided into classes I and II. MHC molecules are extremely polymorphic and they are reported to be evolving under balancing selection, reflecting a diverse repertoire, which is necessary to fight incoming pathogens (Edwards and Hedrick 1998). Recently, variability patterns have been investigated in migratory and widely distributed birds, showing more variability in residents of tropical regions (O'Connor et al. 2018).

Cetaceans are widely distributed aquatic mammals, with representatives ranging from very local and endemic to cosmopolitan and migratory species (Perrin 2009). The variability patterns of MHC Class II DQB (MHC-DBQ) alleles in cetaceans are still uncertain. For example, many allele copies and variations were reported in humpback whales (Baker et al. 2006), while fin whales display little variability at this gene (Trowsdale et al. 1989; Nigenda-Morales et al. 2008). Further, it is suggested that different regimes of selection among cetaceans may be a consequence of different habitats (Villanueva-Noriega et al. 2013). In this context, to better understand the relationship among immunity genes, latitude span, and migration in marine habitats, we investigated a possible pathogen-mediated evolution acting on the variability of MHC DQB locus in cetaceans.

In total, we retrieved 121 sequences available in public databases, including 15 cetacean species with different latitudinal ranges and migration habits. We categorized cetaceans in groups (Table S1) and hypothesized that species residing in tropical waters would have the greatest divergence in MHC DQB sequences, a similar pattern observed in birds and other species from tropical areas (O'Connor et al. 2018).

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00251-020-01171-9) contains supplementary material, which is available to authorized users.

✉ Mariana F. Nery
mariananery@gmail.com

[1] Laboratório de Genômica Evolutiva, Departamento de Genética, Evolução, Microbiologia e Imunologia, Instituto de Biologia, Universidade Estadual de Campinas – UNICAMP, Rua Monteiro Lobato, 255, Cidade Universitária, IB, Bloco H, Campinas, SP CEP 13083-862, Brazil

The maximum likelihood tree of the MHC-DBQ recovered no monophyletic group and pointed to identical alleles among distantly related species as shown in previous studies (Xu et al. 2008, 2009) (Fig. 1). These similarities have been attributed either to trans-species evolution (i.e., identical alleles appear before species divergence and are maintained through balancing selection) or convergent evolution (i.e., similarities arise from common environments rather than ancestry) (Klein 1987; Yeager and Hughes 1999; Lenz et al. 2013). In our results, some species sequences are identical (*Stenella coeruleoalba* and *Tursiops aduncus*, Fig. 1, alleles labeled Stco_4 and Tuad_1), suggesting convergent evolution, as these sequences contain PBR (peptide-binding-sites, i.e., the sites prone to pathogen-mediated selection, Fig. S1) and both share the same habitat. Nevertheless, our tree also shows a cluster with species that do not share similar habitats, such as *S. coeruleoalba*, *T. aduncus*, and *Pontoporia blainvillei* (Fig. 1, alleles labeled Stco_2, Pobl_1, Pobl_2, and Tuad_4 are identical), suggesting the presence of trans-species alleles. Both trans-species and convergent evolution have been reported on immunity genes: trans-species alleles were observed in chimpanzees and humans in MHC class I alleles, whereas convergent evolution was observed in MHC class II alleles from platyrrhine and catarrhine primates' infraorders (Mayer et al. 1988; Kriener et al. 2000). It is important to note that regarding the identical alleles, we discarded the possibility of database mislabeling because the researchers that originally sequenced these alleles had high confidence in the results as they carefully analyzed them to rule out the possibility of PCR artifacts or sample contamination (see Xu et al. 2009).

