Quaternary Science Reviews 226 (2019) 106031

Contents lists available at ScienceDirect

Quaternary Science Reviews

journal homepage: www.elsevier.com/locate/quascirev

Ancient parasite DNA from late Quaternary Atacama Desert rodent middens

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ARTICLE INFO

Article history:
Received 6 August 2019
Received in revised form
17 October 2019
Accepted 21 October 2019
Available online xxx

Keywords:
Climate
DNA metabarcoding
Molecular ecology
Paleoecology
Paleoparasitology

ABSTRACT

Paleoparasitology offers a window into prehistoric parasite faunas, and through studying time-series of parasite assemblages it may be possible to observe how parasites responded to past environmental or climate change, or habitat loss (host decline). Here, we use DNA metabarcoding to reconstruct parasite assemblages in twenty-eight ancient rodent middens (or paleomiddens) from the central Atacama Desert in northern Chile. The paleomiddens span the last 50,000 years, and include middens deposited before, during and after the Central Andean Pluvial Event (CAPE; 17.5–8.5 ka BP). The CAPE was a period of increased precipitation and vegetation change, which we also demonstrate was associated with changes in local rodent taxa. Thirteen parasite taxa (including lice, mites, ticks, nematodes and coccidians) were identified from the middens, nine of which were likely derived from rodent hosts and four from alternative (insect or avian) hosts. The former are consistent with parasites known to infect South American rodent hosts. At our conservative level of high taxonomic rank assignment, the parasites appear to have been resilient to the major perturbations in climate and host taxa associated with the CAPE, and finer taxonomic resolution would be required to detect whether any species turnover occurred within the identified parasite groups. Rodent paleomiddens are fast becoming an unrivaled source of genomic data that can be used to reconstruct past ecosystem change on multiple taxonomic, temporal and spatial scales providing new insights into ecological responses to global change.

1. Introduction

Parasites represent up to half of all animal species on Earth (Dobson et al., 2008; Poulin, 2014) and play important roles within biological systems (Sures, 2003; Broadhurst et al., 2012; Maizels and McSorley, 2016) and ecological interaction networks (Price et al., 1986; Poulin and Morand, 2000; Hudson et al., 2006). It is predicted that future climate change (Dobson et al., 2015; Cizauskas et al., 2017), habitat loss and fragmentation (i.e. through declining host populations) (Bush and Kennedy, 1994; Dunn et al., 2009; Dougherty et al., 2016) could have a major impact on parasite populations globally, with the potential to reduce parasite diversity (Dunn et al., 2009), affect host health (Burge et al., 2014; McCreesh et al., 2015; Spencer and Zuk, 2016) and disrupt ecosystems (Colwell et al., 2012; Wood and Johnson, 2015; Preston et al., 2016). Although environmental and climatic tolerances of different parasite taxa have been incorporated into models used to predict their responses to change (e.g. Ryan et al., 2015; McCreesh et al., 2015; Rose et al., 2015), these tolerances are not well known for many parasites. An alternative approach is provided by paleoparasitology (the study of ancient parasites), which offers a means to look back

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https://doi.org/10.1016/j.quascirev.2019.106031
0277-3791/

into the past and observe directly how parasites responded to previous climate change and host decline events. Insights gained through such research could help improve our understanding about parasite responses to future perturbations, as well as helping to evaluate theoretical models that make predictions about such responses (Wood, 2018).

Ancient parasites can be studied using a variety of different techniques, and their remains can be recovered from a wide range of substrates including museum skins, mummified tissues, coprolites and sediments (Wood, 2018). Visible traces of parasites such as exoskeletons and eggs can be isolated, concentrated, and analysed using microscopy techniques (Reinhard et al., 1986; Schmidt et al., 1992). More recently DNA analyses have proven to be a useful tool for studying ancient parasites. In particular, ancient DNA has helped resolve parasite taxa that may be difficult to identify using microscopy, and has provided insights into haplotype diversity and other genetic aspects of parasite taxa (e.g., Iñiguez et al., 2006; Fernandes et al., 2008; Matheson et al., 2014). Ancient DNA metabarcoding offers the potential to reconstruct entire parasite faunas from fossil assemblages, yet, due to the phylogenetic diversity of parasites, has required the use of universal eukaryote primers. Parasites may represent only a tiny proportion of the total DNA reads obtained using such primers. Therefore, the application of DNA metabarcoding to studying ancient parasite faunas has so far been limited, with just a couple of studies having attempted this approach on avian coprolites from New Zealand (Wood et al., 2013; Boast et al., 2018). Here, we test the suitability of ancient DNA metabarcoding for reconstructing parasite faunas from a novel substrate, ancient rodent middens (or paleomiddens).

Paleomiddens are valuable paleoecological resources in arid environments around the world, where fossil preservation sites are otherwise scarce (Pearson and Betancourt, 2002; Elias, 2007) and appear to provide an ideal substrate for studying ancient parasites (Beltrame et al., 2016). Comprised of vegetative nesting material and coprolites cemented together by hardened crystallised urine, middens offer the potential for preserving remains of both internal (Beltrame et al., 2016) and external parasites of the rodents that formed them. Moreover, whilst individual middens likely represent relatively brief (months to decades) temporal snapshots (Betancourt et al., 2000), collections of multiple middens from certain regions can span long time-periods and thereby provide a resource for testing the responses of organisms to past climate change. In this study we focus specifically on a 50,000 year record of rodent middens from the central Atacama desert in Chile; the study of which has previously revealed responses from plants (Betancourt et al., 2000; Latorre et al., 2002, 2003; de Porras et al., 2017; Díaz et al., 2012, 2019), arthropods (Dézerald et al., 2019) and plant pathogen (Wood et al., 2018) communities to the Central Andean Pluvial Event (CAPE) one of the wettest periods in the last 100,000 years that spanned from 17.5 to 8.5 ka BP (Placzek et al., 2009; Gayo et al., 2012; de Porras et al., 2017). We examine whether any of the parasite taxa detected in the middens also show responses in relation to the CAPE. We hypothesise that parasite faunas will remain relatively stable across the CAPE, as they are largely buffered from the external climate/environment. Several midden-forming rodent species currently inhabit the Atacama. Therefore, quantifying changes in rodent community composition through time is key to understanding any changes in parasite communities. Accordingly, we also attempt to identify the rodent taxa that created each midden in order to control for possible changes in host species through time. We hypothesise that a change in the host taxa during CAPE will result in a change in the parasite taxa detected.

2. Methods

2.1. Parasite DNA

We studied parasite DNA in twenty-eight ancient rodent middens collected from the Calama and Salar de Atacama basins in the central Atacama Desert, northern Chile (Fig. 1). Twenty-four of these came from Cerros de Aiquina (CDA) (~22° 21′ S, 68° 18′ W), three from Lomas de Tilocalar (LDT) (~23° 55′ S, 68° 10′ W), and one from Vegas de Tilocalar (VDT) (~23° 44′ S, 68° 6′ W). The middens ranged in age from 200 to 49,600 14C years and were collected from sites between 2414 and 3355 m above sea level (see Wood et al., 2018 for details). The midden collection sites are currently situated within the prepuna vegetation zone, which is dominated by cushion cacti, xerophytic shrubs and annuals (Díaz et al., 2019). With strongly positive local altitudinal rainfall gradients, the mean annual precipitation of the collection localities ranges from ~0 mm to ~60 mm, and temperatures range from −4° to 32 °C (mean annual temperature 14 °C) (Díaz et al., 2019).

