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New tick records with notes on rickettsial infection from the wildlife of the state of Espírito Santo, southeastern Brazil

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ABSTRACT

This work aimed to report ticks infesting the wildlife among 15 municipalities of the state of Espírito Santo between 2016 and 2021, within the Atlantic Forest biome of southeastern Brazil. A total of 576 tick specimens (187 males, 56 females, 149 nymphs, and 184 larvae) was collected from 41 species of wild vertebrates (two reptiles, nine mammals, and 30 birds). Ticks were identified by morphological or molecular methods into 18 species, being 12, four, one and one of the genera *Amblyomma, Ixodes, Rhipicephalus* and *Ornithodoros*, respectively. *Amblyomma rotundatum* was the only species collected from reptiles. Ticks collected from mammals were identified as *Amblyomma brasiliense, Amblyomma calcaratum, Amblyomma dubitatum, Amblyomma longirostre, Amblyomma nodosum, Amblyomma pacae, Amblyomma sculptum, Amblyomma varium* and *Rhipicephalus microplus. Amblyomma sculptum* was the species found on the widest variety of hosts, collected from four mammal orders and five bird orders. Passeriformes birds were infested by *Amblyomma fuscum, A. longirostre* (also found on non-passerine birds), *A. nodosum, Amblyomma parkeri, Amblyomma romarioi, A. varium* and *Ixodes loricatus.* An adult female of *Ixodes rio* was collected from a Piciformes bird. Seabirds of the order Procellariiformes were infested by *Ixodes percavatus* sensu lato and *Ixodes uriae.* The argasid *Ornithodoros capensis* was collected from an offshore metallic platform that was used by Suliformes seabirds. Rickettsial agents of the spotted fever group, *Rickettsia amblyommatis* and *Rickettsia* sp. strain Pampulha, were detected in the ticks *A. longirostre* [from the Paraguayan hairy dwarf porcupine (*Coendou spinosus*)] and *A. dubitatum* [from the capybara (*Hydrochoerus hydrochaeris*)], respectively. The following nine tick species are reported for the first time in Espírito Santo state: *A. calcaratum, A. fuscum, A. pacae, A. parkeri, A. romarioi, I. loricatus, I. rio, I. uriae*, and *O. capensis*. Although it is also the first report of *I. uriae* in Brazil, we do not consider it established in the country. Multiple new tick-host associations are reported in the present study.

1. Introduction

The tick fauna of Brazil is currently composed by 78 established species, 53 of the Ixodidae family and 25 of the Argasidae family (Soares et al., 2023). Nearly half of these tick species belong to the ixodid genus *Amblyomma,* which is represented in Brazil by 34 established species (Dantas-Torres et al., 2019; Martins et al., 2019; Soares et al., 2023). Despite a relatively diverse tick fauna, knowledge about the natural history of Brazilian ticks is still expanding, reflected during the last 10 years by the constant reports of new hosts and an increase of the geographic area of known species, and by the constant discoveries of new tick species (Muñoz-Leal et al., 2017a; 2020; Martins et al., 2019;

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2020; Teixeira et al., 2020; Soares et al., 2023). Additionally, this evolving scenario raises concerns about tick-borne pathogens, as Brazil currently acknowledges only two bacterial agents (*Rickettsia rickettsii* and *Rickettsia parkeri*) of tick-borne diseases in humans (Rodriguez-Morales et al., 2018; Angerami et al., 2021).

The state of Espírito Santo, with an area of 46,095 km^2, is located in the Atlantic coast of southeastern Brazil. Its entire area is within the Atlantic Forest biome, although much (~85 %) of its natural forest cover has been altered or destroyed during human occupation, especially during the last century (SOS Mata Atlântica, 2019). Nevertheless, the region exhibits considerable biodiversity, particularly within the limited Atlantic Forest Reserves still extant in the area and the coastal Restinga vegetation (Marsden et al., 2001; Moreira et al., 2008; Dario, 2009). This ecological context manifests itself in the presence of a diverse tick fauna, encompassing 20 established species, which constitutes 25 % of the total Brazilian tick fauna (Ogrzewalska et al., 2009; Acosta et al., 2016).

Bacteria of the genus *Rickettsia* are globally associated with ticks in all continents of the world. Many *Rickettsia* species, especially those classified within the spotted fever group (SFG), are tick-transmitted to humans, who manifest different kinds of spotted fever illness, sometimes with fatal outcome (Parola et al., 2013). The state of Espírito Santo is endemic for Brazilian spotted fever, a highly lethal tick-borne disease caused by *R. rickettsii* (Faccini-Martínez et al., 2018). In addition, other five *Rickettsia* species have been reported in ticks from the state of Espírito Santo, as follows: *Rickettsia amblyommatis* in *Amblyomma humerale; Rickettsia bellii* in *Amblyomma aureolatum* and *Amblyomma ovale; Rickettsia felis* in *Rhipicephalus sanguineus* sensu lato (s.l.); *R. parkeri* (strain Atlantic rainforest) in *A. ovale* and *A. aureolatum*; and *Rickettsia rhipicephali* in *Haemaphysalis juxtakochi* (Oliveira et al., 2008; Acosta et al., 2016; 2018; Faccini-Martínez et al., 2020). Among these, only *R. parkeri* is known to be pathogenic to humans; however, no clinical cases have been confirmed in Espírito Santo (Faccini-Martínez et al., 2020). While bacteria of the genus *Borrelia* are globally associated with ticks, there has been no records of these organisms in the state of Espírito Santo, despite of serological reactiveness of vertebrates to *Borrelia burgdorferi* sensu lato (Spolidorio et al., 2010).

