Trends in Parasitology

CellPress

Review

Rethinking community-level avian-haemosporidian studies: gaps and frontiers

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Haemosporidian parasites infect birds globally and impact host fitness, ecology, and evolution. Investigating associations between birds and haemosporidian parasites in a community context is critical for decoding eco-evolutionary dynamics and disease spillover risks. Here, we identify the major biases and gaps in community-level avian-haemosporidian research and propose a framework to advance the field. First, we emphasise the need for standardised sampling, integrative diagnostics, and public availability of metadata and sequence data. Further, we highlight priority research areas for the field, including reducing geographic and taxonomic biases, improving phenological knowledge, and integrating vector sampling. These advances are critical for understanding avian-haemosporidian community assembly and transmission dynamics, and for predicting future disease spillover risks.

Highlights

Community-level studies can uncover host range patterns and evolutionary constraints in avian-haemosporidian systems.

Establishing clear definitions of community boundaries, harmonising sampling and diagnostic protocols, and ensuring transparent reporting and data sharing are critical steps to enable meaningful comparisons across studies and regions.

Integrating cutting-edge molecular and genomic tools with traditional parasitology is needed.

Holistic approaches are needed to view hosts, parasites, and vectors as interconnected in dynamic communities.

Combining ecological, physiological, immunological, and behavioural data within community contexts to achieve mechanistic understanding of infection costs, host competence, and transmission dynamics.

Avian–haemosporidian associations in a community context

Birds (class Aves) are infected by a diversity of **endoparasites** (see Glossary), including helminths, protists, bacteria, and viruses [1,2]. Coupled with this, their ability to fly makes them potential mobile disease reservoirs that can transmit parasites and **pathogens** across regions [3]. Among avian parasites, **dipteran vector-borne haemosporidian blood parasites** (some causing **avian malaria**) represent a diverse and cosmopolitan parasite group [4] and have a long coevolutionary history with their bird hosts [5–8]. The effects of haemosporidian infections on birds can vary between species or individuals, as infections can reduce host fitness by influencing reproduction, survival, and physiology, particularly during **coinfections** [9–12]. Studies on single host species can provide valuable insights into species-specific or population-level bird-haemosporidian associations and the consequences of infections (e.g., [13,14]), but host community-level studies are invaluable for untangling the eco-evolutionary complexity of these interactions [15]. Such insights are critical for elucidating the host ranges of parasites (generalists vs. specialists) and the evolutionary constraints on these associations, ultimately allowing us to predict transmission pathways, **spillover** risk, and parasite persistence.

State of the art in community-level avian-haemosporidian studies

Community-level perspectives have become increasingly important in avian-haemosporidian research (Figure 1A,B), as infections arise from the dynamic interplay between host assemblages, parasite **lineages**, and vector communities (Box 1). These interactions vary across communities due to differences in host composition, parasite strategies, geographic history, and vector

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ecology [18–23]. Variation in vector species composition and feeding preferences further amplifies these differences, reinforcing that no component of the system can be interpreted independently [22,23] (Box 1). Yet many field studies remain centred on single hosts or single parasite lineages, overlooking the community processes that ultimately shape infection outcomes, including the vector component. This fragmented approach limits our ability to identify general rules governing parasite diversity and transmission, underscoring the need to integrate community-level studies and develop a coherent framework for future research.

There has been an increase in attention to certain aspects of community-based haemosporidian parasite research, thanks in part to the widely used data repository, **the MalAvi database** [24] (Figure 1), which has recently been optimised and relaunched (https://malavi-db.github.io/index.html#home). Community-level studies have linked infection patterns with host traits, climate, and community composition [18,19,21,25–28]. However, geographic, taxonomic, and methodological biases remain substantial, limiting comparisons among studies. Moreover, most community-level studies have been carried out in temperate areas, with fewer efforts in tropical (outside the Neotropics; e.g., [26,28]) and subtropical environments (Figure 1A), despite the higher diversity of hosts, parasites, and vectors. Due to methodological constraints (e.g., dependence on **mist-netting**), there is a taxonomic bias towards studies of passerines [29]. Furthermore, studies vary in their sampling design and detection techniques, making comparisons across studies difficult and, in some cases, ineffective.

While previous global avian-haemosporidian reviews [4,7,30,31] and meta-analyses [32,33] have evaluated specific hypotheses related to parasite diversity in certain host groups [31] or impacts of habitat on parasite diversity and prevalence [30], the original studies leveraged for these reviews and meta-analyses generally focus on single or a few closely related species. Inconsistencies in field and molecular methodologies, data curation, and analytical methods in the original studies make it difficult to conduct robust comparisons. The avian-haemosporidian field needs to adopt standardised approaches for sampling hosts, detecting parasites, and, crucially, reporting methods and genetic and ecological metadata. Using these standardised practices in community-level research, we can address major conceptual gaps in the drivers of avian-haemosporidian community assemblages, eco-evolutionary associations, and disease spillover risk, advancing the field beyond descriptive work. In this perspective, we highlight approaches for standardising data collection and sample processing in community-level studies, while discussing strengths and limitations of different methods, and provide a roadmap that identifies conceptual gaps in the field and proposes possible solutions.

Towards a standardised framework for community-level research

Any community-level study involves three critical steps (Figure 2, Key figure). First, a proper sampling methodology is paramount for gathering samples to answer specific research questions and test hypotheses. Second, it is imperative to employ robust parasite screening and diagnostic technologies. Third, we need to report our data in a meaningful way and make all data available, adhering to **findable, accessible, interoperable, and reusable (FAIR) principles** [34].

Standardised sampling for community studies

Studies focusing on avian communities should define community boundaries (Box 1), but this can be difficult, as similar principles might not apply to hosts, vectors, and parasites (Box 1; cf. [15]). However, given that it is not possible to sample all individuals equally within a community or across communities, the parameters defining the community must be made explicit. For example, studies should specify seasonal timing, spatial scale, capture methods, and sampling design,

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as variation in these factors can obscure links among hosts, parasites, and vectors (Box 1). Second, we need to specify the sampling effort, such as the number of mist nets or traps used, and capture effort (e.g., sampling days and time spent capturing birds), which allows us to correct for sampling biases.