According to *p*-distance, we observed greater MHC divergence in the PBR region (as defined on Moreno-Santillan et al. 2016) on alleles of species that reside in tropical zones (Fig. 2A). This result is expected since species and pathogen diversity tend to be greater in low latitudes (Møller 1998; Robar et al. 2010; Bordes et al. 2011). A similar pattern of MHC divergence was found by O'Connor et al. (2018) for birds MHC class I. However, for cetaceans, the differences among tropical species, temperate species, and migratory species were not statistically significant (Table 1). This suggests that the pattern observed for MHC diversity in cetaceans may be influenced by other factors besides pathogen pressure, such as demographics and bottleneck events. The effects of population history and population dynamics indeed have been suggested to reduce MHC diversity in previous studies (Drake et al. 2004; Wan et al. 2006), and they could influence cetaceans as well. For example, the blue whale population at the Gulf of California was affected by whaling, being greatly reduced and recovering afterwards (Moreno-Santillan et al. 2016), and Nigenda-Morales et al. (2008) showed that the population of fin whales in the same location has reduced variability as a consequence of isolation events or may exhibit signs of founder effects rather than whaling. This reduction of population size could reduce MHC diversity without being directly linked to pathogen-mediated selection on a latitudinal gradient; however, it also does not mean that pathogen diversity cannot act alongside demographic factors. For example, history of colonization, drift, and latitudinal gradients of pathogenic pressure have already been suggested to shape genetic variability in frog populations (Cortazar-Chinarro et al. 2018). Another factor that could potentially influence our results is an unbalanced sampling of species as there are more studies and consequently more species sampled from odontocetes (toothed whales) than mysticetes (baleen whales) (Table S1).

PBR *p*-distance was also analyzed comparing coastal and offshore habitats. Offshore species' alleles were more divergent than coastal species, although without statistical significance between them (Table 1). Contact with pathogens in

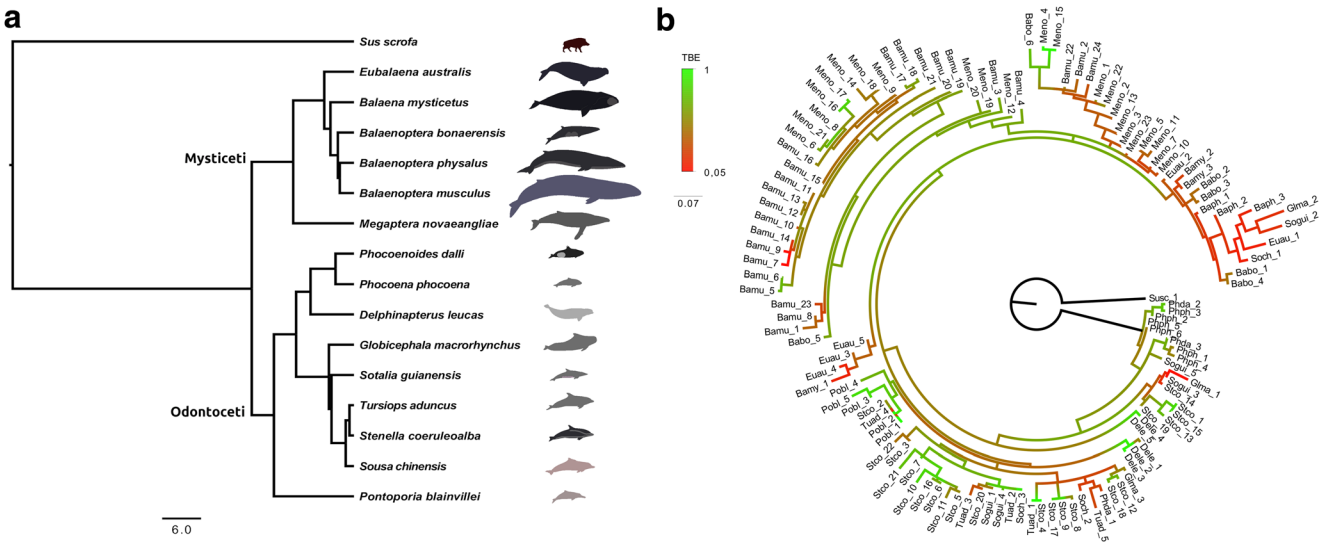

Fig. 1 **a**, **b** Species tree (left) and maximum likelihood tree of nucleotides of all sequences used, colored regarding transfer bootstrap estimations. Values closer to one are greener, and values closer to zero are reddish

