Ticks and Tick-borne Diseases 13 (2022) 101830

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Short communication

Rickettsiaceae in two reptile-associated tick species, *Amblyomma exornatum* and *Africaniella transversale*: First evidence of *Occidentia massiliensis* in hard ticks (Acari: Ixodidae)

Sándor Hornok [a,*], Jenő Kontschán [b], Nóra Takács [a], Anne-Lise Chaber [c], Ali Halajian [d], Sándor Szekeres [a], Attila D. Sándor [a,e], Olivier Plantard [f]

[a] Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest, Hungary
[b] Plant Protection Institute, Centre for Agricultural Research, ELKH, Budapest, Hungary
[c] School of Animal and Veterinary Sciences, Roseworthy Campus, The University of Adelaide, Adelaide, Australia
[d] Research Administration and Development, University of Limpopo, Sovenga, South Africa
[e] Department of Parasitology and Parasitic Diseases, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
[f] INRAE, Oniris, BIOEPAR, Nantes, France

ARTICLE INFO

Keywords:
Ixodidae
Rickettsia
Occidentia
groEL

ABSTRACT

All species of hard ticks associated with reptiles as hosts throughout their life cycle, are currently assigned to genera including *Amblyomma* and *Africaniella*. Among these species, based on literature data, *Africaniella transversale* has never been investigated for the presence of tick-borne pathogens. In this study, seven DNA extracts (two from *A. transversale* and five from *Amblyomma exornatum*) were screened for the presence of important tick-borne protozoa (piroplasms) and bacteria (Anaplasmataceae and Rickettsiaceae) with conventional PCRs and sequencing. A new heat shock protein chaperonin (*groEL*) gene-specific PCR was also developed to identify *Occidentia* spp. in these samples.

In *A. transversale, Occidentia massiliensis* (previously detected in rodent-associated soft ticks) and *Rickettsia hoogstraalii* were present. While the latter was molecularly identical with formerly reported sequences of this rickettsia, the genotype of *O. massiliensis* was new based on sequence and phylogenetic analyses of its *groEL* gene. In *A. exornatum*, a *Rickettsia* genotype closely related to *R. tamurae* and *R. monacensis*, was detected. The *ompA* sequence of this genotype was identical to that of *Rickettsia* sp. Ae-8 reported from *A. exornatum* in a reptile breeding facility in the USA.

These results show that *A. transversale* might carry *O. massiliensis* which (unless having a symbiotic nature in ticks) may originate either from the reptile host of this hard tick species or the rodent prey of reptiles. This is also the first detection of the reptile tick-associated *Rickettsia* sp. Ae-8 (phylogenetically aligning with *R. tamurae, R. monacensis*) in Africa, i.e. within the original geographical range of *A. exornatum*.

1. Introduction

Among hard ticks (Acari: Ixodidae), *Amblyomma* species occur throughout the tropical and sub-tropical regions of the world (Guglielmone et al., 2014). The taxonomy of this genus is currently under revision. Apart from *Amblyomma* sensu stricto, several species formerly listed in this genus were reassigned to a new subfamily (Bothriocrotoninae: Klompen et al., 2002) or to new genera (*Archaeocroton* and *Robertsicus*: Barker and Burger, 2018; *Africaniella*: Hornok et al., 2020). Taxonomically revised species in the latter three genera are comprised of eyeless, reptile-associated ticks.

The primary usage of reptiles as a food source only occurs in tick species formerly, or still, listed in the genus *Amblyomma*, i.e., around 30 species are usual or exclusive parasites of reptiles from the order Squamata (Horak et al., 2006; Guglielmone et al., 2014). While these reptile-associated tick species can occasionally parasitize higher vertebrate hosts (Horak et al., 2018), they are not thought to play any role in the transmission of pathogens. On the other hand, these ticks will readily have access to tick-borne pathogens from reptiles.

This study aimed at screening tick-borne pathogens in connection

* Corresponding author.
E-mail address: Hornok.Sandor@univet.hu (S. Hornok).

https://doi.org/10.1016/j.ttbdis.2021.101830
Received 15 September 2020; Received in revised form 11 September 2021; Accepted 13 September 2021
Available online 23 September 2021
1877-959X/© 2021 The Author(s). Published by Elsevier GmbH. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

with the first phylogenetic analysis of two African, reptile-associated ixodid tick species, *Amblyomma exornatum* and *Africaniella transversale* (Hornok et al., 2020). Both tick species inhabit the Afrotropical zoogeographic region (Guglielmone et al., 2014), and use Squamata as hosts (monitor lizards and pythons, respectively). In this study, specimens of both tick species (five *A. exornatum* and two *A. transversale*) were molecularly investigated for the presence of three groups of important tick-borne pathogens, i.e. Rickettsiaceae, Anaplasmataceae and piroplasms.

