

# **Helminth composition and prevalence of indigenous and invasive synanthropic murid rodents in urban areas of Gauteng Province, South Africa**

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## **Running headline**

Helminths of indigenous and invasive synanthropic murid rodents in urban Gauteng Province, South Africa

## Abstract

Although, synanthropic rodents such as the indigenous species, *Mastomys coucha* and the invasive species, *Rattus norvegicus*, *R. rattus* and *R. tanezumi* are well-known to be hosts to various micro- and macroparasites, their helminth parasite fauna is understudied in South Africa. In an attempt to remediate the situation, the aim of the present study was to investigate the helminth fauna of these sympatric rodent species which were obtained from the informal settlements of Alexandra, Tembisa, Diepsloot and residential suburbs of Pretoria and Hammanskraal, Gauteng Province, South Africa. Helminths were recovered from the urinary bladder, liver and gastrointestinal tract and were identified morphologically and molecularly. Nematodes recovered were all rodent-specific and included *Aspicularis tetraptera*, *Eucoleus* sp., *Heterakis spumosa*, *Mastophorus muris*, *Nippostrongylus brasiliensis*, *Protospirura* sp., *Strongyloides ratti*, *Syphacia obvelata*, *Syphacia muris*, *Trichuris* sp. and *Trichosomoides crassicauda*. *Syphacia obvelata*, a commensal nematode of laboratory rodents, was recovered from indigenous *M. coucha*. Strobilar stages of cestodes recovered included *Hymenolepis diminuta*, *Hymenolepis nana* and *Inermicapsifer madagascariensis*. Metacestodes recovered were strobilocerci of *Hydatigera taeniaeformis* from all three invasive *Rattus* species and coenurostrobilocerci of *Hydatigera parva* from *M. coucha*. An acanthocephalan, *Moniliformis moniliformis*, was recovered from *R. rattus* only. All rodent species examined showed high helminth infection prevalence ( $\geq 70\%$ ) with equal or higher nematode than cestode prevalence. *Mastomys coucha* however, showed significantly lower cestode prevalence than *Rattus* species where they co-occur. Interspecific transmission of helminths likely occurs between invasive and indigenous rodents and these rodents harbour several helminths which have zoonotic implications.

**Keywords:** Rodentia, *Mastomys*, *Rattus*, helminths, diversity, commensal, southern Africa

## Introduction

Synanthropic or commensal species inhabit settlements (Khlyap & Warshavsky, 2010) and live in close association with humans. Urbanization, characterised by high human density, is increasing globally (Gratz, 1999) and therefore allows these species to thrive and when compared to sylvatic wildlife, it has been suggested that synanthropic wildlife is mostly responsible for emerging zoonotic diseases (McFarlane *et al.*, 2012). In addition, urbanization may bring about clumped resource partitioning which has been demonstrated to increase parasite richness through increased contact among wildlife while encroachment into and modification of natural landscapes, promotes contact between humans and wildlife (Wright & Gompper, 2005).

The multimammate mouse, *Mastomys coucha* (Smith, 1836), is an indigenous and common murid rodent species widely distributed in southern Africa (Skinner & Chimimba, 2005) and a known agricultural pest (Smit *et al.*, 2001) that has adopted a synanthropic lifestyle. In urban areas, the species readily enters residential dwellings but does not nest there (Kirsten & von Maltitz, 2005; Kneidinger, 2008). The three matrilineal house rat species of the genus *Rattus* occurring in South Africa (*R. norvegicus*, *R. rattus*, *R. tanezumi*) have long been recognized as true commensal species (Aplin *et al.*, 2011) as they are mostly associated with human habitation. Coincidentally, of the approximately 66 recognized species of *Rattus* worldwide (Musser & Carleton, 2005) these three species are considered to be the most invasive rat species with a cosmopolitan distribution for urban ecosystems (Kosoy *et al.*, 2015).

In Africa, species of *Rattus* and *Mastomys* are frequently implicated in disease epidemiology (Gratz, 1994; Taylor *et al.*, 2008) and in South Africa these rodent species have been identified as hosts and reservoirs of a variety of infectious agents, including viruses (Witkowski *et al.*, 2014), bacteria (Julius *et al.*; 2012; Le Grange, 2014), and parasites

(Collins, 1972; Archer *et al.*, 2011). There is however, a dearth of information regarding their parasitic fauna and particularly helminths. Past helminthological studies which included commensal rodents, were mostly opportunistic and focused on helminth species descriptions and certain taxa (Collins, 1972; Archer *et al.*, 2017) and did not take into consideration the unknown presence of cryptic *R. tanezumi* which is morphologically similar to *R. rattus*. Only recently, an endoparasitic survey in the port city of Durban, Kwa-Zulu Natal Province in South Africa was conducted on commensal *R. norvegicus*, *R. rattus* and *M. natalensis* (Archer *et al.*, 2017).

In South Africa, where commensal species of *Mastomys* and *Rattus* are sympatric (Kirsten & von Maltitz, 2005; Archer *et al.*, 2017), this study provides information on their helminth species composition and prevalence where the rodent species identities were genetically confirmed. Results are discussed in context with the implications to human health and the environment.

