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ORIGINAL PAPER

Additional information about tick parasitism in Passeriformes birds in an Atlantic Forest in southeastern Brazil

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Abstract The habits of birds make them more or less susceptible to parasitism by certain tick species. Therefore, while some bird species are typically found to be intensely infested, others are relatively unaffected. This study investigated the occurrence of ticks in Passeriformes inhabiting an Atlantic Forest fragment in southeastern Brazil, during the dry and rainy seasons, by means of parasitological indexes and multiple correspondence analysis, to determine the factors that influence tick parasitism in these birds. Data were collected on 2391 ticks, all classified in the *Amblyomma* genus, from 589 birds. The ticks identified to the species level were *A. longirostre*, *A. nodosum*, *A. calcaratum*, *A. parkeri*, and *A. ovale*. Thamnophilidae, Conopophagidae, Thraupidae, Dendrocolaptidae, and Platyrinchidae were the families with the highest prevalence. In terms of parasite intensity, the families Conopophagidae, Thamnophilidae, Thraupidae, Furnariidae, and Pipridae stood out with the highest values. Bird species that are generalists regarding eating habits and habitat occupation tended to have higher parasite loads, as did larger species and those inhabiting the understory. The tick prevalence was higher in the dry season than in the rainy season. The majority of the ticks were collected from the head region, mainly around the eyes and in the nape. Also, this work reports 22 new bird-parasite relations.

Keywords Ecology of ticks · Parasitological indexes · Site of infestation · Wild birds

Introduction

Birds occupy a wide range of habitats due to specialization and the ability to adapt to various environmental conditions, as well as the many behavioral traits exhibited during their lifetime (Stotz et al. 1996). These behaviors can depend on age, season of the year, type of foraging, search for mates, construction of nests, and defense against predators (Sick 1997).

Ticks parasitize birds mainly in the immature phases and can be carried long distances, enabling the colonization of new areas (Sonenshine and Stout 1970; Choi et al. 2014). Consequently, birds can act as important dispersers not only of ticks but also of the pathogens transmitted by them, directly (birds as reservoir host) or indirectly (birds carrying infected ticks) (Sonenshine and Mather 1994; Hasle 2013). The impact of tick infestation of wild birds is still relatively under-investigated, particularly with respect to the changes brought by habitat modification/destruction due to human activities. At the extreme, anthropogenic activities can cause the complete disappearance of certain bird species. In this respect, these changes can cause alterations in the distribution of ticks that depends on birds as hosts in their immature stages (Ogrzewalska et al. 2011a). The lack of research means that the importance of ticks as regulators of the life cycle of birds under habitat stress is unknown.

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The study of the parasite dynamics between birds and ticks can help elucidate the effect of human actions in forest fragments. In addition, the hosts are able to visit different areas (cleared, edges, intermediate, and forest interiors), making it possible to monitor the species of ticks that are being dispersed, by bringing wild animals as well as humans and domesticated animals into contact with new parasites.

The Atlantic Forest biome is known for its high biodiversity of birds, with 213 species classified as having restricted endemism (Lima 2014). This biome has been studied by several authors, mainly the tick diversity on Passeriformes (Labruna et al. 2007; Ogrzewalska et al. 2008, 2009a, 2012; Pacheco et al. 2012; Sanches et al. 2013), but they have not quantitatively assessed the host-parasite relation between species of ticks and birds. However, it must be considered that this biome extends from the southern region to the northeast of Brazil, including 17 states and 11 different ecosystems (SOSMA and INPE 2014). It is also worth to mention that the studies of this biome have been conducted in the states of São Paulo, Paraná, Paraíba, Bahia, and Rio Grande do Sul, and the sample of ticks found on birds is mainly restricted to areas of dense ombrophilous and semideciduous seasonal forests (Arzua et al. 2005; Amaral et al. 2013; Labruna et al. 2007; Figueiredo et al. 1999; Ogrzewalska et al. 2008, 2009a, 2011a, b, 2012; Pacheco et al. 2012; Sanches et al. 2013; Lugarini et al. 2014).

To provide more information on this topic, this work reports the results of an inventory of tick species found on Passeriformes in an Atlantic Forest fragment in the state of Minas Gerais, southeastern Brazil, and the main aspects of the birds on which they were found, by means of multiple correspondence analysis.

Materials and methods

Study area

The birds were captured in a fragment of secondary Atlantic Forest covering 56 ha, belonging to a private ranch with 1400 ha (Fazenda Continente), located in the municipalities of Juiz de Fora and Coronel Pacheco, Minas Gerais, Brazil (21°37′ S, 43°21′ W) and elevation of 670–800 m (Fig. 1). The fragment is classified as low montane semideciduous forest (Oliveira-Filho et al. 2005), with surrounding pasture areas. The climate is humid subtropical, with the dry season extending from May to September and the rainy season from October to April.

Capture of the birds

Collection trips were carried out from December 2005 to February 2006 (rainy season) and in June and July 2006 (dry season), for a total of 17 days in rainy season and 16 days in dry season. The birds were captured with mist nets (12 × 3 m with 38 mm mesh), arranged in linear transects of 10 nets at four points within the fragment (Fig. 1), with sampling of a single point on each day. The nets were kept open for approximately 10 h, starting at 6:00–6:30 a.m., for a total sampling effort of 1520 net hours in each season.

The captured birds were identified according to Sick (1997), marked with bands provided by CEMAVE/ICMBio, weighed, and measured. Birds captured again during the same campaign were released as soon as possible. The present work only considered birds of the order Passeriformes, according to the nomenclature and classification of the Brazilian Ornithology Registry Committee (CBRO 2014).

Collection and identification of the ticks

After the identification, banding and measurement, the birds were visually inspected to detect the presence of ticks. The ticks collected were separated according to the following sites: base of the beak, crest, nape, cloaca, back, belly, eye rims, ears, throat, and thighs. Engorged specimens were kept alive in 5-ml plastic syringes cut on the distal part and sealed with hydrophilic cotton. In the laboratory the live ticks were maintained in a controlled climate chamber at 27 °C and humidity higher than 80 %. Unengorged and recently molted ticks were placed in vials containing 70°GL ethanol.

