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Article

Morphological and molecular analysis of *Ornithodoros hasei* and *Ornithodoros* sp. cf. *O. mimon* (Acari: Argasidae) from northeastern Argentina

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Abstract

In this study two species of soft ticks belonging to the genus *Ornithodoros* were recorded in three areas in the province of Corrientes, Argentina. Four larvae were identified as *Ornithodoros hasei* (Schulze, 1935) on *Molossus molossus* (Molossidae) in Paraje Tres Cerros (Department of San Martín), while 56 larvae were identified as *Ornithodoros* sp. cf. *O. mimon* Kohls, Clifford & Jones, 1969 on *Eptesicus furinalis* and *Myotis levis* (Vespertilionidae) in Paraje Galarza (Department of Santo Tomé) and Colonia Carlos Pellegrini (Department of San Martín). These last two locations are found within the Esteros del Iberá eco-region. The known distribution range of *O. hasei* and *O.* cf. *O. mimon* is expanded to include the province of Corrientes. Two new ectoparasite-host associations were recorded for Argentina between *O. hasei* on *M. molossus* and *O.* cf. *O. mimon* on *M. levis.* The morphological differences together with the results of the phylogenetic analysis show that *O. hasei* presents genetic similarity with specimens from Brazil, and in Argentina, with ticks from Santa Fe. Instead, *Ornithodoros* sp. cf. *O. mimon* recorded in Corrientes and Uruguay belong to a different taxon than the *O. mimon* recorded in Brazil and Santa Fe, Argentina. Based on this, *O. mimon* should be considered a complex of species in which the specimens from Corrientes are provisionally considered as *Ornithodoros* sp. cf. *O. mimon* until their taxonomic status can be determined.

Key words: ectoparasites, ticks, larvae, Chiroptera, Corrientes, Argentina.

Introduction

Ticks are non-permanent ectoparasites with a worldwide distribution that parasitize tetrapods terrestrial vertebrates, fliers and even marine reptiles (Hoogstraal, 1985). They are of significant importance for both humans and animals not only for their capacity to produce dermatitis, anemia and other pathologies, but also because they are vectors pathogenic agents (Nava *et al.*2017).

Currently there are over 960 recognized species of ticks distributed over three living families, Argasidae (220 spp.), Ixodidae (740 spp.) and Nuttalliellidae (1 sp.), as well as one fossil, Deinocrotonidae (Peñalver *et al.* 2017; Guglielmone *et al.* 2020; Muñoz-Leal *et al*. 2020).

In the case of the Argasidae family, various species and even whole genera are parasites specific to bats, whereas in the Ixodidae family very few species parasitize bats (Hoogstraal 1985; Hornok *et al.* 2014).

Of the five genera found in the Neotropical Region, only three, *Argas, Ornithodoros* and *Otobius,* are found in the Southern Cone of America. Of these, *Ornithodoros* is the most numerous with 12 taxa (Nava *et al.* 2017). In Argentina they have cited seven species from this genus: *Ornithodoros hasei* (Schulze, 1935)*; Ornithodoros mimon* Kohls, Clifford and Jones, 1969*; Ornithodoros montensis* Venzal, Mangold and Nava, 2019; *Ornithodoros quilinensis* Venzal, Nava and Mangold, 2012; *Ornithodoros rioplatensis* Venzal, Estrada-Peña and Mangold, 2008; *Ornithodoros rostratus* Aragâo, 1911 and *Ornithodoros xerophylus* Venzal, Mangold and Nava, 2015 (Nava *et al*. 2017; Venzal *et al*. 2019). *Ornithodoros hasei* is a species with wide distribution that extends from southern Mexico to Argentina (Guglielmone *et al.* 2003; Nava *et al.* 2007). It is parasite of bats (Emballonuridae, Molossidae, Mormoopidae, Noctilionidae, Phyllostomidae and Vespertilionidae) and also rodents (Cricetidae) (Nava *et al.* 2017). In Argentina it has been found on *Molossops temmincki* (Molossidae), *Histiotus laephotis, Eptesicus diminutus, E. furinalis, Eptesicus* sp. and *Myotis albescens* (Vespertilionidae), in the province of Jujuy and Santa Fe (Nava *et al.* 2007; Colombo *et al.* 2020).