Considering the abundant diversity of wildlife and ticks in the state of Espirito Santo, this work aimed to report ticks infesting wild species of reptiles, birds, and mammals through convenience sampling among multiple areas of the state. In addition, a specific survey of ticks infesting wild birds was carried out in the largest Atlantic Forest Reserve in the state. Finally, some of the collected ticks were tested for the presence of bacteria of the genera *Rickettsia* and *Borrelia*.

2. Materials and methods

Between 2017 and 2021, wild reptiles, birds and mammals were rescued along the state of Espírito Santo and sent to the wildlife rehabilitation center operated by the "Instituto de Pesquisa e Reabilitação de Animais Marinhos" (IPRAM) in Cariacica municipality. Upon arrival, every animal underwent a general physical examination, when attached ticks were collected and stored in microtubes with 100 % ethanol. The date and location (municipality name) of the origin of each rescued animal was annotated. For this study, data were only available for animals infested with ticks.

Additionally, in June and July 2016 free-ranging birds were captured by mist nets in an Atlantic Forest Reserve in Sooretama, state of Espírito Santo. Birds were caught using 20 mist nets (12 m long × 3 m wide, 36 mm mesh) displayed along animal trails (a total of 5 workdays with nets opened from 6:00 am to 12:00 am) inside the forest, resulting in 600 net-hours. Mist nets were checked every 30 min; captured birds were identified to species following Sigrist (2007), examined for the presence of ticks by carefully checking their whole body, banded, and released at the capture site. Most of the collected ticks were stored in 100 % ethanol, while a few engorged immature ticks were stored in dry plastic tubes and observed for molting inside an incubator in the laboratory. For this specific collection of free-ranging birds in Sooretama, we annotated data from every captured bird, either tick-infested or non-infested.

Ticks were morphologically identified to species level following Anastos (1954), Wilson (1970), Marques et al. (2004) and Apanaskevich et al. (2022) for *Ixodes* spp.; Martins et al. (2019) for *Amblyomma romarioi* adults; and Dantas-Torres et al. (2019) for nymphs and adults of the remaining tick species. Due to the lack of literature for reliable morphological identification of the larval stage of many neotropical *Amblyomma* species, some larvae of this genus were morphologically retained as *Amblyomma* sp., except for those that were identified as *Amblyomma longirostre* by direct comparisons with conspecific laboratory-reared larvae and its morphological description by Barros-Battesti et al. (2005). In addition, 55 larval ticks were identified to species level by molecular analysis. For this purpose, larvae were individually submitted to DNA extraction by the guanidine isothiocyanate phenol technique (Sangioni et al., 2005) and tested by a polymerase chain reaction (PCR) assay targeting *a* ≈ 460-bp fragment of the tick mitochondrial 16S rRNA gene, as described (Mangold et al., 1998). PCR products were purified and sequenced with the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) in an automatic sequencer (model ABI 3500 Genetic Analyzer; Applied Biosystems) according to the manufacturer's protocol. The generated sequences were submitted to BLAST analysis (www.ncbi.nlm.nih.gov/blast) to infer the closest identities to tick DNA sequences available in GenBank.

A sample of 137 ticks was selected for testing individually for molecular detection of organisms of the genera *Rickettsia* and *Borrelia*. Extracted DNA samples (same protocol mentioned above) were initially tested by a Taqman real-time PCR assay targeting the rickettsial citrate synthase gene (*gltA*), as described (Labruna et al., 2004; Soares et al., 2012). Positive samples by this Taqman real-time PCR (cycle threshold ≤ 35) were tested by two protocols of conventional PCR, one targeting a 401-bp fragment of the *gltA* gene (Labruna et al., 2004), and another targeting *a* ≈ 630-bp fragment of the rickettsial 190-kDa outer membrane protein gene (*ompA*), as described (Eremeeva et al., 2006). In each set of reactions, negative control tubes containing water and a positive control tube containing *Rickettsia vini* DNA were included. PCR products were DNA-sequenced and submitted to BLAST analyses as described above. For detection of borrelial DNA, all 137 tick samples were tested by a Taqman real-time PCR assay targeting the 16S rRNA gene of organisms of the genus *Borrelia*, as described (Parola et al., 2011). Negative control tubes containing water and a positive control tube containing *Borrelia anserina* DNA were included.

This study has been approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine of the University of São Paulo (protocol 9,959,300,821). Capture of ticks from native fauna was authorized by the Brazilian Ministry of the Environment (permits SISBIO No. 53,767–1 and No. 64,381–2).

