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***Rickettsia* sp. Strain NOD Infecting Ticks (*Amblyomma nodosum*) in an Endemic Area of Spotted Fever in Brazil**

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ABSTRACT: *Amblyomma nodosum* ticks were collected from one collared anteater (*Tamandua tetradactyla*) in the Caatinga biome, Brazil. From one sample, we isolated a *Rickettsia* sp. that was phylogenetically close to *Rickettsia* sp. strain NOD, with 99.9, 100.0, and 99.8% identity for *gltA*, *htrA*, and *ompA* genes, respectively.

Amblyomma nodosum is a neotropic tick species commonly reported in Brazil. Usual hosts of immature stages are Passeriformes, whereas the giant anteater (*Myrmecophaga tridactyla*) and the collared anteater (*Tamandua tetradactyla*) are the hosts of adult stages (Barros-Battesti et al. 2006).

Rickettsia sp. strain NOD (Ogrzewalska et al. 2009), *Rickettsia bellii* (Moerbeck et al. 2016), and *Rickettsia amblyommatis* (= *Rickettsia amblyommii*; Karpathy et al. 2016) are rickettsial organisms known to infect *A. nodosum*. Although there are no records of human infestations by *A. nodosum*, evidence of rickettsial infections and wide distribution of their host species suggest *A. nodosum* as a potential vector in the enzootic cycle of rickettsiae (Guglielmo et al. 2014). Moerbeck et al. (2016) reported a great diversity of ticks and rickettsiae species in this spotted fever (SF)–endemic area, which has a unique characteristic of being composed of forest fragments within the Caatinga biome, a semiarid region of Brazil. We used our data to analyze the potential participation of *A. nodosum* in the epidemic cycle of SF in the region.

In April 2013, 100 adult tick specimens (79 males and 21 females) were collected from a single collared anteater from the municipality of Guaramiranga (4°15'48"S, 38°55'59"W),

state of Ceará, Brazil, an endemic area of SF. Tick specimens were morphologically classified according to Barros-Battesti et al. (2006). Sixteen samples divided into 24 specimens were tested in pools or individually. Eleven samples were individually tested (six samples with one male each and five samples with one female each); two of the females were partially engorged, and three were completely engorged. Five samples were pooled (one sample contained two flat females, one sample had two males, and three samples had three males each). Samples were submitted to DNA extraction (Aljanabi and Martinez 1997) and conventional PCR targeting rickettsial genes: *gltA* CS2 region (CS-78 and CS-323) and CS4 region (CS-239 and CS-1069), *ompA* (Rr190.70F and Rr190.602R), and *htrA* (Rr1175F and Rr2608R) by using published protocols (Regnery et al. 1991; Blair et al. 2004; Labruna et al. 2004). One sample was analyzed for the *cox-1* gene (Folmer et al. 1994). All DNA fragments with expected size were purified by using a DNA Mini Kit (Real Genomics, New Taipei City, Taiwan), and an automated ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, California, USA) was used to sequence the DNA in both directions (Otto et al. 2008). Phylogenetic analysis was performed with a concatenated sequence by using neighbor-joining methods (MEGA 5.2, Tamura et al. 2011) and the Kimura two-parameter model to estimate genetic divergency (Kimura 1980). Bootstrap values were obtained from 1,000 randomly generated trees. Sequences gener-

ated in this study were deposited in GenBank (accession no. KY660046-48).

All ticks were morphologically identified as *A. nodosum*. The sequence of *cox-1* that we obtained was 99.4% (654/658) similar to *A. nodosum* voucher CAS-T028 (accession no. KF200111). All analyzed samples were positive for at least the CS2 fragment of *gltA* gene. The PCR reactions with sample LIC4363I were positive for all primer sets, producing amplicons of expected size. The BlastN analysis (National Center for Biotechnology 2018) showed that *gltA*, *htrA*, and *ompA* sequences were 99.9% (1,055/1,056), 100.0% (394/394), and 99.8% (490/491) similar to *Rickettsia* sp. The NOD sequences (accession nos. EU567177, EU567178, and EU567180, respectively). To improve the phylogenetic analysis, all *gltA* (1,053 base pair [bp]), *htrA* (394 bp), and *ompA* (463 bp) sequences were concatenated (1,910 bp). The resulting tree corroborated with BlastN analysis and showed the bacterium was closely related to *Rickettsia* sp. NOD (Fig. 1).

In our study, morphologic identification was corroborated by molecular analysis, confirming that the tick species responsible for parasitizing of the anteater was *A. nodosum*. In addition, the tick was infected with *Rickettsia* sp. NOD, as previous studies reported (Ogrzewalska et al. 2009). This rickettsial organism may infect different tick species; however, it frequently infects *A. nodosum*, which has a wide distribution in Brazil (Ogrzewalska et al. 2009). Although our study area somewhat resembled the Atlantic rainforest biome, it actually is a semiarid region.

