DNA barcoding of ancient parasites

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SUMMARY

Ancient samples present a number of technical challenges for DNA barcoding, including damaged DNA with low endogenous copy number and short fragment lengths. Nevertheless, techniques are available to overcome these issues, and DNA barcoding has now been used to successfully recover parasite DNA from a wide variety of ancient substrates, including coprolites, cesspit sediment, mummified tissues, burial sediments and permafrost soils. The study of parasite DNA from ancient samples can provide a number of unique scientific insights, for example: (1) into the parasite communities and health of prehistoric human populations; (2) the ability to reconstruct the natural parasite faunas of rare or extinct host species, which has implications for conservation management and de-extinction; and (3) the ability to view in 'real-time' processes that may operate over century- or millenial-timescales, such as how parasites responded to past climate change events or how they co-evolved alongside their hosts. The application of DNA metabarcoding and high-throughput sequencing to ancient specimens has so far been limited, but in future promises great potential for gaining empirical data on poorly understood processes such as parasite co-extinction.

Key words: ancient DNA, archaeology, co-extinction, extinct species, fossils, Holocene, Pleistocene.

INTRODUCTION

Ancient DNA research has evolved markedly since the first pioneering studies were published in the 1980s. While these early studies typically retrieved relatively short regions of mitochondrial DNA from museum, or Holocene age, specimens (e.g. Higuchi *et al*. 1984; Pääbo *et al*. 1988) near-complete genomes are now routinely being sequenced from Pleistocene age remains (Der Sarkissian *et al*. 2015). Moreover, the ever-expanding set of substrates from which ancient DNA has been retrieved (Green and Speller, 2017) is allowing new insights into diverse aspects of the past, such as palaeofaunas (Grealy *et al*. 2015), ancient oral microbiomes (e.g. Weyrich *et al*. 2017), palaeodiets (e.g. Wood *et al*. 2013*a*) and palaeoenvironments (Anderson-Carpenter *et al*. 2011; Parducci *et al*. 2017).

The extraction, amplification, sequencing and analysis of DNA molecules from long-dead organisms present several unique challenges. As a result of natural degradation processes, DNA from old specimens is usually present at a much lower copy number than in recent specimens (Pääbo *et al*. 2004), and is therefore at risk of contamination by modern DNA (Yang and Watt, 2005; Skoglund *et al*. 2014). Moreover, DNA molecules break into shorter fragments over time and accumulate damage that can alter nucleotide sequences (Brotherton *et al*. 2007; Sawyer *et al*. 2012). Early ancient DNA research was hampered by a poor understanding of these issues and several claims of antediluvian DNA (>1 million years old, *sensu* Lindahl, 1993) have since been disproven (Hofreiter *et al*. 2001). Authentication criteria have subsequently been developed to help improve the rigor of ancient DNA research, including the use of specialized ancient DNA facilities, inclusion of negative controls in PCR experiments, ensuring reproducibility and providing independent replication of results (Cooper and Poinar, 2000; Hofreiter *et al*. 2001; Gilbert *et al*. 2005).

Although somewhat challenging, the study of DNA from ancient specimens represents an approach that can provide certain insights about the past that are unattainable by other means (Rizzi *et al*. 2012; Leonardi *et al*. 2017; Cole and Wood, in press). Ancient DNA research provides us with a 'real-time' view of ecological and evolutionary processes that otherwise could only be inferred from current genetic variation (Krause and Pääbo, 2016), or for which the signal in modern gene-pools may be completely obliterated (Orlando and Cooper, 2014). For example, assessment of the genetic diversity within a species through time has been used to reconstruct population dynamics associated with major climatic and environmental change in the past (e.g. Shapiro *et al*. 2004; Rawlence *et al*. 2012) and ancient DNA has provided key evidence for the cryptic extinction of hitherto unknown species diversity (e.g. Boessenkool *et al*. 2009; Soubrier *et al*. 2016). To date, ancient DNA studies have had a significant taxonomic bias towards humans and other vertebrates (see Fig. 1 in Parducci *et al*. 2017), yet there is also the clear