2. Materials and methods

2.1. Specimens used in this study

(1) *Africaniella transversale* (one female, one nymph) collected from *Python regius* imported into United Arab Emirates prior to 2017 (date unknown);
(2) *Amblyomma exornatum* (one female, three nymphs and DNA extract from the legs of a fourth nymph) collected from *Varanus albigularis* in Limpopo Province, South Africa during September 2016.

Ticks were stored in 96% ethanol and were identified under a VHX-5000 digital microscope (Keyence Co., Osaka, Japan) as reported by Hornok et al. (2020). DNA was extracted with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, including an overnight digestion in tissue lysis buffer and Proteinase-K at 56 °C.

2.2. Target groups screened with conventional PCRs

(1) Rickettsiales: Anaplasmataceae

A 350-bp-long fragment of the 16S rRNA gene was amplified with the primers EHR-16sD (5′-GGT ACC YAC AGA AGA AGT CC-3′) and EHR-16sR (5′-TAG CAC TCA TCG TTT ACA GC-3′) (Brown et al., 2001) as reported (Hornok et al., 2018a). These primers were designed to detect genera within Anaplasmataceae, but may also yield PCR products of members from closely related families (Parola et al., 2003).

(2) *Occidentia massiliensis* (Rickettsiales: Rickettsiaceae)

New primers, i.e., Om-groELf1 (5′- AAA AAA GAA ATG TTA GAA GAT ATT GC-3′) and Om-groELr2 (5′-GTA CGT ACW ACT TTA GTT GG-3′) were designed to match the heat shock chaperonin protein encoding *groEL* gene of *O. massiliensis* (note that based on alignments this primer pair may also amplify *Orientia tsutsugamushi*). Based on currently available GenBank data, these primers only align to sequences of these two species, amplifying a 656- and a 669-bp-long fragment of their *groEL* genes, respectively. The reaction volume was 25 µl, which included 3 µl of extracted DNA, and 22 µl of reaction mixture containing 1 unit of HotStarTaq Plus DNA polymerase (5 U/µl), 200 µM of PCR nucleotide mix, 1 µM of each primer and 2.5 µl of 10 × Coral Load PCR buffer (15 mM $MgCl_2$ included). For amplification, an initial denaturation step at 95 °C for 5 min was followed by 40 cycles of denaturation at 95 °C for 20 s, annealing at 50 °C for 30 s and extension at 72 °C for 1 min. Final extension was performed at 72 °C for 5 min.

(3) *Rickettsia* species (Rickettsiales: Rickettsiaceae)

Three consecutive PCRs were used to screen for rickettsiae (Hornok et al., 2018b), for which the target lengths and corresponding primers were as follows:

(a) a 380 bp-long fragment of the citrate synthase (*gltA*) gene with the primers RpCs.877p (5′-GGG GGC CTG CTC ACG GCG G-3′) and RpCs.1258n (5′-ATT GCA AAA AGT ACA GTG AAC A-3′) (Regnery et al., 1991);
(b) an approximately 480-bp-long fragment of the 17 kDa surface antigen gene of *Rickettsia* spp. with primers 17kd1 (5′-GCT CTT GCA ACT TCT ATG TT-3′) and 17kd2 (5′-CAT TGT TCG TCA GGT TGG CG-3′) (Williams et al., 1992); and
(c) a 532-bp-long fragment of the outer membrane protein A (*ompA*) gene of *Rickettsia* spp. was amplified with primers Rr190.70p (5′-ATG GCG AAT ATT TCT CCA AAA-3′) and Rr190.602n (5′-AGT GCA GCA TTC GCT CCC CCT-3′) (Regnery et al., 1991).

(4) Piroplasms (Apicomplexa: Piroplasmida):

The conventional PCR used for the detection of piroplasms was modified from Casati et al. (2006), as reported in Hornok et al. (2014). This method amplifies an approximately 500-bp-long fragment of the 18S rRNA gene of *Babesia* and *Theileria* spp. with the primers BJ1 (forward: 5′-GTC TTG TAA TTG GAA TGA TGG-3′) and BN2 (reverse: 5′-TAG TTT ATG GTT AGG ACT ACG-3′).

