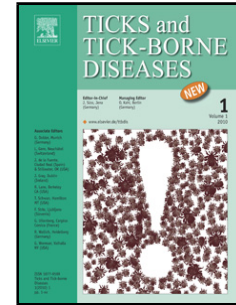


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# Integrative Taxonomy of Afrotropical *Ornithodoros* (*Ornithodoros*) (Acari: Ixodida: Argasidae)

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**Running title:** Afrotropical *Ornithodoros* (*Ornithodoros*) taxonomy

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## Abstract

Afrotropical *Ornithodoros* (*Ornithodoros*) ticks are revised based on qualitative morphology of females and nymphs, as well as tarsus I shape outlines of females measured in a geometric morphometric framework. These lines of evidence corroborate lineages based on 16S rRNA nucleotide sequence data. Four previously unrecognized species are described, along with a

revived *nomen nudum* that was previously considered a synonym. Afrotropical *Ornithodoros* (*Ornithodoros*) now comprise ten species. *Ornithodoros moubata* and *Ornithodoros porcinus* are separated from three other species in southern Africa (*Ornithodoros compactus*, *Ornithodoros phacochoerus* **n. sp.**, *Ornithodoros waterbergensis* **n. sp.**), with *O. porcinus* restricted to central east Africa. Known species boundaries for *Ornithodoros apertus* and *O. compactus* are supported. *Ornithodoros savignyi* are separated from three other species in South Africa and Namibia, with *O. savignyi* restricted to north Africa. Neumann's *Ornithodoros pavimentosus* **nom. rev.** are resurrected from synonymy as a species that occur in Bushmanland, Namaqualand and Namibia, while *Ornithodoros kalahariensis* **n. sp.** occur in Kalahari thornveld, and *Ornithodoros noorsveldensis* **n. sp.** occur in Noorsveld thicket of South Africa. Detailed descriptions are given for each species along with high resolution images and point map distributions. Support is provided for speciation driven by riverine barriers, Pliocene uplift and differential arid tolerance. Exaggerated tarsus I shape in the *O. savignyi* group suggests adaptation to fossorial habits and soil type. Conversely, reduced tarsus I shape in the *O. moubata* group is suggested as an evolutionary consequence of the life history change from soil to warthog burrows. This study represents an integrative (iterative) approach to delimiting Afrotropical *Ornithodoros* (*Ornithodoros*) species, and provides the first application of tarsus I shape outlines in a geometric morphometric framework for testing species boundaries.

Key words: Argasidae; Ornithodoros; African swine fever; human relapsing fever; tarsus; Tampan; Afrotropical; Morphometrics; Shape; 16S; Taxonomy; Procrustes.

## Introduction

Ticks (Ixodida) are comprised of three families, the hard bodied (Ixodidae), soft bodied (Argasidae) and Nuttalliellidae (Guglielmone et al., 2010). Argasidae comprise two subfamilies, Argasinae and Ornithodorinae, with the latter composed of four genera: *Antricola* Cooley & Kohls, 1942; *Ornithodoros* Koch, 1837; *Otobius* Banks, 1912; and *Nothoaspis* Keirans & Clifford, 1975. This follows the schema of Hoogstraal (1985) which we adopt for this work. Subgenera are adopted as designated by Clifford et al. (1964). The type species of *Ornithodoros* is *Ornithodoros savignyi* (Audouin, 1827), commonly known as the sand tampan. This species is morphologically related to *Ornithodoros apertus* Walton, 1962 (east Africa), *Ornithodoros compactus* Walton, 1962 (southern Africa), *Ornithodoros eremicus* Cooley & Kohls, 1941 (North America), *Ornithodoros indica* Rau & Rao, 1971 (India), *Ornithodoros moubata* (Murray, 1877; sensu Walton, 1962) (southern and east Africa), *Ornithodoros*

*procaviae* Theodor & Costa, 1960 (Israel) and *Ornithodoros porcinus* Walton, 1962 (southern and east Africa). Together, they form the subgenus *Ornithodoros* (*Ornithodoros*) (Hoogstraal, 1985), but with possible exception of *O. eremicus* that requires further study (Hoogstraal, 1956). Afrotropical species are important as vectors of African swine fever (*O. porcinus* and *O. moubata* group), human relapsing fever (*O. moubata*) and sand tampan toxicoses (*O. savignyi*) (Mans et al., 2002; Cutler, 2015; Sanchez-Vizcaino et al., 2015).

Historically, Afrotropical *Ornithodoros* (*Ornithodoros*) have posed challenges for species delimitation based on comparative morphology. This is due to few definitive morphological characters that tend towards high variability. Two species were originally described in the 19<sup>th</sup> century based on presence and absence of eyes, separating *O. savignyi* from Egypt and *O. moubata* from Angola (Murray, 1877). *Ornithodoros pavimento*sus Neumann, 1901 was described later based on tarsal and mammillae morphology that distinguished this species from *O. savignyi*. However, Neumann (1901) did not provide a complete description and introduced a *nomen nudum*. Almost half a century later, this species was synonymized with *O. savignyi* for a lack of distinctiveness based on about 1000 specimens that derived from Arabia, Egypt, Libya, South Africa and Sudan (Theiler and Hoogstraal, 1955). Concerning historical biogeography, the wide distribution of sand tampans has led to different hypotheses for their origins in Africa. Hoogstraal (1956) proposed that *O. savignyi* was introduced into Africa from the East via camel routes. Conversely, Theiler (1962) considered an origin in either the Sahelian zones bordering the Sahara in north Africa, or in the Kalahari thornveld of southern Africa. These competing hypotheses have different implications for speciation given the large geographic distances separating semi-desert regions that are amenable to the ecology of these ticks, such as the Arabian, Kalahari, Namib and Sahara deserts.

Walton's (1962) study of *O. moubata* recognized three new species (*O. apertus*, *O. compactus* and *O. porcinus*) based on characters of reproductive biology, developmental time and morphology. Notably, he designated a neotype for *O. moubata* in Groot Marico (South Africa) to replace the lost holotype from Angola – a crucial step in the progress of *Ornithodoros* taxonomy. Two subspecies were recognized among *O. porcinus* that were postulated to have adapted to wild (*O. porcinus porcinus*) and domestic (*O. porcinus domesticus*) environments. Recently, molecular 16S ribosomal RNA data have demonstrated that *O. porcinus* are comprised of paraphyletic lineages that appear geographically restricted (Bastos et al., 2009; Boshoff et al., 2014). These clades correlate to south-eastern, south-western and north-eastern parts of sub-Saharan Africa, raising the possibility that these may represent different species. Moreover, the wide geographic distribution, similar to that of *O. savignyi*, raises similar questions of historical biogeography and speciation in this group. Recently, *O. porcinus*

was detected in Madagascar, but this is probably an introduction due to movement of domestic pigs (Ravaomanana et al., 2010).

The present study investigates taxonomy and species boundaries among Afrotropical *Ornithodoros* (*Ornithodoros*) based on independent data from qualitative morphology (traditional), 16S rRNA sequences and geometric morphometric analysis of tarsus I shape outlines (quantitative morphology). Shape outlines can hold useful clues for taxonomy and species delimitation given that individuals comprising a single species should display more similar morphological shape when compared with individuals from another species. Excluding destabilizing selection, shape outlines will tend toward a central mean among a global population of interbreeding individuals. A geometric morphometric framework 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 morphologically cryptic species complexes with demonstrated success (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. The present approach assumes contemporary formulation of integrative taxonomy that tests species boundary hypotheses in an iterative framework along multiple lines of evidence (Yeates et al., 2011).

## Material and methods

### *Samples and qualitative morphology*

Fresh specimens were collected from a range of localities (Table 1) by burrow excavation and carbon dioxide traps (Nevill, 1964). Additionally, specimens from the Gertrud Theiler Tick Museum (ARC-OVR), South Africa (GTTM) were used for qualitative and quantitative morphology (Table 1). Specimens were identified *a priori* according to the taxonomic keys of Hoogstraal (1956) and Walton (1962), and were categorized by geographic locality. Notably, the type locality for *O. moubata* sensu stricto (Groot Marico) was sampled. Immatures are generally useful for addressing questions of species boundary and relationship because morphological variation is generally more stable in earlier stages of ontogeny. However, larvae were unstudied due to their habit of moulting to nymphs immediately after hatching without taking a bloodmeal (Hoogstraal 1956; Sonenshine et al., 1966). This precludes effective sampling. Moreover, it is not certain that species differences will be present in larvae, due to

their reduced activity and remaining sheltered from the environment within eggs (Sonenshine et al., 1966). Instead, progeny were reared from collected adults, and unfed 1<sup>st</sup> or 2<sup>nd</sup> nymphal instars were sampled and studied qualitatively. Specimens were examined under a Zeiss Discovery.V20 Stereomicroscope for qualitative morphological characters. Terminology generally follows that of Walton (1962), but see Figure 1 for a diagram of morphological features. Complete specimen data are presented in the material examined Table S1 (supplementary material).

#### *Phylogenetic analysis of the 16S ribosomal RNA gene*

Individual samples were cut through the middle of their hind bodies for DNA extraction. Genomic DNA was extracted using the Qiagen Blood Kit according to the manufacturer's instructions. The 16S ribosomal RNA gene was amplified using the 16SF (CTG CTC AAT GAT TTT TTA AAT TGC TGT GG) and 16SR (CCG GTC TGA ACT CAG ATC AAG T) primers (Black and Piesman, 1994). PCR products were sequenced using the 16SR primer by Inqaba Biotec (South Africa) and sequences deposited in GenBank.

For Afrotropical *Ornithodoros* (*Ornithodoros*), sequences were aligned using MAFFT (Q-INS-i, 200PAM / k=2, Gap opening penalty: 1.53) (Kato et al., 2002) and maximum likelihood analysis was performed using IQ-Tree (Nguyen et al., 2015). Analysis included 86 aligned sequences with 282 nucleotide sites, with 105 phylogenetically informative. The optimal substitution model with the best Bayesian information criterion score was selected automatically (HKY+F). The consensus tree was constructed from 100 000 bootstrap trees and nodal support values were calculated from these.

Analysis of 16S rRNA for world *Ornithodoros* was done to test monophyly of Afrotropical *Ornithodoros* (*Ornithodoros*), and 68 sequences comprising 58 outgroup taxa were used to ensure the test for monophyly was robust. Sequences were retrieved from GenBank and aligned using ClustalX (Jeanmougin et al., 1998). Maximum likelihood analysis was performed as above. This analysis included 68 aligned sequences with 339 nucleotide sites, with 232 phylogenetically informative. The optimal substitution model with the best Bayesian information criterion score was selected automatically (HKY+F). The consensus tree was constructed from 100 000 bootstrap trees and nodal support values calculated from these. A number of ticks used in sequencing were likewise used in morphometric analyses to iteratively test species boundaries from 16S rRNA clades (3 *O. compactus*, 3 *O. kalahariensis* **n. sp.**, 3 *O. moubata*, 3 *O. noorsveldensis* **n. sp.**, 4 *O. pavimentosus* **nom. rev.**, 3 *O. phacochoerus* **n. sp.**, 1 *O. waterbergensis* **n. sp.**; *O. savignyi* and *O. porcinus* were unavailable for this iterative procedure, however in the case of *O. porcinus*, a paratype specimen was used).

### *Geometric morphometric analysis of tarsus I shape*

A total of 39 *O. moubata* group and 47 *O. savignyi* group specimens were studied in a geometric morphometric context, including twenty that were sequenced (Table 1). These specimens are stored in alcohol and are housed in the GTTM. Lack of samples led to exclusion of *O. apertus* from morphometric analysis.

Tarsal lobes on left tarsus I 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 taken every 22  $\mu\text{m}$ . Tarsi present matching symmetry and thus symmetric variation (Klingenberg et al., 2002). Tarsal lobe outlines were digitized using COO v41 in the CLIC package by Jean-Pierre Dujardin (available at <http://mome-clic.com/the-clic-package/>) according to 22 landmarks (Figure. S1: supplementary info), and were scaled to a 0.2 mm scale bar. Operational definitions for each landmark are available in Figure 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. A regression of shape on size was performed to quantify allometry, and analysis proceeded using regression residuals to remove shape-size correlates (size correction). 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 and 16S rRNA clades directed *a priori* species hypotheses that were tested in the following geometric morphometric analyses. A discriminant analysis for correct classification and comparison of multivariate means was performed for all pairs of species with 1 000 permutations. Principal components and canonical variates were computed with 10 000 permutation tests for pairwise distances. Canonical variates transform morphospace to maximize differences between groups, and are sensitive to *a priori* species delimitation. This enables a test of group structure based on Mahalanobis distances. In turn, Mahalanobis distance scales among group variation by within group variation, enabling comparison between multivariate group means. This tested hypotheses of species group structure (proxy for species boundaries) based on 16S rRNA data and qualitative morphology, and characterized shape changes between species. The total Afrotropical *Ornithodoros* (*Ornithodoros*) sample was analyzed, as well as the *O. moubata* and *O. savignyi* groups separately.

A pilot study to assess rotational error following Bakkes (2017) was done on five *O. pavimentosus* **nom. rev.** and nine *O. kalahariensis* **n. sp.** specimens on both left and right tarsi with five replicates each. A Procrustes ANOVA was done for individual, side and individual-by-side, with replicate as the error effect and species as the main effect. Individual averages for left side only, were used in discriminant analysis for correct classification and in comparison of multivariate means with 1 000 permutations.

### *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 major river topologies (<http://www.natureearthdata.com/downloads/50m-physical-vectors>), annual precipitation, altitude (<http://www.worldclim.org/current>), as well as soil type (<http://esdac.jrc.ec.europa.eu/content/soil-map-soil-atlas-africa>: Dewitte et al., 2013; Jones et al., 2013) 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 the abiotic environment.

## Results

### *Taxonomy and Species Descriptions*

#### **Family ARGASIDAE** Canestrini, 1890

#### Genus ***Ornithodoros*** Koch, 1837

#### ***Ornithodoros apertus*** Walton, 1962

(Figures 2-5)

*Ornithodoros apertus* Walton, 1962

*Ornithodoros (Ornithodoros) apertus* Walton:Clifford, Kohls & Sonenshine, 1964

Type depository: BMNH (British Museum of Natural History), Holotype: Female

Type locality: Crescent Island, Lake Naivasha, Kenya (0.764316S 36.416119E)

**Etymology.** Not originally stated, however, probably from Latin *apert* -us, -a, -um, adjective (uncovered, unclosed, open) referring to rareness of the species.

**Material examined.** One female (paratype) from Tanzania (Table S1). Paratype: OP3086i; 1 ♀ (GTTM). Label data: '*O. apertus* sp. n. / G.A. Walton. 1961 / Crescent island / Lake Naivasha / Kenya / Heisch & Grainger (1950) / paratypes' (White rectangle).



**Diagnosis.** Females may be distinguished by a combination of (1) eyes absent, (2) posterior dorsal mamillae larger than anterior, (3) post-capitular area without mamillae, (4) metatarsus without lobes, (5) tarsus I long (three times length of trochanter), (6) tarsus I with dorsal lobes small, inconspicuous, pointed.

**Redescription.**

Females

Length 5.0 to 8.0 mm, width 3.5 to 6.0 mm.

**Body** round oval, tightly rounded at anterior and posterior ends, less so posteriorly; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes absent; body colour grey to fawn when alcohol preserved; integument leathery; body setae sparse. **Mamillae** generally opaque with distinct transparent centre, dome-shaped, bearing fifteen indistinct punctations apically, connected to wide dumbbell-shaped pore canals and wide internal cavity, raised and generally well-spaced; anterior and central mamillae small, well-spaced, sub-circular shape, raised, domed apically; posterior mamillae large, tightly packed, sub-circular shape, well-raised, obtusely conical, domed apically; ventral post-capitular area not mammilated, small fine irregularities posteriorly, lacking setae. **Intermamillary space** with cell patterned ridges of fine, vermiform elevations, indistinct anteriorly and centrally, more distinct posteriorly. **Setae** slender, tapering to fine point, occasionally delicately clubbed; anterior setae stout, blunt, delicately clubbed, about 30, reducing posterolaterally; ventral setae on anterior part of supracoxal fold short, blunt, sparse about five between trochanter I and II; setae on anterolateral hump of supracoxal fold short, stout, about six; setae on transversolateral part of supracoxal fold stout, blunt, indistinct. **Genital apron** slightly posterior to post-capitular area, flattened, slightly oval transversely; anterior lip thick, transverse, with about 30 fine striae; posterior lip, notably thick, distinct crescent shape with about 10 coarse striae; post-capitular area longer than wide, generally depressed, comprised of smooth areas proximally, centrally and distally, with fine corrugations, transverse ridge divides depression proximo-centrally, distal integument extending slightly over base of basis capitulum; post-genital area with posterior extension of genital apron posterior lip comprising coarsely corrugated integument folds, about four; mamillae numerous, small, domed, sub-circular, well-spaced.

**Basis capitulum** short, gently undulate, generally flat ventrally, sclerotised; anterolateral humps present; post-hypostomal setae about half length of hypostome, thick basally, tapering in remainder; post-palpal setae small, conical. **Hypostome** large, notched apically, with about 40 large denticles in files of 3:3, 40 small denticles, scattered; cheliceral

apex large, with mobile bicusate tooth, large. **Palp** long, segment II and III with slender setae, five on II, two on III, and smaller setae tapering; segment I with several small spine-like setae.

**Coxae** generally narrow, elongate triangular anteriorly, broader (more equilateral) posteriorly, hemiconical; anterior two-thirds sclerotised, posterior third as mammillate syncoxae, mammillae small, reduced in IV; apex with margin raised, bearing slight constriction centrally; I with posteromedial process stout, rounded, distinct; I, II, III, IV with setae on apical anterior and posterior margins, slender, short, curved; apical marginal hairs longer on II, III, IV. **Trochanter** short, simple with stout, blunt setae; I with setae on anterior margin about two, none posteriorly; II with about three anteriorly, sub-apical, about two posteriorly; III with about one anteriorly, about two posteriorly; IV with two to three anteriorly, about two posteriorly. **Trochanterellus** about length of trochanter; setae on dorsal apex long, on dorsal surface small, clubbed, usually one long, curved seta posterodistally, occasionally absent.

**Legs** long, robust; clubbed setae absent; claws long. **Femur** twice length of trochanter; setae moderately abundant on anterior legs, less posteriorly; I, II with setae on anterior and posterior margin, long, curved, extending towards trochanterellus. **Tibia** long, about twice length of trochanter; setae distinct, curved on apical margin. **Metatarsus** about three times length of trochanter; dorsal lobes absent; setae on ventral margin arising from minor convexities and apical margin. **Tarsus I** about three times length of trochanter; dorsal lobes not prominent, reduced in height, pointed, all separated by about width of three to four lobes; three lobes including a bipartite distal lobe where Haller's organ divides sub-lobes; proximal lobe short, flat distally, thin, vertical axis slightly forwards inclining; middle lobe short, thin, well-curved imparting a shark dorsal fin-like appearance; distal sub-lobes not separated, flat; proximodistal lobe especially wide, short, indistinct contiguous with tarsus; distodistal lobe notably short, thin, contiguous with proximodistal lobe; Haller's organ small; setae on dorsal margin and ventral margin arising from minor convexities, generally flat, curved, short; two setae on apex of proximodistal lobe, short, curved, directed opposite of one another, flat; apical setae arranged concentrically, short, curved, tapering, about four or five.

Nymphs Unknown

**Biogeography.** Apparently rare. Restricted in East Africa (Fig. 9B). Occurring in regions with greater than 900 mm annual precipitation (Fig. S2) and altitude about 1800 m above sea level (Fig. 9B). Associated with porcupine burrows.

**Hosts.** Porcupine: *Hystrix* spp.

**Disease relationships.** Unknown

**Notes.** None.

***Ornithodoros compactus*** Walton, 1962

(Figs 2-8)

*Ornithodoros compactus* Walton, 1962

*Ornithodoros (Ornithodoros) compactus* Walton: Clifford, Kohls & Sonenshine, 1964

Type depository: BMNH, Holotype: Female

Type locality: Niekerkshoop, Kimberley, Northern Cape, South Africa (29.312007S 22.819353E)

**Etymology.** Not originally stated, however, probably from Latin *compact -us, -a, -um*, adjective (close-packed, firm, thick) referring to dense structure of mammillae.

**Material examined.** Three females and three nymphs from South Africa (Table S1). Sequences deposited in GenBank: MF415609, MF415610, MF415611, MF415612, MF415613, MF415614.

**Diagnosis.** Females may be distinguished by a combination of (1) eyes absent, (2) dorsal mammillae flat and tile-like, (3) tarsus I proximal lobe short, thick, rounded to curved, (4) tarsus I middle lobe short, thin, steeply angled, (5) tarsus I proximodistal lobe nearly flat, (6) tarsus I distodistal lobe notably short.

Nymphs may be distinguished by (1) eyes absent, (2) anterior and posterior dorsal mammillae sub-equal size, (3) dorsal mammillae flat and tile-like, (4) basis capitulum short, wide sub-rectangular, and (5) hypostome moderate thickness.

### **Redescription.**

Females

Length 6.0 to 9.0 mm, width 4.5 to 7.0 mm.

**Body** round oval, tightly rounded at anterior and posterior ends; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes absent; body colour dark, grey, reddish when alcohol preserved; integument leathery; setae moderately abundant. **Mammillae** generally opaque with indistinct transparent centre, crenated, bearing one to two distinct punctations apically, all even in size and shape, low and tightly packed; anterior, central and posterior mammillae small, tightly spaced, sub-circular shape, not raised, crenate apically; ventral post-capitular area mammilated, with setae in two lateral groups of four usually. **Intermammillary space** with cell patterned ridges of thick, vermiform elevations, distinct anteriorly, centrally and posteriorly. **Setae** stout, blunt, occasionally clubbed; anterior setae short, slender, tapering, moderately

numerous, about 50, extending posterolaterally almost reaching dorsoventral groove; ventral setae on anterior part of supracoxal fold short, tapering, numerous extending to trochanter II, about twelve between trochanter I and II; setae on anterolateral hump of supracoxal fold slender, about eight; setae on transversolateral part of supracoxal fold short, thick, indistinct. **Genital apron** slightly posterior to post-capitular area, flattened, sub-circular; anterior lip thick, transverse, with about 40 scattered striae; posterior lip, notably thick, half-moon to indistinct crescent shape with about 40 fine striae; post-capitular area wider than long, comprised of finely corrugated area proximally, small, through small mammillate area centrally, large, to flat, rough area distally at capitulum base, large; post-genital area with posterior extension of genital apron posterior lip comprised of finely corrugated integument; numerous mammillae, small, crenated, sub-circular, tightly packed.

**Basis capitulum** short, somewhat undulate, flat ventrally, sclerotised; anterolateral humps absent; post-hypostomal setae more than half length of hypostome, slender; post-palpal setae short, conical, occasionally long and blunt. **Hypostome** elongate slightly, notched apically, with about 17 large denticles in files of 2:2 or 3:3, 40 small denticles scattered; cheliceral apex somewhat truncate, mobile bicusate tooth large. **Palp** long, segment II with four long dorsomedial setae; III with long setae, two; segment I with notably short, blunt, peg-like setae, two or three; all dorsolateral setae short, blunt, peg-like.

**Coxae** elongate triangular anteriorly, broader posteriorly, hemiconical, anterior two-thirds sclerotised, posterior third as mammillate syncoxae, mammillae small, apex with margin raised, bearing slight constriction centrally; I, II, III IV with setae on apical and sub-apical anterior and posterior margins, blunt, short or long, curved, anterior apical marginal hairs longer on II, III, IV; I with posterior setae about six; II with about three; III with about three; IV with about four. **Trochanter** simple with stout, blunt setae; I with setae on anterior margin about three, about two posteriorly; II with about two anteriorly, about two posteriorly; III with about two anteriorly, about two posteriorly; IV with two to three anteriorly, two to three posteriorly. **Trochanterellus** about half length of trochanter; setae on dorsal apex long, on dorsal surface small, clubbed, usually one moderate length, clubbed seta distally, occasionally absent.