3. Results

Ticks were collected from a total of 48 wild hosts that arrived in the IPRAM wildlife rehabilitation center, from 14 municipalities of the state of Espírito Santo (Fig. 1, Table 1). These hosts included two species of reptiles (2 orders, 6 individuals), eight species of non-passerine birds (6 orders, 11 individuals) and nine species of mammals (5 orders, 31 individuals).

During the field work of Sooretama (municipality number 11 in Fig. 1), a total of 129 birds were captured; 119 were of the order Passeriformes (passerines), whereas 10 individual birds were of the order Piciformes (Table 2). Overall, 30.2 % (36/119) passerines were infested by ticks, and only one (10 %) individual of the Picifomes was tick-infested, giving an overall tick prevalence of 28.7 % (37/129). Among the 29 sampled species of Passeriformes, 21 (72 %) were found infested by ticks.

A total of 576 tick specimens was collected in this study, being 243

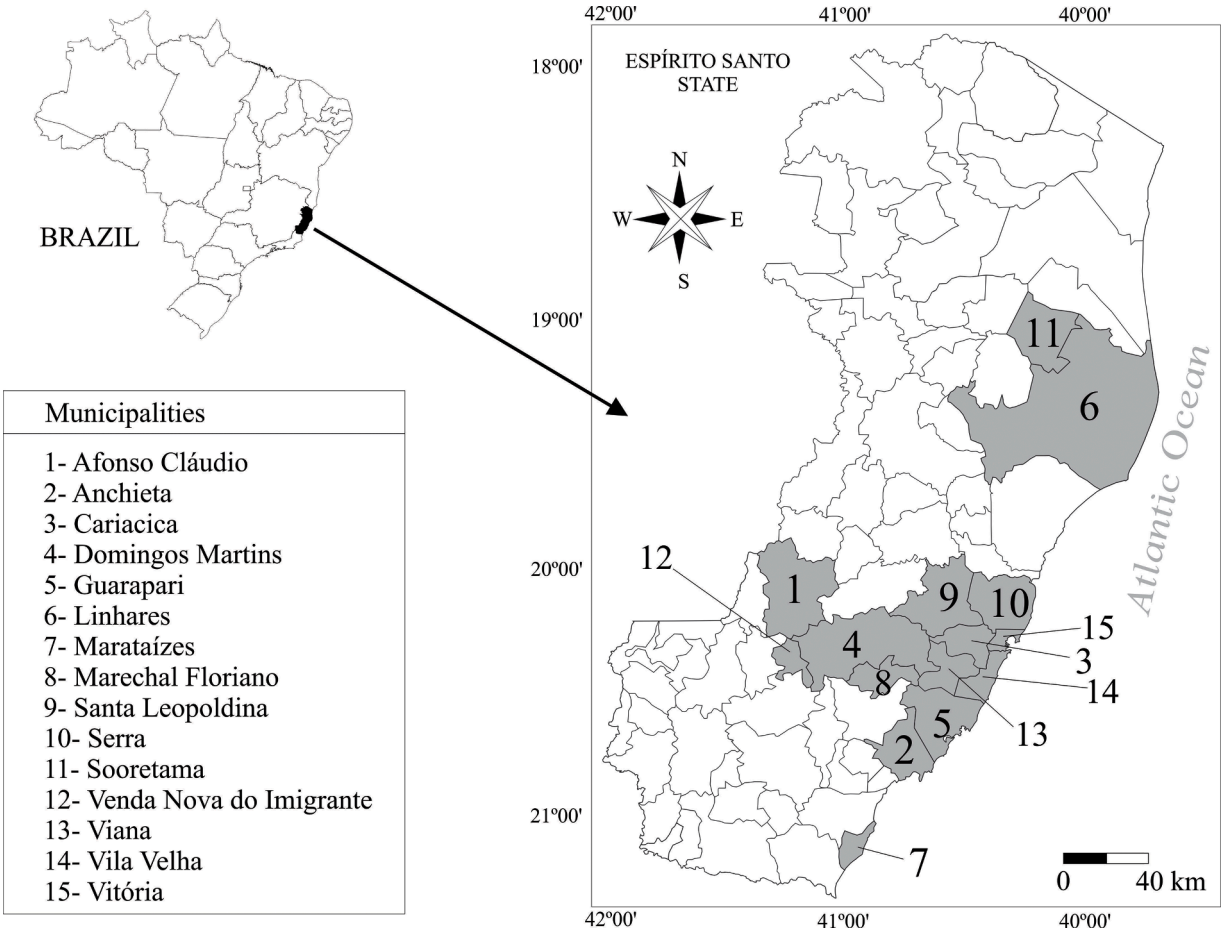

Fig. 1. Localities (numbers 1 to 15) of the state of Espírito Santo, Brazil, where ticks were collected in the present study.

adults (187 males, 56 females), 149 nymphs and 184 larvae. These ticks were identified into 18 species, being 12 of the genus *Amblyomma,* four *Ixodes* species, and one each of the genera *Rhipicephalus* and *Ornithodoros*. All ticks were collected from wild hosts, except for an argasid nymph, identified as *Ornithodoros capensis*. This nymph was opportunistically collected from an offshore metallic platform (~50 km off the coast of Linhares; 19.563 S, 39.254 W) that was being used as roosting site by masked boobies (*Sula dactylatra*) and brown boobies (*Sula leucogaster*).

