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RESEARCH ARTICLE

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Genetic diversity of rodent species sold in South African pet shops

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Abstract

Murid rodents are considered globally important invasive species, yet they are still sold in the pet trade. Little is known about the genetic diversity of traded rodents, and many species are incorrectly identified in the pet trade. We used mitochondrial gene regions to assess the taxonomy and genetic diversity of 149 rodents sold in pet shops across eight South African provinces. We identified a total of 112 specimens as *Mus musculus*, while 31 were *Rattus norvegicus*, and six were identified as the southern African endemic, southern multimammate mouse *Mastomys coucha*. Phylogenetic analyses revealed that the three species were monophyletic. *Mus musculus* and *R. norvegicus* showed higher levels of genetic diversity, with 19 unique mtDNA haplotypes recovered for *M. musculus* and eight haplotypes for *R. norvegicus*. KwaZulu-Natal, Western Cape and Gauteng Provinces had the most unique haplotypes than other provinces. Our findings showed that non-native species are widely distributed in the South African pet trade industry, while *M. coucha* was not widely traded, although recorded in three provinces. This suggests that most provinces comply with the trade regulations on native species, but the threat of invasive rodents to South Africa's unique biodiversity is highlighted.

KEYWORDS
invasion, management, mitochondrial DNA, pet rodent, trade patterns

Résumé

Les rongeurs de type muridés sont considérés comme des espèces envahissantes mondialement importantes, mais ils sont toujours vendus dans le commerce des animaux de compagnie. La diversité génétique des rongeurs commercialisés est peu connue et de nombreuses espèces sont mal identifiées dans le commerce des animaux de compagnie. Nous avons utilisé les régions du gène mitochondrial pour évaluer la taxonomie et la diversité génétique de 149 rongeurs vendus en animalerie dans huit provinces d'Afrique du Sud. Nous avons identifié un total de 112 spécimens comme étant des Mus musculus, tandis que 31 étaient des Rattus norvegicus, et six ont été identifiés comme étant la souris multimammate endémique d'Afrique australe, Mastomys coucha. Des analyses phylogénétiques ont permis de constater que les trois espèces étaient monophylétiques. *Mus musculus et R. norvegicus présentaient des niveaux plus élevés de diversité génétique, avec 19 haplotypes d'ADNmt uniques retrouvés pour M. musculus et huit haplotypes pour R. norvegicus*. Les provinces de KwaZulu-Natal, Western Cape et Gauteng présentaient les haplotypes les plus uniques par rapport aux autres provinces. Les conclusions de notre étude montrent que les espèces non indigènes sont largement distribuées dans l'industrie sud-africaine du commerce des animaux de compagnie, tandis que le M. couchaw n'était pas largement commercialisé, bien qu'il ait été enregistré dans trois provinces. Ces conclusions suggèrent que la plupart des provinces respectent les réglementations commerciales relatives aux espèces indigènes, mais la menace que représentent les rongeurs envahissants pour la biodiversité unique de l'Afrique du Sud est mise en évidence.

1 | INTRODUCTION

Many rodent species have been accidentally or intentionally introduced worldwide through different anthropogenic-mediated pathways (Carpio et al., 2020; Long, 2003). These pathways include transportation of goods (via aeroplanes, trains and ships), food, biological control, fur markets, aesthetics, research and the pet trade (Barun et al., 2011; Measey et al., 2020; Perry et al., 2006). Several rodent species have become invasive through these pathways, for example the Gambian pouched rat (*Cricetomys gambianus*), house mouse (*Mus musculus*) and Norwegian rat (*Rattus norvegicus*) (Engeman et al., 2006; Measey et al., 2020). The latter two are amongst the most common small mammal species kept as pets worldwide (Lankau et al., 2017; Maligana et al., 2020; Mori et al., 2017; Shivambu et al., 2021a). These species are also sold as feeders for other non-native pets such as reptiles (snakes, lizards and turtles), amphibians (frogs and toads) and invertebrates (spiders) (Cartwright et al., 2016; Cooper & Williams, 2014; Kanagarajah et al., 2018; Rawski et al., 2018; Sincage & Hardin, 2015).

Mus musculus and *R. norvegicus* have been introduced to every continent except Antarctica (Atkinson, 1985; Berry, 1968; Vadell et al., 2014), and these pests have decimated seabird populations on numerous islands globally, for example, some of the sub-Antarctic islands (Angel et al., 2009; Rowe-Rowe et al., 1989). *Mus musculus* and *R. norvegicus* have also been implicated in the extinction of several reptile, bird and insect species, especially on islands, through predation of eggs and live animals (Cuthbert & Hilton, 2004; Dagleish et al., 2017; Marris, 2000). These rodent species are important agricultural pests worldwide (Stejskal et al., 2016; Vadell et al., 2010). In addition, both pet or wild rats and mice pose health risks to humans as they spread zoonotic diseases such as salmonellosis and rat-bite fever (Harker et al., 2011; Julius et al., 2021; Rabiee et al., 2018; Stehle et al., 2003). They also cause damage to building infrastructure, fittings and mouldings and household goods by gnawing on them (Garba et al., 2014; Panti-May et al., 2017; Sidorov & Putin, 2010; Yonas et al., 2010). The invasion success of these two rodents is linked to human habitation as they exploit these habitats for food and shelter (Sacchi et al., 2008; Vadell et al., 2010).