Ornithodoros mimon for a long time was only known by its larval description, obtained originally of *Mimon crenulatum* (Phyllostomidae) in Bolivia and *Eptesicus furinalis* (Vespertilionidae) in Uruguay (Kohls *et al.* 1969; Venzal *et al.* 2003, 2004). Subsequently it was registered in Argentina on *Eptesicus diminutus* and *E. furinalis* in the provinces of Salta and Jujuy, *Histiotus macrotus* in Tucumán (Venzal *et al.* 2004), *Myotis albescens* in Catamarca (Trejo *et al.* 2020), and in Brazil it was associated with *Platyrrhinus lineatus* (Phyllostomidae) and humans (Barros-Battesti *et al.* 2011; Labruna *et al.* 2014; Muñoz-Leal *et al.* 2016 a). This species of tick can also be found on marsupials (Didelphidae), rodents (Cricetidae) and birds (Nava *et al.* 2017).

The objective of this work is report the first records of *O. hasei* and *O. mimon* found on bats from northeastern Argentina and to analyze morphological and molecular traits of these specimens.

Materials and Methods

Study area

The samplings were taken in three areas from the Corrientes Province: i) Paraje Tres Cerros Natural Reserve, municipality of La Cruz, department of San Martín (29°06′34.30″S; 56°55′51.92″ O); ii) Paraje Galarza, department of Santo Tomé (28°5'58.80"S; 56°40'3.33"O); iii) Colonia Carlos Pellegrini, department of San Martín (28°31'59.99"S; 57°10'0.02"O). These sites are part of the natural heritage of Corrientes and are within the framework of the Campos y Malezales and Esteros de Iberá ecoregions (Burkart *et al.* 1999).

Paraje Tres Cerros is located in the central east of the province of Corrientes and is characterized by its topographical composition that includes hill formations that is unique within the province. Due to its physiognomic characteristics, the vegetation that grows within the rock formations is like that of the grassy shrub-steppe and hygrophilous forests with a great diversity of microhabitats (Cajade *et al.* 2013). On the other hand, the Esteros de Iberá is situated in the north-central part of the province of Corrientes with about 12,300 km^2 of extension and is part of the larger fluvial paleofan system of the Paraná River that extends over 45.000 km^2 (Neiff and Poid de Neiff, 2005). It is notable for being one of the most extensive and important wetlands in Neotropical America, declared a Provincial Reserve in 1983, a Ramsar Site in 2002 and a National Park in December 2018.

Capture of the host bats

In Paraje Galarza and Colonia Carlos Pellegrini (Esteros del Iberá), the capture of bats was carried out biannually between 2014 and 2017 during the months of the warm season over the course of three nights in each of the two localities, for a total of nine campaigns in the studied sites.

Eight mist-nets were used and relocated every night to cover the largest possible area. The strength of the sample was determined by calculating the meters of net by the number of nights they were used by locality (Pérez-Torres & Ahumada 2004). In total, 78 meters (five 12-meter nets and three 6-meter nets) of net were left out over 27 nights in each locality that implicates a sample strength of 2,106 m in each site. The nets were checked at approximately one-hour intervals, depending on the activity of the bats. The permit for the collection of the bats was granted by the "Direction of Parks and Reservations" of the Province of Corrientes. Likewise, the authorization for the transfer of the specimens to the la Colección Mamíferos Lillo (CML) at the Facultad de Ciencias Naturales e Instituto Miguel Lillo (Universidad Nacional de Tucumán) where they were deposited, was expedited by the "Subsecretary of Fauna and Flora" (Nº 01426/17) that belongs to the "Direction of Natura Resources" (Nº 00001426) of the province.

The specimens from Paraje Tres Cerros were collected for a campaign for a study of the fauna of bats in the area and donated for use by members of the research group from the Vertebrate laboratory at the Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste (Facena-UNNE).

The species of bats were confirmed by specialists at the Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán (San Miguel de Tucumán)–CONICET and at the Fa.CENA (UNNE) and incorporated into the Colección Mamíferos Lillo (CML) and to the Colección de Mastozoología of the Facultad de Ciencias Exactas y Naturales y Agrimensura (CM-FaCENA), respectively.

