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Occurrence of autogeny in a population of *Ornithodoros fonsecai* (Acari: Argasidae)

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ABSTRACT

Ornithodoros fonsecai is an argasid tick that is endemic to Brazil and has been described in the municipality of Bonito, state of Mato Grosso do Sul. Some specimens of this species were found in a cave in the municipality of Nobres, state of Mato Grosso. The specific identification of this population was confirmed by means of morphology and molecular biology. The mitochondrial 16S rDNA partial sequence of this species from Nobres has been deposited in GenBank (MK158949). The objective of this study was to elucidate the biology of *O. fonsecai* from Nobres, and to report autogeny in this tick population. Along three laboratory generations was observed molting of first nymphal instar to the second instar without feeding, a typical behavior of species included in the subgenus *Alectorobius*. The first generation (F1) presented five nymphal instars (N1 to N5), and most of adults emerged through molting of N5. The last nymphal instar of second generation (F2) was N4, but most of adults emerged from N3. In the third generation (F3) the last nymphal instar was N5, with most of the adults emerging from N4. In F2, some females (n = 20) originated from N3 began laying eggs without a blood meal. It was observed that those N3 fed twice before they molted to autogenic females. However, autogenic behavior occurred in relation to third generation females (F3) with specimens originating from N4 (n = 12) that were fed only once as nymphs. This behavior has already been reported as obligatory for the genera *Otobius* and *Antricola*, while it is facultative for one species of genus *Argas* and for four species of genus *Ornithodoros*. However, the present report provides the first record of facultative autogeny for a species of *Ornithodoros* in Brazil.

1. Introduction

Argasid ticks have nidicolous life cycle as a characteristic development, such that they are able to live in different habitats like burrows, holes, bird nests, cracks in rocks, loose soil, bark of trees and caves, and can also be found close to man and domestic animals, inhabiting basements, ceilings, stables, chicken coops and rustic beds (Aragão, 1936; Vial, 2009). They feed on different classes of animal or even several times on the same animal during their life cycle. These ticks are abundant in arid and semi-arid regions (Brites-Neto et al., 2015), but some species have also been found in very humid environments parasitizing amphibians (Barros-Battesti et al., 2015; Muñoz-Leal et al., 2017a).

In general, the life cycle of these ticks includes eggs, larval stage, two to nine nymphal instars and adults (Vial, 2009). Females can lay multiple batches of eggs (gonotrophic cycles). Each batch is normally laid after a blood meal and sometimes after new mating (Hoogstraal, 1985; Landulfo et al., 2012; Ramirez et al., 2016).

The genus *Ornithodoros* Koch, 1844, is the most diversified among the argasids with 131 representative species around the world (Labruna and Venzal, 2009; Vial and Camicas, 2009; Guglielmone et al., 2010; Nava et al., 2010, 2013; Dantas-Torres et al., 2012; Heath, 2012; Venzal et al., 2012, 2013, 2015; Trape et al., 2013; Barros-Battesti et al., 2015; Labruna et al., 2016; Muñoz-Leal et al., 2017a; Dantas-Torres, 2018).

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Sixty-one species are located in the Neotropical region (Venzal et al., 2015; Labruna et al., 2016; Muñoz-Leal et al., 2016a, b). Of these, 19 are distributed in Brazil (Dantas-Torres et al., 2009; Guglielmone et al., 2010; Venzal et al., 2012, 2013; Nava et al., 2013; Martins et al., 2014; Barros-Battesti et al., 2015; Labruna et al., 2016; Wolf et al., 2016; Muñoz-Leal et al., 2017a).

Ornithodoros fonsecai (Labruna and Venzal, 2009) is an argasid tick that was described from larvae collected from the bat species *Peropteryx macrotis* Wagner, 1843, and *Desmodus rotundus* E. Geoffroy, 1810, and from adult ticks collected from the walls of a cave inhabited by these hosts, located in the municipality of Bonito, state of Mato Grosso do Sul, Brazil (Labruna and Venzal, 2009). While collecting these specimens, one of the authors was bitten by this tick with intense pain and skin reaction. This species belongs to the subgenus *Alectorobius* Pocock, 1907, which includes parasitic species of bats. Molecularly, *O. fonsecai* is close to *Ornithodoros dyeri* Cooley and Kohls, 1940 and *Ornithodoros rietcorreai* Labruna, Nava and Venzal, 2016 (Muñoz-Leal et al., 2017a). Morphologically the larvae of these species resemble larvae of *Ornithodoros peruvianus* Kohls, Clifford and Jones, 1969 and *Ornithodoros peropteryx* Kohls, Clifford and Jones, 1969 (Labruna and Venzal, 2009).

