Integrative Taxonomy and Species Delimitation of *Rhipicephalus turanicus* (Acari: Ixodida: Ixodidae)

Authors: DEON K. BAKKES[1,2*], LIDIA CHITIMIA-DOBLER[3], DIKELEDI MATLOA[1], MORNÉ OOSTHUYSEN[1], KOSTA Y. MUMCUOGLU[4], BEN J. MANS[1,5,6], CONRAD A. MATTHEE[2**]

ORCID ID: *0000-0002-5716-0801; **0000-0002-6836-069X

Affiliation: [1]*Gertrud Theiler Tick Museum, Epidemiology, Parasites and Vectors, Agricultural Research Council – Onderstepoort Veterinary Research, Pretoria, 0110, South Africa.* [2]*Evolutionary Genomics Group, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa.* [3]*Bundeswehr Institute of Microbiology, Neuherbergstrasse 11, D-80937 Munich, Germany.* [4]*Parasitology Unit, Department of Microbiology and Molecular Genetics, The Kuvin Center for the Study of Infectious and Tropical Diseases, Hadassah Medical School, The Hebrew University, Jerusalem, Israel.* [5]*The Department of Veterinary Tropical Diseases, University of Pretoria, Pretoria, South Africa.* [6]*Department of Life and Consumer Sciences, University of South Africa, South Africa.*

**Running title**: Integrative taxonomy of *Rhipicephalus turanicus*

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Correspondence: Corresponding author:

Deon K. Bakkes

Gertrud Theiler Tick Museum,

Epidemiology, Parasites and Vectors,

Agricultural Research Council – Onderstepoort Veterinary Research,

Pretoria, 0110, South Africa

Email: BakkesD@arc.agric.za, seventiaguitarist@gmail.com

Phone: +27 12 529 9302

Highlights

- A new African tick species is described that was previously considered part of *Rhipicephalus turanicus* in the Palearctic.
- Past research has shown it can interbreed with a related species, despite differences in morphological and molecular data.
- Speciation likely occurred recently via allopatry as a result of Sahara desert expansion about 7 million years ago.
- Hybridization potential was likely driven by fluctuating divergence and re-integration due to oscillating climate history.
- This is the first application of geometric morphometrics to determine character shape differences between species of ticks.

Graphical abstract
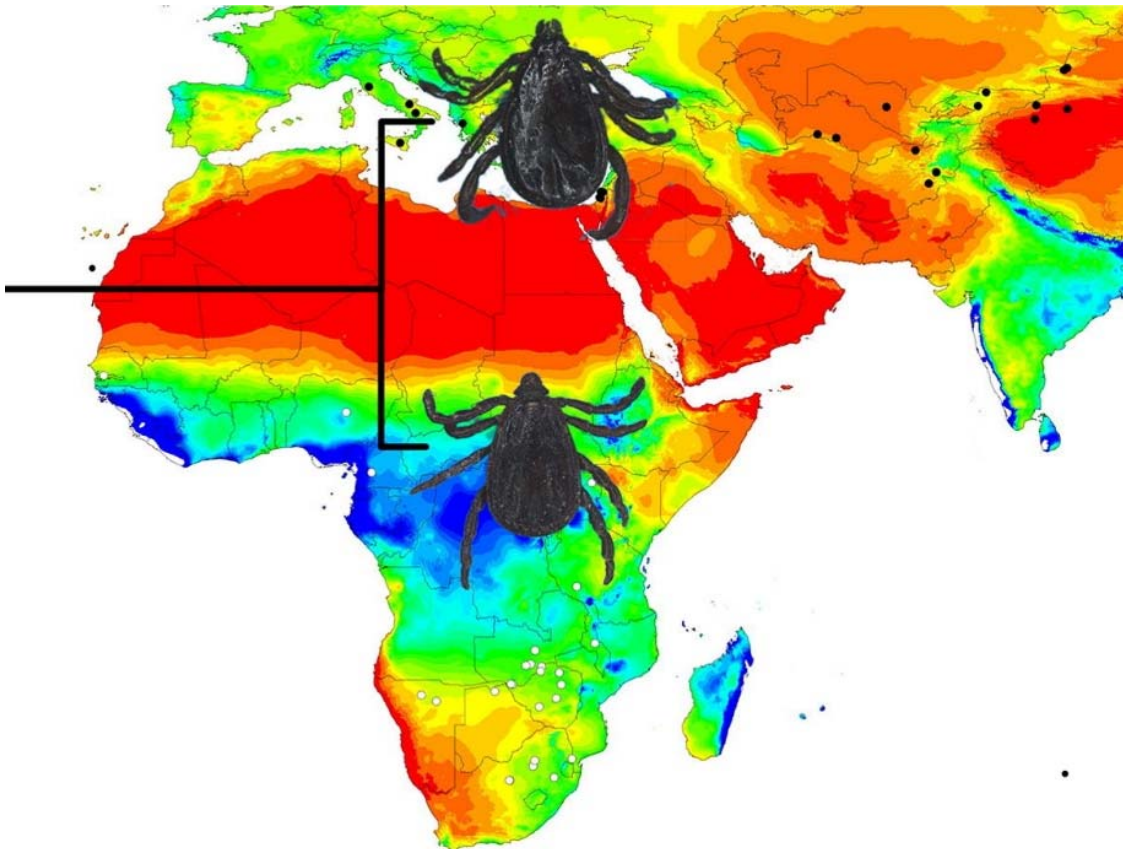
Abstract

Rhipicephalus turanicus ticks are widely distributed across the Palearctic and Afrotropics. These two continental populations display differences in morphological characters that raise the question of a potential species boundary. However, the taxonomic status of these morphologically divergent lineages is uncertain because *R. turanicus* from Cyprus and Zambia have been shown to interbreed and produce fertile hybrids. We employ integrative taxonomy that considers data from mtDNA sequences (12S and 16S rDNA), geographic distribution, traditional (qualitative) morphology, as well as shape outlines of female spiracles and male adanal plates measured in a geometric morphometric framework (quantitative morphology) to resolve this taxonomic issue. Molecular lines of evidence (12S and 16S rDNA) support taxonomic separation between ticks sampled in the Afrotropics and the Palearctic. This is corroborated by qualitative and quantitative morphology. Within the Palearctic, two sub-lineages were recovered based on sequence data that loosely correspond to southern Europe and the Middle-East/Asia. One new species, *Rhipicephalus afranicus* **n. sp.** is described from South Africa with a geographic distribution that extends into east Africa. This leaves *R. turanicus sensu lato* comprised of two lineages located in southern Europe and the Middle-East/Asia. The type locality for *R. turanicus* is in Uzbekistan, thus the Middle-East/Asia lineage is considered *R. turanicus sensu stricto*. Detailed descriptions are provided for *R. afranicus* **n. sp.** and *R. turanicus s. str.* along with high-resolution images. Speciation is attributed to recent Sahara desert expansion that formed a natural barrier to dispersal about 5-7 Mya. However, reproductive potential between these two species suggests that divergence time and mode of speciation were not sufficient for the development of reproductive isolation. We suggest speciation was complicated by divergence and population reintegration events driven by oscillating climate conditions contributing to reticulate

evolution and maintenance of compatibility between reproductive mechanisms. This study represents an integrative (iterative) approach to delimiting *Rhipicephalus* species, and provides the first application of shape outlines for female spiracles and male adanal plates measured in a geometric morphometric framework, applied to testing species boundaries between ticks.

Key words: Tick; Taxonomy; Cryptic Species; Afrotropical; Paleartic; Morphometrics; Morphology; rDNA; Procrustes.

1. Introduction

Rhipicephalinae represent one of six subfamilies of hard-bodied ticks within Ixodidae (Guglielmone et al., 2010). Within Rhipicephalinae, the genus of brown ticks, *Rhipicephalus* Koch, 1844, comprise approximately 75 species mostly confined to the Afrotropics. Taxonomy and evolutionary history of the genus is not well-resolved and *Rhipicephalus turanicus* Pomerantsev, 1940 is a case in point. Morphologically, this species closely resembles *Rhipicephalus sanguineus* (Latreille, 1806) and *Rhipicephalus sulcatus* Neumann, 1908 (Walker et al., 2000). Together these species form part of the *R. sanguineus* group which comprise both Afrotropical and non-Afrotropical species including *Rhipicephalus camicasi* Morel, Moucheti & Rodhain, 1976, *Rhipicephalus leporis* Pomerantsev, 1946, *Rhipicephalus pumilio* Schulze, 1935, *Rhipicephalus pusillus* Gil Collado, 1936, *Rhipicephalus rossicus* Yakimov & Kol-Yakimova, 1911, *Rhipicephalus schulzei* Olenev, 1929 and *Rhipicephalus guilhoni* Morel & Vassiliades, 1963.

Rhipicephalus turanicus have a wide geographic distribution from the Afrotropics to Palearctic with its type locality in Uzbekistan (Filippova, 1997). Closely related *R. sanguineus* are cosmopolitan and previous investigations have suggested that at least

three taxonomic lineages may exist – one associated with tropical climates, one with temperate climates, and a third limited to south-eastern Europe (Dantas-Torres et al., 2013; Hekimoğlu et al., 2016; Zemtsova et al., 2016; Chitimia-Dobler et al., 2017; Coimbra-Dores et al., 2018). Apart from being used as an outgroup in phylogenetic studies of the *R. sanguineus* group (Dantas-Torres et al., 2013; Hekimoğlu et al., 2016; Zemtsova et al., 2016; Chitimia-Dobler et al., 2017) and *Rhipicephalus appendiculatus* and *Rhipicephalus zambeziensis* (Mtambo et al., 2007a, 2007b, 2007c), intraspecific sequence data for Afrotropical *R. turanicus* is limited. Nonetheless, these studies have indicated considerable divergence between Afrotropical and Palearctic *R. turanicus*. This highlights the need for more data to assess the problem of species boundary and test monophyly in *R. turanicus* ticks.

Rhipicephalus turanicus is a three host species with an adult stage that generally parasitizes goats, dogs, cattle, sheep, lions and occasionally horses (Walker et al., 2000; Horak et al., 2018). These ticks have been implicated in transmitting *Babesia* and *Hepatozoon* that are linked to animal diseases (Gianelli et al., 2016; Walker et al., 2000). In Europe, *R. turanicus* has been implicated in transmission of *Theileria equi* (Friedhoff, 1988). Notably, South African specimens were unsuccessful in transmitting *Babesia caballi* and *Theileria equi* to horses (Potgieter et al., 1992; Walker et al., 2000). Geographically distant individuals of *R. turanicus* from Zambia (Afrotropical) and Cyprus (Palearctic) have divergent morphology, but readily interbreed in laboratory environments and produce viable progeny with hybrid vigour, presumably by heterosis (Pegram et al., 1987). Across all laboratory matings, 90% produced offspring, but a higher fecundity was observed in crosses between Zambian and Cypriot individuals (*circa* 5000 eggs in hybrid matings vs *circa* 4000 in same-population matings). Following this, past workers have opted to defend the *R. turanicus* species boundary based on the biological species

concept (Pegram et al., 1987; Walker et al., 2000; Horak et al., 2018). However, the biological species concept has been shown insufficient in certain cases (De Queiroz, 2005, 2007; Sokal and Crovello, 1970), and ticks are no exception (Araya-Anchetta et al., 2013; Kovalev et al., 2015).

Two hypotheses of species boundary may be considered. Either *R. turanicus* in the Afrotropics and Palearctic represent a single morphologically divergent species, or two distinct species exist that are able to hybridize but would not do so in nature due to disjunct distributions. Preliminary data suggest the latter (Dantas-Torres et al., 2013; Hekimoğlu et al., 2016; Mtambo et al., 2007a, 2007b, 2007c; Zemtsova et al., 2016), and if this is true, hybrid vigour may have contributed to introgression and evolutionary reticulation between these two species. In the present study, we test the monophyly of *R. turanicus* based on integrative taxonomy that considers multiple lines of evidence including traditional qualitative morphology, quantitative morphology of shape outlines in female spiracles and male adanal plates (geometric morphometrics), 12S and 16S rDNA molecular sequence data, as well as geographic distribution patterns. Shape outlines of specific morphological features can hold useful clues for taxonomy and species delimitation, given that conspecific individuals should display more similar morphological shapes as opposed to heterospecific individuals. Excluding cases of destabilizing selection, shape outlines are expected to tend toward a central mean within a population of interbreeding individuals. Geometric morphometrics can prove especially useful to quantify such shape distributions between individuals and species given the high resolution afforded by the methods (Adams et al., 2013; Klingenberg, 2010; Mitteroecker and Gunz, 2009; Slice, 2007). These methods can measure statistical distribution of shape variables to determine whether central tendencies overlap between *a priori* hypothesized species. Notably, this has been applied to morphological cryptic species

6

complexes with demonstrated success (Bakkes et al., 2018; Karanovic et al., 2016; Mutanen and Pretorius, 2007; Pretorius and Clarke, 2001, 2000; Villemant et al., 2007). However, convergence can introduce homoplasy to such data, and other lines of evidence that can delimit species boundaries should be employed to corroborate or refute findings. The approach adopted in this study assumes a contemporary formulation of integrative taxonomy that tests a species boundary hypothesis along an iterative framework against multiple lines of evidence (Yeates et al., 2011; Skoracka et al., 2015; Dantas-Torres, 2018).

2. Material and methods

2.1 Samples and qualitative morphology

Fresh adult specimens were collected from a range of localities by dragging (Table S1: Supplementary material). Additional specimens of all life stages were obtained from the Gertrud Theiler Tick Museum (ARC-OVR), South Africa (GTTM) and were used for qualitative and quantitative morphology as well as sequencing in some cases (Table S1). Specimens were identified *a priori* according to established taxonomic characters (Horak et al., 2018; Pegram et al., 1987; Walker et al., 2000), and were categorized by geographic locality. Specimens were examined under a Zeiss Discovery.V20 Stereomicroscope for qualitative morphological characters. Terminology generally follows that of Horak et al. (2018). Complete specimen data are presented in the material examined (Table S1).

2.2 Phylogenetic analysis of 12S and 16S rDNA

Two legs were removed from individual samples for DNA extraction using the prepGEM extraction kit (ZyGEM, Hamilton, New Zealand) according to the manufacturer's

instructions. 16S rDNA was amplified using the 16SF and 16SR primers (Black and Piesman, 1994) and 12S rDNA was amplified using the 12S+1 and 12S-1 primers (Norris et al., 1999). PCR cycles included initial denaturation: 94°C (2min); 40 cycles of 94°C (30s), 45±2°C (30s), 72°C (2min); final elongation: 72°C (2min). PCR products were sequenced at the Central Analytical Sequencing Facility at Stellenbosch University following the Sanger method and by using the 16SR and 12S-1 primers. All sequences were deposited in GenBank (sequences MK158971-MK159007 and MN944860-MN945345). Additional sequences for *R. turanicus*, *R. sanguineus, R. guilhoni, R. camicasi, R. pusillus, R. rossicus, R. pumilio* and outgroups *R. appendiculatus* and *Rhipicephalus evertsi evertsi* were retrieved from GenBank for analysis (Table S1).