**Legs** short; light red-brown; anterior slightly more robust than posterior; clubbed setae numerous on posterior legs; claws short. **Femur** twice length of trochanter; setae numerous on anterior legs, less posteriorly; I, II, III, IV with setae on anterior and posterior margin extending towards trochanterellus, long, but IV short. **Tibia** short, length sub-equal with trochanter; setae distinct, clubbed on apical margin. **Metatarsus** about twice length of trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe; setae on ventral margin arising from minor convexities. **Tarsus I** about twice length of trochanter; dorsal lobes prominent, all separated by about width of two or three lobes; three lobes

including a bipartite distal lobe where Haller's organ divides sub-lobes; proximal lobe short, moderately thick, rounded to slightly curved, vertical axis slightly backwards inclining; middle lobe short, thin, steeply angled, slightly curved; distal sub-lobes well separated; proximodistal lobe wide, short, somewhat indistinct; distodistal lobe notably short, wide; Haller's organ large; setae on dorsal margin and ventral margin arising from minor convexities, mostly long, few short, all thick; two setae on apex of proximodistal lobe, short, thick, directed opposite of one another; apical setae arranged somewhat concentrically, more dorsally than ventrally, medium length, generally few about three or four.

Nymphs (1<sup>st</sup> - 2<sup>nd</sup> instar)

Length 1.1 to 1.4 mm, width 0.8 to 1.0 mm.

**Body** generally rectangular-oval, small, widely rounded at anterior and posterior ends, approximately equal curvature; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes absent; body colour pale when alcohol preserved; integument leathery; dorsal mammillae tile-like and flat, tightly spaced; posterior mammillae flat; setae sparse.

**Basis capitulum** short, wide, sub-rectangular, posterior end with angular corners; sclerotized; anterolateral humps absent. **Hypostome** moderate thickness, slender, not expanded at middle length, truncate apically, denticles numerous. **Palp** long, segmented, bearing setae.

**Coxae** elongate triangular, hemiconical; I, II somewhat swollen; apex with margin raised, bearing constriction centrally. **Trochanter** simple with moderate length, distinctly thinner than coxae. **Trochanterellus** about half length of trochanter.

**Legs** moderate length, slender; pale colour when alcohol preserved; anterior slightly more robust and shorter than posterior; claws short. **Femur** twice length of trochanter, thickening distally; setae numerous and long on anterior legs, less posteriorly. **Tibia** short, length sub-equal with trochanter; setae few. **Metatarsus** slightly longer than trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe, reduced. **Tarsus I** about twice length of trochanter; dorsal lobes prominent; three lobes present including a bipartite distal lobe where Haller's organ divides sub-lobes; Haller's organ small.

**Biogeography.** Moderately spread in South Africa (south-western parts) (Fig. 9B). Occurring in regions with less than 300 mm annual precipitation (Fig. S2).

**Hosts.** Tortoise: *Chersina* spp., *Geochelone* spp., *Homopus* spp., *Psammobates* spp., *Testudo* spp.

**Disease relationships.** Unknown.

**Notes.** None.

***Ornithodoros kalahariensis*** Bakkes, de Klerk & Mans **n. sp.**

(Figs 10-16)

*Ornithodoros savignyi* (Audouin, 1927), *pro parte*.

Type depository: GTTM, Holotype: Female

Type locality: Tosca, North-West Province, South Africa (25.878382S 23.957733E)

**Etymology.** From geographic distribution in the Kalahari Desert, adjective.

**Material examined.** 43 females and 24 nymphs from South Africa and Namibia (Table S1).

Sequences deposited in GenBank: GU355919, KJ133579, MF415635, MF415636, MF415637, MF415638, MF415639.

Type material

Holotype ♀ (deposited in GTTM - OP5059), designated here. Label data: 'Tosca, Northern Cape / South Africa / -25.878382, 23.957733 / 26.ix.2011 / Daniel de Klerk' (White rectangle). 'Holotype / *Ornithodoros kalahariensis* Bakkes / D.K. Bakkes 2017 / ex-OP5041a' (Large red rectangle).

Paratype series designated here: OP5056 (3♀, deposited in GTTM). Label data: 'Droëduin, Northern Cape / South Africa / -27.986705 21.426700 / 2010 / S. Human (White rectangle). 'Paratype / *Ornithodoros kalahariensis* Bakkes / D.K. Bakkes 2017' (Large blue rectangle). Sequences deposited in GenBank: KJ133579, MF415638, GU355919.

OP5060 (2♀, deposited in SAMC). Label data: 'Droëduin, Northern Cape / South Africa / -27.986705 21.426700 / 2010 / S. Human' (White rectangle). 'Paratype / *Ornithodoros kalahariensis* Bakkes / D.K. Bakkes 2017 / ex-OP5042a,b' (Large blue rectangle).

OP5061 (2♀, deposited in USNTC). Label data: 'Tosca, Northern Cape / South Africa / -25.878382, 23.957733 / 26.ix.2011 / Daniel de Klerk' (White rectangle). 'Paratype / *Ornithodoros kalahariensis* Bakkes / D.K. Bakkes 2017 / ex-OP5041b,c' (Large blue rectangle).

**Diagnosis.** Females may be distinguished by a combination of (1) eyes present, (2) eyes sub-equal size, (3) eyes convex, (4) posterior eye peripheral folds indistinct, (5) tarsus I proximal lobe tall, thin, curved, (6) tarsus I middle lobe tall, thin, angled forwards, curved, (7) tarsus I proximodistal lobe tall, distinctly separate from distodistal lobe, (8) tarsus I distodistal lobe notably tall, thick, angled nearly upright.

Nymphs may be distinguished by (1) eyes present, (2) eyes sub-equal size, (3) eyes distinctly convex, (4) basis capitulum expanding basally with (5) rounded posterior corners imparting a sub-oval appearance.

### **Description.**

Females

Length 10.0 to 14.0 mm, width 7.1 to 11.0 mm.

**Body** generally rectangular-oval, widely rounded at anterior and posterior ends; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes present, distinctly convex, peripheral folds indistinct, sub-equal in size; body colour dark, grey, slate when alcohol preserved; integument leathery; setae abundant. **Mammillae** generally opaque with indistinct transparent centre, all sub-equal in size, bearing three to five fine and slightly larger punctations apically; anterior mammillae moderate size, moderately separated, circular to oval shape, slightly raised, flat apically; central mammillae moderate size, moderately separated, circular shape, not raised, flat apically; posterior mammillae moderate size, moderately separated, small dome shaped, slightly raised, rounded apically; ventral post-capitular area mammilated. **Intermammillary space** with cell patterned ridges of fine, vermiform elevations, distinct anteriorly, centrally and posteriorly. **Setae** slender, occasionally clubbed; anterior setae long, slender, numerous, about 250, extending posterolaterally almost reaching dorsoventral groove; ventral setae on anterior part of supracoxal fold long, numerous extending to trochanter III; setae on anterolateral hump of supracoxal fold short, slender, about twenty-three; setae on transversolateral part of supracoxal fold distinct, thick, short. **Genital apron** slightly posterior to post-capitular area, flattened; anterior lip thickened centrally, transverse, with about 70 fine, confluent striae; posterior lip, thick, gentle crescent shape, with about 25 coarse striae; post-capitular area wider than long, comprised of transverse folds proximally, medium mammillae centrally, to flat, densely striate area distally at capitulum base; post-genital area with folded integument just posterior to genital apron, thin, indistinct, with numerous mammillae, small.

**Basis capitulum** short, wider basally, smooth, sclerotized, anterolateral humps absent; post-hypostomal setae two thirds length of hypostome, slender; post-palpal setae minute. **Hypostome** slender, not expanded at middle length, truncate apically, denticles numerous, moderately spaced, not projecting laterally; cheliceral apex robust, mobile bicusate tooth moderately large. **Palp** long, segment I with group of dorsolateral setae, short, blunt, peg-like, about three or four; segment II with four dorsomedial setae distally, three long, apical one slightly longer; segment III with two dorsomedial setae distally, long; all posterolateral setae short, blunt, peg-like.

**Coxae** elongate triangular, hemiconical, anterior two-thirds sclerotised, posterior third as finely mammillate syncoxae; I, II somewhat swollen, II especially so; apex with margin raised, bearing constriction centrally; I, II, III with setae on apical and sub-apical posterior margins, blunt, short, on I numerous, on II and III about five, IV with about five sub-apically, minute. **Trochanter** simple with moderate length, distinctly thinner than coxae, bearing blunt setae on anterior margin; I with setae on anterior margin, nine to twelve, none posteriorly; II with eight to ten anteriorly, two to three posteriorly; III with ten to eleven anteriorly, and two to three posteriorly; IV with six to eight anteriorly, and six to seven posteriorly. **Trochanterellus** about half length of trochanter; setae on posterior aspect, moderate length, sub-apical, one to two.

**Legs** moderately long, slender; light brown-testaceous colour when alcohol preserved; anterior more robust and shorter than posterior; claws short. **Femur** twice length of trochanter, thick distally; setae numerous on anterior legs, less posteriorly; I, II, III with setae on anterior and posterior margin, long, extending towards trochanterellus. **Tibia** short, length sub-equal with trochanter; setae distinct, clubbed on apical margin. **Metatarsus** slightly longer than trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe, reduced; setae on ventral margin arising from minor convexities. **Tarsus I** about twice length of trochanter; dorsal lobes prominent; three lobes present including a bipartite distal lobe where Haller's organ divides sub-lobes; proximal and middle lobes separated by about width of two lobes; proximal lobe tall, thin, curved; middle lobe distinct, tall, thin, angled forwards, curved; distal sub-lobes well separated; proximodistal lobe prominent, tall; distodistal lobe distinctly long, thick, angled upwards at near 90 degrees; Haller's organ small; setae on dorsal margin and ventral margin arising from minor convexities, mostly long, thick; two setae on apex of proximodistal lobe, short, thin, directed opposite of one another; apical setae arranged somewhat concentrically, more dorsally than ventrally, short, thin, generally fewer.

Nymphs (1<sup>st</sup> - 2<sup>nd</sup> instar)

Length 1.6 to 2.1 mm, width 1.2 to 1.6 mm.

**Body** generally rectangular-oval, widely rounded at anterior and posterior ends, more so at posterior end; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes present, distinctly convex, sub-equal in size; body colour light reddish-brown when alcohol preserved; integument leathery; setae abundant.

**Basis capitulum** moderate length, slightly expanded basally, posterior end gently rounded to form a sub-oval shape, sclerotized, anterolateral humps absent. **Hypostome** slender, not expanded at middle length, truncate apically, denticles numerous. **Palp** long, segmented, bearing setae.



**Coxae** elongate triangular, hemiconical; I, II somewhat swollen; apex with margin raised, bearing constriction centrally. **Trochanter** simple with moderate length, distinctly thinner than coxae. **Trochanterellus** about half length of trochanter.

**Legs** moderate length, slender; light brown-testaceous colour when alcohol preserved; anterior more robust and shorter than posterior; claws short. **Femur** twice length of trochanter, thickening distally; setae numerous on anterior legs, less posteriorly. **Tibia** short, length subequal with trochanter; setae few. **Metatarsus** slightly longer than trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe, reduced. **Tarsus I** about twice length of trochanter; dorsal lobes prominent; three lobes present including a bipartite distal lobe where Haller's organ divides sub-lobes; Haller's organ small.

**Biogeography.** Moderately spread in Namibia and South Africa (west-central parts, Kalahari Desert), probably southern Botswana (Fig. 9A). Occasionally sympatric with *O. pavimentosus* **nom. rev.** at Nam and Komaggas, Northern Cape. Occurring in regions with less than 500 mm annual precipitation (Fig. S2). Free living, burrows in sandy soil (arenosols).

**Hosts.** Fowl, dog, horse, goat, cattle, pig, rabbit, human.

**Disease relationships.** Implicated in sand tampan toxicoses.

**Notes.** Specimens of *O. savignyi* from sandveld habitats in northern Kruger National Park (de Vos, 1977) may prove to be either this species or a new species.

***Ornithodoros moubata*** (Murray, 1877 sensu Walton, 1962)

(Figs 2-8)

*Argas moubata* Murray, 1877

*Ixodes monbata* Cobbold, 1879

*Ornithodoros savignyi* var. *caecus* Neumann, 1901

*Ornithodoros jubata* Hertwig, 1922

*Ornithodoros duttoni* Mooser, 1958

*Ornithodoros (Ornithodoros) moubata* (Murray):Clifford, Kohls & Sonenshine, 1964

Type depository: NHM, Neotype: Female, see Walton 1962

Type locality: Groot Marico district, North-West Province, South Africa (24.901676S 26.119925E)

**Etymology.** From indigenous name, moubata, noun.

**Material examined.** 12 females (including paratype) and 27 nymphs from South Africa (Table S1). Sequences deposited in GenBank: KJ133572, MF415625, MF415626, MF415627, MF415628. Paratype: OP3089; 1 ♀ (GTTM). Label data: 'O. moubata (Murray) 1877 / paratypes of neotypes / G.A. Walton, 1961 / African hut, Groot Marico / Transvaal / S. Africa / Bred 1957' (White rectangle).

**Diagnosis.** Females may be distinguished by a combination of (1) eyes absent, (2) anterior and posterior dorsal mammillae sub-equal size, (3) posterior mammillae bulbous shape, tightly spaced, (4) genital apron half-moon to gentle crescent shape, (5) intermammillary space with cell patterned ridges distinct, (6) tarsus I proximal lobe tall, thick, rounded to curved, (7) tarsus I middle lobe tall, moderate width, sub-rectangular to slightly curved, (8) tarsus I proximodistal lobe distinct, (9) tarsus I distodistal lobe distinctly short.

Nymphs may be distinguished by (1) eyes absent, (2) anterior and posterior dorsal mammillae sub-equal size, (3) posterior mammillae bulbous, (4) basis capitulum short, wide sub-rectangular, and (5) hypostome moderate thickness.

### **Redescription.**

Females

Length 5.0 to 12.1 mm, width 3.5 to 7.2 mm.

**Body** generally rectangular-oval, widely rounded at anterior and posterior ends; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes absent; body colour dark, grey, slate when alcohol preserved; integument leathery; setae abundant. **Mammillae** generally opaque with indistinct transparent centre, all sub-equal in size, bearing five or six fine punctations apically; anterior mammillae moderate size, moderately separated, circular to oval shape, somewhat raised, flat apically; central mammillae moderate size, moderately separated, circular shape, somewhat raised, rounded apically; posterior mammillae moderate size, tightly spaced, bulbous shape, somewhat raised, rounded apically; ventral post-capitular area mammilated. **Intermammillary space** with cell patterned ridges of fine, vermiform elevations, indistinct anteriorly, distinct centrally, distinct posteriorly. **Setae** stout, blunt, occasionally clubbed; anterior setae long, slender, numerous, about 100, extending posterolaterally almost reaching dorsoventral groove; ventral setae on anterior part of supracoxal fold long, numerous extending to trochanter III; setae on anterolateral hump of supracoxal fold long, slender, about twelve; setae on transversolateral part of supracoxal fold distinct, thick, blunt. **Genital apron** slightly posterior to post-capitular area, flattened; anterior lip thin, transverse, with about 45 fine striae; posterior lip, thick, half-moon to gentle crescent shape, with about 50 fine striae;

post-capitular area wider than long; comprised of finely corrugated area proximally, through large mammillae centrally, to flat, rough area distally at capitulum base; post-genital area with folded integument just posterior to genital apron, thin, with numerous mammillae, sub-oval, large.

**Basis capitulum** short, smooth, flat ventrally, sclerotised; anterolateral humps absent; post-hypostomal setae two thirds length of hypostome, slender; post-palpal setae minute, stout, conical, occasionally clubbed. **Hypostome** moderately expanded sub-apically, truncate apically; denticles numerous, densely packed, projecting laterally; large angular denticles about 40 in files of 3:3; small denticles about 55, scattered; cheliceral apex robust, mobile bicusate tooth moderately large. **Palp** long, segment I with group of dorsal setae, short, blunt, peg-like, about four or five; segment II with four dorsomedial setae distally, three long, sub-central one slightly shorter; segment III with two dorsomedial setae, long; all short setae blunt, peg-like.

**Coxae** elongate triangular, hemiconical, anterior two-thirds sclerotised, posterior third as mammillate syncoxae, mammillae large; I, II somewhat swollen, II especially so; apex with margin raised, bearing constriction centrally; I, II, III, IV with setae on apical and sub-apical posterior margins, blunt, short; apical marginal hairs slightly longer on II, III, IV; on I about six, II about two apically only, III about four, IV about two. **Trochanter** simple with stout, blunt setae; I with setae on anterior margin four to five, two to three posteriorly; II four to five anteriorly, one to two posteriorly; III with three to four anteriorly, two to three posteriorly; IV with three to four anteriorly, one to two posteriorly. **Trochanterellus** about half length of trochanter; setae on posterior aspect, short, sub-apical, one, occasionally two.

**Legs** moderately long; dark red-brown colour when alcohol preserved; anterior slightly more robust than posterior; claws short. **Femur** twice length of trochanter; setae moderately abundant on anterior legs, sparse posteriorly; I, II, III, IV with setae on anterior and posterior margin extending towards trochanterellus, I, II, III long, IV short. **Tibia** short, length sub-equal with trochanter; setae distinct, clubbed on apical margin. **Metatarsus** about twice length of trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe; setae on ventral margin arising from minor convexities. **Tarsus I** about twice length of trochanter; dorsal lobes prominent, all separated by about width of one lobe; three lobes including a bipartite distal lobe where Haller's organ divides sub-lobes; proximal lobe tall, moderately thick, rounded to curved; middle lobe long, moderate width, sub-rectangular to slightly curved; distal sub-lobes well separated; proximodistal lobe prominent; distodistal lobe notably short, wide; Haller's organ large; setae on dorsal margin and ventral margin arising from minor convexities, mostly long, few short; two setae on apex of proximodistal lobe, short, directed opposite of one another; apical setae arranged somewhat concentrically, more dorsally than ventrally, short, generally many.

Nymphs (1<sup>st</sup> - 2<sup>nd</sup> instar)

Length 1.3 to 1.7 mm, width 0.8 to 1.1 mm.

**Body** generally rectangular-oval, widely rounded at anterior and posterior ends, more so at posterior end; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes absent; body colour light reddish-brown when alcohol preserved; integument leathery; dorsal mammillae bulbous, tightly spaced; posterior mammillae bulbous; setae sparse.

**Basis capitulum** short, wide, sub-rectangular, posterior end with angular corners; sclerotized; anterolateral humps absent. **Hypostome** moderate thickness, slender, not expanded at middle length, truncate apically, denticles numerous. **Palp** long, segmented, bearing setae.

**Coxae** elongate triangular, hemiconical; I, II somewhat swollen; apex with margin raised, bearing constriction centrally. **Trochanter** simple with moderate length, distinctly thinner than coxae. **Trochanterellus** about half length of trochanter.

**Legs** moderate length, slender; pale colour when alcohol preserved; anterior slightly more robust and shorter than posterior; claws short. **Femur** twice length of trochanter, thickening distally; setae numerous and long on anterior legs, less posteriorly. **Tibia** short, length sub-equal with trochanter; setae few. **Metatarsus** slightly longer than trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe, reduced. **Tarsus I** about twice length of trochanter; dorsal lobes prominent; three lobes present including a bipartite distal lobe where Haller's organ divides sub-lobes; Haller's organ small.

**Biogeography.** South Africa (north-western parts), Zimbabwe (Fig. 9B). Unconfirmed historical records include Mozambique, Tanzania, Kenya and Botswana (northern parts), however, these may prove to be different species upon further study. Occurring in regions with greater than 400 mm annual precipitation (Fig. S2), and altitude about 1200 m above sea level (Fig. 9B). Often found in warthog burrows and occasionally in human dwellings.

**Hosts.** Warthog: *Phacochoerus africanus*, porcupine: *Hystrix* spp., pangolin: *Smutsia* spp., pig, sheep, goat, dog, human.

**Disease relationships.** Implicated in human relapsing fever.

**Notes.** Specimens from Maun (Botswana), Ovumboland (Namibia), Franceville (Gabon) appear morphologically aberrant and may represent a distinct species.

***Ornithodoros noorsveldensis*** Bakkes, de Klerk & Mans **n. sp.**

(Figs 10-16)

*Ornithodoros savignyi* (Audouin, 1927), *pro parte*.

Type depository: GTTM, Holotype: Female

Type locality: Allemanskraal, Steytlerville, Eastern Cape, South Africa (33.248351S 24.288374E)

**Etymology.** From habitat affinity to noorsveld, adjective.

**Material examined.** 17 females and 23 nymphs from South Africa (Table S1). Sequences deposited in GenBank: MF415640, MF415641, MF415642, MF415643, MF415644.

Type material

Holotype ♀ (deposited in GTTM - OP5062), designated here. Label data: 'Allemanskraal, Steytlerville / Eastern Cape, South Africa / -33.248351, 24.288374 / 01.i.2011 / Antoine Hayward' (White rectangle). 'Holotype / Ornithodoros / noorsveldensis Bakkes / D.K. Bakkes 2017 / ex-OP5040a' (Large red rectangle).

Paratype series designated here: OP5057 (3♀, deposited in GTTM). Label data: 'Allemanskraal, Steytlerville / Eastern Cape, South Africa / -33.248351, 24.288374 / ??i.2011 / Antoine Hayward' (White rectangle). 'Paratype / Ornithodoros / noorsveldensis Bakkes / D.K. Bakkes 2017' (Large blue rectangle). Sequences deposited in GenBank: MF415640, MF415641, MF415642.

OP5063 (1♀, deposited in SAMC). Label data: 'Allemanskraal, Steytlerville / Eastern Cape, South Africa / -33.248351, 24.288374 / ??i.2011 / Antoine Hayward' (White rectangle). 'Paratype / Ornithodoros / noorsveldensis Bakkes / D.K. Bakkes 2017 / ex-OP5040b' (Large blue rectangle).

OP5064 (1♀, deposited in USNTC). Label data: 'Allemanskraal, Steytlerville / Eastern Cape, South Africa / -33.248351, 24.288374 / ??i.2011 / Antoine Hayward' (White rectangle). 'Paratype / Ornithodoros / noorsveldensis Bakkes / D.K. Bakkes 2017 / ex-OP5040c' (Large blue rectangle).

**Diagnosis.** Females may be distinguished by a combination of (1) eyes present, (2) eyes sub-equal size, (3) eyes flat to slightly convex, (4) posterior eye peripheral folds distinct, (5) basis capitulum short, (6) tarsus I proximal lobe short, thick, angled backwards, (6) tarsus I middle lobe short, moderate width, curved, (7) tarsus I proximodistal lobe short, separate from distodistal lobe, (8) tarsus I distodistal lobe short, thick, angled nearly upright.

Nymphs may be distinguished by (1) eyes present, (2) eyes sub-equal size, (3) eyes distinctly convex, (4) basis capitulum not expanding basally with (5) sharply rounded posterior corners imparting a sub-rectangular appearance.

**Description.**

Females

Length 7.5 to 10.0 mm, width 5.3 to 7.1 mm.

**Body** generally rectangular-oval, widely rounded at anterior and posterior ends; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes present, generally flat or slightly convex, peripheral folds distinct, sub-equal in size; body colour dark, grey, slate when alcohol preserved; integument leathery; setae abundant. **Mammillae** generally opaque with indistinct transparent centre, all sub-equal in size, bearing two to three fine and slightly larger punctations apically; anterior mammillae moderate size, moderately separated, circular to oval shape, slightly raised, flat apically; central mammillae moderate size, moderately separated, circular shape, slightly raised, flat apically; posterior mammillae moderate size, moderately separated, small dome shaped, slightly raised, rounded apically; ventral post-capitular area mammillated. **Intermammillary space** with cell patterned ridges of fine, vermiform elevations, distinct anteriorly, centrally and posteriorly. **Setae** slender, stout, occasionally clubbed; anterior setae long, slender, numerous, about 100, extending posterolaterally almost reaching dorsoventral groove; ventral setae on anterior part of supracoxal fold long, numerous extending to trochanter III; setae on anterolateral hump of supracoxal fold short, slender, about twenty-three; setae on transversolateral part of supracoxal fold distinct, thick, short. **Genital apron** slightly posterior to post-capitular area, flattened; anterior lip thin, transverse, with about 20 coarse, confluent striae; posterior lip, thick, half-moon shape, with about 20 coarse striae; post-capitular area wider than long, comprised of transverse folds proximally, large mammillae centrally and distally to capitulum base; post-genital area with folded integument just posterior to genital apron, thin, with numerous mammillae, medium sized.

