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Comparative phylogeography between parasitic sucking lice and their host the Namaqua rock mouse, *Micaelamys namaquensis* (Rodentia: Muridae)

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To gain a deeper understanding of the mechanisms affecting parasite gene dispersal and subsequent evolution, we investigated mitochondrial and nuclear DNA phylogeographic structures of two ectoparasitic louse species, *Polyplax praomydis* and *Hoplopleura patersoni*, and compared this to their host *Micaelamys namaquensis*. Analyses of mitochondrial and nuclear DNA sequence data derived from 13 geographic populations resulted in the detection of distinct phylogenetic clades within the parasite and host species. Strong support for host–parasite co-divergence was found over larger geographic scales but failed to show complete co-divergence over fine geographic scales. This finding led to the partial rejection of the hypothesis that the evolution of species-specific permanent parasites will mirror the phylogeographic pattern of their host. JANE co-phylogenetic reconstructions support the notion that host switching best explains the discrepancies in geographic patterns. We conclude that host specificity and permanency on the host only plays a partial role in maintaining co-divergences between parasites and their hosts.

ADDITIONAL KEYWORDS: phylogeography – host-parasite systems – South Africa.

INTRODUCTION

Genetic connectivity among populations of the same species across its geographic range can be affected by a number of factors, including the dispersal ability of the organism, their habitat requirements and historical biogeographic events (Kumar & Kumar, 2018). Although the mechanisms affecting gene flow of free-living organisms are reasonably well documented, those affecting parasites are less well-studied and in need of further investigation (Nieberding & Morand, 2006; Morand & Krasnov, 2010). Nevertheless, several parasite studies suggest correlations between gene flow of the parasite and that of their host (Nieberding & Morand, 2006; du Toit *et al.*, 2013; van der Mescht *et al.*, 2015; Engelbrecht *et al.*, 2016; Tobias *et al.*, 2017; Bell *et al.*, 2018; Martinů *et al.*, 2018; Matthee, 2020). To gain a deeper understanding of the mechanisms affecting parasite phylogeography and evolution, comparative studies using multiple species of parasites and hosts (preferably sampled from the same geographic region) can be extremely valuable (also see: Gutiérrez-García *et al.*, 2011; Engelbrecht *et al.*, 2016; Bell *et al.*, 2018; Matthee *et al.*, 2018).

In the present study we document the phylogeographic structures of two ectoparasitic louse species (*Hoplopleura patersoni* Johnson, 1960 and *Polyplax praomydis* Bedford, 1929), which have similar life histories, and compare them to the phylogeographic structure of their rodent host the Namaqua rock mouse, *Micaelamys namaquensis* (A. Smith, 1834). The host species occurs across multiple biomes and diverse habitats in southern Africa, but at the finer geographic scale they prefer rocky outcrops or boulder-strewn hillsides (Skinner & Chimimba, 2005; Fagir *et al.*, 2014). This preferred habitat type leads to a prediction that the species may show strong phylogeographic structuring across their range (see: Matthee & Robinson, 1996; Matthee & Flemming, 2002). Indeed, *M. namaquensis* is characterized by at

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least eight geographically distinct mitochondrial DNA (mtDNA) cytochrome *b* (*Cytb*) lineages throughout the region (Russo *et al.*, 2010). This high level of geographic variation may contribute to the vacillations in the number of recognized *M. namaquensis* subspecies (Roberts, 1951; Meester *et al.*, 1964). The most recent taxonomic investigation suggests at least four geographically distinct lineages confined to phytogeographical zones within southern Africa (Chimimba, 2001).

Previous investigations based on morphological characteristics have shown that *M. namaquensis* is parasitized by three sucking louse species *Hoplopleura aethomydis* Kleynhans, 1969, *H. patersoni* and *P. praomydis* (Durden & Musser, 1994; Fagir *et al.*, 2014). However, a recent investigation failed to detect any *H. aethomydis* on three populations of *M. namaquensis* (Bothma *et al.*, 2020), a scenario most likely attributed to their low prevalence on the host and/or a potentially restricted geographic distribution (Fagir *et al.*, 2014). Pertinent to the focus of this study, significant phylogeographic structure was detected within both louse species sampled from *M. namaquensis* trapped at three geographically distinct sampling sites (Bothma *et al.*, 2020). One lineage (comprising a single sample site) was confined to the northern part of South Africa and another lineage (comprising two sampling sites) to the southern part of South Africa (Bothma *et al.*, 2020). A mtDNA cytochrome oxidase *c* subunit I (*COI*) sequence divergence of 13.1% (± 4.8%) and 14.9% (± 4.7%) separated the geographic clades within *H. patersoni* and *P. praomydis*, respectively (Bothma *et al.*, 2020). The same geographic structure was mirrored in the host, *M. namaquensis*, where the monophyletic northern population was separated from the monophyletic southern population by at least 3.7% (± 1.1%) mtDNA *COI* sequence divergence (Bothma *et al.*, 2020). Moreover, Bothma *et al.* (2020) also produced evidence of significant co-divergence at the higher taxonomic level between *H. patersoni* and *P. praomydis* occurring on four different rodent species (belonging to *Micalaemys* and *Aethomys* genera, respectively), supporting species-specificity between these parasites and their hosts.

