Automatic barcode gap discovery reveals diverse clades of *Rhipicephalus* spp. and *Haemaphysalis* spp. ticks from small mammals in 'Asir, Saudi Arabia

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Abstract

Background: The ixodid tick genera *Rhipicephalus* and *Haemaphysalis* contain several species of medical and/or veterinary importance but their diversity in some regions of the world remains underexplored. For instance, very few modern studies have been performed on the taxonomy of these genera on the Arabian Peninsula.

Methods: In this study, we trapped small mammals in the 'Asir Mountains of southwest Saudi Arabia and collected tick specimens for morphological examination and molecular barcoding, targeting three mitochondrial loci: *coi*, 16S rRNA and 12S rRNA.

Results: We obtained a total of 733 ticks (608 *Haemaphysalis* spp. and 125 *Rhipicephalus* spp.) from 75 small mammal hosts belonging to six species. All tick specimens were immature except for nine adults recovered from a hedgehog (*Paraechinus aethiopicus*). Morphologically, the *Rhipicephalus* ticks resembled *Rhipicephalus camicasi* but the *Haemaphysalis* ticks showed differences in palp morphology compared with species previously described from Saudi Arabia. Phylogenetic analysis and automatic barcode gap discovery identified a novel clade of *Rhipicephalus* sp. representing most of the nymphs. This was most closely related to *Rhipicephalus leporis, Rhipicephalus guilhoni*, and the tropical lineage of *R. sanguineus*. The adult ticks and a small proportion of nymphs clustered with *R. camicasi* sequences from a previous study. Finally, the *Haemaphysalis* nymphs formed two distinct clades that were clearly separated from all reference sequences but closest to some African species.

Conclusions: This high level of tick diversity observed in a single study site of only ~170 km^2, on a relatively small number of hosts, highlights the potential for new tick species to be discovered on the Arabian Peninsula.

Background

The Ixodidae (hard ticks) is by far the most speciose family of ticks, with over 700 validly described species [1]. Until comparatively recently, our understanding of the relationships between tick species was founded almost exclusively on analysis of morphological features. Due to their large and complex genomes, whole nuclear genome data for ticks remains sparse [2] compared with insects of medical and/or veterinary importance and investigations of possible species complexes within morphologically similar tick groups have proceeded slowly. However, molecular confirmation of tick species identity using mitochondrial barcodes and phylogenetic analyses based on concatenated mitochondrial loci; or more recently, nucleotide and amino-acid datasets from whole mitogenomes, have begun to revolutionise both the taxonomic status of closely related species and the higher-level relationships between tick genera and families [3-7].

There have been increasing reports of discordance between morphological features and genetic characteristics within ixodid taxa, including *Ixodes* and *Rhipicephalus*; two of the most intensely studied genera of medical and veterinary importance. For instance, a recent study showed that certain Australian

Ixodes spp. specimens were highly divergent genetically but morphologically indistinguishable, whereas other specimens were morphologically distinct but poorly resolved genetically [8]. Moreover, two of the most important *Rhipicephalus* spp. globally, the Asian blue tick, *Rhipicephalus microplus*, and the brown dog tick, *Rhipicephalus sanguineus*, are each now known to be formed of several distinct lineages, which are becoming recognised as distinct species [9-15]. The highly diverse genus *Haemaphysalis* has been the subject of far fewer molecular studies, although substantial discrepancies between morphology-based classification and molecular characteristics have recently been noted for this taxon too [4, 7, 16]. One generic approach to resolving species diversity using objective molecular criteria is automatic barcode gap discovery (ABGD), which is founded on the principle that the genetic divergence should be smaller within species than between species [17]. This allows a confidence limit to be assigned to intraspecific divergence, thus partitioning gene sequences into bins or operational taxonomic units (OTUs). The ABGD approach and related methods are gaining in popularity in molecular studies of ticks worldwide [18-20].

One geographic region in which the diversity of Ixodidae is underexplored is the Arabian Peninsula. A key to the ticks of Yemen was published by Hoogstraal & Kaiser [21] and for Saudi Arabia by Hoogstraal et al. [22]. Recent reports of ticks from the region have focused primarily on identification of species collected from domestic animals and pathogen screening [23-25], with a smaller number of studies on tick specimens obtained from wild hosts [26-28]. Importantly, to the best of our knowledge, no molecular data from ticks collected from wildlife in Saudi Arabia have been published to date. Here, we identify a novel clade of *Rhipicephalus* spp. ticks feeding on rodents in the 'Asir Mountains of southwest Saudi Arabia, which is molecularly distinct from sympatric specimens that cluster with *Rhipicephalus camicasi*. We also present preliminary evidence for two novel clades of *Haemaphysalis* spp. ticks infesting the same hosts.

Methods

Field site and small mammal trapping

Details of the study site and small mammal collection have been published previously [29]. Briefly, small mammals were trapped overnight in the summers of 2016 and 2017 near three villages (Al Ous', Alogl and Wosanib) on the upper escarpment of the 'Asir Mountains in southwest Saudi Arabia, between the towns of Abha and Muhayil Asir. An additional brief excursion to the same area was undertaken in October 2020. Rodents were identified morphologically with reference to the work of Harrison and Bates [30]. Molecular confirmation was performed by amplification of a cytochrome *b* gene barcode using conventional PCR with primers L14841 and H15149 [31]. Sequences were submitted to the Barcode of Life Data Systems (BOLD) (http://www.boldsystems.org) under project code SSS.

Morphological examination of ticks

Mammal carcasses were examined for ticks with the naked eye and then under a dissecting microscope. Ticks were removed with fine forceps, fixed in 70% ethanol and maintained at 4˚C prior to enumeration.

Approximately 5% of specimens from each host were selected for morphological or molecular analysis, prioritising nymphs over larvae due to the low DNA yields and problems of identification associated with the latter. Semi-engorged immature stages selected for morphological examination were placed in distilled water for 10 min, transferred to a macerating solution (10% potassium hydroxide) and incubated at 37°C for up to 10 min until the cuticle had cleared sufficiently to visualise key morphological features. The specimens were again placed in distilled water for 10 min and then dehydrated serially using 50%, 70%, and 100% ethanol (10 min at each concentration). Finally, specimens were transferred to a glass slide with a drop of DPX mountant (VWR International), covered, and examined using an Axio Imager M2 microscope with ZEN 2011 imaging software (Zeiss). Adult ticks (males only in this study, as females were fully engorged) were examined directly from 70% ethanol under a dissecting microscope without further processing. Morphological features of the ticks were compared with those described in keys and other taxonomic reference works for ticks, focusing on the Middle East, Southern Europe and North Africa [14, 21, 22, 32-38].

DNA extraction, PCR and sequencing

DNA extractions were performed with a DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instructions. In the case of immature ticks, DNA was extracted from the whole specimen, whereas for adults, DNA extractions were performed on the anterior portion only to reduce carryover of the bloodmeal in engorged specimens. The amplification of fragments of three mitochondrial loci (*coi*, 12S rRNA and 16S rRNA) were attempted for each specimen using previously published primers from Low & Prakash [10], Beati and Keirans [39], and Black & Piesman [6], respectively. Expected product sizes were 550 bp for *coi*, 336 bp for 12S rRNA, and 460 bp for 16S rRNA. The PCR assays were performed on a T1 Thermoblock thermocycler (Biometra) using BioMix Red reaction mix (Meridian Bioscience) in 20-ml volumes containing 5 ml DNA template. Following agarose gel electrophoresis, PCR products were purified using a QIAquick PCR Purification Kit (Qiagen) and sequenced in both directions by Eurofins Genomics. Sequences were submitted to GenBank with identifiers MW742686-MW742711 for *coi*, MW756110-MW756125 for 12S rRNA, and MW763030-MW763059 for 16S rRNA.