2.3. Sequencing and phylogenetic analyses

Purification and sequencing were done by Biomi Ltd. (Gödöllő, Hungary). Sequences were compared to GenBank data with the BLASTn program (https://blast.ncbi.nlm.nih.gov). New sequences were submitted to GenBank (*gltA* gene: MN150178 and MN150179 for *Rickettsia hoogstraalii* and *Rickettsia* sp. Ae-8, respectively; 17 kDa gene: MN150180 and MN150181 for *R. hoogstraalii* and *Rickettsia* sp. Ae-8, respectively; *ompA* gene: MN150182 for *Rickettsia* sp. Ae-8; 16S rRNA and *groEL* genes: MN108044 and MT833659, respectively, for *O. massiliensis*). For phylogenetic analyses, sequences from this study and others from GenBank with high coverage (i.e. 99–100% of the fragment length amplified here) were used and resampled 1000 times to generate bootstrap values. Phylogenetic analyses were conducted with the Maximum-Likelihood method, Jukes Cantor and Tamura-3 models (according to the selection of the program) by using MEGA version 7.0.

3. Results

All samples were negative for piroplasms. However, both samples of *A. transversale* were positive in the 16S rRNA gene PCR. Unexpectedly, both sequences were 100% (301/301 bp) identical to that of the originally described (and only known) type strain of *O. massiliensis* (Rickettsiales: Rickettsiaceae), reported from the soft tick *Ornithodoros sonrai* collected in rodent burrows, in Senegal (GenBank: NR_149,220: Mediannikov et al., 2014a). All samples of *Am. exornatum* were negative in this test. Part of the *groEL* gene was also successfully amplified from these two ticks, having only 571/580 bp (98.4%) identity with the corresponding sequence of the type strain (OS18) of *O. massiliensis* (KJ395314). This was also reflected by the results of phylogenetic analysis, because the separation of these two (i.e., hard or soft tick-derived) strains of *O. massiliensis* was highly (100%) supported (Fig. 1).

In addition, from both samples of *A. transversale*, the rickettsia-specific *gltA* PCR yielded a sequence with 100% (328/328 bp) identity to *R. hoogstraalii* (GenBank: MF379281, from *Haemaphysalis parva*). These samples were also positive in the PCR amplifying the 17 kDa antigen gene of rickettsiae. Sequencing confirmed the results of the *gltA* assay, with 100% (378/378 bp) identity to *R. hoogstraalii* (GenBank: FJ767736, from *Haemaphysalis sulcata*). However, amplification of the *ompA* gene fragment was not successful from *A. transversale*.

All DNA extracts of *A. exornatum* were positive in the *Rickettsia gltA* PCR. In these samples, sequencing identified another *Rickettsia* species, which was closely related to *Rickettsia* sp. 10610RCRICK (GenBank: EF662058, from *Ixodes scapularis*), with 99.7% (329/330 bp) *gltA*

