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Original article

Argasid and ixodid systematics: Implications for soft tick evolution and systematics, with a new argasid species list

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ABSTRACT

The systematics of the genera and subgenera within the soft tick family Argasidae is not adequately resolved. Different classification schemes, reflecting diverse schools of scientific thought that elevated or downgraded groups to genera or subgenera, have been proposed. In the most recent classification scheme, *Argas* and *Ornithodoros* are paraphyletic and the placement of various subgenera remains uncertain because molecular data are lacking. Thus, reclassification of the Argasidae is required. This will enable an understanding of soft tick systematics within an evolutionary context. This study addressed that knowledge gap using mitochondrial genome and nuclear (18S and 28S ribosomal RNA) sequence data for representatives of the subgenera *Alectorobius*, *Argas*, *Chiropterargas*, *Ogadenus*, *Ornamentum*, *Ornithodoros*, *Navis* (**subgen. nov.**), *Pavlovskyella*, *Persicargas*, *Proknekalia*, *Reticulinasus* and *Secretargas*, from the Afrotropical, Nearctic and Palearctic regions. Hard tick species (Ixodidae) and a new representative of *Nuttalliella namaqua* (Nuttalliellidae), were also sequenced with a total of 83 whole mitochondrial genomes, 18S rRNA and 28S rRNA genes generated. The study confirmed the utility of next-generation sequencing to retrieve systematic markers. Paraphyly of *Argas* and *Ornithodoros* was resolved by systematic analysis and a new species list is proposed. This corresponds broadly with the morphological cladistic analysis of Klompen and Oliver (1993). Estimation of divergence times using molecular dating allowed dissection of phylogeographic patterns for argasid evolution. The discovery of cryptic species in the subgenera *Chiropterargas*, *Ogadenus* and *Ornithodoros*, suggests that cryptic speciation is common within the Argasidae. Cryptic speciation has implications for past biological studies of soft ticks. These are discussed in particular for the *Ornithodoros (Ornithodoros) moubata* and *Ornithodoros (Ornithodoros) savignyi* groups.

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1. Introduction

Ixodida (ticks) are composed of the families, Argasidae (soft ticks ~200 species), Ixodidae (hard ticks ~ 700 species), and the monotypic Nuttalliellidae (Guglielmone et al., 2010). Compared to soft ticks, hard ticks show greater species diversity with a larger number of species. However, recently there has been an upsurge in the discovery of argasid species, with 26 new species described, resurrected or unique sequences published (Bakkes et al., 2018; Barros-Battesti et al., 2015; Burger et al., 2014a; Dantas-Torres et al., 2012; Dupraz et al., 2016; Heath, 2012; Hornok et al., 2017; Labruna et al., 2016; Muñoz-Leal et al., 2017, 2016; Nava et al., 2013, 2010; Trape et al., 2013; Venzal et al., 2015, 2013, 2012; Vial and Camicas, 2009). Several of these species may be considered cryptic, which raises the possibility that the diversity of soft ticks remains to be fully discovered.

Historically, argasid systematics has been dominated by three main "schools" of scientific thought regarding taxonomic classification: American (Clifford et al., 1964; Hoogstraal, 1985), French (Camicas and Morel, 1977; Camicas, 1998) and Russian (Filippova, 1966; Pospelova-Shtrom, 1946, 1969) (Table 1). In these "schools", classification into tribes, genera and subgenera were based on unique morphological characters (synapomorphies) for various lineages as deduced by expert knowledge. The first formal cladistic analysis based on a matrix of 83 morphological characters (Klompen and Oliver, 1993), which could be considered a fourth classification school, grouped the Argasidae into the subfamilies Argasinae and Ornithodorinae. The Argasinae was considered monogeneric and included: ***Argas*** (subgenera *Argas, Persicargas, Secretargas, Ogadenus, Proknekalia, Alveonasus*), whereas the Ornithodorinae included the genera: ***Carios*** (subgenera *Alectorobius, Antricola, Carios, Chiropterargas, Nothoaspis, Parantricola, Reticulinasus, Subparmatus*), ***Ornithodoros*** (subgenera *Microargas, Ornamentum, Ornithodoros, Pavloskyella, Theriodoros*) and ***Otobius*** (subgenus *Otobius)*. The species lists of Barker and Murrell (2008, 2004) and Horak et al. (2002) used this classification, while Keirans (2009) and Guglielmone et al. (2010) reverted to the scheme of Hoogstraal (1985). In the most recent scheme, the subgenera *Carios* and *Chiropterargas* were moved to Argasinae, the subgenera *Alectorobius*, *Reticulinasus* and *Subparmatus* moved to *Ornithodoros*, while *Antricola* (incl. *Parantricola*), *Nothoaspis* and *Otobius* were retained as valid genera. The subgenera *Alveonasus* and *Proknekalia* were also moved from Argasinae to *Ornithodoros* (Guglielmone et al., 2010).

Morphotaxonomics has benefited from the application of molecular techniques to identify cryptic species in the Acari (Skoracka et al., 2015; Hornok et al., 2017). Thus, molecular data have challenged the current classification scheme. For example, a close genetic relationship has been found between *Alectorobius*, *Antricola* and *Nothoaspis* (Burger et al., 2014a; Estrada-Peña et al., 2010; Klompen and Oliver, 1993; Nava et al., 2009). Moving all members of the subgenus *Alectorobius* to

Table 1
Classification of the Argasidae by various schools of scientific thought regarding taxonomic classification (adapted from Klompen and Oliver (1993) and Burger et al. (2014a, 2014b)). Genera are in bold and subgenera italized. Subgenera in parentheses are included in the above genus or subgenus, but not recognised as valid by the authors of the scheme.

American school Clifford et al. (1964) Hoogstraal (1985) Guglielmone et al. (2010) Clifford et al., 1964; Hoogstraal, 1985; Guglielmone et al., 2010.	Soviet school Filippova (1966) Pospelova-Shtrom (1969) Pospelova-Shtrom, 1946; Filippova, 1966; Pospelova-Shtrom, 1969	French school Camicas and Morel (1977) Camicas (1998) Camicas and Morel, 1977; Camicas, 1998	Cladistic School Klompen and Oliver (1993) Klompen and Oliver, 1993	Proposed classification (current study) Current study
Argasinae	Argasinae	Argasinae	Argasinae	Argasinae
Argas	***Argas***	***Argas***	***Argas***	***Argas***
Argas	*Argas*	*Argas*	*Argas*	*Argas*
Persicargas	*Persicargas*	*Persicargas*	(incl. *Persicargas*)	*Persicargas*
		Carios		
Carios	*Carios*	*Carios*		
Chiropterargas	*Chiropterargas*	*Chiropterargas*		
		Ogadenus		***Ogadenus***
Ogadenus		*Ogadenus*	*Ogadenus*	*Ogadenus*
Secretargas	*Secretargas*	*Secretargas*	*Secretargas*	***Secretargas***
		Proknekalia	*Proknekalia*	***Proknekalia***
			Alveonasus	***Alveonasus***
Microargas				
Ornithodorinae	Ornithodorinae	Ornithodorinae	Ornithodorinae	Ornithodorinae
Otobius	***Otobius***	***Otobius***	***Otobius***	***Otobius***
Ornithodoros (s.l.)	***Alveonasus***	***Alveonasus***		
Alveonasus	*Alveonasus*	*Alveonasus*		
Proknekalia	*Proknekalia*			
	Ogadenus			
	Ornithodoros	***Ornithodoros***	***Ornithodoros (s.s.)***	***Ornithodoros***
Ornithodoros	*Ornithodoros*	*Ornithodoros*	(incl. *Ornithodoros,*	*Ornithodoros,*
Ornamentum	*Ornamentum*	*Ornamentum*	*Ornamentum,*	*Ornamentum,*
			Microargas,	*Microargas*
Pavlovskyella	*Pavlovskyella*	*Theriodoros*	*Pavlovskyella,*	*Pavlovskyella,*
(incl. *Theriodoros*)		(incl. *Pavlovskyella*)	*Theriodoros)*	*Theriodoros*
			Carios (s.l.)	***Carios***
Alectorobius	*Alectorobius*	*Alectorobius*	(incl. *Alectorobius,*	*Alectorobius,*
			Carios, Chiropterargas,	*Carios,*
Reticulinasus	*Reticulinasus*	*Reticulinasus*	*Reticulinasus,*	*Reticulinasus,*
Subparmatus	*Subparmatus*	*Subparmatus*	*Subparmatus,*	*Subparmatus,*
Antricola	*Antricola*	***Antricola***		
Antricola	***Antricola***		*Antricola,*	*Antricola,*
Parantricola	*Parantricola*	***Parantricola***	*Parantricola,*	*Parantricola,*
Nothoaspis		***Nothoaspis***	*Nothoaspis)*	*Nothoaspis*
		Microargas		
				Chiropterargas

Ornithodoros, while retaining the genera *Antricola* and *Nothoaspis*, makes *Ornithodoros* paraphyletic. Analysis of the 16S and 18S rRNA genes supports inclusion of *Alveonasus* in the Argasinae (Black et al., 1997; Klompen and Oliver, 1993; Pienaar et al., 2018). Members of the subgenus *Carios* were moved from Ornithodorinae to Argasinae by Guglielmone et al. (2010), while analyses of 12S and 16S rRNA, as well as mitochondrial cytochrome c oxidase I (COI) indicate that *Argas vespertilionis* (type species for *Carios*) grouped within the Ornithodorinae (Burger et al., 2014a; Hornok et al., 2017; Labruna et al., 2011; Lv et al., 2018). This makes *Argas* paraphyletic as well. A recent molecular study based on nuclear 18S and 28S ribosomal RNA and mitochondrial genome data, placed members of *Alectorobius*, *Antricola* and *Nothoaspis* as a Neotropical clade retaining their subgeneric status (Burger et al., 2014a). Importantly, potential non-neotropical members such as *Chiropterargas* or *Reticulinasus* were not included in this latter study. It appears that confusion and disagreement exists in argasid systematics, with most authors reserving comment on assignment of species to, and relationships between genera (Guzmán-Cornejo et al., 2016). In summary, there is a clear need to revise argasid systematics.

To resolve the apparent challenge within argasid systematics, the current study was undertaken using mitochondrial genome and nuclear (18S and 28S ribosomal RNA) sequence data from representatives of the different argasid tick subgenera. Further, this study was designed to compare molecular data from Afrotropical, Nearctic and Palearctic argasid genera to expand species data coverage. Implications for evolution, biology, biochemistry, speciation and systematics of argasids are discussed. Notably, a revised classification for Argasidae is proposed. Systematic markers for a number of ixodids from South Africa were also sequenced for comparative purposes and to contribute more sequences to the database.

2. Materials and methods

2.1. Tick identification and taxonomy conventions

Ticks were collected as described in the results and discussion. Ethics approval was granted by the ARC-OVR animal ethics committee (AEC 15.01), with Section 20 clearance (12/11/1/1). Relevant permits were obtained for collection in National Parks. Collected ticks were identified based on published keys and descriptions as referenced for individual species. For all species, their 12S rRNA, 16S rRNA or COI sequences were also compared to sequences in GenBank using BLASTN analysis (Altschul et al., 1990) as cross reference for species identification, where ≥96% identity was considered a species match (Table S1). Binomial nomenclature followed the latest proposed tick list (Guglielmone et al., 2010), while subgenera followed Clifford et al. (1964); Hoogstraal (1985) and Klompen and Oliver (1993).

2.2. Next-generation sequencing and assembly of mitochondrial and nuclear markers

For 83 different samples (Table S2), the next-generation sequencing strategy previously described by Mans et al. (2015) was used to obtain sequence data for assembly of full-length mitochondrial genomes, as well as 18S and 28S rRNA markers. Briefly, genomic DNA was extracted from ticks using the QIAamp DNA Blood kit (Qiagen, Valencia, CA, USA), as described before (Mans et al., 2011). Genomic DNA was submitted to the Biotechnology Platform Next Generation Sequencing Service of the Agricultural Research Council (South Africa). Samples were processed using either the Nextera or TruSeq DNA preparation kits (Illumina, San Diego, CA, USA) and sequenced using either the Illumina HiSeq 2500 or MiSeq sequencers. Sequence data were processed using CLC Genomics Workbench v.5.1 software (Qiagen) and quality trimmed (0.001 quality limit) and Nextera adapters removed. Reads were *de novo* assembled using standard assembly parameters [mismatch cost-2, insertion cost-3, deletion cost-3, length fraction-0.9, similarity-0.9, minimum contig length-200, automatic bubble size and a variable word size depending on the specific sample (Table S3)]. The same assembly strategy was employed for datasets obtained from the NCBI sequence read archive (SRA) and Transcriptome Shotgun Assembly (TSA) databases (Table S2). Parameters and statistics for assembly, coverage and total length of contigs as well as GenBank accession numbers are indicated in Tables S3-S6.

2.3. Bioinformatic analysis of nuclear data

The 18S and 28S rRNA genes were separately aligned using iterative alignment with MAFFT taking rRNA secondary structure into account (Q-INS-i) and a 1PAM / k = 2 scoring matrix (Katoh and Standley, 2013). Positions with less than 50% coverage were removed using GBLOCKS (Castresana, 2000). Maximum Likelihood analysis was performed in IQ-Tree 1.5.2 (Nguyen et al., 2015). The most optimal substitution model for each alignment was automatically selected (18S: TVMe + I + G4; 28S: TIM2e + I + G4) and absent genes treated as missing data. An edge-proportional partition model with proportional branch lengths (-spp) was used, to allow different rate parameters for each partition to accommodate different evolutionary rates between partitions. Nodal support was estimated using ultrafast bootstrap (n = 100,000) and the 50% consensus tree was reported.