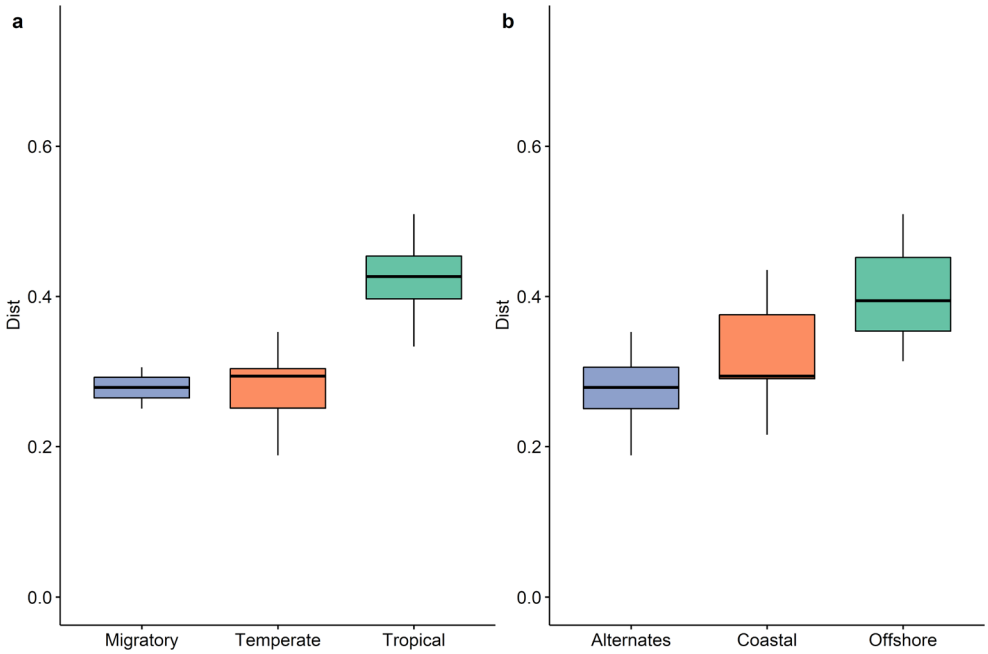

Fig. 2 MHC Class II DQB PBR *p*-distance regarding location. **a** Animals that migrate between both regions (in blue), temperate resident animals (in orange), and tropical resident animals (in green). **b** Animals that alternate (in blue), animals that are coastal (in orange), and animals that are oceanic (in green)

coastal environments is thought to be aggravated by anthropogenic activity, which increases the flux of pathogens such as bacteria or protozoa from land to sea (Miller et al. 2008, 2010). However, information regarding pathogen dynamics in open oceans is still missing, and large-scale epizootics in the ocean environment, such as morbilliviruses epizootics that cause a high number of deaths in cetacean populations (van Bressem et al. 2009) have been reported and could explain the pattern observed. Also, it is likely that populational and phylogenetic factors influence the differences observed, as pointed before to latitudinal gradients.

We also analyzed how latitudinal migration correlates to MHC DQB divergence (Fig. 2A). We found that individuals who migrate to large latitudinal distances exhibited the lowest divergence; however, it is not significantly different from either temperate or tropical species (Table 1). A similar pattern is observed in Fig. 2B for species that alternate between coastal and offshore habitats, which also exhibits the lowest divergence and is not significantly different from either offshore or coastal species (Table 1). The reason for the lowest diversity in MHC in species that exhibits behaviors such as long-distance migration or alternation between biomes could be a consequence of a trade-off between dealing with the exposure to more pathogens and the cost of a strong immune response (especially for long-distance migrations). Nevertheless, as the differences are shown to be non-significant, the answer to this question remains to be statistically established. It is worth to note that we observed a correlation between *p*-distance and latitude and biome indicators; therefore, some pathogen-mediated selection must be taking place on these species. Moreover, positive selection has been already reported in cetacean MHC genes (Moreno-Santillan et al. 2016).