The ticks were identified under a stereoscopic microscope with specific keys for each stage. Larvae were identified to the genus level according to Clifford et al. (1961), because there are no keys to identify larvae of Neotropical ticks. Nymphs and adults were identified to the species level according to Martins et al. (2010) and Onofrio et al. (2006), respectively.

Statistical analysis

The parasitological terms and indices presented follow the proposal of Bush et al. (1997) and were calculated with the QPweb 1.0.8 program (Reiczigel et al. 2013). Comparisons between the mean intensity and mean abundance in the rainy and dry seasons were carried out by the *t*-test or Mann-Whitney test, in the second case when the requirements for the *t*-test were not satisfied, in both cases at significance of $p < 0.05$. The aggregation degree was calculated by the discrepancy index (D) (Poulin 1993) for the interactions with prevalence greater than or equal to 10 %.

Multiple correspondence analysis (MCA), calculated with the FactoMineR package (Lê et al. 2008) of the R program (R Core Team 2014), was used to measure the association of the morphological and behavioral variables of the bird species and the presence/intensity of tick

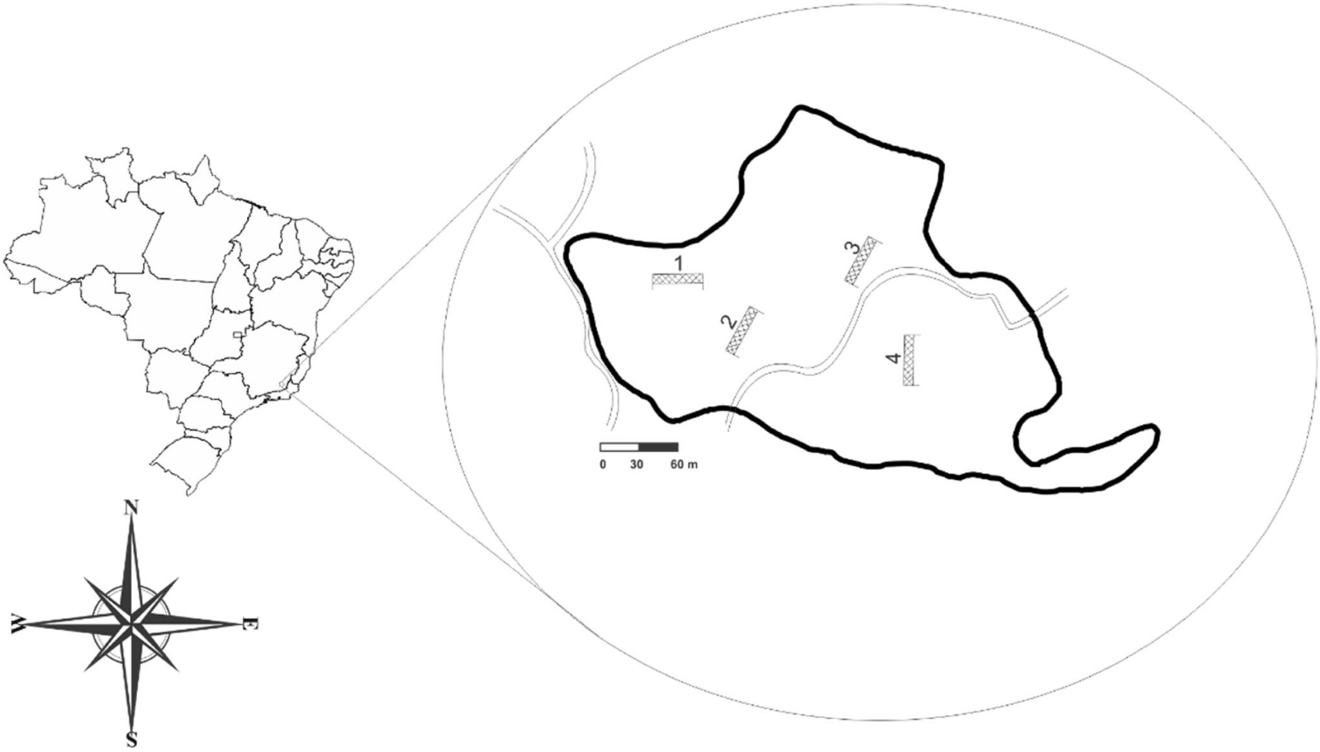

Fig. 1 Forest fragment of Fazenda Continente, Coronel Pacheco-Juiz de Fora, Minas Gerais, Brazil, and arrangement of the sampling points with mist nets. *Sinuous double lines* represent trails within the forest. Adapted from Manhães (2007)

larvae. Ninety-five percent confidence ellipses were obtained by simulation for the coordinates of the variables, and their respective categories were represented in the principal plane. The variables weight, length, and number of larvae were categorized in class intervals. Binary values (yes or no) were considered for the following behavioral variables: habitat occupied (terrestrial, understory, midstory, and canopy) and nest position (on the ground or above ground). Note that certain species can inhabit more than one vertical stratum (e.g., terrestrial, understory, midstory, and canopy). Three categories were used for feeding habit (insectivore, frugivore, and omnivore), and three categories were also used for presence of larvae (none, from 1 to 10 and 11 or more). The last variable, along with the bird families, was treated as supplementary, while the others were treated as active, to verify the structuring action of the other variables in relation to these two. Data on habitat occupation (foraging strata) were taken from Stotz et al. (1996), revised with specific literature (Willis et al. 1983; Sick 1997; Ridgely and Tudor 2009; Sigrist and Brettas 2009; Sigrist 2012). Finally, for nesting place and feeding habits, the following sources were searched: Sick (1997), Sigrist and Brettas (2009), Willis (1979), Lopes et al. (2005), Manhães et al. (2010), Manhães et al. (2005), Stotz et al. (1996), Parrini and Raposo (2008), Ridgely and Tudor (2009), Lill and Ffrench (1970), Aguilar et al. (2000), Godoy (2011), Sigrist (2012), Marini et al. (2007), Auer et al. (2007), and Marini et al. (2002).

