

## SHORT COMMUNICATION

# Relapsing fever *Borrelia* in *Ornithodoros* ticks from Bolivia

### INTRODUCTION

Tick-borne relapsing fever (TBRF), is caused by several species of *Borrelia* spirochetes, which are transmitted to humans through the bites of *Ornithodoros* spp. soft ticks. Wild rodents and insectivores are common reservoir hosts. TBRF is responsible for recurring fever associated with spirochetemia. The epidemiology of TBRF has not been well documented in South America where three endemic ticks are suspected to act as vectors (Guglielmone *et al.*, 2006). Ticks referred as *Ornithodoros talaje* are prevalent in Ecuador, Colombia, Venezuela, Argentina and Brazil, as well as in Guatemala, Panama, Mexico (Guglielmone *et al.*, 2006). This tick was shown to transmit a relapsing fever *Borrelia* in Panama by human experimentation (Bates *et al.*, 1921). It has been associated with '*Borrelia mazzottii*' in Mexico (Davis, 1956). This bacterium has, however, been incompletely described, as neither an isolate nor DNA of this bacterium is available (Davis, 1956). *Ornithodoros rudis* is found in Ecuador, Colombia, Venezuela, Peru, Paraguay and Brazil, and has been associated with '*B. venezuelensis*' (Davis, 1955). Also, a borrelia called '*B. brasiliensis*' has been associated with *O. brasiliensis* in Brazil (Davis, 1952). All three of these borreliae were incompletely described in the 1950s and neither an isolate nor DNA is currently available. In this work, using specific semi-quantitative real-time polymerase chain reaction (PCR) with original primers pairs and probes, we aimed to detect relapsing fever *Borrelia* spp. in ticks collected in Bolivia.

### METHODS

The area of tick collection was rocky outcrops composed of large blocks and located in the Cochabamba valley (alt. 2500 m) in the central zone of the Eastern Cordillera in the Cochabamba Department (Bolivia), close to the town of Cotapachi ( $-17^{\circ}25'47.31''$ ,  $-66^{\circ}17'33.80''$ ). In May 2009, a prototype baited trap combining heat, carbon dioxide and attractive odours was evaluated in Bolivia as a new means to capture triatomine bugs. A total of 35 ticks were also captured, stored in 90% ethanol and sent to Marseille, France. Morphological features indicated that the ticks were argasid ticks (soft ticks). Four specimens were sent to Rafaela, Argentina for expert morphological identification (A.A. Guglielmone). DNA was extracted from each tick using a QIAamp kit (QIAGEN, Hilden, Germany). DNA extracted from a nymph was used in a PCR to amplify and sequence a 460-bp fragment of the tick 16S rRNA-encoding gene as previously described (Mangold *et al.*, 1998; Norris *et al.*, 1996). To confirm the species-level identification of the ticks, a 446-bp sequence of the 16S rRNA-encoding gene was obtained and compared to sequences available in GenBank. The following mitochondrial 16S rDNA sequences of argasid ticks available in the GenBank were used for phylogenetic analyses: *Argas persicus* (GenBank accession number AF001402), *A. monachus* (EU283344), *A. neghmei* (DQ295781), *Ornithodoros puertoricensis* (AF113932), *O. rioplatensis* (EU283343), *O. moubata* (L34328), *O. porcinus porcinus* (L34329) and *O. porcinus domesticus* (L34330). Sequences for *Ornithodoros* spp. collected as larvae from Rodentia: Cricetidae were also

included in the analysis. There is however no 16S rDNA sequence available for *O. talaje*. Phylogenetic relationships were analysed using distance measure, and a neighbour-joining tree was generated from Kimura's two-parameter method. Support for the neighbour-joining topology was tested by boot-strapping over 1000 replicates. The *Argas* sequences were used as outgroups. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (pairwise deletion option).

*Borrelia* DNA was initially detected using specific semi-quantitative real-time PCR with primers (Bor16S3F, AGC CTT TAA AGC TTC GCT TGT AG; Bor16S3R, GCC TCC CGT AGG AGT CTG G) and a probe (Bor16S3P, 6FAM-CCG GCC TGA GAG GGT GAA CGG) that were designed for the amplification of a 148-bp fragment of a 16S rRNA-encoding gene. All PCR reactions were performed using Light Cycler 2.0 equipment and software (Roche Diagnostics GmbH, Mannheim, Germany). Positive samples were used to PCR-amplify a 344-bp fragment of *Borrelia* spp. *flagellin (fla)*. The amplification and sequencing was performed using primers designed for *B. duttonii*:

Bfpbu (5'-GCTGAAGAGCTTGGAAATG-CAACC-3') and Bfpcr (5'-TGATCAGT-TATCATTCTAATAGCA-3') (Fukunaga *et al.*, 2001). The obtained sequences were compared to GenBank.

