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Diversity of ectoparasites associated with *Rhabdomys pumilio* and *Rhabdomys intermedius* (Muridae) in the Fynbos, Succulent Karoo and Nama-Karoo biomes of South Africa

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ABSTRACT

Rodent species within the southern African genus *Rhabdomys* are ecologically flexible, exhibit opportunistic behaviour, and are adapted to a wide range of habitat types. They comprise four morphologically cryptic species that are, however, strictly confined to different vegetation types represented by distinct biomes. Descriptive ectoparasite data on the diversity of lice, fleas, mesostigmatic mites, and ticks are currently limited to *Rhabdomys pumilio* in the Fynbos biome, and narrative data on trombiculid mites are absent. To address these gaps in ectoparasitic knowledge, the present study was extended across a broader geographic scale and included *R. pumilio* from the Succulent Karoo biome, as well as *R. intermedius* occurring in the Nama-Karoo biome. The complete ectoparasite assemblage associated with 237 *Rhabdomys* individuals (171 *R. pumilio* and 66 *R. intermedius*) trapped at 12 localities during the spring–summer seasons of 2023 to 2025 yielded more than 10 000 ectoparasite individuals representing five taxonomic groups: lice, fleas, mesostigmatic mites, trombiculid mites, and ticks. Overall, 46 ectoparasitic species were recorded, comprising one louse species, nine flea species, nine mesostigmatic mite species, five tick species, and 22 trombiculid mite species. *Rhabdomys pumilio* harboured 42 ectoparasite species across the Fynbos and Succulent Karoo biomes, while *R. intermedius* in the Nama-Karoo biome harboured 21 species. The study provides detailed data on the prevalence, and localization on the host body of 14 chigger species described as new in separate taxonomic papers or awaiting description. Additional contributions include 36 new locality records across ectoparasite taxa, 14 new host associations and 24 mtDNA *COI* barcodes for flea species, which complement morphological identifications. This study highlights that more extensive geographic sampling of rodent host species can substantially enhance our understanding of the true diversity of rodent ectoparasites in South Africa.

1. Introduction

South Africa boasts a rich diversity of rodent species, represented by more than 50 extant species encompassing a wide range of life history characteristics (Skinner and Chimimba, 2005). Within this region, the genus *Rhabdomys* (commonly known as the four-striped mouse) comprises four species that are locally abundant and geographically widespread (Skinner and Chimimba, 2005). Historically, *Rhabdomys pumilio* was considered monotypic throughout southern Africa (Skinner and Chimimba, 2005), but cytogenetic and molecular techniques challenged this status and strongly suggest the presence of at least four geographically confined species, with some additional geographic

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sub-clustering (Rambau et al., 2003; du Toit et al., 2012; Ganem et al., 2020). These species are confined to distinct vegetation types (biomes), and pertinent to this study, *R. pumilio* inhabits the predominantly winter rainfall Fynbos and Succulent Karoo biomes, while *Rhabdomys intermedius* occurs in the more xeric Nama-Karoo biome (du Toit et al., 2012; Monadjem et al., 2015; Ganem et al., 2020). These two species are cryptic and most likely display similar life history characteristics, including diet (omnivorous), activity patterns (diurnal and crepuscular), levels of sociality (group-living; Schradin and Pillay, 2005) and synanthropic in behaviour (Froeschke et al., 2010; du Toit et al., 2012; Froeschke and Matthee, 2014).

Current data on the ectoparasite species associated with *Rhabdomys* spp. are mainly limited to taxon-specific monographs for lice, fleas, and mites that were published when the genus *Rhabdomys* was regarded as monotypic (Zumpt, 1961; Ledger, 1980; Durden and Musser, 1994). For example, a tick monograph by Horak et al. (2018) provided data for *R. pumilio* in the Western Cape (Fynbos biome) only and was based on a few localized studies (Matthee et al., 2007, 2010; Froeschke et al., 2013). A more recent regional-scale study spanning different biomes provided species information for fleas originating from *R. bechuanae*, *R. intermedius*, and *R. pumilio* (van der Mescht and Matthee, 2017). None of these studies, however, included trombiculid mites (hereafter referred to as chiggers), which are known habitat specialists that contribute significantly to overall ectoparasite diversity (see Matthee et al., 2020; Stevens et al., 2022; Smith et al., 2023; Little et al., 2024). Barnard et al. (2015) remains the only study to date focusing on some chigger taxa originating from *R. pumilio* at two localities outside Stellenbosch. The true diversity of ectoparasitic taxa associated with *Rhabdomys* is further confounded by cryptic speciation. du Toit et al. (2013) recorded two distinct genetic clades and seven subclades for the louse species *Polyplax arvicanthis* occurring on the four *Rhabdomys* species, while Engelbrecht et al. (2014) found four distinct *Laelaps giganteus* lineages strictly confined to the four *Rhabdomys* species, respectively. Based on these studies, it is clear that molecular barcoding should be considered an essential additional tool in ectoparasite diversity studies (Nadler and de León, 2011; du Toit et al., 2013).

Although ectoparasites are omnipresent, their diversity and abundance may be influenced by host-related factors such as sociality, density, and geographic range (Marshall, 1981; Krasnov et al., 2002; Petney et al., 2004; Lindenfors et al., 2007; du Toit et al., 2013; Engelbrecht et al., 2014) as well as environmental factors such as climate and vegetation type (Altizer et al., 2006; Matthee et al., 2007). The response by ectoparasites to environmental factors are often taxon specific and related to parasite life history traits such as time spent on the host and host specificity. Lice, for instance, are permanent parasites that complete their entire life cycle on a single host and are generally host-specific (Marshall, 1981; Harbison et al., 2009), which makes them more protected against external environmental conditions. In contrast, external environmental factors are likely to have a greater effect on temporary parasites such as ticks and chiggers, as they have several free-living life stages confined to the external environment (Marshall, 1981; López-Pérez et al., 2022). Mesostigmatic mites and fleas are also temporary parasites but differ from ticks and chiggers in that free-living stages occur strictly in the nest of the host (Lehane, 2005), which may partially shelter them from harsh environmental variables such as extreme temperatures and low humidity (Froeschke et al., 2013; López-Pérez et al., 2022). Temporary parasites often have broader host ranges (Zumpt, 1961; Ledger, 1980; Durden and Musser, 1994; Horak et al., 2018), further complicating predictions of the mechanisms influencing ectoparasite diversity. Investigating parasite diversity across a wider range of vegetation types and environmental conditions is therefore likely to provide a more accurate reflection of the ectoparasite diversity associated with rodents. Documenting this diversity is also important from an epidemiological perspective, as several ectoparasite species recorded on *R. pumilio* in the Cape Floral Region (CFR) are known vectors of disease-causing pathogens such as *Babesia canis*, *Nairovirus*, *Bartonella* spp., and *Rickettsia conorii* (Walker, 1991; Lewis et al., 1996; Matthee et al., 2007; Hatyoka et al., 2019).

To address this paucity of data, the present study aimed to develop a better understanding of the ectoparasite diversity associated with two *Rhabdomys* species across the Western Cape Province of South Africa. The objective was to record ectoparasite infestation and diversity associated with *R. pumilio* in the Fynbos and Succulent Karoo biomes and *R. intermedius* in the Nama-Karoo biome of South Africa.

2. Materials and methods

2.1. Study sites and sample collection

A total of 237 *Rhabdomys* spp. individuals were trapped at 12 localities across three biomes ((Fynbos n = 5), Succulent Karoo (n = 4) and Nama-Karoo (n = 3)) in the Western Cape Province (WCP) during the spring and summer seasons in 2023, 2024 and 2025 (Fig. 1; Table 1). This sampling period was selected due to several reasons: a high proportion of adult individuals in the rodent populations, an optimal large proportion of ectoparasites and to facilitate comparisons with previous studies.

