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Deforestation does not affect the prevalence of a common trypanosome in African birds

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ABSTRACT

In spite of numerous reports of avian *Trypanosoma* spp. in birds throughout the world, patterns of the distribution and prevalence of these blood parasites remains insufficiently understood. It is clear that spatial heterogeneity influences parameters of parasite distributions in natural populations, but data regarding avian trypanosomes are scarce. Using microscopy and molecular diagnostic methods, we analysed the variation of prevalence of avian *Trypanosoma* parasites in two widespread African bird species, the yellow-whiskered greenbul *Andropadus latirostris* and the olive sunbird *Cyanomitra olivacea*. In all, 353 birds were captured in pristine forests and agroforest sites in Cameroon and Ghana. Overall, the prevalence of avian trypanosomes was 51.3%. Five morphospecies were reported (*Trypanosoma everetti*, *T. anguiformis*, *T. avium*, *T. naviformis*, *T. ontarioensis*). *Trypanosoma everetti* predominated, representing 98% of all *Trypanosoma* spp. reports, and it was present in both avian hosts. The prevalence of *T. everetti* was significantly less in the yellow-whiskered greenbul (19%) than olive sunbird (83%), and the same pattern of prevalence was reported in these avian hosts at different study sites. We found no interaction between sites and the prevalence of *T. everetti*. For both avian hosts, the prevalence did not differ significantly between pristine forests and agroforests. This indicates the same pattern of transmission at sites with different levels of deforestation and suggests that spatial heterogeneity related to deforestation does not affect the prevalence of avian *Trypanosoma* infections. It is likely that host-related factors, but not environmental conditions favour or reduce these parasite infections in forests of sub-Saharan Africa. Microscopic and PCR-based diagnostics showed the same sensitivity in diagnostics of *T. everetti*. We discuss the implications of these findings for the epidemiology of avian trypanosomiasis in natural populations.

1. Introduction

Avian *Trypanosoma* species (Trypanosomatidae, Kinetoplastidae) are of cosmopolitan distribution. Transmission of these blood parasites occurs globally in countries with warm and cold climates, including the areas located beyond the Arctic Circle (Baker, 1976; Greiner et al., 1975; White et al., 1978; Bennett et al., 1994a; Holmstad et al., 2003; Valkiūnas, 2005; Sehgal et al., 2015). These are successful avian parasites based on their high prevalence in many bird populations worldwide. However, the biology and patterns of distribution of these protists both in avian hosts and blood-sucking insects remain insufficiently understood (Baker, 1976; Kilpatrick et al., 2006; Zídková et al., 2012; Sehgal et al., 2015). Only few studies have addressed the life cycles and specificity of these infections (Bennett, 1970; Baker, 1976; Dirie et al., 1990; Sehgal et al., 2001; Votýpka and Svobodová, 2004; Votýpka et al., 2012), and the development of trypanosomes in avian hosts and blood-sucking insects remains understudied in the great majority of described species. Available data show that avian trypanosomes develop and can be transmitted by blood-sucking dipteran insects belonging to different families of Diptera and dermanyssid mites, but the specificity of certain parasite species to certain vector groups remains insufficiently investigated (Bennett, 1970; Baker, 1976; Molyneux, 1977; Sehgal et al., 2015).

Much research has been carried out on mammalian species of *Trypanosoma*, which can cause severe disease in animals and humans (Taylor et al., 2007; Bezie et al., 2014). This is not a case with avian trypanosomes, which often are relatively benign in their vertebrate hosts (Baker, 1976). This fact may lower the interest of researchers for this group of parasites, which therefore they have

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remained mainly of protistological significance. However, experimental studies suggested that these parasites may cause avian diseases when parasitemia is high; with histopathological changes and splenomegaly (Molyneux, 1973; Molyneux et al., 1983). It remains unclear how often trypanosomes cause disease in wild birds.

Due to broad specificity to avian hosts and trypanosome morphological plasticity, species diversity and taxonomy of these parasites remain debatable (Apanius, 1991; Zídková et al., 2012; Valkiūnas et al., 2011; Sehgal et al., 2015). Limited studies show that the main morphological characters of haematozoic trypomastigotes remain consistent upon repeated experimental passages of the same isolates from different avian hosts, indicating the validity of major readily distinguishable morphospecies (Bennett, 1961, 1970; Molyneux, 1973; Molyneux and Gordon, 1975; Woo and Bartlett, 1982; Sehgal et al., 2015). However, within morphospecies, cryptic speciation certainly exists and requires additional research (Votýpka et al., 2012; Zídková et al., 2012; Sehgal et al., 2015).