The role of *O. fonsecai* as a pathogen transmitter is unknown, but the possibility cannot be ruled out, since it presents aggressive action against humans. However, some species of the subgenus *Alectorobius* in the Neotropical region, such as *Ornithodoros talaje* Guérin-Méneville, 1849, and *Ornithodoros puertoricensis* Fox, 1947, have already been correlated with transmission of recurrent fever and African swine fever in South America, respectively (Hoogstraal, 1985; Endris et al., 1992; Labruna and Venzal, 2009). Up to our knowledge pathogens were not investigated in this tick species.

Autogeny, oviposition without a blood meal, is facultative or obligatory in some of the argasid tick species and was not reported for ixodid species. The species that perform facultative autogeny are: *Argas persicus* Oken, 1818, which is distributed in most African countries, Australia, China, Europe, Asia, Oceania, United States, Paraguay, Argentina and Chile (Muñoz-Leal et al., 2017b); *Ornithodoros lahorensis* Neumann, 1908, which is distributed in Europe and North Africa (Camicas et al., 1998) and in several Asian countries (Estrada-Peña et al., 2018); *Ornithodoros tholozani* (Laboulbène & Mégnin, 1882), which is found in Europe and North Africa (Camicas et al., 1998) and in different locations such as Russia, Iraq, Syria, Central Asia and Tehran (Sharma, 1993); *Ornithodoros tartakovskyi* Olenev, 1931, which is found in Central Asia, from Iran to western China (Turell, 2015), and in Europe and North Africa (Camicas et al., 1998); and *Ornithodoros parkeri* Cooley, 1936, which is found from the United States to the Caribbean (Brites-Neto et al., 2015). The obligatory autogenic argasids are of the genera *Antricola* Cooley and Kohls, 1942, and *Otobius* Banks, 1912 (Estrada-Peña et al., 2008). According to Oliver (1989), it is possible that the genus *Nothoaspis* Keirans and Clifford, 1975 is also autogenic. Species of this genus, in the adult stage, have poorly developed and nonfunctioning mouthparts, turning oviposition without a blood meal crucial (Balashov, 1972; Oliver, 1989). However, there are some exceptions. Females of *O. lahorensis* are obligatorily autogenic in their first gonotrophic cycle, but a blood meal is necessary for subsequent oviposition (Balashov, 1972).

In the present study, we report the occurrence of facultative autogeny in the population of *O. fonsecai* in the municipality of Nobres, which was observed in the last two out of three generations that were studied under laboratory conditions. In addition, we provide the mitochondrial 16S rDNA sequence, in order to further consider the similarity and phylogenetic position of this tick species when compared to other sequences deposited in GenBank.

2. Materials and methods

In 2012, ten nymphs and six adults (three males and three females) of the species *O. fonsecai* were collected from a cave inhabited by two species of bats, *Peropteryx macrotis* and *Desmodus rotundus*. This cave is known as "Gruta da Lagoa Azul" and is located 80 km from the municipality of Nobres (14° 35′ 17″ S, 55° 58′ 0″ W), in the state of Mato Grosso, Brazil. Some of these specimens (1 male and 1 female) were deposited in the Acari collection of the Butantan Institute under the number IBSP 10.571. The remained specimens (ten nymphs and four adults) were used to establish a colony in the laboratory. Ticks were fed on tick bite-naïve rabbits never exposed to acaricides. After feeding, they were kept in a biological oxygen demand (BOD) incubator at 25 °C ± 1 °C and relative humidity of 90% ± 10%. Confirmation of the species was made through morphological comparisons and molecular biology (Labruna and Venzal, 2009; Barros-Battesti et al., 2012).

