Molecular characterization of cosmopolitan and potentially co-invasive helminths of commensal, murid rodents in Gauteng Province, South Africa

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Abstract

Concurrent studies of helminth parasites of introduced and native rodent species are few and miss the opportunity to identify potential co-invasive parasite species. This study employed molecular tools to infer the phylogeny and elucidate the origin of potentially co-invasive parasites of commensal, murid rodents by assessing introduced *Rattus norvegicus*, *Rattus rattus*, *Rattus tanezumi* and native *Mastomys coucha* in Gauteng Province, South Africa. Genotypes of *Nippostrongylus brasiliensis* recovered from *R. norvegicus* are nearly identical to those recovered from elsewhere in the world. The pinworms, *Aspiculurus tetraptera*, recovered from introduced *R. tanezumi* and *R. rattus*, *Syphacia muris* recovered from *R. tanezumi* and *Syphacia obvelata* recovered from indigenous *M. coucha* have affiliations to those recovered of laboratory rodents from the USA and China. *Syphacia obvelata* was previously only known as a commensal endoparasite of laboratory rodents and the *S. muris* genotype recovered from *R. tanezumi* in this study shows an affiliation to a genotype recovered from the same host species in Indonesia which is part of the native range. The study emphasizes the need for surveillance of potential co-invasive species and contributes in documenting genetic diversity of endoparasites of well-known hosts.

Keywords: phylogeography; *Mastomys coucha*; *Rattus*; co-invasive; parasites

Introduction

The origins of introduced *Rattus* species in South Africa can be linked to not only the native range of China and Southeast Asia but also several other geographical areas spanning multiple continents (Bastos et al. 2011) as a consequence of repeated introductions. In addition, Bastos et al. (2011) have demonstrated that commensal *Rattus* species are actively invading and are not present as merely historical introductions and long periods of establishment. While the role of *Rattus* as introduced, invasive species is well-studied, less so are the parasites and other pathogens associated with these introductions (Kosoy et al. 2015).

As a consequence of multiple introductions, it has been estimated that *Rattus rattus* Linnaeus, 1758 has retained 38 % of its parasites in the introduced range (Torchin et al. 2003). Generalist helminths with direct life cycles have better chances to establish in an introduced range (Torchin et al. 2003; Lymbery et al. 2014) as they are not host species specific and do not require an alternative host for their development (Smith and Carpenter 2006). However, helminths with indirect life cycles which have cosmopolitan intermediate and definitive hosts may also be favoured. Non-native parasites that have been introduced with non-native hosts are termed co-introduced parasites and such parasites that infect native hosts in the new distributional range are termed co-invaders (Lymbery et al. 2014). Several studies worldwide have investigated the parasites of introduced *Rattus* spp. (Abu-Madi et al. 2005; Chaisiri et al. 2010; Panti-May et al. 2013; Shimalov 2016) in isolation, while few have simultaneously assessed the parasites of introduced and native rodents to be able to identify potential co-invaders where parasite spill over has occurred (Morand et al. 2015; Diagne et al. 2016).

It is, however, often challenging to distinguish native from non-native parasites in a region (Lymbery et al. 2014) when spill over or spillback events have occurred. In addition, helminth identification only based on morphology may be problematic, with helminths often having controversial taxonomies arising from disparate molecular and morphological phylogenetic analyses (Nkouawa et al. 2016). A molecular approach may overcome these challenges as it is able to identify cryptic diversity that may be associated with geographical origins (Gesy et al. 2014).

The study molecularly characterizes cosmopolitan nematodes and cestodes of the three introduced murids, *Rattus norvegicus* Berkenhout, 1769, *R. rattus* Linnaeus, 1758, *Rattus tanezumi* Temminck, 1844 and native *Mastomys coucha* Smith, 1836 by analysing the nuclear and mitochondrial genes known to differ in their evolutionary rates. The aim is to identify potential co-invasive helminths and their biogeographical origins. Additionally, cryptic diversity of helminths and potential sources thereof are elucidated.

Materials and methods

Rodent sampling regime, helminth recovery and morphological identification

Rodents were sampled from Hammanskraal, Pretoria, Tembisa, Diepsloot and Alexandra in Gauteng Province, South Africa, from August 2010 to September 2011 and May 2012. Procedures on animals were approved by the University of Pretoria Animal Ethics Committee (Permit no. EC025-10). Following euthanization, animals were stored at -20 °C prior to necropsy. Rodent species identities were confirmed by genetic sequence analysis (Julius 2013; Le Grange 2014). Helminth worms were recovered from the gastrointestinal tract (stomach, small intestine (SI), caecum and colon). Upon recovery, helminth specimens were rinsed with phosphate buffered saline (PBS) and subsequently fixed in 70 % glycerol alcohol. Based on morphology, helminth specimens were initially identified to the lowest taxonomic level possible with the aid of published taxonomic keys for nematodes (Anderson et al. 1974) and cestodes (Khalil et al. 1994).