Results

A total of 589 birds were captured from 33 species of the order Passeriformes, distributed in 12 families. Of these, 296 (50.3 %) were caught in rainy season and 293 (49.7 %) in dry season (Table 1). From these birds, 2391 ticks were collected, 2341 (97.9 %) in the larval stage and 50 (2.1 %) in the nymphal stage. All the ticks were classified in the genus *Amblyomma*, Koch 1844. Of the larvae collected, 77 (3.3 %) molted to the nymph stage and were identified as 6 (7.8 %) *Amblyomma longirostre* (Koch, 1844), 26 (33.8 %) *A. nodosum* Neumann, 1899, 33 (42.9 %) *A. calcaratum* Neumann, 1899, 1 (1.3 %) *A. ovale* Koch, 1844, and 11 (14.3 %) *A. parkeri* Fonseca and Aragão, 1952. Of the nymphs collected, four (8 %) molted to the adult stage: one male *A. calcaratum* and two females and one male *A. longirostre*. The other nymphs were identified as 38 (75.8 %) *A. longirostre*, 2 (4 %) *A. nodosum*, 4 (8 %) *A. calcaratum* and 1 (2 %) A. *parkeri*. One nymph was identified as *Amblyomma* sp. because the hypostome has been broken off.

When considering the birds of all families, the prevalence of specimens with ticks was 47.9 %, with corresponding rates of

Table 1 List of the number of birds parasitized by each stage/species of tick in Fazenda Continente, Coronel Pacheco-Juiz de Fora, Minas Gerais, Brazil. *Numbers* in parentheses refer to the total of birds captured of each species

	ll *Amblyomma* sp.	ll *A. longirostre*	ll *A. nodosum*	ll *A. calcaratum*	ll *A. parkery*	ll *A. ovale*	nn *Amblyomma* sp.	nn *A. longirostre*	nn *A. nodosum*	nn *A. calcaratum*	nn *A. parkeri*
Conopophagidae											
Conopophaga lineata (55)	28		4	7		1^a				3	
Dendrocolaptidae											
Campylorhamphus falcularius (3)	3^a							1^a			
Lepidocolaptes squamatus (1)											
Sittasomus griseicapillus (15)	5										
Xiphorhynchus fuscus (4)	3										
Furnariidae											
Anabazenops fuscus (27)	13	1	1^a	1^a	1^a			3	1^a	1^a	
Cranioleuca pallida (1)											
Synallaxis ruficapilla (15)	6										
Parulidae											
Basileuterus culicivorus (44)	21		1^a					1			
Passerellidae											
Arremon taciturnus (14)	4									1^a	
Pipridae											
Chiroxiphia caudata (51)	23	1	1^a					1	1^a		
Manacus manacus (7)	4							1			
Platyrinchidae											
Platyrinchus mystaceus (106)	53	1	1^a	1^a				6			
Rhynchocyclidae											
Corythopis delalandi (24)	6^a										
Hemitriccus diops (1)	1^a										
Leptopogon amaurocephalus (6)	2										
Mionectes rufiventris (48)	14							1^a			
Poecilotriccus plumbeiceps (1)											
Tolmomyias sulphurescens (13)	7							4			
Thamnophilidae											
Dysithamnus mentalis (4)	4										
Pyriglena leucoptera (55)	34							3			
Thamnophilus caerulescens (3)	2			1^a				1			
Thraupidae											
Lanio melanops (40)	22	1	2^a	1^a			1	6			

Table 1 (continued)

	ll *Amblyomma* sp.	ll *A. longirostre*	ll *A. nodosum*	ll *A. calcaratum*	ll *A. parkery*	ll *A. ovale*	nn *Amblyomma* sp.	nn *A. longirostre*	nn *A. nodosum*	nn *A. calcaratum*	nn *A. parkeri*
Saltator similis (1)	1							1			
Tachyphonus coronatus (11)	7	1						1			1[a]
Tangara cyanoventris (3)											
Turdidae											
Turdus albicollis (20)	8	1						2			
Turdus flavipes (3)	2			1[a]							
Turdus leucomelas (1)	1							1			
Turdus rufiventris (3)											
Tyrannidae											
Attila rufus (6)	3							2			
Lathrotriccus euleri (3)											
Myiophobus fasciatus (1)	1										

[a] New report of hosts

23.3 % in rainy season and 72.7 % in dry season. The mean intensity of these larvae in winter was greater than in rainy season ($p<0.05$). The same pattern was observed regarding mean abundance. In both seasons together, the mean intensity was 8.3±15.4 and the mean abundance was 4.0±11.4. With respect to the discrepancy index in the seasons, the values were 0.895 and 0.736 for rainy season and dry season, respectively, with an overall index of 0.822 (Table 2).

Table 1 shows the bird-tick relation and the number of birds parasitized by each tick species/stage. Species of all families were found infested by ticks. The species *Conopophaga lineata* (Conopophagidae) was infested with *A. nodosum*, *A. calcaratum*, and *A. ovale* larvae and one *A. calcaratum* nymph. In the family Dendrocolaptidae, all the species were parasitized by larvae except *Lepidocolaptes squamatus*, and one individual was parasitized by a single *A. longirostre* nymph. In the family Furnariidae, *Anabazenops fuscus* was the species most heavily parasitized by larvae and nymphs of *A. longirostre*, *A. nodosum*, *A. calcaratum*, and one *A. parkeri* larva. Only one individual of *Basileuterus culicivorus* (Parulidae) was parasitized, by a single *A. longirostre* nymph, while on all the other infested birds, only *Amblyomma* sp. and *A. nodosum* larvae were found (identified after molting into nymphs). Five *Arremon taciturnus* (Passerellidae) specimens were parasitized by *Amblyomma* sp. larvae and one by an *A. calcaratum* nymph. Birds of the family Pipridae were parasitized by larvae and nymphs of *A. nodosum* and *A. longirostre* and one *A. longirostre* nymph. The birds *Platyrinchus mystaceus* (Platyrichidae) were parasitized by *Amblyomma* sp., *A. nodosum*, and *A. calcaratum* larvae as well as *A. longirostre* larvae and nymphs. The only individual of *Poecilotriccus plumbeiceps* (Rhynchocyclidae) captured did not carry any ticks, but the other species of this family were infested by *Amblyomma* sp. larvae and *A. longirostre* nymphs. All three species of Thamnophilidae captured were parasitized by *Amblyomma* sp. larvae. *Pyriglena leucoptera* (Thamnophlidae) was the most prevalent bird in this family and also accounted for the largest number of parasitized individuals. In the family Thraupidae, only *Tangara cyanoventris* was free of ticks. All the other species of this family were infested by *Amblyomma* sp. larvae and *A. longirostre* nymphs. *Lanio melanops* was the most prevalent species in this family (41 birds) and also accounted for the largest number of parasitized birds. In the family Turdidae, all the species except *Turdus rufiventris* were infested with *Amblyomma* sp. larvae. Finally, *Lathrotriccus euleri* was the only species of the family Tyrannidae free of ticks.

