A phylogeographic assessment of South African greater cane rats (*Thryonomys swinderianus*): Preliminary insights

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Abstract

The greater cane rat (*Thryonomys swinderianus*) is an African rodent with a wide Sub-Saharan distribution range. This species is viewed as an important protein source in many African countries. These rodents are also regularly viewed as a pest species who frequently raid croplands in agricultural settings. No phylogenetic work has to date been published on *T. swinderianus* from southern Africa. This paper therefore reports the first phylogenetic assessment on the species across the South African distribution range. Thirty samples were sourced from local museum collections, with one direct submission by a member of the public who found a rodent carcass identified as *T. swinderianus* west of its known distribution range in the Eastern Cape Province of South Africa. Two mitochondrial loci previously used in West African studies of this species were used in the current study to assess *T. swinderianus* population genetic diversity and phylogenetic structure across the South African distribution. A comparison to sequence data from West Africa was also performed. A divergence time estimation was conducted to further investigate the evolutionary history of the South African sub-population. Similar genetic diversity estimates were observed for the South African sub-population when compared to the West African datasets. Specimens from the eastern parts of South Africa showed higher genetic diversity estimates, possibly indicative of an initial colonisation site from eastern Africa. Two distinct phylogenetic clades were identified by Bayesian inference, forming distinct West African and South African groups. The divergence estimates showed similar ages for the *T. swinderianus* most recent common ancestor (MRCA) as previously reported. The MRCA estimates for the South African group identified a possible middle to late Pleistocene migratory event from eastern African into southern Africa. Further fine scale sampling across the African distribution range is however needed to provide more accurate assessments for future conservation management planning for the different sub-populations, as needed.

Keywords

Divergence estimates, mitochondrial loci, Pleistocene, rodent phylogenetics

Introduction

The greater cane rat (*Thryonomys swinderianus*) belongs to the family Thryonomyidae, and is one of two species found in this family. The genus is also the sole member of Thryonomyidae (López Antoñanzas et al. 2004). *Thryonomyidae swinderianus* is a widely distributed African rodent, found mainly in the mesic habitats of Sub-Saharan Africa.
This species is well known as an important protein source (Baptist and Mensah 1986; Alexander 1992; Jori et al. 1995; Adu et al. 1999) as well as an important agricultural pest (López Antoñanzas et al. 2004; van der Merwe and Avenant 2004; van Zyl and Delport 2010; Kilwanila et al. 2021). These rodents also form a large part of the African bush-meat trade (Okiwelu et al. 2010; Gaubert et al. 2014), as well as the traditional medicine trade (Kilwanila et al. 2021). A high demand for *T. swinderianus* meat exists in western Africa (Jori et al. 1995; van der Merwe and Avenant 2004). Currently this species is listed as Least Concern by the International Union for Conservation of Nature (IUCN; Child 2016), but continued intensive hunting could have long-term negative effects on population integrity. These rodents are however seen as potential agricultural assets for small scale farmers across Africa, and many West African countries encourage small stock farmers to rear these animals for the meat market (van der Merwe and Avenant 2004).

The most southerly distribution limit for *T. swinderianus* is noted as the Grahamstown district in the Eastern Cape Province of South Africa (Skinner and Chimimba 2005). It is reported by Avenant et al. (2016) that the western distribution range of this rodent species is expanding in South Africa, with sightings in the central regions of the North West Province and western Free State. Recently a cane rat carcass was found west of the southern limit of this species, on a farm between Bedford and Cradock in the Eastern Cape Province of South Africa (W.G. Coetzter, personal observation; Fig. 1). This species is mainly found along drainage lines and rivers where suitable reed beds occur. One of the drivers of range expansion for this species is the ability to adapt to agricultural areas where crops are grown, as well as deforested areas, which form secondary savannah habitats (Jori et al. 1995).

Very little research have been conducted on *T. swinderianus* in southern Africa, with some reports on distribution (van der Merwe and Avenant 2004; Avenant et al. 2016) and physiology (van Zyl and Delport 2010), to name a few. The majority of research on *T. swinderianus* has been performed in western and central Africa (Mustapha et al. 2020; Kilwanila et al. 2021). Phylogenetic and genetic diversity studies are generally scarce, with a number of molecular studies from Ghana (Adenyo et al. 2013; Adenyo et al. 2016; Adenyo et al. 2017a; Adenyo et al. 2017b) and Nigeria (Coker et al. 2017). Several morphological phylogenetic studies using fossil evidence has been conducted on the family Thyromyidae (López Antoñanzas et al. 2004; Kraatz et al. 2013). Much is, however, still to be learnt about the phylogenetic history of this rodent group.