Molecular analysis of helminths

Genomic DNA was extracted using QIAamp DNA minikit (Qiagen, Hilden, Germany) following the manufacturer's protocol to ensure optimal DNA yield. Subsets of strobilar cestode DNA extracts were subjected to molecular identification by polymerase chain reaction (PCR) from individual hosts, *R. norvegicus* (*n* = 5), *R. rattus* (*n* = 10) and *R. tanezumi* (*n* = 5). Cestodes were initially screened using four broad spectrum general invertebrate PCR primer sets characterizing the ribosomal DNA (rRNA) 16S, 18S, 28S and mitochondrial (mtDNA) cytochrome oxidase subunit I (COI) gene regions. Amplification was successful with a published 28S primer set (Whiting et al. 1997) (Table 1) and identified *Hymenolepis diminuta* Rudolphi, 1819, the rat tapeworm. Subsequently, published genus specific primer sets characterising two gene regions were selected based on the number of citations in a literature review and Blast results in GenBank. Resultant published *Hymenolepis*-specific primer sets (Table 1) characterising the mtDNA of the COI (Foronda et al. 2011) and rRNA from the internal transcribed spacer regions (ITS) (Macnish et al. 2002) were successful in targeting ≈ 800 bp fragment each. Reaction conditions were as published in Foronda et al. (2011) and Macnish et al. (2002) respectively, with some minor optimization to minimize non-specific amplification.

One to four adult worms from six morphologically identified nematode taxa each were used for genomic DNA extraction to be representative of each rodent host species where possible (*n* = 16). Successful amplifications were achieved with three overlapping rRNA 18S primer sets (Holterman et al. 2006) and a mtDNA COI primer set (Diagne et al. 2016) (Table 1) yielding fragments of ≈ 1700 bp and ≈ 400 bp,

respectively with reaction conditions as published by Holterman et al. (2006) and Diagne et al. (2016), respectively. PCR was carried out in 25 µl volumes consisting of 1 µl each of forward and reverse primers, 1 U EmeraldAmp® Taq polymerase (Takara, Shiga, Japan) and 3-4 µl DNA template.

All amplified PCR products were viewed with electrophoresis on a 1.5 % agarose gel and purified using Roche PCR Product Purification Kit (Roche Diagnostics, Basel, Switzerland). Amplified PCR products were directly sequenced and proceeded with the BigDye v. 3.1 terminator cycle-sequencing kit (Perkim-Elmer, Waltham, Massachusetts, USA) with each PCR primer. Samples were run on an ABI 3130 sequencer (Applied Biosystems, Foster City, California, USA) and sequence chromatograms were viewed and edited in MEGA 5 (Tamura et al. 2011). Intra-individual variability is known to be associated with the ITS gene regions (Puza et al. 2015). To assess this phenomenon, following Puza et al. (2015), sequence chromatograms were assessed visually for the presence of double peaks in otherwise flawless sequences.

Phylogenetic analyses

Data sets were supplemented with published reference sequences (Table 2) and if these were not available, published or unpublished whole genome sequences were used. *Hymenolepis* (= *Rodentolepis*) *nana* Von Siebold, 1852 was selected as outgroup for the *H. diminuta* phylogeny based on the availability of representative sequences for both gene regions under study. For the nematode phylogeny, outgroup taxa were selected based on Callejón et al. (2013) and Diagne et al. (2016) for their robustness to elucidate the phylogenetic relationships of the diverse nematode taxa present in the dataset. However, the addition of the outgroup taxa for the 18S nematode dataset, yielded highly divergent regions in the alignment. Following Castresana (2000), these large amounts of inferred indels and poor alignments may reduce phylogenetic signal and was consequently removed using gBlocks (Castresana 2000).

JModelTest (Posada 2008) identified HKY+I and TIM3+G as the best fit models of sequence evolution under the Akaike Information Criterion (AIC) for *H. diminuta* and nematode datasets, respectively. Phylogenies were inferred using Maximum Likelihood (ML), Neighbour-Joining (NJ), Bayesian Inference (BI) and, Maximum Parsimony (MP). Nodal support for ML and NJ were assessed by 10,000 non-parametric bootstrap replications performed in MEGA 5 (Tamura et al. 2011) while the BI was run for 10,000,000 MCMC generations in MrBayes v. 2.1.3 (Huelsenbeck and Ronquist 2001) with tree sampling every 100 generations after which 25 % of trees were discarded as burn-in. MP analyses were performed using PAUP 4.0 (Swofford 2002) and based on heuristic search and tree- bisection-reconnection (TBR) and random addition of sequences

(10 replicates) with nodal support determined by 10,000 bootstrap replicates. The majority rule (50 %) consensus tree summarized the results in the event more than one parsimonious tree was saved. Pairwise uncorrected *p*- distances were determined in MEGA5 (Tamura et al. 2011). Nodal support of 75 % and 0.75 and above was reported for bootstrap support and posterior probabilities, respectively. Haplotype analysis was performed in DnaSP v. 5 (Librado and Rozas 2009).

