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Unconventional support for a raptorial niche division between Australaves and Afroaves: The distribution of helminths

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A R T I C L E I N F O

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A B S T R A C T

Deep evolutionary relationships within raptorial niche have recently been challenged. Little is known as to whether birds of the raptorial niche share congruent or host-switching communities of parasites. Here, we analyzed the helminth component communities associated with birds of prey and owls. From 1962 to 2015, we examined 1731 birds of prey and owls in Czechia, and we provide a meta-analysis based on the available literature. Both the analysis of newly examined birds as well as the meta-analysis of previous studies suggested low similarities in the helminth component communities in Strigiformes relative to those in Accipitriformes (Sørensen similarity indices 0.380 in Czechia and 0.324 worldwide) or Falconiformes (0.341 and 0.328), as well as low similarities in the helminth component communities in Falconiformes to those in Accipitriformes (0.366 and 0.413). Globally, 59.6% of helminth species found in Accipitriformes, 39.5% of those in Falconiformes and 38.3% of those in Strigiformes were obligate specialists that were limited to a single examined bird order. Another 11.5%, 12.8% and 8.3% of species had core hosts in only a single order. Only five helminth species infected all three bird orders at a similar prevalence. The differences in prevalence cannot be explained by differences in food composition. We provide detailed information on the prevalence, seasonality, age- and sex-specificity, intensity and lethality of helminth infections. In conclusion, we provide the first systematically collected evidence on the congruence of the helminth distribution and phylogeny of the raptorial niche, which is consistent with its split into Australaves and Afroaves.

1. Introduction

A decade ago, Hackett et al. [1] proposed that the raptorial niche has evolved multiple times in birds. These investigators provided conclusive phylogenetic evidence to support the separation of Falconidae and Accipitridae into distinct clades. They suggested that Falconidae and Cariamidae are positioned close to Psittaciformes and Passeriformes, whereas other members of the raptorial niche (Strigiformes, Accipitridae and Cathartidae) formed a separate cluster [1]. These conclusions were later supported by the dated phylogeny of birds presented by Jetz et al. [2]. Most importantly, these conclusions were corroborated by a genome-scale phylogeny by Jarvis et al. [3]. The genomic analysis provided full bootstrap support for Australaves to contain Falconidae and Cariamidae together with Psittaciformes and Passeriformes and the sister clade Afroaves, which contains Strigiformes, Accipitridae and Cathartidae, together with Coraciiformes, Piciformes and other Coraciimorphae [3]. Based on a large-scale next-generation DNA sequencing project, Prum et al. [4] estimated that the two groups, Australaves and Afroaves, evolved during the Paleocene slightly over 62 million years ago. Strigiformes separated from the lineages that led to today's Accipitridae and Cathartidae, which already existed in the Paleocene slightly over 60 million years ago [4].

This long-term partition of the raptorial niche into several independent evolutionary lineages provides a unique possibility to study the congruence between the host and parasite phylogenies, and between the spectra of parasites that affect each particular host within the niche. There are genera of parasites, which occupy the whole available niche. These genera include, for example, helminths of the *Renicola* genus, which are transmitted by fish. Despite their strict host species specificity, they colonized gulls (*R. lari*), terns (*R. sternae*), grebes (*R. pinguis*), penguins (*R. sloanei*), and other fish-eating birds irrespective of their phylogenetic origin [5]. By contrast, there are examples of congruence between the host and parasite phylogenies, which indicate host and parasite cospeciation [6]. However, parasite communities have received little consideration from ecologists, despite the fact that the study of the factors that determine the composition and structure of

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natural communities has always been at the core of ecology [7].

Due to their global occurrence, shared feeding strategies and ancient divergence dates of the individual families, the birds that occupy the raptorial niche provide an excellent opportunity to study similarities among their associated helminth component communities. Several reports, which have mostly originated from Europe and South America, have addressed detailed composition of these component communities. However, most of these reports were rather descriptive [8–10], they focused on a limited spectrum of host species [11–13], or only on some specific groups of helminths [14,15]. It was only Santoro et al. [16] who noticed that the helminth communities in owls and birds of prey may differ from one another. Despite the differences they found in their study cohort in Calabria, the previous study by Sanmartín et al. [9] did not show any such differences in the helminths of owls and birds of prey from Galicia, Spain. The congruence, structure and pattern of helminth component communities in birds that occupy the raptorial niche have received little attention to date.

In this study, we address the congruence of helminth communities in the Falconidae, Accipitridae and Strigiformes. We analyze the prevalence, intensities of infection and diversity of helminth component communities associated with these taxa in the Czech Republic based on our life-long examination of bird cadavers from 1962 to 2015, and we provide a meta-analysis based on the available literature. Combined, we present the first systematically collected evidence of congruence between host phylogeny and parasite distribution in birds that occupy the raptorial niche.