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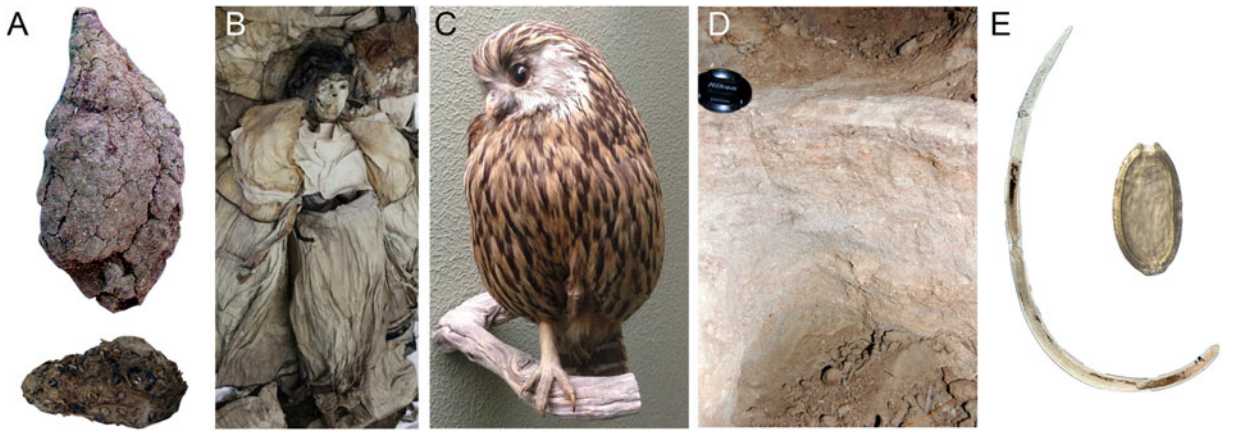


Fig. 1. Some sources of historic or ancient parasite DNA: (A) coprolites and pellets; (B) mummies and burial sediments (from Shin *et al*. 2012, reprinted with permission of Elsevier); (C) museum specimens; (D) sediments, such as from nesting or shelter sites; (E) specimens of ancient parasites or eggs.

potential for ancient DNA to be used to better understand invertebrate communities of the past (e.g. Thomsen *et al*. 2009). Parasites, in particular, are one group of invertebrates that have already received some attention from ancient DNA studies. Here, I review the use of ancient DNA to study ancient parasites and then pay a particular focus on the DNA barcoding of ancient parasites (the amplification and sequencing of short variable DNA regions for the purpose of taxonomic identification). I discuss some of the challenges and options around primer design for DNA barcoding of parasites from ancient samples and explore some of the potentially exciting insights into the evolution and ecology of parasites that ancient DNA barcoding could provide.

ANCIENT PARASITE DNA

The development of the polymerase chain reaction (PCR) technique in the early 1980s paved the way for DNA barcoding studies. The potential for this technique to contribute to the field of palaeoparasitology was quickly realized (Pääbo *et al*. 1989; Hedges and Sykes, 1992), though the first successful attempts to retrieve DNA from historic parasite specimens were not reported until the 1990s. Persing *et al*. (1990) recovered DNA of the *Borrelia burgodorferi* bacterium from museum specimens of deer ticks (*Ixodes dammini*) collected during the 1940s, to demonstrate the likely presence of Lyme disease in invertebrate vectors in the USA prior to its official recognition. The contemporaneous presence of the bacterium within hosts was confirmed through DNA analysis of museum specimens of the white-footed mouse (*Peromyscus leucopus*) by Marshall *et al*. (1994). Four years later DNA of the trypanosome *Leishmania amazonensis* was successfully amplified from taxidermied rodent specimens in museum collections (Araújo *et al*. 1998), and Sallares and Gomzi (1999) reported obtaining *Plasmodium falciparum* DNA sequences from 5th Century Roman skeletons. The temporal range of recoverable parasite DNA was expanded greatly by Guhl *et al*. (1997, 1999) and Ferreira *et al*. (2000), who recovered DNA of the protozoan *Trypanosoma cruzi* (responsible for Chagas disease) from South American mummified humans dating back to 4000 year before present (yrs BP). A few years later this record was extended back to approximately 9000 years BP (Aufderheide *et al*. 2004). This remains the oldest DNA from a parasite with an animal host, though DNA sequences attributed to plant parasites have been recovered from sediment cores extending back 40 000 years (Lydolph *et al*. 2005) (Table 1).

Since the year 2000, ancient parasite DNA has been recovered from a range of different substrates (Fig. 1) including both human (Loreille *et al*. 2001; Iñiguez *et al*. 2003, 2006; Leles *et al*. 2008) and animal (Wood *et al*. 2013*b*; Boast *et al*. 2018) coprolites, the content of cesspits (Myšková *et al*. 2014), mummified tissues (Dittmar *et al*. 2003; Zink *et al*. 2006; Fernandes *et al*. 2008; Nerlich *et al*. 2008), burial sediments (Oh *et al*. 2010*a*, *b*; Shin *et al*. 2013) and permafrost soils (Lydolph *et al*. 2005). Much of this work has focused on extracting parasite DNA from preserved human remains (e.g. human mummies and coprolites), mainly in specimens from Egypt (e.g. Zink *et al*. 2006; Nerlich *et al*. 2008; Hawass *et al*. 2010; Lalremruata *et al*. 2013; Matheson *et al*. 2014), Korea (e.g. Oh *et al*. 2010*a*, *b*; Shin *et al*. 2012, 2013) and South America (e.g. Dittmar *et al*. 2003; Iñiguez *et al*. 2003, 2006; Fernandes *et al*. 2008; Leles *et al*. 2008; Costa *et al*. 2009) (Table 1). Accordingly, the most intensively studied taxa have been those commonly associated with prehistoric human populations in those regions, particularly those responsible for parasitic diseases (e.g.

