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VERSITA

Blood parasites (*Haemoproteus* and microfilariae) in birds from the Caribbean slope of Costa Rica

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Abstract

We studied blood parasites in wild birds within Hitoy Cerere Biological Reserve and Barbilla National Park in Costa Rica during the rainy season of 2004. We examined blood samples from 248 and 114 birds, respectively. Protozoan parasites of the genus *Haemoproteus* as well as microfilariae were found. Prevalence of *Haemoproteus* in birds was 0.8% and 4.4% in Hitoy Cerere and Barbilla, respectively, and differences were significant. Prevalence of infection by microfilariae was 8.1% and 3.5%, respectively, however, differences were not significant. Based on morphological characteristics, we divided microfilariae into two groups and nine morphotypes. In Hitoy Cerere, there were microfilariae of all nine morphotypes whereas in Barbilla we only found two morphotypes.

Keywords

Blood parasites, *Haemoproteus*, microfilariae, birds, Costa Rica

Introduction

Blood parasites (hematozoa) are a heterogeneous group of organisms characterized by the presence of at least some developmental stages in a host bloodstream (Bennett 1987). Since the first description, hematozoa have been found in 68% of all bird species examined (Krone *et al*. 2001). Blood parasites are geographically widely distributed (Valkiunas and Iezhova 2001), however, their prevalence differs in various areas (Bennett and Cameron 1975, Peirce 1989). The most commonly recorded parasites in smears from peripheral blood are unicellular eukaryotic parasites of the genera *Haemoproteus*, *Leucocytozoon*, and *Plasmodium* (Peirce 1989). Of multicellular organisms, nematode motile embryos called microfilariae (Kucera 1982), can occur in bird blood. In most cases pathogenity of bird blood parasites is low and even very high infection is not usually accompanied by clinical symptoms (Bennett *et al*. 1993).

The Neotropical Region is characterized by an extraordinary diversity of organisms. However, studies focused on blood parasites of birds in this region were carried out only sporadically compared with North America or Europe. Existing studies reveal low prevalence of protozoan blood parasites compared with other geographical regions (Bennett *et al*. 1991; Young *et al*. 1993; Valkiunas *et al*. 2003, 2004; Basto *et al*. 2006; Londono *et al*. 2007) and on the other hand high prevalence of microfilariae (Bennett *et al*. 1991, Rodriguez and Matta 2001).

The objectives of this study were (1) to determine the avian hematozoan fauna and describe the diversity of blood parasites in birds of the two lowland locations on the Caribbean slope of Costa Rica; (2) to describe the morphological characteristics and differentiate haemoproteids and avian microfilariae at these locations; (3) to compare the prevalence of haemoproteids and microfilariae in birds among locations studied as well as other Neotropical, mainly Costa Rican locations; and (4) to analyze the host specificity of haemoproteids and microfilariae.

Materials and methods

All the field work was performed in southeastern Costa Rica in the Cordillera de Talamanca mountain range in Limon province. Birds were studied at two locations on the Caribbean slope about 60 km apart that differed in elevation and habitat characteristics. In the first location, the study was carried out in a lowland rainforest within Hitoy Cerere Biological Reserve (9°40´N, 85°05´W) at an elevation of about 100 m. The second study area was a narrow strip of a water-logged pasture lo-

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cated near the Barbilla National Park (9°59′N, 85°27′W) at an elevation of about 570 m. For detailed descriptions of these locations see Dusbabek *et al*. (2007).

Birds were studied in the rainy season between August 17 and September 11, 2004. Dawn-to-dusk mist-netting was conducted to capture as many bird species and individuals as possible to collect blood samples. A line of about 100 m of mist nets were checked at least once an hour. Captured birds were identified, examined and released back into the wild as quickly as possible to minimize stress.

Blood samples used for smears were collected from *vena ulnaris cutanea*. The blood smears were left to dry, then fixed by dipping in methanol, and dried again. In the laboratory, the smears were stained with a combination of stains (May-Grünwald-Giemsa-Romanowski) using a method according to Pappenheim (Lucas and Jamroz 1961). The smears stained were examined microscopically for the presence of blood parasites. First, the whole smear was examined under ×40 magnification for presence of microfilariae. Subsequently, at least 100,000 erythrocytes were examined in each of the smears (Garvin *et al*. 1993) under magnification of ×100 for extracellular protozoa and ×400 for intracellular protozoa. The quantity of erythrocytes was estimated by calculating their numbers in three squares of the eyepiece grid across the smear over an area where the erythrocytes formed a uniform layer without overlapping each other, and by calculating for the total number of squares (Reauz *et al*. 1999). If parasites were found, their number was calculated in a similar manner per 10,000 erythrocytes. The intensity of infection by microfilariae was applied for the whole smear. The intensity of infection was given in five levels (Table I).