In total, ten white rabbits of New Zealand breed (*Oryctolagus cuniculus*), aged 60–120 days and weighing 1.5–2.5 kg, were used as hosts for all developmental stages of the tick specimens. These animals were provided by the central vivarium of the Butantan Institute. The use of these animals was approved by the Butantan Institute's animal ethics committee (protocol 1281/14).

The larvae were placed in a feeding containment chamber that was glued to shaved skin on the back of a rabbit using non-toxic glue. Additionally, a plastic collar was placed around the neck of the animal to limit its movement and to prevent removal of the chamber. The chamber was sealed with adhesive tape and the rabbit was placed in an individual cage at room temperature. Each chamber was observed once a day to collect engorged larvae that had detached naturally. The feeding period was registered and the engorged larvae recovered were placed in labeled glass vials, which were then kept in the BOD incubator under the same conditions as above. The larvae were examined once a day to record the molting period (interval between detachment of the engorged larva and its molting to the next stage).

Groups of 15–30 nymphs were released on each rabbit. For it, was necessary to anesthetize the host with an intramuscular injection of 1 ml of ketamine chlorhydrate (Dopalen; Sespo Indústria e Comércio Ltda, Paulínia, SP, Brazil) and 0.5 ml of xylazine (Anasedan; Sespo Indústria e Comércio Ltda, Paulínia, SP, Brazil). No chamber was used in this case because the nymphs of all instars feed for a few minutes. Again, the feeding time was recorded. Nymphs that failed to attach to the host within 30 min were removed and placed in the BOD incubator. Engorged nymphs of all instars were placed in BOD incubator under the same conditions as described above, and examined once a day to record the molting period. To feed adults, they were submitted to the same conditions as described to nymphs. After this, couples were separated from the others, to record mating, and when it was performed, each female was isolated for observation. The number of fertile egg batches in each gonotrophic cycle was recorded (Landulfo et al., 2012). The biological parameters of the fertilized females, such as preoviposition, oviposition periods and egg incubation period, were observed once a day.

DNA was extracted from two adults using the guanidine isothiocyanate-phenol technique (Sangioni et al., 2005). The DNA samples thus extracted were subjected to the polymerase chain reaction (PCR), targeting a fragment of approximately 460 base pairs (bp) of mitochondrial 16S rDNA (Mangold et al., 1998). The products were purified and sequenced using the same primers as used in the PCR. These sequences were aligned using Clustal X (Thompson et al., 1997) and were adjusted manually using the GeneDoc software, with sequences previously determined for other argasid species that were available in GenBank, and also with sequences from *Ixodes holocyclus* Neumann, 1899, and *Ixodes uriae* White, 1852 (Ixodidae), which were used as outgroups (the accession numbers of all the sequences are shown in the resulting phylogenetic tree). The phylogenetic tree was inferred by means of the maximum parsimony (MP) method using PAUP version 4.0b10, with 500 replicates of random addition taxa and TBR branch swapping (Swofford, 2002). All positions were weighted equally, and Bayesian analysis was performed using MrBayes v3.1.2 with 1,000,000 replicates (Huelsenbeck and Ronquist, 2001). The first 25% of the trees

Table 1
Biological parameters of three *Ornithodoros fonsecai* generations from Nobres, State of Mato Grosso, Brazil, using rabbits as hosts.