Table 2 shows the parasitological indices of the larvae and bird families. For the family Conopophagidae, there was an increase in the prevalence of *Amblyomma* spp. larvae from rainy season to dry season. However, the differences of mean intensity and abundance between seasons were not statistically significant ($p>0.05$). In the family Dendrocolaptidae, the

Table 2 Number of hosts examined (*Ex*), prevalence and number of hosts infested (*Prev%*) (*Inf*), mean intensity (*MI*), mean abundance (*MA*), and discrepancy index (*D*) of larvae of the genus *Amblyomma* in relation to families of Passeriformes birds captured in the rainy season and dry season in the period of December 2005 to February 2006 (rainy season) and June and July 2006 (dry season)

	Rainy season					Dry season					Total				
	Ex	Prev% (Inf)	IM	AM	*D*	Ex	Prev%(Inf)	IM	AM	*D*	Ex	Prev% (Inf)	IM	AM	*D*
Conopophagidae	21	19 (4)	$2.5^{A}\pm2.4$	$0.5^{a}\pm1.4$	0.845	34	76.5 (26)	$9.8^{A}\pm16.7$	$7.5^{b}\pm15.2$	0.687	55	54.5 (30)	8.8±15.8	4.8±12.4	0.782
Dendrocolaptidae	15	26.7 (4)	$3.3^{A}\pm1.7$	$0.9^{a}\pm1.7$	0.75	7	100 (7)	$8.8^{A}\pm9.9$	$8.8^{b}\pm9.1$	0.431	22	50 (11)	6.818±7.6	3.40±6.3	0.719
Furnariidae	25	24 (6)	$9.2^{A}\pm5.4$	$2.2^{a}\pm4.7$	0.799	18	72.2 (13)	$28.3^{A}\pm26.2$	$20.4^{b}\pm25.6$	0.604	43	44.2 (19)	22.3±23.4	9.8±19.0	0.783
Parulidae	24	25 (6)	$2.7^{A}\pm2.7$	$0.7^{a}\pm1.7$	0.825	20	75 (15)	$3.5^{A}\pm3.9$	$2.6^{b}\pm3.7$	0.577	44	47.7 (21)	3.23±3.5	1.5±2.9	0.731
Passerellidae	4	0	0	0	–	10	40 (4)	1.5±0.6	0.6±0.8	0.606	10	28.6 (4)	1.5±0.6	0.4±0.7	0.711
Pipridae	28	3.6 (1)	2*	$0.07^{a}\pm0.4$	–	30	86.7 (26)	6.8±8.0	$5.9^{b}\pm7.9$	0.567	58	46.6 (27)	6.59±8.0	3.1±6.3	0.766
Platyrinchidae	57	29.8 (17)	$3.6^{A}\pm2.9$	$1.0^{a}\pm2.3$	0.806	49	73.5 (36)	$4.9^{A}\pm4.1$	$3.6^{b}\pm4.1$	0.562	106	50 (53)	4.49±3.8	2.2±3.5	0.704
Rhynchocyclidae	49	16.3 (8)	$1.6^{a}\pm0.7$	$0.3^{a}\pm0.7$	0.855	44	50 (22)	$3.5^{a}\pm3.0$	$1.8^{b}\pm2.8$	0.691	93	32.3 (30)	3.03±2.7	1.0±2.1	0.803
Thamnophilidae	35	48.6 (17)	$8.6^{A}\pm10.3$	$4.2^{a}\pm8.3$	0.755	27	85.2 (23)	$11.2^{A}\pm16.4$	$9.5^{b}\pm15.6$	0.656	62	64.5 (40)	10.1±14.0	6.5±12.2	0.73
Thraupidae	24	20.8 (5)	$17.2^{A}\pm18.1$	$3.6^{a}\pm10.4$	0.847	31	83.9 (26)	$16.4^{A}\pm33.6$	$13.7^{b}\pm31.3$	0.699	55	56.4 (31)	16.51±31.4	9.3±24.8	0.783
Turdidae	8	0	0	0	–	19	63.2 (12)	3.5±3.8	2.2±3.4	0.607	27	44.4 (12)	3.5±3.8	1.5±3.0	0.719
Tyrannidae	1	16.7 (1)	1*	0.2*±0.4	0.714	4	75 (3)	12±11	9±10.8	0.444	10	40 (4)	9.25±10.5	3.7±7.7	0.735
Total	296	23.3 (69)	$5.9^{A}\pm8.2$	$1.4^{a}\pm4.7$	0.895	293	72.7 (213)	$9.0^{A}\pm17.0$	$6.6^{b}\pm15.1$	0.736	589	47.9 (282)	8.3±15.4	4.0±11.4	0.822