Sequences were aligned using MAFFT (Q-INS-i, 200PAM / k=2, Gap opening penalty: 1.53) (Katoh et al., 2002). Optimal nucleotide substitution models were selected using BIC calculations in W-IQ-TREE (Trifinopoulos et al., 2016). Nucleotide substitution models were determined as TPM3u+F+G4 (Kimura, 1981) for 16S rDNA and TN+F+G4 for 12S rDNA (Tamura and Nei, 1993). Optimal models and associated parameters were applied to all subsequent analyses. Phylogenetic networks were estimated in SplitsTree v4.14.3 (Huson and Bryant, 2006) using neighbour network analysis with 1000 bootstrap replicates. Phylogenetic network analyses are well suited for cryptic species problems where speciation is complex because the analysis considers reticulate networks that make reticulate evolution explicit (Nakhleh et al., 2005). Analysis for each gene was performed separately with the 12S rDNA dataset including 171 aligned sequences with 256 nucleotide sites, with 63 phylogenetically informative and 163 conserved. The 16S rDNA dataset included 157 aligned sequences with 305 nucleotide sites, with 80 phylogenetically informative and 164 conserved. Pairwise genetic distances were

calculated in MEGA v7.0.14 (Kumar et al., 2016) and then converted to pairwise genetic similarity.

For combined analysis, 12S and 16S rDNA data were concatenated into a single matrix in SequenceMatrix v1.8 (Vaidya et al., 2011) and only included taxa with data for both genes. Separate 12S and 16S rDNA sequences from GenBank were concatenated into single taxa in the data matrix after confirmation that each sequence belongs to the same species and lineage with respect to the 12S and 16S rDNA topologies (Figs 1-2). Furthermore, these sequences were concatenated according to locality (Table S1). Sequences that could not be combined due to lack of reciprocal 12S and 16S rDNA sequences from the same locality for the same species were excluded to minimize taxa with missing data. Thus, the total dataset for combined analysis consisted of 66 taxa. Maximum likelihood inference was done using 1000 bootstrap replicates followed by a thorough maximum likelihood search in RaxML v8.1.20 (Stamatakis, 2014). Bayesian phylogenetic inference was done using two Monte-Carlo Markov chains run simultaneously for 5 million iterations sampling every 200th iteration in MrBayes v3.2.6 (Ronquist and Huelsenbeck, 2003). The first 25% of trees were discarded as burn-in and a majority-rule consensus tree with posterior probabilities was calculated. Tracer v1.6 (Drummond and Rambaut, 2007) was used to assess convergence and effective sampling between all runs, and all ESS values were greater than 200 indicating effective sampling (Drummond et al., 2006).

2.3 Geometric morphometric analysis of male adanal plates and female spiracles

The total sample for morphometric analysis consisted of landmark data for 69 male adanal plates and 76 female spiracles among nine species divided into twelve lineage groups. These included Afrotropical *R. turanicus,* Palearctic *R. turanicus, R. sanguineus*
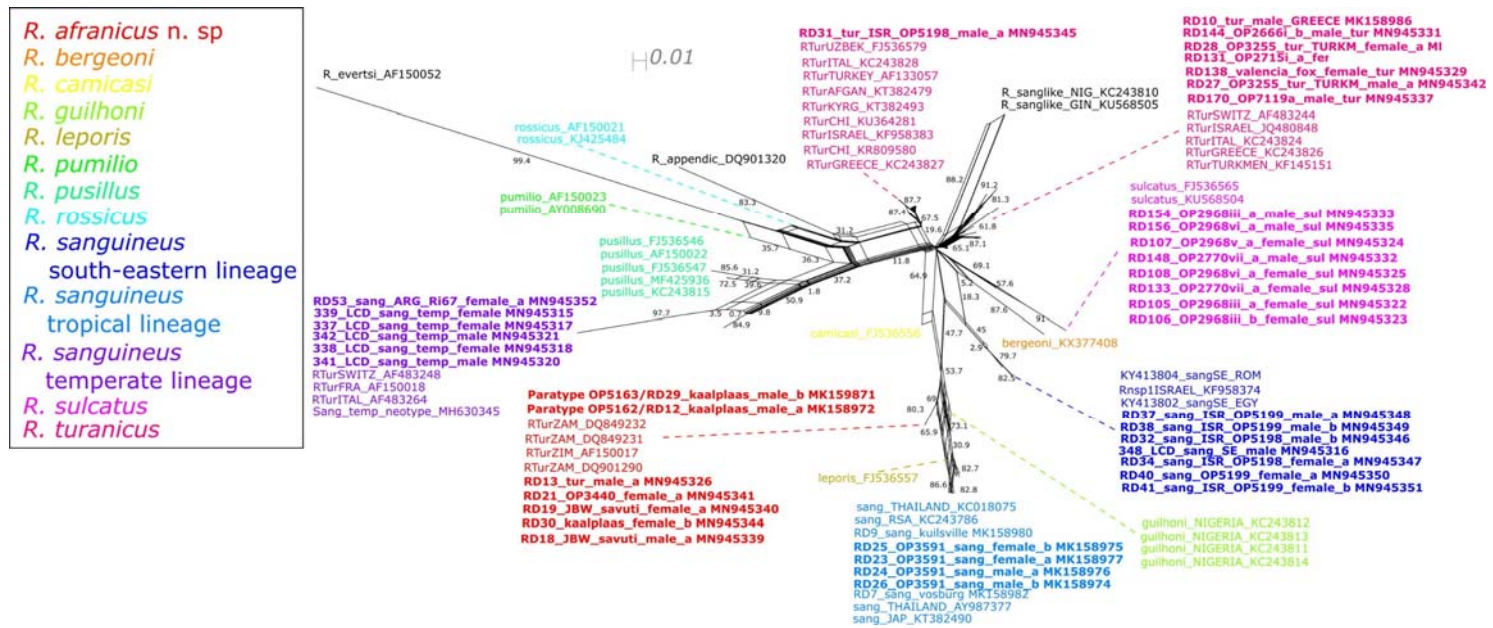
Fig. 1. Phylogenetic splits tree analysis of the *Rhipicephalus turanicus* group 12S rDNA gene using the TN + F + G4 nucleotide substitution model. Indicated are species and lineage names, GenBank accession numbers or study codes (reflected in Supplementary Table S1), country of origin and neighbour network bootstrap support values. Bolded samples were used iteratively in morphometric analyses. Note that between 10 and 12 sequence labels per clade were retained for this figure to increase readability. See Supplementary Fig. S2 for the fully labelled figure.
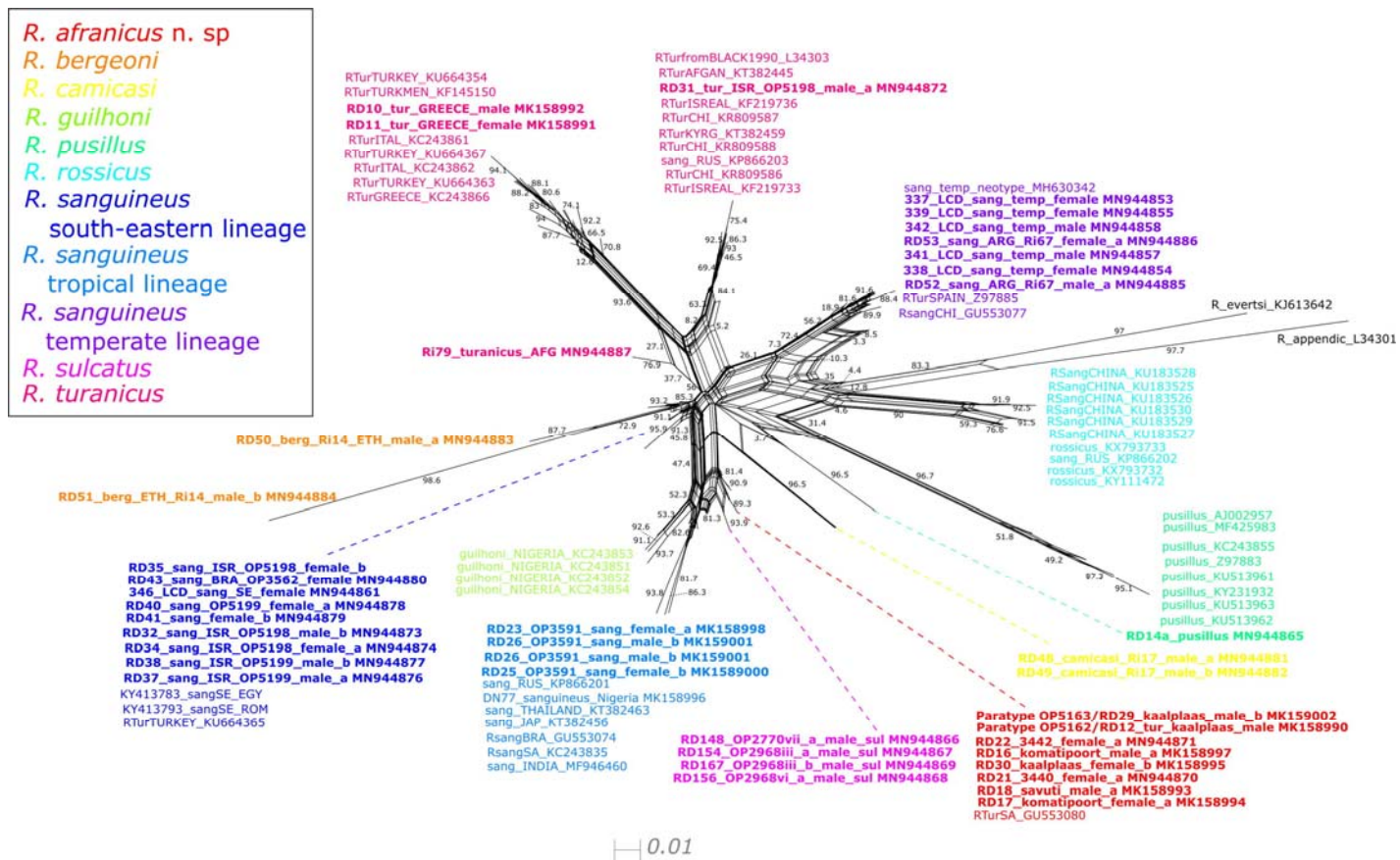
Fig. 2. Phylogenetic splits tree analysis of the *Rhipicephalus turanicus* group 16S rDNA gene using the TPM3u + F + G4 nucleotide substitution model. Indicated are species and lineage names, GenBank accession numbers or study codes (reflected in Supplementary Table S1), country of origin and neighbour network bootstrap support values. Bolded samples were used iteratively in morphometric analyses. Note that between 10 and 12 sequence labels per clade were retained for this figure to increase readability. See Supplementary Fig. S3 for the fully labelled figure.

tropical lineage, *R. sanguineus* temperate lineage, *R. sanguineus* south-east lineage, *R. sulcatus,* R. *guilhoni*, *R. bergeoni*, *R. camicasi*, *R. pusillus*, *R. rossicus* and *R. pumilio.* These specimens have been stored in 96% ethanol and housed in the GTTM (Table S1). Some specimens used in sequencing were likewise used in morphometric analyses to iteratively test species boundaries determined from the mtDNA lineages. Note that a lack of *R. turanicus* samples to represent both Palearctic lineages prevented analysis of their morphometric data separately. Thus, we grouped Palearctic samples together as *R. turanicus sensu lato.* As such, analysis was framed in terms of distinguishing Afrotropical *R. turanicus* from *R. turanicus s. lat.* in the Palearctic*.*

Male adanal plates and female spiracles were photographed on a rotational mount in three replicates following Bakkes (2017). Photographs were taken using a Zeiss AxioCam MRc 5 camera, and were stacked in Zeiss Axiovision v4.8. Each stacked image consisted of between 10 and 20 photographs. Outlines of female spiracles and male adanal plates were digitized using COO v41 in the CLIC package by Jean-Pierre Dujardin (available at http://mome-clic.com/the-clic-package/) according to 15 and 13 landmarks respectively (Fig. S1), and were scaled to a 0.2 mm scale bar. Operational definitions for each landmark are available in Fig. S1. Replicate photographs were digitized in batches by replicate to avoid digitization bias from operator memory. Landmarks were transformed in a Procrustes fit in MorphoJ v1.06d (Klingenberg, 2011) and a covariance matrix was generated. All subsequent analyses were performed on the total twelve-lineage group datasets for males and females, as well as on six-lineage group datasets that focus on the two *R. turanicus*, three *R. sanguineus* and one *R. sulcatus* lineages. All lineages of *R. sanguineus* and *R. sulcatus* were selected for the focused morphometric analyses, as their morphology is closest to, and are often confused with, *R. turanicus.*

A Procrustes ANOVA was done to measure variability in individuals with replicate as the error effect and species as the main effect. Subsequently, observations were averaged by individual. Qualitative morphological characters as well as 12S and 16S rDNA lineages recovered directed *a priori* species hypotheses that were tested in a canonical variates analysis. Canonical variates transform morphospace to maximize differences between groups, and are sensitive to *a priori* species delimitation. This enables a test of *a priori* group structure based on Mahalanobis distances. In turn, Mahalanobis distance scales between-group variation by within-group variation, enabling comparison between multivariate group means. One unit in Mahalanobis distance between groups represents one unit of within-group standard deviation. This enables a test of hypothetical species group structure based on 12S, 16S rDNA data and qualitative morphology (proxies for species boundary), and also serves to characterize shape changes between species (Bakkes et al., 2018; Karanovic et al., 2016; Villemant et al., 2007; Yeates et al., 2011).

2.4 Distribution maps

Point maps of species distributions were made using co-ordinates of digitized locality data from the GTTM (Table S1). Co-ordinates were plotted against current data for annual precipitation in DIVA GIS v7.5.0 (Hijmans et al., 2004). This was done to determine whether distribution patterns may conform to features of physical geography and some aspects of the abiotic environment.