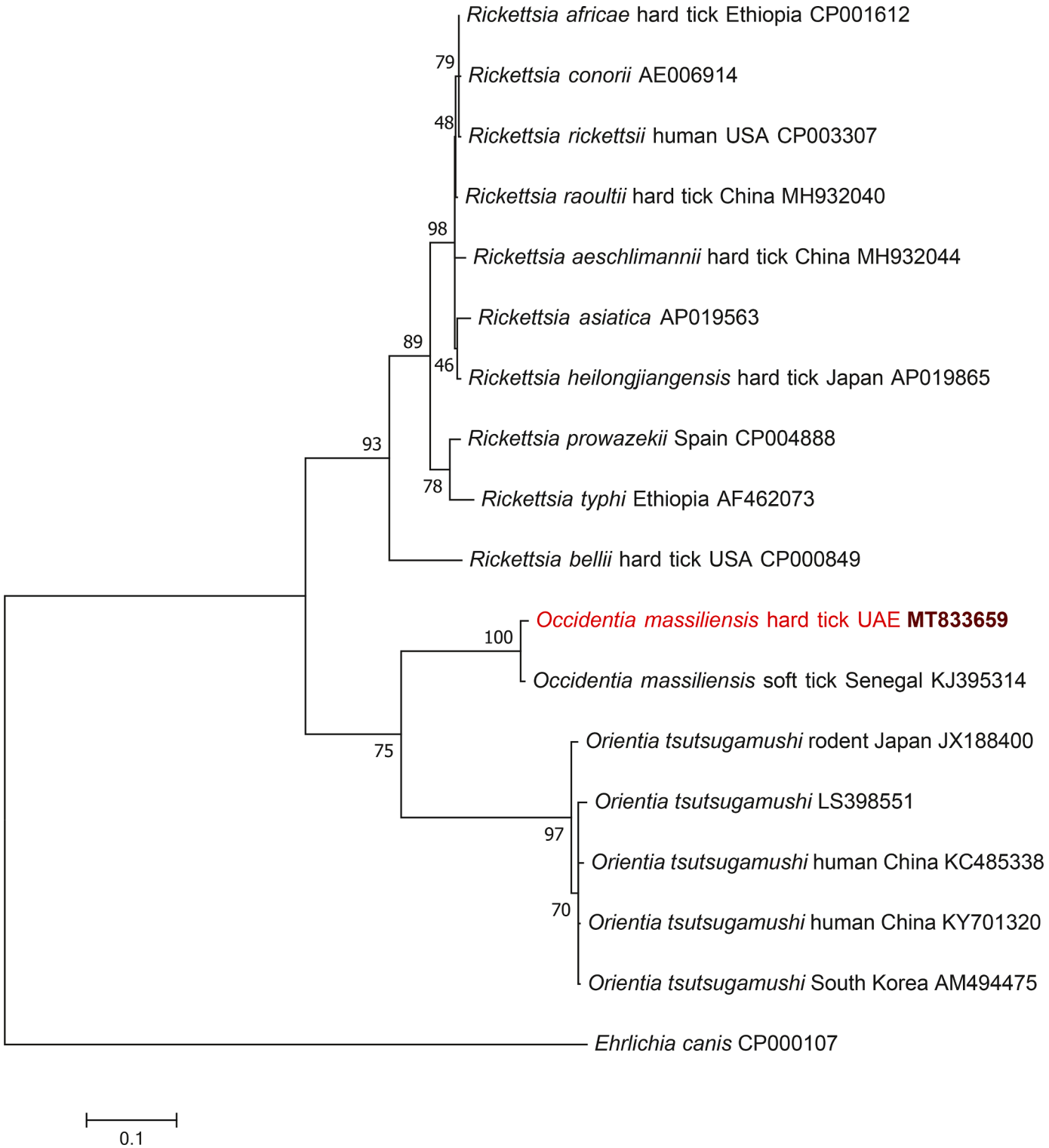

Fig. 1. *GroEL* gene Maximum Likelihood tree (Jukes-Cantor model) of Rickettsiaceae. The species name is followed by the isolation source (if known), the country of origin and GenBank accession number. The sequence from this study is indicated in red font and bold accession number. The scale-bar indicates the number of substitutions per site.

sequence identity. Considering the results of PCRs performed to analyze this species further, both PCRs (for the 17 kDa antigen gene and the *ompA* gene fragments) yielded sequenceable products which were 100% identical (379/379 bp and 470/470 bp, respectively) to those of *Rickettsia* sp. Ae-8 (GenBank: DQ365986 and DQ365985, respectively, also reported from *A. exornatum*). In addition, this rickettsia had 465/470 bp (98.9%) *ompA* sequence identity with the type strain (AT-1) of *R. tamurae* (DQ103259) reported from *Amblyomma testudinarium* ticks in Japan, and the separation of these two rickettsiae was supported by high bootstrap value in the phylogenetic analysis (Fig. 2). These two species clustered together with *Rickettsia monacensis*, forming a sister group to other spotted fever group rickettsiae (Fig. 2).