For Bayesian analysis, the alignments produced were concatenated to produce a supermatrix that comprised 1814 positions. Absent genes were treated as missing data. Bayesian analysis was performed in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003), using a general time reversible (GTR) model of nucleotide substitution with a proportion of invariant sites and a gamma distribution of among site heterogeneity employing the nst = 6 rates = invgamma commands. Four categories were used to approximate the gamma distribution and two runs were performed simultaneously, each with four Markov chains (one cold, three heated) which ran for 15,000,000 generations. The first 4000,000 generations were discarded from the analysis (burnin) and every 100th tree was sampled to calculate a 50% majority-rule consensus tree from 109,983 trees. Nodal values represent the posterior probability that recovered clades exist given the sequence dataset, and were considered significant above 95% (Alfaro et al., 2003).

2.4. Bioinformatic analysis of mitochondrial protein data

Translated protein sequences for the cytochrome oxidase I (COI), cytochrome b (Cytb), NADH dehydrogenase subunit 1 (ND1), NADH dehydrogenase subunit 2 (ND2) and NADH dehydrogenase subunit 4 (ND4) genes were used for phylogenetic analysis (Mans et al., 2012). Additionally, COI sequences from selected soft tick species were included. Multiple alignment for each protein was performed separately using MAFFT with iterative alignment (FFT-NS-i) and the BLOSUM62 amino acid scoring matrix (Katoh and Standley, 2013). All sites with gapped positions in more than 50% of the sequences were removed using GBLOCKS (Castresana, 2000). Maximum Likelihood analysis was performed in IQ-Tree 1.5.2 (Nguyen et al., 2015). An optimal substitution model was calculated for each protein partition: COI (mtART + I + G4), Cytb (mtZOA + F+I + G4), ND1 (mtZOA + F +I + G4), ND2 (mtZOA + F+G4) and ND4 (mtZOA + F+I + G4). Absent protein genes were treated as missing data. An edge-proportional partition model with proportional branch lengths (-spp) was used to allow each partition its own specific rate to accommodate different evolutionary rates between partitions. Nodal support was estimated using ultrafast bootstrap (n = 100,000) and the 50% consensus tree was reported.

For Bayesian analysis, the alignments produced were concatenated to produce a supermatrix that comprised 1427 positions. Bayesian analysis was performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003), with the mtrev amino acid substitution model and a shape prior of 0.77, along with a proportion of invariant sites and a

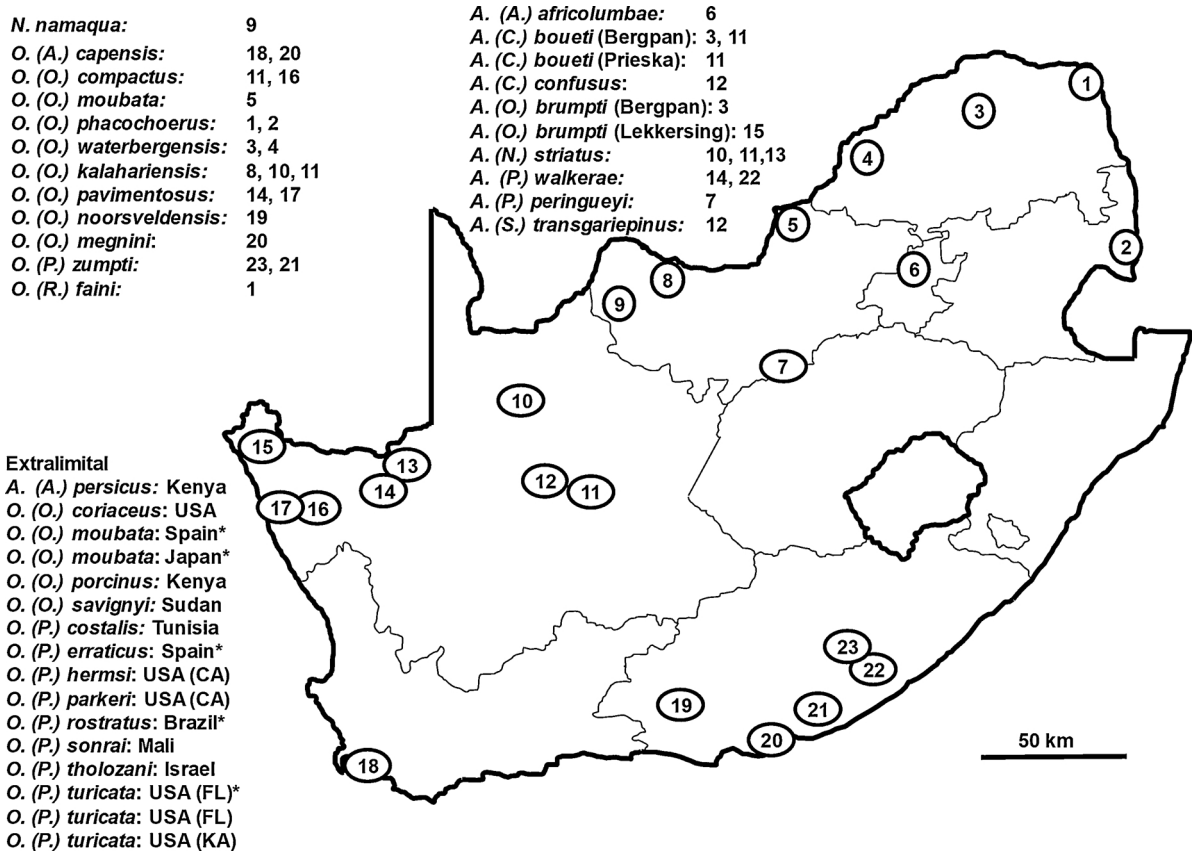

Fig. 1. Collection sites for various soft tick species in South Africa. Also indicated are collections included from outside South Africa as well as *Nuttalliella namaqua*. *Indicate data retrieved and assembled from the SRA database.

gamma distribution of among site heterogeneity employing the nst = 6 rates = invgamma commands. Two runs were performed simultaneously, each with four Markov chains (one cold, three heated) which ran for 5,000,000 generations. Split frequencies were analysed to determine where the data converged and was used to determine the burnin at 25% of the data. Every 100th tree was sampled to calculate a 50% majority-rule consensus tree from 14,959 trees. Nodal values represent posterior probability that the recovered clades exist given the sequence dataset, and were considered significant above 95% (Alfaro, 2003).

2.5. Automatic barcode gap discovery

Nucleotide sequences for the COI gene were aligned using MAFFT yielding an alignment with 1515 bp and no gapped positions. This alignment was submitted to the Automatic Barcode Gap Discovery (ABGD) Server (http://wwwabi.snv.jussieu.fr/public/abgd/) that calculates a barcode gap from the inference of a model-based one-sided confidence limit for intraspecific divergence (Puillandre et al., 2012). Minimum and maximum prior intraspecific divergences (0.001–0.1) were scanned over 10 steps with a minimum gap width (1.0) and distance distribution as Nn bins (20). Pairwise-distances were calculated using the Kimura two parameter model (K2P). Iterative limit inference and gap detection produced results of partitioning data into groups which were predicted to be species units.

2.6. Evolutionary placement algorithm- Poisson tree processes (EPA-PTP)

The Maximum Likelihood tree obtained for the mitochondrial protein sequences was submitted to the Evolutionary placement algorithm-Poisson tree processes (EPA-PTP) server (http://species.h-its.org/ptp/) (Zhang et al., 2013). The tree was rooted with the Pycnogonida and 500,000 Markov Chain Monte Carlo (MCMC) generations were performed, with thinning set at 100. Convergence of MCMC chains was confirmed at a burnin of 0.5.

2.7. Pairwise sequence comparisons of complete mitochondrial genomes

Genus specific mitochondrial genomic sequences were iteratively aligned using the MAFFT server (FFT-NS-i) and the 200PAM / k = 2 scoring matrix. Pairwise sequence identities were calculated from these alignments using Genedoc (Nicholas et al., 1997).

2.8. Molecular clock estimation

Molecular dating analysis was performed using the Maximum Likelihood mitochondrial consensus tree in PhyloBayes 3.3b (Lartillot et al., 2009) using the protein sequence alignment used for the tree construction. The tree was calibrated using fossil data for minimum divergence dates of the Pycnogonida-Chelicerata (501 MYA) (Waloszek and Dunlop, 2002), Xiphosura-Parasitiformes (445 MYA) (Rudkin et al., 2008), Holothyrida-Mesostigmata (130 MYA) (Peñalver et al., 2017), *Carios* (96 MYA) (Klompen and Grimaldi, 2001), *Amblyomma* (100 MYA) (Chitimia-Dobler et al., 2017; Grimaldi et al., 2002) and *Haemaphysalis* (100 MYA) (Chitimia-Dobler et al., 2018). Since overestimation of the molecular clock remains a problem in dating (Duchêne et al., 2014; Rodríguez-Trelles et al., 2002), the terminal extant nodes were constrained by using species replicates where multiple mitochondrial genomes were available. Divergence limits for replicates were estimated using the highest measured substitution rate observed in arthropods (1E-7 substitutions/site/generation) (Haag-Liautard et al., 2008), for the upper limit and calculating a lower limit based on the number of differences observed. A log-normal autocorrelated relaxed clock (-ln) was assumed, with a uniform prior on

divergence times. A gamma prior of mean 2000 and standard deviation of 2000 million years was specified for the age of the root. Two runs were done for each analysis to determine whether convergence had occurred. For each run, approximately twenty five to thrirty thousand cycles were performed, of which the first 2500 were discarded from analysis.

3. Results and discussion

3.1. Tick specimens used in the current study

Argasid ticks were collected over a large region of South Africa as well as extralimitally (Fig. 1; Table S2). *Nuttalliella namaqua* and ixodid ticks were also collected. Primary references used to identify specimens are cited for each species. The collections include:

3.2. Argasinae

Argas (Argas) africolumbae Hoogstraal, Kaiser, Walker, Ledger, Converse and Rice, 1975 (Hoogstraal et al., 1975), collected from Pretoria. *Argas (Chiropterargas) boueti* Roubaud and Colas-Belcour, 1933 (Hoogstraal, 1955), collected in the Northern Cape (Prieska) and northern Limpopo (Bergpan), 985 km apart. *Argas (Chiropterargas) confusus* Hoogstraal, 1955 (Hoogstraal, 1955), collected in the Northern Cape. *Argas (Ogadenus) brumpti* Neumann, 1907 (Hoogstraal, 1956), were collected in the Northern Cape (Lekkersing) and northern Limpopo (Bergpan), ~1400 km apart. *Argas (Persicargas) persicus* (Oken, 1818) (Kaiser and Hoogstraal, 1969), collected in Kenya. *Argas (Persicargas) walkerae* Kaiser and Hoogstraal, 1969 (Kaiser and Hoogstraal, 1969), collected in the Eastern Cape and Northern Cape, ~856 km apart. *Argas (Proknekalia) peringueyi* (Bedford and Hewitt, 1925), representative of the subgenus *Proknekalia* (Hoogstraal, 1985), collected in the North West Province. *Argas (Secretargas) transgariepinus* White, 1846 (Hoogstraal, 1957), the type specimen of the subgenus *Secretargas*, was collected in the Northern Cape near the type locality (Pienaar et al., 2018).

Argas (Navis) striatus Bedford, 1932 (Bedford, 1932), assigned to a new sub-genus (*Navis*) **subgen. nov.**, collected from sociable weaver nests in the Northern Cape, in an area ~180-300 km wide. This new subgenus is proposed given its unique phylogenetic position relative to other subgenera, as described in the current study as well as based on analysis of the 16S rRNA gene (Pienaar et al., 2018). It was never formally assigned to any subgenus (Hoogstraal, 1985), although Camicas et al. (1998) treated it as a member of the subgenus *Argas*. Etymology: *Navis* – Latin for ship, due to its general shape, that includes parallel sides, anterior portion that narrows to a round point, and with its margin transversally striated, raised above its dorsal surface and bisected by the sutural line (Bedford, 1932), giving a distinct appearance of a ship. The only other species that belongs to this subgenus is *Argas (N.) aequalis* (Neumann, 1901), a species known from a single nymph from Tanzania (Neumann, 1908), that was not included in the recent tick list (Guglielmone et al., 2010).

3.3. Ornithodorinae

Ornithodoros (Alectorobius) capensis Neumann, 1901 was sampled from penguins in the Eastern and Western Cape, the original type locality for this species (Neumann, 1901).

Species from the *Ornithodoros (Ornithodoros) moubata* group included *Ornithodoros (Ornithodoros) compactus* Walton, 1962 (Bakkes et al., 2018), from the Northern Cape (South Africa). *Ornithodoros (Ornithodoros) moubata* (Murray, 1877) (Bakkes et al., 2018), collected from the neo-type locality in the Groot Marico district and two colony strains from Spain (Oleaga et al., 2017), and Japan (Ogihara et al., 2015). *Ornithodoros (Ornithodoros) porcinus* Walton, 1962 (Bakkes et al., 2018), collected in West Tsavo, Kenya; *Ornithodoros (Ornithodoros) phacochoerus* Bakkes, de Klerk and Mans, 2018 (Bakkes et al., 2018), collected in the Kruger National Park; and *Ornithodoros (Ornithodoros) waterbergensis* Bakkes, de Klerk and Mans, 2018 (Bakkes et al., 2018), from the Soutpansberg and Waterberg areas.