**Basis capitulum** distinctly short, smooth, sclerotised; anterolateral humps present, small; post-hypostomal setae one third length of hypostome, stout; post-palpal setae minute. **Hypostome** slender, expanded sub-apically, truncate apically; denticles numerous, moderately spaced, not projecting laterally; cheliceral apex robust, mobile bicusate tooth moderately large. **Palp** long, segment I with group of dorsal setae, short, blunt, peg-like, about two to three; segment II with three dorsomedial setae distally, three long, apical one slightly shorter; segment III with one dorsomedial seta distally, long; all dorsolateral setae moderate length, blunt, peg-like.

**Coxae** elongate triangular, hemiconical, anterior two-thirds sclerotised, posterior third as finely mammillate syncoxae; I, II somewhat swollen, II especially so; apex with margin raised, bearing constriction centrally; I, II, III, IV with setae on posterior margins, blunt, short; on I about eight apically and sub-apically, II about three apically, III about five apically, IV about

four apically and sub-apically. **Trochanter** simple with moderate length, bearing blunt setae; I with setae on anterior margin, six to seven, and one to two posteriorly; II with four to five anteriorly, and two to three posteriorly, minute; III with seven to eight anteriorly, and six to seven posteriorly; IV with setae four to five anterodistally, and eight to nine posteriorly. **Trochanterellus** about half length of trochanter; setae on posterior aspect, moderate length, sub-apical, two.

**Legs** generally robust, moderately long; dark brown-testaceous colour when alcohol preserved; anterior more robust and shorter than posterior; claws short. **Femur** twice length of trochanter, thickened distally; setae numerous on anterior legs, less posteriorly; I, II, III, IV with setae on anterior and posterior margin, long, extending towards trochanterellus. **Tibia** short, length sub-equal with trochanter; setae distinct, slender on apical margin. **Metatarsus** sub-equal length with trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe, slightly reduced; setae on ventral margin arising from minor convexities. **Tarsus I** about twice length of trochanter; dorsal lobes prominent; three lobes including a bipartite distal lobe where Haller's organ divides sub-lobes; proximal and middle lobes separated by about width of one lobe; proximal lobe short, thick, sub-rectangular, often angled backwards; middle lobe short, moderately thin, gently curved; distal sub-lobes well separated; proximodistal lobe prominent, short; distodistal lobe short, thick, angled upwards at near 90 degrees; Haller's organ large; setae on dorsal margin and ventral margin arising from minor convexities, mostly long, thick; two setae on apex of proximodistal lobe, short, thin, directed opposite of one another; apical setae arranged somewhat concentrically, more ventrally than dorsally, short, thick, generally fewer.

Nymphs (1<sup>st</sup> - 2<sup>nd</sup> instar)

Length 1.6 to 2.0 mm, width 1.3 to 1.6 mm.

**Body** generally rectangular-oval, widely rounded at anterior and posterior ends, more so at posterior end; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes present, distinctly convex, sub-equal in size; body colour light reddish-brown when alcohol preserved; integument leathery; setae abundant.

**Basis capitulum** short, not expanded basally, posterior end sharply rounded to form a sub-rectangular shape, sclerotized, anterolateral humps absent. **Hypostome** slender, not expanded at middle length, truncate apically, denticles numerous. **Palp** long, segmented, bearing setae.

**Coxae** elongate triangular, hemiconical; I, II somewhat swollen; apex with margin raised, bearing constriction centrally. **Trochanter** simple with moderate length, distinctly thinner than coxae. **Trochanterellus** about half length of trochanter.

**Legs** moderate length, slender; light brown-testaceous colour when alcohol preserved; anterior more robust and shorter than posterior; claws short. **Femur** twice length of trochanter, thickening distally; setae numerous on anterior legs, less posteriorly. **Tibia** short, length sub-equal with trochanter; setae few. **Metatarsus** slightly longer than trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe, reduced. **Tarsus I** about twice length of trochanter; dorsal lobes prominent; three lobes present including a bipartite distal lobe where Haller's organ divides sub-lobes; Haller's organ small.

**Biogeography.** Restricted to South-Africa (south-eastern parts) (Fig. 9A). Only known from a single locality, Allemanskraal, Steytlerville, Eastern Cape. Occurring in a region with about 400 mm annual precipitation (Fig. S2). Free living, burrows in clay rich soils (luvisols) (Fig. 9A).

**Hosts.** Kudu: *Tragelaphus strepsiceros* and other wildlife. Other hosts likely include fowl, dog, horse, goat, rabbit, sheep, cattle, pig, human.

**Disease relationships.** May be implicated in sand tampan toxicoses.

**Notes.** None.

***Ornithodoros pavimento*sus** Bakkes, de Klerk & Mans **nom. rev.**  
(Figs 10-16)

*Ornithodoros pavimento*sus Neumann, 1901, *nomen nudem*

*Ornithodoros savignyi pavimento*sus Neumann: Howard, 1908

*Argas pavimento*sus Bedford, 1932

*Ornithodoros savignyi* (Audouin): Theiler & Hoogstraal, 1955, *pro parte*

Type depository: GTTM, Holotype: Female

Type locality: Komaggas, Northern Cape, South Africa (29.790000S 17.428484E)

**Etymology.** Name revived from Neumann (1901), originally given for cobblestone pavement-like appearance of dorsal mammillae, adjective.

**Material examined.** 36 females and 26 nymphs from South Africa and Namibia (Table S1). Sequences deposited in GenBank: KJ133576, MF415629, MF415630, MF415631, MF415632, MF415633, MF415634.

Type material



Holotype ♀ (deposited in GTTM - OP5065), designated here. Label data: 'Komaggas, Northern Cape / South Africa / -29.790000, 17.428484 / 02.ii.2011 / Daniel de Klerk' (White rectangle). 'Holotype / Ornithodoros / pavimentosus Bakkes / D.K. Bakkes 2017 / ex-OP5039c' (Large red rectangle).

Paratype series designated here: OP5055 (3♀, deposited in GTTM). Label data: 'Nam, Northern Cape / South Africa / -29.451949, 19.201923 / 04.ii.2011 / Ben Mans' (White rectangle). 'Paratype / Ornithodoros / pavimentosus Bakkes / D.K. Bakkes 2017' (Large blue rectangle). Sequences deposited in GenBank: MF415629, MF415630, MF415631.

OP5058 (1♀, deposited in USNTC). Label data: 'Komaggas, Northern Cape / South Africa / -29.790000, 17.428484 / 02.ii.2011 / Daniel de Klerk' (White rectangle). 'Paratype / Ornithodoros / pavimentosus Bakkes / D.K. Bakkes 2017' (Large blue rectangle).

OP5066 (1♀, deposited in SAMC). Label data: 'Komaggas, Northern Cape / South Africa / -29.790000, 17.428484 / 02.ii.2011 / Daniel de Klerk' (White rectangle). 'Paratype / Ornithodoros / pavimentosus Bakkes / D.K. Bakkes 2017 / ex-OP5039b' (Large blue rectangle).

**Diagnosis.** Females may be distinguished by a combination of (1) eyes present, (2) eyes sub-equal size, (3) eyes flat, (4) posterior eye peripheral folds distinct, (5) tarsus I proximal lobe tall, thick, expanded apically, (6) tarsus I middle lobe tall, thick, expanded apically, (7) tarsus I proximodistal lobe tall, distinctly separate from distodistal lobe, (8) tarsus I distodistal lobe tall, thick, angled slightly forward (downward).

Nymphs may be distinguished by (1) eyes present, (2) posterior eye smaller than anterior, (3) eyes flat, (4) basis capitulum distinctly expanding basally with (5) rounded posterior corners imparting a sub-triangular appearance.

### **Description.**

Females

Length 10.5 to 14.2 mm, width 7.1 to 10.2 mm.

**Body** generally rectangular-oval, widely rounded at anterior and posterior ends, anterior expanded as a mildly pronounced bump; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes present, generally flat, slightly convex, peripheral folds distinct imparting a 'sleepy' appearance, sub-equal in size; body colour light brown, fawn when alcohol preserved; integument leathery; setae abundant. **Mammillae** generally opaque with indistinct transparent centre, all sub-equal in size, bearing two to four fine and slightly larger punctations apically; anterior mammillae moderate size, packed tightly, circular to oval shape, not raised, flat

apically; central mammillae moderate size, packed tightly, circular shape, not raised, flat apically; posterior mammillae moderate size, packed tightly, small dome shaped, slightly raised, rounded apically; ventral post-capitular area mammillated. **Intermammillary space** with cell patterned ridges of fine, vermiform elevations, distinct anteriorly, centrally and posteriorly. **Setae** slender, occasionally clubbed; anterior setae long, slender, numerous, about 150, extending posterolaterally almost reaching dorsoventral groove; ventral setae on anterior part of supracoxal fold long, numerous extending to trochanter III; setae on anterolateral hump of supracoxal fold short, slender, about twenty-three; setae on transversolateral part of supracoxal fold distinct, thick, short. **Genital apron** slightly posterior to post-capitular area, flattened; anterior lip thin, gradually thickening centrally, transverse, with about 60 fine, confluent striae; posterior lip, thin, half-moon, with about 30 fine striae; post-capitular area wider than long, comprised of transverse folds proximally, medium mammillae centrally, to flat, coarsely striate area distally at capitulum base; post-genital area with folded integument just posterior to genital apron, thin, indistinct, with numerous mammillae, small.

**Basis capitulum** short, wider basally, slightly rounded, smooth, sclerotised; anterolateral humps absent; post-hypostomal setae one third length of hypostome, stout; post-palpal setae minute. **Hypostome** robust, not expanded at middle length, truncate apically; denticles numerous, moderately spaced, some projecting laterally; cheliceral apex robust, mobile bicusate tooth moderately large. **Palp** long, segment I with group of dorsolateral setae, short, blunt, peg-like, about two; segment II with four dorsomedial setae distally, three long, apical one slightly longer; segment III with two dorsomedial setae distally, long; all dorsolateral setae short, blunt, peg-like.

**Coxae** elongate triangular, hemiconical, anterior two-thirds sclerotised, posterior third as finely mammillate syncoxae; I, II somewhat swollen, II especially so, approaching sub-rectangular shape; apex with margin raised, bearing constriction centrally; I, II, III, IV with setae on apical and sub-apical posterior margins, blunt, short; on I numerous, short; II about seven; III about six; IV about four. **Trochanter** simple with moderate length, short, slender setae; I with setae on anterior margin, six to seven, two to three posteriorly; II with four to five anteriorly, one to two posteriorly, short; III with four to five anteriorly, one to two posteriorly; IV with five to six anteriorly, three to four posteriorly, short. **Trochanterellus** about half length of trochanter; setae on posterior aspect, moderate length, slender, sub-apical, one to two.

**Legs** moderately long, robust; light brown-yellow fawn colour when alcohol preserved; anterior more robust and shorter than posterior; claws short. **Femur** twice length of trochanter, thickened distally; setae numerous on anterior legs, less posteriorly; I, II, III, IV with setae on anterior and posterior margin, long, extending towards trochanterellus. **Tibia** short, length sub-equal with trochanter; setae distinct, slender on apical margin. **Metatarsus** slightly longer than

trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe, reduced; setae on ventral margin arising from minor convexities. **Tarsus I** about twice length of trochanter; dorsal lobes prominent; three lobes including a bipartite distal lobe where Haller's organ divides sub-lobes; proximal and middle lobes separated by about width of one lobe; proximal lobe tall, thick, expanding apically; middle lobe tall, thick, expanding apically; distal sub-lobes well separated; proximodistal lobe prominent, tall; distodistal lobe long, thick, angled upwards at near 70 degrees; Haller's organ small; setae on dorsal margin and ventral margin arising from minor convexities, mostly long, thick; two setae on apex of proximodistal lobe, short, thin, directed opposite of one another; apical setae arranged somewhat concentrically, more dorsally than ventrally, short, thin, generally fewer.

Nymphs (1<sup>st</sup> - 2<sup>nd</sup> instar)

Length 1.5 to 1.8 mm, width 1.2 to 1.5 mm.

**Body** generally rectangular-oval, widely rounded at anterior and posterior ends, approximately equal curvature; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes present, approximately flat, posterior eye smaller than anterior; body colour light reddish-brown when alcohol preserved; integument leathery; setae abundant.

**Basis capitulum** long, distinctly expanded basally, posterior end gently rounded to form a sub-triangular shape, sclerotized, anterolateral humps absent. **Hypostome** slender, not expanded at middle length, truncate apically, denticles numerous. **Palp** long, segmented, bearing setae.

**Coxae** elongate triangular, hemiconical; I, II somewhat swollen; apex with margin raised, bearing constriction centrally. **Trochanter** simple with moderate length, distinctly thinner than coxae. **Trochanterellus** about half length of trochanter.

**Legs** long, distinctly slender; light brown-testaceous colour when alcohol preserved; anterior slightly more robust and shorter than posterior; claws short. **Femur** twice length of trochanter, thickening distally; setae numerous on anterior legs, less posteriorly. **Tibia** short, length sub-equal with trochanter; setae few. **Metatarsus** slightly longer than trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe, reduced. **Tarsus I** about twice length of trochanter; dorsal lobes prominent; three lobes present including a bipartite distal lobe where Haller's organ divides sub-lobes; Haller's organ small.

**Biogeography.** Moderately spread in South Africa (western parts) and Namibia (south and central parts) (Fig. 9A). Sympatric with *O. kalahariensis* n. sp. at Nam and Komaggas, Northern Cape. Occurring in regions with less than 500 mm annual precipitation (Fig. S2). Free living, burrows in gravelly soil (leptosols) (Fig. 9A).

**Hosts.** Cattle, human. Other hosts may include fowl, dog, rabbit, horse, goat, pig.

**Disease relationships.** May be implicated in sand tampan toxicoses.

**Notes.** None.

***Ornithodoros phacochoerus*** Bakkes, de Klerk & Mans **n. sp.**

(Figs 2-8)

*Ornithodoros moubata* (Murray, 1877), *pro parte*.

Type depository: GTTM, Holotype: Female

Type locality: Crocodile Bridge, Kruger National Park, Limpopo Province, South Africa (25.350058S 31.886691E).

**Etymology.** From parasitic affinity to warthogs of the genus *Phacochoerus*, adjective.

**Material examined.** 39 females and 11 nymphs from South Africa (Table S1). Sequences deposited in GenBank: KJ133574, KJ133573, KJ133575, MF415620, MF415621, MF415622, MF415624, MF415623.

Type material

Holotype ♀ (deposited in GTTM - OP5067), designated here. Label data: 'Crocodile Bridge, KNP / Limpopo, South Africa / -25.350058, 31.886691 / 20.ix.2010 / At Dekker' (White rectangle). 'Holotype / *Ornithodoros* / *phacochoerus* Bakkes / D.K. Bakkes 2017 / ex-OP5048f' (Large red rectangle).

Paratype series designated here: OP5053 (3♀, deposited in GTTM). Label data: 'Crocodile Bridge, KNP / Limpopo, South Africa / -25.350058, 31.886691 / 20.ix.2010 / At Dekker' (White rectangle). 'Paratype / *Ornithodoros* / *phacochoerus* Bakkes / D.K. Bakkes 2017' (Large blue rectangle). Sequences deposited in GenBank: MF415620, MF415621, MF415622.

OP5068 (1♀, deposited in SAMC). Label data: 'Crocodile Bridge, KNP / Limpopo, South Africa / -25.350058, 31.886691 / 20.ix.2010 / At Dekker' (White rectangle). 'Paratype / *Ornithodoros* / *phacochoerus* Bakkes / D.K. Bakkes 2017 / ex-OP5048b' (Large blue rectangle).

OP5069 (1♀, deposited in USNTC). Label data: 'Crocodile Bridge, KNP / Limpopo, South Africa / -25.350058, 31.886691 / 20.ix.2010 / At Dekker' (White rectangle). 'Paratype / *Ornithodoros* / *phacochoerus* Bakkes / D.K. Bakkes 2017 / ex-OP5048c' (Large blue rectangle).

**Diagnosis.** Females may be distinguished by a combination of (1) eyes absent, (2) anterior and posterior dorsal mammillae sub-equal size, (3) posterior mammillae conical shape, (4) genital apron thin transverse slit to gentle crescent shape, (5) intermammillary space with cell patterned ridges indistinct, (6) tarsus I proximal lobe short, thin, rounded, (7) tarsus I middle lobe short, thin, sub-rectangular to slightly curved, (8) tarsus I proximodistal lobe nearly flat, (9) tarsus I distodistal lobe short (but longer than *O. moubata*).

Nymphs may be distinguished by (1) eyes absent, (2) anterior and posterior dorsal mammillae sub-equal size, (3) posterior mammillae acutely conical, (4) basis capitulum short, wide sub-rectangular, and (5) hypostome moderate thickness.

### **Description.**

Females

Length 6.1 to 7.9 mm, width 3.8 to 6.5 mm.

**Body** generally rectangular-oval, widely rounded at anterior and posterior ends; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes absent; body colour dark, reddish, slate when alcohol preserved; integument leathery; setae abundant. **Mammillae** small, generally opaque with indistinct transparent centre, widely spaced, all sub-equal in size, bearing one or two punctations apically; anterior mammillae small to medium, widely separated, circular to oval shape, not raised, flat apically; central mammillae small size, widely separated, circular shape, not raised, flat apically; posterior mammillae moderate size, moderately spaced, conical shape marginally, raised, rounded apically; ventral post-capitular area mammilated. **Intermammillary space** with cell patterned ridges indistinct or absent. **Setae** stout, thick, blunt, occasionally clubbed; anterior setae moderately long, stout, about 50, extending posterolaterally almost reaching dorsoventral groove; ventral setae on anterior part of supracoxal fold long, numerous extending to trochanter III; setae on anterolateral hump of supracoxal fold long, slender, about twelve; setae on transversolateral part of supracoxal fold distinct, thick, blunt. **Genital apron** slightly posterior to post-capitular area, flattened; anterior lip thick, transverse, with about 60 fine convoluted striae; posterior lip thin transverse slit-like shape to gentle crescent shape, with about 45 fine striae; post-capitular area slightly longer than wide, comprised of mildly rough area proximally, through small, widely spaced mammillae centrally, to confluent striate area, flat, distally at capitulum base; post-genital area with folded integument just posterior to genital apron, thick, with numerous mammillae, sub-oval, small, widely spaced.

**Basis capitulum** short, somewhat undulating but generally flat ventrally, sclerotised; anterolateral humps absent; post-hypostomal setae two thirds length of hypostome, slender; post-palpal setae minute, stout, conical occasionally clubbed. **Hypostome** uniform width, not expanded, truncate apically; denticles numerous, densely packed, projecting laterally; large, angular denticles about 40 in files of 3:3, small denticles about 55, scattered; cheliceral apex robust, mobile bicuspedate tooth moderately large. **Palp** long, segment I with group of dorsal setae, short, blunt, peg-like, about six or seven; segment II with four dorsomedial setae, three long, sub-distal one distinctly shorter; segment III with two dorsomedial setae distally, long; all dorsolateral setae short, blunt, peg-like.

**Coxae** elongate triangular, hemiconical; anterior two-thirds sclerotised, posterior third as mammillate syncoxa, mammillae small, widely spaced; I, II somewhat swollen, II not especially so; apex with margin raised, bearing constriction centrally; I, II, III, IV with minimal setae on apical anterior and posterior margins, blunt, short, sub-apical setae lacking; I with anterior apical setae about one, about two posteriorly; on II about three anteriorly, about two posteriorly; on III about two anteriorly, about two posteriorly; on IV none anteriorly, about one posteriorly. **Trochanter** simple with stout, blunt setae; I with setae on anterior margin, three to four, about one posteriorly; II with three to four anteriorly, one posteriorly; III with three to four anteriorly, one to two posteriorly; IV with three to four anteriorly, one to two posteriorly. **Trochanterellus** about half length of trochanter; setae on posterior aspect, short, sub-apical, one, occasionally distinctly short.

**Legs** moderately long, slender; dark red-brown colour when alcohol preserved; anterior slightly more robust and shorter than posterior; claws short. **Femur** twice length of trochanter; setae present on anterior legs, sparse, less abundant posteriorly; I, II, III, IV with setae on anterior and posterior margin extending towards trochanterellus, widely spaced, long, IV shorter and almost lacking setae posteriorly. **Tibia** short, length sub-equal with trochanter; setae distinct, clubbed on apical margin. **Metatarsus** about twice length of trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe; setae on ventral margin arising from minor convexities. **Tarsus I** about twice length of trochanter; dorsal lobes prominent, all separated by about width of one lobe; three lobes including a bipartite distal lobe where Haller's organ divides sub-lobes; proximal lobe short, moderately thin, sub-rectangular to rounded; middle lobe thin, short, sub-rectangular to slightly curved; distal sub-lobes well separated; proximodistal lobe moderately prominent, short; distodistal lobe moderate length, wide; Haller's organ large; setae on dorsal margin and ventral margin arising from minor convexities, mostly long, few short; two setae on apex of proximodistal lobe, moderately long, thick, directed opposite of one another; apical setae arranged somewhat concentrically, more dorsally than ventrally, short, thin, generally few.

Nymphs (1<sup>st</sup> - 2<sup>nd</sup> instar)

Length 1.2 to 1.4 mm, width 1.0 to 1.2 mm.

**Body** generally rectangular-oval, widely rounded at anterior and posterior ends, more so at posterior end; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes absent; body colour reddish-brown when alcohol preserved; integument leathery; dorsal mammillae flat, tightly spaced; posterior mammillae conical, acute; setae sparse.

**Basis capitulum** short, wide, sub-rectangular, posterior end with angular corners; sclerotized; anterolateral humps absent. **Hypostome** moderate thickness, slender, not expanded at middle length, truncate apically, denticles numerous. **Palp** long, segmented, bearing setae.

**Coxae** elongate triangular, hemiconical; I, II somewhat swollen; apex with margin raised, bearing constriction centrally. **Trochanter** simple with moderate length, distinctly thinner than coxae. **Trochanterellus** about half length of trochanter.

**Legs** moderate length, slender; pale colour when alcohol preserved; anterior slightly more robust and shorter than posterior; claws short. **Femur** twice length of trochanter, thickening distally; setae numerous and long on anterior legs, less posteriorly. **Tibia** short, length sub-equal with trochanter; setae few. **Metatarsus** slightly longer than trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe, reduced. **Tarsus I** about twice length of trochanter; dorsal lobes prominent; three lobes present including a bipartite distal lobe where Haller's organ divides sub-lobes; Haller's organ small.

**Biogeography.** Moderately spread in South Africa (eastern parts), Swaziland and Mozambique (Fig. 9B). Occurring in regions with greater than 400 mm annual precipitation (Fig. S2) and altitude below 800 m above sea level (Fig. 9B). Often found in warthog burrows.

**Hosts.** Warthog: *Phacochoerus africanus*. Other hosts likely include pig, sheep, goat, dog, human.

**Disease relationships.** May be implicated in transmission of African swine fever.

**Notes.** None.

***Ornithodoros porcinus*** Walton, 1962

(Figs 2-8)

*Ornithodoros porcinus* Walton, 1962

*Ornithodoros (Ornithodoros) porcinus* Walton:Clifford, Kohls & Sonenshine, 1964

*Ornithodoros moubata porcinus* Van der Merwe, 1967

Type depository: NHM, Holotype: Female

Type locality: Dodoma, Tanzania (6.170338S 35.769626E)

**Etymology.** Not originally stated, however, probably from Latin *porcin* -us, -a, -um, adjective (swine, pig, hog) referring to parasitic affinity to domestic pigs.