2. Material and methods

2.1. Sampling

From 1962 to 2015, we examined 1731 individuals from 26 species of birds of prey and owls for the presence of helminths. The examined birds consisted of Falconiformes (485 individuals, 4 species), Accipitriformes (810 individuals, 16 species) and Strigiformes (436 individuals, 8 species). All the specimens originated in the Czech Republic (48°39′N–50°59′N, 12°19′E–18°29′E), primarily from the eastern parts of the country. We obtained these dead birds before they were prepared for the Comenius Museum collection (Přerov, Czech Republic). They consisted primarily of wounded, hunted or poisoned individuals, most of which were sacrificed in local rescue stations due to untreatable wounds; the others were delivered dead directly to the Ornithological Station of the Comenius Museum. Our long-term research was authorized by governmental and local authorities; our most recent permit was issued by the Ministry of the Environment of the Czech Republic on 3-Aug-2009 under No. 11171/ENV/09–747/620/09-ZS 25. Some of the examined cadavers belonged to the species that are considered threatened, both at the regional and global scale. However, the study strictly did not include any vertebrates that would be killed specifically for the research purposes. Because the research did not involve live vertebrates, it was not subject to the approval of the ethics committee or institutional animal care and use committee.

We performed full-body necropsies of the bird specimens as described [17]. We fixed the helminths in 70% ethanol, stained them with borax carmine, transferred them through an alcohol series to xylene and mounted them in Canadian balsam. We observed the nematodes in glycerin solution. We recorded the number of each helminth species in each of the examined host individuals. We recorded the age categories of the birds according to their plumage, which allowed us to distinguish between juveniles (birds hatched during the same calendar year as examined) and adults (birds hatched during the previous calendar year or earlier). We stored representative specimens in the Comenius Museum collections (Přerov, Czech Republic). We published detailed morphological descriptions of some of the first host and country records previously [18]; some of the host-parasite records were previously briefly mentioned in [19]. The nomenclature follows the Fauna Europaea database [20] and the more recently published re-classifications.

2.2. Statistical analyses

We computed the rarefaction curves based on the log gamma function for computing combinatorial terms. To estimate the species richness among the host species, we calculated the Chao-1 estimator, which was corrected for unseen species. We calculated the basic diversity indices for each dataset. These data included the total number of species found, the total number of individuals found, the dominance (expressed as 1 – Simpson index, where 1 indicates the complete domination of a single species, and 0 indicates the equal representation of all taxa), the equitability (the evenness measure, in which the Shannon index is divided by a logarithm of the number of taxa, and the Shannon index itself reflects entropy, ranging from 0 for communities with only a single taxon to high values for communities with many species, each with only a few individuals), Fisher's alpha (diversity measure), and the Berger-Parker dominance index (the number of individuals in the dominant species relative to the total number of individuals). To compare the diversity, we used the Shannon *t*-test with a bias correction term [21]. To compare the species richness, we used the Sørensen similarity index. That similarity index and a Chao-1 estimator were calculated in EstimateS 9.1.0; all the other indices were calculated in PAST v. 2.14. As core host order, we defined the order, in which the respective helminth was at least three-times more prevalent than in compared host orders, which were termed satellite hosts. When the differences in helminth prevalence did not exceed the above criterion, all the host bird orders with the helminth prevalence similar to the maximum observed were defined as core hosts. The significance of differences in these diversity indices was tested by bootstrapping. The significance of differences in the prevalence was tested by X^2 tests. The significance of differences in the infection intensities was tested by unpaired one-tailed *t*-tests.

3. Results

3.1. Characteristics of Czech helminth component communities

We collected a total of 25,268 individuals representing 58 species of helminths, which made up the species-specific component communities of five falconiform species, 16 accipitriform species and eight species of owls. The Chao-1 estimator, which was calculated on the basis of the helminth individuals, suggested that our dataset was nearly complete, and it indicated the presence of 19 helminth species in Falconiformes, 41 species in Accipitriformes and 24 species in Strigiformes in the study area (Table 1), which was also corroborated by the rarefaction analyses (Supplementary Fig. S1). The studied component communities displayed significant differences in dominance, with the highest values found in Strigiformes, followed by Falconiformes and Accipitriformes. The helminth component communities in Falconiformes (but not Accipitriformes) showed higher equitability when compared to that of Strigiformes. The helminth component community in Accipitriformes was associated with the highest values for Fisher's alpha index. All of the above differences were significant at $p < 0.05$ or higher when tested by bootstrapping (Table 1). The Shannon *t*-tests indicated that the diversity of helminths in the three analyzed component communities differed from one another. The Sørensen similarity indices indicated that there was low similarity among the helminth component communities in owls relative to any of the component communities in birds of prey (0.341 and 0.380). Interestingly, a similarly low value for the Sørensen similarity index (0.366) was reached when comparing the helminth component communities of Falconiformes and Accipitriformes (Table 1).