As part of a larger study, numerous blood samples were collected at pristine forest and agroforest sites in Cameroon and Ghana, and information about the distributions of avian *Plasmodium*, *Haemoproteus* and *Leucocytozoon* species at the same study sites was collected and analysed (Bonneaud et al., 2009; Chasar et al., 2009; Loiseau et al., 2010; Iezhova et al., 2010). Several readily distinguishable new morphospecies of avian trypanosomes were found and described (Valkiūnas et al., 2011; Sehgal et al., 2015). Here, we provide prevalence data of avian trypanosomes in two widespread African songbirds, the yellow-whiskered greenbul *Andropadus latirostris* (Pycnonotidae) and the olive sunbird *Cyanomitra olivacea* (Nectariniidae). These passeriform birds are widespread, abundant and relatively easy to sample both in pristine and agroforest sites (Thomas, 1995; Cheke et al., 2001; Bonneaud et al., 2009; Chasar et al., 2009; Smith et al., 2011), providing opportunities for comparative ecological research on their parasites. The aim of the study was to examine whether habitat differences were associated with differences in the prevalence of the predominant avian trypanosome, *Trypanosoma everetti* in common African bird species.

2. Materials and methods

2.1. Study sites and bird sampling

Birds were caught using mist-nets between 27 June and 27 July 2005 in Cameroon and between 7 and 19 June 2007 in Ghana. The methodology of catching birds (with mist nets) was similar at all study sites (see Bonneaud et al., 2009; Loiseau et al., 2010). In Cameroon, birds were caught at four sites. Two sites located in pristine forests (Zoebefame, 02°39.517′ N, 13°23.817′ E and Bobo Camp, 02°39.283′ N, 13°28′.267′ E) and two sites in agroforests (Ndibi, 03°46.00′ N, 12°13.00′ E and Nkwouak, 03°52.017′ N, 13°18.967′ E). All sites were between 600 and 700 m above sea level (asl). Mature forest sites were characterized by a layered closed canopy with tall emergent trees; the sites were located approximately 30 km from the nearest human settlement. Agroforest sites were adjacent to human settlements and had significant disturbance associated with cacao plantations, wood harvesting, burning and various other forms of rainforest habitat degradation. Detailed description of these sites, including habitat characterization using remote-sensing data is presented elsewhere (Bonneaud et al., 2009).

In Ghana, birds were caught at three study sites. Two sites were located in secondary forest, in which between 27% and 41% of tree cover remained (Agumatsa, 07°01.758′ N, 00°33.490′ E; 269 m asl and Abrafo (05°21.171′ N, 01°23.406′ E, 170 m asl). One site (Nkwanta, 05°16.912′ N, 02°38.495′ E, 85 m asl), although a secondary forest, had much lower levels of forest disturbance, with a 64% tree cover present. Detailed description of these sites, including characterization of climate and habitat variables was presented elsewhere (Loiseau et al., 2010).

In all, we examined 353 birds. Among them were 173 olive sunbirds and 180 yellow-whiskered greenbuls. The number of sampled birds was <14 individuals at two study sites in Cameroon (data are not shown). Because of insufficient numbers for reliable statistical analysis of prevalence data, we combined data from different study sites and presented them by nature of their habitat (pristine and agroforests) in Cameroon and Ghana (Table 1).

2.2. Collection of blood samples and their microscopic examination

The blood was taken by puncturing the brachial vein, and two or three blood films were prepared on ready-to-use microscopic glass slides. Blood films were air-dried within 5–15 s after preparation using a battery-operated fan, and fixed in absolute methanol. The blood films were stained in a 10% working solution of a commercially purchased stock solution of Giemsa's stain. Details of preparation and staining of blood films were described by Valkiūnas et al. (2008). For molecular analysis, approximately 50 μl of whole blood was drawn from each bird. The samples were fixed in lysis buffer (Sehgal et al., 2001); they were held at ambient temperature in the field and later at −20 °C in the laboratory.