Stage	Biological parameters	1st Generation	2nd Generation	3rd Generation
Larvae	Feeding period (days)	8.4 ± 0.5 (8-9)	13.5 ± 0.7 (13-14)	9.7 ± 1.0 (7-14)
	Molting period (days)	6.8 ± 0.2 (6-7)	9.7 ± 0.3 (9-10)	4.5 ± d0.7 (3-5)
	N (%) n	170 (98) 167	314 (80) 251	710 (100) 710
N1	Feeding time (min.)	–	–	–
	Molting period (days)	20 ± 0.3 (20-21)	30 ± 0.5 (30-31)	15 ± 1.2 (13-17)
	% of molting	167 (100) 167	251 (100) 251	710 (100) 710
N2	Feeding time (min.)	11.5 ± 1.5 (10-15)	16.5 ± 1.5 (15-25)	16.0 ± 1.0 (12-17)
	Molting period (days)	30 ± 0.3 (30-31)	27 ± 2.0 (19-31)	25 ± 4.0 (14-32)
	% of molting	77 (51) 39	251 (97) 244*	710 (79) 560
N3	Feeding time (min.)	20.5 ± 2.5 (18-25)	20.5 ± 1.5 (10-25)	20.0 ± 1.5 (15-25)
	Molting period (days) - N4	28,7 ± 5.9 (20-32)	25 ± 4.5 (19-34)	29 ± 9.5 (18-37)
	Percentage of molting - N4	39 (90) 35	244 (35) 86	560 (75) 422
	Molting period (days) - ♂	23.5 ± 9.2 (17 - 30)	23.5 ± 15.2 (18 - 160)	0.0
	Molting period (days) - ♀	23.5 ± 12.0 (15 - 32)	23.5 ± 25.0 (26 - 182)	0.0
	% of adult molting	39 (10) 4	244 (65) 158***	0.0
N4	Feeding time (min.)	27.5 ± 3.5 (25-30)	20.5 ± 2.5 (15-25)	20.5 ± 1.5 (20-25)
	Molting period (days) - N5	20.5 ± 3.7 (15-25)	20.5 ± 3.7 (20-25)	20.5 ± 3.7 (18-295)
	Percentage of molting - N5	35 (57) 20	0.0**	299 (45) 134**
	Molting period (days) - ♂	18.5 ± 3.2 (17 - 20)	18.5 ± 3.2 (17 - 20)	45.5 ± 45.2 (28-298)
	Molting period (days) - ♀	20.5 ± 0.5 (19 - 21)	20.5 ± 0.5 (19 - 21)	60.5 ± 35.2 (30-284)
	% of adult molting	35 (43) 15	86 (12) 10	299 (55) 165****
N5	Feeding time (min.)	25.5 ± 5.5 (20-30)	0.0	25.5 ± 1.5 (25-30)
	Molting period (days) - ♂	0.0	0.0	85 ± 35.9 (40-180)
	Molting period (days) - ♀	20.5 ± 0.2 (20 - 21)	0.0	118 ± 49.0 (83 - 153)
	% of adult molting	5 (100) 5	0.0	21 (33) 7

* N3 fed twice before ecdysis to N4; ** 76 N4 died before ecdysis; *** 20 females autogenic; **** 12 females autogenic.

represented burn-in, and the remaining trees were used to calculate Bayesian posterior probability.

3. Results

The biological parameters of the fertilized females, such as pre-oviposition, oviposition periods, egg incubation period, molts and autogenic and non-autogenic data among the three generations are shown in Tables 1 and 2.

3.1. 1st generation

Approximately 170 larvae were fed, and the feeding period, ranged from 8 to 9 days. Most of them (98%) (n = 167) molted to the first nymphal stage (N1) within 6 to 7 days after feeding. It was observed that all N1 molted to N2 after 20 to 21 days in this stage, without a blood meal. However, 90 specimens died subsequently. The remaining 77 specimens in the N2 stage received a blood meal and took 30 to 31 days to molt, resulting in nymphs of the third instar (N3). However, 38 specimens died. The remaining 39 specimens in the N3 stage were fed and molted to the fourth nymphal instar (N4) within a period of 20 to 32 days. All the N4 specimens were fed, and these resulted in only two females and two males, within periods of 17 to 30 days and 15 to 32 days, respectively. Out of the remaining 35 N4 specimens, 20 of them took 15 to 25 days to molt to N5. Fifteen of these molted to adults, resulting in 4 females and 11 males within periods of 19 to 21 days and 17 to 20 days after feeding, respectively. The remaining 5 N5 specimens molted to 5 females within 20–21 days.

The life cycle, starting from eggs that were obtained from specimens collected in the cave of generation F1, to the time of emergence of the first adult from N4 took 146 days, and it took 213 days until the last adult originated from N5.

After feeding, it took two females between 9 and 10 days (pre-oviposition period) to start laying 317 eggs, and it took them 10–15 days subsequently to complete laying all their eggs (oviposition period). The egg incubation period lasted between 18 and 20 days and the larval hatching period took a further 20–32 days, with a hatching rate of 99%.

