GENETICS, EVOLUTION, AND PHYLOGENY - SHORT COMMUNICATION

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Comparative mtDNA phylogeographic patterns reveal marked differences in population genetic structure between generalist and specialist ectoparasites of the African penguin (*Spheniscus demersus*)

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Abstract

To address factors affecting genetic diversity and dispersal of ectoparasites, we compared mitochondrial DNA (mtDNA) population genetic structures of the generalist soft tick *Ornithodoros capensis* to the more host-specific nest flea *Parapsyllus humboldti*. A total of 103 ticks and 92 fleas were sampled at five distinct South African island/mainland African penguin (*Spheniscus demersus*) colonies. With its wide host range, *O. capensis* showed no evidence of significant cytochrome c oxidase subunit I (COI) mtDNA population differentiation among the five sampling sites ($\varphi_{st} = 0.00 \pm 0.004$; $p = 0.80$), as well as a higher level of genetic diversity ($\pi = 0.8\% \pm 0.06\%$) when compared to *P. humboldti*. In contrast, the flea showed significant population structure among most of the same sampling sites ($\varphi_{st} = 0.22 \pm 0.11$; $p \le 0.05$) and a lower level of genetic diversity ($\pi = 0.2\% \pm 0.01\%$). Our findings suggest that despite both parasites being mostly nest bound, *O. capensis* have few barriers to dispersal among island and mainland colonies. However, *P. humboldti* are more dependent on the African penguin for dispersal and thus have more impediments to gene flow among the same colonies. These findings broadly support the SGVH (specialist generalist variation hypothesis) and provide the first evidence for this hypothesis in parasites restricted to seabird colonies.

Keywords *Ornithodoros capensis* · *Parapsyllus humboldti* · South Africa · Specialist generalist variation hypothesis · Population structure · Ectoparasite

Introduction

The factors affecting population structure and genetic diversity of ectoparasites are not well documented. This is primarily due to the varied and complex life history characteristics of parasites and more specifically linked to the dispersal potential of taxa via their hosts (McCoy et al. 2005; Araya-Anchetta et al. 2015; van der Mescht et al. 2015; Engelbrecht et al. 2016). Following predictions from the SGVH (specialist generalist variation hypothesis; Li et al. 2014), it is hypothesized that ectoparasites with a narrow host range (specialists) will be more restricted in their dispersal and will thus have a high degree of population differentiation. In addition, since they will also be exposed to localized stochastic processes (such as local host extinction), these parasites should have a lower level of intraspecific genetic diversity. On the other hand, ectoparasites that have a wider host range (generalists) are more vagile and will thus show less population differentiation and comparably higher levels of genetic diversity (Berkman et al. 2015; Janecka et al. 2016; Matthee et al. 2018). In an attempt to provide additional data to test the validity of the SGVH and particularly to elucidate the mechanisms affecting population structure and genetic diversity of ectoparasites, we compare the population genetic structure of the soft tick *Ornithodoros capensis* to the nest flea *Parapsyllus humboldti*.

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Ornithodoros capensis belongs to the *Ornithodoros capensis* sensu lato species complex, a group of soft ticks that is currently composed of 11 bird-associated morpho-species (see Muñoz-Leal et al. 2017). *Ornithodoros capensis* sensu stricto (hereafter *O. capensis*) have three developmental stages (Sonenshine 1991). Larval ticks spend a few hours on the host to engorge, while nymphal and adult stages feed a couple of minutes (Dupraz et al. 2016). The feeding stage is however so short, that the overwhelming majority of the ticks of various life stages are found in the nests of the hosts (Hoogstraal et al. 1985; Dupraz et al. 2016). Being mostly nest bound, *O. capensis* have restrictions in host-facilitated dispersal (e.g., site philopatry of the host; Crawford et al. 1995) and it is possible that impediments to gene flow can exist among populations (Engelbrecht et al. 2016). However, *O. capensis* has an extensive distribution which includes tropical, subtropical, and southern temperate islands, as well as along the coasts of the Atlantic, Pacific, and Indian Oceans, the Caribbean, Corral seas, and lakes of the East African Rift Valley (Hoogstraal et al. 1985; Dupraz et al. 2016). In addition, *O. capensis* is also considered to be a host generalist that parasitize an array of colonial seabird species, including albatrosses, boobies, cormorants, gulls, penguins, and terns (Hoogstraal et al. 1985; Dupraz et al. 2016). From the wide distribution and broad host range, more gene flow and consequently less population genetic structuring may be evident (van der Mescht et al. 2015; Matthee et al. 2018), particularly as a result of larval dispersal when they feed on highly mobile flighted seabirds. Indeed, several studies investigating phylogeographic structure of ticks parasitizing seabirds showed a lack of phylogeographic structure among localities within regions. For example, the hard tick *Ixodes eudyptidis* showed no phylogeographic structure between localities < 600 km apart (Moon et al. 2015) and a study on *O. capensis* confirmed a lack of regional structure between populations occurring in the Cape Verde Archipelago (Gómez-Díaz et al. 2012).