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Table 1. Examples of studies that have used DNA barcoding approaches to study ancient parasites

Approach	Substrate	Country/region	Parasitic taxon/taxa	Sample age (years before present)	Reference
Taxon-specific barcoding	Animal mummies	Peru	Siphonaptera	1050	Dittmar *et al*. (2003)
	Burial sediments	Korea	*Trichuris trichiura*	558–40	Oh *et al*. (2010*a*)
			Ascaris	558–40	Oh *et al*. (2010*b*)
			Clonorchis sinensi	350–250	Shin *et al*. (2013)
	Head lice	Peru, Chile	*Trypanosoma cruzi*	9000–550	Aufderheide *et al*. (2005)
	Human coprolites	Belgium	*Ascaris*	650–550	Loreille *et al*. (2001)
		Brazil, Chile	*Ascaris*	8800–1050	Leles *et al*. (2008)
		Canary Islands	*Ascaris*	250–150	Botella *et al*. (2010)
		Chile, USA	*Enterobius vermicularis*	6060–1050	Iñiguez *et al*. (2003, 2006)
		Czech Republic	*Enterocytozoon bieneusi*	250–50	Myšková *et al*. (2014)
		Peru, Chile	*T. cruzi*	9000–550	Aufderheide *et al*. (2005)
	Human mummies	Brazil	*T. cruzi*	533	Fernandes *et al*. (2008)
		Canary Islands	*Ascaris*	750	Jaeger *et al*. (2016)
		Chile	*Leishmania donovani*	1000–500	Costa *et al*. (2009)
		Egypt	*Plasmodium falciparum*	4000	Nerlich *et al*. (2008)
			Schistosoma mansoni and *S. haematobium*	3900	Matheson *et al*. (2014)
			P. falciparum	3000–1650	Lalremruata *et al*. (2013)
				3600–3050	Hawass *et al*. (2010)
		Egypt, Nubia	*L. donovani*	4000	Zink *et al*. (2006)
		Korea	*Paragonimus*	400	Shin *et al*. (2012)
		Peru, Chile	*T. cruzi*	4000–550	Guhl *et al*. (1997, 1999), Ferreira *et al*. (2000)
				9000–550	Aufderheide *et al*. (2004)
	Human skeletons	Italy	*P. falciparum*	1450–1550	Sallares and Gomzi (1999)
	Sediment within human skeletons	Brazil	*Ascaris*, *T. trichiura*, *E. vermicularis* and *Strongyloides stercoralis*	250–150	Jaeger and Iñiguez (2014)
	Sediments (various)	Europe, Caribbean	Tapeworms, roundworms, whipworm, pinworm, flukes	7250–0	Côté *et al*. (2016)
	Taxidermied rodents	Brazil	*Leishmania amazonensis*	Recent (1941–1975 AD)	Araújo *et al*. (1998)
Metabarcoding	Bird coprolites	New Zealand	Nematodes, trematodes, apicomplexa	7300–500	Wood *et al*. (2013*b*)
				7300–0	Boast *et al*. (2018)
	Permafrost sediment	Siberia	Plant-parasitic fungi	40 000– 20 000	Lydolph *et al*. (2005)

Ascaris, *P. falciparum*, *T. cruzi*, *Leishmania donovani*) (Table 1).

DNA barcoding of ancient parasites

A wide range of PCR primers exists for the identification of modern parasite taxa. However, the size of the barcode regions amplified by many of these limits their usefulness for ancient samples, where post-mortem fragmentation of DNA molecules is an issue (Allentoft *et al*. 2012). Therefore, primers that amplify shorter regions of DNA tend to have better success with ancient samples, as demonstrated by the fact that around half the primer combinations that have been used to retrieve DNA from ancient parasites amplify regions of 200 bp or less in size (Table 2). This is in contrast to modern DNA barcoding regions, for example, the barcode region widely used for animals that is 658 bp in length (Folmer *et al*. 1994; Hebert *et al*. 2003). However, in several instances relatively long DNA barcode regions (>400 bp) have been successfully amplified from ancient parasite remains (Table 2). It is possible that some ancient parasite remains may include desiccation resistant structures (e.g. thick egg shells, cuticles), and the effect of these on enhancing DNA preservation through reducing exposure of the DNA to physical and chemical degradation processes (Alaeddini *et al*. 2010) may be worthy of future investigation.