Collection and identification of the ticks

The ticks were removed from the fur of the hosts manually using fine point entomological forceps and preserved in 70% ethylic alcohol until they could be identified. Later they were rinsed with a water solution of 20% potassium hydroxide, washed in distilled water for 15–30 minutes and mounted on Hoyer´s medium in semipermanent preparation for optic microscopes (Venzal *et al.* 2013). The measurements were taken for each specimen and recorded in millimeters (mm). The average measurement and range (minimum–maximum) were calculated. For the descriptions, we followed the terminology and the measurements for the larval chaetotaxy from Kohls *et al.* (1965, 1969), Nava *et al.* (2007) and Muñoz-Leal *et al.* (2016 a, b).

We complemented the tick measurements with a phylogenetic analysis by using sequences of the mitochondrial *16S rRNA* gene. For this analysis, larvae were submitted to taxonomic identification. DNA was extracted by using the commercial kit PureLink ™ Genomic DNA Mini Kit (Invitrogen, Germany) following the instructions of the manufacturer. Sequences of the mitochondrial *16S rRNA* gene were obtained according to Mangold *et al.* (1998). The sequences (a ca. 400-bp fragment) were edited using BioEdit Sequence Alignment Editor (Hall 1999) with manual edition whenever it was necessary and aligned with the program Clustal W (Larkin *et al*. 2007). Phylogenetic relationships were assessed with the Maximum-likelihood (ML) method and the best-fitting substitution model was determined with the Bayesian Information Criterion by using MEGA 5 (Tamura *et al.* 2011). GTR model was chosen to create ML trees. Branch support was tested by bootstrap analysis using 1,000 replicates. Sequences of *Argas keiransi* Estrada-Peña, Venzal and González- Acuña, 2003, *Argas monachus* Keirans, Radovsky and Clifford, 1973 and *Argas neghmei* Kohls and Hoogstraal, 1961, were employed as outgroups.

Results

Four hundred thirteen bats were examined for ticks, of which one specimen was collected in Paraje Tres Cerros, 166 in Paraje Galarza, and 246 in Colonia Carlos Pellegrini. Sixteen species belonging to three families were examined: Molossidae (n=149), Phyllostomidae (n=43) and Vespertilionidae (n=221).

Molossus molossus (Pallas, 1776) (Molossidae) (n=1), *Eptesicus furinalis* (d'Orbigny y Gervais, 1847) (n=35) and *Myotis levis* (I. Geoffroy St.-Hilaire, 1824) (Vespertilionidae) (n=2) were found infested with ticks.

Four larvae of *O. hasei* were found on *M. molossus,* 55 larvae of *O. mimon* on *E. furinalis* and one *O. mimon* larva on *M. levis.*

Measurements of specimens appear in Table 1, to describe of the larvae of *O. hasei* and *O. mimon.*