Most birds can be captured using mist nets. However, the effectiveness of mist nets depends on the presence and movement of birds, and mist nets do not reliably capture all species in a community. Mist-netting introduces strong taxonomic and vertical-strata biases and may under-represent aerial, canopy-dwelling, or large-bodied species [35–37]. Alternative capture methods, such as cannon nets, ground whoosh nets, Bal-chatri traps, Swedish goshawk traps, or automatic bow-net traps [36,37], can be used to target bird species that cannot be captured with mist nets (Figure 2).

The sample type (blood, tissue, or feathers) can influence DNA quality and the accuracy of parasite detection [38–40]. Blood is most commonly used, as it captures parasites during the **intraerythrocytic life cycle** [38]. In contrast, tissues may improve detection of **exoerythrocytic stages** [39], while feathers provide a noninvasive but less sensitive alternative [40]. Although it has been shown that different tissue types do not differ in their ability to detect haemosporidians [41], it is advisable to conduct a careful assessment of sample types to identify the optimal option for haemosporidian screening that aligns with the study goals and sampling efforts.

Screening and diagnosis

Only a minimal volume of blood, in most cases well below the recommended upper limit of 1% of body mass, is required for haemosporidian diagnostics [42]. Traditionally, diagnosis of bird haemosporidians has relied on microscopy [38]. However, with advances in molecular technologies, parasite screening has shifted towards barcoding approaches [24,43]. Both techniques have their own advantages and disadvantages, but to move the field forward we need to adopt an integrative approach.

Molecular methods for parasite diagnosis and identification. The most common practice for haemosporidian screening is to barcode a fragment of parasite mitochondrial DNA (mtDNA). Multiple primer sets have been designed, but the most commonly used technique involves a **nested PCR approach** [44,45] targeting 478–480 bp fragments of the cytochrome b gene (*cytb*). Parasites showing even a single bp difference from known sequences in databases (e.g., MalAvi [24]) are considered new lineages, and hence a lineage detected in a single individual should be carefully verified to avoid biases introduced by sequencing errors. Repeated PCRs and sequencing from both ends are highly desirable in ambiguous cases. However, the most commonly used primers for bird haemosporidian detection were developed for European passerines [44,45], and may not be able to detect parasites in distantly related bird clades [43,46]. This advocates evaluating selected primers for haemosporidian screening of a targeted community, which may lead to the use of host taxon-specific primers [46] or a combination of multiple primer sets [47].

Recently, a **multiplexed PCR approach** has been developed that enables simultaneous screening for *Plasmodium, Haemoproteus,* and *Leucocytozoon* infections [48]. This allows for cost-effective molecular detection through initial screening of individuals using multiplexed PCR, followed by barcoding PCR performed only on positive individuals.

Focusing on a single genomic region of the parasites may not provide sufficient taxonomic and phylogenetic resolution, and this warrants multilocus barcoding or genomic-scale sequencing

Glossary

Avian malaria: a mosquito-borne bird disease caused by the haemosporidian genus *Plasmodium*.
Blood smear: a drop of blood spread thinly across a glass slide, then stained and viewed under a microscope.
Coinfection: when a host is simultaneously infected by two or more different pathogens or lineages of the same pathogen.
Dead-end, noncompetent host: a host in which parasites are unable to complete their life cycles.
Dipteran vector-borne: infections transmitted by blood-feeding flies (e.g., mosquitoes, sandflies, biting midges).
Digital droplet PCR (ddPCR): a molecular technique that performs absolute quantification of a targeted DNA molecule.
Ecotone: a dynamic transitional zone where two different ecosystems meet and integrate.
Endoparasites: organisms that live within a host organism's body while gaining resources from the host.
Exoerythrocytic stage: an infectious stage of a parasite that develops and multiplies within host cells outside red blood cells.
Findable, accessible, interoperable, and reusable (FAIR) principles: a set of international guidelines designed to make research data easily findable, accessible, interoperable, and reusable.
Gametocyte: an immature cell that undergoes division to produce gametes.
Haemosporidian blood parasites: intracellular protozoans within the phylum Apicomplexa that infect the blood of terrestrial vertebrates.
Heteroplasmy: the presence of variable copies of mtDNA within a single individual.
Intraerythrocytic life cycle: the period during which blood-borne parasites infect, develop, and multiply within a host's red blood cells.
Lineages: genetically distinct parasites (can represent the same morphospecies).
The MalAvi database: a comprehensive, public reference database dedicated to cataloguing avian haemosporidians [28].
Mist-netting: a field research technique used by ornithologists and bat biologists to capture wild birds and bats in mid-flight.
Multiplexed PCR approach: involves the amplification of multiple DNA targets simultaneously in a single reaction.

[49–51]. However, these approaches can come with added laboratory and sequencing costs. In addition, high-throughput amplicon sequencing allows the simultaneous detection of multiple parasite variants, thereby improving the identification of mixed infections and revealing hidden genetic diversity [52–54]. Specifically, primers that are compatible with the frequently barcoded *cytb* region of haemosporidians are appropriate, as the resulting lineages can be directly compared with databases such as MalAvi [54]. Although high-throughput sequencing could improve detection of mixed infections, potential biases such as **heteroplasmy** [55] and limited knowledge of parasite mtDNA dynamics warrant further validation [56]. Nonetheless, proper methodological comparisons among different sequencing strategies are needed.

Integration of microscopy. The collection and analysis of high-quality **blood smears** are a critical part of haemosporidian research [57]. This is especially true when working in distinct geographic locations and with undersampled bird communities. As the most commonly used barcoding primer sets [44,45] were originally developed to detect parasites in European passerines, we may overlook parasite lineages in samples from other geographic locations. Screening blood smears, therefore, increases confidence in presence/absence data in a given host community. Conversely, PCR may detect nonpatent infections by detecting sporozoites that may be present within the bloodstream of a **dead-end, noncompetent host** [58]. The inclusion of such false host–parasite links can be avoided by screening PCR-positive slides to confirm new host–parasite links through the presence of **gametocytes** [58]. Moreover, detecting a specific parasite lineage across multiple individuals of a host species within a community, even at low parasitaemia, may confirm active local transmission. Coinfecting parasites can also be detected more readily by microscopy, but only if hosts are not infected with multiple lineages of the same morphospecies.

