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GENETIC IDENTIFICATION OF FIVE STRONGYLE NEMATODE PARASITES IN WILD AFRICAN ELEPHANTS (*LOXODONTA AFRICANA*)

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ABSTRACT: African savannah elephants (*Loxodonta africana*) are an ecologically and economically important species in many African habitats. However, despite the importance of elephants, research on their parasites is limited, especially in wild populations. Currently, we lack genetic tools to identify elephant parasites. We present genetic markers from ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) to identify five elephant-specific nematode parasites in the family Strongylidae: *Murshidia linstowi*, *Murshidia longicaudata*, *Murshidia africana*, *Quilonia africana*, and *Khalilia sameera*. We collected adult nematodes from feces deposited by wild elephants living in Amboseli National Park, Kenya. Using both morphologic and genetic techniques, we found that the internal transcribed spacer (ITS) region in rDNA provides a reliable marker to distinguish these species of strongyles. We found no evidence for cryptic genetic species within these morphologic species according to the *cox-1* region of mtDNA. Levels of genetic diversity in strongyles from elephants were consistent with the genetic diversity seen within other strongyle species. We anticipate that these results will be a useful tool for identifying gastrointestinal nematode parasites in elephants.

Key words: African elephant, *cox-1*, genetic markers, intestinal parasites, ITS, mitochondrial DNA, species identification, strongyle nematodes.

INTRODUCTION

The African savannah elephant (*Loxodonta africana*) is a threatened, charismatic, keystone species that is important to many African ecosystems and economies (Western, 1989; Barbier et al., 2009). However, despite the value of elephants, we know relatively little about their parasites (but see: Watve and Sukumar, 1997; Thurber et al., 2011). Both Asian (*Elephas maximus*) and African (*Loxodonta* spp.) elephants are parasitized by a complex of gastrointestinal nematodes in the family Strongylidae, superfamily Strongyloidea. The superfamily Strongyloidea is characterized by a well-developed buccal capsule without teeth or cutting plates, usually surmounted by one or more leaf crowns (corona radiata) in both sexes, and a copulatory bursa in the male. Additionally, the family Strongylidae is characterized by six branches to the dorsal ray (Lichtenfels, 1980). Six genera in this complex are known to infect African elephants: *Choniangium*, *Decrusia*, *Equinurbia*, *Khalilia*, *Murshidia*, and *Quilonia* (Prokopic et al., 1983; Kinsella et al., 2004). If African elephants are like many other host species, strongyle infection may have important consequences for individuals and populations by influencing patterns of co-infection or shaping host population dynamics (e.g., Scott and Dobson, 1989; Cattadori et al., 2007).

Understanding patterns of strongyle infection in elephants is hampered by the fact that species of strongyles are difficult to distinguish using eggs and larvae in fecal samples (Bowman et al., 2003). Strongyle eggs are often morphologically indistinguishable, and successful morphologic identification of larvae generally requires considerable training. While adult worms are morphologically distinct among species, it is often not possible to sample adult worms without destroying the host, which is not a viable option for elephants. In addition, even when adult worms are obtained, morphologically similar strongyles are sometimes divided into cryptic

species (Blouin, 2002), making it especially problematic to rely on morphologic identification to determine patterns of parasite infection in wild populations.

Genetic tools have the potential to help solve these problems. Genetic identification of many parasite groups has been possible using the first or second internal transcribed spacers (ITS) of nuclear ribosomal DNA (rDNA; Gasser et al., 1996a, b; Bott et al., 2009). Genetic tools provide a means by which to identify individual parasites at any stage of the life cycle without morphologic training (Zarlenga and Higgins, 2001; Gasser, 2006). These tools also provide a means of determining cryptic speciation, generally by analysis of mitochondrial DNA regions (mtDNA; Blouin, 2002; Miranda et al., 2008) or ITS region sequences (Chilton et al., 1995).