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Table 2. A selection of PCR primers that have been used for the identification of ancient parasites

Locus	Fw primer	Fw primer sequence (5′–3′)	Rv primer	Rv primer sequence (5′–3′)	Amplicon size (bp)	Taxon/taxa	References
5S	Eva	ACAACACTTGCACGTCTC	Evb	GAATTGCTCGTTTGC	198	*Enterobius vermicularis*	Iñiguez *et al*. (2003)
	Entf	CACTTGCTATACCAACAACAC	Entr	GCGCTACTAAACCATAGAG	420	*E. vermicularis*	Iñiguez *et al*. (2003); Jaeger and Iñiguez (2014)
18S	Asc6	CGAACGGCTCATTACAACAG	Asc7	TCTAATAGATGCGCTCGTC	123	*Ascaris*	Loreille *et al*. (2001); Oh *et al*. (2010*b*); Botella *et al*. (2010); Cleeland *et al*. (2013)
	Asc8	ATACATGCACCAAAGCTCCG	Asc9	GCTATAGTTATTCAGAGTCACC	99	*Ascaris*	Loreille *et al*. (2001); Oh *et al*. (2010*b*); Botella *et al*. (2010); Cleeland *et al*. (2013)
	Asc10	CCATGCATGTCTAAGTTCAA	Asc11	CARAAAWTCGGAGCTTTGGT	147	*Ascaris*	Loreille *et al*. (2001); Oh *et al*. (2010*b*); Botella *et al*. (2010)
	Euk_1391f	GTACACACCGCCCGTC	EukBr	TGATCCTTCTGCAGGTTCACCTAC	~120–170	Nematodes, trematodes, apicomplexa	Boast *et al*. (2018)
	Physa18s243F	TGAATAGCTCTGGCTGATCG	Physa18s343R	CAACCATGGTAGGCACATAAAC	100	*Ascaris*	Botella *et al*. (2010)
	Nem18SF	ATTCCGATAACGARCGAGAC	Nem18SR	CCGCTKRTCCCTCTAAGAAGT	40–120	Nematodes, trematodes, apicomplexa	Wood *et al*. (2013*b*)
	Nem18SlongF	CAGGGCAAGTCTGGTGCCAGCAGC	Nem18SlongR	GACTTTCGTTCTTGATTAATGAA	350–400	Nematodes, trematodes, apicomplexa	Wood *et al*. (2013*b*)
	BMBC-R	GTACACACCGCCCGTCG	NS8	TCCGCAGGTTCACCTACGGA	125	Plant-parasitic fungi	Lydolph *et al*. (2005)
	KW3	TCCAGCTCCAATAGCGTATA	SL43	GAACCACACGTCCTATTC	210	Plant-parasitic fungi	Lydolph *et al*. (2005)
	SR7R	AGTTAAAAAGCTCGTAGTTG	SR5	GTGCCCTTCCGTCAATT	510	Plant-parasitic fungi	Lydolph *et al*. (2005)
	Stro18S-1530F	GAATTCCAAGTAAAACGTAAGTCATTAGC	Stro18S-1630R	TGCCTCTGGATATTGCTCAGTTC	101	*Strongyloides stercoralis*	Jaeger and Iñiguez (2014)
	TT0926F	TTGCGAAAGCATTTGTCAAG	TT1606R	ACGTTTCAACCGATTTCCTG	680	*Trichuris trichiura*	Jaeger and Iñiguez (2014)
	TT1315F	CGAACGAGACTCTGGCCTAC	TT1709R	GTACAAAGGGCAGGGACGTA	390	*T. trichiura*	Jaeger and Iñiguez (2014)
28S	oldflea1a	ACGCGAATAAATCCGTGACA	oldflea9b	ACCTGAGAAACCCGAAAGAT	180	Siphonaptera	Dittmar *et al*. (2003)
	4.8a	ACCTATTCTCAAACTTTAAATGG	7b1	GACTTCCCTTACCTACAT	<200	Insecta	Dittmar *et al*. (2003)
	StroS	TTAGAGTCGTGTTGCTTGGAA	StroAS	GTGCAACTGGCTCTGTATGC	180	*S. stercoralis*	Jaeger and Iñiguez (2014)
AAP13LD	LD2F	AGACAAGCTGGGTCGTAACTC	LD2R	AAGACGACGAGGTAGCCAATAG	131	*Leishmania donovani*	Costa *et al*. (2009)
Adenylate kinase	LD4F	GCTGGAAGTGAAGGTTGTCTGC	LD4R	GATAGATGCGTCCCGACTTGG	220	*L. donovani*	Costa *et al*. (2009)
AMA1	AMA1-f	CCCGCACCACAAGAACAAAAT	AMA1-r	ACTGGACATTTCCCTGATGG	196	*Plasmodium*	Lalremruata *et al*. (2013)
COI	COIf	GTGTTAATATTGCCGGGGTTTGG	COIr	ACCTATAATCATAGTAACCG	207	*Clonorchis sinensis*	Shin *et al*. (2013)