Microscopy has its own challenges, which are twofold. First, there are logistical challenges related to the creation of high-quality smears, including fixing slides as soon as they are dry and staining

Nested PCR approach: a two-step amplification technique designed to drastically increase both the specificity and sensitivity of DNA detection.
Pathogens: any biological agents that cause disease or damage to their hosts.
Quantitative PCR (qPCR): a molecular technique that amplifies and simultaneously measures the amount of a targeted DNA molecule in real time.
Spillover: transmission of a parasite from a primary reservoir host species to a novel or incidental host species. Unlike an evolutionary 'host switch' (which implies permanent establishment and ongoing transmission in the new host), spillover often results in transient infections, varying pathology, or dead-end hosts, though frequent spillover events can act as an ecological bridge towards long-term host switching.
Sporozoites: motile, infective cells formed during the life cycle of haemosporidian parasites.

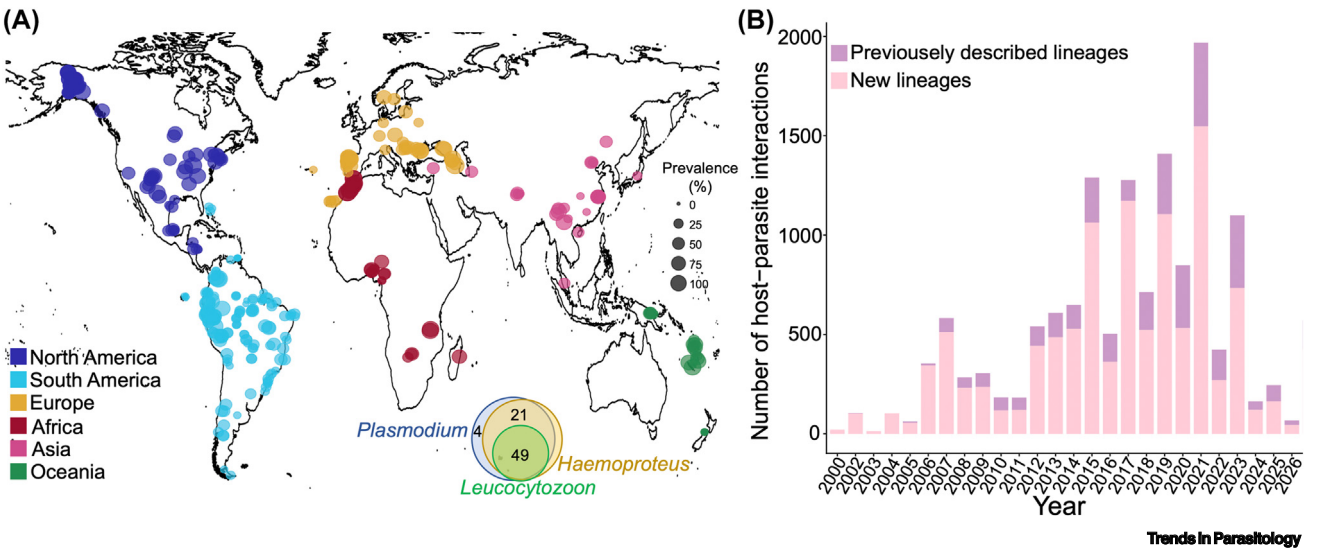


Figure 1. Current state of community-level avian-haemosporidian studies. (A) The bubble plot depicts haemosporidian prevalence (bubble size) in bird communities studied across the globe (community data were extracted from 74 published studies; Box 3 and Table S1). The world map in the background was created with coastline data from Natural Earth (https://www.naturalearthdata.com/). Venn diagrams depict the number of studies investigating the three avian-haemosporidian genera (*Plasmodium, Haemoproteus*, *Leucocytozoon*). (B) The number of bird–haemosporidian associations reported in the MalAvi database over the last 25 years. Described lineages are haemosporidian lineages identified and described in previous studies, while new lineages refer to the first description of a lineage (Note: a lineage is only considered a new lineage in the year it is first described and will be referred to as a described lineage in subsequent years).

them within 1 month of collection [38]. This is even more challenging in humid and tropical areas [59]. Moreover, time and expertise are needed to accurately screen slides. Second, wild-caught birds (e.g., through mist netting) may not represent birds experiencing higher infection intensities, which can lead to missing the detection of parasites using microscopy alone. Thus, to move the field forward, we should adopt an integrative approach combining molecular methods and microscopy to properly screen avian-haemosporidian parasites (Figure 2).

Relevance of quantifying coinfections and their intensity. As molecular approaches have been commonly used in community-based studies to determine infection status, reports of coinfections involving different parasite lineages or even species (as indicated by double peaks in sequencing) are sometimes biased [53]. This lack of coinfection detection may result from PCR primer sensitivity and sequencing bias [53] or from ecological variation among parasite lineages [60,61]. Moreover, positive coinfection results may not be reported due to the impossibility of assigning both lineages correctly. Therefore, in these cases there can be a bias towards reporting only single infections. Molecular method optimisations, however, have identified PCR assays that can guide proper identification of coinfecting genera [47] and can solve issues associated with mixed infections when using the most frequently used primers [45].

Although little is known about the host-specific effects of a given parasite lineage, virulence has been shown to vary among lineages and between bird species [60]. Hosts that sustain high parasitaemia may be more likely to transmit parasites to dipteran vectors, whereas chronically infected hosts may serve as long-term reservoirs [18,62]. Moreover, coinfections might lead to the emergence of costs undetectable in single infections in terms of survival, reproduction, or performance [63,64]. The costs imposed by haemosporidian parasites depend on infection intensity, which is often under-reported in community studies due to the need for **quantitative**

Box 1. What is a 'community' in avian-haemosporidian studies?

The term 'community' is often used in wildlife haemosporidian studies, but rarely is it explicitly defined. Avian-haemosporidian communities consist of three key groups of organisms (hosts, parasites, and vectors), all of which vary in their presence and diversity over time and space (Figure I). Therefore, clarifying what defines a given community, when the community was sampled, and where the community boundaries lie is critical for understanding patterns of haemosporidian parasite diversity. We acknowledge the difficulty of defining exact community boundaries, as natural geographical and temporal limits for host, parasite, and vector communities can vary among one another and may be interdependent or depend on the spatiotemporal scope of the study. For example, the sampled parasite community will be directly associated with the sampled host or vector communities. Nevertheless, providing sufficient and specific detail on the spatiotemporal boundaries of the studied communities will allow for proper comparisons between community-level analyses. Finally, analytical standardisation must accompany field protocols. Studies should explicitly account for imperfect parasite detection (e.g., using occupancy or detection–probability models) and report sample sizes and statistical power to ensure robust prevalence estimates and meaningful cross-study comparisons.