Except for the single specimen of *O. capensis,* all other 575 tick specimens are presented according to stage, species, host and locality in Tables 1 and 2. Most of the collected larvae belonged to the genus *Amblyomma* (138 specimens). Of these, 70 were retained as *Amblyomma* sp. The remaining 68 *Amblyomma* larvae were identified to species level; 19 were identified as *A. longirostre* exclusively by morphology, whereas 49 were molecularly identified to six *Amblyomma* species, as detailed in Table 3. Additionally, six *Ixodes* larvae were confirmed by molecular methods as belonging to the species *Ixodes percavatus* sensu lato (s.l.) or *Ixodes uriae* (Table 3).

Among the 137 tick specimens tested for rickettsial infection, only 9 (6.6 %) ticks yielded rickettsial DNA by both Taqman real-time and conventional PCR assays (Table 4). Six larvae of *A. longirostre*, collected on porcupine (*Coendou spinosus*) from Guarapari municipality, yielded *gltA* (350-bp) and *ompA* (544-bp) partial sequences that were 100 % identical to corresponding sequences of *R. amblyommatis* from GenBank. In addition, two males and one female of *Amblyomma dubitatum* collected on capybara (*Hydrochoerus hydrochaeris*) from Anchieta municipality yielded *gltA* (350-bp) partial sequences that were 100 % identical to corresponding sequences of *Rickettsia* sp. strain Pampulha from GenBank. No product was amplified from these three ticks by the *ompA*-PCR assay. Partial sequences of the rickettsial genes generated in this study have been deposited in GenBank under the accession numbers OR588869 (*R. amblyommatis gltA*), OR588870 (*R. amblyommatis ompA*), and OR588871 (*Rickettsia* sp. *gltA*). None of the 137 ticks yielded amplicon signals by the Taqman real-time assay targeting the genus *Borrelia*.

4. Discussion

A richness of 18 tick species were collected from 41 species of wild vertebrates in the state of Espírito Santo, southeastern Brazil. In addition, two SFG *Rickettsia* species have been molecularly identified in two of the tick species, from which no *Borrelia* DNA was detected.

Amblyomma rotundatum was the only species collected from reptiles, in agreement with its host preference for amphibia and reptiles in the Neotropical region (Guglielmone and Nava, 2010). The two reptile species of the present study, the red-footed tortoise (*Chelonoidis carbonarius*) and the common boa (*Boa constrictor*), have been previously reported as hosts for nymphs and females of *A. rotundatum* (Guglielmone et al., 2021).

Amblyomma sculptum was the species found on the largest richness of hosts, collected from five bird orders (Cariamiformes, Falconiformes, Galliformes, Passeriformes, and Strigiformes) and four mammal orders (Didelphimorphia, Carnivora, Artiodactyla, and Rodentia). Most of these records were for immature stages, which are known to have a broad host range that includes chiefly medium- to large-sized mammals and non-passerine birds (Martins et al., 2016; Guglielmone et al., 2021). On the other hand, the adult stage of *A. sculptum* has a host preference

Table 1
Ticks collected from wild vertebrates in the state of Espírito Santo, Brazil.

Host species (No. specimens)	Tick species	Locality no. [a]	No. tick specimens (No. infested individuals) [b]
REPTILIA			
TESTUDINES			
Chelonoidis carbonarius (1)	*Amblyomma rotundatum*	15	2F, 2 N (1)
SQUAMATA			
Boa constrictor (5)	*A. rotundatum*	3,14,15	7F, 8 N (5)
BIRDS			
CARIAMIFORMES			
Cariama cristata (2)	*Amblyomma sculptum*	3	7 N (1)
	Amblyomma longirostre	2	2 M (1)
	Amblyomma sp.	3	6 L (1)
FALCONIFORMES			
Caracara plancus (1)	*A. sculptum*	14	1 N (1)
GALLIFORMES			
Penelope superciliaris (1)	*A. sculptum*	14	5 L (1)
	Amblyomma sp.	14	5 L (1)
PICIFORMES			
Pteroglossus aracari (1)	*A. longirostre*	5	1 N (1)
Selenidera maculirostris (1)	*A. longirostre*	8	1 N (1)
PROCELLARIIFORMES			
Thalassarche chlororhynchos (3)	*Ixodes percavatus* s.l.	6	8 L (2)
	Ixodes uriae	6	38 L (1)
STRIGIFORMES			
Asio clamator (1)	*A. sculptum*	14	7 N (1)
Pulsatrix koeniswaldiana (1)	*A. longirostre*	12	10 L (1)
MAMMALIA			
DIDELPHIMORPHIA			
Didelphis aurita (1)	*Amblyomma sculptum*	3	1 N (1)
PILOSA			
Bradypus torquatus (3)	*Amblyomma varium*	3,4,9	8 M, 3F, 1 N (3)
Tamandua tetradactyla (4)	*Amblyomma calcaratum*	1,4	13 M (2)
	Amblyomma nodosum	1,3,4	13 M, 7F (4)
CARNIVORA			
Cerdocyon thous (3)	*A. sculptum*	2,3,6	17 N, 2 L (3)
ARTIODACTYLA			
Subulo gouazoubira (2)	*A. sculptum*	3,8	2 N (2)
RODENTIA			
Coendou prehensilis (4)	*Amblyomma longirostre*	15	1 M, 1 N (2)
	Amblyomma dubitatum	4	1 N (1)
	A. sculptum	5	1 N (1)
Coendou spinosus (4)	*A. longirostre*	5,14	2 M, 2F, 2 N, 9 L (4)
	A. dubitatum	7	2 L (1)
Cuniculus paca (1)	*Amblyomma brasiliense*	8	1 N (1)
	Amblyomma pacae	8	1 N (1)
Hydrochoerus hydrochaeris (9)	*A. dubitatum*	2,10,13,14	144 M, 25F, 37 N (6)
	A. sculptum	2,3	4 M, 8F, 18 N (4)
	Amblyomma sp.	2	20 L (2)
	Rhipicephalus microplus	2	1F, 8 N (1)