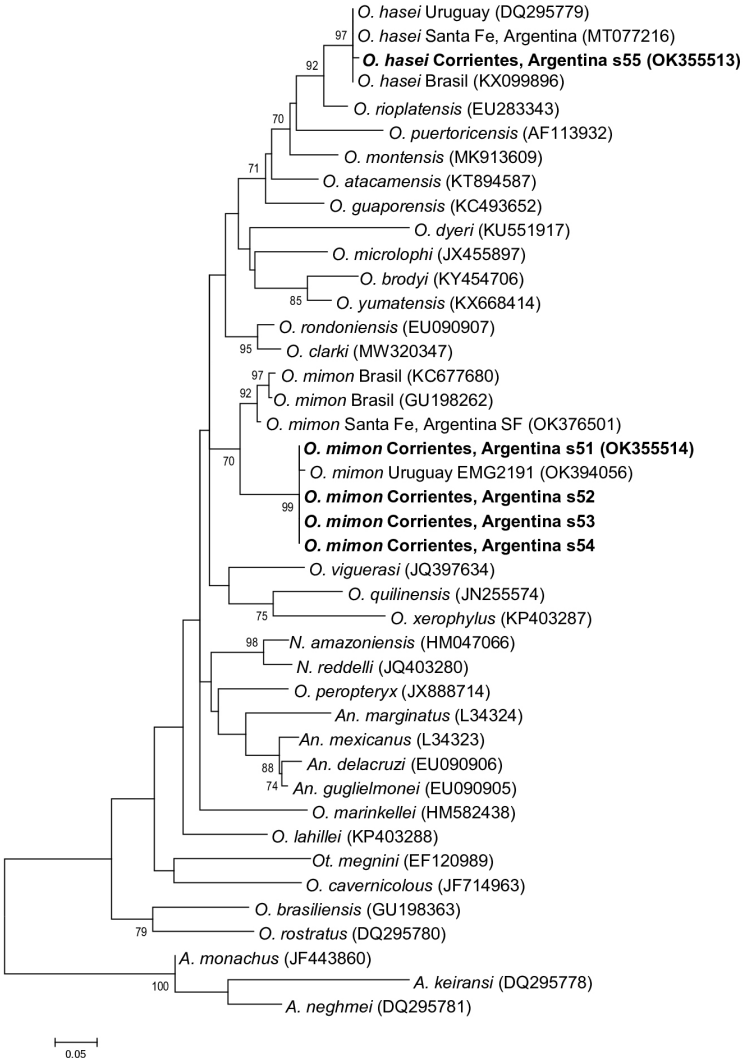

FIGURE 1. Maximum-likelihood tree based on *16S rDNA* partial sequences. Numbers represent bootstrap support generated from 1,000 replications (only values higher than 70 are showed). GenBank accession numbers are indicated in brackets. *An*.: *Antricola*; *O*.: *Ornithodoros*; *N*.: *Nothoaspis*; *Ot*.: *Otobius*; *A*.: *Argas*.

The morphological characteristics used to identify *O. hasei* were based on a piriform dorsal plate, a hypostome with a pointed apex, 3/3 apical detention, 3/3 medial and 2/2 basal. The idiosoma had a semicircular form with 19 pairs of setae on the dorsal surface: seven pairs of anterolateral setae (Al), eight pairs of posterolateral setae (Pl) and four central setae (C). The ventral surface had three pairs of external setae (St), three pairs of circumanal setae (Ca), a pair of postcoxal setae (Pc) and one posteromedian seta (PMS).

Ornithodoros mimon were identified for the presence of a piriform dorsal plate, a hypostome with a blunt apex, 4/4 or 3/3 apical detention, and 2/2 basal. The idiosoma had a subcircular form with 15 to 16 pairs of setae on its dorsal surface: seven pairs of anterolateral setae, five pairs of posterolateral setae (some had the particularity of having six pairs of posterolateral setae) and three pairs of central setae. The ventral surface had eight pairs of setae: three pairs of external setae, three pairs of circumanal setae, one pair of postcoxal setae and one posteromedian seta.

TABLE 1: Measurements (average and range) of the larvae of *Ornithodoros hasei* (n=2) and *Ornithodoros* sp. cf. *O. mimon* (n=19) from Paraje Tres Cerros and Esteros del Iberá (Corrientes, Argentina), respectively.

	O. hasei		*O. mimon*	
	Mm	(Min-Max)	Mm	(Min-Max)
Body length including capitulum	0.172	(0.170–0.173)	0.764	(0.775–1.975)
Body length not including capitulum	0.145	(0.143–0.146)	0.500	(0.500–1.850)
Body width	0.115	(0.109–0.119)	0.871	(0.475–1.350)
Dorsal plate	Pyriform		Pyriform	
Dorsal plate: length	0.211	(0.196–0.225)	0.173	(0.147–0.222)
Dorsal plate: width	0.159	(0.146–0.166)	0.147	(0.137–0.185)
Dorsal setae (pairs): total	19		15–16	
Dorsal setae (pairs): dorsolateral	15		12–13	
Dorsal setae (pairs): central	4		3	
Dorsal anterolateral setae: Al1 length	0.096	(0.095–0.096)	0.103	(0.065–0.120)
Dorsal anterolateral setae: Al2	0.095	(0.095–0.095)	0.099	(0.087–0.120)
Dorsal anterolateral setae: Al3	0.096	(0.095–0.097)	0.099	(0.875–0.122)
Dorsal anterolateral setae: Al4	0.097	(0.097–0.097)	0.098	(0.085–0.120)
Dorsal anterolateral setae: Al5	0.097	(0.090–0.104)	0.097	(0.082–0.112)
Dorsal anterolateral setae: Al6	0.102	(0.097–0.107)	0.097	(0.087–0.115)
Dorsal anterolateral setae: Al7	0.104	(0.097–0.109)	0.093	(0.075–0.115)
Dorsal posterolateral setae: Pl1 length	0.084	(0.082–0.085)	0.088	(0.082–0.102)
Dorsal posterolateral setae: Pl2	0.070	(0.065–0.073)	0.091	(0.075–0.105)
Dorsal posterolateral setae: Pl3	0.079	(0.073–0.085)	0.088	(0.057–0.115)
Dorsal posterolateral setae: Pl4	0.082	(0.080–0.082)	0.085	(0.077–0.102)
Dorsal posterolateral setae: Pl5	0.083	(0.082–0.083)	0.079	(0–0.117)
Dorsal posterolateral setae: Pl6	0.063	(0.061–0.065)	0.031	(0–0.100)
Dorsal posterolateral setae: Pl7	0.066	(0.061–0.070)	-	-
Dorsal posterolateral setae: Pl8	0.063	(0.063–0.064)	-	-
Central setae: C1 length	0.096	(0.095–0.097)	0.085	(0.040–0.105)
Central setae: C2	0.084	(0.080–0.087)	0.071	(0.040–0.095)