Host community

The host community comprises the bird species that harbour haemosporidian parasites. Haemosporidian parasite communities can vary in their diversity and prevalence across host species, space, and time. Consequently, host diversity within a given sampling site shapes parasite diversity by defining the available host environments and transmission opportunities.

Parasite community

The parasite community consists of the assemblage of avian-haemosporidian parasites (e.g., genera *Plasmodium, Haemoproteus*, and *Leucocytozoon*) circulating within the host community. Haemosporidian parasites are defined as distinct 'lineages' based on variation in the partial mitochondrial *cytb* gene, while parasite species are based mainly on morphological traits. The parasite communities are shaped not only by host and habitat specificity, but also by vectors. These communities can therefore vary substantially across geographic space.

Vector community

The vector community comprises the blood-feeding arthropods capable of transmitting avian-haemosporidian parasites. They include mosquitoes, biting midges, hippoboscid flies, and black flies. Haemosporidian-transmitting arthropods differ in host preference, seasonal activity, and vector competence (i.e., their ability to acquire and transmit parasites). Consequently, a given vector species may transmit haemosporidian parasites to only a subset of host species within a given community.

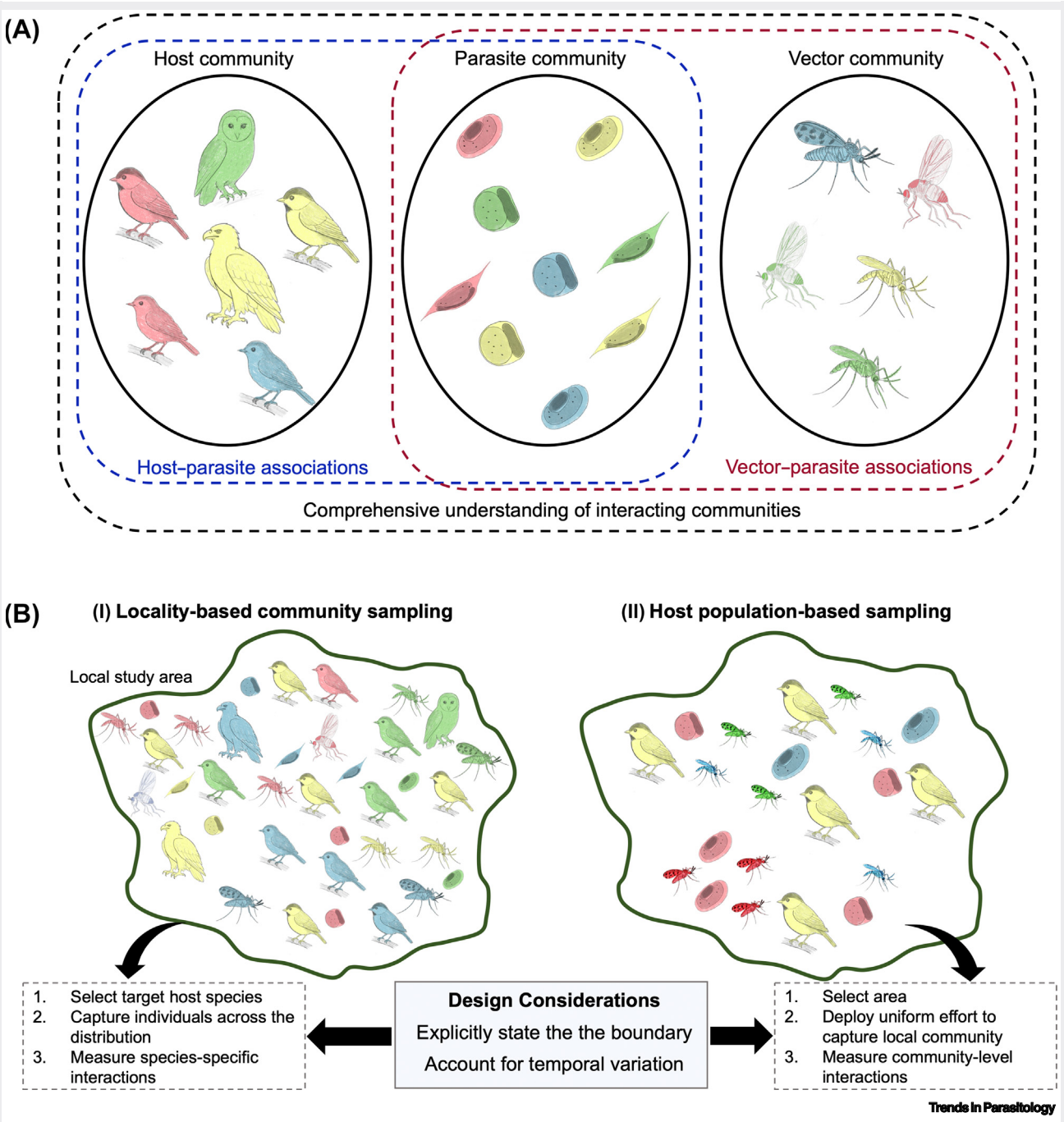
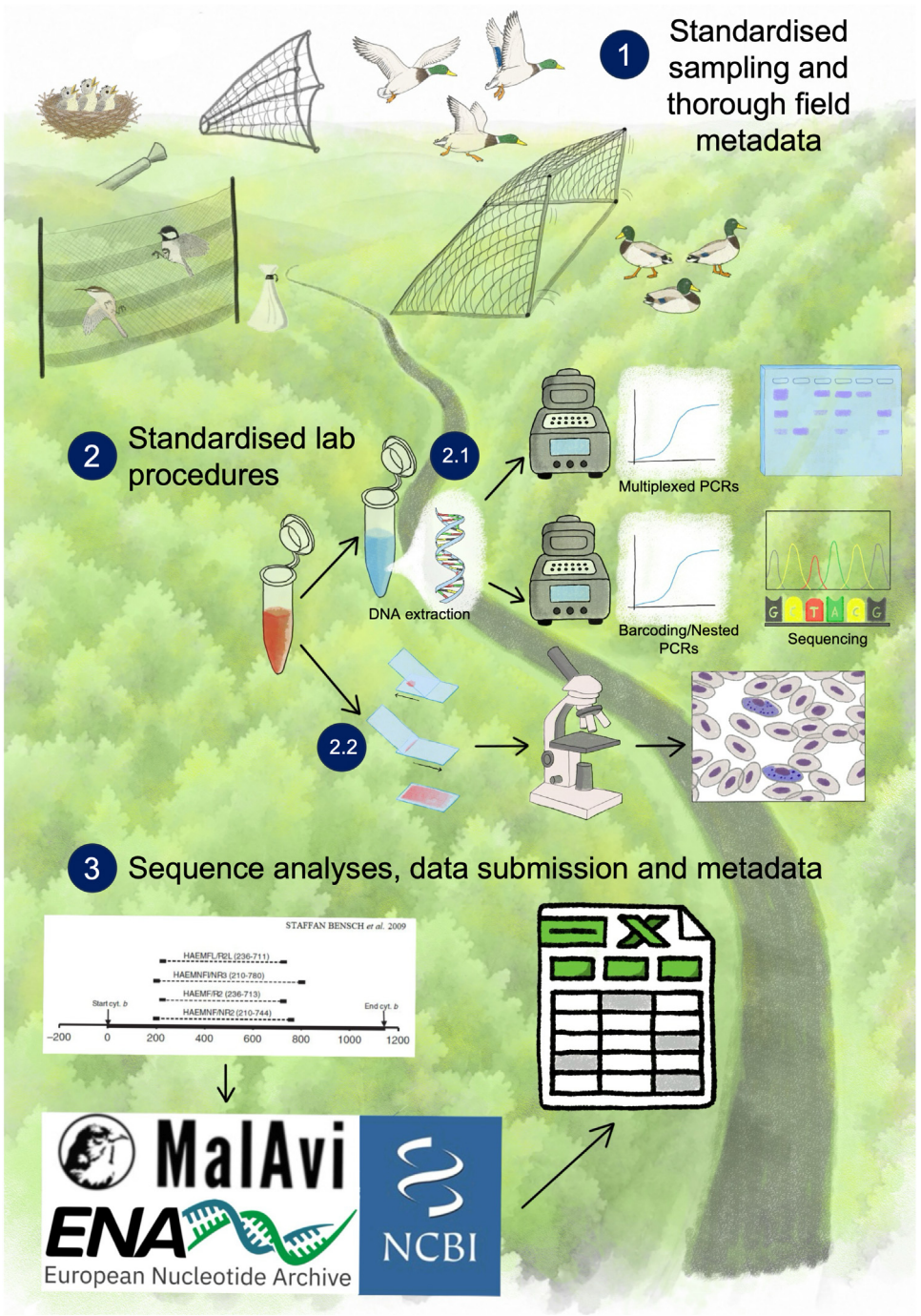


