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Contrasting haemoparasite prevalence in larid species with divergent ecological niches and migration patterns

Radosław Włodarczyk[1], Sandra Bouwhuis[2], Coraline Bichet[3], Patrycja Podlaszczuk[1], Amelia Chyb[1], Piotr Indykiewicz[4], Beata Dulisz[5], Jacek Betleja[6], Tomasz Janiszewski[1] and Piotr Minias[1]

[1]Department of Biodiversity Studies and Bioeducation, Faculty of Biology and Environmental Protection, University of Łódź, Banacha 1/3, 90-237 Łódź, Poland; [2]Institute of Avian Research, Wilhelmshaven, Germany; [3]Centre d'Etudes Biologiques de Chizé, UMR 7372, CNRS-La Rochelle Université, Villiers-en-Bois, France; [4]Department of Biology and Animal Environment, Faculty of Animal Breeding and Biology, Bydgoszcz University of Science and Technology, Mazowiecka 28, 85-084 Bydgoszcz, Poland; [5]Department of Ecology and Environmental Protection, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Plac Łódzki 3, 10-727 Olsztyn, Poland and [6]Department of Natural History, Upper Silesian Museum, Plac Jana III Sobieskiego 2, 41-902 Bytom, Poland

Research Article

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Author for correspondence:
Radosław Włodarczyk,
E-mail: radoslaw.wlodarczyk@biol.uni.lodz.pl

Abstract

Haemoparasites represent a diverse group of vector-borne parasites that infect a wide range of vertebrate hosts. In birds, haemoparasite infection rates may be associated with various ecological and life history traits, including habitat choice, colony size and migration distance. Here, we molecularly assessed the prevalence of 3 main haemoparasite genera (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) in 2 bird species with different habitat preferences and migratory behaviour: black-headed gulls (*Chroicocephalus ridibundus*) and common terns (*Sterna hirundo*). We found that gulls showed a much higher prevalence and diversity of *Plasmodium* or *Haemoproteus* (ca. 60% of individuals infected) than terns (zero prevalence). The prevalence of *Leucocytozoon* was low in both species (<3%). The differences in haemoparasite prevalences may be primarily driven by varying vector encounter rate resulting from different habitat preferences, as black-headed gulls mainly use vector-rich vegetated freshwater habitats, whereas common terns often use vector-poor coastal and brackish habitats. Since common terns migrate further than black-headed gulls, our results did not provide support for an association between haemoparasite prevalence and migratory distance. In gulls, we found a negative association between colony size and infection rates, suggestive of an ideal despotic distribution, and phylogenetic analyses of detected haemoparasite lineages provided evidence for higher host specificity in *Haemoproteus* than *Plasmodium*. Our results suggest that the preference for coastal areas and less vegetated habitats in terns may reduce haemoparasite infection rates compared to other larids, regardless of their migratory distance, emphasizing the role of ecological niches in parasite exposure.

Introduction

Haemoparasites infect a wide range of vertebrate hosts, such as amphibians, reptiles, birds and mammals (Valkiūnas, 2005), thereby being causative agents of malaria and malaria-like diseases in temperate and tropical regions (Valkiūnas and Iezhova, 2018). In birds, there are 3 main genera of haemoparasites, transmitted by different vectors: *Plasmodium* (transmitted by mosquitos), *Haemoproteus* (2 subgenera transmitted by biting midges and louse flies) and *Leucocytozoon* (transmitted by black flies) (Valkiūnas, 2005; Santiago-Alarcon *et al.*, 2012; Lotta *et al.*, 2016; Santolíková *et al.*, 2022). Despite the wide geographic distribution of both vectors and parasites, bird orders show highly variable rates of infection and parasite prevalence (e.g. Atkinson and van Riper, 1991; Scheuerlein and Ricklefs, 2004; Quillfeldt *et al.*, 2011) and infection risk varies considerably across zoogeographical regions (Fecchio *et al.*, 2021). Some avian lineages (e.g. many passerines) are heavily infected, while others (e.g. raptors, storks, cormorants) show low rates of infection (Martinez-Abraín *et al.*, 2004). This interspecific variation is thought to emerge from global and region-scale drivers (Fecchio *et al.*, 2021) linked to differences in both biotic and abiotic factors, such as climate, host population density or feeding behaviour (Sol *et al.*, 2000; Zamora-Vilchis *et al.*, 2012; Sehgal, 2015; Zagalska-Neubauer and Bensch, 2016). Despite interactions between these parasites and their hosts being complex, species with higher infection risk may be subject to stronger parasite-driven selection pressure, which may reduce their competitive ability, body condition, reproductive success or survival (van Riper *et al.*, 1986; Sorci and Møller, 1997; Marzal *et al.*, 2005; Knowles *et al.*, 2010).

