

Bat–bat fly interactions in Central Panama: host traits relate to modularity in a highly specialised network

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Abstract. 1. Recently, network approaches have gained increasing popularity in studies of species interactions. These analyses provide important information about structural and functional organisation, as well as on the dynamics of species interactions. Common model systems for network studies include seed dispersal, pollination, and also parasite interactions.

2. Bat flies (Diptera: Streblidae, Nycteribiidae) are obligate blood-sucking ectoparasites of bats. Resource partitioning allows multiple fly species to co-occur on a single host individual, making them an ideal model system for network analyses.

3. Between 2013 and 2018 in Central Panama, 6528 bats from 53 species were examined for the presence of bat flies. Thereof, we collected a total of 6077 bat flies belonging to 52 species.

4. The resulting interaction network showed a significantly higher specificity ($H_2' = 0.97$) and modularity ($Q = 0.78$) than expected by chance, indicating a very high host specificity of the bat flies. To investigate parasite interactions in the context of host size, host abundance and roosting preferences, we pooled parasite identifications on genus level. The majority of our identified modules were associated with bats using persistent roosting structures. Neither host size nor host abundance appeared to affect module structure. Further, module structure appeared not to be host-phylogeny driven, instead modules were often composed of species known to share roosting structures.

5. Their high host-specificity could put bat flies at risk of extinction in changing environments.

Key words. Bat roosting structures, Chiroptera, host–parasite interactions, Neotropics, network analysis, Streblidae.

Introduction

Species within highly diverse ecosystems interact on a multitude of different levels, forming complex ecological networks. Recently, studies focusing on species interactions using network approaches have gained more and more popularity (Dáttillo & Rico-Gray, 2018; Pellissier *et al.*, 2018). Understanding the

resulting network structures and the underlying mechanisms is fundamental for any ecological study. Network analyses are powerful tools, as they can provide important information about the structural and functional organisation, as well as on the dynamics of species interactions (Olesen *et al.*, 2007; Vázquez *et al.*, 2009). They are commonly used to describe pollinator–plant interactions (Vizentin-Bugoni *et al.*, 2018), seed dispersal (Mello *et al.*, 2011; Escibano-Avila *et al.*, 2018), and also in behavioural studies (Davis *et al.*, 2018).

Bats are used as model organisms in a variety of network analyses. In addition to using network analyses to understand their essential roles as seed dispersers and pollinators (Mello

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et al., 2011, 2019), more recently such analyses were applied to their host–parasite interactions as well (Vázquez *et al.*, 2005; Pinheiro *et al.*, 2016; Zarazúa-Carbajal *et al.*, 2016; Estrada-Villegas *et al.*, 2018). Parasites in general are key components of biodiversity (Christe *et al.*, 2006). They contribute to maintaining stability and integrity of an ecosystem (Brooks & Hoberg, 2001; Hudson, 2007; Frainer *et al.*, 2018) by controlling population sizes of their hosts (Anderson & May, 1979). Parasites may reduce reproduction, increase predation risk, and even alter host morphology and mating behaviour (Frainer *et al.*, 2018).

Bats are highly diverse, especially in tropical regions (Stevens, 2004). This pattern is mirrored by their most conspicuous ectoparasites, the bat flies (Diptera: Streblidae and Nycteribiidae) (Haelewaters *et al.*, 2018). Bat flies are obligate, blood-sucking ectoparasites of bats and currently assigned to two cosmopolitan families, of which the Nycteribiidae are most diverse in the Eastern Hemisphere, particularly the Indo-Malayan and Australasian regions (Haelewaters *et al.*, 2018). Streblidae, on the other hand, are most diverse in the Neotropics (Haelewaters *et al.*, 2018) where they are principally associated with members of the highly diverse leaf-nosed bats (Phyllostomidae) (Wenzel *et al.*, 1966; Wenzel, 1976). They are highly host-specific and most species are associated with just a single host species or occasionally host genus (Wenzel *et al.*, 1966; Wenzel, 1976; Dick & Gettinger, 2005; Estrada-Villegas *et al.*, 2018). Resource partitioning based on the use of different body regions (habitats) on the host together with specific morphological adaptations (Dick, 2005; Hiller *et al.*, 2018) allow the coexistence of various bat fly species on a single host individual (Wenzel *et al.*, 1966; Wenzel, 1976; ter Hofstede *et al.*, 2004; Dick & Gettinger, 2005; Sebastián Tello *et al.*, 2008; Patterson *et al.*, 2009). However, at the moment, no conclusive phylogeny for bat flies is available (Dittmar *et al.*, 2015). Therefore, it remains unclear, whether these morphological adaptations and also the high host specificity are a result of co-evolution or rather of convergent evolution.

A recent study showed a phylogenetic signal in the interaction pattern of fleas and their rodent hosts (Krasnov *et al.*, 2012). However, network studies using bats and their associated bat flies remain scarce (Zarazúa-Carbajal *et al.*, 2016; Barbier & Bernard, 2017; Durán *et al.*, 2019). Standardised descriptors (like specificity or modularity) used in network analyses allow comparing host–parasite interactions between different host populations and provide essential information on this understudied parasite group. Better understanding of host and parasite ecology is also desirable. Especially in fragmented habitats, many parasite species are facing possible extinction threats, due to co-extinctions with their host or because of changes in host behaviour (Carlson *et al.*, 2020; Hiller *et al.*, 2020).

In this study, we present the bat–bat fly interactions obtained during a large-scale bat biodiversity study in Central Panama. We discuss host associations and prevalence of parasitisation. By building a host–parasite network and calculating standardised network metrics we investigated the high specificity of bat–bat fly networks. We obtained a highly modular network, i.e., the subdivision of the network into distinct compartments (Olesen *et al.*, 2007). Further, we used a simplified interaction

matrix of the most diverse and abundant bat family Phyllostomidae and their associated bat flies to identify bat fly modules and associated these with host traits. We expected host abundance, host body mass, and roosting ecology to have an effect on modularity as these are known drivers of parasitisation by bat flies (Patterson *et al.*, 2007, 2008). Finally, we discuss current extinction threats for these highly specialised bat ectoparasites.

Materials and methods

Bat flies were collected between 2013 and 2018 in Central Panama (Fig. 1). The majority of these bat flies originated from a large-scale bat biodiversity and virus ecology project (2013–2015: Brändel *et al.*, 2020; Hiller *et al.*, 2020). Captured bats were kept in the field in soft cotton bags until processing and were released immediately after sampling at the capture site. Bat bags were used only once each capture night and washed afterwards to avoid cross-contamination. All bats were examined for the presence of bat flies. Bat flies were collected, whenever possible, with fine forceps, stored in individually numbered vials containing 96% ethanol, and subsequently identified by the first author (T.H.) in the laboratory, using a stereo-zoom microscope (Olympus SZX9, with 85.5 magnification). Identifications were based on published keys (Wenzel & Tipton, 1966; Wenzel, 1976; Guerrero, 1993, 1994a, 1994b, 1995a, 1995b, 1996, 1997, 1998a, 1998b) and complementary publications (Miller & Tschapka, 2001; Dick, 2013). For current species names of bats and bat flies, we referred to Dick and Graciolli (2013), Graciolli and Dick (2018) and Simmons and Cirranello (2020), respectively. For each bat–bat fly association, we calculated the specificity index as suggested by Dick and Gettinger (2005) to identify non-primary host associations. However, for unfrequently captured bats, relying solely on this specificity index might lead to erroneously excluding “correctly” associated bat flies based on low sample size (especially in oligo- and polyxenous bat fly species). We tried to avoid this mistake by additionally taking published host records into account when deciding which bat–bat fly associates to include in further analyses (Wenzel & Tipton, 1966; Wenzel, 1976; Dick, 2006, 2013).