The Fynbos biome is renowned for its exceptional plant endemism and diversity (Rouget et al., 2004), and in undisturbed areas the vegetation cover is remarkably dense (Mucina and Rutherford, 2006). Rainfall is variable, averaging approximately 480 mm per annum, and occurs mainly during winter (Mucina et al., 2006), while temperatures range from a minimum of 4 °C in July to a maximum of approximately 30 °C in February (Rouget et al., 2004). The Succulent Karoo biome is characterised by sparser vegetation cover due to drier and warmer conditions, with annual rainfall ranging between 100 and 200 mm and summer temperatures reaching up to 44 °C (Mucina et al., 2006; Esler et al., 2015). The Nama-Karoo biome is the most arid of the three and has the sparsest vegetation cover, comprising grassy dwarf shrubs and Karoo bossies, most likely resulting from extreme temperature fluctuations, with daytime highs reaching 44 °C and night-time lows falling below freezing (Mucina et al., 2006).

Sherman live traps baited with a mixture of oats and peanut butter were used for trapping. Adult *Rhabdomys* individuals (>30 g) were placed in separate labelled plastic bags and euthanized with Isoflurane. Each rodent was frozen at −20 °C and then transferred and stored at −80 °C until parasite recovery.

2.2. Parasite removal

Rodent carcasses were thawed and systematically examined for all ectoparasites using fine point forceps and a Leica MZ75 stereomicroscope. All lice, fleas, mesostigmatic mites, ticks, and a subsample (due to fragility) of chigger mites were carefully removed using fine-point forceps. The parasitope (preferred attachment site) of chiggers on the host body (face, ears, body and the base of the tail) was recorded. Each parasite taxon was sorted into its main group and placed into separate tubes containing 100% ethanol for preservation. Specimens were prepared for morphological identification using standard clearing and slide mounting techniques. Mesostigmatic mites and ticks were cleared using lactic acid and gently heated over a candle flame for a few seconds until the specimens cleared. Cleared mesostigmatic mites and ticks were then mounted in polyvinyl alcohol (PVA) medium. Chiggers were mounted directly in PVA without prior clearing. All fleas (males and females) were counted, however only the male individuals that could be identified to species level were mounted and identified. Fleas were placed in 10% potassium hydroxide, dehydrated in a graded ethanol series, cleared in cedar wood oil, and mounted in Canada balsam following the method of Segerman (1995). A representative subsample of male fleas (3 to 5 individuals per morphospecies per locality, depending on availability) was also retained for DNA extraction and species confirmation (see Section 2.3). All ectoparasites were identified morphologically to

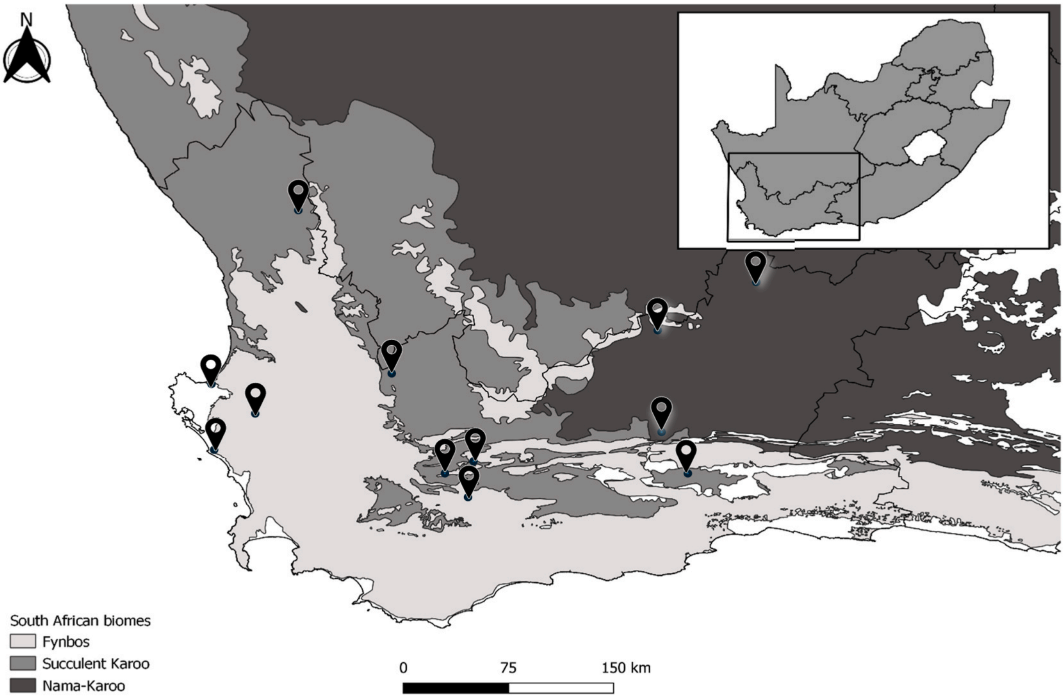

Fig. 1. Sampling localities (n = 12) for *Rhabdomys* spp. in the Fynbos (5) Succulent Karoo (4) and Nama-Karoo (3) biomes in the Western Cape, 2023-2025. Biomes are represented by different shades of grey, sampling localities are indicated by dots.

Table 1
Sampling date, locality information together with GPS coordinates, biome and sample size (N) for two different species of *Rhabdomys* trapped across three biomes in the Western Cape Province, South Africa, 2023-2025.

Date	Locality[a] and (refs code)	GPS coordinates	Biome	*R. pumilio*	*R. intermedius*	N
Dec-2023	Matjiesfontein (Wb)	33°20′28.68″S, 20°30′26.16″E	Fynbos	20	-	20
Oct-2024	Riebeek-Kasteel (Kb)	33°23′37.95″S, 18°53′24.00″E	Fynbos	14	-	14
Oct-2024	Yzerfontein (Bb)	33°18′40.14″S, 18°10′51.69″E	Fynbos	14	-	14
Jan-2025	Dwarskersbos (Dw)	32°41′36.12″S, 18°14′04.09″E	Fynbos	20	-	20
Jan-2025	Touwsrivier (Tw)	33°32′04.01″S, 20°07′06.68″E	Fynbos	20	-	20
Oct-2023	Ceres (Mf)	33°13′45.14″S, 20°34′39.58″E	Succulent Karoo	20	-	20
Nov-2023	Montagu (Ob)	33°43′34.31″S, 20°16′23.59″E	Succulent Karoo	20	-	20
Oct-2024	Vanrhysdorp (Lv)	31°29′42.36″S, 18°54′29.89″E	Succulent Karoo	22	-	22
Oct-2024	Oudtshoorn (Mw)	33°42′44.00″S, 22°02′22.21″E	Succulent Karoo	21	-	21
Nov-2023	Beaufort West (Bf)	32°15′17.98″S, 22°34′27.11″E	Nama-Karoo	-	17	17
Feb-2024	Merweville (Mg)	32°17′36.88″S, 21°39′20.98″E	Nama-Karoo	-	28	28
Oct-2024	Prince Albert (As)	32°59′40.37″S, 21°40′57.64″E	Nama-Karoo	-	21	21
Total				**171**	**66**	**237**

[a] Closest town.

species level where possible, using the following taxonomic reference keys: lice (Ledger, 1980; Durden and Musser, 1994), fleas (Segerman, 1995), and ticks (Walker et al., 2000; Apanaskevich and Horak, 2008; Apanaskevich et al., 2011). Mesostigmatic mites were identified using various taxonomic references (e.g. Till, 1963; Herrin and Tipton, 1976). Chigger identification followed Stekolnikov (2018), Stekolnikov et al. (2025a) and all relevant taxonomic literature cited in these works. This study forms part of a larger study based on sampling ectoparasites from multiple rodent species. Upon completion of the study (December 2028) representative samples of mesostigmatic mites, lice and fleas will be submitted to the respective South African reference facilities (the national mite collection housed at the Plant Protection Institute of the Agricultural Research Council, Pretoria and the Entomology collection at the National Institute for Communicable Diseases, in Johannesburg, respectively). Voucher specimens of chigger mites have already been deposited in the Laboratory of Parasitic Arthropods, Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russia).