2.5 Data Availability

All data and supplementary information associated with this work have been uploaded to Mendeley data: http://dx.doi.org/10.17632/zjmtx7ghx4.1. Sequence data have been deposited in the GenBank database (Accession numbers: MK158971-MK159007, MN944853-MN944887, MN945315-MN945352). Voucher and type specimens are

housed in the Gertrud Theiler Tick Museum – Agricultural Research Council (GTTM), South African National Museum – Iziko Museum (SAMC), and the Berlin Zoological Museum, Museum für Naturkunde (ZMB). See taxonomic treatment for details.

3. Results

3.1 Species boundary between Palearctic and Afrotropical R. turanicus

Bayesian and maximum likelihood analyses of the combined data, as well as neighbour network splits analysis for each gene indicate two evolutionary distinct lineages in samples previously identified as *R. turanicus* (Figs 1-3). These two lineages correspond with geographic distribution in the Palearctic and Afrotropics. The Afrotropical lineage of *R. turanicus* (indicated as *R. afranicus* **n. sp.**; Figs 1-3) forms a strongly supported monophyletic group with *R. sanguineus* (tropical lineage), *R. guilhoni* and *R. sulcatus*, and this clade excludes *R. turanicus* sampled from the Palearctic. Within the Palearctic *R. turanicus* lineage, two sub-lineages were recovered corresponding to the Middle-East/Asia, as well as to southern Europe. For 16S rDNA, pairwise similarity between Afrotropical *R. turanicus* and both southern European and Middle-East/Asian *R. turanicus* lineages was 92.47% and 94.54% respectively, and between southern European and Middle-East/Asian *R. turanicus* was 94.08%. For 12S rDNA, the same values were 95.84% and 95.81%, and between southern European and Middle-East/Asian *R. turanicus* pairwise genetic similarity was 99.7%.

Morphometric analyses reveal distinct shape differences between Palearctic and Afrotropical *R. turanicus* samples. For female spiracles, comparison of multivariate means indicated Mahalanobis distances between Palearctic *R. turanicus* and Afrotropical *R. turanicus* females were large when compared to the other species comparisons (Fig. 4B), and was significantly large to distinguish groups (M.dist.= 6.1214, p < 0.0001).
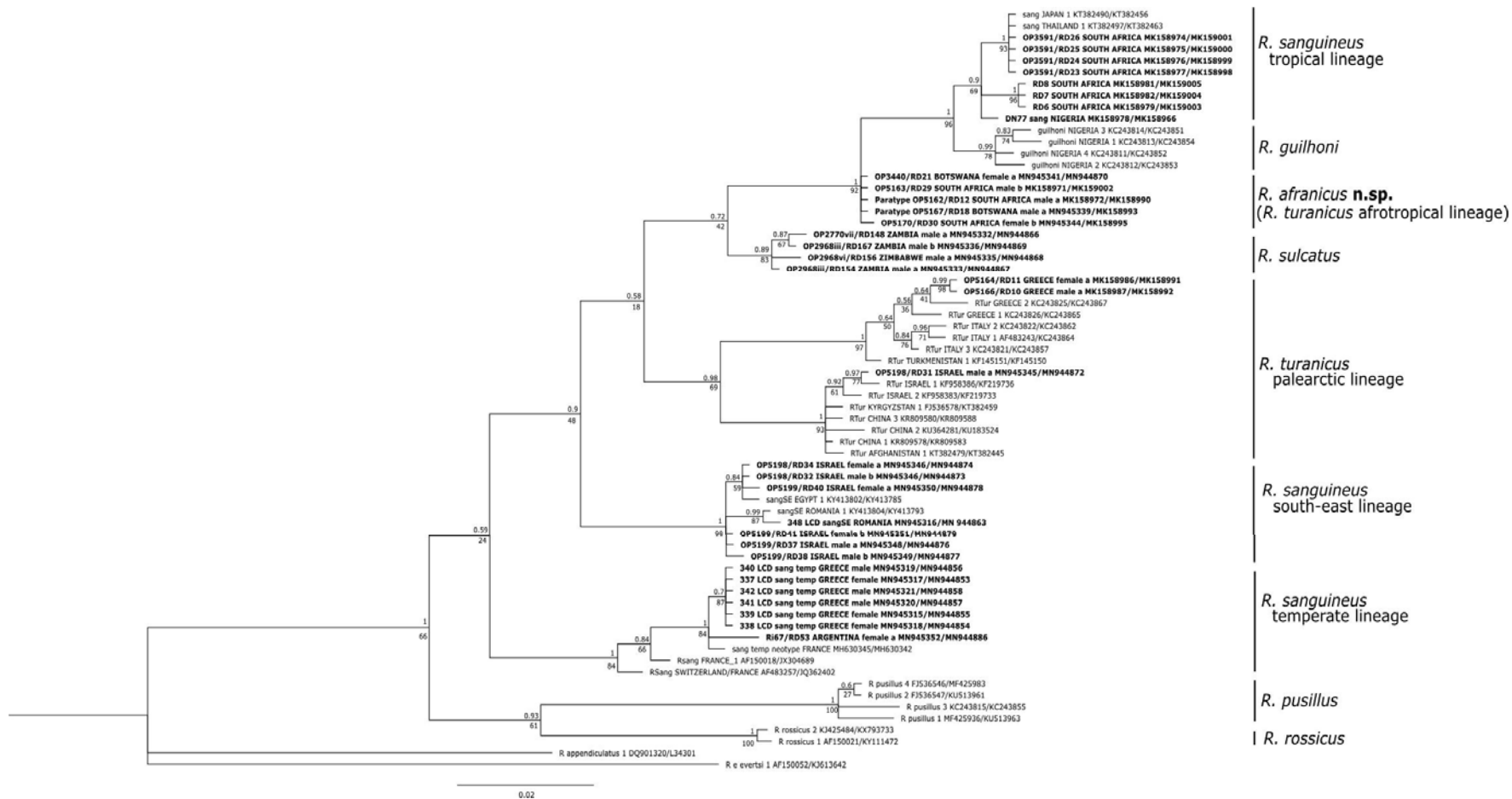
Fig. 3. Consensus tree recovered from Bayesian analysis of the combined *Rhipicephalus turanicus* group dataset. Indicated are species and lineage names, GenBank accession numbers or study codes (left: 12S rDNA, right: 16S rDNA) and country of origin. Nodal support values represent posterior probability (top) and maximum likelihood bootstrap (bottom). Samples in bold refer to sequences generated in this study.
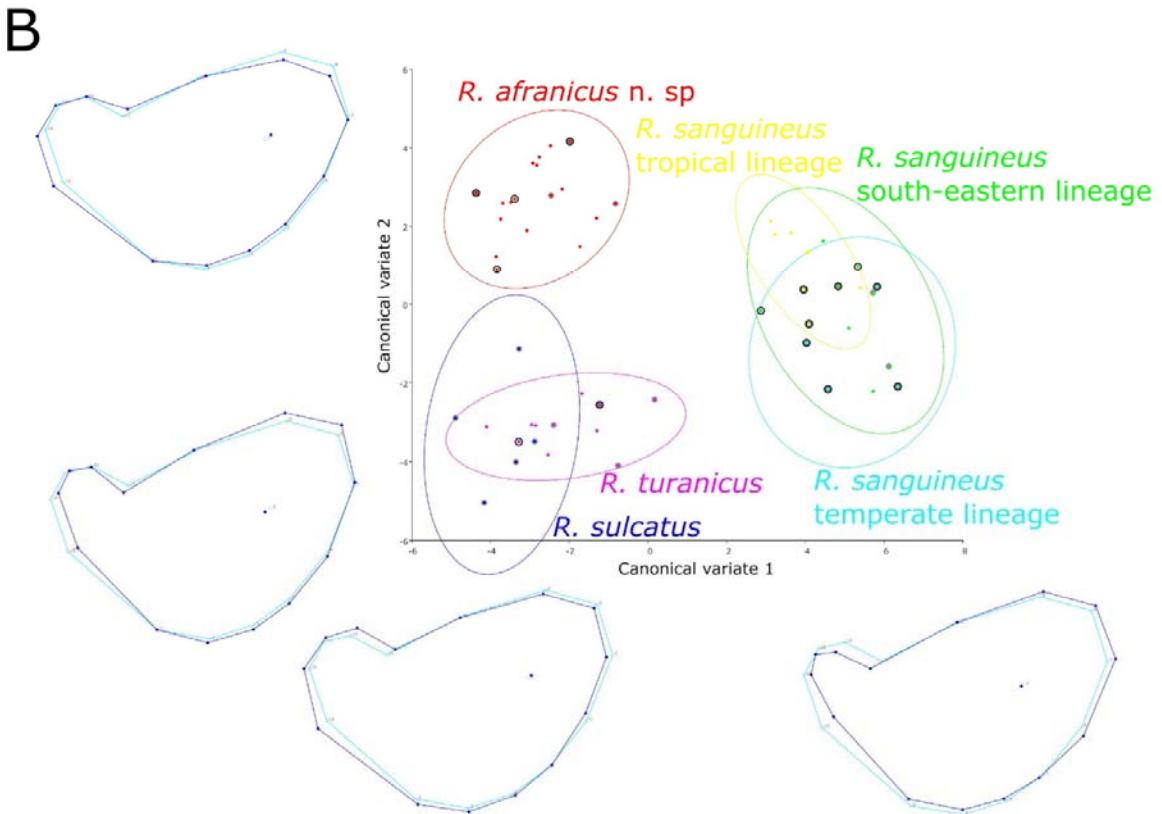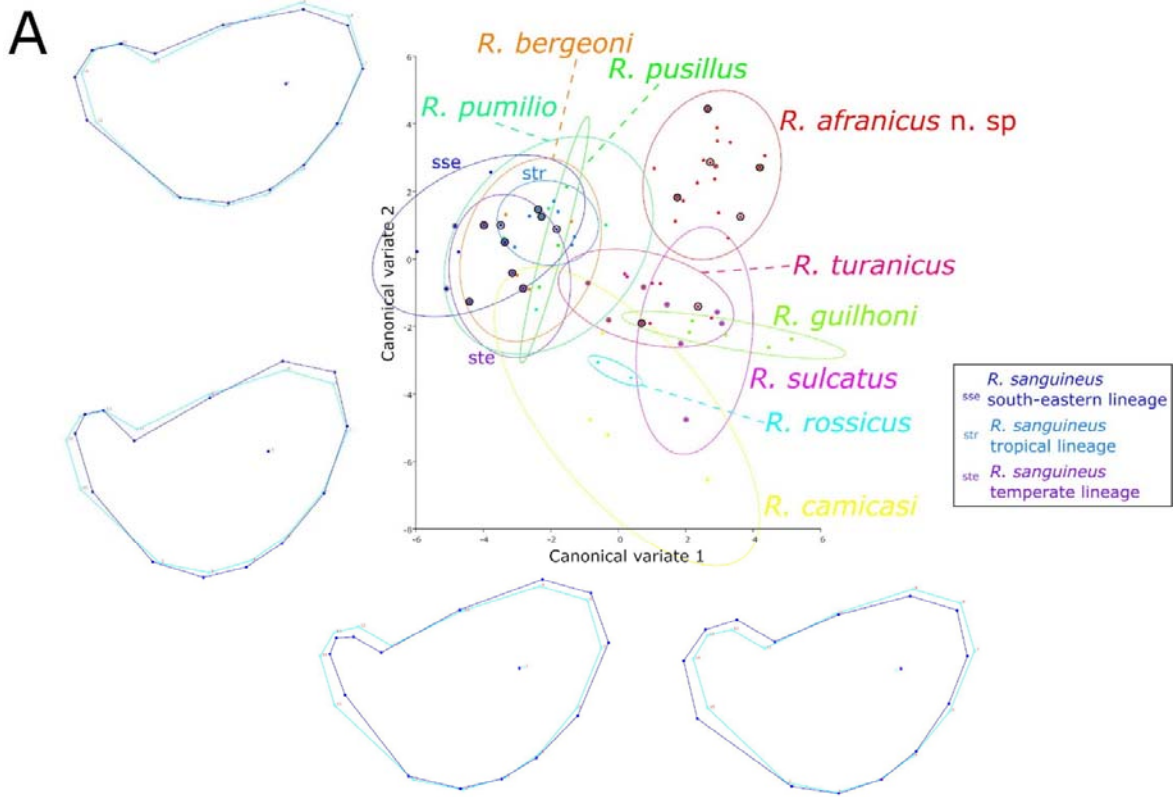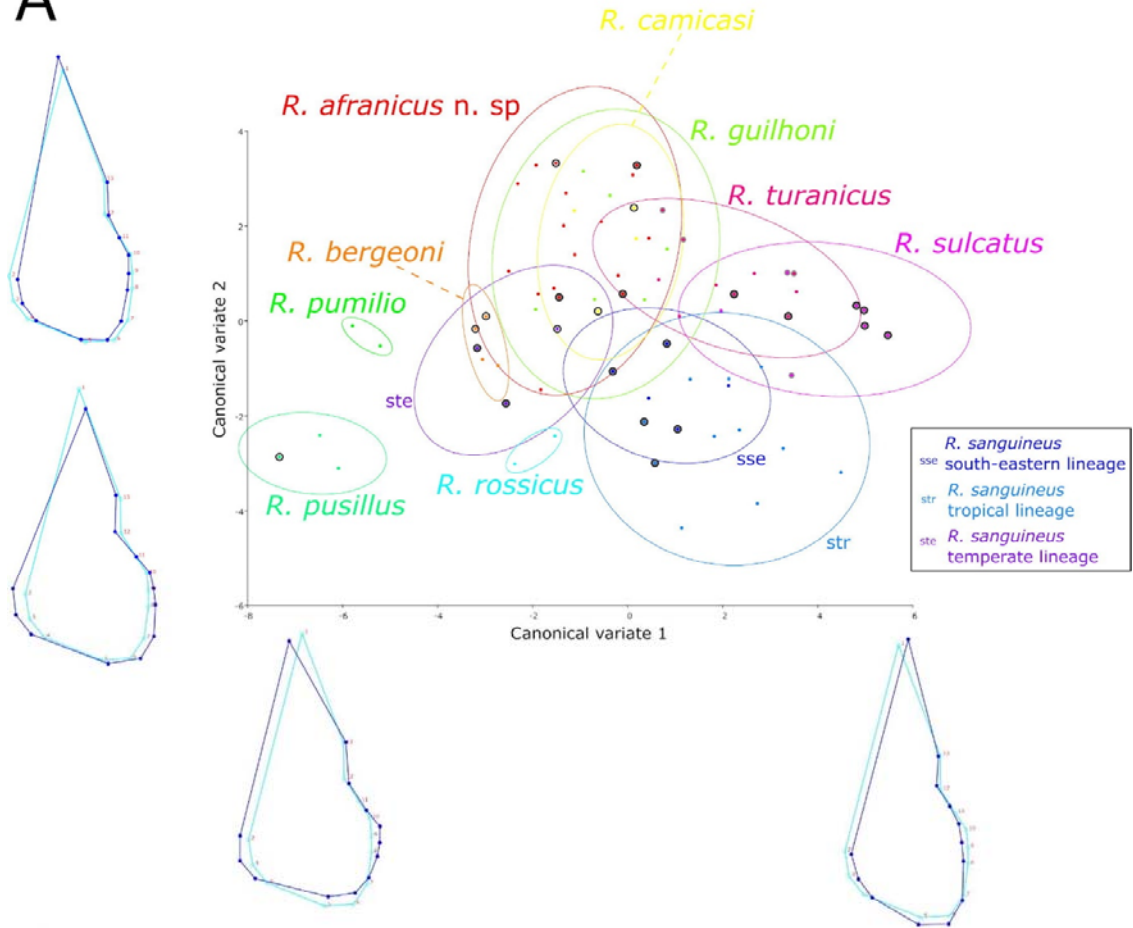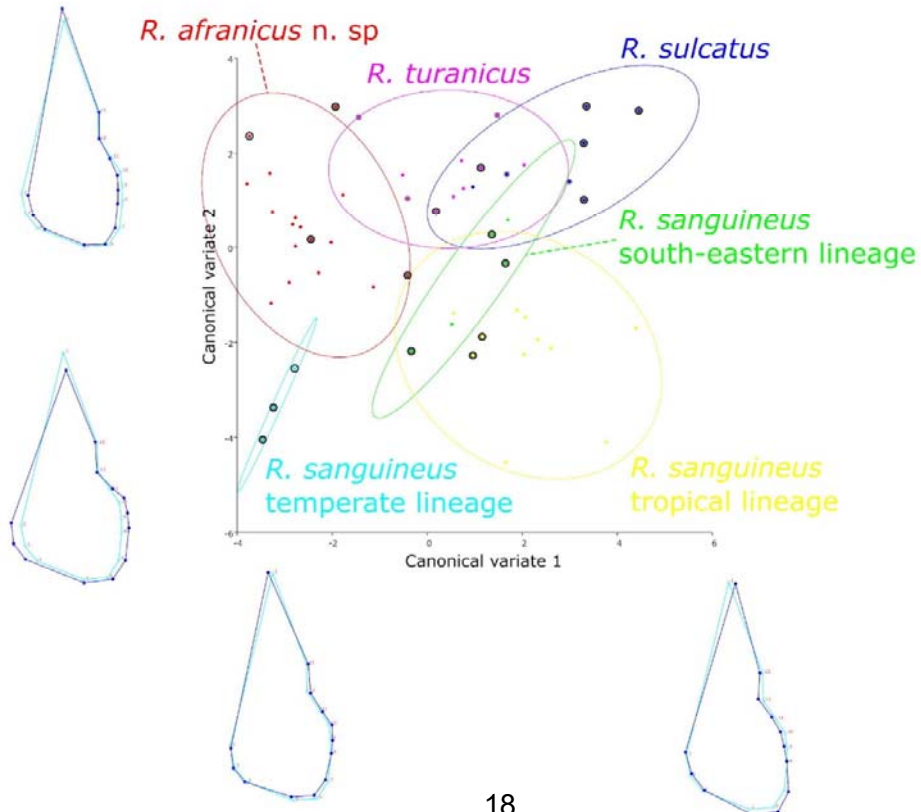
Fig. 4. Canonical variates analysis of female *R. turanicus* group spiracle shape data for the (A) total 12 lineage group and (B) six lineage group datasets. Indicated are axes for canonical variates I and II. Dots represent averages for single specimens. Black and grey rings represent specimens used in 16S or 12S rDNA phylogenetic analysis, respectively. Ellipses represent 95% confidence intervals. Shape changes along Principal Component (PC) axes are to scale at minimum and maximum extents. Light blue (grey) traces represent the mean shape and dark blue (black) traces represent deviation from the mean shape at the given extent.

