Parasite life histories shape their biogeography: a molecular approach



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A thesis submitted for the degree of
Doctor of Philosophy

I declare that this thesis is entirely my own work, and except where otherwise stated, describes my own research. This work has not been submitted for any degree or professional qualification except as specified.

Acknowledgements

I would like to thank my supervisors, Sonya and Beth, for their guidance and support throughout this PhD. Your advice and perspective were greatly appreciated at all stages of the project. I am particularly grateful for your generosity in setting aside time to discuss ideas. I also thank Beth for enabling me to be based at the Natural History Museum (NHM) in London during my PhD. I first became fascinated by parasite dispersal under Beth's supervision during my master's in 2020, and I am very grateful to have been able to continue developing this interest, as well as for her mentorship over the past six years.

My PhD began in unusual circumstances, and I did not meet Sonya in person until I arrived on her doorstep in Australia, ready to start fieldwork. I am very grateful to Sonya and Tim, and their dog Woofler, for making me feel immediately at home. I learned a lot about both research and myself during fieldwork, most importantly that I am scared of snakes. I would also like to thank the people who assisted with fieldwork by identifying field sites and facilitating sample collection, including Jon and the Queensland Bird Research and Banding Group (particularly Dave, who never missed an opportunity to point out a snake-like twig or branch), Judy, Greg, Cat, and Jas.

Thank you to my fieldwork partner, Andrea, who became a close friend. I will be forever nostalgic for our drives down the Pacific Highway, only occasionally failing to turn the headlights on. I am also grateful to the past and present members of the Clegg Lab and the Edward Grey Institute as well as Paddy, Jenny, Eva, Hugh, and Lauren for their camaraderie. I also thank Dave and the Infectious Disease Systems Ecology Group at the NHM for welcoming me to tag along to their meetings and talk analyses as well as Maria and Bibi for their bioinformatic and academic wisdom.

I would like to thank those who facilitated laboratory work, including Lucy and the team at the Natural Environment Research Centre Environmental Omics Facility, as well as the molecular facilities team at the NHM. I also thank Vaidas for a very enjoyable and productive visit to the Nature Research Centre and David for his technical advice, and for many enjoyable Bass-mases.

Thanks to my family for their support and for indulging my interest in the natural world, even when it involved enduring some left-field holiday activities like freezing on a boat in the Norfolk Broads or collecting animal skulls. Thanks also to my sister, Ruth, for always making it clear how proud she is, and her often counterproductive editorial advice. I also thank Shannen and Chris, with whom I lived for much of my PhD and who became a second family, apologies for occasionally filling the freezer with faeces. Finally, I thank my husband, Isaac, for his constant encouragement and support throughout this process. The *aplomb* with which I finish this project would not have been possible without you.

Funding

This study was supported by funding from the UK Natural Environment Research Council (NERC) through a PhD studentship (NE/S007474/1). Fieldwork for this project was supported by an American Ornithological Society Student Research Award and The Biology Eurofins Foundation Fund Award. Laboratory work was supported by the NERC Environmental Omics Facility (NEOF1523). A laboratory visit to the Nature Research Centre, Vilnius in 2024 was supported by the Houghton Trust and a Godfrey-Hewitt Travel Award.

Conference and travel funding was provided by a St Anne's College Travel Grant, which supported attendance at the Society for Integrative and Comparative Biology conference in Phoenix, United States, in 2021; by the Royal Society of Biology, which funded attendance at the Society of Island Biology conference in Sicily, Italy, in 2023; by the British Society for Parasitology (BSP) which supported attendance at the Joint Parasitology Spring Meeting organised by the BSP, German Society for Parasitology, and Swiss Society of Tropical Medicine and Parasitology in Würzburg, Germany in 2025; and the NHM Student Travel Grant supported attendance at the Evolution and Ecology of Infectious Disease conference in South Bend, United States in 2025.

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Publication List

Each chapter has been prepared as a manuscript that is published, under review, or in preparation. All chapters are collaborative, although I undertook the majority of the work in each case.

Chapter 2: Nichols, S., Okamura, B. & Clegg, S. M. Do parasites follow the rules of island biogeography? *In preparation.*

> Conceptualisation: SN and SMC. Investigation: SN identified and screened publications. Analysis and visualisation: SN. Writing: SN wrote the original draft with input from SMC and BO. Supervision: SMC and BO.

Chapter 3: Nichols, S., Estandía, A., Young, C. M., Knowles, L. S., Palinauskas, V., Okamura, B.* & Clegg S. M.*, 2026. Host whole genome sequence data represent an untapped resource for characterising affiliated parasite diversity. *International Journal for Parasitology*, 104768.

> Conceptualisation: SN, SMC and BO. Investigation: SN, AE, CMY and SMC conducted fieldwork to collect samples; SN, AE, LSK and VP conducted lab work to generate the data. Analysis and visualisation: SN analysed the data with input from AE. SN prepared figures to present the data. Supervision: SMC and BO. Writing: SN wrote the original draft with input from BO and SMC. All authors provided feedback on the manuscript during the drafting process.

Chapter 4: Nichols, S., Okamura, B., Estandía, Robertson, B. C. & Clegg, S. M. 2026. Transmission strategy modulates parasite biogeography in an island-colonising bird. *In preparation.*

> Conceptualisation: SN, SMC and BO. Investigation: SMC and BCR conducted fieldwork to collect samples; AE conducted the lab work. Analysis and visualisation: SN analysed and visualised the data. Supervision: SMC and BO. Writing: SN wrote the original draft with input from BO and SMC. All authors provided feedback on the manuscript during the drafting process.

Chapter 5: Nichols, S., Estandía A., Young, C. M., Allnutt, J., Colman, J., Little, J., Little, G., Clegg, S. M.* & Okamura, B.* Life cycle complexity shapes parasite sharing amongst migratory and resident hosts. bioRxiv 2026.01.14.699309; https://doi.org/10.64898/2026.01.14.699309

Conceptualisation: SN, SMC and BO. Investigation: SN, AE, CMY, JA, JC, JL, GL, and SMC conducted fieldwork to collect samples; SN conducted the lab work. Formal analysis: SN analysed and visualised the data. Supervision: SMC and BO. Writing: SN wrote the original draft with input from BO and SMC. All authors provided feedback on the manuscript during the drafting process.

I also provide references for publications that are not included as part of my thesis, but that I contributed to during my PhD:

Briscoe, A.G., **Nichols, S.,** Hartikainen, H., Knipe, H., Foster, R., Green, A.J., Okamura, B. and Bass, D., 2022. High-throughput sequencing of faeces provides evidence for dispersal of parasites and pathogens by migratory waterbirds. *Molecular Ecology Resources*, *22*(4), pp.1303-1318.

Clegg, S.M., Estandía, A., **Nichols, S.,** Robertson, B.C., Sendell-Price, A., Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team, Wellcome Sanger Institute Scientific Operations: Sequencing Operations, Wellcome Sanger Institute Tree of Life Core Informatics team, Tree of Life Core Informatics collective and Blaxter, M.L., 2025. The genome sequence of the silvereye, *Zosterops lateralis* (Latham, 1802)(Passeriformes: Zosteropidae). *Wellcome Open Research*, *10*, p.678.

Gao, K., van der Heide, W., Muijderman, D., **Nichols, S.,** Karwal, C., Kuperus, P. and Groot, A.T., 2025. Ecological immunology: do sexual attraction and immunity trade-off through a desaturase?. *Insect Science*, *32*(1), pp.290-300.

Lee, C.E., Downey, K., Colby, R.S., Freire, C.A., **Nichols, S**., Burgess, M.N. and Judy, K.J., 2022. Recognizing salinity threats in the climate crisis. *Integrative and Comparative Biology*, *62*(2), pp.441-460.

Nichols, S., Ewen, J.G., Gottelli, D., Grueber, C.E., Santure, A.W., Trask, A. and Brekke, P., 2024. Genetic rescue attempt in a small, inbred population of a wild endangered passerine. *Biological Conservation*, *290*, p.110430.

Nichols, S., Ruggeri, P., Pringle, H., Siriwardena, G., Hartikainen, H. and Okamura, B., 2024. Waterbirds and other drivers of endoparasite communities across a hierarchy of spatial scales. *Authorea Preprints*.

The Royal Society, 2025. *Ecological implications of nature offset markets in England: Policy briefing*. Report team: Hazell, J., Jones, C., Lewis, R., **Nichols, S.**, Reynolds, L. X. The Royal Society, September 2025. Available at: https://royalsociety.org/news-resources/publications/2025/nature-offset-markets/

Abstract

Parasites constitute a substantial component of global biodiversity and exert pervasive effects on their hosts, with cascading impacts on ecosystem function and stability. Understanding the processes that shape parasite biogeography is therefore essential. Yet, elucidating these processes can be challenging because parasites rely on their hosts for essential resources. Consequently, host availability and movement constrain the dispersal and distributions of parasites. At the same time, parasites vary widely in their life history traits, such as transmission strategy and life cycle complexity. Variation in parasite life history traits may mediate dispersal, colonisation and persistence success. Therefore, parasite biogeography cannot be inferred from host ecology alone. In this thesis, I first synthesise existing empirical studies which test the factors that shape parasite biogeography. Specifically, I assess whether parasites adhere to the predictions of island biogeography theory. The systematic review reveals inconsistent support for the rules of island biogeography. I show that most studies address single parasite infections and focus on a narrow subset of host-parasite systems, while studies which address diverse parasite assemblages, encompassing varied parasite life history traits, are rare. To address these limitations, I develop and validate an approach which leverages existing whole genome sequence (WGS) data to extract parasites that have been inadvertently captured during host sequencing. I demonstrate that WGS data, which is increasingly freely available, can be re-purposed as a cost-effective and informative means for broad-scale parasite characterisation and unravelling host-parasite interactions. I then apply this method to test island biogeography predictions across varied parasites in the silvereye (*Zosterops lateralis)*, sampled across 25 islands in the South Pacific and mainland Australia. Patterns of biogeography differed markedly according to parasite life histories. Specifically, I found that vector-transmitted parasites were less sensitive to island isolation whereas parasites requiring other secondary hosts or utilising direct transmission strategies showed substantial declines in richness with increasing isolation. Finally, I consider how host movement mediates patterns of parasite biogeography over shorter temporal scales using the partial migrant population of Tasmanian silvereyes (*Z. l. lateralis)*. By comparing resident and migrant individuals within a single host species, I show that parasite sharing is primarily driven by parasites with simple life cycles, while parasites with complex life cycles appear to be constrained by vector or intermediate host availability. My thesis exemplifies the value of molecular approaches for characterising multi-parasite communities and highlights the importance of considering parasite life histories for understanding the processes that shape parasite biogeography.

Chapter 1

Introduction

Parasites pervade every ecosystem and have the potential to drastically alter host population dynamics (Tompkins and Begon, 1999; Tompkins *et al.*, 2002). They directly impact hosts by altering their life histories, physiology, and behaviour (Anderson and May, 1979; Timi and Poulin, 2020; Hall, 2022). Such changes may indirectly shape community structure (Minchella and Scott, 1991) via impacts on competition, predation, and survival. Moreover, the impacts of parasitism may be exacerbated by climate change with some parasites proliferating (Harvell *et al.*, 2002) while others become vulnerable to extinction (Carlson *et al.*, 2017). The changing climate could also lead to phenological mismatches between parasites and their hosts, giving rise to novel interactions and influencing disease dynamics (Singer and Parmesan, 2010; Rohr *et al.*, 2011; Lindgren *et al.*, 2012; Bosch *et al.*, 2018). Similarly, hosts and parasites may respond to changing abiotic conditions by shifting their ranges as they track their respective ecological niches (Lovejoy, 2006). Despite the critical importance of understanding emerging infectious diseases, protecting biodiversity, and maintaining ecosystem health (Hoberg and Brooks, 2015; Rohr *et al.*, 2020), the ecological and evolutionary drivers of parasite biogeography remain poorly understood. This gap reflects the predominance of studies that focus on single parasite taxa and address a narrow range of host-parasite systems, limiting our ability to assess how variation in parasite life history traits shapes patterns of biogeography. Leveraging molecular approaches enables the simultaneous detection of multiple parasite taxa, offering a means to overcome these limitations.

Parasites co-disperse with their hosts

Host and parasite richness often covary at large spatial scales (Krasnov *et al.*, 2004; Kamiya *et al.*, 2014; Poulin, 2014). This spatial covariation arises because parasites depend on hosts for essential resources (Morand, 2015), which constrains parasite dispersal (Barrett *et al.*, 2008; Criscione, 2008; Rehmann *et al.*, 2024). As a result, it is often assumed that the processes shaping macroecological patterns in free-living organisms may be mirrored in parasites. Consequently, the availability, persistence, and movement of hosts will impact the structure of parasite communities. Host movements can occur over different scales of space and time, including range extensions that result in the colonisation of new habitats, such as islands, and regular movements such as seasonal migration. These different contexts of host movement are likely to have varied impacts on patterns of parasite dispersal and distribution.

i. Island colonisation

Island frameworks are utilised extensively for exploring the processes by which free-living organisms colonise and persist in novel habitats (Bonardi et al., 2022; Marino et al., 2024; Roeble et al., 2024; Zhao et al., 2024). The rules of island biogeography are based on the premise that island species richness is determined by geographic features such as degree of isolation and area. This is because large continental or mainland regions act as a source for islands. Consequently, islands are colonised by a subset of mainland species, of which fewer persist due to increased extinction rates, leading to lower equilibrium species richness. Species richness on islands is a function of area and isolation. This is because extinction rates are likely to be lower on larger islands and immigration rates are likely to be reduced on more distant islands (Figure 1). As a result, the lowest equilibrium richness is expected on small, remote islands, following species-area and distance-decay relationships (MacArthur and Wilson, 1967). However, current evidence that parasites adhere to the rules of island biogeography is mixed (Illera *et al.*, 2015; Williamson *et al.*, 2019; Williams *et al.*, 2022).

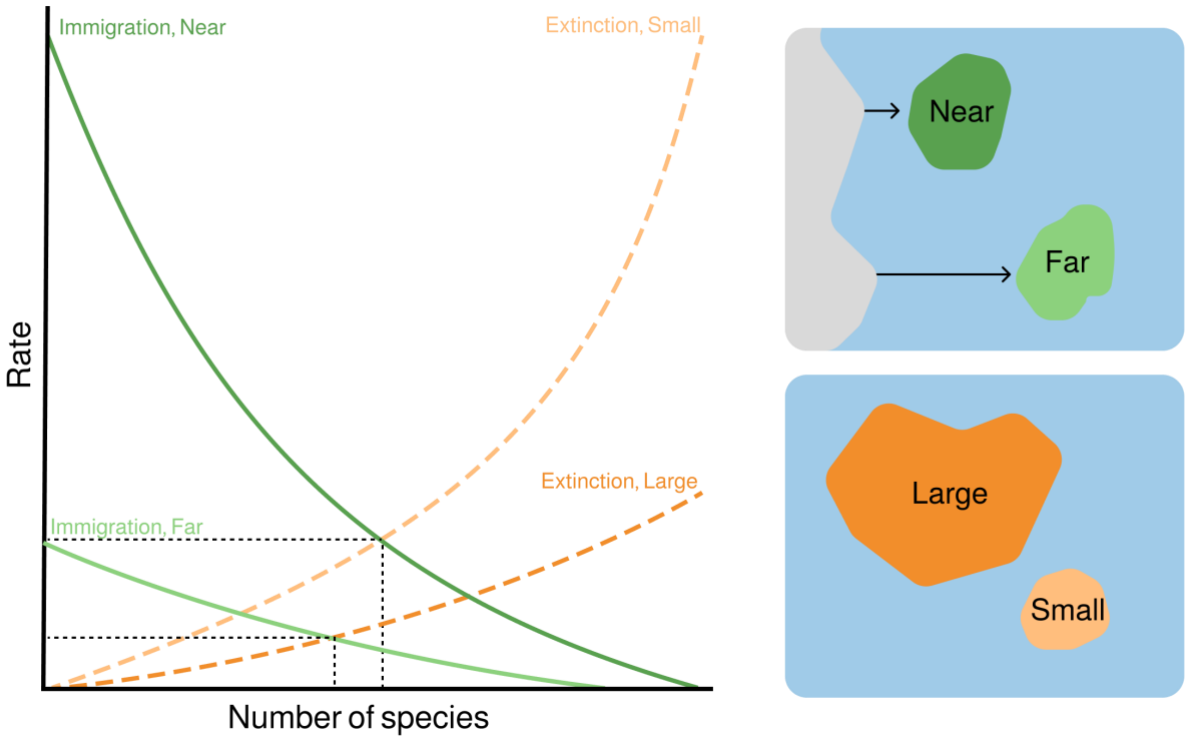

Figure 1. Illustration of the equilibrium theory of island biogeography, adapted from MacArthur and Wilson (1967). Extinction rates (dashed lines) are a function of island area, with extinctions less likely on larger islands (dark orange) and more likely on small islands (light orange). Immigration rates (solid lines) are a function of isolation, with higher immigration on less isolated islands (dark green) and lower immigration on more isolated islands (light green). The intersection of immigration and extinction curves represents the equilibrium species richness, indicated by black dashed lines.

ii. Host migration

Host movements that occur regularly and over shorter temporal scales may also shape parasite biogeography. For example, hosts that undergo seasonal migration may encounter multiple parasite assemblages at stopover or overwintering sites. Such exposure may facilitate transmission, resulting in hosts gaining parasites (Altizer *et al.*, 2011; Leung and Koprivnikar, 2016). These infected hosts may subsequently enable additional parasite transmission, potentially promoting further colonisation of new habitats. Alternatively, parasite pressure may shape migratory behaviour via influences on host physiology. Parasitic infection can be energetically costly, therefore hosts may use migration to avoid or escape parasites (Kendzel *et al.*, 2023). Equally, long-distance movements are costly in themselves. Thus, hosts may be culled during migration due to the detrimental impact of infection on host fitness. Such outcomes would restrict the potential for co-dispersal of parasites with their hosts. The energetically demanding nature of long-distance movement may also lead to trade-offs (Slowinski *et al.*, 2018). In particular, mounting an immunr response is also costly (Bonneaud *et al.*, 2003). Therefore, migratory hosts may invest less in immunity (Eikenaar and Hegemann, 2016), and subsequently have less diverse immune loci (O'Connor *et al.*, 2018). On the other hand, it has been hypothesised that migratory hosts will harbour diverse immune loci to cope with frequent pathogen exposure (Hegemann *et al.*, 2015). These non-mutually exclusive processes may operate in tandem, altering parasite communities across space and time and impacting patterns of parasite biogeography.

Parasite life history traits may impact patterns of biogeography

Reciprocal evolutionary pressures between parasites and their hosts have resulted in the emergence of diverse parasite life history traits such as host specificity, life cycle complexity, and transmission strategy. Such traits are likely to impact parasite colonisation and persistence success, and may interact with one another (Figure 2). Likewise, these varied life history traits will also mediate the impact of host movement on parasite dispersal. Therefore, parasite biogeography cannot be inferred from host biogeography alone (Mazé-Guilmo *et al.*, 2016).

i. Host specificity

Generalist parasites, that can exploit a wide range of hosts, may also occur over a broader geographic range (Amaya-Mejia *et al.*, 2022; Cebrián-Camisón *et al.*, 2025). This is in accordance with the niche-breadth hypothesis, which suggests that generalist parasites are more efficient at colonising host communities (Brown, 1984). Accordingly, generalist parasites are hypothesised to colonise novel environments such as islands more readily (Ishtiaq *et al.*, 2010; Loiseau *et al.*, 2017). It has also been hypothesised that parasites utilising migratory hosts would benefit from being generalist as they are more likely to encounter alternative host species, presenting the opportunity for host switching across migratory routes (Poulin and de Angeli Dutra, 2021). Therefore, breadth of host use is likely to impact the likelihood that parasites will disperse successfully, and hence shape their distributions.

ii. Life Cycle Complexity

Life cycle complexity may also impact parasite colonisation. Parasites with simple life cycles complete development in a single host, whereas those with complex life cycles require multiple hosts. Therefore, complex life cycle parasites may be constrained by the availability of intermediate or definitive hosts (Poulin *et al.*, 2011; Williams *et al.*, 2022). If intermediate hosts are more abundant than definitive hosts, parasites with complex life cycles may have an advantage in achieving colonisation (Choisy et al., 2003). On the other hand, the availability of intermediate hosts has also been shown to restrict parasite dispersal. For example, migratory passerines have been observed to harbour distinct haemosporidian parasite communities that are not shared with

resident hosts at migratory stopover sites, likely because the vectors required for transmission are restricted to different biogeographic regions (Galen *et al.*, 2024). Parasites that possess one or more free-living stages in their life cycles may also disperse independently from their host (Mazé-Guilmo *et al.*, 2016), further complicating patterns of biogeography.

iii. Transmission strategy

Transmission strategy may also impact patterns of parasite dispersal. For example, directly transmitted parasites can often produce encysted stages to achieve protection from changes in environmental conditions (Fayer, 1980; Aguilar-Díaz *et al.*, 2011). These stages can persist in the environment until suitable hosts become available, promoting colonisation (Guernier *et al.*, 2004; Dunn *et al.*, 2010). Parasite transmission strategy can also impose reciprocal effects on the evolution of host migratory behaviour. Host migration impacts parasites by altering contact rates between hosts and transmission opportunities. Conversely, parasites with density-dependent transmission strategies can impose selection on host migratory strategies by favouring behaviours that reduce transmission (Faber *et al.*, 2021).

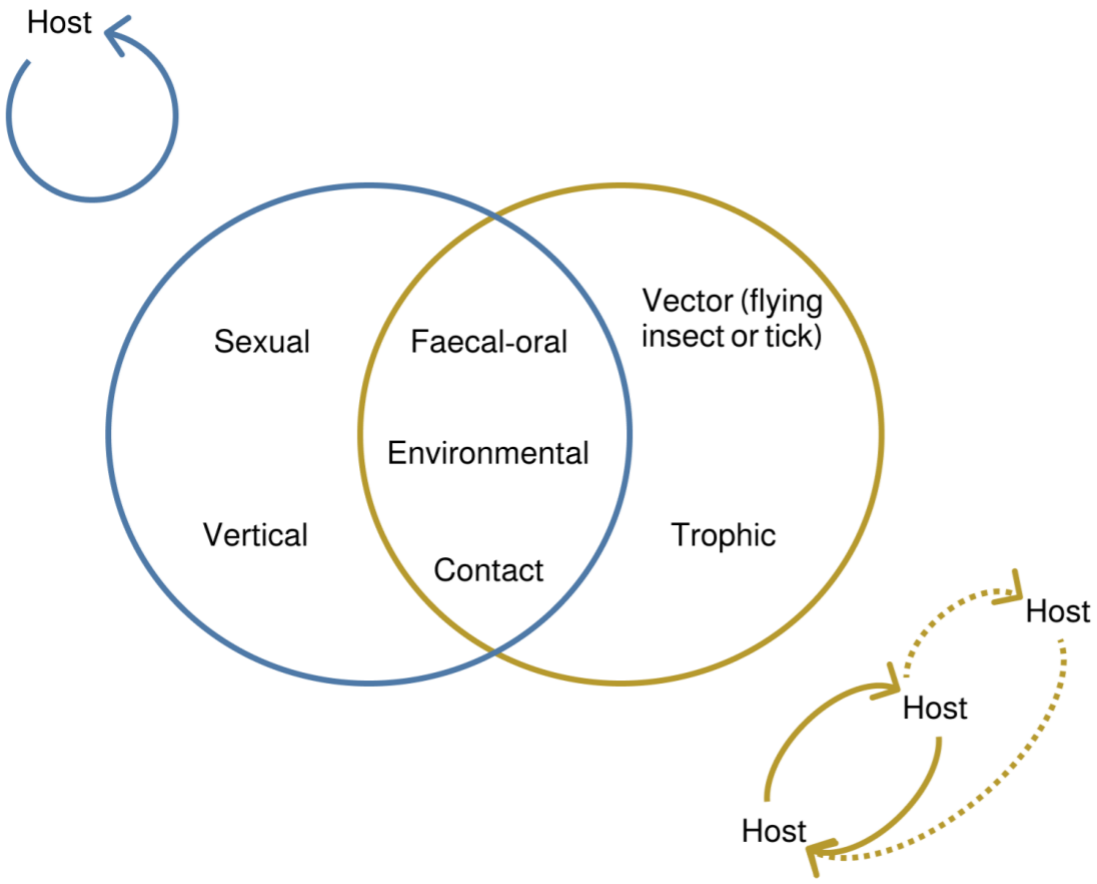

Figure 2. Conceptual illustration of the interaction between parasite life cycle complexity and transmission strategy. Parasites with simple life cycles (blue) complete development within a single host and may be transmitted via sexual and other types of contact, as well as through vertical, faecal-oral, or environmental routes. Parasites with complex life cycles (yellow) require one or more intermediate hosts or vectors and may transmit via trophic interactions or by vectors (flying insects or ticks), as well as by faecal-oral, environmental and contact routes. Different stages of the life cycle may utilise different transmission routes. Transmission strategies appearing in the overlap of the diagram may be utilised by simple or complex life cycles.

Challenges in characterising parasite diversity

Despite the substantial variation in parasite life history traits, ecological studies that assess multiple parasites are uncommon (Hoarau *et al.*, 2020; Huang *et al.*, 2021). This is particularly surprising as co-infections are common in the wild (Petney and Andrews, 1998; Bordes and Morand, 2009). Interactions amongst co-infecting parasites are likely to influence host fitness and transmission success and, ultimately, the patterns of parasite biogeography. Yet, most studies on the macroecology of parasites focus on single-host, single-parasite systems which limits our understanding of how parasite communities are structured in the wild (Hellard *et al.*, 2015).

The tendency to ignore multi-parasite assemblages largely reflects the challenges associated with characterising parasites. It is estimated that most parasite diversity remains undocumented (Dobson *et al.*, 2008). Furthermore, parasites have a cryptic nature, they are patchily distributed, and taxonomic expertise is required to identify them (Dobson *et al.*, 2008; Okamura *et al.*, 2018; de Buron *et al.*, 2025). Conventional parasite surveys include post-mortem examination as well as culturing and/or examination of faecal and blood samples using microscopy (Fallon *et al.*, 2003; Mes *et al.*, 2007; Gouba *et al.*, 2013). However, these methods can be highly invasive and time intensive. In addition, they frequently underestimate parasite diversity (Durso *et al.*, 2010). Such underestimation may be due to low levels of parasitaemia (the number or quantity of parasites per host), transience of observable life cycle stages, and difficulty in culturing parasites (Edgcomb *et al.*, 2002). Molecular approaches offer an alternative and powerful means for detecting multiple parasite genera within a single host (Nadler and de León, 2011). Such methods offer numerous benefits as they are often non-destructive, do not require morphological and taxonomic expertise, and do not rely on detecting specific life cycle stages.

Study system

Birds are increasingly recognised as important parasite dispersers (Okamura *et al.*, 2019; Pulgarín-R *et al.*, 2019; Briscoe *et al.*, 2022; Teitelbaum *et al.*, 2023). The silvereye (*Zosterops lateralis;* Figure 3) is a passerine bird distributed in Australia and islands of the South Pacific, with a latitudinal range spanning -14° to -47° (Figure 4). Silvereyes offer an excellent study system for examining how hosts impact patterns of parasite biogeography for two reasons. First, they demonstrate exceptional dispersal ability and a propensity for rapid population expansion following colonisation (Sendell-Price *et al.*, 2021). As a result, silvereye have successfully colonised a variety of islands, from the large Gondwanan islands of Grand Terre (New Caledonia), as well as the North and South Islands of New Zealand, to small volcanic islands such as those of Vanuatu, French Polynesia and Fiji, as well as coral cays such as Heron Island on the Great Barrier Reef (Mees, 1969).

Silvereyes have colonised islands over a range of timescales. While some colonisations, such as those in Southern Melanesia, Lord Howe Island and Heron Island, are estimated to have occurred thousands or hundreds of thousands of years ago (Estandía *et al.*, 2025), others are more recent. For instance, the historically documented sequential colonisation of the Tasmanian subspecies of silvereye (*Z. l. lateralis)* occurred in the last 200 years. In this sequential colonisation, birds from Tasmania established populations on the South Island and North Island of New Zealand, Chatham Island and Norfolk Island (Mees, 1969). Other populations are the product of recent, human-mediated introductions: Eastham Guild successfully introduced the silvereye to Tahiti during the 1930s (Guild, 1938), and the population has continued to expand across the archipelago (Thibault and Cibois, 2017).

Silvereye colonisations have often involved rapid morphological diversification (Clegg *et al.*, 2002), which has given rise to 17 morphological subspecies. The diversification of subspecies has also resulted in variation in dispersal propensity, with the development of partial migrant, dispersive and totally sedentary populations. The multitude of island populations and the emergence of varied dispersal propensities makes the silvereye a particularly interesting system to address the impact of host movement on parasite biogeography.

The second attraction for using the silvereye as a study system is that their blood parasites have been studied in a biogeographic context previously (Gudex-Cross *et al.*, 2015; Olsson-Pons *et al.*, 2015). However, previous research has predominantly focussed on characterising haemosporidian parasites, which are the causative agent of avian malaria (Clark *et al.*, 2014; Gudex-Cross *et al.*, 2015; Olsson-Pons *et al.*, 2015). Such studies provide considerable background knowledge of the ecology of host populations, but inferences are limited to the few parasites that have been examined previously. Yet, as is common for passerine birds (Parsa *et al.*, 2023), there is also evidence that silvereye harbour a diversity of protozoan parasites with reports of other blood parasites such as *Atoxoplasma* (Laird, 1959) and filarial nematodes (Clark *et al.*, 2016) as well as gastrointestinal parasites such as *Isospora,* a coccidian parasite (Yang et al., 2018). Their generalist diet (Waite *et al.*, 2013) may also enable parasitic infection via multiple routes. Therefore, utilising this system to expand the range of parasites studied may offer considerable insights into patterns of parasite biogeography.



Figure 3. Capricorn silvereye (*Z. l. chlorocephalus*) on Heron Island, Australia. Photograph by Andrea Estandía in April 2022.

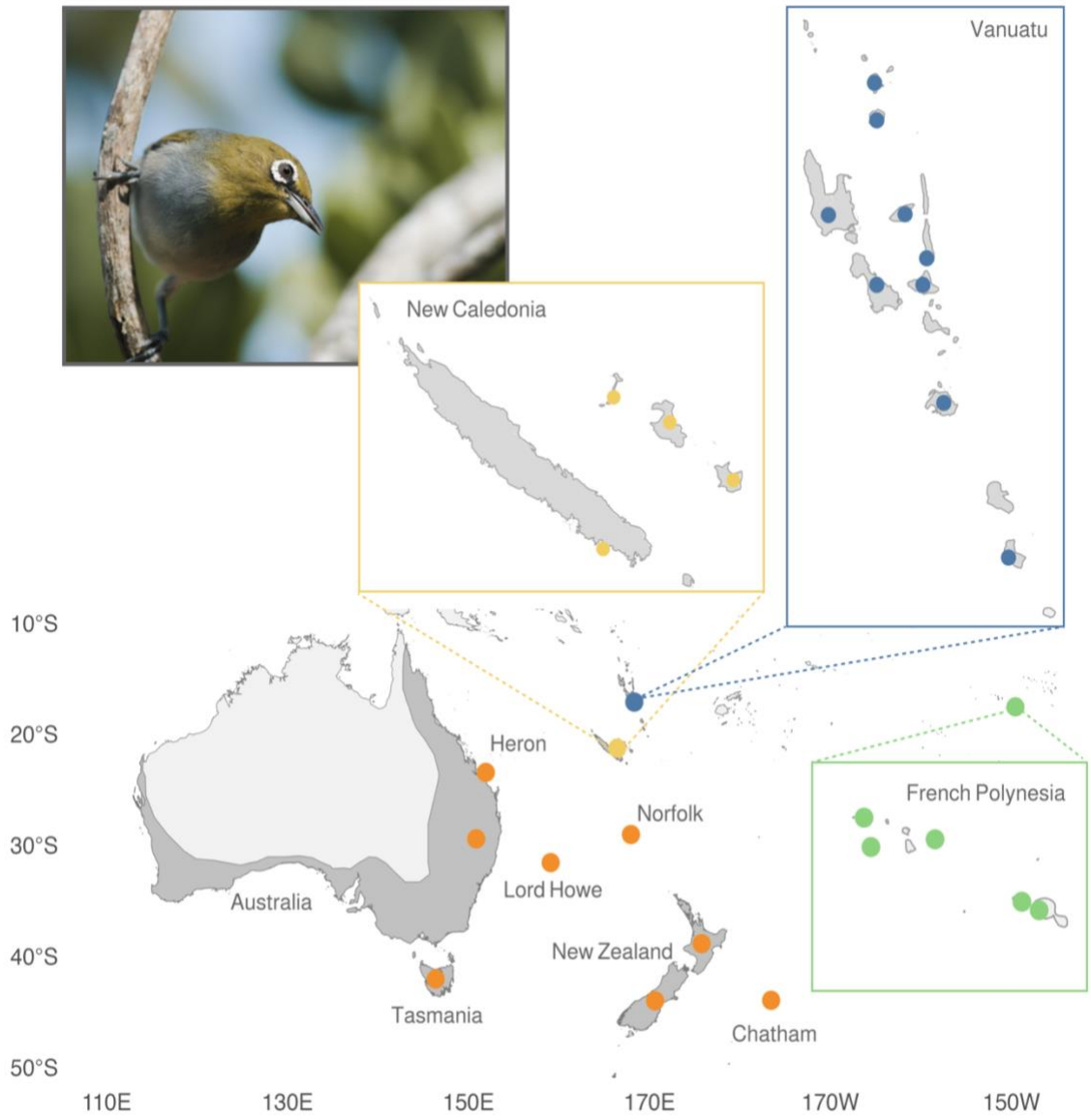

Figure 4. Map showing the silvereye distribution and sampled populations in this thesis. The silvereye (top left) distribution is highlighted in dark grey while dots indicate populations sampled and presented in this thesis, insets indicate New Caledonia (yellow), Vanuatu (blue), and French Polynesia (green).

Thesis aims and rationale

Our understanding of the factors that shape parasite biogeography is limited because most research has focused on single parasite systems and ignores the complexity of parasite life histories. In this thesis, I utilise molecular methods to characterise parasite communities of the silvereye, providing a rare opportunity to investigate the influence of parasite traits on dispersal and distributions as a result of host movements and colonisation of new habitats.

It is often assumed that parasites will conform to the same rules that dictate the biogeography of their free-living hosts. However, parasite dispersal is constrained, as hosts act as resource islands for parasites. Variation in parasite life histories may also differentially impact dispersal and distribution patterns. Chapter 2 aims to evaluate whether parasites conform to the predictions of island biogeography theory and to identify factors that may explain departures from the expected island-mainland, species-area and isolation relationships. To do this, I conduct a systematic review of empirical studies which test the rules of island biogeography in parasites.

In Chapter 2 I highlight the need for approaches that characterise a broad range of parasites. Chapter 3 aims to address this gap by developing a bioinformatic pipeline to extract parasite sequences from existing whole genome sequence (WGS) data of the host. I develop and validate a framework for mining host WGS datasets to detect endogenous eukaryotic parasites that have been inadvertently sequenced, comparing this approach with metabarcoding, as well as more commonly utilised methods such as targeted PCR and microscopy.

Approaches that quantify a broad range of parasites are essential for biogeography studies as it enables the comparison of parasites with varied life histories that may disperse differentially. Chapter 4 aims to apply the approach developed in Chapter 3 to test island biogeography predictions in a range of parasites. I utilise WGS sequences generated from silvereye individuals sampled across mainland Australia and multiple South Pacific islands, testing whether transmission strategy mediates the observed island-mainland, isolation, and species-area relationships.

A final aim is to investigate the role of host movement over a shorter temporal scale in shaping parasite biogeography. Chapter 5 thus focuses on the partial migrant population of silvereye in Tasmania to test how migration influences parasite richness and sharing, and to determine whether parasites with different life cycles differ in dispersal capacity.

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Chapter 2

Do parasites follow the rules of island biogeography?

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Abstract

Parasites represent a major component of global biodiversity, yet the processes that shape their macroecological distributions remain poorly resolved. It is sometimes assumed that parasites will adhere to the same rules that govern the distributions of their free-living hosts, for instance the area-isolation relationships predicted by island biogeography theory. However, drivers of macroecological parasite distributions may deviate from those of free-living organisms due to variation in parasite life histories and reliance on hosts for essential resources. We conducted a systematic review of empirical, peer-reviewed studies that examine patterns of island biogeography in parasites. Specifically, we evaluate support for island biogeography theory and attempt to identify possible factors that may drive deviations from expected patterns. Across host and parasite taxa, evidence for species-area, isolation and island-mainland nestedness effects was highly variable, with no consistent support for island biogeography predictions. Instead, our review provides evidence for methodological biases that have led to a limited range of host-parasite systems being investigated in an island biogeography context. For example, a substantial number of studies have focused on parasites transmitted by flying insect vectors, whose high dispersal capacity is likely to weaken island isolation effects relative to parasites with direct, trophic, or environmental transmission. In addition, despite the tendency for hosts to be infected with multiple parasites in the wild, most investigations focus on distributions of a single parasite taxa and studies which address multi-parasite assemblages are rare. Such biases limit inferences about the mechanisms underlying deviations from expected patterns. Addressing these gaps in the breadth of host-parasite interactions represented in the literature is essential to develop general frameworks for understanding the factors that shape parasite biogeography.

Introduction

Understanding the processes that shape species distributions is essential for anticipating interspecific interactions and predicting their impacts on ecosystem dynamics. Consequently, the biogeography of free-living organisms has long been the focus of macroecological research (Lomolino *et al.*, 2017). In contrast, far less is known about the factors that shape parasite biogeography, despite parasites representing the most abundant mode of eukaryotic life (Dobson *et al.*, 2008; Larsen *et al.*, 2017). At broad spatial scales, parasite and host species richness are often correlated (Krasnov *et al.*, 2004; Kamiya *et al.*, 2014; Poulin, 2014), leading to the expectation that parasite distributions should follow the same general rules that govern their free-living hosts. However, parasites differ fundamentally from free-living organisms due to their reliance on hosts for essential resources (Morand, 2015), creating an asymmetric relationship in which hosts function as islands for parasites (Kuris *et al.*, 1980; Koop *et al.*, 2014; Strona and Fattorini, 2014) thus giving rise to meta-community dynamics which could alter parasite assemblages at the micro- and macro-scales. In addition, parasites co-evolve with their hosts, resulting in a wide range of life history strategies and functional traits (Crossan *et al.*, 2007; Kochin *et al.*, 2010). Therefore, parasite distributions may deviate from the patterns expected in their free-living hosts. Increasing concerns about the potential impacts of changing parasite distributions in the face of climate change (Carlson *et al.*, 2017) and subsequent impacts on ecosystem dynamics (Poulin and Mouritsen, 2006; Polley *et al.,* 2010), underscore the need to elucidate the rules that govern macroecological parasite patterns.

Studies that have tested broad-scale biogeographic rules for parasites often report contradictory results. This includes mixed support for latitudinal diversity gradients (Nunn *et al.*, 2005; Lindenfors *et al.*, 2007; Guilhaumon *et al.*, 2012; Preisser, 2019); Rapoport's rule of larger ranges at higher latitudes (Rohde, 1978; Thieltges *et al.*, 2011; Poulin, 2021) and for the rules of island biogeography (Illera *et al.*, 2015; Williams *et al.*, 2022). According to the latter, continental regions or large land masses act as a source for islands, a subset of species that occur on these source areas, are expected to colonise and persist on islands resulting in lower equilibrium species richness and lowest species richness is expected on small, remote islands, following species-area and distance-decay relationships (MacArthur and Wilson, 1967).

Parasites display substantial functional diversity in traits that, in turn, may impact their colonisation and persistence success (Telfer and Bown, 2012; Cizauskas *et al.*, 2017). These include host specificity, life cycle complexity, transmission strategy, virulence, fecundity, and longevity. It may be assumed that generalist parasites, that can exploit a wide range of hosts, will be less constrained in their ability to colonise and persist on islands than those that are highly host specific (Ishtiaq *et al.*, 2010; Loiseau *et al.*, 2017). Similarly, parasites with complex life cycles may be constrained by the availability of intermediate hosts (Poulin *et al.*, 2011; Williams *et al.*, 2022). Consequently, the impact of life cycle complexity is likely to be context-dependent, as complex life cycles may promote dispersal success in scenarios where the intermediate host is more abundant than the definitive host (Choisy *et al.*, 2003). Transmission strategy may also impact patterns of island biogeography. For example, directly transmitted parasites often produce encysted stages, providing protection from abiotic factors, such as changes in temperature and humidity (Fayer, 1980; Aguilar-Díaz *et al.*, 2011). Such stages can persist in the environment when suitable hosts are unavailable, increasing the chance of establishment (Guernier *et al.*, 2004; Dunn *et al.*, 2010). Other factors such as parasite longevity and environmental tolerance will also impact the opportunities for parasites to encounter viable hosts on islands.

Beyond parasite life history traits, aspects of host ecology on islands may further impact the expected biogeographic patterns for parasites. Island communities and populations differ in predictable ways from those found on a mainland. Island populations often experience density compensation, whereby population density is higher than might be expected on a similar-sized area on the mainland as a result of reduced competition (MacArthur *et al.*, 1972; Wright, 1980). High host density can impact parasite dynamics by increasing host contact rates and driving up prevalence (Fromont *et al.*, 2001; De Bellocq *et al.*, 2002). Furthermore, remote islands are often considered evolutionary refugia from parasites as, according to the rules of island biogeography, their isolated nature may preclude colonisation by a diversity of parasites. Reduced exposure to diverse parasite communities over evolutionary time may reduce selection on host immune loci (Wikelski *et al.*, 2004), potentially providing conditions for the parasites that do successfully colonise to persist at higher prevalences on islands.

Accounting for parasite life histories and host ecology is therefore essential for understanding the processes that shape parasite distributions on islands. In this systematic review, we synthesise empirical studies that have examined parasite communities within the framework of island biogeography. Specifically, we assess the extent to which parasites conform to the rules of island biogeography and compare observed patterns across a range of parasite and host systems. We consider how parasite life history traits may underly deviations from the rules of island biogeography and identify notable gaps in taxonomic, geographic and methodological coverage that impede our understanding of the factors that shape macroecological parasite patterns.