[a] See Fig. 1 for locality names and geographical localization according to the locality numbers in the present table.
[b] L: larvae; N: nymphs; M: males; F: females.

towards fewer species of medium- to large-sized mammals (Martins et al., 2016). This condition was confirmed in the present study, in which adults of *A. sculptum* were collected only from capybaras (*H. hydrochaeris*), whereas larvae and/or nymphs were collected from opossum (*Didelphis aurita*), crab-eating fox (*Cerdocyon thous*), brocket deer (*Subulo gouazoubira*), Brazilian porcupine (*Coendou prehensilis*), capybara, four species of non-passerine birds [red-legged seriema (*Cariama cristata*), crested caracara (*Caracara plancus*), rusty-margined guan (*Penelope superciliaris*), striped owl (*Asio clamator*)] and only one passerine species, the pale-breasted thrush (*Turdus leucomelas*). All these host species associations have been previously reported for *A. sculptum* (Teixeira et al., 2020; Guglielmone et al., 2021).

We report adults and immature stages of *A. longirostre* on porcupines (*Coendou* spp.), which have been reported as main hosts for adults of this tick species, and eventually for nymphs (Guglielmone et al., 2021). On the other hand, passerines are considered the main group of hosts for larvae and nymphs of *A. longirostre* (Guglielmone et al., 2021), corroborating our findings of larvae and/or nymphs of this tick on 10 species of Passeriformes, and on only four species of non-passerines. In fact, *A. longirostre* was the most frequent tick species on passerine birds during our field work in the Atlantic Forest Reserve, similarly to other areas of the Atlantic Forest biome (Labruna et al., 2007; Ogrzewalska et al., 2012; Lugarini et al., 2015; Luz et al., 2017a). According to previous published records of *A. longirostre* (Guglielmone et al., 2021), we report the following tick stage-host records for the first time: larvae on Paraguayan hairy dwarf porcupines (*Coendou spinosus*) and on the birds rusty-margined guan (*P. superciliaris*), violaceous euphonia (*Euphonia violacea*), chestnut-backed antshrike (*Thamnophilus palliatus*) and tawny-browed owl (*Pulsatrix koeniswaldiana*); nymphs on chivi vireo (*Vireo chivi*), black-necked aracari (*Pteroglossus aracari*) and spot-billed toucanet (*Selenidera maculirostris*); and adults on the red-legged seriema (*C. cristata*).

The second most frequent tick species on passerines was *Amblyomma nodosum,* whose nymphs were collected from nine bird species in Sooretama municipality. This result is also corroborated by previous studies that reported passerines as main hosts for immature stages of *A. nodosum,* especially in the Atlantic Forest biome (Ogrzewalska et al., 2009; Lugarini et al., 2015). Differently, the adult stage of *A. nodosum* feeds chiefly on anteaters (Guglielmone et al., 2021), which was also reported in the present study; i.e., adults on the southern tamandua (*Tamandua tetradactyla*). Two of the *A. nodosum*-infested southern tamanduas of this study were co-infested with *Amblyomma calcaratum* in two municipalities (Afonso Claudio and Domingos Martins). These two tick species are ecologically similar, i.e., their adults and immature stages feed chiefly on anteaters and passerines, respectively (Guglielmone et al., 2021). However, we did not find immature stages of *A. calcaratum* on birds in the Sooretama. We report the first records of *A. nodosum* nymphs on gray-hooded Attila (*Attila rufus*), streaked flycatcher (*Myiodynastes maculatus*), white-flanked antwren (*Myrmotherula axillaris*), black-capped becard (*Pachyramphus marginatus*) and white-bellied tanager (*Tangara brasiliensis*).

The third most frequent tick species on passerines was *Amblyomma parkeri*, whose larvae were collected from eight bird species. This tick species is ecologically similar to *A. longirostre,* as they both use porcupines and passerines as main hosts for adults and immature stages, respectively, many times sympatrically in the Atlantic Forest biome (Labruna et al., 2009; Pacheco et al., 2012; Luz et al., 2017a; 2017b). According to previous published records (Guglielmone et al., 2021), we report the first records of *A. parkeri* larvae on gray-hooded Attila (*A. rufus*), blue dacnis (*Dacnis cayana*), short-crested flycatcher (*Myiarchus ferox*), social flycatcher (*Myiozetetes similis*), great kiskadee (*Pitangus sulphuratus*), pale-breasted thrush (*Turdus leucomelas*) and rufous-bellied thrush (*Turdus rufiventris*).

