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Original article

Molecular divergences of the *Ornithodoros sonrai* soft tick species, a vector of human relapsing fever in West Africa

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Abstract

The soft tick *Ornithodoros sonrai* is recognized as the only vector of *Borrelia crocidurae* causing human relapsing fever in West Africa. Its determination has been exclusively based on morphological features, geographical distribution and vector competence. Some ambiguities persist in its systematics and may cause misunderstanding about West African human relapsing fevers epidemiology. By amplifying and aligning *16S* and *18S* rDNA genes in *O. sonrai* specimens collected from 14 distinct sites in Senegal and Mauritania, we showed the existence of four genetically different subgroups that were morphologically and ecologically identified as belonging to the same species. Within *O. sonrai*, intraspecific polymorphism was high (pairwise divergence from 0.2% to 16.4%). In all cases, these four subgroups formed a monophyletic clade sharing a common ancestor with East African soft ticks that transmit *Borrelia duttoni* human relapsing fever. From amplification of the *flagellin* gene of *B. crocidurae* we verified that all subgroups of *O. sonrai* were infected by *B. crocidurae* and may constitute vectors for this pathogen. All *flagellin* sequences were identical, refuting the hypothesis suggesting parallel evolution between *O. sonrai* and *B. crocidurae*. However, differences in infection rates were significant, suggesting different vector competences between subgroups of *O. sonrai*.
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1. Introduction

Ornithodorean soft ticks have global medical importance because of their capacity to transmit human relapsing fever [1]. Each *Borrelia* species causing relapsing fever is considered to be specific to its tick vector [2]. In Africa, three tick-*Borrelia* associations are recognized: (1) *Ornithodoros erraticus* transmits *Borrelia hispanica* in coastal areas of Northern Africa, (2) *Ornithodoros sonrai* (formerly *Alectorobius sonrai*) transmits *Borrelia crocidurae* in West Africa and several arid areas of Northern Africa, and (3) *Ornithodoros moubata*/*Ornithodoros porcinus* transmit *Borrelia duttoni* in East and South Africa [2,3]. Tick species determination has traditionally been based on morphological features, geographical distribution, vector competence and *Borrelia* specificity.

In West Africa, experimental breeding between *O. sonrai* specimens from different geographical areas showed decreased fecundity or was clearly unsuccessful, suggesting

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fundamental divergence within the *O. sonrai* group although specimens were morphologically similar [4]. Therefore, it is difficult to define strict taxonomic barriers within this group or between this group and closely related species such as *O. erraticus*. Taxonomic ambiguities also existed for the associated *Borrelia* pathogens transmitted by *O. sonrai.* The existence of multiple strains of *B. crocidurae* with distinct pathogenic effects was shown although each strain appeared to be perfectly transmitted only by ticks originating from the same geographical area or by ticks and their progeny from which the strain has been isolated [5]. In East and Southern Africa, taxonomic limitations were also demonstrated for determining the *B. duttoni* tick vector; consensus was reached by defining the *O. moubata* complex, including two vector species: *O. moubata* sensu stricto and *O. porcinus*; and two non-vector species: *Ornithodoros apertus* and *Ornithodoros compactus* [6]. Because diseases are traditionally defined by their host–vector–parasite relationships, ambiguities in the systematics of soft ticks and their associated *Borrelia* may have led to misunderstandings and/or confusion about the epidemiology of human relapsing fever in Africa.

Molecular methods have provided useful information on arthropod systematics, especially ticks [7]. However, most studies have focused on hard ticks, using soft ticks as an outgroup for phylogenies. The only analysis of phylogenetic relationships among soft ticks was carried out by using the ribosomal *16S* gene [8]; however, it was applied at the genus level and did not compare species. Additionally, a molecular study was recently conducted on one species from the moubata complex in Central Tanzania [9]; however, the sampling was localized in a single village and did not permit the study of intraspecific divergence.

This paper aims to investigate genetic divergence and relationships within the *O. sonrai* group and to assess their consequences for the epidemiology of West African human relapsing fever. The study was based on the analysis of two ribosomal genes: mitochondrial *16S* rDNA and nuclear *18S* rDNA. The advantages of both molecular markers for phylogenetic analyses have been discussed in previous reviews [10]. The relative ease with which *16S* and *18S* genes can be amplified and their different evolution rates were instrumental in our choice, as they allowed us to measure genetic divergence within the *O. sonrai* group as well as between this group and other closely related groups.

