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Research article

Avian haemosporidians at three environmentally contrasting urban greenspaces

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Abstract

Conversion of natural habitats to urban developments influences the distribution and abundance of wildlife hosts, vectors, and their parasites. Within urban ecosystems, urban greenspaces are usually relics of the original local vegetation, but commonly with the presence of non-native vegetation. These areas are understudied from the parasitological perspective. We estimated avian haemosporidian (*Haemoproteus, Plasmodium, and Leucocytozoon*) prevalence, genetic lineage richness, and both bird and parasite assemblage structure at three environmentally contrasting urban greenspaces (nearctic open scrub forest, neotropical montane cloud forest, palearctic mixed-deciduous ash, oak, and pine forest). There were no bird species in common among the three urban sites, and only one family was shared (Fringillidae). We registered at nearctic and neotropical urban greenspaces 10 haemosporidian lineages; only one lineage (MALERY01) was shared between them. Parasites of the genus *Haemoproteus* were more common in the open scrub urban greenspace than in the montane cloud urban greenspace, whereas those of the genus *Plasmodium* had the opposite tendency. We registered 20 lineages in the palearctic urban greenspace from the genera *Plasmodium, Haemoproteus, and Leucocytozoon*. Prevalence rates were similar among the three urban greenspaces and the assemblage structure of haemosporidian lineages was not significantly different among sites. Furthermore, the three urban greenspaces were composed by phylogenetically diverse parasite communities and were dominated by widespread generalist parasite lineages. Hence, our results suggest that despite contrasting environmental conditions and geographical isolation, there are parallels in the structure of avian haemosporidian communities among the three urban greenspaces.

Key words: urban bird parasitology, urban ecology, haemosporidian parasites, *Plasmodium*, *Haemoproteus*, *Leucocytozoon*

Introduction

Spatial heterogeneity affects community structure and ecological processes. Understanding the influence of different landscape features on host–parasite interactions of wild populations is of particular relevance in a world undergoing rapid environmental changes (Loiseau et al. 2010; Belo et al. 2011). Conversion of natural habitats to urban developments and agro-ecosystems influences the distribution and abundance of wildlife species; thus, it may be a major driver changing parasites ecological dynamics (Bradley and Altizer 2007; Hernández-Lara, González-García, and Santiago-Alarcon 2017). The transmission of infectious diseases is a result of complex interactions between abiotic factors and biotic components, which directly affect

infection prevalence on hosts (Loiseau et al. 2010; Knowles et al. 2014). For example, temperature, altitude and proximity to water bodies are small-scale ecological factors known to influence vectors [e.g. mosquitoes (*Culex*, *Aedes* and *Culiseta*), and black flies (Simuliidae), Isaksson et al. 2013] and/or parasite distribution and abundance (Sehgal et al. 2010; Lachish et al. 2013). Moreover, the same group of parasites infecting different host species may respond differently to the same environmental changes (e.g. forestry practices, Renner et al. 2016), such changes may also alter vector assemblage structure (e.g. Abella-Medrano et al. 2015), which modifies host–parasite–vector dynamics by, for example, changing vector feeding preferences (Santiago-Alarcon et al. 2012). Hence, understanding how parasites respond to changes in their environment is of relevance to develop effective surveillance programs, response procedures in the face of emergent diseases, and urban developments.

In this study, we work with avian haemosporidian parasites, which is a group of vector-born parasites in the order Haemosporida (Phylum Aplicomplexa) and is divided into four genera: *Plasmodium*, *Haemoproteus*, *Fallisia* and *Leucocytozoon* (Valkiūnas 2005; Santiago-Alarcon, Palinauskas, and Schaefer 2012). They have a widespread geographical distribution and are genetically diverse (Bensch, Hellgren, and Pérez-Tris 2009; Clark, Clegg, and Lima 2014). As it is the case for other parasite groups, avian haemosporidians and their vectors are differentially affected by environmental changes, which can lead to the development of novel host–parasite interactions (Santiago-Alarcon et al. 2013; Sehgal 2015). Studies that have compared the prevalence of haemosporidian parasites infecting bird populations at undisturbed versus modified habitats have shown opposite trends depending on the host–parasite system under study (Martin and Boruta 2014; Sehgal 2015).

In Cameroon, Chasar et al. (2009) reported higher prevalence of avian parasites of the genera *Haemoproteus* and *Leucocytozoon* in undisturbed sites, and higher prevalence of *Plasmodium* species in disturbed sites. In contrast, Bonneaud et al. (2009) found higher prevalence of *Plasmodium* species in undisturbed forests compared with agroecosystems in Cameroon. Evans et al. (2009) reported a higher prevalence of *Haemoproteus* and *Plasmodium* species in the majority of rural areas compared with their urban counterparts across different cities in Europe. In contrast, Belo et al. (2011; Brazil) and Hernández-Lara, González-García, and Santiago-Alarcon (2017; Mexico) found higher prevalence of *Plasmodium* and *Haemoproteus* species in an urban site in comparison with preserved, shade-coffee and transition areas. Hence, there is no clear relationship between land use type and prevalence of Haemosporida parasites in birds. This is probably due to the fact that different Diptera families preferentially transmit haemosporidian parasites of different genera (Santiago-Alarcon, Palinauskas, and Schaefer 2012), and vector life cycles are differentially influenced by microclimatic conditions (Isaksson et al. 2013); suggesting that local instead of regional factors mostly govern transmission dynamics of these host–parasite systems (e.g. Santiago-Alarcon et al. 2016). However, no previous study has compared avian parasite communities of urban greenspaces that have contrasting environmental conditions and that are located in distinct biogeographical zones.