Host–parasite interactions have received considerable scientific interest for decades (Loye and Zuk, 1991; Ricklefs *et al.*, 2004; Garcia-Longoria *et al.*, 2019) and avian blood parasites have long been considered an excellent model to study host–parasite dynamics and

coevolution at both the intra- and inter-specific level (Atkinson and van Riper, 1991; Rivero and Gandon, 2018). Although data on host infection rates should best be set within a broad phylogenetic framework to allow robust inferences, research effort has so far, however, been unevenly allocated, focusing mostly on passerine birds (Murdock *et al.*, 2013; Fecchio *et al.*, 2021), with ca. 85% of records within a global database for avian haemoparasites coming from passerines (MalAvi; Bensch *et al.*, 2009). At the same time, information on the prevalence and diversity of blood parasites is still limited in other avian groups (Valkiūnas, 2005; Quillfeldt *et al.*, 2011), possibly reflecting lower research effort in non-passerines, since even the development of molecular techniques that allow highly efficient haemoparasite screening, and resolution of their molecular diversity across divergent avian lineages (Bensch *et al.*, 2000), did not mitigate this phylogenetic bias.

The aim of our study was to assess the prevalence of the 3 main blood parasite genera (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) in 2 colonial bird species from the Laridae family (Charadriiformes): the black-headed gull (*Chroicocephalus ridibundus*) and the common tern (*Sterna hirundo*), and to study a potential link between these prevalences and ecological and life history traits, including habitat choice, colony size and migration distance. So far, data on the occurrence of haemosporidians in Charadriiform birds are scarce (only 0.4% of all records in the MalAvi database), despite a relatively high phylogenetic diversity of this avian group (more than 370 species classified into 90 genera according to Winkler *et al.*, 2015). In fact, the MalAvi database contains no molecular information on haemosporidians detected in our study genera (*Chroicocephalus* and *Sterna*).

Both the black-headed gull and common tern are medium-sized colonial waterbirds with a Holarctic distribution and large population sizes (BirdLife International, 2021). They occupy divergent types of aquatic habitats (rather freshwater in the black-headed gull, both freshwater and marine in the common tern) and feed on a wide range of small prey items including fish, crustaceans, insects and their larvae (Snow and Perrins, 1998). During the breeding season, black-headed gulls prefer shallow, inland waterbodies with abundant vegetation, whereas terns breed mainly on sandy islands lacking plant cover, situated both at the coast and in large river valleys (Snow and Perrins, 1998). Moreover, our study species show strong variation in their migratory behaviour; the common tern being a long-distance migrant (e.g. Kürten *et al.*, 2022) and the black-headed gull migrating only relatively short distances (e.g. Christmas *et al.*, 1986; Jelínek, 2008). This variation is especially apparent in the European populations, where the black-headed gull usually overwinters in Western Europe and in the Mediterranean region, while the common tern spends winter on the coasts of Western Africa, passing across different climatic zones and likely being exposed to diverse local parasite communities. Gulls and terns also differ in their stop-over behaviour during migration. Common terns migrate rather quickly, both over sea and across land, using only few stopover sites located generally at the coast (Kürten *et al.*, 2022). In contrast, black-headed gulls migrate using broad migratory flyways, as they can refuel at diverse stopover sites, often located far from the coast (Cramp and Simmons, 1983; Wernham *et al.*, 2002).

So far, most field research on passerine species has shown that long-distance migrants carry a higher diversity of blood parasites and show higher infection rates than resident individuals (Pérez-Tris and Bensch, 2005), although this general pattern may not necessarily be preserved across all parasite genera or geographical regions (Fecchio *et al.*, 2021). Despite this, phylogenetic diversity of haemoparasites was also positively associated with avian host migratory strategy at the inter-specific level (Jenkins *et al.*, 2012). However, migratory passerines usually fly over land and refuel more often than large bird species (e.g. gulls, ducks, waders), which likely increases their exposure to haemoparasite vectors. Taking this into account, migratory behaviour or habitat preferences during migration, rather than migratory distance *per se*, could explain differences in the species-specific vulnerability to parasite infections in birds.

