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Phylogenomic analysis of seal lice reveals codivergence with their hosts

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Abstract. Lice are considered a model system for studying the process of cospeciation because they are obligate and permanent parasites and are often highly host-specific. Among lice, species in the family Echinophthiriidae Enderlein (Anoplura) are unique in that they infest mammalian hosts with an amphibious lifestyle, i.e. pinnipeds and the river otter. There is evidence that the ancestor of this group infested the terrestrial ancestor of pinnipeds, which suggests these parasites coevolved with their hosts during the transition to marine environments. However, there has been no previous study investigating the phylogenetic relationships among sucking lice parasitizing seals and sea lions. To uncover the evolutionary history of these parasites, we obtained genomic data for *Antarctophthirus microchir* Trouessart and Neumann (from two hosts), *Antarctophthirus carlinii* Leonardi *et al.*, *Antarctophthirus lobodontis* Enderlein, *Antarctophthirus ogmorhini* Enderlein, *Lepidophthirus macrorhini* Enderlein, and *Proechinophthirus fluctus* Ferris. From genomic sequence reads, we assembled > 1000 nuclear genes and used these data to infer a phylogenetic tree for these lice. We also used the assembled genes in combination with read-mapping to estimate heterozygosity and effective population size from individual lice. Our analysis supports the monophyly of lice from pinnipeds and uncovers phylogenetic relationships within the group. Surprisingly, we found that *A. carlinii*, *A. lobodontis*, and *A. ogmorhini* have very little genetic divergence among them, whereas the divergence between different geographic representatives of *A. microchir* indicate that they are possibly different species. Nevertheless, our phylogeny of Echinophthiriidae suggests that these lice have consistently codiverged with their hosts with minimal host switching. Population genomic metrics indicate that louse effective population size is linked to host demographics, which further highlights the close association between pinnipeds and their lice.

Introduction

A very large fraction of all insects are parasites. Phylogenetic studies of insect parasites with respect to their hosts provide opportunities to understand the basis of insect diversification and specialization. In addition, next-generation sequencing technologies provide the opportunity to simultaneously obtain extremely large datasets for both phylogenomic and population genomic approaches (Sweet *et al.*, 2017). In particular, because of their relatively small genomes (*c.* 100–200 Mbp), parasitic lice (Insecta: Phthiraptera) provide an excellent system (Clayton *et al.*, 2016) in which to use these large datasets to study both the pattern of diversification with respect to their hosts and the way in which interactions with the hosts might influence population-level processes. Here we use these datasets to study the process of codiversification and

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Table 1. Seal–louse associations of the family Echinophthiriidae (Anoplura).

Genus	Species	Host
Antarctophthirus	*callorhini*	Northern fur seal
	carlinii[a]	Weddell seal[a]
	lobodontis[a]	Crabeater seal[a]
	mawsoni	Ross seal
	microchir[a]	Steller, Californian, South American[a], Australian[a] and New Zealand sea lion
	ogmorhini[a]	Leopard seal[a]
	trichechi	Walruses
Latagophthirus	*rauschi*	Northern river otter
Lepidophthirus	*macrorhini*[a]	Elephant seals[a]
	piriformis	Monk seals
Echinophthirius	*horridus*	Northern true seals
Proechinophthirus	*fluctus*[a]	Northern fur seal[a]
	zumpti	Southern fur seals

[a]Louse and seal species sampled in this study.

effective population size in the sucking lice (Anoplura) of seals (Pinnipedia).

Sucking lice are obligate, permanent and haematophagous insects, living as ectoparasites in the fur or hairs of their mammalian hosts. Sucking lice in the family Echinophthiriidae Enderlein are unique in that they infest amphibious hosts, such as pinnipeds (walruses, seals and sea lions) and river otters (Durden & Musser, 1994; Leonardi & Palma, 2013). Other aquatic (e.g. whales, dolphins, manatees) or amphibious (e.g. platypus, hippos) mammals do not host lice. The family Echinophthiriidae comprises five genera and 13 species. Genera include *Latagophthirus* Kim and Emerson (one species) from the North river otter, *Proechinophthirus* Ewing (two species) from fur seals, *Echinophthirius* Giebel (one species) from seals in the northern hemisphere, *Lepidophthirus* Enderlein (two species) from monk and elephant seals, and *Antarctophthirus* Enderlein (seven species) from a variety of pinnipeds (Table 1). *Antarctophthirus* is the most diverse genus, and most species are highly host-specific (Durden & Musser, 1994). Species of *Antarctophthirus* are associated with a diversity of pinnipeds, including walruses (Odobenidae Allen), Antarctic seals (Phocidae Gray) and sea lions (Otariidae Gray). Just as their hosts, these lice probably have a terrestrial origin (Kim, 1985). Therefore, these lice evolved many unique morphological, physiological, behavioural and ecological adaptations to cope with the amphibious lifestyle of their hosts. Some of these adaptations include increased density of setae and reduced development time (Kim, 1971; Murray, 1976; Mehlhorn *et al.*, 2002; Leonardi *et al.*, 2012; Leonardi & Lazzari, 2014). These specialized traits, together with host specificity of these lice, suggest that they coevolved with their hosts during the colonization of the marine environment (Kim, 1975, 1985; Kim *et al.*, 1975).