3.2. Differences in helminth component communities at a global scale

We next analyzed whether the low similarity in the species composition of helminth communities in falconiform and accipitriform birds also applied globally. The meta-analysis of 19 previously published studies (including the data presented above) revealed a pattern, which closely resembled the findings based on local cohorts of birds only. In the meta-analysis, we analyzed 4855 infection events caused by 215 helminth species, which were found in 4210 individuals from 50 species of birds of prey and owls that were examined on multiple continents. We analyzed the prevalence of helminths in eight falconiform species, 27 accipitriform species and 15 species of owls. The Chao-1 estimator, which was calculated on the basis of the numbers of infection events, indicated the presence of 127 helminth species in Falconiformes, 167 species in Accipitriformes and 79 species in Strigiformes in the 19 combined studies (Table 1). Both the Chao-1 estimator and the rarefaction analysis (Supplementary Fig. S1) indicated that the dataset was incomplete, and more thorough sampling is needed for the under-researched regions. The component community associated with owls displayed significantly higher dominance than those found in both bird of prey groups. The helminth component communities in Falconiformes but not Accipitriformes showed higher equitability when compared to that of Strigiformes. The helminth component community in Accipitriformes was associated with the highest Fisher's alpha values. Lower Fisher's alpha valueswere associated with Falconiformes, and the Fisher's alpha of helminths in Strigiformes reached just half the value of that associated with helminths in Accipitriformes. All the above differences were significant at $p < 0.05$ or higher when tested by bootstrapping (Table 1). Shannon *t*-tests indicated that the diversity of helminths in owls is significantly lower than the diversity in both the analyzed bird of prey groups. The Sørensen similarity indices indicated low similarity in the helminth component community in owls relative to any of the component communities in the birds of prey (0.328 and 0.324). Only a slightly higher value in the Sørensen similarity index (0.413) was reached when comparing the helminth component communities of Falconiformes and Accipitriformes (Table 1).

Many helminth species were limited to only one of the bird orders under examination (Fig. 1). We found this strictly defined specialization in 59.6% of helminth species in Accipitriformes, with 39.5% of helminth species found in Falconiformes and 38.3% of helminth species in Strigiformes. For a large number of other helminth species, the birds from one order served as the core hosts, whereas the others served only as satellite hosts. These species represented 11.5%, 12.8% and 8.3% of helminth species specialized to Accipitriformes, Falconiformes and Strigiformes, respectively. By contrast, we found only five species, which infected all three bird orders at a similar prevalence. These species included *Centrorhynchus globocaudatus*, *Idiogenes flagellum*, *Cyrnea mansioni*, *Synhimantus laticeps* and the trematode *Echinostoma trivolvis*. A total of 12 species infected the falconiform and accipitriform

Table 1

Helminth infections differ in their species composition, species richness and intensity among Falconiformes, Accipitriformes and Strigiformes. We analyzed the data from this study on the basis of a number of infection events (each helminth species was counted just once per examined host individual) as well as the total helminth individuals found. In addition, we summarized the data from all the previous studies, which listed the numbers of infection events and were known to us ([9,12,13,16,26–38]; this study) and analyzed them using identical methods to those used on the data resulting from this study. The selected species diversity indices are indicated. The significance of differences among the bird orders analyzed here was tested by Shannon *t*-test with a bias correction term and by Sørensen similarity index; the significance of differences in particular diversity indices was tested by bootstrapping.

Order index	Falconiformes	Accipitriformes	Strigiformes	Bootstrapping p (F:A; F:S; A:S)	Total
This study:					
Number of host individuals examined	485	810	436		1731
Number of helminth species recorded	19	41	22		58
Number of host species examined	5	16	8		27
Number of infection events	158	1114	227		1499
Number of helminth individuals recorded	655	22,251	2362		25,268
Calculations based on helminth individuals:					
Chao-1 ± SD	19.3 ± 0.9	41.3 ± 0.9	23.5 ± 2.2		59.5 ± 2.2
Dominance	0.207	0.172	0.320	0.001; 0.001; 0.001	0.138
Equitability	0.684	0.579	0.556	n.s.; 0.001; n.s.	0.605
Fisher's alpha	3.659	4.866	3.354	0.04; n.s.; 0.04	7.093
Berger-Parker dominance index	0.350	0.365	0.541	0.001; 0.001; 0.001	0.233
Shannon *t*-test (t; d_f; p):					
Falconiformes vs. indicated cohort		−3.2; 695.8; 0.001	5.4; 1171.5; < < 0.001		
Accipitriformes vs. indicated cohort			15.3; 2777.9; < < 0.001		
Sørensen similarity index:					
Falconiformes vs. indicated cohort		0.366	0.341		
Accipitriformes vs. indicated cohort			0.380		
All studies combined:					
Number of host individuals examined	1006	2355	854		4214
Number of helminth species recorded	86	156	60		215
Number of host species examined	8	27	15		50
Number of infection events	685	3665	544		4894
Calculations based on infection events:					
Chao-1 ± SD	126.5 ± 19.0	166.8 ± 11.0	79.4 ± 10.7		264.9 ± 17.4
Dominance	0.049	0.054	0.088	0.10; 0.001; 0.001	0.040
Equitability	0.793	0.711	0.736	0.006; 0.001; n.s.	0.723
Fisher's alpha	25.99	33.17	17.22	0.05; 0.001; 0.001	46.07
Berger-Parker dominance index	0.108	0.119	0.188	n.s.; 0.001; 0.001	0.094
Shannon *t*-test (t; d_f; p)					
Falconiformes vs. indicated cohort		−1.8; 1087.7; 0.07	6.8; 1125.5; < < 0.001		
Accipitriformes vs. indicated cohort			9.7; 759.0; < < 0.001		
Sørensen similarity index:					
Falconiformes vs. indicated cohort		0.413	0.328		
Accipitriformes vs. indicated cohort			0.324		

