

An analysis of the gaps in the South African DNA barcoding library of ticks of veterinary and public health importance

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Abstract

Ticks transmit pathogens of veterinary and public health importance. Understanding their diversity is critical as infestations lead to significant economic losses globally. To date, over 90 species across three families have been identified in South Africa. However, the taxonomy of most species has not been resolved due to morphological identification challenges. DNA barcoding through the Barcode of Life Data Systems (BOLD) is therefore a valuable tool for species verifications for biodiversity assessments. This study conducted an analysis of South African tick *COI* barcodes on BOLD by verifying species on checklists, literature, and other sequence databases. The compiled list represented 97 species, including indigenous (59), endemics (27), introduced (2), invasives (1), and eight that could not be classified. Analyses indicated that 31 species (32%) from 11 genera have verified *COI* barcodes. These are distributed across all nine provinces with the Eastern Cape having the highest species diversity, followed by Limpopo, with KwaZulu-Natal having the least diversity. *Rhipicephalus*, *Hyalomma*, and *Argas* species had multiple barcode index numbers, suggesting cryptic diversity or unresolved taxonomy. We identified 21 species of veterinary or zoonotic importance from the Argasidae and Ixodidae families that should be prioritised for barcoding. Coordinating studies and defining barcoding targets is necessary to ensure that tick checklists are updated to support decision-making for the control of vector-borne diseases and alien invasives.

Key words: *COI*, DNA barcoding, ixodidae, pathogen, South Africa, ticks

Introduction

Ticks are obligate external arachnid parasites of the arthropod class Arachnida, belonging to the order Ixodida (Sonenshine 1991). To date, approximately 972 tick species have been described worldwide, with the family Ixodidae (hard-bodied ticks) having 750 species, the Argasidae (soft-bodied ticks) with 221 species, and the monotypic Nuttalliellidae with one species (*Nuttalliella namaqua*) described from different hosts in southern Africa and Tanzania. Nuttalliellidae possess characteristics of both the Argasidae and the Ixodidae the most basal extant lineage of ticks (Guglielmono et al. 2010; Mans et al. 2011). The Ixodidae family with a scutum or hard shield is further classified into two groups based on their morphological and genetic lineage diversity, namely the Prostriata and the Metastriata (Sonenshine 1991). Within the Prostriata is the subfamily Ixodinae, with a single genus *Ixodes* which has 240 known species that include important disease vectors of animals and humans. While the Metastriata includes five subfamilies and 13 genera (Diehl et al. 1982): Amblyomminae (129 species), Bothriocrotoninae (7 species), Haemaphysalinae (164 species), Hyalomminae (25 species), and Rhipicephalinae (81 species) (Black and Piesman 1994;

Klompfen et al. 1996; Nava et al. 2009a). The diversity of this classification demonstrates the taxonomic and phylogenetic complexity of tick taxa. Despite the vast diversity of ticks recorded in South Africa, there is still uncertainty in the taxonomic status and phylogenetic diversity of several species.

Ticks have a global distribution (Magnarelli 2009) and parasitize a wide range of hosts of terrestrial vertebrates, such as birds, reptiles, and mammals (Parola and Raoult 2001; de la Fuente et al. 2008). Abiotic and biotic environmental variables such as humidity, temperature, topography, vegetation types, and rainfall have a significant impact on tick distribution and their abundance in certain areas. Although ticks are extremely tough, hardy, and resilient (Jafarbekloo et al. 2018), environmental conditions have a significant influence on the survival of their four life cycle stages (namely egg, larva, nymph, and adult). Furthermore, the availability of host species also influences tick distribution, as ticks can parasitize specific or different hosts that will either involve one, two, or three hosts (Ixodidae) or multi-hosts (Argasidae) for each of their life cycle stages. As ticks and their life stages depend on the blood meals of their hosts, they tend to be found in greater abundance in areas with high densities of suit-

able hosts. Understanding these ticks' distribution patterns and host associations can provide useful insights into their ecology, potential disease transmission risks, and inform targeted control measures where necessary. This information is critical for numerous other reasons, including understanding their economic impacts on livestock production, identifying potential threats to native species and ecosystems, and developing long-term management strategies.

Ticks are recognized as leading vectors and the second-largest human disease transmitters after mosquitoes. They are important carriers of pathogens that cause infectious diseases (Jongejan and Uilenberg 2004), acting as vectors and reservoirs for the transmission of many zoonotic protozoans, bacteria, and viruses that pose significant health risks to humans and other animals. For example, ticks and tick-borne diseases (TBD) adversely affect approximately 80% of the world's domesticated cattle herds, with an estimated annual cost implication of approximately US\$14–19 billion globally (Kopp et al. 2009). In South Africa, annual financial losses in the agricultural sector due to ticks infestations are estimated between R70 and R550 million (Spickett et al. 2011). However, most of the diversity of ticks remains unknown including the pathogens they harbor. With their high level of adaptability and capacity to live in different environments, ticks are thought to be crucial in the maintenance of TBDs. As the rate of anthropogenic transformation of ecosystems and habitats has increased, most environments have become ideal for the growth of TBDs hotspots. Over the past few decades, there has been a steady increase in the frequency and geographic distribution of TBDs, which has resulted in significant morbidity and occasionally mortality in animals (Jongejan and Uilenberg 2004).