Because samples were collected in remote field locations, culturing of trypanosomes was impractical for diagnostic purposes (Sehgal et al., 2015). All blood samples were examined microscopically. An Olympus BX61 light microscope equipped with Olympus DP70 digital camera and imaging software AnalySIS FIVE was used to examine blood films and to prepare illustrations. Approximately 150 fields were examined at low magnification (400×), and then at least 100 fields were studied at high magnification (1000×). Parasites were identified according to Baker (1956), Valkiūnas et al. (2011) and Sehgal et al. (2015). Intensity of parasitemia was calculated by actual counting of the number of trypomastigotes per 100 microscopic fields of view at 1000×. The statistical analysis was carried out using the 'Statistica 7' package. Prevalences of infections were compared by Yates corrected Chi-square (χ^2) test. A P value of ≤0.05 was considered significant.

2.3. DNA extraction, PCR amplification and sequencing

Samples collected in Ghana were screened by PCR. This was done with the aim to determine parasite lineages and compare the sensitivity of microscopy and PCR detection methods for trypanosomes (Table 2). To obtain total genomic DNA, blood was extracted following a DNeasy kit protocol (Qiagen®, Valencia, California), or the animal tissue protocol provided with the Wizard SV Genomic DNA Purification Kit (Promega Corporation, Madison, WI). The purified DNA was then used in a nested PCR protocol to amplify SSU rRNA DNA (Valkiūnas et al., 2011). PCR reactions and thermal cycling profiles were performed according to Sehgal et al. (2015). We used primers Tryp763 (5′-CATATGCTTGTTCAAGGAC-3′) and Tryp 1016 (5′-CCCCATAATCTCCAATGGAC-3′) for DNA amplification in first PCR. The second set of primers was Tryp99 (5′-TCAATCAGACGTAATCTGCC-3′) and Tryp957 (5′-CTGCTCCTTTGTTATCCCAT-3′). The fragment length was 770 bp. Products positive for infection were visualized on 1% agarose gels. The representative bidirectional sequence of *T. everetti* from yellow-whiskered greenbul sampled in Cameroon was deposited in Genbank (accession AF361430).