Methods

We utilised R version 4.4.2 (R Team, 2024) to assist our literature search and conduct data visualisation. To retrieve peer-reviewed studies that assessed the rules of island biogeography in parasites, we initially searched four databases: Google Scholar, Scopus, ProQuest and PubMed. We used litsearchr to identify keywords for our search term (Grames *et al.*, 2019), with the requirement that article titles or abstracts must contain a minimum of one word from each of three topical groups: parasites ('parasit*', 'disease' and/or 'infection'), islands ('island*' and/or 'archipelago*') and distributions ('biogeograph*', 'diversit*', 'distribution*' and/or 'pattern*'). The asterisk (*) operator was used as a wildcard to include possible variations of keywords. The literature search covered the time period from the earliest available publications to May 2025.

Our initial search yielded 1234 records. Duplicate articles were removed using the *synthesisr* package (Westgate and Grames, 2025), although some manual removal was also required. We screened records according to their titles, followed by their abstracts. We included studies on eukaryotic endoparasites, defined as organisms (helminths or protists) living inside and extracting resources from a host for at least part of their life cycle. Some studies targeted endoparasites alongside other pathogen groups (including ectoparasites, viruses, bacteria and fungal infections). We report results from cases where non-target pathogens were analysed together with endoparasites. However, we note that these non-target pathogens are under-represented in the dataset as they were not specifically targeted in our search term. Our inclusion criteria also required studies to address at least one of the expectations of island biogeography. These could be in the form of island versus mainland comparisons, or effects of island area or isolation (including studies which tested for distance-decay patterns). Regarding the measurement of parasite richness, we considered studies that examined community-level richness, diversity, or genetic diversity at the individual parasite level. Although the rules of island biogeography are defined according to changes in community richness on islands compared to the mainland, we identified a number of studies that tested differences in parasite prevalence. The expectations for parasite prevalence on islands differ from those of richness or diversity because they are determined by different epidemiological processes. The expectations for richness and prevalence are outlined in Table 1.

We excluded studies that applied the rules of island biogeography at the level of individual hosts (e.g. treating hosts as 'resource islands' [Poulin, 2014]). Although there are analogies for species-area with host body size (Guégan *et al.*, 1992; Poulin, 1995), hosts are mobile and therefore do not represent spatially fixed, isolated units, meaning that island-mainland contrasts and isolation effects cannot be tested. Similarly, we excluded studies that applied island biogeography theory to anthropogenically fragmented habitats. Fragmentation typically occurs over shorter timescales, therefore the same eco-evolutionary processes that operate in true island, sky islands, and established lacustrine systems are unlikely to apply. We also did not consider grey literature such as reports, theses or conference proceedings. Finally, we excluded studies conducted in clinical settings that involved treatment interventions as we were interested in understanding the patterns of natural infections in wild hosts. We present a PRISMA diagram to illustrate the process for selecting articles to include in the systematic review (Figure 1). A list of the references included in the systematic review is provided in Table S1.

Table 1. Summary of predicted relationship between parasite community (community-level richness or diversity, genetic diversity at the individual parasite level, community composition, genetic divergence and prevalence) and island features.

Biogeographical Feature	Prediction
Island v Mainland	Higher community-level richness on mainland
	Higher prevalence on island
	Higher genetic diversity for individual parasites on mainland
Island area effects	Increase in community-level richness with area
	Increase in genetic diversity for individual parasites with area
	Decrease in taxon-specific parasite prevalence with increased area
Island isolation and distance effects	Decrease in community-level richness with isolation
	Decrease in genetic diversity for individual parasites with distance
	Community composition difference increases with distance
	Genetic divergence within a parasite taxon increases with distance

For each article, we recorded the following information: year of publication, start and duration of sample collection in years, number of hosts included in the study, location (including continent, country, group of islands [if named], latitude and longitude), geographic extent (the distance between the two furthest points in kilometres [km] as well as the areas of the smallest and largest islands included in the study [km^2]), number of islands, type of island (oceanic, continental, lacustrine or sky islands), host group investigated (bird, mammal [human], mammal [non-human], fish, insect, amphibian, reptile), parasite supergroup (helminths, apicomplexans, diplomonads, trypanosomatids, arthropods, bacteria, ciliophorans, fungi, viruses), lower order of parasite identification (if specified), parasite life cycle complexity (whether parasites use single or multiple hosts and thus have complex or simple life cycles), transmission strategy (vector, trophic, environmental or direct). We also recorded the methodology employed for characterising parasites (i.e. based on morphology or DNA sequences). Finally, we documented which island biogeography expectations were addressed, how the parasite community was characterised (this could be a metric based on genetic diversity, alpha diversity or species richness [referred to broadly as richness]; prevalence; or genetic or community-level divergence for those papers addressing distance-decay relationships), the statistical test used, and whether the study found statistically significant support for each relationship.

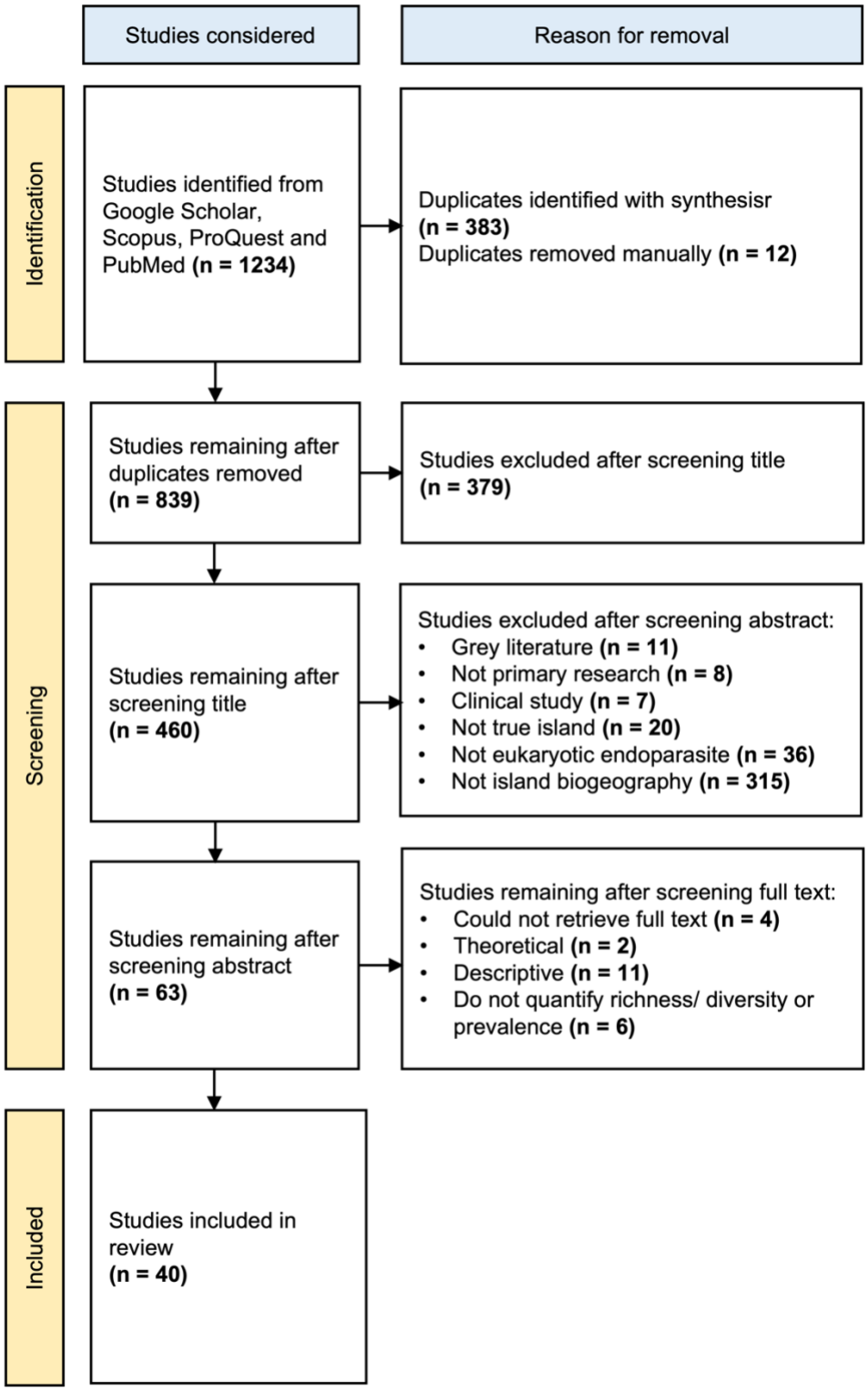

Figure 1. PRISMA flow diagram (Page *et al.*, 2021) illustrating the process of screening records for inclusion in the systematic review and detailing the number of articles excluded at each stage.

Results

We identified 40 peer-reviewed studies that met our inclusion criteria (Figure 2A). The number of studies published per host group varied from 14 on birds, five on fish, five on non-human mammals, seven on humans and six on reptiles. Studies spanned six continents but were geographically clustered. Five of six (83%) studies on reptiles were conducted in Europe, and five of 14 studies on birds were conducted in Macaronesia (36%). Furthermore, despite being the most common host group examined, 100% of studies in bird hosts focused on apicomplexan parasites, and 12 of 14 (86%) exclusively targeted haemosporidian parasites (Figure 2B). On the other hand, no studies characterised apicomplexans in fish, despite these being a common parasite for the host group (Molnár, 2006; Bonacolta *et al.*, 2024; Mamedova and Karanis, 2025). Instead, investigations of fish parasites predominantly targeted helminths. Studies on humans incorporated a broad range of parasites, including apicomplexans, ciliates, diplomonads, helminths and trypansomatids.

We observed a rapid rise in the number of parasite island biogeography studies published in the mid-2000s, with 90% being published after 2007 (Figure 2C), although some sample collection pre-dated the 1960s (Figure 2E). Subsequent to 2005, there have been peaks and troughs in publications, and no further studies have been published since 2023. Sample collection generally took place over relatively short time frames, with 75% of studies collecting data over five years or less (Figure 2E). Oceanic and continental islands were by far the most common island types considered with only three studies on sky islands and one in a lacustrine system (Figure 2D). Studies encompassed a range of spatial scales (Figure 2F), although just two studies took place on the global scale.

Parasites with complex life cycles were more commonly studied than those with simple life cycles, and this was particularly apparent for studies focussing on parasites of humans and birds (Figure 3A). Of these complex life cycle parasites, those transmitted by vectors were most common in both human and bird host studies. Flying insect vector-transmitted parasites also received the most attention overall while direct transmission received the least. Notably, more than half of the studies targeted a single parasite group (Figure 3B). Only three studies targeted more than five parasite taxa, and these all examined parasites in fish and human hosts.

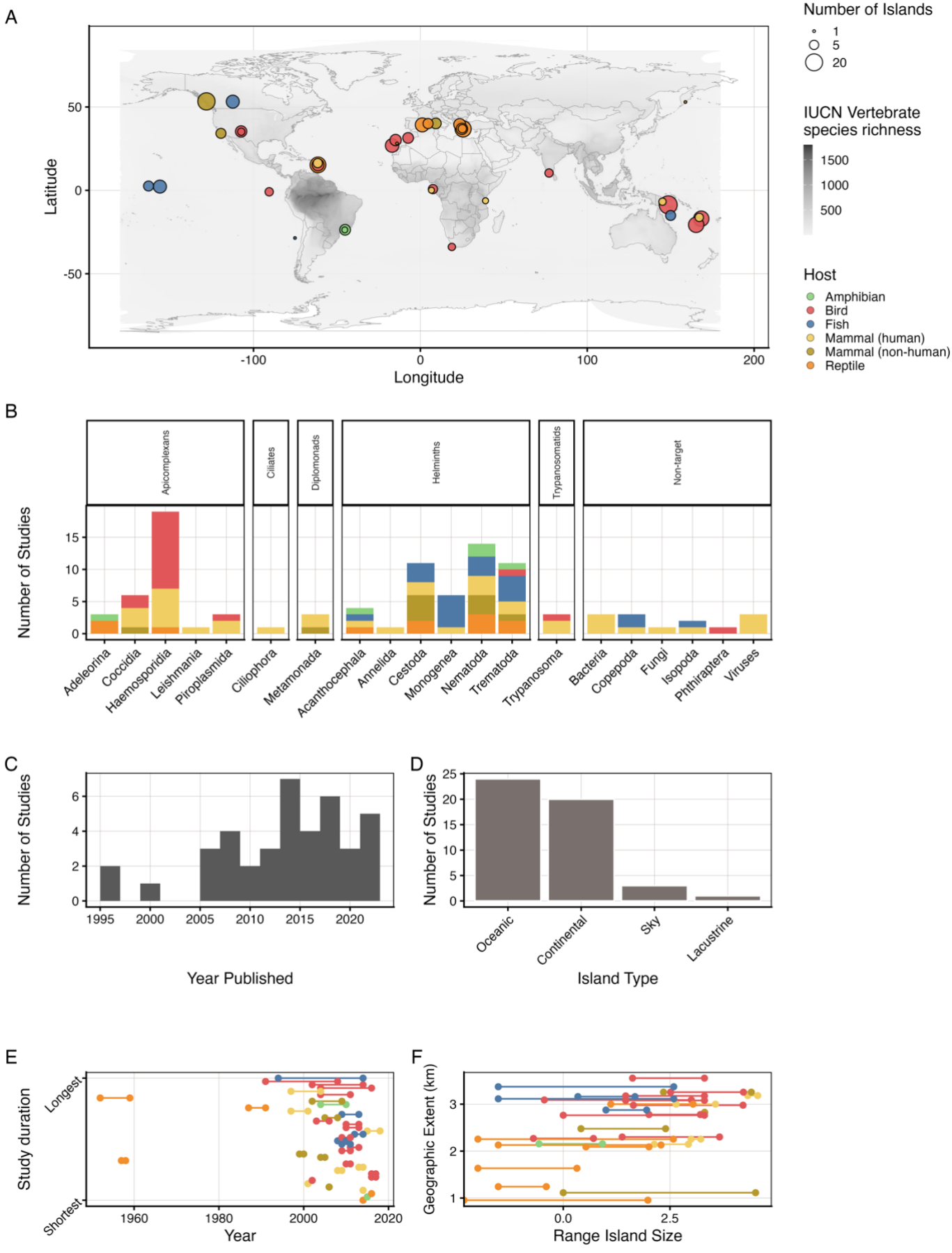
A
Latitude
Longitude
50
0
-50
-100
0
100
200
Number of Islands
1
5
20
IUCN Vertebrate species richness
1500
1000
500
Host
Amphibian
Bird
Fish
Mammal (human)
Mammal (non-human)
Reptile
B
Number of Studies
Apicomplexans
Ciliates
Diplomonads
Helminths
Trypanosomatids
Non-target
Adeleorina
Coccidia
Haemosporidia
Leishmania
Piroplasmida
Ciliophora
Metamonada
Acanthocephala
Annelida
Cestoda
Monogenea
Nematoda
Trematoda
Trypanosoma
Bacteria
Copepoda
Fungi
Isopoda
Phthiraptera
Viruses
C
Number of Studies
Year Published
D
Number of Studies
Oceanic
Continental
Sky
Lacustrine
Island Type
E
Study duration
Longest
Shortest
Year
F
Geographic Extent (km)
Range Island Size

Figure 2. Summary of 40 peer-reviewed studies which test the rules of island biogeography for eukaryotic endoparasites published between 1996 and 2023. A) Sampling location of each study (midpoint of latitude and longitude) excluding global studies. Point size indicates the number of islands included in the study. The map is overlayed with the IUCN vertebrate species richness estimates, requested on 5th December 2025. B) Frequency of studies focusing on taxonomic groups of parasites. C) Frequency of studies published over time. D) Frequency of studies conducted on different types of island. E) Duration of sample collection for each study (single point indicates sampling took place over one year). F) Geographic extent of studies (the log-transformed distance between the two furthest points in the study) against range in island size of studies (the log-transformed areas for the smallest and largest islands included in each study). Colours correspond to host taxa.

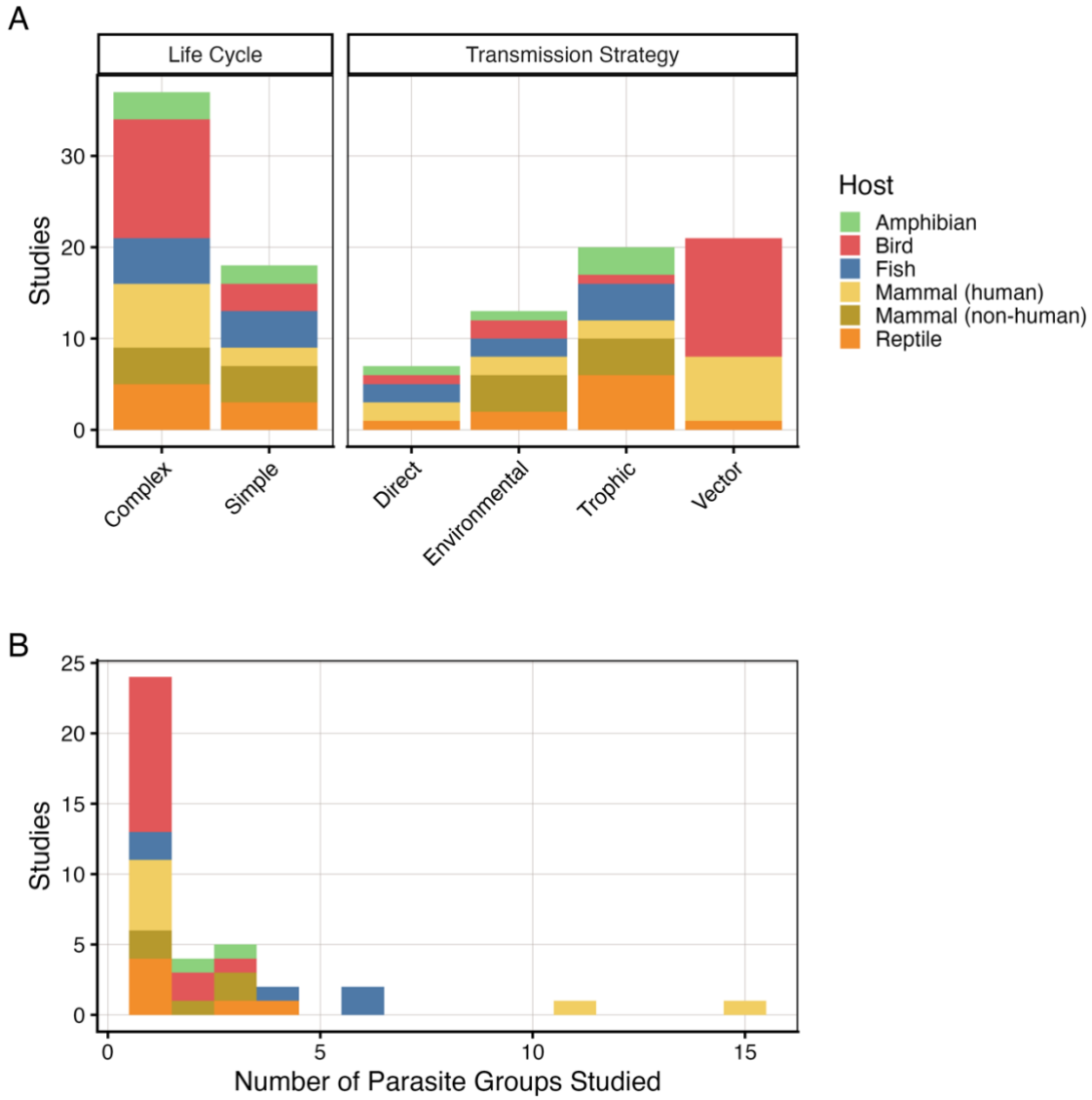

Figure 3. Summary of parasite traits represented in 40 peer-reviewed studies which test the rules of island biogeography for eukaryotic endoparasites published between 1996 and 2023. A) Life cycle and transmission strategies of parasites included in the studies. Life cycle indicates whether parasites use multiple hosts (complex) or a single host (simple). Transmission strategy refers to the mechanism of infection. B) Number of parasites tested per study. Colours indicate host taxa.

Studies that compared parasite communities on islands to the mainland provided mixed results for island biogeography patterns. Parasite richness was consistently lower on islands than the mainland in humans (Figure 4A). However, this pattern was only observed in bird hosts for 33% of cases, with no difference in richness detected by the only analysis that targeted a simple life cycle parasite. For parasites in bird hosts, higher prevalence was documented on mainlands in 11 of 14 (79%) analyses (Figure 4B). Either the reverse pattern or no difference was observed in the two cases where parasites with simple life cycles were examined in bird hosts.

All studies that tested the impact of island area on parasite richness in humans reported an increase with area (Figure 5). The impact of changing island area has not been addressed in parasites of amphibians. A higher proportion of the analyses testing direct and vector-transmitted parasites reported the expected pattern compared to those which utilise trophic or environmental transmission. Differences in parasite prevalence have not been addressed in fish, non-human mammals or humans.

Most studies did not detect a significant effect of isolation on parasite communities (Figure 6). This was particularly notable for humans, as nine of the 16 analyses (from a total of seven studies of human parasites) did not find a significant impact of isolation on richness. There have been no studies published up to our search in May 2025 that addressed the effect of isolation on parasite prevalence in fish, humans or non-human mammals. No studies detected an impact of isolation for the majority of complex and simple life cycle parasites. Distance-decay patterns for parasites have been tested in birds, fish and humans (Figure 7). Most studies show that parasite divergence, measured using genetic variation within particular parasite taxa, or community-level divergence metrics, increased with isolation distance whereas for richness, approximately half of the analyses show no relationship.

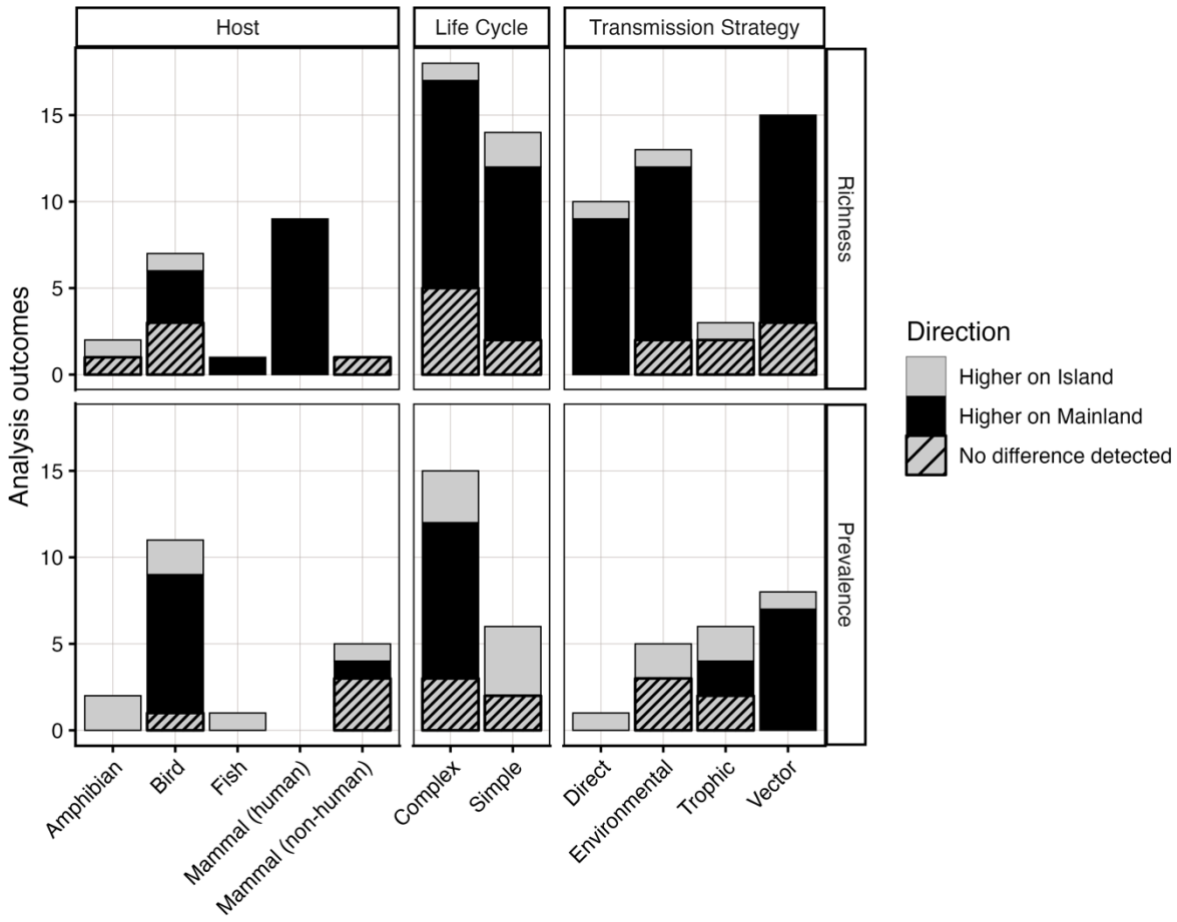

Figure 4. Summary of analysis outcomes reported for 14 of 40 peer-reviewed studies published between 1996 and 2023 that test the difference between parasite communities (richness and prevalence) in mainland compared to island populations for eukaryotic endoparasites according to host groups, life cycle complexity, and transmission strategy. Direction of relationships signified by patterns as follows: solid black indicates that either richness or prevalence was higher on the mainland, grey indicates that richness or prevalence was higher on islands, dashed indicates that no significant difference was observed.

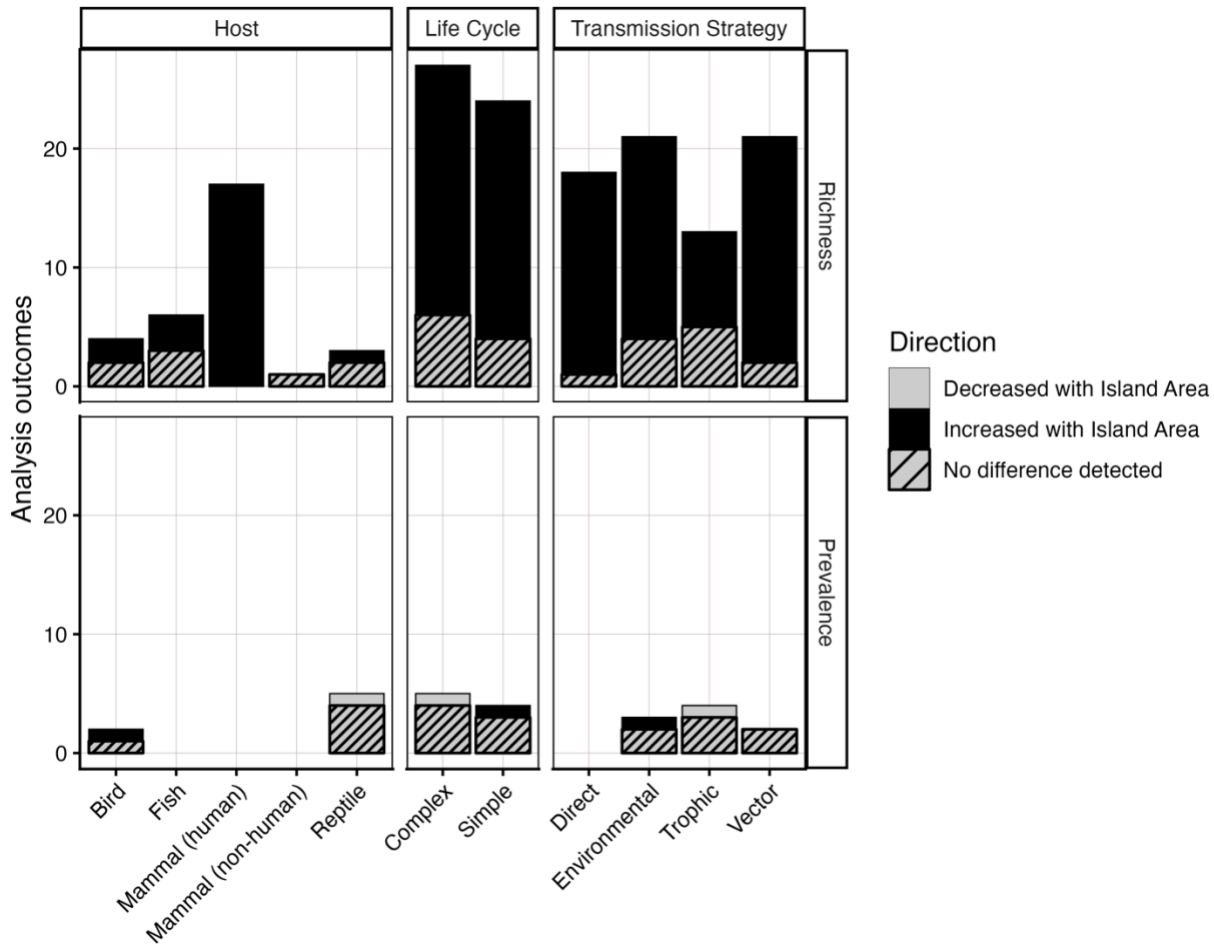

Figure 5. Summary of analysis outcomes reported for 16 of 40 peer-reviewed studies published between 1996 and 2023 that tested the impact of island area on parasite communities in terms of richness and prevalence for eukaryotic endoparasites according to host groups tested, life cycle complexity, and transmission strategy. Direction of relationships signified by patterns as follows: solid black indicates that parasite richness or prevalence increased with island area, grey indicates that parasite richness or prevalence decreased with island area, dashed indicates that no significant difference was detected.

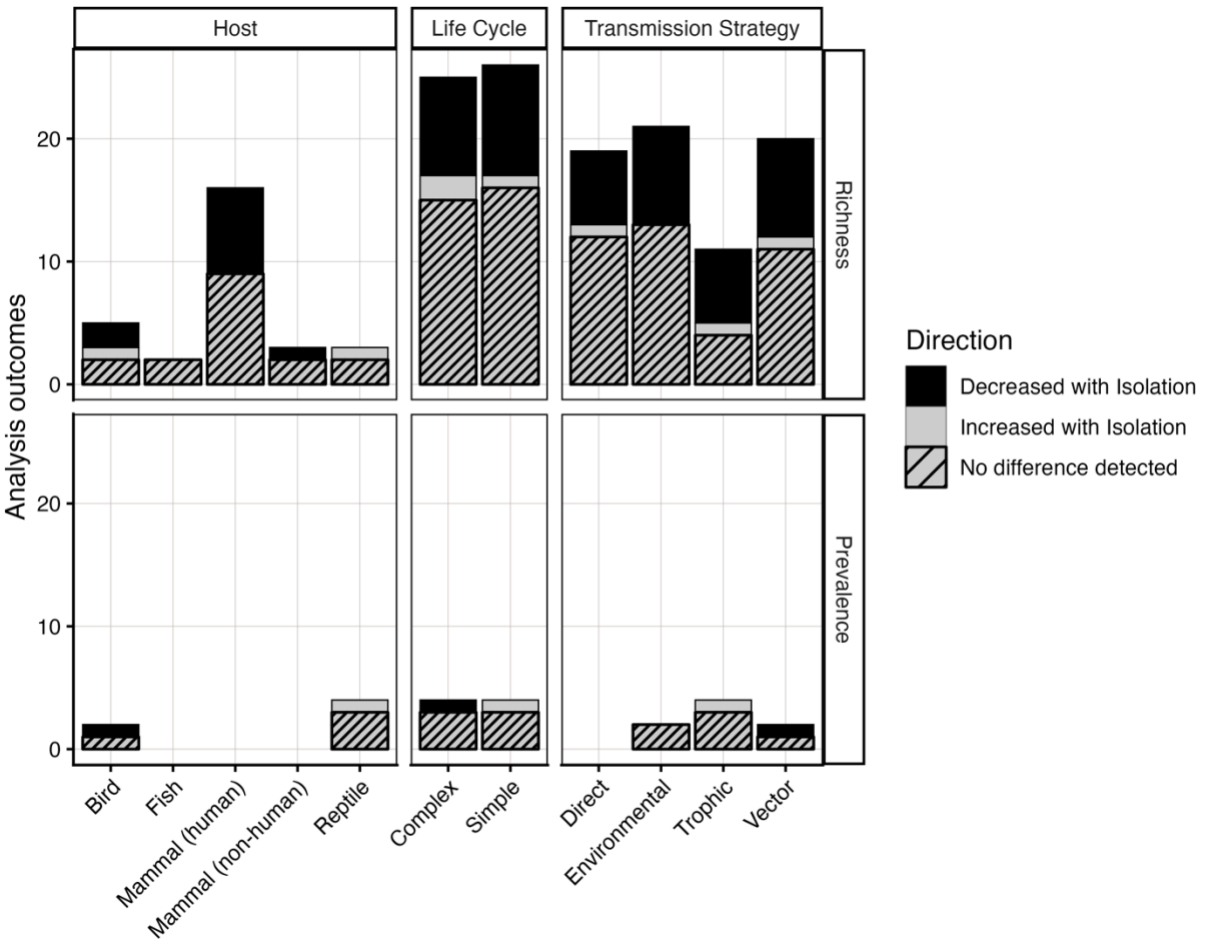

Figure 6. Summary of analysis outcomes reported for 13 of 40 peer-reviewed studies published between 1996 and 2025 that test the impact of isolation on parasite communities (richness and prevalence) for eukaryotic endoparasites according to host groups tested, life cycle complexity, and transmission strategy. Solid black indicates that richness or prevalence decreased with isolation, grey indicates that the opposite pattern was found whereby richness or prevalence increased with isolation, and dashed indicates that no significant difference was observed.

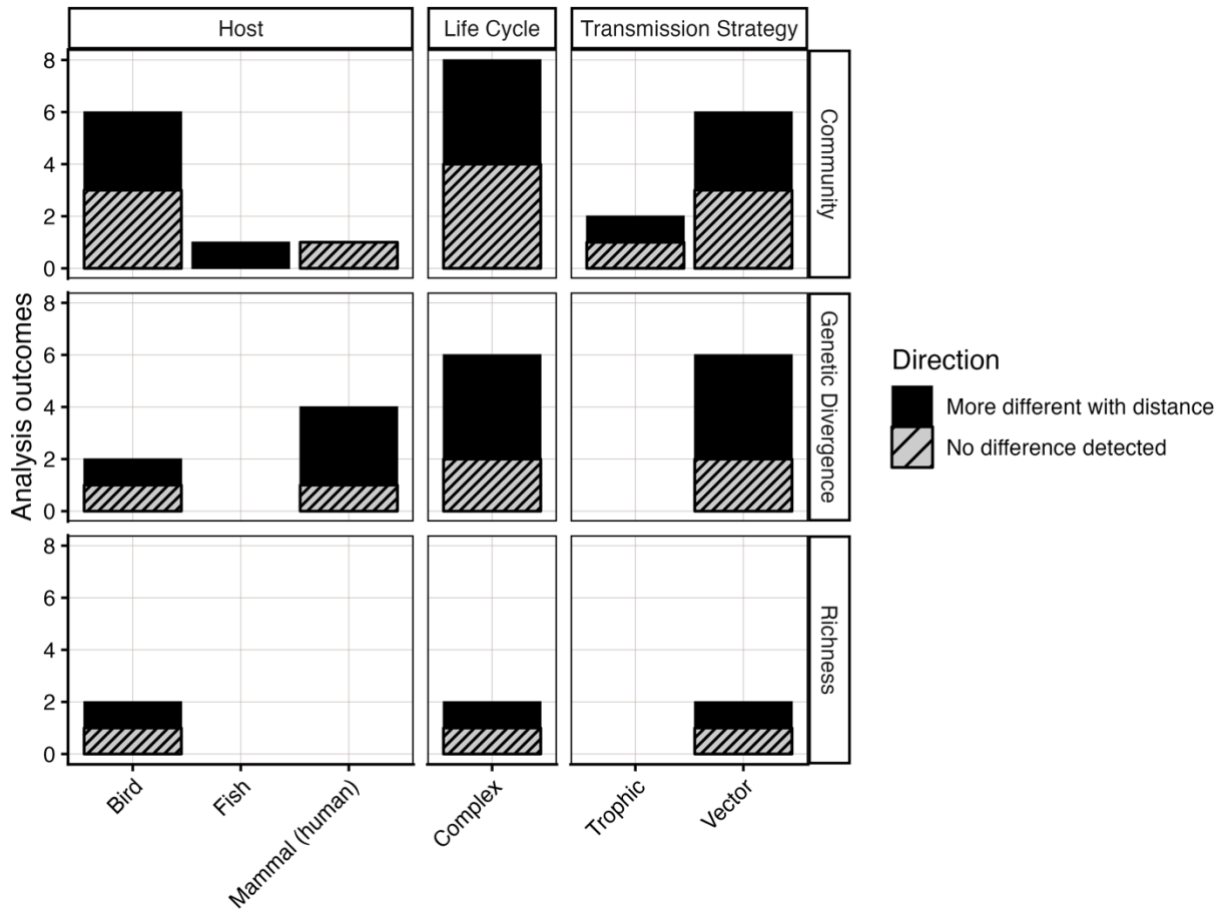

Figure 7. Summary of analysis outcomes reported for 12 of 40 peer-reviewed studies published between 1996 and 2025 that test whether parasite communities (community-level divergence metric, parasite individual-level genetic divergence or community richness) follow distance-decay patterns for eukaryotic endoparasites according to host groups tested, life cycle complexity, and transmission strategy. Solid black indicates that parasite community becomes more distinct or decreases in richness with distance, dashed indicates that no significant effect was observed.

Discussion

Parasites are ubiquitous and can be found in every ecosystem (Lafferty *et al.*, 2006; Dobson *et al.*, 2008), yet there is a dearth of research exploring the factors that shape their distributions. Our review highlights that parasites do not necessarily conform to the rules of island biogeography, despite these patterns being well-documented for free-living taxa. Instead, our analyses suggest that parasite distributions may reflect characteristics of hosts and parasites. However, our results are confounded by sampling biases. These include uneven sampling across host groups, parasite taxa, and geographic regions. Methodological constraints have led to certain parasite and host taxa as well as multi-parasite communities being overlooked. It is therefore essential to expand island biogeography studies to target parasites with varying life history traits and to use approaches that characterise multi-parasite communities to understand the broad-scale processes that shape parasite distributions.

Variation in parasite life history traits is not currently captured in published studies

Parasite life history traits are likely to influence island colonisation success, yet variation in such traits is not represented in published studies. Within our systematic review, birds were the most frequently represented host group. Despite this, adherence to the predictions of island biogeography has almost exclusively been examined using their haemosporidian parasites. These complex life cycle parasites are the causative agents of avian malaria, and rely on bites from flying insect vectors for transmission (Valkiunas, 2004). Similarly, most island biogeography studies on human parasites have focused on those with complex life cycles involving transmission by flying insects. Parasite patterns of distance-decay have rarely been considered outside of complex, vector-transmitted parasites in humans and birds. However, it is likely that parasites transmitted by flying insects will display differing patterns of dispersal in comparison to those utilising other transmission strategies. Colonisation by vector-borne parasites may be constrained by vector availability (Galen *et al.*, 2024), while colonisation by parasites transmitted in the environment, via faecal-oral routes or direct contact, may be less constrained (Williams *et al.*, 2022). However, flying insect vectors likely disperse further than other intermediate hosts of parasites, such as molluscs. Such variation in dispersal capacities of intermediate hosts may thus impact the scale at which patterns of parasite biogeography are observed. These considerations highlight that a more trait-

explicit approach is essential for testing whether parasites adhere to the rules of island biogeography.

Impact of island characteristics on parasite prevalence

Several studies quantified parasite prevalence on islands. The effects of island colonisation on parasite prevalence are likely to differ from impacts on richness because distinct ecological and evolutionary processes operate on islands. For example, predators and competitors of the host are often absent, potentially leading to density-compensation in island host populations (MacArthur *et al.*, 1972). Increased host densities can increase contact rates and result in more opportunities for parasite transmission (Fromont *et al.*, 2001; De Bellocq *et al.*, 2002), resulting in equal or greater prevalence on islands relative to the mainland. Consistent with these expectations, parasite prevalence in non-human mammals is rarely lower on islands in comparison to the mainland (López-Darias *et al.*, 2008; Petrželková *et al.*, 2010). In contrast, elevated or similar prevalence on islands was detected in only two bird studies (Barrientos *et al.*, 2014; Illera *et al.*, 2015), with most studies reporting higher prevalence in mainland populations. This discrepancy may reflect the types of parasites most commonly studied in birds, which are predominantly transmitted by flying insect vectors. Transmission of vector-borne parasites is not directly dependent on primary host density because vector ecology and host-vector encounter rates decouple transmission from simple host density relationships (Dietz, 1988), potentially weakening the effects of host density compensation on parasite prevalence. Such findings highlight that parasite prevalence on islands is shaped by epidemiological processes that depend on parasite life history traits, such as transmission strategy.

Taxonomic and methodological biases in published studies

Parasite diversity is largely undocumented and substantial taxonomic expertise is required for parasite identification (Dobson *et al.*, 2008; Okamura *et al.*, 2018; de Buron *et al.*, 2025). Patterns of host use are also poorly resolved for the majority of known parasite taxa (Dallas *et al.*, 2017; Carlson *et al.*, 2020). As a result, the range of host-parasite systems that have been investigated is circumscribed, and it is therefore unsurprising that our results highlight a bias toward particular host-parasite interactions. For example, apicomplexans, particularly haemosporidians, are commonly studied in birds, while various helminths are targeted in fish.

Biases in methodological approaches may be responsible for the limited taxonomic sampling and the bias toward particular host-parasite interactions. For example, studies of fish often rely on dissection and microscopy to identify macroparasites on the basis of morphology (Dallarés *et al.*, 2016; Williams *et al.*, 2022), whereas studies of birds routinely extract DNA from blood samples to conduct PCR-based screening for haemosporidians (Padilla *et al.*, 2017; Williamson *et al.*, 2019; Amaya-Mejia *et al.*, 2022; Bodawatta *et al.*, 2025). Such methodological biases can cause some host-parasite interactions to be overlooked. Indeed, gregarines, which were previously thought to exclusively parasitise invertebrates (Leander, 2008), have recently been targeted and identified in amphibians (Chambouvet *et al.*, 2016; Chambouvet *et al.*, 2020). Future studies that undertake broad-scale parasite identification would help to elucidate patterns of host use, reveal novel parasite diversity, as well as uncover previously undocumented host-parasite interactions, and will substantially improve our understanding of the processes that drive parasite distributions.