Results

The mtDNA COI dataset for *H. diminuta* consisted of 791 bp fragments comprising 16 in-group taxa of which five were generated in a parallel study (Julius et al. 2017) and 11 are reference sequences (Table 2). The rRNA ITS dataset consisted of 711 bp fragments comprising eight taxa where five (two identical sequences not shown) were generated in this study (GenBank accession nos. MG 322244-6) and three were reference sequences (Table 2). The COI dataset had more conserved (753 bp) and parsimony informative sites as compared to the ITS dataset (662 bp). Tree topologies were generally similar for NJ, ML, BI and MP analyses. The COI phylogeny (Fig. 1a) identified three different genotypes for Gauteng Province, South Africa with good nodal support, while the ITS phylogeny (Fig. 1b) identified at most two different genotypes but with weak nodal support. The MP analysis saved one parsimonious tree for the COI dataset (Tree length (TL) = 191 steps, Consistency index (CI) = 0.97, Retention Index (RI) = 0.87, Rescaled Consistency Index (RC) = 0.84). The MP analysis for the ITS1 gene region retained 13 equally parsimonious trees (TL = 294 steps, CI = 1.00, RI = 1.00 and RC = 1.00). Haplotype analyses of the COI dataset confirmed the presence of three distinct haplotypes with high haplotype- (Hd = 0.7) and nucleotide diversity (Pi = 0.01). One of the haplotypes (UPER84) which was represented by more than one sequence was assessed for intra-individual variability in the ITSI gene region. Intra-individual variability was found to be present, yet represented only one haplotype in the phylogeny. The mtDNA *H. diminuta* genotypes isolated from *R. norvegicus* have affiliations to genotypes from Spain and Madagascar while the genotype isolated from *R. rattus* also has an affiliation to that of Spain.

The nematode COI dataset consisted of 18 in-group taxa of which nine were generated in this study (GenBank accession nos. MG386201-07) and nine are reference sequences (Table 2). The 18S dataset consisted of 16 in-group taxa of which seven were generated in a parallel study (Julius et al. 2017), two in this study (GenBank accession nos. MG356472-3) and seven were reference sequences (Table 2). The parsimony analysis saved one parsimonious tree for both COI (TL = 946 steps, CI = 0.68, RI = 0.71, RC = 0.48) and 18S (TL = 697 steps, CI = 0.82, RI = 0.92, RC = 0.76) datasets, respectively. Phylogenies were similar for COI (Fig. 2a) and

18S (Fig. 2b) gene regions. The 18S phylogeny showed that the *Nippostrongylus brasiliensis* genotype recovered from *R. norvegicus* (Julius et al. 2017) was nearly identical to the genotypes recovered from *R. norvegicus* in the UK and a *Rattus* sp. in Australia, however, despite several attempts in this study, the mtDNA counterpart for the COI gene region did not amplify and subsequent phylogeographic assessments could not be made. The *Syphacia muris* genotype from *R. tanezumi* in Gauteng Province, South Africa had an affiliation to a mtDNA genotype from the same host species in Indonesia and an rRNA genotype from a laboratory rodent in the USA. *Syphacia obvelata* genotypes recovered from indigenous *Mastomys coucha* had nearly identical genotypes as compared to those of an mtDNA genotype of a laboratory rodent in China and an rRNA genotype in the USA. Another pinworm, *Aspiculuris tetraptera* showed a mtDNA genotype which was distinct from a genotype recovered from a laboratory rodent in China but nearly identical to that recovered from a laboratory rodent in the USA for the rRNA gene. As far as it could be established, *Mastophorus muris* has no reference collections available in GenBank in either mtDNA or rRNA gene regions, despite its status as a cosmopolitan nematode (Rojas and Digiani 2003) and as a result, its relationship to *M. muris* from other geographical origins could not be assessed. The genotype of whipworm, *Trichuris* sp., in our study appears to be unique, as revealed by the COI gene region, but this is not the case for the 18S gene region pointing to both *T. arvicolae* and *T. muris* identities.

Uncorrected *p*- distance values showed high levels of similarity in the rRNA gene regions as compared to the mtDNA gene region for both cestode and nematode phylogenies (Table 3). MtDNA isolates of *H. diminuta* in this study differed from the reference collection by 1-3 % while intraspecific differences among the three mtDNA genotypes isolated in this study were 0-3 %. Nematode mtDNA isolates in this study differed with the reference collection by as much as 16 % for *A. tetraptera*, 17 % for *S. muris*, 8% for *S. obvelata* and 17 % for *Trichuris* sp. Intraspecific differences for mtDNA and rRNA respectively, were 0-1 % and 0 % for *A. tetraptera*, 1 % and 0 % for *S. obvelata* and 0% for only mtDNA of *Trichuris* sp. (only one rRNA isolate available). Among the oxyurids isolated in this study, interspecific differences were 4-6 % for rRNA and 11-22 % for mtDNA.

Discussion

The cosmopolitan cestode, *H. diminuta* displays high genetic diversity with 2-3 haplotypes, high haplotype diversity and high mtDNA p-distance, considering the sample size from the introduced rodents examined in this study. These haplotypes are also not closely related (Pi = 0.01). *Hymenolepis diminuta* has an indirect life cycle

and a wide range of arthropod intermediate hosts (Foronda et al. 2011) and this may contribute to the genetic variation shown and facilitate potential co-invasion and establishment of this cestode. It is also known that *Rattus* sp. invasion in South Africa is associated with multiple geographical origins (Bastos *et al*. 2011). The affiliation of Gauteng Province isolates to those from other geographical areas may therefore reflect the origins of the introduced hosts and may also account for the observed genetic variation. The reduced variation in the ITS1 gene region has also been shown elsewhere (Foronda et al. 2011).