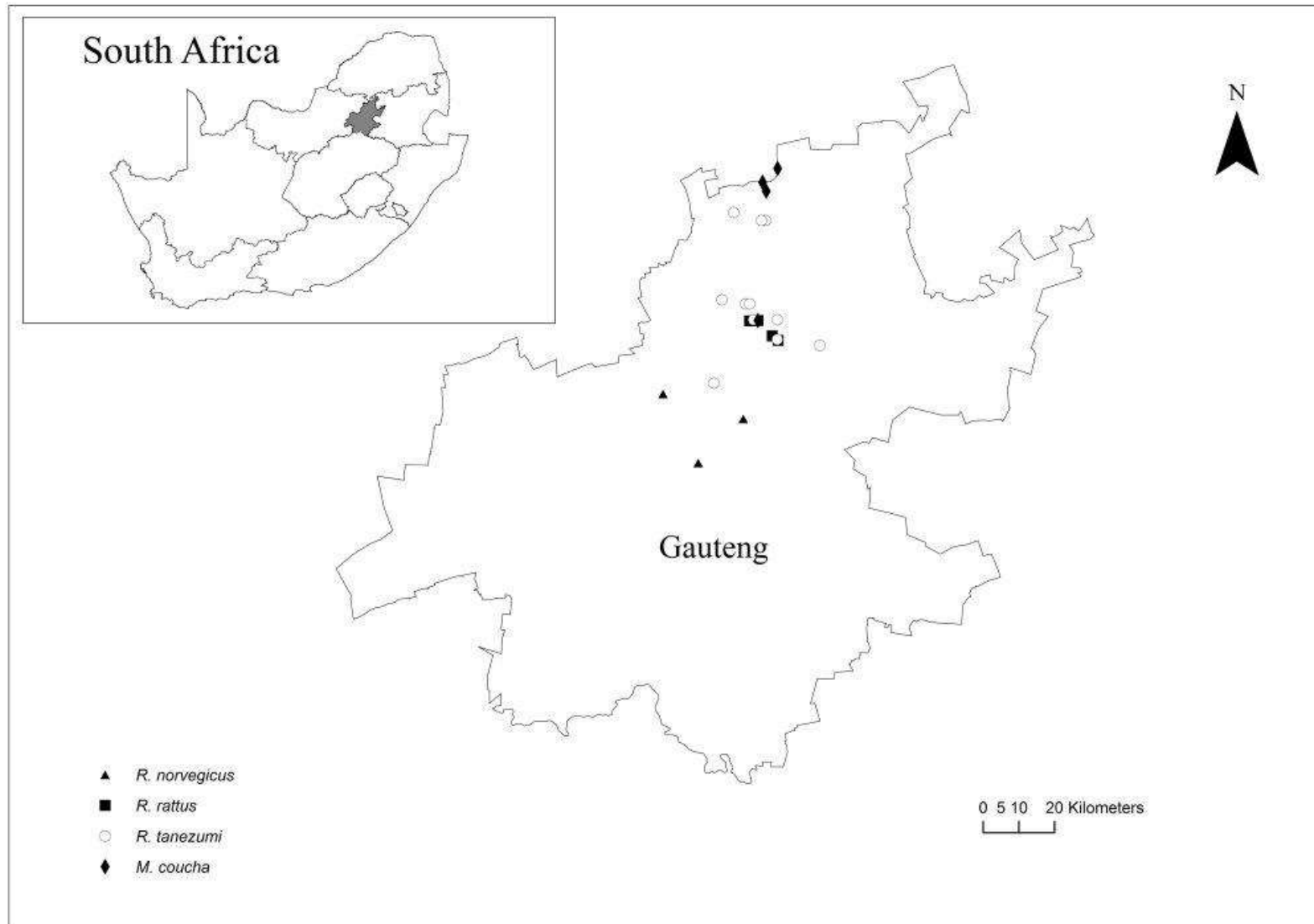
## **Materials and methods**

### *Study site and sampling regime*

Rodents were sampled between September 2010 and September 2011 and in May 2012 with baited (peanut butter, fish and oats) Sherman traps and snap traps. Trapping localities consisted of formal, informal residential homes, school grounds, office-, industrial buildings and smallholdings in Hammanskraal (25°24'S; 28°15'E), Pretoria (25° 45'S; 28° 15' E), Tembisa (26°0' S; 28° 12' E), Diepsloot (25° 56' E; 28° 0' S) and Alexandra (26°6' S; 28° 6' E), Gauteng Province, South Africa (Fig. 1).

### *Rodent capture and helminth recovery*

Following capture, rodents were euthanized, dissected and stored at -20 °C. As part of a parallel study, sample collection was initially prioritized for tissue biopsies and not for



**Figure 1.** Map of Gauteng Province, South Africa, showing sampling localities of indigenous *Mastomys coucha* and invasive *Rattus* species.

helminth recovery and consequently preservation of helminth material was compromised due to repeated freezing and thawing of rodent carcasses. Rodents were identified based on external morphology in accordance with published information and morphologically cryptic species were identified by molecular typing of the mitochondrial cytochrome b (cyt *b*) gene region (Julius, 2013; Le Grange, 2014).

The liver, urinary bladder and gastrointestinal tract (GIT) (stomach, small intestine (SI), caecum and colon) were removed, dissected, and subsequently examined under a stereomicroscope for the presence of helminths. Nematodes, acanthocephalans and metacestodes were counted but not adult cestodes as in many instances only strobilar fragments without scoleces were recovered. Helminth specimens were rinsed with phosphate buffered saline (PBS) and subsequently fixed in 70 % glycerol alcohol.

#### *Morphological identification of helminths*

Cestodes were stained with aceto-alum carmine, cleared in clove oil and mounted in Entellan® (International Institute of Parasitology (IIP), 1996). Nematodes and acanthocephalans were cleared in lactophenol containing Horen's trichrome (International Institute of Parasitology (IIP), 1996). Microscopic examination was conducted with an Olympus BX 50 compound microscope and images taken with an attached Imaging CC12 digital camera (Soft Imaging System, Germany). Scanning electron microscopy (SEM) was conducted with a JEOL 6000F scanning electron microscope following dehydration of specimens in an ethanol series and subsequent drying by the critical point technique. Helminth specimens were identified to the lowest taxonomic level possible with the aid of published taxonomic keys for nematodes (Anderson *et al.*, 1974), cestodes (Khalil *et al.*, 1994) and acanthocephala (Petrochenko, 1971). Voucher specimens will be deposited at the National Collection of Animal Helminths, Onderstepoort Veterinary Institute, Agricultural Research Council, Onderstepoort, South Africa.