The genus of blood protozoa was determined by their morphological traits, according to Valkiunas (1997). Parasite morphology was studied under ×1000 magnification. Measurement was carried out using QuickPHOTOMICRO 2.2 software (www.quickphoto.cz). Statistical assessment of prevalence differences was made using χ^2 test (level of significance $P = 0.05$).

Results

Prevalence and infection intensity of blood parasites

In Hitoy Cerere BR, we examined 248 birds of 48 species belonging to 18 families. Representatives of the genus *Haemoproteus* were recorded in two (0.8%) birds and microfilariae in

Table I. Methods of assessment of infection intensity by blood parasites

Infection intensity	No. of the genus *Haemoproteus*/100 000 erythrocytes	No. of microfilariae in smear in one standard slide
Very low (VL)	<11	1–2
Low (L)	11–100	3–5
Medium (M)	101–1000	6–10
High (H)	1001–10 000	11–20
Very high (VH)	>10 000	>20

Table II. Overview of birds infected with blood parasites in Hitoy Cerere BR and Barbilla NP

		Hitoy Cerere BR			Barbilla NP		
Family	**Species**	**n**	**H**	**MF**	**n**	**H**	**MF**
Columbidae	*Geotrygon montana*	1	–	1	n.t.		
Alcedinidae	*Chloroceryle aenea*[*]	2	–	1	n.t.		
	Chloroceryle americana	4	–	3	n.t.		
Dendrocolaptidae	*Dendrocincla fuliginosa*	1	–	–	1	–	1
Formicariidae	*Formicarius analis*	4	–	1	n.t.		
Pipridae	*Manacus candei*[*]	31	1	–	11	–	–
Tyrannidae	*Attila spadiceus*	1	–	1	2	–	–
	Pitangus sulphuratus	1	–	1	n.t.		
Vireonidae	*Vireo olivaceus*	n.t.			2	1	–
Thraupidae	*Euphonia gouldi*	n.t.			7	4	–
	Habia fuscicauda	10	–	4	n.t.		
	Ramphocelus passerinii	3	–	2	6	–	–
	Tachyphonus delattrii[*]	7	–	1	15	–	3
Emberizidae	*Arremon aurantiirostris*	7	–	2	n.t.		
	Sporophila americana (= *aurita*)	23	1	1	6	–	–
Cardinalidae	*Cyanocompsa cyanoides*	9	–	2	2	–	–
Total		248[**]	2	20	114[**]	5	4

[**]All birds examined at the location; [*]first record of blood parasite in marked species; H – number of specimens positive for *Haemoproteus* spp.; MF – number of specimens positive for microfilariae; n – number of specimens examined; n.t. – not tested.

20 (8.1%) birds (Table II). In Barbilla NP we examined 114 birds of 37 species belonging to 17 families. In five (4.4%) birds, parasites of the genus *Haemoproteus* and in four (3.5%) birds, microfilariae were recorded. When prevalence of infection by the genus *Haemoproteus* was compared, there was a significant difference between Hitoy Cerere BR and Barbilla NP. No significant difference, however, was found when comparing prevalence of microfilariae.

Blood parasites were not recorded in Tinamidae: *Crypturellus soui* (one individual examined); Cathartidae: *Cathartes aura* (1); Accipitridae: *Leucopternis semiplumbea* (1); Columbidae: *Leptotila cassinii* (6); Trogonidae: *Trogon rufus* (2); Bucconidae: *Malacoptila panamensis* (1); Picidae: *Melanerpes pucherani* (1); Furnariidae: *Automolus ochrolaemus* (3), *Sclerurus guatemalensis* (3), *Xenops minutus* (7); Dendrocolaptidae: *Deconychura longicauda* (1), *Glyphorhynchus spiru-*