3.2. 2nd generation

Three hundred and fourteen larvae were fed over a period of between 13 and 14 days. The proportion of engorged larvae recovered was 80%, resulting in 251 engorged specimens. These took between 9 and 10 days to molt to the first instar (N1). All N1 specimens then molted to the second instar (N2), with no blood meal, and the ecdysis period ranged from 30 to 31 days. The N2 specimens were fed and then

Table 2
Parameters of autogenous and non-autogenous females groups of *Ornithodoros fonsecai* from Nobres, State of Mato Grosso, Brazil.

		Weight (mg)		Periods (days)		Eggs	
		At ecdysis	After feeding	Pre-oviposition	Oviposition	N	Incubation period (days)
Autogenous	Female 1	23.8	_	28	21	150	7
	Female 2	24.2	_	26	15	100	12
	Female 3	25.7	_	29	10	80	4
	Female 4	28	_	22	17	180	10
Non-autogenous	Female 1	26.9	82.5	_	_	_	_
	Female 2	21.7	89.3	31	20	450	13
	Female 3	24.8	92.4	_	_	_	_
	Female 4	26.8	88.4	_	_	_	_

N, number of specimens.

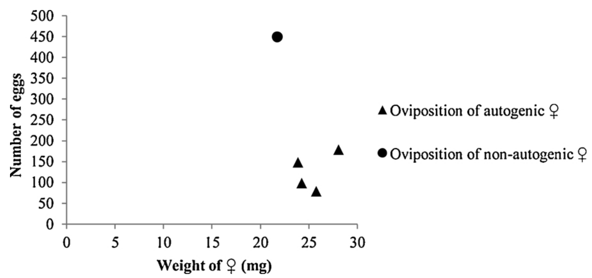

Fig. 1. Individual and egg mass weights of females of *Ornithodoros fonsecai* from Nobres, state of Mato Grosso, Brazil, at ecdysis, and numbers of eggs laid.

molted to N3 after 19 to 31 days. Seven specimens died, and remained 244 were fed. These did molt and therefore were fed again after four months. Out of this total, 98 molted to males, 60 to females and 86 to N4 nymphs, over periods of 18 to 160 days, 26 to 182 days and 19 to 34 days, respectively. All the N4 specimens were fed and ten molted to adults, resulting in 6 males over a period of 20 to 25 days and 4 females over 24 to 32 days. The remainder (n = 76) died after an electrical failure of the incubator. Between 9 and 10 days after copulation, five non-autogenic females started to lay eggs (preoviposition period). Oviposition lasted for 10 to 15 days and the duration of egg incubation was 11 to 20 days. Together, these females laid more than 1000 eggs. The larvae hatching rate was 99%, over a period of 15 to 20 days.

Autogeny was observed in 20 newly molted females from N3. Approximately 200 larvae from three autogenic females were put together to originate the third generation.

The life cycle of generation F2, counting from the oviposition period to the first adult emerging from N3, after four months without molting, was 257 days. The cycle was completed after 339 days, when the last adult emerged from N4.

3.3. 3rd generation

The larvae (n = 710) were fed over a period of between 7 and 14 days and molted within 3 to 5 days after this, to N1. All the N1 specimens molted to N2 without a blood meal, over a period of 13 to 17 days. The majority of the N2 specimens received a blood meal (n = 652). Among these, 560 N2 specimens molted to N3 over a period of 14 to 32 days. Among the N3 specimens, 422 received a blood meal and molted to N4 after 18 to 37 days. A total of 299 N4 specimens received a blood meal and, of these, 106 males and 59 females emerged after 28 to 298 days and 30 to 284 days, respectively. The remaining 134 N4 specimens molted to N5 after 18 to 295 days. Some N5 specimens (n = 21) were fed and a few adults resulted in 5 males within 40 to 180 days and 2 non-autogenic females within 83 to 156 days. One of these females laid around 350 eggs over a period of 31 days (preoviposition period), and the duration of oviposition was 40 days. The egg incubation period lasted for 20 days and the hatching rate was 97%. Twelve autogenic females that were newly molted from N4 were also observed. In this last generation, the life cycle took around 116 days, with adults originating from N4. The last adult emerged 651 days after oviposition.