### *Molecular identification of helminths*

Genomic DNA was extracted using QIAamp DNA minikit (Qiagen) following the manufacturer's protocol with minor adjustments. A subset of cestodes ( $n = 12$ ), one sample from three individuals each of, *M. coucha*, *R. norvegicus*, *R. rattus* and *R. tanezumi* hosts which could not be identified to the species level morphologically, was subjected to molecular identification. For cestode identification by PCR, general universal primer sets, characterising the 28S-D3 ( $\approx 400$  bp) and genus specific primer sets of the COI ( $\approx 800$  bp) gene regions from the ribosomal and mitochondrial genomes were used (Whiting *et al.*, 1997; Foronda *et al.*, 2011). Cosmopolitan nematodes of the Heligmonellidae (heligmonellids) and Oxyuroidea (oxyurids) were initially identified based on morphology but because these groups include numerous morphologically similar species (Durette-Desset & Digiani, 2012; Khalil *et al.*, 2014), they were also subjected to molecular typing using primer sets characterising the ribosomal 18S gene region (Holterman *et al.*, 2006). Polymerase chain reactions (PCR) were carried out in a 25  $\mu$ l volume consisting of 1  $\mu$ l each of forward and reverse primers, 1 U EmeraldAmp® Taq polymerase (Takara) and 3-4  $\mu$ l DNA template. Amplified products were viewed with electrophoresis on a 1.5 % agarose gel and purified using Roche PCR Product Purification Kit (Roche Diagnostics). Identities were confirmed by direct sequencing with aforementioned primers.

### *Helminth molecular and phylogenetic analyses*

DNA sequencing proceeded with a BigDye v. 3.1 terminator cycle-sequencing kit (Perkin-Elmer, USA), with each PCR primer. Samples were run on an ABI 3130 sequencer and sequence chromatograms were viewed and edited in Mega 5 (Tamura *et al.*, 2011) prior to performing BLAST nucleotide searches against the NCBI GenBank database. Helminth species identities were confirmed by phylogenetic analyses and included reference sequences with the maximum sequence similarities in the GenBank database. For each nematode and

cestode alignment, the best-fit model of sequence evolution was selected using the Akaike Information Criterion (AICC) in jModeltest (Posada, 2008). Phylogenies were inferred using Maximum Likelihood (ML) and Neighbour- Joining (NJ) trees with nodal support assessed by 10, 000 non-parametric bootstrap replications also performed in Mega 5 (Tamura *et al.*, 2011), while Bayesian Inference (BI) tree analyses was performed with MrBayes v.2.1.3. and run over 10, '000, '000 generations.

### *Statistical analysis*

The distribution of the parasite communities among the rodent hosts were quantified using mean intensity and prevalence of respective helminths (Bush *et al.*, 1997) and calculated in Quantitative Parasitology (web version) (Reiczigel & Rózsa, 2005). Chi square tests for differences in infection prevalence among rodent species were calculated using Statistica v.12 (Statsoft Inc.). To quantify diversity and abundance of the parasite communities among the rodent hosts, infracommunity parameters (Holmes & Price, 1986) namely Species richness(d), Evenness, Berger-Parker index and Brillouin's index were assessed and were calculated using PAST v.3.12 (Hammer *et al.*, 2001).

## **Results**

### *Helminth composition*

Strobilar stages of the dwarf tapeworm, *Hymenolepis nana* (Hughes, 1941) were recovered from the SI of *R. norvegicus* and those of *Inermicapsifer madagascariensis* (Baylis, 1949; Collins, 1972), were recovered from the SI of *M. coucha* and identified based on morphological criteria. The prevalence of these among the rodent species could not be assessed as all the scoleces could not be recovered and/or the strobilae were too deteriorated for a conclusive identification. Molecular typing of cestodes detected only the presence of the rat tapeworm, *H. diminuta* in *R. rattus* and *R. norvegicus* based on a 791 bp COI mRNA



phylogeny. Sequences were deposited in GenBank (Access no.: KY462775-79) (Supplementary material; Figure S1A). Two taeniid metacestodes were recovered, namely strobilocerci of *Hydatigera taeniaeformis* (syn. *Taenia taeniaeformis*) (Loos-Frank, 2000) from the liver parenchyma of *R. rattus*, *R. norvegicus* and *R. tanezumi* and coenurostrobilocerci of *Hydatigera parva* (syn. *Taenia parva*) (Loos-Frank, 2000) which were attached to the serosal surfaces of the abdominal cavity in *M. coucha* only.

The acanthocephalan, *Moniliformis moniliformis* (Petrochenko, 1971) was recovered from the SI of *R. rattus* only. The nematode composition among the rodent species is summarized in Table 1. A spiruroid, *Mastophorus muris* (Wertheim, 1962; Chabaud, 1975) was found in the SI and stomachs of all three *Rattus* species. The 18S rRNA phylogeny comprising 1155 bp (supplementary material; Figure S1B) and submitted to GenBank (Access no.: KY462823-29), confirmed the morphological identification of *Nippostrongylus brasiliensis* (Tubangui, 1931; Durette-Desset, 1983) recovered from the SI of *R. norvegicus*, *Syphacia muris* (Petter & Quentin, 1976) from the caecums and colons of *R. tanezumi*, *Aspiculuris tetraptera* from *R. rattus* and *R. tanezumi* and *Syphacia obvelata* (Petter & Quentin, 1976) from *M. coucha*. *Syphacia muris* was also present in *R. rattus* based on mounted specimens but a clear sequence could not be obtained possibly due to mixture of *A. tetraptera* in the same host sample. Additionally, molecular typing confirmed the presence of a heligmonellid in the SI of *M. coucha* closely related to *N. brasiliensis* based on the 18S rRNA phylogeny. This heligmonellid has a conspicuous hypertrophied left ala present in both sexes. Body length ranged between 3.60 to 5.18 mm for females and between 2.47 and 2.97 mm for males. Eggs oval 54.37-64.36 (60.31) × 29.36-42.77 (34.77) and spicules of nearly equal lengths with right spicule 353.69-422.74 (401.11) and left spicule 374.02-429.55 (416.84). The heligmonellid and oxyurid nematodes had the highest mean intensity of infection (> 8) (Table 2). *Trichosomoides crassicauda* (Thomas, 1924), recovered from the

**Table 1** Total- and proportional abundance (in parentheses) of helminths \*in four synanthropic murid rodent species in Gauteng Province, South Africa. Oxyurids include both *Aspicularis teraptera* and *Syphacia muris* species