Marine mammals such as pinnipeds are more poorly studied and generally more threatened compared with terrestrial mammals. One in three species of pinnipeds is threatened compared with one in five mammals more generally (Kovacs *et al.*, 2012). Australian sea lions (*Neophoca cinerea* Péron) are particularly at risk, and have been listed as endangered on the IUCN Red List since 2008 (Goldsworthy, 2015). Although some life-history traits of certain pinniped species, such as small breeding colonies, may contribute to their low numbers, human actions have also contributed to their decline (Hamer *et al.*, 2013). Compared with other aspects of their biology, the phylogeny of pinnipeds is relatively well studied and provides an evolutionary framework in which to understand the evolution of their parasitic lice. Molecular analysis supports the monophyletic origin of the Pinnipedia with a basal split between Otarioidea (fur seals and walruses) and Phocoidea (true seals) (Arnason *et al.*, 2006). Current evidence suggests a North American origin for pinnipeds. This was followed by an Atlantic dispersal for phocids and a Pacific dispersal for otariids into the southern hemisphere. Coinciding with this scenario, Kim (1985) suggested that pinniped lice have coevolved with the ancestral Otarioidea and Phocidae, being present in the terrestrial ancestor of pinnipeds. Therefore, the origin and diversification of these lice are likely to be intimately associated with host evolutionary history. However, there have been no prior studies of the phylogeny of pinniped lice.

The primary goal of this study is to analyse the evolutionary history of sucking lice parasitizing seals and sea lions, principally in Patagonia and Antarctica, applying next-generation sequencing approaches. We explore the utility of genome sequencing to resolve the phylogeny of seal lice and to estimate demographic parameters of lice from the same genomic data. Finally, we compare the phylogeny of these lice with that of their hosts in a cophylogenetic framework.

Methods

Genome sequencing

Louse samples were collected with combs or tweezers in the field from their hosts' hind flippers and preserved in 96% ethanol (see details in Leonardi, 2014). When anaesthetization of seals was needed, the animals were immobilized following routine procedures (see details in Wheatley *et al.*, 2006) by people from the Argentinean Antarctic Institute. Collecting lice from the flippers provides a reliable proxy for total louse load, and also reduces host handling time (Leonardi *et al.*, 2018). Antarctic lice were collected in the northern sector of the Danco Coast and 25 de Mayo Island; lice from Australian sea lions and the northern fur seal were sampled by Rebecca McIntosh, Phillip Island Nature Parks, Australia, and Christine Fontaine, The Marine Mammal Center, U.S.A.; finally, lice from South American sea lions were obtained from the Parasitological Collection at Centro Nacional Patagónico, Puerto Madryn. Before DNA extraction, each specimen was photographed at the University of Illinois, Urbana-Champaign, as a voucher. Whole lice were ground up individually (Table S1) in 1.5 mL tubes and genomic DNA was isolated using standard protocols and reagents of the Qiagen QIAamp DNA Micro Kit (Qiagen, Valencia, CA, U.S.A.). The standard protocol was modified to incubate the

specimens in ATL buffer and proteinase K at 55°C for 48 h instead of the recommended 1–3 h, as well as substituting buffer AE with buffer EB. This was done to ensure maximal yield of DNA from the louse remains. Following DNA extractions, we quantified each extraction with a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, U.S.A.) using the manufacturer's recommended protocols and reagents.

Libraries were prepared from these extracts and sequenced with either 100 or 160 bp paired-end reads on an Illumina HiSeq4000 (Albany, New York). The shotgun genomic libraries were prepared with Hyper Library construction kits (Kapa Biosystems, Wilmington, MA, U.S.A.), and were quantitated by qPCR and sequenced on one lane for 151 cycles using a HiSeq 4000 sequencing kit version 1. FASTQ files from sequence data were generated and demultiplexed with BCL2FASTQ v.2.17.1.14. All library preparation, sequencing, and FASTQ file generation was carried out at the W.M. Keck Center (University of Illinois, Urbana, IL, U.S.A.). Raw reads were subsequently deposited to the NCBI GenBank SRA database (Table S1). We also obtained raw reads from two previously sequenced samples: *Antarctophthirus michrochir* Trouessart and Neumann from *Otaria flavescens* Shaw and *Proechinophthirius fluctus* Ferris from *Callorhinus ursinus* Linnaeus (Allen *et al.*, 2017). These libraries were constructed from DNA extracts of pooled individual lice and so are not directly comparable for population genomic parameters with those sequenced for the current study from single individuals. However, these additional samples are included to increase taxon representation in the phylogenomic aspects of this study. For outgroups, we used the previously sequenced genomes from *Haematopinus eurysternus* Nitzsch (hog louse; Allen *et al.*, 2017) and *Pediculus humanus* Linnaeus (human head louse; Allen *et al.*, 2017).