Species from the *Ornithodoros (Ornithodoros) savignyi* group included *Ornithodoros (Ornithodoros) kalahariensis* Bakkes, de Klerk and Mans, 2018 (Bakkes et al., 2018), from the Kalahari; *Ornithodoros (Ornithodoros) noorsveldensis* Bakkes, de Klerk and Mans, 2018 (Bakkes et al., 2018), from the Noorsveld; *Ornithodoros (Ornithodoros) pavimentosus* Neumann, 1901 (Bakkes et al., 2018), from Namaqualand and Bushmanland; and *Ornithodoros (Ornithodoros) savignyi* (Audouin, 1826) (Bakkes et al., 2018) collected in Sudan, which is regionally close to the original type locality in Egypt.

Ornithodoros (Ornamentum) coriaceus Koch, 1844 was obtained from a colony kept at Rocky Mountain Laboratories (RML), California, USA.

For Pavlovskyella, *Ornithodoros (Pavlovskyella) erraticus* (Lucas, 1849) was obtained from a colony kept in Spain (Oleaga et al., 2015). *Ornithodoros (Pavlovskyella) costalis* Diatta, Bouattour, Durand, Renaud and Trape, 2013 (Trape et al., 2013), from Tunisia. *Ornithodoros (Pavlovskyella) hermsi* Wheeler, Herms and Meyer, 1935, from a colony kept at RML. *Ornithodoros (Pavlovskyella) parkeri* Cooley, 1936 SLO strain (San Luis Obispo, California) from a colony kept at RML. *Ornithodoros (Pavlovskyella) rostratus* Cooley, 1936 from the SRA database. *Ornithodoros (Pavlovskyella) sonrai* Sautet and Witkowski, 1943 from Mali (Sautet and Witkowski, 1944). *Ornithodoros (Pavlovskyella) tholozani* (Laboulbène and Mégnin, 1882), the type specimen of *Pavlovskyella*, from a colony kept at RML but originally from Israel. *Ornithodoros (Pavlovskyella) turicata* (Dugès, 1876) FLO strain (Florida) and KAN strain (Kansas) were from colonies kept at RML and another dataset from a transcriptome study (Egekwu et al., 2016). *Ornithodoros (Pavlovskyella) zumpti* Heisch and Guggisberg, 1953 (Heisch and Guggisberg, 1953), collected in the Eastern Cape, ~125 km apart, as the only representative of the subgenus *Pavlovskyella* described in southern Africa (Hoogstraal et al., 1985). *Ornithodoros (Reticulinasus) faini* Hoogstraal, 1960 (Hoogstraal, 1960), collected in a single cave in South Africa previously described (Braack, 1989), which is the only representative of the subgenus *Reticulinasus* described in southern and Eastern Africa (Hoogstraal, 1985).

Otobius (Otobius) megnini (Dugès, 1883) was collected in Port Elizabeth, South Africa (Walker et al., 2003).

3.4. Nuttalliellidae

Nuttalliella namaqua Bedford, 1931 (Nuttalliellidae) (Bedford, 1931), was collected from Heuningvleipan, 640 km from Springbok, the locality previously sampled (Mans et al., 2011, 2012).

3.5. Ixodidae

Ixodid ticks collected in South Africa included *Amblyomma hebraeum* Koch, 1844, *Amblyomma marmoreum* Koch, 1844 and *Amblyomma tholloni* Neumann, 1899 (Hoogstraal, 1956; Robinson, 1926; Theiler and Salisbury, 1959). *Dermacentor rhinocerinus* (Denny, 1843; Hoogstraal, 1956). *Hyalomma rufipes* Koch, 1844 and *Hyalomma truncatum* Koch, 1844 (Hoogstraal, 1956; Apanaskevich and Horak, 2008a, 2008b). *Ixodes rubicundus* Neumann, 1904 and *Ixodes simplex* Neumann, 1906 (Arthur, 1965). *Rhipicentor nuttalli* Cooper and Robinson, 1908 (Theiler, 1961). *Rhipicephalus appendiculatus* Neumann, 1901, *Rhipicephalus (Boophilus) decoloratus* Koch, 1844, *Rhipicephalus evertsi* Neumann, 1897, *Rhipicephalus maculatus* Neumann, 1901, *Rhipicephalus (Boophilus) microplus* (Canestrini, 1888), *Rhipicephalus simus* Koch, 1844 and *Rhipicephalus zambeziensis* Walker, Norval and Corwin, 1981. *Rhipicephalus* species were identified according to Walker et al. (2000) and Walker et al. (2003).

Sequence read archive data were also mined to obtain mitochondrial and nuclear markers for *Amblyomma aureolatum* (Pallas, 1772),

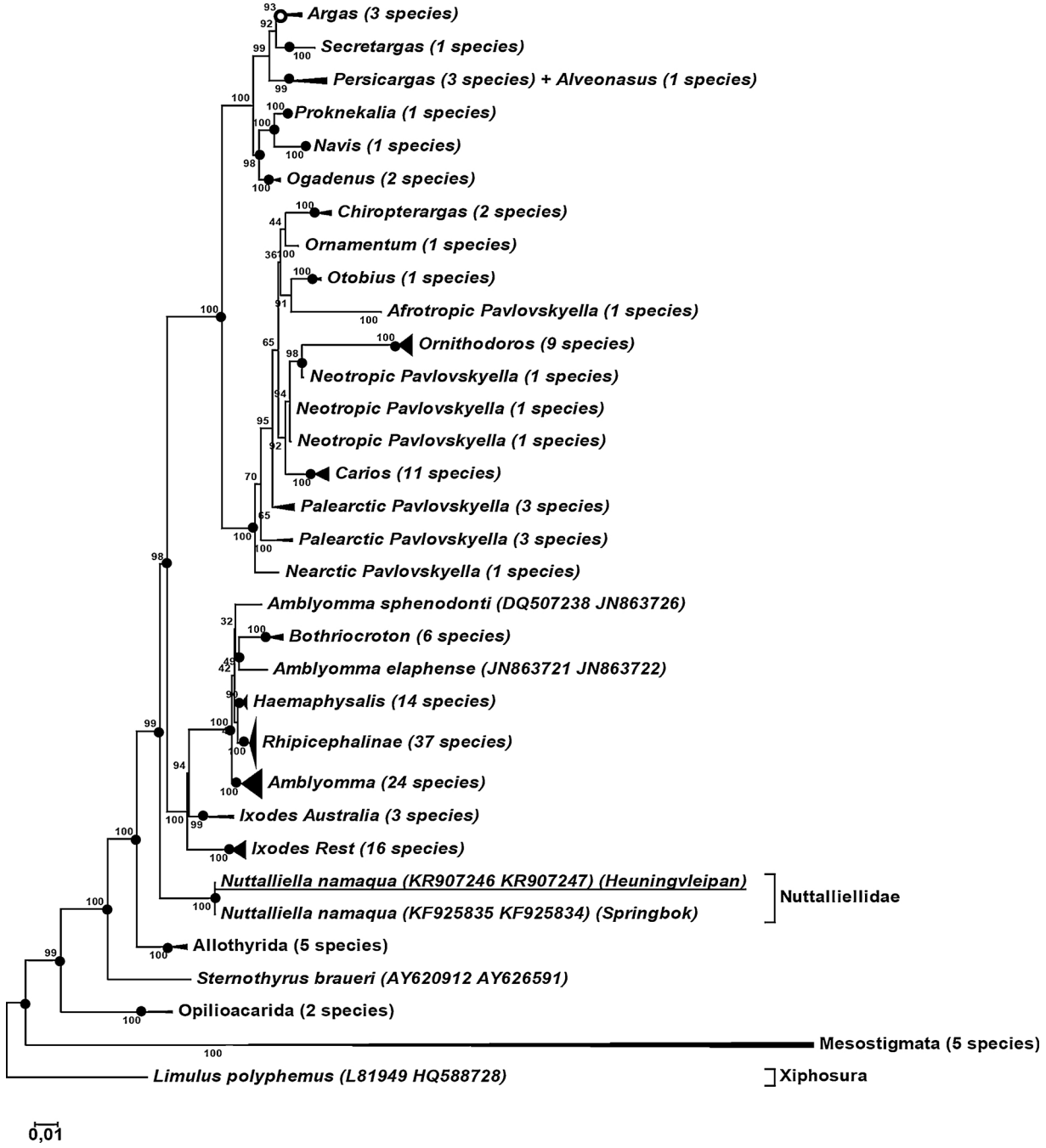

Fig. 2. The 18S-28S phylogenetic analysis for the Ixodida, represented at genus and subgenus level. Nodal support are for Maximum Likelihood analysis, while black dots represent > 95% and white dots > 90% Bayesian posterior probability. Ticks sequenced in the current study are underlined. Accession numbers of 18S and 28S ribosomal sequences are indicated in parentheses.

Amblyomma sculptum (Berlese, 1888), *Dermacentor variabilis* (Say, 1821), *Hyalomma excavatum* Koch, 1844, *Rhipicephalus bursa* Canestrini and Fanzago, 1878 and *Rhipicephalus pulchellus* (Gerstäcker, 1873). In total, 92 different datasets were analysed for which mitochondrial and nuclear markers could be extracted, making this the largest phylogenetic study to date on argasid and ixodid ticks.

3.6. Phylogenetic analysis using nuclear 18S-28S ribosomal RNA datasets

Phylogenetic analysis of combined 18S and 28S genes yielded results similar to previous studies (Burger et al., 2014a; Mans et al., 2015, 2011). Novel or pertinent observations in the current study include: 1) Ixodida are monophyletic with Holothyrida as the closest sister group (Klompen et al., 2007) (Fig. 2). 2) *Nuttalliella namaqua* grouped as a separate lineage at the base of the tick tree in accordance with its designation as a separate family (Mans et al., 2011) (Fig. 2). 3) *Ixodes* from the Afrotropical region group with non-Australian *Ixodes* (Fig. 3). 4) *Ixodes simplex* collected from the Bakwena Cave showed 6 nucleotide differences in the 18S rRNA gene compared to *I. simplex simplex*, that suggest a possible species complex (Hornok et al., 2015), which could include *I.* sp. *incertae* previously described by Arthur (1956) from the same locality, although it is currently not recognized as a valid species (Guglielmone et al., 2010). 5) *Ixodes rubicundus* group in a clade with *Ixodes pilosus* and *Ixodes corwini* (Fig. 3), and all these species are restricted to South Africa (Walker, 1991). 6) Afrotropical *Amblyomma* group within *Amblyomma* in a clade composed of African and South

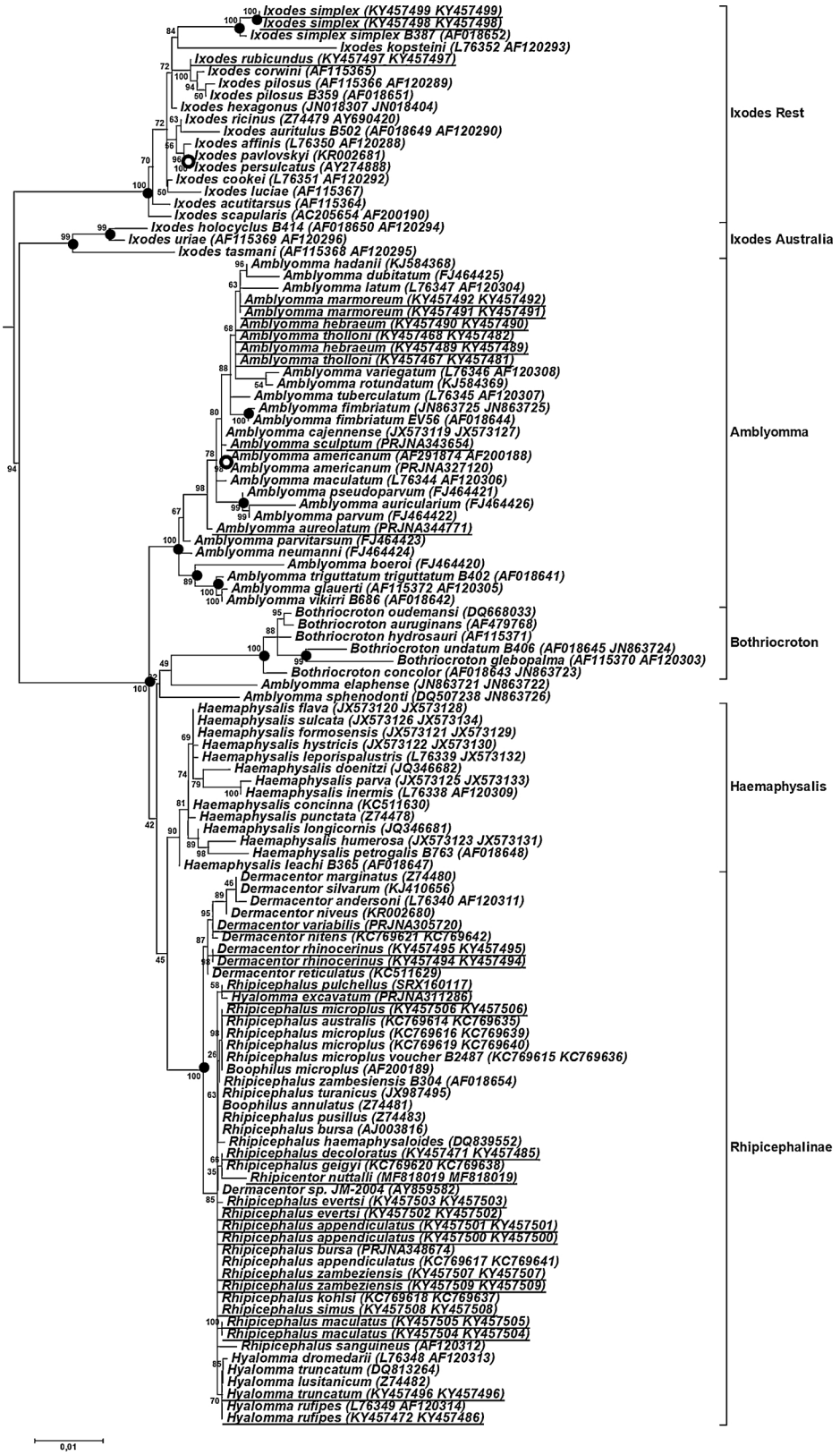

Fig. 3. The 18S-28S phylogenetic analysis for the Ixodidae. Nodal support are for Maximum Likelihood analysis, while black dots represent > 95% and white dots > 90% Bayesian posterior probability. Ticks sequenced in the current study are underlined. Species are represented by their binomial nomenclature (Guglielmone et al., 2010). Accession numbers of 18S and 28S ribosomal sequences are indicated in parentheses.