Helminth	Abundance: Total (%)							
	<i>Rattus norvegicus</i>		<i>R. rattus</i>		<i>R. tanezumi</i>		<i>Mastomys coucha</i>	
<i>Eucoleus</i> sp.	59	(3.957076)	0	(0)	0	(0)	0	(0)
Heligmonellid	0	(0)	0	(0)	0	(0)	1187	(82.5452)
<i>Heterakis spumosa</i>	731	(49.0275)	0	(0)	0	(0)	0	(0)
<i>Hydatigera parva</i>	0	(0)	0	(0)	0	(0)	18	(1.251739)
<i>Hydatigera taeniaeformis</i>	17	(1.140174)	3	(0.564972)	14	(6.666667)	0	(0)
<i>Mastophorus muris</i>	22	(1.47552)	13	(2.448211)	1	(0.47619)	0	(0)
<i>Moniliformis moniliformis</i>	0	(0)	10	(1.883239)	0	(0)	0	(0)
<i>Nippostrongylus brasiliensis</i>	584	(39.16834)	0	(0)	6	(2.857143)	0	(0)
Oxyurids	0	(0)	505	(95.10358)	189	(90)	0	(0)
<i>Protospirura</i> sp.	0	(0)	0	(0)	0	(0)	26	(1.808067)
<i>Strongyloides ratti</i>	0	(0)	0	(0)	0	(0)	5	(0.347705)
<i>Syphacia obvelata</i>	0	(0)	0	(0)	0	(0)	185	(12.86509)
<i>Trichosomoides crassicauda</i>	78	(5.231388)	0	(0)	0	(0)	0	(0)
<i>Trichuris</i> sp.	0	(0)	0	(0)	0	(0)	17	(1.182197)
Total	1491		531		210		1438	

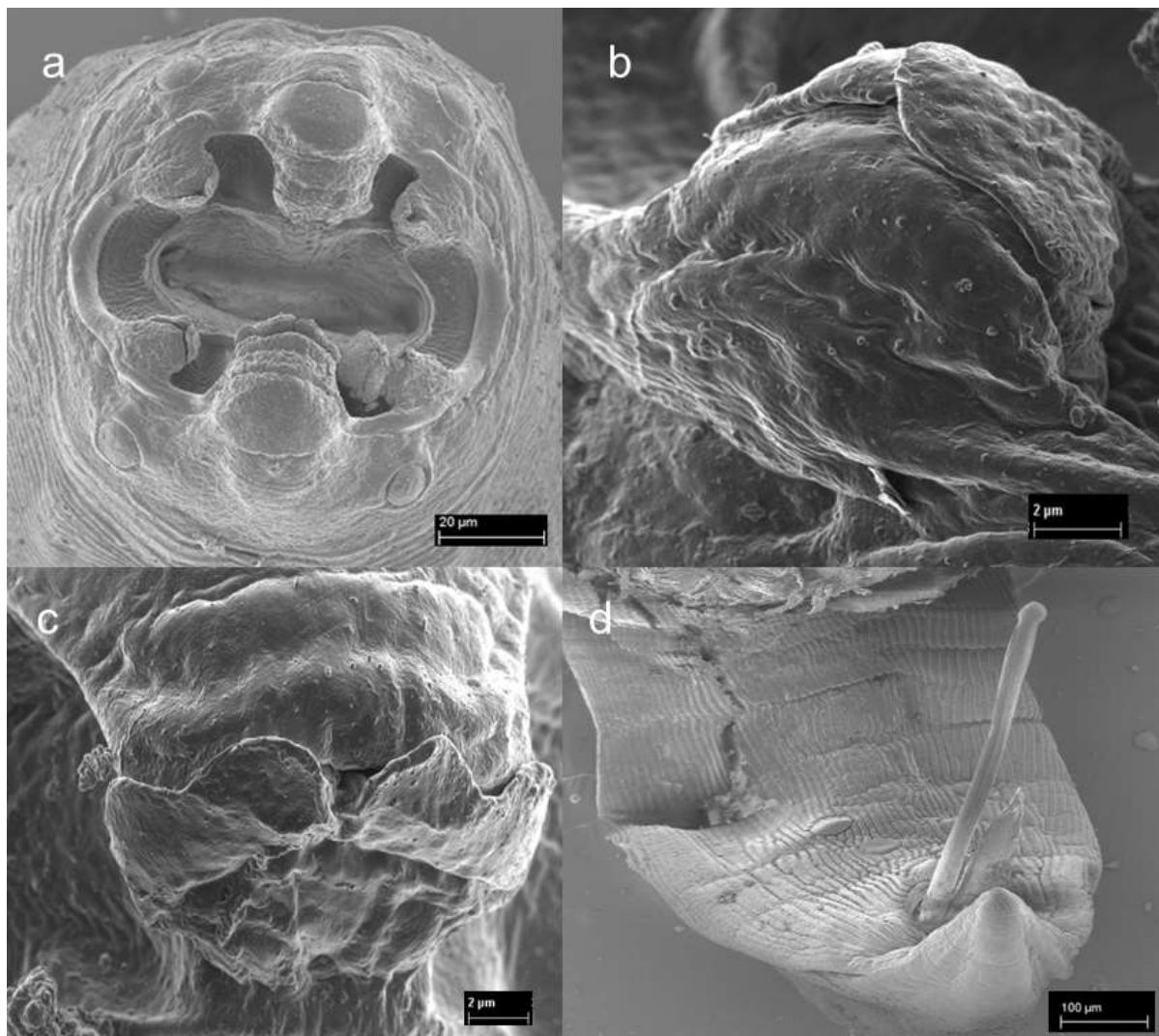
\*Excludes strobilar stages of cestodes

**Table 2.** Mean infection intensity and prevalence of each helminth<sup>†</sup> sampled from synanthropic *Rattus norvegicus*, *R. rattus*, *R. tanezumi* and *Mastomys coucha* in urban areas of Gauteng Province, South Africa with lower and upper CI limits displayed in parenthesis. Oxyurids include both *Aspiculuris tereptra* and *Syphacia muris* species