Overall, our results showed higher allele divergence in MHC for cetaceans from tropical zones compared with temperate zones and migrants and also an increase in oceanic species MHC divergence when compared with coastal and species which alternate between coastal and offshore habitats. Differences, however, were not statistically significant—suggesting influence from other factors besides pathogen-mediated selection. Increasing the sampling to include all cetacean species will certainly help in this matter, and more research on the actual distribution of aquatic pathogens is needed to provide a better picture, especially in marine species. In this context, populational studies may be useful to determine the role of groups in disease transmission and pathogen selection. Moreover, better mapping of pathogen diversity in oceans will contribute to create better conservational policies as well as uncover ecological dynamics.

Method

Data collection One hundred twenty-one DQB sequences from 15 cetacean species were retrieved from GenBank (Sayers et al. 2019), from genomes available at NCBI (NCBI 2016) and our own *Sotalia guianensis* genome (manuscript in preparation) using BLAST (Altschul et al. 1990). In our final dataset, we excluded all species containing only one DQB sequence as we thought it would not be representative. The *Pontoporia blainvillei* sequence labeled as "Pobl_1" was obtained using the primers DQBF (5′-CTGGTAGTTGTGTC

Table 1 BPMM analysis of MHC divergence using species phylogeny

Model			
P-distance~zone	Fixed effects	Posterior mode (CI)	pMCMC
	Migratory	0.2441761 (0.07626899, 0.4116389)	0.004
	Temperate	0.2990626 (0.12438850, 0.4131161)	0.002
	Tropical	0.4712713 (0.18764818, 0.6337848)	< 0.001
	Tropical vs temperate	− 0.1995736 (− 0.3846329, 0.02698707)	0.098
	Migratory vs temperate	− 0.02968676 (− 0.1344075, 0.1030341)	0.862
	Migratory vs tropical	− 0.1529688 (− 0.4153583, 0.06863325)	0.118
	Random effects	Posterior mode (CI)	H^2(CI)
	Phylogenetic variance	0.0005498820 (0.0001865405, 0.002063710)	0.1589339 (0.02784615, 0.7052633)
	Residual variance	0.0009292383 (0.0001581367, 0.007382358)	
P-distance ~ biome	Fixed effects	Posterior mode (CI)	pMCMC
	Alternates	0.2870815 (0.1204612, 0.4675002)	< 0.001
	Coastal	0.2794128 (0.1457435, 0.4653267)	< 0.001
	Offshore	0.3558368 (0.1622210, 0.5325557)	0.002
	Offshore vs coastal	− 0.03548652 (− 0.1650515, 0.04350534)	0.326
	Alternates vs coastal	0.002310635 (− 0.1303704, 0.127347)	0.838
	Alternates vs offshore	− 0.05861948 (− 0.2146571, 0.08995565)	0.426
	Random effects	Posterior mode (CI)	H^2(CI)
	Phylogenetic variance	0.0005346412 (0.0002416185, 0.002385038)	0.1815913 (0.04081635, 0.7373489)
	Residual variance	0.0013558363 (0.0001751623, 0.008268270)	

Gaussian error distribution was used. Credible intervals (CI) in parenthesis. H^2 stands for heritability

TGCACAC) and DQBR (5′-CATGTGCTACTTCA CCAACGG) described by Murray et al. (1995). PCR reaction was carried out in a total volume of 25 μl in which there were 15.3 μl of Milli-Q water, 5 μl Buffer 5xGotaq, 0.5 μl of each primer, 0.5 μl de DNTP, and 0.5 μl de Taq polimerase e 3 μl of DNA. Thermal cycle was accomplished at an initial process of denaturation at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s and a final extension of 72 °C for 8 min. PCR product was visualized on agarose gel of 1.5x concentration, sanger sequencing was carried out on 3730XL Applied Biosystems, and reads were assembled using Geneious 2019.1.3 (https://www.geneious.com).