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TABLE 1.(Continued)

	O. hasei		*O. mimon*	
	Mm	(Min-Max)	Mm	(Min-Max)
Central setae: C3	0.040	(0–0.080)	0.065	(0.042–0.092)
Central setae: C4	0.060	(0.041–0.078)		
Ventral setae (pairs): total	8		8	
Sternal setae: St1	0.055	(0.051–0.058)	0.054	(0.027–0.115)
Sternal setae: St2	0.055	(0.053–0.056)	0.050	(0.022–0.070)
Sternal setae: St3	0.061	(0.061–0.061)	0.051	(0.040–0.077)
Circumanal setae: Ca1	0.050	(0.048–0.051)	0.040	(0.027–0.050)
Circumanal setae: Ca2	0.073	(0.073–0.073)	0.540	(0.037–0.095)
Circumanal setae: Ca3	0.079	(0.078–0.080)	0.071	(0.037–0.092)
Posteromedian seta: PMS	0.034	(0.029–0.039)	0.052	(0.025–0.065)
Postcoxal setae: Pc	0.054	(0.048–0.058)	0.046	(0.020–0.115)
Length of basis capituli (a)	0.176	(0.170–0.180)	0.105	(0–0.165)
Length of basis capituli (b)	0.187	(0.180–0.192)	0.150	(0–0.202)
Length of basis capituli (c)	0.206	(0.078–0.333)	0.061	(0–0.325)
Width of basis capituli	0.206	(0.204–0.207)	0.228	(0.087–0.562)
Posthypostomal setae: Ph1	0.007	(0.007–0.007)	0.009	(0–0.010)
Posthypostomal setae: Ph2	0.013	(0.012–0.014)	0.008	(0–0.012)
Distance of Ph1	0.024	(0.024–0.024)	0.020	(0–0.052)
Distance of Ph2	0.088	(0.085–0.090)	0.099	(0–0.240)
Palpal length	0.201	(0.195–0.207)	0.240	(0.055–0.282)
Length article I	0.060	(0.058–0.061)	0.066	(0.055–0.095)
Length article II	0.090	(0.087–0.092)	0.082	(0.032–0.105)
Length article III	0.069	(0.065–0.073)	0.079	(0.047–0.090)
Length article IV	0.036	(0.036–0.036)	0.043	(0.022–0.070)
Width article I	0.027	(0.026–0.027)	0.037	(0.032–0.042)
Width article II	0.036	(0.036–0.036)	0.044	(0.032–0.047)
Width article III	0.032	(0.031–0.031)	0.041	(0.020–0.047)
Width article IV	0.021	(0.019–0.021)	0.022	(0–0.025)
Setae of palpal article I	1		1	
Setae of palpal article II	4		3–4	
Setae of palpal article III	7		3–4	
Setae of palpal article IV	9		2	
Hypostome: length (d)	0.100		0.201	(0.187–0.210)
Hypostome: length (e)	0.081		0.150	(0.137–0.157)
Hypostome: length (f)	0.082		0.166	(0.157–0.175)
Hypostome: width base (g)	0.024		0.676	(0.055–0.077)
Hypostome: width base (h)	0.022		0.547	(0.037–0.062)
Apex	Pointed		Blunt	
Apical dental formula	3/3		4/4-3/3	

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TABLE 1.(Continued)