Higher taxa	Helminth	Mean Intensity	Prevalence
Acanthocephala	<i>Moniliformis moniliformis</i>	5 [2-5]	0.005 [0.001-0.018]
Cestoda	<i>Hydatigera taeniaeformis</i>	1.36 [1.08-1.76]	0.053 [0.035-0.076]
	<i>Hydatigera parva</i>	6 [1-9]	0.006 [0.002-0.18]
Nematoda	<i>Eucoleus</i> sp.	2.19 [1.74-2.78]	0.068 [0.046-0.098]
	Heligmonellid	18.5 [13.3-29.7]	0.162 [0.128-0.202]
	<i>Heterakis spumosa</i>	6.36 [5.3-7.74]	0.291 [0.248-0.338]
	<i>Mastophorus muris</i>	4 [2.22-5.44]	0.023 [0.011-0.043]
	<i>Nippostrongylus brasiliensis</i>	8.94 [6.23-16.7]	0.167 [0.133-0.207]
	Oxyurids	16 [12.1-22.5]	0.139 [0.107-0.177]
	<i>Protospirura</i> sp.	2.6 [1.1-6.8]	0.025 [0.013-0.046]
	<i>Strongyloides ratti</i>	2.5 [1-2.5]	0.005 [0.001-0.018]
	<i>Syphacia obvelata</i>	12.3 [5.33-30.3]	0.038 [0.022-0.062]
	<i>Trichosomoides crassicauda</i>	3 [2.27-4.35]	0.079 [0.054-0.114]
	<i>Trichuris</i> sp.	5.67 [4-7]	0.008 [0.002-0.022]

\* Excludes strobilar stages of cestodes

bladder, *Heterakis spumosa* (Hartwich, 1974) recovered from SI and caecum and *Eucoleus* sp. (Gibbons, 2010) recovered from the stomach mucosa were only found in *R. norvegicus*. *Trichuris* sp., recovered from the caecum, *Strongyloides ratti* from the SI, and a spiruroid nematode species, *Protospirura* sp. (Yamaguti, 1961) (Fig. 2), recovered from the stomach and SI, were found in *M. coucha* only. The 18S rRNA sequence of *Protospirura* sp. was deposited in GenBank under accession no. KY462830.



**Figure 2.** *Protospirura* sp. (a) Cephalic end, (b) Submedian lobe with denticles, (c) Large lateral lobe with denticles and (d) Ventral side of male caudal end showing spicules

### *Helminth infracommunity structure*

Apart from strobilar stages of cestodes which could not be counted, 3670 nematode, acanthocephalan and metacestode specimens were recovered from 395 rodents. Overall gastrointestinal helminth prevalence was high ( $\geq 70\%$ ) and helminth infracommunities were distinct ( $P < 0.05$ ) for all rodent species namely *R. norvegicus* ( $n = 240$ ), *R. rattus* ( $n = 40$ ), *R. tanezumi* ( $n = 31$ ) and *M. coucha* ( $n = 84$ ) (Table 3). Within rodent species comparisons between nematode and cestode prevalence revealed that *R. norvegicus* ( $\chi^2 = 21.49$ ;  $df = 1$ ;  $n = 228$ ;  $P < 0.05$ ) and *M. coucha* ( $\chi^2 = 47.10$ ;  $df = 1$ ;  $n = 79$ ;  $P < 0.05$ ) displayed higher gastrointestinal nematode than cestode prevalence while within *R. rattus* and *R. tanezumi* these differences were not statistically significant (Fig. 3).

One locality each in Hammanskraal and Pretoria where *Rattus* species and *M. coucha* co-occurred contained sufficient sample sizes for comparative analyses between the invasive and indigenous rodents. At the Hammanskraal locality, *R. tanezumi* ( $n = 6$ ) had similar total helminth and nematode prevalence to *M. coucha* ( $n = 41$ ) while the cestode prevalence of *R. tanezumi* was significantly higher than that of *M. coucha* ( $\chi^2 = 12.00$ ;  $df = 1$ ;  $n = 47$ ;  $P < 0.05$ ). Similarly, at the Pretoria locality *R. rattus* ( $n = 32$ ) and *M. coucha* ( $n = 18$ ) had similar total helminth and nematode prevalence yet the cestode prevalence of *R. rattus* was statistically significantly higher than that of *M. coucha* ( $\chi^2 = 4.52$ ;  $df = 1$ ;  $n = 50$ ;  $P < 0.05$ ). Diversity indices at the Hammanskraal locality (Table 3) showed that *R. tanezumi* had lower alpha diversity (Brillouin's index) than *M. coucha* yet both species have similar species richness ( $d$ ) and are predominantly infected by one or more helminth species (Berger Parker index).