All statistical analyses were conducted using the R statistical environment (R Development Core Team, 2020). First, to investigate prevalence and intensity of infestation with bat flies in relation to roosting preferences of our host bat species, we used generalised linear mixed effects models (R-package *lme4* Bates *et al.*, 2017). Hereby, we modelled the presence of parasites on host individual level as a binary variable (yes/no), while intensity of infestation, also for each host individual, was modelled with a Poisson error distribution and log-link function. In both models we implemented the variable host species as a random effect to correct for different numbers of host individuals per species. The predictor variable roosting preferences was taken for each host species directly from Patterson *et al.* (2007). For bat species not addressed in the study by Patterson *et al.* (2007), we derived the values for roosting preference based on the information given in LaVal & Rodríguez-H. (2002) for Costa Rican bats and Villalobos-Chaves *et al.* (2013) for *Lichonycteris obscura*, following in each case the methodology described by Patterson

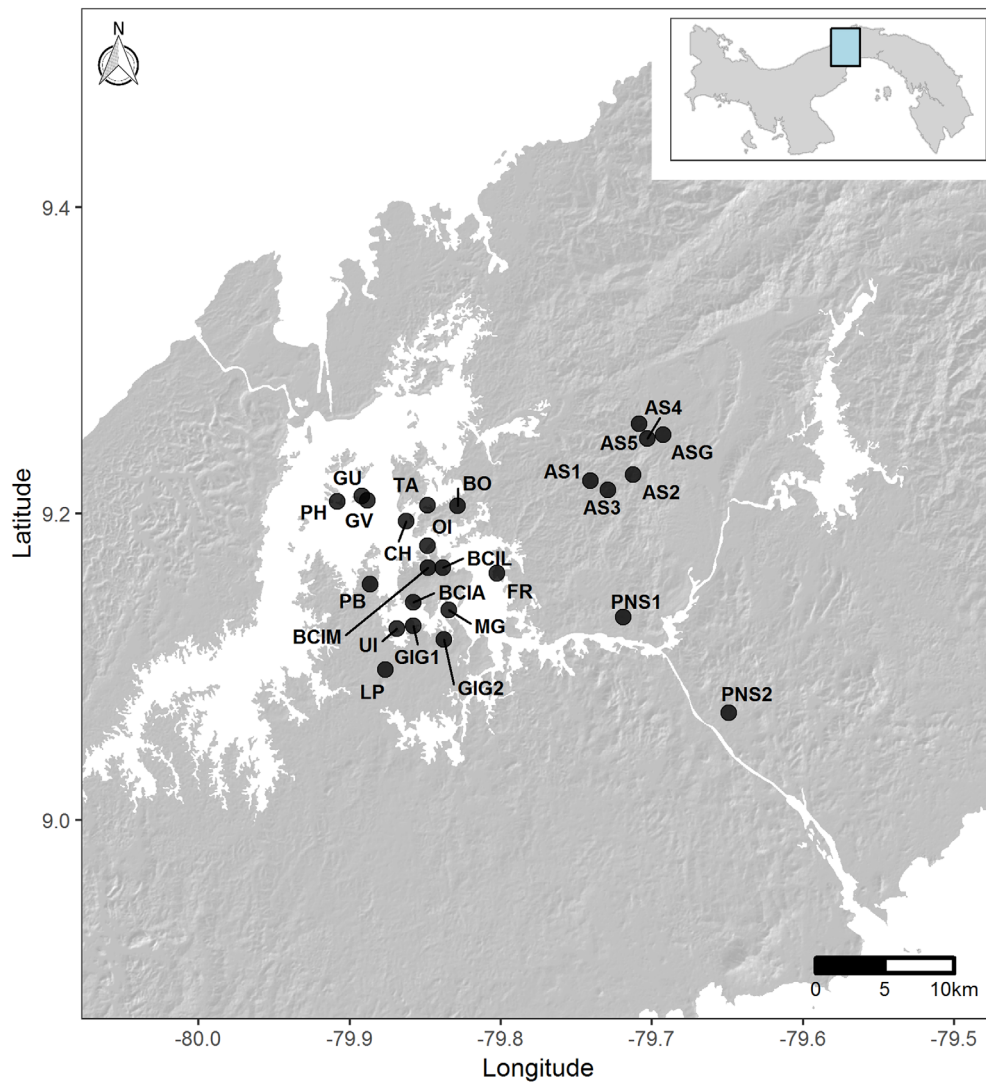


Figure 1. Study area in Central Panama. (AS1: Agua Salud 1, AS2: Agua Salud 2, AS3: Agua Salud 3, AS4: Agua Salud 4, AS5: Agua Salud 5, ASG: Agua Salud garden, BCIA: Barro Colorado Island, Amour trail, BCIL: Barro Colorado Island, Lab Clearing, BCIM: Barro Colorado Island, Miller trail, BO: Bohio, CH: Chicha, FR: Frijoles, GIG1: Gigante 1, GIG2: Gigante 2, GU: Guanabano, GV: Guava, LP: Las Pavas, MG: Mona Grita, OI: Orchid Island, PB: Pena Blanca, PH: Pato Horqueta, PNS1: Parque Nacional Soberania 1, PNS2: Parque Nacional Soberania 2, TA: Tres Almendras, UI: Uroderma Island)

et al. (2007) (Supplementary Table 1). For each candidate model, we calculated pseudo R^2 values to estimate goodness of fit (*r.squaredGLMM()*, R-package *MuMIn*; Barton, 2018). Model results were visualised (*ggpredict()*, R-package *ggeffects*; Lüdtke, 2018) and we decided to overlay values for prevalence and intensity of parasitism on host species level for easier interpretation of the observed trends, however, these values were not used to assess significance. Bat–bat fly associations were then visualised based on an interaction matrix, incorporating both, parasite as well as host abundance (R-packages *igraph*; Csardi & Nepusz, 2006; and *InteractiveGraph*; Vygantas, 2013). We calculated network associated parameters as implemented in the R-package *bipartite*, specifically: network specialisation, modularity (best of

10 replicates, 10E6 steps, method = ‘DormannStrauss’), species specificity index, and generalised specialisation index, (Dormann *et al.*, 2009; Dormann, 2011). The network specialisation index (H_2') ranges from 0 to 1, measures the degree of niche complementarity among species and integrates species-level specialisation across the entire community (Blüthgen *et al.*, 2006). As H_2' is mathematically independent from the total frequency of observations, it allows comparisons of networks differing in size and sampling effort (Blüthgen *et al.*, 2006; Blüthgen, 2010). To investigate which host traits influenced the bat fly modularity, we reduced our dataset to the most commonly captured and most speciose host bat family (Phyllostomidae) and their respective bat flies. We used the interactions (presence/absence) of host bat species with their associated bat

Table 1. Summary of host bats and associated bat fly species addressed in this study.