2. Materials and methods

2.1. Study sites and tick sampling

Specimens of *O. sonrai* were collected between 2002 and 2004 from 14 sites in Senegal and Mauritania, during a prospecting study on tick-borne relapsing fever in West Africa [11] (Fig. 1). These two countries lie within this species' classical distribution area: Sahelian and arid zones of West Africa [12]. Sampling was conducted in variable habitats including wild savannas/steppes, croplands, oases and human dwellings. *O. sonrai* is a strictly endophilic tick inhabiting small mammal burrows. Because it only remains attached to its hosts for a few hours or less to blood feed [12], it was rarely possible to identify its hosts; however, we examined burrows of different sizes and structures to collect *O. sonrai* specimens related to various hosts. Ticks were collected by introducing a flexible tube inside burrows and aspirating their contents using a portable petrol-powered aspirator [13]. The contents were exposed to sunlight on sorting trays, and selected *O. sonrai* specimens were stored in absolute ethanol at 4 °C until determination and DNA extraction.

2.2. Tick determination

Specimens with the following characteristics were considered as *O. sonrai* [14]: elongated body with parallel lateral margins and broadly rounded posterior margin; distinct mammillae on the dorsal integument; hypostome with denticles and palpi slightly exceeding hypostome in length; eyes absent; genital area absent for nymphs and forming a semi-circle apron with smooth surface for males and a transverse slit for females; dorsoventral, preanal and transverse postanal grooves present; subapical dorsal protuberances on tarsi absent; pulvilli absent. The sex and developmental stage were described for each tick.

2.3. DNA isolation and PCR amplification

Twenty-nine *O. sonrai* specimens were used for the genetic analysis. Each tick was washed in three sterile-water baths, air dried and collected in sterile microtubes. Samples were first cooled in liquid nitrogen and individually crushed by shaking with a bead beater (mixer mill MM301, Qiagen). DNA was then isolated and purified using the DNeasy Tissue Extraction Kit (Qiagen) [15].

For each tick, *16S* rDNA was amplified by polymerase chain reaction (PCR) with Tm16S + 1 (5′-CTGCTCAATG ATTTTTTAAATTGC-3′) and Tm16S-1 (5′-CCGGTCTGA ACTCAGATCATGTA-3′) primers designed by Fukunaga et al. [9]. The predicted product size was approximately 475 bp long. Amplification of a variable region of approximately 600 bp from *18S* rDNA was accomplished with NS3 (5′-GCAAGTCTGGTGCCAGCAGCC-3′) and NS4 (5′-CTT CCGTCAATTCCTTTAAG-3′) primers designed by Black et al. [16]. PCRs were performed with the Multiplex PCR Kit (Qiagen) in a 25 μl volume containing 12.5 μl of 2× Multiplex PCR Master Mix (HotStarTaq DNA Polymerase/Multiplex PCR buffer/dNTP Mix), 2.5 μl of 10× Primer Mix (2 μM of each primer/AE buffer), 2.5 μl of 5× Q-solution and 4 μl (40–100 ng/μl) of DNA template. A common initial DNA denaturation step at 95 °C for 15 min was adopted for both PCR reactions. For *16S* rDNA, this step was followed by 10 cycles of 1 min at 92 °C, 1.5 min at 48 °C, and 1.5 min at 72 °C and 32 cycles of 1 min at 92 °C, 1.5 min of 54 °C, and 1.5 min of 72 °C. For *18S* rDNA, the program consisted of 30 cycles of 1 min at 92 °C, 1.5 min at 54 °C, and 1.5 min at 72 °C. A final extension step was carried out for 10 min at 72 °C.