Figure I. Defining the sampling community in wildlife haemosporidian systems. (A) Hosts, parasites, and vectors comprise the sampling community and are bounded by space and time. Links among these groups vary across seasons, years, and geographic contexts. Therefore, the absence of observed interactions may reflect sampling design rather than a true ecological or evolutionary disconnect. (B) Haemosporidian interactions can be investigated with community-centric or host-species-centric approaches. Either approach requires a clear definition of spatial, temporal, and taxonomic boundaries. Such explicit definitions are critical for interpreting patterns of haemosporidian parasite diversity and transmission across space and time. Illustrations are drawn by ©Pablo Quiles Tundidor.

Key figure

Critical steps in a community-level avian-haemosporidian study



(See figure legend at the bottom of the next page.)

PCR (qPCR), **digital droplet PCR (ddPCR)** or microscopy [65,66]. Consequently, prevalence data alone may obscure variation in host competence and transmission potential [67]. Thus, we need to start coupling parasite quantification through either microscopy or molecular methods [48,66] with prevalence data. Moreover, it is important to take temporal aspects of parasite intensities into account, as acute infections usually show higher parasitaemia and associated costs than chronic infections, which vary seasonally in temperate environments [68,69]. Further, infection costs can differ among host species within the same community, potentially shaping population dynamics and parasite transmission [70]. Thus, community-level studies should adopt integrative approaches where both lineage prevalences and intensities are measured.

Data curation and management

Effective data curation and inclusion are essential for ensuring that research outputs remain usable, comparable, and reproducible over time (Figure 2). A key part of this process is standardising metadata and consistently including individual-based supplementary files with variables such as sampling location (e.g., GPS (Global Positioning System) coordinates), collection date and method, host species, sex and age, sequencing methods, gene(s) and primers used for barcoding, success of amplification, assignment to a known lineage, and details concerning microscopy verification. Although genomic methodologies are rapidly advancing within the field [47,51], the lineages defined by the partial *cytb* sequence remain the 'gold standard' that should be carefully followed [24].

Detailed datasets that include uninfected individuals are essential for accurate prevalence estimates. For example, some studies provide the numbers of infected individuals pooled across parasite lineages without reporting the number of uninfected individuals. Consistent data inclusion and formatting enable the integration of datasets from different studies, supporting comparative research and meta-analyses.

Requiring data to be uploaded to public databases like MalAvi [24] is crucial for ensuring standardised lineage nomenclature and avoiding duplicate naming [71]. However, while these short *cytb* barcodes provide an indispensable baseline for lineage tracking, they should not automatically be treated as distinct species, as they lack the resolution needed to resolve deep phylogenies or fine-scale eco-evolutionary dynamics [47]. Moving the field forward will require integrating this standardised MalAvi framework with higher-resolution genomic tools (e.g., full mitogenomes or targeted multilocus amplicon panels) alongside fine-scale environmental and microclimatic data. Finally, to maximise comparability, we highly recommend that researchers follow these sequence standardisation practices while fully reporting metadata for both infected and uninfected individuals in supplementary materials.

Roadmap for going beyond the current state of the art

Despite the recent surge of studies investigating community-level bird-haemosporidian dynamics, there are many major gaps that need to be addressed to drive the field forward. These gaps include, but are not limited to, taxonomic biases and geographic underrepresentation, phenological and life cycle data sparsity, limited integration of vector communities, and the lack of integrated approaches across parasitology, immunology, and host

Figure 2. Road map highlighting the three key steps (1) sample collection and design (note that sampling techniques vary across passerine and nonpasserine taxa, but standardisation lies in reporting uniform sampling effort metrics); (2) laboratory processes for parasite detection; and (3) sequence analyses and data management) that need to be standardised to improve the reproducibility, accessibility and comparability of community-level studies. Illustrations are drawn by ©Pablo Quiles Tundidor.

genomics. Only by filling these gaps can we properly understand the coevolutionary dynamics and spillover risks of these complex bird–haemosporidian–vector interactions.