Phylogenomic analysis

To obtain gene sequence data for analysis, we used a read-mapping approach against existing gene sequences for seal lice. We first obtained sequences of 1022 single-copy protein-coding orthologue genes previously assembled using ATRAM (Allen *et al.*, 2015) from *Antarctophthirus microchir* (Allen *et al.*, 2017). We used the protein-coding portions of these genes in a reference mapping pipeline script (https://github.com/adsweet/louse_genomes/), using BOWTIE2 (Langmead & Salzberg, 2012) to map libraries to these reference gene sequences. After mapping, we sorted the BAM files and created pileup files using SAMTOOLS v.1.7 (Li *et al.*, 2009). To convert the pileup files to VCF flies, we called variants using BCFTOOLS v.1.7 (Li *et al.*, 2009). Sites with sequence coverage less than 5X or greater than 100X, or with Phred quality scores < 28 were filtered using SAMTOOLS. From these files we created consensus sequences for each gene using ambiguity coding for variants.

We aligned nucleotides for each gene separately using PASTA v.1.8.2 (Mirarab & Warnow, 2015). Using a custom Python script, we removed genes that contained fewer than five of the ingroup taxa or less than one outgroup taxon. We then masked sites containing $\geq 40\%$ gaps using TRIMAL v.1.4 (Capella-Gutiérrez *et al.*, 2009). With the aligned data, we performed both an analysis of the concatenated supermatrix and a coalescent analysis of gene trees to produce a species tree. For the concatenated method, we first concatenated all the gene files into a supermatrix using SEQUENCE MATRIX v.1.8 (Vaidya *et al.*, 2011). We performed a maximum likelihood (ML) analysis in RAXML v.8.1.3 (Kozlov *et al.*, 2015), using a GTR + Γ model and 100 rapid bootstrap replicates. Bootstrap support was then summarized on a best tree. For the coalescent analysis, we estimated gene trees for each gene alignment in RAXML using a GTR + Γ model for each gene. A coalescent species tree was estimated from the individual gene trees using ASTRAL v.4.10.6 (Mirarab *et al.*, 2014) with quartet-based local posterior probability support for branches (Sayyari & Mirarab, 2016).

As a comparison with results from nuclear loci, we also assembled sequences from the mitochondrial genes ATP synthase F0 subunit 8 and ATP synthase F0 subunit 6 (ATP), cytochrome *c* oxidase subunit I (COX1), cytochrome *c* oxidase subunit II (COX2), cytochrome *c* oxidase subunit III (COX3), cytochrome *b* (CYTB), NADH dehydrogenase subunit 2 (NADH2), NADH dehydrogenase subunit 4 (NADH4), and NADH dehydrogenase subunit 5 (NADH5) for each library. Specifically, we used ATRAM v.1.3.0 (Allen *et al.*, 2015) to assemble the mitochondrial genes using 10% of the reads from each library using a *Pediculus humanus* reference. We then used BLASTN v.2.8.0 (Benson *et al.*, 2008) to confirm that the data recovered from ATRAM were mitochondrial sequences. Mitochondrial sequences were aligned using PASTA v.1.8.2 to the *P. humanus* genes (Benson *et al.*, 2008; GenBank accessions FJ499476.1, FJ499477.1, FJ499478.1, FJ499479.1, FJ499475.1, FJ499481.1, FJ499483.1 and FJ499484.1). The individual mitochondrial gene alignments were then concatenated. The concatenated mitochondrial gene alignment was then imported into PAUP* (Swofford, 2002) to calculate uncorrected pairwise genetic distances. We also performed a ML analysis from the concatenated mitochondrial gene alignment in RAXML v.8.1.3, using a GTR + Γ model and 100 rapid bootstrap replicates. Bootstrap support was then summarized on the best ML tree.

Cophylogenetic analysis

We used the event-based cophylogenetic method JANE v.4 to compare the louse and seal phylogenies. JANE uses a genetic algorithm (GA) to reconcile host and parasite phylogenies with evolutionary events (cospeciation, host switching, etc.) given a priori costs associated with each event. For our JANE analysis, we used the concatenated louse phylogeny with the outgroups trimmed and the pinniped phylogeny based on a ML analysis of amino acid sequences from Arnason *et al.* (2006). We set the GA parameters to 500 generations and a population size of 1000, and used default event costs (no cospeciation events; one duplication; two duplications and host switches; one loss; and one failure to diverge). We also randomized the tip associations 999 times to test for statistical significance of our optimal score.

Population genomic analysis

To estimate the population genomic diversity in seal lice, we used MLRHO v.2.9 (Haubold *et al.*, 2010; http://guanine.evolbio.mpg.de/mlRho/), a program that can estimate population parameters, such as θ, from individual diploid genome sequences. The parameter θ is defined as the population mutation rate, or $\theta = 4\text{Ne}\mu$, which can be used as an indicator of heterozygosity and effective population size (Meyer *et al.*, 2012). Using MLRHO, we converted pileup files generated from BOWTIE2 to profile (.pro) files for MLRHO for the genome libraries. We did not include *A. michrochir* from *O. flavescens*, or *P. fluctus* from *Callorhinus ursinus* because the libraries from these species were from pooled samples of multiple individuals rather than single individual lice. We then ran MLRHO with maximum distance (M) = 0. As a comparison to θ, we calculated the raw observed heterozygosity of each sample by taking the number of called heterozygous sites for each library divided by the total number of sites.