2.3. Molecular characterisation of flea species (DNA barcoding)

DNA was extracted from 3 to 5 flea individuals per species per locality using the PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific) following the manufacturer's protocol. After DNA extraction, the exoskeleton of each specimen was mounted on a slide for morphological identification. The *COI* gene was amplified using primers Kmt6F (3′-GGAGGATTTGGAAATTGATTAGTTCC-5′ (Zurita et al., 2022) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al., 1994). All PCR reactions were performed in a total volume of 25 μl. Each reaction contained 2.5 μl of genomic DNA, 8 μl of ddH_2O, 1 μl, 0.8 μM of each primer, and 12.5 μl of Taq DNA Polymerase Master Mix Red Ampliqon (Lasec, South Africa). Amplifications were performed using standard PCR cycling conditions on a GeneAmp PCR System 2700 thermal cycler (Applied Biosystems). The thermal cycling conditions for the PCR protocol included an initial denaturation at 96 °C for 2 min followed by 40 cycles of 30 s at 94 °C for denaturation, 30 s at 50 °C for annealing, and 1 min at 72 °C for extension, followed by a final extension of 7 min at 72 °C. Molecular-grade water was used as a negative control and included in all PCR runs. PCR products (5 µl) were visualized on a 1% agarose gel with SYBR Safe DNA stain (Invitrogen) to confirm amplification. 20 µl of PCR products from all positive samples was purified using 1 µl Exonuclease I and 2 µl FastAP Thermosensitive Alkaline Phosphatase (Thermo Fisher Scientific).

Standard bi-directional Sanger sequencing was performed on selected PCR products, using the PCR primers at the Central Analytical Facilities (Stellenbosch University). Geneious v10.2.6 software (www.geneious.com) was used to assemble the forward and reverse sequences. Consensus sequences were aligned with selected published sequences of known flea species from GenBank using the MUSCLE plug-

Table 2

Life stages and infestation parameters of ectoparasite species recorded on two species of *Rhabdomys* (n = 237) across three biomes (Fynbos, Succulent Karoo and Nama-Karoo) in the Western Cape, South Africa, 2023-2025. New locality records (L) for *Rhabdomys* are included.

Species	Larvae (%)	Nymphs (%)	Adult (%)	Sex Ratio (Females/male)	Total count	Mean Abundance (±SE)	Prevalence (%)
Lice							
Polyplax arvicanthis[a]	**-**	**53**	**43**	**0.96:1**	**5388**	**22.7 (± 2.2)**	**82.7**
Fleas			**100**	**1.19:1**	**515**	**2.17 (± 0.22)**	**61.60**
Chiastopsylla carus	-	-	100	1:1.62	34	0.14 (±0.06)	8.02
Chiastopsylla coraxis	-	-	100	0.57:1	55	0.23 (±0.08)	8.02
Chiastopsylla mulleri simplex	-	-	100	1:1	24	0.10 (±0.04)	3.38
Chiastopsylla quadrisetis	-	-	100	1:1	2	0.01 (±0.01)	0.42
Chiastopsylla rossi[L]	-	-	100	1.28:1	248	1.05 (±0.13)	37.13
Dinopsylla ellobius	-	-	100	1:2	3	0.01 (±0.01)	0.84
Dinopsylla tenax[L]	-	-	100	1:1	2	0.01 (±0.01)	0.42
Listropsylla agrippinae	-	-	100	1.95:1	121	0.51 (±0.07)	23.21
Xenopsylla piriei	-	-	100	1.36:1	26	0.11 (±0.03)	6.33
Mesostigmatic mites	**-**	**12**	**88**	**15.09:1**	**1802**	**7.60 (±1.14)**	**81.43**
Androlaelaps dasymys	-	-	100	2:0	2	0.008 (±0.006)	1.27
Androlaelaps fahrenholzi	-	15	85	8.4: 1	753	3.18 (±0.99)	29.11
Androlaelaps rhabdomysi[L]	-	37	63	3.6:1	168	0.71 (±0.15)	24.89
Androlaelaps sp.	-	-	100	0:1	1	0.004 (±0.004)	0.84
Laelaps giganteus[a]	-	-	100	798:0	798	3.37 (±0.34)	61.6
Laelaps horaki[L]	-	-	100	19:0	19	0.08 (±0.06)	1.67
Laelaps nr. *keegani*	-	-	100	5:1	12	0.05 (±0.02)	3.37
Macrochelidae sp.	-	-	100	1:0	1	0.004 (±0.004)	0.84
Mygalonyssus roseinnesi[L]	-	83	17	0.6:1	48	0.2 (±0.007)	7.17
Ticks	**91**	**9**		**-**	**2855**	**12.05 (±2.16)**	**56.54**
Haemaphysalis leachi group	60	40	-	-	42	0.18 (±0.04)	10.97
Hyalomma truncatum	88	12	-	-	185	0.78 (±0.29)	12.24
Ixodes fynbonensis[L]	98	2	-	-	41	0.17 (±0.10)	3.80
Rhipicephalus nr. *follis*	94	6	-	-	295	1.24 (±0.62)	5.91
Rhipicephalus gertrudae group	90	10	-	-	2292	9.67 (±2.06)	44.73
Chiggers	**100**	**-**	**-**	**-**	**-**	**-**	**30.38**
Austracarus campestris[L]	100	-	-	-	-	-	0.42
Herpetacarus alticolus[L]	100	-	-	-	-	-	14.35
Herpetacarus sp. n.[S]	100	-	-	-	-	-	0.42
Herpetacarus spp.	100	-	-	-	-	-	0.42
Hexasternalaea hexasternalaea[L]	100	-	-	-	-	-	2.11
Hypotrombidium subquadratum[L]	100	-	-	-	-	-	6.75
Hyracarus maximus[S]	100	-		-	-	-	1.69
Hyracarus minimus[S]	100	-	-	-	-	-	1.27
Hyracarus oreillyi[S]	100	-	-	-	-	-	1.69
Hyracarus adamsi[S]	100	-	-	-	-	-	1.69
Hyracarus tanneri[S]	100	-	-	-	-	-	0.84
Hyracarus leei[S]	100	-	-	-	-	-	2.95
Hyracarus britti[S]	100	-	-	-	-	-	0.84
Hyracarus lucki[S]	100	-	-	-	-	-	0.42
Hyracarus chicoi[S]	100	-	-	-	-	-	2.95
Hyracarus spp.	100	-	-	-	-	-	3.80
Kayella bothmai[L]	100	-	-	-	-	-	0.42
Matiesacarus capensis [GS]	100	-	-	-	-	-	0.42
Rogeracarus extraordinarius [GS]	100	-	-	-	-	-	0.42
Schoutedenichia lumsdeni[L]	100	-	-	-	-	-	0.84
Schoutedenichia major[L]	100	-	-	-	-	-	0.84
Schoutedenichia morosi[L]	100	-	-	-	-	-	1.26
Schoutedenichia saturnia[S]	100	-	-	-	-	-	1.27
Schoutedenichia spp.	100	-	-	-	-	-	0.42
Zumptrombicula reductiseta[S]	100	-	-	-	-	-	7.59

[S] New species discovered in the present study that were either published in Stekolnikov et al. (2025a); Stekolnikov et al. (2026) or not yet published.