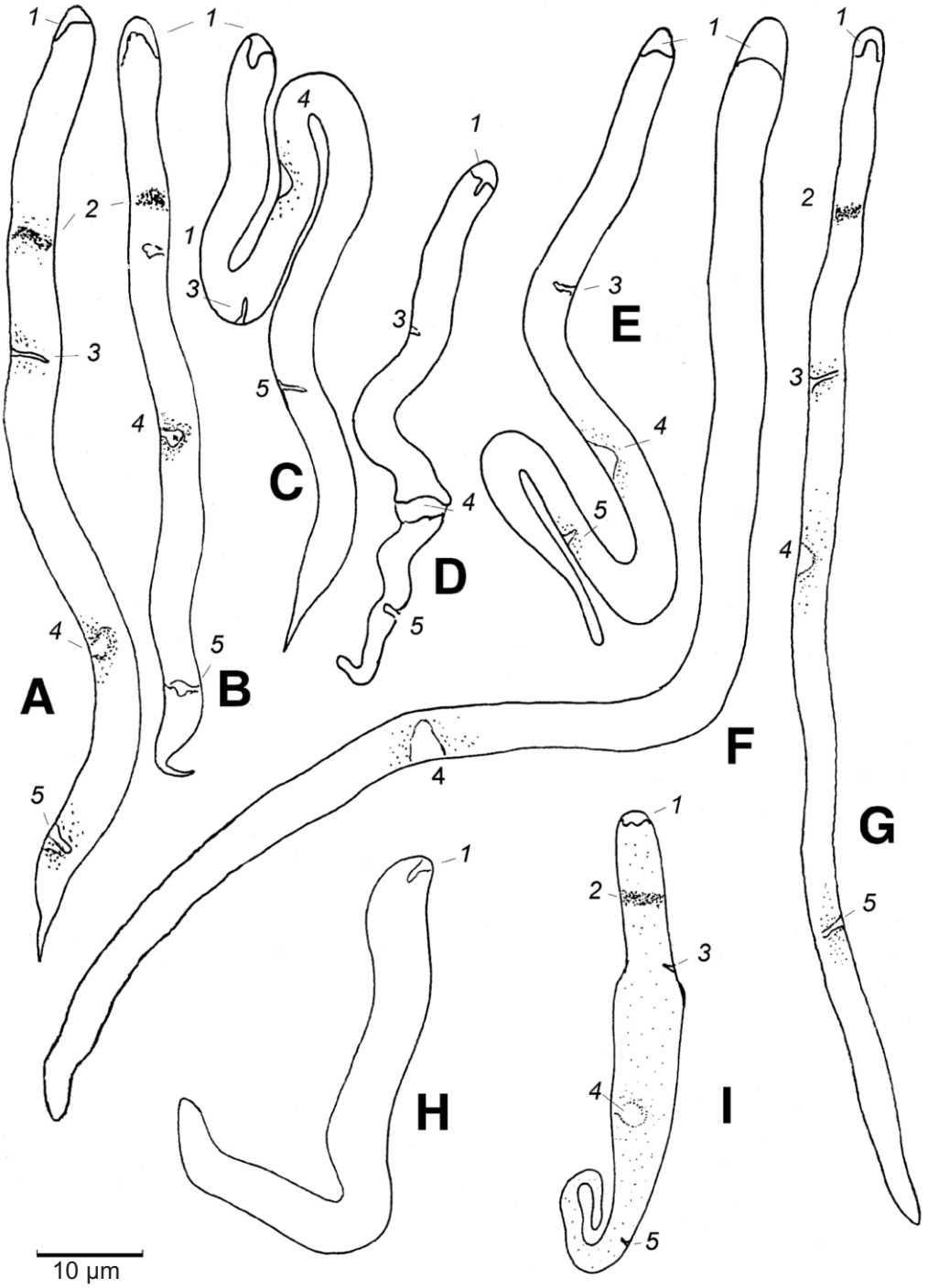

Fig. 1. Morphotypes A, B, C, D, E, F, G, H, and I of microfilariae in Costa Rican birds. 1 – head space, 2 – nerve ring, 3 – excretion pore, 4 – inner body, 5 – anal pore

Table III. Measurement characteristics of microfilariae: morphotypes A, B, C and D