Phylogenetic analysis and automatic barcode gap discovery

All sequences were preliminarily aligned using CLUSTAL X [40] and edited using BioEdit [41]. Phylogenetic relationships were inferred using the Neighbor-Joining method using MEGA X [42]. The Neighbor-Joining bootstrap values were estimated using 1,000 replicates with Kimura's two-parameter model of substitution (K2P distance). Gaps and missing data were eliminated. Statistical congruence was calculated using a partition homogeneity test implemented in PAUP 4.0b10 [43]. No significant differences were found among separate gene regions ($P = 0.800$); hence, *coi*, 12S and 16S sequences were concatenated for further analyses. To assess the genetic divergence of taxa, uncorrected (p) pairwise genetic distances among species were estimated using PAUP 4.0b10 [43]. The species boundary among tick taxa was assigned by automatic barcode gap discovery (ABGD) analysis performed on the

webserver using the Kimura (K80) TS/TV model. Entity recognition was based on the suggested partition at $P = 0.01$ [17].

Results

Material obtained and examined

We obtained 75 small mammal hosts across the three sites, which belonged to six species (Table 1): the Eastern spiny mouse (*Acomys dimidiatus*), king jird (*Meriones rex*), Yemeni mouse (*Myomyscus yemeni*), black rat (*Rattus rattus*), house mouse (*Mus musculus*), and desert hedgehog (*Paraechinus aethiopicus*). These were infested with a total of 733 ticks (608 *Haemaphysalis* spp. and 125 *Rhipicephalus* spp.), all of which were immature except for nine adults (seven males and two females) recovered from the hedgehog. Most subsampled specimens from each host were prioritised for molecular analysis and we focused primarily on *Rhipicephalus* spp. due to its greater potential importance regionally as a disease vector. All specimens subjected to PCR ($n = 42$) generated at least one mitochondrial gene sequence (Table 2). At least two specimens per lifecycle stage of each tick genus were examined morphologically.

Morphological features

Rhipicephalus spp. nymphs displayed variation in the length and shape of the palps as well as the appearance of the scutum, which slightly overlapped coxa III in some individuals only (Fig. 1c and 1d). Nymphs exhibited a highly reduced external spur on coxa I and the internal spur appeared vestigial (Fig. 1d). According to the works of Pegram et al. [35, 36] on the *R. sanguineus* group, these features of the spurs together with the ratio of length-to-width of the capitulum would position these specimens closer in morphology to *Rhipicephalus camicasi* than to *Rhipicephalus turanicus* or *R. sanguineus*. In addition, the adanal plates of the adult males (Fig. 1e) lacked the distinctly concave shape proximal to the anus reported by Nava et al. [14] in their redescription of *R. sanguineus*.

The *Haemaphysalis* spp. nymphs displayed palps that were flared posteriorly (Fig. 2), which according to Hoogstraal et al. [22], is a feature of *Haemaphysalis erinacei* that distinguishes it from *Haemaphysalis sulcata*. However, the ventral spur on palp segment I (Fig. 2b) had a triangular profile unlike that of *H. erinacei*. Since Hoogstraal & Kaiser [21] and Hoogstraal et al. [22] also reported *Haemaphysalis leachi* from the Arabian Peninsula, we consulted the descriptions and re-descriptions of this species and the closely related *Haemaphysalis elliptica* from Africa [32, 37]. The posterior margin of the basis capituli in both of these species is convex, but in some of the specimens from 'Asir, it is straight (compare Fig. 2b and 2c).

Sequence analysis of *Rhipicephalus* spp.

At least one mitochondrial gene sequence was amplified and sequenced successfully from a total of 33 *Rhipicephalus* spp. adult or nymphal tick specimens and one pool of larvae, obtained from two villages and four species of small mammal host (Table 2). The phylogeny based on *coi* indicated that the vast

majority of nymphal specimens belonged to a single, novel clade; this was distinct from all other *Rhipicephalus* spp. included in the analysis (Fig. 3). The novel clade exhibited closest relationships with *R. leporis*, *R. guilhoni*, and the tropical lineage of *R. sanguineus*. In contrast, a single nymph (R25 from host *A. dimidiatus* in Wosanib) clustered with an adult specimen from the current study (H1_2 from host *P. aethiopicus*, also from Wosanib) and previously published sequences from "*R.* cf *camicasi*" from Riyadh Province. The novel lineage was separated from other species by a minimum genetic distance of 2.24% (for *R. leporis*) to a maximum of 15.37% (for *R. simus*) (Additional file 1: Table S1). The ABGD analysis delimited 18 operational taxonomic units (OTUs) and supported the novel clade comprising most nymph specimens (OTU 1) as a distinct taxon (Fig. 3).

For 12S rRNA, the novel lineage was also resolved for all nymphs except R25. The clade differed from other members of the genus with lower genetic distances of 1.84% (for *R. leporis*) to 11.07% for the *R. simus* complex (including an unidentified *Rhipicephalus* sp. from Kenya; Additional file 1: Table S2). The ABGD analysis identified 16 OTUs and although lower interspecific genetic distances were observed, the delimitation analysis demonstrated the novel lineage as a distinct OTU (Fig. 4). The "*R.* cf *camicasi*" specimens from the previous study in Riyadh Province (obtained from camels and a dog) were split into three distinct OTUs, suggesting cryptic diversity in this species. One of these (from a camel) clustered with nymph specimen R25. Interestingly, the pool of six larvae (R29 from Wosanib) was placed in a unique OTU separated from all nymph specimens (Fig. 4). This was most closely related to members of the *R. simus* complex from Africa, especially *R. praetextatus*; indeed, the larval pool was not differentiated from the *R. simus* complex in the PAUP analysis (Additional file 1: Table S2).

In the case of 16S rRNA, the novel lineage was also distantly separated from other members of the genus with genetic distances ranging from 4.13% (for *R. guilhoni*) to 12.27% (for *R. muhsamae*) (Additional file 1: Table S3). A total of 15 OTUs were delimited, one of which was associated with the novel lineage (Fig. 5). *Rhipicephalus* cf *camicasi* comprised two OTUs, populated by adult specimens from *P. aethiopicus*, four nymph specimens and the previously published sequences from specimens collected from camels in Riyadh Province. An incongruence was noted for one of the tick samples, nymph R9_7 from Alogl, which was classified in the novel lineage by *coi* and 12S rRNA genes but clustered with *R.* cf *camicasi* OTU 4 by 16S rRNA (Fig. 5). The pool of larvae (R29) formed its own OTU (#12 in Fig. 5) that was most closely related to a sequence (OTU 13) from an unidentified *Rhipicephalus* sp. collected from a dog in Kenya (GenBank: MN266945).