The flea, *P. humboldti*, shows a marked contrast to *O. capensis*. The species appears to have a narrower host range and is the only known representative of the genus in southern Africa (Segerman 1995). Previous studies have recorded *P. humboldti* on Humboldt Penguins (*Spheniscus humboldti*) in Chile and Peru (Jordan 1942; Smith et al. 2008) and African penguins in Namibia and South Africa (Segerman 1995). Only one other record of *P. humboldti* taken from a rock pigeon (*Columba guinea*) on Dassen Island, South Africa, exists (Segerman 1995) and therefore *P. humboldti* is more host specific compared to *O. capensis.* Although very little information is available on the life history of *P. humboldti* specifically, it is reasonable to assume that similarly to the ticks, all the life stages occur in the host nests (Marshall 1981). Larvae feed on organic matter in the nest and adults visit the host only for a blood meal (Boyd 1951; Bitam et al. 2010). Given the narrower host range, along with the

nest bound nature of *P. humboldti*, limited dispersal is predicted for this taxon. The former life history trait will result in stronger phylogeographic structure when compared to the tick populations who have a broader host range (Li et al. 2014). In support of this hypothesis, the nest bound generalist flea, *Chiastopsylla rossi*, with a relatively narrow host range, showed substantially higher levels of population differentiation when compared to fleas characterized by a broader host range (van der Mescht et al. 2015).

This study is the first to investigate the SGVH on seabird parasites which are both nest bound and spend short periods on their hosts for feeding. The aim of this study is to further clarify the role of host range on the dispersal capacity of nest bound ectoparasites. Since both parasites included in this study are nest bound and spend short periods on their hosts for feeding, but clearly differ in the number of available hosts for dispersal, we hypothesize that populations of *O. capensis* will be genetically less structured than that of *P. humboldti*. Should this be the case, we further predict that *O. capensis* will show a higher level of genetic diversity when compared to *P. humboldti*.

Materials and methods

Sampling

Ornithodoros capensis and *P. humboldti* were collected from *S. demersus* nests between May–July 2016 and 2017. Three islands and two mainland colonies along the coast of South Africa were included (Fig. 1). All collected parasites were stored in 70% ethanol until DNA extraction ((ethical approval was obtained from Stellenbosch University Animal Ethics Committee (SU-ACUD15-00114) who followed the South African National Standard (SANS) for the Care and Use of Animals for Scientific Purposes (SANS 10386:2008). Samples were obtained with the permission of Cape Nature (Research Permit: 0056-AAA007-00191), Division of Environmental Affairs (RES2016/95 and RES2017/02), the Threatened or Protected Species (TOPS) of the Biodiversity Act (07962), and South African National Parks (CRC/2016-2017/038–2015/V1)).