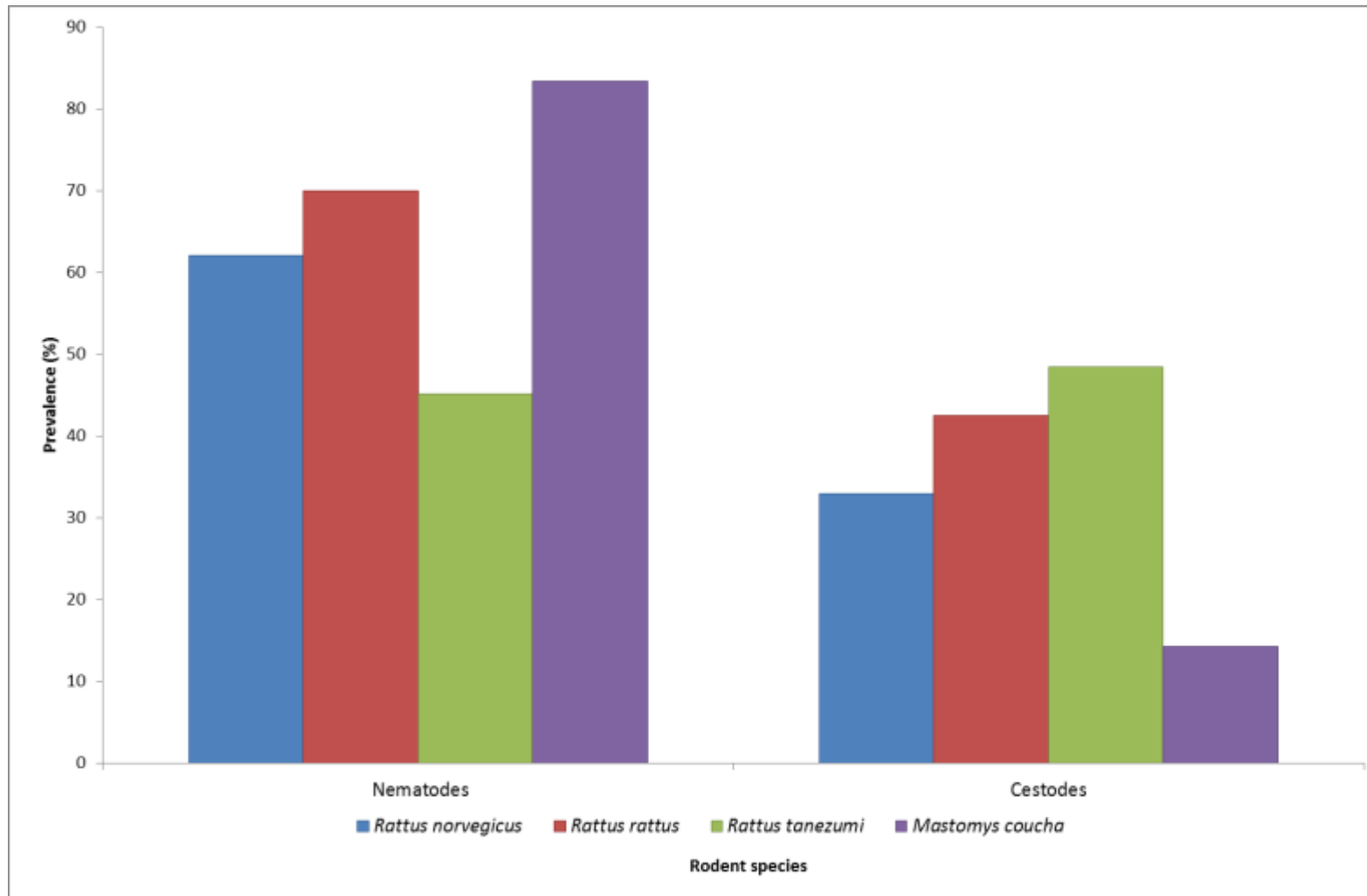
### **Discussion**

The strobilar stage of *I. madagascariensis* has been recorded from islands of the Indian Ocean, Cuba, Venezuela, Thailand, the Philippines, widely from Africa and is typically a

**Table 3.** Infracommunity parameters of the helminth fauna\* of synanthropic indigenous and invasive murid rodents in urban Gauteng Province with lower and upper CI limits displayed in parenthesis

	Rodent species (sample size)	Prevalence (%)	Species richness (d)	Evenness	Berger-Parker index	Brillouin's index
	<i>Rattus norvegicus</i> (240)	69.58	0.68 [0.68-0.68]	0.51 [0.48-0.53]	0.49 [0.47-0.52]	1.1 [1.06-1.14]
All localities	<i>R. rattus</i> (40)	80	0.48 [0.48-0.48]	0.32 [0.30-0.34]	0.95 [0.93-0.97]	0.23 [0.16-0.30]
	<i>R. tanezumi</i> (31)	70.97	0.56 [0.56-0.56]	0.37 [0.33-0.42]	0.9 [0.86-0.94]	0.38 [0.27-0.50]
	<i>Mastomys coucha</i> (84)	85.71	0.69 [0.69-0.69]	0.31 [0.29-0.33]	0.83 [0.81-0.84]	0.61 [0.56-0.66]
Hamman's kraal UP campus	<i>R. tanezumi</i> (6)	100	0.43 [0.43-0.43]	0.42 [0.37-0.48]	0.95 [0.90-0.98]	0.19 [0.09-0.35]
	<i>M. coucha</i> (41)	82.93	0.47 [0.47-0.47]	0.43 [0.40-0.46]	0.85 [0.82-0.88]	0.53 [0.45-0.60]

\*Excludes strobilar stages of cestodes



**Figure 3.** Differences in nematode and cestode prevalence (%) within synanthropic *Rattus norvegicus*, *R. rattus*, *R. tanezumi* and *Mastomys coucha* in urban areas of Gauteng Province, South Africa

parasite of small mammals (Frean & Dini, 2004). This constitutes the first host record from *M. coucha* as the cestode species has already been reported from the congener, *M. natalensis* (Collins, 1972). The species has zoonotic implications and infections in humans were reported from Cuba, Kenya (Baylis, 1949), Zambia (Hira, 1975) and South Africa (Frean & Dini, 2004). Case reports of Frean & Dini (2004) were from two children in the Roodepoort and Benoni areas respectively, forming part of the greater Johannesburg area, Gauteng Province, South Africa. The strobilar stage of *H. nana*, the dwarf tapeworm, is found in sylvatic and laboratory rodents, simian primates and humans (Soulsby & Mönnig, 1982). *Hymenolepis nana* is the most common tapeworm infection in humans as a result of autoinfection and humans being the primary source of infection (Soulsby & Mönnig, 1982; Beaver *et al.*, 1984). There are however, at least two human prevalence reports from children in Gauteng Province (Kark & Le Riche, 1944; Walker *et al.*, 2000). *Hymenolepis diminuta*, the rat tapeworm, is a common parasite of synanthropic murid rodents but is infrequently recovered from humans (Beaver *et al.*, 1984). It has been recovered in human stool samples in South Africa (Fantham & Porter, 1936) but the exact localities cannot be deduced from the publication.

The presence of metacestodes of *H. taeniaeformis* and *H. parva* species confirms the role of synanthropic rodents as intermediate hosts. *Hydatigera taeniaeformis* has a cosmopolitan distribution, and is a common taeniid of the domestic cat (Abuladze, 1970; Loos-Frank, 2000). *Hydatigera taeniaeformis* has recently been discovered to include morphologically cryptic lineages (Lavikainen *et al.*, 2016). Apart from the domestic cat, the definitive host range is broad and includes wild felids, viverrids, genets, mustelids, and wild canids (Abuladze, 1970; Loos-Frank, 2000). Since the metacestode was recovered from invasive *Rattus* species, it is likely to form part of the *H. taeniaeformis* sensu stricto clade which appears to be limited to the liver parenchyma of murid rodents acting as intermediate hosts