Sufficient sequence data were obtained from 10 nymph specimens for a concatenated analysis of *coi*, 12S rRNA and 16S rRNA genes alongside references for *R. sanguineus* (temperate and tropical lineages), *R.* cf *camicasi*, *R. turanicus* and *R. simus*. The novel clade comprised eight specimens and was distinct from all references, demonstrating closest affinity with the *R. sanguineus* tropical lineage (Fig. 6). In concordance with the single-gene trees, specimen R25 clustered with one of two *R.* cf *camicasi* OTUs, whereas the incongruent specimen R9_7 formed its own OTU in proximity to the *R. sanguineus* tropical lineage (Fig. 6). As only short sequences (~200 bases) for 12S rRNA could be obtained from the two adult ticks from *P. aethiopicus,* they were unable to be included in the concatenated analysis. However,

these short sequences exhibited 100% identity with the previously published *R.* cf *camicasi* sequences from Riyadh Province (GenBank MH094506 and MH094507 from camel hosts).

Sequence analysis of *Haemaphysalis* spp.

The *Haemaphysalis* nymph samples collected in this study were resolved robustly into two lineages in the 16S rRNA phylogenetic tree (Fig. 7). While OTU 1 demonstrated a sister relationship with *H. spinulosa* from South Africa (genetic distance, 7.38%), OTU 4 showed closer relationships with *H. muhsamae* and *H. elliptica,* also from sub-Saharan Africa, with genetic distances of 6.77% and 8.17%, respectively (Additional file 1: Table S4). The species delimitation analysis split the Saudi specimens and references into a total of 15 OTUs, with the Saudi nymphs distinctly separated from all other species included in the analysis (Fig. 7). Notably, these two novel OTUs did not segregate by geographic location (Table 2), with OTU 1 containing specimens from both Alogl (*M. musculus* as host) and Alous (*A. dimidiatus* as hosts).

Discussion

In this study, we found heavy tick infestations represented by two genera feeding on small mammals in a relatively small region (approximately 170 km^2) in the 'Asir Mountains. Remarkably, the *Rhipicephalus* and *Haemaphysalis* ticks recovered from these hosts were not only genetically diverse, comprising four and two OTUs respectively, but all but one (*R. camicasi*) of these OTUs appeared to be novel. The strongest evidence for a previously unrecognised taxon was for *Rhipicephalus* OTU 1, which formed a distinct clade in the *coi*, 12S rRNA, 16S rRNA and concatenated analyses. This clade was found on three species of rodent hosts trapped in agricultural areas surrounding the villages of Alogl and Wosanib. It was most closely related to *R. leporis*, *R. guilhoni* and the tropical lineage of *R. sanguineus* (recently identified as *Rhipicephalus linnaei* [44]). Due to the limited number of sequenced mitochondrial markers available for *R. leporis* and *R. guilhoni*, we were only able to include the tropical lineage of *R. sanguineus* and more distantly related *Rhipicephalus* spp. in the concatenated phylogeny, but this analysis clearly separated the novel OTU 1 from the tropical lineage.

Prior phylogenetic analyses have sometimes assigned *R. leporis* and *R. guilhoni* to the same clade as the tropical lineage of *R. sanguineus*, along with *R. camicasi*, depending on the loci included [14, 15, 45-47]. The taxonomy and biogeography of the *R. sanguineus* group are notoriously complex due to their morphological similarity and the tendency for different species or clades to be spread worldwide on domestic hosts. Estrada-Pena et al. [38] consider *R. guilhoni* and *R. camicasi* as tropical species that have invaded Palearctic regions, whereas *R. leporis* appears to be a Palearctic species that has been introduced into sub-Saharan Africa [45]. There are few molecular data available for *R. camicasi* but the sequences provided by Chandra et al. [25] for "*R.* cf *camicasi*" from Riyadh Province are clearly distinct from available references for other *Rhipicephalus* spp. and clustered with a small proportion of our nymph specimens from rodents. To add further to the complexity, *R. camicasi* from Saudi Arabia did not form a single OTU in our analyses, including in the concatenated phylogeny.

Rhipicephalus camicasi was originally described from Northeast Africa in 1976 [33]. It was not included in the tick fauna of Saudi Arabia by Hoogstraal et al. [22], who listed only two native *Rhipicephalus* spp. (*R. sanguineus* and *R. turanicus*), excluding the subgenus *Boophilus*. However, they noted the presence of unidentified *Rhipicephalus* spp. on numerous mammalian hosts, including *A. dimidiatus*, *M. rex* and *M. musculus*. Subsequently, Pegram et al. [36] stated that *R. camicasi* could be found on livestock (ruminants, camels and donkeys) in Yemen and Saudi Arabia without details of specific locations. More recently, *R. camicasi* has been reported from sheep in Makkah Province [48] and from camels and dogs in Riyadh Province [25, 26], as well as from *A. dimidiatus* (as nymphs and larvae) in Ta'if, Makkah Province [27]. To the best of our knowledge, *R. camicasi* has not been reported from a hedgehog host previously worldwide [38]. Our incidental finding of *R. camicasi* on a single *P. aethiopicus* in this study should be followed by a targeted survey to determine if this common and widespread host acts as a vehicle or reservoir to maintain *R. camicasi* populations nationwide.

Very few studies have attempted to identify ticks from small mammal hosts from Saudi Arabia or Yemen previously. However, the classic wild mammal survey of Yemen (which borders 'Asir) by Sanborn & Hoogstraal [49] reported *R. simus*, *R. sanguineus* and *Ornithodoros* sp. from *M. musculus*; *H. leachi* and *R. simus* from *A. dimidiatus*; and *R. simus* and "*Ixodes* sp. nov." from *M. rex*, among a wide range of other hosts examined. Similar host-ectoparasite relationships were recorded by Hoogstraal et al. [22] for Saudi Arabia, with the addition of immature *Hyalomma* spp. observed on all three rodent species. Our finding of *Rhipicephalus* larvae on *M. rex* that appeared to be closely related to the *R. simus* complex supports these early observations of Hoogstraal regarding the introduction of African *Rhipicephalus* spp. into the Arabian Peninsula. Asiry & Fetoh [28] described *R. turanicus* infestations on *A. dimidiatus*, alongside *R. sanguineus* and *R. turanicus* feeding on *R. rattus*, from Ha'il Province in northern Saudi Arabia. Notably, the most recent prior survey by Harrison et al. [27] echoed the work of Hoogstraal et al. [22] in reporting the presence of an unidentified immature *Rhipicephalus* sp. on rodents in Riyadh and Ta'if. It was most common on *M. rex* in Ta'if but was also found on *Meriones lybicus* in Riyadh and in smaller numbers on *Gerbillus nanus* in both locations. Only a single specimen was found on *A. dimidiatus* (in Ta'if), a host species on which it was apparently outcompeted by *R. camicasi* (see above). However, no morphological description (in particular, how the specimens were differentiated from *R. camicasi*) or molecular barcode was provided for this unidentified *Rhipicephalus* sp. Overall, these studies from Arabia highlight distinct differences compared with the wider Middle East, as a recent systematic review reported that *Hyalomma rhipicephaloides* and *Ixodes eldaricus* were the most prevalent ticks found on rodents in the whole region [50].