DNA extraction, PCR and sequencing

DNA extractions were done from either the legs of individual ticks and or whole specimens using a Macherey Nagel genomic DNA extraction kit (Macherey Nagel, Germany), and following the manufacturer's instructions. The mitochondrial cytochrome c oxidase subunit I (COI) region was amplified by polymerase chain reaction (PCR), using published forward (LCO1490) and reverse (HC02198) primers, respectively (Folmer et al. 1994).
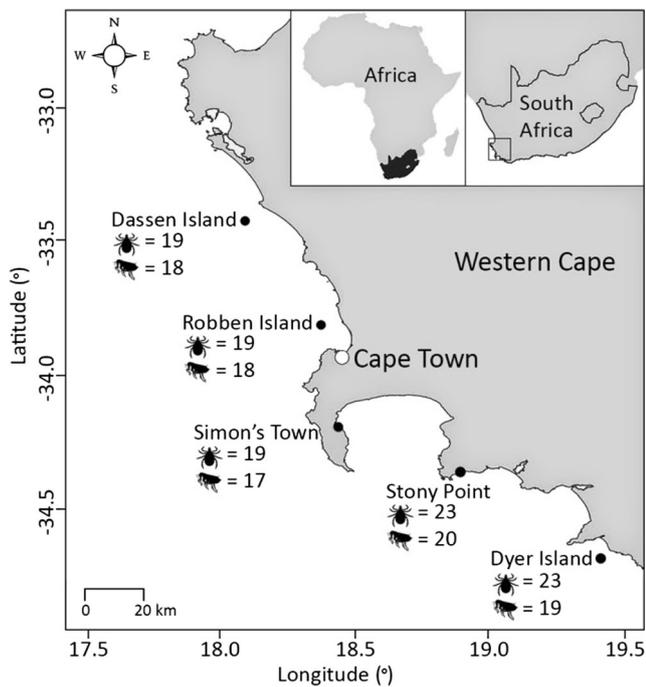
Fig. 1 Map of the selected African penguin colonies ($n = 5$) along the west coast of South Africa. Two mainland (Simon's Town and Stony Point) and three island colonies (Dassen-, Dyer-, and Robben Island) were included. Areas were plotted using GPS coordinates and QGIS open source Geographic Information System (http://qgis.osgeo.org). Black circles indicate sampling localities of both the tick, *Ornithodoros capensis* = [tick symbol] and the flea, *Parapsyllus humboldti* = [flea symbol]. The numbers of ticks and fleas sequenced are given for each taxon at each site

PCR amplifications were performed using the following profile: 94 °C for 3 min, 35 cycles of 40 s at 94 °C, 45 s at 45 °C, 45 s at 72 °C, followed by a final 5-min extension at 72 °C. PCR products were separated on 2% agarose gels stained with ethidium bromide and visualized using a UV light. Sequencing of PCR products were performed using BigDye Chemistry and an ABI 3730 XL DNA Analyzer (Applied Biosystems).
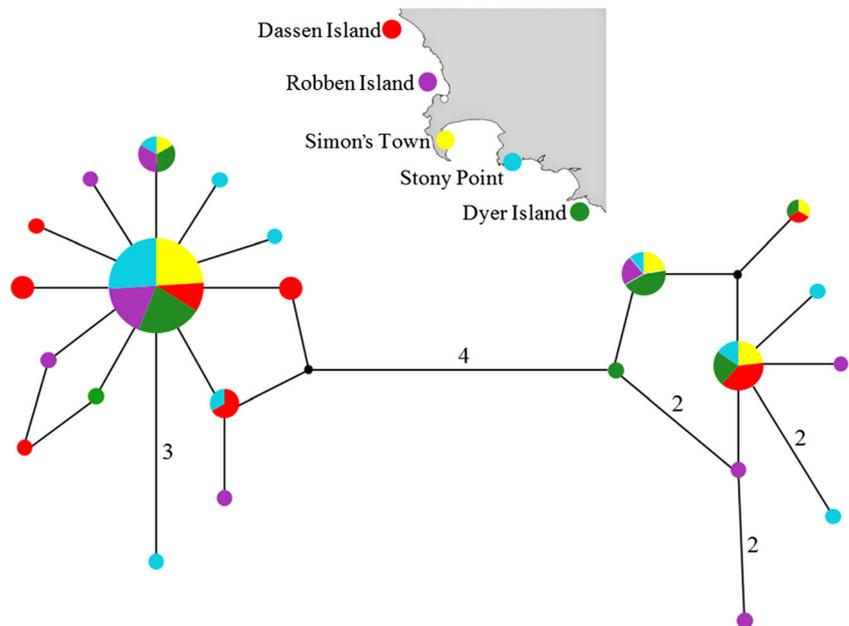
Analyses

All sequences were visually inspected and edited using the program GENEIOUS R7.1 (Biomatters Ltd., Auckland, New Zealand) and aligned using CLUSTAL W (Thompson et al. 1994). To test for the functionality of the fragments sequenced, all sequences were translated to amino acids in EXPASY (Gasteiger et al. 2003). Evolutionary relationships among haplotypes were confirmed independently through statistical haplotype networks in POPART 1.7 (Leigh and Bryant 2015) using a TCS network (Clement et al. 2000). ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010) was used to calculate haplotype diversity (h) and nucleotide diversity (π). To test for differentiation between the various a priori sampling populations, analyses of molecular variance (AMOVA) were performed in ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010). Significance of pairwise φ_{st} values was estimated at the 0.05 level with 10,000 permutation steps.