We developed the first genetic markers to identify five species of elephant-specific strongyle nematodes in three genera: *Murshidia*, *Quilonia*, and *Khalilia*. This is only a first step toward molecular characterization of these genera, as there are currently about 27 species of *Murshidia*, 17 species of *Quilonia*, and 3 species of *Khalilia*. Using adult nematodes collected opportunistically from feces from wild elephants in Amboseli National Park, Kenya, our goals were to: 1) use parasite morphology to determine which species were present in our sample, 2) obtain the first rDNA and mtDNA genetic markers for these species, 3) determine if our sample provided any evidence for cryptic speciation, and 4) compare evolutionary relationships of elephant strongyles predicted by morphology to the evolutionary relationships predicted by genetic sequence data. We hope that our results will prove useful in future studies to understand the patterns of gastrointestinal parasitism in elephants.

MATERIALS AND METHODS

Sample collection

Nematodes were collected during August 2010 from wild elephants living in the Amboseli Ecosystem, Kenya (between 1°37′S and 3°13′S and between 35°49′E and 38°00′E). To sample nematodes, we searched for elephants by driving roads in Amboseli National Park. When we sighted elephants, we approached them in a vehicle and waited for animals to defecate. When possible, individual elephants were identified from physical features recorded in a photographic database maintained by the Amboseli Elephant Research Project (Moss et al., 2011). After the elephants had defecated and moved away, when it was safe to approach the dung pile (usually within 10 min of defecation), we donned latex gloves and manually sorted through the dung to locate nematode worms that were visible to the naked eye. We collected and preserved worms in 95% ethanol. Because worms are often difficult to see in elephant feces, we probably failed to collect worms from all samples where they were present. Moreover, not all nematode species present in the gastrointestinal tract may occur as adults in dung. Despite these limitations, we collected 263 worms from 27 elephants, which were subsequently divided into two categories: worms that were subjected to both morphologic and genetic species identification ($n=156$ worms), and worms that were only subject to genetic species identification ($n=107$).

Morphologic methods to identify species

To identify worm species via morphology, we cleared 156 worms in temporary mounts of 80% phenol and examined them by light microscopy. The anterior ends of a few worms were clipped off with a microscalpel and arranged in an en face view in order to count the number of petals in the leaf crown (corona radiata). Identifications were made using *CIH Keys to the Nematode Parasites of Vertebrates* (Lichtenfels, 1980), a review of the genera *Quilonia* and *Murshidia* by Chabaud (1957), and original descriptions of species (Lane, 1921; Khalil, 1922; Neveu-Lemaire, 1924, 1928).

Genetic methods to identify species

After we obtained a morphologic species identity for the set of 156 worms, we extracted DNA from the entire set of phenol-cleared nematodes (total number of worms=263). Unfortunately, we did not preserve the anterior or posterior ends of our nematodes, and the entire sample was destroyed during DNA extraction. Hence, no specimens were preserved to deposit in a museum collection. DNA was extracted from each worm using a DNeasy Blood and Tissue kit (Qiagen,

Valencia, California, USA) following manufacturer's specifications. DNA was eluted twice in 100 µl of elution buffer provided with the DNA extraction kit. To obtain genetic identities for each worm, we amplified each DNA extract at two loci: the rDNA region spanning ITS1, 5.8S, and ITS2, and a region within the mitochondrial *cox-1* locus. We chose these loci because the rDNA locus is commonly used to distinguish nematode species, while mtDNA provides additional resolution to distinguish cryptic species (Blouin, 2002). Specific PCR conditions were as follows. First, we amplified rDNA from the ITS region using universal nematode primers NC2 (5′-TTAGTTTCTTTTCCTCCGCT-3′) and NC5 (5′-GTAGGTGAACCTGCGGAAGGATCATT-3′; Newton et al., 1998). PCR was carried out in 24-µl reactions containing 2 µl of genomic DNA, 2.4 µl of 10× PCR Buffer (Applied Biosystems, Carlsbad, California, USA), 2.4 µl of 25 mM $MgCl_2$ (Applied Biosystems), 0.16 µl of Amplitaq Gold polymerase (Applied Biosystems), 1.2 µl of each primer (10 mM), and 2.4 µl of deoxynucleotide triphosphates (dNTPs; 2 pmol of each; Invitrogen, Carlsbad, California, USA). Amplification was preceded by a 10-min polymerase activation step at 95 C, followed by 40 cycles of 45 sec each at 55 C annealing, 72 C extension, and 95 C denaturation. These cycles were followed by a 5-min extension step at 72 C.