In the current study, the phylogenetic position of a *T. swinderianus* specimen found outside the reported South African distribution range was assessed via Sanger
sequencing of the mitochondrial cytochrome c oxidase I (COI) and D-loop regions. Additionally, the molecular phylogenetic patterns of the *T. swinderianus* from South Africa, compared to available data from western Africa, was also performed. This study serves as a pilot study to pave the way for future in-depth assessments on the distribution and genetic structure of *T. swinderianus* in southern Africa.

**Materials and methods**

**Ethical considerations**

Ethical clearance was obtained from the Interfaculty Animal Ethics Committee at the University of the Free State, South Africa (Ethics number: UFS-AED2018/0075).

**Sampling**

A farmer from the Cradock district in the Eastern Cape reported the sighting of a *T. swinderianus* carcass on his property. A tail clipping of the carcass was submitted to the Genetics laboratories at the University of the Free State for future analysis. This specimen will be referred to as CR_CDK_01_EC. The sample was stored in 96% ethanol at −20°C. This species has never been seen in this region. The farm is situated in a valley nestled within the Winterberg Mountain range. The habitat consists of mixed vegetation with the Dry Highveld Grassland Bioregion (Mucina and Rutherford 2006) in the valley with a seasonal river draining into the Tarkariver system. The banks of these river systems consist of a mixture of tree and shrub species, including *Acacia (Vachellia)* karroo, *Searstia* sp. and *Lycium* sp. The Tarkariver system in turn runs into the Great-Fish River, which enters the Indian Ocean east of Grahamstown.

Additional samples were sourced from South African museum collections to use in the population genetic and phylogenetic analyses (*n* = 30; Table S1). The museum specimens were sourced from four museums, namely the National Museum (Bloemfontein), Ditsong National Museum of Natural History (Pretoria), Durban Natural Science Museum (Durban) and Amathole Museum (King William’s Town). The museum samples consisted of either dried skins or skull scrapings. Further details on each sample can be obtained in Table S1. Successfully sequenced specimens were grouped according to the provincial region of origin (Fig. 1).

**DNA extraction**

All DNA extractions were performed with the Purelink Genomic DNA Kit (Life Technologies, Carlsbad, CA). The manufacturers protocol was followed for DNA extraction from animal tissues, with additional steps to ensure sufficient DNA quantity and quality as reported by Coetzer and Grobler (2019). A NanoDrop Spectrophotometer ND-1000 was used for DNA quantification. A dedicated ancient/museum DNA laboratory was not available for DNA extraction. All work surfaces and equipment were decontaminated using a 1.25% hypochlorite solution and then a 70% ethanol solution before and after each set of DNA extractions.

**DNA amplification and sequencing**

Partial segments of two mitochondrial regions were targeted for this study. Amplification of a ~650 bp fragment of the cytochrome c oxidase I (COI) gene was performed using primers designed by Gaubert et al. (2014). A fragment of ~500 bp was amplified for the mitochondrial D-loop region using primers reported by Adenyo et al. (2013). PCR setup was performed in a DNA free laboratory, and all working surfaces were decontaminated using a 1.25% hypochlorite solution, prior to setup. Each 10 µl PCR reaction consisted of: ~50 ng template DNA, 5 µl Ampliqon TEMPase Hot Start 2 × Master Mix, 0.4 µM of each primer, 0.5 mg/ml BSA and PCR grade dH₂O to make up the 10 µl. The PCR cycling conditions included an initial denaturation step at 95°C for 3 min, followed by 35 cycles of 95°C for 30 s, 58°C for 40 s and 72°C for 30 s, with a final extension step of 72°C for 1 min. Negative PCR controls were included with each PCR reaction batch to monitor the occurrence of possible contamination.

Amplification success was assessed on a 1% agarose gel. The ExoSAP-IT PCR Product Clean-up kit (Affymetrix Inc., CA, USA) was used for all PCR clean-up procedures. The ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Division, Perkin-Elmer, Foster, CA) was used for all sequencing PCR reactions. Sequencing PCR clean-up was performed with the BigDye XTerminator Purification Kit (Applied Biosystems, CA, USA), followed by sequencing analysis on an ABI 3500 Genetic Analyzer.