Methodological biases have also resulted in paucity of studies that characterise multiple parasite infections. The limited number of multi-parasite studies generally examined parasites in fish or humans. In fish, multiple helminth infections are identified within individual fish hosts during dissections, whereas human studies often rely on databases such as the Global Infectious Disease and Epidemiology Network (GIDEON) which is maintained for the surveillance and diagnosis of human disease (Cashdan, 2014; Jean *et al.*, 2016). Yet, co-infections, involving multiple parasites infecting a single host, are common in nature (Bordes and Morand, 2009; Clark *et al.*, 2016; Reis *et al.*, 2021). Co-infecting parasites may interact, impacting host fitness, parasite transmission, and ultimately parasite distributions (Cox, 2001; Pedersen and Fenton, 2007; Handel and Rohani, 2015). Therefore, future studies should aim to characterise multiple parasite infections per host individual to better understand whether interactions between parasites contribute to macroecological patterns. Molecular approaches such as metabarcoding and metagenomics offer promising avenues for detecting diverse parasite assemblages (Briscoe *et al.*, 2022), particularly as they reduce reliance on specialist taxonomic expertise. However, accurate parasite identifications using these methods depends on the availability of comprehensive and reliable reference sequence databases (Nichols *et al.*, 2026).

Biases in temporal, geographic and host taxonomic range in published studies

Patterns of island biogeography may reflect dispersal ability of the host. For instance, human parasites consistently exhibited the predicted area and mainland-island patterns, yet evidence for an effect of isolation was mixed. This mixed evidence is likely linked to human dispersal behaviour, with global transport networks facilitating long-distance dispersal and subsequently decoupling the relationship between isolation and likelihood of island colonisation. Where isolation impacts were observed, patterns were strongest for those parasites transmitted by flying insect vectors (Jean *et al.*, 2016), possibly because the impact of isolation is more pronounced in the vectors. Birds are also highly mobile and, like humans, seem to similarly obscure isolation-driven patterns (Ishtiaq *et al.*, 2010; Illera *et al.*, 2015). By contrast, less mobile taxa such as reptiles may be more likely to exhibit strong isolation effects. However, studies on reptile hosts remain sparse and geographically restricted (Roca, 1996; Roca *et al.*, 2009; Foufopoulos *et al.*, 2017), limiting our capacity to test island biogeography predictions. These host-mediated effects arise because parasite distributions are shaped not only by host availability, but also by parasite life history traits, highlighting that the rules governing distributions of free-living organisms do not necessarily apply to parasites.

Geographic biases in study locations may also impact the reported patterns of parasite island biogeography to date. Research efforts were heavily clustered in Europe, with almost all studies in reptiles occurring in the Cyclades or Balearic Islands (Roca, 1996; Roca *et al.*, 2009; Foufopoulos *et al.*, 2017). This uneven sampling mirrors patterns documented in parasite discovery more broadly (Poulin and Jorge, 2019). Notably, much of the sampling took place outside the regions where vertebrate species richness is predicted to be greatest (Figure 2A; IUCN, 2025). Considering the prediction that parasite and host species richness are highly correlated (Krasnov *et al.*, 2004; Kamiya *et al.*, 2014; Poulin, 2014), current studies may be overlooking parasite-rich and biogeographically-informative regions. Furthermore, previous work suggests that parasites do not necessarily adhere to the same latitudinal diversity gradients observed in free-living taxa (Nunn *et al.*, 2005; Poulin and Leung, 2011; Preisser, 2019), meaning that the current geographic imbalance in sampling could exacerbate uncertainty regarding global-scale processes. The spatial extent of many studies is also restricted, and we identified few studies that addressed the patterns of island biogeography in lacustrine or sky island settings, preventing the capture of broad-scale patterns. There were just two examples of global studies identified in the review, both of which focus on human parasites (Cashdan, 2014; Jean *et al.*, 2016).

We also observed biases in the temporal extent of studies that have tested the rules of island biogeography in parasites. Many studies conducted sample collection over a single year, despite mounting evidence that parasite diversity and composition can shift markedly over decades as a result of climate change (Wood *et al.*, 2023; Hammoud *et al.*, 2025). Short-term studies are therefore more likely to capture stochastic colonisation-extinction dynamics. As a result, studies of parasite island biogeography which take place over short time scales may not reflect the processes that ultimately determine parasite colonisation and persistence over time. Following the initial increase in studies published after 2007, there have been peaks and troughs in the number of studies published and concerningly, no studies were published after 2023. Continued investigation of the fundamental processes underlying parasite biogeography is essential, particularly as climate change is expected to further influence parasite distributions and persistence (Carlson *et al.*, 2017).

Conclusions

Our systematic review highlights that parasites do not consistently follow the rules of island biogeography that govern their free-living hosts. Instead, their biogeographic patterns are modulated by host dispersal and parasite traits. Future studies should broaden the geographic range of sampling and address the biases in methodological approaches to better incorporate a range of parasite taxa, including multi-parasite communities. Addressing a wider range of parasite life history traits and employing more broad-scale parasite identification approaches would help to develop general frameworks for understanding the factors that shape parasite biogeography.

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Chapter 3

Host whole genome sequence data represent an untapped resource for characterising affiliated parasite diversity

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This chapter has been accepted for publication in the International Journal for Parasitology. The publication is available here, DOI: 10.1016/j.ijpara.2025.104768

Abstract

Parasites are ubiquitous and exert varied ecological and evolutionary pressures on their hosts. Yet, characterising parasite diversity and distributions can be challenging and costly. Leveraging existing data to identify parasites is thus an attractive alternative. High-throughput sequencing (HTS) can generate whole genome sequence (WGS) data which are increasingly freely available in public repositories and represent an untapped resource for characterising parasites affiliated with hosts. In this study, we examine WGS data generated for the silvereye (*Zosterops lateralis*), to identify endogenous eukaryotic parasites that were inadvertently captured during host sequencing. We compared detection of parasite genera by this approach with detection via 18S metabarcoding. Mining WGS data for parasite DNA revealed the broadest range of genera. Results were verified by traditional microscopy of blood slides and conducting a targeted multiplex Polymerase Chain Reaction (PCR) for haemosporidian parasites. Detection of haemosporidians was largely consistent across microscopy, multiplex PCR and WGS data while 18S metabarcoding entirely failed to detect this group of parasites. Our results demonstrate that existing WGS datasets can be used to estimate endoparasite diversity and provide greater insights on diversity than metabarcoding whilst also avoiding the costs and challenges of direct sampling. We provide a framework outlining opportunities and constraints to consider when mining WGS data to identify parasite sequences. The framework particularly stresses the influences of sequencing depth, database completeness, and methodological biases. Our findings demonstrate how repurposing existing WGS data can provide a cost-effective and informative means of unravelling complex host-parasite interactions in future disease ecology studies.

Introduction

Parasites are ubiquitous and diverse (Dobson *et al.*, 2008), imposing significant and varied ecological and evolutionary pressures on their hosts (Penczykowski *et al.*, 2016). However, climate change may alter parasite distributions (Pickles *et al.*, 2013; Carlson *et al.*, 2017; Byers, 2021), with some parasites benefitting (Harvell *et al.*, 2002) while others become vulnerable to extinction (Carlson *et al.*, 2017). These changes could lead to the redistribution of parasites, drastically impacting the landscape of host-parasite interactions and, consequently, altering species interactions throughout ecosystems (Poulin and Mouritsen, 2006; Dougherty *et al.*, 2016; Carlson *et al.*, 2017). Yet, studies that simultaneously quantify a broad range of parasites are relatively uncommon (Hoarau *et al.*, 2020; Huang *et al.*, 2021). In reality, co-infections occur frequently in the wild (Petney and Andrews, 1998; Bordes and Morand, 2009; Vaumourin *et al.,* 2015; Hasik *et al.,* 2024) and parasite interactions may impact host fitness, parasite transmission and ultimately parasite distributions (Cox, 2001; Pedersen and Fenton, 2007; Handel and Rohani, 2015). Nevertheless, most studies focus on single-host, single-parasite systems (Hellard *et al.*, 2015). Our currently poor understanding of parasite distributions and patterns of co-occurrence likely reflects the difficulty of detecting and characterising parasites. These difficulties arise because parasite diversity is largely undocumented, particularly for certain groups, and because parasites are hidden in nature, patchily distributed, and taxonomic expertise is required for identification (Dobson *et al.*, 2008; Okamura *et al.*, 2018; de Buron *et al.*, 2025). In view of these various challenges, it is of considerable interest to explore methodological approaches to simultaneously characterise a broad range of parasites.

Conventional surveys for parasites variously use post-mortem examination, culturing of microbial faecal communities, and microscopic examination of faecal or blood samples (Fallon *et al.*, 2003; Mes *et al.*, 2007; Gouba *et al.*, 2013). However, these often highly invasive and time-intensive methods frequently fail to detect the diversity of parasites present (Durso *et al.*, 2010). This is because levels of parasitaemia (the quantitative content of parasites in the blood, or parasite load) are often low, the appearance of observable life cycle stages is ephemeral, and parasites themselves are often inconspicuous. Furthermore, many protists cannot easily be cultured and their diversity is thus underestimated (Edgcomb *et al.*, 2002). Conventional methods also require specialist taxonomic knowledge, which makes identifying a range of taxa challenging.

Adopting molecular methods to identify parasites could be highly beneficial. Such methods can be non-destructive, do not require expertise in morphological parasite identification, and can reveal a diversity of parasites within a single host (Nadler and de León, 2011). Polymerase Chain Reaction (PCR)-based detection followed by Sanger sequencing may be used to target specific parasite groups (Valkiūnas *et al.*, 2014). The simultaneous detection of multiple parasites can be achieved by high-throughput sequencing (HTS) methods. For example, metabarcoding involves the amplification of a region of DNA and has the potential to reveal information about mixed infections. Commonly used metabarcoding regions are also relatively well represented in reference databases, making it possible to match sequences to taxa (Wylezich *et al.*, 2020). However, this approach requires selection of a primer set to amplify the specific target region by PCR and while many primers claim to be 'universal', none reliably amplify all diversity present in a sample (Pallen, 2014). This is mainly due to amplification bias (Krehenwinkel *et al.*, 2017; Fonseca, 2018).

Untargeted (amplification-free) methods, like shotgun metagenomics, attempt to sequence all the organisms present in a sample, whether the sample is environmental or derived from an individual organism. Shotgun metagenomics allows the detection of a broad range of parasites and disease agents in a single test (Vijayvargiya *et al.*, 2019). This approach has been used to identify eukaryotic pathogens (Briscoe *et al.*, 2022) and antibiotic resistant bacteria (Cao *et al.*, 2020) from avian faecal samples and for diagnosis of specific parasites, such as Malayan filariasis (Gao *et al.*, 2016). However, metagenomics approaches require sufficient sequencing depth to capture the diversity in a sample. Because parasites will likely comprise a small proportion of the sample, sequencing reads will be dominated by the host or environmental contaminants (Wylezich *et al.*, 2019). This is particularly relevant for organisms that have nucleated erythrocytes; such as birds, fish and reptiles (Hartenstein, 2006). Therefore, the cost of generating sequencing data for the primary purpose of parasite identification could be prohibitively expensive. However, HTS data are increasingly generated at scale (Hudson, 2008; Kahn, 2011; Koepfli *et al.*, 2015; Parks *et al.*, 2017) for a range of biological questions (e.g. Lemos *et al.*, 2011; Goldfeder *et al.*, 2017; Posada-Cespedes *et al.*, 2017; Luikart *et al.*, 2019; Nilsson *et al.*, 2019; Baldrian *et al.*, 2022) and are routinely deposited in repositories, making them publicly and freely available. These data provide a resource which could be harnessed for new applications. For example, Franssen *et al.* (2021) surveyed environmental shotgun metagenomics data in public databases for specific pathogen groups. It may be similarly possible to harness other types of genomic data to identify parasites and pathogens.

Whole genome sequencing (WGS) is another HTS method that offers the opportunity to identify parasites and pathogens. The aim of WGS is to generate sufficient sequencing reads to reconstruct the entire genome of the focal organism. A somewhat overlooked feature of WGS data is that, like shotgun metagenomic data, they are produced using untargeted methods. Therefore, any DNA present in a sample is liable to be sequenced, including affiliated parasites that may infect the focal individual. Indeed, WGS data from humans have been harnessed to identify the causative agents of previously idiopathic diseases (Kostic *et al.*, 2011) and to identify pathogens that facilitate some cancers, such as Human papillomavirus (HPV) and *Helicobacter pylori* (Gihawi *et al.*, 2019). Similarly, myxozoan genome sequences have been detected in WGS data of their yellowfin tuna host (Weber et al., 2025). It is therefore increasingly evident that WGS data present an untapped opportunity to investigate the endoparasites of host organisms (Lopes *et al.*, 2017).

The silvereye (*Zosterops lateralis*), is small passerine bird distributed throughout the southwest Pacific. As is common for passerine birds (Parsa *et al.*, 2023), silvereye harbour a diversity of protozoan parasites. Previous research has predominantly focussed on characterising blood parasites from these birds, specifically Haemosporidians which are the causative agent of avian malaria (Clark *et al.*, 2014; Gudex-Cross *et al.*, 2015; Olsson-Pons *et al.*, 2015) although *Atoxoplasma* (Laird, 1959) and filarial nematodes (Clark *et al.*, 2016) have also been documented. Some research also highlights the presence of gastrointestinal parasites such as coccidia like *Isospora* (Yang et al., 2018).

Our aim here is to determine whether WGS data generated from the blood of the host can be used to simultaneously identify a range of affiliated endoparasitic eukaryotes. We then evaluate the performance of WGS and metabarcoding using 18S primers by comparing their ability to characterise a diversity of parasites. In addition, we conducted microscopic examination and targeted multiplex Polymerase Chain Reaction (PCR) to identify a subset of parasites (haemosporidians) to verify and evaluate the consistency and power of detection by WGS and metabarcoding. We summarise insights gained through our analyses by providing a framework of issues to consider when repurposing WGS data to identify affiliated parasites. The framework highlights potential constraints, including sequencing depth, read length, and how the availability of reference sequences can introduce biases in characterising some parasites. While we expect that HTS-based methods, like metabarcoding and mining WGS data, will reveal a greater diversity of

parasites than targeted methods, we predict that they may not identify parasites with the same accuracy. Nevertheless, the ability to characterise parasites from publicly available WGS data could facilitate insights on fundamental and applied questions regarding patterns and distributions of a diverse range of parasites. For example, distributions in space and time or patterns of host use could be revealed. In turn, such insights could provide the impetus for more fine-tuned and targeted studies such as investigating specific host-endoparasite interactions or environmental drivers of parasite macroecology.

Materials and Methods

Sample collection

Mist nets were used to capture birds of the subspecies *Zosterops lateralis lateralis* in Queensland, New South Wales and Tasmania, Australia from May to July 2022. Blood samples were obtained from 68 individuals by pricking the brachial vein with a sterile needle and collecting blood in a glass capillary tube. A small droplet (~ 1-2 µl) of blood was used to prepare a microscopy slide (see below) (Valkiūnas, 2004). Capillary tubes were snapped to size then placed in 1.5 ml microfuge tubes with Queen's lysis buffer. The buffer was modified from Seutin *et al.* (1991) and had a pH of 7.5 with the final concentrations of 0.01 M Tris, 0.01 M NaCl, 0.01 M EDTA and 0.03 M n-lauroylsarcosine (10% w/v). Samples were refrigerated until they could be frozen at -20°C.

Identification of blood parasites via microscopy of blood smears

Microscope slide preparation and screening was carried out according to an adapted protocol (Valkiūnas, 2004). A small droplet of blood (~ 1-2 µl) was smeared onto a glass slide and air-dried for up to 30 seconds, then fixed in absolute methanol for 1-2 min, and air-dried again. Slide staining took place one year after their collection, by submersion in 10% Giemsa stain for 1 hr (concentrated stock solution, pH 7.0-7.2). Despite the delay between fixing and staining, slides were sufficiently informative to identify haemosporidian genera. Initially, each slide was screened using an Olympus BX51 microscope for 10 minutes at x40. This was followed by examining 100 fields of view at x100 oil immersion. The lower magnification for the initial screening enabled a greater area of the slide to be inspected, equating to a minimum of 2 cm^2, thus increasing the likelihood of parasite detection. However, parasites vary in size and while the larger *Leucocytozoon* spp. are detectable at x40, some *Plasmodium* spp. life stages may not be identified unless a higher magnification is utilised. Each field was inspected for 30 seconds. We estimated parasitaemia by counting the number of infected and uninfected erythrocytes in 10 fields of view (Godfrey Jr *et al.*, 1987).

Identification of parasites via molecular methods

DNA extractions were carried out using a phenol-chloroform protocol (Sambrook and Russell, 2006). Approximately 100 µl of blood dissolved in Queen's lysis buffer was incubated overnight with rotation at 55°C with 250 µl DIGSOL extraction buffer (0.02 M EDTA, 0.05 M Tris-HCl (pH 8.0), 0.4 M NaCl, 0.5% SDS) and 10 µl Proteinase K (20 mg/ mL). Following incubation, 250 µl phenol:chloroform:isoamyl alcohol (25:24:1) was added and samples were rotated for 10 minutes and then centrifuged at 10,000 rpm for 10 minutes. At each of the following steps, the aqueous phase was transferred to a new microfuge tube after rotation and centrifugation with addition of: i) phenol:chloroform:isoamyl alcohol as above; ii) 250 µl chloroform:isolamyl alcohol (24:1); and iii) 2 volumes of cold 100% ethanol, 1 volume of 2.5 M ammonium acetate and 1 µl glycogen to precipitate the DNA. The samples were left at -20°C for a minimum of 12 hours, then centrifuged for 10 min at 15,000 rpm at 4°C. The supernatant was discarded, and the precipitate washed with 500 µl cold 70% ethanol. Centrifugation and washing were carried out twice more. The precipitated DNA was left to dry at room temperature. Once dried, the DNA was resuspended in 50 µl of TE (TrisEDTA) buffer (0.01 M Tris-HCL (pH 8.0), 0.0001 M EDTA). A negative extraction control was included per extraction batch. The DNA extracts were used to conduct a multiplex PCR, 18S metabarcoding and generate WGS data.

i. Haemosporidian-specific multiplex PCR

To screen the samples for haemosporidians (*Haemoproteus, Plasmodium and Leucocytozoon*) we used a multiplex PCR assay for avian haemosporidian parasites (Ciloglu *et al.*, 2019). The PCR requires equimolar concentrations of three sets of primers in each reaction (PMF, PMR, HMF, HMR, LMF and LMR) which target and amplify different regions of the parasite genomes. Each reaction included Qiagen 5X Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 1 µl of each primer at 10 µM (final conc. 0.5M), 1 µl DNA (ca. 10 ng/µl) and sterile ddH2O to make a final volume of 10 µl. The thermal cycling conditions were as follows: 95°C for 15 min; followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 59°C for 90 s, and extension at 72°C for 30 s; with a final extension at 72°C for 10 min. All PCR experiments contained one negative (ddH_2O) and one positive control for every 16 samples. For the positive control, we alternated between using a *Haemoproteus* single infection and a *Plasmodium* and *Haemoproteus* mixed infection. The amplified products were resolved using electrophoresis on a 2% agarose gel containing GelRed™ gel stain (Biotium, Inc., Hayward, CA, United States) for 1 h at 90 V. The product size was used to infer

which haemosporidian parasite was present in each sample (*Haemoproteus:* 533 bp, *Plasmodium:* 378 bp, *Leucocytozoon:* 218 bp).

ii. 18S anti-metazoan metabarcoding

Metabarcoding was conducted using a set of 18S anti-metazoan primers, 3NDF (Cavalier-Smith *et al.*, 2009) and the "universal non-metazoan" reverse primer, 18s-EUK1134-R (Bower *et al.*, 2004), which target the V4 region of the 18S SSU rDNA. These primers were chosen for three reasons. First, because the amplified region is conserved across taxa but shows substantial sequence variation between taxonomic groups (Van de Peer *et al.*, 1997; Wuyts *et al.*, 2000; Stoeck *et al.*, 2010). In addition, the region is often used for characterising parasites (Kounosu *et al.*, 2019) therefore it has substantial representation in databases (e.g. NCBI, PR^2), enabling recognition of a broad range of parasites. Finally, the primers have been designed to avoid amplifying metazoans, without this adjustment it is likely that the amplicons would be dominated by reads from the host. The primers were also modified to include annealing sites to be used for indexing. The primer sequences were as follows:

Forward primer:

5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNGCAAGTCTGGTGCCAG-3'

Reverse primer:

5'-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTTAARTTTCASYCTTGCG-3'

PCR1 reactions included Qiagen 5X Multiplex PCR Master Mix, 2 µl of primer at 5 µM (final conc. 0.5M), 1 µl DNA (ca. 10 ng/µl) and sterile ddH2O to make 20 µl final volume. The thermal cycling conditions were as follows: 95°C for 15 min, 35 cycles of 95°C for 30 s, 53°C for 45 s and 72°C for 1 min with a final extension at 72°C for 10 min. Two negative controls were included for every 30 amplifications: a negative PCR control (substituting 1 µl DNA with ddH_2O) and a negative extraction control. Positive PCR reactions produced a ~540 bp product.

Library preparation was carried out by cleaning successfully amplified samples with Promega ProNex Magnetic Beads using a ratio of 1.15X (v/v) beads to product, according to the manufacturer's instructions (Promega, Inc., United States). Dual indexing of each sample was

carried out using Fi5 and Ri7 indexing primers. Reactions were conducted in a total volume of 20 µl that included 5X Meridian Bioscience MyTaq HS Mix, Fi5 and Fi7 primers (at 10 µM), sterile ddH2O and product from PCR1. The PCR cycling conditions were 95°C for 15 min; 8 cycles of 98°C for 10 s, 65°C for 30 s and 72°C for 30 s and a final extension at 72°C for 5 min.

Successful ligation of the index primers was confirmed using the Agilent TapeStation (Agilent Technologies Inc., United States). This was followed by a second clean-up using a 1X (v/v) ratio of Promega ProNex Magnetic Beads. Amplifications were quantified using the Promega QuantiFluor. Pools were created by combining equal ratios of amplicons from each sample according to their concentration (ng/ µl) and repeatedly quantifying by qPCR as follows. Three independent 100- 1000- and 10000-fold dilutions were prepared for each pool using sample dilution buffer (10mM Tris, pH 8.0 and 0.05% Tween 20X). Reactions were prepared with SYBR FAST mix and primers. The thermal cycling conditions were 5 min at 95°C, 35 cycles of 30 s at 95°C and 45 s at 60°C. The pools were subsequently combined and analysed by further qPCR until all the samples were represented in a single pool. The size and quantity of the final product was checked using a High Sensitivity D1000 ScreenTape with the Agilent TapeStation.

The final pool was sent to the Natural Environment Research Council (NERC) Environmental Omics Facility, University of Liverpool, UK for sequencing on a PacBio Sequel II instrument using SMRT cell 8 M, Sequel II Primer V3.1.1.5, Sequel® II Binding Kit V3.1. Library loading was achieved by diffusion, with all the samples loaded onto one SMRT cell. PacBio CCS reads (mean passes = 20, median read quality = Q37) were demultiplexed and Illumina primers were removed from reads using the cutadapt software (Martin, 2011).

The *DADA2* package (Callahan *et al.*, 2016) was used for Amplicon Sequence Variant (ASV) calling. Reads below Q30 were removed and reads with minimum and maximum lengths of 495 bp and 675 bp, respectively, were retained. Following the filtering step, reads were dereplicated to reduce computation time. The error rates were learned, and the samples were subsequently denoised to remove likely sequencing errors. Finally, chimeras were removed.

We downloaded the NCBI nt database version 5 (downloaded on 07/08/2024) (Sayers *et al.*, 2022) and aligned ASVs to this database using the BLASTn algorithm, with the identity threshold set at 97%. For BLAST hits with sequence similarity of 97% or higher, the Lowest Common Ancestor (LCA) was identified using the *BASTA* package (Kahlke and Ralph, 2019). This LCA approach determines the taxonomic assignment for an ASV by examining the hierarchical structure of the taxonomic tree and finding the LCA of all identified taxonomic groups associated with that ASV.

Following taxonomic assignment, we gathered life history information for each detected parasite genus. We searched for information on previously recorded primary hosts according to the categories: bird, other vertebrate, invertebrate, or plant. We also assigned each parasite a dominant transmission strategy: flying insect vector, tick-borne, two-host (i.e. any other complex life cycle with two hosts) or direct transmission.

iii. Generating WGS data and extracting parasite sequences

DNA extractions were quantified with a Qubit 2.0 (Thermofisher Scientific Inc., United States) and were sent to Novogene UK for library preparation and whole genome sequencing, aiming for 10X coverage on the Illumina Novaseq 6000 platform (Figure S1, Illumina, San Diego) and generating 150 bp paired-end reads.

Construction of a pseudochromosome assembly: A pseudochromosome assembly was constructed for use as a reference genome for aligning the raw sequencing data and subtracting host reads. The Chromosembler tool available in Satsuma2 (Grabherr *et al.*, 2010) was used to order and orient scaffolds of the *Z. lateralis melanops* genome assembly (Cornetti *et al.* 2015) (GCA_001281735.1), producing 33 pseudochromosomes assembled according to synteny with the Vertebrate Genome Project's Zebra finch (*Taeniopygia guttata*) genome assembly (GCA_009859065.2) (Rhie *et al.*, 2021). The PathSeq pipeline from the Genome Analysis Toolkit (GATK) (Walker *et al.*, 2018) was used to generate a host k-mer library: k-mers generated from each consecutive position in the reference genome sequence were stored in a hash table. A Burrows-Wheeler Aligner (BWA) image of the reference genome was also generated using the BWA-Maximal Exact Match (MEM) index image file creator in GATK (Li, 2013).

Quality control and removal of host sequences: Raw sequences were trimmed to remove adapter content (10 bp) and low-quality base calls using fastp (Chen *et al.*, 2018). The reads from each individual were then aligned to the pseudo-chromosome assembly using the BWA-MEM algorithm (Li, 2013). Host sequence removal utilises a fast k-mer search of sequence reads with consecutive k-mers from each read checked against the host k-mer library (generated above). Reads were removed if there was at least one k-mer match. Next, the remaining reads were aligned to the host reference image using the BWA-MEM algorithm. Reads with an identity score above 30 were removed. Further quality control was carried out in parallel to host sequence removal using the PathSeq pipeline in GATK (Walker *et al.*, 2018). Reads with an excess of A/T or G/C content (29 out of 30 bases per window; (Benjamini and Speed, 2012) were removed. Low-complexity sequence bases and low-quality bases were masked using the sDUST algorithm (Morgulis *et al.*, 2006). Read ends were further trimmed according to base quality (Q = 15), duplicate sequences and those shorter than 60 bp were removed.

Contiguous sequence assembly: Following host sequence removal, longer contigs were assembled from the remaining sequences using metaSPAdes (Nurk *et al.*, 2017). Two datasets were generated: one containing assembled reads, the other containing the quality filtered, paired-end reads prior to the assembly step. We included the latter dataset because some parasite DNA is likely to be present at extremely low levels in the host DNA and therefore will not have sufficient coverage for assembly, and because reference availability is likely to be limited for longer sequences.

Building a custom reference database of eukaryotic parasite sequences: The accuracy of taxonomic assignment is reliant on reference database availability and completeness (Hleap *et al.*, 2021; Jeunen *et al.*, 2023). To maximise parasite taxon coverage, we compiled a custom database using the pre-built bacteria, Protozoa, UniVec and human databases in Kraken 2 (Wood *et al.*, 2019). We then added additional reference sequences from a variety of sources (Table 1). A complete list of NCBI taxonomic IDs was generated for apicomplexans, fornicata, cercozoans, parabasalids and euglenozoans using TaxonKit (Shen and Ren, 2021). The list was filtered for any IDs labelled as uncultured or environmental. The sequences were subsequently downloaded using Biopython (Cock *et al.,* 2009; version released 05-08-2024). We also downloaded all available genome references for the same taxonomic groups from RefSeq (Pruitt *et al.*, 2007; O'Leary *et al.*,

2016). The PR^2 database was filtered to remove sequences labelled uncultured, environmental, fungal and metazoan. All available genomic references from WormBase ParaSite (Howe *et al.*, 2017) and Eukaryotic Pathogen Genomics Database Resource (EuPathDB) (Warrenfeltz *et al.*, 2018) were also incorporated. Kraken 2 produces a compact hash table of k-mers derived from these reference sequences to be queried against when assigning sequences.

Taxonomic identification: Both the assembled and paired-end sequences derived for each sample were assigned using the custom parasite reference database with Kraken 2 (Wood *et al.*, 2019). We assigned parasites to recorded hosts and dominant transmission strategy as described above for 18S metabarcoding data. Confidence scores for each taxonomic assignment were calculated according to the fraction of k-mers assigned to the final taxonomic identity, its descendants and ascendants, i.e. the RTL score, with Conifer (Silamiķelism, 2020). We applied confidence scores of 0.2 for the assembled dataset and 0.7 for the paired-end dataset. The former score (0.2) reduces the propensity for Kraken 2 to overclassify reads that are not represented in the reference database (Peabody *et al.*, 2015). The latter score of 0.7 was considered highly conservative and hence more suitable for dealing with uncertainty introduced by the relatively short paired-end sequences.

Our processing resulted in two datasets to be used for analysis: 1) parsing filtered paired-end reads directly to Kraken 2 and filtering for 70% confidence (WGS paired-end 70%), and 2) assembling longer contigs from paired-end reads prior to assignment with Kraken 2 and applying a 20% confidence filter (WGS assembled 20%).

Table 1. Summary of reference sequences included in the custom Kraken 2 database.

Source	Details	Sequences obtained (n)
NCBI Genomes	Downloaded via the NCBI Datasets tool, including RefSeq for:	
	Apicomplexans	372
	Cercozoans	5
	Euglenozoa	188
	Fornicata	36
	Parabasalids	10
WormBase ParaSite	Version: WBPS18	4,856,896
EuPathDB	Release 64 12 Jul 2023	281,024
PR^2 database	Release 5.0.0 with metazoan, fungal and uncultured sequences removed.	101,252
NCBI Nucleotide database	All available sequences for apicomplexans, cercozoans, euglenozoa, fornicata and parabasalids.	37,153

There are several alternative approaches that could be adopted to mine WGS data for parasites. For example, sequence reads could be *de novo* assembled without host removal and subsequently characterised using a common algorithm such as BLAST to generate taxonomic identifications. We opted to use a kmer-based approach utilising the BWA-MEM algorithm because it has been optimised for short reads and is computationally less expensive (Walker *et al.*, 2018), making the pipeline highly scalable and affording the opportunity for broad-scale parasite identification.

Assessing parasite identifications from WGS data

Species accumulation curves: These were produced to assess how the sequencing depth of WGS data could impact the number of parasites detected and the confidence scores for taxonomic assignments. Our WGS data aimed to achieve 10X genome coverage although there was some variation (Figure S1). To explore the impact of greater sequencing depths on parasite identification, we downloaded raw WGS data for the Eurasian blackcap (*Sylvia atricapilla*) generated in two studies that aimed to achieve 60X coverage (Ishigohoka *et al.* 2023; Sigeman *et al.* 2020). The data were downloaded from the BioProject IDs PRJEB66075 and PRJNA578893 in NCBI. The samples were processed using the pipeline described above, except that a Eurasian blackcap host reference sequence (generated by the Vertebrate Genomes Project; GCA_009819655.1) was used for host removal. Prior to host filtering, we conducted random sub-sampling to achieve sequence depths of approximately 1X 2.5X, 5X, 10X, 15X, 20X, 30X, 40X and 50X using SAMtools (Li *et al.*, 2009). We used the exact method from the *vegan* package in R (Oksanen, 2019) to plot species accumulation curves for our own WGS data and the blackcap data. We applied the same confidence scores for each dataset as have been employed previously: 0.2 and 0.7.

We used the multiplex PCR data as a reference to ascertain the occurrence of true positive, false positive, true negative and false negative identifications made by mining WGS data for haemosporidian parasites (*Haemoproteus*, *Plasmodium* and *Leucocytozoon)*. We then generated confusion matrices and Area Under the Curve, Receiver Operating Characteristic (AUC ROC) scores using the *caret* package in R (Kuhn, 2011). AUC ROC scores indicate how well a model can distinguish between positive and negative classes. We calculated AUC ROC scores rather than accuracy scores because imbalanced datasets (e.g. most outcomes belong to a single class) may appear to achieve high accuracy by solely predicting the majority class, even if it fails to identify the minority class (Ling *et al.*, 2003). It should be noted that determining infection status via

targeted multiplex PCR can also generate false positive and negative errors (Zehtindjiev *et al.*, 2012; Valkiūnas *et al.*, 2014; Ciloglu *et al.*, 2019). Indeed, previous studies have found that PCR assays can have an accuracy ranging from 57-100% (Freed and Cann, 2006). Nevertheless, PCR results are considered reliable for indicating the true infection status of a sample.

Comparative assessment of different approaches

Methods are variously compared to highlight differences in the detection of parasitic genera. In particular, we assess the number of distinct parasite genera identified per host individual by each method, the total number of genera detected by each method, the frequency with which genera are detected by multiple methods, and among those, the extent to which they are detected in the same samples.

Results

Identification of blood parasites via microscopy of blood smears

Across the 68 individual silvereye samples, microscopic examination of blood smears revealed two genera of blood parasites: *Haemoproteus* in 3.5% of samples and *Leucocytozoon* in 7%. Parasitaemia was < 0.001 for each of the *Leucocytozoon* infections while it ranged from 2-9% for *Haemoproteus*.

Identification of parasites via molecular methods

i. Haemosporidian-specific multiplex PCR

Multiplex PCR detected *Haemoproteus*, *Leucocytozoon,* and *Plasmodium* in 13.6%, 15.2% and 3% of samples, respectively.

ii. 18S anti-metazoan metabarcoding

Metabarcoding with anti-metazoan 18S primers detected a total of six parasite genera. Four of the six genera have previously been reported in birds (*Babesia, Blastocystis, Eimeria* and *Isospora*). *Babesia* are characterised by tick-borne transmission, *Blastocystis* and *Eimeria* by direct modes of transmission, and *Isospora* utilises either a direct or two-host life cycle. We also detected *Theileria*, which is a tick-borne parasite reported in vertebrates, predominantly cattle (Mans *et al.*, 2015), and *Albugo*, which is a directly transmitted parasite of plants (Thines and Voglmayr, 2009).

Assessing parasite identifications from WGS data

Across the 68 samples, a mean of 82,914,641 raw reads was reduced to 368,977 paired-end reads per sample after host removal, quality filtering and removal of single-end reads (Figure 1A). These paired-end reads were assigned directly using Kraken 2, to produce the paired-end dataset. Approximately 5% of reads which remained after host removal could be identified per sample (Figure 1B). Following assembly, a mean of 6335.2 sequences per sample remained, 7% of which were identified on average per sample (Figure 1B).

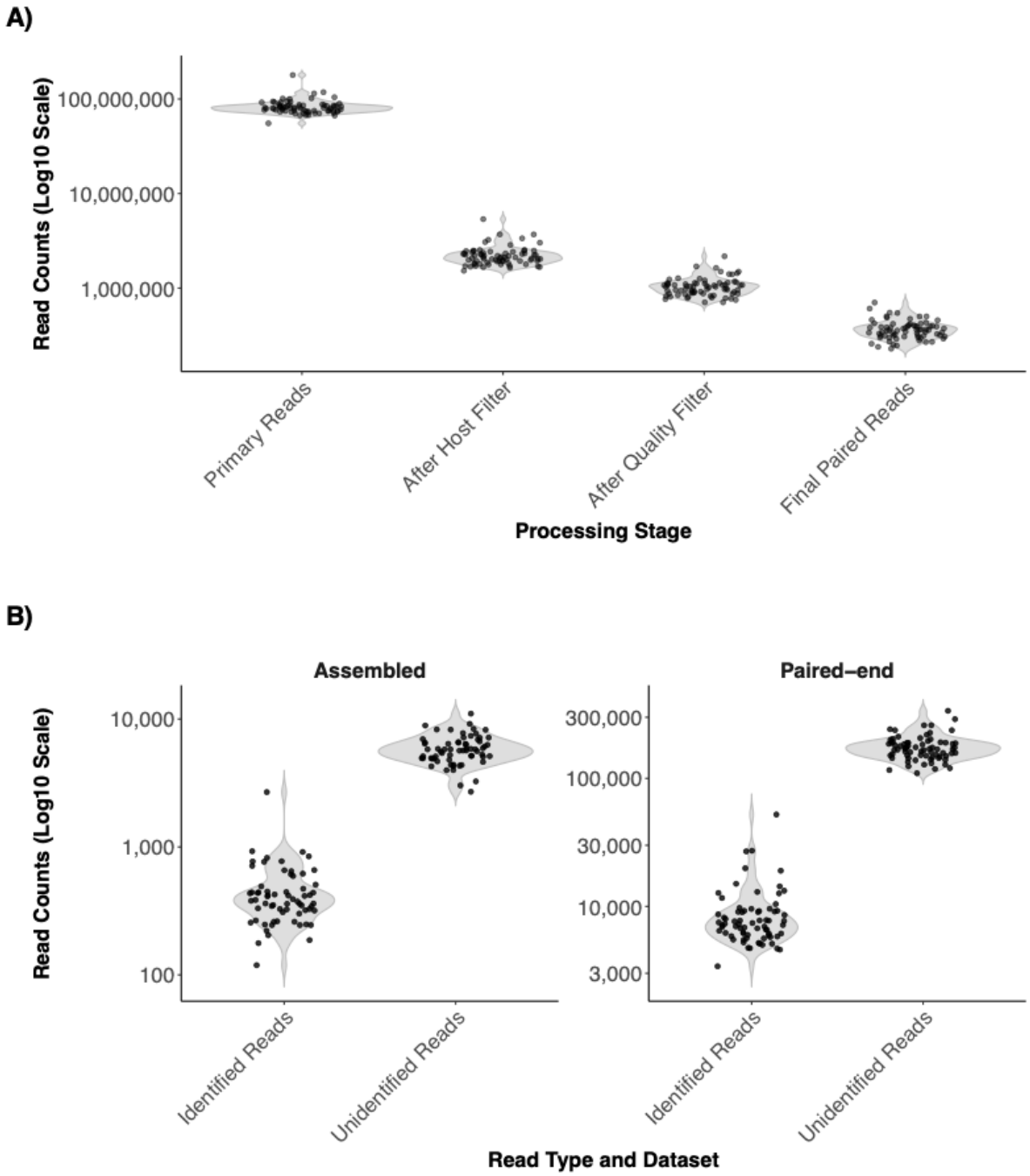

Figure 1. Number of reads retained at each processing step when identifying parasites from WGS data generated from blood samples of 68 silvereye individuals. A) Primary reads (raw reads before host filtering), After Host Filter (removal of host reads), After Quality Filter (reads remaining after quality filtering), and Final Paired Reads (final paired-end reads with single-end reads subtracted). B) The number of reads that could be identified using Kraken 2 for the assembled (left plot) and paired-end reads (right plot) following host filtering.

We detected a total of 73 and 35 parasitic genera for the paired-end and assembled datasets, respectively. We have included a full list of the parasite genera detected, including information on their reported hosts and transmission strategy in Table S1. The number of parasitic taxa identified across both datasets decreased following filtering based on confidence scores. The majority of parasites detected had previously been recorded as parasites of birds (Figure 2). However, several parasites of invertebrates, plants and other vertebrates were recorded in both datasets. After applying a confidence filter of 60%, the parasites recorded in the assembled WGS mining data were all known bird parasites. A variety of transmission strategies were detected for parasites identified in both paired-end and assembled datasets (Figure 2). The diversity of transmission strategies detected in the assembled dataset decreased rapidly following confidence filtering, with only parasites transmitted by flying insect vectors being detected with over 70% confidence.

Species accumulation curves showing the effect of sequencing depth on detection of parasite genera asymptotes by 10X sequencing depth in both blackcaps and silvereyes, even under the highest confidence thresholds (Figure 3). Increasing confidence thresholds lowers the overall value at the asymptote.

When comparing the detection of haemosporidian parasites, the assembled WGS data resulted in fewer false positives than the paired-end data, and the paired-end WGS data detected more true positives than the assembled data. The AUC ROC score, indicating how well a model can distinguish between positive and negative classes, ranged from 0.5 to 0.7 and was generally higher in the paired-end 70% dataset than in the assembled 20% dataset, but scores were similar for detection of *Haemoproteus* (Table 2).