Mitochondrial DNA from the *cox-1* region was amplified using universal primers HCO2198 (5′- TAAACTTCAGGGTGACCAAAAAATCA-3′) and LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′; Folmer et al., 1994). Because these primers did not successfully amplify worms morphologically identified as *Quilonia africana*, we designed a replacement primer for LCO1490, called CO3R (5′-GAATTGGCAAAGCCTGGTT-3′). PCR was carried out in 24-µl reactions containing 2 µl of genomic DNA, 2.4 µl of 10× PCR Buffer (Applied Biosystems), 2.4 µl of 25 mM $MgCl_2$ (Applied Biosystems), 1.2 µl of each primer (10 mM), 2.4 µl of dNTPs (2 pmol of each) (Invitrogen), and 0.32 µl of Amplitaq Gold polymerase (Applied Biosystems). Amplification was preceded by a 10-min polymerase activation step at 95 C, followed by 40 cycles of 1 min each at 42 C annealing, 72 C extension, and 95 C denaturation. These cycles were followed by a 5-min extension step at 72 C.

For both loci, amplification was detected by agarose electrophoresis, and products were cleaned using ExoSAP and sequenced in both directions using Dye Terminator Cycle Sequencing (Applied Biosystems) by the High Throughput Genomics Unit at the University of Washington, Seattle, Washington, USA.

Sequence data were verified by eye using Sequencher™ 4.10 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and analyzed in MEGA 5.0 (Tamura et al., 2007). Ambiguous base calls were noted. The ITS sequences for all individual worms were aligned by ClustalW in MEGA 5.0 (gap opening penalty 15, gap extension penalty 6.66). This alignment was used to determine species identification for worms without morphologic identifications. We constructed a neighbor-joining tree using the number of differences method (Nei and Kumar, 2000) and included all codon positions. All worms included in this data set fell unambiguously into one of five distinct clades, and each clade included morphologically identified worms. Since no other sequence data are available for these species, species assignments for individuals without morphologic identifications were assigned according to the morphologic identification of other individuals in the clade.

Analysis of intraspecific variation

To investigate genetic variation within species (i.e., potential for cryptic species), we calculated intraspecific nucleotide diversity for both the ITS and *cox-1* regions. Sequence data from every individual worm identified were used in this analysis. After alignment by ClustalW, individuals were assigned to a species group in MEGA 5.0, and pairwise sequence comparisons were made within all groups to determine the average number of base-pair differences per site within species. All sites with missing data were excluded from the analysis. Standard error was calculated with 1,000 bootstrap replications.

To facilitate the detection of cryptic species, we investigated haplotype distribution among species using Arlequin 3.5 (Excoffier et al., 2005). Sequences from all the individual worms were included in the Arlequin analysis, and all nucleotide sites with missing data were excluded. We determined the number of unique haplotypes in each species (for both the ITS and *cox-1* regions) and investigated the phylogenetic relationships between these haplotypes in MEGA 5.0, using the same phylogeny construction methods described previously.

Analysis of evolutionary relationships between worm species

To further compare the species we found, we developed a single consensus ITS region sequence for each species, based on the

sequence data from all individual worms sequenced. These consensus sequences ($n=5$) were aligned by ClustalW in MEGA 5.0 (gap opening penalty 15, gap extension penalty 6.66). Phylogenetic relationships were determined in MEGA 5.0 by using the maximum likelihood method based on the Jukes-Cantor model (Jukes and Cantor, 1969), using the strongyle worm *Trichostrongylus colubriformis* as an outgroup. Initial tree(s) for the heuristic search were obtained automatically as follows: When the number of common sites was <100 or less than one quarter of the total number of sites, the maximum parsimony method was used; otherwise, the BIONJ method with a Markov cluster distance matrix was used. Codon positions included were first, second, third, and noncoding. All positions containing gaps and missing data were eliminated.