Despite the extensive research on ticks in South Africa (Horak and Cohen 2001; Horak et al. 2003, 2015; Tønnesen et al. 2004; Spickett et al. 2011; Smit et al. 2023), there is still a gap in knowledge regarding the taxonomy and diversity of all known tick taxa. Globally, DNA barcoding, a genetic-based analytical procedure using targeted gene regions from the mitochondrial DNA (mtDNA) and *Cytochrome c Oxidase I (COI)* for animals, has emerged as a major approach for species identification. DNA barcoding genes rely on having intraspecific diversity being lower than interspecific diversity for species delimitation using the barcode index number (BIN) (Hebert et al. 2003; Ratnasingham and Hebert 2013). The BIN system clusters unique sequences using well-established algorithms to produce operational taxonomic units that closely correspond to species. Although the Barcode of Life Data Systems (BOLD) reference database has grown significantly, with over 19 million specimen records published to date (<https://www.boldsystems.org/>: accessed 13 December 2022), barcode records of South African arthropods (approx. 415 000 for 3390 species) including ticks are very limited. Furthermore, the global taxonomic coverage of published tick records (only 302 species barcoded) notably vary among taxonomic groups and geographic regions due to limited related barcoding research. In addition some tick species records on BOLD have unreliable or unverified taxonomic identifications. Therefore, the main aim of this study was to review and highlight the progress in barcoding of South

African tick species to identify gaps in the BOLD reference library to highlight priorities, including diversity, occurrence, and distribution of ticks. This information can also inform national biodiversity assessments and the monitoring of potential TBD risks or threats to humans and animals.

Materials and methods

A South African checklist of barcoded insect species (class Arthropoda) was downloaded from BOLD (BOLD checklist code: CL-SAART updated on 9 September 2009). This progress checklist included barcode records and specific details for six taxon hierarchical levels (phylum, order, family, genus, species, and subspecies). This progress report also included details on DNA barcoding progress to date for the number of (1) available voucher specimen records on BOLD, (2) specimens with *COI* sequences including those mined from the National Center for Biotechnology Information (NCBI) GenBank database, and (3) specimens with DNA compliant barcodes. A barcode-compliant sequence on BOLD should have both reverse and forward sequences, associated collection metadata, and images. Additionally, we accessed the most recently published South African National Biodiversity Institute (SANBI) Tick Checklist (Opus at SANBI: <http://hdl.handle.net/20.500.12143/8791>; issued 30 March 2023). The two species checklists were then verified by species in terms of valid taxonomic names (phylum, class, order, family, genus, species, and subspecies) before merging the two datasets in Microsoft Excel (365, version 2309) for comparison and/or updates for Ixodida species (Ixodidae, Argasidae, and Nuttalliellidae) only (Supplementary Table S1). The BOLD checklist was filtered to only include information on DNA barcode data of tick specimens collected from South African localities (records with verified GPS coordinates on BOLD and the Global Biodiversity Information Facility (GBIF)).

To further confirm and establish the number of tick species barcoded, a search on the BOLD systems BIN database was done manually using the key terms “Ixodidae”, “Argasidae”, “Nuttalliellidae”, and “South Africa”. DNA barcode information was only extracted from BINs with verified South African localities. Finally, for each tick species barcoded from South Africa, we recorded information on the number of specimen records with (1) DNA sequences, (2) verified barcode sequences, and (3) the number of BINs generated for each species. In cases where a valid South African specimen occurrence record of a barcode from a species that was not listed in the SANBI checklist, this species record was added in the final checklist to the appropriate genera and highlighted as new on the South African tick checklist. The South African status of these new species records was then assessed by verifying whether the record represented a new species, synonym, possible new introduction, or invasive species through literature searches and reviews. Finally, we conducted a literature search for each valid tick species on the merged checklist and documented the following information: known distribution ranges, specific known localities, (or occurrence), residency status in South Africa (i.e., Endemic, Indigenous, cryptogenic, alien, or invasive), and published information on transmitted pathogens supported by the list of notifiable diseases (<https://>

Fig. 1. A flow diagram illustrating the workflow including the search, data verification and inclusion, and statistical data analysis process.

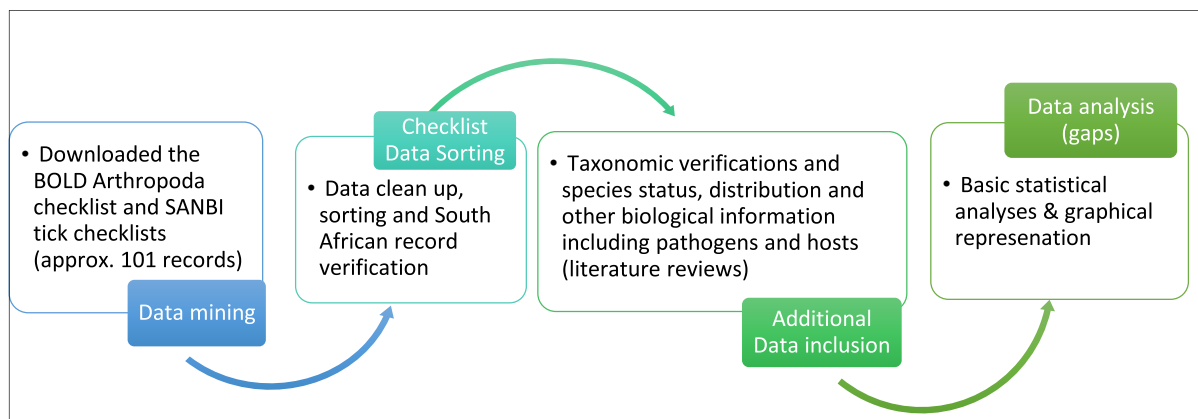
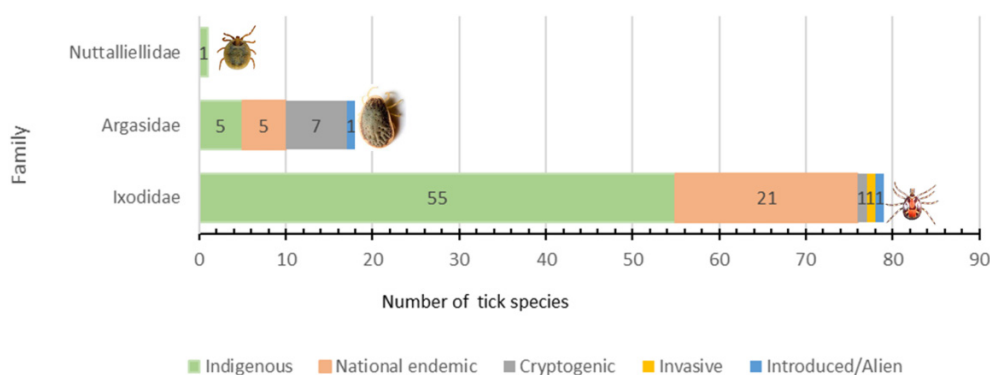