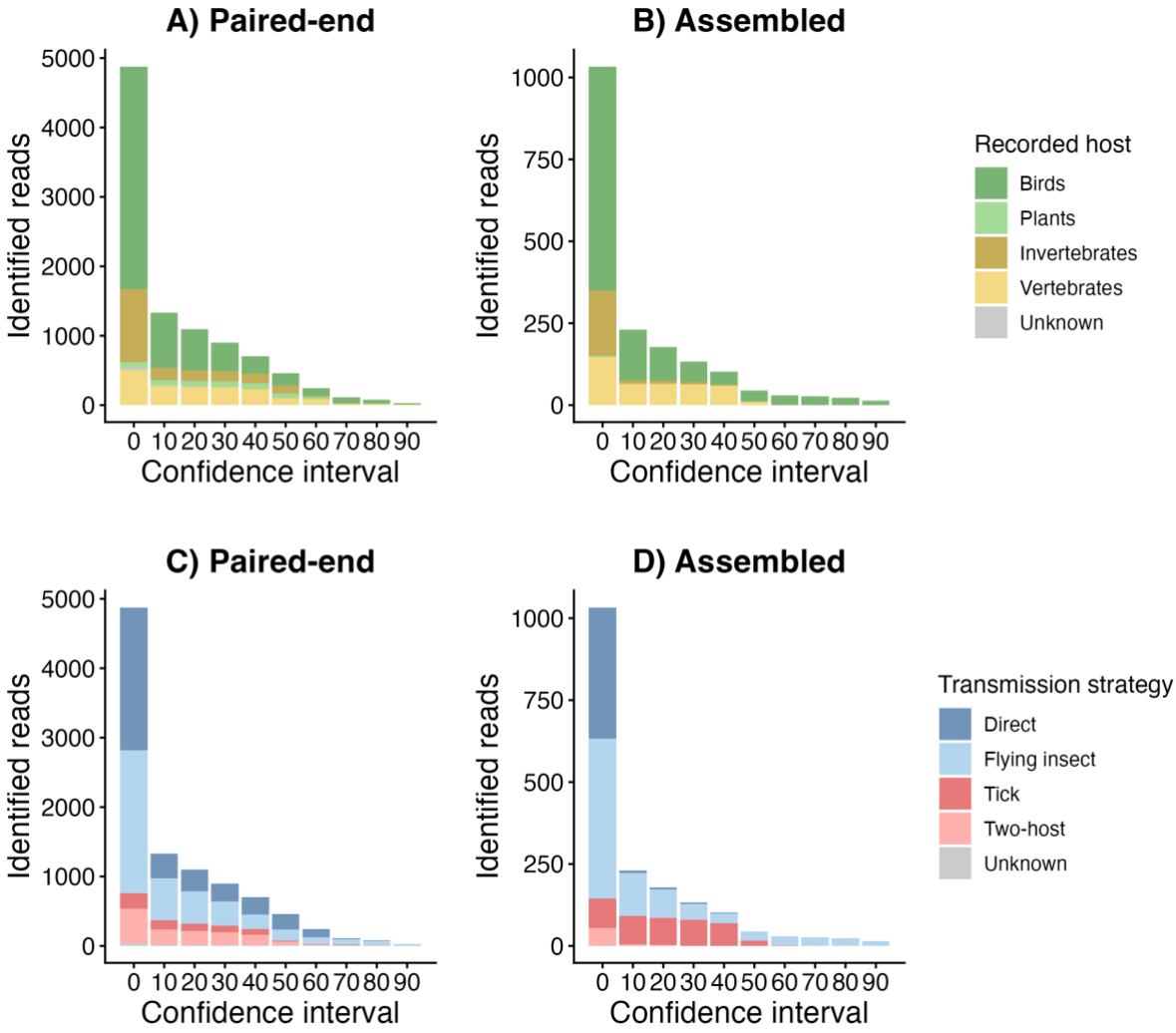

Figure 2. Identified reads at each confidence interval for DNA sequences extracted from WGS data generated from blood samples of 68 silvereye individuals. A) Paired-end reads parsed directly to Kraken 2 following host filtering and quality control, colour indicates parasite host group. B) Reads assembled into longer contigs prior to assignment with Kraken 2, colour indicates parasite host group. C) Paired-end reads parsed directly to Kraken 2 following host filtering and quality control, colour indicates transmission strategy. D) Reads assembled into longer contigs prior to assignment with Kraken 2, colour indicates transmission strategy.

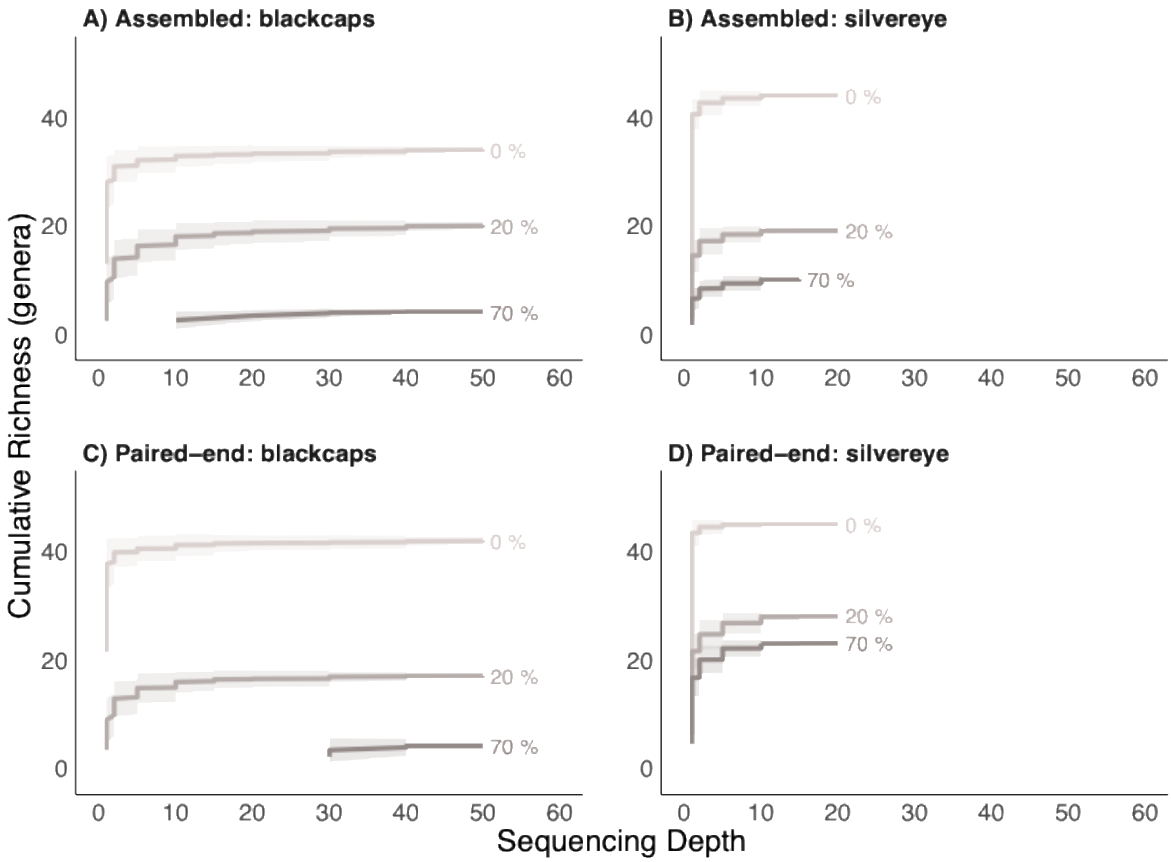

Figure 3. Species accumulation curves showing number of parasite genera detected with increasing sequencing depth for four datasets. A) WGS data assembled into longer contigs before identification with Kraken 2 from 11 blackcaps. B) WGS data assembled into longer contigs before identification with Kraken 2 from 68 silvereye. C) WGS data from 11 blackcaps, paired-end reads parsed directly to Kraken 2. D) WGS data from 68 silvereyes, paired-end reads parsed directly to Kraken 2. Each plot displays the data after applying three confidence thresholds: 0%, 20% and 70%.

Table 2. Occurrence of true positive, false positive, true negative and false negative identifications and AUC ROC scores when characterising *Haemoproteus, Plasmodium* and *Leucocytozoon* spp. from 68 silvereye WGS samples when parsing filtered paired-end reads directly to Kraken 2 and filtering for 70% confidence (WGS paired-end 70%) or assembling longer contigs from paired-end reads prior to assignment with Kraken 2 and applying a 20% confidence filter (WGS assembled 20%), in comparison to multiplex PCR data generated for the same samples.

Dataset	Parasite	True Positive	False Positive	True Negative	False Negative	AUC ROC
WGS Paired-end 70%	*Plasmodium*	1	11	55	1	0.7
	Haemoproteus	3	1	58	6	0.7
	Leucocytozoon	4	4	54	6	0.7
WGS Assembled 20%	*Plasmodium*	0	3	63	2	0.5
	Haemoproteus	3	1	58	6	0.7
	Leucocytozoon	0	0	58	10	0.5

Comparative assessment of different approaches

The number of parasite taxa detected per individual by different methods was variable. Metabarcoding did not detect any parasites in the majority of cases (Figure 4). One or more parasites were detected for most individuals by WGS paired-end datasets with a confidence threshold of 70%. Two parasites were detected in the majority of cases by the WGS assembled dataset with 20% confidence threshold.

The paired-end WGS data detected the highest number of parasite genera (Figure 5). Some genera of parasites were detected by multiple methods (Figure 5), but none were detected by all methods, i.e. there were no cases with incidence recorded across all five methods (Figure 5). It should be noted that some methods, such as the multiplex PCR and microscopy, do not aim to detect a broad range of parasite genera. Figure 6 illustrates incidences when the same parasite genera were detected in the same sample by more than one method. *Haemoproteus* was most consistently detected in the same samples by multiple methods. There was substantial agreement between the detection of *Leucocytozoon* by multiplex PCR and by the paired-end WGS data with a confidence filter of 70%. *Leucocytozoon* was no longer detected in WGS data following the assembly step (Figure 6). Notably, *Babesia* and *Theileria* were both detected by three methods but were usually detected in different samples.

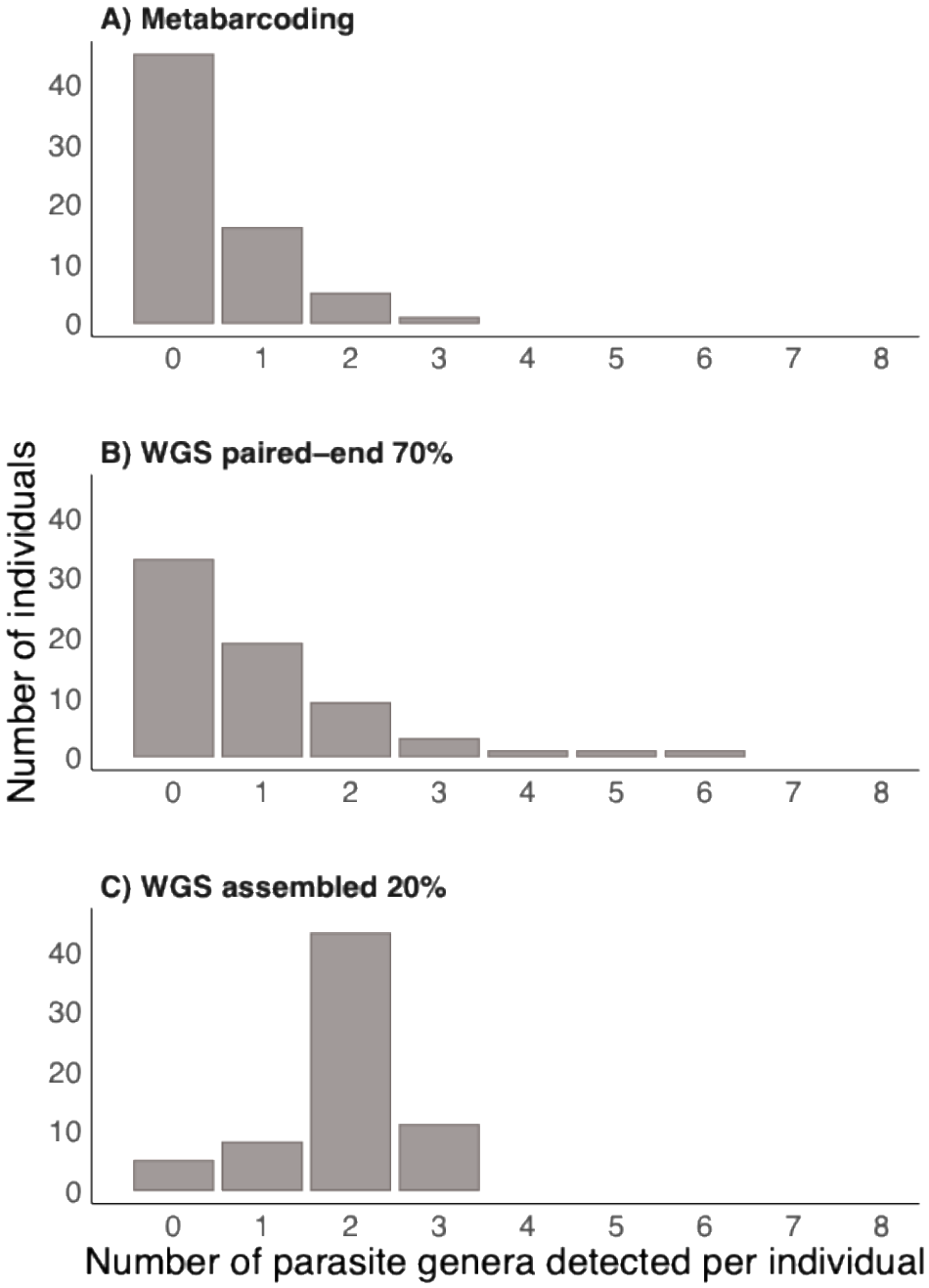

Figure 4. Number of parasite genera detected per individual for 68 silvereye samples for three datasets. A) Metabarcoding data, generated by amplification of the 18S region using anti-metazoan primers. B) WGS paired-end 70%: parasites assigned from paired-end WGS reads identified with Kraken 2 and filtered at 70% confidence. C) WGS assembled 20%: WGS data assembled into longer contigs from the paired-end sequences prior to assignment with Kraken 2 and filtered at 20% confidence.

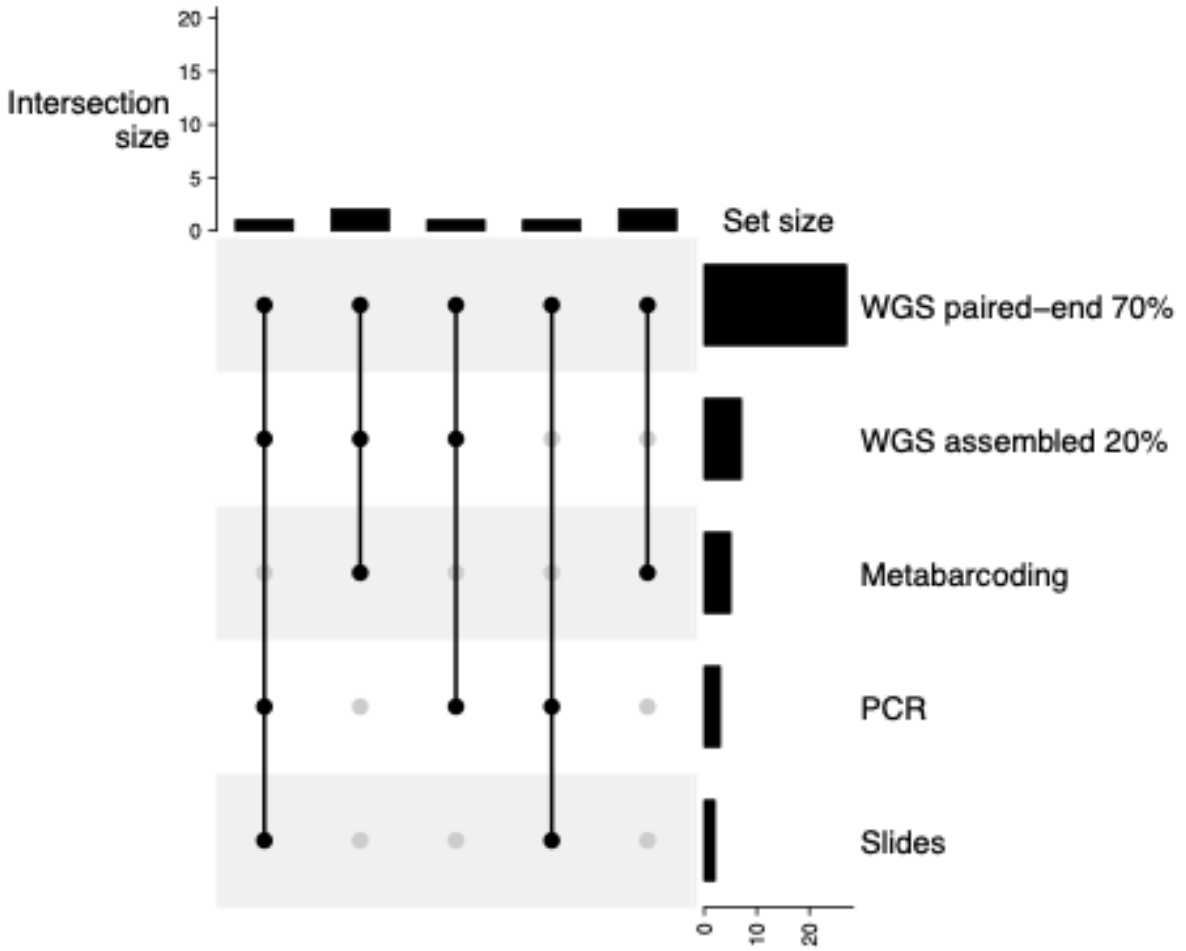

Figure 5. Parasite genera detected for 68 silvereye individuals screened for parasites using five methods: WGS paired-end 70%: parasites assigned from paired-end WGS reads identified with Kraken 2 and filtered at 70% confidence; WGS assembled 20%: WGS data assembled into longer contigs from the paired-end sequences prior to assignment with Kraken 2 and filtered at 20% confidence; Metabarcoding: amplification of the 18S region using anti-metazoan primers; PCR: multiplex PCR for haemosporidian parasites; Slides: inspecting microscopy slides of blood smears. Bar chart on right: set size indicates the total number of parasite genera detected by each method. Bar chart on top: intersection size indicates number of parasite genera detected by multiple methods as indicated by connected black dots. For example, the same two parasite genera were detected in WGS paired-end 70%, WGS assembled 20% and Metabarcoding datasets.

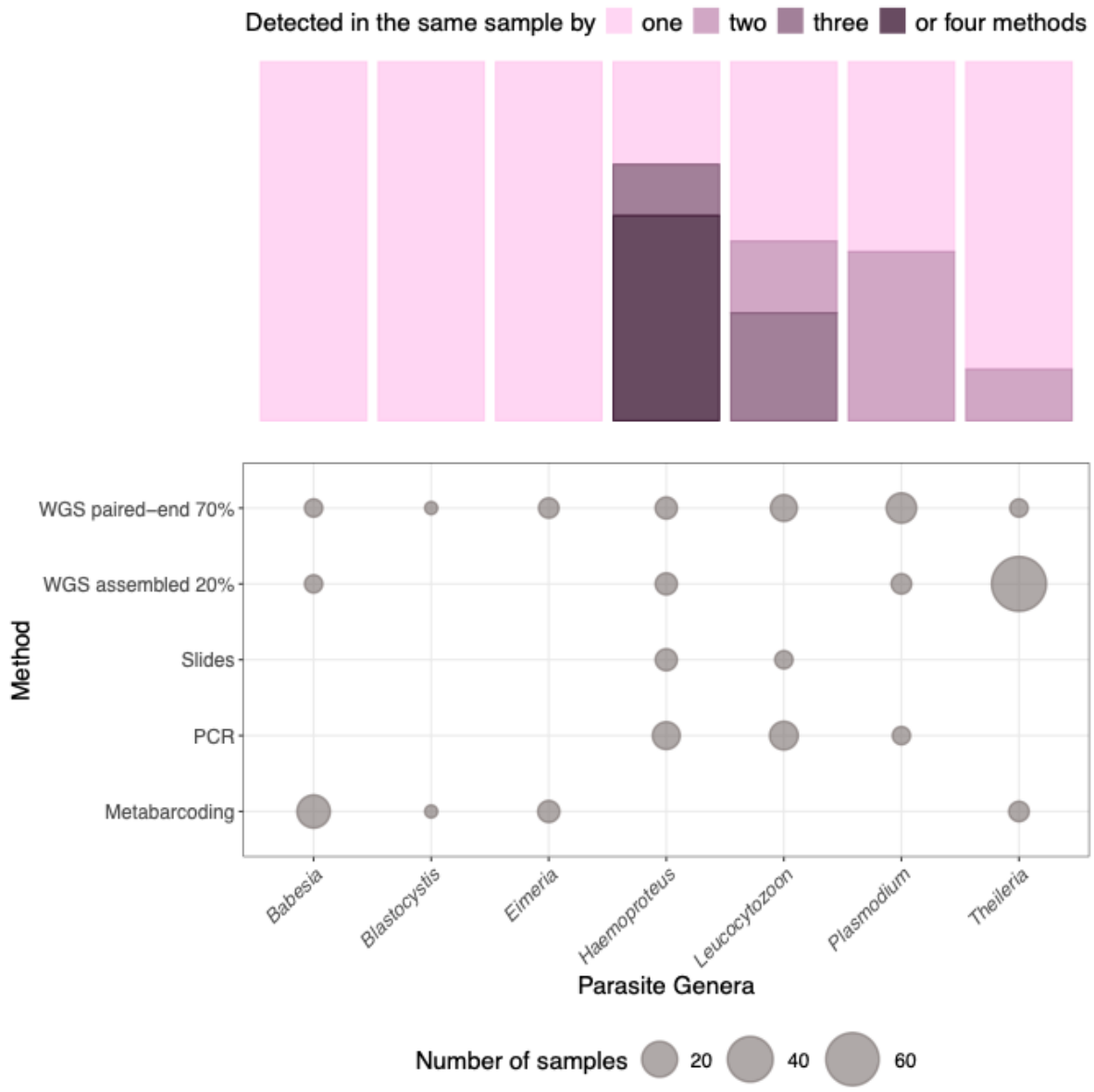

Figure 6. Variation in detection of parasite genera identified in samples from 68 silvereye individuals using five methods. Size of points in grid indicates number of times parasites were recorded per method. Bar chart indicates whether parasites were detected in the same sample by each of the five methods. WGS paired-end 70%: parasites assigned from paired-end WGS reads identified with Kraken 2 and filtered at 70% confidence; WGS assembled 20%: WGS data assembled into longer contigs from the paired-end sequences prior to assignment with Kraken 2 and filtered at 20% confidence; Metabarcoding: amplification of the 18S region using anti-metazoan primers; PCR: multiplex PCR for haemosporidian parasites; Slides: inspecting microscopy slides of blood smears.

Discussion

Eukaryotic endoparasites are detectable in WGS data

WGS data offer a useful resource for effective and broad-scale characterisation of parasite infection via bioinformatic mining for parasite sequences. Our study demonstrates that interrogating WGS data for inadvertently sequenced taxa reveals a greater diversity of parasites (by an order of magnitude) than is revealed by more commonly used methods for broad-scale identification, such as metabarcoding. Furthermore, the approach is effective at detecting a taxonomically wide range of parasite genera with a variety of transmission strategies, as well as identifying cases of co-infections within individuals. The latter may be missed by more targeted approaches, such as a parasite-specific PCR.

Endoparasite diversity revealed by WGS and metabarcoding data

Of the broad-scale detection methods employed in this study, mining WGS data for parasites was more powerful than metabarcoding in terms of the number of parasite genera detected. The assembled and paired-end WGS datasets detected 35 and 73 parasite genera, respectively, compared to the six genera detected by the metabarcoding approach. There are several factors that may explain this discrepancy in the variety of parasites detected. First, the 18S primer design deliberately avoided amplification of metazoans to reduce the representation of host DNA in the sequencing data (Bower *et al.*, 2004). Therefore, we did not expect to capture helminth or other metazoan parasites using metabarcoding, and indeed none were recovered. In contrast, the untargeted WGS approach had no such constraints, enabling detection of a much broader range of parasites. Indeed, the detection of helminths such as *Schistosoma* and *Dibothriocephalus* in WGS data was achieved. However, this explanation does not account for the plethora of non-metazoan parasites detected in WGS data but were not found by metabarcoding.

The WGS approach may have captured a greater range of parasites because it is amplification-free. It is well-established that mixed-template approaches like metabarcoding can suffer from PCR-bias, whereby the polymerase preferentially amplifies sequences with certain characteristics, particularly GC content (Nichols *et al.*, 2018). In particular, GC-rich sequences are expected to

amplify preferentially (Polz and Cavanaugh, 1998). In our study, the metabarcoding approach failed to identify haemosporidian parasites, despite the availability of reference sequences for this region (Harl *et al.*, 2019; Harl *et al.*, 2023). Notably, the GC content of avian haemosporidian parasites is generally low (Nikbakht *et al.*, 2014; Huang *et al.*, 2020). Other parasitic taxa have also been noted to have AT-rich genomes (e.g. *Plasmodium falciparum* [Gardner et al. 2002]; myxozoans [Yang et al. 2014; Faber et al. 2021]; *Strongyloides* spp. [Hunt et al. 2016]). Therefore, it is possible that haemosporidians are not represented in the metabarcoding data because they were outcompeted at the amplification step. Indeed, *Plasmodium* has previously been shown to amplify poorly using a range of 18S primers (Kounosu *et al.*, 2019).

The large difference in the numbers of parasitic genera recovered from WGS compared to metabarcoding is somewhat surprising considering that reference sequences for the 18S ribosomal region are readily available, and they are routinely used for identification (Moon-van der Staay *et al.*, 2001; Guillou *et al.*, 2012; Banos *et al.*, 2018; Harl *et al.*, 2019; Kounosu *et al.*, 2019; Wylezich *et al.*, 2019; De Jonge *et al.*, 2021; Vaulot *et al.*, 2022; Harl *et al.*, 2023). Despite the relatively limited reference sequence availability for DNA fragments mined from WGS, our approach appears to circumvent some of the issues introduced by metabarcoding, allowing for a broad range of parasite diversity to be captured. Reference sequences for WGS will no doubt increase in availability, and this will further improve the utility of the method. The elevated estimate of parasite diversity resulting from the WGS data could also be the result of erroneous identifications, the reasons for which are discussed below (Considerations for repurposing host WGS data to characterise affiliated parasites).

Interestingly, both metabarcoding and WGS data identified several parasites that reside in the intestine, despite being generated from blood samples. For metabarcoding, these 'gut parasites' included *Blastocystis* and the coccidians, *Eimeria* and *Isospora* (Box, 1981; Allen, 1987; Boreham and Stenzel, 1993) which collectively represented the vast majority of identified parasites via metabarcoding. Equally, a number of gut parasites were identified from the WGS data, including *Blastocysitis,* the coccidians *Eimeria*, *Cyclospora*, and *Cryptosporidium* as well as *Entamoeba.* There is increasing evidence that DNA from ingested material or components of the gut microbiome can be detected in the blood. For example, DNA from microbes in the gut can be detected in human blood samples, where it is referred to as circulating microbial DNA (cmDNA) (Damgaard *et al.*,

2015; Zhai *et al.*, 2024). DNA fragments from food items can also be detected in human blood (Spisak *et al.*, 2013). Thus, DNA sourced from the environment, diet or eukaryotic micro-organisms could conceivably enter the blood through translocation from other sites in the body (Tan *et al.*, 2023), or via shedding of parasite DNA into the blood stream. Indeed, it has been suggested that characterising microbiomes from blood rather than faecal samples could help to avoid contamination issues (Zhai *et al.*, 2024). These insights and our results suggest that mining WGS data could be similarly fruitful for identifying a broad range of parasites. However, the detection of parasites in host body compartments that are not associated with the parasite life cycle could also reflect technical artefacts caused by the misclassification of sequencing reads. Further studies to characterise and compare the eukaryotic parasite communities from blood and faecal samples of the same individuals would help to elucidate how reliably gut parasite communities are revealed by analysis of blood.

Consistency of detection by metabarcoding and WGS data

Surprisingly, metabarcoding and WGS seldom provided consistent results in terms of detecting the same parasite genera in the same samples. Sequencing depth provides one possible explanation for this disparity. Low sequence coverage in shotgun metagenomics data has been suggested to result in inconsistent taxonomic identifications of fungal sequences from faecal samples when compared to metabarcoding data (Usyk *et al.*, 2023). Such results may arise if informative regions for distinguishing taxa are not captured at low sequencing depths. However, in the present study (which retained approximately 1% of raw read data compared to the 0.1% recovered by Usyk *et al.* (2023)), species accumulation curves indicated that the parasite community did not change significantly with a greater sequencing depth. Thus, sequencing depth is unlikely to account for the incongruence between the parasites identified in the metabarcoding and WGS data. Similarly, shallow metagenomic sequencing has been shown to be as effective as deep sequencing (Hillmann *et al.*, 2018) and more effective than metabarcoding (La Reau *et al.*, 2023) in capturing community composition in microbiome analyses. These considerations suggest that sequencing depth does not appear to explain the differences observed in the present study.

The elevated parasite richness and the discrepancy in parasites identified in the same samples by the WGS and metabarcoding approaches could also reflect erroneous identifications. In similar shotgun metagenomics studies misalignments with the reference database have been suggested to

result in misidentification of sequences, leading to different taxa being identified in shotgun metagenomics and metabarcoding data (Clooney *et al.*, 2016). However, we found a moderate level of consistency in identifying known haemosporidian parasite infections using the paired-end WGS approach with AUC ROC scores of 0.7 for each genera. Although, it should be noted that utilising the assembled WGS data to identify haemosporidians produced AUC ROC scores of 0.5 for both *Leucocytozoon* spp. and *Plasmodium*, indicating it performed no better than chance in identifying these infections. The metabarcoding approach on the other hand, was unable to identify any haemosporidian parasites. The paired-end WGS approach may thus provide more power in identifying parasites than metabarcoding which further emphasises its consideration for future parasite studies.

Considerations for repurposing host WGS data to characterise affiliated parasites

Our collective results show that mining parasite sequences from WGS data of hosts can reveal a greater diversity of affiliated parasites with potentially more robust estimates than can be gained by commonly used approaches like metabarcoding. Overall, our results highlight how repurposing pre-existing WGS data could allow broad surveys of parasite community composition. However, as the WGS data were originally generated to investigate host biology, the data may not be optimised for characterising parasites. Therefore, there are several caveats and trade-offs to consider on a per-study basis if repurposing such data. These are elaborated below and summarised in Box 1.

Box 1. Factors to consider when implementing a WGS mining pipeline to characterise affiliated parasites.

Consideration	Impact on analysis	Mitigation
Availability of reference sequences	Some parasites undetected if references not available.	Curate reference sequences from a variety of sources.
	Incorrect sequence identification, particularly at lower taxonomic levels. Low confidence for longer sequence assignment as not all kmers match reference.	Restrict analysis to higher taxonomic level, e.g. genera.
	Bias for identifying taxa with longer reference sequences available.	Avoid assembling long reads if few available references.
Read length of raw data	Shorter read lengths less likely to cover diagnostic DNA regions, may match multiple taxa.	Build longer contigs to avoid spurious identifications.
		Use higher confidence scores to filter erroneous IDs.
Sequence coverage for samples	Low parasite abundance (parasitemia), or physical parasite size results in fewer reads and precludes detection.	Greater sequencing depth provides greater cover of parasite genomes, also potentially enabling sufficient coverage for contiguous sequences to be assembled.

The availability and completeness of references for taxonomic assignment are well-recognised limitations of shotgun metagenomics and metabarcoding approaches and have been shown to influence inferences of metagenomics studies (Tessler *et al.*, 2017; Hotaling *et al.*, 2021; Leray *et al.*, 2022; Jeunen *et al.*, 2023; Usyk *et al.*, 2023). The availability of reference sequences may limit or introduce biases in sequence identification. This limitation is exacerbated in parasite studies for several reasons. Parasites are generally challenging to sequence, making them under-represented in databases. This is partially due to the close association between intracellular parasites and host tissues, which can make it difficult to isolate substantial ratios of parasite DNA for sequencing (Palinauskas *et al.*, 2013; Videvall, 2019). In addition, the presence of multiple parasites within a single host can complicate assigning sequences to specific parasite taxa (Galen *et al.*, 2020). Intraspecific and geographic variation of sequences can also be limited in public databases (Hestetun *et al.*, 2020). Furthermore, the sampling effort for parasites is geographically patchy (Poulin and Jorge, 2019) and data for parasites in many regions are simply unavailable. Finally, research effort and therefore reference sequence availability is heavily influenced by the economic importance of taxa (Vallée *et al.*, 2016). As a result many taxa continue to be under-represented in public databases (Galen *et al.*, 2020).

Reliability of sequence information can also be problematic. Taxonomically narrow databases can both increase the rate of false negative identifications and lead to false positives (Keck *et al.*, 2023). These issues may be avoided by using databases with a broad range of sequences, such as the NCBI nucleotide database (Sayers *et al.*, 2022). However, it has been found that large, uncurated databases contain frequent errors (Keck *et al.*, 2023) and next-generation sequencing data uploaded to public databases often contain contaminants, leading to misidentifications (Laurence *et al.*, 2014; Bensch *et al.*, 2021). In situations where reference material is lacking, limiting identification to the genus or a higher taxonomic level may be required, as in the present study.

Our results also indicate a possible trade-off between maximising the captured diversity and ensuring a high level of accuracy in parasite identifications from mining WGS data. In the present study we identified parasites from paired-end and assembled reads. The short 150 bp sequences provided by paired-end reads may not sufficiently capture regions of the genome that enable differentiation between taxa. Therefore, multiple short sequences of the same parasite could hit multiple parasite references, inflating the estimated number of parasites. To minimise such issues,

paired-end reads may be assembled into longer contiguous sequences. Longer sequences confer a better chance of covering informative genomic regions as well as helping to correct sequencing errors by creating consensus sequences between overlapping regions (Ayling *et al.*, 2020). However, improving the accuracy of identifications in this way may also significantly reduce the diversity detected. For example, the lack of genomic references for bird parasites means that the probability of finding a reference of sufficient length to match a longer sequence is low. In addition, some parasites may not have sufficient sequencing coverage to be assembled into longer sequences (Ayling *et al.*, 2020). The sequence coverage can depend on the abundance of parasite in the host (i.e. parasitaemia) and the physical size of parasites. Low parasitaemia infections by small parasites are less likely to be represented in raw sequencing data. For example, *Giardia* is a particularly low parasitaemia infection, resulting in low representation in faecal shotgun metagenomics data and fewer positive identifications (Wylezich *et al.*, 2020). Likewise, in our study blood smears revealed low parasitaemia for *Leucocytozoon* spp. compared to *Haemoproteus* spp. Accordingly, *Leucocytozoon* spp. were not detected in the assembled WGS dataset, despite their detection in the paired-end read WGS datasets, the multiplex PCR data, and by microscopic examination of blood smears. In addition, rarity of genomic references (particularly longer sequences) for *Leucocytozoon* spp. (Omori *et al.*, 2008) could have compounded detection in WGS data. Therefore, while assembling longer sequences can improve confidence in taxonomic identifications, some diversity is likely to go undetected.

A potential solution to avoid the trade-off between maximising captured diversity and the robustness of taxonomic identification is to avoid an assembly step while applying a stringent confidence filter. The developers of Kraken 2 suggest that filtering based on confidence is not necessary to obtain accurate taxonomic identifications (Wood, 2020). However, we found that applying a confidence filter aided in removal of potentially spurious identifications. For example, the number of parasites that are known to use plant or invertebrate hosts, which we presume to be incorrectly identified, were substantially reduced following confidence filtering. It should be noted that confidence scores for longer, assembled sequences will be significantly lower. This might seem counter intuitive as taxonomic assignments based on longer sequences may be expected to confer greater confidence. This is because assembling sequences requires consensus between overlapping fragments, making them less error prone. However, the lack of longer sequences in the reference databases often means that few 'good matches' are available. It is also possible that the detection of non-avian or vertebrate parasites in the host samples is not spurious,

but rather reflects a dearth of knowledge regarding the life cycles and host specificity for many parasites (Dobson *et al.*, 2008). Therefore, it is possible that some of these parasites are yet to be described in avian hosts. However, it is difficult to discern whether detection of DNA belonging to a certain parasite indicates completion of its life cycle in the host. Alternatively, the parasite DNA may have entered the blood from ingested material.

Our framework highlights contradictory decisions that should be considered when repurposing WGS data for parasite identification. For example, while assembling longer contiguous sequences may help to avoid spurious parasite identifications (Ayling *et al.*, 2020), doing so may result in rarer parasites going undetected if sequencing depth or the availability of reference sequences is limited (Tessler *et al.*, 2017; Usyk *et al.*, 2023). In such cases the application of a stringent confidence filter may be preferable. Therefore, it is imperative to consider the aim of the study when designing an analysis pipeline. Indeed, there are some research questions for which the occurrence of false positives or false negatives may be more or less problematic. For example, studies which aim to capture diversity, particularly if much of the taxonomic diversity is unknown, may choose to use a less conservative analysis pipeline. While this may increase the rate of false positives, it will maximise the captured diversity. However, false positives may be problematic in studies that focus on more specific host-parasite interactions and eco-evolutionary dynamics.

Conclusions

We have shown that WGS data, generated with the primary aim of investigating host biology, can be repurposed to reveal information about multiple parasites infecting the host. Utilising such genomic data to quantify affiliated parasites provides an opportunity to conduct broad-scale biogeographic studies, potentially at low additional cost. The capacity to detect co-infections within individuals is a further bonus, together enabling investigations beyond single-host, single-parasite systems (Stewart Merrill *et al.*, 2022) and helping to unravel complex networks of host-parasite and parasite-parasite interactions. Furthermore, the broad taxonomic range of parasites detected could lend itself to exploring the potential of varied parasite life histories to mediate parasite dispersal and distribution patterns. Additional opportunities and outcomes may also arise, for example to investigate novel host-parasite interactions, patterns of host use, or parasite distributions over space and time.

At present, untargeted genomic sequencing data are referred to under a huge variety of names, these include shotgun metagenomics, (Couto *et al.*, 2018), metagenomic shotgun sequencing, genome skimming (Dodsworth, 2015; Vijayvargiya *et al.*, 2019), and WGS (Ng and Kirkness, 2010). Our study highlights that the data resulting from these various approaches can be repurposed for other applications. Although we highlight some limitations to extracting parasite reads from WGS data, it is important to consider the biases associated with well-established methods for quantifying parasite diversity. Future research that adopts a WGS mining approach to characterising parasites should consider the completeness and availability of reference databases, the sequencing depth and read length of WGS data, and the research question to evaluate how best to re-purpose WGS data to characterise affiliated parasites.

Animal studies

Ethical approval was issued to SMC (Griffith University ENV/06/20/AEC) and CY (University of Tasmania 27197). We exported our samples from Australia under permit no. PWS2022-AU-001814 and imported samples to the UK under Authorisation No. ITIMP21.1083. We thank the National Parks and Wildlife Service for New South Wales (Scientific Licence SL102647 from Department of Planning, Industry and Environment, NSW, Australia, issued to SMC), Queensland (Research permit WA0042422 from Department of Environment and Science, Queensland, Australia issued to SMC) and Tasmania (Permit No. FA22367 from Department of Natural Resources and Environment, Tasmania, Australia and Scientific Research permit SR-2022-5283, Hobart City Council, Tasmania, Australia issued to CY) for allowing us access to collecting sites. We recognise ABBBS for issuing a project licence to SMC.

Funding

The study was supported by funding from the UK Natural Environment Research Council (NERC) through a PhD studentship awarded to SN (NE/S007474/1) as well as an American Ornithological Society Student Research Award and The Biology Eurofins Foundation Fund Award granted to SN. The laboratory work was supported by the NERC Environmental Omics Facility (NEOF1523), The following grants were also awarded to AE: Heredity Fieldwork Grant (The Genetics Society), NERC PhD Studentship (NE/S007474/1), The Biology Eurofins Foundation Fund, Hesse Research Award (American Ornithological Society), Santander Travel Award (University of Oxford).

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Chapter 4

Transmission strategy modulates parasite biogeography in an island-colonising bird

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Abstract

Parasites occur in every ecosystem, although their dispersal is often constrained by the availability of hosts or vectors. Here, we explore how variation in parasite life history traits, particularly transmission strategy, may influence their distributions. Specifically, we test whether a variety of parasites adhere to the rules of island biogeography, and whether their distributions vary with transmission strategy. We utilised broad-spectrum parasite detection from existing Whole Genome Sequence (WGS) data to characterise parasites with varying transmission strategies from the blood of a passerine bird, the silvereye (*Zosterops lateralis*), sampled across 25 islands in the South Pacific and from five of the states in mainland Australia. Overall, parasite richness was higher on mainland Australia compared to islands and decreased with distance of islands from the Australian continent. However, these patterns were dependent on transmission strategy. For parasites transmitted by flying insect vectors, richness decreased on islands compared to the mainland. However, increasing isolation from the mainland among islands had little further impact. On the other hand, richness of directly transmitted parasites and those requiring another intermediate host declined sharply with increasing distance from the mainland. These results suggest that while islands may act as an initial barrier to colonisation for parasites relying on flying insect vectors, their highly dispersive vectors may subsequently reduce the impact of increasing isolation distance on richness. Our work underscores the importance of considering parasite life histories and their transmission strategies for understanding the processes that shape parasite communities on islands.

Introduction

Parasitic taxa can account for up to 40% of animal biodiversity in an ecosystem (Lafferty *et al.*, 2006a; Lafferty *et al.*, 2006b; Kuris, 2008) and can thus have profound and varied impacts on ecosystem dynamics (Dobson *et al.*, 2008). They may influence inter- and intra-specific interactions, diversification rates and ultimately global patterns of biodiversity (Ricklefs, 2010; Tompkins *et al.*, 2011; Morand, 2015; Friesen *et al.*, 2020). Therefore, characterising the biogeographic patterns of parasites is important. However, parasites rely on hosts for essential resources (Kuris *et al.*, 1980; Poulin *et al.*, 2011), their distributions are subsequently dictated by host availability and movement. Parasite dispersal can be further complicated by their own life histories, including transmission strategy. How such life history features influence the biogeographic distributions of parasites is unclear and exacerbated by our generally poor knowledge of parasite diversity (Okamura *et al.*, 2018; Carlson *et al.*, 2020) and difficulty in defining the geographic boundaries of their distributions (Pappalardo *et al.*, 2020). To address these limitations, application of molecular methods to provide broad-spectrum characterisation of parasite communities in an island biogeography framework offers a powerful means for advancing our understanding of how life history traits shape parasite distributions.

The rules of island biogeography are based on the premise that a mainland (e.g. a continental region or large land mass) acts as a source for islands. A subset of species is expected to colonise and persist on islands, resulting in lower equilibrium species richness on islands, with the lowest richness on small, remote islands, following species-area and distance-decay relationships (MacArthur and Wilson, 1967). While the framework was developed for free-living organisms, parasite distributions are assumed to behave similarly because parasites are closely associated with their hosts; therefore, island features will similarly constrain colonisation and extinction processes (Poulin and Morand, 2000). Studies have shown the expected pattern of lower parasite diversity on islands for various ecto- and endoparasites in birds (Espinaze *et al.*, 2019), amphibians (Belasen *et al.*, 2019), fish (Dallarés *et al.*, 2016) and mammals (Kornienko and Dokuchaev, 2023). However, conflicting patterns have also been reported in a similarly broad range of parasites and hosts, including birds (Barrientos *et al.*, 2014), amphibians (Aguiar *et al.*, 2020), and insects (Votýpka *et al.*, 2019). Even within one group of well-studied parasites, avian haemosporidians, deviations from island biogeography expectations are frequently reported. In particular, variation in distance-decay

relationships has been observed among haemosporidian genera in South Pacific island white-eyes (Zosteropidae) (Ishtiaq *et al.*, 2010) and for haemosporidian lineage richness patterns in birds of the Gulf of Guinea (Loiseau *et al.*, 2017). These deviations from island biogeography expectations are often attributed to differences in parasite life histories, such as life cycle complexity (Poulin *et al.*, 2011; Williams *et al.*, 2022), or level of host specialisation (Ishtiaq *et al.*, 2010).