We studied summer communities of avian parasites from the order Haemosporida (*Haemoproteus*, *Plasmodium* and *Leucocytozoon*) at three environmentally contrasting urban greenspaces, one nearctic (open scrub forest) and one neotropical (montane cloud forest) in Mexico, and one palearctic (mixed-deciduous ash, oak and pine forest) in Germany. Our aims were: (i) to estimate avian haemosporidian prevalence and genetic lineage richness within and among sites, (ii) to examine the degree of sharing of haemosporidians among sites and (iii) to compare both bird and parasite assemblage structure among sites. Prevalence is a population parameter that in the case of vector-borne parasites depends mostly on external conditions (e.g. temperature, rainfall and humidity), because they directly affect the development, abundance and movement of competent vectors (see Deviche, McGraw, and Greiner 2005). Furthermore, the absence of suitable habitat for vectors in harsh environments like arid and semi-arid areas has been suggested as a reason for low infection rates (Valera et al. 2003). These characteristics may influence parasite infections among biogeographical regions and different habitat types; hence, we expected to find different infection rates at our three sites because of their contrasting environmental conditions: lower prevalence in the open scrub urban greenspace due to the dependence of dipteran vectors for high humidity, in comparison to both the montane cloud and mixed-deciduous urban greenspaces where humidity is not a limiting factor (Santiago-Alarcon et al. 2013; Abella-Medrano et al. 2015). Changes in landscape features (e.g. rainfall and temperature) due to geographic location, as well as changes

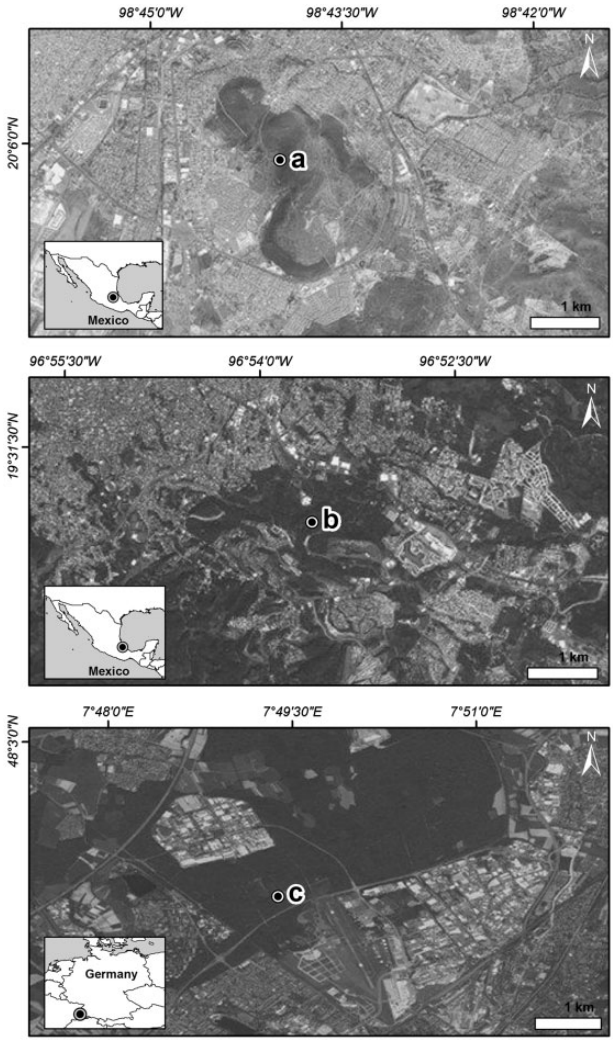

Figure 1. Map showing the location of three studied urban greenspaces: (a) nearctic open scrub (Parque Ecológico Cubitos), (b) neotropical montane cloud (Parque Natura) in Mexico and (c) palearctic mixed deciduous forest (Mooswald Forest) in Germany. Images from Google Earth 7.1, 2016.

in host species composition can affect the structure and composition of parasite assemblages (LaPointe, Atkinson, and Samuel 2012; Pérez-Rodríguez et al. 2013; see Sehgal 2015). Hence, we expected to find different parasite composition (i.e. low parasite sharing) due to the different avian species of the three sites, as a result of geographical isolation (different biogeographical regions, see Methods). Finally, because our three sites are embedded in an urban matrix, they are likely to share the same environmental pressures (e.g. people, cats, buildings) that affect the abundance and distribution of avian hosts (Ortega-Álvarez and MacGregor 2009); consequently, this also affects the abundance of the parasite lineages infecting birds at those same places. Thus, similar anthropogenic pressures should result in a similar abundance structure of both bird and parasite communities regardless of species identity.

Study area, methods and statistical analyses

Study sites

Field work was conducted at three urban greenspaces, which are characterized by being relics of native vegetation:

a. Ecological reserve Parque Ecológico Cubitos (nearctic urban greenspace, 90 ha), located within the city of Pachuca in the state of Hidalgo, Mexico (20°06'N and 98°45'W, at 2265–2420 m.a.s.l.; Fig. 1a). During the dry season (October–April) mean temperature is 12 °C (4.2–24.5 °C) and the driest month is December. In the rainy season (May–September) mean temperature reaches 15.5 °C (9.5–25.4 °C) and the wettest months are July and September. Mean annual precipitation is 367.6 mm (INEGI 2005; Pavón and Meza-Sánchez 2009). The natural vegetation is represented by open scrub forest or arid tropical scrub (Rzedowski 1994) with representative species such as *Agave lechuguilla*, *Hechtia podantha*, *Coryphanta* sp., *Stenocactus* sp., *Senecios praecox*, *Yucca filifera*, *Opuntia streptacantha*, *O. spinulifera*, *O. robusta* and the non-native *Schinus molle*, among others (COEDE 2004). A smaller portion of the park is covered by introduced vegetation: *Cupressus guadalupensis*, *C. macrocarpa*, *Pinus cembroides*, *P. torreyana*, *P. pinceana* and *Ligustrum japonicum* (Zuria and Rendón-Hernández 2010). There are no permanent water bodies or streams. Avifauna at this site is composed by 76 species, 41 considered residents and the rest have different migratory status. Best-represented families were: Trochilidae (8 species), Tyrannidae (10 species), Emberizidae (8 species), Icteridae (8 species) and Parulidae (6 species) (see Zuria, Bravo-Cadena, and Caballero-Quiroz 2009).
b. Ecological reserve Parque Natura (neotropical urban greenspace, 80 ha), located within the urban protected area El Tejar Garnica (130 ha, 19°30'N, 98°44'W, at 1200–1280 m.a.s.l.; Fig. 1b) (Secretaría de Desarrollo Regional 2001). This reserve is within the city of Xalapa in the state of Veracruz, Mexico. During the dry season (November–March) mean temperature is 16.8 °C (11.6–22.0 °C) and the driest month is December. In the rainy season (April–October) mean temperature reaches 20.7 °C (15.4–26.0 °C) and the wettest months are June and September (CONAGUA 2012). Mean annual precipitation is between 1500 and 2000 mm (Secretaría de Desarrollo Regional 2001). Originally, the dominant vegetation of the reserve was montane cloud forest, but currently this vegetation type is only found in isolated patches within the reserve, containing representative species such as *Liquidambar macrophylla* and *Platanus mexicanus* (Corona-López 2006). Shade coffee (*Coffea arabiga*) patches are also present, with some trees associated to them like *Acasia farnesiana*, *Clethra macrophylla*, *Heliocarpus appendiculatus* (Lemoine-Rodríguez 2012) The reserve has permanent streams and springs (Secretaría de Desarrollo Regional 2001). Avifauna at this site is composed by a total of 174 species, 80 species are residents and the rest have different migratory status. Best-represented families were: Parulidae (32 species), Tyrannidae (21 species), Cardinalidae (9 species), Accipitridae (9 species) and Trochilidae (8 species) (see González-García et al. 2014).
c. Urban forest Mooswald (palearctic urban greenspace, 110 ha), located in the upper Rhine valley in the city of Freiburg in Southwestern Germany (48°00'N, 07°51'E, at 275 m.a.s.l.; Fig. 1c). There is no dry season; the driest month is November and the wettest months are June and July. Mean annual precipitation is 1000 mm. Mean annual temperature is 10°C (3-20°C). This forest is a mixed deciduous forest, dominant tree species are *Fraxinus excelsior*, *Quercus robur* and *Q. rubra*; trees found in this forest have an age of about 80–120 years (Marcus Müller, pers. com; see Santiago-Alarcon et al. 2016). This greenspace has water available in small streams and ponds (Santiago-Alarcon et al. 2012). Avifauna at this site is composed by 27 species, 18 species are residents and the rest have different migratory status (Dierschke 2008). Best-represented families were: Picidae (3 species), Paridae (3 species) and Fringillidae (3 species) (Santiago-Alarcon et al. 2016).