Considering the rRNA nematode phylogeny, it appears that the Gauteng Province *N. brasiliensis* isolate cannot be distinguished from isolates of conspecific hosts in the UK and Australia. This may therefore reflect the origins of the nematode and perhaps also that of the host since *N. brasiliensis* was recovered from introduced *R. norvegicus.* However, *N. brasiliensis* has been used as model organism for hookworm infection (Morand et al. 2006) and laboratory maintenance may have reduced genetic diversity. Similar genotypes may therefore not reflect geographical origins but cannot discount potential co-invasion as *R. norvegicus* host genotypes in South Africa have been associated to laboratory rat strains from China (Bastos et al. 2011). *Aspiculuris tetraptera* intraspecific mtDNA sequence differences in this study were higher (0-1 %) than those reported for isolates from laboratory mice (Lou et al. 2015). Intraspecific sequence variation for *S. obvelata* in this study, however, were similar (1 %) to those reported for isolates from laboratory mice (Wang et al. 2015). This is not unexpected as *A. teraptera* was recovered from two different introduced *Rattus* species and *S. obvelata* from only indigenous *M. coucha*. Both *A. tetraptera* and *S. obvelata* Gauteng Province isolates have affiliations to isolates from laboratory rodents from China and the USA for the mtDNA and rRNA phylogenies. Additionally, the Gauteng Province isolate of another pinworm, *S. muris,* was affiliated to a conspecific rat from Indonesia, which is part of the native range, and these geographical affiliations may represent co-invasion. The *M. coucha* associated *S. obvelata* was previously reported by Julius et al. (2017) and may represent parasite spill over since *S. obvelata* was only previously recorded from laboratory rodents as a commensal endoparasite (Hussey 1957). This may be evidence of host switching which is known to occur within the genus *Syphacia* (Okamoto et al. 2009; Weaver et al. 2016). Lack of reference sequences for *Mastophorus muris* may mask identification of introduced taxa because such a cosmopolitan nematode may reveal cryptic diversity when molecularly typed. The difficulty in phylogenetic resolution of *Trichuris* sp. was reported before (Callejón et al. 2013). Along with the historical use of *T. muris* as "dump taxon" for all murid host-related whipworms (Ribas et al. 2013), this complicates species delineations and emphasizes the need of multiple gene region analyses. In this study, the

mtDNA COI phylogeny revealed that the *Trichuris* sp. recovered from *M. coucha* appears to be more closely related to *T. arvicolae* than *T. muris*.

The *H. diminuta* phylogeny was better resolved with the mtDNA COI than the rRNA ITS gene region. Despite dissimilar genetic variation between mtDNA and rRNA, both gene regions were robust in resolving phylogenetic relationships of distantly related nematode taxa. As highlighted by Morand et al. (2015) there is a need for co-phylogeographic studies on invasive rodents and their associated parasites to help reveal invasion routes and distribution. The study therefore adds to this and also contributes to documenting genetic diversity of helminth parasites in South Africa.

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Tables

Table 1. Oligonucleotide sequences of the primer sets used in the PCR amplification of cosmopolitan helminths of murid invasive *Rattus* species and indigenous *Mastomys coucha* in Gauteng Province, South Africa

Primer name (5'-3')	Primer reference	Gene region	Amplicon size (bp)
Cestodes			
28Sa-GACCCGTCTTGAAACACGGA 28sb-TCGGAAGGAACCAGCTACTA	Whiting et al. 1997	28S	≈ 400
Hymenolepis-specific			
COIF- TTGAATTTGCCACGTTTGAATGC COIR- GAACCTAACGACATAACATAATGA	Foronda et al. 2011	COI	≈ 800
ITS1F3-GCGGAAGGATCATTACACGTTC ITS1R3- GCTCGACTCTTCATCGATCCACG	Macnish et al. 2002	ITS	≈ 800
Nematodes			
988F-CTCAAAGATTAAGCCATGC 1912R-TTTACGGTCAGAACTAGGG	Holterman et al. 2006	18S	≈ 900
1096F-GGTAATTCTGGAGCTAATAC 1912R-TTTACGGTCAGAACTAGGG	Holterman et al. 2006	18S	≈ 800
1813F-CTGCGTGAGAGGTGAAAT 2646R-GCTACCTTGTTACGACTTTT	Holterman et al. 2006	18S	≈ 800
DiagF-TTGRTTTTTTGGTCATCCTGARG DiagR-WSYMACWACATAATAAGT ATCATG	Diagne et al. 2016	COI	≈ 400

Table 2. Details of helminth sequences generated in this study and reference sequences used in phylogenetic analyses. The number of helminth samples for each host species is represented by N.