Ecological habits Sequences were divided into the following categories: (i) tropical residents, (ii) temperate residents, (iii) migratory between temperate and tropical areas, (iv) coastal habits, (v) offshore habits, and (vi) alternation between offshore and coastal habits. Species location was defined by the local of collection when it was informed and, when not available, to geographic known range of distribution (Perrin 2009; Jefferson et al. 2015; IUCN/CSG 2019; Myers et al. 2019). Among these species there were two highly migratory, *Balaenoptera musculus* and *Megaptera novaeangliae* that transition between marine temperate and tropical waters for breeding and feeding. Although *Balaenoptera physalus* is migratory, the sequences used in this study came from a resident population in the Gulf of California as stated by Nigenda-Morales et al. (2008) (Table S1).

Phylogenetics and allele divergence analysis Sequences were aligned using MAFFT server v. 7 (Katoh and Standley 2013) and treated with Gblocks v. 0.91b (Castresana 2000). Identical sequences on nucleotide level in the same population were

excluded from the final dataset. After alignment, we used IQ-TREE v. 1.7-beta18 (Nguyen et al. 2015) to generate a maximum likelihood tree implementing the –tbe function as specified by Lemoine et al. (2018). *P*-distance of the PBR region on the amino acid level was measured using MEGA v. 7 (Kumar et al. 2016), where the mean *p*-distance of alleles within each species was calculated. Wu-Kabat statistics were generated with PVS web server (Garcia-Boronat et al. 2008; Díez-Rivero and Reche 2009). All graphics were made with ggplot2 v3.0.0 package (Wickham 2016) and ggpubr v. 0.2.999 (Kassambara 2018) on R v. 3.5.3 (R Core Team 2019).

For statistical analysis controlling for phylogenetic factors, we used the R package MCMCglmm (Hadfield 2010). For modeling divergence on PBR *p*-distance, we used a Gaussian error distribution, we used species phylogeny as a random effect, and we ran our models through 1500 species trees to account for phylogenetic uncertainty. Values were considered significant if credible intervals did not span zero and if pMCMC was below 0.05.

Funding information This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and by FAPESP (2015/18269-1).

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Baker CS, Vant MD, Dalebout ML, Lento GM, O'Brien SJ, Yuhki N (2006) Diversity and duplication of DQB and DRB-like genes of the MHC in baleen whales (suborder: Mysticeti). Immunogenetics 58: 283–296. https://doi.org/10.1007/s00251-006-0080-y

Bordes F, Guégan JF, Morand S (2011) Microparasite species richness in rodents is higher at lower latitudes and is associated with reduced litter size. Oikos 120:1889–1896. https://doi.org/10.1111/j.1600-0706.2011.19314.x

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17:540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Cortazar-Chinarro M, Meyer-Lucht Y, Laurila A, Höglund J (2018) Signatures of historical selection on MHC reveal different selection patterns in the moor frog (*Rana arvalis*). Immunogenetics 70:477–484. https://doi.org/10.1007/s00251-017-1051-1

Díez-Rivero CM, Reche P (2009) Discovery of Conserved Epitopes Through Sequence Variability Analyses. In: Discovery of conserved epitopes through sequence variability analyses. Bioinformatics for Immunomics, vol 3. Springer, New York, pp 95–101. https://doi.org/10.1007/978-1-4419-0540-6_8

Drake GJ, Kennedy LJ, Auty HK et al (2004) The use of reference strand-mediated conformational analysis for the study of cheetah (Acinonyx jubatus) feline leucocyte antigen class II DRB polymorphisms. Mol Ecol 13:221–229. https://doi.org/10.1046/j.1365-294x.2003.02027.x

Edwards SV, Hedrick PW (1998) Evolution and ecology of MHC molecules: from genomics to sexual selection. Trends Ecol Evol 13: 305–311. https://doi.org/10.1016/S0169-5347(98)01416-5