Field procedures

Fieldwork took place in the nearctic urban greenspace in June and July 2010 ($n = 36$), in the palearctic urban greenspace in June and July 2011($n = 216$) and in July 2013 for the neotropical urban greenspace ($n = 30$). In all sites, we used mist nets to capture birds from dawn (5:30 and 6:00) until noon (12:00 and 13:00). All birds were identified to species level, and were banded with a metal ring under federal scientific collecting license (nearctic site: No. FAUT-0174, neotropical site: SGPA/DGVS/05057/13, palearctic site: RPT Tierversuch-Nr.1056 and 55–8853.17/0). We obtained blood from the brachial vein and took up to 30 µl with a micro-capillary tube. We prepared at least one thin blood smear for each bird (Santiago-Alarcon and Carbó-Ramírez 2015). Remaining whole blood was frozen at −20 °C for subsequent molecular analysis. All birds were released at the site of capture.

Light microscopy scanning methods

We scanned blood smears from all sites with a light miscroscope (Nikon Eclipse Ni). First we scanned at low magnification (600×), and when infected we scanned 100 optical fields at high magnification (1000x) to determine relative infection intensity (Valkiūnas 2005; Santiago-Alarcon and Carbó-Ramírez 2015). We identified parasites to species level whenever possible, but this proved difficult in some samples because infection intensity was low or the smears were of low quality (open scrub site) due to staining with Wright–Giemsa and lack of fixation in 100% methanol (see Valkiūnas 2005; Santiago-Alarcon and Carbó-Ramírez 2015, for appropriate blood smear preparation procedures).

Molecular analysis

Blood samples were examined by PCR-based screening techniques. DNA was extracted using the DNeasy Blood and Tissue Kit (QIAGEN); DNA quality was checked running 5 µl of each extraction on 1.2% agarose gels. We used parasite genus-specific primers in a nested PCR method to determine avian haemosporidian presence, and to amplify 540-bp of the mtDNAcyt b

gene from parasites of the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* (Hellgren, Waldenström, and Bensch 2004). All the PCRs were run with a positive (samples from infected birds previously confirmed by sequencing and microscopy) and negative control (ddH_2O), no contamination was detected during the study. For the first or outer PCR reaction, we used a total volume of 10 μl per sample. Each reaction contained 1 μl of Top Taq 10× Buffer (QIAGEN), 6·8 μl of double-distilled H2O, 0·2 mM of dNTPs, 0·15 μM from each 10mM primer, 0·025 U from Top Taq polymerase enzyme (QIAGEN) and 1 μl of DNA template. The second or inner PCR reaction had a total volume of 20 μl per sample, each reagent was doubled compared with the first PCR; we used 2 μl from the first PCR as template for the inner reaction. Thermocycler profile for PCR I was (i) 3 min of initial denaturation at 95 °C, (ii) 20 cycles of 30 s denaturation at 95 °C, 30 s annealing at 50 °C, 45 s elongation at 72 °C and (iii) a 10-min final extension at 72 °C. The second PCR had a similar thermal profile with a change in the annealing temperature to 57 °C and 35 cycles instead of 20.

We ran 4 μl from the second PCR in a 1.5% agarose gel stained with GelRed (BIOTIUM), and visualized it with a UV light source to check for amplification. Positive samples were cleaned with the MinElute PCR Purification Kit (QIAGEN). We sent the samples for bi-directional sequencing at Macrogen, Inc. (Korea). Sequences were assembled, aligned, and edited using the Sequencher 4.1 (GeneCodes, Ann Arbor, MI, USA). Sequences were ∼479 bp long after editing. We obtained four bad sequences and were unable to get a sequence from one other sample. Parasite haplotypes were checked against DNA sequences available in GenBank by using the BLAST algorithm from the NCBI database and also against parasite sequences available in the MalAvi database (Bensch, Hellgren, and Pérez-Tris 2009). Sequences were deposited in GenBank (Accession numbers: KT924369-KT924396).

Phylogenetic analyses

Phylogenetic hypotheses were constructed using the program MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001). Two independent runs were made, with 4 chains in each run for a total of 1 million generations, sampling every 100 generations. The first 2500 trees (25%) were discarded as the 'burn-in'. In total, 7500 trees from each run were used to build our majority-rule consensus tree. We used a GTR + I + G model of molecular evolution with shape parameter $\alpha = 0.66$ and proportion of invariable sites Pinvar = 0.388, as suggested by jModelTest v2.1.7 (Guindon and Gascuel 2003). We used sequences of *Leucocytozoon* parasites to root our consensus phylogram. For our phylogram we used lineages already attached to morphologically described species, seven *Plasmodium* lineages (Valkiūnas et al. 2008, *Plasmodium elongatum*; Valkiūnas et al. 2009, *P. multivacuolaris*, *P. lucens*, *P. parahexameriun*, *P. vaughani*; Mantilla et al. 2013, *P. lutzi*; Walther et al. 2014, *P. homopolare*) and three *Haemoproteus* lineages (Križanauskienė et al. 2006, *Haemoproteus majoris*; Karadjian et al. 2013, *H. syrnii*; Dimitrov et al. 2014, *H. homovelans*).