Different letters in rows mean the difference between seasons. *Capital letters* are the comparisons between mean intensity, and *small letters* are the comparisons between mean abundance. *Asterisk* indicates low variability to perform statistical test; *en dash* indicates the discrepancy index not calculated due the prevalence lower than 10 %

prevalence of *Amblyomma* sp. larvae was 26.7 % and 100 % in the rainy season and dry season, respectively, but without any difference in mean intensity and abundance ($p>0.05$). For the family Furnariidae, there was an increase in both the mean intensity and mean abundance in dry season in relation to rainy season, from 9.2±5.4 to 28.3±26.2 and from 2.2±4.7 to 20.4±25.6 ticks/bird ($p<0.05$), respectively. In the family Parulidae, the prevalences were 25 and 75 % in rainy season and dry season, respectively. Despite the increased prevalence, there were no statistical differences between the mean intensities and mean abundance levels in rainy season and dry season ($p>0.05$). In the family Passerellidae, parasitized birds were only captured in the rainy season, with prevalence of 40 %. For the family Pipridae, there was an increase in prevalence from rainy season to dry season, from 3.6 to 86.7 %. Likewise, there was an increase in abundance in the dry season ($p<0.05$). In the family Platyrinchidae, there was a significant difference ($p<0.05$) between seasons for mean intensity and abundance, with both being higher in the dry season. The same observation applies to the family Rhynchocyclidae. In Thamnophilidae, there was increased prevalence between the seasons, 48.6 % in rainy season and 84.6 % in dry season, but there were no significant differences ($p>0.05$) in mean intensity and abundance. The same was observed for the family Thraupidae. The birds captured of the family Turdidae were only parasitized by larvae in the rainy season, with prevalence of 61.1 %. In Tyrannidae, the prevalence was 40 % and the mean intensity in dry season was 12±11. With respect to the discrepancy indices, there was a greater larval aggregation level in rainy season than in dry season for all the bird families. The lowest aggregation levels were observed for the families Dendrocolaptidae (0.431) and Tyrannidae (0.444), in the dry season, while the highest were for the families Rhynchocyclidae (0.855) and Conopophagidae (0.845), in the rainy season (Table 2).

Figure 2 shows the range of the parasite intensities of the bird species with at least five parasitized individuals and abundance greater than or equal to 10. Parasite intensity greater than 80 ticks per bird was observed in four species. However, except for *A. fuscus, C. lineata, Chiroxiphia caudata, P. leucoptera, L. melanops, Tachyphonus coronatus and T. caerulescens*, the parasite intensity was lower than 20 ticks in the majority of cases, as was also the case for the species not represented in the graph.

In relation to the sites, most ticks were found on the head. Figure 3 shows distribution of ticks on the sites of the head for the bird families captured. The main sites were the nape and around the eyes, while the throat and base of the beak were the places with lowest numbers of ticks.

Figure 4 shows the principal plane after performing MCA, with the arrangement of the categories in their respective variables. The first two axes are responsible for explaining 35.2 % of the inertia (variability) of the data. Considering the confidence ellipses constructed by simulation, only the variables "season" and "weight" (categories 43–54 g and 55 g or more) do not present differences. The variables contributing the most to explain the axis of dimension 1 are "length," "weight," "foraging," and "canopy," while for the axis of dimension 2 they were "weight," "nest," "length," and "terrestrial". In terms of categories, those contributing the most to explain the axis of dimension 1 were "19–23 cm," "55 g or more," "canopy," and "9–13 cm," while those that most explained axis 2 were "soil," "19–30 g," "6–18 g," and "14–18 cm." With respect to correlation with the axes, the categories with the strongest correlation with axis 1 were "19–23 cm," "9–13 cm," "55 g or more," and "omnivore," while for axis 2 they were "19–30 g," "nest above soil," "nest in soil," and "6–18 g." All the variables with binary categories were discriminated by both axes, although the categories of the variable season were near the centroid and the coordinates of the categories canopy and "not canopy" were near 0 on axis 2. For the variable weight, axis 1 separated "6–18 g" from the others, while axis 2 separated 19–30 g and "31–42 g" from the others. For the variable length, axis 1 separated 9–13 cm from the others, and axis 2 separated the category 14–18 cm from the others. Regarding feeding habit, "insectivore" and "frugivore" were separated by axis 1, while insectivore and omnivore were separated by axis 2.

The positioning of the supplementary variables is shown in Fig. 4. There was an overlap of the confidence ellipses between the categories "Dendrocolaptidae" and "Furnarriidae," "Furnariidae" and "Pripidae," "Tyranidae," and "Turdidae" for the variable "family." Axis 1 separated the families "Conopophagidae," "Rynchocyclidae," "Parulidae," "Thamnophilidae," and "Platyrinchidae" from the other families, while axis 2 separated the families Conopophagidae,

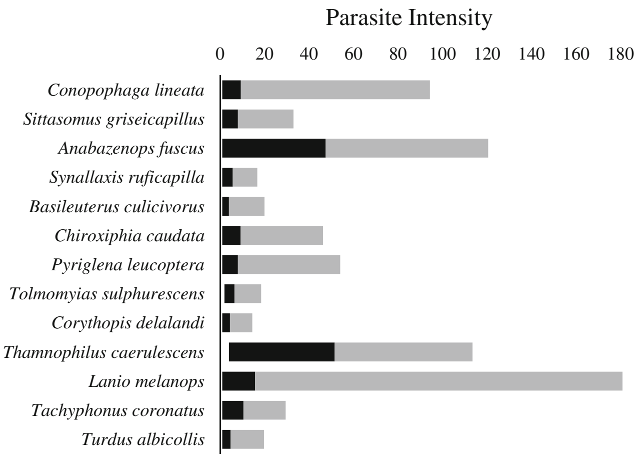

Fig. 2 Range of the tick infestation intensities in birds captured in Fazenda Continente, Coronel Pacheco-Juiz de Fora, Minas Gerais. Black represents 75 % of the data (from the 1st to 3rd quartiles) and gray 25 % (4th quartile)

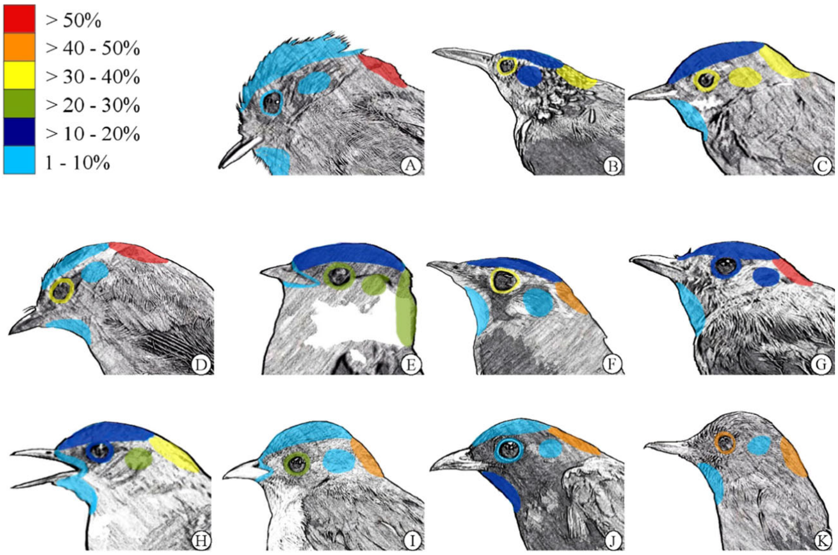

Fig. 3 Relative percentage of distribution of ticks in the head region of bird families. **a** Conopophagidae; **b** Dendrocolaptidae; **c** Furnariidae; **d** Parulidae; **e** Pipridae; **f** Platyrinchidae; **g** Rynchocyclidae; **h** Thamnophilidae; **i** Thraupidae; **j** Turdidae; **k** Tyraniidae