Since eight genetically distinct geographic assemblages have been identified in the host lineage (Russo *et al.*, 2010), and the fact that the two Anoplura louse species included in this study are most likely species-specific and permanently associated with the host (Ledger, 1980; Bothma *et al.*, 2020), it is predicted that the phylogeographic structures of *H. patersoni* and *P. praomydis* will echo that of *M. namaquensis*. However, it is important to realize that permanency on a host, and host specificity, as displayed by the lice included herein, do not necessarily ensure phylogeographic congruence (du Toit *et al.*, 2013). At the micro-evolutionary scale (recent events), differences in demography between parasite and host lineages (Martinů *et al.*, 2018), absence of adaptive divergence (du Toit *et al.*, 2013; Engelbrecht *et al.*, 2016) and differences in the vagility and social behaviour of the host (du Toit *et al.*, 2013), could contribute to a lack of complete phylogeograhic congruence between species-specific permanent parasites and their hosts.

By using wide geographic sampling and data derived from mtDNA and nuclear DNA markers, the aims of this study are to: (1) test for phylogeographic congruence between *M. namaquensis* and two Anoplura sucking louse species associated with it and (2) add to the growing body of literature describing mechanisms involved in parasite dispersal and gene flow. We hypothesize that both *H. patersoni* and *P. praomydis* will show significant phylogeographic congruences with the phylogeographic structure of *M. namaquensis*.

MATERIAL AND METHODS

Host and parasite sampling

A total of 233 *M. namaquensis* individuals were sampled at 13 localities across its geographic range in South Africa (Fig. 1). Sherman-live traps baited with a mixture of peanut butter and oats were used, and sampling occurred under permit numbers: Limpopo, ZA/LP/90994; North West, NW 7705; Eastern Cape, CRO150/17CR and CRO 11/17CR; Northern Cape, FAUNA 0942/2017 and FAUNA 0949/2017; Gauteng, CPF6-0194; Free State, NC. 672/2017; Western Cape, 0056-AAA007-00140) and Stellenbosch University Animal Ethics Committee approval (SU-ACUD16-00190). Trapped hosts of the target species were placed individually in plastic bags (to retain the parasites from each individual separately) and euthanized using an intraperitoneal injection of sodium pentobarbitone (200 mg/kg). Sacrificed individuals were frozen at –20 °C and transferred to the laboratory where muscle/tongue tissue was collected for DNA extraction from the host. All the remains of the material sampled are currently housed in the private collection of SM at Stellenbosch University, Department of Conservation Ecology and Entomology. All DNA is stored in the SUN DNA databank of CAM at the same institution but within the Department of Botany and Zoology.

In the laboratory, all ectoparasites were removed by systematically examining the body of the rodent under a stereoscopic microscope (Leica Microsystems, Wetzlar, Germany) and removing parasites with fine-point forceps. A total of 516 *H. patersoni* and 1043

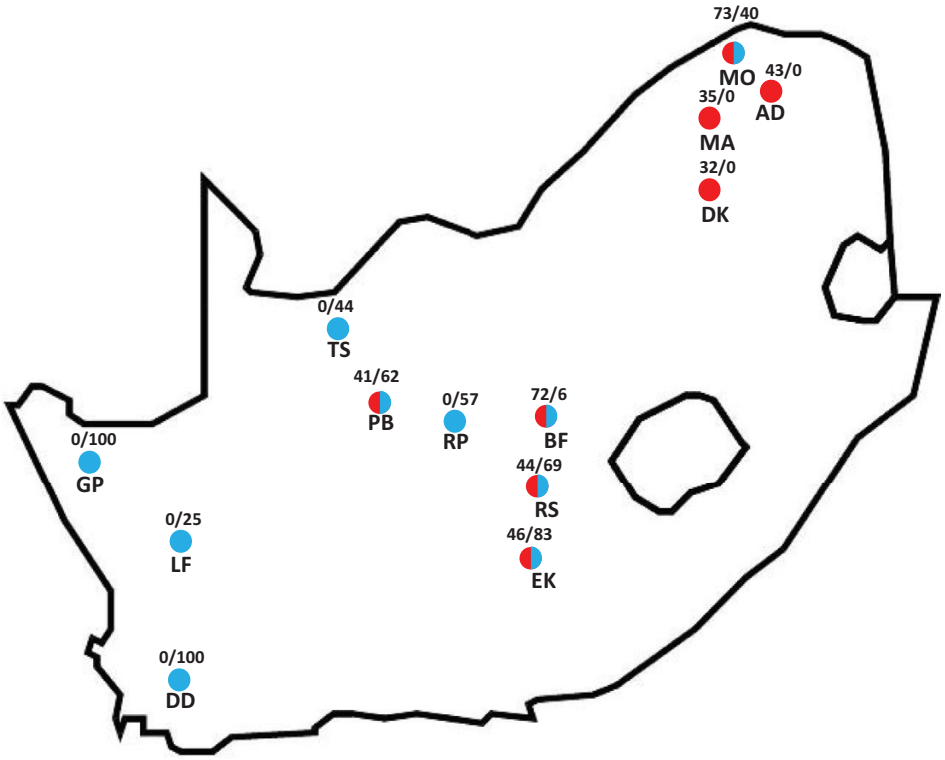

Figure 1. Sampling localities where the host *M. namaquensis* and lice *H. patersoni* (red) and/or *P. praomydis* (blue) were recorded: De Doorns (DD), Loeriesfontein (LF), Goegap (GP), Elandskuil (EK), Rusplaas (RS), Bloemfontein (BF), Rooipoort (RP), Postmasburg (PB), Tswalu (TS), Dinokeng (DK), Marken (MA), Alldays (AD) and Mogalakwena (MO). Numbers indicate the percentage prevalence of *H. patersoni* and *P. praomydis* at each locality.

P. praomydis were removed from 156 hosts (Table 1). All specimens were placed in 100% ethanol. Published species descriptions, as well as taxonomic reference keys, were used to identify the parasites to species level (Paterson & Thompson, 1953; Johnson, 1960; Kleynhans, 1969; Ledger, 1980). Where ten or more lice were collected from the same locality, subsampling was performed by randomly selecting lice from different *M. namaquensis* individuals (Table 1).