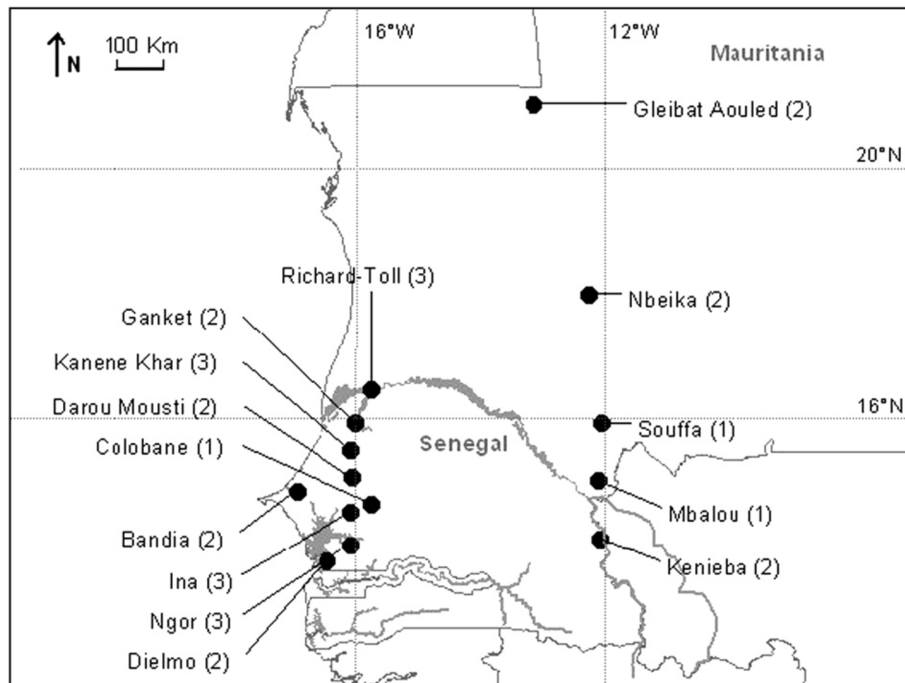

Fig. 1. Sampling sites in Senegal and Mauritania. Numbers following names indicate the number of specimens used for genetic analyses.

The amplified products were detected by electrophoresis on 2% agarose gel in TAE 0.5× buffer (40 mM Tris–acetate, 2 mM EDTA, pH 8.3) and stained with ethidium bromide. Remaining reaction mixtures were stored at −20 °C for direct sequencing. If, following PCR, there were extra bands or a smear around the band, PCR products were separated in a 1.2% agarose gel, the desired band was excised, and the DNA was extracted from the gel (Qiaquick Gel Extraction Kit, Qiagen). Amplified DNA was sequenced using an ABI PRISM 3730xl DNA analyzer (Genome Express, Grenoble, France).

2.4. *Sequence alignment and phylogenetic inferences*

Sequences were edited and manually aligned with Seaview [17]. A heuristic search was conducted using PAUP 4 [18] to generate the most parsimonious trees (MPTs) with random stepwise addition (10 replicates), deletion treated as fifth base, TBR (tree bisection-reconnection) branch swapping and branches with maximum length zero collapsed to yield polytomies. Tree robustness was determined in terms of bootstrap (BS) proportions with 10,000 replicates [19]. Two tick species were used as the outgroup: the hard tick *Ixodes ricinus* from the Ixodidae family and the soft tick *O. moubata* from the Argasidae family and Ornithodorinae subfamily to which *O. sonrai* also belongs. The GenBank accession numbers of their *18S*/*16S* rDNA sequences were Z74479/L34328 and L76355/L34292, respectively. The *16S* rDNA sequence of *O. porcinus porcinus* (GenBank accession number: L34329) was also included to compare genetic divergence within the established moubata complex and the *O. sonrai* group.

2.5. *Detection of* Borrelia *infections*

In each sampled site, *O. sonrai* specimens from each burrow inhabited by this species were tested for *Borrelia* infection. DNA isolation was conducted as above. *Borrelia* detection was based on nested PCR amplification of a 350 bp fragment of the *flagellin* gene. This gene encodes the endoflagellar protein peculiar to *Borrelia*. It is located on the stable linear chromosome of *Borrelia* and its diversity has been used for detecting polymorphism within the *Borrelia burgdorferi* complex [20] and between *Borrelia* strains causing tick-borne relapsing fever in East Africa [9]. The amplification was performed using primers designed for *B. duttoni* [9]: Bfpad (5′-GATCAAGCACAATATAACCATATGCA-3′) and Bfpdu (5′-AGATTCAAGTCTGTTTTGGAAAGC-3′) for the first PCR and Bfpbu (5′-GCTGAAGAGCTTGGAATG CAACC-3′) and Bfpcr (5′-TGATCAGTTATCATTCTAA TAGCA-3′) for the second. Each PCR was performed in a 25-μl volume containing 50 mM KCl, 10 mM Tris–HCl (pH 9), 2 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of each primer and 2.5 units of *Taq* DNA polymerase (Promega). Three microlitres of DNA template was added in the first reaction and 1 μl of the first amplified mix was added in the second. Amplification cycles consisted of an initial DNA denaturation step at 94 °C for 3 min followed by 30 cycles of 40 s at 94 °C, 40 s at 55 °C for the first PCR and 51 °C for the second PCR, and 40 s at 72 °C. A final extension step was carried out for 10 min at 72 °C.