Results

Phylogenetic analysis

The ML phylogenetic analysis in RAXML produced a well-supported tree for the seal lice from 1022 concatenated genes. Most of the nodes received 100% bootstrap support (Fig. 1). The tree produced from individual gene trees using ASTRAL was identical in topology to the ML tree. The ASTRAL tree was also very highly supported, with all branches receiving local posterior probability support of 1.0 (Fig. S1).

Results from mitochondrial sequences for the seal lice are identical to those from nuclear genes (Fig. S2). The phylogenetic relationships recovered are consistent with the whole-genome concatenation and coalescent analysis. The mitochondrial genetic distances among *Antarctophthirus lobodontis* Enderlein from *Lobodon carcinophaga* Hombron and Jacquinot, *Antarctophthirus carlinii* Leonardi *et al.* from *Leptonychotes weddelli* Lesson, and *Antarctophthirus ogmorhini* Enderlein from *Hydrurga leptonyx* Blainville are extremely small (all $< 1\%$; Table 2). This extremely low divergence is in contrast to other divergences among other species (all $> 30\%$) or between two geographically isolated populations of *A. microchir* (25.3%) on different host species.

Cophylogenetic analysis

JANE recovered five cospeciation events, one host switch, and one loss (Fig. 2; Table 3) This amount of cospeciation was far above that expected by chance (observed cost = 3, $P = 0.005$). The single host switch recovered by JANE is from lice on the ancestor of sea lions (*O. flavescens* and *N. cinerea*) to the ancestor of leopard, Weddell, and crabeater seals.

Population genetics

Estimates of θ varied significantly across seal louse individuals (Fig. 3; Table S2). *Lepidophthirus macrorhini* Enderlein from *Mirounga leonina* Linnaeus have the highest θ (0.00 367, 95% CI: 0.003 53–0.003 81) and *A. microchir*_2 from *N. cinerea* have the lowest θ (0.001 07, 95% CI: 0.001 01–0.001 13). Raw heterozygosity of each sample showed similar trends to estimates of θ (Fig. 3). The 95% confidence intervals on θ from *A. microchir*_2, *A. ogmorhini* and *A. carlinii* were overlapping, while those from *A. lobodontis* and *L. macrorhini* did not overlap with anything, indicating significantly higher values of θ.

Discussion

In this study, we generated a molecular phylogenetic tree for pinniped lice (Echinophthiriidae) based on total genomic DNA. This tree was largely congruent with a tree for their seal hosts, indicating a significant amount of codivergence. Lice of Antarctic seals appear to have originated via a host-switching event from sea lions. Population genomic estimates related to effective population size indicated substantial variation among species of lice.

Our results show some general concordance with earlier ideas on the evolution of pinniped lice (Echinophthiriidae). Pioneering morphological phylogenetic studies conducted by Kim (1985, 1988) suggested that the terrestrial ancestors of pinnipeds were already infested by ancestral sucking lice and, consequently, coevolved with the Otariidae and Phocidae. Currently, both morphological and molecular evidence support the monophyletic origin of Pinnipedia (Berta & Wyss, 1994; Arnason *et al.*, 2006; Fulton & Strobeck, 2010; Nyakatura & Bininda-Emonds, 2012). Analysis based on morphological data supports the hypothesis that Pinnipedia is related to Ursidae (Wyss & Flynn, 1993; Berta & Wyss, 1994; Luan *et al.*, 2013), whereas molecular data suggest a relation with an ancestor of extant Mustelidae (Arnason *et al.*, 2006; Eizirik *et al.*, 2010; Nyakatura & Bininda-Emonds, 2012). The presence of *Latagophthirus* in the river otter not only support this idea but also support the hypothesis proposed by Arnason *et al.*, 2006. These authors postulated that pinnipeds evolved from an initial nonmarine phase prior to the colonization of the marine environment; once they entered the sea, they differentiated between Otariidae and Phocidae. Accordingly, Kim (1985, 1988) argued that the evolution of Echinophthiriidae was intimately associated with pinniped evolution and suggested a phylogenetic tree based on morphological data. Unfortunately, we did not have a sample of *Latagophthirus* available to test these hypotheses, but our results from pinniped lice do show consistency with Kim's hypothesis.

Specifically, in our analysis, *P. fluctus* appears as sister of a branch that includes all the *Antarctophthirus* species. Likewise, its host, the northern fur seal *C. ursinus*, is considered the earliest diverging lineage of extant otariid seals (Berta *et al.*, 2018). Kim (1971) and Kim *et al.* (1975) recognized *P. fluctus* as an early-diverging pinniped louse, especially due to the absence of morphological traits characteristic of the family, i.e. the presence