The only native *Haemaphysalis* spp. recorded from Saudi Arabia in Hoogstraal et al. [22] were *H. erinacei* and *H. sulcata*; while in Yemen, *H. leachi* (presumably introduced from Africa) was reported on *A. dimidiatus* [21, 49]. Prior to the emergence of severe fever with thrombocytopenia syndrome virus and the global spread of its vector, *Haemaphysalis longicornis*, molecular analyses of the genus *Haemaphysalis* had been relatively limited [51]. However, sufficient data are available to conclude that neither the morphology nor the 16S rRNA sequences of our *Haemaphysalis* spp. specimens are fully compatible with species previously recorded from Arabia. The two distinct OTUs we identified exhibited closest

relationships with African *Haemaphysalis* spp. (*H. spinulosa*, *H. muhsamae* and *H. elliptica*) that primarily parasitize carnivores or erinaceids in the adult stage and rodents as immature stages [37, 52]. The previous surveys of rodent ticks conducted in Arabia (see above) suggest that *Haemaphysalis* spp. are restricted (or at least more abundant) in Yemen and southern Saudi Arabia compared with more northern regions. Whether the novel *Haemaphysalis* OTUs represent undescribed species native to the southern Arabian Peninsula will require further investigations, including locating adult specimens for comprehensive morphological and molecular analyses.

This first molecular analysis of ticks collected from rodents in the Arabian Peninsula raises many questions about the evolution and distribution of *Rhipicephalus* spp. and *Haemaphysalis* spp. in this understudied region. For instance, the taxonomic status and native geographical range of *R. camicasi* is still poorly defined, especially with respect to its relationship with the tropical lineage of *R. sanguineus*. As highlighted by Hekimoglu et al. [53], Asia Minor and the Middle East constitutes a bridge between Europe and Africa in the evolutionary history of the *R. sanguineus* complex, in which the role of *R. camicasi* remains enigmatic. A limitation of our study was that in order to maximise DNA yields for multiple PCR assays, a portion of each specimen was not retained as a voucher [54] prior to DNA extraction. Hence, it is not clear if *R. camicasi* and *Rhipicephalus* OTU 1 are morphologically distinct in the immature stages, which would be suggested by the work of Harrison et al. [27] - if OTU 1 is indeed the species they recorded from Riyadh and Ta'if. However, the formal characterisation of *Rhipicephalus* OTU 1 will require adult stages to be sampled from the environment or host(s), which of course remain unknown currently. A second limitation of the work presented here is that only short fragments from mitochondrial loci were sequenced. Although mitochondrial *versus* nuclear marker-based phylogenies for ticks are generally congruent [3, 20], nuclear-mitochondrial discordance has been observed within tick species previously [55]. Moreover, the incongruent results between mitochondrial loci for specimen R9_7 could indicate hybridisation between *Rhipicephalus* OTU 1 and *R. camicasi*. Nevertheless, whether or not *Rhipicephalus* OTU 1 is ultimately recognised as a distinct species, our findings suggest a hotspot of tick diversity exists in the 'Asir Mountains that deserves further faunistic, ecological, and genetic investigations.

Conclusions

In a small region of the 'Asir Mountains in southwest Saudi Arabia, small mammals were found to be infested with *Rhipicephalus* spp. and *Haemaphysalis* spp. ticks that formed four and two clades, respectively, by the ABGD method. In addition to two clades of *R. camicasi*-like adult and nymphal ticks and one clade of *R. simus*-like larvae, a novel OTU composed of *Rhipicephalus* nymphs was found infesting three species of rodent hosts. It was related to, but distinct from, *R. leporis*, *R. guilhoni* and the tropical lineage of *R. sanguineus*. Prior ectoparasite sampling from rodents trapped in other regions of Saudi Arabia suggest this clade might constitute a widespread novel species and future studies should focus on locating adult specimens to permit a formal description of the taxon.

Abbreviations

ABGD, automatic barcode gap discovery; OTU, operational taxonomic unit.

Declarations

Ethics approval and consent to participate

Permission for rodent trapping and euthanasia was received from the Saudi Wildlife Authority by co-author ANA before fieldwork commenced. Ethical approval from the University of Liverpool's Animal Welfare and Ethics Review Board was also obtained. Rodents were euthanized by inhaled anaesthetic overdose according to guidelines published by the American Veterinary Medical Association Council on Research (Underwood et al., 2013) and the Canadian Council on Animal Care (Charbonneau et al., 2010). A single individual of the desert hedgehog (*Paraechinus aethiopicus*) was released live after removal of ticks.

Consent for publication

Not applicable.

Availability of data and materials

Sequence data are available from GenBank with identifiers MW742686-MW742711 for *coi*, MW756110-MW756125 for 12S rRNA, and MW763030-MW763059 for 16S rRNA.

Competing interests

None declared.

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Authors' contributions

The study was designed by BLM and ANA. Fieldwork was performed by SQA, HAA, ANA and BLM. Molecular procedures were conducted by SQA and HAA, and morphological studies by SQA and JWM. Sequence analysis was undertaken by VLL. The manuscript was drafted by VLL and BLM and edited by all co-authors. All authors read and approved the final version of the manuscript.

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Tables

Table 1. The number of host species trapped by location.

Village	GPS coordinates	Host species (*n*)					
		A. dimidiatus	*M. rex*	*M. musculus*	*M. yemeni*	*R. rattus*	*P. aethiopicus*
Al Ous'	18.27641, 42.320611	33	0	0	0	1	0
Wosanib	18.315641, 42.211478	10	5	0	2	0	1
Alogl	18.34654, 42.31654	2	13	3	6	0	0

Table 2. Tick specimens examined in this study, origin, and sequences obtained.

						Loci sequenced[#]		
Tick sample ID	**Tick genus**	**Host species**	**Location**	**Year**	**Habitat type**	***coi***	**16S**	**12S**
R11_1	*Rhipicephalus*	*M. rex*	Alogl	2017	Agricultural	Y	Y	N
R11_2						Y	N	N
R11_3						Y	N	N
R12		*M. rex*	Alogl	2017	Agricultural	N	Y	N
R13		*A. dimidiatus*	Alogl	2017	Agricultural	Y	N	Y
R15		*M. rex*	Alogl	2017	Agricultural	Y	Y	Y
R22		*M. yemeni*	Alogl	2017	Montane	Y	N	N
R25		*A. dimidiatus*	Wosanib	2017	Montane	Y	Y	Y
R29*		*M. rex*	Wosanib	2017	Agricultural	N	Y	Y
R3		*M. rex*	Alogl	2017	Agricultural	Y	N	N
R30		*M. rex*	Wosanib	2017	Agricultural	Y	N	N
R39		*A. dimidiatus*	Wosanib	2017	Montane	N	Y	N
R4		*M. rex*	Alogl	2017	Agricultural	N	Y	N
R46_3		*A. dimidiatus*	Wosanib	2017	Montane	N	Y	N
R5_1		*M. rex*	Alogl	2017	Agricultural	Y	Y	Y
R5_2						Y	N	N
R5_3						Y	N	N
R5_4						Y	N	N
R7_1		*M. rex*	Alogl	2017	Agricultural	Y	Y	Y
R7_2						Y	N	N
R7_3						Y	Y	Y
R7_4						Y	N	N
R8		*M. rex*	Alogl	2017	Agricultural	Y	Y	N
R9_1		*M. rex*	Alogl	2017	Agricultural	Y	Y	Y
R9_2						N	Y	N