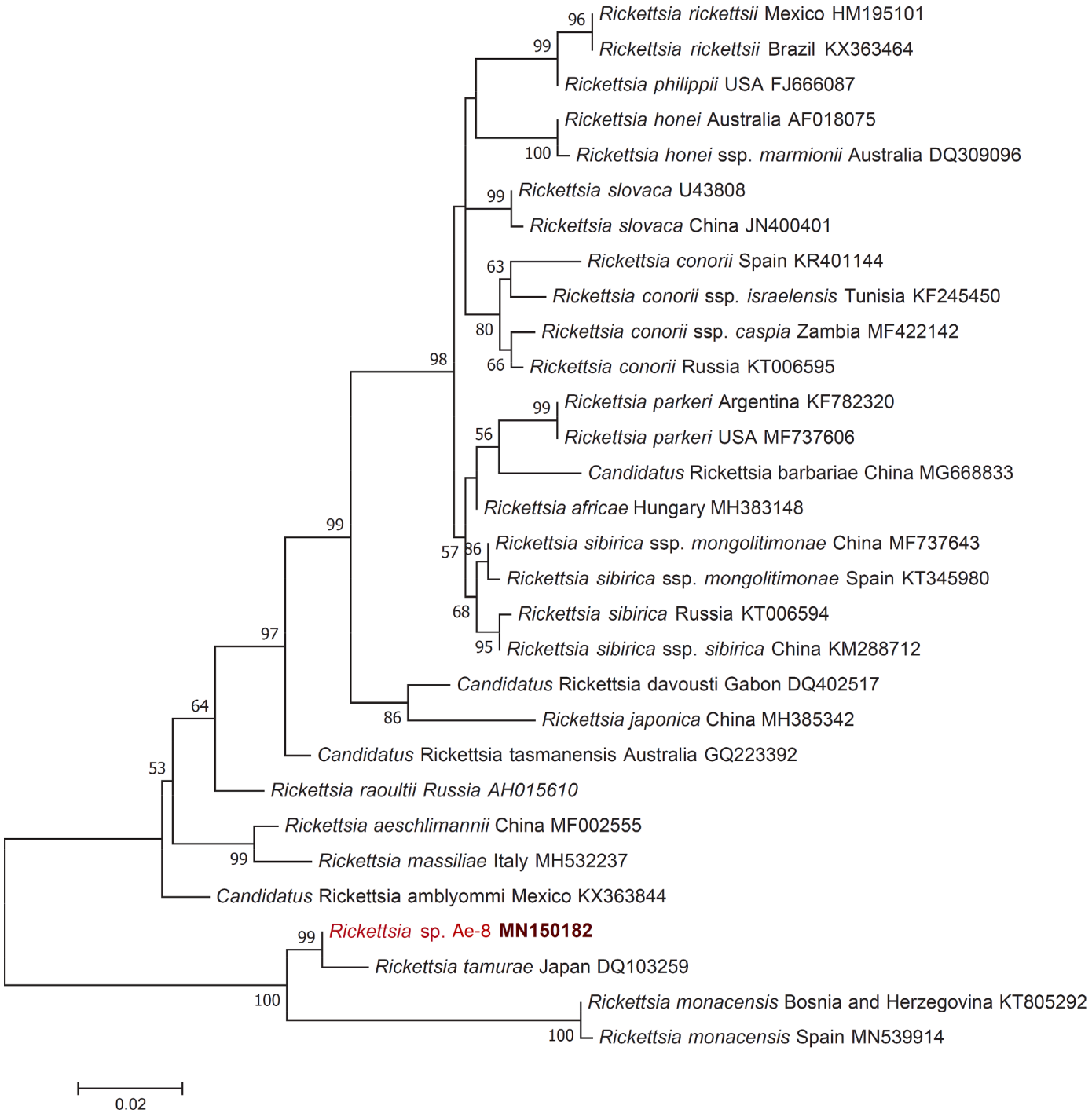

Fig. 2. *OmpA* gene Maximum Likelihood tree (Tamura-3 model) of *Rickettsia* species. Each species name is followed by the country of origin and GenBank accession number. The sequence from this study is indicated in red font and bold accession number. The scale-bar indicates the number of substitutions per site.

4. Discussion

Occidentia massiliensis, isolated and reported previously only from soft ticks collected in rodent burrows in Senegal (Mediannikov et al., 2014a), was identified here in *A. transversale*. To the best of our knowledge, this is the first molecular evidence of *O. massiliensis* occurring in any hard tick species. Because two genetic markers of *O. massiliensis* were amplified from *A. transversale*, it is reasonable to suppose that not only DNA fragments, but also complete microorganisms of this species were present in hard ticks. In this context, the question arises on the exact source of this bacterium in *A. transversale*.

An important geographical coincidence is that Lucas (1845) collected the type specimens of *A. transversale* in Senegal, the same country where *O. massiliensis* was collected from the soft tick species, *O. sonrai* (Mediannikov et al., 2014a). *Ornithodoros sonrai* is a nidicolous soft tick species inhabiting the burrows of small mammals (Mediannikov et al., 2014b) and mostly using rodents as hosts (Logan et al., 1993; Sylla et al., 2004). The main host of *A. transversale*, i.e., ball pythons most often also inhabit rodent burrows or abandoned termite mounds (Murphy et al., 2003; Rizzo, 2014).