Fig. 2. The number of tick species on the South African checklist categorized according to their native status and the percentage of species barcoded per family.



[//nahf.co.za/controlled-and-notifiable-diseases/](http://nahf.co.za/controlled-and-notifiable-diseases/)) host species preference and host specificity (generalist or specialist). All the data that were generated in the spreadsheet including number of barcodes and sequences, BINs, species status, and residency status were assessed to estimate percentages of representations and gaps in the data and plotted into graphs using Microsoft Excel 365 (version 2309) to visualize the results (see the flow diagram of the methodology in Fig. 1).

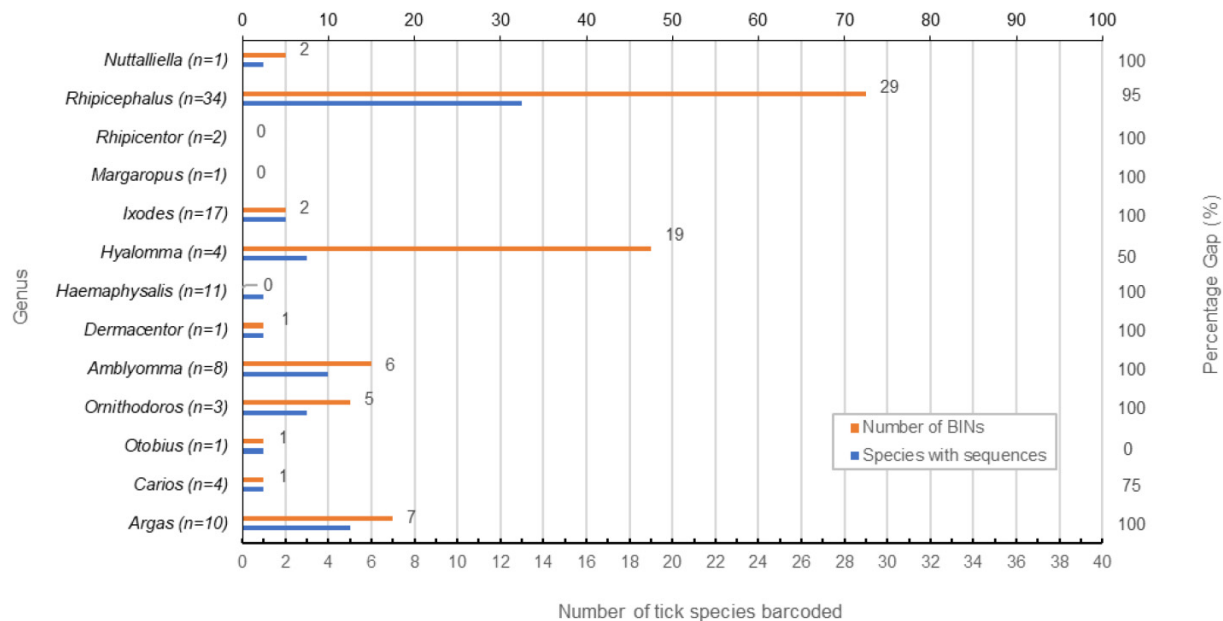
Results

Analysis and comparison of the South African tick checklist (Order: Ixodidae) indicated that all 94 species recorded on the latest SANBI national checklist are included on BOLD. However, BOLD had one additional species, (*Rhipicephalus decoloratus*) that was missing on the SANBI national checklist but included in our analysis. Furthermore, two species (*Argas* sp. *SpringbokSA* and *Ixodes transvaalensis*) recorded in literature that were absent from the checklists were also added to the compilation. Therefore, the final compiled checklist for analysis consisted of 97 unique tick species of which, 27 are endemic (National endemic) to South Africa, while 59 represent either regional or widespread species (indigenous) while

one invasive, and two were alien. We further observed, eight species that could not be verified and classified (cryptogenic) in terms of distribution status (Supplementary Table S1, Fig. 2). These 97 checklist records represent species of Ixodidae (78), Argasidae (18), and Nuttalliellidae (1).