The molecular and bioinformatic methods used in this study follow those detailed by Wood et al. (2018). Briefly, DNA was extracted from 3.45 to 6.03 g subsamples of the paleomiddens and blank controls (one for every batch of extractions) using the PowerMax soil DNA isolation kit (MoBio) in the ancient DNA laboratory at Manaaki Whenua Landcare Research, Lincoln, New Zealand. Subsamples were of bulk midden material, which included a mixture of local vegetation remains and coprolite fragments, cemented together by crystallised urine. Amplicons consisting of a 40–120 bp (in eukaryotes) partial region of the 18S rRNA gene were generated by PCR using the primers Nem18SF and Nem18SR, which were developed for parasite metabarcoding (Wood et al., 2013). These primers were adapted with linker sequences at the 5′ ends, enabling sample-specific indexes and sequencing adapters to be added via a second-round of PCR. Initial PCRs (repeated in triplicate) were performed in 12.5 μL volumes, containing 1 μg/mL BSA, 1× PCR buffer, 2 mM $MgSO_4$, 40 μM dNTPs, 0.4 μM each primer, 1U Platinum HiFi Taq Invitrogen™) and 1 μL DNA extract. The thermocycling program for the initial PCRs was 94 °C for 3 min; 55 cycles of 94 °C for 30 s, 55 °C for 30 s, 68 °C for 45 s; and 68 °C for 10 min. None of the blank extraction and PCR controls, which were included with all amplifications, yielded bands when assessed on 3.5% agarose gels. Second-round PCRs (20 μL volumes) were performed using 1 μL of each amplicon library, 0.75 μL of 2 μM indexed adapters (P5/P7), and iTaq DNA polymerase (Intron) using the manufacturer's reaction mix. The thermocycling conditions for the second-round PCR were 94 °C for 2 min; 7 cycles of 94 °C for 20 s, 55 °C for 10 s, 72 °C for 30 s; and 72 °C for 10 min. Libraries were quantified using a Qubit 2.0 fluorometer, and equal amounts were pooled into several groups of ≤30 μL for purification via a Pippen Prep using a size-selection window of 220–260 bp. After this purification and size-selection step, amplicon groups were quantified and combined in equal amounts into a final amplicon pool, which was sequenced using 2 × 150 bp kit on an Illumina MiSeq (Macrogen, South Korea).

Reads were processed using USEARCH (Edgar and Flyvbjerg, 2015) following the pipeline described by Wood et al. (2018). The nearest 50 matches from the National Centre for Biotechnology Information (NCBI) nucleotide database (https://www.ncbi.nlm.nih.gov/nucleotide/) to each resulting Operational Taxonomic Unit (OTU) were determined using the BLAST algorithm and taxonomic assignments were made using MEGAN v.6.9.1 (Huson et al., 2007) with a minimum LCA score of 50.0 and parsing to matches within 5% of the top score.

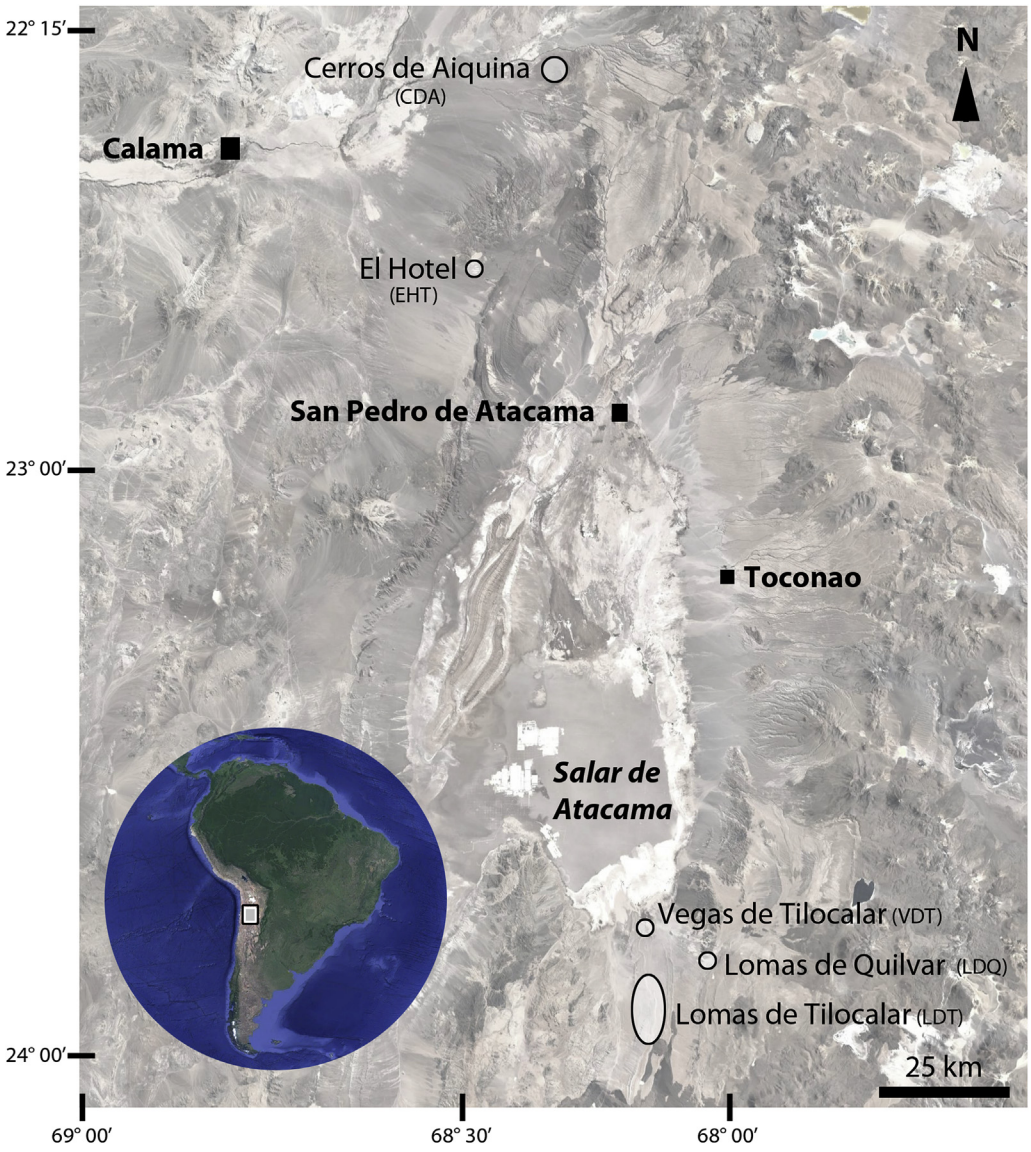

Fig. 1. Map of study area, showing sampling localities (white circles/ovals) mentioned in this paper, together with nearby towns (squares). Scale bar is 25 km.