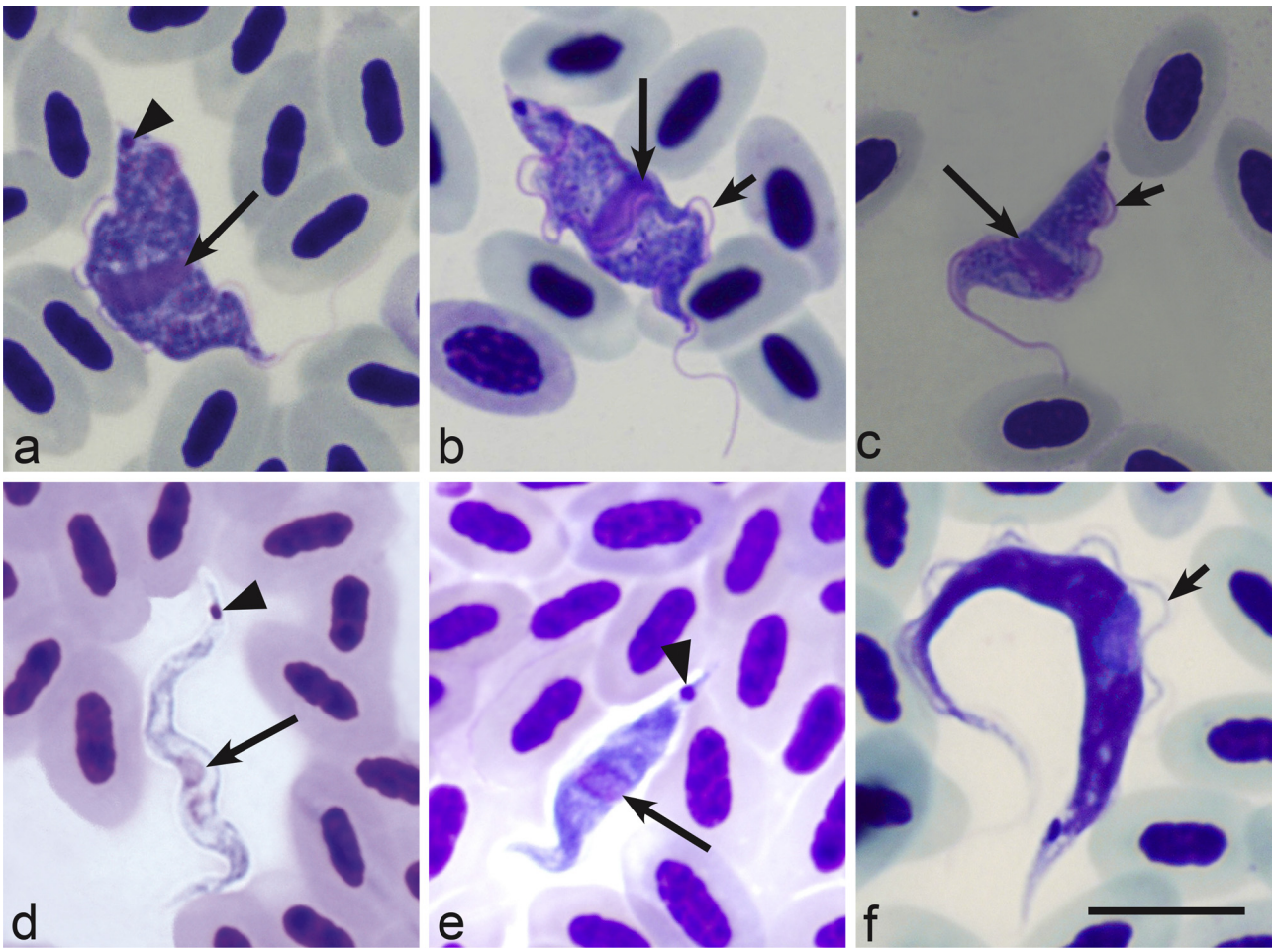

Fig. 1. Haematozoic trypomastigotes of *Trypanosoma everetti* (A, B), *Trypanosoma ontarioensis* (C), *Trypanosoma anguiformis* (D), *Trypanosoma naviformis* (E), and *Trypanosoma avium* (F). Hosts are olive sunbird (A, D, E, F) and yellow-whiskered greenbul (C, B). Long arrows – nuclei of parasites. Short arrows – flagellum. Triangle arrowheads – kinetoplast. Giemsa-stained thin blood films. Bar = 10 μm.

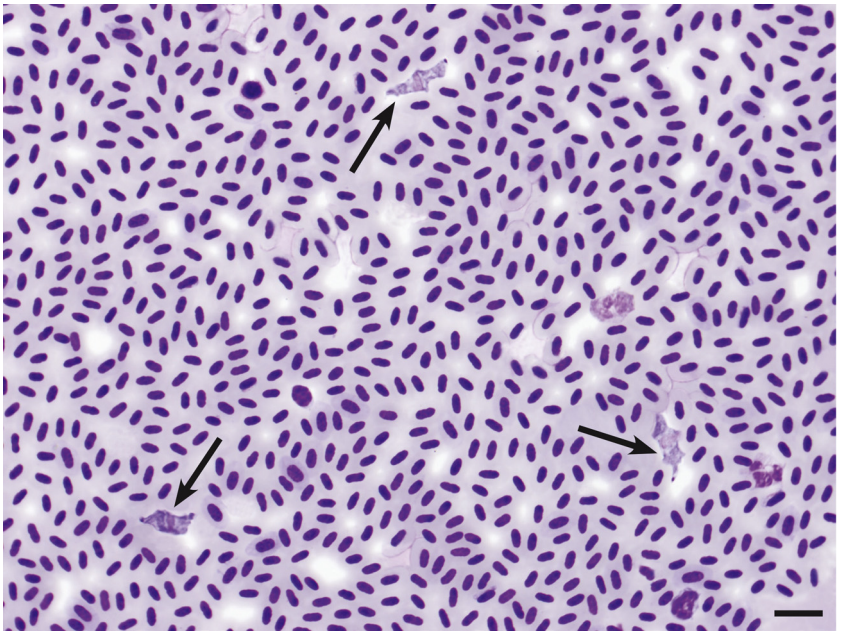

Fig. 2. Intense parasitemia of *Trypanosoma everetti* in peripheral blood of an olive sunbird. Long arrows – trypomastigotes. Giemsa-stained thin blood films. Bar = 10 μm. Note the three parasites present in one microscope field at low magnification (400×), indicating high parasitemia. Due to the often high parasitemia of *T. everetti* in African birds, this infection is straightforward to diagnose both using microscopic and PCR-based methods.