Thirty-one species (32%) from 11 distinct genera (*Amblyomma*, *Argas*, *Carios*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Nuttalliella*, *Ornithodoros*, *Otobius*, and *Rhipicephalus* (*Boophilus*)) had specimen records with verified DNA barcode sequences. Further the DNA barcode sequences were distinct among species. Two genera (*Margaropus* and *Rhipicentor*) had no COI barcode records on BOLD. The 31 distinct tick species (Fig. 3) were, however, represented by 74 BINs indicating high COI genetic diversity as BINs were higher than the number of species barcoded (BIN splits). For example, the genus *Rhipicephalus* on BOLD had 10 unique species represented by 29 BINs suggesting that there might be more COI variation within the genus or cryptic speciation causing misidentifications. At present only two individuals from South Africa have been barcoded from this genus (Supplementary Table S1, Fig. 3). *Argas* with 10 known South African species only had barcode records for five species (50% barcoded) on BOLD that had 7 BINs (14 specimens) also suggesting high COI diversity. Similarly, the genus *Hyalomma* had high genetic diversity be-

Fig. 3. Number of BINs represented by a given number of sequences per genus also indicating the number of known South African species per genus and the percentage of species not barcoded. The number within parentheses represents known species on the South African checklist.



ing represented by three species with 19 BINs but with only two species being barcoded from South Africa. Further, the two South African species accounted for 16 of the 19 BINs indicating high cryptic diversity. Although there were five BINs of *Ornithodoros*, none of these records are from known South African species (Supplementary Table S1, Fig. 3). Similarly, species of *Amblyomma* are also diverse with six BINs on BOLD for four unique species.

The geographical distribution of tick species indicated the presence of Ixodidae species throughout South Africa in all nine provinces while Argasidae species were reported in five and Nuttalliellidae in three (Supplementary Table S2, Fig. 4). The Eastern Cape (EC) province displayed the highest diversity of tick species, followed by Limpopo (L), Mpumalanga (MP), Free State, North West (NW), Western Cape (WC), Northern Cape, and lastly KwaZulu-Natal (KZN).

Host-vector pathogen analysis

The analysis of host preferences among species revealed variations between the Argasidae and Ixodidae families (Supplementary Table S3). For the majority of the Argasidae ticks, limited information was available regarding their host preferences. However, out of the 18 identified Argasidae species, the host species of seven remain unknown while eight were found to generalist infesting birds, reptiles, and mammals. Three species, namely *A. africolumbae*, *A. arboreus*, and *A. walkerae*, exhibited host (bird) specialization. Four tick species from the Argasidae are known to transmit pathogens in South Africa, that could be categorized into three groups based on their relevance as causing (1) controlled diseases, (2) notifiable diseases, and (3) zoonotic pathogens affecting human health (Fig. 5).

Similarly, Ixodidae species also exhibited a broader host range, infesting birds, mammals, and reptiles, with no reports of infestations on amphibian hosts. Notably, most species within the Ixodidae family displayed a generalist behaviour, infesting multiple host species. However, a few species (9) exhibited host specialization, including those of the genus *Amblyomma* (*Am. exornatum*, *Am. latum*, and *Am. (Aponomma) transversal*), *Haemaphysalis* (*H. aciculifer*, *H. colesbergensis*, and *H. cooleyi*), *Ixodes* (*I. theilerae* and *I. transvaalensis*), and *Rhipicephalus* (*R. oculatus*) (Supplementary Table S3). Seven species were associated with the transmission of zoonotic pathogens, seven are vectors of pathogens of veterinary importance, and four transmit controlled diseases affecting human health. *Amblyomma hebraeum* was associated with the transmission of heartwater, benign theileriosis, and African tick-bite fever caused by *Rickettsia africae* (Fig. 5). *Amblyomma marmoreum* and *Am. tholloni* were reported as vectors of heartwater transmitted by *Ehrlichia ruminantium*, and several *Rhipicephalus* species, including *R. appendiculatus*, *R. e. evertsi*, *R. sanguineus*, and *R. zambeziensis* were identified as vectors associated with the transmission of *Theileria* and *Anaplasma* spp. Furthermore, our literature survey implicated *R. decoloratus* and *R. microplus* in the transmission of *Babesia* spp. and *Anaplasma marginale*; however, these species are not barcoded or less barcoded (Supplementary Table S3).

Discussion

South Africa has a high diversity of tick species (estimated >90), with 21 species reported to be endemic being only occur in the country (Horak et al. 2009). Extensive studies using different molecular markers have been done on the genetic characterisation of ticks in South Africa

Fig. 4. The distribution of ticks per province in South Africa. This analysis was based on literature and GBIF records. GBIF data was accessed on the 14th of February 2024. EC = Eastern Cape, FS = Free-state, GP = Gauteng, KZN = KwaZulu-Natal, L = Limpopo, MP = Mpumalanga, NC = Northern Cape, NW = North West, WC = Western Cape; * = Data deficient. The colours represent the occurrence records of each tick species in South African provinces: Green = occurrence in 1–2 provinces (orange = 3–4 provinces), and Red = 5–9 provinces.