Misclassification by cross-validation between these groups was small (12.1% of comparisons). Procrustes ANOVA showed the effect of individual was 8.41 times greater than replicate error, indicating that rotational error was negligible, and that species variation was 4.55 times greater than individual variation. Canonical variate I conclusively distinguished Afrotropical *R. turanicus* from all *R. sanguineus* lineages, but did not provide distinction between Afrotropical *R. turanicus*, Palearctic *R. turanicus* and *R. sulcatus*. Shape change was attributable to (1) width of dorsal prolongation based and (2) width of dorsal prolongation tip. Canonical variate II provided clear distinction for Afrotropical *R. turanicus* from *R. sulcatus* and Palearctic *R. turanicus,* and provided moderate distinction between Afrotropical *R. turanicus* and all *R. sanguineus* lineages. Shape change was attributable to (1) width of dorsal prolongation, (2) vertical angle of dorsal prolongation, and (3) excavation of dorsal margin leading to dorsal prolongation.

A

B

Fig. 5. Canonical variates analysis of male *R. turanicus* group adanal plate shape data for the (A) total 12 lineage group and (B) six lineage group datasets. Indicated are axes for canonical variates I and II. Dots represent averages for single specimens. Black and grey rings represent specimens used in 16S or 12S rDNA phylogenetic analysis, respectively. Ellipses represent 95% confidence intervals. Shape changes along Principal Component (PC) axes are to scale at minimum and maximum extents. Light blue (grey) traces represent the mean shape and dark blue (black) traces represent deviation from the mean shape at the given extent.

For male adanal plates, comparison of multivariate means indicated Mahalanobis distances between Palearctic *R. turanicus* and Afrotropical *R. turanicus* males was small when compared to the other species comparisons (Fig. 5B). However, this distance was significantly large to distinguish groups (M.dist.= 3.6959, p < 0.0001). Misclassification by cross-validation between these groups was small (13.5% of comparisons). Procrustes ANOVA showed the effect of individual was 5.62 times greater than replicate error, indicating that rotational error was negligible, and that species variation was 3.42 times greater than individual variation. Canonical variate I conclusively distinguished Afrotropical *R. turanicus* from *R. sulcatus* and *R. sanguineus* south-east and tropical lineages. Canonical variate I provided moderate distinction between Afrotropical *R. turanicus* and Palearctic *R. turanicus*. However, canonical variate I did not provide distinction between Afrotropical *R. turanicus* and *R. sanguineus* temperate lineage. Shape change was attributable to (1) projection of posteromedial corner, (2) overall width of posterior third, and (3) excavation of medial scallop. Canonical variate II provided clear distinction for Afrotropical *R. turanicus* from *R. sanguineus* temperate lineage and provided moderate distinction between Afrotropical *R. turanicus,* and *R. sanguineus* tropical lineage. Canonical variate II did not provide distinction between Afrotropical *R. turanicus* and *R. sulcatus*, Palearctic *R. turanicus* and *R. sanguineus* south-east lineage.

Shape change was attributable to (1) projection of posterolateral corner, (2) overall width of posterior third, and (3) excavation of medial scallop.

Distribution patterns indicate that Afrotropical *R. turanicus* are limited to regions with 400 mm to 1500 mm annual precipitation. In contrast, Palearctic *R. turanicus* lineages are limited to regions with 100 mm to 1000 mm annual precipitation (Middle-East/Asia lineage: 100 mm to 500 mm; southern Europe lineage: 200 mm to 1000 mm) (Fig. 6). The Sahara desert forms a natural barrier of uninhabitable land between the two species.
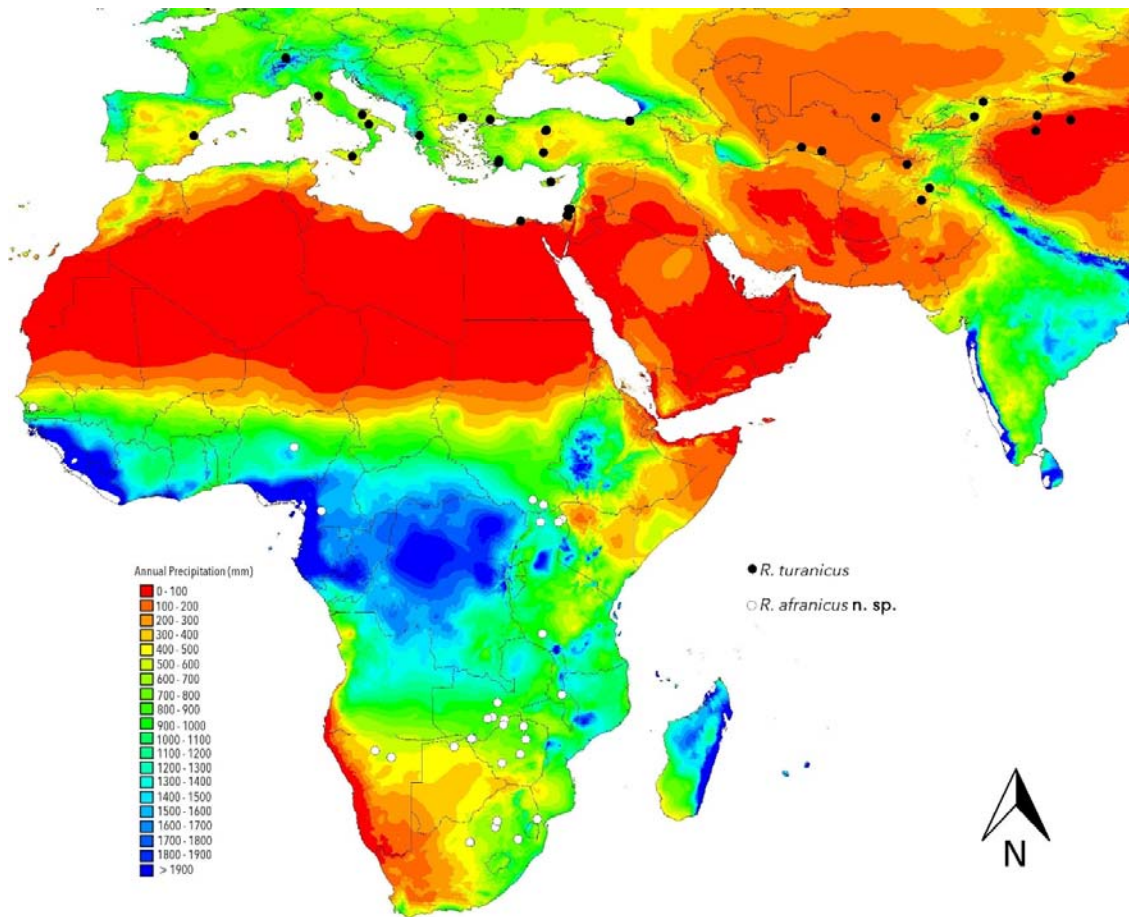
Fig. 6. Point map distribution of *Rhipicephalus afranicus* n. sp. (white) and *Rhipicephalus turanicus* sensu lato (black) against annual precipitation data based on samples studied (Supplementary Table S1).
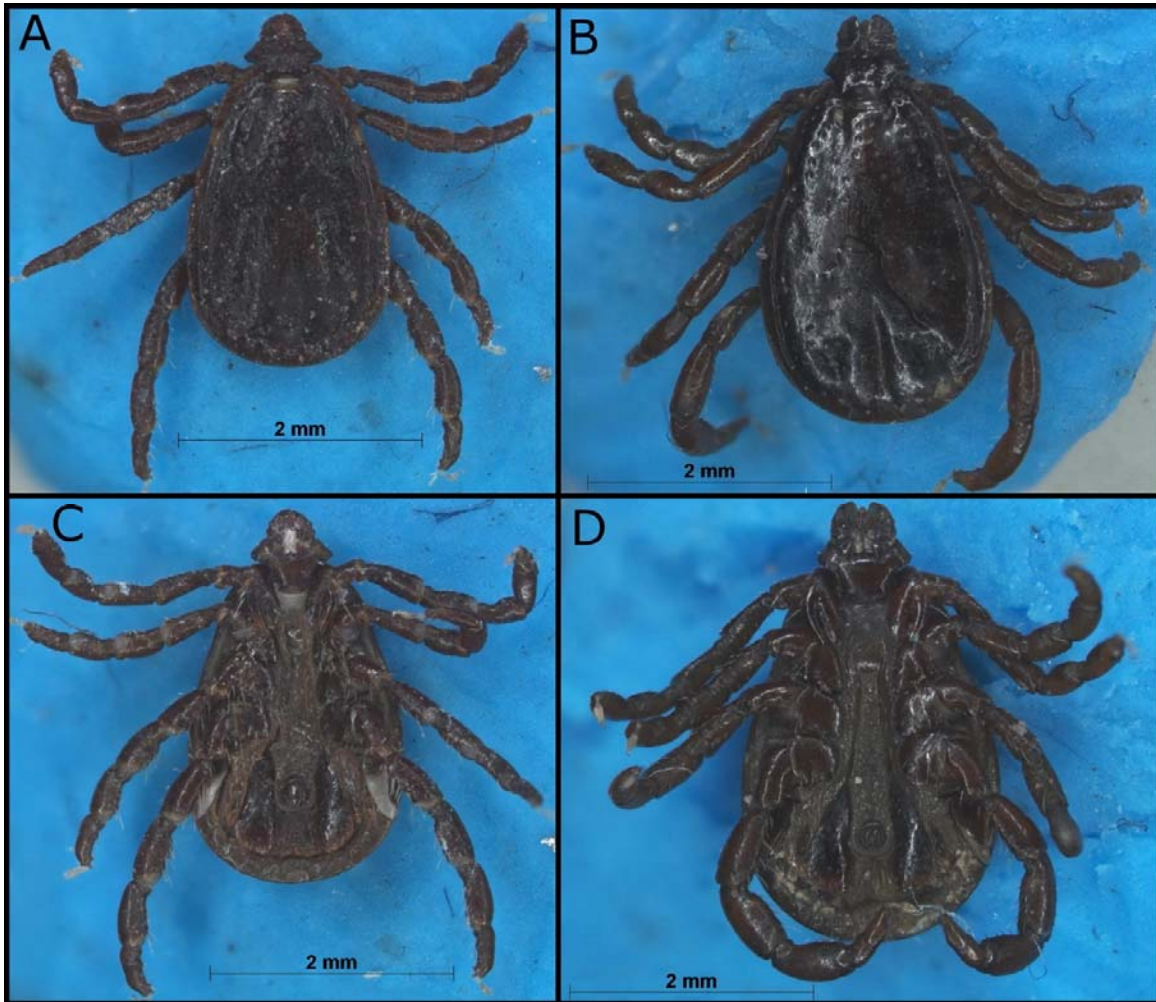
3.2 Taxonomy and species descriptions