**Sequence analysis**

Previously reported COI (*n* = 23; accession numbers: KJ192912–KJ192913, Equatorial Guinea; KJ192933–KJ192949, Ghana; KJ192950–KJ192953 and KJ192955, Nigeria) and D-loop (*n* = 26; accession numbers: AB675385–AB675410, Ghana) sequences were downloaded from GenBank for downstream analyses (Adenyo et al. 2013; Gaubert et al. 2014). Sequence assembly and editing of sequences obtained from the current study were performed with GENEIOUS R9 software (Kearse et al. 2012). All sequences generated from this study were deposited in GenBank (accession numbers: COI, OP121188–OP121208; D-loop, OP121209–OP121231; Table S1). The sequence alignments were performed using the CLUSTALW (Thompson et al. 1994) add-in for GENEIOUS. Summary statistics (number of haplotypes (*h*),
haplotype diversity \((Hd)\), number of polymorphic sites \((S)\), nucleotide diversity \((\pi)\), number of synonymous sites and number of non-synonymous sites) were estimated in DNASP v5.10.01 software (Librado and Rozas 2009).

The most optimal nucleotide substitution model for haplotype alignments of COI and D-Loop were identified prior to phylogenetic tree construction, using the Akaike information criterion (AIC; Akaike 1974) as implemented in JMODELTEST v.2.1 (Darriba et al. 2012). These were identified as HKY +G for both COI and D-loop. Phylogenetic analyses for each gene region were performed via Bayesian inference (BI) in MRBAYES v3.2 (Ronquist et al. 2012).

For comparative purposes, an additional 11 Hystricomorpha and 3 Sciuromorpha species were included (Table 1). The K2P analysis were run for 1 000 bootstraps.

**Divergence dating**

A molecular clock analysis was performed in BEAST2 (Bouckaert et al. 2014) to improve our understanding of the phylogenetic history of *T. swinderianus* using the available data. A partitioned dataset was created, using the COI and D-loop sequences from the South African specimens. Additional COI (Gaubert et al. 2014) and D-loop (Adenyo et al. 2013) sequences were downloaded from GenBank according to geographical region of origin. The outgroup taxa comprised of 14 species, representing four Hystricomorpha families and one Sciuromorpha family as outgroups (See Table 1 for details). The most optimal nucleotide substitution model for the COI data set was identified as GTR +I +G and for the D-loop datasets as HKY+G using JMODELTEST. The lognormal relaxed-clock model, with the calibrated Yule process tree prior was selected. Six calibration dates were used from several sources, including the crown Thryonomyidae group estimate from Kraatz et al. (2013) at 5.6 to 2.6 Mya (Table 2). The simulation was run for 20 million
generations, with a tree sampling frequency of 20,000. Simulation stationarity was assessed using the effective sample size (ESS) values obtained from TRACER v.1.7.1 (Rambaut et al. 2018). The maximum clade credibility (MCC) tree was estimated using TREEANNOTATOR v.2 (Bouckaert et al. 2014) after discarding 10% of the sampled trees. The MCC tree was assessed and divergence dates were retrieved using FIGTREE v.1.4.0 (Rambaut 2014).

Results

DNA concentrations of 9-439.5 ng/µl were obtained from the 31 specimens (Average 260/280 ratio: 1.68; Average 260/230 ratio: 1.33). The sequencing success rate was as expected for museum samples, with 21 specimens successfully sequenced at COI and 23 sequenced at D-Loop. Four samples did not sequence at either COI or D-loop regions.

Genetic diversity of South African T. swinderianus

Due to the small sample size of this preliminary study, all genetic diversity values should be taken with caution. The samples from the KwaZulu-Natal group showed the highest genetic diversity values for both loci (COI, \(H_d = 0.844\), \(\pi = 0.003\); D-loop, \(H_d = 0.972\), \(\pi = 0.006\)) compared to the other South African regions. Assessing the South African sample set as a whole, it was observed that the COI haplotype diversity (\(H_d = 0.724\)) is only slightly lower than that observed for Ghana (\(H_d = 0.814\); Table 3). Six haplotypes were observed among the 21 specimens, with all six haplotypes occurring within the KwaZulu-Natal group. The overall South African D-loop genetic diversity estimates were much lower than observed for Ghana, although there is a large sample size difference to consider. Nine D-loop haplotypes were observed for the South African group, with eight haplotypes observed in KwaZulu-Natal.