Morpho-type	A		B		C		D	
N	4		7		5		5	
Host	*Habia fuscicauda*		*Sporophila aurita*		*Dendrocincla fuliginosa*		*Formicarius analis*	
MW	4.7	(3.0–6.0)	5.3	(5.0–6.0)	5.0	(5.0–6.0)	4.7	(4.5–4.8)
HW	2.6	(2.0–3.0)	2.3	(2.0–3.0)	3.8	(3.0–4.0)	2.7	(2.4–2.8)
TW	1.8	(1.0–2.0)	3.4	(3.0–4.0)	2.2	(2.0–3.0)	2.2	(1.3–2.4)
HS	2.5	(2.0–4.0)	2.9	(2.0–3.0)	3.2	(3.0–4.0)	2.1	(1.9–2.4)
NR	13.9	(5.3–24.8)	20.0	(18.0–23.0)	–	–	9.9	(5.6–14.4)
EP	26.7	(17.2–33.0)	31.3	(28.0–34.0)	29.8	(26.0–33.0)	15.0	(13.1–25.3)
IK	56.0	(45.9–63.9)	46.5	(40.0–50.0)	41.6	(38.0–47.0)	39.4	(35.8–43.0)
AP	74.4	(65.9–78.8)	69.2	(62.0–72.0)	78.0	(65.0–84.0)	57.2	(54.2–64.2)
TS	14.6	(11.1–17.4)	13.3	(10.0–15.0)	40.2	(33.0–44.0)	10.8	(8.7–13.7)
BL	87.8	(78.5–95.8)	82.6	(72.0–87.0)	118.2	(107.0–130.0)	69.9	(63.3–74.3)

All measurements are given in µm, min.-max. values are in parentheses; AP – anal pore; BL – total body length; EP – excretion pore; HS – head space; HW – head width; IK – inner body; MW – maximum width; NR – nerve ring; TS – tail length; TW – tail width.

rus (19), *Lepidocolaptes souleyetii* (1), *Xiphorhynchus guttatus* (6); Thamnophilidae: *Cercomacra tyraninna* (1), *Cymbilaimus lineatus* (1), *Gymnocichla nudiceps* (1), *Gymnopithys leucaspis* (4), *Myrmeciza exsul* (9), *Myrmotherula fulviventris* (4), *Phaenostictus mcleannani* (2), *Thamnistes anabatinus* (2); Pipridae: *Pipra mentalis* (8); Tyrannidae: *Empidonax flaviventris* (1), *E. traillii* (1), *Mionectes oleagineus* (31), *Myiobius sulphureipygius* (1), *Oncostoma cinereigulare* (2), *Terenotriccus erythrurus* (2), *Tyrannus melancholicus* (1); Troglodytidae: *Cyphorhinus phaeocephalus* (1), *Henicorhina leucosticta* (2), *Thryothorus atrogularis* (1), *Th. nigricapillus* (7), *Th. thoracicus* (1); Turdidae: *Turdus grayi* (1); Polioptilidae: *Microbates cinereiventris* (2), *Ramphocaenus melanurus* (3); Parulidae: *Basileuterus* (= *Phaeothlypis*) *fulvicauda* (2), *Seiurus motacilla* (4); Thraupidae: *Mitrospingus cassinii* (4), *Tachyphonus luctuosus* (2), *T. rufus* (1), *Tangara larvata* (2); Emberizidae: *Oryzoborus funereus* (36); Cardinalidae: *Caryothraustes poliogaster* (4), *Saltator grossus* (1), *S. maximus* (4); Icteridae: *Amblycercus holosericus* (3), and *Icterus dominicensis* (1).

In Hitoy Cerere BR, the infection intensity by the genus *Haemoproteus* was VL (1 case) and L (1). In Barbilla NP the infection intensity by the genus was VL (1), L (2), M (1) and VH (1). The highest infection intensity by this genus was recorded in *Euphonia gouldi* in Barbilla NP (20% of erythrocytes infected). In other cases the intensity was lower than 1%. The infection intensity by microfilariae in Hitoy Cerere BR was VL (4 cases), L (5), M (4), H (3) and VH (1), and in Barbilla NP it was VL (1), L (1) and M (2), respectively. The highest infection intensity by microfilariae was recorded in Hitoy Cerere BR in *Formicarius analis* (50 microfilariae in a smear).