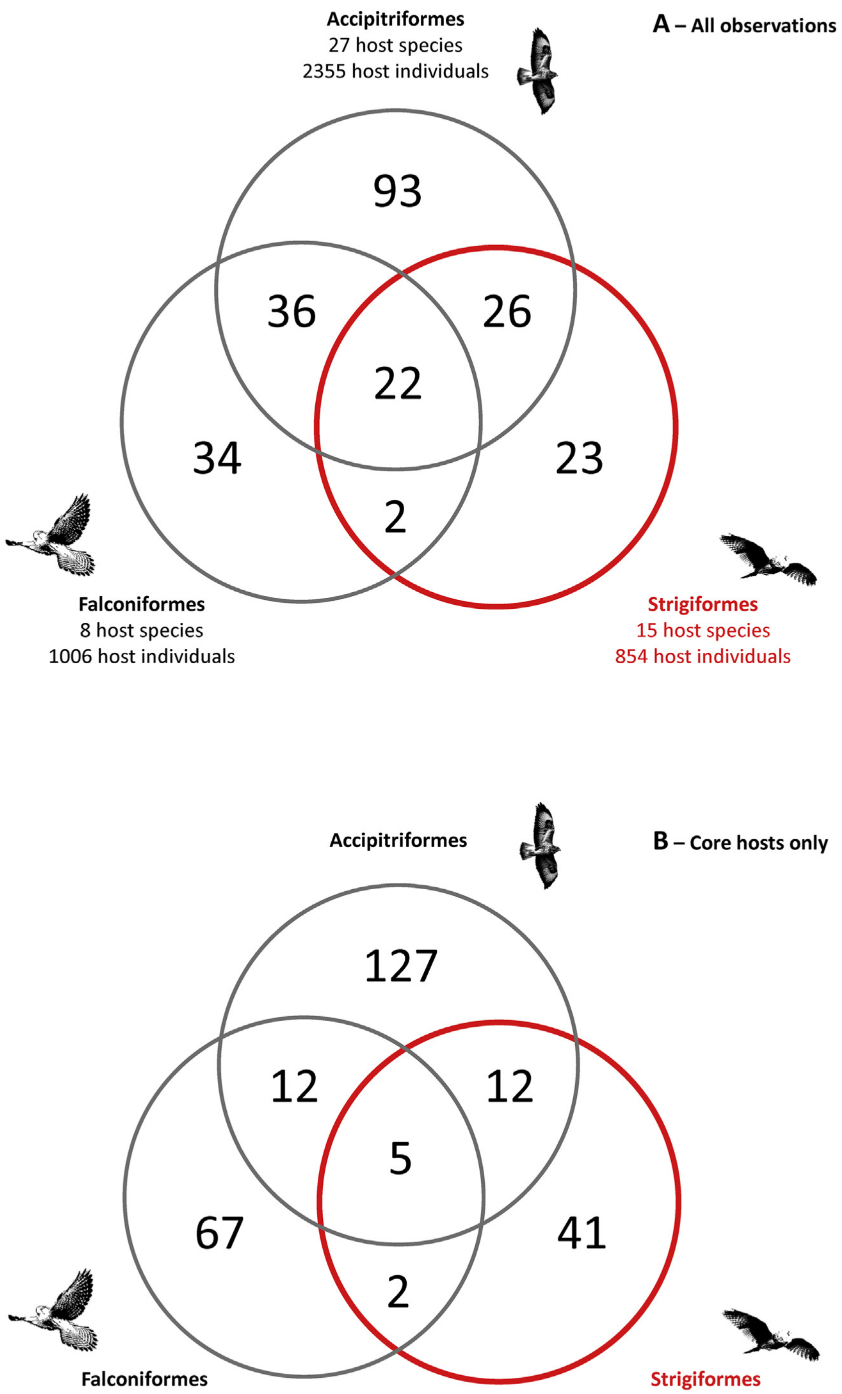

Fig. 1. Host specificity of the helminths infecting the birds of the raptorial niche. Venn diagrams are shown for all studies combined ([9,12,13,16,26–38]; this study). (A) All observations are counted in, which results in relatively low perceived host specificity due to the inclusion of records of helminths in satellite hosts. (B) Observations in core hosts only.

birds of prey at a similar prevalence. Another 12 species infected the accipitriform birds of prey and owls at a similar prevalence. And, finally, only two species infected the falconiform birds of prey and owls at a similar prevalence. Thus, the owls shared five times more species with the Accipitridae than with the Falconidae (Fig. 1).

The CCA analysis (Fig. 2A) suggested that the distribution of helminths among the three examined orders within the raptorial niche can be explained by axis 1 (56.5% of variability), which corresponds to the differences between Accipitriformes and Strigiformes and axis 2 (43.5% of variability), which corresponds to the presence of helminths in Falconiformes. Interestingly, the analysis revealed that there is a discontinuous spectrum of helminths, which would be associated with Falconiformes and Strigiformes but not Accipitriformes, supporting the division of the raptorial niche into Australaves and Afroaves.