Transmission strategies are a potentially important life history trait to consider when aiming to understand patterns of parasite distributions. Parasites with simple life cycles utilise direct transmission strategies that include host contact, faecal-oral routes and environmental contamination (Antonovics *et al.*, 2017). Many directly transmitted parasites can produce encysted stages, with a resistant outer layer that provides protection from adverse environmental conditions, such as temperature extremes and desiccation (Fayer, 1980; Aguilar-Díaz *et al.*, 2011). Encysted stages can persist in the environment when suitable hosts are unavailable, increasing the chance of establishment (Guernier *et al.*, 2004; Dunn *et al.*, 2010). These dormant stages may also be dispersed via zoochory when attached to mobile animals (Coughlan *et al.*, 2017). Directly transmitted parasites spend the majority of their replicating life cycle within a host (Fayer, 1980) and are likely to co-disperse with them, thus their patterns of dispersal are likely to reflect that of their hosts (McGuinness, 1984). As birds show strong adherence to island biogeography expectations (Valente *et al.*, 2020), it follows that directly transmitted parasites of birds will likewise show such patterns.

Parasites with complex life cycles are characterised by a range of transmission strategies. Some are transmitted via bites from ticks or flying insect vectors, others are transmitted trophically, via consumption of additional infected hosts. The ability of such parasites to colonise islands may vary. For example, ticks may co-disperse with birds across extensive distances (Cohen *et al.*, 2015; Sparagano *et al.*, 2015; Loss *et al.*, 2016), carrying parasites with them. Colonisation of islands by parasites disseminated by flying insect vectors may depend on environmental factors such as offshore wind (Clark and Clegg, 2015). Establishment of parasites with complex life cycles may also be more challenged by environmental variation than is the case for directly transmitted parasites. Reliance on multiple hosts may make persistence more precarious as outcomes will depend on local availability of appropriate additional hosts or vectors (Lafferty, 2012; Cizauskas *et al.*, 2017; Wood *et al.*, 2023).

To date, the majority of studies on the patterns of island biogeography in parasites have examined single parasite taxa (e.g. Maria *et al.,* 2009; Santiago-Alarcon *et al.,* 2012; Olsson-Pons *et al.,* 2015; Padilla *et al.,* 2017; Williamson *et al.,* 2019; Amaya-Mejia *et al.,* 2022). This limits our understanding of how parasite life history traits, such as transmission strategy, may impact biogeographic patterns. However, characterising a wider diversity of parasites is challenging, particularly because specific host-parasite interactions are not well documented (Dallas *et al.*, 2017; Farrell *et al.*, 2022). This makes it difficult to select parasite taxa to target. In addition, identification of a broad range of taxa requires expertise in taxonomy, varied methodological approaches, and can incurr a high financial cost (Dobson *et al.*, 2008; Okamura *et al.*, 2018; de Buron *et al.*, 2025). The ability of high-throughput sequencing technologies to generate Whole Genome Sequence (WGS) data at scale (Hudson, 2008) can by-pass these challenges and offers a new avenue for characterising parasites. A relatively neglected feature of WGS data is that they can also capture information about inadvertently sequenced pathogens and parasites infecting the host (Kostic *et al.*, 2011; Nichols *et al.*, 2026). Therefore, in addition to addressing evolutionary questions about free-living organisms, WGS data present an opportunity to simultaneously investigate endoparasites of the host. Utilising WGS data in this way enables the identification of a broad range of affiliated parasites with varied life histories (Nichols *et al.*, 2026).

Here we use WGS data of individual silvereyes, *Zosterops lateralis,* a passerine species that has colonised many islands throughout the South Pacific (Mees, 1969; Lack, 1971; Estandía *et al.*, 2025), to identify inadvertently sequenced parasites. Our aim is to characterise the distribution and diversity of such parasites in island and mainland silvereye populations to illustrate patterns of island biogeography with respect to richness and nestedness of parasite communities across host populations and to explore how transmission strategy may modulate these patterns. We expect that distributions of parasites utilising direct transmission will more closely reflect island biogeography patterns seen in free-living organisms than those with more complex transmission strategies.

Materials and Methods

Study system

The silvereye is a small passerine bird distributed in Australia and islands of the South Pacific (Figure 1). Their distribution spans a latitudinal range of -14° to -47°. Island silvereye populations vary in age from hundreds to hundreds of thousands of years (Clegg *et al.*, 2002; Estandía *et al.*, 2025). They occur on islands ranging in size and geographic isolation, from the large Gondwanan islands of Grand Terre, New Caledonia and the North and South Islands of New Zealand, to small volcanic islands, including those of Vanuatu, and coral cays, such as Heron Island on the Great Barrier Reef.

Sample collection, DNA extraction and sequencing

The blood samples used in this analysis were collected between 1996 and 2019 from 384 individual silvereyes spanning 25 islands of the South Pacific and locations on the Australian continent (Queensland, New South Wales, Australia Capital Territory, South Australia and Victoria). Wild birds were caught using mist nets or traps. Blood was taken using a sterile needle to prick the brachial vein. Blood was collected in a glass capillary tube and placed in a 1.5 ml microfuge tube. The microfuge tubes contained Queen's Lysis Buffer modified from Seutin *et al.* (1991) with a pH of 7.5 and the final concentrations 0.01 M Tris, 0.01 M NaCl, 0.01 M EDTA and 0.03 M n-lauroylsarcosine (10% w/v). Samples were kept at ambient temperature until they could be refrigerated and then frozen at -20°C.

DNA extractions were carried out using a modified phenol-chloroform protocol (Sambrook and Russell, 2006). Approximately 100 µl of blood preserved in Queen's Lysis Buffer was incubated overnight with 250 µl DIGSOL extraction buffer (0.02 M EDTA, 0.05 M Tris-HCl [pH 8.0], 0.4 M NaCl, 0.5% SDS) and 10 µl Proteinase K (20 mg/ mL). Incubation occurred at 55°C with rotation. Following incubation, 250 µl phenol:chloroform:isoamyl alcohol (25:24:1) was added and samples were rotated for 10 minutes then centrifuged at 10,000 rpm for 10 minutes before transferring the aqueous phase to a new microfuge tube. This process was done twice more, first using another 250 µl phenol:chloroform:isoamyl alcohol (25:24:1), then using 250 µl

chloroform:isolamyl alcohol (24:1). Two volumes of cold 100% ethanol, 1 volume of 2.5 M ammonium acetate and 1 µl glycogen were added to the final aqueous phase to precipitate the DNA. The samples were stored at -20°C for a minimum of 12 hours before three rounds of centrifugation for 10 min at 15,000 rpm at 4°C. The supernatant was subsequently discarded and the precipitate washed with 500 µl cold 70% ethanol. The precipitated DNA was left to dry at room temperature. Once dried, the DNA was resuspended in 50 µl of TE (TrisEDTA) buffer (0.01 M Tris-HCL (pH 8.0), 0.0001 M EDTA). A negative extraction control was included for every batch of samples processed (maximum of 24 per batch).

DNA extractions were quantified with a Qubit 2.0 (Thermofisher Scientific Inc., United States) and were sent to Novogene UK for library preparation and whole genome sequencing, aiming for 5X coverage on the Illumina Novaseq 6000 platform (Illumina, San Diego) and generating 150 bp paired-end reads.

Identification of parasite sequences

To characterise parasitic reads from the WGS data, the approach used by Nichols *et al.* (2025) was employed as follows. We used fastp to trim adapter content (10 bp) and remove low-quality base calls from the raw sequence data (Chen *et al.*, 2018). Next, the filtered sequencing data for each sample were aligned to an existing *Z. lateralis* assembly (Estandía *et al.*, 2023) using a Burrows-Wheeler Aligner-Maximal Exact Match (BWA-MEM) algorithm (Li, 2013). A host k-mer library was generated from the existing *Z. lateralis* assembly using the PathSeq pipeline from the Genome Analysis Toolkit (GATK) (Walker *et al.*, 2018). The host k-mer library consists of a hash table, storing k-mers generated from consecutive positions in the reference genome sequence. A BWA image of the reference genome was also generated using the BWA-MEM index image file creator in GATK (Li, 2013). Sequences from each sample were then compared to the host k-mer library using a fast k-mer search. We used the default PathSeq settings to identify reads belonging to the host: reads were removed if there was a minimum of one k-mer match to the host (Walker *et al.*, 2018) and the BWA-MEM algorithm was employed again to align the remaining reads with the host reference image, also generated above. Reads with an identity score above 30 were discarded.

Following host sequence removal, further quality control was carried out with PathSeq (Walker *et al.*, 2018). Reads with an excess of A/T or G/C content (29 out of 30 bases per window [Benjamini and Speed, 2012]), duplicate sequences and those shorter than 60 bp were discarded. Low-complexity sequences and low-quality bases were also masked using the sDUST algorithm (Morgulis *et al.*, 2006). Read ends were further trimmed according to base quality (Q = 15).

While we considered assembling longer contiguous sequences from the remaining sequences, our ability to detect certain parasites decreased following assembly. This may be because some parasite DNA is likely to be present at extremely low levels in the host DNA and therefore will not have sufficient coverage for assembly, and because parasite reference availability is limited for longer sequences (Nichols *et al.,* 2026). Therefore, we opted to use shorter sequences for classification but to impose a stringent confidence filter on the subsequent taxonomic assignments.

Reference database completeness is known to influence the outcome of taxonomic assignments (Hleap *et al.*, 2021; Jeunen *et al.*, 2023). Therefore, we compiled a custom reference database in Kraken 2 to maximise parasite coverage. We utilised the pre-built bacteria, protozoa, UniVec and human databases in Kraken 2. We then compiled additional reference sequences from various sources (Table S1). We downloaded all available genome references for apicomplexans, fornicata, cercozoans, parabasalids and euglenozoans from RefSeq (Pruitt *et al.*, 2007; O'Leary *et al.*, 2016). A complete list of NCBI taxonomic sequence identifiers was generated for the same taxonomic groups using TaxonKit (Shen and Ren, 2021). We used Biopython to download the sequences from NCBI after removing any sequences labelled as 'uncultured' or 'environmental' (Cock *et al.,* 2009; version released 05-08-2024). All genomic references from WormBase ParaSite (Howe *et al.*, 2017) and the Eukaryotic Pathogen Genomics Database Resource (EuPathDB) (Warrenfeltz *et al.*, 2018) were also incorporated. Finally, the PR^2 database was added following filtering to remove sequences labelled uncultured, environmental, fungal and metazoan. Kraken 2 derives a compact hash table of k-mers from these reference sequences to be queried against during sequence assignment.

Sequences were assigned using the custom parasite reference database with Kraken 2 (Wood *et al.*, 2019). Following taxonomic assignment, we searched for information on previously recorded

primary hosts and categorised the primary hosts for each parasite genus as bird, other vertebrate, invertebrate, or plant, accordingly. We removed parasites that have not previously been recorded in birds or other vertebrates. For the detected parasite genera, we collated information from the literature on their life histories which enabled assignment to the following transmission strategies: flying insect vector, tick-borne, transmission involving secondary hosts other than ticks or flying insects (i.e. parasites with complex life cycles) or direct (i.e. parasites with simple life cycles). Table S2 contains a summary of the identified parasites, including available life history information.

Confidence scores for each taxonomic assignment were generated using Conifer (Silamiķelism, 2020). These were calculated according to the fraction of k-mers assigned to the final taxonomic identity, its descendants and ascendants, giving the Root-to-Leaf (RTL) confidence score. We applied a confidence score filter of 0.7 for the paired-end dataset. We previously found that applying a conservative confidence score to relatively short paired-end sequences resulted in fewer apparently spurious parasite identifications than assembling longer contiguous sequences. By utilising short sequences we identified fewer parasites that have not been previously reported in birds; and fairly consistent identification of selected parasites (haemosporidians) while continuing to capture a large amount of diversity (Nichols *et al.*, 2026).

Statistical analysis

Is parasite diversity higher on the mainland compared to islands?

Statistical analysis was conducted in R version 4.4.2 (R Core Team, 2024). To test the island biogeography expectation that parasite richness is higher in mainland compared to island populations, we used the *vegan* package version 2.6-10 to estimate Chao2 richness based on parasite genera detected for each population (Oksanen, 2019). To account for uneven sampling across populations, we conducted random sub-sampling with replacement and repeated the process with 1,000 bootstraps. We sub-sampled to n = 5 to reflect the lower sample sizes for some islands (see Figure 1 for sample sizes). However, Ambae and Vanua Lava had lower sample sizes of n = 3. In these cases where sample size was < 5, Chao2 was estimated without sampling with replacement. Next, a pairwise permutation test with a Bonferroni correction for multiple comparisons was used to compare Chao2 estimates for each island population to the Australian continent. Each comparison was repeated 10,000 times.

We tested whether the Australian continent ('the mainland') represents a regional source for parasite communities in the island populations by looking for evidence of community nestedness. The Nestedness metric based on Overlap and Decreasing Fill (NODF) was generated using the *vegan* package (Oksanen, 2019). To assess whether observed nestedness differed from that expected by chance alone, we compared our matrix to 999 simulations of a row-constrained null model, in which parasite richness on each island (row totals) was conserved across the matrix to account for uneven sampling size, while parasite incidence (column totals) was randomised. We performed the analyses on two datasets: in the first, we included the mainland, and each island population individually; and in the second, we combined data from islands within the French Polynesia and Southern Melanesia archipelagos. We considered them as single units for analysis, as they are geographically clustered.

To examine how variation in parasite communities differs in host individuals from the islands compared to the Australian continent, we implemented an extreme gradient boosting (XGBoost) classification algorithm using the *XGBoost* package 1.7.11.1 (Chen and Guestrin, 2016). We aimed to test whether a host individual's origin (mainland or island) could be predicted according to a 'parasite profile'. The parasite profiles consisted of the number of genera detected in each host individual per transmission strategy. The predicted variable was host origin (i.e. island or mainland). We subsequently extracted importance scores for each of the four transmission strategies in classifying the origin of host individuals. Although XGBoost has been shown to work effectively on imbalanced datasets in the past (Zhao *et al.*, 2018), it is nonetheless likely to cause a decline in model performance if one class is overrepresented (Wang *et al.*, 2020). Therefore, we applied class weighting to the model to account for the imbalanced sample sizes in the island (n = 321) and mainland (n = 48) categories. Specifically, we used the ratio of mainland to island host individuals to adjust the gradient updates during training by increasing the weight of errors made on the underrepresented class (mainland), thereby preventing the model from being biased toward the majority class (island). We examined the distribution of probabilities output by the model to confirm that class imbalance was not causing bias in predictions (Figure S1). We used leave-one-out cross-validation (LOOCV), where one host individual was withheld in each iteration of the model. We calculated Area Under the Receiver Operating Characteristic Curve (AUC ROC) scores to assess the model.

To further illustrate the output of XGBoost, we tested the difference in mean number of parasite genera of each transmission strategy detected per host. We utilised a two-way ANOVA using host origin and transmission strategy as factors, followed by a post-hoc Tukey HSD test to explore pairwise comparisons. We also tested differences between the prevalence per transmission strategy (i.e. pooling parasite genera into four transmission strategies for island and mainland populations) on the island and mainland using four independent Fisher's Exact Tests (one per transmission strategy) and applied a Bonferroni correction to control for multiple comparisons. The adjusted threshold significance was $\alpha = 0.0125$.

Is parasite richness higher on less isolated, larger islands?

To test whether parasite richness increased with island size and decreased with island isolation, we fitted generalized linear models (GLMs) to compare Chao2 richness between islands. For each model, we used the same set of predictor variables: island area, island isolation, and latitude. To calculate island area and isolation, we utilised the Natural Earth 10m landmass data (Kelso and Patterson, 2010; Pebesma, 2018). Areas were computed on the WGS84 ellipsoid (EPSG:4326). We considered two metrics of isolation to capture larger-scale and regional-level isolation. First, we calculated the distance to mainland as the shortest possible distance between the coast of mainland Australia and each island (km). Second, we followed the procedure outlined by Itescu *et al.* (2020) and Weigelt and Kreft (2013), generating a concentric 100 km buffer around each island and calculating the area of landmass it contained, excluding the island itself. This captures a more regional isolation level, which is relevant when a dataset comprises both single isolated islands and islands that fall within archipelagos. Isolation metrics and area varied across several orders of magnitude. To reduce positive skew, we applied a $\log_{10}$ transformation to island area and both measures of isolation prior to modelling. To control for the possibility that parasite communities vary according to latitude (Nunn *et al.*, 2005; Poulin and Leung, 2011), latitude was included as a covariate in the models. Our sampling took place over two decades, over which time parasite communities may be liable to change (Wood *et al.*, 2023). However, we were unable to include sampling year as a random effect because most islands were sampled in a single year, therefore including year as a random factor would prevent models from converging. The models were also weighted to account for uneven sample sizes among populations.

To avoid inclusion of collinear predictor variables in the models, pairwise Pearson correlation coefficients were calculated between each predictor variable to ensure that none exceeded a correlation threshold of $r > 0.7$, which they did not. Variance inflation factors (VIFs) were also computed using the *car* package to quantify multicollinearity (Fox *et al.*, 2007). The moderate correlation between proximate island isolation and island area led to elevated VIF scores (Table S3). Therefore, we excluded regional isolation and recalculated VIF scores. All scores were below two, indicating acceptable levels of collinearity for inclusion in the model.

In the first GLM, we used the $\log_{10}$-transformed mean estimates for Chao2 richness per island, as the response variable and specified a Gaussian distribution of errors. In the second GLM, we classified parasite genera into direct, flying insect vector or other secondary host transmitted parasites and calculated Chao2 richness for each category. We excluded tick-borne parasites from this analysis as the overall richness detected was low (two genera overall), and Chao2 estimates were thus unlikely to be informative. We found that using a $\log_{10}$-transformation was not sufficient to adjust the right-skewed data, so we instead used a gamma distribution of errors to accommodate the resultant non-negative, count-like richness measures. Despite being count-like, Chao2 is in fact a bias-corrected estimate derived from incidence data (Gotelli and Colwell, 2001). Therefore, Chao2 estimates are continuous, non-integer values which cannot be modelled using error distributions that are commonly used for count data. Following model fitting, we used the *DHARMa* package (Hartig and Hartig, 2017) to assess model fit (Figure S2).

For both GLMs, we performed model selection and averaging using the *MuMIn* package (Barton and Barton, 2015). All candidate models were compared based on Akaike's Information Criterion corrected for small sample sizes (AICc). Models within $\Delta\text{AICc} < 2$ of the top-ranked model were considered equally plausible, and we generated model-averaged predictions across this top model set. Model selection tables are included in Tables S5 and S6.

Results

We characterised 48 parasite genera from 384 individual silvereye WGS. Of these, the majority require a secondary host for transmission (44% of total genera detected), followed by equal numbers of directly transmitted genera (26%) and those transmitted by flying insect vectors (26%), and lastly by tick-borne parasite genera (4%). Of the four groups, flying insect vector-transmitted parasites had the highest prevalence, with at least one genus detected in every population.

Is parasite richness higher on the mainland compared to islands?

Parasite richness was generally lower for island populations than on the mainland, with 24 of the 25 island populations having significantly lower Chao2 richness estimates (Figure 1). Heron Island had a significantly higher Chao2 estimate in comparison to the mainland. The distributions of estimates for Heron Island indicate wide variation, suggesting high variability and possibly insufficient power to estimate richness.

The parasite communities of host populations exhibited significant nestedness (Figure 2). In the analysis comparing each island population individually, the NODF was 63.79, substantially higher than expected under the null model (null model mean = 47.32, 95% CI = 43.52-51.30, $p < 0.001$). This corresponds to a standardized effect size of 8.04. The mainland fell at the apex of the nestedness structure, with island parasite communities forming subsets of the genera found on the mainland (Figure 2A). In the analysis that considered islands within Southern Melanesia and French Polynesia at the archipelago level, the observed NODF was 71.68 (null model mean = 59.360, 95% CI = 52.89-65.08, $p < 0.001$) and the standardised effect size was 3.95. However, in this analysis, Southern Melanesia fell at the apex of the nestedness structure (Figure 2B).

According to LOOCV, the XGBoost model had a classification accuracy of 82% and an AUC ROC score of 86%, indicating that the parasite profile, i.e. the number of parasite genera detected per individual host per transmission strategy, provides discriminatory power to identify host samples sourced from an island versus the mainland. Feature importance scores extracted from the model indicated that flying insect vector-transmitted parasites were most useful in this

distinction (Figure 3A). Furthermore, the mean number of genera detected per individual host was significantly higher for mainland than island populations for direct (ANOVA, mean difference = 0.49 95% CI = 0.38-0.59, $p < 0.001$) and flying insect vector-transmitted parasites (ANOVA, mean difference = 1.35, 95% CI = 1.04-1.66, $p < 0.001$) whereas there was no significant difference for parasites transmitted by ticks (ANOVA, mean difference = 0.03, 95% CI = -0.28-0.35, $p = 0.999$) or other secondary hosts (ANOVA, mean difference = 0.08, 95% CI = -0.28-0.47, $p = 0.996$; Figure 3B). The prevalences of directly transmitted (Fisher's Exact Test, Odds Ratio (OR) = 8.07, 95% CI = 2.03-70.12, Bonferroni-adjusted $p < 0.001$), other secondary host transmitted (Fisher's Exact Test, OR = 4.61, 95% CI = 2.30-9.80, Bonferroni-adjusted $p < 0.001$), and tick-borne (Fisher's Exact Test, OR = ∞, 95% CI = 3.17-∞, Bonferroni-adjusted $p < 0.001$) parasites were higher on the mainland than in the island populations. No difference was found for flying insect vector-transmitted parasites between island and mainland populations (OR = ∞, 95% CI = 0.06-∞, Bonferroni-adjusted $p = 1.00$).

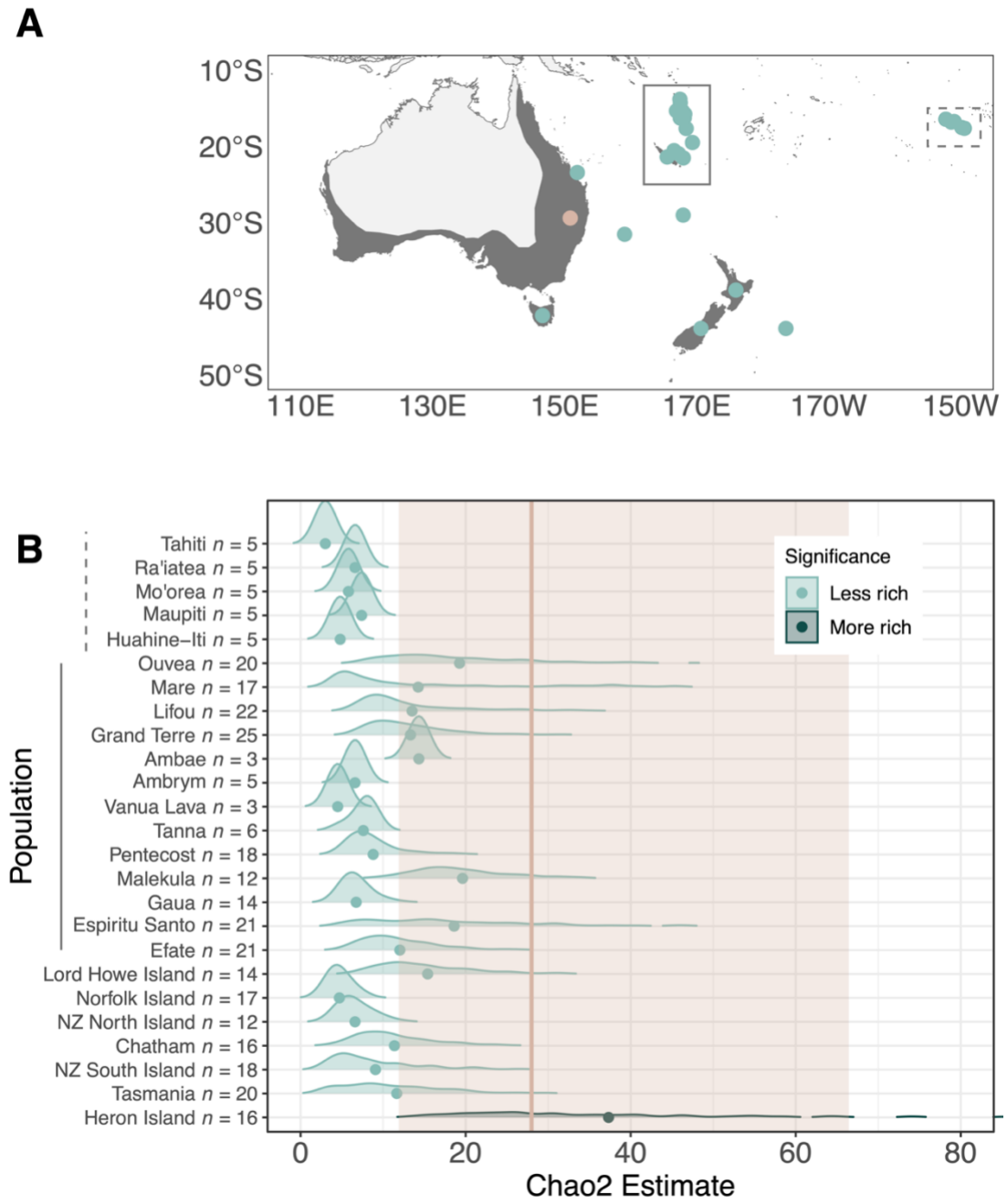

Figure 1. A) Map of sampled mainland (beige) and island (turquoise) populations with dark grey shading indicating the silvereye range. B) Chao2 richness estimates with random subsampling with replacement to n = 5 with 1,000 bootstraps, the colours of each distribution indicate whether Chao2 richness was significantly more rich (darker) or less rich (lighter) from the mean Chao2 estimate on the mainland, indicated by the beige vertical line and shaded 95% Confidence Intervals (CI).

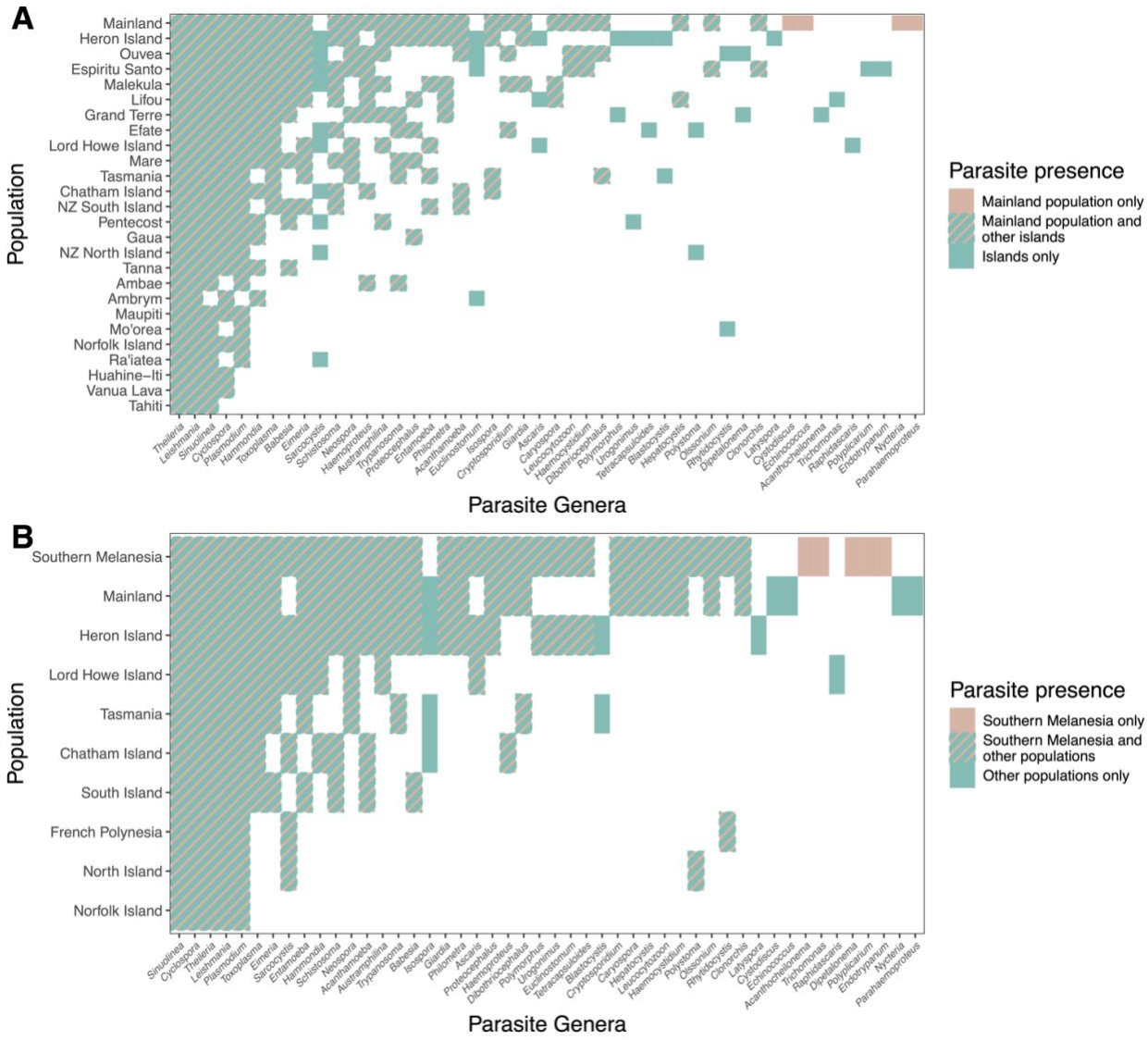

Figure 2. Nestedness structure amongst A) the mainland and each island population and B) the mainland, single islands, and the Southern Melanesia and French Polynesia archipelagos (grouping islands within each archipelago).

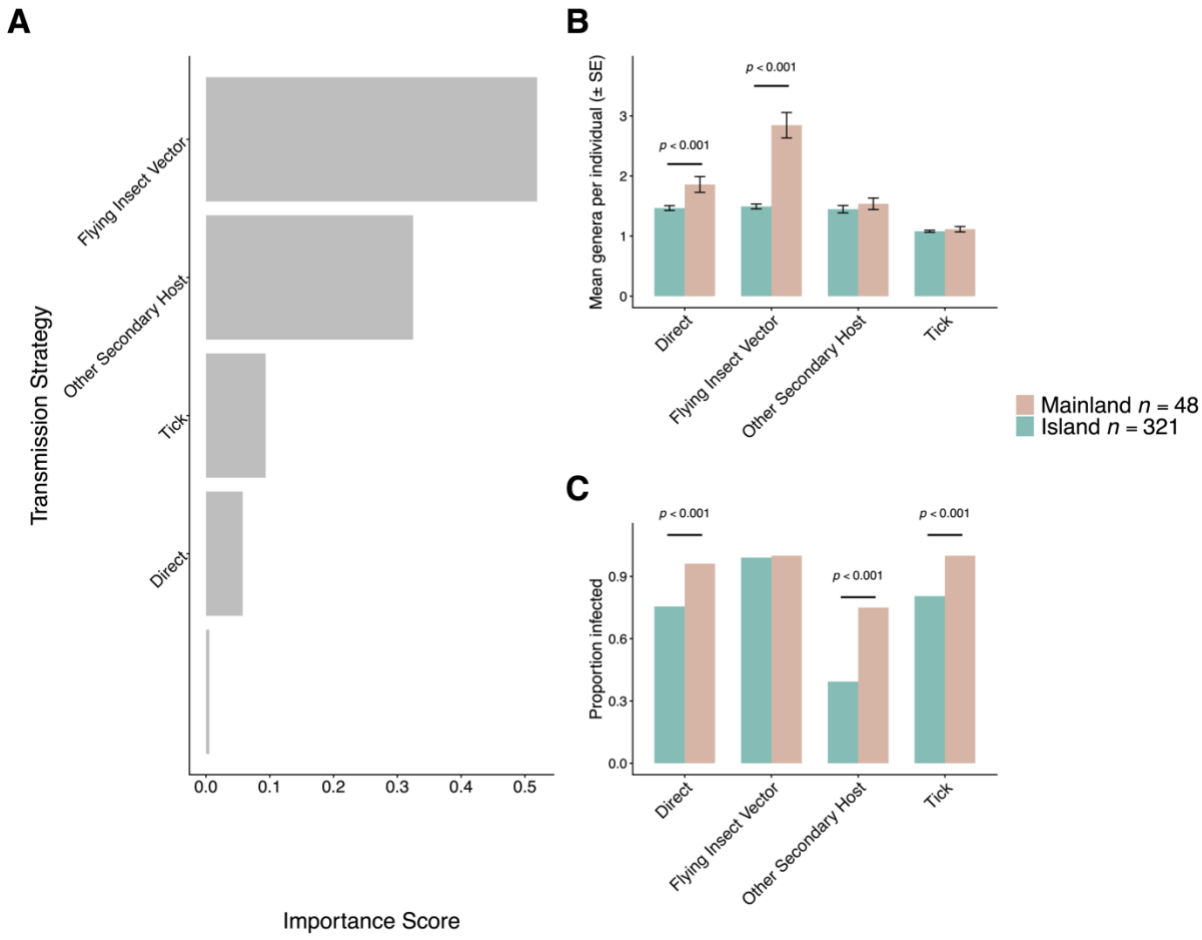

Figure 3. A) Importance scores extracted from XGBoost when classifying 384 individual hosts into island and mainland origins according to their parasite profile (number of parasite genera per transmission strategy detected per individual host) B) mean number of genera of parasites detected per individual host according to transmission strategy, p values generated using an ANOVA and Tukey HSD test and C) proportion of individual hosts sampled from the mainland and island populations with parasites detected per transmission strategy, p values derived from Fisher's Exact Test with Bonferroni correction.

Is parasite richness higher on less isolated, larger islands?

For total Chao2 richness per island, two models were averaged based on AICc (Table S4; 1). Island area was included in one of the two models, and because the models were similarly weighted, island area was attributed a weighted importance score of 0.51, while latitude and isolation from the Australian continent were attributed weighted importance scores of 1. While there was support for including all three variables in the models, isolation from the Australian continent had the most substantial effect size, with richness decreasing as isolation from the Australian continent increased (Figure 4A). Although island area and latitude did explain some of the variation in the data, it was not possible to discern the direction of the relationships.

For Chao2 richness per transmission strategy on each island, a single top model was selected based on AICc scores (Table S5; 2). The top model included every variable, although it was not possible to discern the direction of the relationship between Chao2 richness and island area, isolation from the Australian continent, and latitude, as the 95% CI encompassed zero. However, we found substantial variation between Chao2 richness for each transmission strategy: with directly transmitted parasites having the overall highest richness, followed by parasites transmitted by other secondary hosts and finally, flying insect vector-transmitted parasites. We also found that the relationship between Chao2 richness and isolation from the Australian continent was dependent on transmission strategy (Figure 4B), with distance having almost no impact on parasites that rely on flying insect vectors but a substantial decline in richness for parasites utilising direct transmission strategies and those involving other secondary hosts. Table 3 summarises the results obtained from each of the statistical tests performed.

Table 1. Model-averaged estimates for GLM predicting total Chao2 richness based on parasite genera detected per island population. Estimates, standard errors, 95% CI and weighted importance scores (WIP) are based on model averaging across the top two models (ΔAICc < 2; Table S4). The parameters included in each model (1 and 2) are indicated by black dots.

Parameter	Model 1	Model 2	β	SE	95% CI	WIP
(Intercept)	•	•	5.704	0.893	(3.954, 7.454)	NA
$\log_{10}$ Island Area (km^2)	•		-0.014	0.031	(-0.075, 0.047)	0.51
Latitude	•	•	0.014	0.009	(-0.004, 0.033)	1.00
$\log_{10}$ Isolation from the Australian Continent (km)	•	•	-0.385	0.110	(-0.601, -0.169)	1.00
df	5.00	4.00				
logLik	-966.88	-968.50				
AICc	1946.91	1946.99				
delta	0.00	0.08				
weight	0.51	0.49				

Table 2. Model estimates from the top GLM predicting Chao2 richness per transmission strategy on each of 25 islands. Estimates, standard errors, 95% CI and weighted importance scores (WIP) are based on the top model (ΔAICc < 2; Table S5). The parameters included in each model are indicated by black dots.

Parameter	β	SE	95% CI	WIP
(Intercept)	1.828	1.078	(-0.345, 4.236)	
$\log_{10}$ Island Area (km^2)	0.015	0.039	(-0.065, 0.094)	1.00
Latitude	0.005	0.008	(-0.010, 0.019)	1.00
$\log_{10}$ Isolation from the Australian continent (km)	-0.117	0.140	(-0.430, 0.174)	1.00
Transmission Strategy: Direct	3.946	1.482	(0.941, 6.895)	1.00
Transmission Strategy: Other Vertebrate Host	3.089	1.437	(-0.011, 6.179)	1.00
$\log_{10}$ Island Area (km^2) x Direct Transmission	0.032	0.053	(-0.072, 0.137)	1.00
$\log_{10}$ Island Area (km^2) x Other Secondary Host Transmission	-0.067	0.053	(-0.181, 0.049)	1.00
$\log_{10}$ Isolation from the Australian continent (km) x Direct Transmission	-0.571	0.201	(-0.984, -0.154)	1.00
$\log_{10}$ Isolation from the Australian continent (km) x Other Secondary Host Transmission	-0.283	0.192	(-0.716, 0.148)	1.00
df	11.00			
logLik	-1790.03			
AICc	3606.53			
delta	0.00			
weight	0.91			

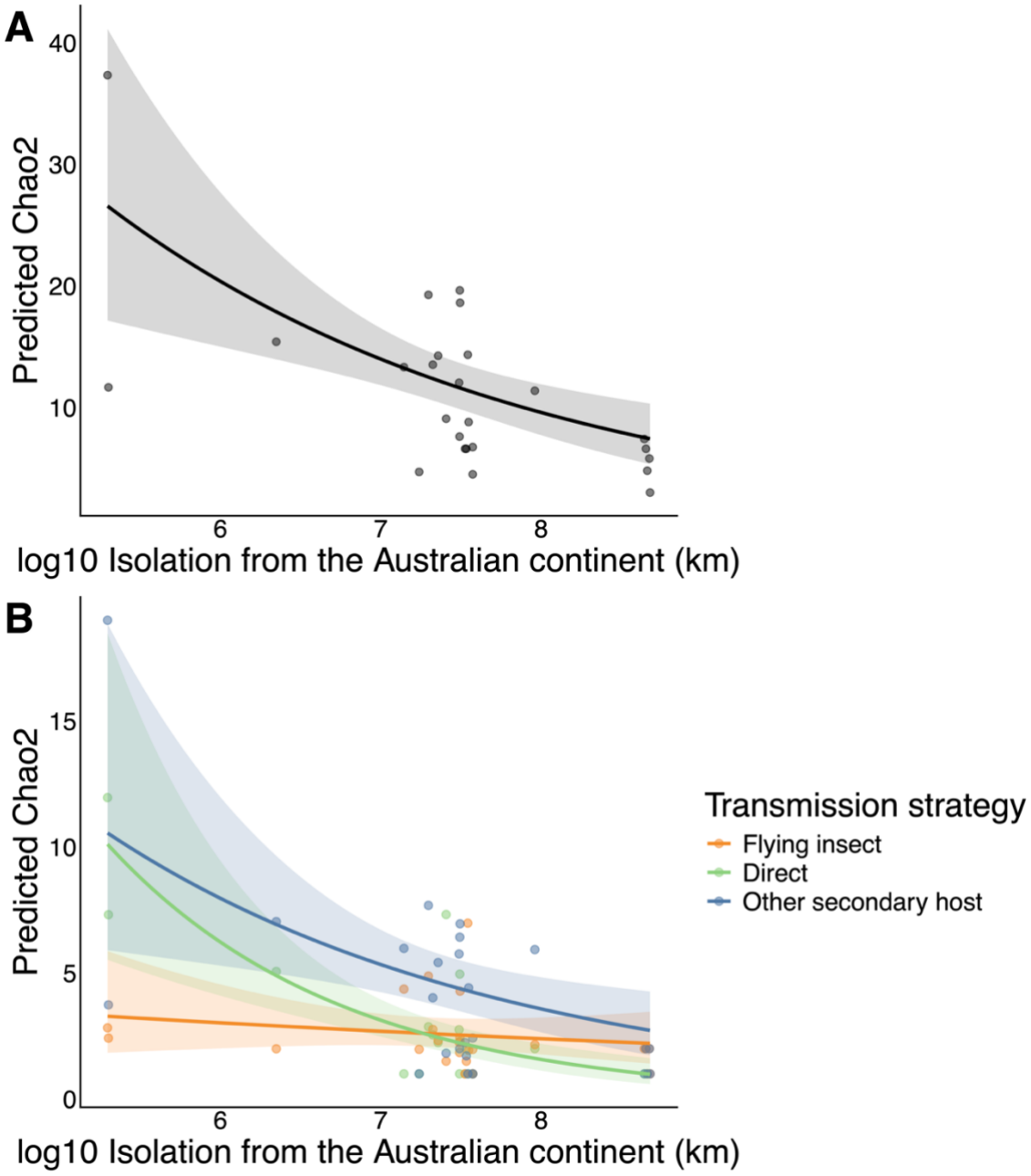

Figure 4. Predicted Chao2 richness as a function of isolation from the Australian continent for A) total Chao2 richness per island and B) per transmission strategy. Predictions are based on an average of the top two models with < 2 AICc (A) or the top GLM according to AICc values (B). Points represent observed islands used to generate predictions.

Table 3. Summary of results obtained from each comparison made (island vs mainland or island vs island) for different groups of parasites.