Tick species	EC	FS	GP	KZN	L	MP	NC	NW	WC	Tick species	EC	FS	GP	KZN	L	MP	NC	NW	WC
<i>Argas africanus</i>										<i>Ixodes drakensbergensis</i>									
<i>Argas arboreus</i>										<i>Ixodes elongatus</i>									
<i>Argas eboris</i>										<i>Ixodes myotomys</i>									
<i>Argas peringueyi</i> *										<i>Ixodes neitzi</i>									
<i>Argas streptopelia</i> *										<i>Ixodes pilosus</i>									
<i>Argas striatus</i>										<i>Ixodes procaviae</i>									
<i>Argas theileri</i>										<i>Ixodes rhabdomysae</i>									
<i>Argas walkerae</i>										<i>Ixodes spiculae</i> *									
<i>Argas zumpti</i>										<i>Ixodes theileri</i>									
<i>Argas sp. SpringbokSA</i>										<i>Ixodes transvaalensis</i>									
<i>Carios boueti</i>										<i>Margaropus winthemi</i>									
<i>Carios confusus</i> *										<i>Rhipicephalus bicornis</i>									
<i>Carios cordiformis</i> *										<i>Rhipicephalus nuttalli</i>									
<i>Carios faini</i>										<i>Rhipicephalus arnoldi</i>									
<i>Ornithodoros compactus</i>										<i>Rhipicephalus appendiculatus</i>									
<i>Ornithodoros porcinus</i>										<i>Rhipicephalus decoloratus</i>									
<i>Ornithodoros zumpti</i>										<i>Rhipicephalus microplus</i>									
<i>Otobius megnini</i>										<i>Rhipicephalus capensis</i>									
<i>Amblyomma exomatum</i>										<i>Rhipicephalus distinctus</i>									
<i>Amblyomma hebraeum</i>										<i>Rhipicephalus evertsi evertsi</i>									
<i>Amblyomma latum</i>										<i>Rhipicephalus evertsi mimeticus</i>									
<i>Amblyomma marmoreum</i>										<i>Rhipicephalus exophthalmos</i>									
<i>Amblyomma nuttalli</i>										<i>Rhipicephalus foliis</i>									
<i>Amblyomma sylvaticum</i>										<i>Rhipicephalus gertrudae</i>									
<i>Amblyomma tholloni</i>										<i>Rhipicephalus glabroscutatum</i>									
<i>Amblyomma (Aponomma) transversale</i>										<i>Rhipicephalus kochi</i>									
<i>Dermacentor rhinoceros</i>										<i>Rhipicephalus longiceps</i> *									
<i>Haemaphysalis aciculifer</i>										<i>Rhipicephalus lounsburyi</i>									
<i>Haemaphysalis colesbergensis</i>										<i>Rhipicephalus lunulatus</i>									
<i>Haemaphysalis cooleyi</i>										<i>Rhipicephalus maculatus</i>									
<i>Haemaphysalis elliptica</i>										<i>Rhipicephalus muelhensi</i>									
<i>Haemaphysalis hoodi</i>										<i>Rhipicephalus neumanni</i>									
<i>Haemaphysalis hyracophila</i>										<i>Rhipicephalus nitens</i>									
<i>Haemaphysalis parmata</i>										<i>Rhipicephalus oculatus</i>									
<i>Haemaphysalis pedetes</i>										<i>Rhipicephalus oreotragi</i>									
<i>Haemaphysalis silacea</i>										<i>Rhipicephalus sanguineus</i>									
<i>Haemaphysalis silacea</i>										<i>Rhipicephalus simpsoni</i>									
<i>Haemaphysalis zumpti</i>										<i>Rhipicephalus simus</i>									
<i>Hyalomma glabrum</i>										<i>Rhipicephalus sulcatus</i>									
<i>Hyalomma truncatum</i>										<i>Rhipicephalus theileri</i>									
<i>Hyalomma marginatum</i>										<i>Rhipicephalus tricuspis</i>									
<i>Hyalomma rufipes</i>										<i>Rhipicephalus turanicus</i>									
<i>Ixodes alluaudi</i>										<i>Rhipicephalus warburtoni</i>									
<i>Ixodes aulacodi</i>										<i>Rhipicephalus zambeziensis</i>									
<i>Ixodes bakeri</i>										<i>Rhipicephalus zumpti</i>									
<i>Ixodes bedfordi</i>										<i>Rhipicephalus punctatus</i> *									
<i>Ixodes catherinei</i>										<i>Rhipicephalus glabroscutatus</i>									
<i>Ixodes cavipalpus</i>										<i>Nuttalliella namaqua</i>									
<i>Ixodes cornini</i>																			

(Bakkes et al. 2020; Mofokeng et al. 2021; Wyk et al. 2022; Hornok et al. 2023). Although these studies have increased our knowledge on different aspects of tick biology (including their taxonomy, ecology, systematics, host-vector relationships, and disease dynamics), studies are still lacking on the DNA barcoding of ticks. This study reports a total of 97

species in South Africa (Supplementary Table S1) belonging to the families Ixodidae, Argasidae, and Nuttalliellidae after verification of checklists. This finding contrast with previous published numbers (Horak et al. 2009), which estimated 106 species (80 ixodid, 25 argasid, and *Nuttalliella namaqua*) occurring in South Africa. This discrepancy in estimates of

Fig. 5. Vector-pathogen analysis based on available records for selected tick species used in this study (Aeschlimann 1967; Bezuidenhout 1988; Cutler 2006; de Castro 1997; De Vos et al. 2004; Ergonul and Whitehouse 2007; Gothe et al. 1986; Hoogstraal 1985, 1979; Horak et al. 2018, 2015; Kleiboeker and Scoles 2001; Labuda and Nuttall 2008; Ledger et al. 2021; Lewis et al. 1996; MacKenzie and Norval 1980; Madder et al. 2014; Mans et al. 2012, 2011; Mitani et al. 2004; Mumcuoglu et al. 2005; Noda et al. 1997; Norval and Horak 2004; Penzhorn and Chaparro 1994; Penzhorn et al. 1994; Rebaudet and Parola 2006; Swanepoel 1994; Turell et al. 1994; Walker et al. 2003). Pathogens that cause Zoonotic diseases*, pathogens that cause diseases of veterinary importance**, pathogens that cause controlled diseases***, and notifiable diseases****. <https://nahf.co.za/controlled-and-notifiable-diseases/>. Note A = Argas; Am = Amblyomma; H = Haemaphysalis; Hy = Hyalomma.