[GS] New genera and species discovered in the present study and published in Stekolnikov et al., 2025

[L] New locality records (also see Supplementary Table 3 for complete list).

[a] Cryptic linages are present in these taxa (du Toit et al., 2013; Engelbrecht et al., 2014).

in (Edgar, 2004) within Geneious. The final alignment of flea sequences consisted of 24 new *CO1*sequences from this study, and published sequences of *Xenopsylla brasiliensis* (MH142413) and *Phthiropsylla agenoris* (KM891005). The phylogenetic tree was constructed using Bayesian Inference (BI) using MrBayes version 2.2.3 (Huelsenbeck and Ronquist, 2001) plug-in in Geneious Prime v10.2.6. The best-fitting model was T92+I, as determined by MEGA X. The Markov chain Monte Carlo (MCMC) model was run using four chains of 1.1 million generations, with subsampling every 200 generations. The first 25% of trees were discarded as "burn-in," and the remaining trees were used to construct a consensus tree.

2.4. Data analysis

Analysis of data were limited to the calculation of prevalence and mean abundance of ectoparasite taxa and species recorded in the present study. These calculations following Bush et al. (1997). Prevalence was defined as the proportion of hosts infected with a particular parasite species, while mean abundance was calculated as the total number of individuals of a species divided by the total number of hosts examined, regardless of infection status. All computations were performed in Microsoft Excel using species counts per biome (except for the chiggers, for which presence and absence data were used).

3. Results

3.1. Ectoparasite species and their infestation parameters on Rhabdomys spp

A total of 237 *Rhabdomys* individuals were trapped across the Fynbos, Succulent Karoo, and Nama-Karoo biomes and examined for ectoparasites (Table 1). Species identity was assigned following du Toit et al. (2012) (*R. pumilio* = 171 and *R. intermedius* = 66). In total, 10560 ectoparasite individuals (excluding chiggers) were recorded. Lice were the most abundant (5388) followed by ticks (2855) (Table 2). An overall number of 46 ectoparasitic species (based on morphology) was recorded. Important to note however, this value is most likely an underrepresentation of the true species richness since cryptic lineages have been identified in both the louse *P. arvicanthis* (du Toit et al., 2013) and the mite species *L. giganteus* (Engelbrecht et al., 2014). Since the cryptic species have not been formally described nor sequenced in this study, all *P. arvicanthis* and *L. giganteus* have been treated monotypically (Supplementary Table 1). Chiggers were the most speciose taxon (22 species), followed by fleas and mites (nine species each) (Table 2). Lice were the most prevalent and abundant taxon (82.7%, 22.7 (±2.2)), followed by mesostigmatic mites (81.43%, 7.60 (±1.14)) (Table 2).

Only one cryptic louse species, *P. arvicanthis*, (82.7%, 22.7 (±2.2)), was recorded on the two *Rhabdomys* species (Table 2). The sex ratio was almost equal (0.96:1). Nine flea species, representing four genera, were recorded on *Rhabdomys* included herein, with a prevalence of 61.60% and abundance of 2.17 (±0.22) (Table 2). Five species belonged to the genus *Chiastopsylla*. Based on the morphological identification of male fleas, *C. rossi* (37.13%, 1.05 (±0.13)) was the most prevalent and abundant flea species, followed by *Listropsylla agrippinae* (23.21%, 0.51 (±0.07)) (Table 2). Fleas were predominantly female (sex ratio of 1.19:1). A total of nine mesostigmatic mite species were recorded on *Rhabdomys*, with a mite overall prevalence of 81.43% and abundance of 7.60 (±1.14) (Table 2). The *Androlaelaps* and *Laelaps* genera were the most species rich, with respectively four and three species. Adult females were predominantly recorded on the rodents (sex ratio of 15.09:1). *Laelaps giganteus* was the most prevalent and abundant species (61.6%, 3.37(±0.34)) followed by *Androlaelaps fahrenholzi* (29.11%, 0.51 (±0.07)) (Table 2). Four tick genera represented by five species were recorded (Table 2). Ticks had an overall prevalence of 56.54%, with *Rhipicephalus gertrudae* group being the most prevalent and abundant tick species (44.73%, 9.67 (±2.06)). *Hyalomma truncatum* (12.24%) was the second most prevalent tick while the second most abundant tick species was *Rhipicephalus* nr. *follis* (1.24 (±0.62)). For ticks in general, larvae were the most common life stage (91%), followed by nymphs (9%) (Table 2). Chiggers were represented by ten genera, with an overall prevalence of 29.96% (Table 2). *Herpetacarus alticolus* (14.35%) was the most prevalent chigger species followed by *Zumptrombicula reductiseta* (4.64%). Two genera and 14 new species were already described (Stekolnikov et al., 2025a, 2026) or still awaiting description for some included in the present study (Table 2). Sixteen new locality records were noted, with *H. alticolus* present at five localities (Table 2, Supplementary Tables 2 and 3). Chiggers were predominantly recorded on the rodent body (17 species) followed by the ear (10 species) and the tail base (9 species) (Table 3).

3.2. Ectoparasite species recorded on R. pumilio and R. intermedius

Forty-two ectoparasite species were recorded on *R. pumilio* (across the Fynbos and Succulent Karoo biomes) and the species were represented by one louse species, nine flea species, nine mesostigmatic mite species, 18 chigger species and five tick species. *Rhabdomys pumilio* is a new host record for one mesostigmatic mite (*Mygalonyssus roseinnesi*) and two chigger species (*Hypotrombidium subquadratum* and *Rogeracarus extraordinarius*) (Table 4). The total number of ectoparasite taxa was very similar between the Fynbos (29) and Succulent Karoo (30). Ectoparasite taxa abundance and prevalence values varied between localities (Supplementary Table 2) and biomes, with no consistent biome pattern recorded among *R. pumilio* in the Fynbos and Succulent Karoo biomes (Table 4, Supplementary Table 3).

Rhabdomys intermedius hosted 21 ectoparasite species that comprised

Table 3
Parasitope preference given as proportion of chigger species (n = 25) recorded on *Rhabdomys* spp. (n = 237) across three biomes (Fynbos, Succulent Karoo and Nama-Karoo) in the Western Cape, 2023-2025.