We present two host records for *A. romarioi,* collected from two passerines: one larva infesting the black-cheeked gnateater (*Conopophaga melanops*) and one nymph infesting the plain-winged woodcreeper (*Dendrocincla turdina*). This tick species was described in 2019, and until the present study, all host records for larvae were on 25 species of passerines, whereas the few host records for nymphs and adults were restricted to the black-fronted titi monkey (*Callicebus nigrifrons*)

Table 2
Ticks collected from wild birds in the Atlantic Forest Natural Reserve of Sooretama municipality, state of Espírito Santo, Brazil, in June–July 2016.

Birds					Ticks			
Family [a]	Species	No. infested/ No. captured	Prevalence[b] (%)	Mean intensity [c]	Species	No. larvae	No. nymphs	No. females
Dendrocolaptidae	*Dendrocincla turdina*	1/4	25	1	*Amblyomma romarioi*		1	
Conopophagidae	*Conopophaga melanops*	1/4	25	4	*A. romarioi*	1		
					Amblyomma sp.	3		
Fringillidae	*Euphonia violacea*	1/3	33	1	*Amblyomma longirostre*	1		
Galbulidae	*Galbula ruficauda*	0/2	0					
Pipridae	*Manacus manacus*	4/12	33	1.8	*A. longirostre*	5	1	
					Amblyomma nodosum		1	
Rhynchocyclidae	*Tolmomyias poliocephalus*	1/3	33	3	*Amblyomma parkeri*	3		
Thamnophilidae	*Thamnophilus palliatus*	2/2	100	2	*A. longirostre*	4		
	Myrmotherula axillaris	1/1	100	2	*A. nodosum*		2	
Thraupidae	*Dacnis cayana*	1/3	33	2	*A. parkeri*	1		
					Amblyomma fuscum	1		
	Tachyphonus coronatus	4/8	50	1.3	*A. longirostre*		1	
					A. nodosum		2	
					Amblyomma varium		1	
					Ixodes loricatus		1	
	Tangara brasiliensis	2/8	25	1	*A. nodosum*		2	
	Thraupis palmarum	0/1	0					
Tityridae	*Pachyramphus marginatus*	1/6	17	2	*A. nodosum*		2	
Trochilidae	*Phaethornis idaliae*	0/1	0					
	Thalurania glaucopis	0/6	0					
Turdidae	*Turdus albicollis*	1/3	33	1	*A. longirostre*		1	
	Turdus amaurochalinus	0/2	0					
	Turdus leucomelas	4/9	44	10.8	*A. longirostre*	2		
					A. nodosum		2	
					A. parkeri	2		
					Amblyomma sculptum		1	
					Amblyomma sp.	36		
	Turdus rufiventris	3/13	23	4	*A. longirostre*	3	1	
					A. parkeri	8		
Tyrannidae	*Attila rufus*	2/4	50	3.5	*A. nodosum*		5	
					A. parkeri	2		
	Hylocharis sapphirina	0/1	0					
	Myiarchus ferox	1/2	50	3	*A. parkeri*	3		
	Myiarchus tuberculifer	0/2	0					
	Myiodynastes maculatus	2/5	40	1.5	*A. longirostre*		2	
					A. nodosum		1	
	Myiozetetes similis	1/2	50	3	*A. parkeri*	3		
	Pitangus sulphuratus	1/2	50	1	*A. parkeri*	1		
	Rhytipterna simplex	0/1	0					
Vireonidae	*Vireo chivi*	1/1	100	1	*A. longirostre*		1	
Xenopidae	*Xenops minutus*	1/8	13	2	*A. longirostre*		1	
					A. nodosum		1	
Picidae	*Melanerpes flavifrons*	0/4	0					
	Picumnus cirratus	0/4	0					
	Veniliornis affinis	1/2	50	1	*Ixodes rio*			1
TOTAL		37/129	28.7	3.0		79	30	1

[a] All the bird families belong to the order Passeriformes, except for Picidae (Piciformes).
[b] Prevalence of tick infestation: No. infested hosts/ No. captured individuals x 100.
[c] Mean intensity of tick infestation: Total No. ticks / No. infested hosts.

(Martins et al., 2019; Ramírez et al., 2020). We add the black-cheeked gnateater (*C. melanops*) as the twenty-sixth host passerine species for the larval stage of *A. romarioi*, whose nymph is reported here for the first time on a passerine.

We report adults of *Amblyomma varium* on the maned sloth (*Bradypus torquatus*), in agreement with the statement that sloths (Pilosa: Bradypodidae, Choloepodidae) are main hosts for the adult stage of this tick species (Guglielmone et al., 2021; Bernardes et al., 2022). Most of the host records of the immature stages of *A. varium* have been on birds, especially Passerifomes (Guglielmone et al., 2021). Here, we add the ruby-crowned tanager (*Tachyphonus coronatus*) as a new passerine host of the *A. varium* nymphal stage, which was also collected from a maned sloth.