Species	N (bats)	Parasitised	#Samples	Mean int. (no cont.)	n (BF)	SI	Abrev.
Emballonuridae							
<i>Cormura brevirostris</i> (Wagner, 1843)	7	0	0				Cbre
<i>Rhynchonycteris naso</i> (Wied-Neuwied, 1820)	2	0	0				Rnas
<i>Saccopteryx bilineata</i> (Temminck, 1838)	8	0	0				Sbil
<i>Saccopteryx leptura</i> (Schreber, 1774)	20	0	0				Slep
Molossidae							
<i>Molossus molossus</i> (Pallas, 1766)	85	0	0				Mmol
Mormoopidae							
<i>Pteronotus gymnotus</i> (J.A. Wagner, 1843)	1	1	1	2.0			Pgym
<i>Nycterophilus parnelli</i> (Wenzel, 1966)					2	0.05	Npar
<i>Pteronotus mesoamericanus</i> (Smith, 1972)	87	83	74	6.2			Pmes
<i>Nycterophilus parnelli</i> (Wenzel, 1966)					36	0.92	Npar
<i>Paratrachobius dunni</i> * (Curran, 1935)					1	0.01	Pdun
<i>Trichobius yunker</i> (Wenzel, 1966)					423	0.99	Tyun
<i>Trichobius sparsus</i> (Kessel, 1925)					1	1	Tspa
Natalidae							
<i>Natalus mexicanus</i> (Miller, 1902)	3	2	2	2.0			Nmex
<i>Nycterophilus natali</i> (Wenzel, 1966)					2	1	Nnat
<i>Speiseria ambigua</i> * (Kessel, 1925)					1	0.01	Samb
<i>Trichobius galei</i> (Wenzel, 1966)					2	1	Tgal
Noctilionidae							
<i>Noctilio albiventris</i> (Desmarest, 1818)	20	20	15	10.8			Nalb
<i>Noctiliostrebla maa</i> i (Wenzel, 1966)					6	1	Nmaa
<i>Paradyschiria parvuloides</i> (Wenzel, 1966)					156	1	Ppar
<i>Noctilio leporinus</i> (Linnaeus, 1758)	6	6	4	5.75			Nlep
<i>Noctiliostrebla traubi</i> (Wenzel, 1966)					12	1	Ntra
<i>Paradyschiria lineata</i> (Kessel, 1925)					11	1	Plin
Phyllostomidae							
<i>Artibeus jamaicensis</i> (Leach, 1821)	3173	1498	1283	2.0			Ajam
<i>Nycterophilus parnelli</i> * (Wenzel, 1966)					1	0.03	Npar
<i>Metaspermus pseudopterus</i> (Coquillett, 1907)					60	0.98	Mpse
<i>Aspidoptera phyllostomatis</i> (Perty, 1833)					1197	1.00	Aphy
<i>Megistopoda aranea</i> (Coquillett, 1899)					1264	0.99	Mara
<i>Neotrichobius delicatus</i> * (Machado-Allison, 1966)					1	0.14	Ndel
<i>Paratrachobius dunni</i> * (Curran, 1935)					1	0.01	Pdun
<i>Trichobius yunker</i> * (Wenzel, 1966)					1	0.00	Tyun
<i>Trichobius joblingi</i> * (Wenzel, 1966)					1	0.00	Tjob
<i>Artibeus lituratus</i> (Olfers, 1818)	422	41	35	1.2			Alit
<i>Aspidoptera phyllostomatis</i> * (Perty, 1833)					1	0.00	Aphy
<i>Mastoptera guimaraesi</i> * (Wenzel, 1966)					1	0.02	Mgui
<i>Megistopoda aranea</i> * (Coquillett, 1899)					3	0.00	Mara
<i>Paratrachobius longicrus</i> (Miranda Ribeiro, 1907)					36	0.97	Plon
<i>Carollia castanea</i> H. (Allen, 1890)	354	153	123	1.8			Ccas
<i>Strebla guajiro</i> (García & Casal, 1965)					3	0.10	Sgua
<i>Speiseria ambigua</i> (Kessel, 1925)					60	0.56	Samb
<i>Trichobius yunker</i> * (Wenzel, 1966)					3	0.01	Tyun
<i>Trichobius joblingi</i> (Wenzel, 1966)					156	0.18	Tjob
<i>Carollia perspicillata</i> (Linnaeus, 1758)	543	372	278	2.7			Cper
<i>Strebla guajiro</i> (García & Casal, 1965)					26	0.90	Sgua
<i>Aspidoptera phyllostomatis</i> * (Perty, 1833)					1	0.00	Aphy

(continued)

Table 1. (continued)