Results and discussion

A total of 103 *O. capensis* individuals, derived from the nests of five African penguin colonies, were successfully sequenced (Fig. 1, Table 1). No stop codons were evident in the data and 23 distinct haplotypes were obtained from 534 base pairs of the mtDNA COI gene fragment (Table 1). All individual sequences were deposited in Genbank (Accession numbers: MK104352 - MK104454). The statistical haplotype network reflected a lack of geographic population structure for *O. capensis* (Fig. 2). The number of haplotypes per population ranged from five to 10 (Table 1) and the most common haplotype was present at all five colonies and represented 48.54% of the total number of individuals (Fig. 2). The second most common haplotype represented 12.62% of the total number of individuals and this haplotype was shared between four of the five colonies: Dassen Island, Dyer Island, Simon's Town, and

Table 1 Genetic diversity summary statistics for *Ornithodoros capensis* (ticks) and *Parapsyllus humboldti* (fleas) sampled at the five different sites. Abbreviations indicate the number of mtDNA (COI) sequences (n), the number of nests parasites were sampled from (n nests), haplotype diversity (h) (± standard deviation), nucleotide diversity (π) (± standard deviation) and the number of unique haplotypes (nh)

Sampling site	n (n nests)		h (± SD)		π (± SD)		nh	
	Ticks	Fleas	Ticks	Fleas	Ticks	Fleas	Ticks	Fleas
Dassen Island	19 (8)	18 (9)	0.87 (±0.05)	0.31 (±0.13)	0.009 (±0.001)	0.001 (±0.0003)	8	3
Dyer Island	23 (15)	19 (10)	0.74 (±0.08)	0.61 (±0.13)	0.008 (±0.0009)	0.002 (±0.0006)	7	7
Robben Island	19 (10)	18 (10)	0.78 (±0.1)	0.77 (±0.08)	0.007 (±0.002)	0.003 (±0.0006)	9	6
Simon's Town	19 (12)	17 (7)	0.59 (±0.12)	0.47 (±0.12)	0.007 (±0.001)	0.001 (±0.0004)	5	3
Stony Point	23 (12)	20 (12)	0.69 (±0.11)	0.66 (±0.07)	0.007 (±0.002)	0.002 (±0.0003)	10	5
All sites	103 (57)	92 (48)	0.74 (±0.04)	0.68 (±0.04)	0.008 (±0.0006)	0.002 (±0.0002)	23	14

Fig. 2 MtDNA (COI) statistical haplotype network for the tick, *Ornithodoros capensis*. The circle sizes represent relative frequencies of haplotypes. Branch lengths connecting haplotypes are drawn proportional to the number of substitutions among haplotypes. It is also indicated where more than a single site separate haplotypes. The colors correspond to localities as indicated in the inset

Stony Point (Fig. 2). The level of genetic variation measured by nucleotide diversity (π) was estimated at 0.8% ($\pm$ 0.06%; Table 2). AMOVA analysis detected non-significant genetic structure among penguin colonies, with an overall φ-st value of 0 ($\pm$ 0.004) and $p = 0.80$ (Table 2). All the variation (100%) was found within populations, and pairwise φ-st comparisons between geographic localities were not significant in all instances (Table 2). These results confirm the prediction that the generalist *O. capensis* will show limited evidence for population differentiation among the five penguin colonies. This holds true despite the fact that *O. capensis* is mainly nest bound and nymphal and adult stages attach for very short periods on the host (Dupraz et al. 2016). We propose that the dispersal of soft ticks among colonies is mostly facilitated by the wide host range of the tick and in particular through the larval stages. Larvae attaches to the host for longer periods than other life stages (Dupraz et al. 2016) and appears to be the dominant life stage on African penguins (Espinaze unpublished data). In addition, flighted seabird species such as cormorants (*Phalacrocorax* spp.) and gulls (*Larus dominicanus*)