To understand patterns of genetic variation between species, we calculated interspecific nucleotide diversity (in both the ITS and *cox-1* regions). Sequence data from every individual worm identified were used in this analysis. Individuals were assigned to a species group in MEGA 5.0, and pairwise sequence comparisons were made between all groups to determine the average number of base-pair differences per site between species. The standard error was calculated from 1,000 bootstrap replications.

RESULTS

Species identification

We observed 183 defecations from 156 individual elephants, and we collected nematodes from 17% (27 of 156) of these elephants. Of the 263 worms collected from 27 individual elephants, 156 worms were subject to morphologic identification. Of these, we identified 147 worms to species level and 9 worms to genus. These morphologic identities revealed five species: 87 worms (55.8%) were identified as *Murshidia linstowi* (Khalil, 1922), 46 worms (29.4%) were *Murshidia longicaudata* (Neveu-Lemaire, 1928), 7 worms (4.5%) were *Quilonia africana* (Lane, 1921), 6 worms (3.8%) were *Murshidia africana* (Lane, 1921), and 1 worm (0.6%) was *Khalilia sameera* (Neveu-Lemaire, 1924). Characteristics used to differentiate species in both sexes included the number of petals in the corona radiata and the shape and length of the esophagus. In addition, the shape and length of the spicules and the shape and length of the dorsal ray of the bursa were used in males; and the position of the vulva, uterine morphology, and tail length were used in females.

We obtained useable DNA sequence data in both the *cox-1* and ITS regions for 151 of the 156 morphologically identified individuals. For the remaining five individuals (2 *M. linstowi*, 1 *M. longicaudata*, and 2 *Q. africana*), the resulting DNA sequences were not clear enough to identify species, as the chromatograms contained evidence for multiple nucleotides at the majority of bases. These five individuals were excluded from further analyses. The resulting usable DNA sequences represent the first ITS and *cox-1* sequences for these nematode genera and species

DNA sequences from these morphologically identified worms revealed a high level of agreement between morphologic and genetic species identifications. Specifically, genetic identifications for 150 of the 151 individuals matched their morphologic identifications perfectly. One individual was morphologically identified as *M. longicaudata* but was a genetic match to worms identified as *M. linstowi*. This is likely the result of human error and unlikely to suggest species confusion. This individual was also excluded from further analyses.

We also sequenced the subset of 107 worms not subjected to morphologic identification at both the ITS and *cox-1* loci. We were unable to obtain clean sequence data for two of these worms. The remaining individuals ($n=105$) were assigned to a species group based on their position in the ITS sequence–based neighbor-joining tree. We did not find evidence for additional species in this subset of worms, as all of these worms fell unambiguously into a clade with morphologically identified worms.