2.2. Statistical methods

Statistical analyses were implemented in R v.3.5.3 (R Core Team, 2019). Differences in overall parasite taxonomic richness, Shannon diversity (calculated using the *vegan* package; Oksanen et al., 2011) and relative abundance of parasite DNA reads between CAPE middens (those with ages between 17.5 and 8.5 ka BP) and non-CAPE middens (those with ages <8.5 ka BP or >17.5 ka BP) were assessed using a Welch's two sample *t*-test (two-sided), which is robust to unequal variances. These analyses were repeated for two groups that included those parasite taxa likely to have been from rodent hosts, and those that were likely to be from other host taxa (see results section for details of taxa).

Differences in the relative abundances of individual parasite taxa were assessed using the same method as for overall relative abundance. Binary data, a feature of presence absence data, requires a different statistical approach to the conventional methods used above. We therefore used a generalised linear model with a Bernoulli distribution to test differences in the frequency of occurrence of individual parasite taxa between CAPE and non-CAPE middens.

In all cases analyses were repeated without midden CDA-593A, which due to its relatively old age (49,600 ± 3600 radiocarbon years BP) is a temporal outlier within the sampled middens.

2.3. Rodent DNA

To examine temporal changes in the composition of midden-forming rodent communities we attempted to identify the rodent species that formed each midden. A short (85 bp) region of the rodent cytochrome *b* (*cyt b*) gene was amplified from midden DNA extracts using the primers H14927 and L14841 (Kuch et al., 2002). The PCR mastermix and cycling conditions were as for the initial 18S PCRs above. Amplicons were visualised on 4% agarose gels, purified and sequenced in both directions on an ABI377XL sequencer (Macrogen, South Korea). Amplification and sequencing of the *cyt b* fragment was successful for just 7 of the 28 middens. To increase our sample size we sequenced the fragment from a further six radiocarbon dated rodent middens from the Calama and Salar de Atacama basins (Table S1). Sequences are provided in a

supplementary fasta file to this study.

The sequences obtained from the middens ranged from 85 to 49 bp (shorter lengths were due to the trimming of low-quality base calls from the ends of sequences). These were aligned using Muscle (Edgar, 2004) via Geneious 10.2.6 (https://www.geneious.com) with reference sequences from midden-forming rodent taxa presently found in the local or adjacent regions. These included representatives of all the midden-forming rodent genera known in the Atacama (*Phyllotis*, *Abrocoma*, *Lagidium* and *Octodontomys*) (Table S2). Any reference sequences that had undetermined bases (N) within the 85 bp region were deleted from the alignment. The final alignment (containing 103 reference sequences and 13 new

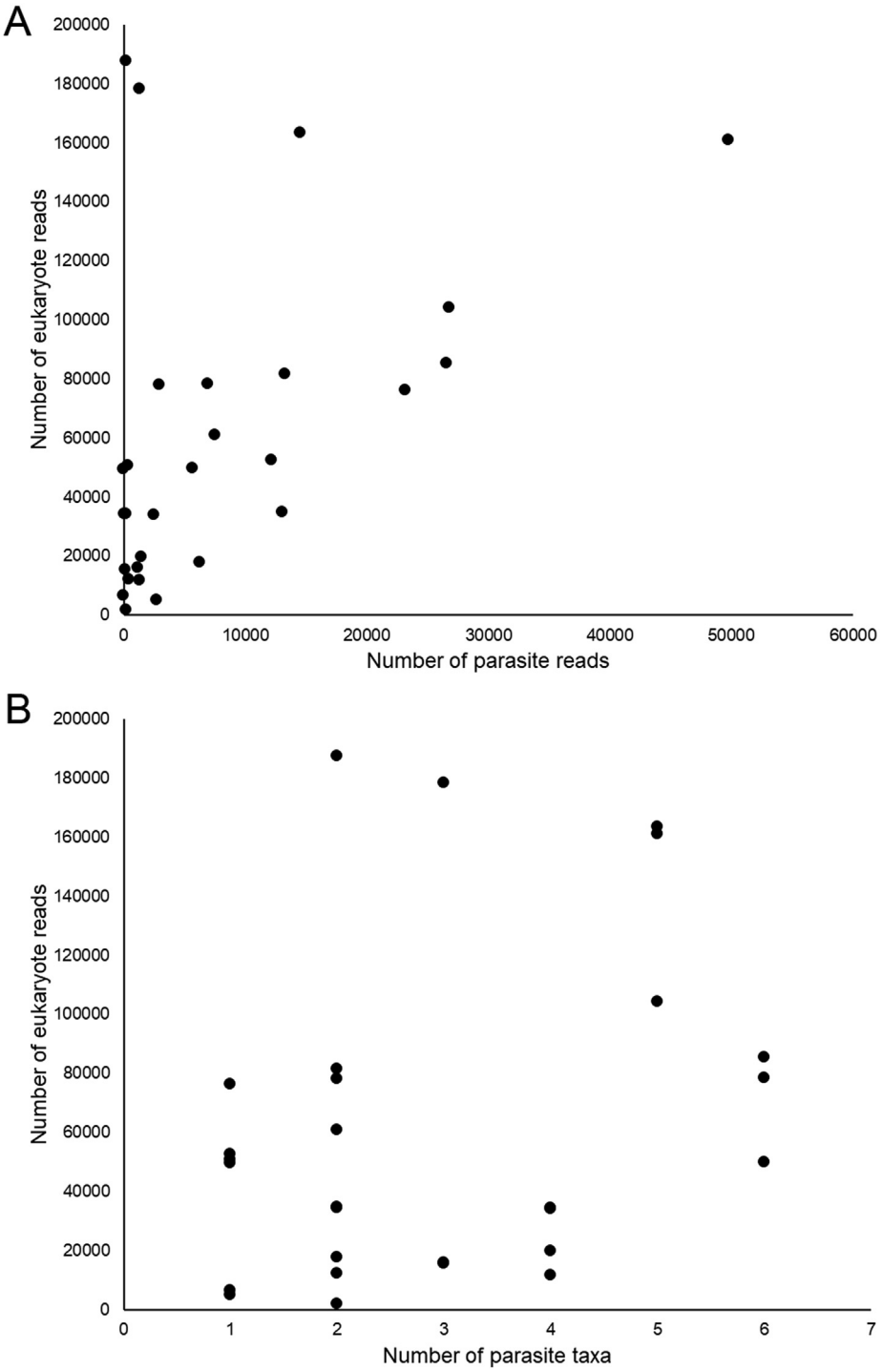

Fig. 2. Relationship between: A, number of parasite reads and total eukaryote reads recovered from each midden, and; B, number of parasite taxa and total eukaryote reads recovered from each midden.

midden sequences) was used to create a minimum spanning haplotype network (Bandelt et al., 1999) in PopART v.1.7 (Leigh and Bryant, 2015).