Phylogenetic assessment

The Bayesian inference (BI) trees for COI and D-loop both identified two clearly separated clades, dividing West African and South African specimens (posterior probability = 1; Fig. 2). The pairwise K2P analyses showed higher mean genetic distances between the South African and West African T. swinderianus groups (COI K2P = 0.112, SE = 0.015; DL K2P = 0.117, SE = 0.017), than what was observed for other species pairs tested. All three pairwise genetic distances calculated for the representative *Hystrix* species at both COI and DL were substantially lower that what was observed for the two T. swinderianus groups. A similar result was also observed for the more distantly related *Marmota* sp. included in this analysis (Table S2).

No clear geographic structuring within South Africa was observed within either sequence datasets. From the haplotype network analysis, it can however be observed that there are four COI haplotypes unique to the KwaZulu-Natal group (Fig. 3a). One COI haplotype was shared between the KwaZulu-Natal and Eastern Cape groups, and another was shared by all three groups. A similar trend was observed for the D-loop dataset, with six unique haplotypes recorded for KwaZulu-Natal. One

<table>
<thead>
<tr>
<th>Group</th>
<th>COI</th>
<th>D-Loop</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Number of sequences</td>
<td>h</td>
</tr>
<tr>
<td>South Africa</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td>Eastern Cape</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Free State</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>KwaZulu-Natal</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Gauteng</td>
<td>—</td>
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<tr>
<td>Western Africa</td>
<td>23</td>
<td>8</td>
</tr>
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<td>Coastal Savanna*</td>
<td>8**</td>
<td>5**</td>
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<tr>
<td>Forest*</td>
<td>3**</td>
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</tr>
</tbody>
</table>

* Ghanaian agro-ecological zones from Adenyo et al. (2013).
** COI values estimated from Gaubert et al. (2014). Agro-ecological zones extrapolated from locality information on GenBank.

Table 3. Summary statistics calculated for COI and D-loop sequences generated from South African *Thryonomys swinderianus* specimens in the current study, as well as sourced sequences from Adenyo et al. (2013) and Gaubert et al. (2014). Number of sequences, number of haplotypes (\(h\)), haplotype diversity (\(H_d\)), number of polymorphic sites (\(S\)) and nucleotide diversity (\(\pi\)) estimates are provided.
unique haplotype was identified for the Free State group, containing 17 mutation. No COI sequence was obtained for this specimen. One D-loop haplotype was present in all South African groups, with another shared between the KwaZulu-Natal and Eastern Cape groups (Fig. 3b).

**Phylogenetic position of specimen CR_CDK_01_EC**

Specimen CR_CDK_01_EC collected from the Eastern Cape, neatly grouped with the rest of the South African *T. swinderianus* specimens confirming the species identity. The haplotype network analyses, placed specimen CR_CDK_01_EC within the main haplotype cluster for both loci (Fig. 3). The COI cluster contained all the Free State specimens and one KwaZulu-Natal specimen. The D-loop cluster containing specimen CR_CDK_01_EC consists of specimens from all regions sampled, with all but one Free State specimen also in this cluster. No clear phylogenetic position within the South African clade was observed during the BI analyses.

**Divergence dating**

Divergence date estimates are provided in Table 4, with the letters on Fig. 4 corresponding to the node names in
the table. The most recent common ancestor (MRCA) of Phiomorpha rodents were estimated at 33.935 million years ago (Mya) (95% highest posterior density (HPD) = 29–40.991 Mya). The MRCA of *Thryonomys* was calculated at 2.919 Mya (95% HPD = 2.6–4.154 Mya), which was only older than the date estimated for the split between *Hystrix africaeaustralis* and *Hystrix cristata* (0.308 Mya; 95% HPD = 0.047–0.653) (Table 4). The MRCA of the South African *Thryonomys* clade was estimated at 0.199 Mya (95% HPD = 0.069–0.373 Mya) and the MRCA for the West African clade was calculated as slightly older at 0.277 Mya (95% HPD = 0.122–0.476 Mya).
Discussion