Statistical analyses

We used the software Quantitative Parasitology 3.0 (Reiczigel and Rózsa 2005) to calculate infection prevalence and its 95% confidence intervals (CI) for each site. Estimates of infection prevalence and CI take into account sample size; CI estimation is based on resampling methods (e.g. bootstrap), avoiding the problems that normal theory faces when dealing with skewed distributions, particularly for small sample sizes (Rózsa, Reiczigel, and Majoros 2000; Reiczigel and Rózsa 2005). Moreover, CIs using resampling methods are not necessarily symmetric, which reflects how accurately the sample estimate of prevalence characterizes the true population prevalence as a function of sample size (Rózsa, Reiczigel, and Majoros 2000; Reiczigel 2003). Confidence intervals were calculated with the Sterne's exact method (Reiczigel 2003). We used Fisher's exact test (Rózsa, Reiczigel, and Majoros 2000) to compare infection prevalence of *Plasmodium* and *Haemoproteus* parasites between sites.

Bird diversity was compared between sites at the community level using records only from birds captured in mist nets (i.e. no census point counts were made); we did the same for parasitized birds only, and for haemosporidian lineages. We used rank-abundance curves to assess assemblage structure and compare between sites for bird communities, parasitized birds and haemosporidian lineages. This approach represents the species abundance distribution of a community graphically, allowing us to describe the diversity of an assemblage (Magurran 2004). To evaluate statistical differences of the evenness/dominance slopes between urban forests we performed ANCOVAs, and values were $\log_{10}$ transformed before analysis (Ortega-Álvarez and MacGregor-Fors 2009). To complement the rank-abundance analysis, we used diversity order 2 (^2D) using MVUE estimator (Minimum variance unbiased estimator), which favors very abundant species and can be interpreted as the number of dominant species in the community (Chao, Chiu, and Hsieh 2012).

Results

Prevalence

In the nearctic site ($n = 36$), we found an infection prevalence of 50.0% (95% CI: 33.4–66.6%, Fig. 2; Table 1). Of the infected birds, 28% of birds were infected with *Plasmodium* parasites and 72% with *Haemoproteus* parasites; prevalence was significantly different between the two genera in this urban greenspace ($P = 0.02$).

In the neotropical site ($n = 30$), we found and infection prevalence of 46.7% (95% CI: 29.8–65.2%, Fig. 2; Table 1). Of the infected birds 71.4% were infected with *Plasmodium* parasites and 28.6% with *Haemoproteus* parasites; prevalence between the two genera in this urban greenspace was significant ($P = 0.05$).

In the palearctic site ($n = 216$), we found and infection prevalence of 33.3% (95% CI: 25.4–37.9%, Fig. 2; Table 1); birds were evenly infected with *Plasmodium* (30.5%), *Haemoproteus* (33.3%) and *Leucocytozoon* (36.2%) parasites. We did not detect significant differences in infection prevalence among any of the three parasite genera at this site ($P \geq 0.59$).

There were no differences in prevalence between the nearctic and neotropical urban greenspaces ($P = 0.81$). Although we found significant difference between the palearctic and the neotropical sites, prevalence rates were similar among the three sites given the observed overlap in their 95% CIs (Fig. 2). There was a significant difference in prevalence for *Haemoproteus* parasites between the nearctic and neotropical urban greenspaces ($P = 0.03$; nearctic site 72.2% and neotropical site 28.6%), also between the nearctic and palearctic urban greenspaces ($P = 0.006$; nearctic site 72.2% and palearctic site 33.3%), but no significant difference between neotropical and palearctic sites ($P = 1.0$; neotropical site 28.6% and palearctic site 33.3%). For *Plasmodium* genus, there was a significant difference in prevalence between the neotropical and nearctic urban greenspaces ($P = 0.03$; nearctic site 27.8% and neotropical site 71.4%), also between the neotropical and palearctic sites ($P = 0.006$; neotropical

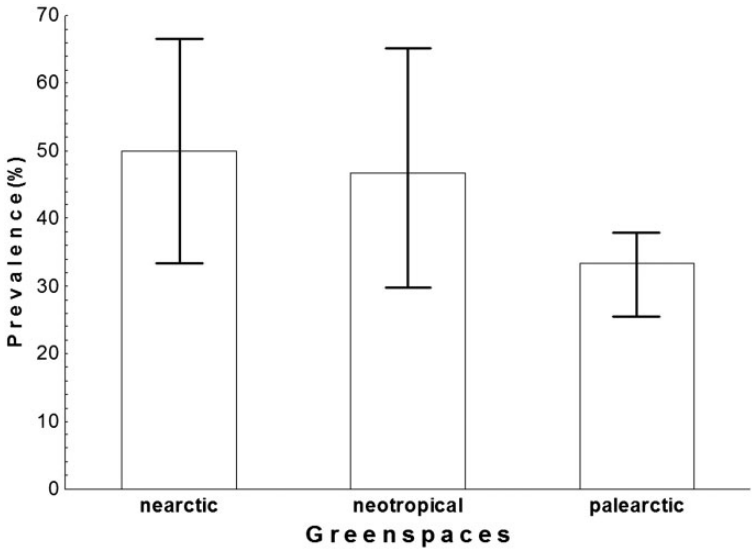

Figure 2. Prevalence and 95% CIs of the three urban greenspaces.

Table 1. Data on sampling sites, family, and species of birds infected with haemosporidian lineages