In South Africa, *M. musculus* and *R. norvegicus* distributions are associated with high-density urban areas (Bastos et al., 2011; Shivambu et al., 2021b; Taylor et al., 2008). The introduction of *M. musculus* has been linked with the early shipping to South Africa, while *R. norvegicus* is thought to have arrived via Asian and European shipping routes (Measey et al., 2020). Presently, these non-native rodents dominate the pet trade industry as they are sold as both pets and feeders in the country (Maligana et al., 2020; Shivambu et al., 2021a). Native rodent species, such as the Natal multimammate mouse *Mastomys natalensis* and southern multimammate mouse *Mastomys coucha*, are also used for pet trade purposes in South Africa (du Plessis et al., 2016). Tracking trade in these species is complicated because *M. natalensis* and *M. coucha* are similar morphologically but can be accurately distinguished using molecular analyses (Bastos et al., 2005; Kneidinger et al., 2014).

Unfortunately, many species are incorrectly identified or mislabelled in the pet trade as a result of cryptic species and the lack of taxonomic expertise by the traders (Gehring et al., 2018; Gerson et al., 2008; Maligana et al., 2020; Nelufule et al., 2020; Sanders et al., 2008; Shivambu et al., 2020). Consequently, the lack of correct taxonomic information on traded species poses challenges in enforcing regulations regarding their shipment into a foreign country (Lankau et al., 2017). In addition, accurate species identification is important for effective conservation, especially for endangered species, which may be traded as pets (Mishra et al., 2017; Nagy et al., 2012; Wenner et al., 2012). Taxonomic uncertainty also limits the ability to assess accurately the scale of species used in the pet trade industry (Ng et al., 2016; Strecker et al., 2011).

Presently, populations of *M. coucha*, *M. musculus*, *M. natalensis* and *R. norvegicus* are not threatened by the pet trade industry as these species are all listed as least concern (Cassola, 2016;

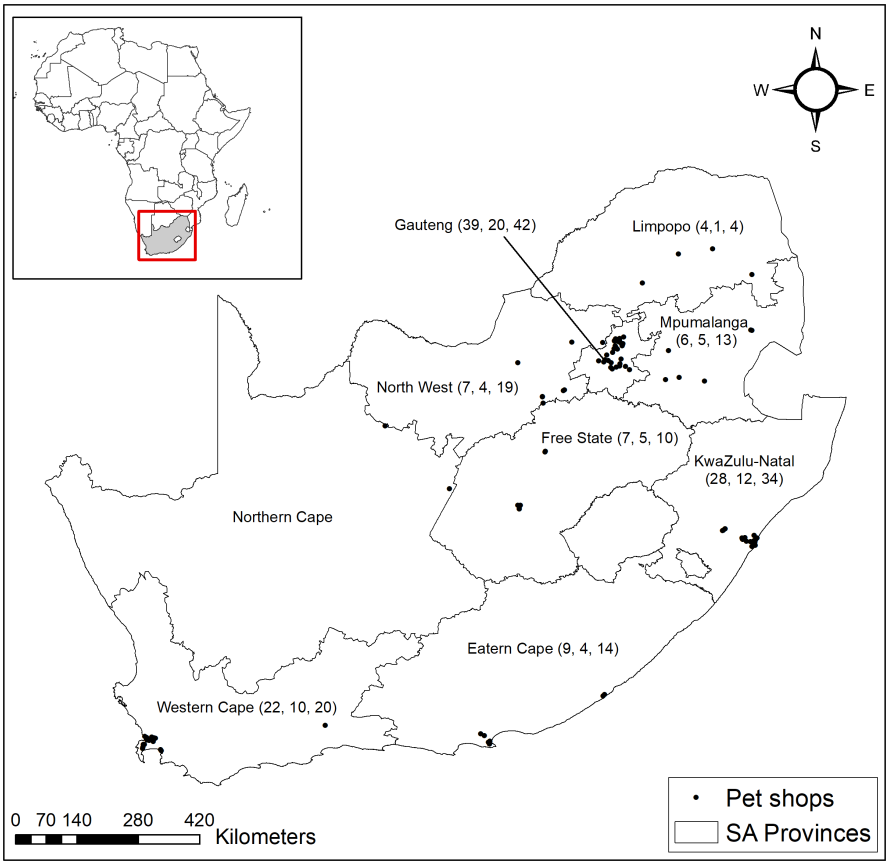

FIGURE 1 Map of South Africa showing physical pet shops visited in September 2019. Numbers in parentheses indicate the total number of shops per province, the number of shops selling frozen rodents and the number of samples gathered.

TABLE 1 Details of primers used to amplify each gene region in the current study. The size of the amplified PCR fragment and details of cycling conditions used are also provided. References for primers used are provided below the table

Region	Primer name	Primer sequence (5′-3′)	Amplified fragment size	PCR conditions
Cyt b (1)	L14723 (F) H15915 (R)	CCA ATG ACA TGA AAA ATC ATC GTT TCT CCA TTT CTG GTT TAC AAG AC	890 bp	1. 96°C for 20 s 2. 96°C for 12 s, 49°C for 25 s, 72°C for 60 s (X2) 3. 96°C for 12 s, 47°C for 20 s, 72°C for 55 s (X5) 4. 96°C for 12 s, 45°C for 15 s, 72°C for 50 s (X35) 5. 72°C for 1 min
CO1 (2)	LCO1490 (F) HCO2198 (R)	GGT CAA CAA ATC ATA AAG ATA TTG G TAA ACT TCA GGG TGA CCA AAA AAT CA	637 pb	1. 94°C for 1 min 2. 94°C for 30 s, 50°C for 30 s, 72°C for 60 s (X35) 3. 72°C for 10 min
16S rRNA (3)	16SA (F) 16SB (R)	CGC CTG TTT ATC AAA AAC AT CCG GTC TGA ACT CAG ATC ACG T	433 bp	1. 94°C for 2 min 2. 94°C for 30 s, 50°C for 45 s, 68°C for 60 s (X35) 3. 68°C for 5 min
CR (4)	N777 (F) DLH1 (R)	TAC ACT GGT CTT GTA AAC C ATC CTC TCT CTG CAG CAC ATT TCC	433 bp	1. 94°C for 3 min 2. 94°C for 30 s, 48°C for 30 s, 68°C for 1:30 s (X35) 3. 68°C for 10 min

Note: References: [1] Ducroz et al. (2001), [2] Folmer et al. (1994), [3] Palumbi et al. (1991), [4] Alpers et al. (2004).