DNA EXTRACTION, POLYMERASE CHAIN REACTION (PCR) AND SEQUENCING

Total genomic DNA was extracted from hosts and lice using the protocols provided in the Nucleospin Tissue kit (Macherey-Nagel, Duren, Germany; Table 1). For both *M. namaquensis* and the louse taxa, the mtDNA *COI* gene was amplified and sequenced using standard published procedures (Bothma *et al.*, 2020). Nuclear DNA data were generated for the carbamoyl-phosphate synthetase 2, aspartate transcarbamylase and dihydroorotase (*CAD*), nuclear elongation factor 1 alpha (*EF-1α*) and interphotoreceptor retinoid binding protein (*IRBP*) genes for *P. praomydis*, *H. patersoni* and *M. namaquensis*, respectively (see: Bothma *et al.*, 2020). Sanger sequences were visualized in GENEIOUS v.9.1 (https://www.geneious.com) and aligned with Clustal W using default parameters (Thompson *et al.*, 1994).

DATA ANALYSIS

Statistical parsimony haplotype networks were constructed in POPART v.1.7 (Clement *et al.*, 2002) to illustrate the intraspecific evolutionary relationships among host, as well as parasite, individuals. Significance of connections were obtained from TCS v.1.21 (Clement *et al.*, 2000). Standard molecular diversity measures were calculated in DNAsp v.6.12.1 (Rozas *et al.*, 2017). Three-level hierarchical analyses of molecular variance (AMOVA; Excoffier *et al.*, 1992) were conducted for the mtDNA and the nuclear DNA data in ARLEQUIN v.3.5.2.2 (Excoffier & Lischer, 2010). Geographic assemblages identified by the haplotype networks were used as priors to define the groups within *M. namaquensis*, *P. praomydis* and *H. patersoni*.

Bayesian phylogenetic trees in MrBayes v.3.2.6 (Ronquist *et al.*, 2012) and maximum likelihood trees in RAxML v.1.5 (Stamatakis, 2006) were used to depict the deeper evolutionary relationships among clades. Since

Table 1. The number of *M. namaquensis* trapped and screened, the total abundance of lice per locality and the number of hosts and lice used for molecular analyses for each locality. The locality abbreviations correspond to Figure 1

Locality	Number of *M. namaquensis* caught and screened	Number of hosts with lice	Number of lice (*H. patersoni*/*P. praomydis*	Number of *M. namaquensis* used in analyses	Number of lice used in analyses (*H. patersoni*/*P. praomydis*)
DD	2	2	0/6	2	0/5
LF	8	2	0/15	2	0/8
GP	6	6	0/6	1	0/6
EK	35	31	69/362	10	15/19
RS	36	31	82/436	16	14/18
RP	23	13	0/34	13	0/16
PB	29	23	69/157	15	9/19
TS	9	4	0/8	4	0/8
BF	18	14	83/6	14	22/6
DK	25	9	49/0	4	7/0
MA	20	7	61/0	4	13/0
AD	7	3	10/0	3	7/0
MO	15	11	93/19	10	10/12
Total	233	156	516/1043	98	97/121

the nuclear DNA data did not provide any conflicting resolution when compared with the mtDNA results, the two gene fragments for each species were combined and analysed in a single dataset. Best-fit models of sequence evolution for each gene fragment and codon position was calculated using jModelTest v.3.7 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012) in PAUP v.4 (Swofford, 2002). The AIC criterion was applied to select among models (Akaike, 1973; Burnham & Anderson, 2004). *Micaelamys granti* (Wroughton, 1908) and its associated *Hoplopleura* and *Polyplax* lice were used as closely related outgroups in the analyses (Bothma *et al.*, 2020). The Bayesian analyses were all partitioned by gene and codon, and parameters were unlinked across partitions. Each analysis included two parallel Markov chain Monte Carlo (MCMC) simulations comprising four chains each that ran for ten million generations. Trees were sampled every 100th generation and 25% of the initial sampled trees were discarded as burn-in. The remaining trees were visualized in FigTree v.1.4.3 (Rambaut & Drummond, 2015) to obtain posterior probabilities for nodes. The same partitions were employed for the maximum likelihood analyses, but in this instance, 1000 bootstrap repetitions were performed to obtain confidence in the nodes. The best-fit models of evolution were again specified for different partitions. Trees were again visualized in FigTree v.1.4.3 (Rambaut & Drummond, 2015).

Geographic co-divergence analyses

Geographic co-divergences between the geographic clades detected in *M. namaquensis* and those detected in *H. patersoni* were investigated by topology-based reconciliation in JANE v.4 (Conow *et al.*, 2010). Since merely two clades were detected in *Polyplax*, this taxon was not included in the analyses [see: Bothma *et al.* (2020) showing complete co-divergence between these two clades]. The selection of *H. patersoni* OTUs included in this analysis was based on monophyletic groupings found in the parasite and the host, and these OTUs were specifically chosen to emphasize the conflict between parasite and host geographic patterns. Tree topologies based on the mtDNA haplogroups were constructed for *M. namaquensis* and *H. patersoni* using tree editor imbedded in JANE v.4 (Conow *et al.*, 2010). In these co-divergence analyses, the standard Vertex cost model with the cost scheme: failure to diverge = 1, loss = 1, duplication followed by host-switch = 2, duplication = 1, co-divergence = 0, was implemented (Conow *et al.*, 2010). The genetic algorithm was set to 1000 generations and a population size of 300. Statistical significance of the solutions was evaluated by random tip mapping and the randomization of the parasite topology, where the statistical algorithm was again set to 1000 generations, a population size of 300 and including a sample size of 1000 [as in Engelbrecht *et al.* (2016)].