American species (Fig. 3). 7) *Dermacentor rhinocerinus* group within *Dermacentor* (Fig. 3). 8) The subgenus *Ornithodoros* group within a monophyletic clade (Bakkes et al., 2018) (Fig. 4). 9) *Pavlovskyella* group within Ornithodorinae, but are paraphyletic (Fig. 4). 10) *Chiropterargas* group within Ornithodorinae in a monophyletic clade (Fig. 4), that excludes *Carios* sensu Klompen and Oliver (1993). 11) *Argas (C.) boueti*

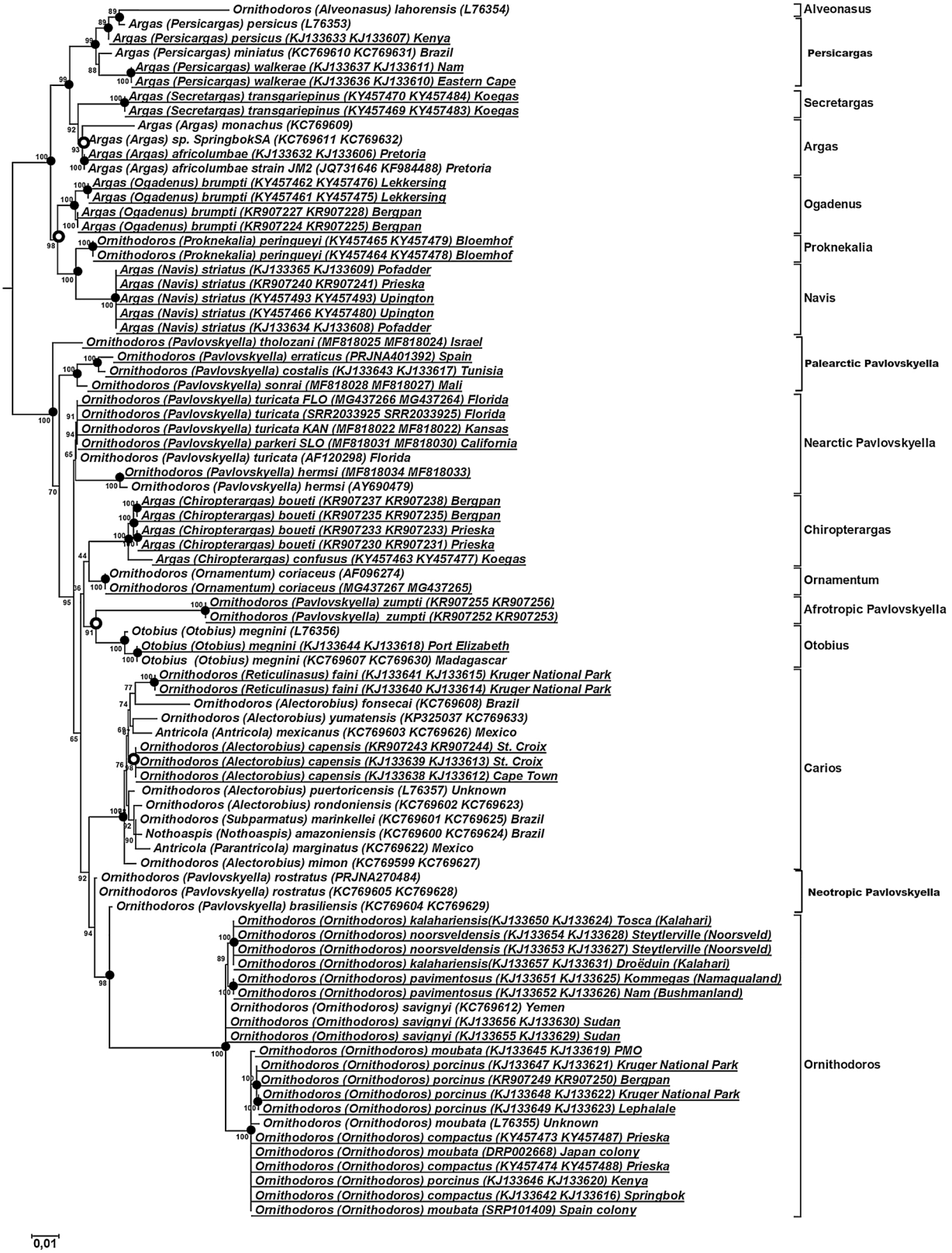

Fig. 4. The 18S-28S phylogenetic analysis for the Argasidae. Nodal support are for Maximum Likelihood analysis, while black dots represent > 95% and white dots > 90% Bayesian posterior probability. Ticks sequenced in the current study are underlined. Species are represented by their binomial nomenclature (Guglielmone et al., 2010) and subgeneric designation according to Hoogstraal (1985). Accession numbers of 18S and 28S ribosomal sequences are indicated in parentheses. Origins for various sequences are also indicated.

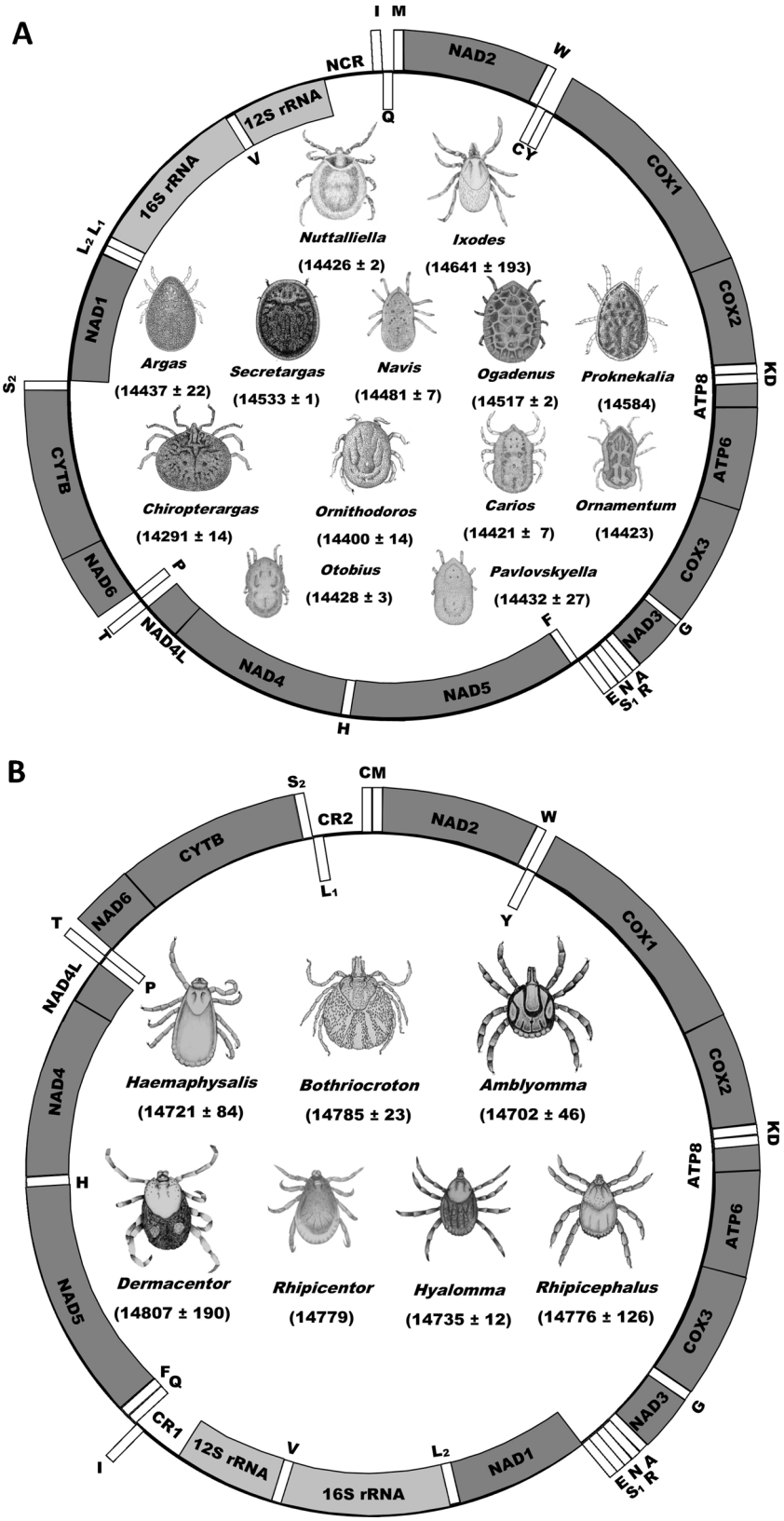

Fig. 5. Maps of the mitochondrial gene arrangement for Argasidae, Metastriates, Prostriates, and Nuttalliellidae. Genes on the outside are on the forward strand, while genes on the inside are on the reverse or complementary strand. Protein genes are in dark grey, ribosomal genes in light grey and transfer RNA in white boxes. Also indicated are mitochondrial genome sizes and size variation where known (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

group in two well supported monophyletic clades, suggesting multiple species (Fig. 4). 12) Members of the genus *Carios* sensu Klompen and Oliver (1993), form a monophyletic clade in Ornithodorinae and includes the subgenera *Alectorobius*, *Antricola*, *Nothoaspis*, *Parantricola*, *Reticulinasus* and *Subparmatus* (Fig. 4). 13) The subgenera *Alveonasus*, *Argas*, *Navis*, *Ogadenus*, *Persicargas*, *Proknekalia* and *Secretargas* group in Argasinae (Fig. 4). 14) The subgenera *Argas* and *Persicargas* are not monophyletic due to inclusion of *Secretargas*. 15) The previously monotypic subgenus *Ogadenus* form two well supported clades, suggesting multiple species (Fig. 4).

3.7. Mitochondrial genome structure

Argasid and *Ixodes* mitochondrial genomes analysed in this study showed the genome arrangement as previously described for argasids, *N. namaqua* and prostriates (Fig. 5). This corresponds to the ancestral arrangement for arthropods, as found in the horseshoe crab (Black and Roehrdanz, 1998; Burger et al., 2014a; Campbell and Barker, 1998; Lavrov et al., 2000; Mans et al., 2012; Montagna et al., 2012; Shao et al., 2004, 2005). Metastriate ticks analysed in the present study had the mitochondrial genome arrangement as previously reported for metastriates (Fig. 5) (Black and Roehrdanz, 1998; Burger et al., 2014b, 2013, 2012; Campbell and Barker, 1998).