Taxonomic biases and geographic under-representation

Large-scale biogeographical sparsity. Geographic under-representation is a major issue in avian-haemosporidian research, which strongly influences our global perspective. Community-level studies are more extensive in Europe and North and South America (Figure 1A) compared to other regions, largely due to easier access to sampling sites, greater funding opportunities, and logistical convenience. However, some parts of Africa, parts of Asia (e.g., Central and Southeast Asia), and Oceania remain understudied (Figure 1A) despite their high biodiversity [4,8]. Even in regions that have been more extensively studied, data remain imbalanced due to unequal sampling effort between countries or suboptimal placement of sampling sites relative to the size of the study area. Moreover, in some of these areas, specific lineages have not been characterised (e.g., in MalAvi). These limitations make comparative community-level analyses challenging and result in fragmented insights regarding parasite distribution and/or host range. While studies of haemosporidian diversity with broad geographic coverage can be found [72,73], they either centre around one geographic region (e.g., the Americas) or focus only on a single host species [74,75]. Thus, to reduce our fragmented understanding of biogeographic trends, we need to advocate for community-level studies that encompass broad geographic coverage and turn our attention to previously unstudied areas. To achieve this, it is essential to foster equitable global partnerships that support local research infrastructure, build screening capacity within under-represented regions (e.g., Africa, Asia, and Oceania), and secure dedicated funding models that empower local scientists to lead community-level studies in their own regions.

Aligning evolutionary and ecological processes with fine-scale environmental drivers. Ecological factors play a key role in shaping host–parasite communities [76], and, therefore, areas such as transition zones or **ecotones** often experience increased interactions among species [77], resulting in unique community assemblages (Figure 3). Similarly, habitat alteration due to anthropogenic activities can shape these communities by increasing contact among species that were previously isolated [78,79]. Similar to ecotones, fragmented or altered habitats can act as zones of elevated evolutionary pressure, as the risk of parasite spillover to new host species is higher [80] (Figure 3). These transition zones may act as filters for hosts, parasites, and vectors [81], creating new opportunities for interactions in adjacent, similar environmental patches. Consequently, areas with similar ecological factors that are separated by a transition zone may have distinct parasite networks (Figure 3). However, there is limited knowledge (but see [35]) about how natural and human-induced habitat alterations and transitional zones impact bird–haemosporidian interactions. Such investigations will provide invaluable insights into fine-scale effects of environmental changes on eco-evolutionary dynamics between birds and haemosporidian parasites.

Birds and vectors are not distributed homogeneously along the vertical axis, with species utilising specific forest strata, leading to differential host–parasite associations in canopy and understory habitats [35]. However, most researchers focus on sampling the lower strata due to methodological constraints, thereby biasing the sampling of haemosporidian parasites. Despite the challenges associated with sampling in higher forest strata, we need to design community-level studies that sample birds and vectors inhabiting all forest heights through the use of canopy netting for birds [82] and aerial collection of vectors [83].

Gathering samples from existing programmes and facilities. Bird ringing programmes are an underused resource for avian-haemosporidian research (but see [84]). Coordinated national and international ringing authorities such as EURING (European Union for bird ringing), AFRING

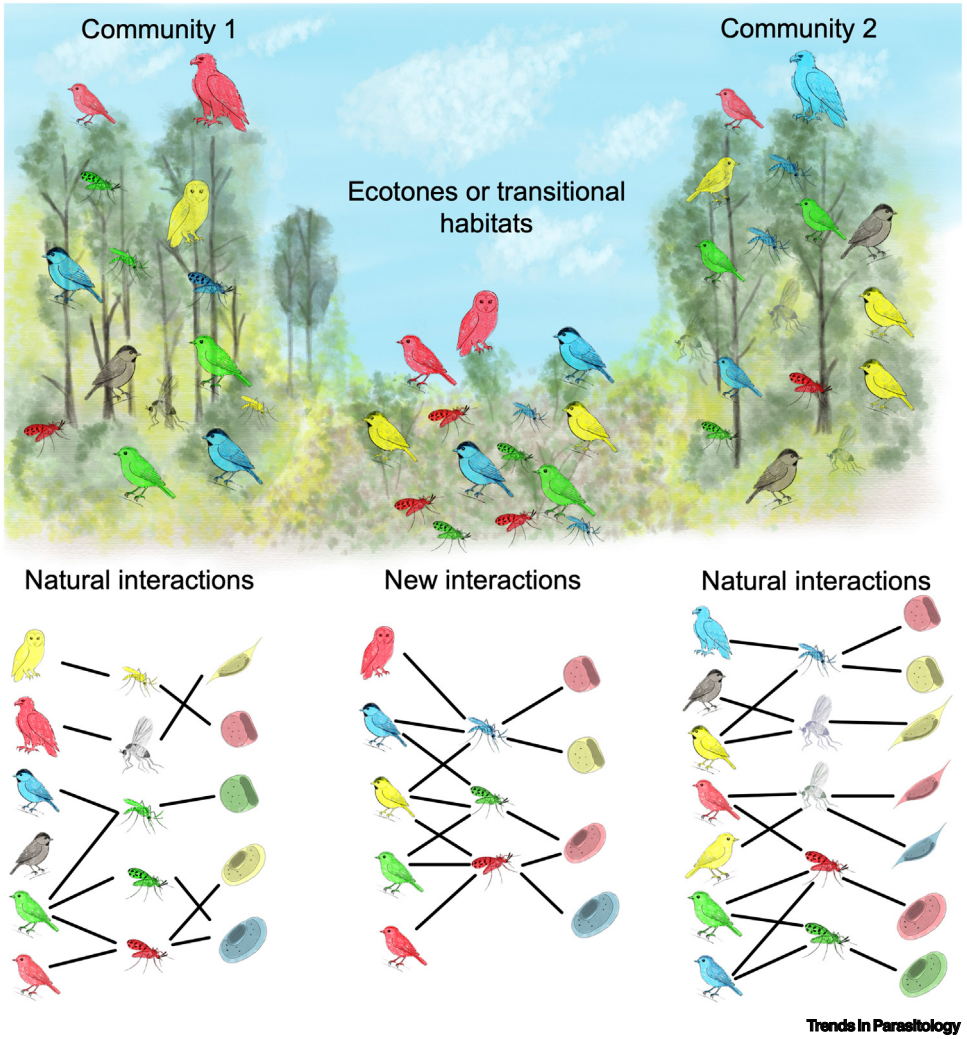


Figure 3. Fine-scale impacts of habitat changes (e.g., ecotones, transitional zones, and fragmentation) on bird–vector–haemosporidian associations. The top panel illustrates shifts in bird and vector communities in an ecotone or a transition zone between two habitats (communities 1 and 2). The bottom panel illustrates a hypothetical scenario of how fine-scale habitat change can influence tripartite interactions between birds, vectors, and haemosporidian parasites. Illustrations are drawn by ©Pablo Quiles Tundidor.