RESULTS

Identification of sucking lice associated with *Micaelamys namaquensis*

In the present study, only *H. patersoni* and *P. praomydis* were recorded on *M. namaquensis*. *Hoplopleura patersoni* was distributed across most of the country (eight localities), but show either lower prevalence or complete absence from some of the central and the extreme south-western localities that are dryer in climate (Fig. 1). In contrast, *P. praomydis* shows a low prevalence in the more mesic northern part of the country and was only recorded from a single location outside of the Central/SW clade (Fig. 1).

Molecular data

MtDNA and nuDNA sequence data were generated for 98 *M. namaquensis*, 121 *P. praomydis* and 97 *H. patersoni* individuals across the range of *M. namaquensis* (Table 1). All the sequences generated were translated to proteins to confirm the absence of stop codons. The population data used in this study were deposited in GenBank (accession numbers: *COI M. namaquensis*: MT424221–MT424318; *IRBP M. namaquensis*: MT424486–MT424559; *COI P. praomydis*: MT424100–MT424220; *CAD P. praomydis*: MT424399–MT424485; *COI H. patersoni*: MT424004–MT424099; *EF-1α H. patersoni*: MT424319–MT424398).

Diversity indices

The nucleotide diversity within *M. namaquensis* was 0.02 (± 0.01) for the mtDNA and 0.00 (± 0.00) for the nuDNA, while the haplotypic diversity was 0.95 (± 0.01) for the mtDNA and 0.60 (± 0.06) for the nuDNA. Within *P. praomydis*, the nucleotide diversity was 0.03 (± 0.01) for the mtDNA and 0.00 (± 0.00) for the nuDNA, whilst the haplotypic diversity was 0.82 (± 0.02) for the mtDNA and 0.29 (± 0.06) for the nuDNA. The nucleotide diversity within *H. patersoni* was 0.07 (± 0.01) for the mtDNA and 0.00 (± 0.00) for the nuDNA, while the haplotypic diversity was 0.91 (± 0.01) for the mtDNA and 0.00 (± 0.00) for the nuDNA.

Micaelamys namaquensis host phylogeographic structure

Significant intraspecific differentiation among sampling sites was present within *M. namaquensis*. Three distinct mtDNA lineages were recovered that could not be connected with 95% confidence (Fig. 2A). All individuals trapped at the nine central and south-western localities in South Africa belong to the first haplogroup (Central/SW; Fig. 2A, D). Individuals trapped at a single locality in the far north of the country [Mogalakwena (MO)] belong to the second haplogroup (NE 1; Fig. 2A, D). The third haplogroup (NE 2) is also situated in the northern regions of the country and includes three localities (Fig. 2A, D). Mitochondrial DNA sequence divergences between the three haplogroups ranged from 3.6% (± 0.4%; 13 mutational steps), between the central/SW and NE 1 haplogroup, to 4.0% (± 1.1%; 18 mutational steps), between the geographically closer NE 1 and NE 2 haplogroups (Fig. 2A). The nuDNA TCS network for *M. namaquensis* shows a mixture of private haplotypes at several localities but also a large amount of haplotype sharing among sampling sites (all haplotypes could be connected with 95% confidence; Fig. 3). By using the Timef model (nst = 6; rates = equal) the concatenated mtDNA and nuDNA dataset reveals significant posterior probability and high bootstrap support for the monophyly of the three *M. namaquensis* clades (Supporting Information, Appendix S1). The three-level hierarchical analyses of molecular variance indicated that the majority of the genetic variation among *M. namaquensis* sampling sites were between haplogroups (54.1%; $P < 0.05$) followed by among populations within haplogroups (35.0%; $P < 0.05$). The high level of structure among *M. namaquensis* sampling sites was also reflected in the high ΦST value of 0.89 ($P < 0.05$) for the mtDNA (Table 2) and 0.71 ($P < 0.05$) for the nuclear data (Table 2). Significant pairwise mtDNA Φst values among all sampling localities (where more than five individuals were included) confirm a low level of population connectivity among geographic populations (Supporting Information, Appendix S2).

Hoplopleura patersoni phylogeographic structure

Similar to the host, the mtDNA TCS network for *H. patersoni* also reveals three haplogroups that could not be connected with 95% confidence (Fig. 2B). The first haplogroup (central/SW) represented all the *H. patersoni* individuals collected from *M. namaquensis* individuals trapped at localities in central and south-west South Africa (central/SW). This pattern is 100% congruent with what has been found in the host (Fig. 2A, D). There are also two separate haplogroups in the north-eastern part of South Africa, but the geographic positioning of these clades differ from the host pattern (Fig. 2A, B, D). *Hoploplura patersoni* individuals from Mogalakwena (MO), Marken (MA) and Alldays (AD) form a single haplogroup (NE 1; Fig. 2B), whilst individuals from Dinokeng (DK) form part of the second northern