**Material examined.** 22 females (including paratypes) and 12 nymphs from Tanzania, Rwanda/Burundi, DRC, Malawi, Kenya and Uganda (Table S1). Sequences deposited in GenBank: KJ133595. Subspecies not treated here, refer to Walton (1962). Paratype: OP3087i; 1 ♀ (GTTM). Label data: 'Holo-allotype' (White rectangle). 'O. porcinus domesticus / subsp. n. Walton, 1961 / South mixed feeding race / African hut M'Noho / Mwitikira, Dodoma / Taganyika Bred 1957' (White rectangle). OP3087ii; 1 ♀ (GTTM). Label data: 'O. porcinus domesticus / subsp. n. Walton, 1961 / African hut, Kangeta / Meru, Kenya / Kenya / Bred 1956' (White rectangle). OP3087iii; 1 ♀ (GTTM). Label data: 'O. porcinus domesticus / subsp. n. Walton, 1961 / South E. African Race / Host Fowl, African hut / Tiwi, Digo, Kenya / Bred 1957 G.A. Walton / Paratypes' (White rectangle). OP3088ia; 1 ♀ (GTTM). Label data: 'O. porcinus porcinus / subsp. n. / G.A Walton, 1961 / Warthog burrow / Mwitikira, Dodoma / Taganyika XI.1956 / paratypes' (White rectangle).

**Diagnosis.** Females may be distinguished by a combination of (1) eyes absent, (2) posterior dorsal mammillae larger than anterior, (3) genital apron sub-triangular to gentle crescent shape, (4) post-capitular area with mammillae, (5) intermammillary space with cell patterned ridges distinct, (6) tarsus I proximal lobe tall, thick, rounded, (7) tarsus I middle lobe tall, thick, sub-rectangular to slightly curved, (8) tarsus I proximodistal lobe distinct, (9) tarsus I distodistal lobe short.

Nymphs may be distinguished by (1) eyes absent, (2) posterior dorsal mammillae larger than anterior, (3) basis capitulum notably wide but short, sub-rectangular, and (4) hypostome moderate thickness.

### Redescription.

Females

Length 6.8 to 16.0 mm, width 4.4 to 10.5 mm.

**Body** generally rectangular-oval, widely rounded at anterior and posterior ends; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes absent; body colour brown to fawn when alcohol



preserved; integument leathery; setae abundant. **Mammillae** generally opaque with distinct transparent centre, generally smooth, bearing numerous microscopic punctations apically, connected by minute pore canals to internal cavity, tall, narrow; anterior mammillae moderate size, moderately separated, circular to oval shape, raised slightly, gently rounded apically; central mammillae moderate size, moderately separated, sub-circular shape, somewhat raised, gently rounded apically; posterior mammillae large in size, tightly packed, obtusely conical shape, somewhat raised, domed apically; ventral post-capitular area mammilated, mammillae crenated. **Intermammillary space** with cell patterned ridges of fine, vermiform elevations, less distinct anteriorly, distinct centrally, distinct posteriorly. **Setae** stout, blunt, tapering, usually not clubbed; anterior setae short, tapering, about 45, extending posterolaterally almost reaching dorsoventral groove; ventral setae on anterior part of supracoxal fold moderate length, generally sparse, extending to trochanter II; setae on anterolateral hump of supracoxal fold short, tapering, sparse, about eight, extending towards trochanter III; setae on transversolateral part of supracoxal fold indistinct, short, blunt. **Genital apron** slightly posterior to post-capitular area, flattened; anterior lip thick, transverse, with about 35 fine striae; posterior lip, thick, sub-triangular to crescent shape, bounded laterally by oblique posterolateral folds, with about 45 fine striae; post-capitular area longer than wide, comprised of smooth area proximally, through medium and crenated mammillae centrally, to transversely striate area distally at capitulum base; post-genital area with folded integument just posterior to genital apron, thin, about three folds, with numerous mammillae, sub-circular, medium, crenated.

**Basis capitulum** distinctly short, flat ventrally, sclerotized with rough texture; anterolateral humps present; post-hypostomal setae half length of hypostome, stout; post-palpal setae minute, conical. **Hypostome** moderately expanded sub-apically, truncate apically; denticles conical, numerous, densely packed, not projecting laterally; large denticles about 30, less dense, small denticles about 65, in files of 3:3; cheliceral apex robust, mobile bicusate tooth large, occasionally with up to five cusps. **Palp** long, segment I with group of dorsolateral setae, short, thin, minute, about four; segment II with four dorsomedial setae distally, long thick, sub-distal distinctly short; segment III with two dorsomedial setae distally, long, thin; all dorsolateral setae short, blunt, peg-like.

**Coxae** elongate triangular, hemiconical, anterior two-thirds sclerotised, posterior third as mammillate syncoxa, mammillae medium; I, II somewhat swollen, II especially so; apex with margin raised, bearing constriction centrally; I with setae on apical posterior margin, short, about three, none anteriorly, none sub-apically; II, III with setae on apical anterior margin, about two, and posterior margin, about three, short, none sub-apically; on IV about two anteriorly, about two posteriorly, none sub-apically. **Trochanter** simple with stout, blunt setae; I with setae on anterior margin two to three, none posteriorly; II with two to three anteriorly,

one to two posteriorly, short; III with one to two anteriorly, one to two posteriorly, short; IV with two to three anteriorly, one posteriorly. **Trochanterellus** about half length of trochanter; setae on posterior aspect, short, sub-apical, one, occasionally two.

**Legs** moderately long, robust; fawn to brown colour when alcohol preserved; anterior slightly more robust than posterior; claws short. **Femur** twice length of trochanter; setae moderately numerous on anterior legs, sparse posteriorly; I, II, III, IV with setae on anterior and posterior margin extending towards trochanterellus, long, but shorter on IV. **Tibia** short, length sub-equal with trochanter; setae distinct, curved on apical margin. **Metatarsus** about twice length of trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe, small; setae on ventral margin arising from minor convexities. **Tarsus I** about two and a half times length of trochanter; dorsal lobes prominent, all separated by about width of two lobes; three lobes including a bipartite distal lobe where Haller's organ divides sub-lobes; proximal lobe tall, moderately thick, sub-rectangular to rounded; middle lobe tall, moderately thick, sub-rectangular to slightly curved; distal sub-lobes well separated; proximodistal lobe prominent, wide; distodistal lobe short, wide; Haller's organ moderate size; setae on dorsal margin and ventral margin arising from minor convexities, mostly long, few short; two setae on apex of proximodistal lobe, short, directed opposite of one another; apical setae arranged somewhat concentrically, less dorsally than ventrally, short, generally few.

Nymphs (1<sup>st</sup> - 2<sup>nd</sup> instar)

Length 1.6 to 1.8 mm, width 1.2 to 1.5 mm.

**Body** generally rectangular-oval, widely rounded at anterior and posterior ends, approximately equal curvature; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes absent; body colour brownish when alcohol preserved; integument leathery; dorsal mammillae domed, moderately spaced; posterior mammillae larger than anterior, domed; setae sparse.

**Basis capitulum** short, notably wide, sub-rectangular, posterior end with angular corners; sclerotized; anterolateral humps present. **Hypostome** moderate thickness, slender, not expanded at middle length, truncate apically, denticles numerous. **Palp** long, segmented, bearing setae.

**Coxae** elongate triangular, hemiconical; I, II somewhat swollen; apex with margin raised, bearing constriction centrally. **Trochanter** simple with moderate length, distinctly thinner than coxae. **Trochanterellus** about half length of trochanter.

**Legs** moderate length, slender; pale colour when alcohol preserved; anterior slightly more robust and shorter than posterior; claws short. **Femur** twice length of trochanter, thickening distally; setae numerous and long on anterior legs, less posteriorly. **Tibia** short, length sub-equal with trochanter; setae few. **Metatarsus** slightly longer than trochanter; dorsal

lobes prominent, dorsoapical margin developed as additional lobe, reduced. **Tarsus I** about twice length of trochanter; dorsal lobes prominent; three lobes present including a bipartite distal lobe where Haller's organ divides sub-lobes; Haller's organ small.

**Biogeography.** Moderately spread in Tanzania, Kenya, Rwanda and Burundi (Fig. 9B). Occurring in regions with greater than 600 mm annual precipitation (Fig. S2). Often found in warthog burrows and human dwellings.

**Hosts.** Warthog: *Phacochoerus africanus*, elephant: *Loxodonta africana*, human, pig.

**Disease relationships.** Implicated in transmission of African swine fever.

**Notes.** Walton (1962) records a biologically unique strain of this species in South Africa ('Gravelotte strain') with a longer diapause, but these records probably refer to *O. phacochoerus* n. sp. instead.

***Ornithodoros savignyi*** (Audouin, 1827)

(Figs 10-16)

*Argas savignyi* Audouin, 1827,

*Ornithodoros morbillosus* Gerstäcker, 1873

*Argas schinzii* Berlese, 1889

*Ornithodoros tunisiacum* Garibaldi, 1935

*Ornithodoros schinzii* Berlese: Leeson, 1953

*Ornithodoros (Ornithodoros) savignyi* (Audouin): Clifford, Kohls & Sonenshine, 1964

Type depository: Muséum National d'Histoire Naturelle, Paris (MNHN), Holotype: Sex unknown

Type locality: Egypt

**Etymology.** Named after French zoologist Marie Jules César Lelorgne de Savigny, a colleague of Jean Victoire Audouin who travelled to Egypt as part of the French Scientific Expedition (1798-1801) that collected the original type specimen.

**Material examined.** 28 females and 17 nymphs from Egypt, Sudan and Somaliland (Table S1). Sequences deposited in GenBank: KJ133577, KJ133578, MF415645, MF415646.

**Diagnosis.** Females may be distinguished by a combination of (1) eyes present, (2) posterior eye larger, (3) eyes convex, (4) posterior eye peripheral folds indistinct, (5) tarsus I proximal lobe tall, thick, rounded, (6) tarsus I middle lobe tall, thick, sub-rectangular to curved, (7) tarsus

I proximodistal lobe short, fused with distodistal lobe, (8) tarsus I distodistal lobe tall, thin, angled nearly upright.

Nymphs may be distinguished by (1) eyes present, (2) posterior eye larger than anterior, (3) eyes flat, (4) basis capitulum expanding basally with (5) rounded posterior corners imparting a sub-triangular appearance.

### **Redescription.**

Females

Length 9.2 to 12.0 mm, width 5.5 to 7.9 mm.

**Body** generally rectangular-oval, widely rounded at anterior and posterior ends; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes present, distinctly convex, peripheral folds indistinct, posterior eye slightly larger than first; body colour dark, grey, slate when alcohol preserved; integument leathery; setae abundant. **Mammillae** generally opaque with indistinct transparent centre, all sub-equal in size, bearing one or two fine and slightly larger punctations apically; anterior mammillae moderate size, moderately separated, circular to oval shape, not raised, flat apically; central mammillae moderate size, moderately separated, circular shape, not raised, flat apically; posterior mammillae moderate size, moderately separated, small dome shaped, slightly raised, rounded apically; ventral post-capitular area mammilated. **Intermammillary space** with cell patterned ridges comprising coarse, vermiform elevations, indistinct anteriorly, distinct centrally, distinct posteriorly. **Setae** stout, blunt, occasionally clubbed; anterior setae long, slender, numerous, about 200, extending posterolaterally almost reaching dorsoventral groove; ventral setae on anterior part of supracoxal fold long, numerous extending to trochanter III; setae on anterolateral hump of supracoxal fold long, slender, about twenty-three; setae on transversolateral part of supracoxal fold distinct, thick, short. **Genital apron** slightly posterior to post-capitular area, flattened; anterior lip thick, transverse, with about 55 coarse, confluent striae; posterior lip, thick, gentle crescent shape, with about 30 coarse striae; post-capitular area wider than long, comprised of medium mammillae scattered proximally and centrally, to flat, striate area distally at capitulum base; post-genital area with folded integument just posterior to genital apron, thin, with numerous mammillae, sub-oval, small.

**Basis capitulum** short, wider basally, mildly undulating, sclerotised; anterolateral humps absent; post-hypostomal setae one third length of hypostome, stout; post-palpal setae minute. **Hypostome** expanded at middle length, truncate apically; denticles numerous, moderately spaced, not projecting laterally; cheliceral apex robust, mobile bicusate tooth

moderately large. **Palp** long, segment I with a single dorsal seta, short, blunt, peg-like; segment II with three dorsomedial setae distally, two long, basal one slightly shorter; segment III with two dorsomedial setae, long; all short setae blunt, peg-like.

**Coxae** elongate triangular, hemiconical, anterior two-thirds sclerotised, posterior third as mammillate syncoxae, mammillae fine; I, II somewhat swollen, II especially so; apex with margin raised, bearing constriction centrally; I, II, III, IV with setae on apical and sub-apical posterior margins, blunt, short; apical marginal hairs slightly longer on II, III; on I about fourteen; II about eleven; III about eight; IV about six. **Trochanter** simple with moderate length, blunt setae; I with setae on anterior margin, eleven to twelve, posteriorly three to four; II with six to seven anteriorly, one to two posteriorly; III with four to five anteriorly, two to three posteriorly; IV with one to two anteriorly, four to five posteriorly. **Trochanterellus** about half length of trochanter; setae on posterior aspect, short, sub-apical, one to three.

**Legs** moderately short, robust; light brown-slate colour when alcohol preserved; anterior more robust, shorter than posterior; claws short. **Femur** twice length of trochanter; setae numerous on anterior legs, less posteriorly; I, II, III, IV with setae on anterior and posterior margin extending towards trochanterellus, long, short on IV posterior margin. **Tibia** short, length sub-equal with trochanter; setae distinct, clubbed on apical margin. **Metatarsus** slightly longer than trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe, reduced; setae on ventral margin arising from minor convexities. **Tarsus I** slightly longer than trochanter; dorsal lobes prominent, all separated by about width of one lobe; three lobes including a bipartite distal lobe where Haller's organ divides sub-lobes; proximal lobe tall, thick, sub-rectangular to rounded; middle lobe distinctly tall, moderately thick, sub-rectangular to curved; distal sub-lobes not well separated, often fused; proximodistal lobe not prominent, short, appears to arise from distodistal lobe; distodistal lobe long, narrow, angled upwards at near 90 degrees; Haller's organ minute; setae on dorsal margin and ventral margin arising from minor convexities, mostly long, thick; two setae on apex of proximodistal lobe, short, thin, directed opposite of one another; apical setae arranged somewhat concentrically, more dorsally than ventrally, short, thick, generally many.

Nymphs (1<sup>st</sup> - 2<sup>nd</sup> instar)

Length 1.6 to 2.0 mm, width 1.1 to 1.5 mm.

**Body** generally rectangular-oval, widely rounded at anterior and posterior ends, posterior end wider; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes present, approximately flat, posterior eye larger than anterior; body colour light reddish-brown when alcohol preserved; integument leathery; setae abundant.

**Basis capitulum** long, distinctly expanded basally, posterior end gently rounded to form a sub-triangular shape, sclerotized, anterolateral humps absent. **Hypostome** slender, not expanded at middle length, truncate apically, denticles numerous. **Palp** long, segmented, bearing setae.

**Coxae** elongate triangular, hemiconical; I, II somewhat swollen; apex with margin raised, bearing constriction centrally. **Trochanter** simple with moderate length, distinctly thinner than coxae. **Trochanterellus** about half length of trochanter.

**Legs** moderate length, slender; light brown-testaceous colour when alcohol preserved; anterior slightly more robust and shorter than posterior; claws short. **Femur** twice length of trochanter, thickening distally; setae numerous on anterior legs, less posteriorly. **Tibia** short, length sub-equal with trochanter; setae few. **Metatarsus** slightly longer than trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe, reduced. **Tarsus I** about twice length of trochanter; dorsal lobes prominent; three lobes present including a bipartite distal lobe where Haller's organ divides sub-lobes; Haller's organ small.

**Biogeography.** Widespread in Egypt, Sudan, Somaliland, Saudi Arabia and Yemen (Fig. 9A). Occurring in regions with less than 500 mm annual precipitation (Fig. S2). Free living, burrows in sandy soils (arenosols) (Fig. 9A).

**Hosts.** Camel, fowl, dog, horse, goat, cattle, pig, rabbit, human.

**Disease relationships.** May be implicated in sand tampan toxicoses.

**Notes.** Specimens from Yemen may represent a distinct species compared with Sudanese samples sequenced in this study based on genetic distance in 16S sequences. Samples from Somaliland and Egypt are slightly different from those in Sudan, with anterior eye larger than posterior eye, and tarsus I with proximodistal and distodistal lobes not as closely fused.

***Ornithodoros waterbergensis*** Bakkes, de Klerk & Mans **n. sp.**

(Figs 2-8)

*Ornithodoros moubata* (Murray, 1877), *pro parte*

Type depository: GTTM, Holotype: Female

Type locality: Lephalale, Limpopo Province, South Africa (23.775083S 27.481694E)

**Etymology.** From geographic distribution near the Waterberg mountain range, adjective.

**Material examined.** 8 females and 29 from South Africa (Table S1). Sequences deposited in GenBank: MF415615, MF415616, MF415617, MF415618, MF415619.

## Type material

Holotype ♀ (deposited in GTTM - OP5073), designated here. Label data: 'Lephalale, Limpopo / South Africa / -23.775083, 27.481694 / 21.viii.2012 / Ben Mans' (White rectangle). 'Holotype / Ornithodoros / waterbergensis Bakkes / D.K. Bakkes 2017 / ex-OP5043a' (Large red rectangle).

Paratype series designated here: OP5074 (1♀, deposited in GTTM). Label data: 'Lephalale, Limpopo / South Africa / -23.775083, 27.481694 / 21.viii.2012 / Ben Mans' (White rectangle). 'Paratype / Ornithodoros / waterbergensis Bakkes / D.K. Bakkes 2017 / ex-OP5042a' (Large blue rectangle). Sequence deposited in GenBank: MF415615.

OP5075 (2♀, deposited in SAMC). Label data: 'Bergpan / Limpopo, South Africa / -22.956677, 29.316727 / 14.ii.2014 / Daniel de Klerk' (White rectangle). 'Paratype / Ornithodoros / waterbergensis Bakkes / D.K. Bakkes 2017 / ex-OP5046a,b' (Large blue rectangle).

OP5076 (2♀, deposited in USNTC). Label data: 'Bergpan / Limpopo, South Africa / -22.956677, 29.316727 / 14.ii.2014 / Daniel de Klerk' (White rectangle). 'Paratype / Ornithodoros / waterbergensis Bakkes / D.K. Bakkes 2017 / ex-OP5046c,d' (Large blue rectangle).

**Diagnosis.** Females may be distinguished by a combination of (1) eyes absent, (2) anterior and posterior dorsal mammillae sub-equal size, (3) posterior mammillae bulbous shape, moderately spaced, (4) genital apron distinct crescent shape with rounded corners, sometimes narrow triangular, (5) intermammillary space with cell patterned ridges distinct, (6) tarsus I proximal lobe short, thin, rounded to curved, (7) tarsus I middle lobe short, thick, sub-rectangular to curved, (8) tarsus I proximodistal lobe nearly flat, (9) tarsus I distodistal lobe short (but longer than *O. moubata*).

Nymphs may be distinguished by (1) eyes absent, (2) posterior dorsal mammillae obtusely conical, (3) basis capitulum long, anterior slightly wider, and (4) hypostome notably thin.

## Description.

### Females

Length 6.6 to 11.0 mm, width 4.5 to 8.5 mm.

**Body** generally rectangular-oval, widely rounded at anterior and posterior ends; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes absent; body colour dark, reddish grey when alcohol preserved; integument leathery; setae abundant. **Mammillae** generally opaque with

indistinct transparent centre, all sub-equal in size, bearing numerous microscopic fine punctations apically; anterior mammillae small and moderate sized, size distribution erratic imparting a messy appearance, moderately separated, circular to oval shape, somewhat raised, slightly rounded apically; central mammillae small and moderate sized, size distribution erratic, moderately separated, circular shape, somewhat raised, rounded apically; posterior mammillae moderate size, moderately spaced, bulbous shape, somewhat raised, rounded apically; ventral post-capitular area mammillated. **Intermammillary space** with cell patterned ridges of fine, vermiform elevations, distinct anteriorly, distinct centrally, distinct posteriorly, sometimes entirely absent. **Setae** thick, blunt, straight; anterior setae long, slender, numerous, about 130, extending posterolaterally almost reaching dorsoventral groove; ventral setae on anterior part of supracoxal fold stout, numerous extending to trochanter III; setae on anterolateral hump of supracoxal fold long, slender, about nineteen; setae on transversolateral part of supracoxal fold indistinct, short. **Genital apron** slightly posterior to post-capitular area, flattened; anterior lip thick, transverse, with about 30 coarse striae; posterior lip, thick, distinct crescent shape with rounded corners, occasionally narrow triangular, bearing about 30 fine striae; post-capitular area longer than wide, comprised of finely corrugated area proximally, through large mammillae centrally, to transverse folds distally, about two, at capitulum base; post-genital area with folded integument just posterior to genital apron, thin, slightly extended, with numerous mammillae, sub-oval, medium sized.

**Basis capitulum** short, transversely corrugated ventrally, sclerotised; anterolateral humps present; post-hypostomal setae two thirds length of hypostome, slender; post-palpal setae stout, conical. **Hypostome** moderately expanded sub-apically, truncate apically; denticles large, numerous, densely packed, projecting laterally; large, angular denticles about 30 in files of 3:3, small denticles about 40, scattered proximally; cheliceral apex robust, mobile bicusate tooth moderately large. **Palp** long, segment I with group of dorsal setae, short, blunt, peg-like, about six or seven; segment II with four dorsomedial setae, long, sub-distal short; segment III with two dorsomedial setae distally, long; all dorsolateral setae short, blunt, peg-like.

**Coxae** elongate triangular, hemiconical, anterior two-thirds sclerotized, posterior third as mammillate syncoxae, mammillae large; I, II somewhat swollen, II especially so; apex with margin raised, bearing constriction centrally; I, II, III IV with setae on apical anterior and posterior margins, blunt, short, straight; sub-apical setae lacking; apical marginal hairs longer on II, III, IV; on I about three anteriorly, about three posteriorly; on II four to six anteriorly, about four posteriorly; on III four to five anteriorly, three to four posteriorly; on IV two to three anteriorly, three to four posteriorly. **Trochanter** simple with stout, blunt setae; I with setae on anterior margin four to six, one posteriorly; II with three to five anteriorly, one posteriorly; III with three to four anteriorly, one to two posteriorly; IV with three to five anteriorly, three to four



posteriorly. **Trochanterellus** about half length of trochanter; setae on posterior aspect, long, sub-apical, one to three, number variable.

**Legs** moderately long; dark red-brown colour when alcohol preserved; anterior slightly more robust than posterior; claws short. **Femur** twice length of trochanter; setae numerous on anterior legs, sparse posteriorly; I, II, III, IV with setae on anterior and posterior margin extending towards trochanterellus, long, IV shorter. **Tibia** short, length sub-equal with trochanter; setae distinct, curved, slender. **Metatarsus** about twice length of trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe; setae on ventral margin arising from minor convexities. **Tarsus I** about two and a half times length of trochanter; dorsal lobes prominent, all separated by about width of one lobe; three lobes including a bipartite distal lobe where Haller's organ divides sub-lobes; proximal lobe short, thin, rounded to curved, often angled slightly backwards; middle lobe short, thick, sub-rectangular to curved, sometimes distinctly so, angled slightly forwards; distal sub-lobes indistinctly separate; proximodistal lobe weakly prominent; distodistal lobe moderately short, narrow; Haller's organ large; setae on dorsal margin and ventral margin arising from minor convexities, mostly long, few short; two setae on apex of proximodistal lobe, short, directed opposite of one another; apical setae arranged somewhat concentrically, more dorsally than ventrally, short, indistinct.

Nymphs (1<sup>st</sup> - 2<sup>nd</sup> instar)

Length 1.2 to 1.5 mm, width 0.9 to 1.1 mm.

**Body** generally rectangular-oval, widely rounded at anterior and posterior ends, curvature approximately equal; dorsoventral groove between legs III and IV; supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; eyes absent; body colour reddish-brown when alcohol preserved; integument leathery; dorsal mammillae flat, tightly spaced; posterior mammillae conical, obtuse; setae sparse.

**Basis capitulum** long, slightly wider anteriorly, posterior end with angular corners; sclerotized; anterolateral humps absent. **Hypostome** thin, slender, not expanded at middle length, truncate apically, denticles numerous. **Palp** long, segmented, bearing setae.

**Coxae** elongate triangular, hemiconical; I, II somewhat swollen; apex with margin raised, bearing constriction centrally. **Trochanter** simple with moderate length, distinctly thinner than coxae. **Trochanterellus** about half length of trochanter.