Here, we sampled 623 black-headed gulls and common terns from several Central European breeding populations and used molecular approaches for haemoparasite detection and identification. As infected individuals could be non-randomly distributed across populations and infection rates could be associated with the size of social groups, we also tested for an association between haemoparasite prevalence and colony size in black-headed gulls.

Materials and methods

Sample collection

We collected samples from 2 breeding colonies of common terns and 7 breeding colonies of black-headed gulls. One tern colony was located at the North See coast in northern Germany, Wilhelmshaven (690–740 breeding pairs), and the second in central Poland, at Jeziorsko reservoir (260–380 breeding pairs) (Fig. 1). All gull colonies were scattered across different regions of Poland (Fig. 1) and the number of breeding pairs varied between 100 and 2800 per colony. Samples from the common tern were collected in 2014–2019, whereas black-headed gulls were sampled exclusively in 2018. In total, we collected 483 samples from terns and 140 samples from gulls (Table 1). Blood samples were obtained from adult breeders, either *via* puncture of the ulnar vein with a disposable needle (following capture) or non-invasively, using artificial eggs with bloodsucking bugs (*Dipetalogaster maximus*) (for common terns from Wilhelmshaven, details in Becker *et al.*, 2006). This method allows to obtain blood samples without any negative impact on genetic material (DNA), which can be used as a high-quality source for molecular analyses (e.g. Vedder *et al.*, 2021). Samples were stored in 95% ethanol or phosphate-buffered saline before DNA extraction.

DNA extraction, amplification and sequencing

Nuclear DNA was extracted from blood samples using Bio-Trace DNA Purification Kits (EURx, Gdansk, Poland). We used a nested polymerase chain reaction (PCR) approach to amplify the cytochrome b (Cyt b) gene according to the methodology developed by Hellgren *et al.* (2004), which allows a parallel detection of *Leucocytozoon*, *Plasmodium* and *Haemoproteus*. Moreover, it allows the detection of parasites in different phases of infection even during subpatent infections with low intensity of parasites present in peripheral blood (Hellgren *et al.*, 2004). In the first step, the conserved primers HaemNFI and HaemNR3 were used to amplify the Cyt b gene across all parasite genera. In the second PCR reaction, we used primers that allowed specific amplifications of Cyt b in *Haemoproteus*/*Plasmodium* (HaemF and HaemR2) and *Leucocytozoon* (HaemFL and HaemR2L) (Hellgren *et al.*, 2004). All PCRs were conducted in 20 μL total volume, which contained 2 μL of template DNA, 10 μL of DreamTaq PCR Master Mix (Thermo Fisher Scientific Inc., Waltham, MA, USA), 0.5 μL of each primer, 1.4 μL of 25 mM $MgCl_2$ and 5.4 μL of water. Each PCR run contained a positive control (DNA from previously genotyped individuals with a confirmed infection) and non-template negative control (ddH_2O). The thermal profile of the PCR reactions followed Hellgren *et al.* (2004), although we increased the number of cycles in the second PCR to 35 and used 2 μL of the product from the first PCR as a template for the second one.