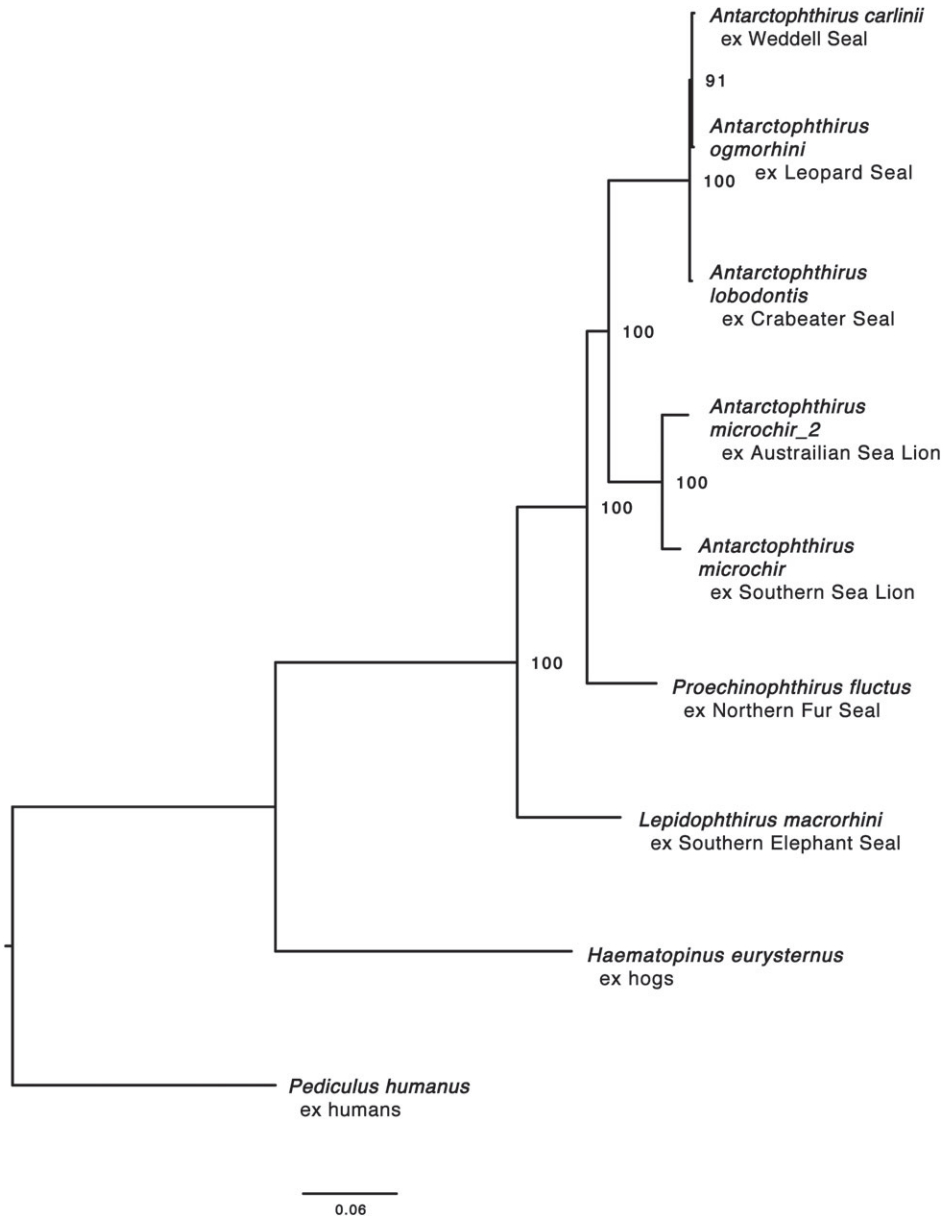

Fig. 1. Seal louse phylogeny from a partitioned maximum likelihood analysis based on a concatenated sequence alignment of 1022 nuclear genes. Bootstrap support values are indicated at each node, and branch lengths are scaled to nucleotide substitutions per site, as indicated by the scale bar below the phylogeny. Shown are: Weddell seal, *Leptonychotes weddelli*; leopard seal, *Hydrurga leptonyx*; crabeater seal, *Lobodon carcinophaga*; Australian sea lion, *Neophoca cinerea*; South American sea lion, *Otaria flavescens*; northern fur seal, *Callorhinus ursinus*; and southern elephant seal, *Mirounga leonina*.

of scales and the high development of the second and third pairs of legs. The genus *Proechinophthirus* contains two species, *P. fluctus* from the northern fur seal, and *P. zumpti* Werneck from the southern fur seals in the genus *Arctocephalus* Cuvier. At present, *P. zumpti* has only been described from the Cape fur seal *A. pusillus* Schreber, its type host, and the South American fur seal *A. australis* Zimmermann (Castro *et al.*, 2002). A review of the species infesting the remaining six species of fur seals is needed to understand the phylogenetic relations between *Proechinophthirus* and *Antarctophthirus*.