**Legs** moderate length, slender; pale colour when alcohol preserved; anterior slightly more robust and shorter than posterior; claws short. **Femur** twice length of trochanter, thickening distally; setae numerous and long on anterior legs, less posteriorly. **Tibia** short, length sub-equal with trochanter; setae few. **Metatarsus** slightly longer than trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe, reduced. **Tarsus I** about

twice length of trochanter; dorsal lobes prominent; three lobes present including a bipartite distal lobe where Haller's organ divides sub-lobes; Haller's organ small.

**Biogeography.** Restricted to South Africa (north-western parts) (Fig. 9B). Occurring in regions with greater than 400 mm annual precipitation (Fig. S2) and altitude above 700 m above sea level (Fig. 9B). Often found in warthog burrows.

**Hosts.** Warthog: *Phacochoerus africanus*. Other hosts likely include pig, sheep, goat, dog, human.

**Disease relationships.** May be implicated in African swine fever.

**Notes.** None.

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#### *Phylogenetic analysis of the 16S ribosomal RNA gene*

Data from 16S rRNA serve well to distinguish new species and test monophyly of groups. However, these data perform poorly in determining interrelationships between species and genera. Thus, we only interpret our findings insofar as they are useful to test monophyly and distinct species.

Phylogenetic analysis of Afrotropical *Ornithodoros* (*Ornithodoros*) indicated distinct clades in both *O. savignyi* and *O. moubata* groups (Fig. 17). In the *O. savignyi* group, four main clades were observed that correspond with geographic localities, namely North Africa, Kalahari/Bushmanland, Namaqualand/Namibia and Noorsveld (Eastern Cape, South Africa). Pairwise genetic distances within clades range from 98-100% identity, while between clades range from 80-90% identity, suggesting distinct species. In the *O. moubata* group, five main clades were observed, of which three confirmed Walton's (1962) species from Northern Cape, South Africa (*O. compactus*), North West, South Africa, Zimbabwe and Malawi (*O. moubata*) and east Africa (*O. porcinus*). Two unique clades, closely related to *O. porcinus*, represent ticks from the Soutpansberg/Waterberg area (western Limpopo province, South Africa) and the lowveld of east South Africa (Kruger National Park in east Limpopo province and north Kwa-Zulu Natal, South Africa, as well as Swaziland). Members of these clades were previously identified as *O. porcinus porcinus* (Bastos et al., 2009; Boshoff et al., 2014). Pairwise genetic distances within these clades range from 97-100% identity, while comparisons between clades range from 87-90% identity for *O. compactus*-*O. porcinus* clades, 86-91% identity for *O. moubata*-*O. porcinus* clades, and 95% for *O. moubata*-*O. compactus*. Pairwise genetic distances for the *O. porcinus* sensu lato clades range from 91-95% identity with the

Soutpansberg/Waterberg and east Africa/east South Africa clades, while the east Africa and east South Africa clades showed 91-92% sequence identity. Genetic distances within the *O. moubata* group were not as large as those observed in the *O. savignyi* group, however most differences are large enough ( $\leq 95\%$  identity) to suggest that different species exist.

For analysis of world *Ornithodoros*, Afrotropical *Ornithodoros* (*Ornithodoros*) were monophyletic with high bootstrap support (Fig. 18). Genetic distances between the various lineages are similar to distances between other recognized argasid species, again suggesting different species in *O. moubata* and *O. savignyi* groups. Data from 16S rRNA indicate that *Argas* and *Ornithodoros* are paraphyletic according to the current classification scheme of Guglielmo et al. (2010). This is due to (1) *Antricola*, *Nothoaspis* and *Otobius* that group within *Ornithodoros*, and (2) *Ornithodoros lahorensis* that group within *Argas*. This issue has been discussed before (Nava et al. 2009; Estrada-Peña et al. 2010), and corroboration of these observations by larger datasets and alternative genes will likely necessitate a reclassification of the Argasidae to present a better natural classification based on robust phylogenetic analysis.

#### *Geometric morphometric analysis of tarsus I shape*

To further test whether the Afrotropical *Ornithodoros* (*Ornithodoros*) clades represent different species, morphometric analyses were performed on shape outlines of tarsus I. Comparison of multivariate means indicated Procrustes and Mahalanobis distances were largest in comparisons between species from different species groups (average P.dist.= 0.087, M.dist.= 10.071), smaller in comparisons among the *O. savignyi* group (average P.dist.= 0.070, M.dist.= 7.616), and smallest among comparisons from the *O. moubata* group (average P.dist.= 0.047, M.dist.= 5.120). Moreover, these distances were all significant ( $p < 0.05$ ). Misclassification in cross-validation was most frequent in species comparisons from the *O. savignyi* group (average = 16.17%), less frequent in *O. moubata* group comparisons (average=12.40%), and least frequent in comparisons between different groups (average=2.60%). Largest number of misclassifications (>20% of sample) include *O. savignyi*-*O. kalahariensis* **n. sp.** (39%), *O. phacochoerus* **n. sp.** -*O. moubata* (25%), *O. pavimentosus* **nom. rev.** -*O. kalahariensis* **n. sp.** (24%), *O. compactus*-*O. waterbergensis* **n. sp.** (23%), and *O. porcinus*-*O. phacochoerus* **n. sp.** (22%).

In the total sample, canonical variate I conclusively distinguished between *O. moubata* and *O. savignyi* groups (Fig. 19A). Shape change was attributable to (1) height of all lobes and (2) excavation between lobes. Canonical variate II provided some separation in the *O. savignyi* group, but generally less in the *O. moubata* group, with shape change attributable to (1) proximity (excavation) between distal sub-lobes, (2) angle of distodistal lobe, (3) width of

middle lobe and (4) width of proximal lobe. In the *O. savignyi* group sample, canonical variate I distinguished between the subgroups of (1) *O. pavimentosus* **nom. rev.** + *O. kalahariensis* **n. sp.** and (2) *O. savignyi* + *O. noorsveldensis* **n. sp.** (Fig. 19B). Shape change was attributable to (1) width of proximal lobe, (2) width of middle lobe, (3) height of proximodistal lobe, (4) height of distodistal lobe and (5) angle of distodistal lobe. Canonical variate II distinguished *O. savignyi*, and shape change was attributable to (1) proximity between distal sub-lobes, (2) width of distodistal lobe, (3) height of proximodistal lobe and (4) height of middle lobe. In the *O. moubata* group sample, canonical variate I distinguished three groups: *O. compactus*; *O. waterbergensis* **n. sp.** + *O. phacochoerus* **n. sp.**; and *O. moubata* + *O. porcinus* (Fig. 18C). Shape change was attributable to (1) size of proximal lobe, (2) width of middle lobe, (3) height of middle lobe, (4) curvature of middle lobe and (5) excavation between distal sub-lobes. Canonical variate II distinguished *O. phacochoerus* **n. sp.** + *O. porcinus* from *O. waterbergensis* **n. sp.** + *O. moubata* leaving *O. compactus* as intermediate. Shape change was attributable to (1) height of middle lobe, (2) width of middle lobe, (3) height of distodistal lobe and (4) width of proximal lobe.

The pilot study to assess rotational error, showed that error was minimal with the effect of species being larger than all other effects in a Procrustes ANOVA (Table 2). Moreover, fluctuating asymmetry was marginally greater than replicate error (Table 2). No misclassifications were found from cross validation (not shown). In the complete dataset, the effect of individual was thirteen times greater than replicate error, and effect of species was twelve times greater than individual (Table 2).

#### *Distribution maps*

Comparison of distribution patterns with annual precipitation and soil type demonstrated positive correlation in the *O. savignyi* group (Figs 9A, S2). Similarly, *O. moubata* group distribution correlated positively with altitude, major river topology and annual precipitation (Figs 9A, S2). However, distribution of *O. savignyi* group species did not correlate with altitude and major river topology, and likewise *O. moubata* group species did not correlate with soil type (data not shown).

For precipitation, species of the *O. savignyi* group were limited to regions of less than 500 mm annual precipitation, while *O. moubata* group species were limited to regions of greater than 300 mm annual precipitation (Fig. S2). This is with exception of *O. compactus* that was limited to regions of less than 200 mm annual precipitation. Topologies of major rivers bisected the *O. moubata* group in geographic regions congruent with 16S rRNA and tarsus I shape data (Fig. 9B). For altitude, *O. waterbergensis* **n. sp.** + *O. moubata* were separated from *O. phacochoerus* **n. sp.** that occurred below 700 m above sea level (Fig. 4B). Moreover, the

Drakensberg mountain range provided further separation between these species. For soil type, *O. pavimentosus* **nom. rev.** was generally restricted to leptosols, *O. savignyi* and *O. kalahariensis* **n. sp.** to arenosols, and *O. noorsveldensis* **n. sp.** to a single locality having luvisols (soil type obscured on Fig. 9A).

## Discussion

Lack of distinctive morphology between species has caused historical confusion in identification and species delimitation of *Ornithodoros* ticks. Molecular data aid in determining *a priori* species hypotheses that may be further tested by independent lines of evidence in an iterative framework commonly known as integrative taxonomy (Yeates et al., 2011). Additionally, we introduce a novel set of methods that quantify shape outline data between individuals and species. These methods discriminate between statistical distributions of shape variables in hypothesized species to determine whether or not central tendencies overlap. This revision serves to update the taxonomy of Afrotropical *Ornithodoros* (*Ornithodoros*) based on three lines of evidence that can test species boundaries. Phylogenetic relationships based on 16S rRNA sequence data detected four previously hidden lineages amongst the *O. moubata* and *O. savignyi* groups that are congruent with geographic regions. These were interpreted as possible new species, and were iteratively tested along lines of qualitative morphology (traditional) and quantitative analysis of tarsus I shape outlines in a geometric morphometric framework.

### *Species relationships, autapomorphies and synapomorphies*

Investigation of qualitative morphology provided characters that corroborate 16S rRNA lineages as well as groups from tarsus I shape outlines. However, some homoplasious states in *O. moubata* group nymphal basis capituli can be observed when compared against 16S rRNA lineages. This indicates complexity in morphological change during speciation that may relate to differential rates of morphological evolution. Below, we synthesize findings from female as well as nymphal morphology. Immature stages are useful to determine species relationships because they are less affected by changes that accrue during ontogeny due to environment and nutrient uptake.

Eyes present is a synapomorphy for *O. savignyi*, *O. pavimentosus* **nom. rev.**, *O. noorsveldensis* **n. sp.** and *O. kalahariensis* **n. sp.**, but female *O. savignyi* have larger posterior eyes that are phylogenetically autapomorphic. The remaining three species have eyes sub-equal in females as a synapomorphy. *O. pavimentosus* **nom. rev.** seem most closely related to *O. savignyi* in sharing nymphs with broad sub-triangular basis capituli as well as having flat eyes. However, *O. pavimentosus* **nom. rev.** are autapomorphic in having smaller posterior

eyes as nymphs, and distinct peripheral eye folds as adults. Nymphs of *O. kalahariensis n. sp.* and *O. noorsveldensis n. sp.* have eyes sub-equal and distinctly convex as synapomorphies, but *O. noorsveldensis n. sp.* are autapomorphic in having notably short basis capituli as adults. These characters display good congruence with 16S rRNA lineages.

Eyes absent is a synapomorphy for *O. moubata*, *O. compactus*, *O. porcinus*, *O. apertus*, *O. phacochoerus n. sp.* and *O. waterbergensis n. sp.*. As nymphs, a short, wide, sub-rectangular basis capitulum unites *O. moubata*, *O. compactus* and *O. phacochoerus n. sp.* (homoplasious compared with 16S rRNA lineages), while an even wider basis capitulum distinguishes *O. porcinus*, and a long basis capitulum distinguishes *O. waterbergensis n. sp.* *O. compactus* have uniform tile-like mammillae as an autapomorphy in nymphs and mature females, whereas *O. moubata* have bulbous mammillae instead. Uniting *O. moubata*, *O. compactus*, *O. phacochoerus n. sp.* and *O. waterbergensis n. sp.* are posterior mammillae that are sub-equal in size compared with anterior mammillae. Conversely, *O. porcinus* are autapomorphic with distinctly larger posterior mammillae in nymphs and adults. *O. apertus* have similarly large posterior mammillae, placing them close to *O. porcinus*, but are autapomorphic in having long legs as well as reduced tarsus I dorsal lobes in females. Unfortunately, *O. apertus* nymphal data are unavailable for basis capituli comparison. *O. waterbergensis n. sp.* and *O. phacochoerus n. sp.* are united by having generally conical posterior mammillae in nymphs, but *O. phacochoerus* have these smaller and more acute. Moreover, *O. phacochoerus n. sp.* are autapomorphic in having a thin slit-like genital apron in females. In contrast, *O. waterbergensis n. sp.* females are automorphic with a gentle crescent shaped genital apron that has rounded corners.

#### *Ornithodoros savignyi* group

In the case of the *O. savignyi* group, clades correspond to defined ecoregions in Africa, namely (1) the Kalahari, Nama and succulent Karoo (Bushmanland), (2) Namaqualand and semi-desert regions in Namibia, (3) Noorsveld thicket in the Eastern Cape, and (4) Sahel regions bordering the Sahara (Fig. 9A). This corroborates previous morphological differences noted between Noorsveld and Egyptian specimens (Theiler, 1962). Moreover, data from 16S rRNA suggest a split in these lineages that place *O. pavimentosus nom. rev.* + *O. savignyi* and *O. kalahariensis n. sp.* + *O. noorsveldensis n. sp.* in separate clades (Fig. 17). This supports Theiler's (1962) hypothesis of origin in the Namib or Kalahari with subsequent dispersal to north Africa on the one hand, and to Noorsveld on the other. These species occur in regions with less than 500 mm annual precipitation, suggesting adaptation to arid habitats. Indeed, physiological studies suggest the same (Lees, 1947; Cloudsley-Thompson, 1975). Hypothetically, speciation may have been driven by cycles of desertification resulting in flux of

island deserts and unsuitable habitat to induce vicariance of large populations (Senut et al., 2009). However, Hoogstraal's (1956) hypothesis of dispersal along camel routes from Timbuktu to India may yet be plausible for the north African lineage. More extensive sampling from west Africa to India is needed to test this given the biased species richness in southern Africa. We predict more species should exist in these regions.

Biogeography in *O. savignyi* group species may be limited by soil type given the soil burrowing life history of these species. Positive correlation between geographic distributions with either leptosols (gravelly), arenosols (sandy) or luvisols (fine clay) (Fig. 9A) suggest geographic distribution constrained by soil type. Moreover, dorsal lobes of tarsus I in *O. savignyi* group species are generally much larger than their *O. moubata* group counterparts (Figs 5, 13, 19). Hypothetically, this represents morphological adaptation to burrowing in *O. savignyi* group species where larger lobes provide a greater vantage point for digging into soils, as in fossorial arthropods such as dung beetles or mole crickets (Brown, 1974; Villani et al., 1999; Tong et al., 2005) – however, this remains to be empirically tested. Lobes may also be linked to preventing leg cuticle damage from abrasion by large soil particles, which would increase water loss and enable pathogen infection (Lees, 1947; Cloudsley-Thompson, 1975, 1991). Presumably, tarsal lobes can divert abrasive soil particles away from sensitive areas. This stands in contrast with *O. moubata* group species that live in burrows with little to no need for digging. As such, reduced lobes would have decreased developmental costs for a feature that was no longer useful following the life history switch to warthog burrows.

Tarsus I lobe shapes in *O. savignyi* group species also seem correlated with soil type, specifically in terms of particle size. The largest lobes are found in *O. pavimentosus* **nom. rev.** which occur in gravelly soil. This may suggest greater digging power with increased surface area to exert pressure on surrounding soil matter. Additionally, we speculate larger lobes may afford greater protection against abrasive particles due to reduced spaces between lobes that can exclude these particles. Conversely, *O. noorsveldensis* **n. sp.** have the smallest lobes of the group and occur in fine clay soils where abrasion is less of a risk, and where large lobes would hinder ease of movement. Lobes of *O. kalahariensis* **n. sp.** and *O. savignyi* represent an intermediate, and these species occur in sandy soils with particle size likewise intermediate between gravel and clay.

Neumann's (1901) *O. pavimentosus* **nom. rev.** was confirmed as a valid species based on 16S rRNA data, eye morphology and tarsus I shape outlines (Figs 11, 13, 17, 19). These morphological differences were noted by Neumann in his description despite inciting *nomen nudum*. This makes it puzzling that the species was synonymized with *O. savignyi* (Theiler and Hoogstraal, 1955). However, that study investigated few localities among the *O.*

*pavimentosus* **nom. rev.** distribution range, meaning the few *O. pavimento* **nom. rev.** specimens that were likely examined, must have appeared as incidental outliers.

#### *Ornithodoros moubata* group

In the *O. moubata* group, *O. moubata* is limited to Zimbabwe, Malawi and the North West province of South Africa, while *O. compactus* is limited to the Northern Cape of South Africa (Fig. 9B), as in previously described distributions (Walton, 1962; Horak et al., 2006). These two species form a monophylum with high support that excludes the remainder of *O. moubata* group species (Fig. 17). They share 95% sequence identity but are morphologically distinct. The peculiar biology of *O. compactus* feeding on tortoises probably did not drive speciation, but rather resulted from recent adaptation when the warthog population was decimated by European settlers in the early 19<sup>th</sup> century (Van der Merwe, 1967). Instead, speciation was likely driven by geographic separation of warthog hosts between the Northern Cape and Kalahari regions. Indeed, the Orange river separates the distributions of *O. compactus* and *O. moubata* (Fig. 9B).

An origin for the *O. moubata* group in the Kalahari basin is supported given the basal position of the *O. moubata* + *O. compactus* lineage (Fig. 18). This is similar to the scenario postulated for the *O. savignyi* group above. Additionally, monophyly of Afrotropical *Ornithodoros* (*Ornithodoros*) indicates this may apply to the entire Afrotropical lineage, with subsequent speciation and dispersal into Africa. Climatically, *O. moubata* group species occur in regions with greater than 300 mm annual precipitation, with exception of *O. compactus* (Fig. S2). This suggests lower arid tolerance than *O. savignyi* group species, as well as adaptation to less arid environments (Peirce, 1974). Differential adaptation to arid and less arid habitats in the common ancestor of Afrotropical *Ornithodoros* (*Ornithodoros*) may have contributed to initial divergence of these clades. However, these conclusions are tentative given that new species may be found which could reject these hypotheses if they are phylogenetically basal.

In the *O. porcinus* clade, *O. porcinus* is limited to east Africa and Madagascar, *O. phacochoerus* **n. sp.** to the eastern side of southern Africa (Kruger National Park and Kwa-Zulu Natal, South Africa and Swaziland) and *O. waterbergensis* **n. sp.** to the western side of Limpopo, South Africa (Fig. 9B). Speciation may have been linked to partial host isolation. Indeed, reduced gene flow among common warthog (*Phacochoerus africanus*) populations indicate three distinct populations limited to southern, east and west Africa (Muwanika et al., 2003). This likely separated southern and east African populations of the common ancestor for the *O. porcinus* clade. Additionally, two major river systems separate these regions (Fig. 9B). The Limpopo and Zambezi rivers enclose the region where *O. moubata* occur (albeit with population expansion past the head of the Limpopo). As such, the lineage of east African *O.*



*porcinus* is isolated from the South African lineages. This is similar to the *O. compactus* and *O. moubata* distributions mentioned above (Fig. 9B). The riverine barrier hypothesis has been considered a driver of speciation for various terrestrial animals in the Afrotropics (Scott et al., 2004; Voelker et al., 2013). Although warthog movement may not be totally affected, large rivers could hinder attachment of ticks during river crossings, especially when considering the rapid feeding duration of *Ornithodoros* ticks (Oliver, 1989; Vial, 2009).

Speciation in the Limpopo region is unique however, given no major river bisects the distribution of *O. waterbergensis* **n. sp.** and *O. phacochoerus* **n. sp.** (Fig. 9B). The Waterberg, Soutpansberg and Drakensberg mountain ranges may form natural barriers separating bushveld and lowveld from one another (Fig. 9B). Significant tectonic uplift occurred in the Pliocene (about 5 Mya) to form the great escarpment that separates extant lowveld and bushveld. This has been considered an important driver of speciation in north east South Africa (Bentley et al., 2014; Stanley and Bates, 2014). Overall, geographic distribution appears localized for lineages that make up these species. However, breakdown of historical barriers may have occurred due to human movement of domestic suids and human parasitism by these ticks, leading to possible admixture of lineages. Possible causes of these should be investigated to contribute to management of tick-borne diseases such as African swine fever and human relapsing fever.

### Conclusion

The 16S rRNA gene has been extensively used in tick studies to delimit, identify and classify new and cryptic species. It has seen recent successes in Argasidae taxonomy for delimitation and description of new species (Labruna et al., 2008; Venzal et al., 2008; Nava et al., 2010; Dantas-Torres et al., 2012; Venzal et al., 2012; Nava et al., 2013; Trape et al., 2013; Venzal et al., 2013; Barros-Battesti et al., 2015; Venzal et al., 2015; Dupraz et al., 2016; Labruna et al., 2016; Muñoz-Leal et al., 2016; Muñoz-Leal et al., 2017). Since Guglielmone and colleagues (2010) compiled the taxonomy that reported 193 argasid species, the present study and other new species unavailable at that time, bring the known species count for argasids to 216. Further research on Argasidae systematics will likely uncover additional species, and 16S rRNA analyses will play an important role in delimiting lineages and providing voucher sequences.

Tarsus I shape outlines provide a novel way of testing species boundaries under the assumption that conspecific individuals should have similar shape compared to heterospecific individuals, and display central tendency that can delimit groups. Tarsus I shape outlines between species of *O. moubata* and *O. savignyi* groups clearly demonstrate this. Variation within each group was less pronounced, but still significant for species delimitation. Principal

component analyses of body and setal measurements have been used to support and differentiate closely related argasid species (Nava et al., 2013; Venzal et al., 2015; Labruna et al., 2016). Likewise, geometric morphometric analyses of landmark data and body shape outlines have been used to differentiate members of cryptic species groups (Dupraz et al., 2016), as well as island populations (Dujardin et al., 2014). As such, outline based geometric morphometrics show great potential, albeit underappreciated, for studying arthropod shape to answer biological research questions. The present study represents the first use of geometric morphometric analyses of tarsus I shape outlines, and demonstrates it is a useful measure to differentiate species of Afrotropical *Ornithodoros* (*Ornithodoros*).

Future work on Afrotropical *Ornithodoros* (*Ornithodoros*) species should consider larval morphology for additional characters to support species boundaries. Other studies should explore population dynamics and test vector competence to determine the role of Afrotropical *Ornithodoros* (*Ornithodoros*) ticks in transmission of African swine fever and human relapsing fever. Additionally, more species of *Ornithodoros* may present themselves with future collections. Given the difficulty of identification between *Ornithodoros* species, future samples should be validated by either molecular analysis or geometric morphometric analysis of tarsus I shape.

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### **Conflict of Interest**

The authors declare no conflict of interest.

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## Figure Captions

**FIGURE 1.** Schematic diagram of morphological features of *Ornithodoros* (*Ornithodoros*) ticks. Specimen: *O. phacochoerus* female, Pafuri, Kruger National Park, South Africa, OP5047b.

**FIGURE 2.** Dorsal (left) and ventral (right) habitus photos of *O. moubata* group females. *Ornithodoros compactus*, Klein Noute, South Africa, OP5160 (A); *O. moubata*, Groot Marico, South Africa, OP5044a (B); *O. phacochoerus* n. sp., holotype, Crocodile Bridge, Kruger National Park, South Africa, OP5067 (C); *O. porcinus*, paratype, Mwitikira, Dodoma, Tanzania, OP3088ia (D); *O. apertus*, paratype, Lake Naivasha, Kenya, OP3086 (E); and *O. waterbergensis* n. sp., holotype, Lephalele, South Africa, OP5073 (F).

**FIGURE 3.** Comparative morphology of posterior mammillae in *O. moubata* group females. *Ornithodoros compactus* (A); *O. moubata* (B); *O. phacochoerus* n. sp. (C); *O. porcinus* (D); *O. apertus* (E); and *O. waterbergensis* n. sp. (F). Specimen data as in Fig. 5.