Family IXODIDAE Koch, 1844

Genus *Rhipicephalus* Koch, 1844

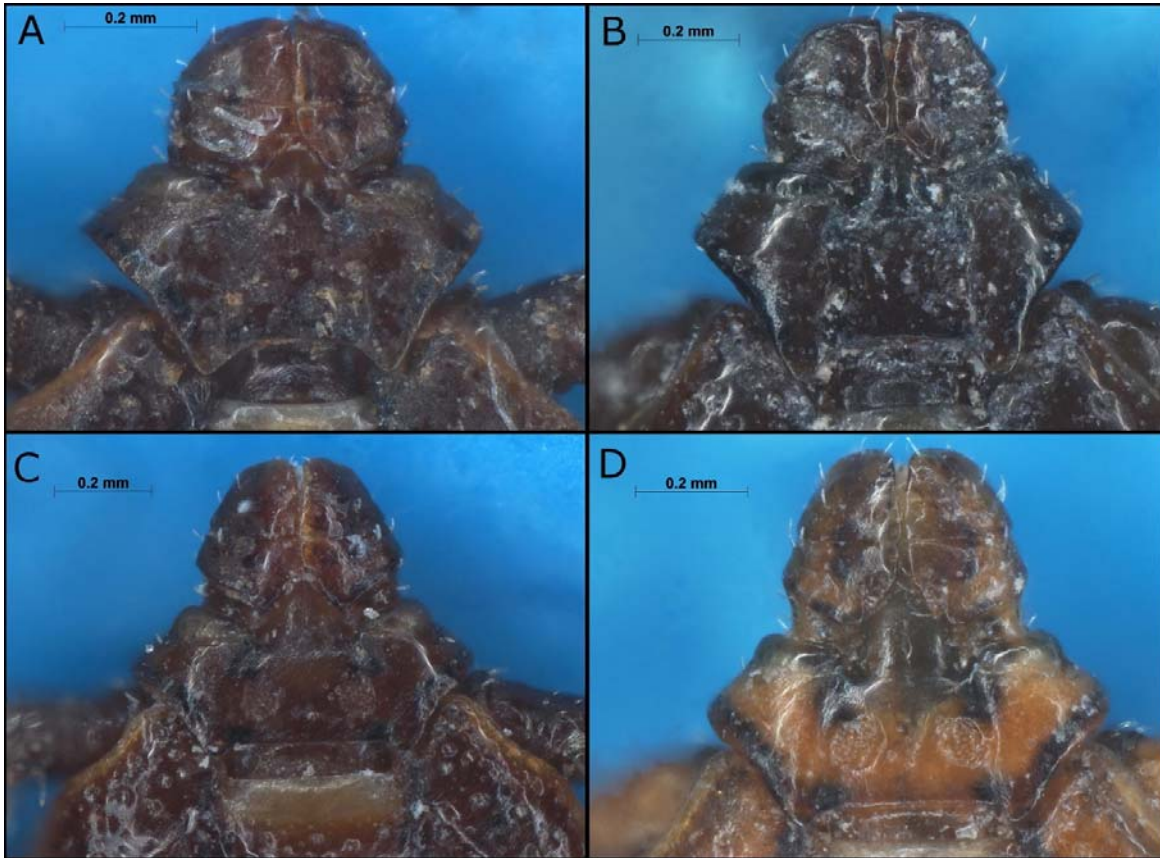*Rhipicephalus afranicus* Bakkes **n. sp.**

(Figs 7-15)

Fig. 7. Dorsal and ventral habitus photos of male *Rhipicephalus afranicus* n. sp. (A, C) and *Rhipicephalus turanicus* sensu stricto (B, D). Specimen data: *R. afranicus* n. sp. (A, C) – OP5172 Holotype, Kaalplaas, vegetation, March 2018; *R. turanicus* sensu stricto (B, D) – OP3255/RD27, Turkmenistan, *Canis lupus familiaris* (Dog), April 1988.
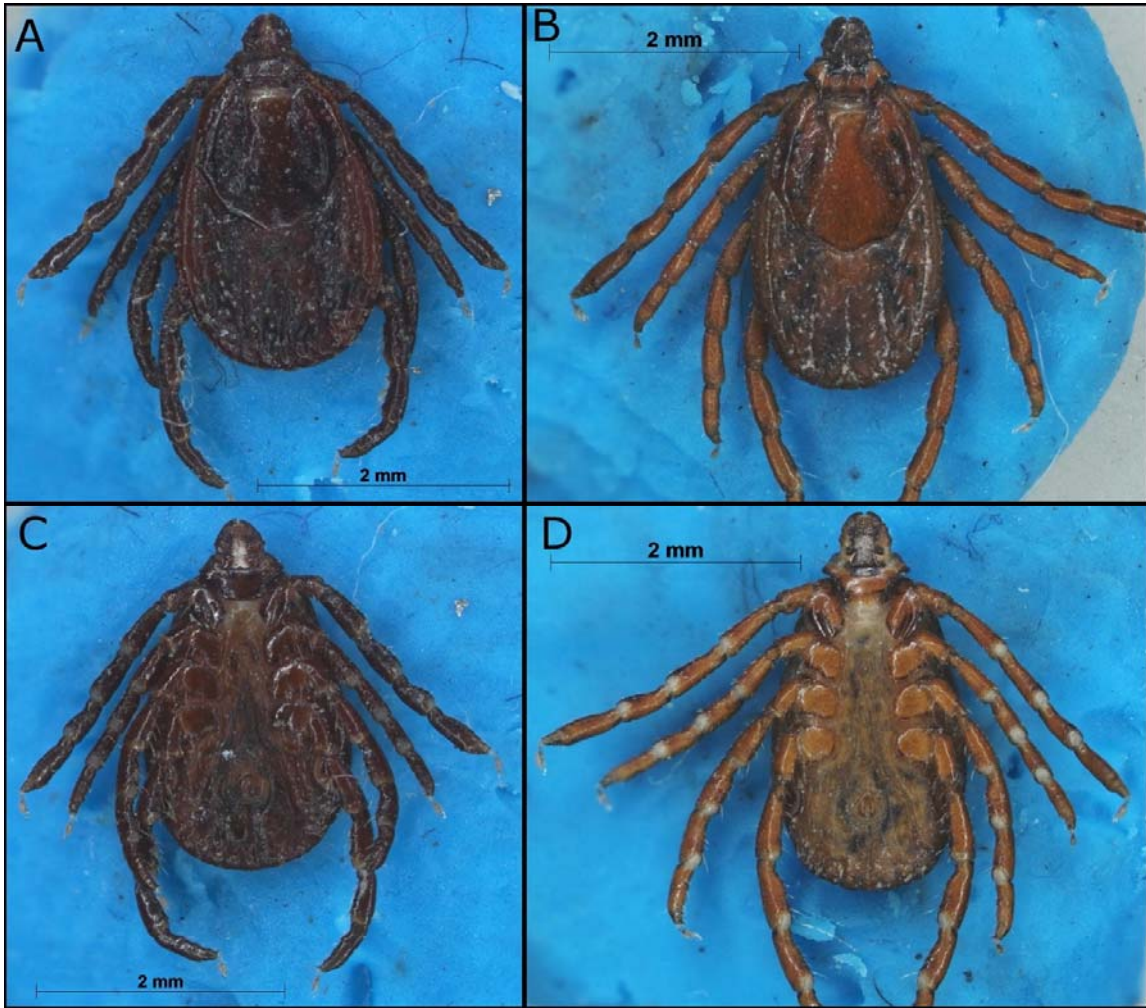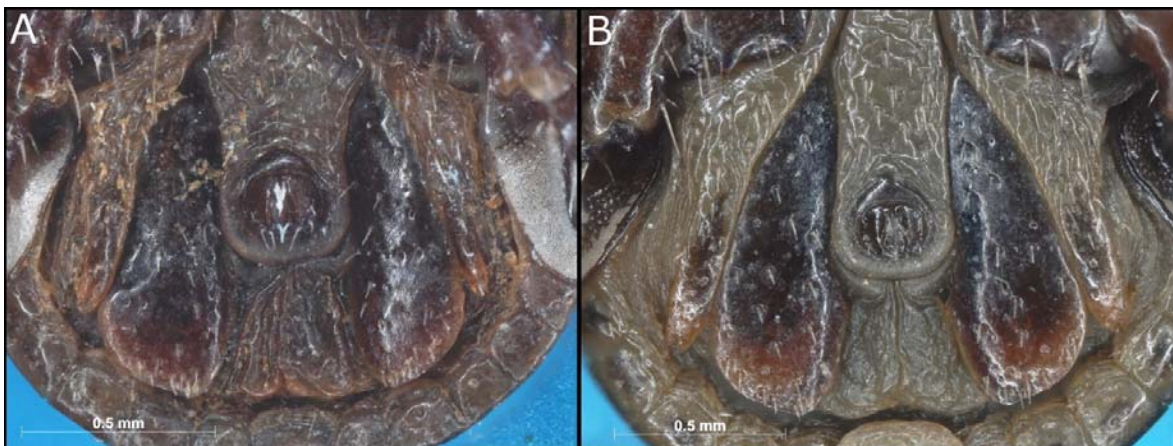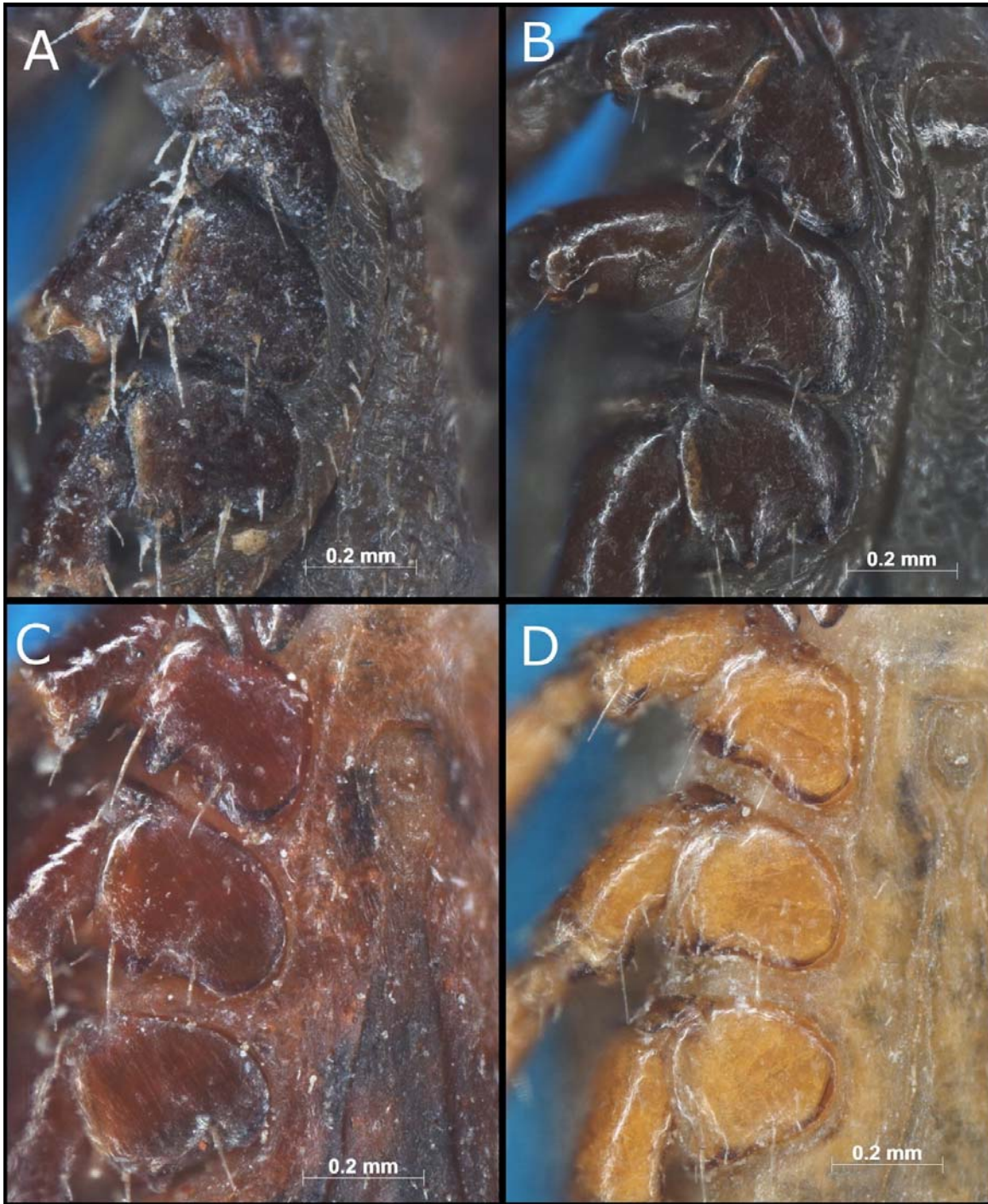
Fig. 8. Dorsal and ventral habitus photos of female *Rhipicephalus afranicus* n. sp. (A, C) and *Rhipicephalus turanicus* sensu stricto (B, D). Specimen data: *R. afranicus* n. sp. (A, C) – OP5173 Allotype, Kaalplaas, vegetation, March 2018; *R. turanicus* sensu stricto (B, D) – OP3255/RD28, Turkmenistan, *Canis lupus familiaris* (Dog), April 1988.

Fig. 9. Comparative morphology of basis capituli in male and female *Rhipicephalus afranicus* n. sp**.** (A, C) and *Rhipicephalus turanicus* sensu stricto (B, D). Specimen data: *R. afranicus* n. sp. male (A) – OP5172 Holotype, Kaalplaas, vegetation, March 2018; *R. turanicus* sensu stricto male (B) – OP3255/RD27, Turkmenistan, *Canis lupus familiaris* (Dog), April 1988. *Rhipicephalus afranicus* n. sp. female (C) – OP5173 Allotype, Kaalplaas, vegetation, March 2018; *R. turanicus* sensu stricto female (D) – OP3255/RD28, Turkmenistan, *Canis lupus familiaris* (Dog), April 1988.

Fig. 10. Comparative morphology of adanal plates in male *Rhipicephalus afranicus* n. sp. (A) and *Rhipicephalus turanicus* sensu stricto (B). Specimen data: *R. afranicus* n. sp**.** (A) – OP5172 Holotype, Kaalplaas, vegetation, March 2018; *R. turanicus* sensu stricto (B) – OP3255/RD27, Turkmenistan, *Canis lupus familiaris* (Dog), April 1988.