Among *Antarctophthirus* species, we found two clades, one including lice from Antarctic seals and another grouping the two *A. microchir* from the and the Australian sea lions. Antarctic seals (Lobodontini) diverged from elephant seals approximately 7 Ma and dispersed in the Atlantic, along the coast of South America and colonizing Antarctica at least 3.4 Ma (Berta *et al.*, 2018). Extant Lobodontini is represented by four species: the Ross seal (*Ommatophaca rossi* Gray), the crabeater seal (*Lobodon carcinophaga*), the Weddell seal (*Leptonychotes weddelli*) and the leopard seal (*Hydrurga leptonyx*). There is no

Table 2. Uncorrected pairwise distances of seal lice calculated from mitochondrial genes, ATP synthase F0 subunit 8 and ATP synthase F0 subunit 6 (ATP), cytochrome c oxidase subunit I (COX1), cytochrome c oxidase subunit II (COX2), cytochrome c oxidase subunit III (COX3), cytochrome b (CYTB), NADH dehydrogenase subunit 2 (NADH2), NADH dehydrogenase subunit 4 (NADH4), and NADH dehydrogenase subunit 5 (NADH5). Louse–seal associations are as follows: *Antarctophthirus microchir*_2 from *Neophoca cinerea*, *Antarctophthirus microchir* from *Otaria flavescens*, *Antarctophthirus lobodontis* from *Lobodon carcinophaga*, Antarctophthirus carlinii from *Leptonychotes weddelli*, *Antarctophthirus ogmorhini* from *Hydrurga leptonyx*, *Lepidophthirus macrorhini* from *Mirounga leonina*, *Proechinophthirus fluctus* from *Callorhinus ursinus*, *Pediculus humanus* from humans.

	1	2	3	4	5	6	7	8
1 *Lepidophthirus macrorhini*	–							
2 *Antarctophthirus lobodontis*	0.483 577	–						
3 *Antarctophthirus carlinii*	0.474 036	0.014 422	–					
4 *Antarctophthirus ogmorhini*	0.477 025	0.015 122	0.002 142	–				
5 *Antarctophthirus microchir*_2	0.487 899	0.446 788	0.446 136	0.447 311	–			
6 *Proechinoph-thirus fluctus*	0.458 936	0.446 417	0.446 945	0.447 940	0.466 009	–		
7 *Antarctophthirus microchir*	0.462 107	0.417 867	0.414 683	0.415 110	0.334 312	0.445 899	–	
8 *Pediculus humanus*	0.449 981	0.522 456	0.515 452	0.517 670	0.526 871	0.493 251	0.506 248	–

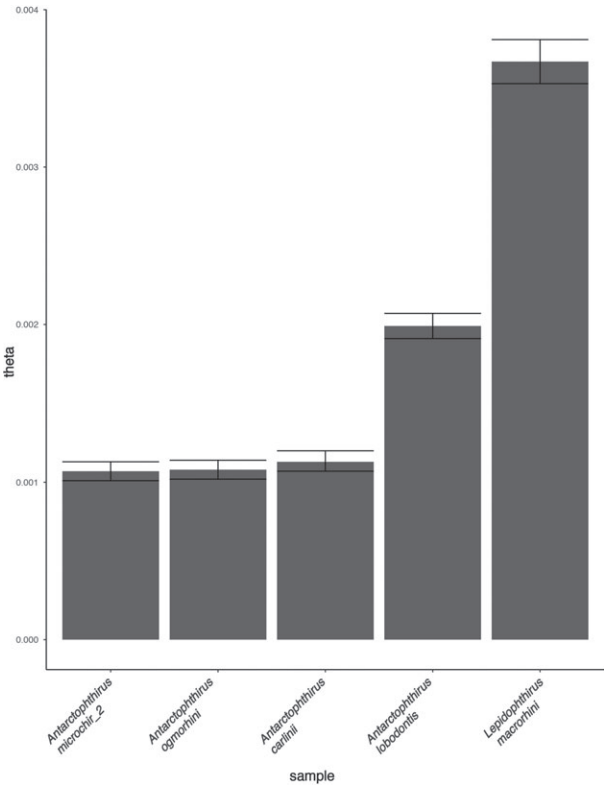

Fig. 2. Estimates of θ based on mapped genomes of seal lice: *Antarctophthirus microchir*_2 ex *Neophoca cinerea*, *Antarctophthirus lobodontis* ex *Lobodon carcinophaga*, *Antarctophthirus carlinii* ex *Leptonychotes weddelli*, *Antarctophthirus ogmorhini* ex *Hydrurga leptonyx* and *Lepidophthirus macrorhini* ex *Mirounga leonina*. Bars indicate 95% confidence intervals.

fossil record for these species, with the exception of *O. rossi*. However, it seems plausible that the four lineages diversified during the Holocene (Berta *et al.*, 2018). As currently described, each species of Lobodontini is infested by a unique and specific louse species of *Antarctophthirus*. However, the genetic divergences among *A. lobodontis*, *A. carlinii* and *A. ogmorhini* are so small that they may likely be a single species. Morphological differences do exist among these described species. It could be that this morphological variation persists in the face of sufficient gene flow among louse populations on different host species to make these lice genetically relatively homogeneous. Alternatively, the extremely low number of generations per year (see later) on these hosts may lead to reduced substitution rates in these lice relative to other lice. Given that we have only sampled one individual louse per species, more individuals are needed to draw definitive conclusions about the status of these species.