Species diversity of microfilariae

We identified nine morphologically distinct forms of parasitic unsheated microfilariae from 21 infected birds. Since only a few adult filariae have been described from these birds so far, these microfilariae are referred to with letters. Morphometric data are in Tables III–V; illustrations in Figure 1. We divided

Table IV. Measurements of microfilariae: morphotypes E, F and G

Morpho-type	E		F		G	
N	6		13		6	
Host	*Arremon aurantiirostris*		*Geotrygon montana*		*Chloroceryle americana*	
MW	4.0	(3.0–5.0)	5.8	(5.0–6.0)	3.2	(2.0–4.0)
HW	2.8	(2.0–3.0)	4.7	(4.0–6.0)	3.0	(2.0–4.0)
TW	2.2	(2.0–3.0)	4.0	(3.0–5.0)	2.2	(2.0–3.0)
HS	3.7	(3.0–4.0)	–		2.7	(2.0–4.0)
NR	–		–		20.0	(16.0–26.0)
EP	31.0	(27.0–35.0)	–		37.5	(33.0–44.0)
IK	43.0	(38.0–48.0)	107.8	(92.0–118.0)	60.3	(50.0–74.0)
AP	71.7	(65.0–75.0)	–		93.0	(79.0–98.0)
TS	37.7	(36.0–40.0)	–		30.2	(27.0–34.0)
BL	99.8	(81.0–115.0)	160.9	(146.0–185.0)	123.2	(107.0–132.0)

See Table III for explanations.

Table V. Measurements of microfilariae: morphotypes H and I

Morpho-type	H	I	
N	1	4	
Host	*Pitangus sulphuratus*	*Chloroceryle americana*	
MW	4.7	5.5	(5.0–6.0)
HW	3.7	3.0	(3.0)
TW	2.5	2.0	(2.0)
HS	–	1.0	(1.0)
NR	–	8.3	(7.0–11.0)
EP	–	16.0	(15.0–17.0)
IK	–	34.0	(32.0–36.0)
AP	–	44.3	(44.0–45.0)
TS	–	20.8	(18.0–24.0)
BL	47.8	65.0	(62.0–69.0)

See Table III for explanations.

the microfilariae into two groups (I with three subgroups 1–3; II) and nine types:

Group I. Body width of microfilariae is the same without narrowing followed by widening in the front (= cephalic) part, the outer line is continuous, without distinctive changes. Morphotypes A-H:

Subgroup 1 (morphotypes A, B, C). The posterior end of the body gradually tapers; the tail is conical with a sharp end. These forms were recorded in Passeriformes (Emberizidae, Thraupidae, and Dendrocolaptidae).

Subgroup 2 (morphotypes D, E, F). The posterior end of the body gradually tapers; the tail is conical with a distinctly rounded end. These forms were recorded in Passeriformes (Formicariidae – morphotype D, Emberizidae – morphotype E) and Columbiformes (Columbidae – morphotype F).

Subgroup 3 (morphotypes G, H). The posterior end of the body slightly tapers or it is completely without tapering, the end of the tail is widely rounded. These forms were recorded in Coraciiformes (Alcedinidae – morphotype G) and Passeriformes (Emberizidae – morphotype H).

Group II. The body of microfilariae is narrower in its front part, and then it distinctly tapers. The tail tapers and has a sharp end. This form – morphotype I was only recorded in the genus *Chloroceryle* (Coraciiformes, Alcedinidae).

Morphometrical descriptions and differentiations of microfilariae

Morphotype A. Hosts: *Tachyphonus delatrii*, *Habia fuscicauda* (Thraupidae). Body length >100 μm; tail length 15–21 μm (mean 19.2 μm). Fixed points in % of body length-head space 1.5–3.5% (mean 2.3%), nerve ring 12.0–18.3% (14.7), excretory pore 24.8–27.5% (26.0), inner body 63.1–71.2% (66.9), anal pore 83.2–85.6% (84.1), tail length 14.4–16.8% (15.9).

Morphotype B. Host: *Sporophila aurita* (Emberizidae). Body length <100 μm; tail length 10–15 μm (mean 13.7 μm). Fixed points in % of body length-head space 2.3–4.2% (mean 3.2%), nerve ring 21.2–26.4% (24.2), excretory pore 35.3–39.1% (38.1), and inner body 48.2–61.7% (56.5), anal pore 81.9–86.1% (83.6), tail length 13.9–18.1% (16.4).

Morphotype C. Host: *Dendrocincla fuliginosa* (Dendrocolaptidae). Body length >100 μm; tail length 33.0–44.0 μm (mean 40.2 μm). Fixed points in % of body length-head space 2.3–3.2% (mean 2.7%), nerve ring – not measured, excretory pore 24.3–25.7% (25.2), inner body 33.9–36.2% (35.2), anal pore 60.7–71.8% (65.9), tail length 28.2–39.3% (34.1).