	O. hasei		*O. mimon*	
	Mm	(Min-Max)	Mm	(Min-Max)
Median dental formula	3/3		2/2	
Basal dental formula	2/2		2/2	
Denticles in hypostomal row 1	18		11–14	
Denticles in hypostomal row 2	16		9–13	
Denticles in hypostomal row 3	10		4–7	
Denticles in hypostomal row 4	-		1–5	
Torsus I: length	0.155	(0.146–0.163)	0.196	(0.160–0.230)
Torsus I: width	0.049	(0.048–0.048)	0.077	(0.067–0.100)

(a) Length of basis capituli to Ph1, (b) Length of basis capituli to insertion of hypostome, (c) Length of basis capituli to final hypostome, (d) Measured to point to Ph1, (e) Measured to point to inferior toothed portion, (f) Measured to point to insertion of hypostome in basis capituli, (g) Measured in basis portion of hypostome, (h) Measured in medial portion of hypostome.

Through the phylogenetic analysis, we could confirm the presence of *O. hasei* in Paraje Tres Cerros and of *O. mimon* in the Paraje Galarza and Colonia Carlos Pellegrini (Esteros del Iberá). The phylogenetic analysis also confirms that the *O. hasei* larvae from Corrientes (sample 55, GenBank accession number: OK355513) belong to the same group that *O. hasei* from Santa Fe Province in Argentina (MT077216), Uruguay (DQ295779) and Brazil (KX099896). On the other hand, the sequences obtained from the *O. mimon* larvae (samples 51–54, GenBank accession number: OK355514) matched with the sequences of *O. mimon* from Uruguay (sample EMG2191, GenBank access number: OK394056), and they are different from those of *O. mimon* from Brazil (GenBank access number: KC677680, GU198262) and Santa Fe province from Argentina (sample SF, GenBank accession numbers: OK376501) (Fig. 1).

Discussion

The larval stages of two soft ticks, *O. hasei* and *Ornithodoros* sp. cf. *O. mimon*, were found in bats, constituting new records for the northeast region of Argentina. In addition, new host - parasite associations are presented between *O. hasei* and *M. molossus* and *Ornithodoros* sp. cf. *O. mimon* and *M. levis.*

The morphological traits of *O. hasei* and *Ornithodoros* sp. cf. *O. mimon* from Corrientes Province coincide in general terms with those determined by Kohls *et al.* (1965, 1969) in the original description of the species. The results of the taxonomic measures presented in this work are similar to those cited by Muñoz-Leal *et al.* (2016) in Brazil. In comparison with the *O. hasei* of the Argentine northwest presented by Nava *et al*. (2007) there is a small variation in terms of the length of the hypostome, being shorter in specimens from Corrientes. Molecular analyzes confirm that *O. hasei* from Corrientes is phylogenetically related to *O. hasei* from Brazil, Uruguay, and in Argentina, with ticks from Santa Fe.

As for *Ornithodoros* sp. cf. *O. mimon*, there are differences in the number of setae between specimens from Corrientes and those specimens collected in other regions. In these specimens, the number of dorsal setae is greater (12-13 pairs) than the number of setae exhibited by the specimens described by Khols *et al.* (1969) and those mentioned by Barros-Battesti *et al*. (2011) and Muñoz-Leal *et al.* (2016) for specimens from Brazil. These morphological differences, along with the results of the phylogenetic analysis, suggest that *O. mimon* from Corrientes and Uruguay could belong to a different taxon than *O. mimon* from Brazil and central Argentina (Santa Fe). Based on this, it could

be argued that the taxon *O. mimon* represent a species complex. For that reason, it is considered that the population from Corrientes should be named as *Ornithodoros* sp. cf. *O. mimon* until its taxonomic status will be determined.

Recently, Colombo *et al.* (2020) found "*Candidatus* Rickettsia wissemanii" in specimens of *O. hasei* from the center of the country. As this rickettsia belongs to the group of the spotted fevers (SFG), additional study is indispensable to be able to verify the presence of this pathogen in ticks in this part of the country. Even though the *O. mimon* capacity to transmit pathogens has not been studied up to this point, bites from this species can cause the death of the host (Hoogstraal 1985) and, in humans, can cause severe inflammatory reactions (Labruna *et al.* 2014; Nava *et al.* 2017). Given this, it is important to continue studying both argasidae species as *Ornithodoros* sp. cf. *O. mimon* can bite humans and *O. hasei* has been found infected with a rickettsia from SFG.

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