Amplified products were detected by electrophoresis as above. A negative control was included in each nested PCR analysis to monitor contamination and false-positive amplification. Sensitivity of detection was ensured by comparison

with reference samples (DNA amplification from rodents' blood experimentally infected by *B. crocidurae*). Two amplified products from each *O. sonrai* subgroup identified by phylogenetic analysis were directly sequenced, using an ABI PRISM 3730xl DNA analyzer (Genome Express, Grenoble, France), in order to determine the species of *Borrelia* and to detect genetic polymorphism.

3. Results

3.1. Morphological and ecological characteristics

All sampled specimens had morphological characteristics corresponding to *O. sonrai* [14], suggesting they belonged to the same species. For the 29 specimens used in the genetic analysis, developmental stages, sex and habitat are indicated in Table 1. Hosts could only be directly identified at three sites: *Mus musculus* in Richard-Toll human dwellings, *Mastomys erythroleucus* in Bandia savannas and *Arvicanthis niloticus* in Darou Mousti savannas, confirming the diversity of burrows and hosts sampled for *O. sonrai.*

Table 1
Biological and ecological characteristics of the *O. sonrai* specimens used in genetic analyses

Subgroup	Location	Stage/sex	Habitat	*Borrelia* infection
1	Dielmo A	F	Human dwelling	−
	Dielmo B	F	Human dwelling	−
	Bandia A	F	Savannah	−
	Bandia B	F	Savannah	−
	Ina A	F	Human dwelling	−
	Ngor A	F	Human dwelling	−
	Ngor B	F	Cropland	+
2	Richard-Toll A	F	Human dwelling	+
	Richard-Toll B	M	Human dwelling	+
3	Ganket A	M	Cropland	−
	Ganket B	NN	Cropland	−
	Kanene Khar A	F	Cropland	+
	Kanene Khar B	NN	Cropland	−
	Darou Mousti A	F	Savannah	−
	Darou Mousti B	NN	Savannah	−
	Colobane A	M	Cropland	−
	Gleibat Aouled A	NN	Steppe	−
	Gleibat Aouled B	NN	Steppe	−
	Souffa A	M	Human dwelling	−
	Kenieba A	F	Human dwelling	−
	Kenieba B	NN	Human dwelling	+
	Mbalou A	M	Human dwelling	−
	Nbeika A	M	Oasis	−
	Nbeika B	F	Oasis	−
4	Ina B	M	Human dwelling	−
	Ina C	F	Cropland	−
	Ngor C	NN	Human dwelling	−
	Richard-Toll C	M	Human dwelling	−
	Kanene Khar C	F	Human dwelling	+

Individual specimens are separated in four subgroups identified in the phylogenetic analysis. The capital letter following the location's name specifies the individual tick. We distinguished three classes of stage/sex (F = Female, M = Male, NN = Nymph) and five classes of habitat (human dwelling, savannah, steppe, cropland, oasis). *Borrelia* infection is indicated by + or − (+: positive and −: negative).

3.2. Sequence analysis

16S rDNA was amplified for 29 *O. sonrai* specimens; the amplification of *18S* rDNA was successful for 27 of the 29 specimens (one in Bandia and one in Ngor failed). The GenBank accession numbers are DQ234703−DQ234748 and DQ250439−DQ250448. The average nucleotide composition was 37.0% A, 8.5% C, 16.3% G and 38.2% T and 26.0% A, 22.3% C, 27.3% G and 24.4% T for *16S* and *18S* rDNA, respectively.

For the 414 bp aligned fragment of *16S* rDNA, 196 sites were variable and 112 sites were parsimony-informative for the whole dataset (meaning that a difference at one site was represented at least twice by different sequences). Within the *O. sonrai* group, 93 sites were variable and 19 were parsimony-informative (Fig. 2). The high polymorphism of *16S* rDNA within this species mainly resulted from the existence of a homogeneous subgroup of five individuals that diverged markedly from the other *O. sonrai* specimens by 57 substitutions, eight deletions and seven insertions; however, it shared 13 parsimony-informative substitutions with them (Fig. 2). Sequence divergence ranged from 0.2% to 16.8% within the *O. sonrai* group (0.2−4% when the most divergent group was excluded) and was 6.5% within the moubata group (between *O. moubata* and *O. p. porcinus*). It ranged from 20% to 26.7% between both groups and increased little when we finally compared the two groups to *I. ricinus* (24.3−25.4% between *I. ricinus* and *O. moubata*/*O. p. porcinus* and 22.6−27.3% between *I. ricinus* and *O. sonrai*).