Garcia-Boronat M, Diez-Rivero CM, Reinherz EL, Reche PA (2008) PVS: a web server for protein sequence variability analysis tuned to facilitate conserved epitope discovery. Nucleic Acid Res 36: W35–W41. https://doi.org/10.1093/nar/gkn211

Gaston KJ (2000) Global patterns in biodiversity. Nature 405:220–227. https://doi.org/10.1038/35012228

Guernier V, Hochberg ME, Guégan J-F (2004) Ecology drives the worldwide distribution of human diseases. PLoS Biol 2:e141. https://doi.org/10.1371/journal.pbio.0020141

Hadfield JD (2010) MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. J Stat Soft 33:1–22. https://doi.org/10.18637/jss.v033.i02

IUCN/CSG (2019) IUCN – SSC Cetacean Specialist Group. https://iucn-csg.org. Accessed 3 Oct 2019

Jefferson TA, Webber MA, Pitman RL (2015) Marine mammals of the world: a comprehensive guide to their identification. Elsevier Science

Kassambara A (2018) Ggpubr: "ggplot2" based publication ready plots

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. https://doi.org/10.1093/molbev/mst010

Klein J (1987) Origin of major histocompatibility complex polymorphism: the trans-species hypothesis. Hum Immunol 19:155–162. https://doi.org/10.1016/0198-8859(87)90066-8

Kriener K, O'hUigin C, Tichy H, Klein J (2000) Convergent evolution of major histocompatibility complex molecules in humans and New World monkeys. Immunogenetics 51:169–178. https://doi.org/10.1007/s002510050028

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Lemoine F, Domelevo Entfellner J-B, Wilkinson E, Correia D, Dávila Felipe M, de Oliveira T, Gascuel O (2018) Renewing Felsenstein's phylogenetic bootstrap in the era of big data. Nature 556:452–456. https://doi.org/10.1038/s41586-018-0043-0

Lenz TL, Eizaguirre C, Kalbe M, Milinski M (2013) Evaluating patterns of convergent evolution and trans-species polymorphism at MHC immunogenes in two sympatric stickleback species. Evolution 67: 2400–2412. https://doi.org/10.1111/evo.12124

Mayer WE, Jonker M, Klein D, Ivanyi P, van Seventer G, Klein J (1988) Nucleotide sequences of chimpanzee MHC class I alleles: evidence for trans-species mode of evolution. EMBO J 7:2765–2774. https://doi.org/10.1002/j.1460-2075.1988.tb03131.x

Miller MA, Miller WA, Conrad PA, James ER, Melli AC, Leutenegger CM, Dabritz HA, Packham AE, Paradies D, Harris M, Ames J, Jessup DA, Worcester K, Grigg ME (2008) Type X Toxoplasma gondii in a wild mussel and terrestrial carnivores from coastal California: new linkages between terrestrial mammals, runoff and toxoplasmosis of sea otters. Int J Parasitol 38:1319–1328. https://doi.org/10.1016/j.ijpara.2008.02.005

Miller MA, Byrne BA, Jang SS, Dodd EM, Dorfmeier E, Harris MD, Ames J, Paradies D, Worcester K, Jessup DA, Miller WA (2010) Enteric bacterial pathogen detection in southern sea otters (*Enhydra lutris nereis*) is associated with coastal urbanization and freshwater runoff. Vet Res 41:1. https://doi.org/10.1051/vetres/2009049

Møller AP (1998) Evidence of larger impact of parasites on hosts in the tropics: investment in immune function within and outside the tropics. Oikos 82:265. https://doi.org/10.2307/3546966

Moreno-Santillan DD, Lacey EA, Gendron D, Ortega J (2016) Genetic variation at exon 2 of the MHC class II DQB locus in blue whale (Balaenoptera musculus) from the Gulf of California. PLoS One 11: e0141296. https://doi.org/10.1371/journal.pone.0141296