R9_28						Y	Y	Y
R9_3						Y	Y	N
R9_4						Y	Y	Y
R9_5						N	Y	Y
R9_6						Y	Y	Y
R9_7						Y	Y	Y
R9_8						Y	N	Y
H1_1		*P. aethiopicus*	Wosanib	2020	Montane	N	Y	Y^
H1_2						Y	Y	Y^
R3a_1	*Haemaphysalis*	*A. dimidiatus*	Al Ous'	2016	Montane	NA	Y	NA
R3a_2						NA	Y	NA
R3a_3						NA	Y	NA
R3a_7						NA	Y	NA
R6a	*Haemaphysalis*	*A. dimidiatus*	Al Ous'	2016	Montane	NA	Y	NA
R20a	*Haemaphysalis*	*A. dimidiatus*	Al Ous'	2016	Montane	NA	Y	NA
R44_1	*Haemaphysalis*	*M. musculus*	Alogl	2017	Montane	NA	Y	NA
R44_2						NA	Y	NA

[#]Y, sequence obtained; N, sequence not obtained; NA, sequence amplification not attempted.

*All sequences were obtained from individual nymphs except for R25, which was a pool of six larvae.

^Sequences obtained were too short (~200 bases) to include in phylogenetic analyses.

Figures

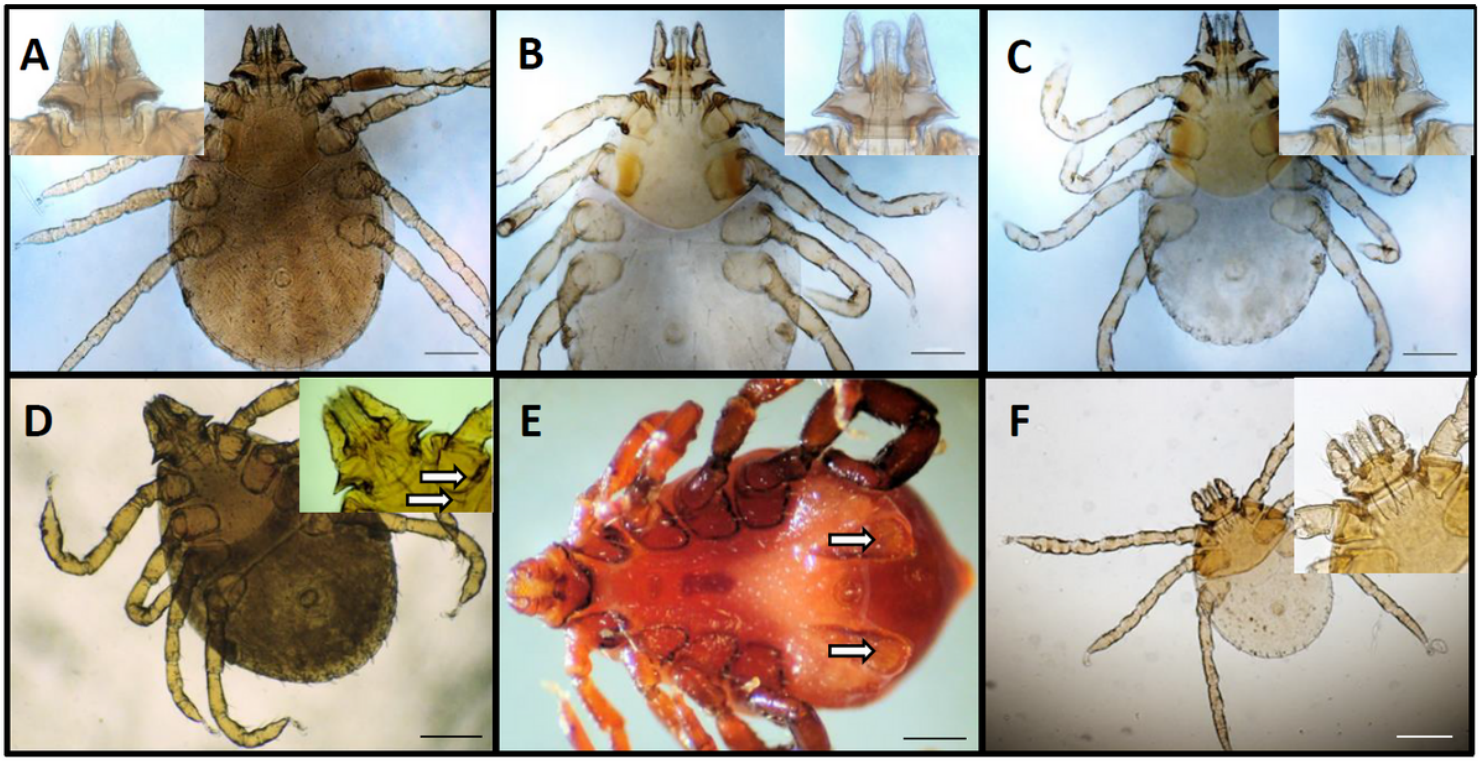

Figure 1

Morphology of Rhipicephalus spp. ticks from 'Asir. A – C Nymphs from Alogl (A, C) and Al Ous' (B) displaying variation in the shape of the palps (insets) and extent of the dorsal shield. D Nymph from Wosanib. Inset shows poorly define spurs (arrows) on coxa I. E Adult male from Wosanib. Note shape of adanal plates (arrows). F Larva from Wosanib. Inset displays details of the gnathostome. All scale bars, 200 µm; except in E, 500 µm.

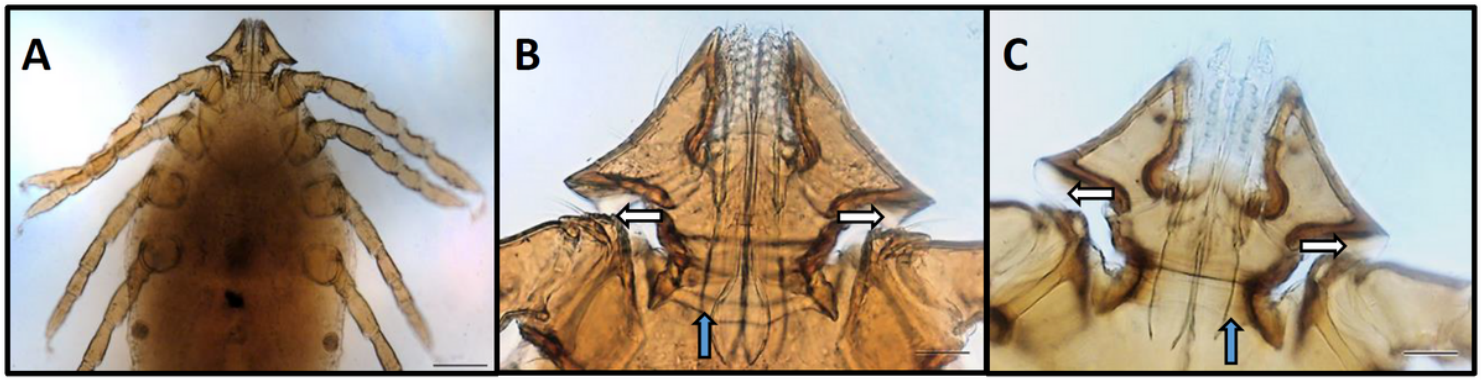

Figure 2

Morphology of Haemaphysalis nymphs from Al Ous'. A Overview of a specimen displaying the posteriorly flared palps. B Detail of the gnathostome from A. Note the triangular spurs on palp segment I (white arrows) and convex posterior margin to basis capitulum (blue arrow). C A different specimen displaying spurs on palps (white arrows) and straight posterior margin to basis capitulum (blue arrow). Scale bars, 200 µm (A); 50 µm (B, C).