3.8. Phylogenetic analysis of mitochondrial markers

Phylogenetic analysis of mitochondrial markers gave results that were largely consistent with previous analysis (Burger et al., 2014a; de Lima et al., 2018; Mans et al., 2012). Observations pertinent to the current study include: 1) Monophyly of Ixodida, with three clades representing the different tick families (Mans et al., 2012) (Fig. 6). 2) Ixodidae are monophyletic (Burger et al., 2014b, 2013, 2012; Mans et al., 2012) (Fig. 6). 3) The genus *Ixodes* is monophyletic, with Australian and non-Australian lineages forming separate clades (Fig. 7). 4) Afrotropical *Amblyomma* grouped within *Amblyomma* in a clade composed of African and South American species (Fig. 7). 5) Rhipicephalinae form a monophyletic clade with *Dermacentor* and *Rhipicentor* basal to other rhipicephalines (Murrell et al., 2001; Barker and Murrell, 2004; Burger et al., 2014b) (Fig. 7). 6) *Hyalomma* group within Rhipicephalinae basal to *Rhipicephalus* as previously proposed (Murrell et al., 2001; Barker and Murrell, 2008) (Fig. 7). 7) All *Rhipicephalus* species form a monophyletic group (Barker and Murrell, 2008, 2004) (Fig. 7). 8) *Rhipicephalus sanguineus* (Latreille, 1806), *R. simus* and *Rhipicephalus turanicus* Pomerantzev, 1940 form a monophyletic clade (*R. sanguineus* group), and *R. appendiculatus* and *R. zambeziensis* forms a monophyletic clade (*R. appendiculatus* group) (Barker and Murrell, 2008) (Fig. 7). These two clades are basal to other *Rhipicephalus* as described previously (Burger et al., 2014b). 9) *Rhipicephalus maculatus* and *R. pulchellus* form a monophyletic clade (*R. pulchellus* group) (Barker and Murrell, 2004) (Fig. 7). 10) *Rhipicephalus bursa* and *R. evertsi* form a monophyletic clade (*R. bursa* group) basal to members of the former genus, now subgenus *Boophilus* (Barker and Murrell, 2004) (Fig. 7). 11) *Rhipicephalus* (*Boophilus)* species forms a monophyletic clade within *Rhipicephalus* (Burger et al., 2014b) (Fig. 7). Addition of *R. (B.) decoloratus* has now completed representation of the members of this subgenus. 12) *Rhipicephalus (B.) microplus* from South Africa also groups with genotypes obtained from Brazil as previously proposed (Burger et al., 2014b) (Fig. 7). 13) Monophyly of Argasidae, comprising Argasinae and Ornithodorinae (Burger et al., 2014a; Mans et al., 2012) (Fig. 8). 14) Grouping of *Argas. (P.) persicus* and *A. (P.) walkerae* in a clade with other members of *Argas* and *Persicargas* within Argasinae (Fig. 8). 15) Monophyly of the subgenera *Argas*, *Ogadenus* and *Persicargas* (Fig. 8). 16) Grouping of the subgenera *Navis* **subgen. nov.**, *Proknekalia* and *Secretargas* in a monophyletic clade within Argasinae to the exclusion of *Argas, Persicargas* and *Ogadenus* (Fig. 8). 17) Multiple clades suggesting multiple species within the previous monotypic subgenus *Ogadenus* (Fig. 8). 18) Grouping of the subgenus *Chiropterargas* within Ornithodorinae, but not in the clade formed by *Carios* sensu Klompen and Oliver (1993), as well as presence of multiple species in *A. (C.) boueti* (Fig. 8). 19) Grouping of the subgenera *Alectorobius*, *Antricola*, *Carios*, *Nothoaspis*, *Reticulinasus* and *Subparmatus* in a clade formed by *Carios* sensu Klompen and Oliver (1993) (Fig. 8). 20) Grouping of the subgenus *Pavlovskyella* within Ornithodorinae, but in separate clades for different geographic lineages, indicating paraphyly (Klompen and Oliver, 1993) (Fig. 8). 21) Monophyly of the subgenus *Ornithodoros*, with different species of the *O. (O.) savignyi* and *O. (O.) moubata* groups, as supported by 16S rRNA and morphometric analysis (Bakkes et al., 2018) (Fig. 8).

3.9. Species delimitation

The mitochondrial gene tree suggests that species groups exist, which may be cryptic. To obtain an estimation of which clades represent unique species, the Automatic Barcode Genetic Distance Discovery (ABGD) (Puillandre et al., 2012) and the evolutionary placement algorithm- Poisson tree processes (EPA-PTP) (Zhang et al., 2013) servers were used for species delimitation (Collins and Cruickshank, 2013). Using an aligned COI subset and the ABGD server, 109 species groups were recognized and corresponded to phylogenetic designations for the various clades (Table S7). Similar results were obtained using the EPA-PTP server for the ML tree obtained from the mitochondrial dataset, which yielded 95 species (Table S7). These species designations allow interpretation of pairwise sequence comparisons of the whole mitochondrial genomes, where pairwise nucleotide identities below 94% indicate different species and identities of 96% or above indicate the same species (Table S8). Pairwise identities of 95% based on mitochondrial genome alignment seem to designate a barcoding gap in ticks, while identities from 93%–94% may indicate cryptic species.

Species delimitation, pairwise comparisons and phylogenetic analyses indicated that at least four different species exist among ticks that were previously designated as *O. (O.) savignyi* (Theiler and Hoogstraal, 1955). These species correspond to geographic areas, notably the Noorsveld of the Eastern Cape [*O.* (*O.*) *noorsveldensis*], Namaqualand and Bushmanland [*O.* (*O.*) *pavimentosus*], the Kalahari [*O.* (*O.*) *kalahariensis*], and north Africa [*O.* (*O.*) *savignyi*]. Of these, the north African samples represent the type locality and are widely distributed across north Africa and the Middle East, most probably due to dispersal along the camel trade routes (Hoogstraal, 1956). The presence of at least three different species in southern Africa, suggests that multiple species may be present in isolated semi desert-like areas across Africa (Leeson, 1953). Taxonomic differentiation based on classical morphology and morphometric analysis support the validity of these new species (Bakkes et al., 2018).

In the *O. (O.) moubata* group, five distinct species were observed, notably *O. (O.) compactus*, *O. (O.) moubata*, *O. (O.) phacochoerus*, *O. (O.) porcinus*, and *O. (O.) waterbergensis*. The original mitochondrial genome of *O. (O.) moubata* and the Japan colony form a unique lineage that groups with *O. (O.) compactus*. Conversely, the *O. (O.) moubata* colony from Spain groups with *O. (O.) moubata* from the type locality.

In the subgenus *Ogadenus*, at least two species exist in this previously monotypic subgenus (Hoogstraal, 1985). Given that both putative *Ogadenus* species from this study occur in southern Africa, and that the original type specimen was described from the Ogaden region in Ethiopia (~4000 km north from Soutpansberg), it is likely that South African specimens are not *A. (O.) brumpti* sensu stricto, but represent two new species instead. The biology of this subgenus comprising various hosts (birds, mammals and reptiles), various ecotypes (caves, rocks and termite mounds), rapid feeding of nymphs and adults, and its secretive nature, suggests limited dispersal by host migration (Hoogstraal, 1985, 1956). As such, multiple species in this genus may be expected. Using a distance of ~1400 km from the current study as

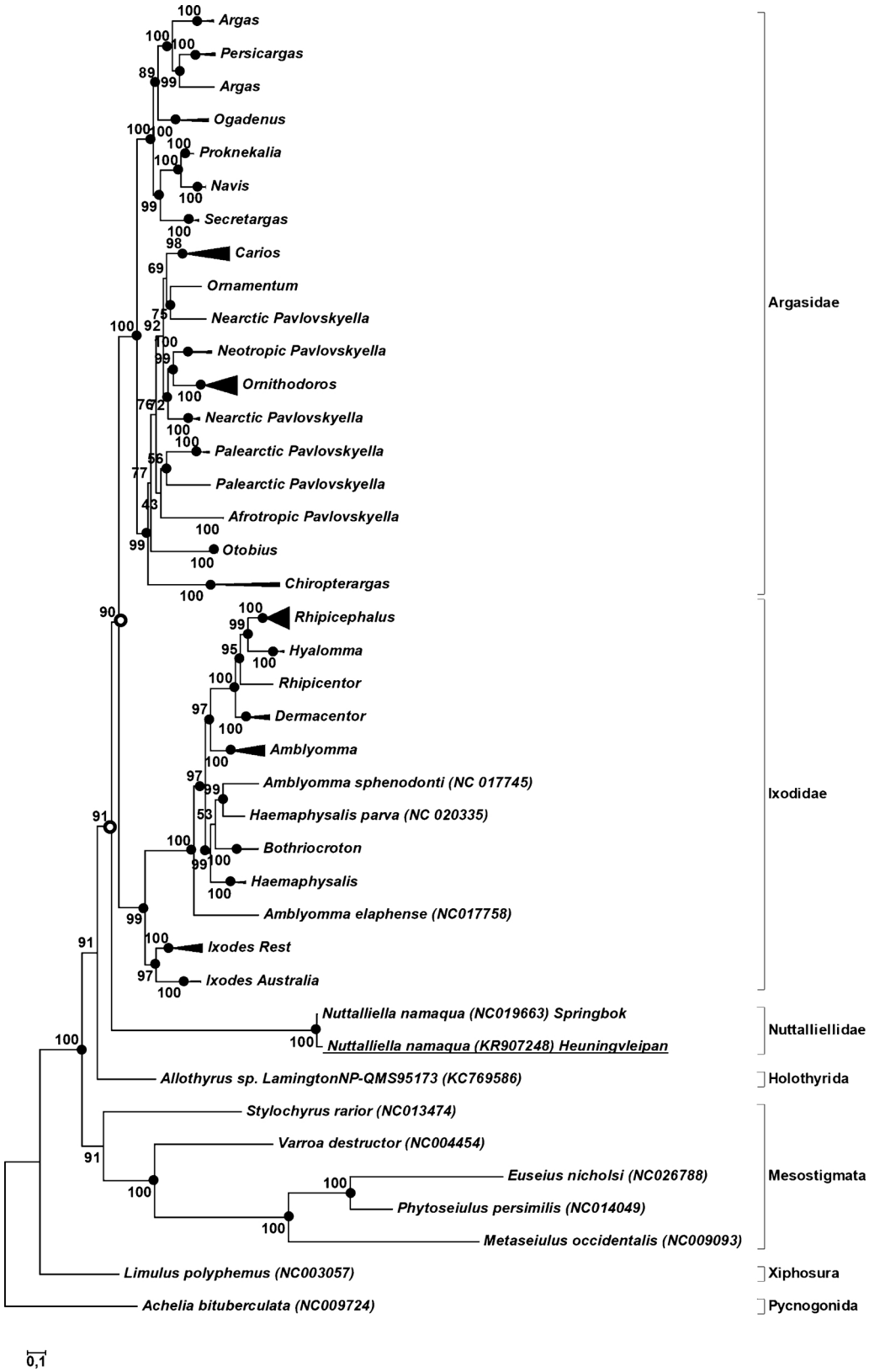

Fig. 6. The mitochondrial protein phylogenetic analysis for the Ixodida, represented at genus and subgenus level. Nodal support are for maximum likelihood analysis, while black dots represent > 95% and white dots > 90% Bayesian posterior probability. Ticks sequenced in the current study are underlined. Accession numbers of mitochondrial genomes are indicated in parentheses.

proxy for geographic isolation, the number of existing species may be greater than 10 given the widespread distribution of this genus across Africa (Hoogstraal, 1956).

Based on COI data there may also exist cryptic species in *A. (S.) transgariepinus*. The current study collected specimens near the type locality, while larvae were collected previously from the Soutpansberg

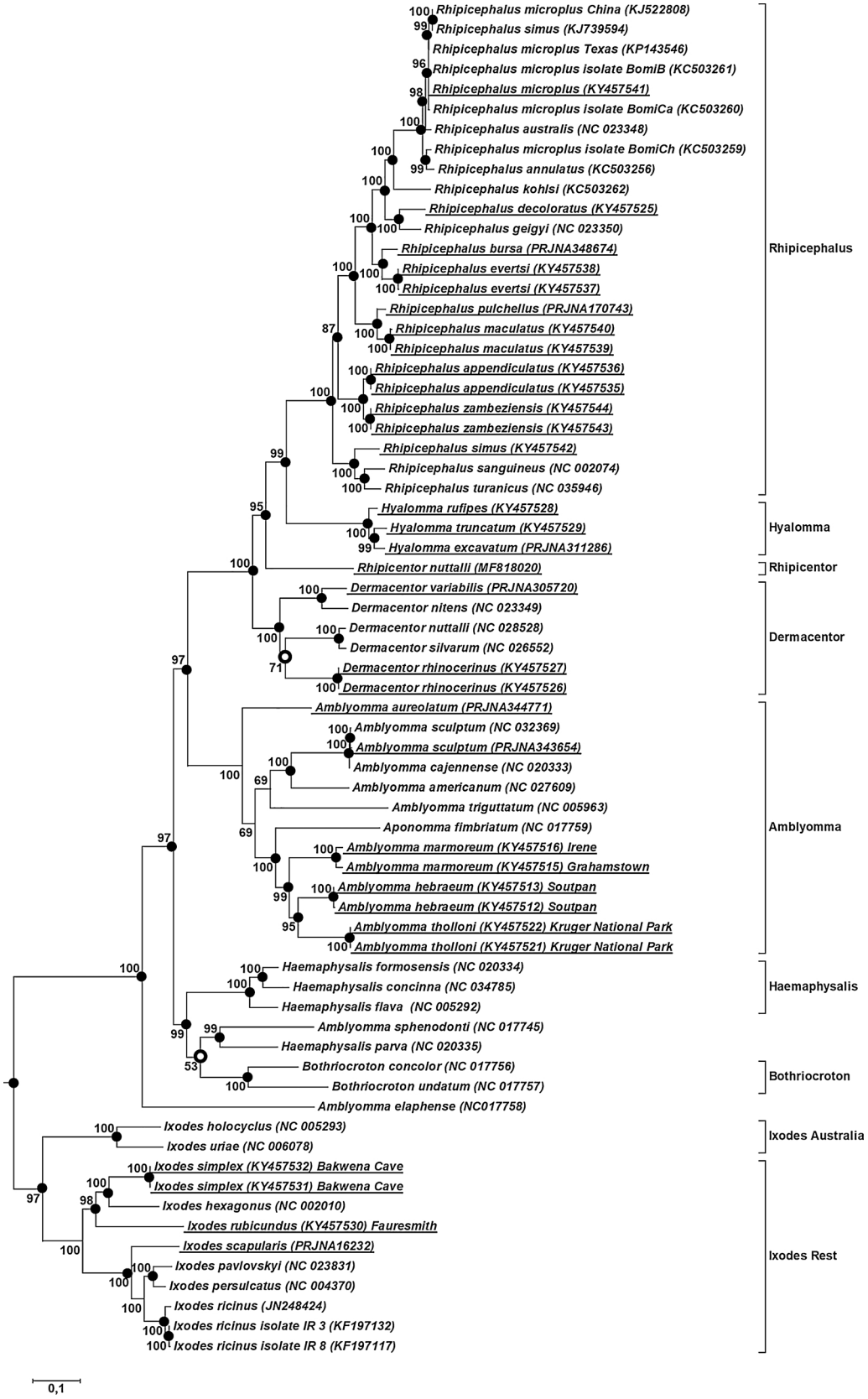

Fig. 7. The mitochondrial protein phylogenetic analysis for the Ixodidae. Nodal support are for Maximum Likelihood analysis, while black dots represent > 95% and white dots > 90% Bayesian posterior probability. Ticks sequenced in the current study are underlined. Species are represented by their binomial nomenclature (Guglielmone et al., 2010). Accession numbers of mitochondrial genomes are indicated in parentheses.