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Table 2. (*Cont.*)

Locus	Fw primer	Fw primer sequence (5′–3′)	Rv primer	Rv primer sequence (5′–3′)	Amplicon size (bp)	Taxon/taxa	References
	As-Co1F	TTTTTTGGTCATCCTGAGGTTTAT	COX1R	GCCCGAGAGTCAAGATCCAT	199	*Ascaris*	Botella *et al*. (2010)
	COX1F	GGATCTTGACTCTCGGGCTTA	As-Co1R	ACATAATGAAAATGACTAACAAC	248	*Ascaris*	Botella *et al*. (2010)
	EvPRA	GGAGGTGTTTGGTCATTTGG	EvPRB	CGTCCCCCTATCAAAGTCAA	198	*E. vermicularis*	Jaeger and Iñiguez (2014)
Cytb	Asc1	GTTAGGTTACCGTCTAGTAAGG	Asc2	CACTCAAAAAGGCCAAAGCACC	142	*Ascaris*	Loreille *et al*. (2001); Leles *et al*. (2008); Oh *et al*. (2010*b*); Jaeger and Iñiguez (2014); Jaeger *et al*. (2016)
IMP dehydrogenase	LD1F	TGCGTGCGTGTGTGTGACATC	LD1R	TACGTCAGTCCATCGCCCCG	118	*L. donovani*	Costa *et al*. (2009)
ITS	F1f	CGATTCTAGTTCCGTCATCT	F1r	CCACTCATTAAGCCTAGCACTG	319	*C. sinensis*	Shin *et al*. (2013)
	F2f	TCGGTATGCTCGCTTCCGTTG	F2r	GGCGTTATCAGTCGTACCCGG	298	*C. sinensis*	Shin *et al*. (2013)
	ITS2f	GGTACCGGTGGATCACTCGGCTC GTG	ITS2r	TATGCTTAAATTCAGCGGGT	497	*C. sinensis*	Shin *et al*. (2013)
	EBITS3	GGTCATAGGGATGAAGAG	EBITS4	TTCGAGTTCTTTCGCGCTC		*Enterocytozoon bieneusi*	Myšková *et al*. (2014)
	EBITS1	GCTCTGAATATCTATGGCT	EBITS2.5	ATCGCCGACGGATCCAAGTG	390	*E. bieneusi*	Myšková *et al*. (2014)
	INT580F	TTTCACTCGCCGCTACTCAG	INT580R	TGCAGTTAAAATGTCCGTAGT		*Encephalitozoon* spp.	Myšková *et al*. (2014)
	MSP-3	GGAATTCACACCGCCCGTCVYTAT	MSP-4A	CCAAGCTTATGCTTAAGTYMAARGGGT		*Encephalitozoon* spp.	Myšková *et al*. (2014)
Minicircle molecule of kinetoplastid mtDNA		TGGTTTTGGGAGGGGSSKTCAAM TTT		TATATTACACCAACCCCAATCGAACC	121	*Trypanosoma cruzi Leishmania*	Ferreira *et al*. (2000); Zink *et al*. (2006)
	S35	AAATAATGTACGGGKGAGATGCAT GA	S36	GGTTCGATTGGGGTTGGTGTAATATA	330	*T. cruzi*	Guhl *et al*. (1997, 1999)
	LD3F	GGAGTAGCCTCAGGACTTTAG	LD3R	TAGGTACACTCTATCAGTAGCAC	184	*L. donovani*	Costa *et al*. (2009)
MSP1 K1 alleles	Msp1K1-f	GAAGAAATTACTACAAAAGGTG	Msp1K1-r	CACTTTACCTCGTTCAAATACTTCATCT	240	*Plasmodium*	Lalremruata *et al*. (2013)
NAD1	NAD1F	CTCCTCTGAATTCTTCGGAAA	NAD1R	CAGAAAACCCAATCAAACACA	152	*Ascaris*	Botella *et al*. (2010); Jaeger *et al*. (2016)
	MH5F	TATGAGCGTCATTTATTGGG	As-NDR	GCATCACAATAGCCAACAAATAC	~400	*Ascaris*	Jaeger and Iñiguez (2014)
*Sm*1–7	Primer1	GAAAATCGTTGTATCTCC	Primer2	GGTGACCTGCCTAAAAATAC	121	*Schistosoma mansonii*	Matheson *et al*. (2014)
SSUrRNA	P1fw	TCTCCATGAGACGCGTTACC	P1rv	GAACGACTCCTGCTTAGGAC	189	*T. trichiura*	Oh *et al*. (2010*a*); Myšková *et al*. (2014)
	P2fw	GCTCGTAGTTGGATTGCGGATGT	P2rv	TCCATGCACCACGGTTCAAGC	187	*T. trichiura*	Oh *et al*. (2010*a*)
	F1	TTCTAGAGCTAATACATGCG	R1	CCCATTTCCTTCGAAACAGGA	1325[a]	*Cryptosporidium* spp.	Myšková *et al*. (2014)
	F2	GGAAGGGTTGTATTTATTAGATA AAG	R2	CTCATAAGGTGCTGAAGGAGTA	826–864[a]	*Cryptosporidium* spp.	Myšková *et al*. (2014)
TPI fragment	GIAF1	AAATIATGCCTGCTCGTCG	GIAR1	CAAACCTTITCCGCAAACC	605[b]	*Giardia* spp.	Myšková *et al*. (2014)
	GIAF2	CCCTTCATCGGIGGTAACTT	GIAR2	GTGGCCACCACICCCGTGCC	530[b]	*Giardia* spp.	Myšková *et al*. (2014)