Fig. 11. Comparative morphology of coxae II–IV in male and female *Rhipicephalus afranicus* n. sp. (A, C) and *Rhipicephalus turanicus* sensu stricto (B, D). Specimen data: *R. afranicus* n. sp. male (A) – OP5172 Holotype, Kaalplaas, vegetation, March 2018; *R. turanicus* sensu stricto male (B) – OP3255/RD27, Turkmenistan, *Canis lupus familiaris* (Dog), April 1988. *Rhipicephalus afranicus* n. sp. female (C) – OP5173 Allotype, Kaalplaas, vegetation, March 2018; *R. turanicus* sensu stricto female (D) – OP3255/RD28, Turkmenistan, *Canis lupus familiaris* (Dog), April 1988.
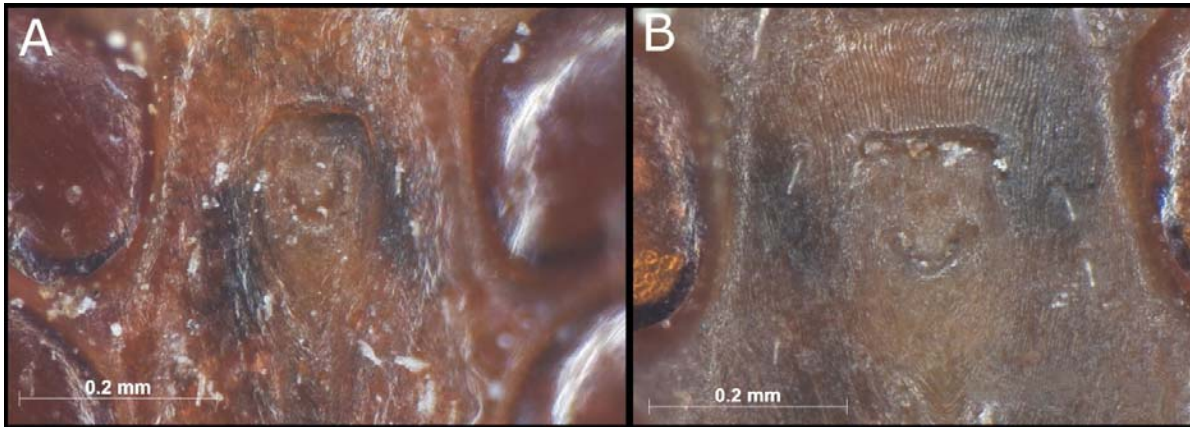
Fig. 12. Comparative morphology of genital aperture in female *Rhipicephalus afranicus* n. sp. (A) and *turanicus* sensu stricto (B). Specimen data: *R. afranicus* n. sp. (A) – OP5173 Allotype, Kaalplaas, vegetation, March 2018; *R. turanicus* sensu stricto (B) – OP3255/RD28, Turkmenistan, *Canis lupus familiaris* (Dog), April 1988.

Fig. 13. Comparative morphology of spiracles in female *Rhipicephalus afranicus* n. sp. (A) and *Rhipicephalus turanicus* sensu stricto (B). Specimen data: *R. afranicus* n. sp. (A) – OP5173 Allotype, Kaalplaas, vegetation, March 2018; *R. turanicus* sensu stricto (B) – OP3255/RD28, Turkmenistan, *Canis lupus familiaris* (Dog), April 1988.
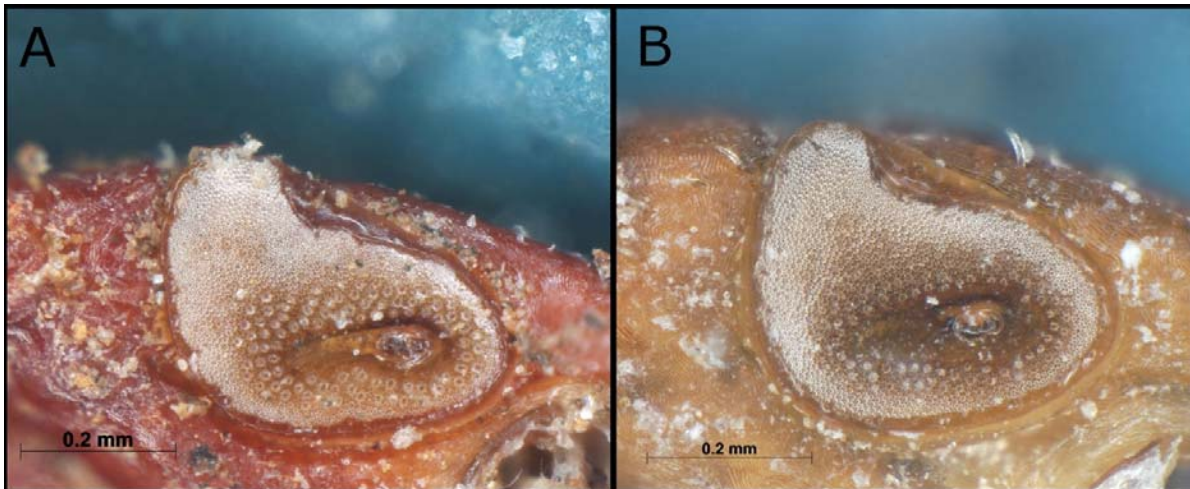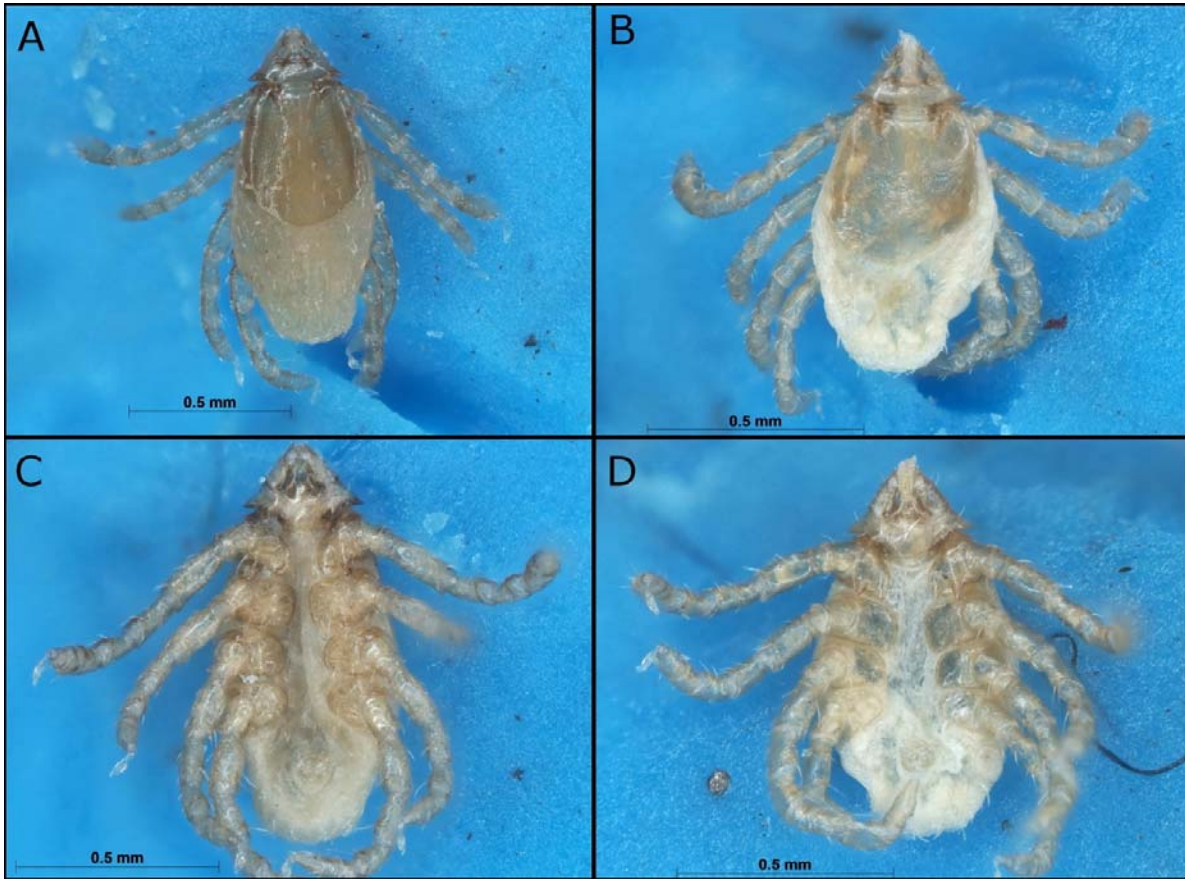
Fig. 14. Dorsal and ventral habitus photos of nymphal *Rhipicephalus afranicus* n. sp. (A, C) and *Rhipicephalus turanicus* sensu stricto (B, D). Specimen data: *R. afranicus* n. sp. (A, C) – OP5194, Mukulaikwa, Zambia, laboratory reared; *R. turanicus* sensu stricto (B, D) – OP5195, Cyprus, Greece, laboratory reared.
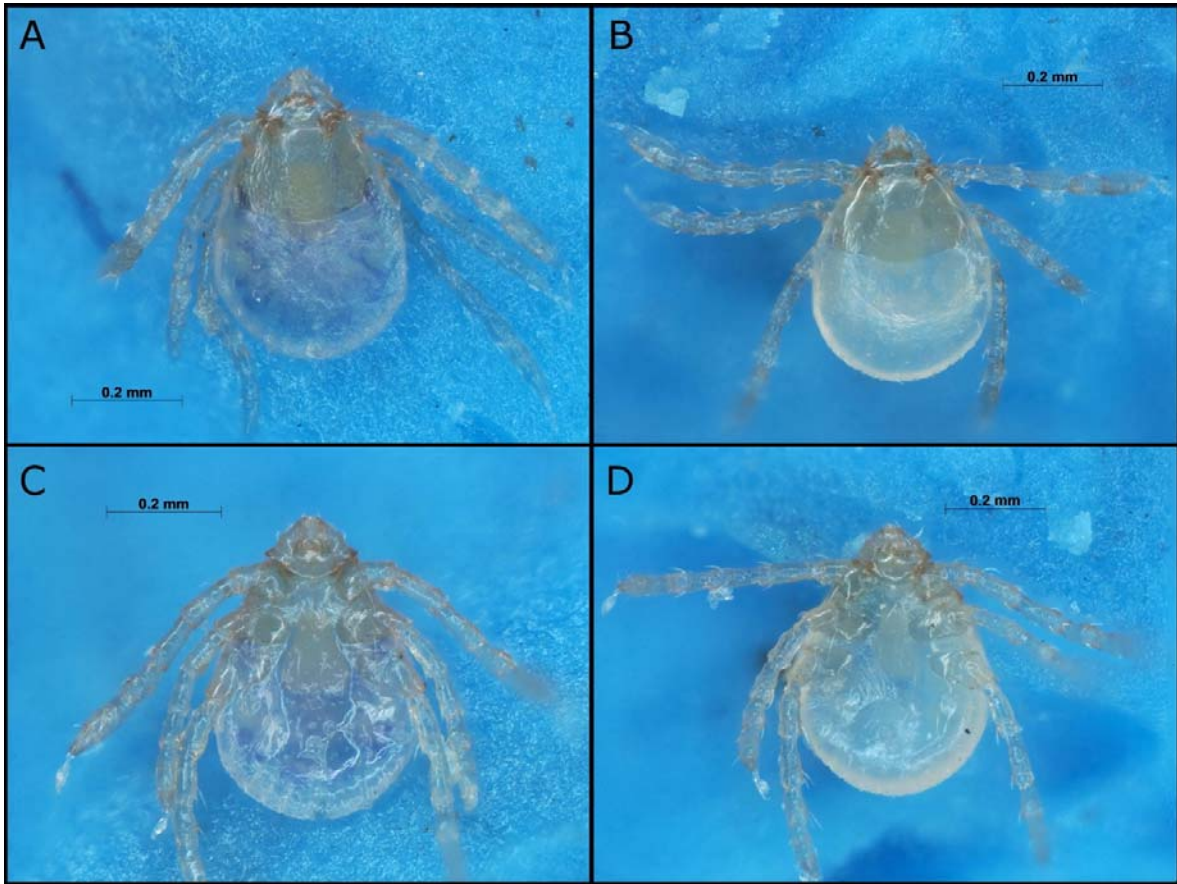
Fig. 15. Dorsal and ventral habitus photos of larval *Rhipicephalus afranicus* n. sp. (A, C) and *Rhipicephalus turanicus* sensu stricto (B, D). Specimen data: *R. afranicus* n. sp. (A, C) – OP5194, Mukulaikwa, Zambia, laboratory reared; *R. turanicus* sensu stricto (B, D) – OP5195, Cyprus, Greece, laboratory reared.

ZooBank LSID: zoobank.org:act:B4417059-1998-41D5-AA4C-841571751094

Synonymy.

Rhipicephalus turanicus Pomerantsev, 1940, *pro parte.*

Type depository: GTTM, Holotype: Male

Type locality: Kaalplaas, Onderstepoort, South Africa (25.627227S 28.147016E)

Etymology. From geographic distribution in the Afrotropics, Latin –*icus*, adjective (belonging to, derived from), as well as from similarity to *R. turanicus* in morphology.

Material examined. Forty-seven specimens from South Africa, Zimbabwe, Botswana, Namibia, Zambia, Malawi, Tanzania, Uganda, Sudan, Cameroon and Nigeria (Table S1).

Type material

Holotype ♂ (deposited in Gertrud Theiler Tick Museum, Onderstepoort – GTTM; OP5172) designated here. Allotype ♀ (deposited in GTTM; OP5173) designated here. Holotype and Allotype not sequenced in order to preserve specimen integrity.

Paratype series designated here: OP5162/RD12 (♂ deposited in GTTM) Genbank: 12S rDNA - MK158972, 16S rDNA - MK158990; OP5163/RD29 (♂ deposited in GTTM) Genbank: 12S rDNA - MK158971, 16S rDNA - MK159002; OP5174 (2♂2♀, deposited in South African National Museum, Iziko Museum, Cape Town - SAMC); OP5175 (2♂2♀, deposited in Berlin Zoological Museum, Museum für Naturkunde, Berlin - ZMB).

Specimen data for all types: 'Kaalplaas, Onderstepoort, South Africa / -25.627227 28.147016 / iii.2018 / dragged from vegetation / Lidia Chitimia-Dobler & Deon Bakkes.

Description.