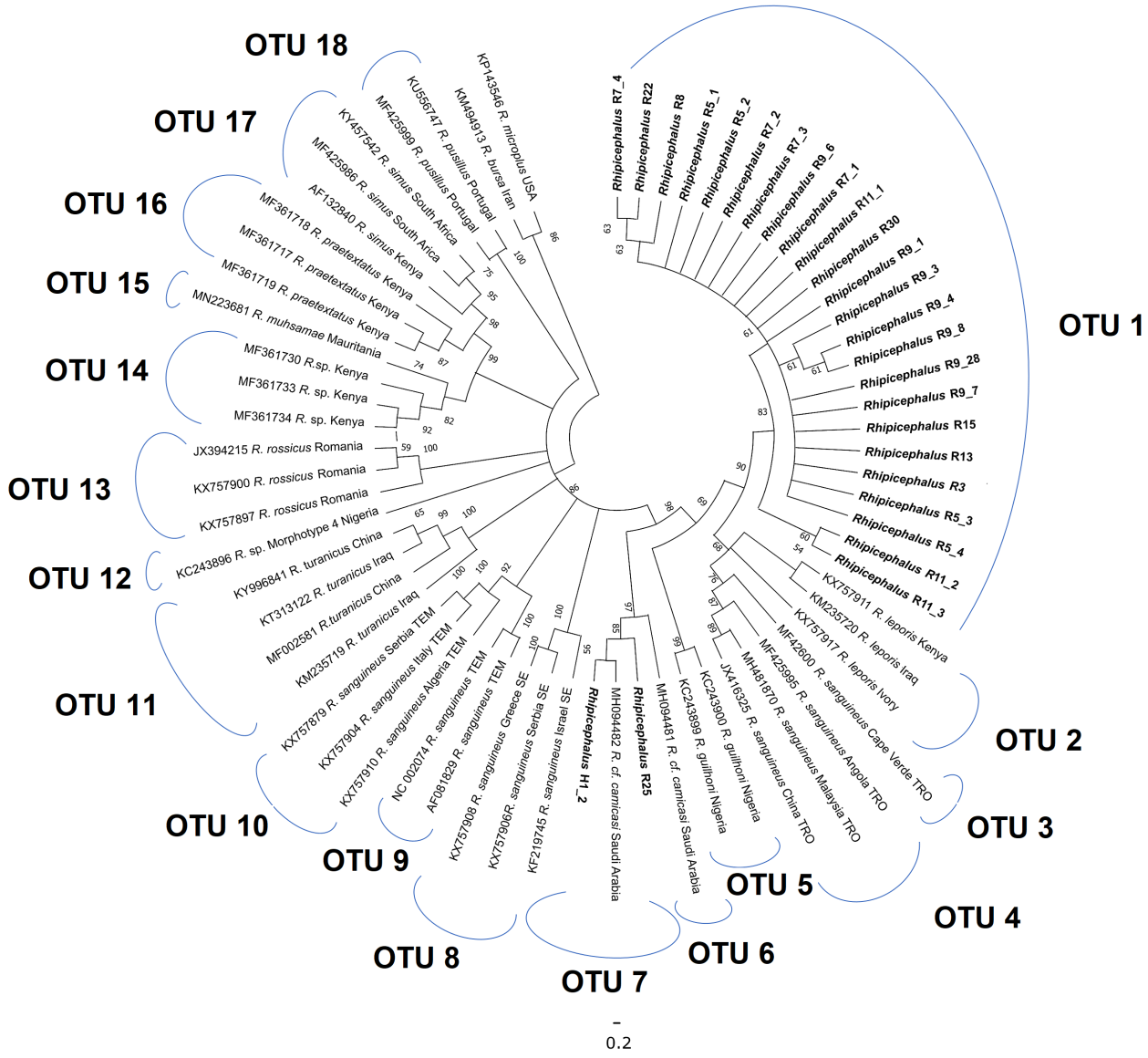

Figure 3

Neighbour-joining phylogenetic tree of Rhipicephalus taxa based on 254 bp of coi sequences. Bootstrap values are shown on the branches. Sequences generated from the present study are indicated in bold type. TRO = tropical lineage, TEM = temperate lineage and SE = south-eastern Europe lineage.