For the 535 bp aligned fragment of *18S* rDNA, 82 sites were variable, and 19 sites were parsimony-informative for the whole dataset. Within the *O. sonrai* group, only six sites were variable and one site was parsimony-informative; this gene is not informative within *O. sonrai*.

3.3. Phylogenetic inferences

The PAUP analysis yielded two MPTs that were 220 steps in length for *16S* rDNA, with a consistency index of 0.966 and a retention index of 0.949. The strict consensus tree is presented in Fig. 3. The whole tree is not illustrated, but only the branch topology to focus on large, well-supported subdivisions.

Clearly, all *O. sonrai* specimens were outside the other tick species involved in the analysis, especially *O. moubata*/*O. p. porcinus* belonging to the same Ornithodorinae subfamily. Their monophyletic origin was also confirmed by *18S* rDNA that included all *O. sonrai* in the same species with 100% BS support (result not shown).

Within this species, four genetically distinct subgroups of *O. sonrai* were identified (Fig. 3). The differentiation of the markedly divergent subgroup from the other *O. sonrai* specimens was confirmed by a 100% BS value. The distinction of another subgroup was also upheld with a 100% BS value.

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                                                  16SrDNA                                                       18S rDNA
                                         111111111111111111111111111111111222222222222222222222222222233333333333           12
Position            112223444555666667899011122222233333444445555667788999000001111223333444557888888923344555667         699930
                    241260357014034568059456901347903567013593468285956234025683456491345045176345678082604459379         702336

1  Dielmo A         GTAAGTTAGATAA-ATGAAGAGATGTGGGGG-TAGTTATAAGTTAAATTA-TTATAAGT----ATTAATGGG-TAATAGATCATTGGGAGTCA         TTTGCG
   Dielmo B         .............................................................................................         ......
   Bandia A         .......C...........................................A.........................................         ......
   Bandia B         ...................................................A.........................................         Absent
   Ina A            ...................................................A.........................................         C.A...
   Ngor A           ...................................................A.........................................         Absent
   Ngor B           ...................................................A.........................................         C.A...

2  Richard-Toll A   ..GG....................A....A...T...G........G...AA....G.C..........A.T.C.......T.A..A......         CC...A
   Richard-Toll B   ..GG....................A....A...T...G........G...AA....G.C..........A.T.C.......T.A..A......         CC...A

3  Ganket A         ..GG...................CA....AA...A.....G.....G.C......................T.C.......T....A......         CC...A
   Ganket B         ..GG...................CA....AA...A.....G.....G.C......................T.C.......T....A......         CC...A
   Kanene Khar A    ..GG...................CA....AA...A.....G.....G.C......................T.C.......T....A......         CC...A
   Kanene Khar B    ..GG...................CA....AA...A.....G.....G.C......................T.C.......T....A......         CC...A
   Darou Mousti A   ..GG...................CA....AA...A.....G.....G.C......................T.C.......T....A......         CC...A
   Darou Mousti B   ..GG...................CA....AA...A.....G.....G.C......................T.C.......T....A......         CC...A
   Colobane A       ..GG...................CA....AA...A.....G.....G.C......................T.C.......T....A......         CC...A
   Gleibat Aouled A ..GG...................CA....AA...A.....G.....G.C......................T.C............A......         CC...A
   Gleibat Aouled B ..GG...................CA....AA...A.....G.....G.C......................T.C............A......         CC...A
   Souffa A         ..GG...................CA....AA...A.....G.....GC.......................T.C............TA.....         CC..A.
   Kenieba A        ..GG....................A....AA...A.....G.....G....A...................T.C............A......         CC.C..
   Kenieba B        ..GG....................A....AA...A.....G.....G....A...................T.C............A......         CC.C..
   Mbalou A         ..GG....................A....AA...A.....G.....G....A...................T.C............A......         CC..A.
   Nbeika A         A.GG....................A....AA...A.....G.....G....A...................T..............A....T.         CC.C..
   Nbeika B         A.GG....................A....AA...A.....G.....G....A...................T..............A....T.         CC.C..