Species	N (bats)	Parasitised	#Samples	Mean int. (no cont.)	n (BF)	SI	Abrev.
		<i>Mastoptera minuta</i> * (Costa Lima, 1921)			2	0.01	Mmin
		<i>Megistopoda aranea</i> * (Coquillett, 1899)			1	0.00	Mara
		<i>Speiseria ambigua</i> (Kessel, 1925)			44	0.41	Samb
		<i>Trichobius joblingi</i> (Wenzel, 1966)			657	0.76	Tjob
		<i>Trichobius dugesioides</i> * (Wenzel, 1966)			1	0.00	Tdug
		<i>Trichobius anducei</i> (Guerrero, 1998)			24	1	Tand
<i>Centurio senex</i> (Gray, 1842)	7	0	0				Csen
<i>Chiroderma villosum</i> (Peters, 1860)	27	0	2				Cvil
		<i>Aspidoptera phyllostomatis</i> * (Perty, 1833)			1	0.00	Aphy
		<i>Megistopoda aranea</i> * (Coquillett, 1899)			1	0.00	Mara
<i>Chrotopterus auritus</i> (Peters, 1856)	1	1	1	2.0			Caur
		<i>Trichobius dugesioides</i> (Wenzel, 1966)			2	0.01	Tdug
<i>Dermanura rava</i> (Miller, 1902)	141	21	21	1.2			Drav
		<i>Neotrichobius stenopterus</i> (Wenzel & Aitken, 1966)			26	0.55	Nste
<i>Dermanura watsoni</i> (Thomas, 1901)	260	17	16	1.3			Dwat
		<i>Neotrichobius stenopterus</i> (Wenzel & Aitken, 1966)			21	0.45	Nste
<i>Desmodus rotundus</i> (E. Geoffroy, 1810)	41	30	21	4.7			Drot
		<i>Strebla wiedemanni</i> (Kolenati, 1856)			53	1	Swie
		<i>Trichobius parasiticus</i> (Gervais, 1844)			46	0.88	Tpar
<i>Diaemus youngii</i> (Jentink, 1893)	1	1	1	18.0			Dyou
		<i>Strebla diaemi</i> (Wenzel, 1966)			12	1	Sdia
		<i>Trichobius parasiticus</i> (Gervais, 1844)			6	0.12	Tpar
<i>Glossophaga soricina</i> (Pallas, 1766)	86	28	27	1.6			Gsor
		<i>Speiseria ambigua</i> * (Kessel, 1925)			1	0.01	Samb
		<i>Trichobius uniformis</i> (Curran, 1935)			26	1	Tuni
		<i>Trichobius dugesii</i> (Townsend, 1891)			18	1	Tdug
<i>Glyphoncyteris daviesi</i> (Hill, 1964)	1	0	0				Gdav
<i>Lamproncyteris brachyotis</i> (Dobson, 1878)	10	9	9	5.4			Lbra
		<i>Trichobius joblingi</i> (Wenzel, 1966)			49	0.06	Tjob
<i>Lichonycteris obscura</i> (Thomas, 1895)	2	0	0				Lobs
<i>Lonchophylla robusta</i> (Miller, 1912)	1	1	1	12.0			Lrob
		<i>Eldunnia breviceps</i> (Curran, 1934)			6	1	Ebre
		<i>Trichobius lonchophyllae</i> (Wenzel, 1966)			6	1	Tlon
<i>Lonchorhina aurita</i> (Tomes, 1863)	73	45	26	2.0			Laur
		<i>Strebla altmanni</i> (Wenzel, 1966)			52	1	Salt
<i>Lophostoma brasiliense</i> (Peters, 1866)	1	1	1	3.0			Lbras
		<i>Mastoptera minuta</i> (Costa Lima, 1921)			3	0.01	Mmin
<i>Lophostoma silvicolium</i> (d'Orbigny, 1836)	113	78	69	4.5			Lsil
		<i>Pseudostrebla riberoi</i> (Costa Lima, 1921)			4	1	Prib
		<i>Strebla kohlsi</i> (Wenzel, 1966)			11	1	Skoh
		<i>Mastoptera minuta</i> (Costa Lima, 1921)			281	0.94	Mmin
		<i>Trichobius silvicolae</i> (Wenzel, 1976)			16	1	Tsil

(continued)

Table 1. (continued)

Species	N (bats)	Parasitised	#Samples	Mean int. (no cont.)	n (BF)	SI	Abrev.
<i>Macrophyllum macrophyllum</i> (Schinz, 1821)	5	5	5	3.2			Mmac
		<i>Trichobius macrophylli</i> (Wenzel, 1966)			16	1	Tmac
<i>Micronycteris hirsuta</i> (Peters, 1869)	34	2	0				Mhir
<i>Micronycteris microtis</i> (Miller, 1898)	33	2	3	1.0			Mmic
		<i>Strebla alvarezi</i> (Wenzel, 1966)			2	1	Salv
		<i>Megistopoda aranea</i> * (Coquillett, 1899)			1	0.00	Mara
<i>Micronycteris minuta</i> (Gervais, 1856)	1	0	0				Mimin
<i>Gardnerycteris crenulatum</i> (E. Geoffroy, 1803)	32	22	20	1.8			Gcre
		<i>Basilina tiptoni</i>			35	1	Btip
<i>Phylloderma stenops</i> (Peters, 1865)	8	8	8	7.9			Pste
		<i>Strebla christinae</i> (Wenzel, 1966)			63	0.97	Schr
<i>Phyllostomus discolor</i> (Wagner, 1843)	34	34	34	6.7			Pdis
		<i>Strebla hertigi</i> (Wenzel, 1966)			33	0.97	Sher
		<i>Trichobioides perspicillatus</i> (Pessôa & Galvão, 1937)			99	1	Tper
		<i>Trichobius costalimai</i> (Guimarães, 1938)			96	1	Tcos
<i>Phyllostomus hastatus</i> (Pallas, 1767)	29	29	27	7.4			Phas
		<i>Strebla hertigi</i> (Wenzel, 1966)			1	0.03	Sher
		<i>Strebla mirabilis</i> (Waterhouse, 1879)			55	0.93	Smir
		<i>Mastoptera guimaraesi</i> (Wenzel, 1966)			43	0.98	Mgui
		<i>Mastoptera minuta</i> (Costa Lima, 1921)			13	0.04	Mmin
		<i>Trichobius longipes</i> (Rudow, 1871)			89	1	Tlong
		<i>Trichobius dugesioides</i> * (Wenzel, 1966)			1	0.00	Tdug
<i>Platyrrhinus helleri</i> (Peters, 1866)	28	0	2				Phel
		<i>Megistopoda aranea</i> * (Coquillett, 1899)			2	0.00	Mara
<i>Tonatia bakeri</i> (Williams, Willig & Reid, 1995)	79	46	43	3.8			Tbak
		<i>Strebla christinae</i> * (Wenzel, 1966)			2	0.03	Schr
		<i>Strebla galindoi</i> (Wenzel, 1966)			153	1	Sgal
		<i>Trichobius</i> new sp.			8	1	T.sp.nov
<i>Trachops cirrhosus</i> (Spix, 1823)	59	57	52	6.6			Tcir
		<i>Strebla mirabilis</i> (Waterhouse, 1879)			4	0.07	Smir
		<i>Speiseria ambigua</i> * (Kessel, 1925)			2	0.02	Samb
		<i>Trichobius dugesioides</i> (Wenzel, 1966)			335	0.99	Tdug
<i>Trinycteris nicefori</i> (Sanborn, 1949)	13	10	10	2.7			Tnic
		<i>Parastrebla handleyi</i> (Wenzel, 1966)			22	1	Phan
		<i>Strebla obtusa</i> (Wenzel, 1976)			5	1	Sobt
<i>Uroderma bilobatum</i> (Peters, 1866)	418	141	92	1.4			Ubil
		<i>Metasmsus pseudopterus</i> * (Coquillett, 1907)			1	0.02	Mpse
		<i>Paratrichobius dunni</i> (Curran, 1935)			94	0.98	Pdun
		<i>Paratrichobius longicrus</i> * (Miranda Ribeiro, 1907)			1	0.03	Plon
		<i>Trichobius urodermae</i> (Wenzel, 1966)			28	1	Turo
<i>Uroderma magnirostrum</i> (Davis, 1968)	1	0	0				Umag
<i>Vampyriscus nymphaea</i> (Thomas, 1909)	24	1	1	1.0			Vnym

(continued)

Table 1. (continued)