[a] Sizes reported in Xiao *et al*. (1999).
[b] Sizes reported in Sulaiman *et al*. (2003).

While most studies on ancient parasite DNA have applied PCR primers with high specificity (often genus-level) to detect parasite taxa of interest (taxon-specific barcoding approach in Table 1), DNA metabarcoding using universal primers together with cloning or high-throughput sequencing have also revealed a diversity of parasitic taxa in some ancient samples (e.g. Sallares and Gomzi, 1999; Lydolph *et al*. 2005; Wood *et al*. 2013*b*; Boast *et al*. 2018) (Table 2). However, the need for ancient DNA metabarcoding primers to target relatively short DNA regions means that it is not usually possible to achieve the same degree of taxonomic resolution typical of modern specimens, and sequences may only be able to be attributed to genera or families of parasite taxa (e.g. Wood *et al*. 2013*b*). Moreover, as parasites represent a diverse and polyphyletic assemblage of taxa, creating parasite-specific ancient metabarcoding primers is also a challenging prospect. Universal, or domain-specific, metabarcoding primers such as Nem18SF/Nem18SR, Nem18SlongF/Nem18SlongR (Table 2) and Euk_1391f/EukBr (Amaral-Zettler *et al*. 2009) target relatively short regions and can detect a broad range of parasite taxa (Wood *et al*. 2013*b*; Boast *et al*. 2018), but are not specific to parasites (Fig. 2). The taxonomic biases of universal primers can be tested using reference sequence databases (e.g. Fig. 2) and are important to understand when interpreting the sequencing results for reconstructing ancient parasite communities.

APPLICATIONS AND INSIGHTS

Parasite detection and community reconstruction

DNA barcoding provides a molecular means for detecting the presence of parasite taxa in ancient samples. Most studies of ancient parasites so far have used barcoding specifically for this application. Often DNA barcoding can compliment other techniques, such as to confirm the identity of a taxon identified by microscopic analysis (e.g. Loreille *et al*. 2001; Shin *et al*. 2013). However, DNA barcoding can also provide the sole evidence for the presence of a parasite in the absence of microscopic evidence (e.g. Leles *et al*. 2008), particularly for microbial parasitic taxa where microscopic remains may not preserve (e.g. Ferreira *et al*. 2000; Zink *et al*. 2006). Metabarcoding allows entire ancient parasite communities to be reconstructed, and when applied to multiple host species can provide insights into parasite characteristics such as host-specificity (Wood *et al*. 2013*b*; Boast *et al*. 2018). Information about the parasites that once inhabited now rare or extinct species potentially has important implications for species conservation and de-extinction, particularly considering the range of benefits conferred to hosts from their parasites (Maizels and McSorley, 2016; Spencer and Zuk, 2016; Wood *et al*. 2017; Selbach *et al*. 2018).