The eggs of pinniped lice do not survive being submerged (Murray, 1976; Leonardi & Lazzari, 2014), which is the main restriction on the survival of lice on amphibious hosts. As a consequence, lice reproduction can only occur when their hosts remain on land for a substantial amount of time (*c*. 10 days), thus constraining the number of louse generations per year by the duration of haul-out periods of their hosts (Aznar *et al.*, 2009; Leonardi & Lazzari, 2014). Moreover, transmission between hosts depends on physical contact between hosts (Demastes *et al.*, 1998; Toloza *et al.*, 2009; Galloway, 2012). Therefore, seal louse transmission is only possible during the seals' haul-out periods (Kim, 1975; Leonardi *et al.*, 2013). Opportunities for louse dispersal are thus affected by this behaviour (Murray & Nicholls, 1965; Murray *et al.*, 1965; Kim, 1972, 1975; Leonardi *et al.*, 2013). Because of these biological constraints, the major transmission of Antarctic seal louse species occurs from cows to pups during nursing, as pups are infested a few hours after birth (Kim, 1972). Horizontal transmission among pups seems to be important in species where pups or juveniles form close congregations (Kim, 1972), whereas transmission among adults could play a minor role (Kim, 1975). These characteristics would reduce gene flow. Although all seal lice are likely to be affected by these constraints, the time for louse reproduction is even shorter on Antarctic seals than on sea lions in more temperate regions. This limited number of generations per year may lead to a decrease in substitution rates compared with other lice, and thus the relatively low genetic divergence, although it is not clear if this difference is

Table 3. Results of a JANE analysis comparing pinnipeds and their lice.

Number of cospeciation events	Number of duplications	Number of duplications and host switches	Number of losses	Failures to diverge	Cost
5	0	1	1	0	3

sufficient to completely explain the patterns of divergence we found.

In contrast to lice from different Antarctic seal hosts, we found a high genetic distance between the two samples of *A. microchir* from the South American and Australian sea lions (33.4% mitochondrial divergence). This species has been described as a parasite from all five extant species of sea lions: the Steller's and Californian sea lions from North America (Ferris, 1934), the South American sea lion (Leonardi *et al.*, 2009), the Australian sea lion (McIntosh & Murray, 2007) and the New Zealand sea lion (Trouessart & Neumann, 1888). Members of Echinophthiriidae are mainly host-specific (i.e. they infest a single host species), with two exceptions: *Echinophthirius horridus* from northern true seals (genera *Cystophora* Erxleben, *Erignathus* Gill, *Halichoerus* Nilsson and *Phoca* Linnaeus) and *A. microchir* (Kim, 1985). Kim (1985) suggested that *A. microchir* constitutes a complex of cryptic species that are morphologically indistinguishable. In their re-description of the species infesting the South American sea lion, Leonardi *et al.* (2009) could not differentiate between *A. microchir* from several host species. The results of this study confirm the hypothesis proposed by Kim (1985) that *A. microchir* comprises several cryptic species from different hosts.

According to our phylogenetic tree, *L. macrorhini* is recovered as sister to all other species of pinniped lice. This position does not reflect the host evolutionary history. Among the family Phocidae, elephant seals are included in the clade Mirounga. The finding of a piece of a rostrum similar to *Mirounga* from the early Pleistocene (2.6 Ma) in New Zealand was considered by several authors (Boessenecker & Churchill, 2016; Berta *et al.*, 2018)