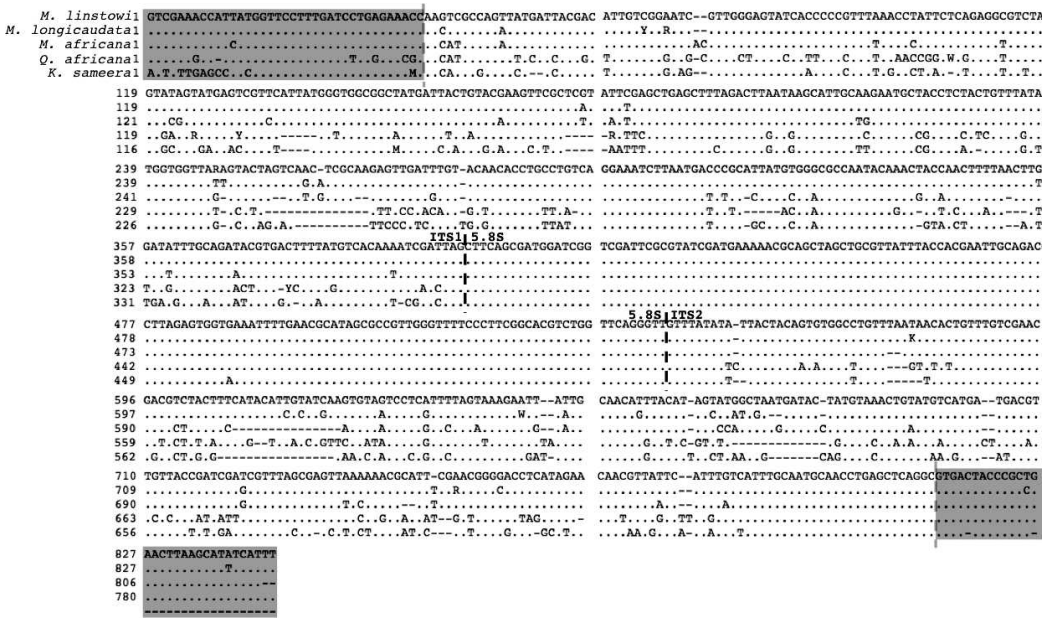

FIGURE 1. Alignment of ribosomal DNA consensus sequences for five species of strongyle nematode parasites in African elephants (*Loxodonta africana*), collected in Amboseli National Park, Kenya, in 2010, including three *Murshidia* species (*M. linstowi*, *M. longicaudata*, and *M. africana*), one *Quilonia* species (*Q. africana*), and one *Khalilia* species (*K. sameera*). Consensus sequences were determined for each species and aligned in MEGA 5.0. A period represents conserved nucleotides; a dash represents a gap. ITS1, 5.8S, and ITS2 regions were determined by alignment with sequences from other strongyle nematodes, *Trichostrongylus colubriformis* (accession number: HQ389232) and *Haemonchus contortus* (accession number: HQ389229). The shaded regions were included in the consensus sequences but excluded from further analyses because these data were missing for many individuals. Ambiguous base calls and/or sites of intraspecific variation are noted with standard International Union of Pure and Applied Chemistry base symbols.

In total, we obtained a genetic species identity for 255 worms; 177 worms were identified as *M. linstowi*, 63 as *M. longicaudata*, 8 as *M. africana*, 6 as *Q. africana*, and 1 as *K. sameera.* Of the 27 elephants for which we were able to collect adult worms, 100% were infected with strongyles of the genus *Murshidia*. Of these 27 elephants, 19% were also infected with *Q. Africana*, and 4% *with K. sameera*.

All sequence data were deposited in GenBank (accession numbers JN252508–JN252693). Consensus ITS region sequences were developed for each species based on the sequence data from all individual nematodes (Fig. 1). Summary characteristics of these consensus sequences are presented in Table 1.

Intraspecific variation

To determine whether any of the nematode species we found might be divided into cryptic species, we examined intraspecific variation in both rDNA and mtDNA regions (Table 2). In rDNA, the ITS1 region was the most variable, followed by the ITS2 region. We observed very little variability in the 5.8S region of our samples (data not shown). Intraspecific nucleotide diversity in the entire ITS region ranged from 0% to 0.27% across species. Intraspecific nucleotide diversity was higher in the mtDNA *cox-1* region than the ITS region, ranging from 0.81% to 2.2% across species. In both regions, *M. africana* showed the lowest level of intraspecific nucleotide diversity, and *Q. africana* had the highest levels (Table 2).

TABLE 1. Information on ribosomal DNA sequences, including the sequence length in base pairs (bp) and the percent of guanidine and cytosine (GC%), from five species of parasitic nematodes in African elephants (*Loxodonta africana*).

	Overall		ITS1		5.8S		ITS2	
Species	Length (bp)	GC%	Length (bp)	GC%	Length (bp)	GC%	Length (bp)	GC%
Murshidia linstowi	843	41.3	398	41.2	146	50	299	37.1
Murshidia longicaudata	839	41.8	398	40.5	146	50	295	39.3
Murshidia africana	821	43.0	395	42.5	146	50	280	40.0
Quilonia africana	792	42.6	359	45.4	146	50	287	35.3
Khalilia sameera	753	45.0	369	45.8	146	49.3	238	40.8

The levels of intraspecific variation we detected in both the ITS and *cox-1* regions are consistent with the levels of variation seen within other nematode species (Blouin, 2002). However, due to increased variability in the mtDNA, we also investigated the distribution of *cox-1* haplotypes for the presence of distinct clades within species, which would suggest the possibility of cryptic species. We found no distinct groups of haplotypes within a species, and clear evidence for differentiation between species (Table 3 and data not shown), consistent with the levels of inter- and intraspecific variation in the *cox-1* region (Tables 2b and 3b).