Comparison and parasite group	Parasite measure	Direction
Island vs mainland		
Total	Richness	Higher on mainland than islands
	Nestedness	Islands nested within mainland
Direct (simple)	Richness	Higher on mainland than islands
	Prevalence	Higher on mainland than islands
Flying insect vector (complex)	Richness	Higher on mainland than islands
	Prevalence	No difference
Other Secondary Host (complex)	Richness	No difference
	Prevalence	Higher on mainland than islands
Tick (complex)	Richness	No difference
	Prevalence	Higher on mainland than islands
Island vs Island (isolation)		
Total	Richness	Declines with increasing isolation
Direct (simple)	Richness	Declines with increasing isolation
Flying insect vector (complex)	Richness	No difference
Other Secondary Host (complex)	Richness	Declines with increasing isolation
Island vs Island (area)		
Total	Richness	No difference
Direct (simple)	Richness	No difference
Flying insect vector (complex)	Richness	No difference
Other Secondary Host (complex)	Richness	No difference

Discussion

By extracting parasite DNA from existing low-coverage WGS data of hosts, we empirically demonstrate how parasite distributions reflect the expected patterns of island biogeography. Overall, parasite species richness was higher on mainland Australia compared to island populations and, among island populations, parasite richness decreased with distance from the continental mainland. Taken together with our nestedness analysis, it appears that parasite diversity in the region is sourced from both the Australian continent and Melanesian archipelagos. Notably, we found that distribution patterns were differentially driven by parasites utilising varied transmission strategies.

Isolation and parasite life history traits influence parasite richness and prevalence

Our results clearly demonstrate that transmission strategies influence parasite patterns of island biogeography. For directly transmitted, simple life cycle parasites, we found lower parasite richness in island populations, with richness declining with distance from the continental mainland. These results indicate that when the primary dispersal mechanism is co-dispersal with the host, island isolation acts as a dispersal filter simultaneously for both host and parasite. Although studies which test the rules of island biogeography for directly transmitted parasites in birds are rare, reduced richness of directly transmitted helminths with distance from the nearest island has previously been shown for fish parasites within the Pacific Line Islands (Williams *et al.*, 2022) and is also demonstrated by reduced haplotype diversity with distance from the mainland in the nematode parasite, *Heligmosomoides polygyrus* infecting mice in the Mediterranean (Nieberding *et al.*, 2006).

Parasites with complex life cycles showed varied patterns of island biogeography. Complex life cycle parasites that utilise intermediate hosts are expected to be more limited in their establishment success than directly transmitted parasites (Cizauskas *et al.*, 2017; Wood *et al.*, 2023), and this should lead to stronger patterns of island biogeography. However, we detected no significant difference overall in richness between island versus mainland populations for such parasites, and although richness decreased with increasing island isolation, the effect was weaker than for directly transmitted parasites. A possible explanation for these patterns is that parasites with complex life cycles may be able to utilise a paratenic host (a host that can harbour the parasite in a dormant

state). This may facilitate persistence in newly colonised environments as parasites do not require immediate availability of definitive hosts (Morand *et al.*, 1995; Parker *et al.*, 2015; Cable *et al.*, 2017). Therefore, rather than being a limitation, intermediate host use could enhance opportunities for colonisation and persistence, thereby contributing to the formation of stronger patterns of island biogeography.

Parasites transmitted by flying insect vectors exhibited a reduction in genus richness on islands compared to the mainland, yet this richness did not decline further with increasing island isolation. The overall reduction in richness on islands followed expectations as successful transmission requires a suitable vertebrate host and compatible flying insect vector (Spurgin *et al.*, 2012). Variation in the dispersal propensities and flight mechanisms of flying insects (Compton, 2002), combined with the relatively limited availability of habitat types on islands (McGuinness, 1984; Turner and Tjørve, 2005) may restrict diversity of vectors able to establish, thereby constraining the diversity of vector-dependent parasites (Robert *et al.*, 2011). However, the observed patterns suggest that for the subset of parasites whose vectors can traverse long distances, island isolation likely exerts a weaker influence on colonisation success. We note that wind-assisted dispersal of *Anopheles mosquitos* can promote travel over 300 km in a 9 hour flight period (Huestis *et al.*, 2019). This finding has important implications for future disease projections under climate change. In particular, the dispersal of flying insect vectors may be augmented globally due to lower air densities resulting from warming temperatures (McManus, 1988; Spiridonov and Ćurić, 2021) and changing wind patterns (Kling and Ackerly, 2020). Consequently, highly dispersive flying insect vectors may be increasingly able to colonise remote geographic reaches, thereby creating new opportunities for transmission.

In contrast to parasite richness, prevalence of flying insect vector-transmitted parasites remained similarly high on the mainland and the islands. A pattern of high prevalence of island parasites has been reported previously (Lindenfors *et al.*, 2007; Illera *et al.*, 2015; Dybing *et al.*, 2018) and reflects a well-established biogeographic trend for free-living organisms whereby a subset of species that occur on islands often reach higher population densities (MacArthur *et al.*, 1972). Elevated prevalence of parasites on islands may therefore be supported by high host densities, which facilitate frequent transmission opportunities (Fromont *et al.*, 2001; De Bellocq *et al.*, 2002).

Island area and parasite richness

Although area was important in explaining differences in parasite richness among islands, we did not detect a positive correlation between richness and island area, as expected under the rules of island biogeography. Our study investigated a wide range of island areas, from Heron Island, which is just 0.29 km^2, to much larger islands, such as the North and South Islands of New Zealand, which are ~114,000 and ~150,000 km^2, respectively. Despite being the smallest in our dataset, Heron Island hosted the richest parasite community. This finding may reflect the Small Island Effect (SIE), which describes how the relationship between species richness and island area can be decoupled for small islands (Lomolino and Weiser, 2001). The mechanisms underlying this effect may include elevated or reduced habitat diversity on small islands (Sfenthourakis and Triantis, 2009; Chen *et al.*, 2020), edge effects (Lomolino and Weiser, 2001), or disequilibrium between colonisation and extinction events. Indeed, the proximity of Heron Island to the mainland (~80 km) likely bolsters the parasite community via frequent colonisation opportunities and episodes of offshore winds have previously been associated with the appearance of *Plasmodium* infection in the silvereye population on the island (Clark and Clegg, 2015). Accordingly, Heron Island was positioned immediately below mainland Australia in the nestedness analysis, suggesting that parasites readily colonise from the mainland. Therefore, for small islands, stochastic processes and ecological factors may have a greater impact on richness than area-dependent relationships predicted by classical island biogeography.

Notably, several parasite genera detected on Heron Island were not detected on the Australian mainland, this may partly reflect a lower sampling effort on the mainland. However, the identity of these parasites suggests that they could have entered the silvereye parasite community via alternative sources. Indeed *Sarcocystis* (Gjerde *et al.*, 2018; Sato *et al.*, 2022), *Euclinostomum* (Abro et al., 2016; Shamsi et al., 2021) and *Polymorphus* (Kuklina and Kuklin, 2021; Galaktionov and Atrashkevich, 2023) are all reported to parasitise seabirds. Heron Island supports high-density breeding populations of Black Noddies (*Anous minutus*) and Wedge-tailed Shearwaters (*Ardenna pacifica*) (Hill *et al.*, 1997; Dyer *et al.*, 2005). These produce substantial amounts of guano. Black Noddies alone are estimated to produce over one hundred tonnes of guano annually on Heron Island (Johnson, 1995). The abundance of guano likely provides opportunities for parasite transmission as the silvereyes routinely forage in Black Noddy nests, as well as consume food items that have been regurgitated by noddies. In summary, Heron Island's elevated parasite richness

despite its small area likely reflects the combined influence of a unique avian community assemblage, which includes copius seabirds, and frequent immigration from the mainland.

Sources of parasite fauna

The nestedness analysis suggests that the Australian mainland functions as the dominant source of parasites for the sampled island populations. However, the detection of several parasite genera on the islands but not on the Australian mainland indicates that multiple source populations may be involved in determining regional parasite assemblages. When analysed at the archipelago scale, Southern Melanesia occurred at the apex of the hierarchical structure of nestedness. New Guinea is a major source for much of the regional avifauna in Southern Melanesia (Diamond, 1970; Filardi and Moyle, 2005; Jønsson *et al.*, 2010; Jønsson *et al.*, 2011), and it follows that movements of birds from New Guinea have provided a source of parasite diversity in Southern Melanesia.

Host colonisation history likely further influences the parasite assemblages detected across islands. Silvereyes have colonised islands throughout the South Pacific over a range of timescales. Some populations in Southern Melanesia are estimated to have colonised hundreds of thousands of years ago, while others are the product of recent, human-mediated introductions. During the late 19th and early 20th centuries, numerous non-native bird species were introduced to French Polynesia (Thibault and Cibois, 2017). In the 1930s, one aviculturalist, Eastham Guild sourced over 7000 individuals of 59 species from around the world to test whether they would acclimatise and establish in French Polynesia (Guild, 1940). Following their introduction, 11 species, including the silvereye, became permanently established, all 11 species on Tahiti and nine on Mo'orea (Thibault and Cibois, 2017). It is possible that the low parasite richness observed more broadly across French Polynesia reflects recent host colonisations, and the island populations may not have reached equilibrium in the establishment of host-parasite interactions.

Caveats

The limited understanding of the breadth of host associations and patterns of host use in our system makes it challenging to empirically assess how host specificity influences parasite distributions across islands. A previous study found that host specificity of parasites significantly

decreased on islands when comparing the outcomes of 28 studies on 16 species of mammal (De Bellocq *et al.*, 2002). Therefore, it is likely that being a generalist confers a benefit to parasites colonising islands. However, there is limited information on the factors that shape host specificity, and levels of specificity are liable to change with environmental variability (Wells and Clark, 2019). Furthermore, parasite specificity is likely to vary with transmission strategy. For example, the transmission of tick-borne parasites will be further complicated as ticks themselves have complex life cycles (Troughton and Levin, 2007; Apanaskevich *et al.*, 2014) and display varying levels of host specificity (Froeschke *et al.*, 2013). Similarly, patterns of specificity have been shown to differ between birds and insect vectors in haemosporidians (Njabo *et al.,* 2011). There has been a sustained interest in characterising the degree of host specificity for some avian parasites (Bensch *et al.*, 2000; Schrenzel *et al.*, 2005; Križanauskienė *et al.*, 2006; Schoener *et al.*, 2013). Such work may help to elucidate the impact of host specialisation on island colonisation in the future.

Re-purposing WGS data to characterise affiliated parasites involves some trade-offs. While the approach enables detection of a broad range of parasites, some diversity may be overlooked due to the limited availability of published reference sequences. Furthermore, the lack of longer or complete parasite genome reference sequences means that assembly of short reads into longer contiguous sequences yields fewer matches. Yet the use of short sequences introduces the possibility of assignment to an incorrect reference. To combat these collective issues, we used short sequences rather than assembling longer sequences, but applied a stringent confidence filter for identifications, and classified only to the genus level (Nichols *et al.*, 2026). Our approach reflects the decision to maximise diversity capture, despite introducing some false-positive identifications.

Conclusions

By leveraging existing WGS data from an island-colonising bird, we provide evidence that large-scale parasite distributions follow the rules of island biogeography, but these distributions are mediated by aspects of parasite life history. This suggests that inconsistent biogeographical patterns revealed in previous studies that focus on particular parasite groups (e.g. Illera *et al.,* 2015; Jean *et al.,* 2016; Votýpka *et al.,* 2019) can be at least partially explained by variation in life history, an insight that is only revealed by examining a broad range of parasites. While the increasing availability of parasite sequence data in public databases will enhance analyses of distribution patterns, a concerted effort is also needed to gather information on parasite ecology and life history

traits (Cizauskas *et al.*, 2017). Such integrative data will enable a more nuanced understanding of parasite distributions over space and improve forecasts of future distributional changes, including the spread of disease agents.

Acknowledgements

We are grateful to the many people who facilitated this work. We thank the chiefs and landholders of Vanuatu and New Caledonia for granting access to field sites. We are grateful to those who assisted with fieldwork and/or logistics for fieldwork: N. Clark, R. Black, D. Treby, J. LeBreton, F. Cugny , W. Waheoneme, O. Hebert and A. Rouquie, G. Kakue (New Caledonia); F. Robertson and C. Sendell-Price (Heron Island); S. Geiger, O. Boissier, R. Hills, S. Totterman, O. Drew, K. Ser, W. Ser, J. Saksak, A. Phillimore (Vanuatu), I.P.F. Owens, N. Clark, F. Robertson, M. Massaro, D. Potvin (Australian mainland); J. Kikkawa (Norfolk Island); I.P.F. Owens (Lord Howe Island); P. Park, A. Fletcher, P. Gray, F. Robertson (Tasmania); P. Schweigman, D. Onely, F. Robertson (New Zealand); M. Bell (Chatham Island); N. Davies, O. Grant, C. Sendell-Price (Moorea, French Polynesia). We also thank D. Bass and M. Kamouyiaros for advice and discussions about the bioinformatics required to extract parasite sequences from the data.

The work was conducted under permits from the Direction de l'Environnement Province Sud and Direction Du Développement Economique (New Caledonia and Loyalty Islands with thanks to G. Kakue); Vanuatu Environment Unit letters of permission and permits provided by E. Bani and we further thank D. Kalfatak and T. Tiwok for their assistance (Vanuatu); Délégations régionales à la recherche et à la technologie (French Polynesia); Lord Howe Island Board; Environment Australia (Norfolk Island); Queensland Department of Environment and Resource Management; Parks and Wildlife Service (New South Wales with thanks to M. Massaro); Parks and Wildlife Service (Tasmania); New Zealand Department of Conservation Te Papa Atawhai (New Zealand); Australian Bird and Bat Banding Scheme project (SMC), and New Zealand National Banding Office (BCR) .

Animal studies

Ethics clearances were provided by University of Queensland ethics committee (ZOO/165/94/ARC, ZOO/520/96/ARC/PHD, ZOO/520/97/ARC/PHD), Griffith University ethics committee (ENV/01/12/AEC, ENV/07/16/AEC, ENV/06/20/AEC, ENV/24/13/AEC) to SMC, Charles Sturt University AEC to BCR and M. Massaro and Otago University AEC to BCR.

Funding

We thank the funders of this work: the Department of Zoology, Oxford start-up and OUP John Fell Fund grant to SMC to support sequencing; Marsden Fund New Zealand to BCR and SMC to support fieldwork on mainland Australia and Heron Island and sequencing; National Geographic Society Committee for Research and Exploration Grant (9383-13) to SMC to support fieldwork in New Caledonia; Natural Environment Research Council (NERC) postdoctoral fellowship to SMC to support fieldwork in Vanuatu and New Caledonia; Percy Sladen Memorial fund to SMC to support fieldwork in French Polynesia; SN was supported by a NERC studentship (NE/S007474/1); AE was supported by a NERC studentship (NE/S007474/1) and a St John's College Graduate Scholarship.

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Chapter 5

Life cycle complexity shapes parasite sharing amongst migratory and resident hosts

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This chapter is available as a pre-print, here: DOI: 10.64898/2026.01.14.699309

Abstract

Migratory animals are potentially important parasite dispersers. However, understanding how host migration influences parasite distributions can be challenging because variation in parasite life histories, ecologies, and host use can impact dispersal potential. Furthermore, studies of parasite dispersal via host migration often compare parasites in different species of migrant and resident hosts, despite differences in host phylogeny, ecology, and exposure history, which can complicate ascertaining the impact of migration on parasite communities and patterns of sharing. The Tasmanian population of silvereye (*Zosterops lateralis lateralis*) exhibits partial migration. Some members of the population overwinter on the Australian mainland, where they co-occur with year-round residents of another silvereye subspecies (*Z. l. cornwalli).* Utilising targeted PCR, metabarcoding and high-throughput sequencing, we characterise a broad range of parasite genera from blood and faecal samples in Tasmanian resident, migrant and mainland resident birds. Greater parasite richness was documented in migrant individuals, which may reflect exposure to varied parasite faunas at stopovers or during overwintering. The increased richness was primarily driven by infections of parasites with simple life cycles. Reduced sharing of parasites with complex life cycles amongst migratory and resident host populations may be explained by ecological constraints in establishing transmission, such as vector or secondary host availability. Our study exemplifies the utility of molecular methods for characterising multi-parasite systems and makes use of a single host species to provide insights on patterns of parasite distributions and sharing that result from migratory movements. Knowing how parasite life histories constrain or promote dispersal via regular host migration will be essential for predicting future patterns of pathogen dispersal under climate change and shifting migratory regimes.

Introduction

Migratory animals embarking on seasonal long-distance movements typically encounter a variety of habitats and organisms, exposing them to a wide array of pathogens and parasites. Hosts may consequently lose, swap or gain parasites as a result of their seasonal migratory movements (Clay, 2003; Clark *et al.*, 2015). Such migration-related exposure to parasites can profoundly impact the dynamics of infectious disease (Altizer *et al.*, 2011). Yet, the impact of migration on parasite dispersal and distribution remains difficult to predict, particularly because processes that cause migratory hosts to gain and lose parasites may be happening concurrently, and because similar patterns of parasite distributions can arise via different mechanisms (Peacock *et al.*, 2020). With global climate change altering the timing, routes, and scale of animal migrations (Malcolm *et al.*, 2002; Mills, 2005; Bussière *et al.*, 2015; Kristensen *et al.*, 2015), elucidating the impact of host migration on parasite distribution has become increasingly urgent (Garamszegi, 2011; Fritzsche McKay and Hoye, 2016).

Several hypotheses have been established regarding dynamics of parasites in migratory hosts. Hosts may gain a diverse parasite community via exposure to multiple parasite assemblages during stopovers or upon arrival, i.e. "migratory exposure" (Koprivnikar and Leung, 2015; Leung and Koprivnikar, 2016). Parasites may also alter migratory host behaviours by imposing energetic costs which lead to trade-offs among functions (DeGroote and Rodewald, 2010). For example, physiological trade-offs with the immune system (Eikenaar and Hegemann, 2016), may result in less variation at the immune loci (O'Connor *et al.*, 2018), and consequently higher parasite loads in migratory animals (Altizer *et al.*, 2011). Contrastingly, migrants have also been hypothesised to have evolved varied immune loci as a response to frequent parasite exposure (Hegemann *et al.*, 2015), resulting in them having a less diverse parasite community. Alternatively, the energetic cost of migration may lead to behavioural trade-offs. Thus, the "migratory escape" hypothesis proposes that periodic migration enables hosts to avoid the accumulation of parasites (Kendzel *et al.*, 2023). The detrimental impact of infection on host fitness may also lead to loss of parasites from host populations via "migratory culling" (Bartel *et al.*, 2011; Slowinski *et al.*, 2018). Such evolutionary responses from hosts may, in turn, lead to changes in parasite phenology (Ishtiaq and Renner, 2020), transmission, or survival strategies (Poulin and de Angeli Dutra, 2021). Disentangling the effects of these non-mutually exclusive hypotheses is challenging, particularly as they may operate

in tandem with different parasite genera imposing opposing effects within the same host (Gutiérrez *et al.*, 2019).

Empirical studies that compare parasite diversity in migratory and resident birds have produced varied results. Greater parasite richness in migratory compared to resident host species have been documented for nematodes (Koprivnikar and Leung, 2015; Leung and Koprivnikar, 2016); whereas for haemosporidians, the causative agents of avian malaria, results are often contradictory. Some studies have found higher richness in migratory bird species (de Angeli Dutra *et al.*, 2021a) while others have found no detectable difference between resident and migrant species (Ricklefs *et al.*, 2017).

Quantifying parasite diversity in migratory hosts is important for understanding the potential for migration-facilitated parasite sharing. In terms of sharing at the host species community level, studies have yielded mixed results. Perrin *et al.* (2025) compared 697 bird species and found that migratory species shared many *Haemoproteus* and *Plasmodium* lineages with resident species encountered along the African-Eurasian and Americas flyways, implying that migrants may disperse and/or acquire parasites during their journeys. Another study, based on 896 bird species sampled across South America, revealed that communities with a higher proportion of migrant birds supported higher haemosporidian lineage richness (de Angeli Dutra *et al.*, 2021b). In contrast, other studies have found that various species of co-occurring migratory and resident Passeriformes harbour largely distinct haemosporidian communities at stopover and overwintering sites in South and North America (Pulgarín-R *et al.*, 2019; Soares *et al.*, 2020; Galen *et al.*, 2024). Such results may reflect differences in vector availability or competency across the migrant hosts' annual cycle. For example, Galen *et al.* (2024) examined haemosporidian parasites in 63 species of birds at a stopover site in Pennsylvania (United States) and found that migratory birds shared a core set of haemosporidians with residents. However, migratory bird species also carried several unique haemosporidian lineages associated with different biogeographic regions that were characterised by different vector communities. This suggests that vector ecology may preclude transmission for some haemosporidian parasites. Such results highlight that dispersal of parasites with complex life cycles may be constrained by the availability of secondary hosts or vectors. Therefore, it is important to assess parasites with differing life cycles when considering the impact of migration on parasite sharing.

To date, studies that attempt to quantify parasite diversity and subsequent sharing between resident and migrant hosts often rely on species-level comparisons. Such comparisons do not necessarily account for differences in host phylogeny, ecology, and exposure history, which may confound efforts to resolve the effects of migration on parasite communities (Binning *et al.*, 2022). Partial migrant systems, in which individual-level variation in migratory behaviour occurs, provide a valuable opportunity to understand parasite transmission dynamics during migration in a single host species. Although partial migration is relatively common in birds (Lundberg, 1988; Chapman *et al.*, 2011), distinguishing migrants from non-migrants without individual tracking can be challenging. However, this issue can be avoided by strategically sampling during the migratory period, when migrant and resident birds self-sort. Furthermore, systems in which migrating individuals encounter another morphologically distinct subspecies during the migratory period enable further resident and migrant comparisons to be made. Such systems offer a tractable framework for studying parasite sharing between resident and migratory populations.

The Tasmanian population of silvereye (*Zosterops lateralis lateralis*), a passerine bird of Australia and the southwest Pacific, undergoes partial migration (Chan and Kikkawa, 1997). During the winter, some individuals remain on the Tasmanian breeding grounds while others cross the Bass Strait to overwinter on the Australian mainland, where they co-occur with year-round residents of another morphologically distinct subspecies of silvereye (*Zosterops lateralis cornwalli)*. We take advantage of this system of resident and partial migrant silvereye subspecies to understand how migration shapes parasite communities. By utilising high-throughput sequencing (HTS) methods we characterise a broad range of parasite genera with varied life histories detected in host faeces and blood. This enables us to directly address whether differences in parasite richness between resident and migrant birds depend on life cycle complexity, and if some parasites are more broadly shared between host groups than others.

Materials and Methods

Sample collection

We conducted sampling from May to July 2022 when the Tasmanian population of silvereye (*Z. l. lateralis*) undergoes partial migration (Chan and Kikkawa, 1997). During May and June, samples from overwintering migratory individuals were collected in Queensland (QLD) and New South Wales (NSW) on the Australian mainland. We also collected samples from individuals of the year-round resident subspecies, *Z. l. cornwalli,* which were present in the same area as migrants. Individuals of the two subspecies were discriminated by plumage differences (Little *et al.*, 2020). Samples from individual Tasmanian residents were collected during June 2022. Figure 1A illustrates the sampling locations and partial migrant route.

Faecal samples were collected according to a protocol adapted from Knutie and Gotanda (2018). Birds were captured using a mist net and subsequently placed in a ventilated, flat-bottomed paper bag lined with two sterile weigh boats covered by wire mesh that acted as a perch (Figure S1). Individual birds were left in the bag for approximately three minutes to defecate. Faecal samples were transferred to 1.5 ml microfuge tubes containing 1 ml 100% ethanol. A blood sample was taken by pricking the brachial vein with a sterile needle and collecting the resulting blood droplet in a glass capillary tube. Capillary tubes were snapped to size then placed in 1.5 ml microfuge tubes with Queen's lysis buffer (0.01 M Tris, 0.01 M NaCl, 0.01 M EDTA and 0.03 M n-lauroylsarcosine [10% w/v], pH 7.5, modified from Seutin *et al.* [1991]). All samples were refrigerated until they could be frozen at -20°C. Birds were fitted with an individually numbered band provided by the Australian Bird and Bat Banding Scheme. A suite of morphometrics were measured with dial calipers ±0.1 mm (tarsus length as well as bill length, depth and width) and a flat rule ±1 mm (wing length and tail length). Weight was measured with a Pesola scale. Birds were then released.

DNA extractions

Faecal DNA extractions were carried out using the Qiagen DNeasy PowerSoil Pro Kit (Qiagen, Germany) according to the manufacturer's instructions. Blood extractions were conducted using a modified phenol-chloroform protocol (Sambrook and Russell, 2006). Samples were incubated

overnight at 55°C with rotation, adding approximately 100 µl of blood to 250 µl DIGSOL extraction buffer (0.02 M EDTA, 0.05 M Tris-HCl [pH 8.0], 0.4 M NaCl, 0.5% SDS) and 10 µl Proteinase K (20 mg/ mL). Digested samples were added to 250 µl phenol:chloroform:isoamyl alcohol (25:24:1) and rotated for 10 minutes followed by centrifugation at 10,000 rpm for 10 minutes. The aqueous phase was transferred to a new microfuge tube; rotation and centrifugation were repeated with the addition of 250 µl phenol:chloroform:isoamyl alcohol (25:24:1). This step was then repeated with the addition of 250 µl chloroform:isolamyl alcohol (24:1); and 2 volumes of cold 100% ethanol, 1 volume of 2.5 M ammonium acetate and 1 µl glycogen to precipitate the DNA. Samples were stored at -20°C for at least 12 hours before further centrifugation for 10 min at 15,000 rpm at 4°C, the precipitate was washed with 500 µl cold 70% ethanol. The process of centrifugation and washing was done twice more. The precipitated DNA was left to dry at room temperature before resuspension in 25 µl of TE (TrisEDTA) buffer (0.01 M Tris-HCL [pH 8.0], 0.0001 M EDTA). Each batch of 24 samples included one negative extraction control.

Sexing

Silvereyes are sexually monomorphic (Wilson and Kikkawa, 1988), therefore individuals were sexed genetically. We used two sets of markers: P2D-P8 (Dawson *et al.*, 2012) and Z002B (Dawson, 2007). PCR amplification was conducted in a total volume of 2 µl, consisting of 1 µl QIAGEN Multiplex PCR mix (5X, QIAGEN Inc., Germany) and 1 µl of primer mix, containing 5 µM of each primer, ddH_2O and ~15 ng of DNA, obtained by allowing 1 µl of resuspended DNA extract to evaporate. The PCR conditions involved an initial denaturation stage at 95°C for 15 min, followed by amplification for 35 cycles (94°C for 30 s, 48°C [P2D-P8] or 56°C [Z002B] for 90 s and 72°C for 60 s, and a final extension for 30 min at 60°C). PCR products were diluted in ddH_2O to a ratio of 1:2500-1:5000 and analysed on an ABI 3730 48-capillary DNA Analyser using formamide and GeneScan™-500 ROX size-standard (Applied Biosystems, Warrington, UK). Alleles were scored using GENEMAPPER v 5 software (Applied Biosystems, California, United States).

18S anti-metazoan metabarcoding

A set of 18S anti-metazoan primers, 3NDF (Cavalier-Smith *et al.*, 2009) and the "universal non-metazoan" reverse primer, 18s-EUK1134-R (Bower *et al.*, 2004), were used to amplify the V4

region of the 18S SSU rDNA. We chose these primers because the 18S region is highly conserved across taxa, but shows substantial variation among taxonomic groups (Van de Peer *et al.*, 1997; Wuyts *et al.*, 2000; Stoeck *et al.*, 2010). Furthermore, this region is frequently used to characterise parasites (Kounosu *et al.*, 2019), providing comparatively good representation in reference databases compared to other regions. The primers were designed to avoid amplifying metazoans as otherwise the resultant sequencing reads would likely be dominated by the host. The primers were modified to include annealing sites for indexing. The primer sequences were as follows:

Forward primer:

5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNGCAAGTCTGGTGCCAG-3'

Reverse primer:

5'-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTTAARTTTCASYCTTGCG-3'

PCR1 reactions had a final volume of 20 µl and included Qiagen 5X Multiplex PCR Master Mix (Qiagen, Germany), 2 µl of primer at 5 µM (final conc. 0.5M), 1 µl DNA (ca. 10 ng/µl) and sterile ddH2O. The thermal cycling conditions were as follows: 95°C for 15 min, 35 cycles of 95°C for 30 s, 53°C for 45 s and 72°C for 1 min with a final extension at 72°C for 10 min. Two negative controls were included for every 30 amplifications: a PCR control (using 1 µl ddH2O instead of DNA) and an extraction control (using the negative controls generated during DNA extractions). PCR reactions produced a ~540 bp product.

Library preparation and sequencing

Successfully amplified samples were cleaned with Promega ProNex Magnetic Beads using a ratio of 1.15X (v/v) beads to product, according to the manufacturer's instructions (Promega, Inc., United States). Each sample was dual indexed using a PCR reaction that included 5X Meridian Bioscience MyTaq HS Mix, Fi5 and Fi7 primers (at 10 µM), sterile ddH2O and product from PCR1 in a total volume of 20 µl. The PCR conditions were 95°C for 15 min; 8 cycles of 98°C for 10 s, 65°C for 30 s and 72°C for 30 s and a final extension at 72°C for 5 min.

We used the Agilent TapeStation (Agilent Technologies Inc., United States) to confirm the index primers had successfully ligated and subsequently conducted a second clean-up using a 1X (v/v) ratio of Promega ProNex Magnetic Beads. The concentration of cleaned product (ng/µl) was

quantified using the Promega QuantiFluor. Pools were created by combining equal ratios of amplicons from each sample which were further quantified by qPCR. A 100- 1000- and 10000-fold dilution was prepared for each pool with sample dilution buffer (10mM Tris, pH 8.0 and 0.05% Tween 20X) and SYBR FAST mix and primers. The thermal cycling conditions were 5 min at 95°C, 35 cycles of 30 s at 95°C and 45 s at 60°C. Pools were further amalgamated and quantified by qPCR until a single pool remained. A High Sensitivity D1000 ScreenTape was used to ascertain the size and quantity of the final product using an Agilent TapeStation.

The final pool was sequenced on a PacBio Sequel II at the Natural Environment Research Council Environmental Omics Facility, University of Liverpool, UK. Library loading was achieved by diffusion, with all the samples loaded onto one SMRT cell 8 M, using the Sequel II Primer V3.1.1.5, Sequel® II Binding Kit V3.1. PacBio CCS reads (mean passes = 20, median read quality = Q37) were demultiplexed and Illumina primers were removed using the cutadapt software (Martin, 2011).

Calling ASVs and taxonomic assignment

The *DADA2* package (Callahan *et al.*, 2016) was used to clean and call Amplicon Sequence Variants (ASVs) from the data. Reads with a minimum quality score of Q30 and those within the minimum (475 bp) and maximum (675 bp) lengths were retained. Following filtering, reads were dereplicated. Dereplicating reduces computation time by amalgamating unique sequences, while retaining information about their abundance in the dataset. The samples were subsequently denoised using the learned error rates to remove likely sequencing errors. Chimeras were also removed.

Taxonomic identification of metabarcoding data is often based on sequence similarity. However, sequence similarity ignores evolutionary context (Czech *et al.*, 2022) and is known to give rise to erroneous identifications (Koski and Golding, 2001), particularly when there are limited reference sequences available (Barbera *et al.*, 2018). For these reasons, we utilised phylogenetic placement to taxonomically assign ASVs. Phylogenetic placement requires a reference tree which represents sequences spanning the genetic diversity likely to be present in a sample (Czech *et al.*, 2022). We used general 18S anti-metazoan primers; therefore, we needed to include a range of eukaryotic

sequences. To do this, we utilised the 211 sequences curated by Rapp and Wolf (2024) spanning the eukaryotic tree of life, supplemented with additional parasitic sequences that we have previously identified in silvereyes (Nichols *et al.*, 2026). A full list of accession numbers used to build the tree is available in Table S1. We trimmed the reference sequences to encompass the region amplified by our primers using cutadapt (Martin, 2011) and subsequently removed any sequences that exceeded 1000 bp as these were assumed to be erroneous (expected amplicon size is 600 bp). Next, we created a multiple sequence alignment (MSA) using MAFFT (Katoh and Standley, 2013) and trimmed aligned sequences using trimAL (Capella-Gutiérrez *et al.*, 2009), removing any alignment columns where more than 50% of sequences had gaps. We used ModelTest-NG to select a substitution model (GTR + G4; Darriba *et al.*, 2020) and inferred a phylogenetic tree using RAxML-NG (referred to as a maximum likelihood [ML] tree) (Kozlov *et al.*, 2019). The alignment was reduced for identical sequences and a ML tree search was conducted with 25 parsimony and 25 random starting trees. Felsenstein bootstrap proportion (FBP) was calculated from 1000 bootstrap replicates to assess node support (Felsenstein, 1985). We did not root the tree as this is not required for phylogenetic placement (Czech *et al.*, 2022). Next, we aligned ASVs to the reference MSA using PaPaRa (Berger and Stamatakis, 2012), placed them onto the reference tree using EPA-ng (Barbera *et al.*, 2018) and finally, converted placements to taxonomic assignments with gappa (Czech *et al.*, 2020). Taxonomic assignments with a Likelihood Weight Ratio (LWR) of 0.7 were retained for further analysis.

After taxonomic assignment, we used the *microDecon* package to identify and remove potential contaminants, using the 26 sequenced negative controls generated during the PCR and extraction steps as references (McKnight *et al.*, 2019). To account for variation in sequencing depth amongst samples, we standardised read depth by rarefying each sample to a minimum sample coverage of 0.9, following the approach recommended by Chiu and Chao (2016).

We searched the available literature to compile life history information for each of the identified parasite genera (Table S2). Specifically, we collated information on previously recorded primary hosts according to the following categories: bird, other vertebrate, invertebrate or plant. We excluded parasites whose life cycle has not been elucidated in vertebrates. Similarly, we recorded information about the parasite life cycle, categorising parasites into simple (requiring one host) or complex (requiring multiple hosts).

Haemosporidian-specific multiplex PCR

Despite the availability of reference sequences in public databases, 18S metabarcoding was unable to detect haemosporidians (*Haemoproteus, Plasmodium* and *Leucocytozoon*). This could be because amplification of mixed template PCRs is biased against AT-rich sequences (Polz and Cavanaugh, 1998) and haemosporidian genomes are known to be AT-rich (Outlaw and Ricklefs, 2010). Therefore, we used a multiplex PCR assay which utilises equimolar concentrations of three sets of primers, targeting different genomic regions of each parasite, these are: PMF, PMR, HMF, HMR, LMF, and LMR (Ciloglu *et al.*, 2019). Each reaction had a final volume of 10 µl and included Qiagen 5X Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 1 µl of each primer at 10 µM (final conc. 0.5M), 1 µl DNA (ca. 10 ng/µl) and sterile ddH_2O. The thermal cycling conditions were as follows: 95°C for 15 min; followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 59°C for 90 s, and extension at 72°C for 30 s; with a final extension at 72°C for 10 min. We included one negative (ddH_2O) and one positive control for every 16 samples. For the positive control, we alternated between using DNA extracts taken from birds with a confirmed *Haemoproteus, Leucocytozoon,* and a mixed *Haemoproteus* and *Plasmodium* infection. Amplified products were resolved using electrophoresis for 1 h at 90 V on a 2% agarose gel containing GelRed™ gel stain (Biotium, Inc., Hayward, CA, United States). The product size was used to infer which genera were present in each sample (*Haemoproteus:* 533 bp, *Plasmodium:* 378 bp, *Leucocytozoon:* 218 bp). For some analyses, we used presence-absence data for the parasite genera detected per individual by our metabarcoding approach and combined it with the resulting information on haemosporidian incidence.

Statistical analysis

ASV-based analyses

All statistical analyses were conducted in R version 4.4.2 (R Core Team, 2024). To test whether migrant birds carry a greater richness of parasites than their resident conspecifics, we estimated richness for each individual using the standardised ASV data. We estimated observed richness (number of ASVs detected) and asymptotic Chao1 richness per sample type (faecal, blood and combined) using the *iNEXT* package (Hsieh *et al.*, 2016). To quantify whether the migrant population had compositionally distinct parasite communities compared to Tasmanian and mainland resident populations, we calculated Local Contribution to Beta Diversity (LCBD). LCBD can be used to quantify proportional contributions to overall beta diversity within the dataset

(Legendre and De Cáceres, 2013). To compute LCBD, we generated a Jaccard dissimilarity matrix from the standardised ASVs which we had converted to presence-absence data, and calculated LCBD using the *Adespatial* package (Dray *et al.*, 2018). Differences in parasite richness (Chao1 and observed richness) as well as community distinctiveness (LCBD) among the populations were assessed using Kruskal-Wallis tests and subsequent post hoc Dunn's tests. We used Holm-adjusted p-values to account for multiple comparisons.

Next, we tested for differences in the parasite communities sampled in mainland resident and Tasmanian migrant populations in QLD and NSW. Southern to central-eastern QLD is considered the limit for silvereye migration (Robertson, 1971; Lane, 1972; Chan, 2005). Therefore, birds sampled in QLD are more likely to have reached their overwintering sites than those sampled in NSW. Consequently, a difference in parasite community richness between the two states (QLD and NSW) could help to elucidate when parasites are gained during the migratory cycle. We calculated Chao1 and observed richness for mainland residents and Tasmanian migrants sampled in each state. Then, we fit two generalised linear models (GLM) with a negative binomial error distribution using Chao1 and observed richness as the response variables. We included the following factors as possible predictors: sex, date of sampling, population and sampling state. We included an interaction term between population and sampling state to test whether parasite richness in the Tasmanian migrants differed between the states.

Network-based analysis

We built a bipartite network using the presence-absence data derived from metabarcoding and the haemosporidian-specific PCR. The bipartite network enables us to explore whether parasite life cycle complexity influenced patterns of sharing among populations. We split the parasite genera into two categories depending on their life cycle complexity. To test whether parasite life cycles had an impact on richness of parasites infecting the three host populations, we used the networks to calculate scaled degrees per life cycle complexity (i.e. the number of links from each bird to parasite genera with simple or complex life cycles, standardised according to the number of total parasite genera in the network). We fitted a GLM using scaled degrees as the response variable, implemented with a binomial error distribution. The following factors were included as possible predictors: sex, date of sampling, population. We included an interaction term between parasite life cycle complexity and population to test whether parasites with simple life cycles more readily

infected migrants. To account for repeated measures per host individual (for simple and complex parasite genera), we report cluster-robust standard errors. Host individual could not be included as a random effect because effective variation was ~0, indicating no detectable within-individual variation.

We included sex and date of sampling in all the models. This is because previous work has shown sex-specific variation in parasite prevalence (Moore and Wilson, 2002; Isomursu *et al.*, 2006; Rodriguez *et al.*, 2021; García *et al.*, 2023) and migratory behaviour (Medeiros *et al.*, 2012; Baert *et al.*, 2018; Pedersen *et al.*, 2019). We included date of sampling to account for potential seasonal variation in parasite dynamics, as we sampled birds from May to July, during the Austral autumn and winter. Parasite prevalence (Deviche *et al.*, 2001; Cosgrove *et al.*, 2008; Tavares-Dias *et al.*, 2014), load and diversity have been observed to vary in migrant hosts over time, with late arrivals reported as having both higher (López *et al.*, 2013; Emmenegger *et al.*, 2018) and lower (Mijele *et al.*, 2016) parasite burdens in different systems.

Body condition has also been shown to vary with parasite infection status (Scheuerlein and Ricklefs, 2004; Jiménez-Peñuela *et al.*, 2019) as well as over the course of migration (Jones *et al.*, 2002) and with migratory distance (Møller, 2008). Therefore, it is of interest to test whether host body condition impacted individual parasite communities. However, we were unable to include body condition in our GLMs due to missing weight data for 29 individuals. Therefore, to address whether body condition could have impacted our results we conducted a separate GLM with binomial error distribution using scaled degrees (extracted from the bipartite network, representing proportion of total parasite genera detected per host individual) as the response variable. We included body condition, measured by extracting the residuals from an Ordinary Least Squares regression of body mass (g) by tarsus length (cm) (Brown, 1996); sex; population; and date of sampling as possible predictors.

To avoid inclusion of collinear predictor variables in the models, variance inflation factors (VIFs) were computed using the *performance* package to quantify multicollinearity (Lüdecke *et al.*, 2021). All predictor variable scores were below three, indicating acceptable levels of collinearity for

inclusion in the models (Table S3). Model fit was assessed using the *performance* package (Figure S2, S3, S4 and S5).

We performed model selection and averaging using the *MuMIn* package (Barton and Barton, 2015). All candidate models were compared based on Akaike's Information Criterion corrected for small sample sizes (AICc), and model selection tables are presented in Table S4, S5, S6 and S7. Models within ΔAICc < 2 of the top-ranked model were considered equally plausible and we generated model-averaged predictions across this top model set.

To interpret the output of our model selection and averaging approach, we consider the weighted importance (WIP) value, which reflects how frequently a predictor appears in the top-ranked models, weighted by the Akaike weights of those models. A WIP close to 1.0 indicates that the variable is consistently included in high-support models, and likely helps to improve model fit. The 95% CI indicates the range of possible values for the coefficient estimate. When the 95% CIs include zero, there is insufficient information in the data to rule out a positive or negative effect, and the biological interpretation for that predictor is therefore less clear.

We tested whether certain parasite genera tended to infect a broader range of host individuals than would be expected by chance. We used the bipartite network to calculate proportional similarity per parasite genera (i.e. specialisation within the network, measured as similarity between host use and availability). We compared the z-scores for proportional similarity to the range of z-scores generated in a fixed-fixed null model. In a fixed-fixed null model, the number of parasite infections and host individuals remains the same, but the incidence of infection is randomly allocated in the network.

Results

We identified parasites in 168 individuals comprising 53 Tasmanian residents, 56 Tasmanian-origin migrants and 59 mainland residents (Figure 1B). Eight parasite genera with life cycles characterised in vertebrate hosts were recovered (Table S2): five from metabarcoding analysis and three haemosporidian genera from targeted PCR. Three parasite genera are considered to have simple life cycles (*Eimeria* (López-Osorio *et al.*, 2020), *Isospora* (Fayer, 1980; Box, 1981; Parsa *et al.*, 2023), and *Atoxoplasma* (Levine, 1982)) and five to have complex life cycles (*Haemoproteus, Plasmodium, Leucocytozoon* (Valkiunas, 2004), *Lankesterella* (Keckeisen *et al.*, 2024), and *Hepatozoon* (Bennett *et al.*, 1992)).