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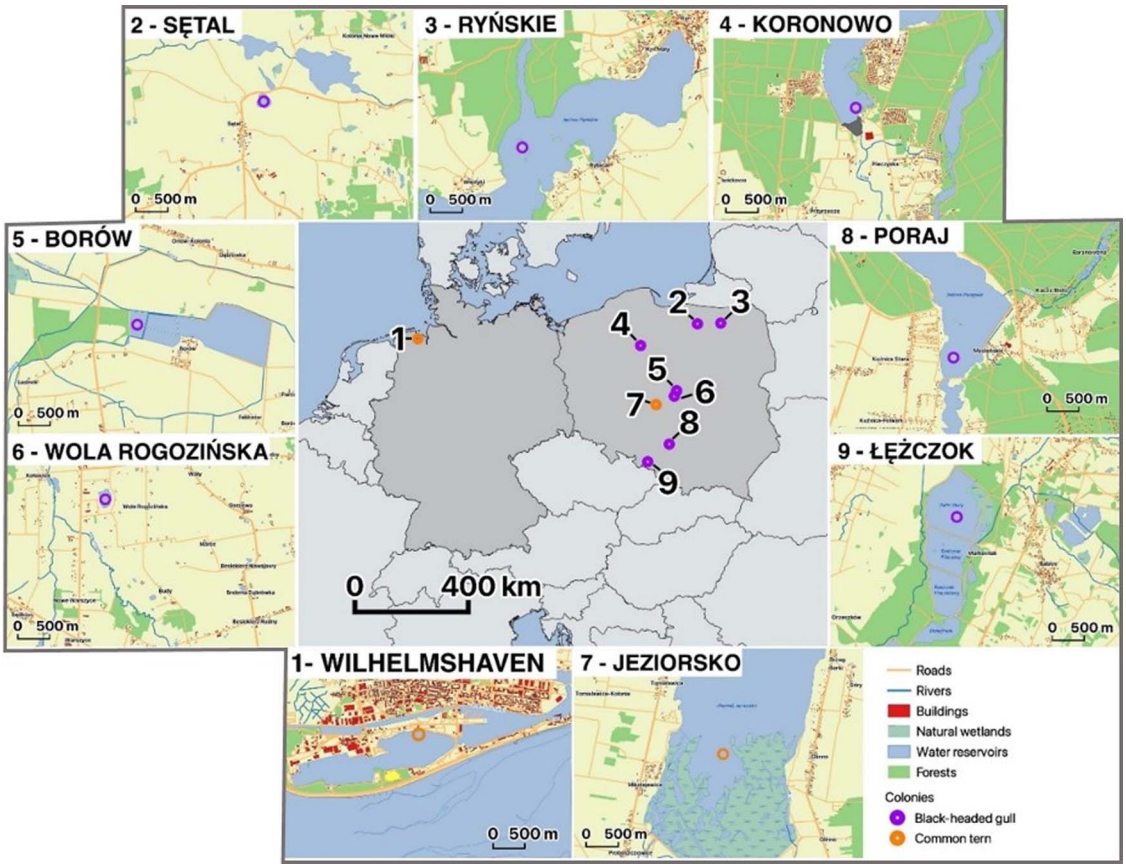

Fig. 1. Location of black-headed gull (violet) and common tern (orange) colonies used for sampling. Maps created based on templates from OpenStreetMap.

Table 1. Occurrence of haemoparasites in black-headed gulls and common terns at 9 sites across Poland and Germany

Species	Population	PCR amplifications			Molecular lineage identification		
		N_{amp}	*Leucocytozoon*	*Haemoproteus/Plasmodium*	N_{seq}	*Haemoproteus*	*Plasmodium*
Black-headed gull	Sętal	20	0	13 (65%)	7	6	1
	Ryńskie	20	0	13 (65%)	2	1	1
	Koronowo	20	0	13 (65%)	7	5	2
	Borów	20	2 (10.0%)	15 (75%)	10	7	3
	Wola Rogozińska	20	0	7 (35%)	4	1	4
	Poraj	20	0	13 (65%)	9	8	1
	Łężczok	20	1 (5.0%)	9 (45%)	3	3	0
	Total	140	3 (2.1%)	83 (59.3%)	42	31 (44%)1	12 (17%)[1]
Common tern	Wilhelmshaven	322	0	0	0	–	–
	Jeziorsko	161	2 (1.3%)	0	0	–	–
	Total	483	2 (0.4%)	0	0	–	–

Sample size (N_{amp}) and the number (proportion) of infection-positive individuals positive for infection (as confirmed with PCR amplifications) are shown for each location. Sample size of birds selected for sequencing (N_{seq}) and the number of individuals positive for either *Haemoproteus* or *Plasmodium* infection (as confirmed with molecular lineage identification) are also shown.
[1]Sample sizes include coinfections.

Amplicons were electrophoresed on a 1.5% agarose gel stained using the SYBR Safe DNA Gel Stain (Thermo Fisher Scientific) at 132 V for 20 min to detect parasite-specific bands. All positive samples were confirmed with an electrophoresis of amplicons from independent PCRs to avoid false positives.