3. Results

3.1. Identification of parasite taxa

Every midden yielded DNA reads that could be attributed to at least one parasite taxon. Overall, there was a positive relationship between the total number of eukaryote reads obtained for each midden and the number of reads attributed to parasites (Fig. 2a), but not to the taxonomic richness of parasites (Fig. 2b). We obtained DNA reads from thirteen unique parasite groups, nine of which are likely to have been derived from rodent hosts and four that are likely to have had alternative hosts (Fig. 3; 4). Each of these groups of parasites are described separately below.

Anoplura. Just two reads from a single midden were assigned to Anoplura (sucking lice) (Fig. 4). The nearest matching genus *Linognathoides* (99% identity) is specific to the rodent family Sciuridae (Durden and Musser, 1994) and has not been identified from South American rodents. One lice genus (*Abrocomaphthirus*) within the same family as *Linognathoides* (Polyplacidae) has been recorded from the native South American rodent *Abrocoma cinerea* (Durden and Webb, 1999). However, we could not test the similarity between the reads we obtained and 18S sequences of *Abrocomaphthirus* as there were no reference sequences for this genus in the NCBI database.

Acari. Reads from five middens were assigned to the mite genus *Oryzomyopus* (Fig. 4), which is associated with rodent nests (Houck, 1994) and has previously been recorded living in the tail hair follicles of South American rodents (Fain, 1967).

Ixodidae. Reads from two middens were assigned to the family Ixodidae (hard ticks) (Fig. 4), but no further taxonomic resolution was possible. Physical remains of Ixodidae have previously been recorded from Atacama rodent middens (Dézerald et al., 2019).

Argasidae. Reads from thirteen middens were assigned to the family Argasidae (soft ticks) (Fig. 4). Argasidae are widespread in dry environments, and have previously been recorded from rodent middens in the central Atacama (Dézerald et al., 2019). Most reads assigned to Argasidae could not be resolved to lower taxonomic ranks, but reads from one midden resolved to *Ornithodoros rostratus*, which occurs widely in South America. The genus *Ornithodoros* is found on rodents and other burrow-inhabiting or cave-dwelling animals (Venzal and Estrada-Peña, 2006; Vial, 2009) and multiple species of *Ornithodoros* are known from the Atacama (Venzal et al., 2012; Munoz-Leal et al., 2016).

Strongylida. Reads from one midden were assigned to the parasitic nematode suborder Strongylida (Fig. 4). The most common OTU assigned to Strongylida matched with 100% identity to a wide range of gastrointestinal parasitic genera that shared identical sequences for the barcode region (*Cyathostoma*, *Necator*, *Ancylostoma*, *Austrostrongylus*, *Haemonchus*, *Petrovinema*, *Cylicocyclus*, *Chabertia*, *Cyclodontostomum*, *Herpetostrongylus*, *Nicollina*, *Strongylus* and *Labiostrongylus*).

Spirurida. Reads from 14 middens were assigned to taxa within the nematode order Spirurida. While most of these reads were assigned at the order level (9 middens), some resolved the lower taxonomic ranks of superfamily Filarioidea (1 midden) and family Onchocercidae (8 middens) (Fig. 4). Spiruda (and Onchocercidae) infect a wide range of animals, including mostly mammals and birds but also amphibians, and cause a wide range of diseases in humans. Spurids are known to infect Sigmodontid rodents (Kinsella, 1974), and there are records of Onchocercidae infecting *Phyllotis* and other rodents in Chile and Argentina (Notaernicola and Navone, 2011; Landaeta-Aqueveque et al., 2014). The nearest matches (98% identity for 97% coverage) to the midden OTU include sequences from *Pelecitus* (Onchocercidae), *Mastophorus* (Spirudae), *Onchocerca* (Onchocercidae), *Loa* (Onchocercidae) and *Dirofilaria* (Onchocercidae).

Ascaridida. Reads from three middens were assigned to the nematode order Ascaridida (Fig. 4). The identity of the host responsible for the presence of Ascaridida in the middens is equivocal. This is because the nearest matches to the midden reads were from the genera *Ortleppascaris* and *Terranova* (100% identity) whilst other Ascaridida genera were at $\leq$ 98%. Neither of these

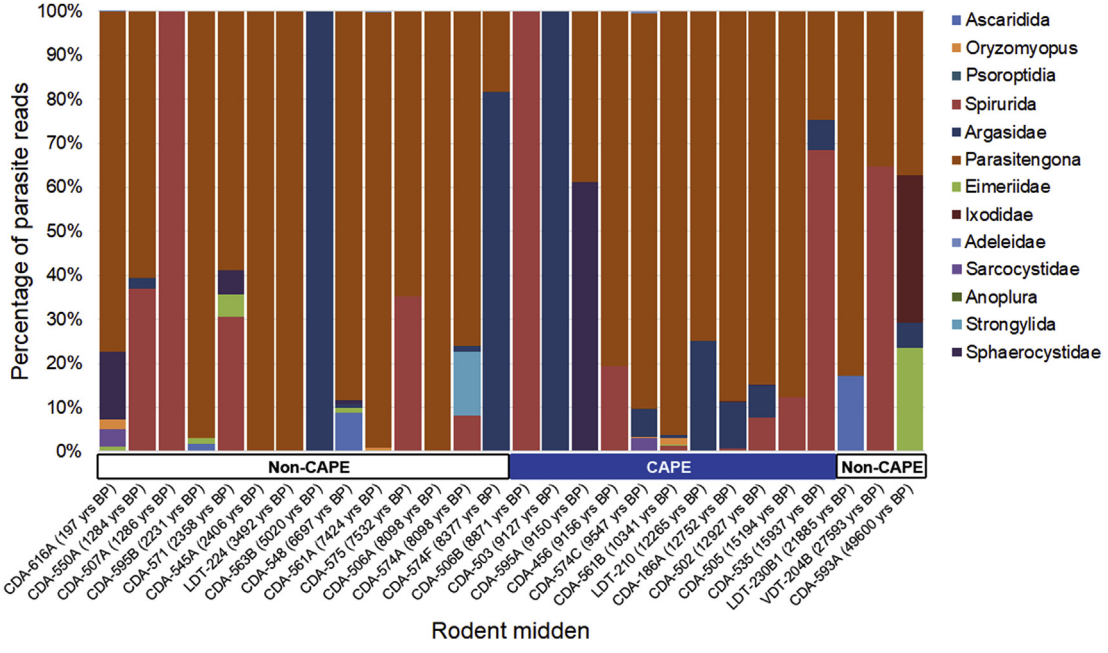

Fig. 3. Relative abundance of DNA reads assigned to parasite taxa within each of the rodent middens. Note that prefixes of midden codes relate to collection locality (see Fig. 1).