Helminths	N	Host species	Gene region	GenBank acc. no.	Reference
Cestodes					
Hymenolepis diminuta	3	*Rattus rattus*	COI	KY462775-7	Julius et al. 2017
	2	*Rattus norvegicus*	COI	KY462778-9	Julius et al. 2017
	1	*R. norvegicus*	COI	AF314223	Von Nickisch-Rosenegk et al. 2001
	2	*R. rattus*	COI	LC063184, LC063186	Nkouawa et al. 2016
	1	*R. norvegicus*	COI	LC063185	Nkouwa et al. 2016
	5	*R. rattus*	COI	JN288042-3 JN288045-7	Foronda et al. 2011
	2	*R. norvegicus*	COI	JN288044, JN288048	Foronda et al. 2011

	1	*R. rattus*	ITS1	MG322244	This study
	2	*R. norvegicus*	ITS1	MG322245-6	This study
	2	*R. rattus*	ITS1	JN258038-9	Foronda et al. 2011
	1	*R. norvegicus*	ITS1	AF461125	Macnish et al. 2002
Nematodes					
Nippostrongylus brasiliensis	1	*R. norvegicus*	COI	AP017690	Unpublished
	1	*R. norvegicus*	18S	KY462825	Julius et al. 2017
	1	*R. norvegicus*	18S	AF036597	Blaxter et al. 1998
	1	*R. norvegicus*	18S	AJ920356	Chilton et al. 2006
Aspiculuris tetraptera	1	*R. rattus*	COI	MG386201	This study
	1	*Rattus tanezumi*	COI	MG386202	This study
	1	Laboratory rat	COI	KT764937	Wang et al. 2016
	1	*R. rattus*	18S	KY462827	Julius et al. 2017
		R. tanezumi	18S	KY462828	Julius et al. 2017
	1	*Mus musculus*	18S	EF464551	Feldman and Bowman 2007
Syphacia muris	1	*R. tanezumi*	COI	MG386203	This study
	1	*R. tanezumi*	COI	LC038089	Dewi and Hasegawa 2015
	1	unknown	COI	AP017697	Unpublished
	1	*R. tanezumi*	18S	KY462829	Julius et al. 2017
	1	Laboratory mouse	18S	EF464553	Feldman and Bowman 2007
Syphacia obvelata	2	*Mastomys coucha*	COI	MG386204-5	This study
	1	Laboratory mouse	COI	KF738499	Wang et al. 2015
	1	Laboratory mouse	COI	GQ260133	Li et al. 2009
	2	*M. coucha*	18S	KY462826	Julius et al. 2017
	1	Laboratory mouse	18S	EF464554	Feldman and Bowman 2007

Trichuris sp.	2	*M. coucha*	COI	MG386207	This study
	1	House mouse	COI	KU575062	Wasimuddin et al. 2016
	1	*R. rattus*	COI	KU575069	Wasimuddin et al. 2016
	1	*Microtus arvalis*	COI	FR851281	Callejón et al. 2013
	1	*M. coucha*	18S	MG356472	This study
	2	*Mus domesticus*	18S	HF586907	Callejón et al. 2013
		Myodes glareolus	18S	HF586908	Callejón et al. 2013
Mastophorus muris	1	*R. norvegicus*	COI	MG386206	This study
	1	*R. norvegicus*	18S	MG356473	This study

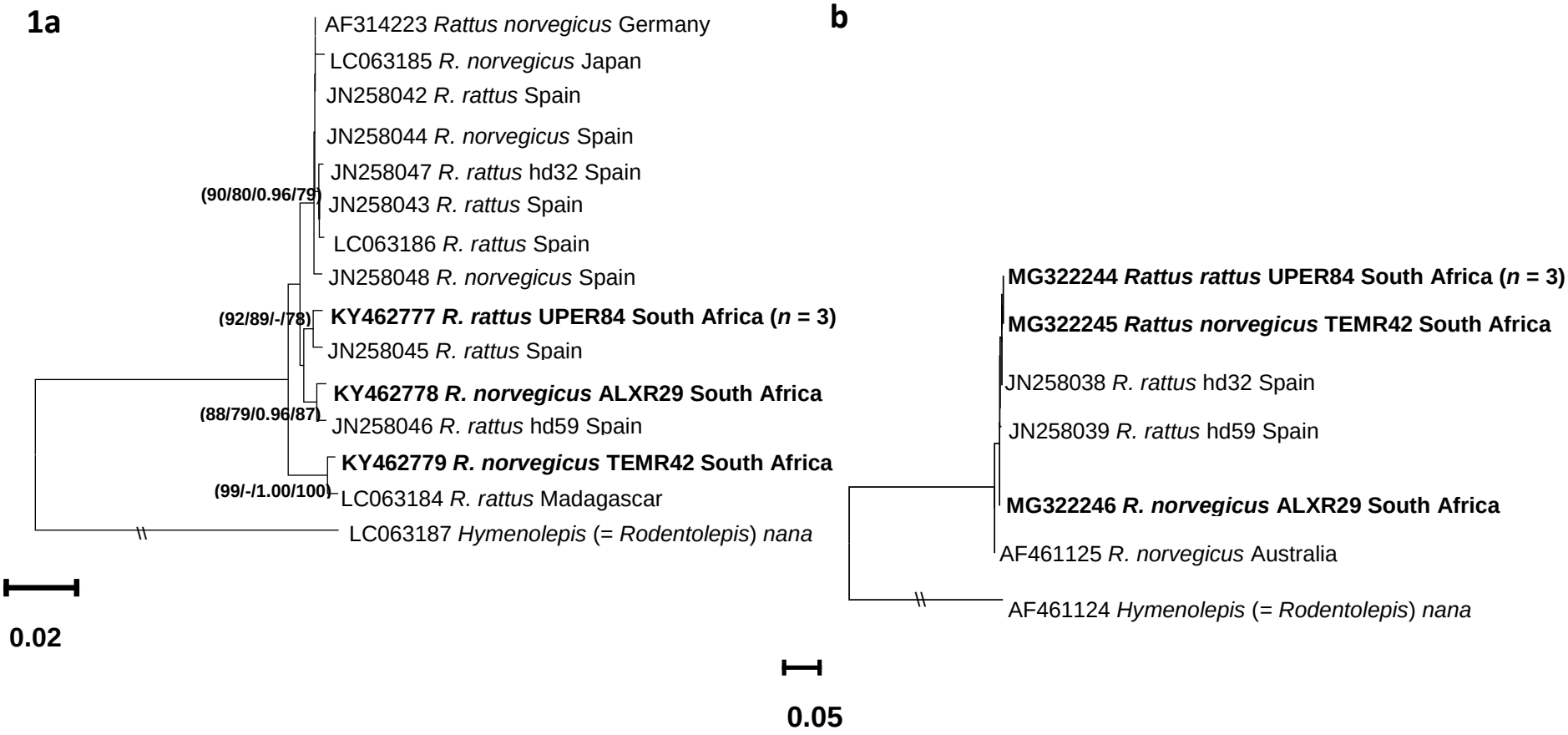
Table 3. Uncorrected *p*- distance values between helminth isolates recovered in this study from Gauteng Province, South Africa, and nearest matches from GenBank for 18S, ITS and COI gene regions, respectively