To identify haemoparasite lineages, we selected all samples positive for *Leucocytozoon* ($n = 5$) and 50% of samples positive for *Haemoproteus*/*Plasmodium* ($n = 42$). We expected this sample size to facilitate a reliable characterization of haemoparasite lineage composition in our study species, allowing for cost optimization at the same time. All amplicons were sequenced in both forward and reverse directions. All sequences were assembled, edited and aligned in Geneious 10.0.5 software (Biomatters Ltd., Auckland, New Zealand). Next, we blasted all unique sequences against reference haemoparasite sequences from the MalAvi database (Bensch *et al.*, 2009). Morphospecies and molecular lineages

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were identified based on maximum and 100% pairwise nucleotide similarity, respectively. Sequences showing any nucleotide mismatches with available data were recognized as novel lineages and submitted to MalAvi. Phylogenetic clustering of query and reference lineage sequences was conducted using Bayesian inference method in MrBayes 2.2.4. (Huelsenbeck and Ronquist, 2001) installed as a plugin in Geneious 10.0.5. The analysis was run with 120 000 chain length, 20 000 burn-in length and a general time-reversible (GTR) substitution model.

Statistical analyses

Differences in prevalence rate between species and parasite genera were tested with *G* tests. An association between parasite prevalence and colony size of gulls was tested using a Spearman's rank correlation coefficient (*r*). All analyses were performed with Statistica 12 (StatSoft, Tulsa, OK, USA).

Results

All 3 haemoparasite genera were detected in the black-headed gull, whereas only *Leucocytozoon* was recorded in the common tern (Table 1). The overall prevalence of *Haemoproteus*/*Plasmodium* in gulls was 59.3%, being significantly greater than the 2.8% prevalence of *Leucocytozoon* ($G = 64.13$, $P < 0.001$). *Plasmodium* infection was less common compared to *Haemoproteus* infection (17 *vs* 44% prevalence, as estimated with molecular lineage identification). The prevalence of *Leucocytozoon* in the common tern was marginally low (<0.5%; Table 1) and not significantly different from the prevalence in the black-headed gull ($G = 1.61$, $P = 0.205$). Within the black-headed gull, there were significant differences in *Haemoproteus*/*Plasmodium* infection rate between colonies ($G = 4.86$, $P = 0.028$) and there was a negative correlation between haemoparasite prevalence rate and colony size ($r = -0.87$, $N = 7$, $P = 0.011$; Fig. 2).

Phylogenetic analyses of *Cyt b* sequences obtained from our samples revealed an occurrence of multiple lineages within each parasite genus (Fig. 3). All detected *Haemoproteus* and *Leucocytozoon* lineages were identical to, or showed highest pairwise similarity to, lineages previously described in other larid hosts. A *Haemoproteus* lineage dominant within our sample (96% of infections) was not found in MalAvi database, but showed highest pairwise similarity (99.5%) to the LARCRA01 lineage described previously in the Caspian gull (*Larus cachinnans*). This lineage was classified as *Haemoproteus* (*Parahaemoproteus*) *valkiūnasi* (98.6% similarity to FREAND01; Merino *et al.*, 2012). The second *Haemoproteus* lineage (CREFUR01) was previously reported only in tropical larid species (e.g. swallow-tailed gull *Creagrus furcatus*) and classified as *Haemoproteus jenniae* (Levin *et al.*, 2012).

Two detected *Leucocytozoon* lineages were also previously reported in the Caspian gull, but they showed a host specificity within our samples, as the CIAE02 lineage was found exclusively in the black-headed gull, while the LARCAC01 lineage was found in the common tern (Fig. 3). These lineages showed highest pairwise similarity to *Leucocytozoon californicus* (99.5% similarity with FASPA02; Walther *et al.*, 2016) and *Leucocytozoon polynuclearis* (96.6% similarity with COLAUR01; Groff *et al.*, 2022).

Plasmodium parasites showed slightly higher lineage diversity ($N = 3$), and the most common lineage (SGS1, *Plasmodium reticulum*; Palinauskas *et al.*, 2007) found in gulls (67% of *Plasmodium* infections) was previously reported, among the others, in 4 larid species, 2 of them (Caspian gull and herring gull *Larus argentatus*) breeding within the range of our study species. Another lineage (LINN1, *Plasmodium matutinum*; Valkiūnas *et al.*, 2017) was previously reported in an avian host from the order of Charadriformes (Atlantic puffin *Fratercula arctica*) (Fig. 3). The third lineage (DONANA03) was identical to sequences identified only in a vector species (*Culex* sp.) (Fig. 3). This lineage showed high pairwise similarity (95.8%) to *Plasmodium circumflexum* (TURDUS1; Palinauskas *et al.*, 2007).