To find if the species within the raptorial niche use different food sources, we next analyzed whether the differences in the helminth spectra within the raptorial niche can be explained simply by food composition. We assigned the estimated food composition to each species in the raptorial niche, and we categorized it into the following food categories: mammals, birds, fish, reptiles, amphibians, insects, carrion, and other food sources (e.g., other invertebrates and plants). The CCA analysis (Fig. 2B-C) revealed a complex pattern with axis 1

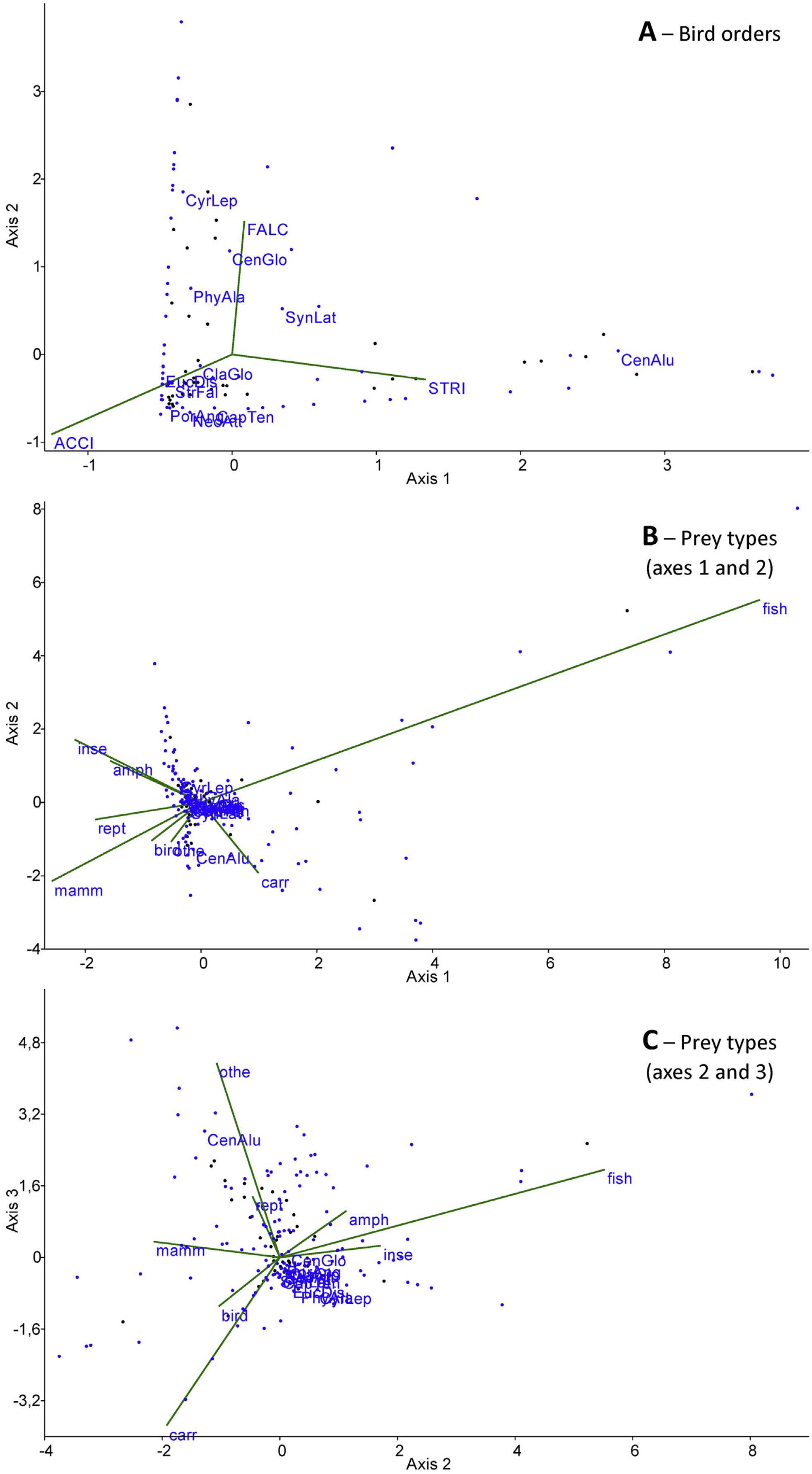

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Fig. 2. The CCA analysis of the distribution of helminths in birds of the raptorial niche. (A) Distribution of helminths among the three examined orders of host birds. (B-C) Distribution of helminths according to the food composition of each analyzed host species, with axes 1 and 2 shown in (B) and axes 2 and 3 shown in (C). For the acronyms used cf. Table S14.

explaining only 28.0% of the variability, axis 2 explaining 17.0% of the variability and axis 3 explaining 16.5% of the variability. The analyses revealed that only the fish, other food sources and carrion are associated with distinct effects on the host specificity of helminths. Importantly, all the dominant helminth species (i.e., those found in 100 or more examined birds worldwide), with the exception of *Centrorhynchus aluconis*, were not associated with any food category (Fig. 2B-C).