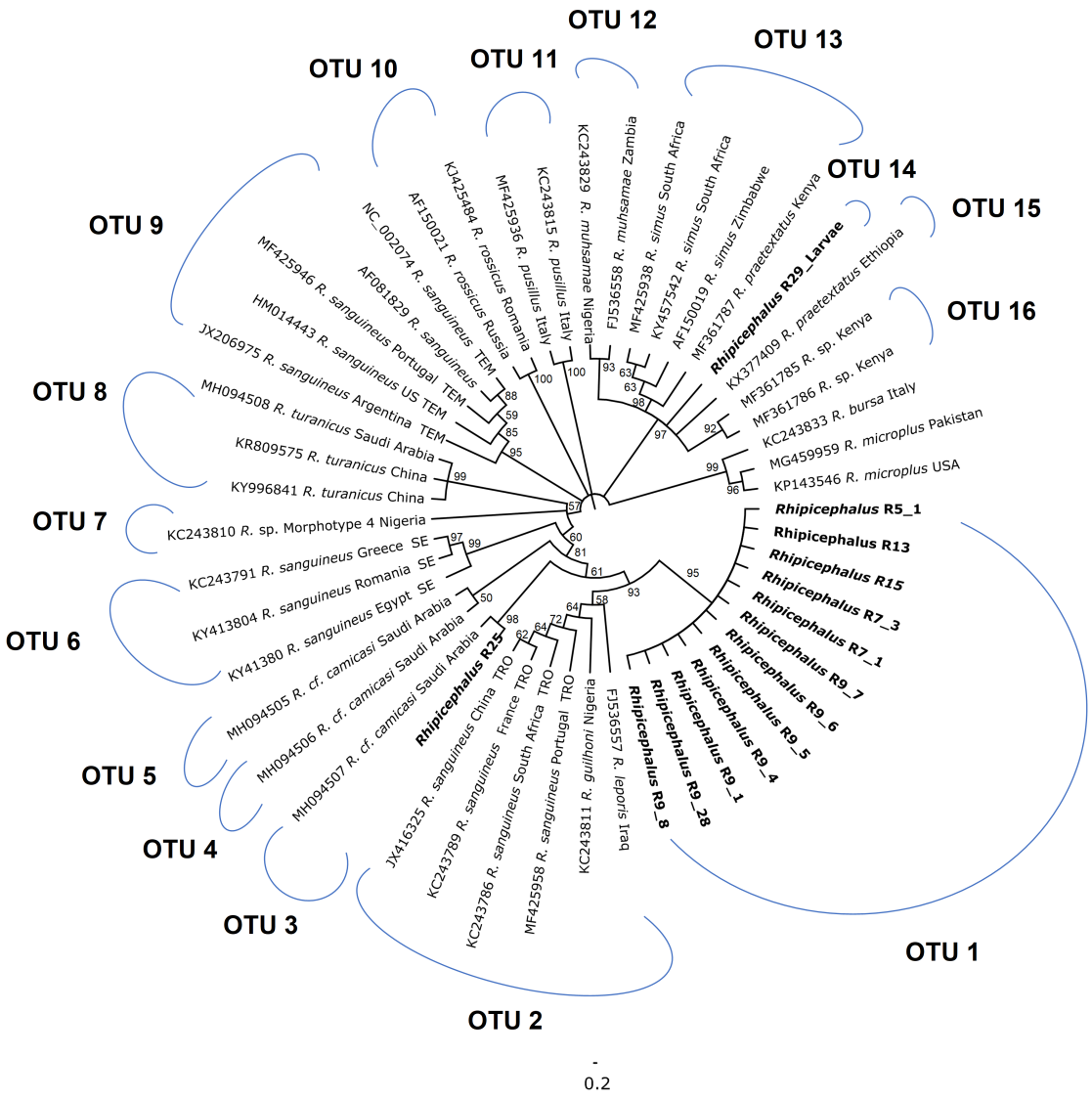

Figure 4

Neighbour-joining phylogenetic tree of Rhipicephalus taxa based on 222 bp of 12S rRNA sequences. Bootstrap values are shown on the branches. Sequences generated from the present study are indicated in bold type. TRO = tropical lineage, TEM = temperate lineage and SE = south-eastern Europe lineage.

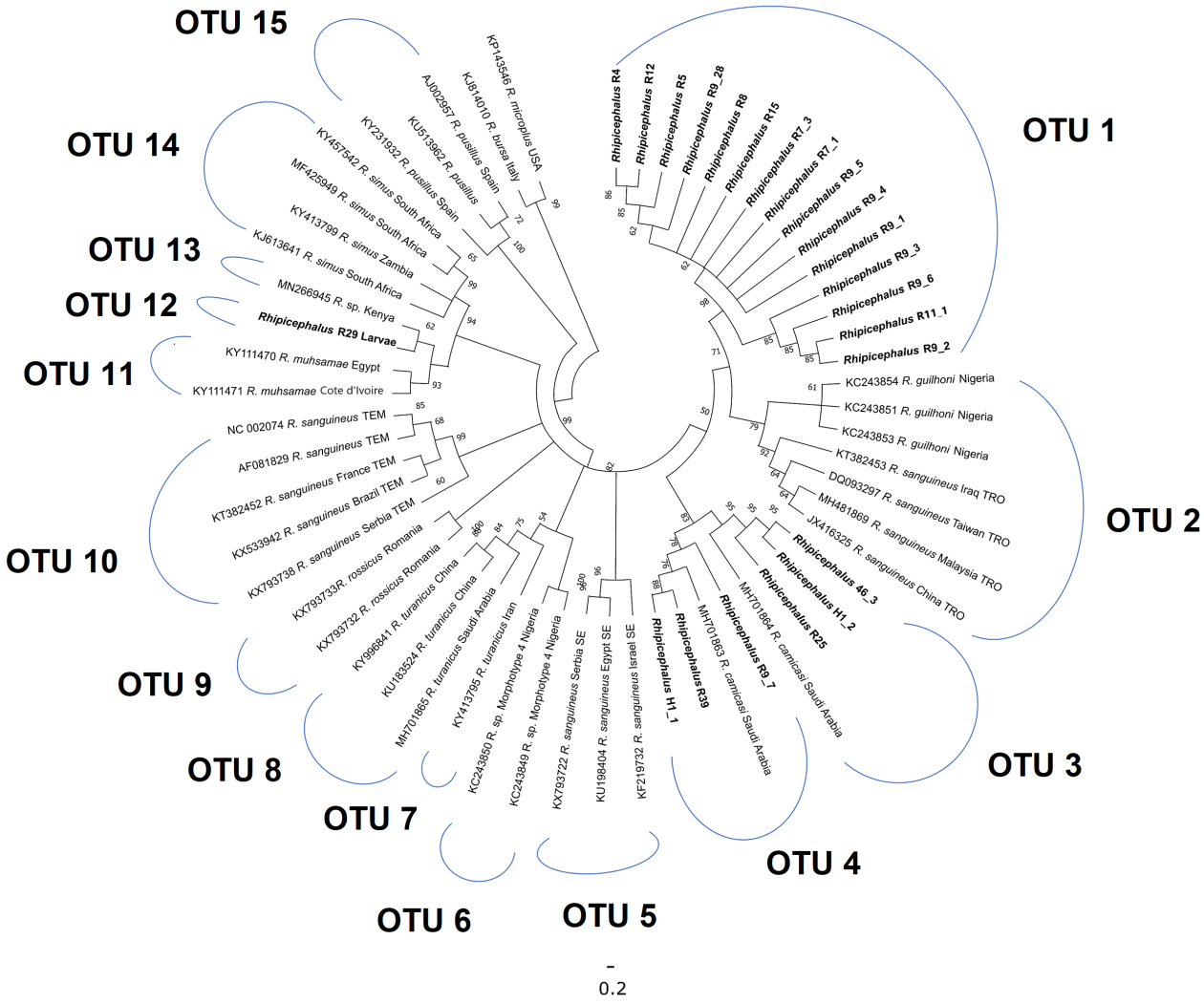

Figure 5

Neighbour-joining phylogenetic tree of Rhipicephalus taxa based on 236 bp of 16S rRNA sequences. Bootstrap values are shown on the branches. Sequences generated from the present study are indicated in bold type. TRO = tropical lineage, TEM = temperate lineage and SE = south-eastern Europe lineage.