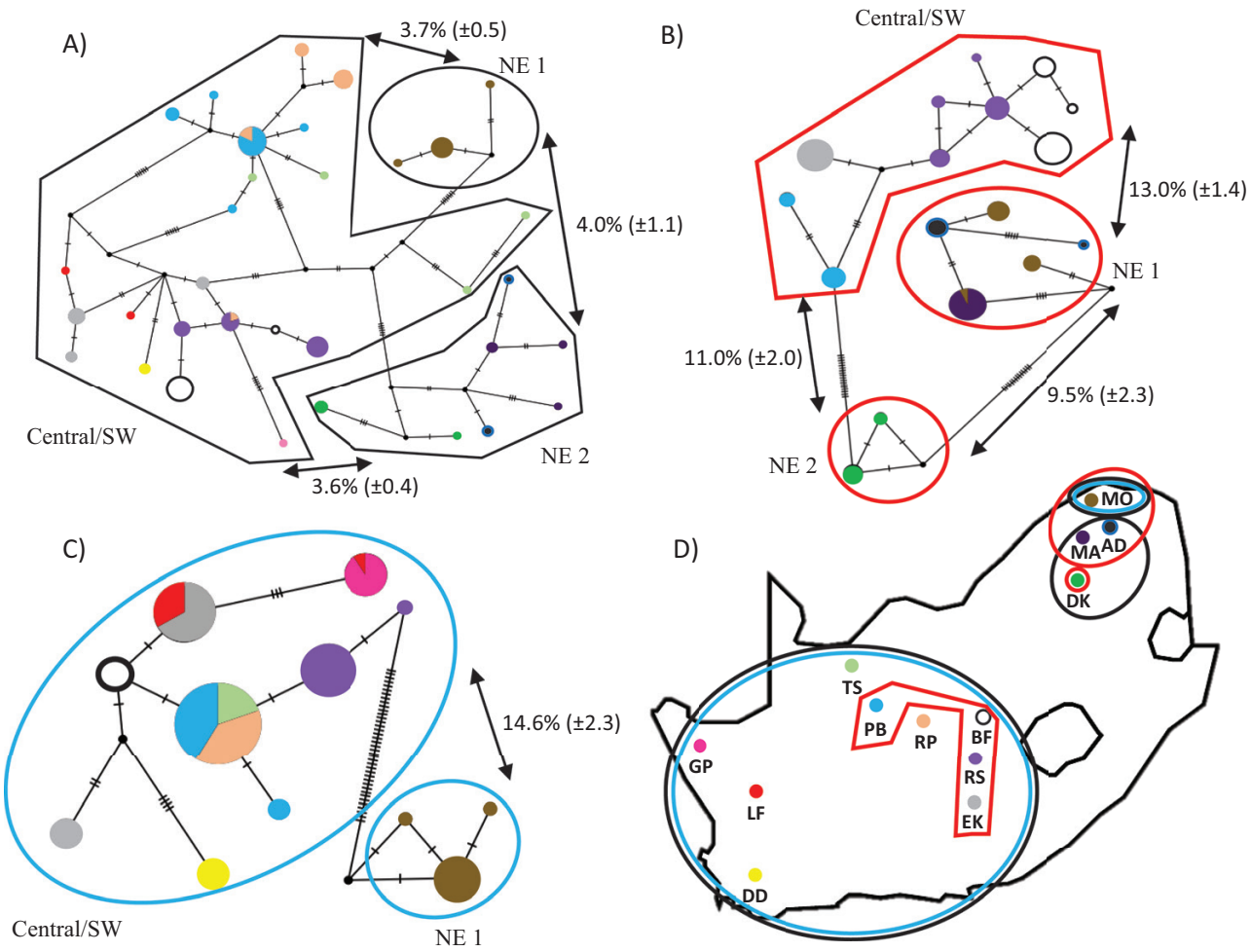

Figure 2. MtDNA haplotype networks for: A, *M. namaquensis*; B, *H. patersoni*; C, *P. praomydis*. Haplogroups that could not be connected with 95% confidence are identified by shapes/circles. The average percentage *COI* sequence divergence between the different haplotypes and the number of mutational steps between haplogroups are indicated. The geography of haplogroups are indicated in (D), where black, red and blue circles indicate the haplogroups of *M. namaquensis*, *H. patersoni* and *P. praomydis* separately. Colours of sampling localities correspond to the colours used in the haplotype networks (A–C).

haplogroup (NE 2; Fig. 2B). As expected for parasites, the genetic differentiation between the *H. patersoni* haplogroups is much higher than that seen between the *M. namaquensis* haplogroups and range from 9.5% (± 2.3%; 17 mutational steps), between NE 1 and NE 2, to 13.0% (± 1.4%; 35 mutational steps), between the central/SW haplogroup and the NE 1 haplogroup (Fig. 2B). The nuDNA TCS network is unable to provide any insights because all *H. patersoni* individuals are identical for the *EF-1α* gene. For the *H. patersoni* phylogeny the TrNef+G model (nst = 6, rates = gamma) of sequence evolution was assigned for the first and third codons, whilst the K81 model (nst = 6, rates = equal) of sequence evolution was assigned to the second codon. The phylogenetic analyses reveal significant nodal support for the monophyly of all the *H. patersoni* individuals collected from *M. namaquensis* and there is also significant nodal support for the monophyly of the three clades within *H. patersoni* (Supporting Information, Appendix S1). The three-level hierarchical analyses of molecular variance indicated that at the mtDNA level, 85.7% ($P < 0.05$) of the variation is found among haplogroups and another 10.2% ($P < 0.05$) is found among localities within haplogroups (Table 2). A high level of phylogeographic structure for this parasite species is reflected in the high and significant mtDNA Φst value 0.96 ($P < 0.05$). Limited gene-flow among localities is further supported by significant pairwise Φst values among all sampling localities (Supporting Information, Appendix S2).