4  Ina B            .CGGACA.ACGGGTGAAGTTGAG.AGAATTATC..C-.AG.AAGT-GC.G..AGCG.AATATATCAGGGAATA.G------TG.CATAGCG.G         C.....
   Ina C            .CGGACA.ACGGGTGAAGTTGAG.AGAATTATC..C-.AG.AAGT-GC.G..AGCG.AATATATCAGGGAATA.G------TG.CATAGCG.G         C.A...
   Kanene Khar C    .CGGACA.ACGGGTGAAGTTGAG.AGAATTATC..C-.AG.AAGT-GC.G..AGCG.AATATATCAGGGAATA.G------TG.CATAGCG.G         CC...A
   Ngor C           .CGGACA.ACGGGTGAAGTTGAG.AGAATTATC..C-.AG.AAGT-GC.G..AGCG.AATATATCAGGGAATA.G------TG.CATAGCG.G         C.A...
   Richard-Toll C   .CGGACA.ACGGGTGAAGTTGAG.AGAATTATC..C-.AG.AAGT-GC.G..AGCG.AATATATCAGGGAATA.G------TG.CATAGCG.G         CC...A
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Fig. 2. Variable sites for 16S rDNA and 18S rDNA among the *O. sonrai* specimens. Individual specimens are separated into four subgroups identified in the phylogenetic analysis. The capital letter following the location's name specifies the individual tick.

Then two other subgroups were differentiated with a 62% bootstrap value.

Genetic divergence did not correlate with habitat characteristics, sex or developmental stage (Table 1). However, the three first and most closely related subgroups of *O. sonrai* reflected geographical clusters (Fig. 3): (1) the "Dielmo, Ngor, Ina and Bandia" subgroup was located in central-western Senegal, (2) the "Richard-Toll" subgroup was located in north-western Senegal along the Senegal River, and (3) the "Colobane, Darou Mousti, Kanene Khar, Ganket, Kenieba, Mbalou, Souffa, Nbeika and Gleibat Aouled" subgroup was located in northern and eastern Senegal and Mauritania (Fig. 3). Conversely, the representatives of the most divergent subgroup came from different geographical clusters (Ina,

Fig. 3. Branch topologies characterizing the strict consensus tree of the two most parsimonious trees obtained from 414 aligned positions of 16S rDNA from 29 *O. sonrai* specimens. Bootstrap values over 60% (based on 10,000 replicates) appear in bold. *O. sonrai* subgroups are located on the right-side of the map: full circles, triangles and a square for the three first geographical subgroups and dotted arrows for the fourth subgroup.

Ngor, Richard-Toll and Kanene Khar) although they showed strictly identical sequences (Figs. 2 and 3).

3.4. Detection of Borrelia *infections*

Of the 668 ticks tested by amplifying the *flagellin* gene, 206 (30.8%) were found infected by *Borrelia.* For all tested ticks, females (37.5%) were infected more than males (30.7%) and nymphs (26.3%) but differences were not significant ($p = 0.146$ by Kruskal–Wallis test). Infection rates, calculated for the three most related subgroups of *O. sonrai* separated into distinct geographical clusters differed significantly ($p = 0.0057$ by Kruskal–Wallis test); 35.8% of infected ticks were detected in central-western Senegal, 21.8% in north-eastern Senegal and Mauritania, and 45.8% for Richard-Toll along the Senegal River.

Of the 29 specimens used for the genetic analysis, six ticks belonging to the four different subgroups of *O. sonrai* were infected by *Borrelia* (Table 1). Sequencing amplified fragments of *flagellin* (276 bp) from these ticks did not reveal any genetic divergence among examined sequences (DQ234749 in GenBank); all sequences differed by two nucleotides from the *B. crocidurae* sequence originating from Barbour's collection (X75204 in GenBank), by 12 nucleotides from the *B. hispanica* sequence (BHU28498 in GenBank) and by 3–23 nucleotides from *B. duttoni* sequences produced by Fukunaga's team and Backenson (D82859, B28497, AB057545, AB113313–AB113314, AB105117–AB105133, AB105169–AB105170, AB057546–AB057548 in GenBank). Our sequence diverged by six nucleotides from the ORI *B. crocidurae* strain published by Backenson (U28496 in GenBank) but different experts retrospectively considered this strain a peculiar one that might not originate in West Africa. Then, we only found one or two nucleotide differences between our sequence and those of *B. duttoni* recently published by Cutler (DQ346831–DQ346837).