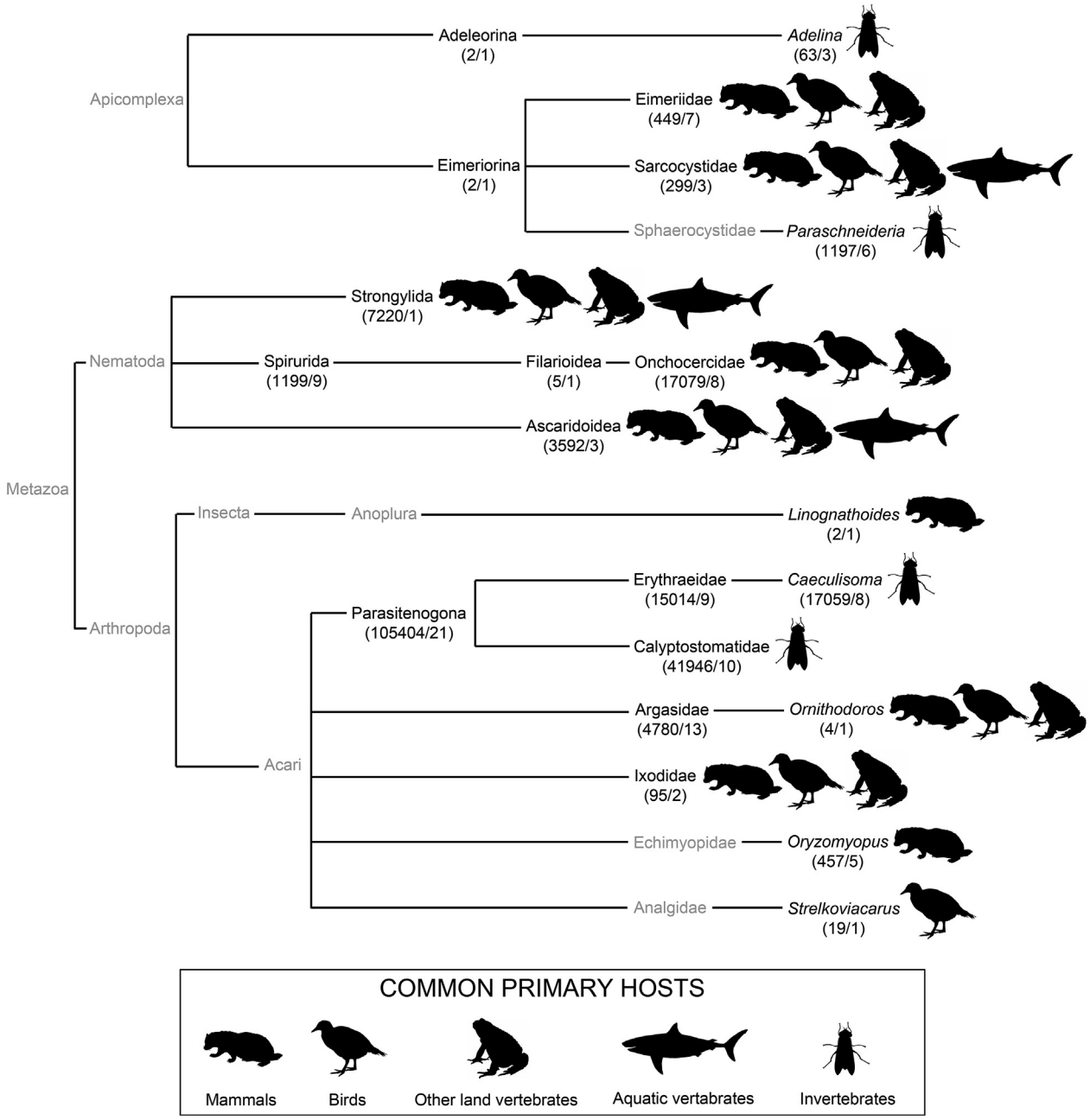

Fig. 4. Phylogenetic relationships of parasite taxa identified from DNA sequences from rodent middens, central Atacama Desert, Chile. Numbers in brackets refer to the total number of reads assigned to each taxon and number of middens in which reads assigned to that taxon were recovered. The most common primary hosts for terminal taxa are shown.

genera appear to have natural mammalian hosts, though *Terranova* infections have been recorded in humans. *Ortleppascaris* has been recorded from crocodilians (Sprent, 1978; Zhao et al., 2016) and the cane toad (*Rhinella marina*) (Pereira e Silva et al., 2014), whilst *Terranova* is known from crocodilians and fish (see Table 3 in Shamsi and Suthar, 2016). As reads assigned to Ascaridida occurred in three different midden samples of different ages (Fig. 4) we suggest that the reads could represent a rodent parasite, but one for which there are currently no reference DNA sequences available in the NCBI database.

Eimeriorina. Two families were resolved within the parasitic apicomplexan suborder Eimeriorina (Fig. 4). Reads from seven middens were assigned to Eimeriidae, but provided no finer taxonomic resolution. Reads from three middens were assigned to Sarcocystidae, and had 100% identity to reference DNA sequences from several genera within the family (*Toxoplasma*, *Neospora*, *Besnoitia*, *Nephroisospora* and *Hammondia*).

Parasites from non-rodent hosts. We resolved four additional parasite taxa that are more likely to have come from invertebrates or other animals than from the rodents that formed the middens (Fig. 4). The first was the mite suborder Psoroptidia, identified from one midden. Psoroptida originated as bird parasites, with a secondary radiation of mammal parasites within the group (O'Connor and Houck, 1994). However, the Psoroptida sequence from the middens was a closest match (100–98% identity) to sequences from the genus *Strelkoviacarus*; a feather mite (Analgidae) which has previously been recorded on birds in central Chile (Fuentes-Castillo et al., 2016).

Table 1
Differences in parasite assemblage metrics between CAPE and non-CAPE Atacama rodent middens using Welch's two sample *t*-test (two-sided).

Parasite group		t	df	p
Taxonomic richness				
All parasites	All middens	0.302	19.567	0.766
	49,600 yr midden excluded	0.396	20.09	0.696
Rodent parasites	All middens	0.445	14.57	0.663
	49,600 yr midden excluded	0.596	14.697	0.560
Non-rodent parasites	All middens	0.172	21.146	0.865
	49,600 yr midden excluded	0.059	20.783	0.953
Overall Shannon diversity				
All parasites	All middens	−0.275	26	0.786
	49,600 yr midden excluded	0.143	24.893	0.888
Rodent parasites	All middens	0.037	17.524	0.971
	49,600 yr midden excluded	0.292	16.908	0.774
Non-rodent parasites	All middens	0.304	11.621	0.767
	49,600 yr midden excluded	0.252	12.084	0.805
Overall relative read abundance				
All parasites	All middens	−2.228	25.777	0.035*
	49,600 yr midden excluded	−2.42	24.495	0.023*

Reads from six middens were assigned to Sphaerocystidae, and had a 100% identity match to the sequence of *Paraschneideria metamorphosa* (now within Actinocephalidae) and were more distant to other taxa. However, *P. metamorphosa* appears be the only species with a reference sequence available within the genus or family. Therefore, it is perhaps more appropriate to assign the midden reads to Eugregarinorida, which are parasitic protists commonly found in insects and worms.