**FIGURE 4.** Comparative morphology of anteroventral aspect in *O. moubata* group females. *Ornithodoros compactus*, female, Klein Noute, South Africa, OP5051b (A); *O. moubata* (B); *O. phacochoerus* n. sp. (C); *O. porcinus* (D); *O. apertus* (E); and *O. waterbergensis* n. sp. (F). Specimen data as in Fig. 5.

**FIGURE 5.** Comparative morphology of tarsus I in *O. moubata* group females. *Ornithodoros compactus*, Klein Noute, South Africa, OP5051a (A); *O. moubata* (B); *O. phacochoerus* n. sp. (C); *O. porcinus* (D); *O. apertus* (E); and *O. waterbergensis* n. sp. (F). Specimen data as in Fig. 5.

**FIGURE 6.** Dorsal (left) and ventral (right) habitus photos of *O. moubata* group nymphs. *Ornithodoros compactus*, Klein Noute, South Africa, OP5051 (A); *O. moubata*, Groot Marico, South Africa, OP5044 (B); *O. phacochoerus* n. sp., Crocodile Bridge, Kruger National Park, South Africa, OP5048 (C); *O. porcinus*, Tsavo West, Tanzania, OP5045 (D); and *O. waterbergensis* n. sp., Lephalele, South Africa, OP5043 (E).

**FIGURE 7.** Comparative morphology of posterior mammillae in *O. moubata* group nymphs. *Ornithodoros compactus* (A); *O. moubata* (B); *O. phacochoerus* n. sp. (C); *O. porcinus* (D); and *O. waterbergensis* n. sp. (E). Specimen data as in Fig. 9.

**FIGURE 8.** Comparative morphology of anteroventral aspect in *O. moubata* group nymphs. *Ornithodoros compactus* (A); *O. moubata* (B); *O. phacochoerus* n. sp. (C); *O. porcinus* (D); and *O. waterbergensis* n. sp. (E). Specimen data as in Fig. 9.

**FIGURE 9.** Point map distribution of *Ornithodoros savignyi* group against soil type data (A), and *Ornithodoros moubata* group against altitude data (B). Circles represent localities of collections studied morphologically, triangles represent localities of previously published 16S sequences.

**FIGURE 10.** Dorsal (left) and ventral (right) habitus photos of *O. savignyi* group females. *Ornithodoros kalahariensis* n. sp., holotype, Tosca, South Africa, OP5059 (A); *O. noorsveldensis* n. sp., holotype, Allemanskraal, Steytlerville, South Africa, OP5062 (B); *O. pavimentosus* nom. rev., holotype, Komaggas, South Africa, OP5065 (C); and *O. savignyi*, El Obeid, Sudan, OP5038c (D).

**FIGURE 11.** Comparative morphology of anterior eye (left) and posterior eye (right) in *O. savignyi* group females. *Ornithodoros kalahariensis* n. sp. (A); *O. noorsveldensis* n. sp. (B); *O. pavimentosus* nom. rev. (C); and *O. savignyi* (D). Specimen data as in Fig. 5.

**FIGURE 12.** Comparative morphology of anteroventral aspect in *O. savignyi* group females. *Ornithodoros kalahariensis* n. sp. (A); *O. noorsveldensis* n. sp. (B); *O. pavimentosus* nom. rev. (C); and *O. savignyi* (D). Specimen data as in Fig. 5.

**FIGURE 13.** Comparative morphology of tarsus I in *O. savignyi* group females. *Ornithodoros kalahariensis* n. sp. (A); *O. noorsveldensis* n. sp. (B); *O. pavimentosus* nom. rev. (C); and *O. savignyi* (D). Specimen data as in Fig. 5.

**FIGURE 14.** Dorsal (left) and ventral (right) habitus photos of *O. savignyi* group nymphs. *Ornithodoros kalahariensis* n. sp., Droeduin, South Africa, OP5042 (A); *O. noorsveldensis* n. sp., Allemanskraal, Steytlerville, South Africa, OP5040 (B); *O. pavimentosus* nom. rev., Nam, South Africa, OP5037i (C); and *O. savignyi*, Wad Medani, Sudan, OP2958i (D).



**FIGURE 15.** Comparative morphology of eyes in *O. savignyi* group nymphs, left eye is anterior eye. *Ornithodoros kalahariensis* n. sp. (A), *O. noorsveldensis* n. sp. (B), *O. pavimentosus* nom. rev. (C) and *O. savignyi* (D). Specimen data as in Fig. 16.

**FIGURE 16.** Comparative morphology of anteroventral aspect in *O. savignyi* group nymphs. *Ornithodoros kalahariensis* n. sp. (A), *O. noorsveldensis* n. sp. (B), *O. pavimentosus* nom. rev. (C) and *O. savignyi* (D). Specimen data as in Fig. 16.

**FIGURE 17.** Phylogenetic analysis of the 16S rRNA gene for Afrotropical *Ornithodoros* (*Ornithodoros*). Alignment used 282 nucleotide sites, with 105 phylogenetically informative. Indicated are species names, GenBank accession numbers, first author, publication date, locality or origin and country of origin. General geographic distributions are indicated per clade and correspond with respective species designation. Sequences with no author name were sequenced during the study.

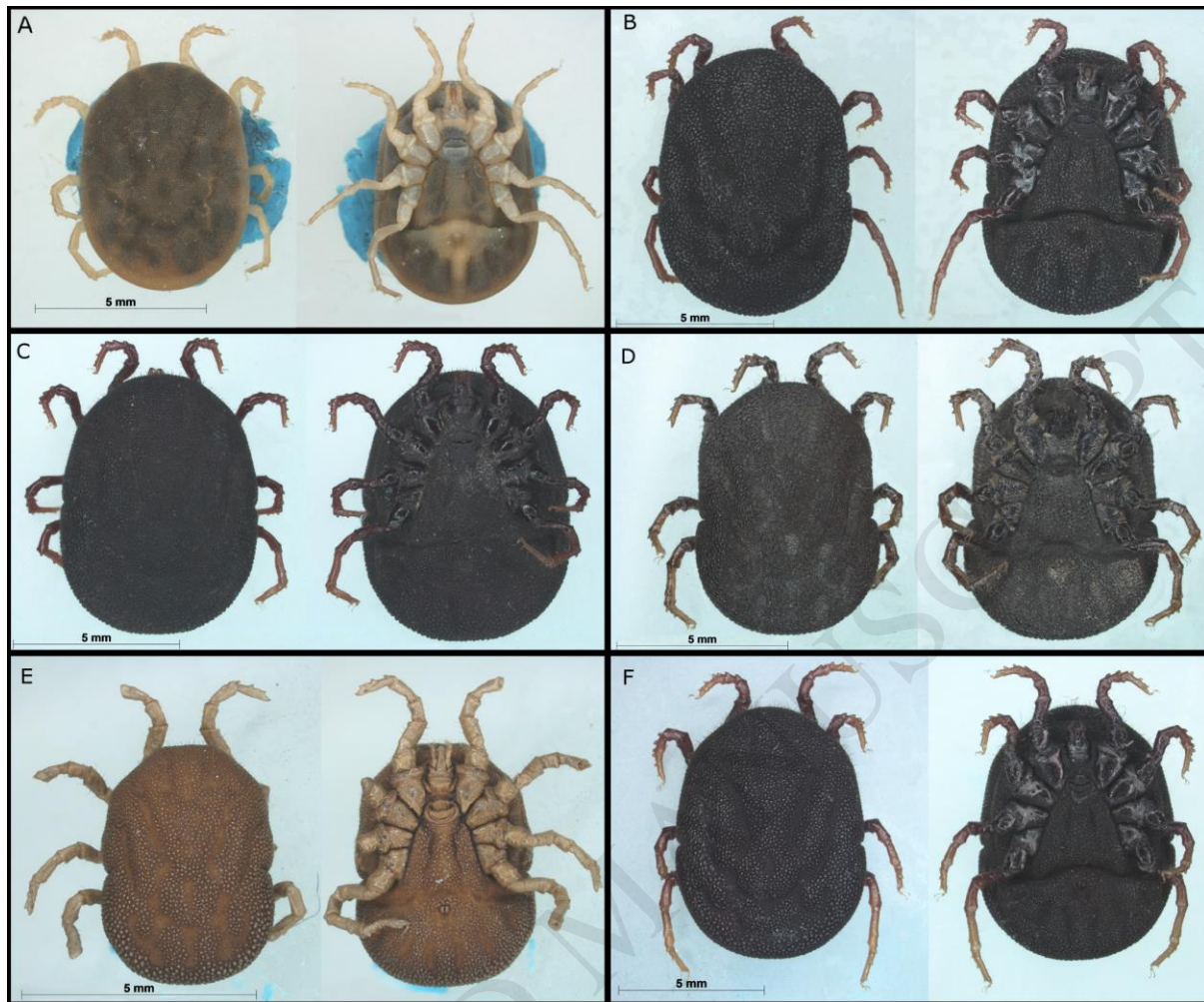
**FIGURE 18.** Phylogenetic analysis of the 16S rRNA gene for world *Ornithodoros*. Alignment used 339 nucleotide sites, with 232 phylogenetically informative. The maximum likelihood consensus tree derived from 100 000 bootstraps with nodal support values are indicated along with species names and GenBank accession numbers. Afrotropical *Ornithodoros* (*Ornithodoros*) are in bold. Genus names are presented according to Guglielmone et al. (2010), while subgenera are according to Clifford et al. (1964). **FIGURE 19.** Canonical variates analysis of total Afrotropical *Ornithodoros* (*Ornithodoros*) sample (A), *Ornithodoros savignyi* group (B), and *Ornithodoros moubata* group (C). Indicated are axes for canonical variates I and II. Dots represent averages of single specimens. Black-ringed dots represent specimens used in both sequencing and morphometrics. Starred dot represents paratype specimen for *O. porcinus*. Ellipses represent 95% confidence. Shape changes along PC axes are to scale at minimum and maximum extents. Light blue traces represents mean shape and dark blue traces represent deviation from mean shape at given extent.