4. Discussion

Our genetic analysis of *16S* rDNA showed high polymorphism within the *O. sonrai* group in Senegal and Mauritania although all specimens were morphologically and ecologically identified as belonging to the same species. Pairwise divergence (from 0.2% to 16.8%) was higher than observed within the moubata complex (6.5%), where *O. moubata* and *O. porcinus* are considered two different species [8,9], and was also higher than observed within *Ixodes scapularis* hard ticks (2.3%) that exhibit extreme variations in morphology, host usage, development time and behavior [21]. Our results are high in comparison to previous intraspecific studies of terrestrial invertebrates and vertebrates based on analysis of *16S* rDNA, which generally found divergence values of <5% [22–26]. However, such high values were detected within sedentary species such as the endophilic trapdoor spider, *Aptostichus simus* (6–12%) [27] or within species with different populations separated by substantial distances or pronounced geographical barriers, e.g. the *Buthus occitanus* scorpion of the Mediterranean area (10%) [28].

Phylogenetic inferences on *16S* rDNA confirmed high divergence within the *O. sonrai* group and determined four subgroups, with different levels of divergence. However, our analysis showed that all subgroups of *O. sonrai* formed a monophyletic clade; it was also confirmed by *18S* rDNA, which is considered a useful marker at the family and genus levels [10]. The *O. sonrai* group could have shared a common ancestor with *O. moubata* and *O. porcinus* ticks that transmit relapsing fever in East Africa. Three of the four subgroups identified within *O. sonrai* reflected geographical clusters; however, genetic divergence did not increase as a function of geographical distance between sampling sites, refuting the hypothesis that suggests genetic isolation is caused by the species' low dispersal abilities. No correlation was found with habitat or individual characteristics and other hypotheses will be investigated to explain geographical patterns. In addition, the last most divergent subgroup did not reflect any geographical patterns and was distributed in all the various visited areas.

Exclusion of this most divergent subgroup in our analysis greatly decreased pairwise divergence, equaling the same magnitude observed in the *O. moubata* complex (0.2–4%). This subgroup formed a homogeneous separate group from other *O. sonrai* specimens as it was evolutionary frozen and showed numerous peculiar substitutions and deletions/insertions corresponding to frameshift mutations while no differentiation was noticed for *18S* rDNA. It may not correspond to the *16S* rDNA mitochondrial gene but to a pseudogene (i.e. a nuclear paralog copy of mitochondrial gene) [29]. However, other arthropods such as *Anopheles*, *Caenorhabditis* or *Drosophila* present no or few pseudogenes and our amplification results showed no PCR ghost band neither sequence ambiguity, denying the pseudogene hypothesis [30].

O. sonrai is recognized as the only vector of *B. crocidurae*, the pathogenic agent causing human relapsing fever in West Africa [2]. The detection of tick infection by amplification of the *flagellin* gene confirmed that all subgroups of *O. sonrai* were infected by *Borrelia* and could potentially transmit the pathogen to humans. However, significant differences in infection rates between distinct geographical zones may suggest different vector competences between subgroups of *O. sonrai*. Considering the apparent high specificity between soft ticks and their *Borrelia* [2] and reports of greater transmission of *B. crocidurae* strains by ticks from which strains had been isolated [5], we could expect a parallel evolution between *O. sonrai* and *B. crocidurae* and similar genetic divergence within both organisms. While high polymorphism was observed within *O. sonrai*, the sequencing of *flagellin* fragments of *Borrelia* coming from different geographical areas and different *O. sonrai* subgroups did not show any genetic divergence. Because of limited differences between our sequences and previously published sequences of *B. crocidurae* and other related species such as *B. duttoni*, further investigations on *Borrelia* and their tick vectors have to be conducted with more informative molecular markers.

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Contributors: J.F. Trape designed and supervised the study. F. Renaud was responsible for molecular assays and phylogenetic analysis. L. Vial and G. Diatta conducted tick collection. L. Vial, P. Durand, C. Arnathau and L. Halos were responsible for tick and pathogen genotyping. L. Vial conducted phylogenetic analysis with the assistance of P. Durand. L. Vial, P. Durand and F. Renaud wrote the paper. All authors saw and approved the final manuscript.

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