Species	Prevalence (%)	Parasitope			
		Face	Ear	Body	Tail base
Austracarus campestris	0.42	-	-	-	100
Herpetacarus alticolus	14.35	12.50	63.54	23.96	-
Herpetacarus sp. n.	0.42	16.67	-	83.33	-
Herpetacarus spp.	0.42	100	-	-	-
Hexasternalaea hexasternalaea	2.11	-	100	-	-
Hypotrombidium subquadratum	6.75	-	21.13	67. 61	11.27
Hyracarus maximus	1.69	-	100	-	-
Hyracarus minimus	1.27	-	51.72	48.28	-
Hyracarus oreillyi	1.69	-	-	78.72	21.28
Hyracarus adamsi	1.69	-	-	80	20
Hyracarus tanneri	0.84	16.67	-	83.33	-
Hyracarus leei	2.95	-	-	56.25	43.75
Hyracarus britti	0.84	31.03	-	68.97	-
Hyracarus lucki	0.42	60	-	40	-
Hyracarus chicoi	2.95	-	19.40	55.22	25.37
Hyracarus spp.	3.80	16.67	8.33	58.33	16.67
Kayella bothmai	0.42	-	-	100	-
Matiesacarus capensis	0.42	-	100	-	-
Rogeracarus extraordinarius	0.42	-	-	50	50
Schoutedenichia lumsdeni	0.84	-	100	-	-
Schoutedenichia major	0.84	-	-	100	-
Schoutedenichia morosi	1.26	-	-	57.14	42.86
Schoutedenichia saturnia	1.27	-	50	50	-
Schoutedenichia spp.	0.42	-	100	-	-
Zumptrombicula reductiseta	7.59	-	8.33	68.06	23.61
Total number of species	25	7(5[a])	12 (10[a])	18 (17[a])	10(9[a])

[a] Number of separate species, without *Herpetacarus* spp., *Hyracarus* spp., and *Schoutedenichia* spp.

Table 4
Mean abundance (MA) and prevalence (P) of ectoparasite species recorded on *R. pumilio* (Fynbos (FB) and Succulent Karoo (SK)) and *R. intermedius* (Nama-Karoo (N-K)) in the Western Cape, 2023-2025.

Species	*R. pumilio* – FB and SK		*R. pumilio* - FB		*R. pumilio* - SK		*R. intermedius* – N-K	
	MA (±SE)	P (%)	MA (±SE)	P (%)	MA (±SE)	P (%)	MA (±SE)	P (%)
Lice								
Polyplax arvicanthis	24.94 (±2.68)	85.38	13.49 (±2.03)	77.27	37.13 (±4.75)	93.98	16.95 (±3.87)	75.76
Fleas	**2.38 (±0.25)**	**68.42**	**2.24 (±0.37)**	**63.64**	**2.53 (±0.33)**	**73.49**	**1.64 (±0.47)**	**43.94**
Chiastopsylla carus	0.21 (±0.09)	11.11	0.26 (±0.16)	11.36	0.13 (±0.04)	10.84	-	-
Chiastopsylla coraxis	0.05 (±0.02)	4.68	-	-	0.11 (±0.04)	9.64	0.67 (±0.31)	16.67
Chiastopsylla mulleri simplex	0.14 (±0.06)	4.68	-	-	0.29 (±0.12)	9.64	-	-
Chiastopsylla quadrisetis	0.01 (±0.006)	0.58	-	-	0.02 (±0.02)	1.20	-	-
Chiastopsylla rossi	1.19 (±0.16)	40.94	1.30 (±0.245)	44.32	1.08 (±0.21)	37.35	0.67 (±0.23)	27.27
Dinopsylla ellobius	0.02 (±0.008)	1.17	0.01 (±0.01)	1.14	0.02 (±0.02)	1.20	-	-
Dinopsylla tenax	0.01 (±0.01)	0.58	0.02 (±0.02)	1.14	-	-	-	-
Listropsylla agrippinae	0.61 (±0.09)	32.16	0.58 (±1.13)	29.55	0.65 (±0.12)	34.94	0.24 (±0.13)	10.61
Xenopsylla piriei [H−Ri]	0.14 (±0.05)	8.19	0.07 (±0.04)	4.55	0.22 (±0.09)	12.05	0.03 (±0.02)	3.03
Mites	**8.36 (±1.53)**	**80.36**	**10.86 (±2.89)**	**77.27**	**5.71 (±0.75)**	**83.13**	**5.64 (±0.88)**	**83.33**
Androlaelaps dasymys	0.01 (±0.01)	1.17		-	0.02 (±0.02)	2.41	-	-
Androlaelaps fahrenholzi [H−Ri]	4.18 (±1.36)	36.26	7.23 (±2.60)	51.14	0.94 (±0.34)	20.48	0.59 (±0.33)	9.09
Androlaelaps rhabdomysi [H−Ri]	0.74 (±0.15)	26.90	0.23 (±0.08)	12.50	1.28 (±0.28)	42.17	0.64 (±0.36)	18.18
Androlaelaps sp.	0.01 (±0.01)	0.58	0.01 (±0.01)	1.14	-	-	-	-
Laelaps giganteus	3.10 (±0.43)	53.80	3.15 (±0.68)	48.86	3.05 (±0.50)	59.04	4.06 (±0.51)	80.30
Laelaps horaki	0.11 (±0.08)	1.75	-	-	0.23 (±0.16)	3.61	-	-
Laelaps keegani	0.07 (±0.03)	4.09	0.02 (±0.02)	1.14	0.12 (±0.06)	7.23	-	-
Macrochelidae sp.	0.01 (±0.01)	0.58	0.01 (±0.01)	1.14	-	-	-	-
Mygalonyssus roseinnesi [H−both]	0.15 (±0.05)	5.26	0.22 (±0.09)	7.95	0.07 (±0.06)	2.41	0.35 (±0.22)	10.61
Ticks	**12.56 (±2.85)**	**45.61**	**20.53 (± 4.91)**	**59.09**	**4.08 (±2.45)**	**31.33**	**10.72 (±2.35)**	**84.84**
Haemaphysalis leachi group	0.19 (±0.05)	11.11	0.19 (±0.06)	13.64	0.18 (±0.09)	8.43	0.15 (±0.07)	10.60
Hyalomma truncatum [H−Ri]	0.25 (±0.13)	7.02	0.47 (±0.25)	12.5	0.01 (±0.01)	1.20	2.17 (±0.97)	25.76
Ixodes fynbonensis	0.24 (±0.13)	5.26	0.47 (±0.25)	10.22	-	-	-	-
Rhipicephalus nr. *follis*	1.60 (±0.85)	7.02	3.10 (±1.63)	13.64	-	-	0.33 (±0.32)	3.03
Rhipicephalus gertrudae group	10.28 (±2.74)	31.58	16.30 (±4.74)	35.22	3.89 (±2.41)	27.71	8.09 (±2.12)	78.79
Chiggers	-	**32.75**	-	**19.32**		**46.99**		**24.24**
Austracarus campestris [H−Ri]	-	-	-	-	-	-	-	1.52
Herpetacarus alticolus [H−Ri]	-	16.37	-	3.41	-	30.12	-	9.09
Herpetacarus sp. n.	-	0.58	-	1.14	-	-	-	-
Herpetacarus spp.	-	0.58	-	1.14	-	-	-	-
Hexasternalaea hexasternalaea [H−Ri]	-	-	-	-	-	-	-	7.58
Hypotrombidium subquadratum [H−both]	-	7.02	-	1.14	-	13.25	-	6.06
Hyracarus maximus	-	2.34	-	-	-	4.82	-	-
Hyracarus minimus	-	1.75	-	-	-	3.61	-	-
Hyracarus oreillyi	-	2.34	-	4.55	-	-	-	-
Hyracarus adamsi	-	2.34	-	-	-	4.82	-	-
Hyracarus tanneri	-	1.17	-	2.27	-	-	-	-
Hyracarus leei	-	4.09	-	-	-	8.43	-	-
Hyracarus britti	-	1.17	-	2.27	-	-	-	-
Hyracarus lucki	-	0.58	-	1.14	-	-	-	-
Hyracarus chicoi	-	0.58	-	-	-	1.20	-	9.09
Hyracarus spp.	-	5.26	-	4.55	-	6.02	-	-
Kayella bothmai [H−Ri]	-	-	-	-	-	-	-	1.52
Matiesacarus capensis	-	0.58	-	-	-	1.20	-	-
Rogeracarus extraordinarius [H−Rp]	-	0.58	-	-	-	1.20	-	-
Schoutedenichia lumsdeni [H−Ri]	-	-	-	-	-	-	-	3.03
Schoutedenichia major	-	1.17	-	2.27	-	-	-	-
Schoutedenichia morosi	-	1.75	-	-	-	3.61	-	-
Schoutedenichia saturnia	-	1.17	-	2.27	-	-	-	1.52
Schoutedenichia spp.	-	-	-	-	-	-	-	1.52
Zumptrombicula reductiseta	-	10.53	-	1.14	-	20.48	-	-
Total number of species		44(42[a])		31(29[a])		31(30[a])		22(21[a])