Species	N (bats)	Parasitised	#Samples	Mean int. (no cont.)	n (BF)	SI	Abrev.
	<i>Paratrachobius salvini</i> complex sp. (Wenzel, 1966)				1	1	PsalB
<i>Vampyressa thylene</i> (Thomas, 1909)	54	5	5	1.2			Vthy
	<i>Neotrachobius delicatus</i> (Machado-Allison, 1966)				6	0.86	Ndel
<i>Vampyrodes major</i> (Allen, 1908)	139	0	2				Vmaj
	<i>Megistopoda aranea</i> * (Coquillett, 1899)				1	0.00	Mara
	<i>Trichobius joblingi</i> * (Wenzel, 1966)				1	0.00	Tjob
<i>Vampyrum spectrum</i> (Linnaeus, 1758)	1	0	0				Vspe
Thyropteridae							
<i>Thyroptera tricolor</i> Spix, 1823	3	0	0				Ttri
Vespertilionidae							
<i>Eptesicus furinalis</i> (d'Orbigny, 1847)	1	0	0				Efur
<i>Lasiurus ega</i> (Gervais, 1856)	1	0	0				Lega
<i>Myotis albescens</i> (E. Geoffroy, 1806)	1	0	0				Malb
<i>Myotis nigricans</i> (Schinz, 1821)	19	11	11	2.3			Mnig
	<i>Basilina anceps</i> (Guimarães & d'Andretta, 1956)				12	1	Banc
	<i>Basilina</i> sp.				10	1	Bsp.
	<i>Basilina ferrisi</i> (Schuurmans Stekhoven, 1931)				3	1	Bfer
<i>Rhogeessa tumida</i> H. (Allen, 1866)	15	0	0				Rtum

(N(bats) = number of bats captured; n(BF) = number bat flies collected; SI = specificity index following Dick and Gettinger (2005); * = non-primary association).

fly genera (or species complexes within the genus *Trichobius*) and identified the modules of this interaction web. Next, we built a trait-space by calculating the distance-based functional diversity index (FD package; Laliberté *et al.*, 2014) of our bat–bat fly matrix and individual host traits (relative host abundance and body mass; Brändel *et al.*, 2020; as well as roosting preferences; Patterson *et al.*, 2007) (Supporting Information Table 1). Three rather rare bat species were not captured during the standardised capture efforts of the large-scale biodiversity project but only during opportunistic netting sessions later in the study period (*Diaemus youngi*, *Lonchorhina aurita*, *Lonchophylla robusta*) (Brändel *et al.*, 2020). We assigned them for our analyses the same host abundance value as for a bat species represented by only a single individual. Finally, we used the obtained module assignment of each bat species to visualise the location of each module in our trait-space.

Results

During this study, we checked 6528 bats belonging to 53 species and 8 bat families for the presence of bat flies. We collected a total of 6077 bat flies from 2801 infested bats. Occasionally, bats were just marked as positive for bat flies ($n = 476$), either when

flies escaped during sampling or when during nights with very high numbers of bat captures, we had to process the animals very quickly to ensure their wellbeing. This approach allowed us to obtain reliable data for overall prevalence (number of infested bats/number of examined bats), even when bats were released directly at the mist net without processing (almost exclusively individuals of the common species *A. jamaicensis* and *Carollia* spp.). Prevalence was highest in bat species using more persistent roosting structures (e.g., caves, hollow trees) and lowest in bat species roosting in leaf tents or open in the vegetation (many Stenodermatinae and most Emballonuridae) (Table 1, Fig. 2) (GLMM, pseudo- $R^2 = 0.69$, $P < 0.001$). Intensity (number of all bat flies on an infested bat) was also positively correlated with roost persistence, mirroring the pattern obtained for prevalence (GLMM, pseudo- $R^2 = 0.68$, $P = 0.001$) (Table 1; Fig. 2).

We identified a total of 6077 individual bat flies belonging to 52 species (Table 1). We collected the bat fly *Strebla obtusa* from *Trinyciteris nicefori* for the first time in Panama. On *Molossus molossus* a single individual of the ectoparasitic bug *Hesperoctenes* sp. (Hemiptera: Polyctenidae) was collected but not further included in the quantitative analysis. We further noticed 161 bat flies infected with ectoparasitic fungi of the order Laboulbeniales (Haelewaters *et al.*, 2017; Liu *et al.*, 2020).

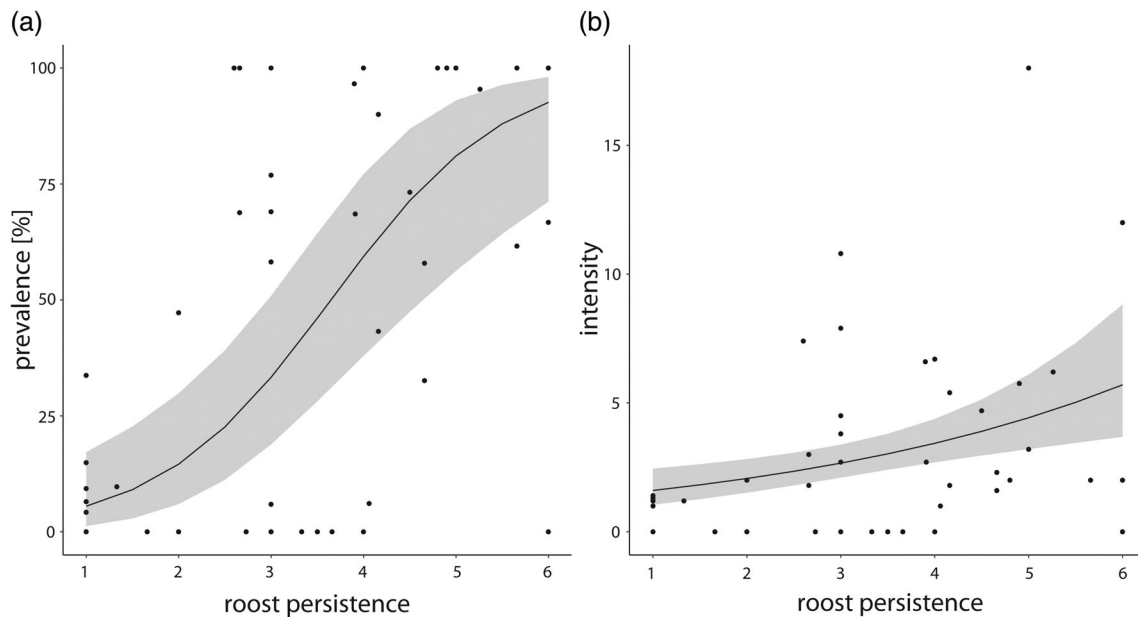


Figure 2. Visualisation of model results for (a) prevalence (%) and (b) intensity (#bat flies/bat) of parasitism plotted against the roost persistence. Shaded areas reflect 95% confidence intervals. For easier visual interpretation, prevalence and mean intensity for each host species are overlaid; however, these values were not used to assess significance.