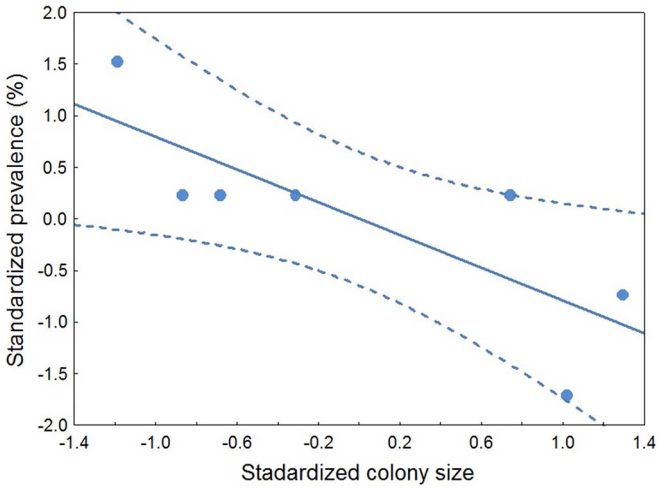

Fig. 2. Relationship between haemoparasite infection rate and colony size in the black-headed gull. For the purpose of presentation, both variables were standardized to *z* scores, so that the correlation coefficient equals the regression slope (shown with its 95% confidence intervals).

Finally, our analyses revealed 3 cases of gulls being co-infected by different haemoparasite genera. Specifically, we recorded 2 cases of *Haemoproteus*/*Leucocytozoon* coinfections and 1 *Plasmodium*/*Haemoproteus* coinfection.

Discussion

We found considerable differences in blood parasite prevalence between 2 larid species, and the pattern may be primarily driven by variation in habitat selection rather than differences in distance covered during migration. The black-headed gull, which mostly breeds in vegetated freshwater habitats, showed a much higher prevalence and diversity of haemoparasites than the common tern, which breeds in coastal areas and less vegetated freshwater habitats. More than half of all examined gulls (59.3%) were infected with *Haemoproteus* or *Plasmodium*, and we recorded several cases of co-infections in this species. In contrast, we detected only 1 genus of haemoparasite (*Leucocytozoon*) in the common tern, with marginally low prevalence (<0.5%). Molecular lineage identification indicated that *Haemoproteus* in gulls was more prevalent when compared to *Plasmodium*. We also found among-colony variation in gull infection rates, as infected individuals were more prevalent in smaller colonies.

Despite limited empirical data, haemoparasite prevalence in Laridae seems to be rather low compared to that in many other bird orders, especially passerines (e.g. Bensch *et al.*, 2009; Dunn *et al.*, 2017; Fecchio *et al.*, 2021; Ilahiane *et al.*, 2022). However, even within larids, infection rates can be strongly variable at the intra- and inter-specific level (Quillfeldt *et al.*, 2011). Our results are highly consistent with existing information, suggesting that gulls (Larinae) in general show much higher infection rates than terns (Sterninae). Different populations of yellow-legged gull (*Larus michahellis*), for example, showed *Haemoproteus* prevalences between 39 and 100% (Ruiz *et al.*, 1995; Bosch *et al.*, 1997). In contrast, most examined tern species showed zero prevalence of *Plasmodium* and *Haemoproteus* (as reviewed by Quillfeldt *et al.*, 2011 and Fecchio *et al.*, 2021), although minor infection rates were reported in little terns (*Sternula albifrons*)

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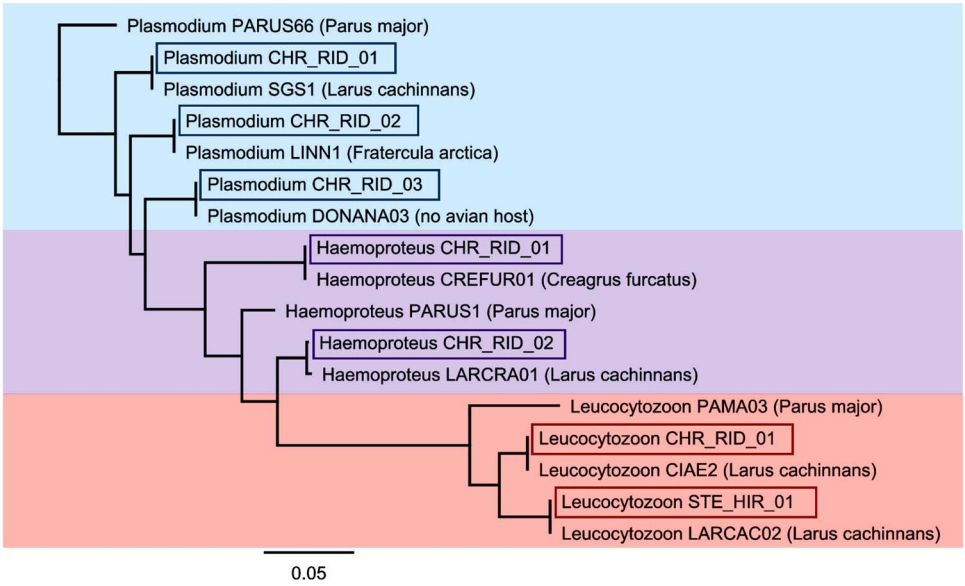