Table 1
Prevalence of *Trypanosoma everetti* in two bird species in pristine forest and agroforest sites in Cameroon (July 2005) and Ghana (July 2007).

Sites and country	Bird species	No. examined	No. infected[a]
Pristine forest			
Cameroon			
	Andropadus latirostris	22	1 (4.5)[b]
	Cyanomitra olivacea	28	13 (46.4)
	Overall	50	14 (28.0)
Ghana			
	A. latirostris	70	20 (28.6)
	C. olivacea	63	62 (98.4)
	Overall	133	82 (61.7)
Total		183	96 (52.5)
Agroforest			
Cameroon			
	A. latirostris	48	9 (18.7)
	C. olivacea	41	32 (78.0)
	Overall	89	41 (46.1)
Ghana			
	A. latirostris	40	5 (12.5)
	C. olivacea	41	36 (87.8)
	Overall	81	41 (50.7)
Total		170	82 (48.2)
Grand total		353	178 (50.4)

[a] Microscopic examination data.
[b] Percentage of birds positive is given in parentheses.

Table 2
Comparison of sensitivity of PCR-based and microscopic examination in detection of *Trypanosoma everetti* in two species of birds sampled in Ghana (July 2007).

Bird species	Number of examined	Number of positive by both methods	Number of positive		*P*-value[b]
			PCR	Microscopy	
Andropadus latirostris	110	27(24.5)[a]	24 (21.8)	25 (22.7)	0.9770
Cyanomitra olivacea	104	101 (97.1)	100 (96.2)	98 (94.2)	0.9980

[a] Percentage is given in parenthesis.
[b] *P*-value according to the Yates corrected Chi-square test.

3. Results

Overall prevalence of avian trypanosomes was 51.3%. Five morphospecies were reported: *Trypanosoma everetti* (Fig. 1A,B), *Trypanosoma anguiformis* (Fig. 1D), *Trypanosoma naviformis* (Fig. 1E), *Trypanosoma ontarioensis* (Fig. 1C), and *Trypanosoma avium* (Fig. 1F). All *Trypanosoma* species were scattered among study sites. *Trypanosoma everetti* predominated (overall prevalence was 50.4%), representing 98% of all *Trypanosoma* spp. reports. Additionally, only *T. everetti* was present in both avian hosts. For this reason, we focused on this parasite species for this study.

Overall, *T. everetti* was significantly more prevalent in olive sunbirds (82.7%) than in yellow-whiskered greenbul (19.4%) ($\chi^2 = 46.9$, $P < 0.001$). Interestingly, the same pattern in the prevalence between these avian hosts remained in pristine and agroforest sites both in Cameroon and Ghana ($\chi^2 > 8.7$, $P < 0.01$), for each country and forest type (Table 1). In other words, the prevalence of *T. everetti* in olive sunbirds was over 3 fold greater than in the yellow-whiskered greenbul at each forest type independent of the level of deforestation.

There were no differences discernable in prevalence of *T. everetti* in olive sunbirds and yellow-whiskered greenbuls within each bird species in pristine forest and agroforest sites both in Cameroon and Ghana ($\chi^2 < 2$, $P > 0.24$, for each species at each study site) (see Table 1). The test values for pristine forest versus agroforest within each country and within each bird species were also insignificant ($\chi^2 < 1.8$, $P > 0.18$, for each country and within each bird species). These data show absence of an interaction between the site and each host species for the prevalence of *T. everetti* infection.

Intensity of *T. everetti* parasitemia was relatively high in both avian hosts. Between 1 and 200 trypomastigotes (in average 4 ± 1.3 parasites per 100 fields at high magnification) were seen in blood films. Microscopic and PCR-based diagnostic methods showed the same sensitivity in detection of this infection (Table 2).