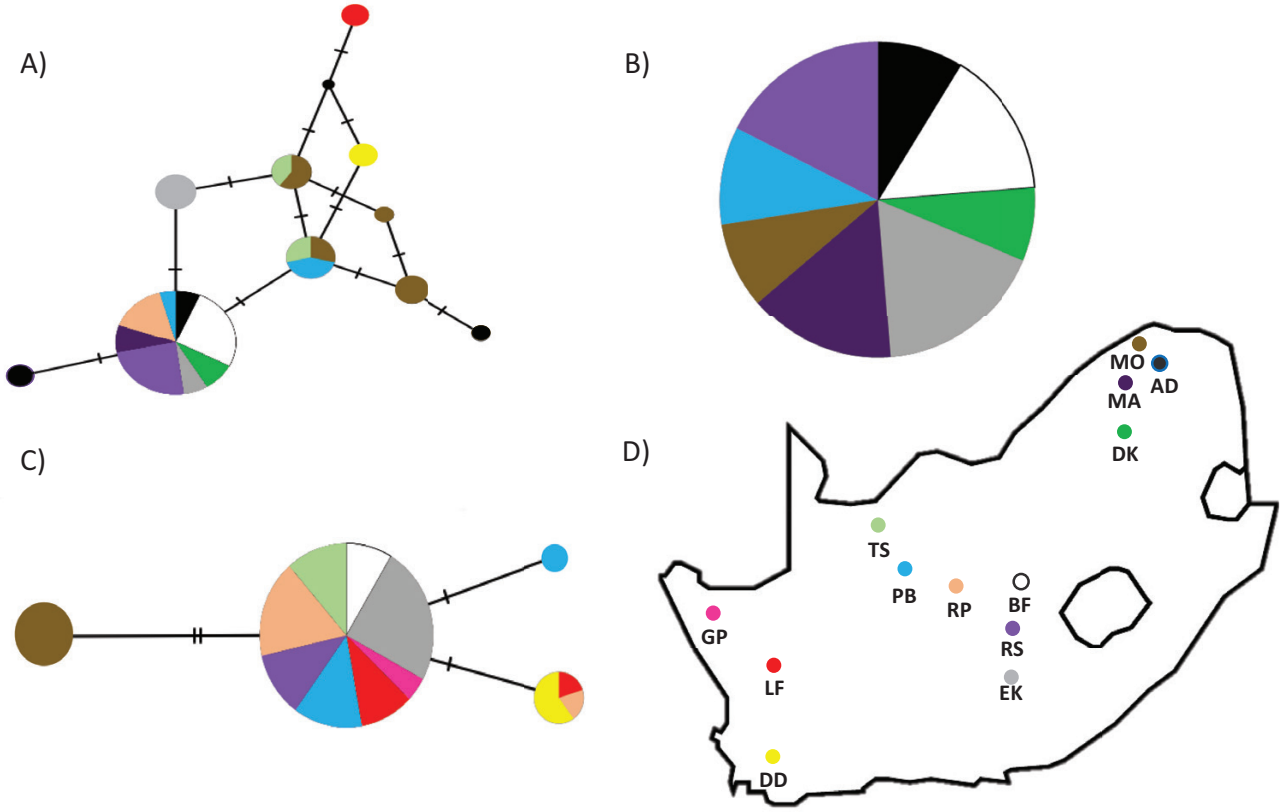

Figure 3. NuDNA haplotype networks for: A, *M. namaquensis*; B, *H. patersoni*; C, *P. praomydis*. Haplotype colours correspond to the colours of the different localities from where the samples were collected in (D).

Table 2. Results from three-level hierarchical analyses of molecular variance for the mtDNA and nuclear DNA datasets of *M. namaquensis*, *H. patersoni* and *P. praomydis*. Statistically significant values ($P < 0.05$) are indicated with *

Species	Fixation index			Variation (%)		
	ΦST	ΦSC	ΦCT	Among haplogroups	Among localities within haplogroups	Within localities
mtDNA						
M. namaquensis	0.89*	0.76*	0.54*	54.1	35.0	10.9
H. patersoni	0.96*	0.72*	0.86*	85.7	10.2	4.1
P. praomydis	0.99*	0.85*	0.94	93.6	5.5	0.9
nuDNA						
M. namaquensis	0.71*	0.63*	0.22*	21.7	49.3	29.0
H. patersoni	NA	NA	NA	NA	NA	NA
P. praomydis	0.97*	0.53*	0.93	92.9	3.8	3.4

POLYPLAX PRAOMYDIS PHYLOGEOGRAPHIC STRUCTURE

The mtDNA TCS network for *P. praomydis* reveals two haplogroups that could not be connected with 95% confidence (Fig. 2C). All *P. praomydis* individuals from all nine localities sampled in the central and western parts of the country form part of the same haplogroup (central/SW; Fig. 2C). This pattern is consistent to what has been found in the host and for *H. patersoni*. The second *P. praomydis* haplogroup (NE 1) only includes individuals from Mogalakwena (MO; Fig. 2C) and

correspond to the host NE1 clade (Fig. 2A). These two haplogroups are separated by a mtDNA sequence divergence of 14.6% (± 2.3%; 40 mutational steps). Although all *P. praomydis* individuals could be connected with 95% confidence in the nuDNA TCS network, the two haplogroups (retrieved in the mtDNA data) differ by two mutational steps (Fig. 3C). The TrNef+G model (nst = 6; rates = gamma) of sequence evolution was assigned for the first and third codons, whilst the K81 model (nst = 6; rates = equal) was assigned to the second codon. The concatenated mtDNA and nuDNA analysis reveals significant posterior probability and high bootstrap support for both the monophyly of the NE 1 lineage and the central/SW lineage (Supporting Information, Appendix S1). Analyses of molecular variance support significant differentiation among the haplogroups of *P. praomydis* with 93.6% ($P < 0.05$) of the mtDNA variation assigned to this category and a further 5.5% ($P < 0.05$) assigned to variation among localities within haplogroups (Table 2). A similar picture is obtained for the nuclear DNA analyses where 92.9% ($P < 0.05$) of the variation is confined to variation between haplogroups (Table 2). *Polyplax praomydis* populations show a near absence of gene flow among sampling sites [mtDNA Φst of 0.99 ($P < 0.05$); nuclear Φst of 0.97 ($P < 0.05$); Table 2]. Significant mtDNA pairwise Φst values also support the differentiation among almost all sampling localities (Supporting Information, Appendix S2). Non-significant pairwise Φst values are confined to pairwise comparisons between Tswalu (TS), Postmasburg (PB) and Rooipoort (RP) sampling localities (Supporting Information, Appendix S2).