Granjon, 2016; Musser et al., 2016; Ruedas, 2016). However, these species' trade poses health risks to humans as they are reservoirs for zoonotic diseases (Bastos et al., 2005; Harker et al., 2011; Julius et al., 2021; Lecompte et al., 2006; Rabiee et al., 2018; Skinner & Chimimba, 2005). In addition, non-native rodents may become invasive through pet trade releases and escapes in South Africa. For example, Maligana (2018) observed colonies of red-eyed albino rats typical of laboratory and pet rat strains living in feral conditions in Alexandra township, South Africa. A study by Julius (2013) based on Cyt b gene also revealed that *R. norvegicus* haplotype was closely linked to both wild and laboratory strains, suggesting possible introduction from Indonesia by escaped laboratory rats, subsequently

TABLE 2 Genetic variability of the mitochondrial DNA regions (16S rRNA, CO1, Cyt b and CR) and combined data set of the rodent species sold in the South African pet shops

Variables	*Mastomys coucha*					*Mus musculus*					*Rattus norvegicus*				
	16S rRNA	CO1	Cyt b	CR	Combined	16S rRNA	CO1	Cyt b	CR	Combined	16S rRNA	CO1	Cyt b	CR	Combined
Total number of individuals	5	5	6	6	6	112	71	93	112	112	31	2	25	31	31
Total base pair	525	637	890	433	2485	525	637	890	433	2485	525	637	890	433	2485
Conservative site	506	620	888	425	2439	504	604	868	417	2393	502	619	870	424	2415
Variables sites	0	6	0	2	8	9	33	22	12	76	3	0	20	4	27
Parsimonious informative sites	0	0	0	0	0	4	13	9	7	33	1	0	6	3	10
Singleton site	0	6	0	2	8	5	20	13	5	43	2	0	14	1	17
Nucleotide composition (%)															
T (%)	28.1	30.0	27.8	31.9	29.5	30	29.7	30	30.1	30.0	27.5	29.7	27.6	31.5	29.1
C (%)	19.2	26.0	26.7	26.8	24.7	18.3	24.7	26.7	25.2	23.7	19.3	26.8	30.3	25.8	25.6
A (%)	33.7	29.4	32.5	30.9	31.6	32.4	29.8	30.6	33.3	31.5	33.9	27.8	29.4	31.0	30.5
G (%)	19.0	14.6	13.0	10.4	14.3	19.3	15.8	12.7	11.4	14.8	19.3	15.7	12.6	11.7	14.8

forming a distinctive haplotype in South Africa. This suggests that these rats may have high-genetic diversity that may increase their establishment, spread and adaption to new habitations, as reported for other invasive species with high genetic variation (Stepien et al., 2005). Assessing the genetic structure of non-native species is useful in determining the origin or source of the introduced populations and evaluating the rate of invasion success (Campbell et al., 2019; Collins et al., 2002). In this study, we used four mitochondrial markers (Cytochrome b (Cyt b), cytochrome oxidase subunit 1 (CO1), 16S ribosomal RNA (16S rRNA) and the hypervariable control region (CR)) to identify and assess the genetic diversity of captive rodent populations in South Africa. We also investigated the geographic distribution pattern of rodent species' genetic diversity to determine the sources of breeding stocks.

2 | METHODS

2.1 | Sample collection

Pet shops sell frozen and live rodents as food for predator pets such as snakes, lizards and tarantulas. We surveyed a total of 122 pet shops which sell small mammals across South Africa in September 2019. Only 61 shops sold frozen rodents, and therefore, we purchased between two to five frozen mice and rats from each pet shop (Figure 1). Overall, 156 frozen rodent samples were purchased, 34 rats and 122 mice. Frozen rodents were used instead of living rodents due to ethical restrictions, but the same rodents are bred for both feeding and pet purposes (Shivambu, 2019, pers. comm.). Rodent samples were sealed in zip-up bags, stored in a car portable fridge and transported to the University of KwaZulu-Natal, Pietermaritzburg campus for genetic analyses.

2.2 | DNA extraction and polymerase chain reaction (PCR)

We dissected the rodents for tissue samples using surgical blades and forceps. To avoid contamination, we used disposable scalpels and disinfected them with 99% ethanol and flame. We harvested liver and stored these samples in 1.5 ml Eppendorf tubes filled with 99% ethanol for molecular analyses. Genomic DNA was successfully extracted from 156 liver tissue samples using the Omega Biotek extraction Kit (Norcross, Georgia), following the manufacturer's standard protocol. The extracted DNA was stored in a −80°C freezer until further analyses. We amplified the DNA extracts using polymerase chain reactions (PCRs) targeting four mitochondrial gene regions: Cyt b, CO1, 16S rRNA and CR. These gene regions were chosen as they have been reliably used to resolve mammalian phylogenies (Nicolas et al., 2012; Pun et al., 2009). The PCRs were done in a reaction volume of 14.5 µl containing 5.25 µl of double-distilled water (ddH$_2$O), 6.25 µl OneTaq®—2X Master Mix with Standard Buffer (New England Biolabs, Ipswich, MA, USA) or