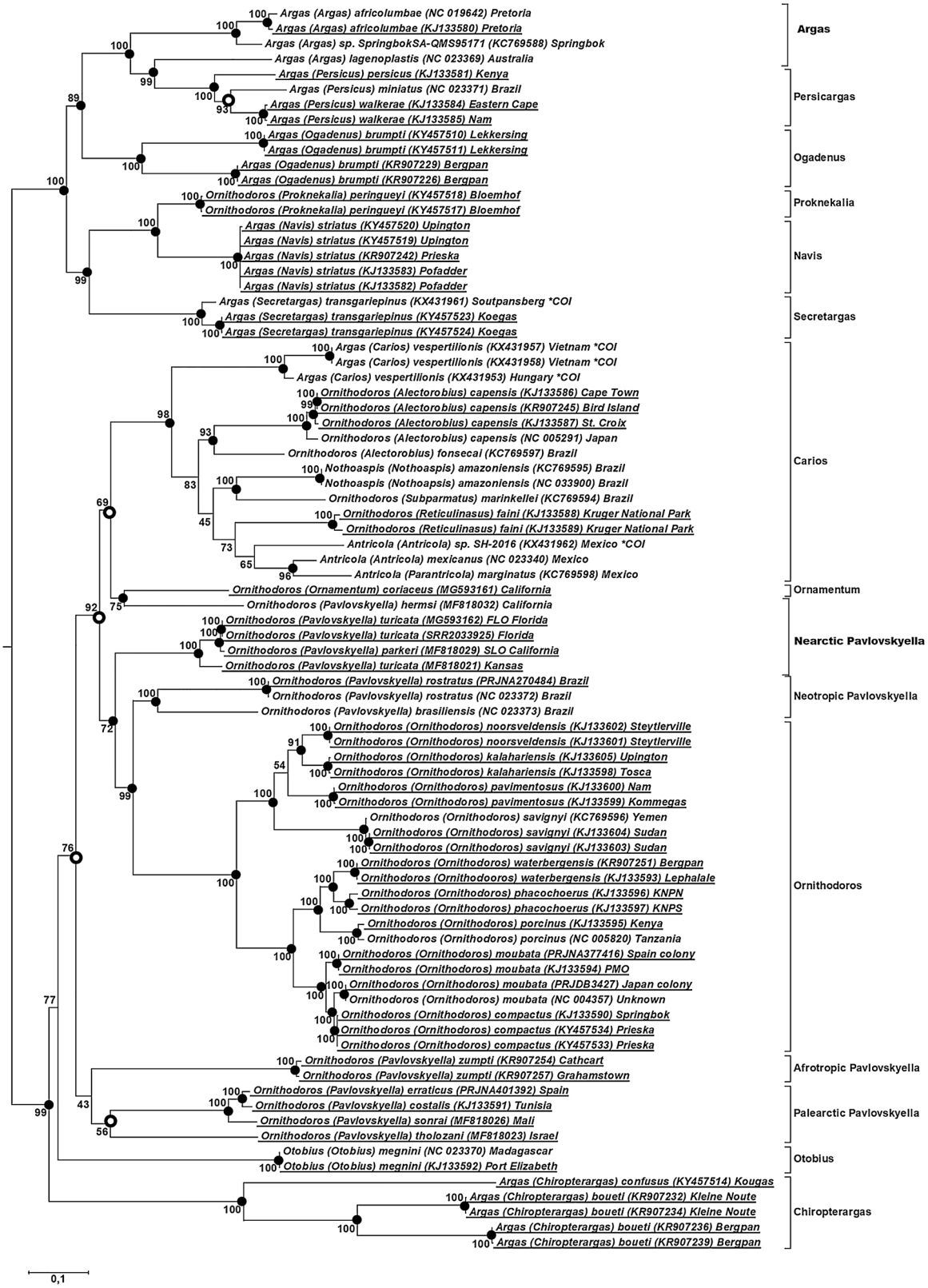

Fig. 8. The mitochondrial protein phylogenetic analysis for the Argasidae. Nodal support are for Maximum Likelihood analysis, while black dots represent > 95% and white dots > 90% Bayesian posterior probability. Ticks sequenced in the current study are underlined. Species are represented by their binomial nomenclature (Guglielmone et al., 2010) and subgeneric designation according to Hoogstraal (1985). Accession numbers of the mitochondrial genomes are indicated in parentheses. Origins for various sequences are also indicated.

area, ~1000 km apart (Hornok et al., 2017). Given the wide distribution of this tick species across the Afrotropic and Palearctic regions, multiple cryptic species may be expected, even if the wide distribution of this species has been explained by dispersal of larvae with migrating bats (Hoogstraal, 1985).

In *Chiropterargas*, two different species exist within the *A. (C.) boueti*

group and may reflect the different races previously described for this species (Hoogstraal, 1955). While these species were sampled at two distinct geographic localities, they were sympatric at Kleine Noute as evidenced by 16S rRNA sequencing (B.J. Mans, unpublished observation). The type for *A. (C.) boueti* was designated from Egypt (Hoogstraal, 1955), and so these two species may represent new species distinct from *A. (C.) boueti* s. s.

Within *Carios*, *C. (R.) faini* may represent two cryptic species that were sampled from the same cave where this tick was previously identified (Braack, 1989). Analysis of 16S rRNA (results not shown), indicated that one of the sequences clustered with *C. (R.) faini* sampled from Uganda (Schuh et al., 2016). Since the type locality of this species is in the Congo (Hoogstraal, 1960), we cannot be sure that the type locality has been sampled. *Carios (A.) capensis* from South Africa and Japan (Shao et al., 2004; Ushijima et al., 2003), may also represent cryptic species. The *Carios capensis* complex comprises several distinct species from the northern and southern hemispheres, and may form distinct races in support of a possible cryptic complex (Dupraz et al., 2016). *Carios vespertilionis* may also be a cryptic group, as suggested previously (Hornok et al., 2017).

For ixodids, potential cryptic species may exist for *A. marmoreum* collected in Gauteng and Eastern Cape, which is a species known to show morphological variation (Theiler and Salisbury, 1959). The cryptic species complex composed of *Rhipicephalus* (*Boophilus*) *annulatus*, *Rhipicephalus* (*Boophilus*) *australis* and *R.* (*B.*) *microplus* is well known (Burger et al., 2014b). A mitochondrial genome from China classified as *R. simus* (Xu et al., 2016), is most probably *R. (B.) microplus*, since *R. simus* occurs exclusively in southern Africa (Walker et al., 2000). The mitochondrial genome reported for *R. simus* in the current study, clusters in a clade with *R. sanguineus* and *R. turanicus* as previously observed (Barker and Murrell, 2004). In *Dermacentor*, *Dermacentor nuttalli* and *Dermacentor silvarum* belong to the same species, as previously suggested (Kulakova et al., 2014). The sequences of *A. sculptum* and *Amblyomma cajennense* s.l. also belong to the same species, probably being *A. sculptum*, as recently described (de Lima et al., 2017; Nava et al., 2014).

Nuttalliella namaqua from Springbok and Heuningvleipan (sampled 640 km apart) may also be cryptic species. Given the wide distribution from South Africa to Tanzania, this could suggest that other species may exist for this family (Mans et al., 2015, 2014). Even so, the sequence similarities suggest that this family will not be species rich and given its inferred ancient history in relation to other tick families, may still be considered a relic with close affinities to the ancestral tick lineage (Mans et al., 2016, 2012, 2011).

3.10. Species delimitation and implications for biological studies

The presence of different species for ticks previously considered as single species has significant implications for various biological studies. As such, a very large body of biochemical and molecular work has been performed on *O. (O.) savignyi* and *O. (O.) moubata*. In the case of *O. (O.) savignyi*, the majority of work was reported for samples from the Kalahari in southern Africa which are now considered a distinct species [*O. (O.) kalahariensis*] as compared with the north African *O. savignyi* s.s. (Bakkes et al., 2018). Different proteins such as apyrase (Mans et al., 1998; Stutzer et al., 2009), barium sulphate adsorption proteins (BSAPs) (Ehebauer et al., 2002), fXaI (Gaspar et al., 1996; Joubert et al., 1998), savignin (Mans et al., 2002a; Nienaber et al., 1999), savignygrin (Mans et al., 2002b) and tick salivary gland proteins 1–4 (TSGP1-TSGP4) (Mans and Ribeiro, 2008a, 2008b; Mans et al., 2008, 2003, 2001) would now describe functions for *O. (O.) savignyi* sensu lato and not for *O. (O.) savignyi* s.s. The sensu stricto species would have its own orthologs with inferred similar functions. The question arises regarding the ability of various species from the *O. (O.) savignyi* group to cause sand tampan toxicoses, since this has only been recorded for the Kalahari species from South Africa, and ticks from the Lake Chad region (Mans et al., 2002c). The risk for various species to act as vectors for Alkhurma hemorrhagic virus should also be determined since infected ticks have only been identified in Saudi Arabia (Charrel et al., 2007).

In the case of *O. (O.) moubata*, the origin of tick samples for various biochemical studies is fairly obscure. Tick anticoagulant peptide (TAP), disagregin and moubatin were isolated from ticks obtained from South Africa (Karczewski et al., 1994; Waxman and Connolly, 1993; Waxman et al., 1990). However, it is unlikely that these ticks derived from the type locality (Groot Marico district). Rather, it is plausible that the ticks were sourced from the Kruger National Park or Waterberg areas (B.J. Mans, discussions with State Veterinarians), which represent localities for *O. (O.) phacochoerus* and *O. (O.) waterbergensis*, respectively. Ticks used for the characterization of the complement inhibitor OMCI were originally from Kenya (Bell-Sakyi et al., 2009; Nunn et al., 2005) and would probably represent *O. (O.) porcinus*. Ticks used to characterize apyrase, TSGP1, TSGP4 and enolase (Baranda et al., 2000, 1997; Díaz-Martín et al., 2015, 2013, 2011; Manzano-Román et al., 2016; Oleaga et al., 2007) derived from a colony kept at the Institute for Animal Health (Pirbright), which originally was established using ticks from the London School of Hygiene and Medicine (Rennie et al., 2000), or from Zambia (Wilkinson et al., 1988), or Malawi (Oleaga et al., 2017). This colony represents *O. moubata* s.s. as indicated in the current study.

The origin of ticks used to characterize alpha-macroglobulin-like glycoprotein, cystatins, Dorin M, ferritin and lysozyme (Grunclová et al., 2006, 2003; Kopáček et al., 2003, 2000, 1999; Kovár et al., 2000; Rego et al., 2005; Salát et al., 2010; Saravanan et al., 2003) is unknown. Characterization of actin, defensin, ecdysteriod receptor, retinoid X receptor and vitellogenin (Horigane et al., 2010, 2008, 2007a, 2007b; Nakajima et al., 2001) derived from ticks that group closely to *O. (O.) compactus*, but they probably represent a unique lineage. Most of the biological functions described above would be for *O. moubata* s.l. and cannot be assigned to specific species.