Africaniella transversale completes its entire on-host life cycle in association with pythons (Lucas, 1845), on which it feeds once in each

stage and has never been reported from rodents. Similarly, *O. sonrai* is known to use reptilian hosts, even snakes occasionally (Sylla et al., 2004) and it feeds several times in the nymphal and adult stages. Therefore, we consider it likely that *O. massiliensis* transferred from *O. sonrai* to *A. transversale* via reptiles (or when co-feeding on reptiles). Similarly, given the strong association of pythons with their main prey items (rodents), including co-habitation, based on the present results, the susceptibilities of both pythons and rodents to *O. massiliensis* are equally possible. However, the latter seems more likely, since the type species of the closely related genus, *Orientia* (i.e., *O. tsutsugamushi*) also infects rodents (Cosson et al., 2015). In summary, the host range of *O. massiliensis* and its association with soft or hard ticks as vectors or reservoirs await further clarification.

In addition to *O. massiliensis*, we also detected *R. hoogstraalii* in *Af. transversale*. This rickettsia was originally described from ticks of the genus *Haemaphysalis* (Duh et al., 2010), to which *A. transversale* is closely related (Hornok et al., 2020). Taken together, these are the first microorganisms demonstrated from *A. transversale*.

The rickettsia detected here for the first time in *A. exornatum* in Africa, was previously reported in the same tick species from exotic reptiles (*Varanus olivaceus*) kept in the USA (Reeves et al., 2006). On the other hand, based on its *gltA* gene, this species is also very closely related (with only 1 bp difference) to the *Rickettsia* sp. circulating in *Ixodes* ticks in Maryland, USA (GenBank: EF662058) (Swanson and Norris, 2007). The presence of the same rickettsia in all examined *A. exornatum* ticks might be related to the transovarial origin and symbiotic nature of these microorganisms, as proposed earlier (Reeves et al., 2006; Swanson and Norris, 2007).

This is the first detection of a rickettsia associated with the reptile tick, *A. exornatum* in Africa, the original geographical range of its carrier. Interestingly, an imported case of this rickettsia was also reported from Japan, but with a shorter, 461 bp sequence (AB795206), amplified from *Amblyomma latum* originating from Madagascar (Andoh et al., 2015). It is also noteworthy that both *Rickettsia* species, shown here to be phylogenetically closely related to *Rickettsia* sp. Ae-8, are known to be associated with ticks specialized to feed on lizards (Squamata: Varanidae, Lacertidae): *Rickettsia tamurae* in Japan (Fournier et al., 2006) and *R. monacensis* in Europe (Sánchez-Montes et al., 2019), respectively. This suggests a common evolutionary origin of these three rickettsiae related to their host-associations.

Author statement

Sándor Hornok: Conceptualization, Writing, editing. **Jenő Kontschán, Nóra Takács**: Methodology, Visualization, Investigation. **Anne-Lise Chaber, Ali Halajian**: sample collection, investigation. **Sándor Szekeres**: methodology, software. **Attila D. Sándor:** financial support, conceptualization. **Olivier Plantard**: conceptualization, supervision, editing.

Acknowledgements

ADS, SH, NT and SS were supported by the National Research, Development and Innovation Office, Hungary (grant number NKFIH-132794). Thanks to Prof. W. J. Luus-Powell (SARChI Research Chair, University of Limpopo) for supporting AH with lab facility to check the road-killed monitors.

References

Andoh, M., Sakata, A., Takano, A., Kawabata, H., Fujita, H., Une, Y., Goka, K., Kishimoto, T., Ando, S., 2015. Detection of *Rickettsia* and *Ehrlichia* spp. in ticks associated with exotic reptiles and amphibians imported into Japan. PLoS ONE 10, e0133700. https://doi.org/10.1371/journal.pone.0133700.

Barker, S.C., Burger, T.D., 2018. Two new genera of hard ticks, *Robertsicus* n. gen. and *Archaeocroton* n. gen., and the solution to the mystery of Hoogstraal's and Kaufman's "primitive" tick from the Carpathian Mountains. Zootaxa 4500, 543–552. https://doi.org/10.11646/zootaxa.4500.4.4.