## RESULTS

Morphological identification of ticks determined that they belonged to the *Ornithodoros talaje* species group (Acari: Ixodida: Argasidae) (Guglielmone *et al.*, 2006). For the phylogenetic analysis, there were a total of 431 positions in the final dataset. There is, however, no 16S rDNA sequence available for *O. talaje* itself. A phylogenetic tree using a neighbour-joining analysis showed a high bootstrap value (99%) supporting the group of *Ornithodoros* sp. from Bolivia and two *Ornithodoros* sp. from Argentina (Mangold, personal unpublished data), both branching with members of the *O. talaje* group including *O. puertoricensis* and *O. rioplatensis* (Fig. 1). The sequence of the 16S rRNA gene fragment from Bolivian ticks has been deposited under the GenBank accession number is JF895756.

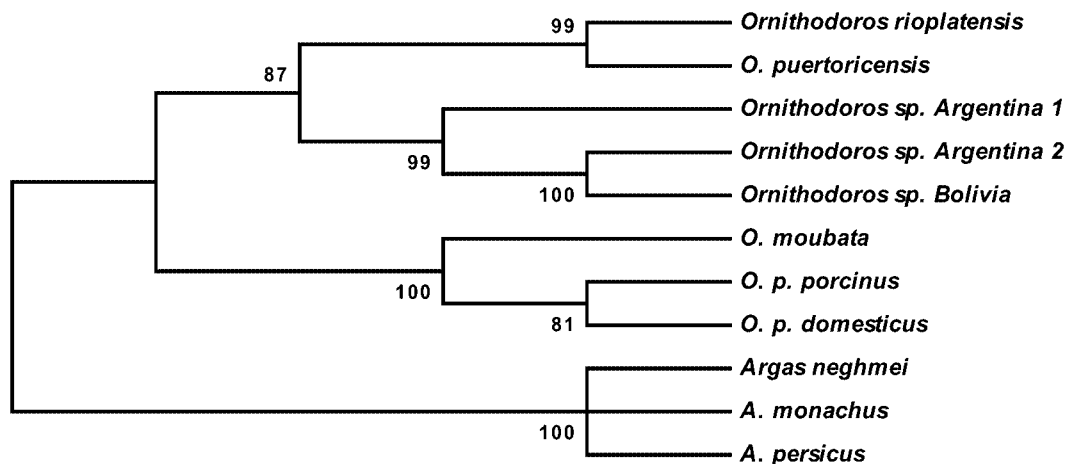


FIG. 1. Phylogenetic tree (neighbour-joining analysis) for 13 taxa within *Ornithodoros* ticks, derived from mitochondrial 16S rRNA gene sequences. The sequence of the 16S rRNA gene fragment from Bolivian ticks branches with members of the *O. talaje* group including *O. puertoricensis* and *O. rioplatensis*.

Two ticks tested positive for *Borrelia* DNA with a  $C_t$  (cycle number at the threshold level of log-based fluorescence) value lower than 36 (approximately 10–20 copies of spacer). These samples were used to PCR-amplify a 344-bp fragment of *Borrelia* spp. *flagellin* (*fla*).

The sequences obtained were identical (GenBank HM583797) and appeared to differ from all corresponding sequences available in GenBank. Those of *Borrelia duttonii* and *B. crociduræ* were the closest

with 92.4% identity. In a neighbour-joining phylogenetic tree based on the alignments of 302 bp of *fla*, the sequence clustered with those of relapsing fever *Borrelia* spp (Fig. 2).

## DISCUSSION

Our baited traps captured ticks as well as triatomine bugs, revealing their potential as 'generalist' lures for different haematophagous