The distinct difference between the types A, B, C are expressed in combination of tail and body length and in related fixed point values (in %) of excretory pore, inner body, and anal pore.

Morphotype D. Host: *Formicarius analis* (Formicariidae). Body length <100 μm; tail length 13.7–21.4 μm (mean 17.4 μm). Fixed points in % of body length-head space 2.3–3.2% (mean 2.7%), nerve ring – not measured, excretory pore 24.3–25.7% (25.2), inner body 33.9–36.2% (35.2), anal pore 60.7–71.8% (65.9), tail length 28.2–39.3% (34.1).

Morphotype E. Host: *Arremon aurantiirostris* (Emberizidae). Body length >80 and <120 μm; tail length 36.0–40.0 μm (mean 37.7 μm). Fixed points in % of body length-head space 2.7–4.9% (mean 3.7%), nerve ring – not measured, excretory pore 26.7–30.4% (28.5), inner body 37.6–41.7% (39.6), anal pore 64.4–68.7% (67.0), tail length 33.0–35.6% (34.3).

Morphotype F. Host: *Geotrygon montana* (Columbidae). Body length >100 μm; tail length 36.0–40.0 μm (mean 33.7 μm). From the fixed points, only the position of the inner body was identified, with value 62.0–65.1% (mean 63.7%).

Microfilariae in morphometrical subgroup I/2 (types D-F) differ clearly in body length, head and tail width, inner body position and related fixed points in combination.

Morphotype G. Hosts: *Chloroceryle americana*, *Chloroceryle aenea* (Alcedinidae). Body length >100 μm; tail length 25.0–34.0 μm (mean 30.2 μm). Fixed points in % of body length-head space 1.5–3.1% (mean 2.2%), nerve ring 12.1–

Table VI. Occurrence of microfilariae morphotypes in individual bird species

Family	Species	Morphotype	Location
Columbidae	*Geotrygon montana*	F	HC
Alcedinidae	*Chloroceryle aenea*	G, I	HC
	Chloroceryle americana	G, I	HC
Dendrocolaptidae	*Dendrocincla fuliginosa*	C	HC, B
Formicariidae	*Formicarius analis*	D	HC
Tyrannidae	*Attila spadiceus*	A	HC
	Pitangus sulphuratus	H	HC
Thraupidae	*Habia fuscicauda*	A	HC
	Ramphocelus passerinii	E	HC
	Tachyphonus delattrii	A	HC, B
Emberizidae	*Arremon aurantiirostris*	E	HC
	Sporophila americana (= *aurita*)	B	HC
Cardinalidae	*Cyanocompsa cyanoides*	C	HC

Location: HC – Hitoy Cerere BR, B – Barbilla NP.

20.0% (16.4), excretory pore 27.7–36.4% (30.7), inner body 43.1–57.8% (49.4), anal pore 73.8–79.3% (76.1), tail length 20.7–26.2% (24.7).

Morphotype H. Host: *Pitangus sulphuratus* (Tyrannidae). Body length 41–56 µm (mean 47.8 µm); no other values were obtained.

In the morphological subgroup I/3 (morphotypes G and H), there are the most significant differences among all the microfilarial types in the body length. Type H is the smallest microfilaria species in the studied collection.

Morphotype I. Host: *Chloroceryle americana* (Alcedinidae). Body length <100 µm; tail length 18.0–24.0 µm (mean 20.8 µm). Fixed points in % of body length-head space 1.4–1.6% (mean 1.5), nerve ring 11.1–15.9% (12.6), excretory pore 22.7–27.0% (24.6), inner body 50.0–57.1% (52.4), anal pore 65.2–71.0% (68.2), tail length 29.0–34.8% (31.8).

The microfilariae occurrence of the above mentioned morphotypes of individual bird species is given in Table VI.

Host specificity of microfilariae

Diversity characterized by the presence of microfilariae morphotypes was higher in Hitoy Cerere BR (9 taxa) than in Barbilla NP (2 taxa). Two morphotypes: C in the host *Dendrocincla fuliginosa* and A in *Tachyphonus delatrii*, occurred in both locations. Close specific relationship of microfilariae to families and genera of host birds is represented by the following morphotypes (Table VI): F (*Geotrygon*, Columbidae), G and I (*Chloroceryle*, Alcedinidae), B (*Sporophila*, Emberizidae), D (*Formicarius*, Formicariidae) and H (*Pitangus*, Tyrannidae). We can consider this group of microfilariae to be representative of six specialist nematode taxa. A wider host spectrum is represented by morphotypes: C (*Dendrocinla*, Dendrocolaptidae; *Cyanocompsa*, Cardinalidae), E (*Ramphocelus*, Thraupidae; *Arremon*, Emberizidae), and A (*Attila*, Tyrannidae; *Tachyphonus*, Thraupidae). Morphotypes A, C, and E of microfilariae are probably represented by generalist nematode taxa.