Reads assigned to the mite suborder Parasitengona were recovered from all 24 middens, and represented the majority of all reads assigned to parasites (Fig. 3). Within these reads some lower taxonomic ranks were resolved: Erythraeidae (including *Caeculisoma*), Calyptostomatoidea (including *Calyptostoma*) and Microtrombidiidae. Although the adults of Erythraeidae and Microtrombidiidae are free-living predators, the larvae are parasites of arthropods (Wohltmann, 2000).

Reads from three middens were assigned to the coccidian family Adeleidae. These reads had a closest match (100% identity) to sequences of *Adelina*, which commonly parasitise insect hosts (Kopečna et al., 2006).

3.2. Temporal trends in parasite occurrence and abundance

There were no statistically significant differences between CAPE and non-CAPE middens in terms of parasite taxonomic richness or Shannon diversity index (Table 1). Only the total relative abundance of parasite reads differed significantly (Table 1), with a lower mean value during the CAPE. We found no significant differences between CAPE and non-CAPE middens for any individual parasite taxon in terms of relative read abundance or occurrence frequency (Table 2).

3.3. Midden-forming rodents

Some Atacama midden-forming rodent taxa could be identified based on haplotypes of the short *cyt b* region sequenced in this study, but several taxa share identical haplotypes and therefore cannot be distinguished (Fig. 6). Three haplotypes were obtained from the sampled rodent middens. The first, H1, was obtained from 7 middens (CDA-561A, EHT-478B, LDT-223, LDT-226B, CDA-616A, LDT-235, VDT-204B) and shared 100% identity only to reference sequences from *Phyllotis xanthopygus rupestris* (Fig. 6). The second, H2, was obtained from 5 middens (CDA-561B, CDA-574C, EHT-453B1, CDA-456, CDA-186A) and included reference sequences of *Phyllotis limatus*, *P. xanthopygus*, *P. x. vaccarum*, and *P. x. rupestris* (Fig. 6). The sequence obtained from an Atacama rodent midden by Kuch et al. (2002) also fell within haplotype H2. A third haplotype, H3, was obtained from a single midden and was shared by a reference sequence of *Abrocoma cinerea* (Fig. 6).

Haplotype H1 was recovered from middens spanning from the present day back to ~28,000 years BP (Fig. 7). It was the only haplotype obtained from middens deposited outside CAPE. In contrast, haplotypes H2 and H3 were only obtained from middens deposited during the CAPE. Although based on a limited sample size, these results indicate that a greater diversity of midden-forming rodents may have inhabited the Calama and Salar de Atacama basins during the CAPE. The results also appear to indicate a replacement of the H2 haplotype with haplotype H1 occurred in this region sometime between 10,000 and 9000 years BP (Fig. 7).

As both the depositing species and parasite assemblages were characterised in only seven middens (those from Cerros de Aiquina, Lomas de Tilocalar and Vegas de Tilocalar in Fig. 7), the small sample size prevented analysis of the direct correlation between hosts and parasites. However, two observations can be made based on the available data. First, apart from relative read abundance, there were no significant differences observed in the parasite faunas between CAPE and non-CAPE middens, despite there being increased host diversity during the CAPE (Fig. 7). Second, and perhaps coincidentally, the first appearances of two apicomplexan families (Sarcocystidae and Eimeriidae) in the middens (Fig. 5) both occur around the time of transition between H2 and H1 rodent haplotypes (Fig. 7).

4. Discussion

Since rodent middens were first identified as a valuable resource for Quaternary research in the arid parts of South America and especially the Atacama Desert (Betancourt and Saavedra, 2002; Latorre et al., 2002), study of these deposits has given insights into past climate change within these regions during the late Quaternary (Latorre et al., 2006; Quade et al., 2008; Mujica et al., 2015), as well as the long-term trends and responses to climate change in plants (Latorre et al., 2002, 2003; 2006; Díaz et al., 2012, 2019; de Porras et al., 2017), plant pathogens (Wood et al., 2018), arthropods (Dézerald et al., 2019) and parasites (Beltrame et al., 2016). Although there have been several studies of late Quaternary parasite faunas from South American rodent coprolites, here we provide just the second study from paleomiddens, extending the temporal range of midden parasite remains from the Holocene back into the late Pleistocene. Our DNA metabarcoding data have allowed us to identify a wide range of both internal and external parasites preserved in these samples, supporting the contention that they provide a valuable resource for paleoparasitological analyses (Beltrame et al., 2016). With excellent preservation of ancient DNA (Kuch et al., 2002; Wood et al., 2018; Díaz et al., 2019), rodent middens from South America will also provide a valuable resource for more detailed paleogenomic studies of prehistoric parasites in future. Similarly, rodent middens from other continents including North America, Australia, Africa, Asia and the Middle East (Elias, 2007) likely represent important, but unexplored, resources for paleoparasitological studies.

Extensive microscopic analyses of parasite remains in Holocene rodent coprolites from South America (Argentina) have previously identified just two key gastrointestinal parasite groups: nematodes and cestodes (Sardella and Fugassa, 2009a, 2009b; 2011; Sardella et al., 2010; Beltrame et al., 2012, 2013; 2014, 2018; 2019). These parasite groups are well-suited to microscopic studies, as they have relatively large and characteristic eggs that preserve well in coprolites and can be abundant in some samples. The previous

Table 2
Differences in individual parasite taxa metrics between CAPE and non-CAPE Atacama rodent middens assessed using a generalised linear model with a presence/absence approach.

		statistic	p
Anoplura			
Relative read abundance	All middens	1.00	0.341
	49,600 yr midden excluded	1.00	0.341
Occurrence frequency	All middens	−0.928	0.354
	49,600 yr midden excluded	−0.892	0.372
Oryzomyopus			
Relative read abundance	All middens	−0.683	0.501
	49,600 yr midden excluded	−0.729	0.474
Occurrence frequency	All middens	−1.02	0.307
	49,600 yr midden excluded	−0.951	0.342
Ixodidae			
Relative read abundance	All middens	−0.774	0.449
	49,600 yr midden excluded	1.00	0.341
Occurrence frequency	All middens	−0.320	0.749
	49,600 yr midden excluded	−0.004	0.997
Argasidae			
Relative read abundance	All middens	1.57	0.147
	49,600 yr midden excluded	1.57	0.148
Occurrence frequency	All middens	−1.445	0.148
	49,600 yr midden excluded	−1.63	0.103
Strongylida			
Relative read abundance	All middens	−1.00	0.332
	49,600 yr midden excluded	−1.00	0.333
Occurrence frequency	All middens	0.423	0.673
	49,600 yr midden excluded	0.458	0.647
Spirurida			
Relative read abundance	All middens	−1.45	0.165
	49,600 yr midden excluded	−1.47	0.163
Occurrence frequency	All middens	−1.149	0.250
	49,600 yr midden excluded	−1.008	0.313
Ascaridida			
Relative read abundance	All middens	−1.26	0.226
	49,600 yr midden excluded	−1.26	0.227
Occurrence frequency	All middens	1.053	0.293
	49,600 yr midden excluded	1.095	0.274
Eimeriidae			
Relative read abundance	All middens	−1.91	0.073
	49,600 yr midden excluded	−1.61	0.128
Occurrence frequency	All middens	1.456	0.145
	49,600 yr midden excluded	1.284	0.199
Sarcocystidae			
Relative read abundance	All middens	−0.201	0.842
	49,600 yr midden excluded	−0.243	0.810
Occurrence frequency	All middens	0.222	0.824
	49,600 yr midden excluded	0.276	0.783
Psoroptida			
Relative read abundance	All middens	1.00	0.341
	49,600 yr midden excluded	1.00	0.341
Occurrence frequency	All middens	−0.928	0.354
	49,600 yr midden excluded	−0.892	0.372
Sphaerocystidae			
Relative read abundance	All middens	−1.18	0.257
	49,600 yr midden excluded	−1.18	0.255
Occurrence frequency	All middens	0.336	0.737
	49,600 yr midden excluded	0.417	0.677
Parasitengona			
Relative read abundance	All middens	−1.45	0.16
	49,600 yr midden excluded	−1.61	0.12
Occurrence frequency	All middens	0.471	0.638
	49,600 yr midden excluded	0.406	0.685
Adeleidae			
Relative read abundance	All middens	−0.201	0.842
	49,600 yr midden excluded	−0.249	0.806
Occurrence frequency	All middens	0.223	0.824
	49,600 yr midden excluded	0.276	0.783