3.3. Prevalence and intensities of helminth infections

For the Czech dataset, we quantified the species-specific prevalence (Tables S1-S3) and intensities of the infections (Tables S4-S7). In Falconiformes and Strigiformes, obligate falconiform and strigiform specialists displayed mean frequencies (0.007 ± 0.005 individuals per host, range 0.002–0.016, and 0.097 ± 0.114 individuals per host, range 0.002–0.310, respectively) that were lower than those present in two or more examined bird orders. The species shared by falconiform and accipitriform birds of prey displayed a mean frequency of 0.019 ± 0.020 individuals per host in Falconiformes, and the species shared by strigiform and accipitriform birds displayed a mean frequency of 0.107 ± 0.114 in Strigiformes. The helminths that infected all three orders of birds were the most abundant ones in both Falconiformes (mean frequency 0.086 ± 0.139 individuals per host) and Strigiformes (mean frequency 0.291 ± 0.747 individuals per host). However, we did not observe this relationship in Accipitriformes. The mean frequency of helminths infecting Accipitriformes only was 0.325 ± 0.593 individuals per host (range 0.001–2.103), which was lower than the mean frequency of species shared by Accipitriformes and Strigiformes (1.063 ± 2.544 individuals per accipitriform host) and for all three examined bird orders (0.949 ± 1.993 individuals per accipitriform host); it was higher than the mean frequency of species shared by Accipitriformes and Falconiformes (0.056 ± 0.077 individuals per accipitriform host) (Table S8).

The differences in the mean infection frequencies were caused by both differences in the relative prevalence and intensities of infection. In Falconiformes, the mean intensity of infection by obligate helminth specialists was 1.9 ± 1.1 helminth individuals per host, whereas the helminths shared by Falconiformes and Accipitriformes reached a mean intensity of 10.0 ± 10.1 individuals per host. The mean intensity of infection by generalists was 4.8 ± 4.8 individuals per host. In the other two bird orders, the mean intensities of infections by obligate specialists were not lower than those of the others (Table S8). The relative prevalence of obligate specialists was low in Falconiformes and Strigiformes (0.3 ± 0.1% and 0.6 ± 1.0%, respectively), while it was higher by one order of magnitude in the generalists (2.0 ± 3.1% and 7.4 ± 12.0%, respectively). In Strigiformes, the relative prevalence of obligate specialists and generalists was similar to one another (2.2 ± 4.2% and 2.6 ± 4.0%, respectively; Table S8).

3.4. Lethal infections

Despite the fact that the vast majority of trematode infections are not considered lethal, trematode infection was considered a likely cause of death in 28 of the examined 1727 host birds (Table S9). All but one of these cases were associated with large numbers of the causative species. The only exception was *Euparadistomum falconi*, seven individuals of which caused a lethal infection in *Falco tinnunculus*. In total, we identified 10 species of helminths as being capable of causing the death of the infected birds of prey and the owls. Among them were one acanthocephalan species (*C. aluconis*), one tapeworm (*Cladotaenia globifera*), four roundworm species (*Cyathostoma americana*, *Microtetrameres cloacitectus* and *M. oshmarini*, and the *Porrocaecum depressum/angusticolle* species complex) and four trematodes (*E. falconi*, *Neodiplostomum attenuatum*, *Parastrigea flexilis* and *Strigea strigis*). The most common were the lethal infections *by S. strigis* in *Asio otus* (seven cases) and by *Porrocaecum* spp. in *Accipiter nisus* and *Buteo buteo* (in a total of four cases). The other species caused only one or two lethal infections. However, some such infections may still be of veterinary significance. These include the only recorded infection of *Strix aluco* by *M. oshmarini*, which turned out to be lethal, and similarly, the only recorded infection of *Athene noctua* by *C. americana*, which was lethal as well. There were also only two records of *E. falconi* in *F. tinnunculus*, one of which was lethal. In addition, the infections of *B. buteo* by *M. cloacitectus* and of *Circus aeruginosus* by *P. flexilis* were recorded in only a few cases (10 and 14, respectively), but two of these cases each (20% and 14%) were lethal (Table S9).

In addition to the single causative agents, we also uncovered seven lethal cases of simultaneous infections by large numbers of two to four helminth species. These cases included various combinations of *C. globifera*, *C. spathula*, *N. attenuatum*, *P. depressum/angusticolle*, *S. falconis* and *T. excavata*. In particular, the intensities of infection by *N. attenuatum*, *P. depressum/angusticolle* and *T. excavata* were extremely high in these combined infections. We recorded the highest infection intensities by these three species in host individuals that were affected by combined infections. Hosts infected with a single helminth species tended to have lower intensities of infection than those infected with multiple species. We assumed that the multimorbid individuals represented immunocompromised hosts.

In some cases, the infection severity was intensified by harsh weather conditions and limited food availability, such as that in all seven *A. otus* individuals that were infected lethally by *S. strigis*, which were retrieved in the winter or early spring at wintering grounds where dozens of long-eared owls spent their winter period. They displayed muscle atrophy, followed by death. Lethal *C. americana* infections obstructed the trachea, bronchi and bronchioles, and the infected bird asphyxiated. Lethal *C. globifera* infection caused an obstruction of the intestine by tapeworms, which caused muscle atrophy followed by death. Lethal *E. falconi* infection caused an enormous enlargement of the gallbladder due to the presence of large trematodes inside and the likely poor liver function caused muscle atrophy, followed by death. Lethal *M. cloacitectus* and *M. oshmarini* infections caused the inflammation of the proventriculus; these birds were unable to fly due to exhaustion when arriving at the rescue station. Lethal *P. depressum* infections caused the destruction of the intestinal wall, which was translucent; the infected birds had atrophic muscles, were unable to fly and died within several days after they were brought to a rescue station. All the other lethal infections caused muscle atrophy, followed by death.