3.4. Autogeny

Autogenic behavior was observed only in the second and third generations. In F2, mate and fertile eggs were produced from some females (n = 20) that had recently molted from N3. The preoviposition and oviposition periods of all the autogenic females of this generation were 19 to 25 days and 13 to 24 days, respectively. The incubation period took 9 to 15 days, and approximately 800 eggs were produced, with a hatching rate of 90%. The larval hatching period took 14 to 24 days. In F3, autogenic females that had recently molted from N4 (n = 12) were isolated and were mated over a period of 2 to 3 days, with two males each. They produced approximately 900 eggs, with a hatching rate of 99%. The preoviposition and oviposition periods were 25 to 40 days and 9 to 25 days, respectively. The egg incubation period was between 4 and 20 days.

The difference between the generations F2 and F3 was that the N3 nymphs of the 2nd generation fed, but did not molt within the next four months.

The N3 nymphs were fed again and then they molted to females. These started to lay eggs without feeding. The females of generation F3 were fed once as nymphs, and were able to perform autogeny. Even so, in both generations, non-autogenic females producing fertile eggs were also observed (n = 5 and n = 1 in F2 and F3, respectively). In the first generation, only non-autogenic females were obtained (n = 2).

In order to compare the influence of autogeny, a test was done using females only from the third generation. These were separated into two groups (autogenic and non-autogenic), with four females that had recently molted in each group (Table 2). Females of the autogenic group were weighed individually (Fig. 1) one day after molting, and then isolated, each with two males to mate with. After three days, the females were separated and were then kept in individual vials, for daily observation to verify oviposition. In the autogenic group all females laid eggs and the preoviposition and oviposition periods lasted for 22 to 29 days and 10 to 21 days, respectively. They laid 510 eggs, of which 70% were viable. The incubation period was between 4 and 12 days. The females of the non-autogenic group were weighed before and after feeding (Table 2). In the same day of feeding, they were placed with two males each to copulate. Subsequently, they were kept under the same conditions as the other group. Only one female laid about 450 eggs, 31 days after mating (preoviposition period), and the duration of oviposition was 20 days. The egg incubation period lasted for 13 days and the hatching rate was 97%.

It is important to emphasize that in all generations, a single gonotrophic cycle was seen both for autogenic and non-autogenic females, and that none of the autogenous females received any blood meal after the first oviposition.

3.5. Molecular and phylogenetic analyses

The sequences obtained from two specimens of *Ornithodoros* from Nobres were similarity and only one of them was deposited in GenBank (MK158949) (Fig. 2). The sequence of *O. fonsecai* from Nobres showed 99% similarity to *O. fonsecai* (KX781699). Phylogenetic trees based on maximum parsimony and Bayesian methods showed similar topology and grouped the sequences from *O. fonsecai* in the same branch (100% bootstrap and 1.0 *a posteriori* possibility).

4. Discussion

Ornithodoros fonsecai was successfully reared in the laboratory for three generations.

The nymphal stage of the first generation (F1) included five instars (N5), as did the third generation (F3). In the second generation (F2), the last nymphal instar was N4. However, it is important to note that in F2, all the N3 specimens that fed still had not molted after four months, and were then offered another meal. These results corroborate those of Oliver (1989), who reported that a single nymphal instar might occasionally feed twice before completing the molt to the next stage if its feeding was interrupted or the amount of blood needed to enable molting was not absorbed. In addition, this author stated that the number of nymphal instars varied within the species or between different species.

In the present study, it was noticed that in generation F1 most of the adults emerged from N5 (80%); in F2, most emerged from N3 (94%); and in F3, most emerged from N4 (95.9%). In the first generation, females and males emerged together, and the majority from N5 was male.