Calculating the specificity index following Dick and Gettinger (2005) and reviewing published host–parasite records, we identified 35 non-primarily associated bat fly individuals (0.6%) (Table 1; Fig. 3), which were excluded from subsequent analyses. Network specialisation was high ($H_2' = 0.97$, significantly different from random association $t = -1989.1$, $P < 0.001$), which confirms a high host specificity. Additionally, we detected a high modularity in our bat–bat fly network ($Q = 0.78$; Supporting Information Fig. 1, significantly higher than random association, $P < 0.05$). Generalised specialisation indices ($d'_{\text{mean}} = 0.77$) as well as species specificity indices for individual bat fly species ($ssi_{\text{mean}} = 0.98$) were both high (Supporting Information Table 2), indicating a high level of host specificity at the parasite species level.

Pooling the bat flies of phyllostomid bats on genus level and for *Trichobius* on species-complex level lowered the modularity ($Q = 0.63$, significantly higher than random association, $P < 0.05$) and enabled us to investigate parasite interactions in context to host size, host abundance and roosting preferences (Fig. 4). The majority of our identified modules were associated with bats using persistent roosting structures. As such, neither host size nor host abundance appeared to affect module structure. Modules associated with bats using more ephemeral roosts were clearly separated by host size or host abundance.

Discussion

We present here the second largest collection of bat flies in Central America, consisting of over 6000 individuals, and exceeded only by the Smithsonian Panama survey (Wenzel & Tipton, 1966). Including the Smithsonian Panama survey, this is the fifth study

investigating bat–bat fly associations in Panama (González *et al.*, 2004; Estrada-Villegas *et al.*, 2018; Walker *et al.*, 2018). Meticulous parasite sampling in the field resulted in a high-quality dataset emphasising the high host specificity of bat flies.

Bat–Bat Fly associations

By calculating the specificity index following Dick and Gettinger (2005), we identified 35 bat fly individuals (0.6%) in 31 samples most likely to be contaminations and excluded them from further analysis. In the majority of these cases (21/31 samples), when parasites were erroneously associated with non-primary host bats (Table 1), the primary host was captured during the same sampling night. This suggests that these likely contaminations occurred during handling of the bat, during sample collection or while the bats were still in the net, as some bat fly species, especially *Speiseria ambigua*, fly off easily when disturbed (Dick & Gettinger, 2005; Dick, 2007). In the remaining 10 cases, the primary host was not handled during the sampling night and even multiple parasite individuals were recollected from the non-primary host (three cases). To explain these associations with certainty is difficult. They might represent extremely rare, but ‘natural’ alternate host–parasite associations, probably obtained while using the same roosting structures as the primary host (Dick, 2007). However, the occasional occurrence of a single bat fly individual on a non-primary host in the absence of its primary host does not necessarily document a primary association. Therefore, extreme caution is necessary when interpreting uncommon host–parasite associations, particularly on single host individuals, to avoid introducing erroneous host–parasite interactions into the literature. The record of an undescribed species of

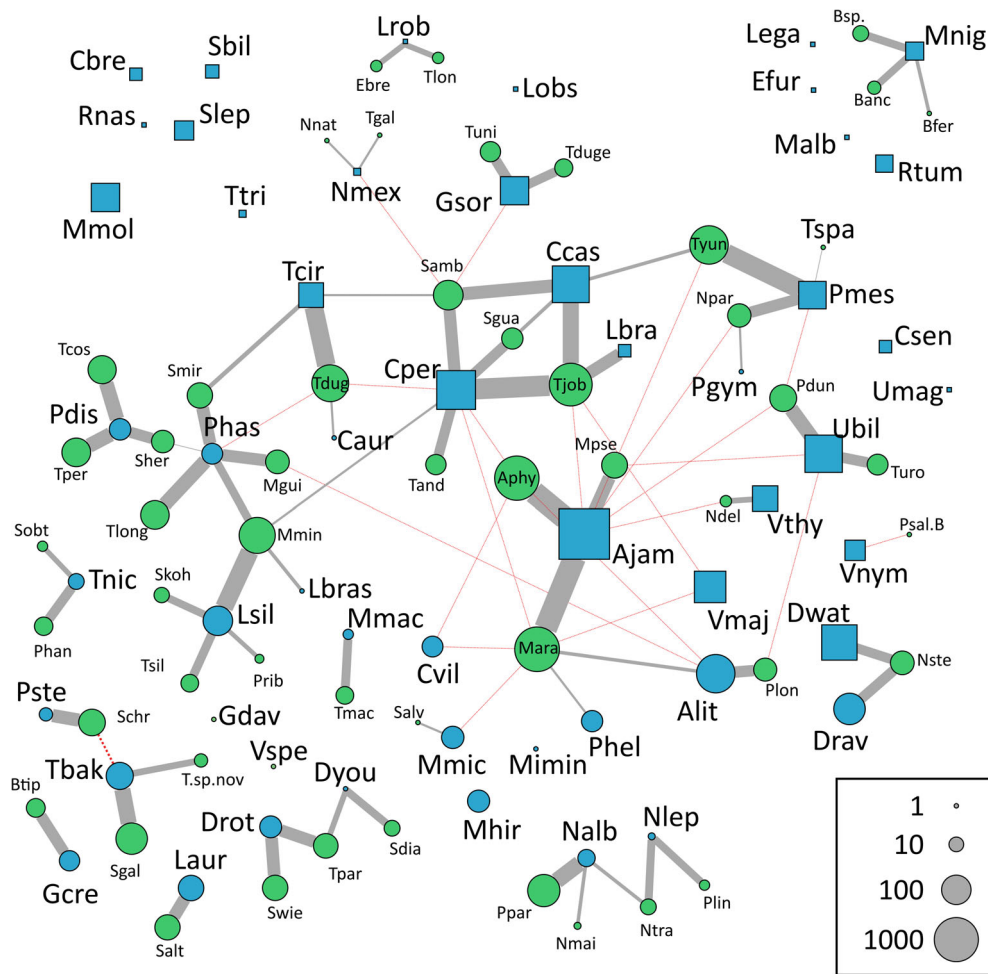


Figure 3. Interaction web of bat–bat fly associations. Width of interactions and symbols are logarithmic transformed. Interactions in red dotted lines are interactions considered non-primary associations. (For species abbreviations see Table 1.)

Paratrichobius on the small frugivore *Vampyriscus nymphaea* is unusual. However, host associations in this bat fly species complex are still unresolved (Wenzel, 1976), and therefore we decided to include this record in our analysis.

During the large-scale ectoparasite study by Wenzel and Tipton (1966) in Panama, four of the here reported 52 bat fly species were not yet reported. Two of them (*Strebla obtusa* and *Trichobius anducei*) were described only later from Venezuela by Wenzel (1976), who also revoked synonymy of the species pair *Neotrichobius delicatus* and *N. stenopterus*. The occurrence of *T. anducei* was suggested to be restricted to the Amazon region by Guerrero (1998a). However, recently, it was reported also from Panama (Walker *et al.*, 2018) and Costa Rica (TH, unpublished data) based on morphological characteristics, and probably it occurs also in Belize (ter Hofstede *et al.*, 2004). The fourth species, a yet undescribed member of the *Trichobius dugesii* species-complex, is known from Costa Rica, Honduras and Panama and currently awaits revision (Miller & Tschapka, 2001; Dick, 2013; Estrada-Villegas *et al.*, 2018). Compared to Wenzel *et al.* (1966), we increased the number of detected bat fly species in Panama from 66 to 70 for Streblidae,

and from 7 to 8 for Nycteribiidae. Given the wide distribution ranges of some bat species as well as their associated bat fly species (e.g., *T. joblingi*, *M. aranea*), it is highly recommended to implement genetic methods in future studies. Only this method promises accurate comparisons of host–parasite associations between different regions or countries.