Helminths	**18S**	**ITS**	**COI**
Hymenolepis diminuta	-	0.0	0.01-.0.03
Nippostrongylus brasiliensis	0.0	-	-
Aspiculuris tetraptera	0.0	-	0.15-0.16
Syphacia muris	0.01	-	0.06-0.17
Syphacia obvelata	0.0	-	0.05-0.08
Trichuris sp.	0.0	-	0.12-0.17
Oxyurids	0.04-0.06	-	0.11-0.22

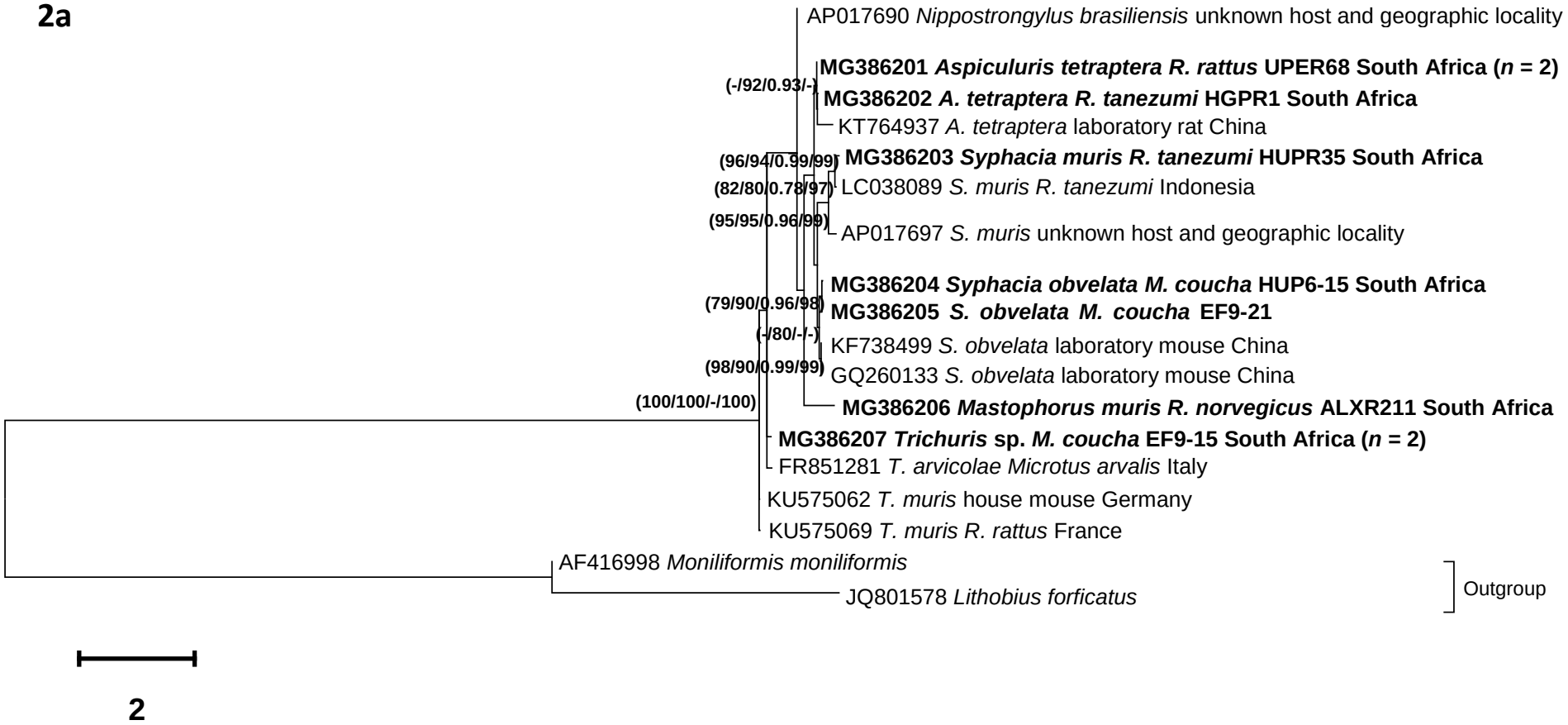
Figure legends

Fig. 1 *Hymenolepis diminuta* phylogeny based on samples recovered from commensal, invasive *Rattus* species in Gauteng Province, South Africa, as characterized by partial fragments of the (a) mitochondrial (mtDNA) COI and (b) ribosomal (rRNA) ITS gene regions. Sequences originating from South Africa are indicated in bold type to be distinguished from reference sequences. Taxon names include the GenBank accession no., host species, specimen code, or haplotype name (when available) and geographic locality. Nodal support of ≥75 % and ≥ 0.75 are displayed and indicated in parentheses above or below the relevant node in the order Neighbour Joining, Maximum Likelihood, Bayesian Inference and Maximum Parsimony. *Hymenolepis* (= *Rodentolepis*) *nana* was used as outgroup taxon.