Fig 1



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Fig 2



ACCEPTED

Fig 3

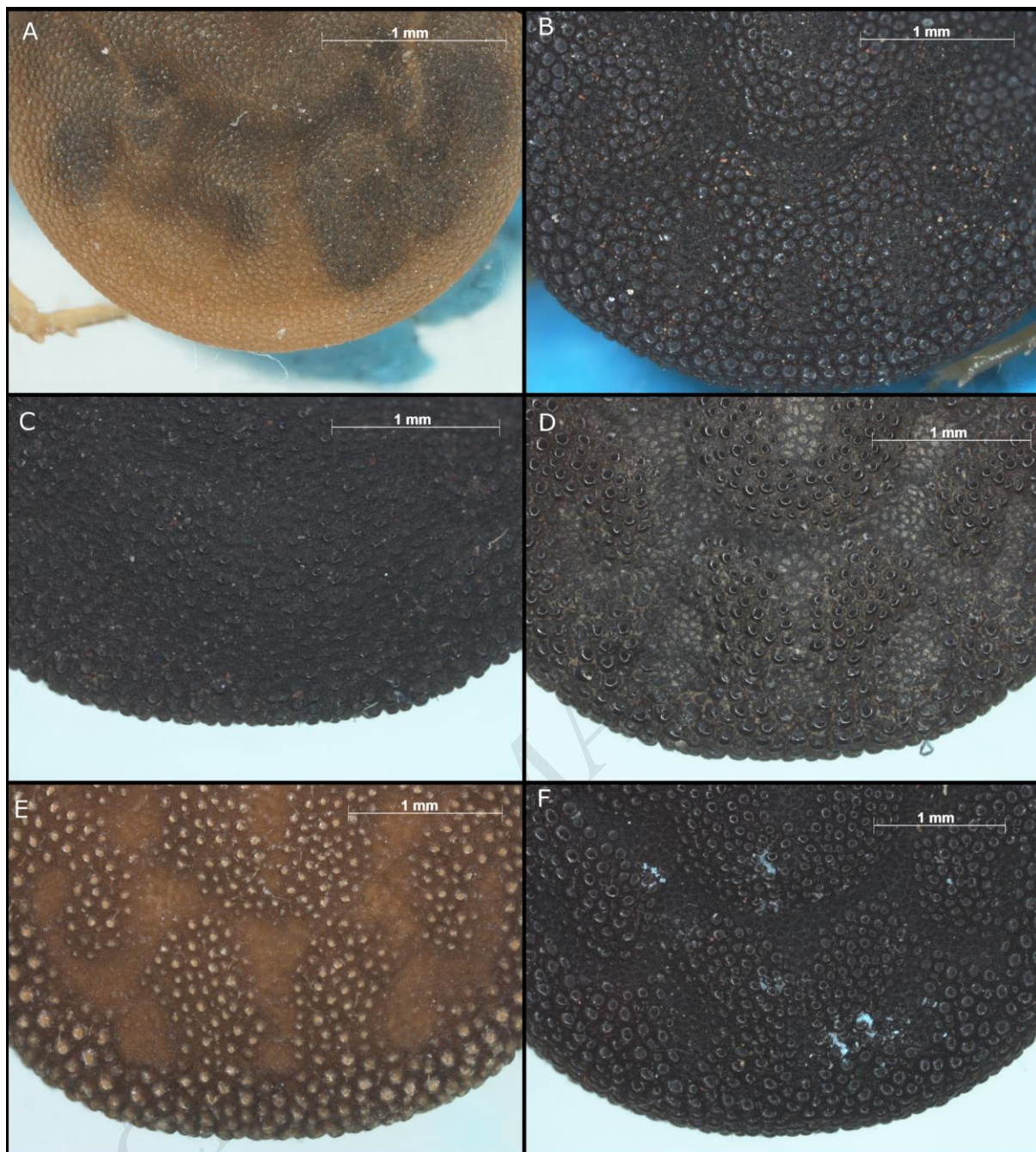


Fig 4

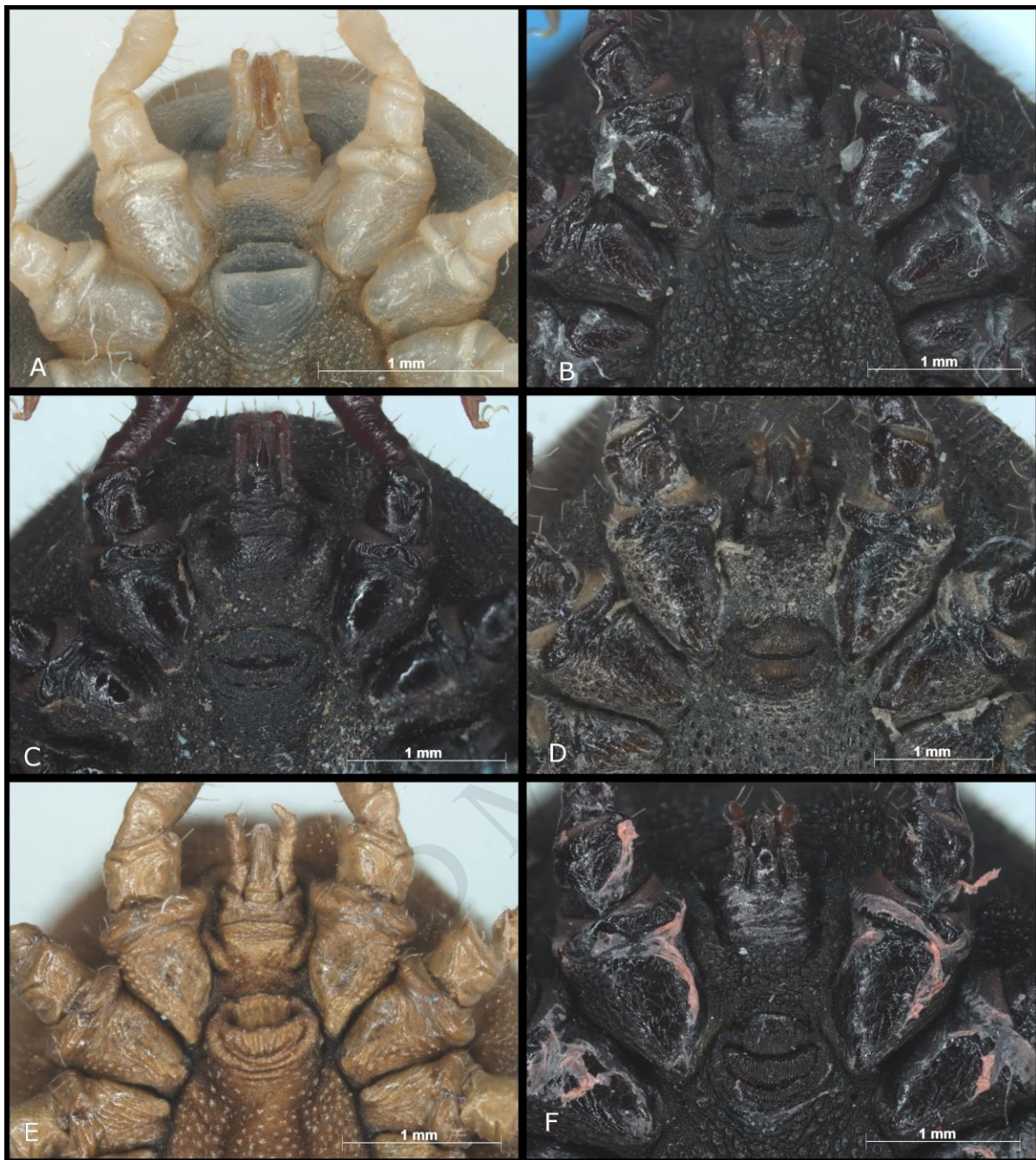


Fig 5

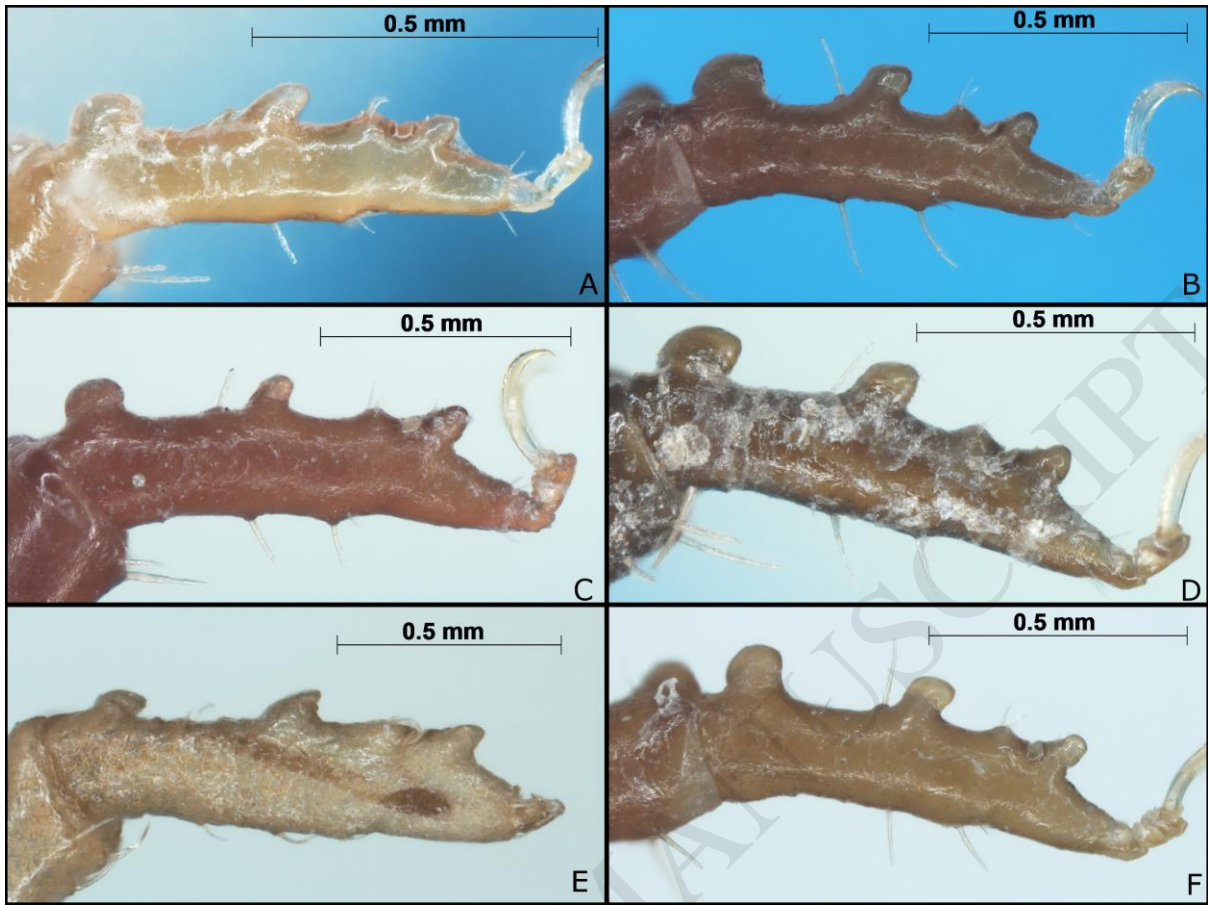


Fig 6

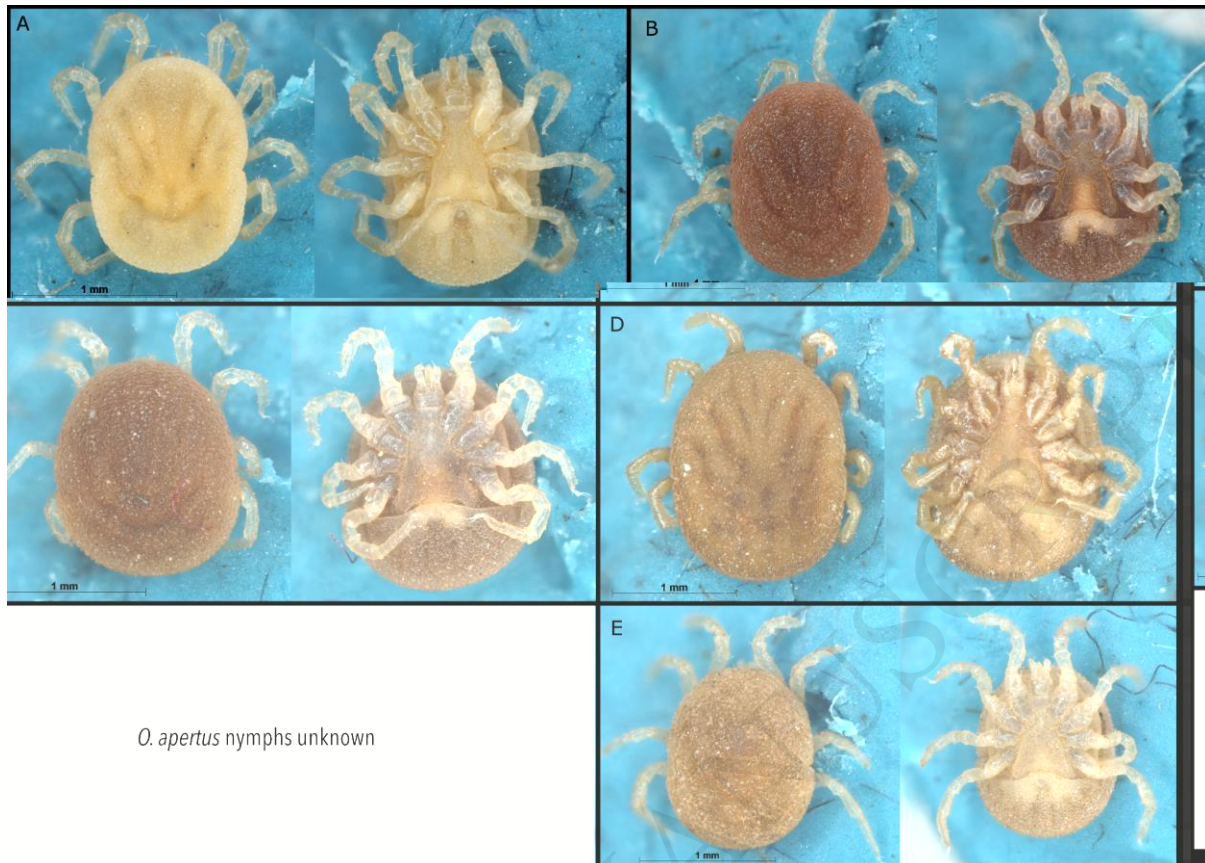


Fig 7

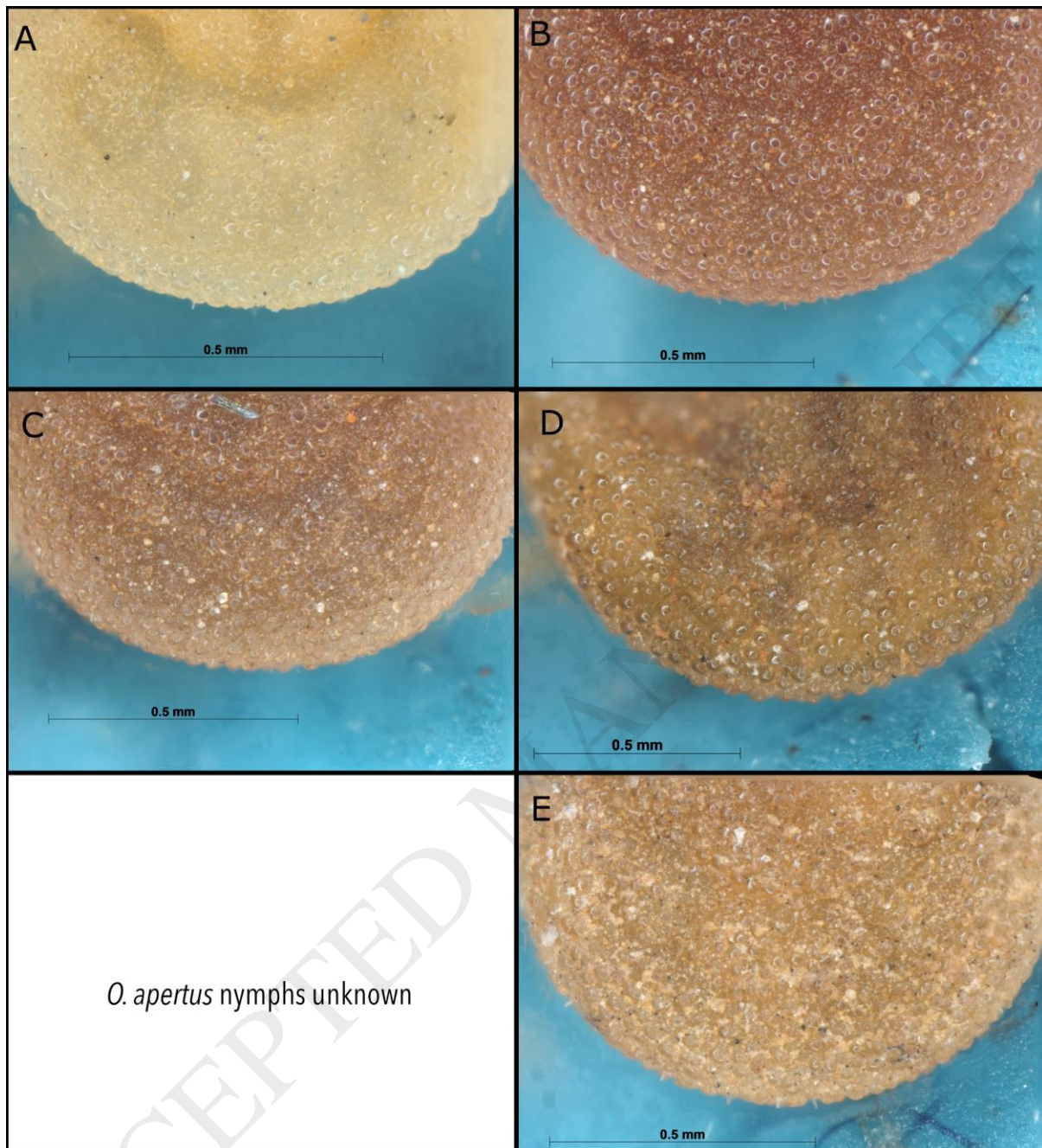
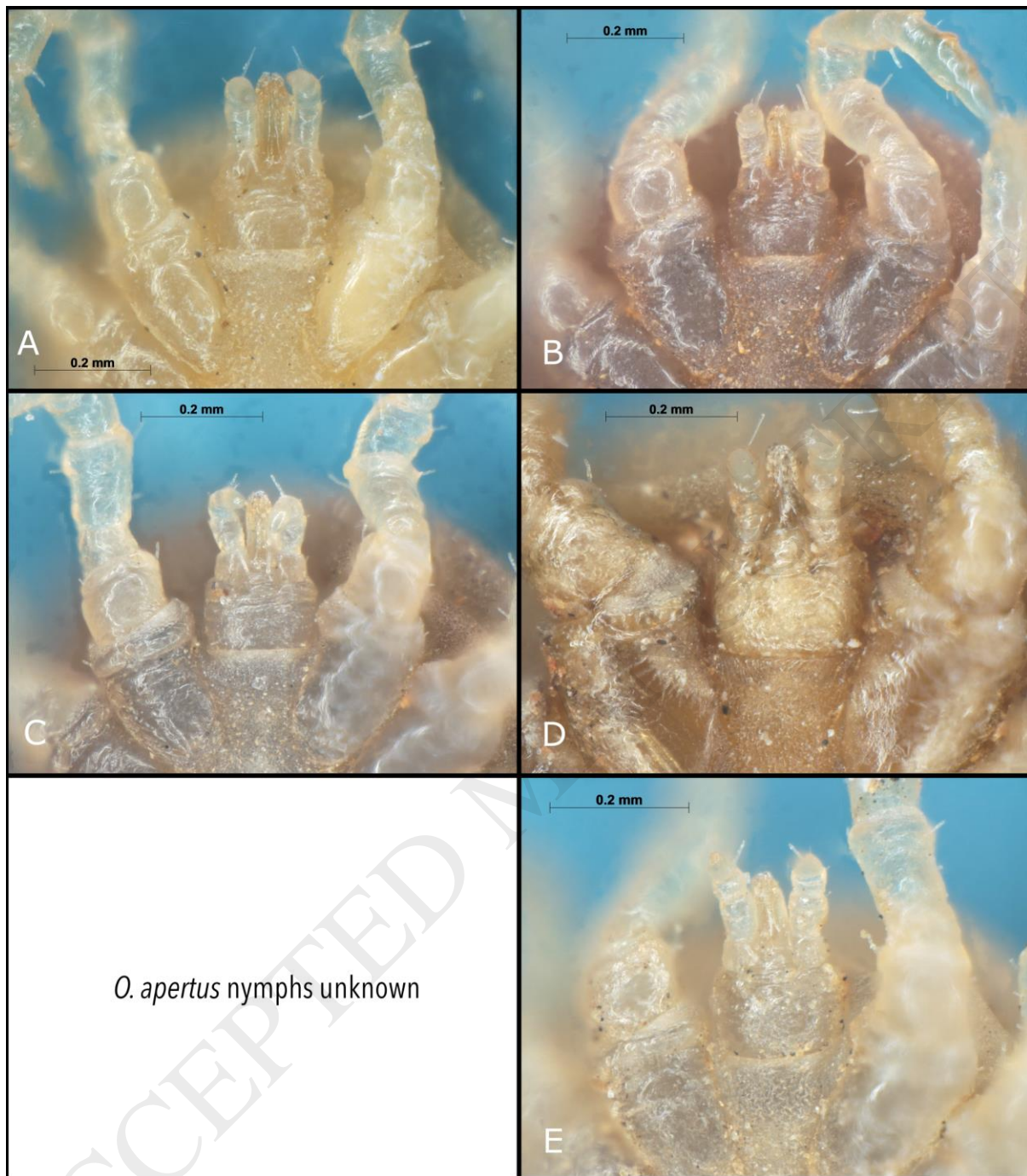




Fig 8



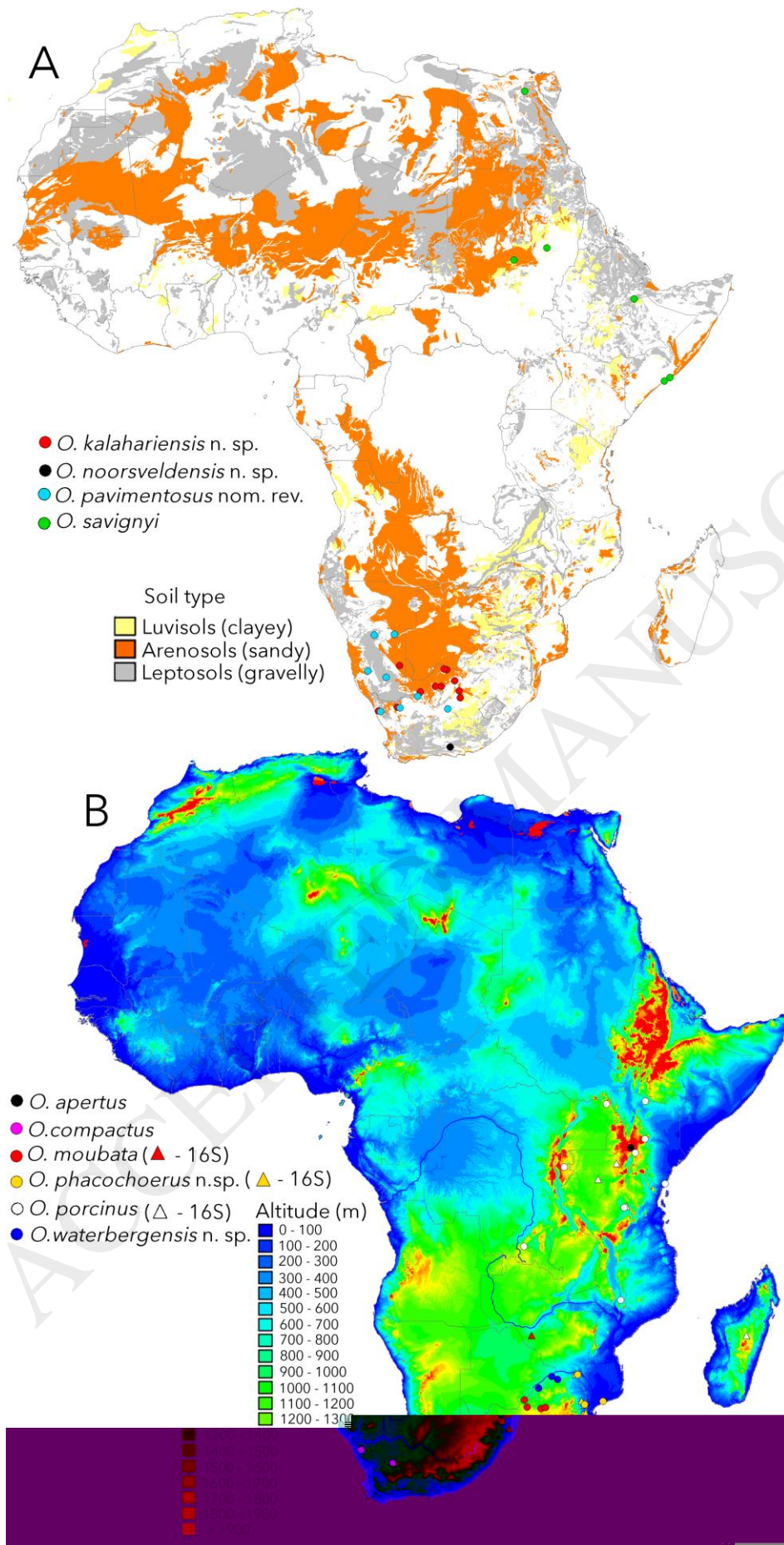
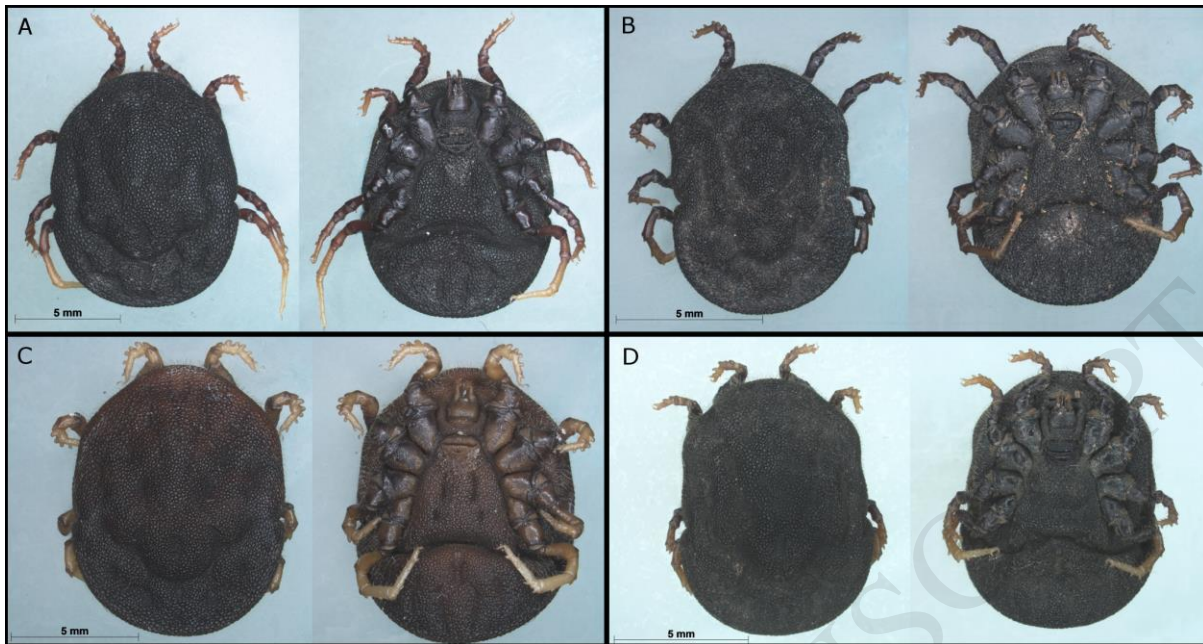


Fig 10



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Fig 11

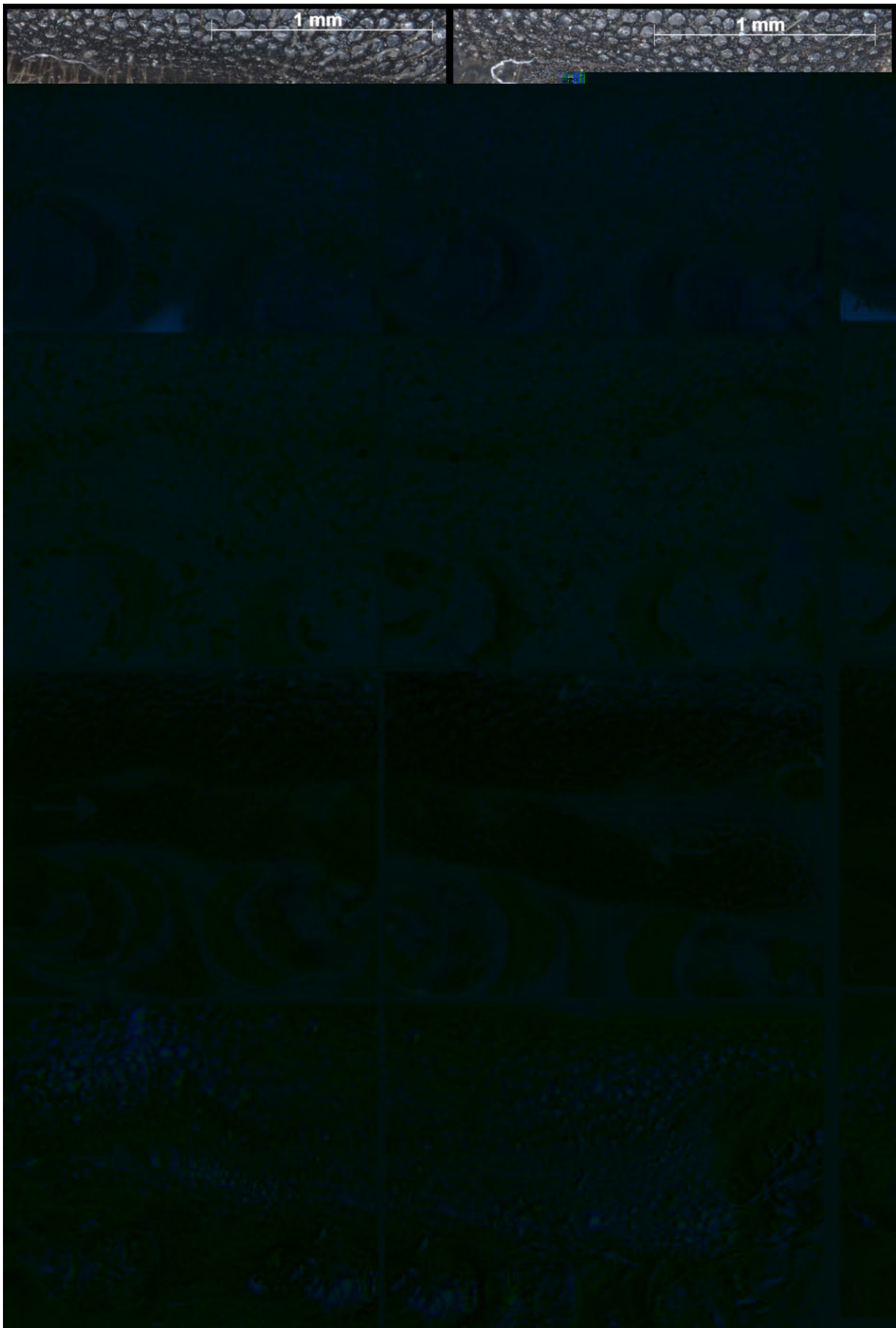


Fig 12



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Fig 13

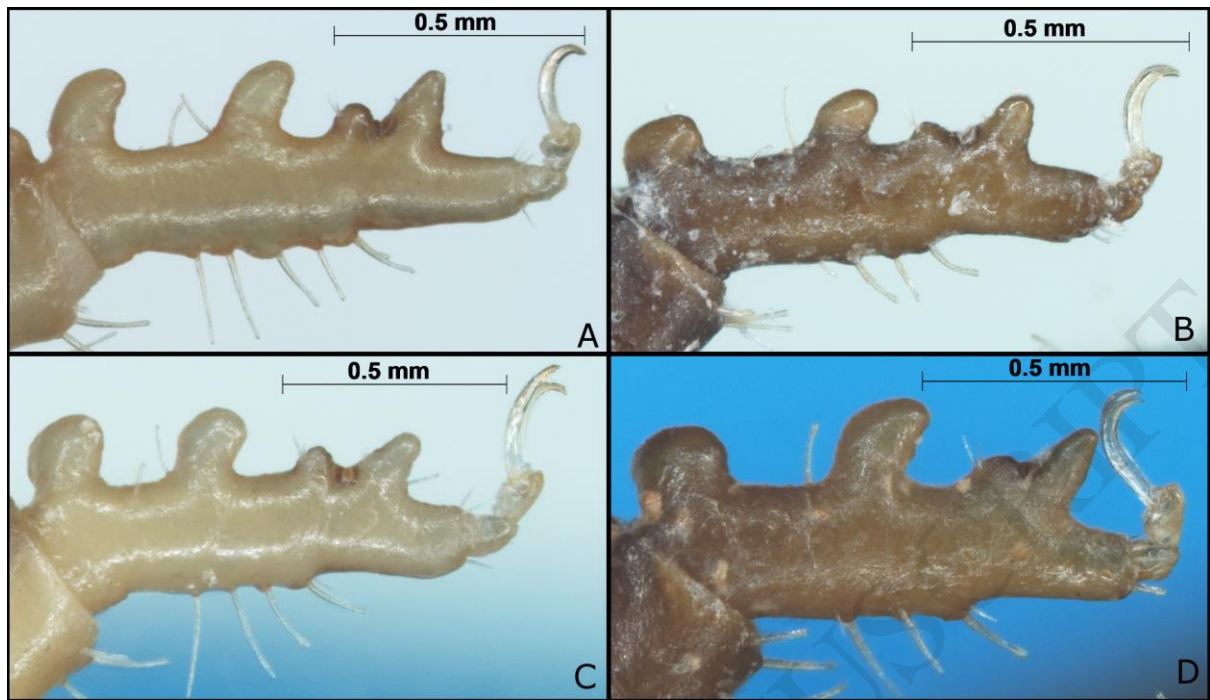
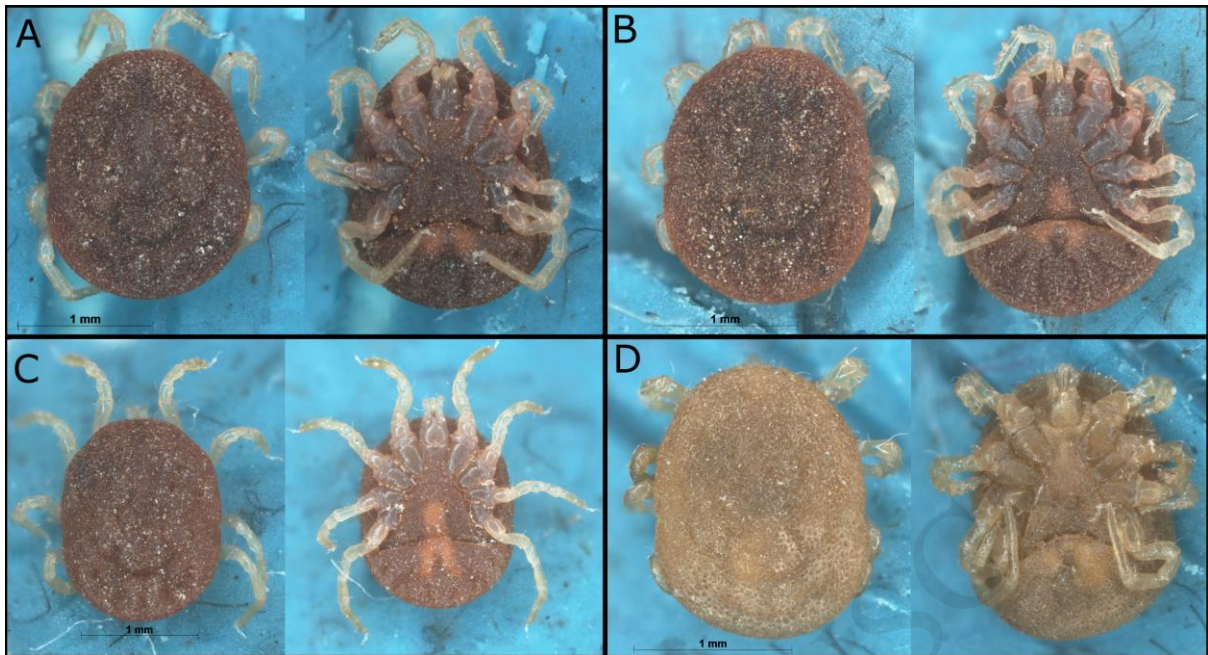