Evolutionary relationships

We expected that worm species that were members of the same morphologically defined genus (e.g., *Murshidia*) would be more genetically similar than worms with more distant morphologic relationships. In support, the phylogeny in Figure 2 shows that the three *Murshidia* species were more closely related to each other than they were to *Q. africana* or *K. sameera.* Similarly, for both mtDNA and rDNA, nucleotide diversity between

TABLE 2. The average number of pairwise differences (and standard error, SE[a]) between DNA sequences from ribosomal DNA (ITS1, 5.8S, and ITS2) and mitochondrial DNA (*cox-1*), from five species of parasitic nematodes in African elephants (*Loxodonta africana*).

a)	ITS1, 5.8S, ITS2			
Species	*1*	*2*	*3*	*4*
1. *Murshidia linstowi*				
2. *Murshidia longicaudata*	0.0303 (±0.058)			
3. *Murshidia africana*	0.0659 (±0.0087)	0.0675 (±0.0083)		
4. *Quilonia africana*	0.1883 (±0.0143)	0.1922 (±0.0145)	0.1800 (±0.0137)	
5. *Khalilia sameera*	0.1990 (±0.0144)	0.2040 (±0.0143)	0.1783 (±0.0134)	0.1966 (±0.0138)
b)	*cox-1*			
Species	1	2	3	4
1. *M. linstowi*				
2. *M. longicaudata*	0.0269 (±0.0051)			
3. *M. africana*	0.1032 (±0.0113)	0.1022 (±0.0111)		
4. *Q. africana*	0.1100 (±0.0121)	0.1123 (±0.0121)	0.1190 (±0.0126)	
5. *K. sameera*	0.1893 (±0.0150)	0.1892 (±0.0148)	0.1885 (±0.0145)	0.1458 (±0.0136)

[a] SE calculated from 1,000 bootstrap replicates.

TABLE 3. Number of unique haplotypes and nucleotide diversity (π) in DNA sequences from (a) the ribosomal ITS region, and (b) the mitochondrial *cox-1* gene, from five species of parasitic nematodes in African elephants (*Loxodonta africana*).

a)

Species (No. individuals)	No. unique haplotypes[a]	ITS nucleotide diversity[b]
Murshidia linstowi (177)	3	0.0001 (±0.00001)
Murshidia longicaudata (63)	22	0.0025 (±0.00110)
Murshidia africana (8)	1	0
Quilonia africana (6)	5	0.0027 (±0.00123)
Khalilia sameera (1)	1	N/A

b)

Species (No. individuals)	No. unique haplotypes[a]	*cox-1* nucleotide diversity[b]
M. linstowi (177)	121	0.0082 (±0.00131)
M. longicaudata (63)	20	0.0085 (±0.00176)
M. africana (8)	6	0.0081 (±0.00187)
Q. africana (6)	6	0.0220 (±0.00395)
K. sameera (1)	1	N/A

[a] Excludes data from any nucleotide site with missing information in any individual.

[b] SE calculated from 1,000 bootstrap replicates.

members of the genus *Murshidia* was lower than nucleotide diversity between *Murshidia* spp. and members of other genera (Table 2).

DISCUSSION

The results of this study provide the first DNA sequences for five species of strongyles known to infect elephants worldwide. We found strong concordance between morphologic and genetic species identities and no evidence for cryptic species within these groups. Though there are many strongyle species known to infect elephants that we did not detect in our study, our results represent a first step toward a method for genetic detection of patterns of parasite infection in elephants.