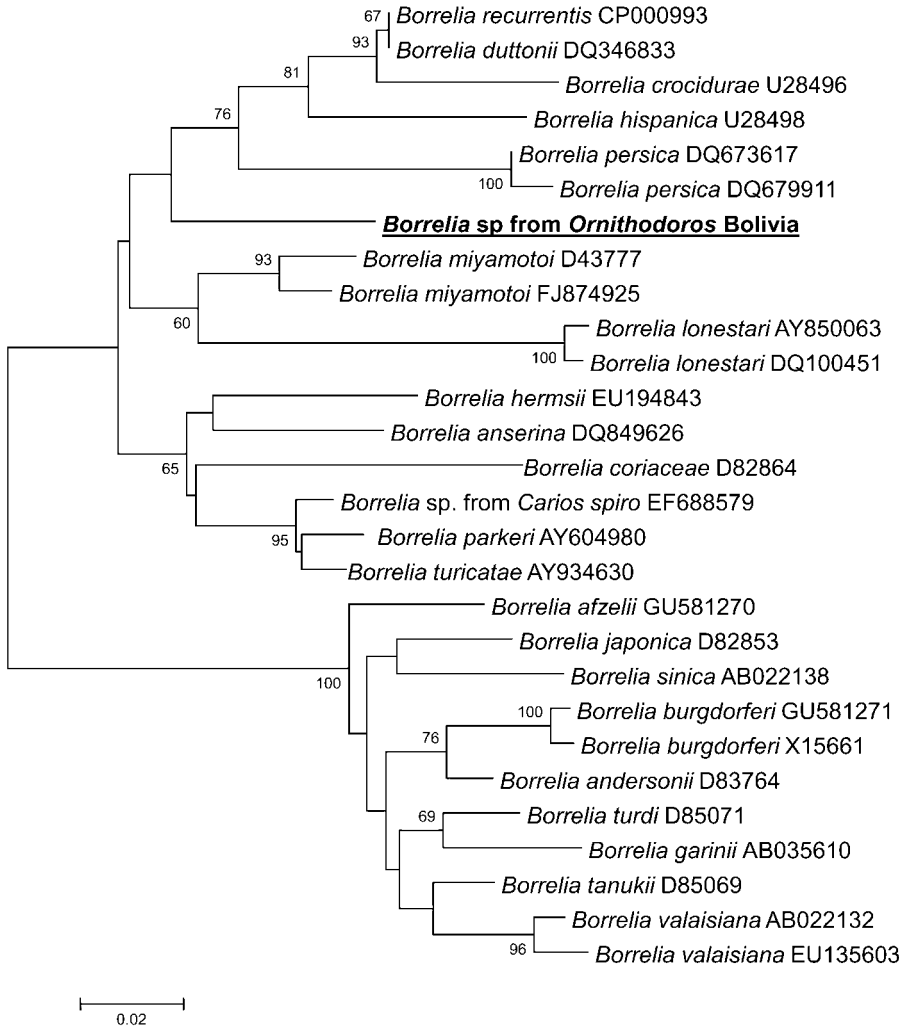


FIG. 2. Phylogenetic tree based on aligned 302-bp sequences of *fla* and constructed by the neighbour-joining method. The numbers in the nodes represent bootstrap values when above 60. The tree shows the position of *Borrelia* identified in *Ornithodoros talaje* group ticks from Bolivia. The GenBank accession number is indicated at the end of the line for each sequence.

arthropods (Ryelandt *et al.*, 2011). *O. talaje* ticks have been reported to bite humans (Guglielmone *et al.*, 2006). However, most ticks previously named as 'talaje' in the literature have been ascribed to other species, and no new collections of 'talaje' are known. Thus, the previously wide distributional range of *O. talaje* recorded in the literature may actually be restricted to Central America. Given the results of our phylogenetic analysis, our Bolivian ticks may represent a new species belonging to the 'talaje' group with other closely related species (Venzal *et al.*, 2008). The systematics of *Argasidae* remains, however, controversial (Estrada-Peña *et al.*, 2010).

The true burden of TBRF deserves more attention, particularly in tropical and subtropical areas, although it was recently shown to remain a significant problem in Africa (Cutler *et al.*, 2009). Little information is available about the epidemiology of TBRF in Bolivia. In 1994, the results of a seroepidemiological survey of borrelioses conducted in the Cordillera Province, south-eastern Bolivia, were published (Ciceroni *et al.*, 1994). *Borrelia turicatae* and *B. parkeri*, two agents of TBRF in North America, were used as antigens in an indirect immunofluorescent assay, and sera were also tested after absorption with a *Treponema phagedenis* extract to eliminate cross-reactivity to common antigens of several bacteria. Anti-*B. turicatae* and anti-*B. parkeri* antibodies were detected in 16.1% and 8.2% of the serum samples tested, respectively, and confirmed after absorption in 1.3% and 1.0%, respectively.

The identification of DNA of a novel TBRF agent in ticks collected in the field does not definitely mean that this has significance for human health. We were unable to determine whether the *Borrelia* detected in the present study was the incompletely described '*Borrelia mazzottii*' (Davis, 1956). Although the borrelia detected here deserves further investigation, our results confirm the possibility of the presence of TBRF in Bolivia. Specific surveys in patients with fever of unknown origin

are warranted, particularly with the help of molecular tools, to estimate the public health importance of TBRF in Bolivia.

**ACKNOWLEDGEMENTS.** The authors acknowledge F. Noireau and his research team (IIBISMED, Cochabamba, Bolivia) for their valuable technical assistance during the field work. The field work in Bolivia was supported by the Agence Nationale de la Recherche, the Centre National de la Recherche Scientifique and the University of Tours (France). We thank Juline Collin for technical assistance, and Claudio R. Lazzari for his support.

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Received 13 February 2011,

Revised 15 May 2011,

Accepted 13 June 2011

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