Discussion

Considering the great diversity of both environments and organisms occurring in the Neotropical Region, it is difficult to compare results obtained in different seasons and from different localities. In spite of that, when assessing the research results from this region, we can find common features. One of them is the low prevalence of protozoan blood parasites ranging from 1.4% to 19.9% (Bennett and Borrero 1976; White *et al*. 1978; Bennett *et al*. 1980; Sousa and Herman 1982; Bennett *et al*. 1991; Young *et al*. 1993; Rodriguez and Matta 2001; Valkiunas *et al*. 2003, 2004; Matta *et al*. 2004; Basto *et al*. 2006; Londono *et al*. 2007). The results of our study (prevalence 0.8 and 4.4% in Hitoy Cerere BR and Barbilla NP, respectively) correspond with these values. Another common feature of most studies of avian hematozoa in the Neotropical Region is minimal prevalence or even absence of representatives of the genera *Leucocytozoon*, *Plasmodium* and *Trypanosoma*, which we did not record either.

The low prevalences of the representatives of the genus *Haemoproteus* found indicate either low density or the absence of possible vectors in the study areas. When the results from both locations were compared, there was a significant difference in prevalence of the genus *Haemoproteus*. This may due to separate location characteristics in terms of differing densities of possible vectors. In this study, we did not determine species of the genus *Haemoproteus*. Despite the fact that we had samples with medium and very high infection intensity, it was not possible to find unequivocally determining morphological parameters to classify the parasite into a species according to Valkiunas (1997).

Infection prevalence by microfilariae in birds of the Neotropical Region ranges from 0.6 to 10.5% (Bennett and Borrero 1976; White *et al*. 1978; Bennett *et al*. 1980; Sousa and Herman 1982; Bennett *et al*. 1991; Young *et al*. 1993; Rodriguez and Matta 2001; Valkiunas *et al*. 2003, 2004; Matta *et al*. 2004; Basto *et al*. 2006). In this case the results of our study (prevalence 6.9 to 3.4% in Hitoy Cerere BR and Barbilla NP,

respectively) correspond with these values. In the species *Chloroceryle americana* (Alcedinidae) and *Tachyphonus delatrii* (Thraupidae), it is the first record of microfilariae. According to Bartlett and Greiner (1986), adult stages identified in *Chloroceryle americana* are *Pelecitus helicinus*.

The tendency of increasing infection prevalence by microfilariae, and its ratio to prevalence of parasites of the genus *Haemoproteus* spp. in later studies are noteworthy. After 2000, all the above mentioned studies show prevalence of microfilariae equal or higher compared with *Haemoproteus* spp.; whereas in former studies it was reversed. Beside the differences in bird species diversity, environmental conditions and the time of collecting samples, the differences in prevalence can also be related to methods of taking blood and examining of samples. Prevalence and infection intensity can be much higher if the samples are taken from the pulmonary artery (Holmstad *et al*. 2003). Circadian rhythm can also influence occurrence of microfilariae in peripheral blood, and subsequently have an influence on the results of individual studies as well (Sehgal *et al*. 2005).

Morphometric species characteristics of Neotropical microfilariae in birds do not enable species determination. In spite of the fact that records of adult filariae necessary for species determination and classification are missing, we can at least classify some microfilariae based on their morphology and the values of fixed points. In the Neotropical Region, microfilariae are not only more plentiful, but also their morphology is much more varied compared with records from the Palearctic Region (Supperer 1958, Lopez-Caballero 1978, Kucera 1982, Cano-Martil *et al*. 1989, Lopez-Caballero *et al*. 1992, Hauptmanova *et al*. 2004).