4. Discussion

Trypanosoma everetti was originally described from the black-rumped waxbill *Estrilda troglodytes* in Nigeria (Molyneux, 1973), and then reported in numerous bird species throughout the world (Bennett et al., 1982; Bishop and Bennett, 1992), with the majority of reports from African passeriform birds and European migrants wintering in Africa (Bennett et al., 1994a; Sehgal et al., 2001). This trypanosome belongs to the group of small (<30 μm in length in average) non-striated avian trypanosomes with the kinetoplast situated close to the posterior end of the body. This is one of the most distinctive and readily recognized of the avian trypanosomes (Molyneux, 1973; Bennett et al., 1994b; Valkiūnas et al., 2011), particularly due to its well-distinguished free flagella and irregular cell shape resembling a leaf or a kite in outline (Fig. 1A,B) rather than the usual spindle shape associated with trypanosome morphology (compare Fig. 1A,B with C-F). Due to readily distinguishable morphological characters (Fig. 1), a developed molecular characterisation (Sehgal et al., 2001) and broad geographic and host distributions, *T. everetti* is a convenient model organism for ecological and evolutionary studies of avian trypanosomes.

The key results of this study are that (1) the prevalence of *T. everetti* was high in two species of passeriform birds belonging to the Pycnonotidae and Nectariniidae, (2) the prevalence did not differ significantly between these host species in pristine forests and agroforests, indicating the same patterns of transmission at sites with different levels of deforestation and suggesting that spatial

heterogeneity related to deforestation does not affect the prevalence of avian *Trypanosoma* infections in sub-Saharan Africa, (3) prevalence of *T. everetti* was several fold greater in the olive sunbird than in the yellow-whiskered greenbul both in pristine and agroforest sites, suggesting that host-related factors, but not environmental conditions related to deforestation favour or reduce this parasite infection in forests in sub-Saharan Africa, (4) microscopic and PCR-based diagnostics were of the same sensitivity in detecting *T. everetti*. These findings can be explained using available information about vectors and peculiarities of transmission of avian trypanosomes.

The most unexpected result was that both undisturbed forest and agroforest sites display the same prevalence of *T. everetti* infection, indicating that forest density and structure do not play a significant role in the transmission or maintenance of this infection in sub-Saharan Africa. Spatial heterogeneity often affects parasite transmission due to complex interactions between biotic and abiotic factors. Climatic conditions and habitat characteristics usually play a significant role because they contribute to changes in vector and host diversity and abundance, resulting in changes in parasite transmission dynamics. Infections by avian blood parasites of the order Haemosporida markedly vary in space, including pristine and agroforest sites (Bensch and Akesson, 2003; Wood et al., 2007; Svensson and Ricklefs, 2009; Bonneaud et al., 2009; Chasar et al., 2009; Sehgal, 2015). Effects of landscape characters on the prevalence of avian *Haemoproteus* and *Plasmodium* parasites have been investigated both over narrow (Wood et al., 2007) and broad scales (Chasar et al., 2009) revealing complex relationships of different haemosporidian infections and habitat. For example, Bonneaud et al. (2009), Chasar et al. (2009) and Loiseau et al. (2010) examined olive sunbirds and yellow-whiskered greenbuls previously at the same study sites. It was demonstrated that deforestation affects host–haemosporidian parasite interactions in African birds in terms of parasite diversity and distribution, sometimes resulting in opposing trends in prevalence of *Haemoproteus* and *Plasmodium* lineages in the same bird populations. Wood et al. (2007) reported that *Plasmodium* parasite prevalence significantly fluctuated in a temperate woodland population of blue tit *Cyanistes caeruleus* by up to several-fold over one kilometer, indicating that habitat structure is important character in avian malaria transmission. However, that was not the case in this study and can be explained by the broad specificity of avian trypanosomes to both their vectors and avian hosts, a character which readily distinguishes this infection from haemosporidian parasites.