[H−Rp] New host record for *R. pumilio*.
[H−Ri] New host record for *R. intermedius*.
[H−both] New host records for both rodent species.
[a] Number of separate species, without *Herpetacarus* spp., *Hyracarus* spp., and *Schoutedenichia* spp.

of one louse species, four flea species, four mesostigmatic mite species, eight chigger species and four tick species. *Rhabdomys intermedius* is a new host record for one flea (*Xenopsylla piriei*), three mesostimatic mites (*A. fahrenholzi*, *Androlaelaps rhabdomysi* and *M. roseinnesi*), one tick species (*H. truncatum*) and six chigger species (*Austracarus campestris*, *Herpetacarus alticolus, Hexasternalaea hexasternalaea, Hypotrombidium subquadratum, Kayella bothmai* and *Schoutedenichia lumsdeni)* (Table 4). Across ectoparasite taxa, 36 new locality records were noted

(Supplementary Table 3).

3.3. *Molecular characterisation of host and flea species*

A total of 24 *COI* sequences were successfully generated for seven flea species (GenBank Accession Numbers PZ126503-PZ126526). Phylogenetic analysis of the *COI* sequences revealed the presence of four distinct groups that represent four genera (*Chiastopsylla*, *Xenopsylla*, *Listropsylla*, or *Dinopsylla*) as supported by the morphological identifications (Fig. 2).

4. Discussion

4.1. *Ectoparasite diversity*

The study recorded a high diversity of ectoparasites, comprising 46 taxa (44 nominate species and two tick species groups that could not be identified with certainty). This includes 36 new locality records and 14 new host records for *Rhabdomys* ectoparasites and reveals the presence of 13 species described (or awaiting description) as new to science. These findings support the prediction that the current ectoparasite diversity associated with rodent species in the genus *Rhabdomys* is underestimated, and that investigating parasite diversity across a wider range of vegetation types and environmental conditions provides a more accurate reflection of the ectoparasite assemblages associated with this rodent genus. The high diversity associated with *R. pumilio* and *R. intermedius* is most probably related to the ability of these rodents to adapt to and exploit diverse landscapes (Matthee et al., 2007; Froeschke et al., 2013; van der Mescht and Matthee, 2017). This elevated ectoparasite diversity aligns with previous research conducted on *R. pumilio* in the CFR (Matthee et al., 2007, 2010; Froeschke et al., 2013) and with a regional study that recorded 21 flea species on several *Rhabdomys* species (van der Mescht and Matthee, 2017). More specifically, Matthee et al. (2007) recorded 33 ectoparasite species on *R. pumilio* (n = 510) at several inland localities, while Matthee et al. (2010) recorded 20 species on *R. pumilio* (n = 41) at a single coastal locality.

In the present study, *P. arvicanthis* was the only louse species recorded (based on morphology), and it was among the most prevalent and abundant ectoparasite species on *Rhabdomys* overall (82.7%), as well as individually on *R. pumilio* (85.38%) and *R. intermedius* (75.76%). The high occurrence of *P. arvicanthis* is supported by previous studies that reported prevalences of 59.20% and 69.43% on *R. pumilio* in the CFR (Matthee et al., 2007, 2010). The prevalence of *P. arvicanthis* differed among biomes, with the highest prevalence recorded in the Succulent Karoo (93.98%), followed by the Fynbos (77.27%) and the Nama-Karoo (75.76%). The occurrence of this louse in all three biomes and at consistently high infestation levels suggests that *P. arvicanthis sensu stricto* is tolerant of a wide range of environmental conditions.

Nine flea species were recorded in the present study. This is comparable to the eight flea species recorded on *R. pumilio* in the CFR (Matthee et al., 2007), but fewer than the 14 species recorded on *R. pumilio* sampled across the Fynbos (six localities) and Succulent Karoo (five localities) by van der Mescht and Matthee (2017). This difference may be attributed to the larger rodent sample size (250 individuals) in the latter study compared to the present study (Fynbos: 88; Succulent Karoo: 83). Fleas were more prevalent on *R. pumilio* in the present study (68.42%) than in the CFR study by Matthee et al. (2007) (46.7%), possibly reflecting the larger geographic extent of the present study relative to the more localised sampling of the earlier work. In the present study, three (*Chiastopsylla coraxis*, *C. rossi* and *Listropsylla agrippinae*) of the four flea species recorded on *R. intermedius* had previously been reported from this host (van der Mescht and Matthee, 2017). That study also recorded *Xenopsylla eridos* on *R. intermedius*; however, this species was absent in the present study and was replaced by *X. piriei*. It is likely

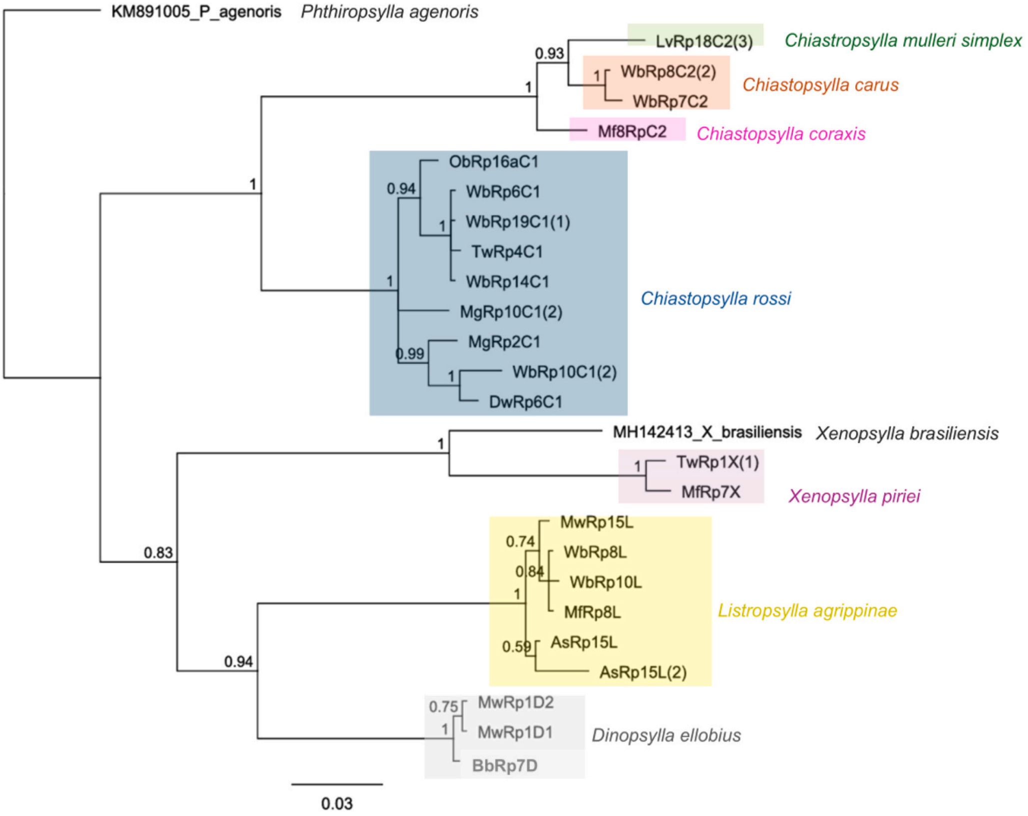

Fig. 2. Bayesian inference (BI) of partial *COI* gene sequences of fleas recorded on *Rhabdomys* spp. Posterior probabilities (>0.70) are indicated at nodes. Accession numbers are indicated for published sequences (*Phthiropsylla agenoris* and *Xenopsylla brasilliensis*) from GenBank. The *COI* sequence of *Phthiropsylla agenoris* is used as an outgroup.