EmeraldAmpMax PCR Master Mix (Takara Bio Inc, Kusatsu, Shiga, Japan), 0.5 μl of each oligonucleotide primer and 2 μl DNA template. Each set of PCR reactions included a negative control (no template control) to detect contamination of reagents. The PCR products were visualised with 1.5% TBE agarose gel, and Quick-Load® 1 kb DNA Ladder (New England Biolabs) was used to estimate the size of amplicons. The primer details, cycling conditions and the amplified fragment size for each gene region are listed in Table 1. Overall, out of 156 samples, 149 were successfully amplified, with three and four failed samples for rats and mice, respectively. For Sanger sequencing, we sent PCR products to the Central Analytical Facility (CAF), Stellenbosch University, or the KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP), University of KwaZulu-Natal, South Africa. All sequences were BLASTed against the NCBI GenBank database (www.ncbi.nlm.nih.gov/blast) to verify sequences and assess taxonomic assignment.

2.3 | Phylogenetic analyses

We edited sequence chromatograms and aligned each gene region separately using ClustalW 2.0 (Larkin et al., 2007). After computational alignment, we manually optimised sequence alignments in BioEdit 7.2.6 (Hall, 2005) to ensure homology. We estimated the length of each final alignment, the number of conserved sites and the number of parsimonious sites using Mega 6 (Tamura et al., 2013). Haplotype number (h), haplotype diversity (HD) and nucleotide diversity (π; SD) were estimated using DnaSP 5.10.1 (Rozas et al., 2017). We deposited the sequences generated in the present study in GenBank (accession numbers: MZ353018-MZ353519).

We constructed phylogenies using two model-based methods, maximum likelihood and Bayesian inference. The best-fit substitution model for each gene region was selected based on the Akaike information criterion (AIC) using JModeTest 2.1.6 (Darriba et al., 2012) on the CIPRES server (Miller et al., 2010). The best-fitting substitution models were GTR for Cyt b, Tim3+G for CO1, Tim2+G for 16S rRNA and Tpm3uf+I for CR. We conducted phylogenetic analyses on each gene region separately and thereafter combined the sequence data for the four mitochondrial markers into a single dataset and inferred the phylogeny using a partitioned approach. Phylogenies from the analyses of individual gene regions were compared for conflict before we conducted the combined analyses.

We performed maximum likelihood analyses (ML) using Garli 2.0 (Zwickl, 2006). We used MrBayes 3.2.7a (Ronquist et al., 2012) to conduct Bayesian inference (BI). Both programs were run on the CIPRES server. For maximum likelihood, branch support was assessed using 1000 bootstrap replicates. We constructed consensus trees using the 50% majority rule method in CONSENSE in the PHYLIP package (Felsenstein, 2005).

For the BI, two independent runs, each consisting of four Markov Monte Carlo Chains (MCMC), were run for 20 million generations. To confirm the convergence of MCMC, we used Tracer 1.7 (Rambaut et al., 2018). Convergence was assumed when the effective sample size (ESS) values were all >200. Thereafter, 20% of initial trees were removed as burn-in from the tree file before consensus trees were

TABLE 3 Genetic diversity indices for three rodent species (*Mastomys coucha*; *Mus musculus* and *Rattus norvegicus*) sold in South African pet shops based on mitochondrial DNA gene regions (16S rRNA, CO1 and Cyt b), hypervariable control region (CR) and combined data set

	Number of individuals (*n*)	Number of haplotypes (h)	Haplotype diversity (Hd)	Nucleotide diversity (π, SD)	Tajima's D
Mastomys coucha					
16S rRNA	5	1	0.00	0.00	–
CO1	5	3	0.70	0.001	−0.97 ($p > 0.10$)
Cyt b	6	1	0.00	0.00	–
CR	6	1	0.00	0.00	–
Combined	4	3	0.83	0.00	−0.70 ($p > 0.10$)
Mus musculus					
16S rRNA	112	5	0.27	0.001	−0.09 ($p > 0.10$)
CO1	71	15	0.56	0.003	−1.88 ($p > 0.05$)
Cyt b	93	11	0.56	0.001	−1.75 ($p > 0.05$)
CR	112	3	0.26	0.003	0.11 ($p > 0.05$)
Combined	51	19	0.74	0.003	−1.55 ($p > 0.10$)
Rattus norvegicus					
16S rRNA	31	2	0.49	0.0009	1.47 ($p > 0.10$)
Cyt b	25	9	0.76	0.004	−0.79 ($p > 0.10$)
CR	31	3	0.52	0.002	0.91 ($p > 0.10$)
Combined	21	8	0.76	0.003	0.76 ($p > 0.10$)