Human parasites

DNA barcoding studies of ancient human remains, including mummified tissues (e.g. Hawass *et al*. 2010) and coprolites (e.g. Botella *et al*. 2010), and sediments associated with human burials (e.g. Oh *et al*. 2010*a*), have revealed a wealth of information about the parasites of prehistoric human populations. Human parasites have been the focus of the majority of palaeoparasitological work (including DNA barcoding) to date, and several in-depth reviews of prehistoric human parasites are available on this topic (e.g. Araújo and Ferreira, 2000; Gonçalves *et al*. 2003; Araújo *et al*. 2008; Anastasiou and Mitchell, 2013; Côte and Le Bailly, in press). Briefly, such studies have provided important evidence for the prevalence of different parasitic diseases within prehistoric populations (e.g. Aufderheide *et al*. 2004; Jaeger *et al*. 2016), including instances where prevalence rates have remained remarkably constant over several millennia (e.g. Aufderheide *et al*. 2004) or have been subsequently altered by landscape modification (e.g. Lalremruata *et al*. 2013). By combining DNA barcoding analysis of tissues, coprolites and ectoparasites from the same mummified individuals, it has also been possible to gain insights into potential prehistoric transmission pathways for human parasites (Aufderheide *et al*. 2005). Moreover, the detection of certain parasites within mummies or coprolites from indigenous cultures can provide information on the timing of parasite dispersal events relating to prehistoric human migration events (Araújo *et al*. 2008).

Co-evolution and climate change

With DNA dating back to the Middle Pleistocene (ca. 450–800 000 years ago) having been sequenced (Willerslev *et al*. 2007; Orlando *et al*. 2013), and a theoretical preservation limit of DNA of about 1 million years (Allentoft *et al*. 2012), there is plenty of temporal scopes to study long-term processes (occurring on century- or millennial-timescales) involving parasites. For example, it is thought that many parasites may be prone to decline or extinction in the face of future climate change, yet how different parasite taxa might respond to this threat is not well understood (Cizauskas *et al*. 2017). Some insights into these responses might be gained through studying parasite DNA within temporal sequences of host remains (e.g. coprolites) that span past periods of climate change. Moreover, co-evolution of parasites and their hosts is another long-term process for which ancient DNA may provide informative insights. Araújo and Ferreira

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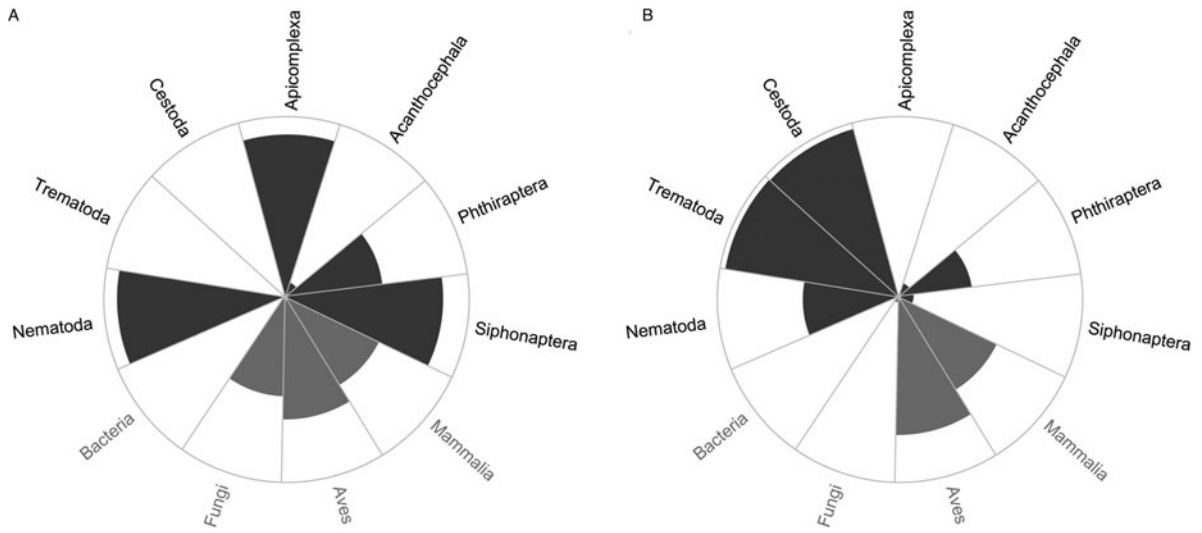


Fig. 2. *In silico* performance of PCR primers that have been used for 18S rRNA metabarcoding of ancient parasite communities: (A) Nem18Slong and (B) Nem18S (Wood *et al*. 2013*b*; Boast *et al*. 2018). Each segment represents 0–100% coverage (matches to eligible sequences) of sequences of that particular taxonomic group in the RefNR collection of the SILVA SSU r126 database. Analyses were performed using TestPrime 1.0 (https://www.arb-silva.de/search/testprime/). A maximum of two nucleotide mismatches per primer was allowed. Parasitic groups are shown in dark grey, while selected non-target groups are shown in light grey.