that flea diversity associated with *R. intermedius* is currently underestimated, as both the present study and that of van der Mescht and Matthee (2017) were based on only two to three localities in the Nama-Karoo. The dominance of *C. rossi* and *L. agrippinae* on *R. pumilio* in the present study supports previous records from the CFR (Matthee et al., 2007) and broader regional patterns for *R. pumilio* (van der Mescht and Matthee, 2017). *Chiastopsylla rossi* was also the most common flea species on *R. intermedius*, followed by *C. coraxis* and *L. agrippinae*. Both *C. rossi* and *L. agrippinae* have wide host ranges that include at least three *Rhabdomys* species (*R. bechuanae*, *R. intermedius* and *R. pumilio*), as well as other co-occurring rodents such as the vlei rat (*Otomys irroratus*), bush Karoo rat (*O. unisulcatus*), and Namaqua rock mouse (*Micaelamys namaquensis*) (Segerman, 1995; van der Mescht and Matthee, 2017). *Chiastopsylla coraxis* appears to prefer more xeric habitats such as the Nama-Karoo and Succulent Karoo (Segerman, 1995; van der Mescht and Matthee, 2017), which may explain its higher occurrence on *R. intermedius*. According to Segerman (1995), *O. unisulcatus* is the primary host of *C. coraxis*, with *Rhabdomys* species acting as secondary hosts. Both *M. namaquensis* and *O. unisulcatus* were trapped at the same localities as *R. pumilio* and *R. intermedius* in the present study. The occurrence of *X. piriei* on *R. pumilio* confirms earlier records (Segerman, 1995), although this species was not recorded in previous empirical studies on *R. pumilio* or *R. intermedius* (Matthee et al., 2007, 2010; van der Mescht and Matthee, 2017). In the present study, *X. piriei* occurred mainly in the Succulent Karoo and Nama-Karoo, suggesting adaptation to xeric environments, a pattern consistent with the absence of records from the more mesic southern CFR (Segerman, 1995). Five flea species (*C. carus*, *C. mulleri simplex*, *C. quadrisetis*, *Dinopsylla ellobius* and *D. tenax*) were recorded exclusively on *R. pumilio*. *Chiastopsylla carus* and *C. mulleri simplex* have previously been recorded on *R. pumilio* (Segerman, 1995; van der Mescht et al., 2016; van der Mescht and Matthee, 2017). *Dinopsylla ellobius* exhibited broader environmental tolerance, occurring on *R. pumilio* in both the Fynbos and Succulent Karoo, whereas *D. tenax* was confined to the Fynbos, consistent with earlier findings (Segerman, 1995; van der Mescht and Matthee, 2017). *Dinopsylla ellobius* also shows a broader host range, including gerbils (*Gerbilliscus* spp.), *Otomys* spp., multimammate mice (*Mastomys* spp.) and *Rhabdomys* spp., whereas *D. tenax* appears more host-specific to *O. unisulcatus* and *R. pumilio* (Segerman, 1995). The presence of *C. quadrisetis* on *R. pumilio* may be incidental, as *O. unisulcatus* is its primary host (Segerman, 1995) and was trapped at most localities. This study provides two new locality records for *C. rossi* and *D. tenax*. Three flea species (*D. ellobius*, *C. rossi* and *X. piriei*) recorded in this study are of veterinary and medical importance due to their potential role in transmitting *Yersinia pestis* (Segerman, 1995).

Nine mesostigmatic mite species were recorded on *Rhabdomys* spp., including *R. pumilio*. Species richness and prevalence on *R. pumilio* (80.36%) and *R. intermedius* (83.33%) were comparable to previous studies conducted in the CFR (Matthee et al., 2007, 2010). The high prevalence of *Laelaps giganteus* on *R. pumilio* (53.80%) and *R. intermedius* (80.30%) is consistent with earlier findings (Matthee et al., 2007, 2010; Froeschke et al., 2013) and with molecular evidence demonstrating strong co-divergence between *L. giganteus* and four *Rhabdomys* species in South Africa (Engelbrecht et al., 2016). *Androlaelaps fahrenholzi* was the most abundant mesostigmatic mite species on *R. pumilio*, particularly within the Fynbos biome. *Rhabdomys intermedius* represents a new host record for *A. fahrenholzi*. This association is supported by previous studies on *R. pumilio* in the CFR (Matthee et al., 2007, 2010). *Androlaelaps fahrenholzi* has a broad host range and wide African distribution (Tipton, 1960; Zumpt, 1961; Till, 1963; Matthee et al., 2007), with recent records from Zambia indicating close associations with *Lemniscomys striatus* and *Lophuromys stanley* (Babyesiza et al., 2023). *Androlaelaps rhabdomysi* was common on both *R. pumilio* (26.90%) and *R. intermedius* (18.18%). *Rhabdomys pumilio* in the CFR is the type host for this species (Matthee et al., 2007), and *R. intermedius* represents a new host record. The species appears to have a broad host range, having been recorded at 25% prevalence on *M. namaquensis* across South Africa (Stevens et al., 2022). Its occurrence across all three biomes further highlights its wide geographic distribution. In the present study, *Mygalonyssus roseinnesi* (formerly placed in *Ornithonyssus*) was recorded on both *R. pumilio* and *R. intermedius*. This species was previously recorded from *Rhabdomys bechuanae* (then considered *R. pumilio*) at Goshen in the Cape Province (Shepherd and Narro, 1983). Both *R. pumilio* and *R. intermedius* therefore represent new host records. *Mygalonyssus roseinnesi* has also been recorded on *M. namaquensis* (Shepherd and Narro, 1983; Stevens et al., 2022). *Laelaps horaki* was recorded only once on *R. pumilio* at Montagu in the Succulent Karoo. *Rhabdomys pumilio* in the CFR is the type host for this species (Matthee and Ueckermann, 2009). The present study provides 10 new locality records for *A. rhabdomysi* in the Western Cape (Matthee and Ueckermann, 2008; Stevens et al., 2022) and one new locality record for *L. horaki* in the Succulent Karoo.