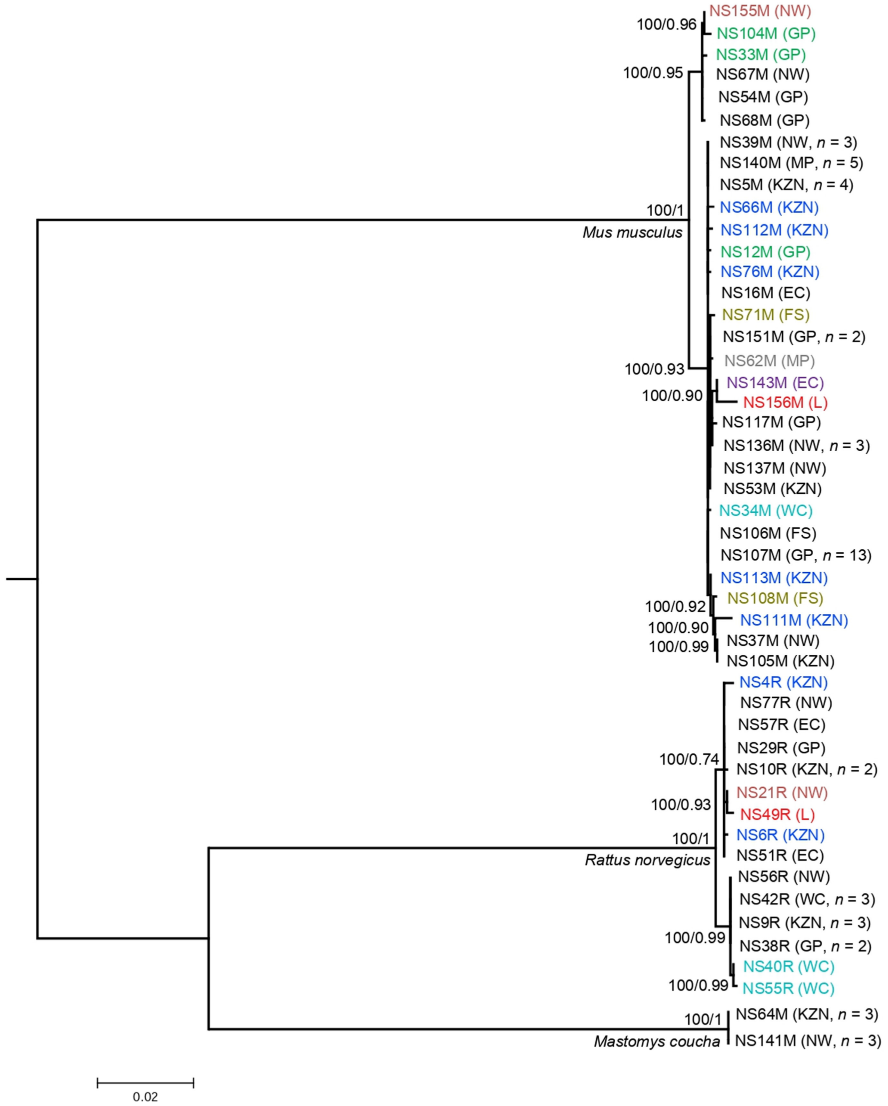

FIGURE 2 Most likelihood phylogeny constructed from rodents collected from South African pet shops. The combined data included four mtDNA gene regions, 16S ribosomal RNA (16S rRNA), cytochrome oxidase subunit 1 (CO1), mtDNA cytochrome *b* (Cyt b) and hypervariable control region (CR). Nodal support values on branch nodes denote the maximum likelihood bootstrap (>50%) and Bayesian posterior probability (>0.6) support values. The number of identical sequences is shown in parentheses, and the colour-coded taxa show unique individuals. The provinces where samples were collected are abbreviated as follows: EC, Eastern Cape; FS, Free State; GP, Gauteng Province; KZN, KwaZulu-Natal; L, Limpopo; MP, Mpumalanga; NW, North West; and WC, Western Cape.

constructed. We assessed branch support using posterior probability values. All trees (ML and BI) were midpoint rooted, and bootstrap and posterior probability values were annotated onto the most likely tree.

Haplotype networks were also used to examine the genetic diversity of rodents. Median-joining network analysis was constructed for each gene region and the combined dataset using PopART 1.7 (Leigh & Bryant, 2015).

3 | RESULTS

3.1 | Genetic diversity

In total, 149 PCR products for the four mtDNA loci were successfully sequenced from the 156 rodent specimens. However, 94% of the *R. norvegicus* samples failed to amplify for CO1 gene region despite several attempts in this study. Poor amplification success might be due to poor primer binding. Using BLAST searches, specimens were identified as *M. coucha* ($n = 6$), *M. musculus* ($n = 112$) and *R. norvegicus* ($n = 31$). The nucleotide composition and genetic variables for each species differed for the four gene regions (Table 2). The most variable mtDNA region was the CO1 in *M. coucha* (6 variable characters) and *M. musculus* (33 variable characters), while Cyt b was the most variable gene region in *R. norvegicus* (20 variable characters). The most conserved mtDNA region was 16S rRNA, with the least number of variable characters in all three species. The final aligned dataset, including all three rodents and all four gene regions, was 2485 bp in length. The combined dataset for *M. coucha* contained only eight variable sites, while *M. musculus* had 76 variable sites and 33 parsimonious sites. Lastly, the combined dataset for *R. norvegicus* had 27 variable sites and 10 parsimonious sites (Table 2).