Amblyomma dubitatum was the most abundant and prevalent tick species among the nine sampled capybaras, in agreement with the finding that capybaras are the main hosts of all parasitic stages of this tick species (Luz et al., 2019). Interestingly, we collected nine specimens of the cattle tick, *Rhipicephalus microplus*, from one capybara, a finding that may indicate that the infested capybara frequented cattle pastures, the primary habitat of free-living stages of *R. microplus* in Brazil (Labruna et al., 2005). Our findings of one *Amblyomma pacae* nymph and one *Ambyomma brasiliense* nymph on a lowland paca (*Cuniculus paca*) agree with previous reports of nymphs of these two tick species on lowland pacas (Guglielmone et al., 2021), although this is the first report of *A. pacae* in the state of Espírito Santo.

Two tick species, *Amblyomma fuscum* and *Ixodes loricatus,* are here reported for the first time on birds. The former, a larva on the blue dacnis (*D. cayana*); the latter, a nymph on the ruby-crowned tanager

Table 3
Tick larvae identified to species level by molecular analysis of mitochondrial 16S rDNA partial sequences in the present study.

No. larval specimens	Municipality	Haplotype name (GenBank accession no.)	Highest% identity with GenBank available 16S rDNA sequences
2	Anchieta	Asc1-ES (OR584065)	100 % = *Amblyomma sculptum* MK059459
5	Vila Velha	Asc2-ES (OR584066)	100 % = *A. sculptum* MK059459
2	Marataízes	Adub-ES (OR584067)	99.5 % = *Amblyomma dubitatum* DQ858955
23	Sooretama	Apar-ES (OR584068)	100 % = *Amblyomma parkeri* JN573300
1	Sooretama	Arom-ES (OR584069)	100 % = *Amblyomma romarioi* MN857742
1	Sooretama	Afus-ES (OR584070)	100 % = *Amblyomma fuscum* KU894375
5	Venda N. Imigrante	Alo1-ES (OR584071)	99.8 % = *Amblyomma longirostre* JN573303
5	Guarapari	Alo2-ES (OR584072)	100 % = *A. longirostre* JN573303
5	Sooretama	Alo2-ES (OR584072)	100 % = *A. longirostre* JN573303
1	Linhares	Ipe1-ES (OR584073)	99.5 % = *Ixodes percavatus* s.l. MT072697
2	Linhares	Ipe2-ES (OR584074)	99.3 % = *I. percavatus* s.l. MT072697
1	Linhares	Iur1-ES (OR584075)	100 % = *Ixodes uriae* AF549859
2	Linhares	1ur2-ES (OR584076)	99.8 % = *I. uriae* AF549859

(*T. coronatus*). Previous host records for immature stages of both tick species have been mainly on small mammals, namely opossums and rodents (Guglielmone et al., 2021).

Before this study, the tick genus *Ixodes* was never reported from the state of Espírito Santo (Acosta et al., 2016). In addition, we also found a female of *Ixodes rio* on the red-stained woodpecker (*Veniliornis affinis*). Previous records of *I. rio* from Brazil were reported as *Ixodes auritulus,* until an extensive study that split the later taxon into nine distinct species, of which only *I. rio* was confirmed to occur in Brazil (Apanaskevich et al., 2022).

Among the 30 species of birds found infested by ticks in this study, one seabird species, the Atlantic yellow-nosed albatross (*Thalassarche chlororhynchos*), was found infested by larvae of two typical seabird tick species: *I. percavatus* s.l. and *I. uriae.* These infested albatrosses were rescued on the beach of Linhares municipality, in agreement with a recent study that reported several individuals of Atlantic yellow-nosed albatross infested by *I. percavatus* s.l. larvae and nymphs in the southeastern coast of Brazil, including Linhares (Labruna et al., 2020). Because *I. percavatus* s.l. is not established in Brazil, Labruna et al. (2020) proposed that the origin of the infested albatrosses was the Tristan da Cunha Archipelago, in the South Atlantic Ocean. Our finding of *I. uriae* on the same species of albatross, although it is the first of this tick species in Brazil, probably also originated from the Tristan da Cunha Archipelago in the South Atlantic Ocean, where the Atlantic yellow-nosed albatross breeds and where *I. uriae* is known to occur (Muñoz-Leal and González-Acuña, 2015). There is no evidence that *I. uriae* is established in Brazil.

The finding of an *O. capensis* nymph on an offshore platform could be linked to the booby (*Sula* spp.) population that uses it as a roosting site, since the only previous records of this tick in Brazil were from nesting sites of *Sula* spp. in the Queimada Grande Island, off the coast of the state of São Paulo, and from the São Pedro and São Paulo Archipelago, in the central equatorial Atlantic Ocean (Muñoz-Leal et al., 2017b). Although *Sula* spp. do not nest off the coast of the state of Espírito Santo (Efe, 2004), several other seabird species have been reported as hosts for

Table 4
Results of PCR analyses for the presence of rickettsial infection in ticks in the state of Espírito Santo, Brazil.