Parasite richness varied among the populations (Figure 2). There were significant differences among populations when comparing ASV sequences extracted from faecal samples for Chao1 ($H_{df=2} = 6.91, p = 0.032$) and observed richness ($H_{df=2} = 12.32, p = 0.002$) as well as when combining both sample types for Chao1 ($H_{df=2} = 7.69, p = 0.021$) and observed richness ($H_{df=2} = 6.05, p = 0.049$). However, no significant difference was observed for blood parasites for Chao1 ($H_{df=2} = 0.43, p = 0.807$) or observed richness ($H_{df=2} = 1.82, p = 0.402$). There was also a significant difference in LCBD (i.e. proportional contributions to overall beta diversity) for each population for faecal ($H_{df=2} = 8.00, p = 0.018$) and the combined samples ($H_{df=2} = 5.75, p = 0.056$) but not blood ($H_{df=2} = 0.22, p = 0.895$). The Tasmanian migrant population had higher richness than the Tasmanian residents for Chao1 faecal and combined samples as well as observed richness for faecal samples. The mainland residents also had higher richness than the Tasmanian residents for faecal and combined samples according to observed richness and when samples were combined according to Chao1 (Figure 2). Table S8 summarises the pairwise comparisons generated with post-hoc Dunn's tests.

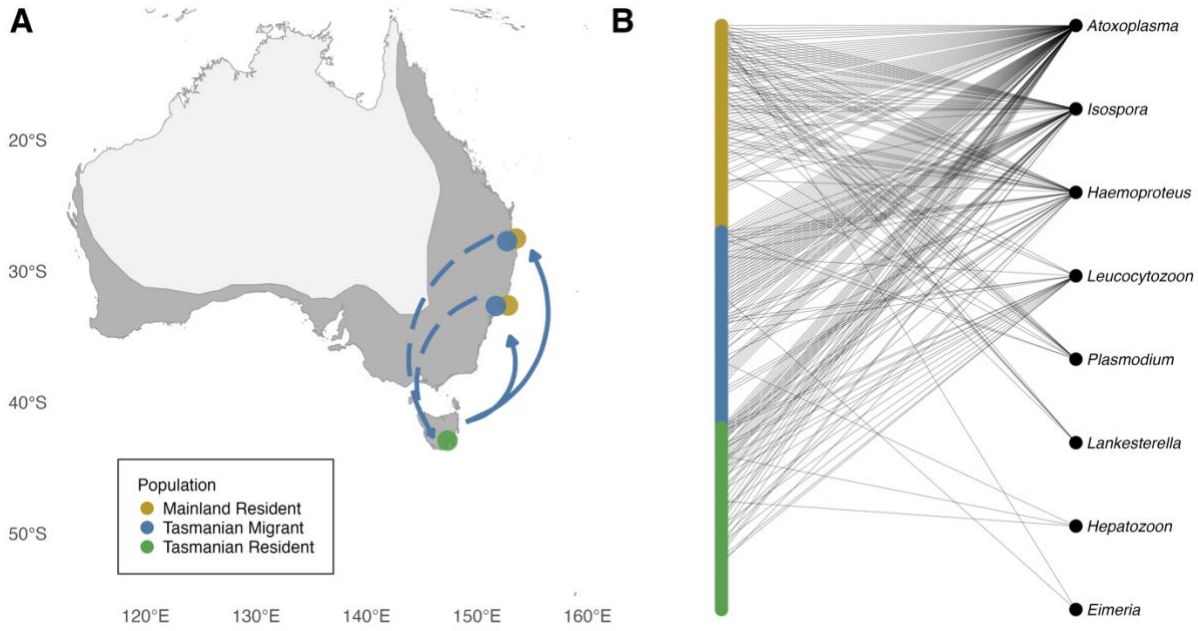

Figure 1. A) Map showing silvereye distribution in Australia (dark grey) and sampling locations (indicated by circles) for Tasmanian residents (green, n = 53), Tasmanian migrants (blue, total n = 56, comprising New South Wales [NSW] = 41 and Queensland [QLD] = 15) and mainland residents (yellow, total n = 59, comprising NSW = 43 and QLD = 16), the blue arrows indicate the north-south migrant movement, Tasmanian migrants were sampled after they arrived on the mainland (direction of travel indicated by solid blue line), before their presumed return to Tasmania (dashed line). B) Corresponding bipartite network constructed for 8 parasite genera and 168 silvereye individuals from the three silvereye populations.

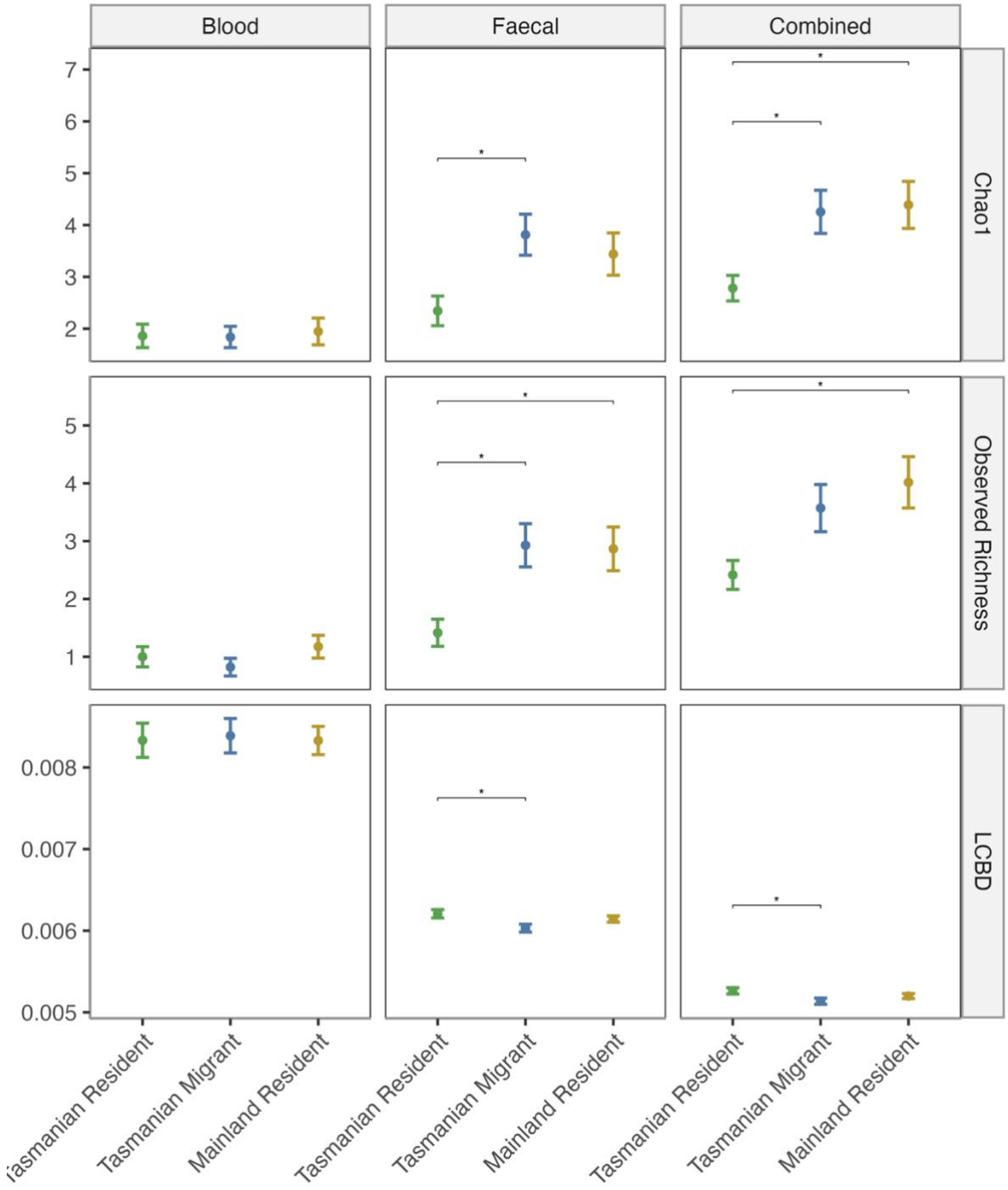

Figure 2. Mean estimated Chao1, Observed Richness and Local Contribution to Beta Diversity (LCBD), (± SE) for each population (Tasmanian residents, Tasmanian migrants and mainland residents) according to standardised ASV data extracted from blood and faecal samples as well as both types of samples combined. Significant population comparisons indicated by square brackets and asterisks, calculated according to post-hoc Dunn's test with Holm correction ($P < 0.05$).

When testing the difference in parasite richness between mainland residents and Tasmanian migrants for each state, we identified three and four models within AICc < 2 for each model utilising Chao1 and observed richness as the response variable, respectively (Table S4 and S5). Both models found that state was an important predictor of parasite richness attributing a WIP of 1 (Table 1). In both cases, richness was higher in the NSW population (Figure 3). However, neither top model set retained the interaction term between state and population, implying that there was no difference in richness detected in the Tasmanian migrants in each mainland state. Both sets of models also supported the inclusion of sex as a predictor, with a WIP of 0.20 (Chao1) and 0.37 (observed richness) although there was some uncertainty about the scale and direction of the effect.

We found four models within < 2 AICc when testing whether parasite life cycle had an impact on richness of parasites infecting the three host populations (Table S6). Life cycle complexity and population were included in all five of the top models, with both explanatory variables having a WIP of 1. Simple life cycle parasites infected more individuals than complex life cycle parasites (Figure 4A). The mainland residents carried the most parasite infections, and Tasmanian residents and migrants carried the least (Figure 4B), although there was some uncertainty in estimating this effect (Table 2). The interaction term between population and life cycle complexity was also included in three of the four top models and had a WIP of 0.82. The number of simple life cycle parasites infecting Tasmanian residents was substantially lower than migrants or mainland residents (Figure 4C). Sampling date and sex were included in one of the top models for each, and were attributed WIP scores of 0.19 and 0.18, respectively. However, the 95% CI indicated some uncertainty in ascertaining the size and direction of their effect on parasite infections. We did not find substantial evidence that body condition impacted the parasite infections detected in individual birds (Table S9).

The proportional similarity analysis indicated that three parasites (*Lankestrella, Plasmodium and Leucocytozoon)* were less evenly shared across the network than would be expected under a fixed-fixed null model (Figure 5). These three parasites have complex life cycles. *Eimeria* was more widely distributed in the network than would be expected by chance; however, there were only two cases where *Eimeria* was detected (Figure 1B).

Table 1. Model-averaged estimates for General Linear Model predicting Chao1 and observed richness for Tasmanian migrants (TM) and mainland residents (MR) sampled in New South Wales (NSW) and Queensland (QLD). Coefficient estimates (β), standard errors (SE), 95% cluster robust confidence intervals (95% CI) and weighted importance scores (WIP) are based on model averaging across the top three (Chao1) and four (richness) models, i.e. the change in small-sample corrected Akaike Information Criterion was within 2 (ΔAICc < 2). The parameters included in each model are indicated by black dots. The intercept represents the predicted richness when all predictor variables are equal to zero or their reference (baseline) for categorical variables. For each model in the top set, we also present degrees of freedom (df), Log-likelihood (logLik), AICc, the difference in AICc from the top model (delta) and the Akaike weight, which reflects the relative support for each model.

Response variable: Chao1							
Parameter	1	2	3	β	SE	95%CI	WIP
(Intercept)	•	•	•	1.715	0.125	(1.471, 1.960)	
Sampling State (QLD)	•	•	•	-0.763	0.183	(-1.122, -0.403)	1.00
Sex (Male)		•		-0.185	0.155	(-0.488, 0.118)	0.32
Migratory status (MR)			•	0.105	0.158	(-0.205, 0.416)	0.20
df	3.00	4.00	4.00				
logLik	-167.68	-166.94	-167.45				
AICc	341.71	342.49	343.49				
delta	0.00	0.78	1.79				
weight	0.48	0.32	0.20				

Response variable: Richness								
Parameter	1	2	3	4	β	SE	95%CI	
(Intercept)	•	•	•	•	5.333	0.630	(4.097, 6.568)	
Sampling State (QLD)	•	•	•	•	-3.165	0.732	(-4.599, -1.730)	1.00
Migratory status (MR)		•		•	0.765	0.711	(-0.629, 2.159)	0.38
Sex (Male)			•	•	-0.762	0.715	(-2.163, 0.639)	0.37
df	3.00	4.00	4.00	5.00				
logLik	-193.16	-192.60	-192.61	-191.89				
AICc	392.64	393.75	393.77	394.62				
delta	0.00	0.10	1.12	1.97				
weight	0.40	0.23	0.23	0.15				

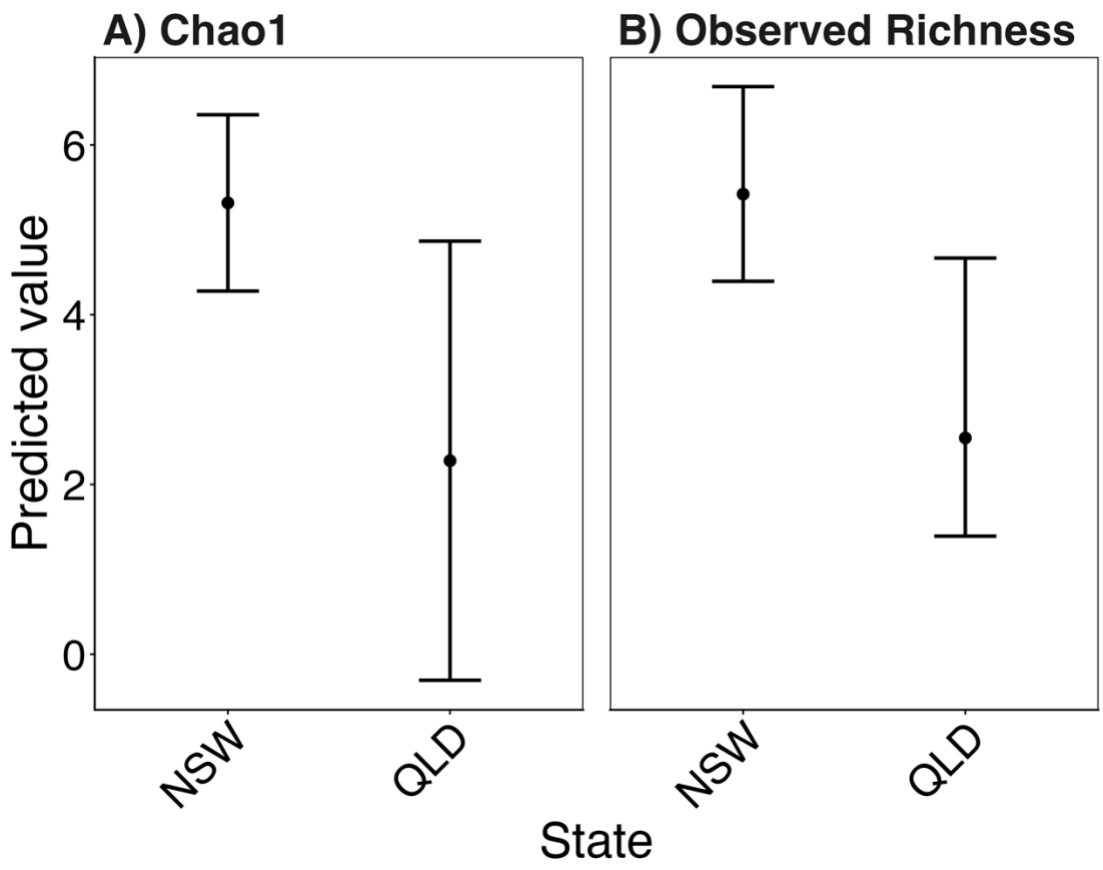

Figure 3. Marginal predicted effects extracted from GLM testing difference in A) Chao1 and B) observed richness for Tasmanian migrants and mainland residents sampled in New South Wales (NSW) and Queensland (QLD). Predictions are based on an average of the top three (Chao1) and four (richness) models within < 2 AICc.

Table 2. Model-averaged estimates for General Linear Model predicting scaled degrees (extracted from the bipartite network, representing proportion of parasite genera with simple and complex life cycles detected per host individual). Coefficient estimates (β), standard errors (SE), 95% cluster robust confidence intervals (95% CI) and weighted importance scores (WIP) are based on model averaging across the top four models, i.e. the change in small-sample corrected Akaike Information Criterion was within 2 (ΔAICc < 2). The parameters included in each model are indicated by black dots. The intercept represents the predicted scaled degrees when all predictor variables are equal to zero or their reference (baseline) for categorical variables. For each model in the top set, we also present degrees of freedom (df), Log-likelihood (logLik), AICc, the difference in AICc from the top model (delta) and the Akaike weight, which reflects the relative support for each model.

Parameter	1	2	3	4	β	SE	95% CI	WIP
(Intercept)	•	•	•	•	-2.518	0.343	(-3.190, -1.846)	NA
Life cycle complexity (Simple)	•	•	•	•	2.015	0.356	(1.317, 2.713)	1.00
Population (Mainland Resident)	•	•	•	•	0.520	0.340	(-0.146, 1.186)	1.00
Population (Tasmanian Resident)	•	•	•	•	0.140	0.396	(-0.636, 0.916)	1.00
Life cycle complexity (Simple) × Population (Mainland Resident)	•	•		•	-0.700	0.390	(-1.465, 0.064)	0.82
Life cycle complexity (Simple) × Population (Tasmanian Resident)	•	•		•	-0.977	0.423	(-1.806, -0.148)	0.82
Sampling Date		•			-0.001	0.002	(-0.006, 0.003)	0.19
Sex (Male)				•	-0.068	0.158	(-0.378, 0.242)	0.18
df	6.00	4.00	7.00	7.00				
logLik	-311.13	-313.90	-310.98	-311.05				
AICc	634.53	635.92	636.33	636.45				
delta	0.00	1.39	1.80	1.92				
weight	0.37	0.19	0.15	0.14				

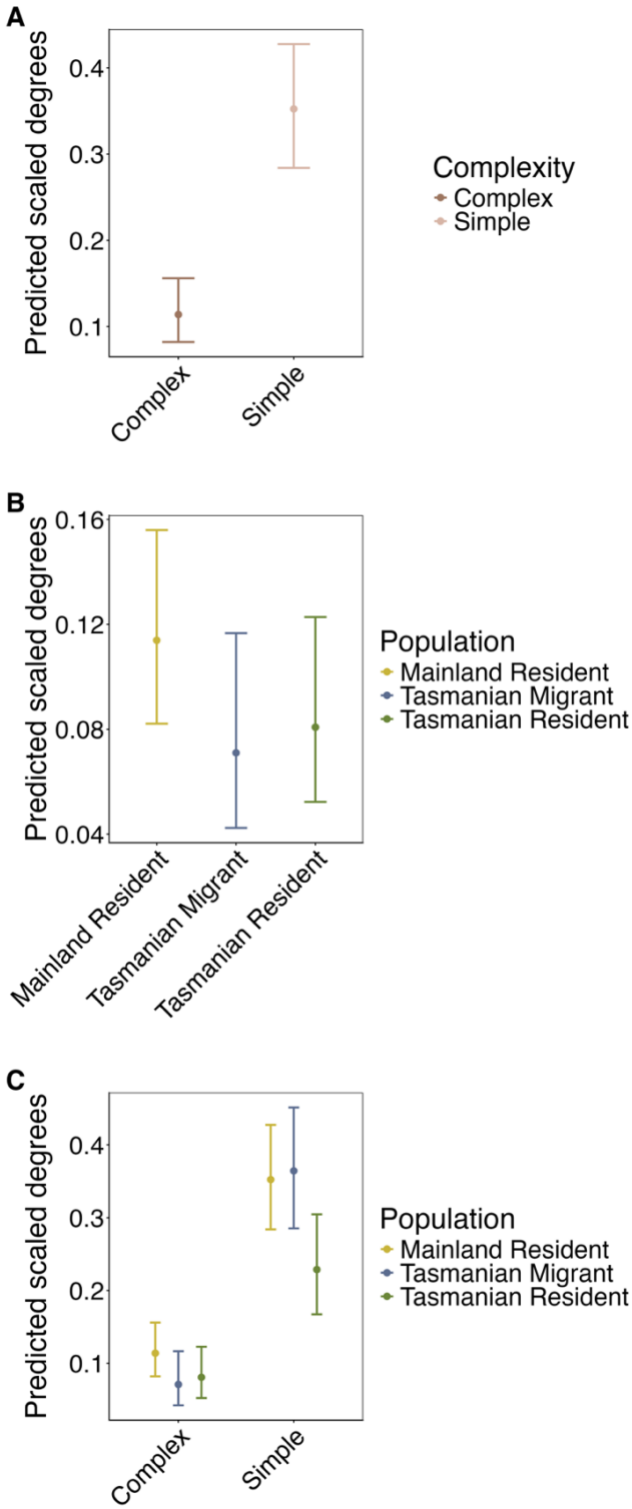

Figure 4. Marginal predicted effects of A) life cycle complexity, B) population and C) the interaction between life cycle complexity and population on scaled degrees (i.e. extracted from the bipartite network, representing proportion of total parasite genera with simple and complex life cycles detected per host individual). Predictions are based on an average of the top four models within < 2 AICc.

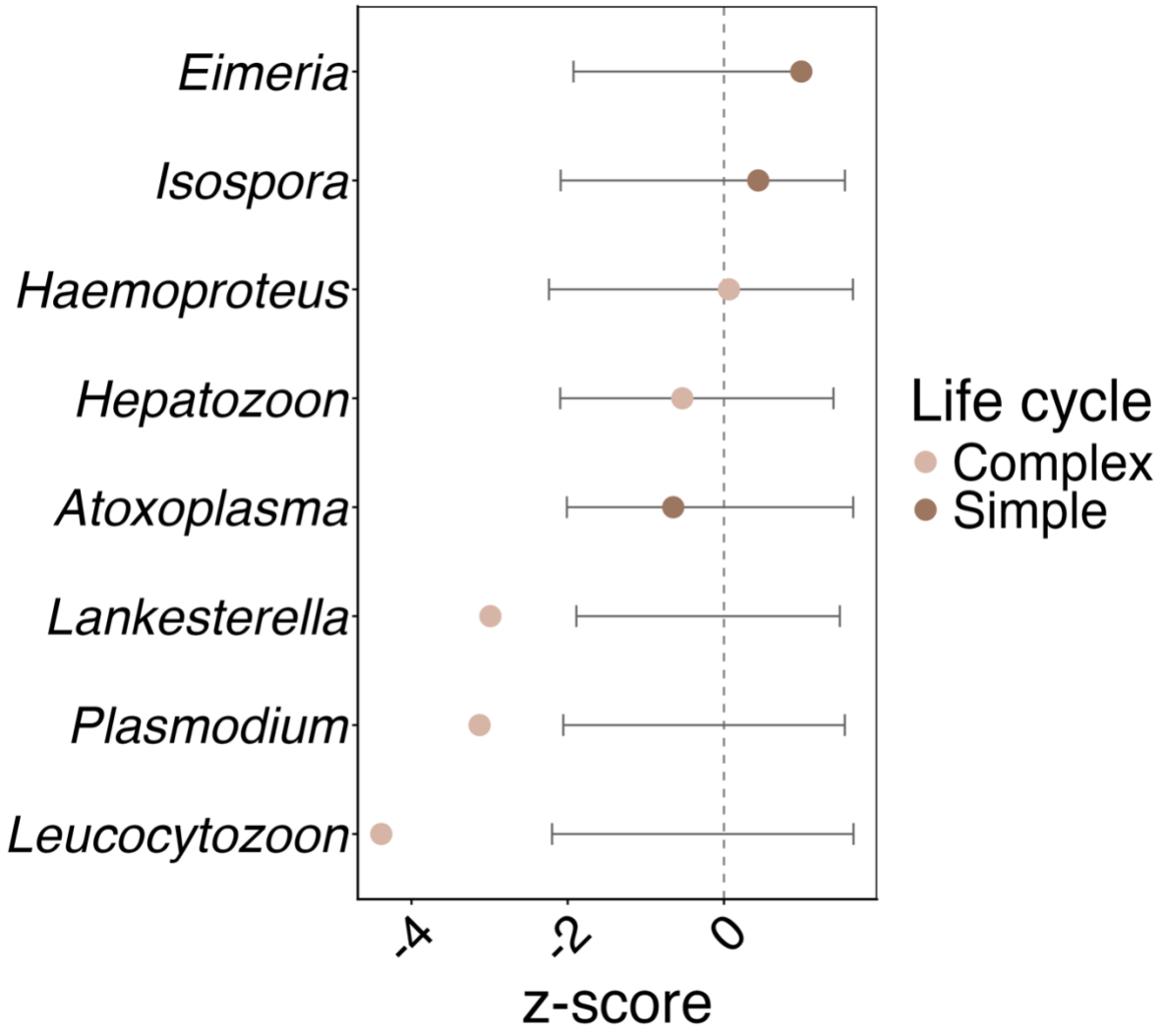

Figure 5. Proportional similarity z-scores for each parasite genera in the bipartite network mapping infections among 168 individuals and eight parasite genera compared to a fixed-fixed null model. Error bars indicate the range of z-scores generated in a fixed-fixed null model and the dashed line is the null expectation. Points show the observed proportional similarity values for each parasite genus and colours indicate parasite life cycle complexity.

Discussion

By characterising a range of parasites from blood and faecal samples in a partial migrant system, we have revealed that parasite sharing facilitated by migration is influenced by life cycle complexity. While many parasite genera are shared by all three populations, migrant individuals from Tasmania appear to acquire parasite richness during the migratory period, supporting the 'migratory exposure' hypothesis. This pattern is largely driven by parasites with simple life cycles which are more readily acquired by Tasmanian migrants than those with complex life cycles. Furthermore, several of the complex life cycle parasites that we detected appear to be less readily shared between the populations. Our results suggest that migrant birds may be gaining parasites from the residents they encounter on the mainland, with hosts sampled in the same mainland states displaying similar levels of parasite richness, despite differing in their migratory behaviour. Collectively, our findings from the partial migrant silvereye system expand our understanding of how transmission dynamics influence parasite sharing across the migratory range.

Parasite sharing amongst migrants and residents is influenced by parasite life cycles

The availability of secondary hosts or vectors may constrain dispersal of parasites with complex life cycles. We detected higher parasite richness in the faeces and blood sampled from the mainland resident and Tasmanian migrant populations compared to the Tasmanian resident population. Our network analysis confirmed that these richness patterns are driven by the increase in infections of parasites with simple life cycles (particularly *Atoxoplasma* and *Isospora*) which are notably elevated in mainland resident and Tasmanian migrant populations. This result implies that complex life cycle parasites are less readily shared between hosts during the migratory cycle, a pattern observed previously and often attributed to vector availability (Hellgren *et al.*, 2007; Pulgarín-R *et al.*, 2019; Soares *et al.*, 2020; Galen *et al.*, 2024).

In the present study, *Leucocytozoon* spp. were less frequently shared among silvereye hosts than would be expected by chance. Notably, fewer infections were detected in mainland resident than Tasmanian individuals. Previous work has used *Leucocytozoon* infections to identify immigrant birds for population monitoring (Vysotsky and Valkiūnas, 2001). This is possible because *Leucocytozoon* is transmitted by black flies (Diptera: Simuliidae) (Pramual *et al.*, 2020; Žiegytė and Bernotienė,

2022), which rely on fast flowing water for larval development (Crosskey, 1993; Adler *et al.*, 2004) and have a relatively limited dispersal distance of up to 10 km (Walsh *et al.*, 2023). As a result, local-scale variation in vector availability impacts parasite distributions. The differing habitats of sample collection sites in Tasmania and mainland Australia may thus differentially support *Leucocytozoon* transmission. Assemblages of helminth parasites have similarly been used to track host populations, as their transmission depends on the availability of intermediate hosts such as molluscs or arthropods (Sheehan *et al.*, 2016). These findings highlight that migration is more likely to facilitate the spread of simple life cycle parasites, while distributions of parasites with complex life cycles may be constrained by local ecological conditions that govern the presence and abundance of their intermediate or final hosts (Benesh *et al.*, 2022). Nonetheless, it is evident that several of the complex life cycle parasites detected in our study were broadly shared amongst the three host populations (e.g. *Haemoproteus* and *Hepatozoon*).

Differences in parasite richness became more pronounced when parasites identified in the blood and faecal samples for each host were combined, although differences among the three populations were not evident when comparing blood samples alone. The elevated richness detected in faecal samples may reflect differences in parasite life history strategies. Vector-borne parasites, which are transmitted by bites, spend part of their life cycle in host blood cells, potentially making them more readily detected in blood (Valkiūnas and Atkinson, 2020). In contrast, gut-associated parasites are more likely to rely on environmental transmission, such as excretion and ingestion of transmission stages via faecal-oral routes (Cevallos and Farthing, 1993). Such environmental transmission could better facilitate parasite dispersal during migration (Briscoe *et al.*, 2022; Teitelbaum *et al.*, 2023). Our results show that *Atoxoplasma* and *Isospora*, two parasites characterised by faecal-oral transmission, are widely shared between the populations. However, it should be noted that some parasites were detected in both the faeces and blood. This was surprising as their life cycles imply restriction to a specific body compartment. The detection of parasites in host body compartments that are not associated with the parasite life cycle could reflect limitations in the availability of reference sequences (Hleap *et al.*, 2021; Nichols *et al.*, 2026). Alternatively, it may add to the evidence that gut-associated DNA from microbes or food can be detected in the blood (Spisak *et al.*, 2013; Damgaard *et al.*, 2015; Zhai *et al.*, 2024).

Migrant birds gain parasites

Previous efforts to infer the directionality of parasite sharing resulting from migratory host movements have relied on multi-species comparisons of migratory and resident species at sites where they co-occur (Pulgarín-R *et al.*, 2019; de Angeli Dutra *et al.*, 2021b; Perrin *et al.*, 2025). Such analyses are often confounded by interspecific differences in evolutionary and ecological histories (Binning *et al.*, 2022) and limited by sampling single points in migratory cycles. These complications may underly contradictory patterns of parasite richness and sharing between migratory and resident hosts (Ricklefs *et al.*, 2017; Teitelbaum *et al.*, 2018; Perrin *et al.*, 2025). In our single host species study, Tasmanian migrants caught on the mainland harboured a substantially richer parasite community as measured by Chao1 and observed richness than Tasmanian resident silvereyes. The ability to compare Tasmanian migrants to their resident 'counterfactual' population allows us to infer that Tasmanian migrants gained parasites from mainland residents, as the latter supported richer parasite communities than Tasmanian residents. Taken together, our results provide evidence in support of the "migratory exposure" hypothesis (Altizer *et al.*, 2011) but could be further bolstered by staged sampling along the migratory route of both resident and migrating birds.

The "migratory exposure" hypothesis would further predict that parasite richness should increase with migration distance due to prolonged opportunity for exposure (Gregory, 1990; Figuerola and Green, 2000). However, we observed a higher overall parasite richness in Tasmanian migrants sampled in NSW compared to QLD, despite the shorter distance travelled by migrants. It is possible that these results are explained by "migratory culling", i.e. the death of birds that carry heavy parasite loads (Bartel *et al.*, 2011; Slowinski *et al.*, 2018), resulting in the sampled population in QLD having lower parasite richness. However, parasite richness and load for mainland resident and Tasmanian migrants were comparable in both states. This suggests that the pattern is not due to the loss of heavily infected individuals from the population. Previous work has suggested that host site use, rather than migratory distance, explained changes in helminth parasite richness in Charadriiform birds. We may similarly infer that parasite communities are determined by ecological factors operating at the local scale, causing Tasmanian migrants and mainland residents which are sampled in the same state to be more similar in their parasite richness than individuals belonging to the same population but sampled in different states.

There is a dearth of research exploring the rate at which host individuals gain and lose parasites (Rigaud *et al.*, 2010; Donaldson *et al.*, 2024). It is therefore notable that, despite Tasmanian residents and migrants belonging to the same subspecies and co-occurring throughout most of the year, parasite communities were more similar between Tasmanian migrants and mainland residents. In addition, Tasmanian residents harboured the most distinct parasite communities. This is evidenced by the similarly elevated parasite richness in Tasmanian migrants and mainland residents compared to Tasmanian residents. Our LCBD analysis also showed that Tasmanian residents were characterised by a more distinctive parasite assemblage while those of the migrant and mainland resident host populations were largely indistinguishable. These results suggest that parasites are gained and lost by migratory birds over short timescales. Parasite life histories may further influence the rate of parasite gain and loss at the host level (Donaldson *et al.*, 2024). For example, complex life cycle parasites could require prolonged periods of incubation, limiting the opportunity for transmission. Future research should consider whether transmission of parasites with complex life cycles is constrained by the length of time that migrants and residents co-occur.

Conclusions

There are many factors that may influence the likelihood that parasites are shared as a consequence of host migration. Parasite traits can play an important role in facilitating successful sharing. These include parasite specificity (Clark and Clegg, 2017; de Angeli Dutra *et al.*, 2021c), life cycle complexity (as shown here), and life cycle flexibility, such as the ability to use paratenic hosts or to prolong stages of the life cycle (Auld and Tinsley, 2015). Virulence could also reduce host survival or migratory success, thereby limiting dispersal (Shaw *et al.*, 2018; Peacock *et al.*, 2020). Furthermore, the time required to clear infections or mount an immune response could reduce the opportunity for parasite sharing among hosts (and may also prevent successful detection of the parasite; Buehler *et al.*, 2010; Hegemann *et al.*, 2018). Parasite dispersal may thus be simultaneously influenced by a suite of factors whose varying influences should be considered when interpreting differences in parasite communities across migratory systems.

Our empirical evidence supports the "migratory exposure" hypothesis, whereby migrant birds are exposed to diverse parasite faunas during migration, resulting in a greater parasite richness in migratory birds compared to their resident counterparts. It appears that these patterns are mediated by life cycle complexity as infections with simple life cycles are particularly common in

Tasmanian migrants compared to Tasmanian residents. The tendency for parasites with complex life cycles to be less readily shared throughout the network may reflect availability of their vectors or secondary hosts. Our evidence also suggests that parasite communities are determined on a local scale, with populations sampled in the same state having more similar parasite communities than those of the two Tasmanian populations. Collectively our results suggest that migration-mediated parasite sharing is constrained by the ecology and life history of the parasite. More broadly, our results emphasise the value of partial migrant systems for disentangling the ecological and evolutionary drivers of parasite transmission, and they underscore the importance of integrating host movement, parasite life history, and environmental context in future predictions of parasite spread.

Acknowledgements

We are grateful to the many people who assisted us in identifying field sites and facilitating sample collection. In particular, we would like to thank Will Goulding, Sylvia Alexander, Ian Yeo, Brendan Doohan and many members of the Queensland Bird Research and Banding Group (Queensland); Susanne Callaghan, Alan Stuart, Rob Kyte and the National Parks and Wildlife Services, Hunter Central Coast Branch (New South Wales); and Karina Sorrell, Menna Jones and Kat Stuart (Tasmania). We would also like to thank Vaidas Palinauskas for providing positive haemosporidian DNA extracts for use in the multiplex PCR. Thanks also to Lucy Knowles, Ewan Harney and the staff at the NERC Environmental Omics Facility (NEOF) for providing technical support for laboratory and bioinformatic work.

Animal Studies

Ethical approval was issued to SMC (Griffith University ENV/06/20/AEC) and CY (University of Tasmania 27197). We exported our samples from Australia under permit no. PWS2022-AU-001814 and imported samples to the UK under Authorisation No. ITIMP21.1083. We thank the National Parks and Wildlife Service for New South Wales (Scientific Licence SL102647 from Department of Planning, Industry and Environment, NSW, Australia, issued to SMC), Queensland (Research permit WA0042422 from Department of Environment and Science, Queensland, Australia issued to SMC) and Tasmania (Permit No. FA22367 from Department of Natural Resources and Environment, Tasmania, Australia and Scientific Research permit SR-2022-5283, Hobart City Council, Tasmania, Australia issued to CY) for sampling permissions. We recognise ABBBS for issuing a project licence to SMC.

Funding

The study was supported by funding from the Natural Environment Research Council (NERC) through a PhD studentship awarded to SN (NE/S007474/1) as well as an American Ornithological Society Student Research Award and The Biology Eurofins Foundation Fund Award granted to SN. Lab work was supported by a student training grant awarded to SN and BO by the NEOF (NEOF1523). The contribution by AE was facilitated by a NERC funded PhD studentship (NE/S007474/1), Heredity Fieldwork Grant (The Genetics Society), Eurofins

Foundation Fund, Hesse Research Award (American Ornithological Society), Santander Travel Award (University of Oxford).

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Chapter 6

General discussion

Understanding the processes that shape species distributions has long been a central focus in ecology and evolutionary biology. Despite their global abundance and ubiquity (Lafferty et al., 2006; Dobson et al., 2008), parasites have received substantially less attention than free-living organisms in terms of elucidating these processes. Parasite biogeography has often been interpreted using the general rules established for free-living organisms, with the implicit assumption that parasites broadly track the distributions of their host (Krasnov *et al.*, 2004; Thieltges *et al.*, 2011; Poulin, 2021). This thesis demonstrates that such assumptions oversimplify the nature and impacts of host-parasite interactions. The factors that shape parasite biogeography are complicated by asymmetry of host-parasite interactions and parasite life history traits that may influence dispersal, establishment, and persistence. Yet, most studies to date focus on single parasite systems and address a limited diversity of host-parasite interactions, overlooking these complexities. In this thesis, I utilise molecular methods to characterise diverse parasite assemblages and demonstrate that variation in life history traits plays a crucial role in shaping parasite biogeography.

Parasite life history traits mediate biogeographic patterns

Focusing on a limited range of parasite taxa risks obscuring broader insights into drivers of their biogeographic patterns. In Chapter 2, I found that studies testing whether parasites conform to the rules of island biogeography have not observed consistent island-mainland, species-area or isolation relationships as predicted by theory (MacArthur and Wilson, 1967). Instead, reported patterns varied widely among host-parasite associations (Illera *et al.*, 2015; Jean *et al.*, 2016; Williams *et al.*, 2022). These inconsistencies are striking given the relatively narrow taxonomic range of hosts and parasites represented in the existing literature. Within birds, the most extensively investigated host group identified in my systematic review, studies have overwhelmingly focused on the island biogeography of haemosporidian parasites (e.g. Loiseau *et al.*, 2017; Padilla *et al.*, 2017; Williamson *et al.*, 2019; Amaya-Mejia *et al.*, 2022). These parasites are the causative agents of avian malaria and rely on bites from flying insect vectors for transmission (Valkiunas, 2004). As a result, conclusions and generalisations about parasite island biogeography are biased toward parasites that employ a single transmission strategy. Yet, Chapters 4 and 5 illustrate that parasites with different life history traits, such as transmission strategy and life cycle complexity, respond differently to isolation and host movement, highlighting the importance of incorporating a diversity of parasite traits in future biogeographical analyses.

In my studies of parasites in the silvereye, I found that parasite biogeography was shaped by host movement, but importantly the effects were dependent on parasite life history traits and the timescale over which movement occurred. Consistent with this, patterns of parasite distributions differed in an island-colonisation context (Chapter 4), compared to those recovered from individuals undergoing seasonal migratory movements (Chapter 5). The discrepancy observed across timescales may reflect extinction dynamics, whereby broader-scale island colonisation patterns exclude parasites that colonise but go extinct, whereas parasites introduced during seasonal migration may be transient and lost before long-term persistence is achieved.

My investigations further suggest that parasite life history traits differentially impact dispersal patterns in these contrasting contexts. Under island colonisation scenarios, parasites transmitted by flying insect vectors were less constrained by isolation distance, with richness remaining

constant as distance from the mainland increased. Hosts involved in range expansion and colonisation of new habitats may not necessarily arrive with parasites (MacLeod *et al.*, 2010; Phillips *et al.*, 2010). Therefore, highly dispersive vectors may confer an advantage, enabling parasites to establish following host colonisation as they are able to overcome geographic barriers. On the other hand, the increased parasite richness resulting from seasonal migration was primarily driven by parasites with simple life cycles. Furthermore, parasites with complex life cycles, many of which were transmitted by flying insect vectors, were less readily shared by migratory and resident hosts. Recurrent and transient movements associated with seasonal migration may facilitate parasite introduction but impose different ecological constraints on establishing transmission pathways (Poulin, 2011). Consequently, over shorter timescales, local ecological conditions, such as the immediate availability of vectors, play an important role in parasite distributions (Soares *et al.*, 2020; Galen *et al.*, 2024). Accounting for the differential impacts of parasite life history traits is critical for understanding parasite community assembly and for predicting how parasite distributions may respond to ongoing changes in host ranges, migration patterns, and environmental conditions.

Parasites with differing life histories may also utilise different mechanisms for dispersal and colonisation that, in turn, may impact their distributions. Complex life cycle parasites may disperse at various stages in their life cycles. There is evidence that haemosporidian parasites are more likely to disperse via their vertebrate hosts than their invertebrate vectors (Maria *et al.*, 2009). Further, Prugnolle *et al.* (2005) found little evidence that schistosomiasis dispersed via its intermediate molluscan hosts. However, there is evidence that infected ticks traverse extensive distances with their vertebrate hosts (Zamoto-Niikura *et al.*, 2012; Scott, 2015), and this may enable ticks, the intermediate host in the life cycle, to further encounter and infect their primary hosts. Apart from these examples, the mechanisms of parasite dispersal are poorly resolved. To forecast changes in parasite distributions, it will be important to better understand mechanisms underlying colonisation. Such research may facilitate the management and control of emerging infectious diseases whereby interventions target specific stages in the parasite life cycle.

Opportunities and limitations for molecular approaches in parasite biogeography

The taxonomic biases identified in host-parasite systems represented in published studies are arguably the product of methodological constraints. In Chapter 3, I employed multiple molecular and traditional methods to characterise parasite communities, including 18S metabarcoding, targeted PCR, microscopy and the development and validation of a bioinformatic pipeline to extract parasite sequences from existing host whole genome sequencing (WGS) data. By comparing parasite detection across these approaches, this thesis demonstrates both the strengths and weaknesses of different methodologies and highlights the value of characterising multiple parasites to overcome long-standing limitations in parasite biogeography research.

Broad-spectrum molecular approaches could be particularly valuable for addressing the gaps in our understanding of parasite host range and patterns of host use. Host specificity is likely to impact patterns of parasite biogeography. Previous work suggests that specialist parasites may be less common in insular settings, as generalist parasites have an advantage during colonisation and establishment (De Bellocq *et al.*, 2002). Generalist parasites may also be more capable of host switching during the annual cycle of migration, increasing the likelihood that they will be dispersed by migratory birds (Poulin and de Angeli Dutra, 2021). There has been a sustained interest in characterising the degree of host specificity for some avian parasites (Bensch *et al.*, 2000; Schrenzel *et al.*, 2005; Križanauskienė *et al.*, 2006; Schoener *et al.*, 2013). However, there is limited information on the factors that shape host specificity in general, and levels of specialism are liable to change under variable environments (Wells and Clark, 2019). Furthermore, the tendency for certain host-parasite associations to be targeted for parasite biogeography studies, as highlighted in the systematic review (Chapter 2), limits our knowledge of patterns of host use. Thus, it is difficult to empirically assess the impact of host specificity on parasite distributions across landscapes. Future studies using molecular approaches that do not target specific parasite taxa, such as those employed in this thesis, would generate valuable baseline data on host-parasite associations. Such research may also reveal insights about generalist and specialist parasites.