Tick species	African swine fever****	Anaplasma bovis	Anaplasma marginale**	Babesia bigemina**	Babesia bovis**	Babesia caballi**	Babesia canis**	Babesia occultans	Babesia rossi	Babesia theileri	Babesia traubmanni	Babesia vogeli	Borrelia anserina	Borrelia duttoni	Borrelia tilling	Crimean-Congo hemorrhagic fever virus*	Ehrlichia canis	Ehrlichia ruminantium**	Hepatozoon canis	Nyamanyi virus	Pretoria virus	Quarantini virus*	Rickettsia africana*	Rickettsia conorii*	Rickettsia aeschlimannii*	Rickettsia sibirica mongolotimonae*	Rickettsia massillae*	Theileria equi**	Theileria mutans	Theileria parva***	Theileria separata	Theileria taurotragi	Theileria velifera	West Nile virus****		
<i>A. africanum</i>																																				
<i>A. arboreus</i>																																				
<i>O. porcinus</i>																																				
<i>O. zumpti</i>																																				
<i>Am. hebraeum</i>																																				
<i>Am. marmoreum</i>																																				
<i>Am. tholoni</i>																																				
<i>H. elliptica</i>																																				
<i>Hy. rufipes</i>																																				
<i>Hy. marginatum</i>																																				
<i>Hy. truncatum</i>																																				
<i>R. appendiculatus</i>																																				
<i>R. decoloratus</i>																																				
<i>R. microplus</i>																																				
<i>R. e. evertsi</i>																																				
<i>R. e. mimeticus</i>																																				
<i>R. sanguineus</i>																																				
<i>R. simus</i>																																				
<i>R. tricuspis</i>																																				
<i>R. turanicus</i>																																				
<i>R. zambeziensis</i>																																				

numbers of species can be attributed to revisions due to advances in taxonomic knowledge and molecular identification techniques that have resulted in the reclassification of tick species, accounting for inconsistencies among the two studies.

COI Subunit 1 is considered as the approved genetic marker for DNA barcoding animal species identification as was observed in our analysis. This is due to its high discriminatory power of closely related species, the availability of extensive reference databases, making it a valuable tool for accurate species delimitation and the discovery of species. The analysis of the barcoding library revealed that species belonging to *Rhipicephalus* (*R. appendiculatus*, *R. arnoldi*, *R. microplus*, *R. evertsi*, *R. glabroscutatus*, *R. maculatus*, *R. muelhensi*, *R. sanguineus*, *R. turanicus*, and *R. zambeziensis*) and *Hyalomma* (*Hy. glabrum*, *Hy. truncatum*, and *Hy. rufipes*) genera are the most barcoded among South African species. Of these species, *R. appendiculatus*, *R. microplus*, *R. evertsi*, *R. sanguineus*, *R. turanicus*, and *R. zambeziensis* have been associated with disease transmission causing economic losses in the agricultural sector (Baker et al. 1989; de Castro 1997; Walker 2000; Norval and Horak 2004;

Nyangiwe et al. 2017). However, *Rhipicephalus* species are difficult to identify morphologically as many species share similar features. It is noteworthy that while past research has primarily focused on the four endemic species and four indigenous species due to the economic losses they cause in the livestock sector (Horak et al. 2009, 2015; Nyangiwe et al. 2017; Sonenshine 2014; Makwarela et al. 2023), a total of 20 tick species have been implicated as human tick species in South Africa (Hornok et al. 2002) highlighting their importance in the transmission of zoonotic diseases.

Similarly the genus *Hyalomma*, has had a lot of unclear taxonomic classifications in the past that could be verified using DNA barcoding. For instance, *H. rufipes*, previously listed as a subspecies of *H. marginatum* has since been listed as a valid species (Apanaskevich and Horak 2008), while *H. marginatum turanicum* previously synonymised is now reinstated as *H. glabrum* (Apanaskevich and Horak 2006). In such instances, it is ideal to incorporate molecular techniques including DNA barcoding, using multiple genes or methods to inform species delineation and taxonomic re-evaluation (Amrutha et al. 2023).

Hyalomma species such as *H. truncatum* and *H. rufipes*, are important to study because they are also involved in the transmission of pathogens that are either zoonotic (*Rickettsia conorii*, Crimean-Congo haemorrhagic fever virus) or of veterinary importance (*Anaplasma marginale*, *Babesia caballi*, *Theileira parva*, *Theileira equi*) (Makwabela et al. 2023; Horak et al. 2018). Although some specimen records for these species have been uploaded on BOLD, we observed that the two genera (*Rhipicephalus* and *Hyalomma*) have diverse species that were represented by more than one BIN. This often occurs when intraspecific variation cannot be assessed adequately due to high genetic variation or due to misidentification of records submitted to BOLD for closely related cryptic taxa.