Brown, G.K., Martin, A.R., Roberts, T.K., Aitken, R.J., 2001. Detection of *Ehrlichia platys* in dogs in Australia. Aust. Vet. J. 79, 554–558. https://doi.org/10.1111/j.1751-0813.2001.tb10747.x.

Casati, S., Sager, H., Gern, L., Piffaretti, J.C., 2006. Presence of potentially pathogenic *Babesia* sp. for human in *Ixodes ricinus* in Switzerland. Ann. Agric. Environ. Med. 13, 65–70.

Cosson, J.F., Galan, M., Bard, E., Razzauti, M., Bernard, M., Morand, S., Brouat, C., Dalecky, A., Bâ, K., Charbonnel, N., Vayssier-Taussat, M., 2015. Detection of *Orientia* sp. DNA in rodents from Asia, West Africa and Europe. Parasit. Vectors 8, 172. https://doi.org/10.1186/s13071-015-0784-7.

Duh, D., Punda-Polic, V., Avsic-Zupanc, T., Bouyer, D., Walker, D.H., Popov, V.L., Jelovsek, M., Gracner, M., Trilar, T., Bradaric, N., Kurtti, T.J., Strus, J., 2010. *Rickettsia hoogstraalii* sp. nov., isolated from hard- and soft-bodied ticks. Int. J. Syst. Evol. Microbiol. 60, 977–984. https://doi.org/10.1099/ijs.0.011049-0.

Fournier, P.E., Takada, N., Fujita, H., Raoult, D., 2006. *Rickettsia tamurae* sp. nov., isolated from *Amblyomma testudinarium* ticks. Int. J. Syst. Evol. Microbiol. 56, 1673–1675. https://doi.org/10.1099/ijs.0.64134-0.

Guglielmone, A.A., Robbins, R.G., Apanaskevich, D.A., Petney, T.N., Estrada-Peña, A., Horak, I.G., 2014. The Hard Ticks of the World. Springer, Dordrecht, The Netherlands, p. 738.

Horak, I.G., McKay, I.J., Henen, B.T., Heyne, H., Hofmeyr, M.D., De Villiers, A.L., 2006. Parasites of domestic and wild animals in South Africa. XLVII. Ticks of tortoises and other reptiles. Onderstepoort J. Vet. Res. 73, 215–227.

Horak, I.G., Heyne, H., Williams, R., Gallivan, G.J., Spickett, A., Bezuidenhout, J.D., Estrada-Peña, A., 2018. The Ixodid Ticks (Acari: Ixodidae) of Southern Africa. Springer, Cham, Switzerland, p. 676.

Hornok, S., Mester, A., Takács, N., Fernández de Mera, I.G., de la Fuente, J., Farkas, R., 2014. *Re*-emergence of bovine piroplasmosis in Hungary: has the etiological role of *Babesia divergens* been taken over by *B. major* and *Theileria buffeli*? Parasit. Vectors 7, 434.

Hornok, S., Horváth, G., Takács, N., Farkas, R., Szőke, K., Kontschán, J., 2018a. Molecular evidence of a badger-associated *Ehrlichi*a sp., a *Candidatus* Neoehrlichia lotoris-like genotype and *Anaplasma marginale* in dogs. Ticks Tick Borne Dis. 9, 1302–1309. https://doi.org/10.1016/j.ttbdis.2018.05.012.

Hornok, S., Baneth, G., Grima, A., Takács, N., Kontschán, J., Meli, M.L., Suter, V., Salant, H., Farkas, R., Hofmann-Lehmann, R., 2018b. Molecular investigations of cat fleas (*Ctenocephalides felis*) provide the first evidence of *Rickettsia felis* in Malta and *Candidatus* Rickettsia senegalensis in Israel. New Microbes New Infect. 25, 3–6. https://doi.org/10.1016/j.nmni.2018.05.001.

Hornok, S., Kontschán, J., Takács, N., Chaber, A.-L., Halajian, A., Abichu, G., Kamani, J., Szekeres, S., Plantard, O., 2020. Molecular phylogeny of *Amblyomma exornatum* and *Amblyomma transversale*, with reinstatement of the genus *Africaniella* (Acari: ixodidae) for the latter. Ticks Tick Borne Dis. 11, 101494 https://doi.org/10.1016/j.ttbdis.2020.101494.