(the African bird ringing project), and the North American Bird Banding Program provide access to millions of birds annually across broad taxonomic, spatial, and seasonal ranges [85]. Migration stations, constant effort sites (CES), and winter ringing schemes offer standardised, multispecies sampling opportunities and longitudinal data on abundance, productivity, and survival [86]. Furthermore, partnerships with bird banding programmes, such as Monitoring Avian Productivity and Survivorship and Monitoring Neotropical Migrants in Winter, both governed by the Institute of Bird Populations (https://www.birdpop.org/), can provide opportunities for establishing long-term community-level studies. However, many programmes rely on volunteer ringers lacking formal training in blood or tissue collection, while licencing restrictions and associated costs may limit widespread training. Thus, either dedicated sampling training (e.g., citizen science efforts that have an initial training component) or collaborations with trained personnel are needed to ensure ethical and standardised collection procedures.

Rehabilitation centres can further complement sampling by providing access to under-represented taxa such as raptors, nocturnal birds, seabirds, shorebirds, and cryptic species [87–89]. These centres also allow large-scale and, in some cases, longitudinal screening at relatively low cost. However, birds admitted to rehabilitation centres represent a nonrandom subset of the community, often under-representing small passerines and potentially introducing artificial host–parasite associations through infections acquired during rehabilitation [90]. Additional spatial and temporal biases may arise from uneven distribution of centres, human population density, collision-prone infrastructure, and seasonal recruitment patterns. Likewise, dead birds submitted for necropsy provide opportunities to detect lesions and exoerythrocytic stages in internal organs missed by blood smears or PCR from live birds [91,92], although these data share many of the same taxonomic, spatial, temporal, and detection biases. Consequently, data derived from rehabilitation centres and necropsies should be treated as complementary surveillance layers rather than unbiased representations of wild bird communities.

Museum collections (both blood and whole specimens) are another available resource for acquiring samples from birds across space and time [41,93]. Given that specimen collections for museum expeditions use methods beyond mist netting to capture individuals (e.g., shooting), they provide access to bird species that cannot be captured with standard methods [93]. This can help sample near-complete bird communities in a given area. Moreover, as museums house samples collected across many years, in some cases many decades, they represent an invaluable source for investigating long-term temporal changes in haemosporidian parasites. Even though these blood and tissue collections can be used for molecular detection of haemosporidians, museum tissue samples may not be ideal for haematology. This is because current museum preservation methods (e.g., in ethanol) can impact the cellular integrity of tissues. Thus, we need to generate new collaborations with museums to capitalise on their expeditions to create endoparasite-focused collections that include blood smears and proper preservation of tissues for haematology. Taken together, forming research partnerships with museums can strengthen our understanding of the spatiotemporal dynamics of haemosporidian associations in bird communities.

Sparsity of phenological and life cycle data

Most community-level studies are carried out during the breeding season, with fewer studies focusing on migration [94,95] or wintering grounds [55]. Moreover, there are even fewer longitudinal (capture–recapture) studies throughout the year in wild birds that could help us to better understand infection dynamics in natural conditions. Host behaviour and biochemistry (and therefore susceptibility to infection) can drastically shift before, during, and after a breeding attempt [96]. While temperate bird communities may show some degree of breeding synchronicity [97], this is not necessarily the case in the tropics [98]. As a result, host susceptibility to haemosporidian infections or relapses in infection may be drastically different at different times of the year, depending on the host species. This could skew observed patterns of prevalence and host breadth for haemosporidian lineages. One solution to tackle this is to capitalise on ringing and monitoring programmes along migration flyways (see Gathering samples from existing programmes and facilities).

There is also a lack of insights into how host–parasite interactions change throughout the host life cycle. Because bird community sampling is typically opportunistic, studies mainly focus on adults, leaving early-life infections poorly understood. Sampling nestlings is challenging because nests can be difficult to locate and access, mortality is often high, and some species (e.g., passerines) must be sampled very early, before patent infections become detectable, due to the prepatent developmental stage occurring in organs prior to the appearance of gametocytes in blood [99]. Consequently, nestling studies are often restricted to larger species that remain in nests longer [100].

Nevertheless, because infections are recently acquired and infection sites are known, nestlings provide valuable models for studying local host–parasite interactions, provided that sampling protocols are noninvasive and rigorously designed to avoid impairing reproductive success or nest survival.

Limited integration of vector communities

Since avian–haemosporidian interactions are shaped by haematophagous dipteran vectors (Box 1), studies focusing only on birds and parasites provide an incomplete picture and may limit our ability to predict ecological drivers of transmission and spillover risk (Figure 3). Integrating vector communities into avian-haemosporidian studies is therefore essential.

Appropriate trapping methods are essential for effectively sampling vector communities, which can vary across small spatial scales and microhabitats [101–103] (Box 2). Integrating vector and bird sampling is therefore necessary to accurately characterise host–vector–parasite interactions. Complementary tools such as blood meal metabarcoding and environmental DNA can improve vector identification and detection of cryptic species [106,107]. Vector competence should also be assessed through targeted approaches, including salivary gland dissections and molecular analyses to detect sporozoites [58,108]. However, these methods remain underdeveloped for many nonmosquito vectors and require specialised expertise [109]. Seasonal variation in vector abundance, infection prevalence, and parasite transmission, together with hosts' migratory status, should also be considered [95,110]. We acknowledge that sampling vectors and screening them for haemosporidian parasites can be challenging and requires specialised knowledge. However, we need to design integrative and interdisciplinary studies in which ornithologists and vector specialists come together to sample both bird and vector communities to accurately characterise vectors and their role in avian–haemosporidian transmission.