Fig. 3. Bayesian phylogenetic tree of haemoparasite cytochrome b sequences isolated from black-headed gulls (CHR_RID) and common terns (STE_HIR). Reference sequences (blast hits with maximum pairwise identity and 1 randomly selected lineage from the great tit *Parus major*) were retrieved from MalAvi database (Bensch *et al*., 2009). Node tips are labelled with the parasite genus, reference lineage name (MalAvi) and previously reported avian host species. The tree was rooted in *Plasmodium* PARUS66 lineage. Clusters corresponding to each haemoparasite genus were marked in different colours, and all sequences generated in this study were marked in frames.

(Kairullaev, 1986), Forster's terns (*Sterna forsteri*) (Coatney, 1938) and gull-billed terns (*Sterna nilotica*) (Fecchio *et al*., 2021). *Haemoproteus larae* was also detected in a Kazakhstan population of the common tern (Kairullaev, 1986).

Higher infection rates in gulls than terns are inconsistent with the migration hypothesis (Alerstam *et al*., 2003; Møller *et al*., 2011), assuming that long-distance migration (as performed by the common tern) should increase exposure to diverse parasites (and their vectors). Thus, our results clearly indicate that migration is not a key factor in parasite transmission within our study system, despite extensive comparative evidence showing that migrant bird species harbour greater diversity of parasites compared to non-migratory taxa (e.g. Ricklefs *et al*., 2005; Jenkins *et al*., 2012; Ricklefs *et al*., 2016). Consequently, it seems reasonable to conclude that other ecological characters, such as habitat preferences, should primarily determine differences in haemoparasite prevalence between our study species. Common terns often spend a large part of their annual cycle in coastal habitats and breed in more open areas with little or no vegetation (e.g. sandy islands or beaches). In contrast, black-headed gulls prefer inland swampy habitats with shallow stagnant water and abundant macrophyte or reed plants (Snow and Perrins, 1998). The latter habitats are often associated with large numbers of mosquitos, which are crucial for transmission of *Plasmodium* haemoparasite (Valkiūnas, 2005), and which should enhance high local infection rates in birds. In Poland, gull colonies are often located within lowland river valleys that represent a mosaic of oxbow lakes and river creeks, which are also suitable for other haemoparasite vectors (e.g. black flies or biting midges). Such habitats, rich in a variety of biting insects, can increase the probability of infection with *Leucocytozoon* or certain *Haemoproteus* lineages (e.g. from *Parahemoproteus* subgenus; Santiago-Alarcon *et al*., 2012). For example, a breeding colony of yellow-legged gulls and herring gulls located in habitats with high densities of blackflies (Vistula valley) showed unusually high haemosporidian (mostly *Leucocytozoon*) prevalence (up to 95%) in both species (Zagalska-Neubauer and Bensch, 2016). Haemoparasite prevalence in passerines and pigeons varied with vector abundance, regardless of host species (Apanius *et al*., 2000; Sol *et al*., 2000) and exposure to vectors was also recognized as the main factor responsible for the habitat-related differences in malaria occurrence among Charadrii shorebirds (Mendes *et al*., 2005). Specifically, species from freshwater inland habitats had significantly higher prevalence of blood parasites than species occupying marine coastal habitats (Mendes *et al*., 2005). Low haemoparasite prevalences in marine environments were also reported for another long-distance migrant, the Eleonora's falcon (*Falco eleonorae*) (Gutiérrez-López *et al*., 2015). It is worth noting that seasonal peaks in vector abundance are likely to coincide with the breeding season of many European avian species, when resources are allocated to reproduction and host immune function can be reduced (Cosgrove *et al*., 2008; Knowles *et al*., 2009). This may result in higher host susceptibility to infection and, thus, habitat choice during the breeding season should drive variation in infection rates between species. Consistent with this prediction, common terns from the only coastal colony within our dataset (Wilhelmshaven, Germany) showed zero haemoparasite prevalence, despite the largest number of birds ($N = 322$) screened.