We detected a strong correlation of prevalence and intensity of parasitisation with roost persistence. As shown by Patterson *et al.* (2007), the use of more persistent roosts by the host bats, such as hollow trees or caves, favours both prevalence and intensity of parasitisation. Values for mean intensity in our study are similar to those reported from Venezuela (Patterson *et al.*, 2007); however, we found a distinctly higher prevalence for most species. This is surprising, because while bats in Venezuela were euthanised and screened for parasites in the laboratory, we collected parasites from live bats, a method that should be more prone to underestimating intensity and prevalence. Comparing our results with other studies from Belize, Brazil, Colombia, or Mexico reveals the plasticity in the overall parasitisation prevalence by bat flies. The prevalence in a single host species varied up to fivefold between studies, without consistent pattern over all host species (ter

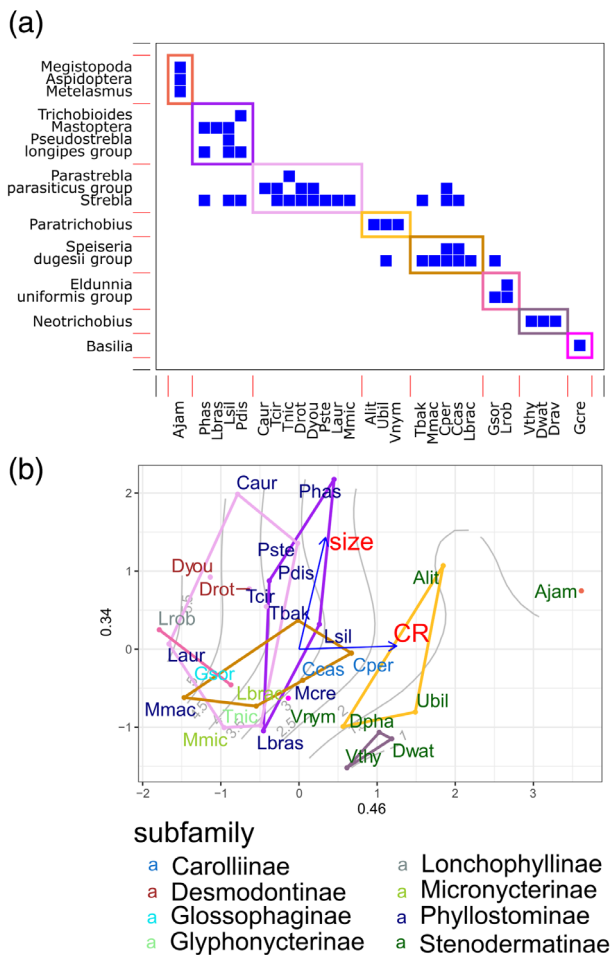


Figure 4. Modular structure of the host–parasite interaction network of phyllostomid bats with their associated bat flies on genus or species group level a) and position of modules in a trait space based on host species characteristics (weight = size, capture rate = CR, roost persistence = contour line). Colour of modules in (a) corresponds to modules in trait space (b). (For species abbreviations see Table 1.)

Hofstede *et al.*, 2004; Cuxim-Koyoc *et al.*, 2015; Calonge-Camargo & Pérez-Torres, 2018; Urbietta *et al.*, 2019). However, in general, there is a shortage of studies reporting robust results on prevalence and intensity of parasitisation, as bat flies are frequently collected rather opportunistically and often the number of checked host individuals is rather small for most bat species. We would welcome future studies to report more extensively and in a standardised way on prevalence and intensity of parasitisation to promote the knowledge on host parasite ecology and to establish a baseline for studies on bat–bat fly biogeography.

Host specificity

The encountered bat–bat fly interactions were highly specific, with 99.4% of all bat fly individuals associated with

their primary host. This value is comparable to studies from Panama, Venezuela and Paraguay (Wenzel, 1976; Dick & Gettinger, 2005; Estrada-Villegas *et al.*, 2018; Walker *et al.*, 2018) and underlines the accuracy of sample collection. The interpretation of the specificity index suggested by Dick and Gettinger (2005) is straightforward. However, for bat fly species parasitising various host species, care must be taken, especially if the host species is rarely captured (e.g., in this study *Chrotopterus auritus* or *Lophostoma brasiliense*) to avoid erroneously excluding correct host–parasite associations. Therefore, host abundance, as well as prevalence and intensity of parasitisation need to be considered when interpreting the obtained indices.

The network specialisation index (H_2') ranges from 0 to 1 and measures the degree of niche complementarity among species and integrates species-level specialisation across the entire community (Blüthgen *et al.*, 2006). In our case, the network specialisation was, as expected, very high ($H_2' = 0.97$) and distinctly higher than in similar studies throughout Latin America ($H_2' = 0.73$; Hernández-Martínez *et al.*, 2019; $H_2' = 0.67$; Durán *et al.*, 2019). Until now only few studies have applied network analysis techniques to bat–bat fly associations and several of these did not include H_2' -values on network level, thus complicating further comparisons (Rivera-García *et al.*, 2016; Zarazúa-Carbajal *et al.*, 2016; Fagundes *et al.*, 2017). Interaction specialisation on species level is described by d' , an indicator of the exclusiveness of a species' niche (Blüthgen, 2010). However, for highly specialised interactions, in which several parasite species are associated with one host species, the interpretability of d' is rather limited, as the host abundance is considered when calculating d' (Dormann *et al.*, 2009; Blüthgen, 2010).

The high host specificity resulted also in a highly modular interaction network. The calculated modularity ($Q = 0.78$) is considerably higher than commonly detected, for instance, in pollination networks (mean modularity $Q = 0.52$ ($n = 51$), Olesen *et al.*, 2007) or in other host–parasite networks (e.g., bat–bat flies: $Q = 0.61$, Fagundes *et al.*, 2017, mean modularity $Q = 0.65$ ($n = 69$), Júnior *et al.*, 2020, or Neotropical fish – endoparasites: $Q = 0.65$, Bellay *et al.*, 2015). Reducing the host species to members of the bat family Phyllostomidae allowed us to use high-quality predictor variables (body mass, relative abundance and roosting preferences) in a subsequent analysis, which were shown to affect parasitism by bat flies (Patterson *et al.*, 2007, 2008). The network modularity ($Q = 0.63$) was lower than in the full network and consisted of eight modules. Although some bat and bat fly taxa were associated with partners outside their modules (e.g. *Strebla* spp.), none were categorised as ‘connectors’ (i.e., linking different modules) or as ‘module or network hubs’ (i.e., species highly linked within their module or the entire network, respectively) (Olesen *et al.*, 2007). This again emphasises the specificity of this host–parasite network. Adding module associations to our trait space based on host characteristics, we found a clear separation of modules associated with bats using more stable roosting sites and bats using tents or foliage for roosting. Host size and abundance appeared to be of less importance. Roosting in hollow trees or caves offers the most stable conditions for bat flies to reproduce (Patterson *et al.*, 2007) and comprises the evolutionarily oldest roosting behaviour, very

common in contemporary phyllostomid bats (Garbino & Tavares, 2018). It is therefore not surprising that the majority of the modules were associated with bats roosting in stable environments. Hereby, bat flies of the genus *Strebla* were only associated with bat species roosting in stable environments, while bats associated with *Strebla* were further separated into three modules, each characterised by bat flies of a distinct *Trichobius* species complex. This observation highlights the importance of stable roosting conditions in the evolution and shaping of bat–bat fly associations.