Integrated approaches to parasitology, immunology, and host fitness

Haemosporidian infections can reduce host energy, physiology, reproduction, performance, and survival [13,14,111], although mortality effects are difficult to assess in the wild, since corpses are seldom found and tested [112]. Infection costs vary among host species depending on immune responses, life-history constraints, parasite lineage, infection stage, and parasitaemia [113,114], highlighting the need for integrative community-level approaches combining ecology, parasitology, behaviour, and emerging molecular tools such as transcriptomics, single nucleotide polymorphism analysis, microbiome characterisation, and metabarcoding. Experimental studies remain essential to quantify infection costs and transmission dynamics, yet most focus on single host or parasite species [115–117]. Community-level experimental systems, including

Box 2. Trapping approaches for dipteran vectors

The role of vectors in transmitting avian-haemosporidian parasites remains only partially understood, although their importance is clear. Many studies on parasite–vector interactions depend on limited trapping methods and may identify potential vectors in the field without distinguishing between infected and infectious individuals [101], or conduct PCR on the entire vector without confirming the presence of infectious sporozoites in the salivary glands. Trap attractiveness varies among vector species and with feeding habits, thus relying solely on a single trap type may overlook crucial information about vector roles or fail to detect a competent vector at a given site [104]. Common trapping techniques include encephalitis virus surveillance traps, CDC (Centers for Disease Control and Prevention) miniature light traps (baited with dry ice to generate CO_2 as an attractant), gravid traps (usually with grass-infused water as an attractant), standing red box traps (provide a covered environment in which mosquitoes can rest), and net traps (baited with attractants such as dry ice or live birds) [101]. To effectively capture a broad spectrum of vector species, using multiple trap types is crucial [101]. In addition to the trap types, it is also crucial to sample vectors across the full day to account for differences in daily activity levels among vector species and groups. Research should also confirm the presence of **sporozoites** in the salivary glands and include vector competence assays and field studies, as the presence of sporozoites alone does not confirm parasite transmission [105]. Without employing various trapping methods tailored to different vector species and considering individual infection status, it is difficult to reliably determine the vectors' roles in avian–haemosporidian transmission.

Box 3. What is the status of published avian-haemosporidian community-level studies?

We performed a literature search in April 2026 to gather community-level studies of avian haemosporidians. Initial title, keyword, and abstract screening was performed using the methodology for systematic reviews and meta-analyses established in the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) framework [16,17]. A systematic search of the available literature on associations between birds and haemosporidian communities was performed in the Web of Science (WoS), Scopus, PubMed Central (PMC), and MalAvi databases. We focused on studies published between January 2000 and April 2026.

To target community-level avian-haemosporidian research, the search string was structured using the following Boolean keywords within the Title, Abstract, and Keyword fields (TITLE-ABS-KEY): (haemosporid* OR 'blood parasites' OR plasmodium OR haemoproteus OR leucocytozoon) AND (bird* OR avian OR 'bird community' OR 'avian community' OR 'host-parasite community') AND (lineage* OR 'genetic lineage*' OR diversity OR 'parasite diversity') AND NOT ('Plasmodium falciparum' OR 'human malaria' OR Anopheles OR Aedes).

We retrieved 987 articles from WoS, 1206 from Scopus, 29 from PMC, and 95 from MalAvi. After removing duplicates, a total of 493 studies remained for full-text screening. For this stage, we developed custom inclusion criteria, where the study needed to (i) be an original article (excluding reviews and meta-analyses), (ii) focus on wild bird communities, (iii) use a molecular detection method to screen for haemosporidians and sequence the *cytb* gene, and (iv) involve capturing birds without selecting particular species or genera. Ultimately, 143 studies met these criteria. From these studies, we extracted individual community-level datasets (many studies have more than one geographic community) and their GPS locations. Some studies did not include the complete screened bird communities in their supplementary data or did not provide exact GPS locations of the communities. For these, we contacted authors for the missing data, and studies for which we could not obtain data from the authors were omitted. Overall, we were able to recalculate prevalence data from 82 studies representing 543 communities (see Table S1 in the supplemental information online). Of these, we used only prevalence from communities with at least 4 sampled species (416 communities from 74 studies) for generating Figure 1A.

Outstanding questions

Do avian–haemosprodian parasite interactions vary across broader geographic regions?

How do fine-scale environmental changes influence tripartite eco-evolutionary dynamics and disease spillover risks between avian hosts, dipteran vectors, and haemosporidian parasites?

What physiological, immunological, or ecological mechanisms drive differences in infection costs among host species within a community?

How does host phenology influence avian and haemosporidian parasite associations?

seminatural aviaries, could allow controlled manipulation of infection status, vector exposure, and environmental conditions [118]. Long-term monitoring and ringing programmes further provide valuable opportunities to link infection dynamics with demographic, behavioural, and fitness data across environmental contexts. Experimental medication approaches may also be informative, although antimalarial drugs can themselves alter host physiology and behaviour [64,119]. Thus, to further the field by evaluating infection costs at the community level, we need to design studies integrating long-term community-level data, manipulation experiments in the wild, and emerging molecular tools.

Concluding remarks

Community-level studies are essential for understanding the eco-evolutionary dynamics of avian–haemosporidian interactions. Our synthesis highlights the need to move beyond fragmented, single-host or single-lineage approaches towards standardised and integrative frameworks that explicitly consider hosts, parasites, and vectors as interconnected components. Future research should prioritise coordinated and longitudinal sampling designs, the systematic integration of vector data, and the use of emerging molecular and genomic tools alongside traditional parasitological methods. Importantly, combining ecological, physiological, immunological, and behavioural data within community contexts will allow a more mechanistic understanding of infection costs, host competence, and transmission dynamics. Experimental and long-term monitoring approaches, especially those linked to existing monitoring programmes, offer valuable opportunities to bridge the gap between observational patterns and causal processes (see Outstanding questions). However, implementation of these experimental and long-term projects in a community context requires navigating substantial ethical approval processes, long-term funding constraints, and logistical hurdles. Ultimately, advancing community-level research will require collaborative and interdisciplinary efforts supported by shared methodological standards that are consistently reported. Such integrative approaches will be key for identifying the drivers of host–parasite communities, predicting responses to environmental change, and assessing the risks of pathogen emergence and spillover.

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Declaration of interests

The authors declare no competing interests.

Supplemental information

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