In our study, haemoparasite prevalence was negatively related to colony size in the black-headed gull. Haemoparasites are unlikely to be transmitted directly between birds and, thus, infection rates should not increase with host density (or social group size), as expected for contagious diseases (Brown, 2016). Instead, higher haemoparasite prevalence in gulls from smaller colonies fits the model of an ideal despotic distribution, where less dominant individuals of poor phenotypic quality are relegated to less attractive breeding sites (Fretwell, 1972; Drzewińska-Chańko *et al*., 2021). Small colonies are often located in suboptimal habitats, which may be associated with higher vector densities and thus pose a higher risk of infection. Alternatively, individuals infected during the pre-breeding season may be in a relatively poor condition and not be able to effectively compete for nesting sites in large colonies, which are better protected against predators (via communal defence) and are likely to have better access to food resources (Ward and Zahavi, 1973; Krause and Ruxton, 2002; Jungwirth *et al*., 2015). An important role of intraspecific competition during settlement decisions was demonstrated for a number of colonial birds, including gulls and terns (e.g. Oro,

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2008; Minias, 2014; Indykiewicz *et al.*, 2019). Despite these considerations, we are aware that the robustness of our findings is limited by a small number of sampled gull colonies ($N = 7$) and our analyses should be replicated across more colonies and more diverse geographical locations to confirm our conclusions. To test a possible role of habitat selection for host–parasite interactions in larids, it would be also valuable to study mixed breeding colonies of terns and gulls from a single location.

Our phylogenetic analyses of *Cyt b* sequences revealed low molecular diversity of haemoparasites within our sample, as we recorded only 2–3 lineages per parasite genus. The majority of examined black-headed gulls was infected with a single dominant lineage of *Haemoproteus*, consistent with patterns observed in other gull species (e.g. Kram *et al.*, 2012; Zagalska-Neubauer and Bensch, 2016). Blood parasites are generally known to be evolutionarily conserved in terms of host choice, although recent molecular studies suggest that host switching may occur relatively frequently in some lineages (Bensch *et al.*, 2000; Ricklefs and Fallon, 2002). Comparative analyses of diverse bird species suggest that *Haemoproteus* tends to be more host-specific than *Plasmodium*, which is recognized as a host generalist (Beadell *et al.*, 2004; Dimitrov *et al.*, 2010). This is consistent with our molecular analyses, as all *Haemoproteus* (and *Leucocytozoon*) lineages detected within our sample were previously identified in other larid species. In contrast, 2 out of 3 *Plasmodium* lineages isolated from black-headed gulls matched lineages previously found in other non-passerines (alcids *Alcidae*) or not detected in avian hosts.

In conclusion, this is one of the few studies providing information on the prevalence and molecular diversity of haemoparasites in representatives of Laridae family. It reveals contrasting infection rates between 2 species with divergent ecological niches (habitat choice), although long migratory distance did not enhance higher haemoparasite prevalence. We conclude that larids may show great diversity in haemoparasite infection rates and that the ecological factors that drive this variation should be further explored under a broader phylogenetic coverage. Moreover, the general pattern of marginal infection rate of haemosporidians in terns suggests a possible presence of unique immunological or immunogenetic adaptations (e.g. structure of immune receptors) that needs further examination.

Data availability. All sequences generated and used in this study have been deposited in MalAvi database, while the single novel sequence has also been submitted to GenBank (Haemoproteus CHR_RID_02 (ON950078)).

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Author contributions. R. W., S. B., C. B. and P. M. designed the study, analysed the data and wrote the manuscript; P. I., B. D., J. B., T. J. and P. M. collected data; R. W., P. P. and A. Ch. performed laboratory analyses; all authors revised the manuscript.

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Ethical standards. All applicable institutional and/or national guidelines for the care and use of animals were followed and all blood sampling procedures were conducted with permission of the Local Bioethical Commission for Experiments on Animals and Regional Environmental Protection Directorate in Łódź and Bydgoszcz, Poland.

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