The mitochondrial genomes derived from the *O. (P.) turicata* synganglion dataset (Egekwu et al., 2016; Zhu et al., 2016) and from the RML derived FLO strain show 98% identity to the *O. (P.) parkeri* SLO strain. These genomes [including *O. (P.) parkeri*] show only 85% identity to the mitochondrial genome of *O. (P.) turicata* from Kansas. Conversely, COI and Cytb data from *O. (P.) turicata* from Texas (Travis Co.) show 99% identity to the Kansas strain (results not shown), while the Kansas strain shows 100% identity to the *O. (P.) turicata* 16S rRNA (L34327) (Black and Piesman, 1994). The 16S rRNA from *O. (P.) parkeri* SLO, *O. (P.) turicata* FLO and *O. (P.) turicata* synganglion all shows 99% identity in the 16S rRNA gene for *O. (P.) parkeri* (EU009925) obtained from a transcriptome previously sequenced (Francischetti et al., 2008), of which samples originally derived from California (Oliver et al., 1984). BLAST analysis of 154 nucleotide sequences obtained from this latter transcriptome, showed 100% identity to contigs from the *O. (P.) turicata* synganglion transcriptome (results not shown). The *O. (P.) turicata* from Florida are therefore morphologically cryptic variants of *O. (P.) parkeri*. This may explain the curious distribution pattern of *O. (P.) turicata* that is disjunct between the mid-West and Florida in the United States of America (Donaldson et al., 2016). Moreover, the suggestion resurfaces that *O. (P.) turicata* from Florida should be considered a subspecies, *O. (P.) turicata americanus* (Beck et al., 1986). The possibility that humans introduced *O. (P.) parkeri* into Florida, possibly via the pet trade, should be considered (Krysko et al., 2011). These data also suggest that an in-depth population genetic study of both *O. (P.) parkeri* and *O. (P.) turicata* across their distribution ranges is necessary to delineate their true geographic distributions. The tick vector specificity of *Borrelia* species has been used as a means to identify tick vectors, specifically in regard to *O. (P.) parkeri* and *O. (P.) turicata* (Davis, 1952, 1942). *Borrelia turicatae* was previously identified in Florida in a dog, based on phylogenetic analysis of the combined genes for 16S rRNA, periplasmic flagella (*flaB*), gyrase (*gyrB*) and glycerophosphodiester phosphodiesterase (*glpQ*) (Schwan et al., 2005). There

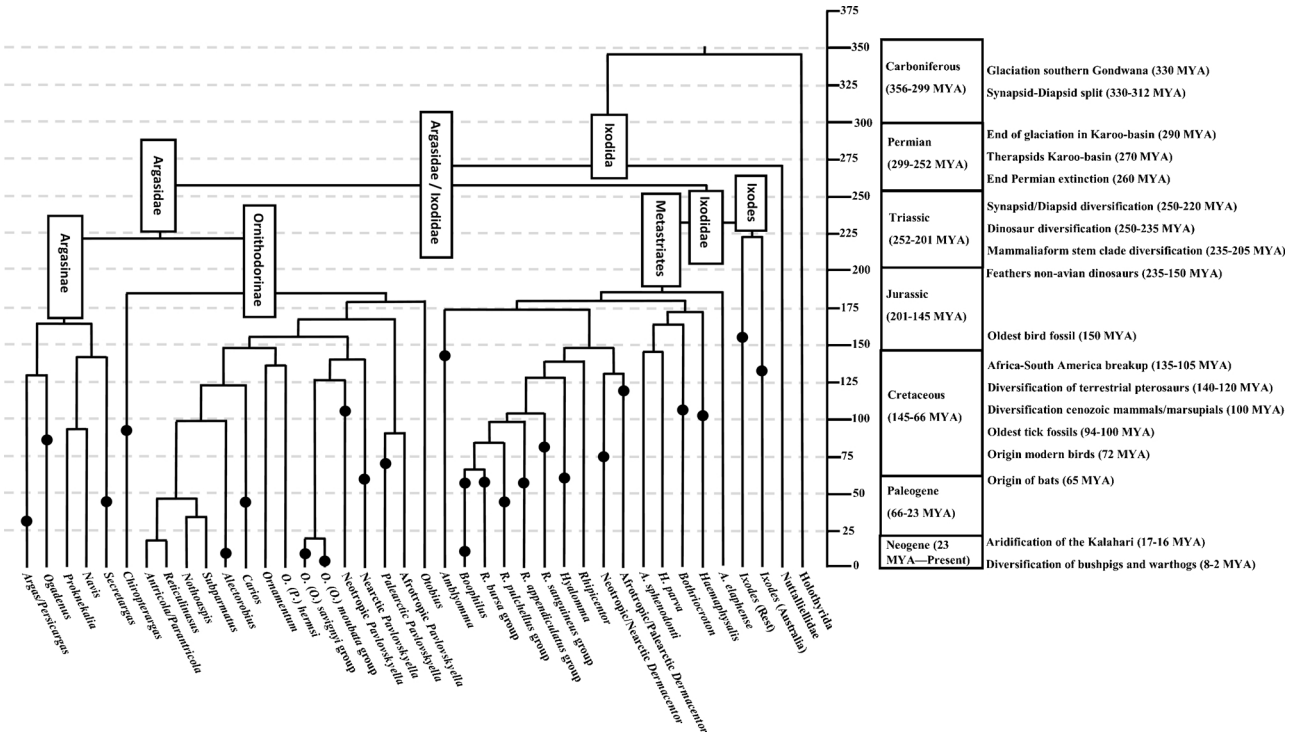

Fig. 9. Molecular clock dating of the Ixodida. Indicated is the chronogram for the mitochondrial topology. The time scale was derived from the GSA Geologic Time Scale v 4.0 (Walker et al., 2012). Pertinent events possibly related to tick evolution are indicated on the right. Black dots indicate the minimum age of divergence within a lineage.

is therefore a possibility that *O. (P.) turicata* may also occur in Florida. Alternatively, the dog could have relapsed six years after it relocated to Florida from Colorado due to immunosuppression (Breitschwerdt et al., 1994). While long-term relapse from relapsing fever *Borrelia* is uncommon, chronic cases have been reported (Lange et al., 1991).

Ornithodoros (*P.*) *costalis* and *O.* (*P.*) *erraticus* grouped as one species using the ABGD server. Pairwise sequence identity of the full mitochondrial genome also indicated 95% identity, making it a borderline cryptic complex. However, comparison of 16S rRNA sequences showed that *O.* (*P.*) *erraticus* gave as best hit *O.* (*P.*) *costalis* (98% identity) (Table S1), while only showing 81% identity to *O.* (*P.*) *erraticus* (KC311539). This would imply that the *O. (P.) erraticus* colony from Spain is *O.* (*P.*) *costalis* instead. A variety of studies that used this colony reported molecular data that should be considered as derived from *O.* (*P.*) *costalis*. These include data on antigen characterization, proteomic and transcriptomic analysis and vaccine development (Astigarraga et al., 1997; Baranda et al., 2000, 1997; Manzano-Román et al., 2007, 2006; Oleaga et al., 2015, 2018, 2007).

Molecular sequence data reported for R. (*B.*) *microplus* may derive from *R.* (*B.*) *microplus* or *R.* (*B.*) *australis*, if data were generated from Australian ticks in the latter case (Mans et al., 2016). The genome of *R. microplus* was sequenced using specimens from a population causing an outbreak in the U.S.A. (Barrero et al., 2017). *R. microplus* was detected infesting cattle in the U.S.A in 1912 (Bishopp, 1913). It was suspected that the infested cattle arrived in Florida through commerce from a source in the Caribbean. Recent analyses on *R. microplus* causing outbreaks in the U.S.A. showed molecular sequences that matched more closely those of samples from Cambodia and the Philippines (Goolsby et al., 2016). Current scientific knowledge indicates that *R. microplus* evolved originally in Southeast Asia (Low et al., 2015).

This study allowed taking another approach to address challenges with tick taxonomics. The *O.* (*P.*) *erraticus* species complex was only recently resolved (Trape et al., 2013), similar to the *O.* (*O.*) *moubata* and *O.* (*O.*) *savignyi* groups (Bakkes et al., 2018). It appears that the *R.* (*B.*) *microplus* complex is still being resolved (Burger et al., 2014b; Csordas et al., 2016), while the issues around *O.* (*P.*) *parkeri/turicata* need to be resolved. Linking biochemical and molecular data to the correct species, will enable us to reconstruct evolution of protein function during the adaptation of ticks to blood-feeding, and correctly assign orthologs for phylogenomic studies (Mans et al., 2016). It has been suggested that voucher 16S rRNA or COI sequences be submitted or referenced for every biochemical study and laboratory strain used in the future, to enable the placement of biochemical data within a phylogenetic context (Mans et al., 2016). As the current study indicates, submission of voucher sequences should be a priority for any future published study, especially for argasids where cryptic species complexes may exist.

3.11. Molecular dating

Three main periods have been considered for the origin of ticks. An older origin in Australia during the Devonian (408-362 MYA) that coincides with the appearance and terrestrialization of labyrinthodont amphibians as archetypal hosts (Barker et al., 2014). Currently, no supporting evidence for this date exists, beyond a consideration that ticks originated in Australia.

A younger origin in the Cretaceous (~130 MYA) was proposed, based mainly on the lack of tick fossils in Lebanese amber (~130 MYA), the oldest amber deposits that contain a parasitiform mite, and on the presence of tick fossils in Burmese and New Jersey amber (~100 MYA) (Klompen and Grimaldi, 2001; Peñalver et al., 2017). However, fossil evidence can only provide minimum ages, while the relative paucity of tick fossils in amber makes the “absence as evidence” argument tenuous. By comparison, a similar example concerns pseudoscorpions where the majority of fossils are found in amber with minimum age ~135-100 MYA (comparable to the proposed tick date of origin). Three older fossils found in shale that date to 392 MYA pushed their origins back ~260 million years from the amber estimates (Harms and Dunlop, 2017). Harvestmen show the same fossil paucity with an estimated older date of origin (Selden et al., 2016). The more recent origin hypothesis also implies that ticks rapidly diverged into the three tick

families as well as the major lineages (Argasinae, Ornithodorinae, Ixodinae, Metastriata) within a period of less than 30 million years, an implication not supported by genetic distances observed in molecular trees. The widespread phylogeography of tick genera is problematic for a young origin, especially in Australia, as it would imply that ticks dispersed after the breakup of Gondwanaland (McLoughlin, 2001), and then populated the major continents. This is especially troublesome for the fast feeding Ornithodorinae that are nidicolous, but do not attach to their host for days, making rapid dispersal problematic. Dispersal biology of soft ticks remains to be fully understood (Kada et al., 2017).

A Late Carboniferous-Early Permian origin (320-290 MYA) has also been considered based on phylogeographic considerations and molecular clock analyses (Jeyaprakash and Hoy, 2009; Mans et al., 2012, 2011). These analyses used fossils as minimum ages to calibrate nodes, but did not place an upper age limit on nodes. We utilized this latter approach in the current study (Fig. 9; Table S9), which yielded interesting implications:

1) The origin of the ancestral lineage to ticks and Holothyrida was dated in the Early Carboniferous (348 ± 31 MYA), while the origin of Ixodida was dated at 273 ± 22 MYA. This fits with an origin in the Early Permian and supports the original proposition that ticks originated in the Karoo basin of Gondwanaland with potential archetypal hosts being therapsids (Mans et al., 2011). The predilection of extant *N. namaqua* for mammals and lizards, may suggest that apart from therapsids, early diapsids may also have been hosts (Mans et al., 2014).
2) The ancestral lineage to hard and soft ticks diverged in the Late Permian (~260 ± 21 MYA). Most recent estimates for the Permian extinction of terrestrial animals is 260 MYA (Day et al., 2015), implying that ticks would have originated during or just after the Permian extinction, and suggest a possible reason for the paucity of ancestral lineages and Nuttalliellidae in fossils (Mans et al., 2011). Divergence of tick families may have been driven by rapid recovery of terrestrial vertebrates after the Permian extinction (Botha and Smith, 2006).
3) Argasid and ixodid lineages both diverged in the Triassic, for argasids 223 ± 20 MYA and ixodids 234 ± 18 MYA, leading to the splits between Argasinae-Ornithodorinae and Prostriates-Metastriates, respectively. In the case of argasids, the emergence of feathered dinosaurs may have contributed to their divergence (Chiappe and Dyke, 2002), while divergence of mammaliaform stem clades could have driven ixodid divergence (Luo, 2007).
4) Argasinae (164 ± 29 MYA) diverged in the middle Jurassic which is close enough to the oldest bird fossil (150 MYA) to suggest that diversification of early birds may have driven divergence in argasines. The lineage leading to *Argas/Persicargas* only diverged in the Late Paleogene (29 ± 9 MYA). Since this latter group comprises ~75% of all Argasinae (Table S10), it implies that the majority evolved recently in the Neogene. Divergence of *Argas/Persicargas* also occurred well after the origin of modern birds (~72 MYA) (Prum et al., 2015), which implies that *Argas*, *Navis* and *Proknekalia* adapted to bird hosts independently. The position of *Ogadenus*, *Secretargas*, *Proknekalia* and *Navis* relative to *Argas*, suggests that Argasinae evolved in the Afrotropical region.
5) *Secretargas* (47 ± 27 MYA) adapted to bats after their origin 65 MYA (Teeling et al., 2005). Remarkably, other bat infesting lineages also show divergence around this time, in *Antricola/Nothoaspis/Reticulinasus* (46 ± 12 MYA) and *Carios* s.s. (45 ± 21 MYA), suggesting that bat diversification may have driven evolution in these lineages. On the other hand, *Chiropterargas* (90 ± 20 MYA) originated before the origin of bats and may have originally parasitized other flying reptiles such as pterosaurs.
6) The previously monotypic *Ogadenus* shows an ancient diversification (84 ± 26 MYA) relative to other Argasinae, which suggests it might resemble the ancestral form. Host specificity of extant species for reptiles and mammals suggests that these may have been ancestral hosts to the Argasinae.
7) Ornithodorinae diverged in the middle Jurassic (186 ± 18 MYA) and placement of *Chiropterargas*, as well as Afrotropical and Palearctic *Pavlovskyella* at the base of the tree suggests that Ornithodorinae evolved in Gondwanaland. Given that both Argasinae and Ornithodorinae seemed to have originated in this area, it seems likely that Argasidae originated in the Afrotropical region of Gondwanaland as well.
8) Subsequent divergences seem to split the tree into several different groups, with primarily significant divergences in *Carios* and a lineage composed of Nearctic and Neotropical *Pavlovskyella*, as well as Afrotropical *Ornithodoros*. *Carios* s.l. Klompen and Oliver (1993), diverged 122 ± 10 MYA ago, which suggests that adaptation to bats occurred multiple times independently in this lineage. However, it is possibile that this lineage adapted to terrestrial flying pterosaurs, which showed major divergence 140-120 MYA (Andres et al., 2014). Under this scenario, a host switch from pterosaurs to bats would have occurred during the K–T extinction event and the origin of bats (Andres et al., 2014; Teeling et al., 2005). *Carios* s.l. most probably originated in the Neotropical or Nearctic region with subsequent dispersal to the Old World as observed for *Carios* s.s. and *Reticulinasus*, which coincide with historical bat dispersal (Amador et al., 2018; Teeling et al., 2005).
9) A clade composed of *Ornamentum* and *O. hermsi* (Nearctic *Pavlovskyella*) grouped separately from other Nearctic *Pavlovskyella*. Bootstrap support to consider all Nearctic *Pavlovskyella* monophyletic is low for either nuclear or mitochondrial markers. The American school (Clifford et al., 1964; Hoogstraal, 1985) considered *O. hermsi* part of the *Pavlovskyella*, while the French school considered *O. hermsi* a novel subgenus (Sbg. nov.1 Morel) in *Alectorobius* (Camicas and Morel, 1977; Camicas, 1998).
10) Divergence of Nearctic *Pavlovskyella* from Neotropic *Pavlovskyella* and Afrotropical *Ornithodoros* occurred 141 ± 14 MYA. The latter two lineages diverged 127 ± 17 MYA, which coincides with the final breakup of South America and Africa (McLoughlin, 2001; Seton et al., 2012), which suggests that these lineages were separated by continental drift. Nearctic *Pavlovskyella* probably colonized North America via the Palearctic. The above scenarios suggest possible reasons for paraphyly in the *Pavlovskyella*, i.e. different divergence times, vicariance and continental drift. It also indicates an origin in the Afrotropical region, with divergence and dispersal to the Palearctic and then the Nearctic, and dispersal to Australia via Antarctica and to the Neotropics during the Africa/South America breakup. To test these hypotheses sampling of the Nearctic, Palearctic and Australian lineages are particularly necessary.
11) Divergence of Afrotropical *Ornithodoros* (*Ornithodoros*) occurred recently (19 ± 5 MYA) and coincides with the aridification of the Kalahari (17-16 MYA). The origin of the *O.* (*O.*) *savignyi* group (11 ± 1.7 MYA) coincides with continual desertification of the Kalahari and the origin of the Sahara desert (8-7 MYA) (Thomas and Shaw, 1993). The origin of the *O.* (*O.*) *moubata* group (4.2 ± 1 MYA) coincides with the origin of wild suids in Africa and diversification of bushpigs and warthogs (8-2 MYA) (Frantz et al., 2016; Muwanika et al., 2003). Phylogenetic patterns for both groups suggest that these lineages evolved in southern Africa before dispersal to east and north Africa (Bakkes et al., 2018). Divergence of the *O.* (*O.*) *moubata* group also coincides with the origins of major rivers and tectonic uplift in the Pliocene, and these are suggested as drivers of speciation in this group (Bakkes et al., 2018).
12) Prostriates diverged ~224 ± 18 MYA into the Australasian and Rest of the World *Ixodes*. For Australasian lineages, given the date of divergence (134 ± 32 MYA), dispersal most probably occurred