occur in close proximity to penguin nests (Espinaze personal observation) and in this case will facilitate the dispersal of *O. capensis*. Soft ticks are also resilient in their ability to survive (they can persist unfed for several weeks or months, Sonenshine 1991, 1993; they do not require a host in the nest, Duffy 1983, 1988; although nidicolous, they can survive under vegetation and rocks, Sonenshine 1993). It has been documented that nidicolous hard ticks (that attach to the host for longer periods) can survive transoceanic dispersal among seabird colonies (Moon et al. 2015). In addition, it has been found that the ear cavity and feathers of penguins provide insulation from the saline in seawater to a nidicolous tick, *I. eudyptidis* (Moon et al. 2015). Murray and Vestjens (1967) also established that nidicolous hard ticks (*I. uriae*) can survive immersion in water for several months (also see Sutherst 1971). In the present study, it thus seems reasonable to conclude that soft tick larvae have few barriers to gene flow among island and mainland colonies of seabirds.

A total of 92 *P. humboldti* individuals were sequenced and revealed 14 haplotypes for a 442 base pair mtDNA COI gene

Table 2 Population pairwise φ-st matrix for *Ornithodoros capensis* (above the diagonal) and *Parapsyllus humboldti* (below the diagonal) among sampling regions generated by AMOVA for mtDNA (COI). Significant values are indicated in italics ($p < 0.05$)

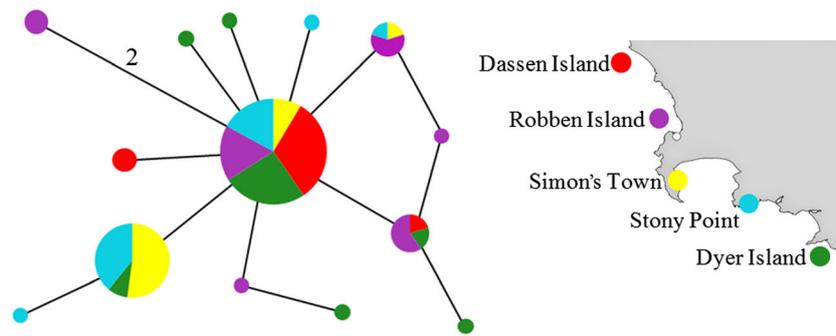|	Simon's Town	Dassen Island	Dyer Island	Robben Island	Stony Point
Simon's Town	–	0.00	0.00	0.00	0.00
Dassen Island	*0.53*	–	0.00	0.00	0.00
Dyer Island	*0.32*	0.01	–	0.00	0.01
Robben Island	*0.37*	*0.08*	*0.04*	–	0.00
Stony Point	0.01	*0.29*	*0.14*	*0.23*	–

Fig. 3 MtDNA (COI) statistical haplotype network for the flea, *Parapsyllus humboldti*. The circle sizes represent relative frequencies of haplotypes. Branch lengths connecting haplotypes are drawn proportional to the number of substitutions among haplotypes. It is also indicated where more than a single site separate haplotypes. The colors correspond to localities as indicated in the inset

fragment (Table 1). All individual sequences were deposited in Genbank (Accession numbers: MK104260 - MK104351). The statistical haplotype network reflected several shared haplotypes throughout the range and thus no visible geographic population structure (Fig. 3). The number of haplotypes per population ranged from three to seven (Table 1). The most common haplotype was present at all five penguin colonies and represented 51.1% of the total number of individuals (Fig. 3). The second most common haplotype represented 25% of the total number of individuals but this haplotype was only shared between Dyer Island, Simon's Town, and Stony Point (Fig. 3). In sharp contrast to the pattern obtained for the more generalist *O. capensis*, the nucleotide diversity (π) was much lower at 0.2% ($\pm$ 0.01%; Table 2) and AMOVA analysis detected significant genetic structure for *P. humboldti* among penguin colonies, with an overall φ-st value of 0.22 ($\pm$ 0.11%; $p = 0.00$). The variation among and within populations was 21.53% ($\pm$ 0.11%) and 78.47% ($\pm$ 0.39%) respectively. Most importantly, the pairwise φ-st comparisons between geographic localities were significant between all colonies, except Dassen Island and Dyer Island, as well as Simon's Town and Stony Point (Table 2).