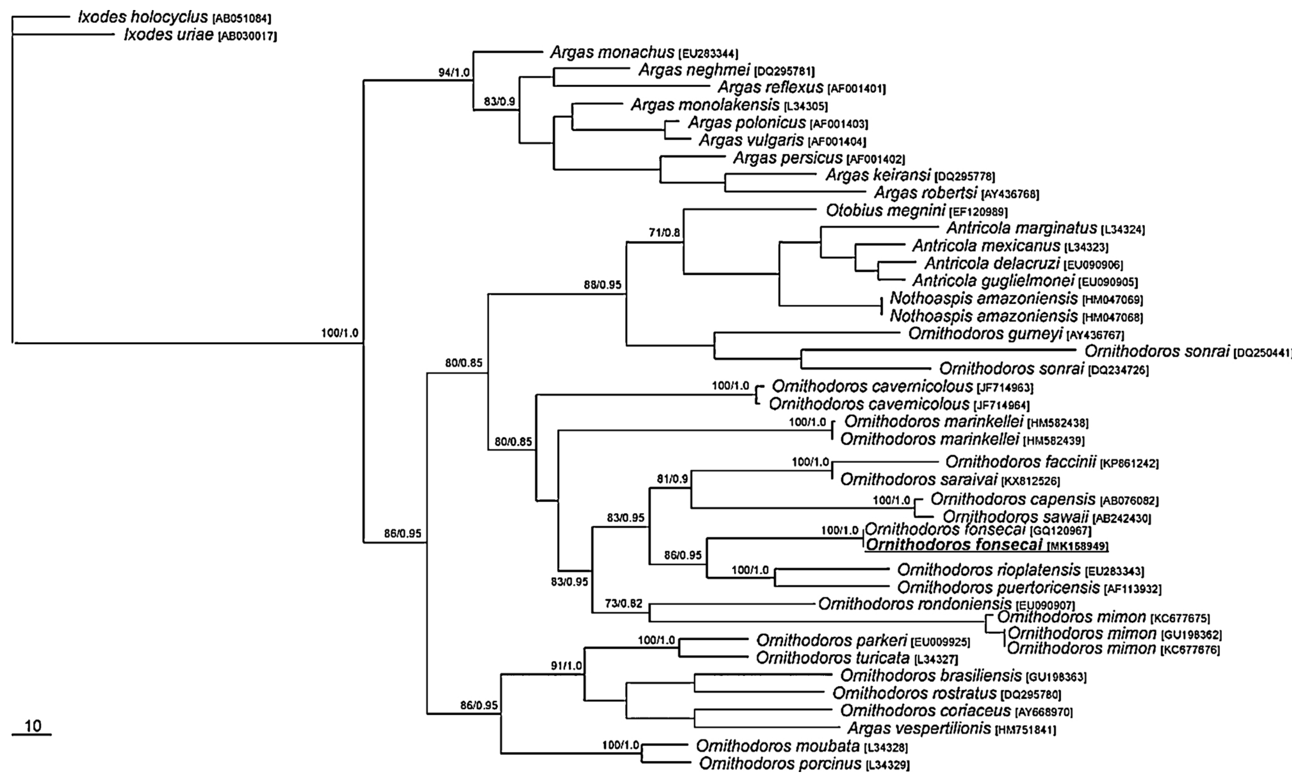

Fig. 2. Phylogenetic tree inferred from a partial sequence of the 16SrRNA mitochondrial gene of tick species of the genera *Ornithodoros* and *Argas*, using *I. holocyclus* and *I. uriae* as outgroups. The numbers at nodes are the values supported for the major branches (bootstrap/posterior probability; 500 replicates) using the maximum parsimony and Bayesian methods, respectively.

This was repeated in the second generation, in which the females and males emerged from N3 and males formed the majority. Lastly, in F3, males and females were again obtained at the same time, with predominance of males from N4. These results are different from those quoted by Oliver (1989), who reported that males generally reach adulthood with one nymphal instar fewer than do females. However, the initial nymphal instars usually produce more males than females, while females reach adulthood at later nymphal stages, as showed by *O. parkeri* (Pound et al., 1986). It is important to note that many variables were involved for the adequate adaptation of the species in laboratory conditions, which may have influenced the low number of adults in F1.

Ornithodoros fonsecai larvae pre-feeding period was of 25–30 days, while that of nymphs and adults 20–30 days. Previous studies on the *Alectorobius* group (Schumaker and Barros, 1995; Landulfo et al., 2012) indicated that these intervals are sufficient for successful argasid engorgement.