Most filarial parasites fall within the families Aproctidae and Splendidofilariidae (Sonin 1966, 1968; Anderson 2000). Within the family Aproctidae, nematode species in Nearctic and Neotropical Regions belong to genera *Aproctella* Cram, 1931, *Aproctiana* Skrjabin, 1934, *Cardiofilaria* Strom, 1937, *Paronchocerca* Peters, 1936 and *Pelecitus* Railliet et Henry, 1910. In the Splendidofilariidae, genera found in Nearctic or Neotropical Regions are *Splendidofilaria* Skrjabin, 1923, *Eufilariella* Sonin, 1956, *Ornithofilaria* Gonnert, 1937, *Parornithofilaria* Sonin, 1965, and *Vagrifilaria* Augustine, 1937.

Based on morphological and morphometrical features, types A, B and C (group I, subgroup 1) probably belong to genera *Aproctella* or *Eufilariella*. Some species of the genus *Aproctella* (e.g. *A. stoddardi* Cram, 1931) are generalists parasitizing 14 families of six orders of birds (Galliformes, Charadriiformes, Psittaciformes, Strigiformes, Piciformes and Passeriformes). Species of the genus *Eufilariella* are more specialized.

According to morphological features, morphotypes D, E, F (group I, subgroup 2) showed a congeneric relationship with the genera *Aproctiana*, *Aproctella*, *Cardiofilaria*, *Paronchocerca* and *Pelecitus*. Forms D and E probably belong to the genera *Aproctella*, *Paronchocerca*, *Pelecitus* or *Vagrifilaria*. The genus *Cardiofilaria* can be excluded due to its significantly bigger length of microfilariae (more than 300 μm). Form F definitely belonged to genus *Pelecitus*, which includes the generalistic species *P. helicinus* (Molin, 1860), and was found in 18 host families in seven orders (Galliformes, Psittaciformes, Coraciiformes, Cuculiformes, Trogoniformes, Piciformes and Columbiformes). According to Bartlett and Greiner (1986), within the Columbiformes of the Neotropical Region, *P. helicinus* was found in *Columbina picui* (in Brazil) and in *P. barusi* Coy Otero, 1982 was found in *Zenaida macroura* (Cuba). Microfilariae found in *Geotrygon montana* are morphometrically different from *Eulimdana clava* (Wedl, 1856), a cosmopolitan parasite of pigeons (Bartlett *et al*. 1985). Microfilariae *P. helicinus* from the anterior vagina are 185–205 μm long; with bluntly rounded extremities, a maximum width of 4/5 of the anterior body 5–6 μm, tapering to 2–3 μm at the caudal extremity (Bartlett *et al*. 1985). We do not consider the morphotype G to be conspecific with *P. helicinus*.

Morphotypes G and H (group I, subgroup 3) belong morphometrically to the genera *Eufilaria*, *Ornithofilaria*, *Parornithofilaria* or *Splendidofilaria*. Morphotype G is similar to *Parornithofilaria brasiliense* (Yeh, 1957) found in *Rhamphastos dicolorus* (Piciformes, Bucerotidae), *P. flexivaginalis* (Jones, 1961) in *Corvus brachyrhynchus* and *P. striatospicula* (Hibler, 1964) in *Pica pica* (both Passeriformes, Corvidae). Microfilariae of all these species are longer than 100 μm (138–181). Morphotype H – the smallest microfilaria from our collection is morphometrically very similar to *Ornithofilaria* (= *Splendidofilaria*) species. Holarctic microfilariae of the genus *O. algonquinensis* Anderson, 1955 found in *Hirundo rustica* and *Riparia riparia* (Passeriformes, Hirundinidae) have the smallest body length (61–77 μm; Anderson 1955, Sonin 1966). However, due to the different geographical distribution and hosts species we cannot consider these microfilariae to be conspecific.

Microfilariae of the group II (type I) are characterized by their body shape, and based on recent data available we cannot place them in any systematic group. We hypothesize that, based on the high prevalence of host records in the family Alcedinidae, it may be possible to get adult specimens in the future and hence describe a new taxon.

Our results of morphometric studies of microfilariae, using dedicated software, indicate that using measurable features and "fixed points" enables the characterization of species richness of viviparous filariids in avian hosts, not only for basic ecological studies, but also for goal-directed systematic parasitological research. It is possible that host switching might be common in heteroxenous nematodes, since insect vectors may feed on several bird species, and thus theoretically spread parasites readily among different avian hosts. We found that viviparous filariids are frequently prevalent in one or two avian species. Our results correspond with the data by Sehgal *et al*. (2005) on host specificity of parasitic nematode microfilariae in African rainforest birds.

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