Ixodes is the second largest tick genus in southern Africa, and the largest genus in the family Ixodidae globally. According to Guglielmone et al. (2010), this genus has approximately 240 species, with its taxonomy still being poorly known. Tick species in this genus are commonly found in reptiles, birds and mammals host species (Guzmán-Cornejo and Robbins 2010). A number of *Ixodes* species are of significant medical and veterinary importance as they transmit pathogens that cause disease in both wild and domestic animals, including humans (Guzmán-Cornejo and Robbins 2010). Our results show that none of the South Africa species belonging to this genus have been barcoded to date. This is of concern as there are still gaps regarding their species diversity and taxonomic classification including their role in diseases transmission. Due to the complexity in this genus, DNA barcoding is essential and may play a critical role for this particular genus to also assist with resolving the current taxonomic ambiguities that have been reported (Anstead et al. 2011; Burger et al. 2012; Blanco et al. 2013).

The results further revealed a significant gap in the availability of genetic information for tick species belonging to two genera, *Rhipicephalus* and *Margaropus*, on BOLD and other sequence databases. Although, *Rhipicephalus* has only two recognized species globally, *R. nuttalli* and *R. bicornis* (Theiler 1961), there was no sequence data for either of these species on BOLD. Similarly, the genus *Margaropus* comprising of three known species, *M. reidi*, *M. wileyi*, and *M. winthemi*, with the latter being the only species occurring in South Africa (Barker and Murrell 2004) had limited *COI* barcode data. This suggests that there is a significant gap in the barcoding library for tick species in the two genera. Although the tick species belonging to these two genera are not known to transmit any diseases, their impact on animal health should not be overlooked. Madder et al. (2014) reported that large infestations of *M. winthemi*, can lead to significant deaths of zebra ferals. Additionally, *R. nuttalli* has been associated with paralysis in dogs suggesting that understanding the diversity of these species is necessary.

According to our results, 11 species of the genus *Haemaphysalis* genera occur in South Africa. However, this genus has no *COI* barcode data with only one sequence of *H. hoodi* deposited in BOLD. *Haemaphysalis elliptica* known as the southern African yellow dog tick has not been barcoded, although it plays an important role in the transmission of *Rickettsia africae* a zoonotic pathogen, as well as a vector of *Babesia rossi*, the causative agent of canine babesiosis in dogs (Madder et

al. 2014). In the past, *H. elliptica* was wrongly identified as *H. leachi*, but it has since been reinstated as a valid species (Apanaskevich and Horak 2006; Madder et al. 2014; Horak et al. 2018). When extracting mtDNA *COI* data on BOLD for the current study, we could not obtain any sequences linked to *H. elliptica*; however, we could only find a specimen of *H. leachi* associated with a BIN from Nigeria (BOLD: AEG9405). This highlights the importance of conducting South African research on DNA barcoding to fill the gaps in the missing genetic data for these particular tick species.

This study further revealed the presence of one invasive tick species, *R. (Boophilus) microplus*, and two alien species, *R. evertsi mimeticus* and *Otobius megnini* in South Africa. *Rhipicephalus microplus* known as the Asian blue tick is one of the notable ticks species infesting livestock in many parts of the world (Coetzer and Tustin 2004). According to Madder et al. (2011), *R. microplus* was introduced in South Africa through cattle imported from Asia, via Madagascar and has since extended its distribution (Tønnesen et al. 2004). In certain parts of Africa, the invasive cattle tick species has been able to replace and displace the closely related native tick species, *R. decoloratus*, also known as the African blue tick (Madder et al. 2011; Tønnesen et al. 2004). *Rhipicephalus e. mimeticus*, also known as the Namibian red-legged tick, is indigenous to Namibia and Angola, and is reported to have been introduced into South Africa on more than one occasion (Walker 2000). Spickett et al. (2011) speculated about the geographical expansion of this tick whether it would establish itself in the country, and their findings indicated that the expansion has occurred. However, this species has no barcoding data on BOLD. *Otobius Megnini* or the spinose ear tick, is regarded as an economically important soft tick as it parasitizes various hosts including, livestock, horses as well as humans (Jensen et al. 1982; Nava et al. 2009b). In horses, the tick species causes horse ootacariasis which may cause serious injury and occasionally death (Hall 1923; Rich 1957). The tick species was introduced from America and has established itself in many parts of the country (Theiler and Salisbury 1958). According to Nava et al. (2009a) and Walker et al. (2003), the tick species can easily be transported by hosts into new locations, potentially increasing its distribution.

Our results indicate that there is a gap in DNA barcoding of ticks based on BOLD. This finding is a significant indicator of why it is important to conduct surveys and research on tick distribution and the role they play in disease transmission. Sonenshine et al. (2006) reported that, tick distributions are essential for establishing tick-borne disease prevalence and the ecological conditions that ticks are exposed to.

Host-vector analysis

South Africa is known for its remarkably diverse species richness, and climatic conditions, that support a wide range of ticks and a high prevalence of tick-borne pathogens (Frawley et al. 2024; Horak et al. 2018; Ledwaba et al. 2022; Makwabela et al. 2023). For survival, ticks require the presence of a host to maintain their life cycle. According to Tälleklint and Jaenson (2014) the distribution of ticks infesting cattle depends on host availability species and vegetation.

In another study done by Hoogstraal and Aeschlimann (1982) they stated that, most tick species in the family Ixodidae have a strong preference of their host, meaning that they are confined to the areas where their host is normally found. Furthermore, Horak et al. (2015) mentioned that cattle and the larger wildlife species are the preferred hosts of the adults of a multitude of ixodid tick species in South Africa. For example, *Am. marmoratum* and *Am. hebraeum*, are associated with various host species including bovines, reptiles, mammals, and avian hosts (Horak et al. 2018; Ledwaba et al. 2022) making them generalists with the exception of *Am. exornatum*, a specialist tick of reptiles, specifically infesting monitor lizards (Hoogstraal 1956; Mofokeng et al. 2021).