We used a novel method to determine morphologic and genetic characteristics of the same nematode. Other methods entail removing the anterior and posterior ends of the adult worm and fixing them for morphologic study while retaining the

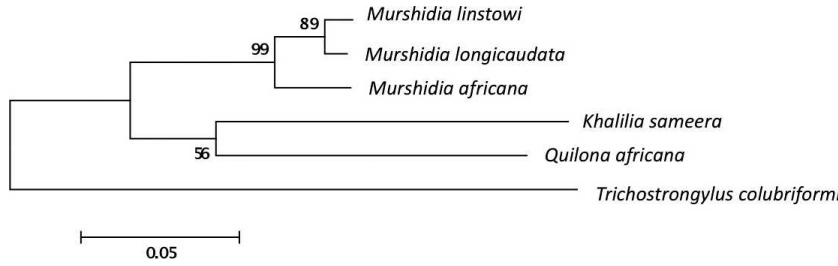

FIGURE 2. Genetic comparison of five species of African elephant (*Loxodonta africana*) strongyle nematode parasites, using *Trichostrongylus colubriformis* as an outgroup. Elephant parasites were collected in Amboseli National Park, Kenya, in 2010. A maximum likelihood tree was derived from consensus sequences of all individual worms sequenced for each species. The percentage of trees in which the associated taxa clustered together is shown next to the branches (1,000 bootstrap replicates). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

midregion of the nematode for DNA extraction (e.g., Hoste et al., 1995). Moreover, we found that it is possible to extract and amplify DNA from worms cleared in 80% phenol, which may be useful in future studies.

Among the three strongyle genera we detected, *Murshidia* was the most common. There are two possible explanations for why *Murshidia* spp. were more common than members of the other genera. First, some unique feature of the natural history of *Murshidia* spp. may make them more likely to be detected as an adult outside the host than *Q. africana* or *K. sameera.* For instance, if *Murshidia* spp. form weaker attachments to the intestinal wall than other strongyles, they may be more likely to occur as adults in elephant dung. Alternatively, perhaps *Murshidia* spp. actually have a higher prevalence of infection in the Amboseli elephants than *Q. africana* or *K. sameera*. If so, this pattern differs from the only other study to measure the abundance of strongyle species in African elephants. Thurber et al. (2011) used morphologic methods to identify L-3 larvae from fecal samples collected from elephants living in Etosha National Park, Namibia, and found that *Quilonia* spp. were the most common, followed by *Khalilia* spp., and then *Murshidia* spp. If the difference in patterns of parasite species abundance between our study and that of Thurber et al. represents a true difference in parasite prevalence, this suggests that different strongyle species predominate in different habitats and populations. More accurate estimates of parasite prevalence in Amboseli are needed to confirm this result.

We identified the strongyles infecting the elephants in our study using genetic markers. In particular, we found that the rDNA region spanning ITS1, 5.8S, and ITS2 provides a good genetic marker for distinguishing among the five strongyle species we detected: *M. linstowi*, *M. longicaudata*, *M. africana*, *Q. africana*, and *K. sameera*. Although there is some intraspecific variation in rDNA for these species, this variation was much less than the variation observed between species, making rDNA a reliable and relatively invariant marker for species identity. These results are consistent with many other studies that have used ITS regions to distinguish among closely related nematode species (e.g., Hoste et al., 1995; Nguyen et al., 2001). In contrast, we found much higher levels of genetic diversity in mtDNA, with intraspecific nucleotide diversities ranging from 0.8% in *M. linstowi* to 2.5% in *Q. africana.* These levels are consistent with the high mtDNA diversity documented in many strongyle species (Blouin, 1998) and do not indicate the presence of cryptic species. The development of these genetic markers will allow researchers to identify some common strongyle parasites in elephants without necessarily receiving training in parasite morphology. A logical next step is to develop a method to extract strongyle DNA from elephant fecal samples and design species-specific primers to amplify unique parasite species. Such a method has been used in other systems (Bott et al., 2009) and has the potential to transform our understanding of strongyle infection in elephants.

Our results are a vital first step in using genetic tools to detect patterns of parasite infection in elephants. Future work using these genetic tools has the potential to greatly improve our understanding of these patterns, with implications for understanding variation in elephant health and population dynamics.

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