Co-phylogeny

All parasite and host individuals belonging to the CSW clade form a strongly supported monophyletic group (96% bootstrap support; 1.00 posterior probability; Supporting Information, Appendix S1). Conflicts between the parasite and host trees are all confined to the four localities found in the NE1 and NE2 clades (Supporting Information, Appendix S1). The JANE co-phylogenetic reconstruction between *M. namaquensis* and *H. patersoni* reveals that the most parsimonious solution with a total cost of three, includes three co-divergences, one host switch and one loss (Fig. 4). Both of the statistical analyses indicated non-significant co-phylogeny between the *M. namaquensis* and *H. patersoni*, with $P = 0.39$ and $P = 0.27$ for random tip mapping and random parasite tree, respectively.

DISCUSSION

Recent co-phylogeny research on the rodent genera *Aethomys* and *Micaelamys* and their sucking lice, *Polyplax* and *Hoplopleura*, indicated significant co-divergences between these obligate parasites and their hosts (Bothma *et al.*, 2020). The co-divergences were attributed to potential host specificity of the parasites who are associated with their hosts on a permanent basis, but factors such as host distribution patterns and the limited vagility of the host could not be excluded as reinforcing mechanisms involved (limited host contact will restrict host switching).

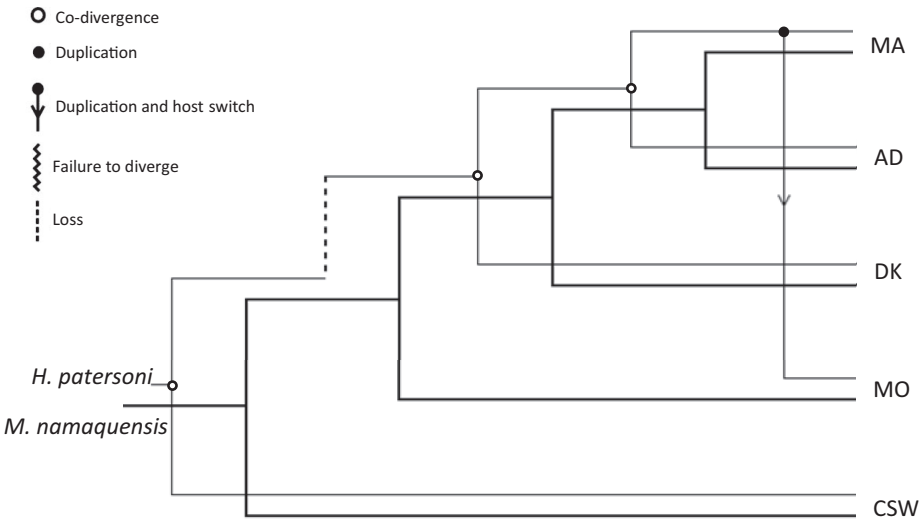

Figure 4. Phylogenetic reconciliation of *H. patersoni* and *M. namaquensis* retrieved from JANE after the five types of evolutionary events (legend) was tested for. The locality abbreviations refer to the localities in Figure 1 and CSW refers to the central/south-western haplogroup.

The focus of this phylogeographic study is on more recent evolutionary events (geographic co-divergence within species) and it is notable that strong signs of host–parasite co-divergence are again present over larger geographic scales (both parasite species and the host show strong congruences in patterns between the northern clades on the one hand and the central/SW clade on the other; Fig. 2). Unfortunately, *Polyplax* lice were only recovered from a single locality in the northern regions of the country and fine-scale geographic structure in this region can thus only be inferred by comparing *Hoplopleura* patterns to the host. In the latter instance, we detected incongruences in patterns between parasite and host (NE1 and NE2 clades; Fig. 2) and we, therefore, have to reject the hypothesis that *Hoplopleura* co-diverged fully with *M. namaquensis*.

The reason for the inconsistencies in patterns between parasite and host are most likely due to host switching in the absence of adaptive evolution between *Micaelamys* and their *Hoplopleura* lice (also see: du Toit *et al.*, 2013). This finding emphasizes the fact that permanency on the host, and species-specificity, do not guarantee co-divergences between parasites and their hosts. In our study, host contact is needed for host switching to happen and we predict that this will often happen, especially where host taxa are evolutionary closely related (also see: du Toit *et al.*, 2013; Martinů *et al.*, 2018). Our findings further highlight the notion that predicting co-divergences between parasites and hosts are complex and can depend on multiple host- and parasite-related features (Nieberding & Morand, 2006; du Toit *et al.*, 2013; van der Mescht *et al.*, 2015; Engelbrecht *et al.*, 2016; Tobias *et al.*, 2017; Bell *et al.*, 2018; Martinů *et al.*, 2018). For reasons not yet determined, it is noteworthy to point out that two louse species with similar life histories, and who are both species-specific to the same host (Bothma *et al.*, 2020), do not show similar distribution patterns across the landscape. Within the same genetic lineages (Central/SW) there is a clear absence of *H. patersoni* in the region where it is driest and hottest (arid west of South Africa; Fig. 1), and this may point to intrinsic differences among parasite species that can also influence their evolutionary path (also see: Martinů *et al.*, 2020).