For *M. musculus*, DnaSP analysis recovered the most haplotypes in CO1 and Cyt b genes, 15 and 11, respectively (Table 3). The genetic diversity for both these gene regions was 0.56, with CO1 having higher nucleotide diversity than Cyt b (Table 3). The combined data set for *M. musculus* recovered a total of 19 unique haplotypes, with high haplotypic diversity (0.74) and low nucleotide diversity ($p = 0.003$) (Table 3). Tajima's D was negative for all the gene regions except for CR in *M. musculus*, but it was not significant when

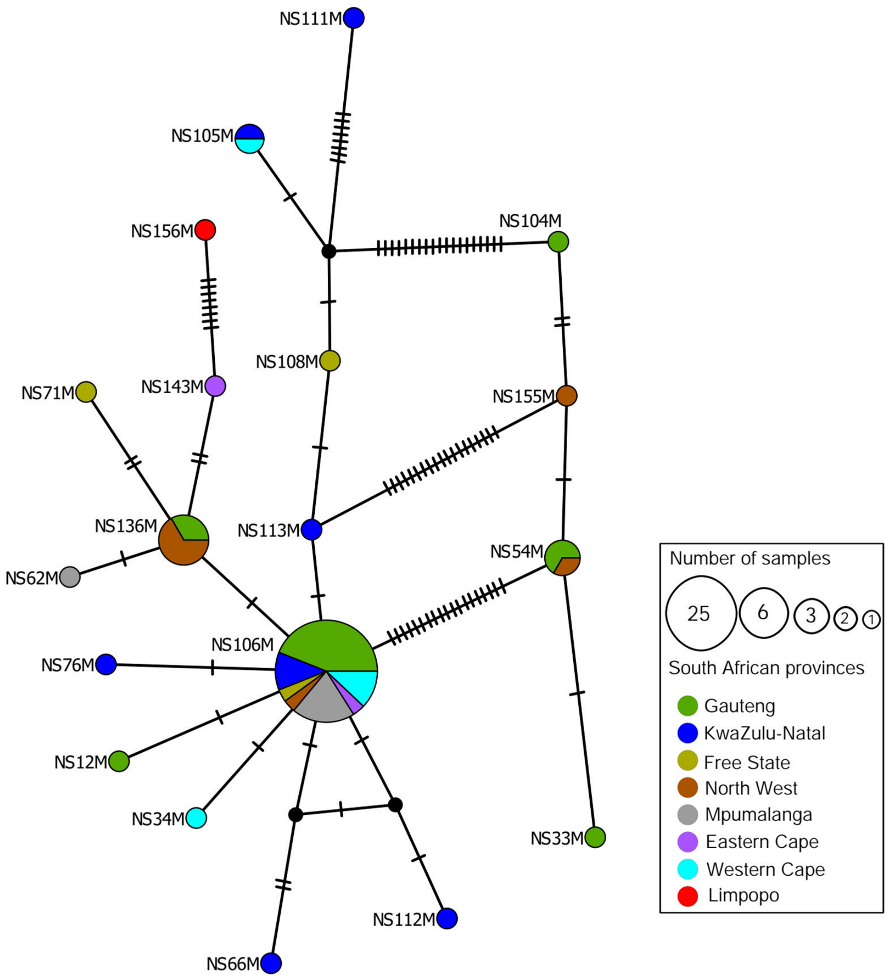

FIGURE 3 Median-joining haplotype network constructed using combined data from four mtDNA gene regions (16S rRNA, CO1, Cyt b and CR) based on 51 sequences of *Mus musculus* sold in South African pet shops.

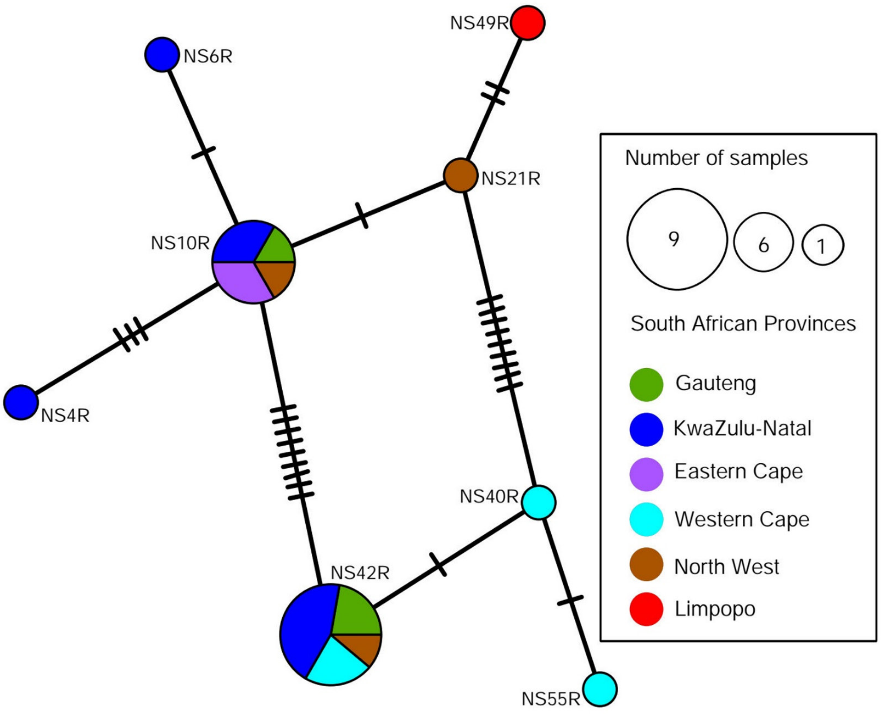

FIGURE 4 Median-joining haplotype network constructed using combined data from three mtDNA gene regions (16S rRNA, Cyt b and CR) based on 21 sequences of *Rattus norvegicus* sold in South African pet shops.

all the mtDNA regions were combined (Table 3). For *R. norvegicus*, DnaSP analysis recovered more haplotypes in the Cyt b gene, with a total of nine unique haplotypes. The haplotypic diversity was high (Hd = 0.76), with a low nucleotide diversity (p = 0.004) (Table 3). The same analysis recovered a total of eight unique haplotypes when the dataset was combined. The haplotypic diversity (Hd = 0.76) was similar to Cyt b; however, the nucleotide diversity (p = 0.003) was lower for the combined dataset (Table 3). Tajima's D was negative for Cyt b but positive for 16S rRNA, CR and combined data, with all not significant at the $p < 0.005$ level (Table 3). For *M. coucha*, DnaSP analysis recovered the most haplotypes in the CO1 gene and combined data, each representing three haplotypes (Table 3). The haplotypic diversity was 0.70 for the CO1 gene and 0.83 for combined data; however, the nucleotide diversity was lower for both, $p = 0.001$ and $p = 0.00$, respectively. Tajima's D was negative for both CO1 and combined data, and it was not significant (Table 3).