Different groups of avian haemosporidians are strictly specific to vectors. For example, species of avian *Plasmodium*, *Haemoproteus* (*Parahaemoproteus*), *Haemoproteus* (*Haemoproteus*) and *Leucocytozoon* complete sporogony and are transmitted only by species of Culicidae, Ceratopogonidae, Hippoboscidae and Simuliidae, respectively (Garnham, 1966; Bukauskaitė et al., 2015). In other words, transmission of these infections occurs only if certain vector species belonging to certain genera and families are present at a study site. This is entirely different with avian trypanosomes, which can be transmitted by blood-sucking arthropods belonging to different families (Bennett, 1961, 1970; Fallis et al., 1973; Baker, 1976; Molyneux, 1977; Miltgen and Landau, 1982; Votýpka and Svobodová, 2004). For example, experimental studies showed that the same isolate of *T. avium* could actively use species of two dipteran families (Culicidae and Simuliidae) as vectors and be transmitted to 11 species of birds representing 7 avian families and 4 avian orders (Bennett, 1970). Additionally, the same parasite developed in species of *Culicoides*, *Chrysops* and *Ornithomyia*, but the role of these insects in transmission remained unclear. Dermanyssid mites can be involved in the transmission of some avian trypanosomes as well (Baker, 1976). Habitat change often leads to a change in the density or diversity of certain groups of blood-sucking insects. For example, deforestation has impacts on the diversity of mosquito species (Beck et al., 1994; Tadei et al. 1998; Reiter and LaPointe, 2007), which could change from zoophilic to anthropophilic with ecological changes, resulting in a decrease in parasite prevalence in the original hosts (Vittor et al., 2006). Such changes in vector populations influence the transmission of haemosporidians (Bonneaud et al., 2009; Chasar et al., 2009; Loiseau et al., 2010; Sehgal et al., 2011), but could be less important for the transmission of avian trypanosomes. Specificity of avian trypanosomes to vector species seems to be greater than to avian hosts (Apanius, 1991), but remains unidentified for the great majority of described avian trypanosomes, including *T. everetti*. Additional field studies combined with experimental research are needed for better understanding transmission of this parasite.

Overall the prevalence of *T. everetti* was at least 3-fold less in the yellow-whiskered greenbul than olive sunbird both in pristine sites and agroforests (Table 1). Intrinsic factors associated with the hosts, such as behaviour or innate resistance can be responsible for differences in parasite infection prevalence in different birds (Ots and Horak, 1998; Bonneaud et al., 2009; Loiseau et al., 2010). Marked differences in prevalence between yellow-whiskered greenbul and olive sunbirds inhabiting same forests might be due to the trade-offs that occur between investment in their immune system and reproduction (Apanius et al., 1994; Tomas et al., 2007). However, experimental data about *T. everetti* or other avian trypanosomes are absent on these issues. With avian trypanosomes, the behaviour of birds in regard to association with vectors is an important component in transmission because these parasites are transmitted by contaminative rather than by inoculative routes. In previous studies, avian trypanosomes multiplied and produced infective small metacyclic infective stages in the mid-gut and hind-gut of simuliids and mosquitoes, and resulted in effective transmission by contamination of the bird's scarified epithelium by infected insect feces or via ingestion of vectors (Baker, 1976; Desser et al., 1975; Votýpka and Svobodová, 2004). This route of transmission was demonstrated experimentally through the ingestion of black flies and mosquitoes, and by contamination of damaged skin and via conjunctiva (Bennett, 1970; Desser et al., 1975; Votýpka and Svobodová, 2004; Votýpka et al., 2012).

At first glance, the greater prevalence of infection in olive sunbirds compared to yellow-whiskered greenbuls seemed unexpected because the former species is mainly nectivorous and the second insectivorous (Cheke et al., 2001; Mackworth-Praed and Grant, 1973; Smith et al., 2011). However, olive sunbirds eat insects and feed nestlings with them as well (Cheke et al., 2001). High prevalence of trypanosomes in olive sunbirds suggests that blood-sucking insects might represent a part of this bird diet and/or these birds are extensively exposed to their bites. Additional ecological studies are needed to better understand these issues. It is difficult to rule out that the olive sunbirds might be more attractive to blood-sucking insects than yellow-whiskered greenbuls, as occurs in some other bird species (Hamer et al., 2009). Interestingly, the prevalence of *Haemoproteus* and *Plasmodium* infections has been reported to be higher in olive than in yellow-whiskered greenbuls in tropical Africa (Sehgal et al., 2001; Valkiūnas et al., 2005), indicating greater exposure of the former species to vector bites, which are the main route of haemosporidian transmission (Bonneaud et al., 2009; Chasar et al., 2009; Loiseau et al., 2010). Iezhova et al. (2010) reported that prevalence of *Haemoproteus cyanomitrae* reached 21% in olive sunbird at some study sites in Ghana, indicating heavy exposure to bites of biting midges, which transmit avian haemoproteids belonging to *Haemoproteus* (*Parahaemoproteus*) (Bukauskaitė et al., 2015; Žiegytė et al., 2016). Further field and experimental studies are needed to clarify the transmission of *T. everetti* in relation to the feeding behaviour of their avian hosts and their susceptibility to vector bites.