from Gondwanaland to Australia via Antarctica (McLaughlin, 2001; Seton et al., 2012), with establishment and diversification of Australasian lineages coinciding with introduction and diversification of dinosaurs (Agnolin et al., 2010). Divergence of the Rest of the World *Ixodes* occurred 154 ± 43 MYA suggesting an origin in the Afrotropical region, before dispersal to the Nearctic, Palearctic and Neotropics.

13) Metastriates originated ~186 ± 16 MYA. *Bothriocroton* and *Haemaphysalis* diverged from *Amblyomminae* and *Rhipicephalinae* ~180 ± 15 MYA, and from each other ~164 ± 16 MYA. Amblyomminae and Rhipicephalinae diverged ~174 ± 15 MYA. These dates allow sufficient room for dispersal to Neotropical, Nearctic and Australian continents. The time of origin for *Amblyomma* (144 ± 12 MYA), suggests that divergence between African and South American groups occurred due to continental rifting.
14) Diversification of *Dermacentor* (131 ± 14 MYA), with a split into a Nearctic/Neotropical and Afrotropical/Palearctic clades suggest that the breakup of Gondwanaland assisted in this diversification. An Afrotropical origin for *Dermacentor* has been proposed (Barker and Murrell, 2004). However, distribution data as well phylogenetic analysis suggest that this clade originated in either the Afrotropics or Palearctic region.
15) Diversification for *Hyalomma* was estimated in the Early Paleocene (63 ± 18 MYA). This differed from a recent study that estimated a date of divergence at 36 MYA instead, which was constrained by a fossil calibration between 35–50 MYA (Sands et al., 2017). However, the *Hyalomma* fossil used for calibration was shown to be a rake-legged mite (Acariformes) and therefore not suitable for calibration (Chitimia-Dobler et al., 2017). It should be noted that *Hyalomma* samples analyzed in the current study could be considered derived rather than basal species (Sands et al., 2017). The origin of *Hyalomma* may likely be older than 63 MYA if the divergence of the *Hyalomma/Rhipicephalus* clade is considered (128 ± 12 MYA). This would have an impact on phylogeographic hypotheses. Distribution of *Hyalomma* mainly in Afrotropical and Palearctic regions has suggested a potential origin in the Palearctic with subsequent introduction into the Afrotropics (Sands et al., 2017).
16) *Rhipicephalus* mainly occurs in the Afrotropics, with all basal lineages also Afrotropical, suggesting an origin in the Afrotropics (Murrell et al., 2001; Barker and Murrell, 2004). Diversification of *Rhipicephalus* (104 ± 12 MYA) occurred well after breakup of Gondwanaland and may explain its occurrence mainly in the Afrotropics. The former genus *Boophilus* lies within *Rhipicephalus*, but form a monophyletic group, suggesting that one-host biology evolved once for this group. Their divergence is dated at 58 ± 11 MYA, and the basal positions are all of Afrotropical origin, suggesting again that this lineage evolved in the Afrotropics after which dispersal occurred to the Palearctic and Australasian regions. The *R. microplus* complex originated 28 ± 8 MYA and includes *R. annulatus*, *R. australis* and *R. microplus*. The relatively old divergence date for various *R. microplus* genotypes (11 ± 5 MYA) suggests that these may be different species.

Molecular clock dating remains a controversial subject area that is dependent on accurate fossil calibrations, phylogenies and algorithms used (Duchêne et al., 2014; Kumar, 2005; Rodríguez-Trelles et al., 2002; Warnock et al., 2012, 2015; Warnock et al., 2017). The paucity of tick fossils may hamper accurate calibrations (Chitimia-Dobler et al., 2018; Dunlop, 2010), while the discovery of new ancient fossils has the potential to increase minimum estimates dramatically. An existing phylogenetic framework may change with the addition of new species or more genetic data, while new algorithms and evolutionary models may also impact on divergence date estimates (Warnock et al., 2017). The implications addressed in this study should be considered in this light. It is not, however, expected that additional data or new algorithms will impact the current proposed scenarios on an orders of magnitude scale. Discovery of new fossils that significantly increase our minimum estimates would in contrast have major implications for an older origin hypothesis. For the moment, the soft-bodied nature of ticks may limit us to amber deposits with minimum estimates within the 100–150 MYA range (Dunlop, 2010; Peñalver et al., 2017). For the moment these minimum estimates fit well with the molecular clock estimates.

3.12. Argasidae classification and systematics

Both nuclear and mitochondrial analyses support the cladistic analysis of Klompen and Oliver (1993) in broad terms. The only significant difference is placement of *Chiropterargas* outside *Carios* sensu Klompen and Oliver (1993). *Carios vespertilionis* groups within *Carios* s.l. (Klompen and Oliver, 1993) with good support, even if only based on COI sequences. This suggests that the classification scheme of Klompen and Oliver (1993) should be maintained until more data can support the movement of *Carios*. If *Carios s.s.* were to move, it is likely that it will form a separate clade within Ornithodorinae, similar to *Chiropterargas*. The remaining clade cannot be considered as "Neotropical Ornithodorinae" (Burger et al., 2014a), because *Reticulinasus* is comprised of bat-infesting species from the Afrotropical, Australasian, Indomalayan and Palearctic regions (Hoogstraal, 1985). If *Carios* moves to another clade, *Alectorobius* (Pocock, 1907) would be the valid name for this group.

The current data do not support monophyly of *Pavlovskyella*, as previously indicated by Klompen and Oliver (1993). From an evolutionary perspective, this may imply that *Pavlovskyella* lineages originated at different times and geographic localities. This suggests that many more lineages need to be elevated to genus level within Ornithodorinae if classification is to represent evolutionary history. Even so, it may be more prudent to retain the subgeneric classification (Hoogstraal, 1985; Klompen and Oliver, 1993), until sampling of a more representative set of *Pavlovskyella* can be achieved that would include Afrotropical, Indomalayan, Nearctic, Neotropical, Palearctic and Australasian lineages. The current data suggest that *Pavlovskyella* separated into clades congruent with continental distributions, which would suggest that divergence may have been precipitated by continental drift. As such, to avoid confusion when discussing the subgenus, reference can be made to their ecoregion of origin, i.e. Afrotropical *Pavlovskyella*.

While the current classification scheme (Guglielmone et al., 2010) reverts back to Hoogstraal (1985) (a reversal of 25 years), its roots may be traced to Horak et al. (2002), where a consensus in both tick lists was reached based on community agreement. Given the reality that soft tick systematics may only be resolved over several decades and the current state of uncertainty in argasid systematics, it is proposed that the argasid classification scheme be updated in real-time, as scientific knowledge progresses using the most up-to-date evolutionary models possible. It is proposed that a new revised argasid tick list, which could include a revised ixodid list as well, be published every five years, for periodic updates of current scientific knowledge and consensus.

For Argasinae with divergent clades, each having unique biological triats (Hoogstraal, 1985), it is proposed that the subgenera *Alveonasus*, *Navis*, *Ogadenus*, *Proknekalia* and *Secretargas* should be raised to genus level. Therefore, the general classification by Klompen and Oliver (1993) remains with these modifications: **Argasinae**: ***Alveonasus*** (*Alveonasus*), ***Argas*** (*Argas*, *Persicargas*), ***Navis*** (*Navis*), ***Ogadenus*** (*Ogadenus*), ***Proknekalia*** (*Proknekalia*) and ***Secretargas*** (*Secretargas*). **Ornithodorinae**: ***Carios*** (*Alectorobius, Antricola, Carios, Nothoaspis, Parantricola, Reticulinasus, Subparmatus*), ***Chiropterargas*** (*Chiropterargas*), ***Ornithodoros*** (*Microargas, Ornamentum, Ornithodoros, Pavlovskyella, Theriodoros*) and ***Otobius*** (*Otobius*). This proposition also contemplates that, until molecular data from specific subgenera are

obtained, the cladistic classification of Klompen and Oliver (1993) be maintained. Furthermore, subgeneric classifications should be retained because these clearly differentiate lineages with different biology and geographic origin. A new list is included herein as reference (Table S10), to enhance the designation of subgeneric classifications from the previous tick list by Guglielmone et al. (2010), which was based on Hoogstraal (1985).

3.13. Future directions in tick systematics

The current study contributed mitochondrial genomes for 55 tick species. This is a significant contribution to the existing 55 mitochondrial genomes available for unique tick species, bringing the number of unique tick mitochondrial genomes to 101, ~10% of all known tick species. It may be concluded that tick mitochondrial genomics are now becoming a matured science, since the first mitochondrial genomes was sequenced in 1998 with more than 20 original contributions to this field (Black and Roehrdanz, 1998; Burger et al., 2012, 2013; Burger et al., 2014a, b; Carpi et al., 2016; de Lima et al., 2017; de Lima et al., 2018; Guo et al., 2016; Jeyaprakash and Hoy, 2009; Liu et al., 2013; Mans et al., 2012, 2015; McCooke et al., 2015; Mikryukova et al., 2016; Mitani et al., 2004; Montagna et al., 2012; Shao et al., 2004, 2005; Williams-Newkirk et al., 2015; Xu et al., 2016). A relevant question is whether tick mitochondrial genomics should remain a future focus area for tick systematics. The current study and others cited above has indicated that mitochondrial genomes are usefull to investigate species and higher level relationships in ticks. While representation of the majority of tick genera has been obtained, some important genera are still absent, while members from all geographic regions has not been sampled yet. Mitochondrial genome sequencing will as such remain a viable and economic recourse to study tick systematics (Mans et al., 2015). Additional nuclear markers will in future become available as more tick transcriptomes and genomes are sequenced (Mans et al., 2016). Such phylogenomic approaches will be important to confirm ribosomal RNA and mitochondrial based systematic analysis. In line with this, the first phylogenomic analysis based on tick transcriptome data has been published which recapitulated to a large extent the results from mitochondrial systematic studies (Landulfo et al., 2017).

4. Conclusions

This study contributed the largest number of 18S and 28S ribosomal and mitochondrial genome markers to date for argasid ticks. Additionally, a number of novel ixodid markers were sequenced. This confirmed the general sequencing strategy previously proposed to generate markers using next-generation sequencing and extends its use to ixodid ticks as well (Mans et al., 2015). The systematic analysis reported here supports the original cladistic classification of Klompen and Oliver (1993), with minor modifications. However, the recognition of subgenera may be more appropriate to understand the evolutionary history of various argasid lineages. Results presented here also document evidence for cryptic and novel soft tick species, and highlight the importance of voucher sequences for biochemical and ecological studies. Molecular dating linked to a systematic framework suggests possible explanations for the evolution of various lineages. The approach reported here provided a systematic framework to interpret biochemical, transcriptomic and genomic analyses. As such, this study serves as a reference for future attempts to unify morphological and molecular data to refine the systematics of the Ixodida.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2018.09.010.

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