Site	Family	Species	Sample size (*n*)	Infected birds	MalAvi database lineages
Nearctic	Columbidae	*Columbina inca* (*Col inc*)	13	6	MALERY01
	Mimidae	*Toxostoma curvirostre* (*Tox cur*)	3	3	AMMAUR01 ZOCAP08
	Emberizidae	*Melozone fusca* (*Mel fus*)	2	1	ZOCAP08
		Spizella atrogularis (*Spiz atro*)	2	1	MALERY01
	Icteridae	*Icterus wagleri* (*Ict wag*)	1	1	MALERY01
		Icterus parisorum (*Ict par*)	3	3	MOLATE01 SEIAUR01 CYCYA02
	Fringillidae	*Haemorhous mexicanus* (*Hae mex*)	3	1	–
	Passeridae	*Passer domesticus* (*Pass dom*)	3	2	MALERY01
Neotropical	Cracidae	*Ortalis vetula* (*Ort vet*)	1	1	Bad sequence
	Troglodytidae	*Thryothorus maculipectus* (*Thr mac*)	1	1	MALERY01
	Turdidae	*Catharus aurantiirostris* (*Cat aur*)	2	1	CATUST05
	Parulidae	*Basileuterus culicivorus* (*Bas cul*)	6	1	MALERY01
	Emberizidae	*Arremon brunneinucha* (*Arr bru*)	2	2	BAEBIC02
		Arremonops rufivirgatus (*Arr ruf*)	3	3	BAEBIC02
		Aimophila rufescens (*Aim ruf*)	1	1	BAEBIC02
	Fringillidae	*Cyanocompsa parellina* (*Cya par*)	4	3	PADOM11
		Euphonia hirundinacea (*Eup hir*)	3	1	LOXLEU01
Palearctic	Paridae	*Parus major* (*Par maj*)	43	20	SYAT05, PARUS4, SGS1, PARUS22, CWT4, CB1
	Phylloscopidae	*Phylloscopus collybita* (*Phy coll*)	5	1	Bad sequence
	Acrocephalidae	*Acrocephalus palustris* (*Acr pal*)	1	1	Bad sequence
	Sylviidae	*Sylvia atricapilla* (*Syl atr*)	18	6	SYAT01, SYAT02 see Santiago-Alarcon et al. (2011)
	Muscicapidae	*Muscicapa striata* (*Mus str*)	3	1	ANLAT12
		Erithacus rubecula (*Eri rub*)	61	5	LINN1, ROBIN1, ANLAT16, STUR1
	Turdidae	*Turdus merula* (*Tur mer*)	21	13	LINN1, SYAT05, TURDUS2, TUFAL02
		Turdus philomelos (*Tur phi*)	12	12	SYAT05, TUFAL02, SYAT32, STUR1, TURDUS1
	Fringillidae	*Fringilla coelebs* (*Fri coe*)	16	12	CCF1, CCF2, SYAT32
		Coccothraustes coccothraustes (*Coc coc*)	1	1	Bad sequence

Uninfected bird species (the number of birds in parenthesis) detected at each site: **(Nearctic) Picidae**: *Picoides scalaris* (*Pic sca*, 1); **Hirundinidae**: *Hirundo rustica* (*Hir rus*, 1); **Troglodytidae**: *Thryomanes bewickii* (*Thr bew*, 2), *Campylorhynchus brunneicapillus* (*Cam bru*, 2). **(Neotropical) Trochilidae**: *Campylopterus curvipennis* (*Cam cur*, 3); **Tyrannidae**: *Tolmomyias sulphurescens* (*Tol sul*, 1); **Cardinalidae**: *Habia rubica* (*Hab rub*, 2), *Habia fuscicauda* (*Hab fus*, 1). **(Palearctic) Picidae**: *Dendrocopos medius* (*Den med*, 1), *Dendrocopos major* (*Den maj*, 4); **Paridae**: *Poecile palustris* (*Poe pal*, 4), *Cyanistes caeruleus* (*Cya cae*, 6); **Aegithalidae**: *Aegithalos caudatus* (*Aeg cau*, 1); **Sittidae**: *Sitta europaea* (*Sit eur*, 3); **Certhiidae**: *Certhia familiaris* (*Cer fam*, 3); **Throglodytidae**: *Troglodytes troglodytes* (*Tro tro*, 10); **Sylviidae**: *Sylvia borin* (*Syl bor*, 3).

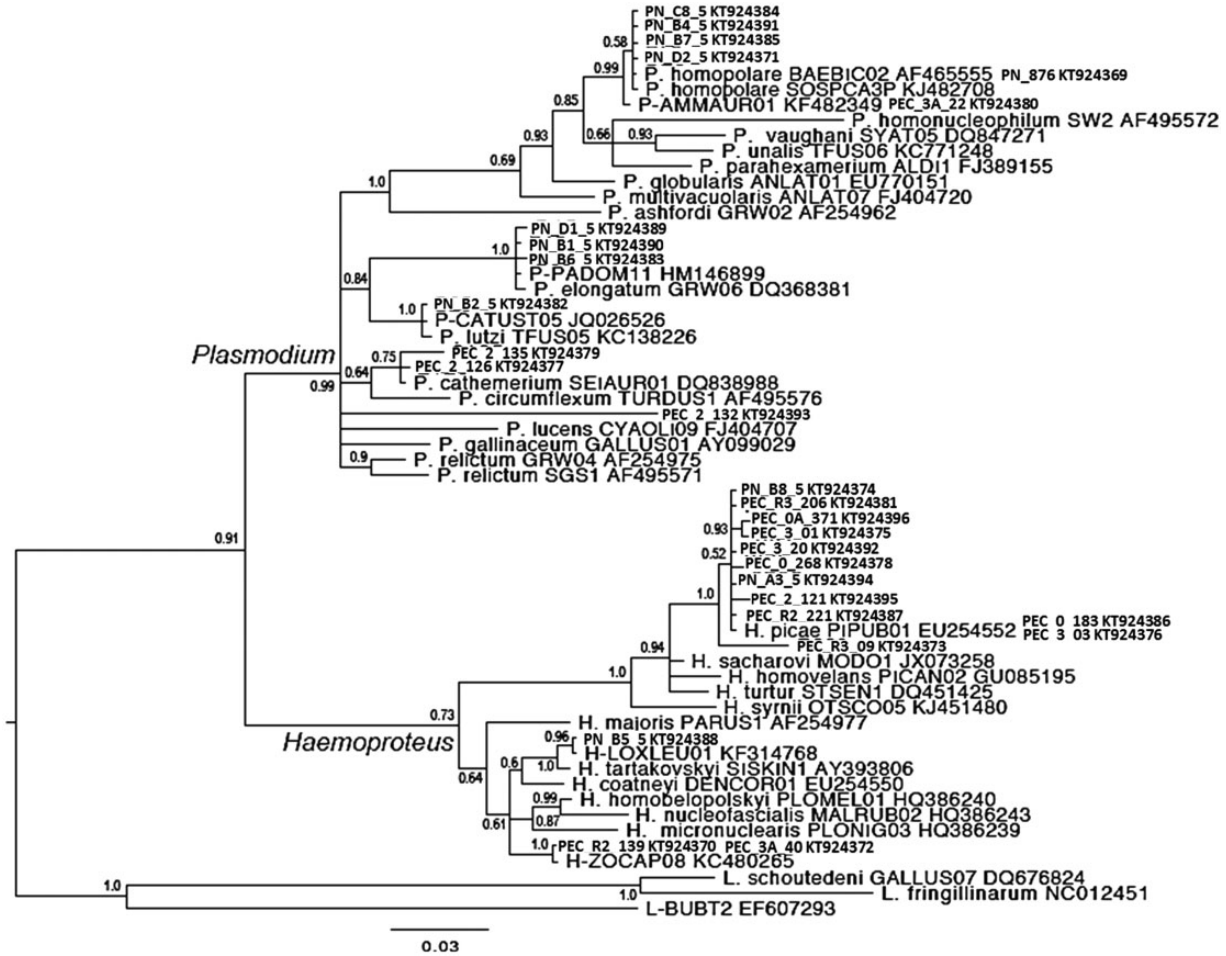

Figure 3. Phylogram showing phylogenetic relationships of haemosporidian lineages detected in the nearctic and neotropical urban greenspaces in relation to other lineages already attached to a morphospecies. Lineages recorded in this study have the prefix PN or PEC (PN = neotropical urban greenspace, PEC = nearctic urban greenspace) and are in smaller font size. GenBank accession numbers of all sequences are indicated after morphospecies and lineage name. *Leucocytozoon schoutedeni* and *L. fringillinarum* were used as outgroup. Numbers along branches correspond to node support values from Bayesian analysis.