paleoparasitological assessment of a South American rodent midden (Beltrame et al., 2016) also identified nematode eggs, but sampled only coprolites from within the midden. The more extensive parasite assemblage revealed by our bulk DNA metabarcoding approach suggests that rodent middens can preserve a wider array of taxa than those present in coprolites, including external parasites (e.g., lice, ticks, mites) likely to be preserved within the nesting material component of the midden matrix. Moreover, DNA can detect parasite taxa which, although likely present in coprolites, can be difficult to identify from ancient

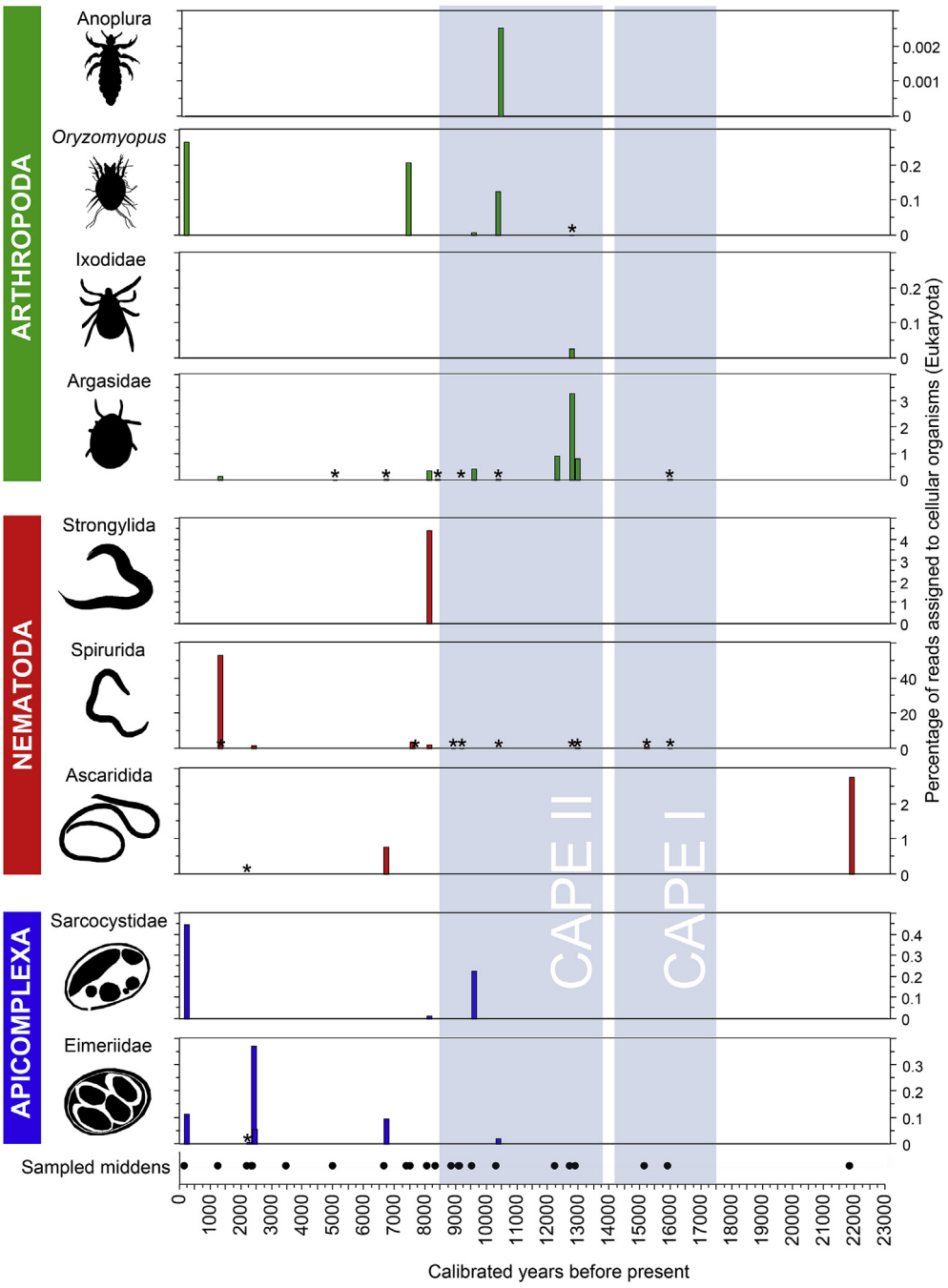

Fig. 5. Relative abundance of reads attributed to rodent parasites from rodent middens <23,000 years old, central Atacama Desert. Asterisks represent samples were the taxon was present but at low abundance.

samples using microscopy due to their relatively small size and low likelihood of morphological preservation (e.g., Apicomplexans).

The parasite assemblages identified from the rodent middens using DNA metabarcoding were biogeographically consistent with those which would be expected from such samples. Where it was possible to resolve lower taxonomic ranks (e.g., family or genus) of mammalian parasites they had often been recorded from South American rodents or the Atacama region previously (e.g. *Ornithodoros, Oryzomyopus*), although we cannot rule out the possibility that some of the parasite taxa identified in the middens could have originated from non-midden forming mammals using the sites. The identity of parasites with non-mammalian hosts were also consistent with what might be expected from rodent middens. For example, a likely feather mite is consistent with feathers having been found previously in rodent middens (Markgraf et al., 1997; Latorre et al., 2005). Moreover, the detection of insect parasites was also not unexpected. Arthropod remains are common in middens, and some insect species may even occupy rodent nests on a semi-permanent basis (Dézerald et al., 2019).