Klompen, H., Dobson, S.J., Barker, S.C., 2002. A new subfamily, Bothriocrotoninae n. subfam., for the genus *Bothriocroton* Keirans, King & Sharrad, 1994 status amend. (Ixodida: ixodidae), and the synonymy of *Aponomma* Neumann, 1899 with *Amblyomma* Koch, 1844. Syst. Parasitol. 53, 101–107.

Logan, T.M., Wilson, M.L., Cornet, J.P., 1993. Association of ticks (Acari: ixodoidea) with rodent burrows in northern Senegal. J. Med. Entomol. 30, 799–801.

Lucas, M.H., 1845. Sur une nouvelle espèce d'Arachnide qui appartient au genre *Ixodes*, et qui vit dans le contour interne de la cavité orbitaire de *Python sebae*, Duméril et Bibron (*Coluber sebae*, Gmelin). Annal. Soc. Entomol. France (Sér. 2) 3, 61–65.

Mediannikov, O., Nguyen, T.T., Bell-Sakyi, L., Padmanabhan, R., Fournier, P.E., Raoult, D., 2014a. High quality draft genome sequence and description of *Occidentia massiliensis* gen. nov., sp. nov., a new member of the family Rickettsiaceae. Stand. Genomic Sci. 9, 9. https://doi.org/10.1186/1944-3277-9-9.

Mediannikov, O., Diatta, G., Kasongo, K., Raoult, D., 2014b. Identification of bartonellae in the soft tick species *Ornithodoros sonrai* in Senegal. Vector Borne Zoonotic Dis. 14, 26–32. https://doi.org/10.1089/vbz.2013.1326.

Murphy, J.B., Schlager, N., Hutchins, M. (Eds.), 2003. Grzimek's Animal Life Encyclopedia, 2nd ed. Reptiles, Gale Group, Farmington Hills, MI, pp. 419–428. Vol. 7.

Parola, P., Cornet, J.P., Sanogo, Y.O., Miller, R.S., Thien, H.V., Gonzalez, J.P., Raoult, D., Telford III, S.R., Wongsrichanalai, C., 2003. Detection of *Ehrlichia* spp., *Anaplasma* spp., *Rickettsia* spp., and other eubacteria in ticks from the Thai-Myanmar border and Vietnam. J. Clin. Microbiol. 41, 1600–1608. https://doi.org/10.1128/JCM.41.4.1600-1608.2003.

Reeves, W.K., Durden, L.A., Dasch, G.A., 2006. A spotted fever group *Rickettsia* from an exotic tick species, *Amblyomma exornatum* (Acari: ixodidae), in a reptile breeding facility in the United States. J. Med. Entomol. 43, 1099–1101. https://doi.org/10.1603/0022-2585(2006)43[1099:asfgrf]2.0.co;2.

Regnery, R.L., Spruill, C.L., Plikaytis, B.D., 1991. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J. Bacteriol. 173, 1576–1589. https://doi.org/10.1128/jb.173.5.1576-1589.1991.

Rizzo, J.M., 2014. Captive care and husbandry of ball pythons (*Python regius*). J. Herpetol. Med. Surg. 24, 48–52.

Sánchez-Montes, S., Isaak-Delgado, A.B., Guzmán-Cornejo, C., Rendón-Franco, E., Muñoz-García, C.I., Bermúdez, S., Morales-Diaz, J., Cruz-Romero, A., Romero-Salas, D., Dzul-Rosado, K., Lugo-Caballero, C., Colunga-Salas, P., Becker, I., 2019. *Rickettsia* species in ticks that parasitize amphibians and reptiles: novel report from Mexico and review of the worldwide record. Ticks Tick Borne Dis. 10, 987–994. https://doi.org/10.1016/j.ttbdis.2019.04.013.

Swanson, K.I., Norris, D.E., 2007. Co-circulating microorganisms in questing *Ixodes scapularis* nymphs in Maryland. J. Vector. Ecol. 32, 243–251.
Sylla, M., Pourrut, X., Faye, N., Bâ, K., Cornet, J.P., Camicas, J.L., 2004. Argasidae (Acari: ixodida) parasite of wild and domestic animals in Senegal: 1-Review and distribution. Acarologia 44, 137–150.
Williams, S.G., Sacci, J.B., Schriefer, M.E., Anderson, E.M., Fujioka, K.K., Sorvillo, F.J., Barr, A.R., Azad, A.F., 1992. Typhus and typhus-like rickettsiae associated with opossums and their fleas in Los Angeles county. California. J. Clin. Microbiol. 30, 1758–1762.