Murray BW, Malik S, White BN (1995) Sequence variation at the major histocompatibility complex locus DQ beta in beluga whales (*Delphinapterus leucas*). Mol Biol Evol 12:582–593. https://doi.org/10.1093/oxfordjournals.molbev.a040238

Myers PRE, Parr CS, Jones T et al (2019) The Animal Diversity Web. https://animaldiversity.org. Accessed 3 Oct 2019

NCBI Resource Coordinators (2016) Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 44(D1): D7–D19. https://doi.org/10.1093/nar/gkv1290

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268–274. https://doi.org/10.1093/molbev/msu300

Nigenda-Morales S, Flores-Ramirez S, Urban RJ, Vazquez-Juarez R (2008) MHC DQB-1 polymorphism in the Gulf of California fin whale (*Balaenoptera physalus*) population. J Hered 99:14–21. https://doi.org/10.1093/jhered/esm087

O'Connor EA, Cornwallis CK, Hasselquist D, Nilsson JÅ, Westerdahl H (2018) The evolution of immunity in relation to colonization and migration. Nat Ecol Evol 2:841–849. https://doi.org/10.1038/s41559-018-0509-3

Perrin WF (ed) (2009) Encyclopedia of marine mammals, 2nd edn. Academic Press, Burlington

R Core Team (2019) R: A language and environment for statistical computing

Robar N, Burness G, Murray DL (2010) Tropics, trophics and taxonomy: the determinants of parasite-associated host mortality. Oikos 119: 1273–1280. https://doi.org/10.1111/j.1600-0706.2009.18292.x

Rohde K (2002) Ecology and biogeography of marine parasites. In: Advances in Marine Biology. Elsevier

Sayers EW, Agarwala R, Bolton EE, Brister JR, Canese K, Clark K, Connor R, Fiorini N, Funk K, Hefferon T, Holmes JB, Kim S, Kimchi A, Kitts PA, Lathrop S, Lu Z, Madden TL, Marchler-Bauer A, Phan L, Schneider VA, Schoch CL, Pruitt KD, Ostell J (2019) Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 47:D23–D28. https://doi.org/10.1093/nar/gky1069

Stearns SC, Koella JC (eds) (2008) Evolution in health and disease, 2nd edn. Oxford University Press, Oxford

Trowsdale J, Groves V, Arnason A (1989) Limited MHC polymorphism in whales. Immunogenetics 29:19–24. https://doi.org/10.1007/BF02341609

van Bressem M, Raga A, Di Guardo G et al (2009) Emerging infectious diseases in cetaceans worldwide and the possible role of environmental stressors. Dis Aquat Org 86:143–157. https://doi.org/10.3354/dao02101

Villanueva-Noriega MJ, Baker CS, Medrano-González L (2013) Evolution of the MHC-DQB exon 2 in marine and terrestrial mammals. Immunogenetics 65:47–61. https://doi.org/10.1007/s00251-012-0647-8

Wan Q-H, Zhu L, Wu H, Fang S-G (2006) Major histocompatibility complex class II variation in the giant panda (*Ailuropoda melanoleuca*). Mol Ecol 15:2441–2450. https://doi.org/10.1111/j.1365-294X.2006.02966.x

Wickham H (2016) ggplot2: elegant graphics for data analysis. Springer

Xu S, Chen B, Zhou K, Yang G (2008) High similarity at three MHC loci between the baiji and finless porpoise: trans-species or convergent evolution? Mol Phylogenet Evol 47:36–44. https://doi.org/10.1016/j.ympev.2007.05.026

Xu SX, Ren WH, Li SZ, Wei FW, Zhou KY, Yang G (2009) Sequence polymorphism and evolution of three cetacean MHC genes. J Mol Evol 69:260–275. https://doi.org/10.1007/s00239-009-9272-z

Yeager M, Hughes AL (1999) Evolution of the mammalian MHC: natural selection, recombination, and convergent evolution. Immunol Rev 167:45–58. https://doi.org/10.1111/j.1600-065X.1999.tb01381.x