Amblyomma nodosum has been widely reported in the Americas: Argentina, Bolivia, Brazil, Colombia, Costa Rica, Guatemala, Mexico, Nicaragua, Panama, Trinidad and Tobago, US, and Venezuela (Barros-Battesti et al. 2006; Mukherjee et al. 2014). In Brazil, it is reported in 11 states (Espírito Santo, Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Paraná, Rio de Janeiro, Rio Grande do Sul, Rondônia, Santa Catarina, and São Paulo) that encompass three biomes (Atlantic Forest, Cerrado, and Pantanal;

Barros-Battesti et al. 2006). Our report extends the distribution of this tick species into Ceará, northeastern Brazil, and within the Caatinga biome, mature stages of *A. nodosum* parasitized *T. tetradactyla*, which is consistent with previous studies (Barros-Battesti et al. 2006). There is no record of human infestation by *A. nodosum* (Guglielmo et al. 2014), but it has been previously reported parasitizing a dog (*Canis familiaris*; Mazioli et al. 2012). Our results may indicate the possibility for epidemic and enzootic cycles of rickettsiae. Immature stages of this arthropod have been reported parasitizing birds (Passeriformes; Ogrzewalska et al. 2009). Although we did not collect birds, their role as spatial dispersers of potential vectors of rickettsia must be considered, because that would facilitate the establishment and circulation of species of rickettsia in new areas (Ogrzewalska et al. 2009). Thus, immature stages of *A. nodosum* may be important for increasing the dispersion of *Rickettsia* sp. NOD, whereas mature stages would serve to maintain this bioagent in an area.

Amblyomma nodosum lives in preserved or minimally anthropically impacted areas, as do its vertebrate hosts (Ogrzewalska et al. 2009). This scenario may explain the species presence in a fragment of Atlantic rainforest within the Caatinga. Thus, the dispersion of immature stages by birds and the colonization of new areas by adult specimens on anteaters may contribute to the establishment of its enzootic cycle in forest fragments.

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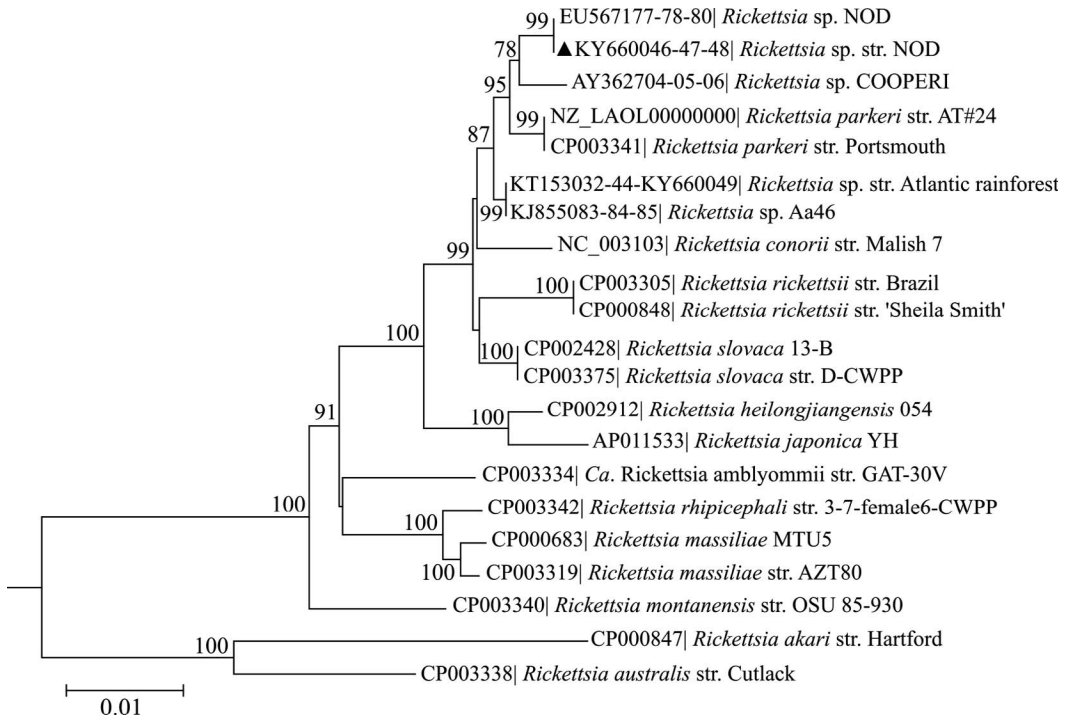


FIGURE 1. Phylogenetic tree of concatenated spotted fever group rickettsia *gltA*, *htrA*, and *ompA* genes constructed by neighbor-joining method with the Kimura two-parameter evolution model. GenBank accession nos. are presented at the beginning of each rickettsial species. The numbers at nodes are the bootstrap values obtained from 1,000 resamplings. Bootstrap values below 70% are not presented. The triangle indicates the sequences obtained in this study from a *Rickettsia* sp. that was phylogenetically close to *Rickettsia* sp. strain NOD obtained from ticks (*Amblyomma nodosum*) recovered from the collared anteater (*Tamandua tetradactyla*) in northeastern Brazil.

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