Males (voucher numbers: OP5172, OP5162, OP5163, OP5174, OP5175)
Length 2.6 to 3.2 mm, width 1.6 to 2.1 mm

Basis capitulum hexagonal, short, wide. Lateral angles slightly obtuse projecting at anterior third of length. Cornua present, short, broadly triangular. Palps short, sub-triangular. Body reddish-brown. Conscutum elongate, ovate, broadest at coxae IV with anterolateral margin convex. Lateral idiosoma expanded when engorged. Eyes flat, dorsally bordered by a row of medium punctations. Cervical grooves convergent and deep anteriorly, shallow and divergent posteriorly. Cervical fields slightly sunken with numerous small punctations present imparting a rugose appearance, lateral grooves

bordered by a row of large, confluent punctations. Marginal grooves enclosing first festoon and reaching level of coxa III, bordered by a row of sparse, large punctations. Posteromedial groove short, oval, rugose. Posterolateral grooves approximately half the length of posteromedial groove, rugose. Small punctations numerous, a few large punctations sparsely scattered in four irregular rows as well as on scapulae.

Legs reddish brown, slender, slightly thicker posteriorly. Coxae approximately equal in size. Coxa I elongate with small anterior process and posteromedial spur broad with triangular tip, posterolateral spur narrow, tapering to rounded tip. Coxa II sub-triangular with posteromedial spur minute, broad, flange-like and posterolateral spur moderate size, broad, triangular. Coxa III sub-rectangular with posteromedial spur broad, flange-like and posterolateral spur short, narrow, triangular. Coxa IV sub-rectangular with posteromedial spur minute, triangular and posterolateral spur short, narrow, triangular. Adanal plates elongate, triangular with slight medial concavity and posterolateral convexity. Posterior third slightly more wide than long, imparting a stunted appearance. Accessory plates short, reaching level of posterolateral convexity. Spiracles elongate, sub-ovate with dorsal prolongation short, broad.

Females (description voucher numbers: OP5173, OP5174, OP5175)

Length 2.6 to 3.1 mm, width 1.5 to 2.2 mm (Unengorged)

Basis capitulum hexagonal, short, wide. Lateral angles rectangular, projecting at mid-length. Cornua present, short, broadly triangular. Porous areas sub-ovate, small, separated by a distance slightly less than twice their width. Palps short, sub-triangular, stalked on article I. Body reddish-brown. Scutum elongate, ovate, broadest at mid-length with anterolateral margin convex, posterior margin sinuous with most posterior point slightly pronounced. Eyes flat, dorsally bordered by a row of medium punctations.

29

Cervical grooves convergent and deep anteriorly, shallow and divergent posteriorly. Cervical fields slightly sunken with numerous confluent punctations, not reaching posterolateral scutal margins. Lateral grooves distinct, bordered by a row of large punctations. Small punctations numerous, large punctations moderately scattered across scutum.

Legs reddish brown, slender, slightly thicker posteriorly. Coxae approximately equal in size. Coxa I elongate with posteromedial spur broad with triangular tip, posterolateral spur narrow, tapering to pointed tip. Coxa II sub-rectangular with posteromedial spur short, broad, flange-like and posterolateral spur moderate size, triangular. Coxa III sub-rectangular with posteromedial spur short, broad, flange-like and posterolateral spur short, narrow, triangular. Coxa IV sub-rectangular with posteromedial spur minute, arcuate and posterolateral spur short, broad, triangular. Genital aperture U-shaped with lateral margins slightly diverging anteriorly. Spiracles broadly sub-triangular with rounded angles, dorsal prolongation short, thick proximally, tapering to rounded tip distally.

Nymphs (description voucher numbers: 5 specimens from OP5194)

Length 0.7 to 1.1 mm, width 1.0 to 1.2 mm (Unengorged)

Basis capitulum hexagonal, almost flat, wide. Lateral angles acute, projecting at mid-length. Cornua absent. Palps short, sub-triangular. Body yellowish-brown. Scutum elongate, ovate, broadest at posterior third with anterolateral margin convex, posterior margin rounded. Eyes flat, located at posterior third of scutum. Cervical grooves convergent and deep anteriorly, shallow and divergent posteriorly. Cervical fields, narrow, distinctly sunken, not reaching posterolateral scutal margins. Lateral grooves distinct.

Legs transparent brown, slender. Coxae approximately equal in size. Coxa I sub-rectangular with spurs moderately separated. Posteromedial spur short, broad, triangular and posterolateral spur long, broad, triangular. Coxa II sub-rectangular with posteromedial spur absent and posterolateral spur moderate size, broad, triangular. Coxa III sub-rectangular with posteromedial absent and posterolateral spur short, narrow, triangular. Coxa IV sub-rectangular with spurs absent.

Larvae (description voucher numbers: 5 specimens from OP5194)

Length 0.4 to 0.6 mm, width 0.3 to 0.5 mm (Unengorged)

Basis capitulum hexagonal, almost flat, width almost equal to posterior margin of scutum. Lateral angles acute, projecting at mid-length. Cornua absent. Palps short, sub-triangular. Body yellowish-brown. Scutum narrow, triangular, widest posteriorly with anterolateral margin convex, posterior margin rounded. Eyes flat, located at posterior corner of scutum. Cervical grooves convergent and deep anteriorly, shallow and divergent posteriorly. Cervical fields, narrow, not distinctly sunken, not reaching posterolateral scutal margins. Lateral grooves indistinct.

Legs transparent brown, slender. Coxae approximately equal in size. Coxa I sub-rectangular with posteromedial spur moderate size, broad, flange-like and posterolateral spur absent. Coxa II sub-rectangular with posteromedial spur short, broad, flange-like and posterolateral spur absent. Coxa III sub-rectangular with posteromedial spur minute, triangular and posterolateral spur absent.

Biogeography. Afrotropical (Fig. 6). Most collections are from southern and east Africa with limited records from west Africa. Generally, occurring in regions with annual precipitation between 400 mm and 1500 mm.

Hosts. Three host life cycle. Host data from Walker et al. (2000), Horak et al. (2018) and GTTM records. Black-backed Jackal (*Canis mesomelas*), Black-footed Cat (*Microfelis nigripes*), Hare (*Lepus* sp.), Leopard (*Panthera pardus*), Red Lechwe (*Kobus leche*), Cattle (*Bos taurus*), Stanley's Bustard (*Ardeotis denhami jacksoni*), Marabou Stork (*Leptoptilos crumeniferus*), Grant's Gazelle (*Nanger granti*), Common Ostrich (*Struthio camelus*), Spotted Thick-knee (*Burhinus capensis*), Domestic Dog (*Canis lupus familiaris*), Serval Cat (*Leptailurus serval*), Sheep (*Ovis aries*), Mountain Zebra (*Equus zebra*), Plains Zebra (*Equus quagga*), Genet (*Genetta* sp.), Side-striped Jackal (*Canis adustus*), Lion (*Panthera leo*), African Wildcat (*Felis silvestris*), Eland (*Taurotragus* sp.), Goat (*Capra aegagrus hircus*), Horse (*Equus caballus*), Cat (*Felis catus*), Cheetah (*Acionyx jubatus*), African Wild Dog (*Lycaon pictus*), Crested Francolin (*Dendroperdix sephaena*), African Grass Owl (*Tyto capensis*), Aardwolf (*Proteles cristatus*), Bat-eared Fox (*Otocyon megalotis*), Black-bellied Bustard (*Lissotis melanogaster*), Southern Pale Chanting Goshawk (*Melierax canorus*), Secretary Bird (*Sagittarius serpentarius*), Greater Kudu (*Tragelaphus strepsiceros*), Gemsbok (*Oryx gazella*), Red Hartebeest (*Alcelaphus caama*), Cape Fox (*Vulpes chama*), African Aardvark (*Orycteropus afer*), Kori Bustard (*Ardeotis kori*).

Rhipicephalus turanicus Pomerantsev, 1940

(Figs 7-15)

Synonymy.

Rhipicephalus secundus Feldman-Muhsam, 1952

Rhipicephalus sulcatus Morel and Vassiliades, 1963 *pro parte*

Rhipicephalus turamicus Uzakov 1964 *nomen nudum, lapsus*

Type depository: ZIAC, Lectotype: Male

Type locality: Uzbekistan

Etymology. From geographic distribution in the Turan (Persian, meaning the region north of the Amu-Darya river and east of the Caspian Sea - Uzbekistan and Turkmenistan). Latin –*icus*, adjective (belonging to, derived from).

Material examined. Thirty-six specimens from Turkey, Israel, Egypt, Greece, Afghanistan, Turkmenistan (Table S1).

Type material

Types not examined due to museum collection inaccessibility, but samples from Turkmenistan and Afghanistan are taken to represent *R. turanicus sensu stricto* (*sensu* Filippova, 1997) as they are closest to the type locality in Uzbekistan.

Redescription.

Males (description voucher numbers: OP3255/RD27, OP5198/RD31)
Length 2.8 to 3.0 mm, width 1.6 to 2.2 mm

Basis capitulum hexagonal, short, wide. Lateral angles slightly obtuse projecting at anterior third of length. Cornua present, medium length, rounded. Palps short, sub-triangular. Body reddish-brown. Conscutum elongate, ovate, broadest at coxae IV with anterolateral margin convex. Lateral idiosoma expanded when engorged. Eyes flat, dorsally bordered by a row of medium punctations. Cervical grooves convergent and deep anteriorly, shallow and divergent posteriorly. Cervical fields slightly sunken with few small punctations imparting a smooth appearance, lateral grooves bordered by a row of large, punctations. Marginal grooves enclosing first festoon and reaching level of coxa III, bordered by a row of sparse, large punctations. Posteromedial groove short, oval, rugose.

Posterolateral grooves approximately half the length of posteromedial groove, rugose. Small punctations few imparting a smooth appearance, a few large punctations sparsely scattered in four irregular rows as well as on scapulae.

Legs reddish brown, slender, slightly thicker posteriorly. Coxae approximately equal in size. Coxa I elongate with small anterior process and posteromedial spur broad with triangular tip, posterolateral spur narrow, tapering to rounded tip. Coxa II sub-triangular with posteromedial spur broad, flange-like and posterolateral spur moderate size, triangular. Coxa III sub-rectangular with posteromedial spur broad, flange-like and posterolateral spur minute, triangular. Coxa IV sub-rectangular with posteromedial spur moderate size, triangular and posterolateral spur moderate size, narrow, triangular. Adanal plates elongate triangular with slight medial concavity centrally and convexity posterolaterally. Posterior third slightly more long than wide, imparting a slender appearance. Accessory plates short, reaching level of posterolateral convexity. Spiracles elongate, sub-ovate with dorsal prolongation short, broad.

Females (description voucher numbers: OP3255/RD28, OP5198/RD34)
Length 2.7 to 3.1 mm, width 1.5 to 2.3 mm (Unengorged)

Basis capitulum hexagonal, short, wide. Lateral angles rectangular, projecting at mid-length. Cornua present, short, broadly triangular. Porous areas sub-ovate, moderate size, separated by a distance approximately equal to their width. Palps short, sub-triangular, stalked on article I. Body reddish-brown. Scutum elongate, ovate, broadest at mid-length with anterolateral margin convex, posterior margin sinuous with most posterior point distinctly pronounced. Lateral idiosoma expanded when engorged. Eyes flat, dorsally bordered by a row of medium punctations. Cervical grooves convergent and deep anteriorly, shallow and divergent posteriorly. Cervical fields slightly sunken with

numerous confluent punctations, not reaching posterolateral scutal margins. Lateral grooves distinct, bordered by a row of large punctations. Small punctations numerous, large punctations sparse across scutum.

Legs reddish brown, slender, slightly thicker posteriorly. Coxae approximately equal in size. Coxa I elongate with posteromedial spur broad with triangular tip, posterolateral spur narrow, tapering to pointed tip. Coxa II sub-rectangular with posteromedial spur short, broad, flange-like and posterolateral spur short, broad, triangular. Coxa III sub-rectangular with posteromedial spur short, broad, flange-like and posterolateral spur minute, narrow, triangular. Coxa IV sub-rectangular with posteromedial spur minute, arcuate and posterolateral spur minute, broad, triangular. Genital aperture widening U-shape with lateral margins distinctly diverging anteriorly. Spiracles broadly sub-triangular with rounded angles, dorsal prolongation short, moderate thickness proximally, tapering to rounded tip distally.

Nymphs (description voucher numbers: 5 specimens from OP5195)

Length 0.9 to 1.2 mm, width 0.8 to 1.0 mm (Unengorged)

Basis capitulum hexagonal, almost flat, wide. Lateral angles acute, projecting at mid-length. Cornua absent. Palps short, sub-triangular. Body yellowish-brown. Scutum broad, circular, widest at posterior third with anterolateral margin convex, posterior margin rounded. Eyes flat, located at posterior third of scutum. Cervical grooves convergent and deep anteriorly, shallow and divergent posteriorly. Cervical fields, broad, slightly sunken, not reaching posterolateral scutal margins. Lateral grooves distinct.

Legs transparent brown, slender. Coxae approximately equal in size. Coxa I sub-rectangular with spurs distinctly separated. Posteromedial spur short, broad, triangular

and posterolateral spur long, broad, triangular. Coxa II sub-rectangular with posteromedial spur absent and posterolateral spur minute, triangular. Coxa III sub-rectangular with spurs absent. Coxa IV sub-rectangular with spurs absent.

Larvae (description voucher numbers: 5 specimens from OP5195)

Length 0.4 to 0.6 mm, width 0.3 to 0.5 mm (Unengorged)

Basis capitulum hexagonal, almost flat, width distinctly shorter than posterior margin of scutum. Lateral angles acute, projecting at mid-length. Cornua absent. Palps short, sub-triangular. Body yellowish-brown. Scutum broad, triangular, widest posteriorly with anterolateral margin convex, posterior margin rounded. Eyes flat, located at posterior corner of scutum. Cervical grooves convergent and deep anteriorly, shallow and divergent posteriorly. Cervical fields, broad, distinctly sunken, not reaching posterolateral scutal margins. Lateral groove indistinct.

Legs transparent brown, slender. Coxae approximately equal in size. Coxa I sub-rectangular with posteromedial spur moderate size, broad, flange-like and posterolateral spur absent. Coxa II sub-rectangular with posteromedial spur short, broad, triangular and posterolateral spur absent. Coxa III sub-rectangular with posteromedial spur short, triangular and posterolateral spur absent.

Biogeography. Palearctic (Fig. 6). Generally, occurring in regions with annual precipitation between 100 mm and 1000 mm. This wide range may be a result of differential arid tolerance in the two lineages observed in *R. turanicus*.