(2000) also recognized this potential, stating that the ability to compare ancient and modern parasite genomes would help resolve aspects of parasite origins, evolution, dispersion, virulence and pathogenicity. While examples of this exist for microbial pathogens of humans (e.g. Bos *et al*. 2011) and plants (e.g. Haas *et al*. 2009), analyses of ancient parasite genomes have yet to be published.

Co-extinction

The extinction of a species is seldom a discrete event. Ever since Darwin mused on the inter-related fates of Malagasy orchids and their specialist moth pollinators (Darwin, 1862), scientists have been aware of how the extinction of one species might create a ripple effect throughout an ecosystem, precipitating secondary declines among associated obligate mutualists, predators, prey or parasites (Brodie *et al*. 2014). In extreme instances, the extinction of one species can directly cause the 'co-extinction' of others that were ecologically dependent upon it (Stork and Lyal, 1993; Brodie *et al*. 2014). Parasites are a group considered particularly prone to co-extinction (Bush and Kennedy, 1994; Dunn *et al*. 2009) as they can be highly sensitive to population declines and fragmentation of their host species (Bush and Kennedy, 1994; Poulin and Morand, 2004; Lafferty, 2012). Given the diversity of parasites that inhabit every animal species, and that each of these parasite communities contain a certain proportion of host-specific taxa (Poulin and Morand, 2004; Strona and Fattorini, 2014), it is perhaps no surprise that some authors have claimed a greater number of species may be lost through the process of co-extinction than through all other primary causes (e.g. hunting, habitat destruction) combined (Koh *et al*. 2004; Dunn *et al*. 2009).

Parasite co-extinction is therefore clearly an important process in terms of biodiversity loss, yet a robust estimate of the relative magnitude of co-extinction remains elusive (Dunn, 2009; Dunn *et al*. 2009; Colwell *et al*. 2012). The development of such an estimate has been impeded by a paucity of empirical data for the co-extinction process (Dunn *et al*. 2009; Colwell *et al*. 2012), partly a result of difficulties in studying a process that can occur over decades or centuries (Brooks *et al*. 1999; Pimm and Raven, 2000). Ecological network models have therefore become the principal tool for studying the co-extinction process, yet their ability to make accurate predictions remains to be critically tested. This is exemplified by significant disagreement between the high rates of co-extinction predicted in most theoretical studies (e.g. Koh *et al*. 2004; Bascompte and Jordano, 2007; Vieira *et al*. 2013; Vieira and Almeida-Neto, 2015) and the relatively few co-extinctions that have been observed in nature (Dunn *et al*. 2009; Powell, 2011; Strona *et al*. 2013); giving rise to the so-called 'paradox of missing co-extinctions'.

Ancient DNA barcoding has a lot to offer in this regard, and parasites may be the ideal group on which to study the co-extinction process. The ability to amplify and sequence the DNA of parasites from ancient faunal remains (such as those shown in Fig. 1) provides a new means for reconstructing the

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parasite diversity within declining host-species through time, essentially providing a 'real-time' view of how parasite communities have responded to changes in host densities in the past (Wood *et al*. 2013*b*; Boast *et al*. 2018). Empirical data from palaeoparasitology could, therefore, help strengthen our understanding the co-extinction process, leading to improved modelling of ecological networks, and better predictions about future risk of co-extinction in threatened species (Dunn *et al*. 2009; Moir *et al*. 2010; Lafferty and Hopkins, 2018).

Concluding remarks

Numerous studies have now proven DNA barcoding to be an effective technique for detecting parasite taxa in ancient remains. A wide range of barcode regions has been used for identifying ancient parasite taxa, with PCR primers ranging from those with genus-level specificity to more universal primers with metabarcoding applications (Table 2). The ability to identify parasites in ancient remains, and to characterize how parasite faunas have changed through time, can provide insights into parasite responses to climatic and environmental change, habitat fragmentation (i.e. declining host populations) and processes such as co-evolution and co-extinction. High-throughput sequencing has yet to be applied in a widespread and systematic way to characterizing ancient parasite communities, yet the detection of parasite DNA in studies using such approaches demonstrates its potential (e.g. Khairat *et al*. 2013; Rivera-Perez *et al*. 2015; Côté *et al*. 2016; Boast *et al*. 2018).

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