Light parasitemia is a prominent obstacle in field studies of avian trypanosomes (Baker, 1976; Apanius, 1991; Sehgal et al., 2001, 2015). These parasites can persist and probably multiply in bone marrow, making them difficult to observe in the circulation. Usually, few trypomastigotes are present in blood films, and they are difficult to find using microscopic examination. Laboratory diagnostic methods (haematocrit centrifuge or cultivation) are more sensitive in the detection of avian trypanosomes, but often are impractical in remote areas (Bennett, 1962; Zídková et al., 2012; Sehgal et al., 2015). Additionally, culture requirements and a medium capable of culturing all isolates of avian trypanosomes are lacking. Molecular characterisation of few avian *Trypanosoma* species has been developed (Sehgal et al., 2001; Zídková et al., 2012). This study reveals that the intensity of *T. everetti* trypomastigotes in peripheral blood is relatively high in tropical African passerines, with several parasites often observed in one field of microscope at magnification of ×400 (Fig. 2). This can explain the high efficacy of both microscopic and PCR-based detection methods in this study (Table 2). The high parasitemia also is a secondary indication of active transmission of this infection in African birds. It remains unclear why *T. everetti* is so different from trypanosomes of the *T. avium* group and other small trypanosomes parasitizing passeriform birds in respect of the parasitemia intensity. Other reported *Trypanosoma* infections were of significantly lighter parasitemia during this study, with few trypomastigotes seen in blood films. That might be related to possible multiplication in avian hosts or peculiarities of development in vectors of *T. everetti*, which needs further research.

Many bird species are intercontinental migrants making the question about transmission areas of parasites difficult to approach. However, the transmission of *T. everetti* certainly occurs not only in Africa, but also in Europe because this infection has been reported in European bird species, which do not migrate to sub-Saharan Africa. For example, *T. everetti* was prevalent in great tits, *Parus major*, in Fennoscandia (Bennett et al., 1994b). This indicates the involvement both of African and European vector species in the transmission of this infection providing opportunities to address *T. everetti* and its ecology in many bird communities in the Old World.

Trypanosomes remain a neglected group of avian blood parasites (Valkiūnas et al., 2011; Sehgal et al., 2015). The level of cryptic speciation in these organisms remains insufficiently understood, and it is probable that prominent cryptic diversity exists (Baker, 1976; Zídková et al., 2012). However, molecular data also indicate both broad geographical and host ranges of the same haplotypes of avian trypanosomes (Sehgal et al., 2001; Zídková et al., 2012), supporting conclusions of microscopic examination about the presence of *T. everetti* in numerous avian host species across Africa (Molyneux and Gordon, 1975; Bennett et al., 1994a; Sehgal et al., 2001). Further studies are needed for better understanding biodiversity of these blood parasites.

In conclusion, this study confirms that *T. everetti* is prevalent in sub-Saharan African passeriform birds (Bennett et al., 1994a; Sehgal et al., 2001; Valkiūnas et al., 2005), with similar prevalence reported in the same avian hosts both in disturbed and non-disturbed forests. We show that forest type has no effect on bird–*T. everetti* interactions, probably due to the broad specificity and involvement of many species of avian hosts and numerous species, and possibly several genera and even families of dipteran vectors in its transmission. Our work suggests that avian trypanosomes cannot be recommended as indicators of environmental disturbance related to deforestation because transmission shows the same patterns both in pristine and markedly disturbed agroforests in tropical Africa. However, *T. everetti* is a good model for research aimed at better understanding the biology and transmission of avian trypanosomes. This blood parasite is cosmopolitan, easy to diagnose both by microscopic and PCR-based methods, and importantly, intensity of parasitemia of *T. everetti* is relatively high in African passeriform birds, providing opportunities to use microscopic and PCR-based detection methods in parallel. The latter opportunity is important in distinguishing possible co-infections of different species of trypanosomes, a prominent obstacle in molecular diagnostics of co-infections of blood parasites using general primers in wildlife (Bernotienė et al., 2016).

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