Thamnophilidae, Pipridae, Thraupidae, and Furnariidae from the remaining families (Fig. 5a). With respect to the presence and number of larvae, there was an overlap between the categories "no larvae" and "1–10 larvae." These categories were near the centroid and were separated by axes 1 and 2 of the category "11 or more larvae" (Fig. 5b).

Fig. 4 Representation of the confidence ellipses of the active variables in relation to the axes of dimensions 1 and 2

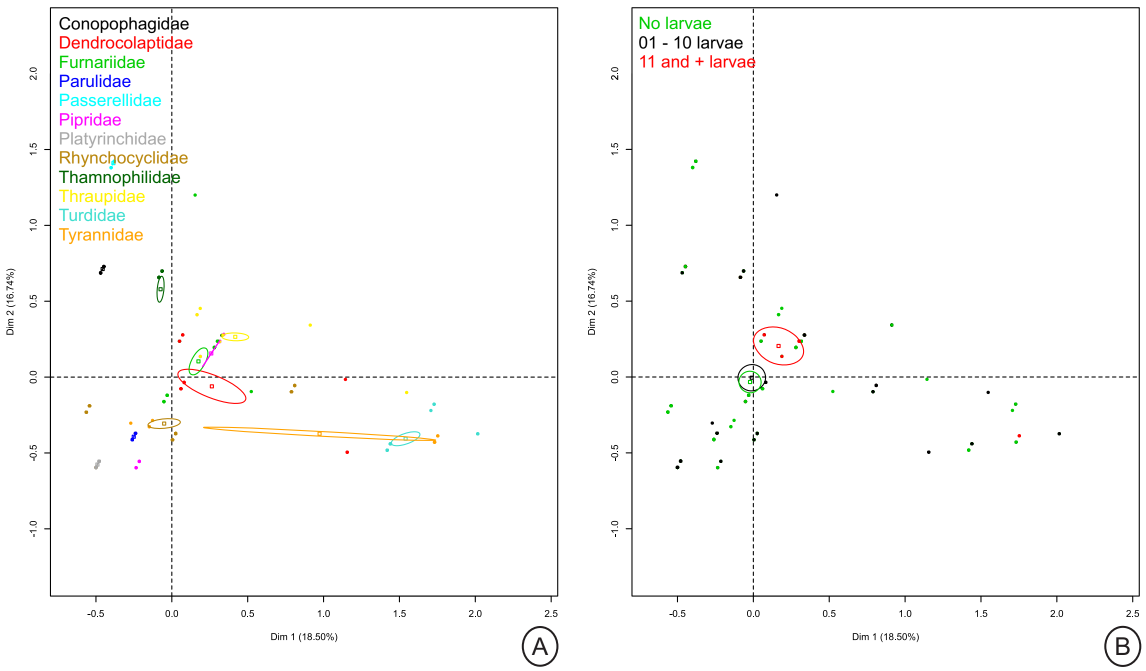

Fig. 5 Representation of the confidence ellipses of the supplementary variables. **a** "family" and **b** "larvae"

Discussion

The most common tick species collected in this study were *A. longirostre*, *A. nodosum*, and *A. calcaratum*, as also reported (Labruna et al. 2007; Ogrzewalska et al. 2008, 2009a, 2012; Luz et al. 2012; Pacheco et al. 2012; Sanches et al. 2013). The exception was *A. aureolatum* (Pallas, 1772), also reported by previous authors but not found in this survey. Birds are important hosts to maintain the life cycle of these parasites during immature stages and they also act as tick dispersers (Onofrio et al. 2006; Guglielmone et al. 2014). This dependence leads to particular strategies of the ticks to maintain this relationship of a morphological, physiological, reproductive, or behavioral nature, such as choice of attachment site, feeding behavior, and control of host defense mechanisms (Dusbabek 2002). The behavior of birds is important to the success of these strategies, especially the likelihood of contact with the parasite. Therefore, some bird species are more susceptible to parasitism than others because the chance of a tick finding a host depends on the latter's behavior. In this respect, the logical expectation would be the repetition of host-parasite interactions in similar forest fragments. However, the record of 22 new host-parasite relationships can be the result of ecological differences among the various fragments of the Atlantic Forest, influencing the behavior of ticks, mainly regarding microhabitats (Estrada-Peña and De La Fuente 2014) and/or preferred host, although it is not possible to rule out some deficiency in the sampling effort to explain the absence of tick species reported in other Atlantic Forest fragments.

Lack of knowledge of the mammalian fauna of Fazenda Continente prevents us from specifying the hosts used by the adult stages of the ticks collected. However, these species have preferences for certain groups of mammal hosts. In the adult stage, *A. longirostre* mainly parasitizes Rodentia, particularly the genera *Coendou* and *Sphiggurus*, which are arboreal rodents (Oliveira and Bonvicino 2006; Onofrio et al. 2006; Nava et al. 2010). According to the data obtained by capturing arboreal Passeriformes, Labruna et al. (2007) considered the free-living stages of this species as an occupant of this microhabitat. In the present study, *A. longirostre* larvae and/or nymphs were found on 9 of the 12 families of birds captured. This wide range of bird families acting as hosts of *A. longirostre* has also been reported on Atlantic Forest (Arzua et al. 2005; Labruna et al. 2007; Ogrzewalska et al. 2009a, b; Lugarini et al. 2014).