3.5. Age- and sex-specific differences in the helminth component communities

We analyzed the age- and sex-specific differences of the nine most frequently examined bird species ($n \geq 32$ examined individuals each) (Table S10). We found that in all the examined species, birds in their first calendar year hosted similar or lower numbers of helminth species than the older birds. However, the mean infection intensities were not necessarily lower in birds during their first calendar year. When analyzing all the host birds together, the age-dependent differences in infection intensities were significant only in *Baruscapillaria falconis* (1y: 20.1 ± 32.3 individuals, adults: 5.7 ± 11.4 individuals, *t*-test $p = 0.004$). However, the prevalence of *B. falconis* did not differ between the first-year birds and adults (X^2 test $p > 0.05$). The age-dependent prevalence of infection were significantly higher in first year birds than the adults in *Cladotaenia circi*, *Neodiplostomum spathoides*, *P. flexilis* and *Tylodelphys excavata*, and lower in *C. globifera*, *Cyrnea leptoptera*, *N. attenuatum* and *P. depressum* (X^2 test $p < 0.05$ each; Table S11).

We found that the female birds hosted a broader spectrum of helminths than the males, and they were also associated with higher mean infection intensity (Table S10). When focusing on the most frequently examined bird species, the above-mentioned pattern in the broader host spectrum that was detected in females was present in seven of the eight bird species that were positive for helminths (the only exception was *F. tinnunculus*). The mean intensities of infection differed among the examined bird species; it was mostly similar in both the examined sexes of each given species with the exception of *C. aeruginosus* and *S. aluco*, in which the females had noticeably higher mean infection intensities. In all the host birds together, the sex-dependent differences in infection intensities were significant only in *B. falconis* (M: 3.8 ± 5.7 individuals, F: 8.5 ± 16.3 individuals), *P. depressum* (M: 5.7 ± 9.9 individuals, F: 12.1 ± 33.4 individuals) and *Synhimantus laticeps* (M: 5.6 ± 5.9 individuals, F: 2.9 ± 2.1 individuals) (*t*-test F vs. M $p < 0.05$ each). The sex-dependent prevalences of infection by *C. aluconis*, *C. globocaudatus*, *C. circi* and *C. leptoptera* were significantly higher in host males, while those by *N. attenuatum* and *Strigea falconis* were higher in host females (X^2 test $p < 0.05$ each; Table S11).

3.6. Intra-annual differences in the helminth component communities

We tested whether the distribution of abundant helminth species displayed any seasonal patterns. Some species, including *B. falconis*, *Porrocaecum spirale* and *Synhimanthus laticeps*, were found throughout the year at a stable prevalence (X^2 test $p > 0.05$ each when tested against the expected values derived from the numbers of birds examined each month). By contrast, the others displayed unimodal distribution, with abundant records in the summer only (*C. globocaudatus* and *C. leptoptera*) or in winter only (*C. globifera*, *N. attenuatum*, *S. falconis* and *S. strigis*). The *Porrocaecum* spp. had bimodal distribution with peak abundance during the spring and autumn months.

The intensities of infection were either similar throughout the whole year, or their peaks co-incided with the prevalence peaks. The infection intensities peaked during the summer for *C. globocaudatus* and the winter for *S. strigis*. Bimodal distributions of infection intensity peaks were identified in *N. attenuatum*. The intensities of infection by *S. falconis* peaked in May and October, but its prevalence was highest in February and March (Table S12).

3.7. New host records

In total, 29 of the host-parasite interactions identified over the course of this study consisted of new host records globally (Table S13). Several host-parasite records were rather incidental; these most likely include, for example, two cases of infection with the mammalian tapeworm *Taenia taeniaeformis*, which may resulted from recent ingestion of an infected rodent instead of representing established infection cases.

4. Discussion

It is now broadly accepted that the raptorial niche has evolved multiple times in birds. However, because this claim is based on studies published from 2008 onwards, little is known about the convergent evolution of their anatomical and histological characters or their symbionts and parasites. Here, we focused on the congruence of helminth communities in the Falconiformes, Accipitriformes and Strigiformes. There are numerous country-specific checklists of helminth species that are often stratified according to the host species, and there are global databases of host-parasite records, such as the one compiled in 1998–2003 by D. Gibson but that is no longer updated.[1] There were numerous studies published on the helminths of birds of prey, but most of them focused on a single bird species or were severely limited by the number of host individuals under examination. Only two studies previously focused on the similarities in helminth communities in birds of the raptorial niche. In this regard, Sanmartín et al. [9] stated that the helminth communities in raptorial niche birds are homogeneous from a qualitative point of view, with very few species that are specific for individual hosts or host groups. They also claimed that the helminth communities observed by them showed a clear relationship to a diet of the host birds. However, the investigators already noticed that even the specialists in vertebrate prey, such as *A. nisus*, have three highly prevalent helminth species, which are transmitted by invertebrates only [9]. Based on the results of the present study, and consistent with the observations by Sanmartín et al. [9] and Santoro et al. [10], we assume that the major prey type may not necessarily be responsible for the whole helminth spectrum found in the respective species, nor for the dominant helminth species found here. In focusing on the above-mentioned *A. nisus*, this species is thought to be a carnivore which preys nearly exclusively on small birds that may form up to 97% of its prey. However, various mammals, reptiles, amphibians, insects and carrion can be eaten as well [22–25]. Thus, these minor parts of the diet are important from an epidemiological point of view. As we show in the CCA analysis, which accounted for the diet types that were weighed according to their estimated proportion in a diet of each respective host species, the specialization in a particular prey (except fish) is not a major driver of infection by specific helminths (Fig. 2B–C). We assume that it is only caused by facultative specialization in the major prey type for most of the analyzed birds. For example, 32 of the 46 analyzed bird species consume insects, but only five of them consume insects as the dominant form of their prey. Similarly, 32 of the 46 analyzed bird species consume reptiles, but the average share of reptiles out of the whole food intake profile does not exceed 20% in any of the analyzed species.