site 71.4% and palearctic site 33.3%), but no significant difference between nearctic and palearctic urban greenspaces ($P = 1.0$; nearctic site 27.8% and palearctic site 33.3%).

Host–parasite assemblage structure and composition

We identified a total of 29 *Haemoproteus*, 14 *Plasmodium*, and 10 *Leucocytozoon* lineages (Table 1). We detected 30 mixed infections only in the palearctic urban greenspace (see Santiago-Alarcon et al. 2011, 2016). For the nearctic and neotropical urban greenspaces we recorded a total of three parasite lineages of *Haemoproteus* and seven of *Plasmodium* and no sample was positive for parasites of the *Leucocytozoon* genus (Table 1). In the palearctic urban greenspace we recorded nine parasite lineages of *Haemoproteus*, four of *Plasmodium*, and seven of *Leucocytozoon* (Table 1). In the nearctic urban greenspace, we recorded the *Haemoproteus* lineages MALERY01 and ZOCAP08, and the *Plasmodium* lineages AMMAUR01, CYCYA02, MOLATE01, and SEIAUR01 (Table 1). We identified lineages belonging to three morphospecies in the nearctic site: *Haemoproteus picae*, *Plasmodium homopolare*, and *P. cathemerium* (Fig. 3). In the neotropical urban greenspace we recorded the *Haemoproteus* lineages MALERY01 and LOXLEU01, and the *Plasmodium* lineages CATUST05, BAEBIC02 and PADOM11 (Table 1). We identified lineages belonging to five morphospecies in the neotropical site: *Plasmodium homopolare*, *P. elongatum*, *P. lutzi*, *Haemoproteus picae* and *H. tartakovskyi* (Fig. 3). In the palearctic urban greenspace a larger array of genetic parasite lineages of the three genera were recorded, the most important from *Haemoproteus* are SYAT01 and SYAT02 belonging to the *H. parabeloposlkyi* morphospecies, and SGS1 *Plasmodium* lineage belonging to the pathogenic avian malaria morphospecies *P. relictum* (Table 1) (for more details see Santiago-Alarcon et al. 2011, 2016).

According with the diversity order 2 index, the bird communities of the nearctic and palearctic urban greenspaces have the same effective number of bird species, but the neotropical urban greenspace was two times as diverse (Fig. 4a). All bird species captured in each urban greenspace have been previously reported on the local bird list, and reflect the known bird diversity and composition at each site (nearctic site: Zuria, Bravo-Cadena, and Caballero-Quiroz 2009; neotropical site: González-García et al. 2014; MacGregor-Fors et al. unpublished; and palearctic site: Santiago-Alarcon et al. 2016). Considering the bird communities for the three sites, we found that they do not share any bird species, where *Columbina inca* dominated the community of the nearctic site, *Basileuterus culicivorus* and *Cyanocompsa parellina* of the neotropical site and *Erithacus rubecula* and *Parus major* of the palearctic site (Fig. 4a, 4b). Regarding Haemosporidian lineages, we found that nearctic and neotropical urban greenspaces have

Table 2. Geographic distribution of lineages and morphospecies reported in this study

LINEAGES	MORPHOSPECIES	BIOREGION
AMMAUR01	*Plasmodium*	South America
BAEBIC02	*Plasmodium homopolare*	North America, South America
CATUST05[a]	*Plasmodium lutzi*[a]	North America, South America
CYCYA02	*Plasmodium*	South America
LINN1[a]	*Plasmodium*	Asia, Europe, Oceania
MOLATE01	*Plasmodium*	North America
PADOM11	*Plasmodium elongatum*[a]	Africa, Asia, Europe, North America, Oceania, South America
SEIAUR01	*Plasmodium cathermerium*[a]	North America
SGS1	*Plasmodium relictum*[a]	Asia, Africa, Europe, Oceania, South America
SYAT05[a]	*Plasmodium vaughani*	Africa, Asia, Europe, North America, Oceania
TURDUS1	*Plasmodium circumflexum*[a]	Africa, Asia, Europe
CCF1	*Haemoproteus*	Africa, Europe
CCF2	*Haemoproteus*	Africa, Asia, Europe
CWT4	*Haemoproteus majoris*	Asia, Europe
LOXLEU01	*Haemoproteus*	North America
MALERY01	*Haemoproteus picae*	North America
PARUS1	*Haemoproteus majoris*	Africa, Asia, Europe, North America
ROBIN1	*Haemoproteus attenuates*	Africa, Asia, Europe
SYAT01, SYAT02	*Haemoproteus parabelopolskyi*	Africa, Asia, Europe
SYAT32	*Haemoproteus*	Europe
ZOCAP08	*Haemoproteus*	North America, South America
ANLAT12	*Leucocytozoon*	Africa, Europe
ANLAT16	*Leucocytozoon*	Africa
CB1	*Leucocytozoon majoris*	Europe, North America,
PARUS22	*Leucocytozoon*	Africa, Asia, Europe
STUR1	*Leucocytozoon*	Europe
TUFAL02	*Leucocytozoon*	South America

The information was accessed on the MalAvi database and all appropriate references are contained therein.
[a]Reported in urban areas.

the same effective number of lineages, but the palearctic was more diverse (Fig. 4c). For haemosporidian lineage composition, we found that nearctic and neotropical site shared one *Haemoproteus* lineage (MALERY01; Fig. 4c), which was the dominant one at the nearctic site. This lineage infected a total of six species of birds (nearctic = 4; neotropical = 2) and 11 individuals (Table 1). *Plamodium* SYAT05 was the dominant lineage in the palearctic site (Fig. 4c).