The adults of *A. calcaratum* and *A. nodosum* parasitize anteaters (Xenarthra: Myrmecophagidae), as reported by Onofrio et al. (2006); Guglielmone et al. (2014). In the case of this study, the likely host is *Myrmecophaga tridactyla* (Xenarthra: Dasypodidae) due to the phytophysionomic characteristics of Fazenda Continente. *M. tridactyla* feeds on insects in the soil or in trees and rests in tree hollows and armadillo burrows (Medri et al. 2006). The usual hosts of the immature stages of *A. calcaratum* and *A. nodosum* are Passeriformes (Labruna et al. 2007), mainly birds that feed on or near the ground, as seen by Ogrzewalska et al. (2009a, b) in an Atlantic Forest area in the state of São Paulo, and Tolesano-Pascoli et al. (2010) and Luz et al. (2012) in Cerrado (savanna) areas. *C. lineata* was the main species associated with larvae of these two species in Fazenda Continente (Table 1). This bird captures prey in the soil, leaf litter, and trunks and branches near the ground, the same microhabitats used by *M. tridactyla* (Willis et al. 1983). However, Pascoal et al. (2013) observed the presence of *A. nodosum* in birds that feed in both the soil and arboreal strata in Cerrado areas and inferred that this species can be present above the ground. Moreover, we also recorded association of *A. nodosum* and arboreal birds in Fazenda Continente.

The main hosts of *A. parkeri* adults are rodents of the family Erethizontidae, most of which have arboreal habits (Oliveira and Bonvicino 2006; Onofrio et al. 2006; Guglielmone et al. 2014), while Passeriformes are among the hosts of the immature stages (Onofrio et al. 2006; Guglielmone et al. 2014). Reports of *A. parkeri* in birds in Brazil are restricted to larvae in the Atlantic Forest biome in the state of São Paulo, where they have been found in various families of Passeriformes, as also observed in the present work. The presence of *A. parkeri* (in *T. coronatus*) reported here is a first report for nymphs of this species in Passeriformes in Brazil. The immature stages of *A. ovale* mainly infest rodents and marsupials, with only rare reports in Passeriformes (Onofrio et al. 2006; Ogrzewalska et al. 2009a; Luz et al. 2012; Guglielmone et al. 2014). Only larvae of *A. ovale*, firstly report of this interaction in Brazil, were found in *C. lineata*.

The prevalence of larvae was greater in the dry season than the rainy season (Table 2). Due to the similar frequencies of birds captured in the dry and rainy seasons, there was an overlap of the confidence ellipses between the categories "dry" and "rainy." However, an association between the dry season and high intensities was observed (Fig. 4). Although all the larvae listed in Table 2 were only identified to the genus level, it is plausible to assume that the majority of the individuals belong to the three main species identified (*A. longirostre*, *A. calcaratum*, and *A. nodosum*). The predominance of larvae and nymphs of *Amblyomma* spp. in birds in the dry season in the Neotropical region has been widely reported (Marini et al. 1996; Labruna et al. 2007, 2009; Luz et al. 2012; Amaral et al. 2013), as was also observed herein (Table 2). Higher parasite intensities were also observed in this season (Fig. 2). Furthermore, the higher discrepancy indices of larvae in dry season coincided with bird families whose species had large amplitude of mean intensity (Fig. 2). The Rhynchocyclidae was an exception: its high value of *D* indicates low prevalence in this season, since the discrepancy index increases as the prevalence decreases (Poulin 1993). The ecological indices related to mean intensity, abundance, prevalence, and discrepancy of families were influenced by the variations of the average intensities and abundances between some species. For example, *A. fuscus* had high average intensity, while the other species of the same family (Furnariidae) had low average intensities or were not parasitized at all, as occurred with the families Thamnophilidae and Thraupidae (Fig. 2 and Table 2). This fact points to the variation in susceptibility to parasitism of species of the same family of birds, although in general, species that are closely related phylogenetically tend to present similar traits regarding habitat exploitation and, hence, susceptibility to parasites. However, a more detailed exploratory analysis of the data collected here, considering species instead of families, is not possible because the variation of the sample size at the species level would result in imprecise comparisons.

When attached, ticks are vulnerable to removal by the host through grooming (scratching and preening) as part of a wide behavioral repertoire to avoid or remove parasites (Clayton et al. 2010). Regarding birds, preening is the most important, way to remove parasites (Marshall 1981). This behavior can explain why the majority of ticks were found on the head, specifically the nape and around the eyes (Fig. 3). In addition, birds also use their feet to remove parasites on the head (Marshall 1981), but this behavior is probably not as efficient for removal of ticks, mainly from the nape, a region that is hard to reach when using the feet to scratch. On the other hand, the area surrounding the eyes is preferred by ticks probably because of its intense vascularization and may be less scratched by birds due to its skin sensitivity. In general, all the families had a similar pattern of tick distribution on the head leading to the supposition that this behavior by ticks is not taxon-specific.

This study assumed the hypothesis that different bird species have different probabilities to be infested by tick larvae, depending on specific variables for each bird species/taxon, such as type of habitat occupied, foraging habit, nesting place, size, and weight. With respect to habitat occupation, it is necessary to point out that the capture with mist nets is selective, because the birds caught will more often be Passeriformes in flight in the understory and part of the midstory. Therefore, all the birds captured will inhabit at least one of the two vertical vegetation strata and are principally insectivorous (Dunn and Ralph 2004). Thus, although the use of mist nets is the most often used capture method, the sample obtained will not fully reflect all the birds, or even the Passeriformes, in a given forest fragment. Furthermore, it is necessary to have a consistent and current database regarding bird habits, since new information is regularly reported in the literature. Despite the effort to gather the latest information, there will naturally be samples bias that can influence the statistical tests.