Hosts. Three host life cycle. Host data from Filippova (1997), Walker et al. (2000) and GTTM records. Cattle (*Bos taurus*), Domestic Dog (*Canis lupus familiaris*), Sheep (*Ovis aries*), Goat (*Capra aegagrus hircus*), Cat (*Felis catus*), Donkey (*Equus africanus*), Pig

(*Sus scrofa domesticus*), Bactrian Camel (*Camelus bactrianus*), Red Deer (*Cervu elaphus*), Markhor (*Capra falconeri*), East Caucasian Tur (*Capra caucasia cylindricornis*), Urial (*Ovis orientalis vignei*), Black-tailed Gazelle (*Gazella subgutturosa*), Onager (*Equus hemionus*), Wild Boar (*Sus scrofa*), Grey Wolf (*Canis lupus*), Golden Jackal (*Canis aureus*), Red Fox (*Vulpes vulpes*), European Badger (*Meles meles*), Amur Leopard (*Panthera pardus orientalis*), European Wildcat (*Felis silvestris*), Jungle Cat (*Felis chaus*), Marbled Polecat (*Vormela peregusna*), European Hedgehog (*Erinaceus europaeus*), Southern White-breasted Hedgehog (*Erinaceous concolour*), Long-eared Hedgehog (*Hemiechinus auritus*), European Hare (*Lepus europaeus*), Cape Hare (*Lepus capensis*), Long-clawed Ground Squirrel (*Spermophilus leptodactylus*), Yellow Ground Squirrel (*Spermophilus fulvus*), Egyptian Vulture (*Neophron percnopterus*), Short-eared Owl (*Asio flammeus*), Rook (*Corvus frugilegus*), Hoopoe (*Upupa epops*), Calandra Lark (*Melanocorypha calandra*), Common Blackbird (*Turdus merula*), Brown Rat (*Rattus norvegicus*), Turkestan Rat (*Rattus pyctoris*), Short-tailed Bandicoot Rat (*Nesokia indica*), House Mouse (*Mus musculus*), Wood Mouse (*Apodemus sylvaticus*), Yellow-necked Mouse (*Apodemus flavicollis*), European Snow Vole (*Chionomys nivalis*), Social Vole (*Microtus socialis*), European Water Vole (*Arvicola terrestris*), Grey Dwarf Hamster (*Cricetulus migratorius*), Great Gerbil (*Rhombomys opimus*), Libyan Jird (*Meriones libycus*), Tristram's Jird (*Meriones tristrami*), Persian Jird (*Meriones persicus*), Small Five-toed Jerboa (*Allactaga elater*), Brandt's Hedgehog (*Paraechinus hypomelas*), Gueldenstaedt's Shrew (*Crocidura gueldenstaedtii*), Common Bent-wing Bat (*Miniopterus schreibersii*), Crested Lark (*Galerida cristata*), European Green Lizard (*Lacerta viridis*), Sand Lizard (*Lacerta agilis*), Indian Crested Porcupine (*Hystrix indica*).

Notes. Two lineages are observed among *R. turanicus*. One lineage is distributed in the Middle-East/Asia, while the other is in southern Europe (Figs 3,6). These two lineages

seem to correspond to differential arid tolerance in these environments based on annual precipitation (Fig. 6). These represent separately evolving lineages that may be shown as different species upon further investigation. If true, *R. turanicus s. str.* (*sensu* Filippova, 1997) would be represented by the Middle-East/Asian lineage. It is possible that the synonym *R. secundus* Feldman-Muhsam, 1952 represents the southern Europe lineage because samples associated with this name have been collected from Italy, Israel, Iraq, Turkey, Serbia and France (Feldman-Muhsam, 1953; Feldman-Muhsam, 1952; Hoogstraal, 1956; Paperna and Giladi, 1974).

Differential diagnosis. Males of *R. afranicus* **n. sp.** may be distinguished from *R. turanicus* by (1) adanal plates slightly shorter with posterior third more wide than long [*R. turanicus* = longer with posterior third more long than wide, (2) adanal plates with posteromedial tip indistinct [*R. turanicus* = posteromedial tip slightly distinct], (3) conscutum more punctate [*R. turanicus* = less punctate], and (4) cornua short, broadly triangular [*R. turanicus* = medium length, rounded].

Females of *R. afranicus* **n. sp.** may be distinguished from *R. turanicus* by (1) spiracles with dorsal prolongation thick proximally [*R. turanicus* = thin proximally], (2) spiracles with excavation between dorsal prolongation and spiracle dorsal margin indistinct [*R. turanicus* = excavation distinct], (3) spiracles with dorsal prolongation angled slightly posteriorly [*R. turanicus* = angled near-perpendicularly], (4) scutum with sinuous posterior margin bearing mild pronouncement [*R. turanicus* = distinct pronouncement], and (5) scutum bearing many large punctations among numerous small punctations [*R. turanicus* = few large punctations].

Nymphs of *R. afranicus* **n. sp.** may be distinguished from *R. turanicus* by (1) scutum elongate, ovate [*R. turanicus* = broad, ovate], (2) cervical fields narrow, distinctly sunken

[*R. turanicus* = broad, slightly sunken], and (3) coxa I with spurs moderately separated [*R. turanicus* = distinctly separated].

Larvae of *R. afranicus* **n. sp.** may be distinguished from *R. turanicus* by (1) basis capitulum width almost equal to posterior margin of scutum width [*R. turanicus* = distinctly shorter than posterior margin], (2) scutum narrow triangular [*R. turanicus* = broad triangular], and (3) cervical fields narrow, slightly sunken [*R. turanicus* = broad, distinctly sunken].

Differential disease relationships.

R. afranicus **n. sp.** **–** Experimentally shown as a vectors of *Babesia trautmanni* to domestic pigs in South Africa (Lopez-Rebollar & De Waal, 1994), referring to *R. afranicus* **n. sp.**. However, *R. afranicus* **n. sp.** do not readily parasitize pigs in Africa, making this finding potentially more applicable to *R. turanicus* in Europe, but requires further vector competency testing.

R. turanicus s. lat. **–** Suspected vector of *Babesia caballi* and *Theileria equi* to horses in Europe (Enigk, 1943; Friedhoff, 1988). South African ticks were unsuccessful in transmitting infections to horses (Potgieter et al., 1992). As such, this finding refers to *R. turanicus s. lat.* in Europe. Experimentally shown as a vector of *Babesia canis* to domestic dogs in India (Achuthan et al., 1980), and of *Hepatozoon canis* to dogs in Italy (Gianelli et al., 2016). Implicated as a vector of Q fever and Siberian tick typhus in Europe and the middle east (Balashov & Daiter, 1973; Berdyev, 1980). Whether these refer to the southern European, Middle-East/Asian or both lineages in each case is uncertain.

4. Discussion

Phylogenetic relationships based on 12S and 16S rDNA sequence data provided evidence for at least two distinct lineages among what are traditionally known as *R. turanicus* (Figs 1-3)*.* As such, the hypothesis of *R. turanicus* as a single monophyletic species is refuted. To deal with the resulting paraphyly, a new taxon, *R. afranicus* **n. sp.**, is established to represent all Afrotropical *R. turanicus*. Pairwise genetic distance data for both 12S and 16S rDNA are below 95% similarity for these two clades. This value is generally considered a threshold of conspecificity for these genes in ticks (Bakkes et al., 2018; Chitimia-Dobler et al., 2017; Lado et al., 2018; Li et al., 2018; Mans et al. 2019). More comprehensive sampling of *R. turanicus* in the current study corroborates the observations of Dantas-Torres et al. (2013) and Coimbra-Dores et al. (2018) based on similar molecular data. This species boundary was tested against qualitative morphology (traditional) and quantitative analysis of shape outlines in female spiracles and male adanal plates (Figs 4,5). These lines of evidence confirmed *R. afranicus* **n. sp.** as a distinct species. Furthermore, two lineages among *R. turanicus* were recovered (Figs 1-3) that appear to correlate with two different regions, as well as two regimes of annual rainfall in southern Europe and the Middle-East/Asia (Fig. 6). These may prove to be distinct species upon further investigation and may refer to the synonym *R. secundus*. Samples associated with the name *R. secundus* have been collected from Italy, Israel, Iraq, Turkey, Serbia and France (Feldman-Muhsam, 1953; Feldman-Muhsam, 1952; Hoogstraal, 1956; Paperna and Giladi, 1974). For the purposes of this study however, the focus is testing the species boundary of Afrotropical populations of *R. turanicus* against Palearctic *R. turanicus s. lat.,* as such we refrain from examining the possibility of two species within Palearctic *R. turanicus*. We provide full descriptions for both *R. afranicus* **n. sp.** and *R. turanicus s. str.* (Middle-East/Asian lineage).

Disjunct distribution patterns between *R. afranicus* **n. sp.** and *R. turanicus s. lat.* (Fig. 6) support a species boundary based on allopatric speciation (Mayr, 1947). An obvious geographic barrier separating these two tick lineages is the Sahara desert that probably served as the vicariant agent for speciation about 5-7 Mya (Schuster et al., 2006; Zhang et al., 2014). However, this region is characterized by humid and arid cycles, and the oscillating climatic history of the Sahara can result in complex evolutionary patterns (Gonçalves et al., 2018). Indeed, generalist tick species (*e.g. Hyalomma*) that utilize a variety of hosts for dispersal, do not show consistent vicariance patterns across the Sahara desert, and instead, several intercontinental dispersals between the Afrotropics and Palearctic have been shown (Sands et al., 2017). If this holds true for *R. afranicus* **n. sp.** and *R. turanicus s. lat.*, it implies the initial expansion of the Sahara desert (7-5 Mya) acted as a catalyst for initial divergence, followed by population re-integration events driving reticulate evolution as a result of oscillating climatic history.

Reproductive potential between *R. turanicus* from Cyprus and *R. afranicus* **n. sp.** from Zambia under laboratory conditions (Pegram et al., 1987), challenges the species boundary proposed here. Fertile hybrids display marked heterosis and increased fecundity that produces approximately 20% more offspring than same-population matings do (Pegram et al., 1987). However, it is uncertain whether or not these were *bona fide R. turanicus* samples (Guglielmone et al., 2014). Morphology of *R. afranicus* **n. sp.** is an exact match with Zambian *R. turanicus* in Pegram et al. (1987), but morphology between *R. turanicus s. str.* (Middle-East/Asia) is a close, but not exact match with Cypriot *R. turanicus* in Pegram et al. (1987) (Figs 7-10, 12-15). These Cypriot *R. turanicus* likely refer to the southern European lineage recovered among *R. turanicus s. lat.* (Figs 1-3). As such, further investigation is required to determine exact species status of *R. turanicus s. lat.* in the Palearctic. Nevertheless, hybridization between *R. afranicus* **n. sp.** from

Zambia and at least one lineage of *R. turanicus s. lat.* has been demonstrated (Pegram et al., 1987).

Hybridization in ticks is not well understood, yet fertile hybrids between closely related tick species outside of the *R. sanguineus* group have been documented before (Zivkovic et al., 1986; Rees et al., 2003; Kovalev et al., 2015). Within the *R. sanguineus* group, fertile hybrids have been observed between lineages in North America and the Mediterranean, but these lineages produce infertile hybrids when Afrotropical lineages are involved (Levin et al., 2012). Likewise, lineages from Argentina and Brazil produce infertile hybrids (Szabó et al., 2005), as do Brazilian and French lineages (Nava et al., 2018). These reproductive incompatibilities are in line with distinct *R. sanguineus* group lineages distributed in temperate and tropical regions where Brazilian and African samples are closely related, and conversely Argentinian, North American and European samples are closely related (Szabó et al., 2005; Coimbra-Dores et al., 2018). Moreover, *R. turanicus* and the closely related *R. sulcatus* produce infertile hybrids (Pegram et al., 1987). This makes fertile hybrids produced by *R. afranicus* **n. sp.** from Zambia and *R. turanicus s. lat.* from Cyprus highly unusual for the *R. sanguineus* group. Such anomalous hybridization suggests recombination of these two geographically distant, and genetically distinct lineages lead to hybrid vigour (Edmands, 2002; Matter et al., 2014). However, for this process to cross distinctly divergent species boundaries is puzzling (Figs 1-3), and suggests there may be unknown pre- or post-zygotic reproductive mechanisms occurring at the cellular level that remain compatible between these lineages despite evolutionary divergence. This warrants further investigation into gamete compatibility, oogenesis and cytogenetics that form pre- and post-zygotic reproductive mechanisms between these species.

Given corroboration of the four independent lines of evidence in this study (mtDNA, male adanal plates, female spiracles and disjunct distribution) it is reasonable to consider *R. afranicus* **n. sp.** as a distinct and diagnosable species (De Queiroz, 2007) that is able to hybridize with at least one lineage among *R. turanicus s. lat.* under laboratory conditions. Pre- and post-zygotic reproductive isolation is expected to evolve faster when driven by direct selection rather than neutral evolution and drift (Edmands, 2002). As such, an alternative explanation for these two species must consider their hybridization potential due to (1) insufficient evolutionary time for development of pre- or post-zygotic reproductive barriers, and (2) a lack of direct selection against hybrids that would differentiate pre- and post-zygotic reproductive mechanisms (Edmands, 2002; De Queiroz, 2007). These may be due to allopatric speciation, driven by Sahara desert expansion, that would form an environmental barrier to the formation of hybrids before a physiological barrier would evolve, limiting selection against hybrids during speciation (Edmands, 2002; Douady et al., 2003). An additional consideration is the oscillating climate history in the Sahara desert (Gonçalves et al., 2018) that would have facilitated population reintegration events after initial divergence. Taken together, a lack of direct selection against hybrids to conserve reproductive mechanisms following rapid allopatry (Douady et al., 2003), likely acted in concert with fluctuating allopatric speciation and population re-integration events driven by environmental oscillation (Gonçalves et al., 2018). This would drive reticulate evolution between *R. afranicus* **n. sp.** and *R. turanicus s. lat.* to maintain compatibility of reproductive mechanisms and facilitate hybridization potential even after divergence.

These findings indicate that speciation in ticks may be complicated by localized historical introgression events during divergence (Maddison, 1997; Arnold, 2004). As such, evolution may have been reticulate, where lineages diverged and recombined due to

rapid changes in external factors such as climate and host shifts before divergence finalized (incomplete lineage sorting). More recently, global human-mediated movement of *R. sanguineus* group ticks associated with dogs may also play a role (Gray et al., 2013). In either case, introgression (both recent and historical) may explain current confusion associated with morphologically cryptic species among ticks that have highly structured lineages. As demonstrated in this paper, future studies on *R. sanguineus* group taxonomy should employ multiple independent lines of evidence in combination with tracing causality of evolutionary events in order to stabilize the taxonomy of this group.

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