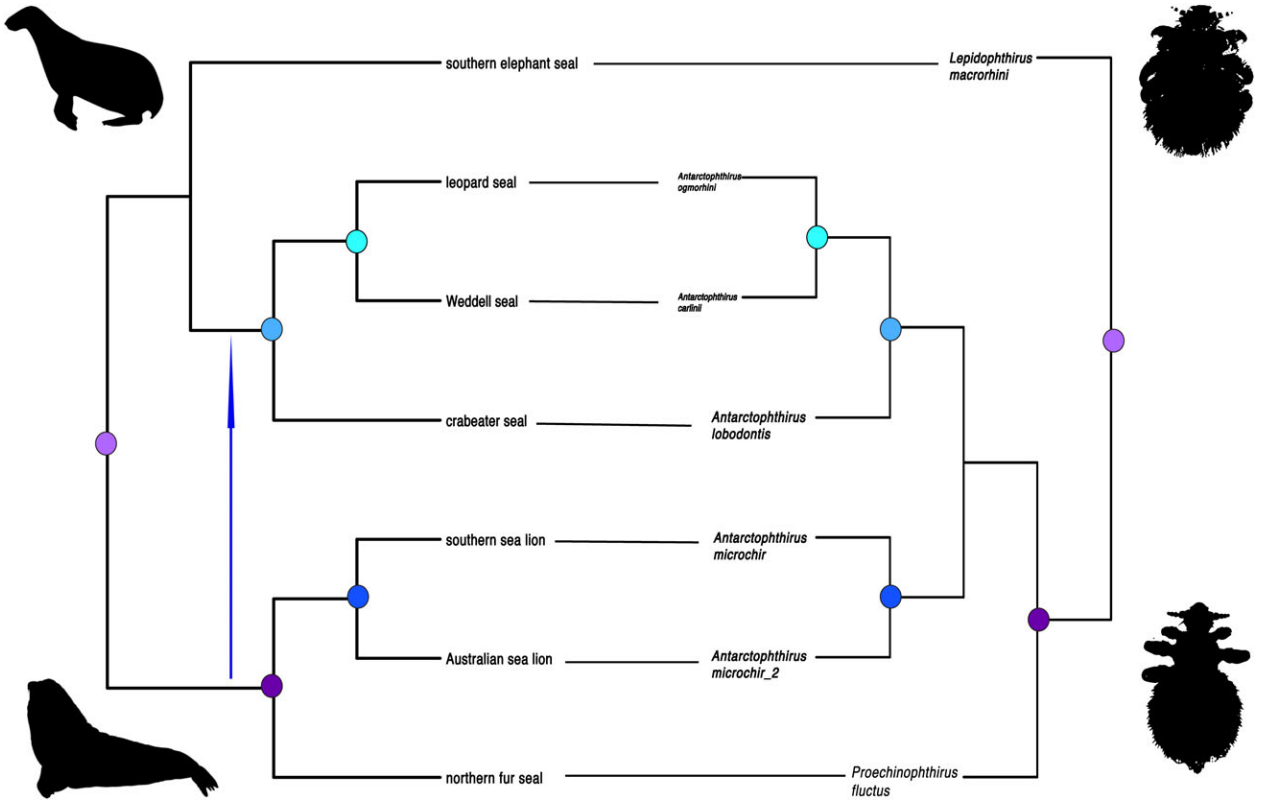

Fig. 3. Tanglegram comparing the evolutionary histories of seals and sea lions (left) and their lice (right). The host phylogeny is adapted from Arnason *et al.* (2006). The louse phylogeny is adapted from the concatenated tree in this study. Coloured circles above nodes indicate cospeciation events recovered from Jane. The arrow indicates the host-switching event recovered by JANE. Seal silhouettes are from PhyloPic (http://phylopic.org/; seal courtesy of Jakovche, license link: https://creativecommons.org/licenses/bysa/3.0/; sea lion courtesy of Steven Traver). [Colour figure can be viewed at wileyonlinelibrary.com].

as evidence that Miroungini originated in the southern hemisphere. This hypothesis is also supported by the molecular analysis of Arnason *et al.* (2006). However, Koretsky (2001) argued that *Mirounga* is actually related to *Cystophora*, a hypothesis originally proposed by King (1966) based on morphological characters. The position of *L. macrorhini* in the phylogenetic tree suggests that to fully understand the phylogenetic position of *L. macrorhini*, other species of Echinophthiriidae need to be included in a future phylogenetic study. Specifically, to understand the evolutionary history of phocid lice, future analysis should include species of *Echinophthirius* and *L. piriformis* Blagoveshtchensky from monk seals.

Finally, we estimated θ, the population mutation rate, which is a method of describing genetic diversity in a population and is directly proportional to effective population size. The relative ranking of estimates of θ for each louse species is consistent with host abundance. The highest value was obtained for *L. macrorhini* from the southern elephant seal, which is the host species in our dataset with the greatest geographical distribution (Hindell & Perrin, 2009). The second highest θ was for *A. lobodontis* from the crabeater seal, one of the most abundant pinnipeds in the world (Southwell *et al.*, 2012). The lowest estimate of θ was for *A. microchir* from the Australian sea lion, which is noteworthy considering that the Australian sea lion is an endangered species (IUCN, 2018). Like their pinniped hosts, these parasites are understudied, even though they can potentially provide insights into the health and abundance of their hosts. Understanding the evolutionary and demographic history of parasites of these pinnipeds can be informative for developing effective conservation strategies of the hosts.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Specimen information, extraction results, library preparation details, Illumina sequencing statistics, and raw sequence data deposition for louse samples.

Table S2. Results from MLRHO estimated from genome sequences of seal lice. Raw heterozygosity values are also indicated. *Antarctophthirus microchir_2* ex *Neophoca cinerea*, *Antarctophthirus lobodontis* ex *Lobodon carcinophaga*, *Antarctophthirus carlinii* ex *Leptonychotes weddelli*, *Antarctophthirus ogmorhini* ex *Hydrurga leptonyx*, *Lepidophthirus macrorhini* ex *Mirounga leonina*.

Fig. S1. Seal louse phylogeny from a coalescent ASTRAL analysis of 1022 nuclear gene trees. Local posterior probability support is indicated at each node, and internal branches are scaled as indicated by the scale bar below the phylogeny. Weddell seal, *Leptonychotes weddelli*; leopard seal, *Hydrurga leptonyx*; crabeater seal, *Lobodon carcinophaga*; Australian sea lion, *Neophoca cinerea*; southern sea lion, *Otaria flavescens*; northern fur seal, *Callorhinus ursinus*; southern elephant seal, *Mirounga leonina*.

Fig. S2. Seal louse maximum likelihood tree estimated from mitochondrial gene sequences, mitochondrial genes, ATP synthase F0 subunit 8 and ATP synthase F0 subunit 6 (ATP), cytochrome *c* oxidase subunit I (COX1), cytochrome *c* oxidase subunit II (COX2), cytochrome *c* oxidase subunit III (COX3), cytochrome b (CYTB), NADH dehydrogenase subunit 2 (NADH2), NADH dehydrogenase subunit 4 (NADH4), and NADH dehydrogenase subunit 5 (NADH5). Bootstrap support values are indicated at each node, and branch lengths are scaled to nucleotide substitutions per site, as indicated by the scale bar below the phylogeny.

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