Confirmation of a westerly expansion

The placement of specimen CR_CDK_01_EC with specimens from the Free State group was unexpected, as the Grahamstown region which is the excepted southern boundary of the *T. swinderianus* distribution is but 120 km south-east from this site. It was thus originally thought that cane rats travelled along the Great Fish river system, which enters the Indian ocean east of Grahamstown, and travelled to where the specimen was found in the Cradock district (Figure 5). The haplotype network, however, point to a possible migration event southward from the Free State Province. This is feasible since there have been an anecdotal report from van der Merwe and Avenant (2004) of a *T. swinderianus* sighting along the Leeu River valley, which is an tributary of the Caledon river in the eastern Free State. The Caledon river in turn flows into the Orange river. This region is known for large agricultural developments utilising irrigation from the Gariep dam situated in the Orange River (Nieuwoudt et al. 2004). Further south of the Gariep dam are several crop fields fed from either borehole irrigation or water from the Orange-Fish River irrigation system (W.G. Coetzer, personal observation). It can therefore be argued that cane rats could have migrated south from the eastern Free State along these river systems and patches of cropland, eventually ending up along the Fish River system near Cradock.

The use of *T. swinderianus* as a human protein source, from either bushmeat or captive-bred sources, could also potentially contribute to the occurrence of these animals outside their known distribution ranges. It is suggested by Avenant et al. (2016) that the observation of this species in the central Free State Province could be linked to human mediated translocations.

It is clear that *T. swinderianus* populations are continuing to expand their range in a westerly direction in South Africa, corroborating a previous report by van der Merwe and Avenant (2004). van der Merwe and Avenant (2004) provided the first reports of this westerly range expansion of *T. swinderianus* in South Africa, warning of the possible negative effects this could have on agricultural practices in the region. This can further be observed from museum collections outside of the current distribution range in Kwa-Zulu-Natal (Figure 1). Following these authors’ recommendations, it is important for conservation management authorities to closely monitor this range expansion. These rodents are known to cause damage to crops across Africa (Ewer 1969; van der Merwe and Avenant 2004; van Zyl and Delport 2010), therefore appropriate human-animal conflict solutions should be developed before the problem become too big. Additional sampling at a fine scale across the distribution range could provide better insights into the phylogeographic structure of this species, shedding more light on possible range expansion and colonisation events.

Figure 5. An elevational map depicting the Great Fish River (pink) system in relation to the new extralimital sample from the Eastern Cape Province. The Orange River system is shown in orange, with the location of the Gariep Dam also indicated in blue.
The addition of nuclear as well as adaptively linked loci would further benefit future studies.

Genetic diversity and phylogenetic structure

This study is the first to report on *T. swinderianus* genetic diversity and phylogenetic structure from South Africa. The high level of genetic diversity, and the occurrence of unique haplotypes, in the eastern regions of South Africa supports an ancient colonisation event from East Africa southwards into South Africa. Similar trends have been observed in other mammal species in southern Africa including African mole-rats (*Heliophobius* sp.; Faulkes et al. 2004), samango monkeys (*Cercopithecus* sp.; Lawes 1990; Linden et al. 2020), vervet monkeys (*Chlorocebus* spp.; Turner et al. 2016), and greater kudu (*Tragelaphus strepsiceros*; Jacobs et al. 2022) to name a few. The small sample size of the current study could have attributed to the lack of significant genetic structuring within the South African distribution range.

The haplotype diversity levels observed for the South African group was only slightly lower than that observed from data obtained from Adenyo et al. (2013) and Gaubert et al. (2014) for west African populations. The absence of any shared haplotypes for either the COI or D-loop regions between western and southern Africa, could point to an ancient common ancestry and independent evolutionary processes driving mutation rates in western vs southern Africa. This was further supported by the high K2P value observed between the two *T. swinderianus* lineages. This observation points to possible separate colonisation events into western and southern Africa, from eastern or north-eastern Africa, as fossil records from east Africa suggest this region being the origin for the genus (*Winkler 1992; Winkler et al. 2010; Kraatz et al. 2013*).

Divergence dating

The topology of the maximum clade credibility (MCC) tree obtained from BEAST reflects the known higher taxonomic groupings of the Rodentia taxa used, as compared to other reports (Fig. 4; Upham and Patterson 2015; Swanson et al. 2019). It is worth noting that several contradicting phylogenetic topologies for the Hystricomorpha families have been reported in the past, which for one could be an artefact of the molecular marker used (Huchon et al. 2000; Honeycutt et al. 2007; Heritage et al. 2016; Swanson et al. 2019).