Comparing rank/abundance curves, we found that bird communities (using either all birds captured in mist nets or only those that were parasitized) between nearctic and neotropical urban greenspaces are structured in a similar way as there was not statistical differences between their slopes (all birds: ANCOVA: $F_{1,15} = 0.002$, $P = 0.96$; parasitized birds: ANCOVA: $F_{1,5} = 0.50$, $P = 0.51$); however, the palearctic site has a bird community that is more evenly structured compared to the other two sites (all birds: ANCOVA: $F_{1,22} = 7.93$, $P = 0.01$ and $F_{1,21} = 8.01$, $P = 0.01$, respectively; parasitized birds: ANCOVA: $F_{1,8} = 12.86$, $P = 0.007$ and $F_{1,7} = 17.64$, $P = 0.004$, respectively; Fig. 4a, 4b). Finally, haemosporidian lineages abundance structure was similar among the three sites, indicating that they are dominated by a few parasite lineages (nearctic-neotropical: ANCOVA: $F_{1,3} = 0.28$, $P = 0.63$; palearctic-nearctic: ANCOVA: $F_{1,14} = 0.70$, $P = 0.42$; palearctic-neotropical: ANCOVA: $F_{1,15} = 0.0029$, $P = 0.96$; Fig. 4c).

Only one bird family was shared among sites (Fringillidae), indicating the uniqueness of bird communities at each location (Table 1). We recorded parasites only from the *Haemoproteus* genus from individuals of the Fringillidae family in the three urban greenspaces, LOXLEU01 lineage from individuals of the neotropical site, CCF1, CCF2 and SYAT32 in the palearctic site (Table 1), and at the nearctic site the infection was recorded only by microscopy, so no genetic lineage is available.

Discussion

Host–parasite assemblage structure

Although our study sites are located at different geographical areas, and have contrasting abiotic (e.g. temperature, humidity, rainfall, altitude) and biotic factors (e.g. vegetation), including different bird community composition, we found that birds and their blood parasites are similarly structured in these three urban greenspaces: similar infection prevalence, similar assemblage structure particularly for parasite lineages and a phylogenetically diverse parasite community (see Fig. 3; Santiago-Alarcon et al. 2011, 2016 for details of the palearctic site). At this point, it is important to consider such similarities among urban greenspaces with caution because we still have low sample sizes for the neotropical and nearctic sites. Hence, we suggest a more thorough sampling of urban greenspaces in the neotropical and nearctic and an increase in sampling urban green locations across different geographical areas; only the inclusion of control sites (i.e. well preserved native vegetation) will provide a clear understanding of how parasite communities respond to the urbanization process.

There are also some differences in the number of haemosporidian lineages among sites, however, when looking closer at each forest we observe that in the neotropical site, lineages of the *Plasmodium* genus were the most common, whereas lineages of the *Haemoproteus* genus were the most common in the nearctic site, while in the palearctic site the three genera of

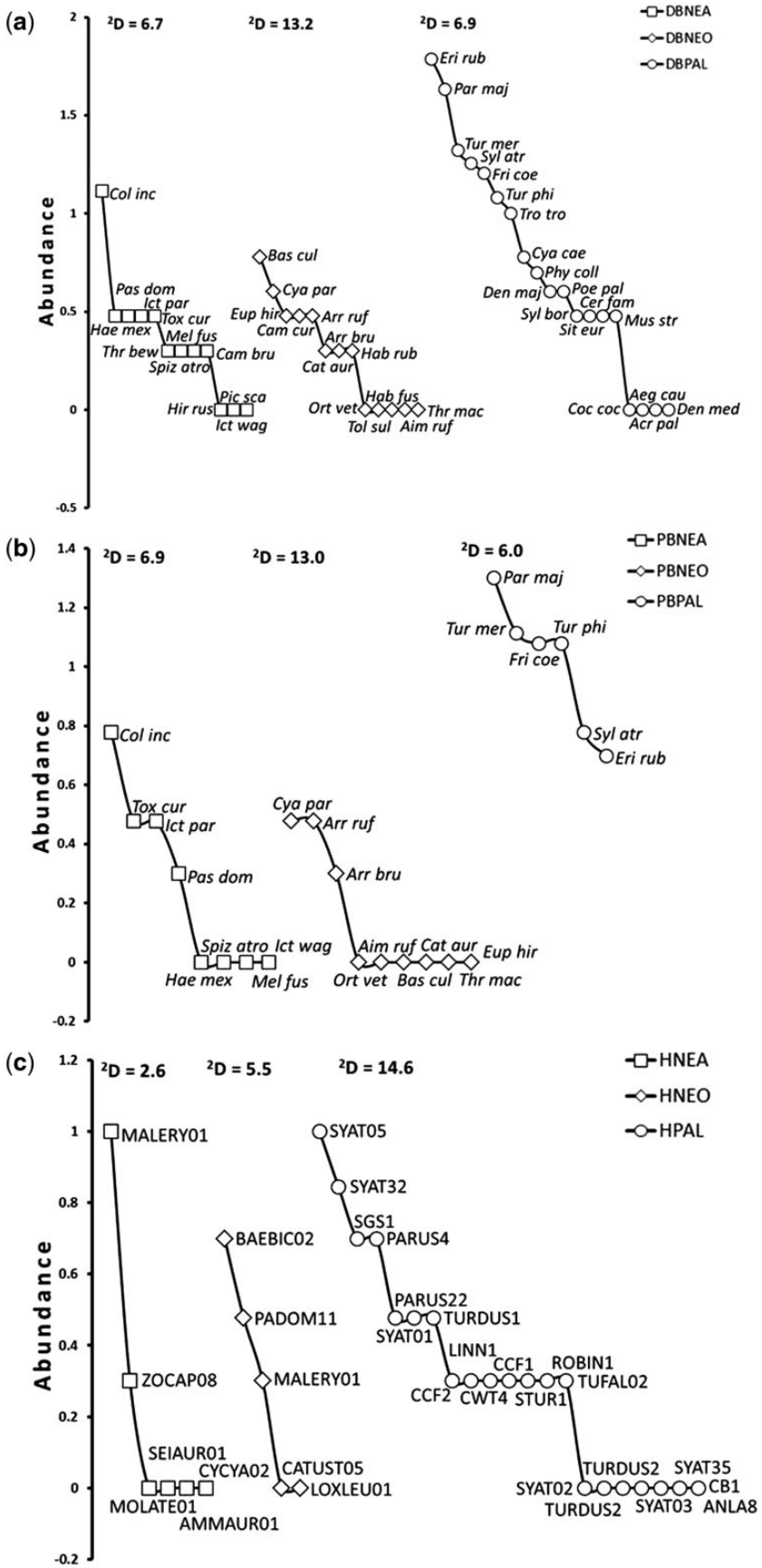

Figure 4. Rank/abudance plots: (a) bird communities (DBNEA, diversity of birds in the nearctic site; DBNEO, diversity of birds in the neotropical site; DBPAL, diversity of birds in the palearctic site), (b) parasitized bird species (PBNEA, parasitized birds in the nearctic site; PBNEO, parasitized birds in the neotropical site; PBPAL, parasitized birds in the palearctic site) and (c) haemosporidian lineages (HNEA, haemoporidians in the nearctic site; HNEO, haemosporidians in the neotropical site; HPAL, haemosporidians in the palearctic site). ^2D, diversity order 2 (see Methods). Codes for bird species are given in Table 1.