The feeding profile of the larval stage that was observed in this study was similar to what has been reported in the literature, given that *O. fonsecai* remained fixed to its host for many days, in all generations. Moreover, it was observed that the N1 specimens did not need to feed in order to molt to N2. This had previously been reported in relation to the species *Ornithodoros mimon* Kohls, Clifford and Jones, 1969 (Landulfo et al., 2012) and *Ornithodoros amblus* Chamberlin, 1920 (Khalil and Hoogstraal, 1981), and is a behavior that is commonly seen among *Alectorobius* species. On the other hand, in *O. talaje* (Schumaker and Barros, 1995), N1 specimens needed to have a blood meal in order to molt to N2, thus differing from other species of this subgenus. Landulfo et al. (2012) observed two generations of *O. mimon* in which nymphal instars of up to N3 were produced and adults started to emerge from N2. The life cycle of generation F1, from its larval stage to the hatching of larvae of generation F2, took 167 days, while the larva-to-larva cycle of F2 was completed in approximately 146 days. The specie *O. amblus* reached seven nymphal instars, and the first adults were produced through molting from N4. The life cycle was completed from 63 and 401 days, counting from oviposition of the initial females to oviposition of the females of generation F1 (Khalil and Hoogstraal, 1981). Lastly, Schumaker and Barros (1995) observed the life cycle of *O. talaje* and found that there were five nymphal instars, with adults emerging at different instars, such as males from N3 and females from N4. The life cycle lasted 53 to 849 days, with adults that emerged after the third molt.

In comparison with some species of the subgenus *Pavlovskyella* Pospelova-Shtrom, 1950, such as *Ornithodoros brasiliensis* Aragão, 1923, Ramirez et al. (2016) observed two generations of this species and noted that the larvae molted to the first nymphal instar without having had a blood meal. These authors also obtained five nymphal instars, such that most of the adults emerged through molting of N3, but females emerged more from N4 and N5. The life cycle from egg to egg in F1 was 378 days, and it was 324 days in F2.

Ribeiro et al. (2013) studied the life cycle of *Ornithodoros rostratus* Aragão, 1911, and obtained six nymphal instars such that emergence of adults started from molting of N3. First-instar nymphs that had not been fed molted to N2. The duration of the cycle ranged from approximately 66 to 136 days. The first nymphal stage of *Ornithodoros erraticus* (Lucas, 1849), an European species, needed to feed in order to molt to N2. Five instars of nymphs were observed, such that only males emerged through molting of N3 and females subsequently emerged through molting of N4. The life cycle was completed within a maximum of 154 days, from parent oviposition to F1 oviposition (Shoura, 1987).

Autogeny is a behavior whereby females oviposit without a blood meal. Feldman-Muhsam (1973) observed three species of the genus *Ornithodoros* that displayed autogeny: *O. parkeri*, *O. tartakovskyi* and *O. tholozani*, for which the observed percentages of autogenic females were 68%, 82% and 63%, respectively. There was a positive association between the weight of the female at ecdysis and autogenicity, such that the largest females were always autogenous, and the smaller females were generally non-autogenic. An important correlation between the weight of the female after emergence through molting and the number of eggs deposited by the autogenic females of all three species was also observed. In the present study, the percentages of autogenic females of *O. fonsecai* were 31.2% in F2 and 19.6% in F3. The test conducted in the present study showed that even the smallest females exhibited autogeny. Furthermore, the autogenous females laid more eggs than did those of greater weight. This was discordant with what was observed by Feldman-Muhsam (1973).

A study on autogeny in *O. tholozani* showed that autogenic females had completed four instars as nymphs and were larger than non-autogenic females that had emerged from the third nymphal instar (Feldman-Muhsam and Havivi, 1973). Autogenic females of the F2 generation of *O. fonsecai* originated from N3 nymphs that had fed twice before molting. These females probably used their remaining nutritional reserves from their last nymphal stage, to perform oviposition. On the other hand, in the F3 generation, the autogenous females emerged through molting of N4 and fed once as nymphs.

Despite the reproductive differences of *O. fonsecai* from Nobres, there is no phylogenetic evidence to allow the segregation of this population. The high sequence similarity values and topologies based on the 16S mitochondrial gene obtained in this study confirm to be the same species.

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