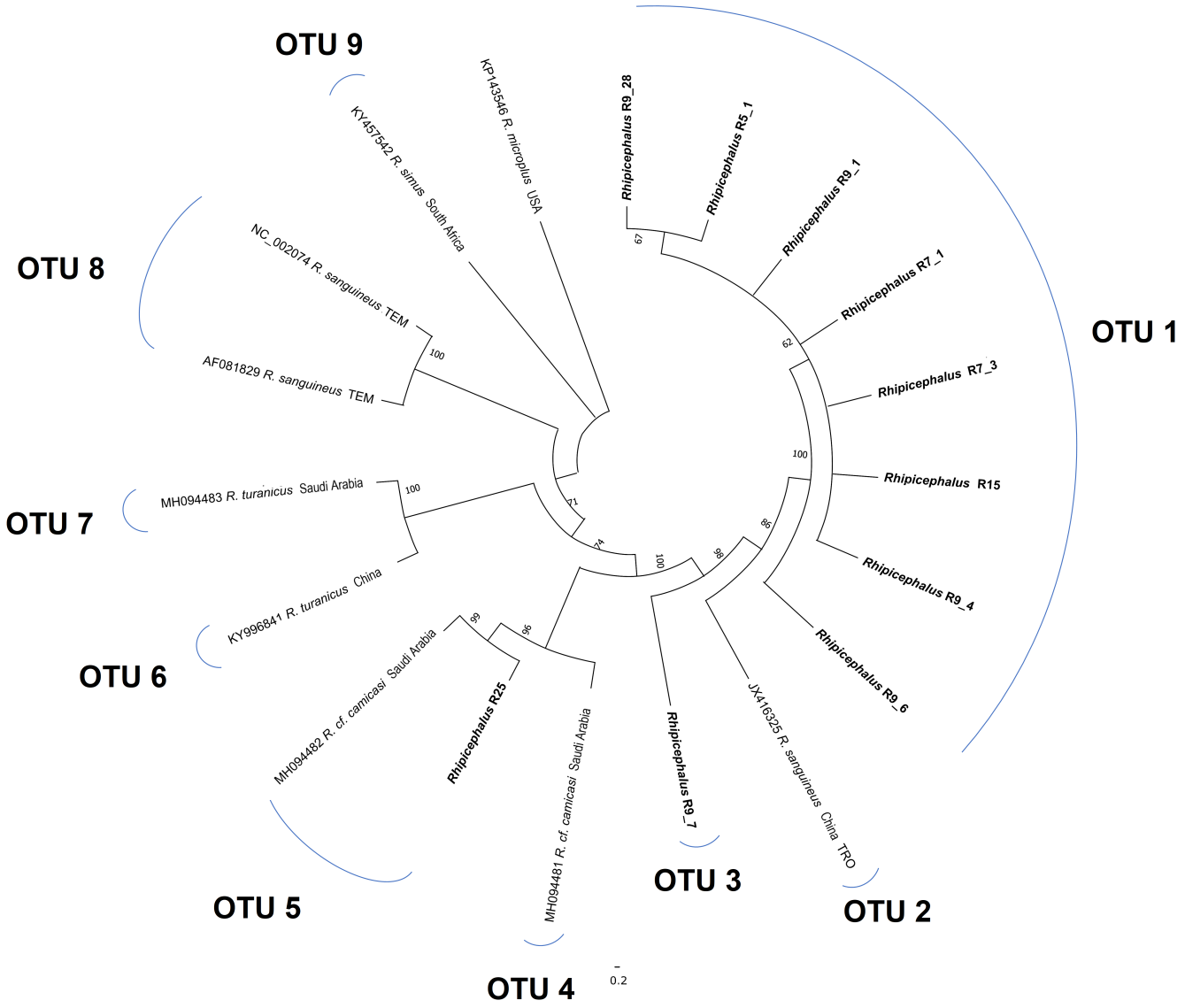

Figure 6

Neighbour-joining phylogenetic tree of Rhipicephalus taxa based on 716 bp of concatenated coi + 12S rRNA + 16S rRNA sequences. Bootstrap values are shown on the branches. Sequences generated from the present study are indicated in bold type. TRO = tropical lineage, TEM = temperate lineage and SE = south-eastern Europe lineage.

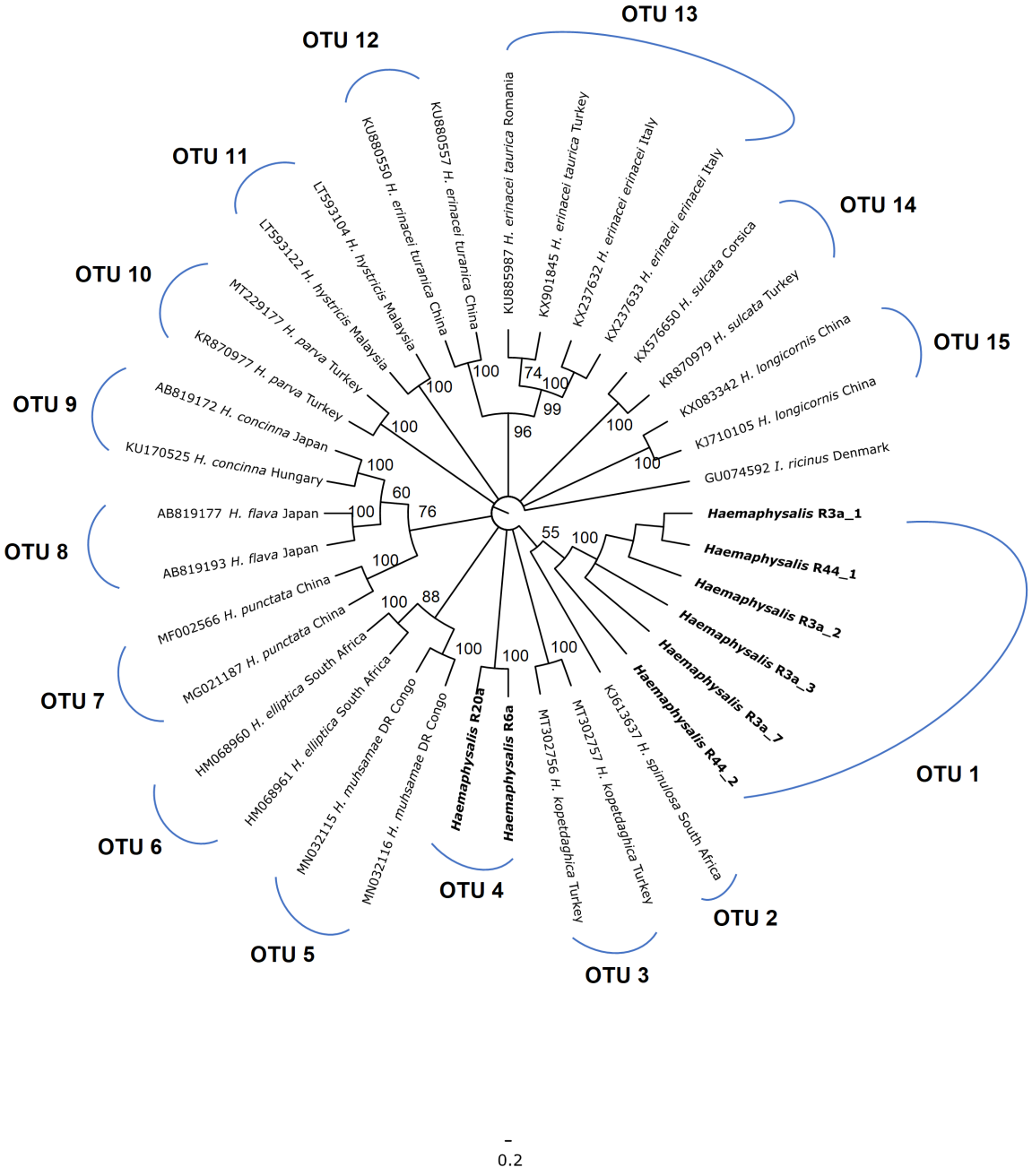

Figure 7

Neighbour-joining phylogenetic tree of Haemaphysalis taxa based on 329 bp of 16S rRNA sequences. Bootstrap values are shown on the branches. Sequences generated from the present study are indicated in bold type.

Supplementary Files

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