haemosporidians are equally represented. This might reflect the presence of different dominant and competent vectors at each place due to the local environmental conditions, which might favor breeding sites for different Diptera families (e.g. Simuliidae favored by high humidity and running water, whereas Ceratopogonidae can thrive in dry conditions with ephemeral water bodies and succulent vegetation) (Valkiūnas 2005; Krama et al. 2015; Svodoba et al. 2015).

Our results show that there is not a significant difference in prevalence between the neotropical and the nearctic urban greenspaces, which does not support our initial prediction because the dry conditions in the open scrub site were expected to prevent the successful development of haemosporidian vectors, lowering parasite infection. However, this can be due to the low sample sizes, because when we compared the palearctic site with the nearctic one we did find significantly lower parasite prevalence in the open scrub environment (i.e. nearctic site). Alternatively, it is important to mention that the months of capture at the three places were the wettest for each urban greenspace (rainy season). Therefore, similar infection rates may be related to common abiotic factors present during that season of the year, but such pattern of infection might not hold year-round.

We did not find any bird species shared among sites, only one bird family (Fringillidae) at the three locations, and only one parasite lineage (MALERY01) between the nearctic and neotropical urban greenspaces. Such differences in parasite composition can be related to the dissimilar avian composition given their separate biogeographical histories and contrasting environmental conditions (e.g. different plant communities, temperature, humidity, presence/amount of water), which is akin to what happens on parasite assemblages along altitudinal gradients (Galen and Witt 2014; González et al. 2014). For example, Durrant et al. (2006) found that there were only three *Plasmodium* lineages shared among birds between the tropical and temperate biomes of South America. Thus, there are geographical restrictions in haemosporidians distributions, particularly for those lineages that do not infect widespread and/or invasive hosts. A geographical restriction pattern is observed even for those parasites infecting migratory birds (Hellgren et al. 2007). For instance, many lineages of *Haemoproteus* and *Leucocytozoon* infecting migratory birds flying between Europe and Africa had restricted transmission areas (i.e. low parasite lineage sharing between biogeographical zones, Hellgren et al. 2007).

Interestingly, the only haemosporidian lineage shared (MALERY01) belongs to the *Haemoproteus picae* morphospecies and is a geographically widespread generalist infecting several bird families, genera, and species (e.g. *Aphelocoma coerulescens*, *Corvus corax*, *Cyanocitta cristata*, *Dendrocitta formosae*, *Garrulus glandarius*, *Melanerpes erythrocephalus*, *Pica pica*; Valkiūnas 2005). This finding is relevant because parasites of the *Haemoproteus* genus have been considered more specific than parasites of the *Plasmodium* genus (Atkinson and van Riper 1991). However, recent studies have demonstrated that both genera can include generalists and specialists (Svensson-Coelho et al. 2013, 2014; Olsson-Pons et al. 2015).

Haemosporidian lineages: geographic distribution and urban areas

From the haemosporidian lineages, and their corresponding morphospecies, reported in this study, some of them are known to be widely distributed; however, only a few have been previously reported in urban areas (Table 2). *Plasmodium lutzi* and its CATUST05 lineage were found in an urban area, characterized by vegetation patches in Colombia (González et al. 2015; see also Mantilla et al. 2013) (Table 2). Lineages LINN1 and SYAT05 were reported infecting European blackbirds (*Turdus merula*) in an urban location of France (Bentz et al. 2006) (Table 2). *Plasmodium elongatum* is the most widely distributed from parasites recorded in this study, it was reported in suburban areas including parks, cemeteries, and residential communities infecting several avian hosts (i.e. *Dumetella carolinensis*, *Melospiza melodia* and *Cardinalis cardinalis*) in USA (Madeiros, Hamer, and Ricklefs 2013; Thurber et al. 2014) (Table 2). *Plasmodium relictum*, a world-wide distributed malaria parasite, has been found infecting birds in urban areas, including House Finch (*Haemorhous mexicanus*) in Mexico City (Hewitt 1940), House sparrow (*Passer domesticus*) along a gradient of urbanization, characterized by agriculture, woodland, meadow and shrubland in France (Bichet et al. 2013), and six other avian hosts (*Calandrella rufescens*, *Turdoides altirostris*, *T. caudatus*, *Pycnonotus leucogenys*, *Phoenicurus phoenicurus*, *Passer domesticus*) in different areas within and around Baghdad City (Mohammas and Al-Moussawi 2012). *Plasmodium circumflexum* has been reported in three bioregions (Table 2), more recently in suburban areas of the USA (Thurber et al. 2014). Finally, *Plasmodium cathemerium* has been reported only in North America, including urban areas of Mexico (Hewitt 1940) and USA (Madeiros, Hamer, and Ricklefs 2013; Thurber et al. 2014) (Table 2).

The high haemosporidian lineage richness found in these urban sites, might reflect the fact that such places are relicts of the original vegetation at each area. This type of urban greenspaces still contain bird species not found in heavily built areas, possibly carrying parasites not infecting species of birds considered typical in these areas (e.g. House sparrows, Rock pigeons). However, due to the isolation of such areas within the urban environment, it is likely that parasite communities get simplify through time due to losses of both competent hosts and vectors; the velocity of such a process will depend on the size and management of the site (i.e. connectedness to other similar patches).

Conclusions

Although we must keep in mind the low sample sizes of the nearctic and neotropical urban greenspaces, this is the first study to compare avian haemosporidian communities of urban greenspaces at three distinct biogeographical regions. Our results suggest that despite contrasting environmental conditions and geographical isolation, there are ecological parallels in the three urban greenspaces: 1) similar haemosporidian infection prevalence, 2) same parasite abundance structure, 3) phylogenetically diverse parasite communities, and 4) communities dominated by widespread generalist parasite lineages.

Data availability statement

Data sets used for analyses in this study are available upon request to any of the corresponding authors.

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