Fig 14



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Fig 15

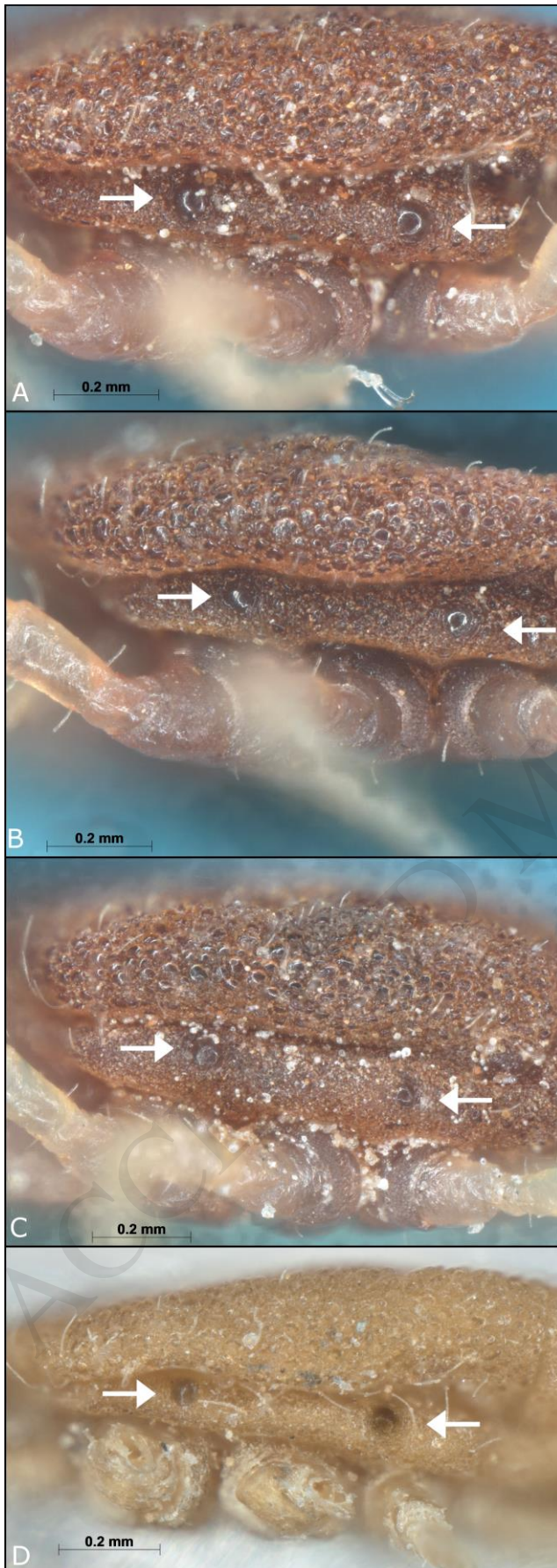
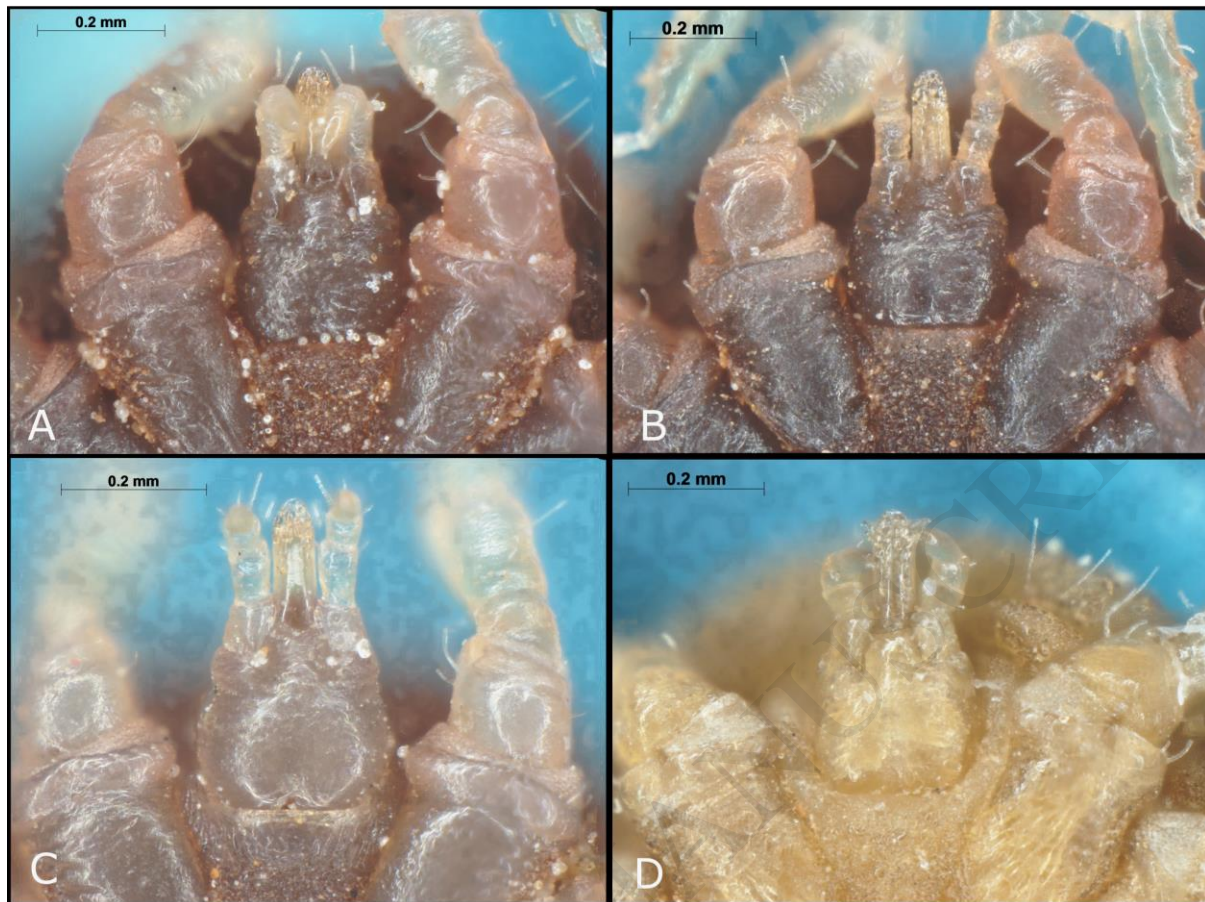




Fig 16



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Fig 17

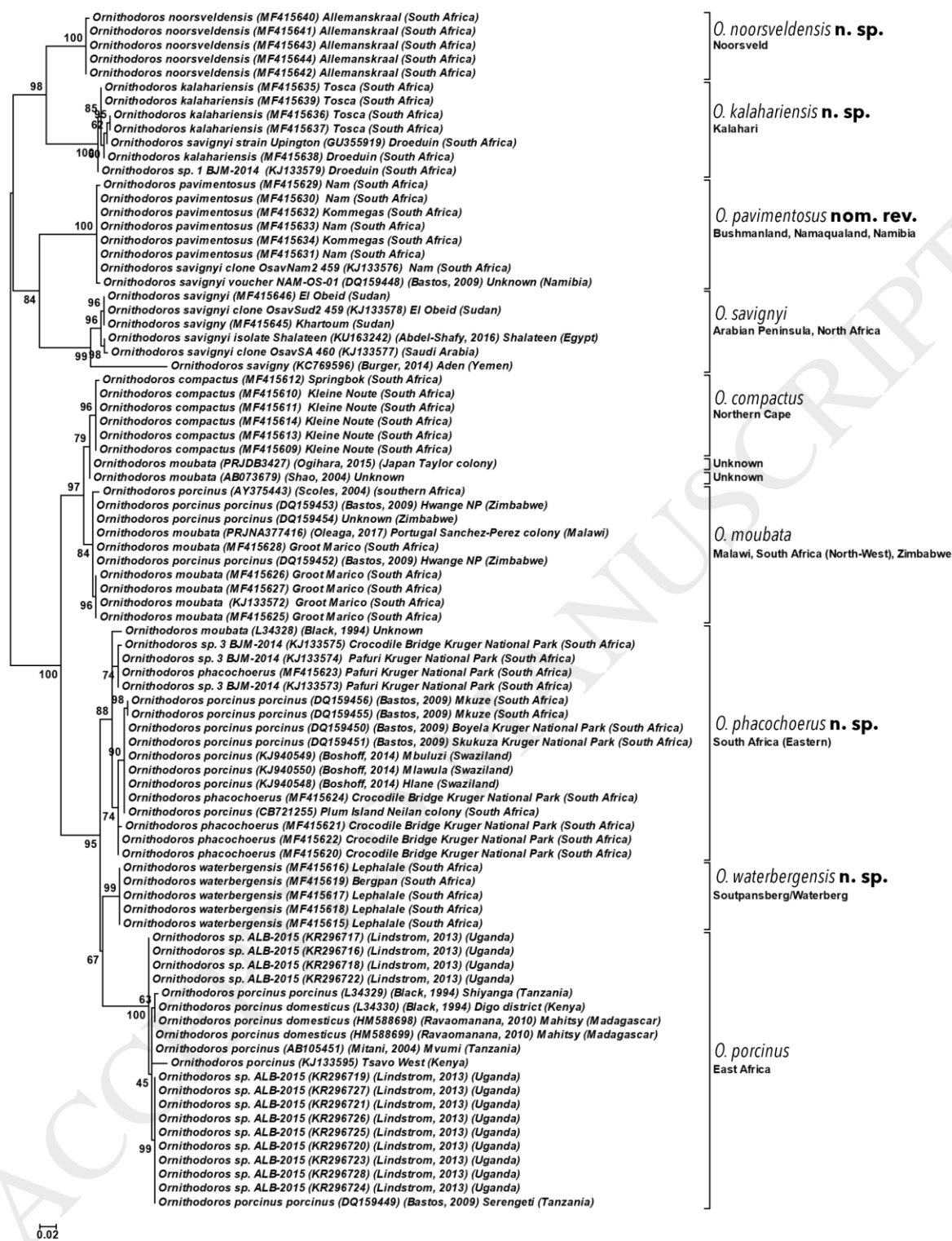


Fig 18

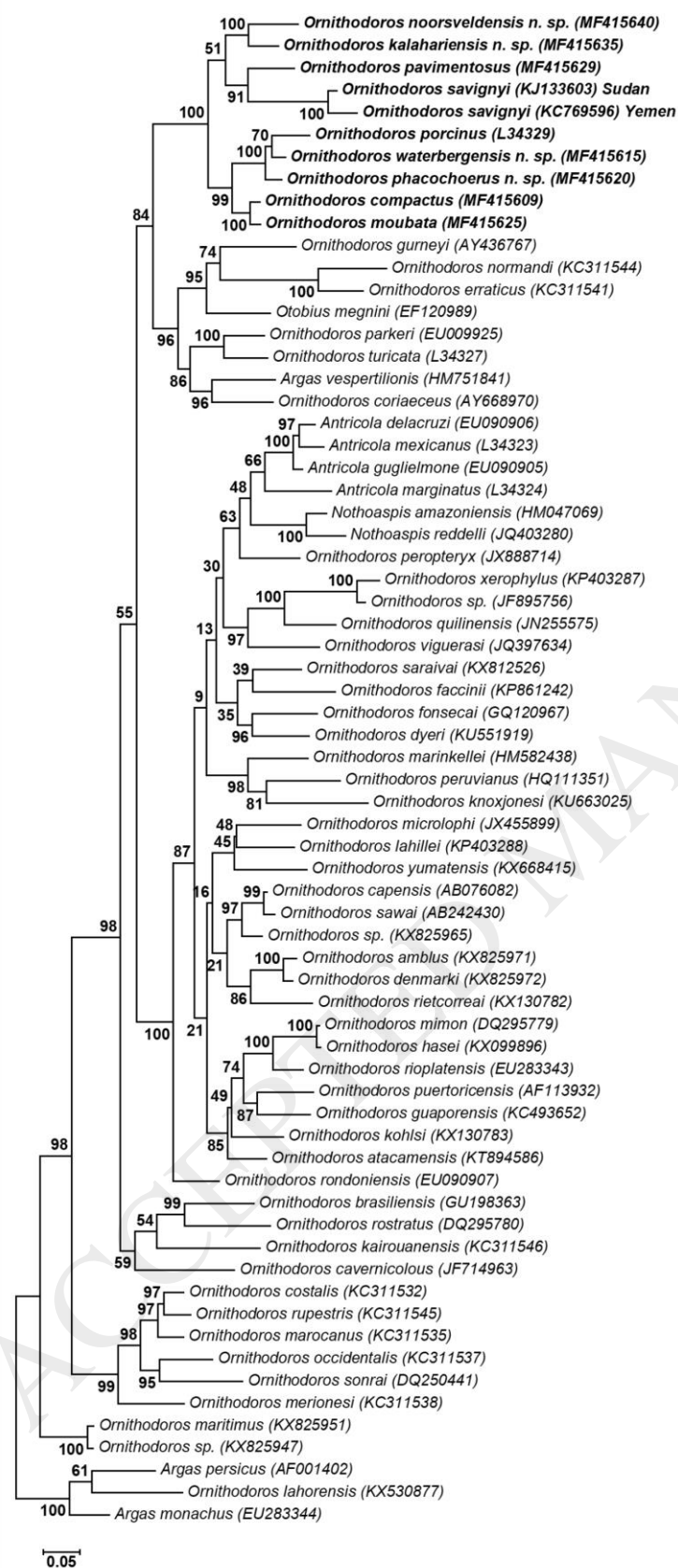
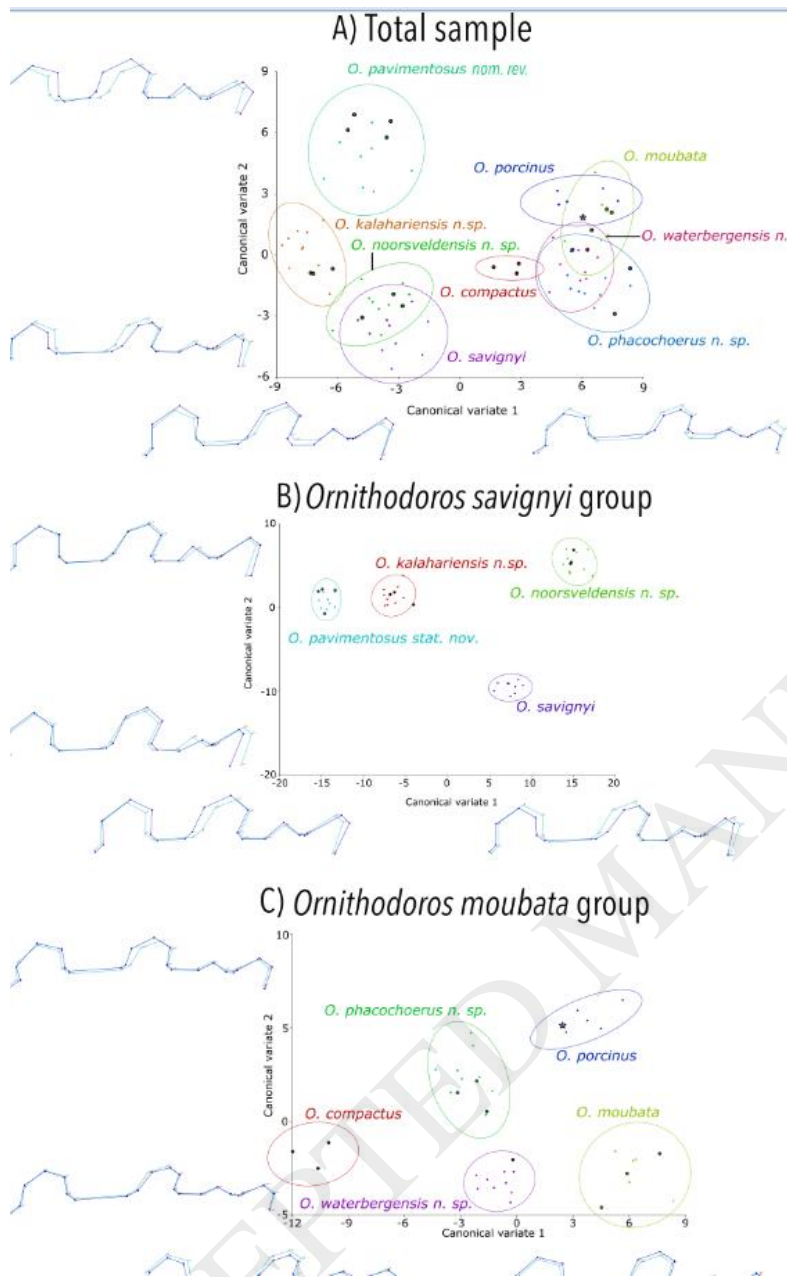


Fig 19



**Table 1.** Specimens used for 16S molecular phylogeny and tarsus I shape outline morphometric analyses.

Species	OP GTTM Code (morphometrics)	GenBank Code (16S phylogeny)	Locality	S/N	E
<i>O. compactus</i>	OP5051a	MF415611	Klein Noute	-31.376720	21.05672 6
	OP5051b	MF415613	Klein Noute	-31.376720	21.05672 6
	OP5051c	MF415614	Klein Noute	-31.376720	21.05672 6
		MF415609	Klein Noute	-31.376720	21.05672 6
		MF415610	Klein Noute	-31.376720	21.05672 6
		MF415612	Springbok	-29.672027	17.87715 2
<i>O. kalahariensis</i> n. sp.	OP5037ib		Nam	-29.451949	19.20192 3
	OP5039a		Kommagas	-29.790000	17.42848 4
	OP5039d		Kommagas	-29.790000	17.42848 4
		MF415635	Tosca	-25.878382	23.95773 3
	OP5041a		Tosca	-25.878382	23.95773 3
	OP5041b	MF415636	Tosca	-25.878382	23.95773 3
	OP5041c	MF415637	Tosca	-25.878382	23.95773 3
	OP5041d	MF415639	Tosca	-25.878382	23.95773 3
	OP5042a		Droeduin	-27.986705	21.42670 0
	OP5042b		Droeduin	-27.986705	21.42670 0
	OP5056a	MF415638	Droeduin	-27.986705	21.42670 0
	OP5056b		Droeduin	-27.986705	21.42670 0
	OP5056c		Droeduin	-27.986705	21.42670 0
	<i>O. moubata</i>	OP2413iiia		Wolhuterskop	-25.716437
OP2413iiib			Wolhuterskop	-25.716437	27.70472 1
OP5044a			Groot Marico (Type locality)	-24.901676	26.11992 5
OP5044b			Groot Marico (Type locality)	-24.901676	26.11992 5
OP5044c			Groot Marico (Type locality)	-24.901676	26.11992 5
		MF415625	Groot Marico (Type locality)	-24.901676	26.11992 5
OP5054a		MF415626	Groot Marico (Type locality)	-24.901676	26.11992 5

	OP5054b	MF415627	Groot Marico (Type locality)	-24.901676	26.11992 5
	OP5054c	MF415628	Groot Marico (Type locality)	-24.901676	26.11992 5
<i>O. noorsveldensis</i> n. sp.	OP3023iia		Allemanskraal	-33.248351	24.28837 4
	OP3023iib		Allemanskraal	-33.248351	24.28837 4
	OP3023iic		Allemanskraal	-33.248351	24.28837 4
	OP3023iid		Allemanskraal	-33.248351	24.28837 4
	OP3023iie		Allemanskraal	-33.248351	24.28837 4
	OP3023iif		Allemanskraal	-33.248351	24.28837 4
	OP5040a		Allemanskraal	-33.248351	24.28837 4
	OP5040b		Allemanskraal	-33.248351	24.28837 4
	OP5040c		Allemanskraal	-33.248351	24.28837 4
		MF415640	Allemanskraal	-33.248351	24.28837 4
		MF415641	Allemanskraal	-33.248351	24.28837 4
	OP5057a	MF415642	Allemanskraal	-33.248351	24.28837 4
	OP5057b	MF415643	Allemanskraal	-33.248351	24.28837 4
	OP5057c	MF415644	Allemanskraal	-33.248351	24.28837 4
<i>O. pavimentosus</i> nom. rev.	OP2419a		Wittenhorst, Namibia	-26.598394	18.14341 8
		MF415629	Nam	-29.451949	19.20192 3
	OP5037a		Nam	-29.451949	19.20192 3
	OP5037c		Nam	-29.451949	19.20192 3
	OP5037d		Nam	-29.451949	19.20192 3
	OP5037e		Nam	-29.451949	19.20192 3
	OP5055a	MF415630	Nam	-29.451949	19.20192 3
	OP5055b	MF415631	Nam	-29.451949	19.20192 3
	OP5055c	MF415633	Nam	-29.451949	19.20192 3
		MF415632	Kommagas	-29.790000	17.42848 4
	OP5039b		Kommagas	-29.790000	17.42848 4
	OP5039c		Kommagas	-29.790000	17.42848 4
	OP5058a	MF415634	Kommagas	-29.790000	17.42848 4
	OP5050a		Luderitz, Namibia	-26.004386	16.41552 3
<i>O. phacochoerus</i> n. sp.	OP5047a		Pafuri	-22.491673	31.23848 7
	OP5047b		Pafuri	-22.491673	31.23848 7
		MF415623	Pafuri	-22.491673	31.23848 7

	OP5048b		Crocodile Bridge	-25.350068	31.88669 1
	OP5048c		Crocodile Bridge	-25.350068	31.88669 1
	OP5048d		Crocodile Bridge	-25.350068	31.88669 1
	OP5048e		Crocodile Bridge	-25.350068	31.88669 1
	OP5048f		Crocodile Bridge	-25.350068	31.88669 1
	OP5048h		Crocodile Bridge	-25.350068	31.88669 1
	OP5048g		Crocodile Bridge	-25.350068	31.88669 1
	OP5053a	MF415620	Crocodile Bridge	-25.350068	31.88669 1
	OP5053b	MF415621	Crocodile Bridge	-25.350068	31.88669 1
	OP5053c	MF415622	Crocodile Bridge	-25.350068	31.88669 1
<i>O. porcinus</i>		KJ133595	Tsavo west, Kenya	3.631648	37.70003 2
	OP2645iia		???, Ruanda-Urundi	-2.644323	29.93816 4
	OP2645iib		???, Ruanda-Urundi	-2.644323	29.93816 4
	OP2645iic		???, Ruanda-Urundi	-2.644323	29.93816 4
	OP2645iid		???, Ruanda-Urundi	-2.644323	29.93816 4
	OP2645iie		???, Ruanda-Urundi	-2.644323	29.93816 4
	OP3088ia (paratype)		Mwitikira, Tanzania	-6.519769	35.66242 7
<i>O. savignyi</i>		MF415645	Khartoum, Sudan	15.487265	32.62519 9
	OP2775iia		El Manswiya, Egypt	29.407931	31.39574 4
	OP2775iib		El Manswiya, Egypt	29.407931	31.39574 4
	OP2958ia		Wad Medani, Sudan	14.425954	33.53979 3
	OP2958ib		Wad Medani, Sudan	14.425954	33.53979 3
	OP2958iia		Merca Station, Somalia	1.718481	44.76028 8
	OP2958iib		Merca Station, Somalia	1.718481	44.76028 8
		MF415646	El Obeid, Sudan	13.293591	30.36134 0
	OP5038a		El Obeid, Sudan	13.293591	30.36134 0
	OP5038b		El Obeid, Sudan	13.293591	30.36134 0
	OP5038c		El Obeid, Sudan	13.293591	30.36134 0
	OP5038d		El Obeid, Sudan	13.293591	30.36134 0
<i>O. waterbergensis n. sp.</i>	OP5043a		Lephalale	-23.775079	27.48170 4
	OP5043b		Lephalale	-23.775079	27.48170 4
		MF415615	Lephalale	-23.775079	27.48170 4
		MF415616	Lephalale	-23.775079	27.48170 4

	MF415617	Lephalale	-23.775079	27.48170 4
OP5052a	MF415618	Lephalale	-23.775079	27.48170 4
OP5046a		Bergpan	-22.956677	29.31672 7
OP5046b		Bergpan	-22.956677	29.31672 7
OP5046c		Bergpan	-22.956677	29.31672 7
OP5046d		Bergpan	-22.956677	29.31672 7
OP5046e		Bergpan	-22.956677	29.31672 7
	MF415619	Bergpan	-22.956677	29.31672 7
OP5049a		Tonash	-22.666625	28.74998 3
OP5049b		Tonash	-22.666625	28.74998 3

**Table 2.** Procrustes ANOVA of *Ornithodoros* tarsus I shape from three replicates on left side only (above), and five replicate pilot study on left and right sides (below).

Effect	Sum of Squares	Mean Squares	Degrees of Freedom	F	p
Species	0.69474083	0.0021710651	320	12.29	<0.0001
Individual	0.54406027	0.0001766429	3080	13.47	<0.0001
Replicate Error	0.09024474	0.0000131170	6880	-	-
Species	0.20339420	0.0053524790	38	13.73	<0.0001
Individual	0.17776111	0.0003898270	456	4.65	<0.0001
Side	0.00956014	0.0002515826	38	3.00	<0.0001
Individual x Side (Fluctuating Asymmetry)	0.04144306	0.0000838928	494	4.64	<0.0001
Replicate Error	0.03845811	0.0000180724	2128	-	-