Five tick species were recorded on *Rhabdomys* spp. and *R. pumilio*, and four species on *R. intermedius*. Species richness was lower than the nine and 10 species previously recorded on *R. pumilio* in the CFR (Matthee et al., 2007, 2010). The larger sample size and greater habitat diversity (natural and remnant fragments) in the study by Matthee et al. (2007) may explain this difference. In addition, Matthee et al. (2010) sampled a pristine coastal Fynbos reserve with high wildlife diversity, including antelope species that serve as essential hosts for many tick life cycles (Horak et al., 2018). *Rhipicephalus gertrudae* group was the most prevalent taxon on *R. pumilio* (31.58%) and *R. intermedius* (78.79%), occurring in all three biomes. A similar pattern was observed for *Haemaphysalis leachi* group. The difficulty of identifying tick larvae and nymphs to species level due to morphological stasis is well documented (Walker et al., 2000; Smith et al., 2023). High prevalences of both tick groups are consistent with previous CFR studies (Matthee et al., 2007, 2010; Froeschke et al., 2013). *Hyalomma truncatum* was common on *R. pumilio* in the Fynbos and on *R. intermedius*, supporting previous associations with the Fynbos biome (Matthee et al., 2007, 2010) and suggesting tolerance of more xeric habitats (Stevens et al., 2022). *Ixodes fynbonensis* was recorded on *R. pumilio* at a single Fynbos locality. As *R. pumilio* in the CFR is the type host (Apanaskevich et al., 2011), this species may show biome specificity, although further study is required. Ticks are major vectors of human and animal pathogens (de la Fuente et al., 2008), and in South Africa both *H. leachi* group and *H. truncatum* are of medical and veterinary importance (Walker, 1991; Hoogstraal, 1979; Penzhorn et al., 2020).

The present study is the first to conduct species-level identifications of chiggers associated with *Rhabdomys* spp. Earlier work by Barnard et al. (2015) identified chiggers only to genus level. Chiggers accounted for almost 50% (22 species) of the total ectoparasite diversity, with 18 species on *R. pumilio* and eight on *R. intermedius*. Barnard et al. (2015) recorded seven morphospecies from two CFR localities. Given that chiggers are often habitat specialists (Shatrov and Kudryashova, 2006), the higher species richness observed here likely reflects the broader geographic sampling across multiple biomes. Similar patterns have been documented in other rodent studies in South Africa (Stevens et al., 2022; Smith et al., 2023; Little et al., 2024) and elsewhere (Antonovskaia et al., 2024; Binh et al., 2020; Stekolnikov et al., 2022; Liu et al., 2024, 2025). Fourteen species are new to science, with thirteen recently described (Stekolnikov et al., 2025a, 2026) and one awaiting description. Sixteen new locality records and eight new host records were documented, reinforcing concerns that limited research attention and taxonomic expertise constrain knowledge of ectoparasite diversity (Stevens et al., 2022). The genus *Hyracarus* was the most species-rich, represented by nine species, all of which were described as new to science. *Hyracarus* is endemic to Africa, with recent discoveries on South African rodents (Stekolnikov et al., 2025a, 2025b). *Schoutedenichia* was the second most species-rich genus, with four species, including the newly described *S. saturnia*. Host and locality records for additional chigger taxa further expand known distributions. Although chiggers are generally habitat-specific, some species exhibit broader tolerance. In this study, *Herpetacarus alticolus* showed the widest distribution, occurring across all three biomes. Numerous new species, locality records and host associations were documented, substantially expanding knowledge of chigger diversity on *Rhabdomys* spp. Most chigger species were recorded on the body, followed by the ear and tail base, consistent with findings by Barnard et al. (2015). In contrast, studies conducted largely in summer rainfall regions reported a preference for the ear (Fagir et al., 2014; Matthee et al., 2020; Stevens et al., 2022; Smith et al., 2023; Little et al., 2024). Although speculative, this may indicate an environmental influence on parasitope preference, warranting further investigation.

4.2. Flea genetic confirmation

This study presents the first *COI* barcodes for species in the genera *Chiastopsylla*, *Listropsylla* and *Dinopsylla*, as well as the first sequences for *X. piriei*. The phylogenetic pattern corresponded closely with documented morphological differences. The separation of *C. rossi* from *C. mulleri simplex*, *C. carus* and *C. coraxis* aligns with the *numae* and *mulleri* group divisions described by Segerman (1995). *Chiastopsylla rossi* is characterised by a sclerotised interantennal groove extending to the occiput and by seven to eight dorsal notches on the hind tibiae, whereas *mulleri* group species lack the groove and possess only six notches. The phylogenetic separation of *X. piriei*, *L. agrippinae* and *D. ellobius* reflects distinct morphological traits. *Xenopsylla piriei* lacks a genal and pronotal comb and has a long, flat medium lamella and strongly curved penile rods with a digitoid tip.

Within *Chiastopsylla*, two monophyletic, well-defined clusters were obtained in the phylogenetic analyses. One clade contained *C. mulleri simplex*, *C. carus*, and *C. coraxis* and the second clade contained sequences that support the morphological identification of *C. rossi* (Fig. 2). Newly generated sequences for *Xenopsylla piriei* clustered with a reference sequence *X. brasiliensis.* Differences among sequences were also evident in the varying branch lengths observed between sister taxa, suggesting genetic divergence within otherwise morphologically similar groups.

The monophyletic grouping of *X. piriei* with the *Xenopsylla brasiliensis* reference sequences provides further support for the morphological species identifications as species in the genus *Xenopsylla* and specifically within the species groups comprising *X. brasiliensis,* and *X. piriei*. This assemblage is morphologically characterised by a combination of the following features: suture between the metasternum and metepisternum well marked; hind coxa with the posterior-apical margin strongly concave; genal lobe smoothly rounded; eye nearly always well developed and pigmented and the absence of a genal and pronotum comb. *Listropsylla agrippinae* retains a pronotal comb but lacks a genal comb and has short, thick lamellae and stout penile rods, while *D. ellobius* shows a strongly developed pronotal comb, broadly sclerotised lamellae and reduced, asymmetrical penile rods (Segerman, 1995). These diagnostic characters corroborate the genetic separation observed, confirming the reliability of morphological identification across collection localities.

Differences among sequences were also evident in the varying branch lengths observed between sister taxa, suggesting genetic divergence within otherwise morphologically similar groups.

5. Conclusion

This study documents a high diversity of ectoparasite species associated with only two *Rhabdomys* species in South Africa. The discovery of numerous undescribed chigger species and multiple new host and locality records highlight the largely unexplored nature of ectoparasite diversity within this mite family. The generation of 24 *COI* barcodes spanning four flea genera represents a significant advance in developing reference libraries to support morphological identification. Comprehensive assessments of rodent ectoparasite diversity require extensive geographic sampling combined with integrative approaches incorporating both alpha taxonomy and molecular systematics. Building on to foundational biodiversity research, future studies should aim to include comparable sample sizes across multiple biomes and seasons to facilitate more in-depth statistical analyses and inferences on the ecological factors that shape ectoparasite infestations across the landscape.

CRediT authorship contribution statement

Lola Singo: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Conrad A. Matthee:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Funding acquisition. **Mamohale E. Chaisi:** Writing – review & editing, Validation, Supervision, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Alexandr A. Stekolnikov:** Writing – review & editing, Methodology, Investigation. **Edward A. Ueckermann:** Writing – review & editing, Methodology, Investigation. **Sonja Matthee:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Ethics and permits

The study was approved by Animal Care and Use Ethics Committee of Stellenbosch University (reference numbers: ACU-2022-17062, ACU-2024-30885). A Section 20 permit from Department of Agriculture, Land Reform and Rural Development (no:12/11/1/7/5 (b) (6091 MG), as well as a collection permit from Cape Nature (Western Cape Provincial Nature Conservation authority) CN44-87-27373 and CN44-87-33283.

Declaration of generative AI and AI-assisted technologies in the writing process

After the preparation of this work the author(s) used ChatGPT in order to improve readability and language in certain sections of the manuscript. After using this tool/service, the author(s) responsibly reviewed and edited the content as needed.

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Declarations of interest

We declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2026.101217.

Data availability

All data generated and/or analysed during this study are presented within this article. Additional datasets are available from the corresponding author upon reasonable request. Genbank sequences will be available.

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