Although phylogenetically closely related bats often share similar bat fly species (Wenzel *et al.*, 1966), we did not detect a clear pattern for a host-phylogeny - driven module structure. However, bats roosting in leaf tents or foliage all belong to the subfamily Stenodermatinae and are associated with parasites not shared with cave/hollow tree roosting phyllostomid bats. While stenodermatines are further commonly associated with just a single bat fly species, the very common *A. jamaicensis* is associated with three bat fly genera (Wenzel *et al.*, 1966). Although *A. jamaicensis* may use foliage and tents for roosting, it is frequently found in hollow trees and also caves (Morrison, 1979; Rodríguez *et al.*, 2007) that offer more stable conditions for parasite reproduction and thus perhaps leading also to associations with more parasite species. Patterson *et al.* (2007) showed a clear correlation of the persistency of the roosting structure and the number of associated bat fly species.

Another important variable to be considered when interpreting our module structure is which bat species are regularly observed roosting together. Bats of the genera *Lophostoma* and *Phyllostomus* can share the same roost (termite nests) (Tuttle, 1970; Reid, 1997) and are combined into one module with unique bat fly associations (*Mastoptera*, *Trichobius longipes*-complex). The same case can be observed for typical cave/hollow tree roosting species such as *T. cirrhosus*, *C. auritus*, *L. aurita*, *D. rotundus*, and *D. youngi*. They are all associated with various *Strebla* species and members of the *Trichobius parasiticus*-complex. Although our dataset does not cover all bat – bat fly interactions known from the literature for the Neotropics (Wenzel *et al.*, 1966; Wenzel, 1976; Dick & Gettinger, 2005; Gracioli *et al.*, 2019), we see a clear grouping of bat species using the same roosting structures. Adding records from the literature might make this observation even clearer. While *A. jamaicensis* is the only stenodermatine bat species in our dataset associated with three bat fly species from three different genera, these exact same genera are reported for bats of the genus *Sturnira* (Wenzel *et al.*, 1966). Hereby, both *A. jamaicensis* and *Sturnira*-species are also reported to use hollow trees for roosting (Reid, 1997). This suggests that bat–bat fly associations are probably more strongly influenced by roost sharing than by the phylogenetic relationship of their hosts or by co-evolutionary history (Wenzel *et al.*, 1966; Gracioli & de Carvalho, 2012), differing from the common pattern in most host–parasite systems in mammals (Krasnov *et al.*, 2012).

The high host specificity together with the dependency on their host bat's roosting structure renders bat flies highly vulnerable to habitat alterations and sub-sequential changes in host communities. Several studies show that some bat species dependent on old-growth forests go locally extinct with increasing anthropogenic habitat disturbance and forest fragmentation (Meyer *et al.*, 2009; Brändel *et al.*, 2020). Esser *et al.* (2019) found a

similar pattern for host–tick associations, with local host–tick co-extinctions in forest fragments. Bellay *et al.* (2020) have shown that the risk of extinction is much higher in ectoparasites than in endoparasites, as ectoparasites are often more host-specific. Therefore, bat flies might be at high risk of extinction in a changing environment. Not only host–parasite co-extinctions in forest fragments, but also parasites associated with common generalist bat species which can adapt to forest fragmentation show lower prevalence and intensity in anthropogenically influenced areas (Hiller *et al.*, 2020), perhaps due to an altered roosting ecology. Especially in light of increasing efforts for parasite conservation (Carlson *et al.*, 2020), this highly specialised host–parasite system offers a unique model system to study the effects of habitat change on host–parasite communities.

The underlying reasons for this high host specificity are still not well understood. While bat flies can survive in captivity on non-primary host species (Overal, 1980; Caire *et al.*, 1985), they prefer their primary host, when given the choice (Wenzel & Tipton, 1966; Overal, 1980; Esbérard *et al.*, 2005). Also, in roosts like caves that are often shared by various bat species and where newly emerged bat flies need to search for a possible host individual, host specificity remains high (personal observation). Bat flies orientate themselves hereby by various host cues such as carbon dioxide, body heat, and body odours (Loureño & Palmeirim, 2008). However, how bat flies discriminate their preferred host remains unknown. In Panama, individuals of *Phyllostomus hastatus* are parasitised by *Strebla mirabilis* and also share to some degree another bat fly species (*S. hertigi*) with the sister species *P. discolor* (Wenzel & Tipton, 1966; this study). Further north, in Costa Rica and Nicaragua, *S. mirabilis* is then completely replaced by *S. hertigi* on *P. hastatus*, although it still occurs on *Trachops cirrhosis* (Wenzel & Tipton, 1966). The why and how this host switch occur remains, however, still unknown, especially as all three bat species and both parasite species occur sympatrically throughout their geographic distribution (Barquez & Diaz, 2015; Barquez *et al.*, 2015; Miller *et al.*, 2015). The host specificity of these two parasite species for instance is high when seen on country level, however, over a larger geographic region they appear to become less host specific.

In conclusion, we found a highly modular interaction network, illustrating the extraordinarily high specialisation of bat flies on their respective host bats. Further, by pooling the associated bat fly species into higher taxonomic levels, we found roosting structure used by the host bats having an effect on modularity. Specifically, bat species clustering into modules based on associated parasite taxa are also commonly observed roosting together. Their high host-specificity might put bat flies at risk of extinction in changing environments. However, while studies focusing on bat–bat fly interactions have increased in numbers in recent years, studies investigating bat fly ecology and natural history are still rare, but highly important for parasite conservation (Carlson *et al.*, 2020). Of particular value are highly standardised studies on bat–bat fly associations with measures put in place that minimise cross contaminations, such as the single use of bat bags and pre-prepared labelled vials during a capture night. These bat fly collections contain not only information on prevalence and intensity of infestation but also on the distribution of

species, which could depict a first step to conserve this complex host–parasite system.

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Data availability statement

The data that supports the findings of this study are available in the supplementary material of this article

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplementary Fig. 1 Modular structure of the host – parasite interaction network of all bat species captured during this study with their associated bat fly species. (For species abbreviations see Supplementary Table 1.)

Supplementary Table 1

Supplementary Table 2

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