(Lavikainen *et al.*, 2016). A prevalence of 5.3% ( $n = 311$ ) and mean intensity of 1.36 (Table 2) of *H. taeniaeformis* in *Rattus* species from this study was comparable to that of a study in Mexico, where *Rattus* species are also invasive (Panti-May *et al.*, 2013). While, *H. taeniaeformis* infection is common in *R. norvegicus* and *R. rattus* (Abuladze, 1970), prevalence in *R. tanezumi* outside the native range is also confirmed from the present study. Since there are only two cases of infections with strobilocerci reported in humans, *H. taeniaeformis* is not regarded as a public health concern (Sterba & Barus, 1976). The strobilar stage of *H. parva* has been reported in some wild felids (*Felis silvestris silvestris*, *F. s. lybica*), genets (*Genetta* spp.) and the striped polecat (*Ictonyx striatus*) from Africa and Europe (Jones & Pybus, 2001). The intermediate host range of the polycephalic metacestode of *H. parva*, also referred to as a coenurostrobilocercus, appears to be restricted to murid rodents (Mas-Coma & Feliu, 1977; Loos-Frank, 2000) and has been recovered in this study from *M. coucha* which is a new intermediate host record. Other known intermediate hosts are the congener *M. natalensis* and other rodent species, *Mus musculus*, *M. minutoides*, *Apodemus sylvaticus*, *Aethomys chrysophilus*, *Micaelamys namaquensis* (Skinner & Chimimba, 2005), *Thallomys paedulus* and *Rhabdomys pumilio* (Jones & Pybus, 2001).

The acanthocephalan *Moniliformis moniliformis*, is a cosmopolitan and common parasite of *R. rattus* and *R. norvegicus* with cockroaches acting as intermediate hosts (Schmidt, 1971). A recent study in Kwa-Zulu Natal Province, South Africa reported it from *R. norvegicus* only (Archer *et al.*, 2017) while the present study found it only in *R. rattus*. Human infections are rare but may present with gastrointestinal disease symptoms and have been reported from several countries (Schmidt, 1971; Andres *et al.*, 2014).

All nematodes recovered in the study were rodent-specific and non-zoonotic. *Nippostrongylus brasiliensis* was first described from *R. norvegicus* in Brazil and is believed to have spread throughout the world with invasive rodents (Mawson, 1961; Smales, 1997).

*Syphacia muris* was first described from a laboratory rat while *S. obvelata*, common in laboratory mice, was long used as a model for pinworm infection for chemotherapeutic studies against human enterobiasis (Hussey, 1957). As a result *S. obvelata* may have spread throughout the world with laboratory rodents eventually spreading to indigenous rodents such as *M. coucha*. The unidentified heligmonellid recovered from only *M. coucha* might belong to the genus *Heligmonina* based on the presence of a hypertrophied left ala (Durette-Desset *et al.*, 2008) but other more distinguishing characteristics need to be examined for a conclusive identification. *Mastophorus muris* was present in all three *Rattus* species. The nematode has a cosmopolitan distribution with cockroaches acting as intermediate hosts (Verster & Brooker, 1970) and its presence is therefore not unexpected in urban areas. Recorded from indigenous wild hosts in only Argentina, Australia and Madagascar (Rojas & Digiani, 2003), it appears to infect mostly invasive, synanthropic rodents (Smales, 1997). *Protospirura* sp. was recovered from only indigenous *M. coucha* and may represent a new species but further work is needed to elucidate its taxonomy. *Protospirura chabaudi* was so far the only species in the genus to be described from Africa and it was described from *R. rattus* in Congo (now the Democratic Republic of Congo (DRC)) (Vuylsteke, 1964). *Protospirura chabaudi* lacks denticles on the large lateral lobe of the pseudolabia (Vuylsteke, 1964) while the *Protospirura* sp. in this study clearly contains two denticles (Fig 2a-c) on each lateral lobe of the pseudolabia. With the exception of *P. muricola*, which has various definitive hosts and an almost global distribution (Smales *et al.*, 2009), *Protospirura* species appear to be geographically restricted. An example is *P. siamensis* which has been recorded from South-East Asia only (Ribas *et al.*, 2012) although recovered from synanthropic, invasive *R. tanezumi* which has an almost global distribution (Kosoy *et al.*, 2015). *Protospirura muricola* has spicules of similar length (Smales *et al.*, 2009) while the *Protospirura* sp. recovered in this study has spicules of markedly unequal lengths (Fig. 2d)., *Protospirura muricola*

however, was surprisingly not recorded in the present study although it was reported from several small mammal taxa, including *M. coucha* in the DRC and appears to be widespread in Africa (Smales *et al.*, 2009).

In conclusion, synanthropic, invasive *Rattus* species when compared to synanthropic, indigenous *M. coucha*, pose a higher risk to public health in urban areas of Gauteng Province, South Africa as they harbour helminths with zoonotic implications at a higher prevalence which may become more relevant under poor hygienic conditions. Where *R. tanezumi* and *M. coucha* co-occurred, *R. tanezumi* showed lower helminth diversity and equal species richness to *M. coucha*. Nevertheless, both synanthropic invasive and indigenous rodents act as both definitive and intermediate hosts to helminths that can infect humans and domestic animals. The presence of a cosmopolitan nematode in an indigenous rodent which originate from laboratory or wild, globally invasive rodent hosts demonstrates the co-invasive potential of helminth parasites. Furthermore, molecular techniques were useful in supplementing and confirming morphological identities of the identified helminths and revealing the phylogenetic relationships of unidentified helminths but would require additional work i.e. more gene regions and larger, more comprehensive databases for genetic sequence comparison to make further inferences. It is anticipated that the genetic sequences resulting from this study would supplement databases and be helpful for future work.

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## **Conflict of interest**

None

## **Ethical standards**

Rodents were sampled with permission from the Gauteng Directorate of Nature Conservation (Permit number: CPF6 0032) and processed with approval granted by the Animal Ethics Committee, University of Pretoria (Ethics clearance number: EC025-10).