Compared to the pattern obtained for the more generalist *O. capensis*, lower levels of genetic diversity, and the significant structure obtained for *P. humboldti* between most colonies, confirm the SGVH hypothesis. Parasites with narrower host ranges will show stronger signals of population differentiation and lower levels of genetic diversity, when compared to more generalist species (Li et al. 2014; Matthee et al. 2018). Several reasons can be put forward to explain the phylogeographic structure obtained for *P. humboldti*. First, fleas compared to soft ticks are more dependent on the presence of the host in the nest to survive (Marshall 1981) and flea numbers are known to fluctuate seasonally (Marshall 1981). Second, the narrow host range contributes strongly towards the phylogeographic structure obtained, particularly in the context of the documented tendency for the host, *S. demersus*, to show site philopatry (Crawford et al. 1995). Third, previous studies on bird fleas have shown that several thousand fleas can occur

in the nests of tits, *Parus* species (Jurik 1974) and of house martins, *Delichon urbica*; however, juveniles leaving the nest were found to carry on average only one or two fleas, up to a maximum of 10 (Marshall 1981). For *P. humboldti*, more limited host dispersal coupled to a narrow host range, contributes to the notion that during host dispersal, nest parasites with a narrow host range (that spend the majority of their time off the host) often miss the boat and the few that do occur on the dispersing host, drown on arrival (MacLeod et al. 2010).

This study provides additional evidence to support the SGVH for ectoparasites who are dependent on their host for dispersal. Previous studies on free-living vertebrate species partly supported the SGVH (Berkman et al. 2015; Janecka et al. 2016; Martinossi-Allibert et al. 2017) and in all instances, habitat specialization was put forward as the main contributor for a higher level of geographic population structure and or a lower level of intraspecific genetic diversity. In the case of parasites, however, host range seems to be the main driver in obtaining genetic patterns conforming to the SGVH.

Compliance with ethical standards

Ethical approval was obtained from Stellenbosch University Animal Ethics Committee (SU-ACUD15-00114) who followed the South African National Standard (SANS) for the Care and Use of Animals for Scientific Purposes (SANS 10386:2008).

Conflict of interest The authors declare that there is no conflict of interest.

References

Araya-Anchetta A, Busch JD, Scoles GA, Wagner DM (2015) Thirty years of tick population genetics: a comprehensive review. Infect Genet Evol 29:164–179

Berkman LK, Nielsen CK, Charlotte LR, Heist EJ (2015) Comparative genetic structure of sympatric Leporids in southern Illinois. J Mammal 96:552–563

Bitam I, Dittmar K, Parola P, Whiting MF, Raoult D (2010) Fleas and flea-borne diseases. Int J Infect Dis 14:667–676

Boyd EM (1951) The external parasites of birds: a review. Wilson Bull 63:363–369

Clement M, Posada DCKA, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1659

Crawford RJM, Williams AJ, Hofmeyer JH, Klages NTW, Randall RM, Cooper J, Dyer BMCY (1995) Trends of African penguin Spheniscus demersus populations in the 20th century. S Afr J Mar Sci 16:101–118

Duffy DC (1983) The ecology of tick parasitism on densely nesting Peruvian seabirds. Ecology 64:110–119

Duffy DC (1988) Ticks among the seabirds. Living Bird Q 7:8–13

Dupraz M, Toty C, Noël V, Estrada-Peña A, González-Solís J, Boulinier T, Dujardin J, McCoy K (2016) Linking morphometric and genetic divergence with host use in the tick complex, Ornithodoros capensis sensu lato. Infect Genet Evol 46:12–22

Engelbrecht A, Matthee S, Matthee CA (2016) Limited dispersal in an ectoparasitic mite, Laelaps giganteus, contributes to significant phylogeographic congruence with the rodent host, Rhabdomys. Mol Ecol 25:1006–1021

Excoffier L, Lischer HEL (2010) Arlequin Suite Ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3: 294–299

Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD, Bairoch A (2003) ExPASy: the proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Res 31:3784–3788