The present study is based on the analysis of the largest amount of birds of prey and owls ever examined. However, the present study was limited to a narrow geographic area in central Europe. Thus, to be able to generalize the study conclusion, we also performed a meta-analysis of all the available quantitative data to date on the prevalence of helminths in birds of prey and owls known to us, which consisted of 20 studies that were published in the English, Russian, Polish and Czech languages [9,12,13,16,26–38]. These studies focused on host birds from four continents, including Europe (former Czechoslovakia, Czechia, Finland, Germany, Great Britain, 2× Italy, Poland, Russia, Slovakia, Spain and Ukraine), Asia (Kazakhstan), North America (5× United States of America) and South America (Chile). The analyzed host birds originated from the present study (41.0%), other studies conducted in Europe (50.6%), the USA (7.5%) and elsewhere (0.9%). Clearly, the currently available information on helminths from birds in the raptorial niche is strongly Euro-centric, and it needs to be supplemented with data from the tropics in particular. These data provide a solid basis for analyzing the congruence of the helminth distribution and the phylogeny of the raptorial niche, particularly the split into Australaves and Afroaves. Given that there are very few specific helminth species that are considered as obligate specialists in individual host species or genera [9,10], it was quite surprising that the analysis of the Czech dataset led to the finding of very low similarities in the helminth communities from the three bird orders of the raptorial niche (Table 1). As Krasnov et al. [39,40] noted, the locally perceived specialization of the parasite could be refuted when examining geographically diversified cohorts of hosts. Nevertheless, the meta-analysis performed in this study led to the same conclusions as the analysis of the single Czech

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[1] http://www.nhm.ac.uk/research-curation/scientific-resources/taxonomy-systematics/host-parasites/

dataset. The helminth communities associated with Falconiformes and Accipitriformes are clearly distinct from one another. They differ somewhat in species richness, with that of helminths in Accipitriformes being the richest and that of helminths in Strigiformes being the least species-rich. The helminth community in Strigiformes also has the highest dominance and lowest Fisher's alpha, which is consistent with previous observations that were published using analyses of local datasets, for example, from Spain [9].

Helminths are increasingly recognized for their significant but hidden diversity [41,42]. Of the estimated total of 75,000–300,000 helminth species, 3% to 5% are threatened with extinction in the next few decades [41]. A large part of these predicted extinctions will be due to the simplification of food webs, because approximately three quarters of the links in the food webs likely involve a parasitic species. The finding that the birds of the raptorial niche host helminths with much more stringent host specificity than previously thought further highlights the importance of preserving these food webs to facilitate the survival of threatened helminth species. There are already bird species that are completely free of helminths even though they contained high helminth loads just decades ago. Among these are typically the mallards *Anas platyrhynchos*, given grain by hunters or fishermen (J. Sitko & P. Heneberg, unpubl.). The food webs of raptors are also subject to change, and numerous birds of prey are now most commonly found at waste dumps (e.g., *Milvus migrans*, *Cathartes aura*, *Necrosyrtes monachus* and *Neophron percnopterus*) [43–47]. For some birds of prey, such as *Gyps fulvus* and *M. migrans*, the waste dumps form important migration stopovers and even winter quarters [48,49]. Further research should thus address the possible simplification and changes in the helminth diversity in their hosts, which display changing or dichotomous feeding strategies.

5. Conclusions

Parasites provide many ecosystem services, including the regulation of their intermediate and definitive hosts` populations [50], and regulation of pollutant concentrations [51,52]. Here, we found that despite only a limited number of helminths are host species-specific, these organisms form distinct communities within Accipitriformes, Falconiformes and Strigiformes. The finding of extensive differences in the species composition of these communities, particularly in Accipitriformes and Falconiformes, provides the first systematically collected evidence of congruence in the distribution of helminths and the phylogeny of the raptorial niche, which is consistent with its split into Australaves and Afroaves. Certain helminth infections in birds from the raptorial niche were lethal, particularly when present in combination with harsh weather conditions and limited food availability. Our findings challenge the current view of the helminth community in birds from the raptorial niche. Thus, the concept of a uniform community with species occurring randomly in various host species in direct relation to the primary prey that are taken by the respective host species should no longer be considered valid in birds from the raptorial niche.

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