Our findings revealed that various tick genera, including *Amblyomma*, *Rhipicephalus*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Rhipicephalus*, *Otobius*, and *Ornithodoros*, and some species in the *Argas*, infest both wild and domestic animals with incidental findings in humans (Horak et al. 2018). Horak et al. (2002) recorded 20 species of ticks that feed on humans in South Africa. However, species in these genera are not well represented on BOLD, and they are not barcoded. *Haemaphysalis* species, *H. elliptica*, *H. hoodi*, *H. hyracophila*, and *H. Zumpti*, are generalist as they are associated with various host species. Similarly with tick species in the *Hyalomma* genera, *Hy. truncatum*, *Hy. rufipes*, and *Hy. marginatum*, resemble the same characteristics of being generalists, as they infest various hosts including mammals, birds as well as humans according to our analysis (Walker et al. 2003).

The *Margaropus* genus contains only three species, *M. reidi*, *M. wileyi*, and *M. winthemi*, with the latter being the only species occurring in South Africa (Walker et al. 2003). This tick species is known as the South African winter horse tick or beady-legged tick, as it parasitizes particularly horses, zebras, eland as well as cattle (Madder et al. 2014). The *Rhipicephalus* genus is the largest genus in Southern Africa (Walker 1991). According to Walker (2000), various species belonging to this genus in Africa are vectors of pathogens affecting domestic and wild animals, and rarely humans. Our results show that tick species belonging to this genus are generalists, mostly reported in mammals, birds as well as humans, with the exception of two specialist species: *R. oculatus* a parasite of hares and *R. arnoldi* a parasite of hares and rabbits (Horak et al. 2018). Argasidae species, such as *A. walkerae* and *O. compactus* were noted as specialists as they are associated with avian and reptile hosts, respectively. *Otobius megnini* was associated with infestations in mammals (horses, cattle, sheep, goats, dogs, donkeys, mules, cats, and occasionally infesting humans).

These findings highlight the diversity in host preferences among tick species, with some exhibiting a high degree of specialization, while others are more generalist in their host associations.

Geographical distribution of ticks

This study identified 30 prevalent tick species belonging to 9 genera in South Africa based on occurrence record data. These genera include *Amblyomma*, *Argas*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Margaropus*, *Ornithodoros*, *Otobius*, and *Rhipi-*

cephalus (Supplementary Table S2, Fig. 3). Furthermore, our data showed variations in the diversity of tick species across different provinces, with EC having the highest number of species ($n = 47$) followed by KZN ($n = 42$), WC ($n = 40$), and L ($n = 36$) (Supplementary Table S2). This high diversity can be attributed to the diverse range of ecological conditions and habitats present in these regions. These provinces have a variety of biomes, including grasslands, savannas, forests, Albany thicket, and coastal regions, which provide suitable environments for different ticks and their respective hosts (Horak et al. 2009, 2018; Makwarela et al. 2023; Nyangiwe et al. 2011). Therefore these provinces should be prioritised for sampling for barcoding assessments of tick diversity. Provinces with a higher diversity of wildlife, livestock, are more likely to harbour a greater variety of tick species, as many ticks exhibit host preferences or require specific hosts to complete their life cycles (Horak et al. 2015; Léger et al. 2013). Moreover, the widespread distribution of these tick species across multiple provinces is likely attributed to the continuous trade of animals (legal and illegal) between and within provinces (Cumming 2002; Horak et al. 2015). These also highlights the need for monitoring (including barcoding) of wildlife trade with regards to vector distributions and pathogen transmission. In contrast, MP and the NW had the least number of tick species. This may be influenced by factors such as habitat fragmentation, urbanization, and agricultural practices that could potentially limit the diversity and abundance of tick hosts (Dantas-Torres 2015).

For most of the Argasidae we could not find comprehensive information regarding their occurrence and distribution in the country suggesting that this family is less understudied. However, we only obtained data for three species *A. walkerae*, *O. compactus*, and *O. megnini*, which revealed a wide distribution across the country with occurrences reported in four or more provinces. Notably, the distribution of *O. megnini*, has expanded to other provinces since its first introduction in 1898 (Madder et al. 2014; Theiler and Salisbury 1958). The scarcity of information available for the majority of Argasidae ticks in South Africa highlights the need for targeted research and documentation of their diversity. Only one species *O. megnini* has been barcoded in South Africa.

Conclusion

Several genetic studies have been conducted on ticks, focusing on their distribution, disease transmission, and identification methods. While these findings are significant, tick species involved with disease transmission are still underrepresented on BOLD. According to Dantas-Torres and Otranto (2016), the Ixodidae family are the most common vectors of pathogens that are significant worldwide in both veterinary and medical health. Further, the Argasidae family taxonomy remains incomplete due to the lack of consensus on taxonomic guidelines for morphological identification. These taxonomic issues and incomplete geographic coverage highlight the need for additional tick research and molecular characterization to understand their diversity and all potential implications for public health. Integrating molecular data such as DNA barcodes with ecological and epidemiological infor-

mation can enhance bio-surveillance to assess disease risks for control and/or eradication implementation. This is crucial for developing targeted control strategies, mitigating disease transmission risks, and safeguarding both animal and human health. In conclusion, the findings of this study emphasize the importance of prioritizing the building of DNA barcoding species reference libraries of tick species, particularly those implicated in disease transmission.

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Data availability

The tick checklist is available on OPUS <http://hdl.handle.net/20.500.12143/8791>.

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