3.2 | Phylogenetic analyses

The phylogenies produced by maximum likelihood and Bayesian analyses were consistent. As a result, the most likely phylogeny is presented with bootstrap and posterior probability support values (Figures 2 and S1–S4). The phylogenies for both the individual and combined data sets revealed three distinct clusters, which were confirmed by BLAST as belonging to *M. coucha*, *M. musculus* and *R. norvegicus* (Figures 2 and S1–S4). The three species formed monophyletic clades on topologies produced by individual analyses of 16S rRNA, CO1, Cyt b, CR and the combined data set (Figures 2 and S1–S4). All three monophyletic clades were supported with high bootstrap support values in all phylogenies (Figures 2 and S1–S4). The 16S RNA phylogeny revealed a total of eight different *M. musculus* genotypes and two genotypes for *R. norvegicus* (Figure S1). The CO1 gene region phylogeny recovered 21 different genotypes for *M. musculus* and a single genotype for *R. norvegicus* (Figure S2). In Cyt b phylogeny, *M. musculus* had 11 different genotypes, while *R. norvegicus* had eight different genotypes (Figure S3). In the CR gene region, the phylogeny recovered three different genotypes for *M. musculus* and *R. norvegicus* (Figure S4). The combined datasets recovered a total of 21 different *M. musculus* genotypes, eight different *R. norvegicus* genotypes and a single genotype for *M. coucha* (Figure 2). Within the species lineages, the genotypes for both *M. musculus* and *R. norvegicus* were separated into two well-supported clades (Figure 2).

3.3 | Haplotype network

Haplotype diversity was limited within *M. coucha*, and haplotype networks were not constructed for this species. The most abundant haplotype found in *M. musculus*, NS106M appeared to be the parent haplotype in 16S rRNA, Cyt b, CO1 and CR data (Figure S5). Haplotype, NS54M, was also ancestral to other unique haplotypes represented by five provinces (Figure S5). However, some of the haplotypes that grouped together with this haplotype were separated in Cyt b (Figure S5). Interestingly, Cyt b had an additional abundant haplotype, NS136M, which was the parent haplotype for the Mpumalanga Province haplotype, NS62M (Figure 3). Gauteng, North West and KwaZulu-Natal Provinces had the most number of haplotypes in 16S rRNA, Cyt b, CO1 and CR data sets (Figure 3).

The analysis of *M. musculus* combined dataset recovered a starburst haplotype network (Figure 3). There was no clear geographically correlated pattern. The central haplotype (NS106M) was present in all provinces except Limpopo (Figure 3). Another common haplotype was NS136M, which was found in six individuals, but this haplotype was only recorded from the North West and Gauteng Provinces (Figure 3). A unique haplotype from Limpopo Province was distantly linked by eight mutational steps to this haplotype. KwaZulu-Natal Province had a high number of haplotypes (n = 7); of these, five were unique and not found in any of the other provinces (Figure 3). KwaZulu-Natal Province also shared a unique haplotype, NS105M, with the Western Cape Province. The haplotypes for KwaZulu-Natal Province were all closely related, with haplotypes separated by single mutations, except for haplotypes NS66M and NS111M, which were separated from other haplotypes by two and eight mutational steps, respectively (Figure 3). Gauteng Province had six haplotypes, of which two were shared with North West Province (Figure 3). The haplotypes shared with the North West Province were separated by single and 19 mutational steps (Figure 3).

According to the haplotype network analysis for *R. norvegicus*, haplotype NS10R appeared to be the parent haplotype in 16S rRNA, Cyt b and CR networks (Figure S6). Another common haplotype, NS42R, was shared by KwaZulu-Natal, Western Cape and Gauteng Provinces in all three data sets (Figure S6). In Cyt b gene, NS42R was the parent haplotype for the three Western Cape Province unique haplotypes, separated by single and six mutational steps (Figure S6). Haplotype NS10R was separated from NS42R by a single mutational step in 16S rRNA, while in Cyt b and CR, it was separated by five and two mutational steps, respectively. Western Cape and KwaZulu-Natal Provinces had more unique haplotypes in Cyt b than in 16S rRNA and CR (Figure S6).

The analysis of *R. norvegicus* combined dataset recovered a total of eight unique haplotypes, which showed a subtle geographic structure (Figure 4). NS10R and NS42R were present in four provinces (Figure 4). NS10R was the ancestral haplotype to two KwaZulu-Natal Province haplotypes and North West Provinces haplotypes. The North West Province haplotype, NS21R, was the ancestral haplotype to a Limpopo Province haplotype NS49R (Figure 4). These haplotypes were separated from NS10R by single, two and three mutational steps. NS42R was the ancestral haplotype to two unique Western Cape Province haplotypes, separated by a single mutation. KwaZulu-Natal and Western Cape Provinces had more unique haplotypes when compared with other provinces (Figure 4).