In the case of *Micaelamys* and the two louse species studied herein, it seems reasonable to argue that the lack of geographic overlap between hosts' lineages imposed a similar lack of gene flow between the central/SW clades and the northern clades detected in both louse species. The central/SW clades and northern clades are separated by at least 3.7% mtDNA sequence divergence in the host, and 11.0% and 14.6% between clades within *H. patersoni* and *P. praomydis*, respectively (Fig 2). From an evolutionary perspective these clades are confined to different phytogeographical zones, which can play a role in the divergences of the host (Chimimba, 2001; Russo *et al.*, 2010). There is no opportunity for host switching over such large geographic scales and the co-divergence between parasites and hosts are supported by the mean estimated time of the divergences for host and lice that all range from 2.3 Mya to 3.3 Mya (Bothma *et al.*, 2020; also see Russo *et al.*, 2010).

In the northern part of South Africa, the host is characterized by two geographically close mtDNA clades (NE1 and NE2) that differ by 4.0% sequence divergence and that also form two strongly supported monophyletic entities on the phylogenies (Supporting Information, Appendix S1). In the case of *H. patersoni*, there are also two monophyletic clades in this same region, differing by at least 9.5% sequence divergence, but the geographic orientation of these clades does not correspond to that of the host (Fig. 2). The lack of significant phylogeographic co-divergence between *Micaelamys* and *H. patersoni* in this region most likely points to a lack of adaptive divergence (or co-evolution) between the parasite and their host. Previous studies have shown that certain morphological characters of lice evolve to suit the host better. For example, the body size of lice often correlates positively with the body size of its host (Morand *et al.*, 2000) and the diameter of the tibiotarsal claw, which lice use to grasp the hair of their host, significantly correlates to the hair diameter of their host (Cannon, 2010). Such adaptations could result in lice being unable to switch hosts as the adaptive features would be detrimental to their survival on other less optimal hosts. However, in this study, the close intraspecific evolutionary history between the geographically close host lineages most likely precluded such adaptational differences between the louse clades, making host switching and survival possible. Indeed, the JANE analyses suggested that the lack of phylogeographic congruence can most likely be attributed to a duplication and one host switch in that area (Fig. 4).

Unfortunately, the nuclear data for *H. patersoni* failed to support any pattern (a single haplotype was found), which is most likely due to the slower rate of evolution when compared to the mtDNA. However, it is notable that the nuclear DNA data for *M. namaquensis* do not strongly support the three-clade mtDNA genetic structure found. Although this could be due to incomplete lineage sorting in the nuclear DNA data, the potential for male-biased dispersal in *M. namaquensis* cannot be excluded. It has been recorded that *M. namaquensis* males disperse across a wide area during the breeding season, whilst females remain in their relatively small, discrete, contiguous areas (Fleming & Nicolson, 2004). As a result, it is possible that the mtDNA may

not reveal true host movements and subsequent gene flow, particularly over shorter geographic distances such as that found between the NE1 and NE2 clades. If this argument holds, then it is likely that male-host dispersal, particularly during the breeding season when testosterone levels are elevated (Hughes & Randolph, 2001), can facilitate host switching in the parasite (also see: Matthee *et al.*, 2010). An alternative explanation for the conflict between parasite and host phylogeographic structure in the north of the country is local parasite extinction due to dramatic fluctuations in host population sizes that may be accompanied by the effects of straggling (see: Rozsa, 1993; Rivera-Parra *et al.*, 2017). Supplementary sampling and the addition of faster-evolving nuclear markers are needed to resolve these hypotheses more fully.

The significant co-divergences between louse clades at the geographic level support the notion that the taxonomy of the lice occurring on *Micaelamys* is in need of revision. The magnitude of sequence distances among the well-supported *H. patersoni* and *P. praomydis* clades provide strong support for high levels of cryptic diversity in ectoparasites (also see: de León & Nadler, 2010; Nadler & de León, 2011; Perkins *et al.*, 2011; du Toit *et al.*, 2013; Engelbreght *et al.*, 2014). For example, the sequence divergences of 11.0% and 9.5% among *H. patersoni* lineages approach the 15% interspecific divergences detected between *Hoplopleura emphereia* Kim, 1965 and *H. reithrodontomydi*s Ferris, 1951 that parasitize the genera *Peromyscus* Gloger, 1841 and *Reithrodontomys* Giglioli, 1873, respectively (Sánchez-Montes *et al.*, 2016). Additional data from faster evolving nuclear markers, coupled to a thorough morphological investigation, would be required to fully resolve the taxonomy of *H. patersoni* and *P. praomydis* collected from *M. namaquensis*.

The present study supports the notion that the evolutionary history of the host can have a direct influence on the evolutionary history of obligatory permanent ectoparasites. However, it also suggests that obligate ectoparasite taxa who are host-specific (Bothma *et al.*, 2020), often lack adaptive divergence, especially at shallow evolutionary timescales (and among closely related host species; du Toit *et al.*, 2013). These findings emphasize the fact that co-divergence signals at the phylogeographic level do not provide evidence for co-evolution between parasites and their hosts (when opportunities present themselves, host switching seems to be prominent resulting in a lack of co-divergence) and that multiple factors play a role in parasite evolution.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Appendix S1. Bayesian and maximum likelihood topology for *M. namaquensis* (A), *H. patersoni* (B) and *P. praomydis* (C). Nodal support indicated by posterior probabilities above and bootstrap values below nodes. Names on the right indicate haplogroups that could not be connected with 95% confidence in the TCS network. The outgroups, *M. granti* and its associated *Hoplopleura*- and *Polyplax* lice are indicated and used as outgroups in the respective topologies.

Appendix S2. Supplementary tables providing MtDNA Φst values.