Gómez-Díaz E, Morris-Pocock JA, González-Solís J, McCoy KD (2012) Trans-oceanic host dispersal explains high seabird tick diversity on Cape Verde islands. Biol Lett 8:616–619

Hoogstraal H, Wassef HY, Hays C, Keirans JE (1985) Ornithodoros (Alectorobius) spheniscus n. sp. [Acarina: Ixodoidea: Argasidae: Ornithodoros (Alectorobius) capensis group], a tick parasite of the Humboldt penguin in Peru. J Parasitol 71:635–644

Janecka JE, Tewes ME, Davis IA, Haines AM, Caso A, Blankenship TL, Honeycutt RL (2016) Genetic differences in the response to landscape fragmentation by a habitat generalist, the bobcat, and a habitat specialist, the ocelot. Conserv Genet 17:1093–1108

Jordan K (1942) On Parapsyllus and some closely related genera of Siphonaptera. Eos 18:7–29

Jurik M (1974) Bionomics of fleas in bird's nests in the territory of Czechoslovakia. Acta Sc Nat Brno 8:1–54

Leigh JW, Bryant D (2015) PopART: full-feature software for haplotype network construction. Methods Ecol Evol 6:1110–1116

Li S, Jovelin R, Yoshiga T, Tanaka R, Cutter AD (2014) Specialist versus generalist life histories and nucleotide diversity in Caenorhabditis nematodes. Proc R Soc Lond B 281:2013–2858

MacLeod CJ, Paterson AM, Tompkins D, Duncan RP (2010) Parasites lost – do invaders miss the boat or drown on arrival? Ecol Lett 13: 516–527

Marshall AG (1981) The ecology of ectoparasitic insects. Academic, London; New York

Martinossi-Allibert I, Clavel J, Ducatez S, Le Viol I, Teplitsky C (2017) Does habitat specialization shape the evolutionary potential of wild bird populations? J Avian Biol 48:1158–1165

Matthee CA, Engelbrecht A, Matthee S (2018) Comparative phylogeography of parasitic Laelaps mites contribute new insights into the specialist-generalist variation hypothesis (SGVH). BMC Evol Biol 18:131

McCoy KD, Boulinier T, Tirard C (2005) Comparative host-parasite population structures: disentangling prospecting and dispersal in the black-legged kittiwake Rissa tridactyla. Mol Ecol 14:2825–2838

Moon KL, Banks SC, Fraser CI (2015) Phylogeographic structure in penguin ticks across an ocean basin indicates allopatric divergence and rare trans-oceanic dispersal. PLoS One 10:e0128514

Muñoz-Leal S, Dias RA, Abrahão CR, Labruna MB (2017) The Ornithodoros capensis group (Acari: Argasidae): a morphological diagnosis and molecular characterization of O. capensis sensu stricto from Queimada Grande Island, Brazil. Syst Appl Acarol 22:28–41

Murray MD, Vestjens WJM (1967) Studies on the ectoparasites of seals and penguins III. The distribution of the tick Ixodes uriae White and the flea Parapsyllus magellanicus heardi de Meillon on Macquarie Island. Aust J Zool 15:715–725

Segerman J (1995) Siphonaptera of southern Africa. Handbook for the identification of fleas. Publications of the South African Institute for Medical Research, No. 57, Johannesburg

Smith KM, Karesh WB, Majluf P, Paredes R, Zavalaga C, Hoogesteijn Reul A, Stetter M, Braselton WE, Puche H, Cook RA (2008) Health evaluation of free-ranging Humboldt penguins (Spheniscus humboldti) in Peru. Avian Dis 52:130–135

Sonenshine DE (1991) Life cycles of ticks. In: Sonenshine DE (ed) Biology of ticks. Oxford University Press, New York, pp 51–66

Sonenshine DE (1993) Ecology of nidicolous ticks. In: Sonenshine DE (ed) Biology of ticks. Oxford University Press, New York, pp 66–91

Sutherst RW (1971) An experimental investigation into the effects of flooding on the ixodid tick Boophilus microplus (Canestrini). Oecologia 6:208–222

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Van der Mescht L, Matthee S, Matthee CA (2015) Comparative phylogeography between two generalist flea species reveal a complex interaction between parasite life history and host vicariance: parasite-host association matters. BMC Evol Biol 15:105