The divergence dates estimated here are, however, in line with previous studies. The accuracy of the estimated *Thryonomys* divergence dates were assessed by comparing estimates of well-known taxa to the literature. The MRCA date estimated for Phiomorpha in this study is slightly younger than estimates reported by Upham and Patterson (2015), although these estimates do overlap with the confidence intervals calculated in that study. The Phiomorpha date calculated in the current study further overlaps with the confidence intervals of estimates from Sallam et al. (2009), Upham and Patterson (2012) and Huchon et al. (2007). The divergence estimates at lower taxonomic ranks were generally much younger than previously published. An example is the divergence date for the *Atherurus/Hystrix* split (4.38; 95% HPD = 3.582-5.656). This date estimate is relatively younger than what has been reported by others (8.6-13.6 Mya; Sobrero et al. 2014; Upham and Patterson 2015; Kumar et al. 2017). These lower divergence estimates could be an artefact of the markers used, and should be taken into consideration when viewing the results (Mueller 2006; Swanson et al. 2019). Additional markers could provide more reliable results.

The divergence time estimates for the *T. swinderianus* clades from the current study is younger than the divergence date estimations by Kraatz et al. (2013), based on thryonomyd fossil records from the United Arab Emirates and assessments of previous publications. Early *Thryonomys* sp. fossils identify the origin of the genus in East Africa from the late Miocene (6 – 5.6 Mya) (Haile-Selassie et al. 2004; Manthi 2007; Wesselman et al. 2009). The estimated divergence date for the South African *T. swinderianus* clade indicate the occurrence of a common ancestor during the late Pleistocene. The occurrence of *Thryonomys* sp. fossils in southern Africa were observed from the late Pleistocene at 0.126 to 0.0117 Mya (Brain 1981; Winkler et al. 2010; ICS 2022). This is slightly younger than the molecular date estimated from this paper, although the 95% HPD does overlap with the fossil ages. The mid- to late Pleistocene consisted of oscillations of wetter and drier conditions in Africa, leading to possible habitat fragmentation and the formation of unique habitats (Steele 2013; Hoag and Svenning 2017). Eastern Africa, specifically, underwent severe drought conditions starting in the middle Pleistocene (Hoag and Svenning 2017). This in turn would lead to population isolation and differentiation due to reduce gene flow and genetic drift. Further studies on the phylogenetic structure of *T. swinderianus* utilising specimens from across Africa would provide further details on the evolution of Africa’s second largest rodent. Such information could be useful for conservation authorities to establish conservation plans in areas where the species is experiencing anthropogenic pressures which could have long-term detrimental effects.

Conclusions

The continued westward expansion of *T. swinderianus* in South Africa could become a problem for agricultural farmers in the affected regions. Close observation of this species is therefore needed to properly plan future conservation measures. The results from this study further identified clear phylogenetic differentiation between southern and western African *T. swinderianus* groups. This result warrants further investigation into the phylogenetic history of this species. A wider sample pool from across the
distribution range, with fine scale sampling in specific geographic regions can provide further insights into the evolutionary histories of the *T. swinderianus* sub-populations across Africa. Intense hunting practices observed in some African countries can put stress on local *T. swinderianus* populations. Further phylogenetic information will therefore be useful for conservation authorities to ensure the continued survival of the species in its distribution range, as these regional populations should possibly be managed as unique management units.

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I would like to thank the museum curators from the National Museum (Bloemfontein), Ditsong National Museum of Natural History ( Pretoria), Durban Natural Science Museum (Durban) and Amathole Muse um, (King William’s Town) who assisted with sourcing samples for this project. I also would like to thank the Department of Genetics, University of the Free State (UFS) for laboratory space used during my time there. I would also like to acknowledge the University of Fort Hare for the provision of resources and time to complete this project. The study was supported by research incentive funds provided by the University of the Free State (UFS).

**References**


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Supplementary Material 1

Table S1

Authors: Coetzer WG (2023)
Data type: .xlsx
Explanation notes: Information concerning each sample used in the current study as acquired from each collection. NCBI GenBank accession numbers are provided for each sequence obtained. Specimen were no sequences were obtained is indicated by “-.
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/vz.73.e94111.suppl1

Supplementary Material 2

Table S2

Authors: Coetzer WG (2023)
Data type: .xlsx
Explanation notes: Estimates of evolutionary divergence over sequence pairs between groups for COI and D-Loop alignments using using the Kimura 2-parameter model (Kimura 1980).
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/vz.73.e94111.suppl2