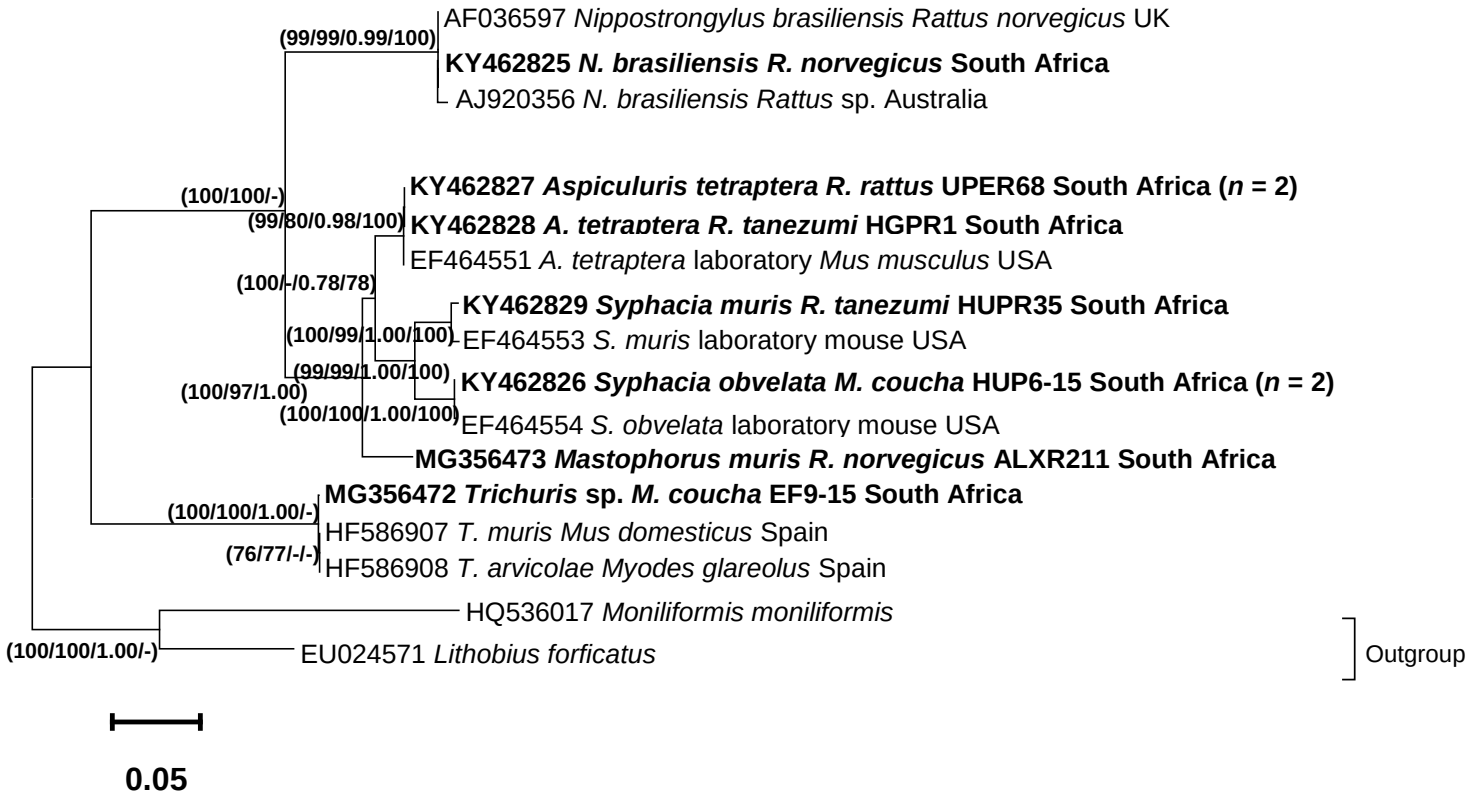
Fig. 2 Phylogenetic relationships of nematode helminths sampled from commensal, invasive *Rattus* species and indigenous *Mastomys coucha* in Gauteng Province, South Africa, based on partial fragments as characterized by the (a) mitochondrial (mtDNA) COI and (b) ribosomal (rRNA) 18S phylogenies. Sequences originating from South Africa are indicated in bold type to be distinguished from reference sequences. Taxon names include the GenBank accession no., helminth species, host species, specimen code (when available), and geographic locality. Nodal support of ≥75 % and ≥ 0.75 are displayed and indicated in parentheses above or below the relevant node in the order Neighbour Joining, Maximum Likelihood, Bayesian Inference and Maximum Parsimony. An acanthocephalan (*Moniliformis moniliformis*) and arthropod (*Lithobius forficatus*) were used as outgroup taxa.