4 | DISCUSSION

We used four mitochondrial DNA regions to identify and assess the genetic variation of the captive rodent populations in South African pet shops. We identified three species, namely *M. coucha*, *M. musculus* and *R. norvegicus*. *Mastomys coucha* is native to South Africa, and it has been reported to be used for pet trade purposes together with morphologically similar *M. natalensis* (du Plessis et al., 2016). However, our study found that *M. coucha* was the only native species available for sale in two provinces, and we found no evidence of *M. natalensis* being traded. The identification of *Mastomys* species is impossible based on the morphological appearance, but molecular analyses appear to be useful in identifying this rodent species (Kruppa et al., 1990; Smit et al., 2001). It is possible that only *M. coucha* is sold and not *M. natalensis*, given that limited studies have identified rodent species sold in South African pet shops based on molecular analyses. A previous study by Kruppa et al. (1990) in Germany found that experimental animals assumed to be *M. natalensis* were all identified as *M. coucha*.

Based on the results of the phylogenetic analyses and BLAST searches, *M. coucha* was the least widely sold species when compared to the non-native *M. musculus* and *R. norvegicus*. The latter two species were sold in more than five provinces. Present South African legislation does not permit South African native species to be traded (DEA, 2016), but the widespread trade in invasive rodents is a concern. Both *M. musculus* and *R. norvegicus* formed monophyletic clades. However, both of these species exhibited below-species genetic diversity. This suggests that the pet shop owners breed or source different rat and mouse varieties, which may have led to sampling genetically different populations. There are more than 20 rat and mouse strains used in laboratories around the world (Aitman et al., 2008; Atanur et al., 2013; Tsang et al., 2005). As a result, some of these strains could have been introduced to the pet trade industry as most pet rats and mice are donated to pet shops to be used as pets (Baumans et al., 2007; Carbone et al., 2003). This finding was supported by a study by Maligana et al. (2020), which found that samples of *M. musculus* and *R. norvegicus* were affiliated with the laboratory strains.

Mus musculus haplotype analyses revealed that most individuals belonged to one highly abundant haplotype (NS106M) that was found in all the provinces. Haplotype networks for both separate and combined genes did not display a clear geographically correlated pattern. CO1 and Cyt b had higher genetic diversity than 16S rRNA and CR, but singletons represented most haplotypes in these two genes. KwaZulu-Natal, Gauteng and North West Provinces had more unique haplotypes in all the gene regions, with some of the haplotypes being ancestral to unique haplotypes found in other provinces. Unique haplotypes indicate that there may have been several introductions of new strains into the South African pet market. Four unique haplotypes in Free State, Limpopo, Eastern Cape and Mpumalanga Provinces were closely related, suggesting that pet shop owners may source stock from Gauteng and North West Provinces. Our result also found a negative Tajima's D for *M. musculus* specimens, suggesting a steady population expansion.

For *R. norvegicus*, individuals belonged to two haplotypes which are not present in all the provinces. The haplotype network suggests a subtle geographic structure. Further sampling may recover additional shared haplotypes between the provinces. Even though few samples were analysed, the haplotype analyses recovered eight haplotypes in the combined dataset and nine in the Cyt b gene. Interestingly, the number of Cyt b haplotypes identified in our relatively small sample of *R. norvegicus* recovered more haplotypes than that recorded from wild populations of this species in South Africa (Bastos et al., 2011). This could be because of the introduction of new strains in the pet trade industry. Genetic diversity is important as it enables the species to respond to threats such as predators, parasites, diseases and environmental changes (Chen et al., 2012; Nguiffo et al., 2019). Consequently, if *M. musculus* and *R. norvegicus* haplotypes escape or are intentionally released into the wild, they may be able to overcome such threats, given that their haplotypes are genetically different. Although the pet trade is not cited as an invasion pathway for *R. norvegicus* in South Africa, rats with markings typical of laboratory and pet rat strains were observed living in feral conditions in Alexandra Township, South Africa (Maligana, 2018). This suggests there may have been several past escapes and releases of these traded rodents in South Africa.

5 | CONCLUSIONS

Our study revealed that most rodents sold in the pet trade belong to the invasive species *M. musculus* and *R. norvegicus*. Very few representatives of the native species *M. coucha* were confirmed, and all individuals were closely related. In contrast, significant below-species genetic diversity was recorded from *M. musculus* and *R. norvegicus*. *Mus musculus* haplotype analysis did not show a clear geographical pattern, while the *R. norvegicus* haplotype network showed a subtle geographic structure. Western Cape, Gauteng, North West and KwaZulu-Natal Provinces had the highest number of unique haplotypes not shared with any of the other provinces. This suggests that these provinces are introducing or breeding varieties of these non-native species into the pet trade industry. The introduction and breeding of these non-native species should be regulated because the continued introduction of species with high-genetic diversity may influence their ability to become established if released into the wild. This is a valid concern because accidental escapes and intentional releases may occur occasionally. These results can be used when implementing management strategies regarding the trade of these species.

AUTHOR CONTRIBUTIONS

Ndivhuwo Shivambu, Colleen T. Downs and Sandi Willows-Munro conceptualised and did the sample design. Colleen T. Downs sought funding. Ndivhuwo Shivambu implemented the study. Ndivhuwo Shivambu and Tinyiko C. Shivambu collected and prepared the samples. Ndivhuwo Shivambu, Tinyiko C. Shivambu and Sandi Willows-Munro analysed the data. Ndivhuwo Shivambu wrote the manuscript

draft while Tinyiko C. Shivambu, Sandi Willows-Munro and Colleen T. Downs edited the manuscript.

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CONFLICTS OF INTEREST

The authors declare neither conflict of interest nor competing interests.

DATA AVAILABILITY STATEMENT

Sequence data for this study are available on NCBI GeneBank (Accession numbers: MZ353018-MZ353519).

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