The results of the MCA discriminated, by axes 1 and 2, the category "11 or more larvae" from the categories representing "no infestation" or "up to 10 larvae." In this respect, it is possible to note the existence of an association between the variables treated as active in the solution and the presence, magnitude, or absence of infestation. The morphological variables weight and length were among those that contributed most to explain both axes, indicating that larger and heavier birds are associated with more intense infestation by tick larvae. In relation to axis 1, omnivorous birds were associated with more intense larval infestation. Regarding habitat occupation, the categories canopy and terrestrial were associated with greater parasite intensities on axes 1 and 2, respectively. These results indicate that birds with generalist habits, whether referring to habitat occupation or foraging habit, are more susceptible to high tick larval loads. Because different tick species occupy different microhabitats, generalist birds can be more susceptible to parasitism by more than one tick species, which might have contributed to the high parasite intensity values. In comparison with other studies carried out in Atlantic Forest areas, Santolin et al. (2012) found higher prevalence of ticks in omnivorous birds that feed in the soil, while Marini et al. (1996) found greater prevalence in insectivorous birds. In relation to vertical vegetation strata, high parasite intensities were associated with the understory, while the midstory was associated with low intensity or no parasitism; but opposing results have been published by Lugarini et al. (2014) who have not found any relationship between host trophic category or foraging strata and prevalence of *A. longirostre* and *A. nodosum*. Regarding the association between bird families and tick infestation intensity, axis 2 better explained this relationship: two clusters (bird families) with high parasite intensity (Conopophagidae, Thamnophilidae, Thraupidae, Furnariidae, and Pipridae) and low intensity or no parasitism (Parulidae, Rhynchocyclidae, Dendrocolaptidae, Tyrannidae, Passerelidae, and Platyrinchidae). The first group is predominantly made of families with species that feed or nest in the soil, with one species of the family Conopophagidae (*C. lineata*) being the one that spend most time foraging in this habitat. *L. melanops* (Thraupidae) and *A. fuscus* (Furnariidae) are species that use the understory but also feed on the ground (Sick 1997).

An exception is *T. coronatus*, which despite the high parasite intensity, has not been reported by other authors as inhabiting the soil (Sick 1997; Stotz 1996; Sigrist 2012). *P. leucoptera* (Thamnophilidae), a species with high parasite intensity, forages at low altitude and follow army ants on the ground (Willis 1979), a behavior that could make this species more susceptible to tick infestation. The association of this species with ticks is common in the Neotropical region, supporting this hypothesis (Labruna et al. 2007; Ogrzewalska et al. 2012; Pacheco et al. 2012). *T. leucomelas* (Turdidae) and *X. fuscus* (Dendrocolaptidae), whose families were associated with low or nil parasite intensities, also feed in the soil and follow army ants, but with less frequency. In this case the absence of ticks in this survey can be related to the small number of individuals captured. However, there are reports of tick infestation of these species, although without detailed data on parasite prevalence and intensity (Ogrzewalska et al. 2011b, 2013; Pacheco et al. 2012; Pascoal et al. 2013; Sanches et al. 2013; Torga et al. 2013). Conversely, *A. taciturnus* (Passerellidae), *C. delalandi* (Rhyncocyclidae), and *T. rufiventris* (Turdidae), which also forage on the ground, were not intensely parasitized, except for *T. rufiventris* (Table 1). However, since only a few individuals of *T. rufiventris* were captured (four), it is not possible to relate parasitism quantitatively with the habits of this species. Nevertheless, tick infestation of *T. rufiventris* is common (Arzua et al. 2003, 2005; Labruna et al. 2007; Luz et al. 2012; Ogrzewalska et al. 2012; Sanches et al. 2013). An exception to the group of families associated with high parasite loads, in Figs. 4 and 5, is the Pipridae, which forages on the understory and midstory but can exhibit anting behavior and mating rituals on the ground as well the species captured in this survey (Sick 1997). Nevertheless, these behaviors are infrequent and mainly occur in the rainy season when the number of ticks is lowest.

Platyrinchus mystaceus (Platyrinchidae) was the species captured in greatest number in both seasons, accounting for 50 % of the total prevalence but the intensity of parasitism in this species was low. Reports of parasitism by ticks on this species are common in the literature (Labruna et al. 2007; Ogrzewalska et al. 2008, 2009a, 2012; Sanches et al. 2013). This bird is small, with average weight of 10 g, which can explain the smaller number of ticks in relation to the other species with higher prevalences (e.g., *C. lineata*, *A. fuscus*, *P. leucoptera*, and *L. melanops*). A similar pattern of infestation was seen in *B. culicivorus* (Parulidae). Very few individuals of the family Tyrannidae were captured, which prevents us to make inferences about the prevalence and intensity of tick infestations, although there are reports of this species carrying ticks in others regions (Arzua et al. 2005; Labruna et al. 2007; Luz et al. 2012; Sanches et al. 2013).

Birds that build nests in trees appear to be more susceptible to infestation by *A. longirostre* (Labruna et al. 2007). However, the variable "nest," on axis 2, showed association of the category "soil" with the category "11 or more larvae". This fact can be related to parasitism by tick species found more often in the soil, such as *A. nodosum* and *A. calcaratum*. In turn, the nil or low parasite intensities (up to 10 larvae) were associated with birds that build nests above the ground. However, the search behavior and parasitism by ticks in the nest require further studies.

A particularity of Fazenda Continente is the high prevalence and intensity of ticks infesting birds in relation to other areas studied in Brazil (Ogrzewalska et al. 2009a, 2010, 2011a; Tolesano-Pascoli et al. 2010; Luz et al. 2012; Santolin et al. 2012; Pascoal et al. 2013; Sanches et al. 2013). This is probably due to its ecological characteristics because the forest fragment is small (about 56 ha) and is surrounded by extensive pastures, with abrupt interruption of vegetation into the grazing area. This forested area is much smaller than other forest fragments investigated from the Atlantic Forest. According to Ogrzewalska et al. (2011a), tick prevalence and intensity tends to be higher in smaller than larger fragments, probably as a result of a large number of birds with generalist habits.

Finally, this imbalance might influence the transmission of zoonotic agents, since the proximity of wildlife to humans and domesticated animals increases with deforestation and uncontrolled land use. For example, species of *Rickettsia* of spotted fever group in Brazil have been found in *A. longirostre*, *A. calcaratum*, and *A. nodosum* collected from birds. Although reports of parasitism of humans are restricted to *A. longirostre* (in rare cases), it is important to monitor possible vectors and hosts on a regular basis as a preventive measure (Ogrzewalska et al. 2009b, 2013; Pacheco et al. 2012).

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