Tick species	Locality no. [a]	No. PCR positive / no. tested ticks (% infection)	Tick stage/sex [b]	*Rickettsia* species	Closest identity in GenBank according to rickettsial gene (Accession no.)	
					gltA	*ompA*
Amblyomma dubitatum	13	0/20 (0)	M			
A. dubitatum	2	3/3 (100)	2 M,1F	*Rickettsia* sp. strain Pampulha	100 % (JN676158)	
A. dubitatum	14	0/10 (0)	N			
A. dubitatum	7	0/2 (0)	L			
Amblyomma fuscum	11	0/1 (0)	L			
Amblyomma longirostre	11	0/5 (0)	L			
A. longirostre	12	0/5 (0)	L			
A. longirostre	5	6/6 (100)	L	*Rickettsia amblyommatis*	100 % (MG712729)	100 % (KX137901)
A. longirostre	2	0/1 (0)	M			
A. longirostre	14	0/1 (0)	F			
Amblyomma nodosum	3	0/10 (0)	6 M,4F			
A. nodosum	11	0/2 (0)	N			
Amblyomma parkeri	11	0/15 (0)	L			
Amblyomma romarioi	11	0/1 (0)	L			
Amblyomma rotundatum	3	0/4 (0)	2F,2N			
Amblyomma sculptum	2	0/19 (0)	1 M,2F,14 N,2L			
A. sculptum	14	0/10 (0)	5 N,5L			
A. sculptum	3	0/2 (0)	N			
Amblyomma varium	4	0/4 (0)	M			
A. varium	9	0/2 (0)	M			
A. varium	3	0/1 (0)	M			
Ixodes percavatus s.l.	6	0/3 (0)	L			
Ixodes uriae	6	0/10 (0)	L			
Total		9/137 (6.6)				

[a] See Fig. 1 for locality names and geographical localization according to the locality numbers in the present table.
[a] L: larvae; N: nymphs; M: males; F: females.

O. capensis outside Brazil (Kohls et al., 1965). Therefore, the presence of established populations of *O. capensis* in Espírito Santo remains to be confirmed.

Two SFG agents, *R. amblyommatis* and *Rickettsia* sp. strain Pampulha, were detected in the ticks *A. longirostre* and *A. dubitatum*, respectively. These two agents are considered of unknown pathogenicity to humans or animals (Parola et al., 2013; Karpathy et al., 2016). There are numerous records of *R. amblyommatis* infecting different tick species, including *A. longirostre,* from different regions of Brazil and other American countries (Karpathy et al., 2016; Estrada-Peña et al., 2021). However, previous records of *R. amblyommatis* in the state of Espírito Santo were restricted to *A. humerale* (Acosta et al., 2016). On the other hand, we provide the first report of *Rickettsia* sp. strain Pampulha in the state, since previous studies reported this rickettsial agent only from the adjacent states of Minas Gerais and Rio de Janeiro, also infecting *A. dubitatum* ticks (Guedes et al., 2011; Almeida et al., 2011; Spolidorio et al., 2012). Finally, although *R. rickettsii* has been associated with human cases of Brazilian spotted fever in Espírito Santo (Faccini-Martínez et al., 2018), our failure to detect this agent in ticks could be explained by the extremely low infection rates (<1 %) of its main vector in Brazil, *A. sculptum* (Luz et al., 2019; Angerami et al., 2021).

5. Conclusions

Until the present study, the tick fauna of the state of Espírito Santo was known to contain 20 tick species (Acosta et al., 2016). The following nine tick species are reported for the first time in Espírito Santo: *A. calcaratum, A. fuscum, A. pacae, A. parkeri, A. romarioi, I. loricatus, I. rio, I. uriae*, and *O. capensis*. In addition, we provide multiple new tick-host associations. Because all Brazilian records of *I. percavatus* s.l. and *I. uriae* have been exclusively on a seabird species (*T. chlororhynchos*) that does not nest in Brazilin territory, we exclude them from the list of established tick species in the state of Espírito Santo. The establishment of *O. capensis* remains to be confirmed in the state of Espírito Santo. With the present study, the tick fauna of Espírito Santo is updated to 27 species, 35 % (27/78) of the Brazilian tick fauna. This species richness is highly representative if one considers that the whole area of the state of Espirito Santo (46,095 km^2) represents only 0.5 % of the Brazilian mainland (8510,000 km^2). Because Espírito Santo still bears many remnants of the original Atlantic Forest biome, the present richness of tick species (and consequently tick-borne *Rickettsia* spp.) is an indication of the importance of the state for wildlife conservation.

CRediT authorship contribution statement

Igor da Cunha Lima Acosta: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **Isaias Roveri Garcia:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **Hermes Ribeiro Luz:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – review & editing. **Maria Carolina de Azevedo Serpa:** Data curation, Investigation, Methodology, Writing – review & editing. **Thiago Fernandes Martins:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Ralph Eric Thijl Vanstreels:** Conceptualization, Data curation, Investigation, Methodology, Resources, Visualization, Writing – review & editing. **Marcelo B. Labruna:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

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