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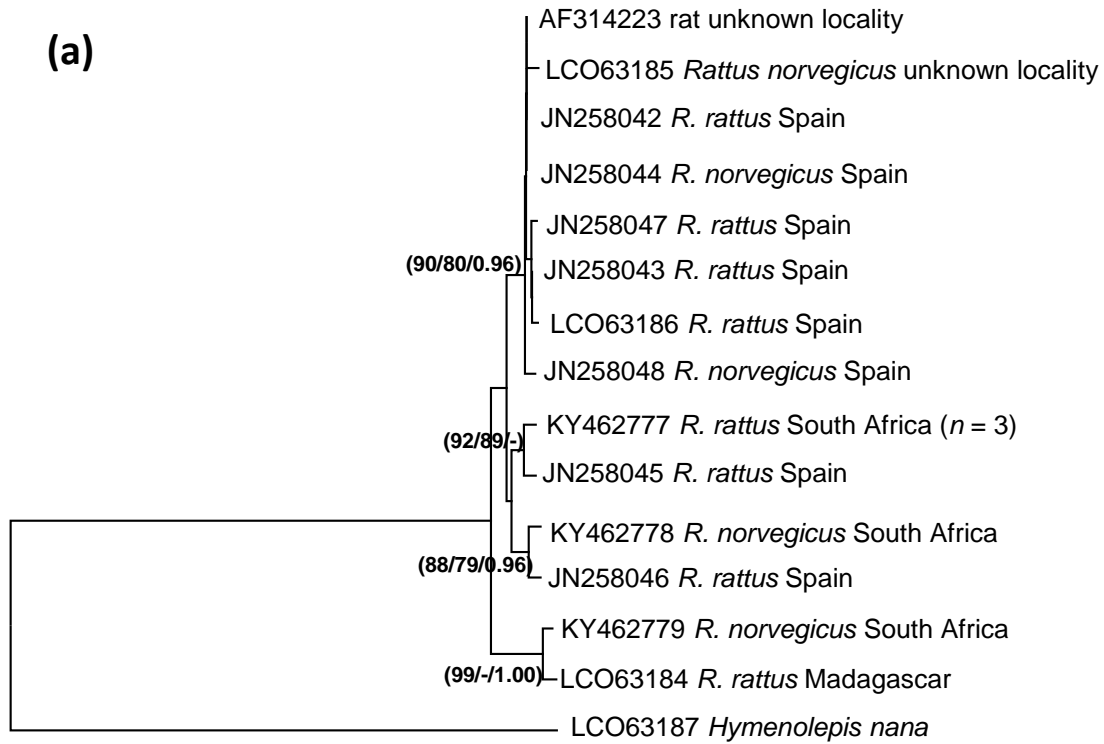
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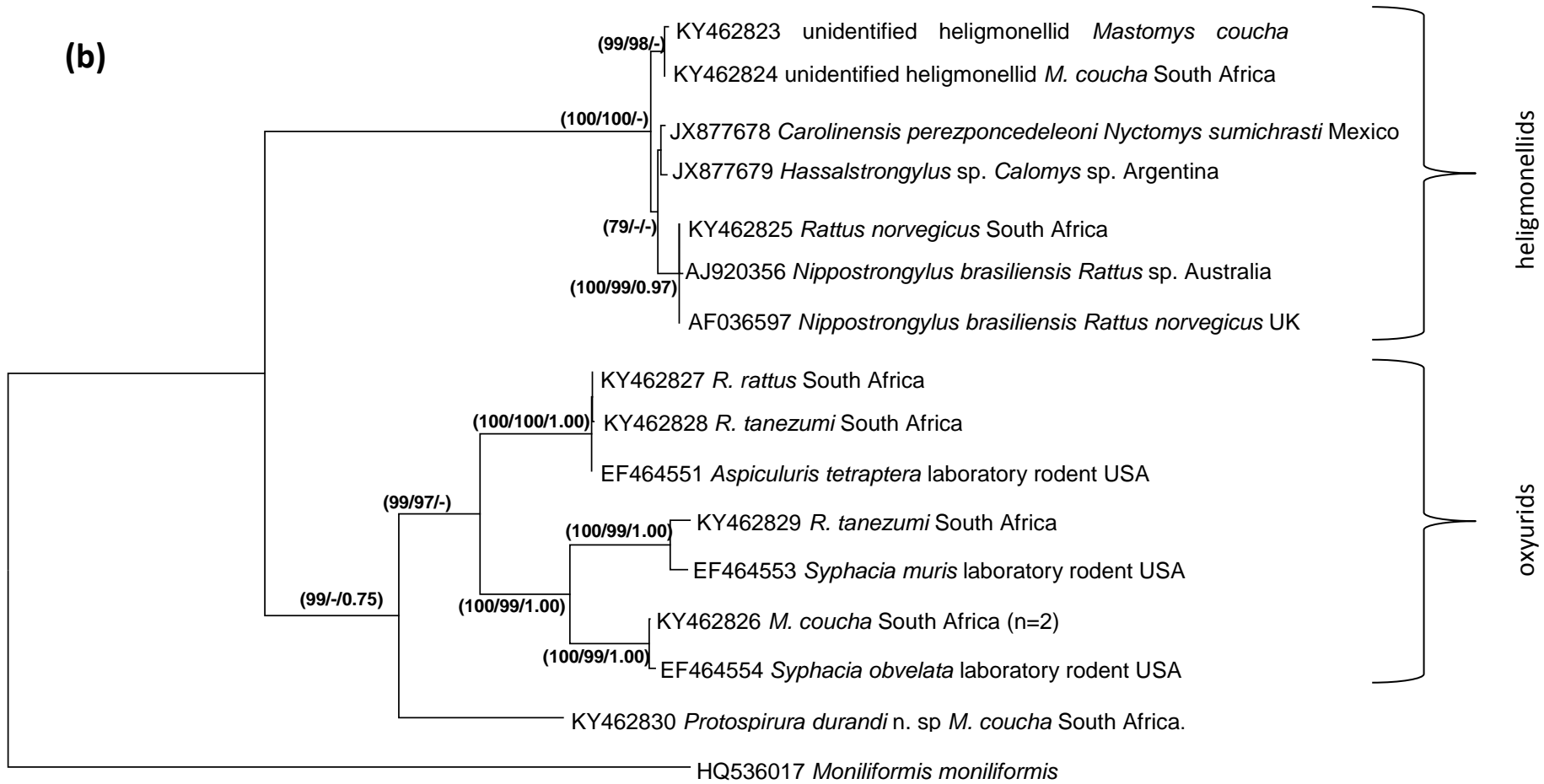
Supplementary Material

(a)



0.02

(b)



**Figure S1.** Phylogenies based on partial fragments of the COI gene region of cestode, *Hymenolepis diminuta* **(a)** and partial fragments of the 18S gene region of heligmonellid, oxyurid and *Protospirura durandi* n. sp. nematodes **(b)** from commensal murid rodents in Gauteng Province, South Africa