1a
AF314223 Rattus norvegicus Germany
LC063185 R. norvegicus Japan
JN258042 R. rattus Spain
JN258044 R. norvegicus Spain
JN258047 R. rattus hd32 Spain
(90/80/0.96/79)
JN258043 R. rattus Spain
LC063186 R. rattus Spain
JN258048 R. norvegicus Spain
(92/89/-/78)
KY462777 R. rattus UPER84 South Africa (n = 3)
JN258045 R. rattus Spain
KY462778 R. norvegicus ALXR29 South Africa
(88/79/0.96/87)
JN258046 R. rattus hd59 Spain
KY462779 R. norvegicus TEMR42 South Africa
(99/-/1.00/100)
LC063184 R. rattus Madagascar
LC063187 Hymenolepis (= Rodentolepis) nana
0.02
b
MG322244 Rattus rattus UPER84 South Africa (n = 3)
MG322245 Rattus norvegicus TEMR42 South Africa
JN258038 R. rattus hd32 Spain
JN258039 R. rattus hd59 Spain
MG322246 R. norvegicus ALXR29 South Africa
AF461125 R. norvegicus Australia
AF461124 Hymenolepis (= Rodentolepis) nana
0.05

2a


AP017690 Nippostrongylus brasiliensis unknown host and geographic locality
MG386201 Aspiculuris tetraptera R. rattus UPER68 South Africa (n = 2)
(-/92/0.93/-)
MG386202 A. tetraptera R. tanezumi HGPR1 South Africa
KT764937 A. tetraptera laboratory rat China
(96/94/0.99/99)
MG386203 Syphacia muris R. tanezumi HUPR35 South Africa
(82/80/0.78/97)
LC038089 S. muris R. tanezumi Indonesia
(95/95/0.96/99)
AP017697 S. muris unknown host and geographic locality
MG386204 Syphacia obvelata M. coucha HUP6-15 South Africa
(79/90/0.96/98)
MG386205 S. obvelata M. coucha EF9-21
(-/80/-/-)
KF738499 S. obvelata laboratory mouse China
(98/90/0.99/99)
GQ260133 S. obvelata laboratory mouse China
(100/100/-/100)
MG386206 Mastophorus muris R. norvegicus ALXR211 South Africa
MG386207 Trichuris sp. M. coucha EF9-15 South Africa (n = 2)
FR851281 T. arvicolae Microtus arvalis Italy
KU575062 T. muris house mouse Germany
KU575069 T. muris R. rattus France
AF416998 Moniliformis moniliformis
JQ801578 Lithobius forficatus
Outgroup
2

b


AF036597 *Nippostrongylus brasiliensis Rattus norvegicus* UK
(99/99/0.99/100)
KY462825 *N. brasiliensis R. norvegicus* South Africa
AJ920356 *N. brasiliensis Rattus* sp. Australia
(100/100/-)
KY462827 *Aspiculuris tetraptera R. rattus* UPER68 South Africa (*n* = 2)
(99/80/0.98/100)
KY462828 *A. tetraptera R. tanezumi* HGPR1 South Africa
EF464551 *A. tetraptera* laboratory *Mus musculus* USA
(100/-/0.78/78)
KY462829 *Syphacia muris R. tanezumi* HUPR35 South Africa
(100/99/1.00/100)
EF464553 *S. muris* laboratory mouse USA
(99/99/1.00/100)
KY462826 *Syphacia obvelata M. coucha* HUP6-15 South Africa (*n* = 2)
(100/97/1.00)
(100/100/1.00/100)
EF464554 *S. obvelata* laboratory mouse USA
MG356473 *Mastophorus muris R. norvegicus* ALXR211 South Africa
MG356472 *Trichuris* sp. *M. coucha* EF9-15 South Africa
(100/100/1.00/-)
HF586907 *T. muris Mus domesticus* Spain
(76/77/-/-)
HF586908 *T. arvicolae Myodes glareolus* Spain
HQ536017 *Moniliformis moniliformis*
(100/100/1.00/-)
EU024571 *Lithobius forficatus*
Outgroup
0.05