In contrast to assemblages of plants (Díaz et al., 2019) and plant-pathogens (Wood et al., 2018), which showed major responses to the CAPE, this period of increased precipitation had little effect on our parasite data. Just one taxon, the soft-bodied ticks (Argasidae), showed any hint of changes in occurrence frequency and/or relative

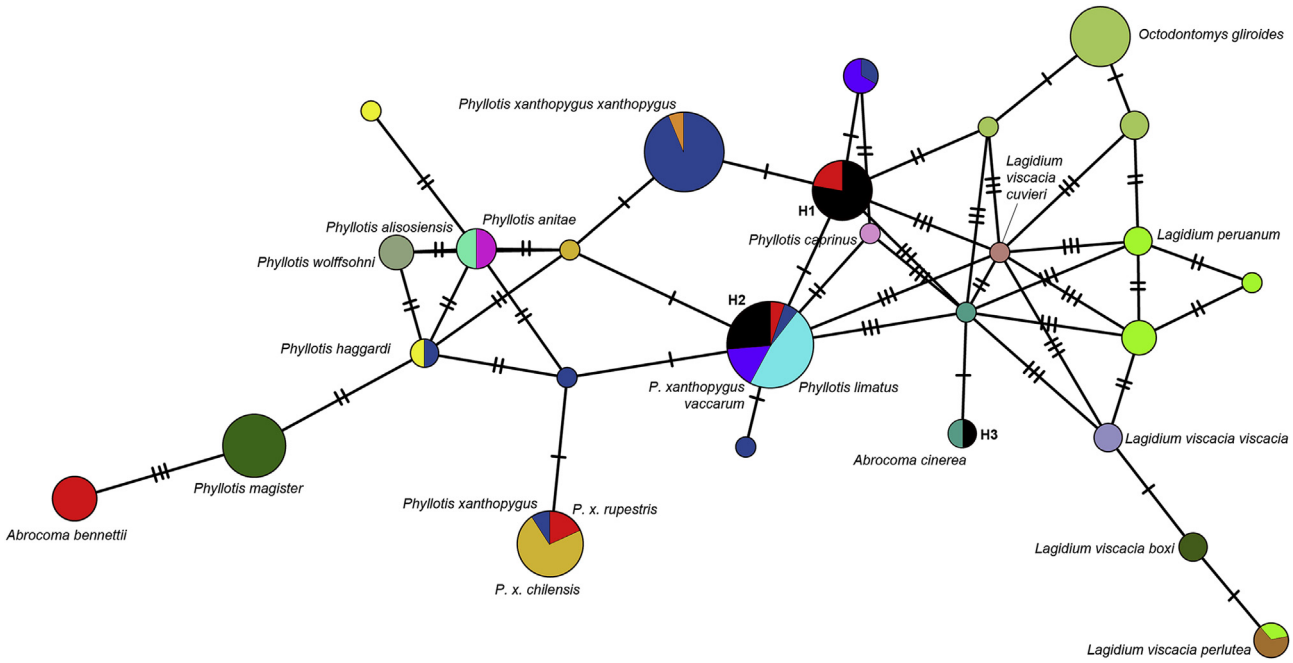

Fig. 6. Haplotype network based on the 85bp region of cytochrome *b* amplified with primers H14927 and L14841 (Kuch et al., 2002). Sequences generated in this study from ancient middens are represented by black, while colours represent reference sequences for genera that include Atacama midden-forming rodent species. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

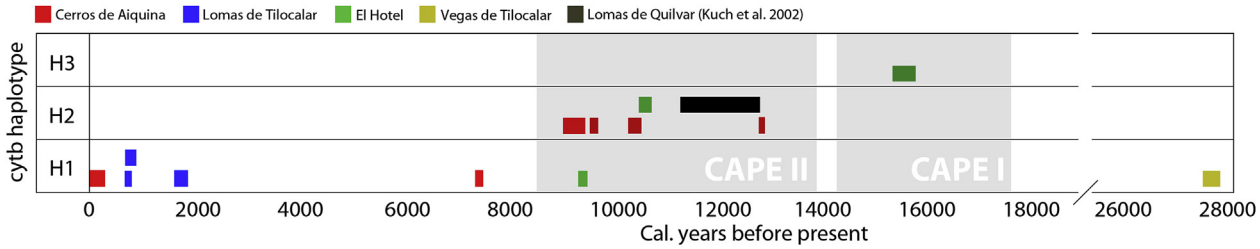

Fig. 7. Spatio-temporal distribution of rodent cytochrome *b* haplotypes from Atacama middens. Each bar represents a single midden, and the width of the bar reflects the 95.4% confidence range of the calibrated radiocarbon age of the midden.

abundance during the CAPE (Fig. 5) but this was not statistically significant. Moreover, we found evidence for a greater diversity of rodent taxa in paleomiddens formed during the CAPE (three haplotypes) compared to those formed outside this event (one haplotype), and a possible turnover event between two of these rodent taxa around 10,000 to 9000 years BP. The apparent resilience of the parasites to changes in host taxa and climate could be explained by two factors: 1) the low taxonomic resolution that parasite taxa were resolved to, and; 2) parasite-sharing between closely-related midden-forming rodent taxa (i.e. the most common haplotypes H1 and H2) (e.g. González-Acuña et al., 2005). While organisms such as plants and plant-pathogens are directly influenced by local climate conditions, the relationship between climate and parasites may be more complex. Internal parasites may only be exposed to external climate conditions for part of their life-cycle, and even external parasites (particularly nidicolous species) can be largely buffered from external climate conditions (Vial, 2009). However, external climate influences microclimate conditions (e.g. of burrows), and therefore local climate conditions remain a major driver of parasite distributions, even in nidicolous parasites (Vial, 2009). In future, improving the degree of taxonomic resolution (ideally to species) of both the parasites and the host rodents may reveal patterns that are presently obscured in our current dataset. Due to the fragmented nature of ancient DNA, such advances would likely require metagenomic approaches combined with targeted enrichment of parasite and rodent DNA.

5. Conclusions

We have shown that diverse assemblages of parasite taxa can be reconstructed from paleomiddens using aDNA metabarcoding. At a broad-scale, parasites from Atacama rodent middens appear to have been resilient to major changes in climate and host rodent taxa over the past 50,000 years. However, improved taxonomic resolution is necessary, and could resolve species-level turnover within the parasite groups identified by our study. The results provide further evidence that paleomiddens are fast becoming an unrivalled source of genomic data that can be used to reconstruct past ecosystem change on multiple taxonomic, temporal and spatial scales with unprecedented detail.

Data availability

The demultiplexed unassembled fast read files are available at https://doi.org/10.7931/Y9AJ-9368.

Declaration of competing interest

None.

Acknowledgements

F.P.D. was funded by a FONDECYT Postdoctorado # 3150616, iBio Iniciativa Científica Milenio-MINECON and Fondo de Desarrollo de Areas Prioritarias (FONDAP) Centre for Genome Regulation (15090007). C.L acknowledges funding from Aporte de Financiamiento Basal (AFB-170008) and J.R.W. and J.M.W. acknowledge Core funding for Crown Research Institutes from the New Zealand Ministry of Business, Innovation and Employment's Science and Innovation Group.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.quascirev.2019.106031.

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