Molecular approaches that simultaneously identify multiple parasites also present the opportunity to consider the possible interactions among different parasites within a single host individual. Co-infections are ubiquitous in wild animals (Petney and Andrews, 1998). Interactions among co-infecting parasites may impact host fitness, parasite transmission and ultimately parasite distributions (Cox, 2001; Pedersen and Fenton, 2007; Handel and Rohani, 2015). Despite this, most studies on the evolution and ecology of parasites continue to focus on single-parasite systems. Thus, the effects of multiple and concomitant infections are only beginning to be understood (Vaumourin *et al.*, 2015; Pike *et al.*, 2023). Empirical work has documented both facilitative and antagonistic interactions among parasites. For example, synergistic interactions between soil transmitted helminths (Fleming *et al.*, 2006) have been shown to modulate the immune system and thus facilitate concomitant infections (Corrêa-Oliveira *et al.*, 2002). There is also evidence of competition between helminth infections, resulting in decreased worm intensities during co-infection (Fleming *et al.*, 2006). Similarly, protozoan (*Eimeria*) and nematode infections have been seen to interact competitively (Knowles *et al.*, 2013). The dynamics of such interactions on patterns of parasite dispersal are yet to be explored. Such interactions could have important implications for overall patterns of biogeography.

While this thesis demonstrates that molecular approaches are necessary to capture the breadth of parasite diversity, it also highlights important limitations presented by these methods. In particular, the availability, quality and completeness of reference databases can bias taxonomic assignments and inflate rates of false negative identification (Tessler *et al.*, 2017; Leray *et al.*, 2022; Usyk *et al.*, 2023). These constraints are pronounced for parasites, which remain under-represented in public databases. Furthermore, large un-curated databases can introduce false positives via contamination or mislabelled sequences (Laurence *et al.*, 2014; Bensch *et al.*, 2021; Keck *et al.*, 2023). The discrepancies observed between parasites detected using the WGS approach and metabarcoding in this thesis likely reflect, at least in part, these database limitations. Yet, paired-end WGS data showed moderate concordance with haemosporidian infections detected using microscopy and targeted PCR, providing some confidence in the ability of this approach to detect true infections. These results highlight the importance of maintaining taxonomic expertise for validating molecular inferences, as over reliance on molecular approaches would risk the erosion of such skills for ground truthing data. Progress in the field of parasite biogeography depends on the integration of molecular approaches with traditional parasitological expertise, as well as sustained investment in the generation, curation, and long-term maintenance of high-quality reference databases. Such

efforts are foundational for advancing our understanding of parasite diversity and its biogeographic patterns yet are increasingly threatened by limitations in long-term funding (Fernandez-Prada *et al.*, 2025).

Conclusions

This thesis demonstrates that the factors shaping parasite biogeography are complex. Parasite distributions are mediated by their reliance on hosts for essential resources, but such impacts are context-dependent, as life history traits can alter parasite dispersal, establishment and persistence success. By integrating evidence across island colonisation and seasonal migration, this work shows that traits such as transmission strategy and life cycle complexity fundamentally shape parasite biogeography across spatial and temporal scales.

To date, methodological constraints have shaped our understanding of parasite biogeography. By applying and comparing multiple molecular and traditional approaches, including the repurposing of host whole genome sequencing data, this thesis illustrates how broad-spectrum parasite detection can overcome taxonomic and sampling biases. Such approaches may be used for future applications to characterise patterns of host use as well as the impact of co-infections. Overall, this thesis supports a trait-based approach for understanding parasite biogeography. Integrating parasite life histories into future analyses is essential for improving predictions of parasite and pathogen distributions under a changing climate as host ranges, migration patterns, and environmental conditions continue to be in flux.

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Supplementary Material A

Do parasites follow the rules of island biogeography?

Table S1. List of publications (DOI, year published and title) included in the systematic review.

DOI	Year	Title
10.2307/2389202	1996	Malarial parasites (*Plasmodium*) of Anolis lizards: Biogeography in the Lesser Antilles
NA	1996	The effect of some factors on the helminth parasite infracommunities of *Podarcis lizards* in the Balearic Islands (Western Mediterranean)
10.1017/S0031182001007727	2001	Parasite (*Schistosoma mansoni*) and host (*Biomphalaria glabrata*) genetic diversity: population structure in a fragmented landscape
10.1111/j.1365-2699.2006.01512.x	2006	Parasites and the island syndrome: the colonization of the western Mediterranean islands by *Heligmosomoides polygyrus* (Dujardin, 1845)
10.1111/j.1366-9516.2006.00279.x	2006	Potential spread of introduced black rat (*Rattus rattus*) parasites to endemic deer mice (*Peromyscus maniculatus*) on the California Channel Islands
10.1016/j.actatropica.2007.05.012	2007	Genetic diversity and gene flow of humans, *Plasmodium falciparum*, and *Anopheles farauti* s.s. of Vanuatu: Inferred malaria dispersal and implications for malaria control
10.2478/s11686-008-0036-5	2008	Helminth parasites in native and invasive mammal populations: comparative study on the Barbary ground squirrel *Atlantoxerus getulus* L. (Rodentia, Sciuridae) in Morocco and the Canary Islands
10.1111/j.1365-2699.2009.02180.x	2009	Biogeographical patterns of blood parasite lineage diversity in avian hosts from southern Melanesian islands
10.1163/156853809789647176	2009	Parasitic infracommunities of the Aegean wall lizard *Podarcis erhardii* (Lacertidae, Sauria): isolation and impoverishment in small island populations
10.1645/GE-1655.1	2009	Feather mites and internal parasites in small ground finches (*Geospiza fuliginosa*, Emberizidae) from the Galápagos Islands (Ecuador)
10.1111/j.1365-2656.2011.01837.x	2011	Host phylogeography and beta diversity in avian haemosporidian (Plasmodiidae) assemblages of the Lesser Antilles
10.1016/j.parint.2011.07.007	2012	Monorchiids (Platyhelminthes: Digenea) of chaetodontid fishes (Perciformes): Biogeographical patterns in the tropical Indo–West Pacific
10.1017/S0031182011002319	2012	Seasonal and biogeographical patterns of gastrointestinal parasites in large carnivores: wolves in a coastal archipelago
10.1111/geb.12105	2013	Evolution of parasite island syndromes without long-term host isolation
10.1371/journal.pone.0106752	2014	Biogeography of Human Infectious Diseases: A Global Historical Analysis
10.1016/j.jaridenv.2014.03.005	2014	Biogeography of haemo- and ectoparasites of an arid-land bird, the Trumpeter finch
10.1645/14-534.1	2014	Size, Time, and Asynchrony Matter: The Species–Area Relationship for Parasites of Freshwater Fishes
10.1111/1365-2656.12354	2015	Differences in host-species relationships and biogeographic influences produce contrasting patterns of

		prevalence, community composition and genetic structure in two genera of avian malaria parasites in southern Melanesia
10.1111/jbi.12548	2015	Unforeseen biogeographical patterns in a multiple parasite system in Macaronesia
10.1002/ece3.1452	2015	Genetic structure in insular and mainland populations of house sparrows (*Passer domesticus*) and their haemosporidian parasites
10.1186/s13071-015-0892-4	2015	Patterns of genetic variation and life history traits of *Zeuxapta seriolae* infesting *Seriola lalandi* across the coastal and oceanic areas in the southeastern Pacific Ocean
10.1111/geb.12393	2016	An equilibrium theory signature in the island biogeography of human parasites and pathogens
10.1016/j.meegid.2016.06.001	2016	Genetic diversity and population structure of *Plasmodium falciparum* over space and time in an African archipelago
NA	2017	Effects of island characteristics on parasitism in a Mediterranean lizard (*Podarcis erhardii*): a role for population size and island history?
10.1111/jbi.13060	2017	Insularity effects on the assemblage of the blood parasite community of the birds from the Gulf of Guinea
10.1016/j.ijpara.2018.11.012	2018	Ecology, not distance, explains community composition in parasites of sky-island Audubon's Warblers
10.1111/gcb.14159	2018	Human impacts decouple a fundamental ecological relationship — The positive association between host diversity and parasite diversity
10.1016/j.meegid.2017.11.028	2018	Nationwide genetic surveillance of *Plasmodium vivax* in Papua New Guinea reveals heterogeneous transmission dynamics and routes of migration amongst subdivided populations
10.1017/S0031182018002159	2019	Parasite diversity associated with African penguins (*Spheniscus demersus*) and the effect of host and environmental factors
10.3389/fevo.2018.00236	2019	Long-term habitat fragmentation is associated with reduced MHC IIB diversity and increased infections in amphibian hosts
10.1098/rspb.2019.0439	2019	Geographical and host-species barriers differentially affect generalist and specialist parasite community structure in a tropical sky-island archipelago
10.1017/S0022149X20000620	2020	Can differences between continental and insular habitats influence the parasites communities associated with the endemic frog *Haddadus binotatus*?
10.1007/s00442-021-04854-6	2021	Detecting turnover among complex communities using null models: a case study with sky-island haemosporidian parasites
10.21425/F5FBG49428	2021	Biogeographic patterns of blood parasitism in the Aegean wall lizard (*Podarcis erhardii*) across the Cycladic Islands
10.1111/1365-2656.13693	2022	Life history mediates the association between parasite abundance and geographic features
10.1007/s00436-022-07490-y	2022	Prevalence and diversity of avian haemosporidian parasites across islands of Milne Bay Province, Papua New Guinea

10.3390/d15010099	2023	Cestode Diversity of Shrews on the Kamchatka Peninsula and Paramushir Island
10.3390/ani13040723	2023	What Influences the Prevalence and Intensity of Haemoparasites and Ectoparasites in an Insular Lizard?
10.7554/eLife.90173	2023	Strong isolation by distance and evidence of population microstructure reflect ongoing *Plasmodium falciparum* transmission in Zanzibar

Supplementary Material B

Host whole genome sequence data represent an untapped resource for characterising affiliated parasite diversity

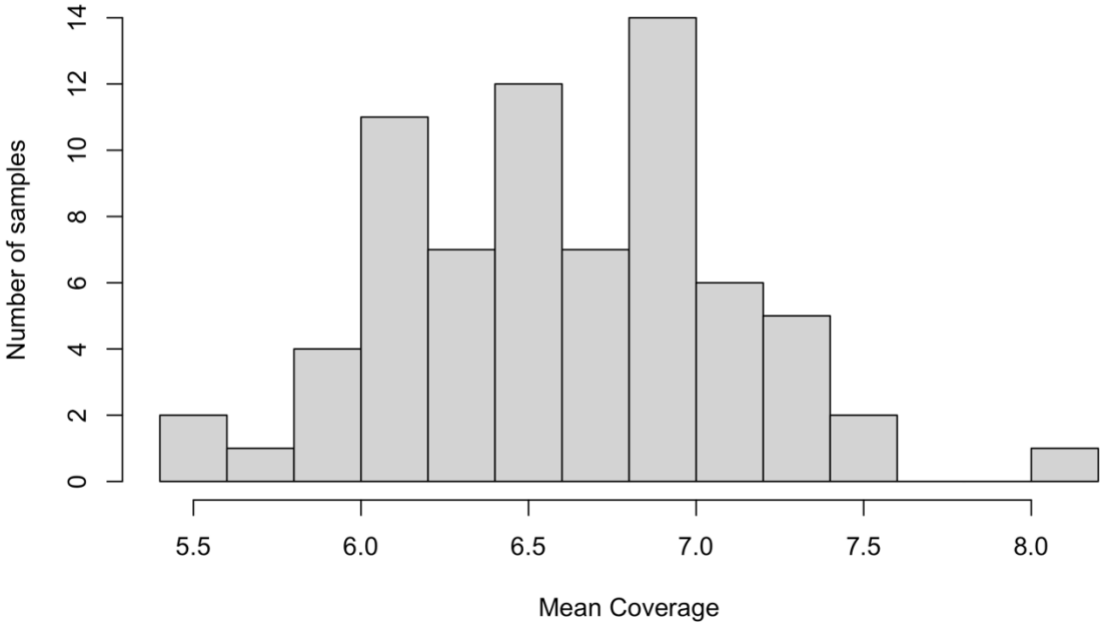

Figure S1. Distribution of mean sequence coverage per individual WGS sample generated from 68 individual silvereye.

Table S1. Number of identified reads belonging to different parasite genera extracted from WGS data generated from blood samples of 68 silvereye individuals. Paired-end reads parsed directly to Kraken 2 following host filtering and quality control, filtered at 70% confidence (WGS Paired-end 70%) and reads assembled into longer contigs prior to assignment with Kraken 2, filtered at 20% confidence (WGS Assembled 20%). For each parasite, the dominant transmission strategy and previously recorded host is listed.

Genus	WGS Assembled 20%	WGS Paired-end 70%	Transmission Strategy	Host
Ascogregarina	13	29	Direct	Invertebrate
Babesia	2	52	Tick-borne	Bird
Besnoitia	1	39	Flying Insect	Bird
Cardiosporidium	14	52	Direct	Bird
Crithidia	17	71	Direct	Invertebrate
Cryptosporidium	12	57	Direct	Bird
Cyclospora	2	61	Direct	Bird
Eimeria	34	71	Direct	Bird
Endotrypanum	26	34	Flying Insect	Other Vertebrate
Haemoproteus	43	69	Flying Insect	Bird
Hammondia	16	61	Other Host	Other Vertebrate
Herpetomonas	31	70	Direct	Invertebrate
Hexamita	5	28	Direct	Bird
Kipferlia	1	31	Unknown	Unknown
Leishmania	66	71	Flying Insect	Bird
Leptomonas	6	71	Direct	Invertebrate
Lotmaria	6	42	Direct	Invertebrate
Neospora	12	48	Other Host	Bird
Novymonas	4	54	Direct	Invertebrate
Plasmodium	51	71	Flying Insect	Bird
Platyproteum	5	71	Other Host	Invertebrate
Porcisia	2	19	Flying Insect	Other Vertebrate
Porospora	1	31	Other Host	Invertebrate
Pratylenchus	4	71	Direct	Plant
Sarcocystis	9	53	Other Host	Bird
Strigomonas	23	67	Direct	Invertebrate
Theileria	65	71	Tick-borne	Other Vertebrate
Toxoplasma	9	64	Other Host	Bird
Trichomonas	13	46	Direct	Bird
Tritrichomonas	62	68	Direct	Bird
Trypanosoma	64	71	Flying Insect	Bird
Trypanozoon	1	10	Flying Insect	Bird
Vickermania	12	55	Direct	Invertebrate

Wallacemonas	53	71	Direct	Invertebrate
Zelonia	30	71	Flying Insect	Other Vertebrate
Austramphilina	0	3	Other Host	Other Vertebrate
Blastocystis	0	1	Direct	Bird
Cephalenchus	0	2	Direct	Plant
Clonorchis	0	4	Other Host	Other Vertebrate
Cystodiscus	0	1	Other Host	Other Vertebrate
Cystoisospora	0	31	Direct	Bird
Cytauxzoon	0	1	Tick-borne	Other Vertebrate
Dibothriocephalus	0	1	Other Host	Bird
Echinobothrium	0	1	Unknown	Other Vertebrate
Echinococcus	0	2	Other Host	Other Vertebrate
Entamoeba	0	2	Direct	Bird
Giardia	0	22	Direct	Bird
Goussia	0	71	Direct	Other Vertebrate
Gregarina	0	5	Direct	Invertebrate
Haemocystidium	0	4	Flying Insect	Other Vertebrate
Hepatocystis	0	24	Flying Insect	Other Vertebrate
Jaenimonas	0	6	Direct	Invertebrate
Leucocytozoon	0	8	Flying Insect	Bird
Longidorus	0	3	Unknown	Plant
Nosema	0	1	Direct	Invertebrate
Nycteria	0	3	Flying Insect	Other Vertebrate
Olssonium	0	2	Unknown	Other Vertebrate
Parahaemoproteus	0	1	Flying Insect	Bird
Paratylenchus	0	2	Unknown	Plant
Philometra	0	1	Other Host	Other Vertebrate
Polystoma	0	1	Direct	Other Vertebrate
Proteocephalus	0	1	Other Host	Other Vertebrate
Raphidascaris	0	1	Other Host	Other Vertebrate
Rhabdias	0	1	Other Host	Other Vertebrate
Rhytidocystis	0	7	Other Host	Other Vertebrate
Schistosoma	0	1	Other Host	Bird
Selenidium	0	4	Unknown	Invertebrate
Sinuolinea	0	71	Other Host	Other Vertebrate
Spironucleus	0	6	Direct	Bird
Tetracapsuloides	0	1	Other Host	Other Vertebrate
Trichodorus	0	1	Unknown	Plant
Tylencholaimellus	0	1	Unknown	Plant
Xiphinema	0	3	Direct	Plant

Supplementary Material C

Transmission strategy modulates parasite biogeography in an island-colonising bird

Table S1. Summary of reference sequences included in the custom Kraken 2 database.

Source	Details	Sequences obtained (n)
NCBI Genomes	Downloaded via the NCBI Datasets tool, including RefSeq for:	
	Apicomplexans	372
	Cercozoans	5
	Euglenozoa	188
	Fornicata	36
	Parabasalids	10
WormBase ParaSite	Version: WBPS18	4,856,896
EuPathDB	Release 64 12 Jul 2023	281,024
PR^2 database	Release 5.0.0 with metazoan, fungal and uncultured sequences removed.	101,252
NCBI Nucleotide database	All available sequences for apicomplexans, cercozoans, euglenozoa, fornicata and parabasalids	37,153

Table S2. Parasite genera, transmission strategy and recorded host identified from 384 individual silvereye whole genome sequences.

Genus	Transmission strategy	Host	Number detected
Acanthamoeba	Direct	Birds	7
Acanthocheilonema	Flying Insect	Birds	2
Ascaris	Direct	Birds	3
Austramphilina	Other Secondary Host	Vertebrates	11
Babesia	Tick	Birds	31
Blastocystis	Direct	Birds	2
Caryospora	Direct	Birds	4
Clonorchis	Other Secondary Host	Vertebrates	2
Cryptosporidium	Direct	Birds	7
Cyclospora	Direct	Birds	114
Cystodiscus	Other Secondary Host	Vertebrates	1
Dibothriocephalus	Other Secondary Host	Birds	5
Dipetalonema	Flying Insect	Vertebrates	4
Echinococcus	Other Secondary Host	Vertebrates	1
Eimeria	Direct	Birds	37
Endotrypanum	Flying Insect	Vertebrates	1
Entamoeba	Direct	Birds	8
Euclinostomum	Other Secondary Host	Birds	6
Giardia	Direct	Birds	7
Haemocystidium	Flying Insect	Vertebrates	12
Haemoproteus	Flying Insect	Birds	44
Hammondia	Other Secondary Host	Vertebrates	116
Hepatocystis	Flying Insect	Vertebrates	3
Isospora	Other Secondary Host	Birds	8
Latyspora	Other Secondary Host	Vertebrates	1
Leishmania	Flying Insect	Birds	361
Leucocytozoon	Flying Insect	Birds	15
Neospora	Other Secondary Host	Birds	11
Nycteria	Flying Insect	Vertebrates	3
Olssonium	unknown	Vertebrates	3
Parahaemoproteus	Flying Insect	Birds	4
Philometra	Other Secondary Host	Vertebrates	9
Plasmodium	Flying Insect	Birds	145
Polymorphus	Other Secondary Host	Birds	2
Polyplicarium	Direct	Vertebrates	1
Polystoma	Direct	Vertebrates	2
Proteocephalus	Other Secondary Host	Vertebrates	6
Raphidascaris	Other Secondary Host	Vertebrates	1
Rhytidocystis	Other Secondary Host	Vertebrates	2

Sarcocystis	Other Secondary Host	Birds	12
Schistosoma	Other Secondary Host	Birds	17
Sinuolinea	Other Secondary Host	Vertebrates	247
Tetracapsuloides	Other Secondary Host	Vertebrates	2
Theileria	Tick	Vertebrates	308
Toxoplasma	Other Secondary Host	Birds	63
Trichomonas	Direct	Birds	1
Trypanosoma	Flying Insect	Birds	19
Urogonimus	Other Secondary Host	Birds	3

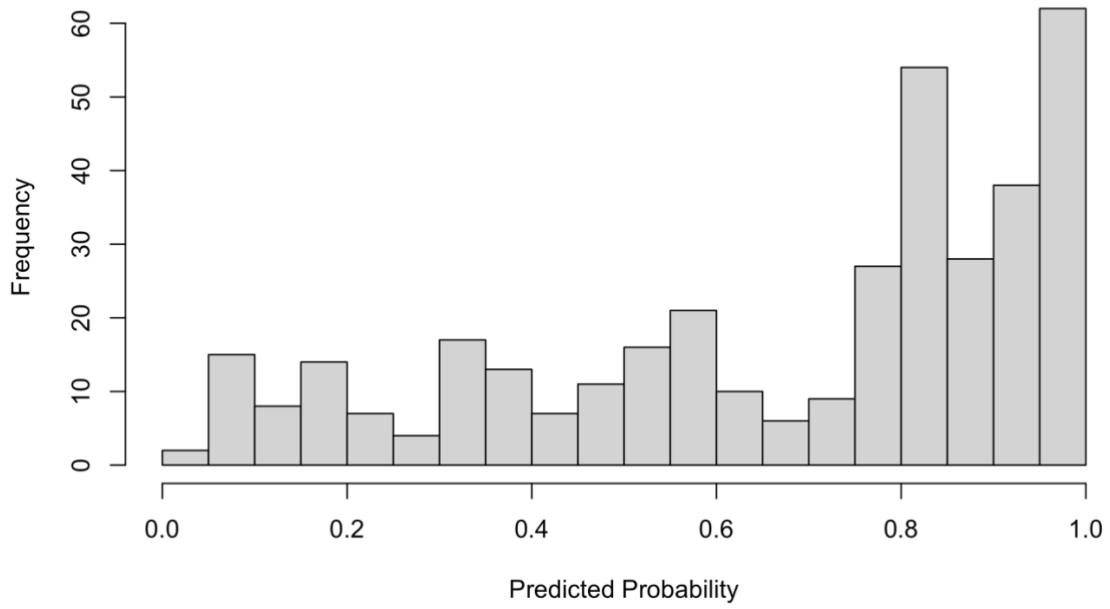

Figure S1. Distribution of predicted probabilities output from XGBoost when classifying 384 individual silvereye into origin groups (i.e. island or mainland) according to their parasite profile (number of parasite genera per transmission strategy detected per individual host).

Table S3. Pairwise variance inflation factors (VIFs) and Pearson's correlation coefficients calculated for each predictor variable included in the GLMs.

Variable	VIF (excluding Proximate Isolation)	VIF	log_{10} Island Area (km^2)	log_{10} isolation from the Australian continent (km)	Latitude	Population Size
log_{10} Island Area (km^2)	1.42	4.21	0.66	-0.09	-0.43	0.37
log_{10} Regional Island Isolation (km^2)	NA	3.2		0.24	0.13	-0.02
log_{10} Isolation from the Australian continent (km)	1.59	1.59			0.4	-0.51
Latitude	1.47	2.13				-0.33
Population Size	1.59	1.72				

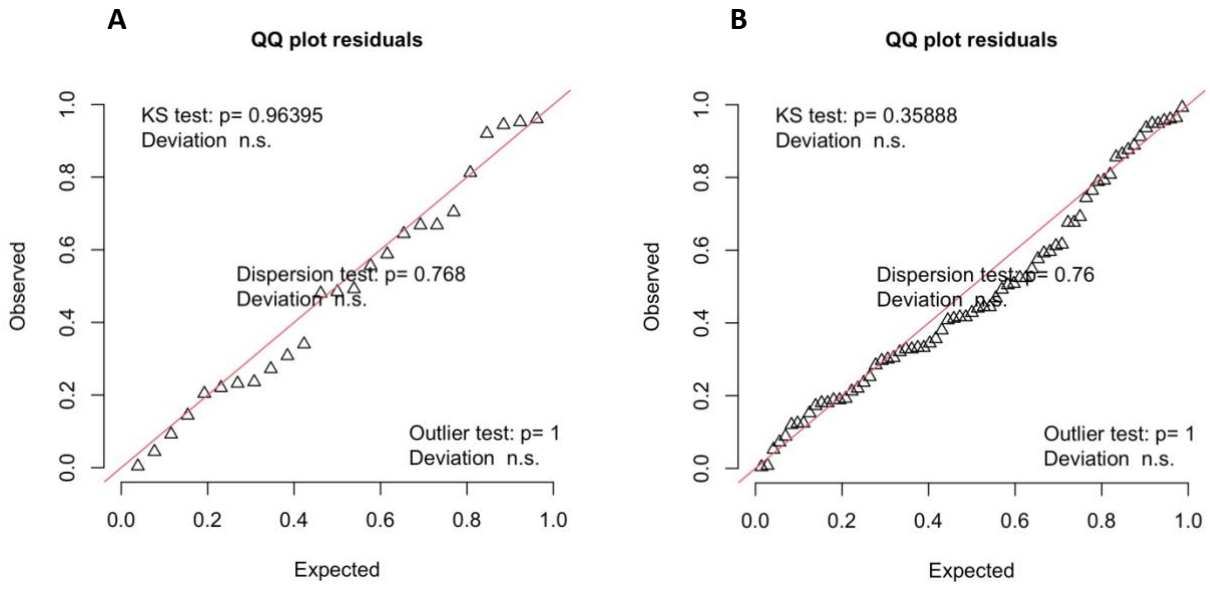

Figure S2. Residual plots for A) GLM predicting total Chao2 richness based on parasite genera detected per island population and B) GLM predicting total Chao2 richness based on parasite genera detected per island population per transmission strategy.

Table S4. Model selection results for GLM predicting total Chao2 richness based on parasite genera detected per island population according to $\log_{10}$ Island Area in km² (Area), Latitude, $\log_{10}$ isolation from the Australian continent in km (Isolation). Each row represents an individual model and includes parameter estimates (intercept and coefficients), degrees of freedom (df), log-likelihood (logLik), Akaike Information Criterion corrected for small sample size (AICc), delta AICc (ΔAICc), and Akaike weights (Weight). NA values indicate that the parameter was not included in the model.

Intercept	Area	Latitude	Isolation	df	logLik	AICc	ΔAICc	Weight
5.67	-0.01	0.01	-0.38	5	-966.88	1946.91	0.00	0.51
5.74	NA	0.02	-0.39	4	-968.50	1946.99	0.08	0.49
5.10	-0.03	NA	-0.33	4	-983.12	1976.25	29.33	0.00
5.13	NA	NA	-0.36	3	-990.69	1988.51	41.60	0.00
2.93	-0.06	NA	NA	3	-1036.45	2080.05	133.13	0.00
2.97	-0.05	0.00	NA	4	-1036.18	2082.36	135.44	0.00
2.75	NA	0.01	NA	3	-1056.55	2120.24	173.33	0.00
2.57	NA	NA	NA	2	-1060.08	2124.71	177.79	0.00
5.67	-0.01	0.01	-0.38	5	-966.88	1946.91	0.00	0.51
5.74	NA	0.02	-0.39	4	-968.50	1946.99	0.08	0.49
5.10	-0.03	NA	-0.33	4	-983.12	1976.25	29.33	0.00
5.13	NA	NA	-0.36	3	-990.69	1988.51	41.60	0.00
2.93	-0.06	NA	NA	3	-1036.45	2080.05	133.13	0.00
2.97	-0.05	0.00	NA	4	-1036.18	2082.36	135.44	0.00
2.75	NA	0.01	NA	3	-1056.55	2120.24	173.33	0.00
2.57	NA	NA	NA	2	-1060.08	2124.71	177.79	0.00

Table S5. Model selection results for GLM predicting total Chao2 richness based on parasite genera detected per island population according to $\log_{10}$ Island Area in km² (Area), Latitude, $\log_{10}$ isolation from the Australian continent in km (Isolation), Transmission strategy (TS), $\log_{10}$ Island Area (km²) x Transmission strategy (Area x TS), $\log_{10}$ isolation from the Australian continent (km) x Transmission strategy (Isolation x TS). Each row represents an individual model and includes parameter estimates (intercept and coefficients), degrees of freedom (df), log-likelihood (logLik), Akaike Information Criterion corrected for small sample size (AICc), delta AICc (ΔAICc), and Akaike weights. NA values indicate that the parameter was not included in the model.

Intercept	Area	Latitude	Isolation	TS	Area x TS	Isolation x TS	Df	Log Likelihood	AICc	ΔAICc	Weight
1.83	0.02	0.01	-0.12	+	+	+	11	-1790.03	3606.53	0.00	0.91
1.59	0.01	NA	-0.10	+	+	+	10	-1793.71	3611.10	4.57	0.09
1.64	NA	NA	-0.09	+	NA	+	7	-1815.94	3647.65	41.12	0.00
1.75	NA	0.00	-0.10	+	NA	+	8	-1815.26	3648.85	42.32	0.00
1.62	0.00	NA	-0.09	+	NA	+	8	-1815.61	3649.54	43.01	0.00
1.76	0.01	0.00	-0.11	+	NA	+	9	-1814.31	3649.56	43.03	0.00
4.07	0.02	0.00	-0.42	+	+	NA	9	-1843.36	3707.68	101.15	0.00
3.87	0.01	NA	-0.41	+	+	NA	8	-1845.99	3710.31	103.78	0.00
4.19	NA	0.00	-0.43	+	NA	NA	6	-1859.35	3732.00	125.47	0.00
4.07	NA	NA	-0.42	+	NA	NA	5	-1860.78	3732.47	125.94	0.00
4.19	0.00	0.00	-0.43	+	NA	NA	7	-1859.34	3734.46	127.93	0.00
4.07	0.00	NA	-0.42	+	NA	NA	6	-1860.60	3734.51	127.98	0.00
4.54	NA	0.01	-0.44	NA	NA	NA	4	-1964.11	3936.82	330.29	0.00
4.51	-0.01	0.00	-0.43	NA	NA	NA	5	-1963.71	3938.34	331.81	0.00
4.31	-0.01	NA	-0.41	NA	NA	NA	4	-1966.19	3940.98	334.45	0.00
4.32	NA	NA	-0.43	NA	NA	NA	3	-1968.00	3942.35	335.82	0.00
0.75	0.00	-0.01	NA	+	+	NA	8	-1978.53	3975.37	368.84	0.00
0.92	0.01	NA	NA	+	+	NA	7	-1988.03	3991.84	385.31	0.00
1.05	-0.05	-0.01	NA	+	NA	NA	6	-1998.73	4010.78	404.25	0.00
1.21	-0.03	NA	NA	+	NA	NA	5	-2011.20	4033.33	426.80	0.00
0.85	NA	-0.01	NA	+	NA	NA	5	-2025.76	4062.44	455.91	0.00
0.97	NA	NA	NA	+	NA	NA	4	-2028.68	4065.98	459.45	0.00
1.42	-0.05	-0.01	NA	NA	NA	NA	4	-2082.77	4174.14	567.61	0.00
1.59	-0.04	NA	NA	NA	NA	NA	3	-2091.97	4190.30	583.77	0.00
1.21	NA	0.00	NA	NA	NA	NA	3	-2116.07	4238.50	631.97	0.00
1.30	NA	NA	NA	NA	NA	NA	2	-2117.23	4238.64	632.11	0.00
1.83	0.02	0.01	-0.12	+	+	+	11	-1790.03	3606.53	0.00	0.91
1.59	0.01	NA	-0.10	+	+	+	10	-1793.71	3611.10	4.57	0.09
1.64	NA	NA	-0.09	+	NA	+	7	-1815.94	3647.65	41.12	0.00
1.75	NA	0.00	-0.10	+	NA	+	8	-1815.26	3648.85	42.32	0.00
1.62	0.00	NA	-0.09	+	NA	+	8	-1815.61	3649.54	43.01	0.00

1.76	0.01	0.00	-0.11	+	NA	+	9	-1814.31	3649.56	43.03	0.00
4.07	0.02	0.00	-0.42	+	+	NA	9	-1843.36	3707.68	101.15	0.00
3.87	0.01	NA	-0.41	+	+	NA	8	-1845.99	3710.31	103.78	0.00
4.19	NA	0.00	-0.43	+	NA	NA	6	-1859.35	3732.00	125.47	0.00
4.07	NA	NA	-0.42	+	NA	NA	5	-1860.78	3732.47	125.94	0.00
4.19	0.00	0.00	-0.43	+	NA	NA	7	-1859.34	3734.46	127.93	0.00
4.07	0.00	NA	-0.42	+	NA	NA	6	-1860.60	3734.51	127.98	0.00
4.54	NA	0.01	-0.44	NA	NA	NA	4	-1964.11	3936.82	330.29	0.00
4.51	-0.01	0.00	-0.43	NA	NA	NA	5	-1963.71	3938.34	331.81	0.00
4.31	-0.01	NA	-0.41	NA	NA	NA	4	-1966.19	3940.98	334.45	0.00
4.32	NA	NA	-0.43	NA	NA	NA	3	-1968.00	3942.35	335.82	0.00
0.75	0.00	-0.01	NA	+	+	NA	8	-1978.53	3975.37	368.84	0.00
0.92	0.01	NA	NA	+	+	NA	7	-1988.03	3991.84	385.31	0.00
1.05	-0.05	-0.01	NA	+	NA	NA	6	-1998.73	4010.78	404.25	0.00
1.21	-0.03	NA	NA	+	NA	NA	5	-2011.20	4033.33	426.80	0.00
0.85	NA	-0.01	NA	+	NA	NA	5	-2025.76	4062.44	455.91	0.00
0.97	NA	NA	NA	+	NA	NA	4	-2028.68	4065.98	459.45	0.00
1.42	-0.05	-0.01	NA	NA	NA	NA	4	-2082.77	4174.14	567.61	0.00
1.59	-0.04	NA	NA	NA	NA	NA	3	-2091.97	4190.30	583.77	0.00
1.21	NA	0.00	NA	NA	NA	NA	3	-2116.07	4238.50	631.97	0.00
1.30	NA	NA	NA	NA	NA	NA	2	-2117.23	4238.64	632.11	0.00
1.83	0.02	0.01	-0.12	+	+	+	11	-1790.03	3606.53	0.00	0.91
1.59	0.01	NA	-0.10	+	+	+	10	-1793.71	3611.10	4.57	0.09
1.64	NA	NA	-0.09	+	NA	+	7	-1815.94	3647.65	41.12	0.00
1.75	NA	0.00	-0.10	+	NA	+	8	-1815.26	3648.85	42.32	0.00
1.62	0.00	NA	-0.09	+	NA	+	8	-1815.61	3649.54	43.01	0.00
1.76	0.01	0.00	-0.11	+	NA	+	9	-1814.31	3649.56	43.03	0.00
4.07	0.02	0.00	-0.42	+	+	NA	9	-1843.36	3707.68	101.15	0.00
3.87	0.01	NA	-0.41	+	+	NA	8	-1845.99	3710.31	103.78	0.00
4.19	NA	0.00	-0.43	+	NA	NA	6	-1859.35	3732.00	125.47	0.00
4.07	NA	NA	-0.42	+	NA	NA	5	-1860.78	3732.47	125.94	0.00
4.19	0.00	0.00	-0.43	+	NA	NA	7	-1859.34	3734.46	127.93	0.00
4.07	0.00	NA	-0.42	+	NA	NA	6	-1860.60	3734.51	127.98	0.00
4.54	NA	0.01	-0.44	NA	NA	NA	4	-1964.11	3936.82	330.29	0.00
4.51	-0.01	0.00	-0.43	NA	NA	NA	5	-1963.71	3938.34	331.81	0.00
4.31	-0.01	NA	-0.41	NA	NA	NA	4	-1966.19	3940.98	334.45	0.00
4.32	NA	NA	-0.43	NA	NA	NA	3	-1968.00	3942.35	335.82	0.00
0.75	0.00	-0.01	NA	+	+	NA	8	-1978.53	3975.37	368.84	0.00
0.92	0.01	NA	NA	+	+	NA	7	-1988.03	3991.84	385.31	0.00

Supplementary Material D

Life cycle complexity shapes parasite sharing amongst migratory and resident hosts

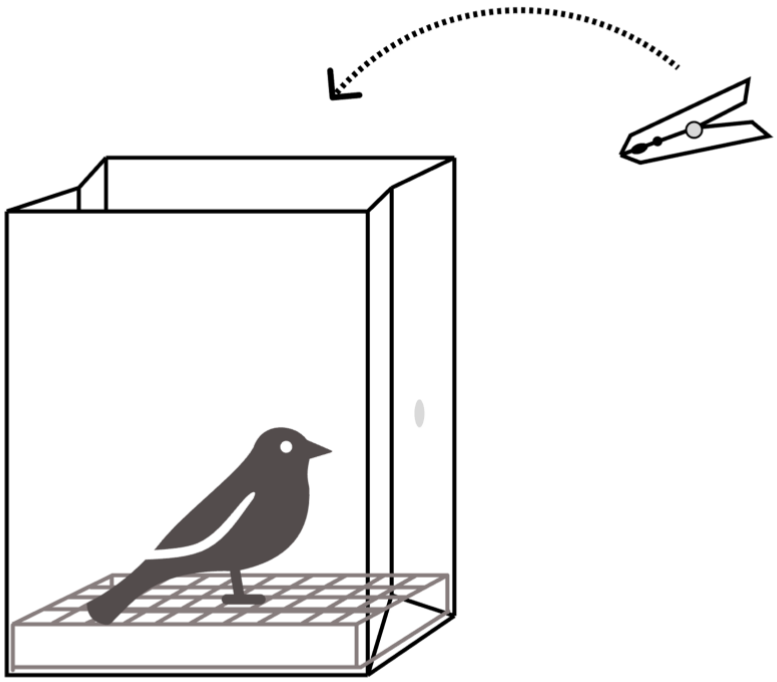

Figure S1. Illustration of faecal sampling protocol: flat-bottomed paper bag lined with sterile weigh boats covered with wire mesh, ventilated by puncturing the sides of the paper bag. Pegs were used to secure the top of the bag.

Table S1. List of accession numbers used to build the maximum-likelihood phylogenetic tree inferred using RAxML-NG. These accession numbers were adapted from Rapp and Wolf (2024).

Accession	Genus
AF029763.1	*Balantidium*
AF043355.1	*Bangia*
AF043364.2	*Pseudobangia*
L26189.1	*Erythrotrichia*
AF139462.1	*Rhodochaete*
U25637.1	*Ichthyophonus*
L29455.1	*Choanoflagellate*
AF394528.1	*Microsporidium*
U33180.1	*Psorospermium*
D63696.1	*Aspergillus*
X04971.1	*Neurospora*
X54864.1	*Pododpora*
U07367.1	*Plasmodium*
X12708.1	*Pneumocystis*
X15590.1	*Fragaria*
GU476424.1	*Eucalyptus*
X74753.1	*Genicularina*
KM020091.1	*Uronema*
X77692.1	*Euglypha*
U09048.1	*Alexandrium*
X59604.1	*Babesia*
EF551335.1	*Babesia*
KT361081.1	*Cytauxzoon*
MT904044.1	*Cytauxzoon*
MT904037.1	*Cytauxzoon*
XR_009718807.1	*Theileria*
XR_696411.1	*Theileria*
FJ717705.1	*Babesia*
DQ200887.1	*Babesia*
MH615006.1	*Hepatozoon*
MG136687.1	*Hepatozoon*
KF939628.1	*Hepatozoon*
KX507249.1	*Haemogregarina*
KX507246.1	*Haemogregarina*
U16159.1	*Neospora*
EF472967.1	*Toxoplasma*
XR_001974082.1	*Toxoplasma*
L37415.1	*Toxoplasma*
XR_003828656.1	*Besnoitia*

AY665399.2	*Besnoitia*
GU479632.1	*Besnoitia*
KU341121.1	*Sarcocystis*
MH590230.1	*Sarcocystis*
KU341123.1	*Sarcocystis*
KU180248.1	*Lankesterella*
MF167545.1	*Lankesterella*
AY331569.1	*Atoxoplasma*
KT224380.1	*Isospora*
KY801685.1	*Isospora*
AF080614.1	*Eimeria*
DQ136185.1	*Eimeria*
EF210323.1	*Eimeria*
AF080611.1	*Lankesterella*
L31922.1	*Babesia*
AF129883.1	*Ophriocystis*
AJ535150.1	*Achnanthes*
AJ535158.1	*Amphora*
AF374482.2	*Thalassiosira*
AJ535152.1	*Pseudogomphonema*
AJ535155.1	*Sellaphora*
X77703.1	*Rhaphoneis*
AJ535140.1	*Thalassionema*
X74752.1	*Staurastrum*
AY216906.1	*Grammatophora*
Y10569.1	*Lithodesmium*
X85396.1	*Thalassiosira*
X85395.1	*Skeletonema*
AF374481.2	*Thalassiosira*
AF525667.1	*Lampriscus*
AJ535188.1	*Pleurosira*
AF123595.1	*Bolidomonas*
X85404.2	*Aulacoseira*
AJ535186.1	*Aulacoseira*
AJ535175.1	*Leptocylindrus*
L27634.1	*Labyrinthuloides*
X85399.2	*Lauderia*
M54939.1	*Lagenidium*
X85402.2	*Melosira*
AF164133.1	*Gastrostyla*
AF164128.1	*Pleurotricha*
AF164127.1	*Paraurostyla*
AF164135.1	*Cyrtohymena*

X53486.1	*Oxytricha*
AF164122.1	*Oxytricha*
U23936.1	*Allomyces*
Z14142.1	*Palmaria*
AF132294.1	*Balbiania*
AF026040.1	*Audouinella*
AF342743.1	*Nemalionopsis*
L26190.1	*Gelidium*
U33133.1	*Halymenia*
U09621.1	*Rhodymenia*
U09618.1	*Plocamiocolax*
M33639.1	*Gracilariopsis*
L26183.1	*Ceramium*
Z14139.1	*Ahnfeltia*
AF006089.1	*Rhodogorgon*
Z36893.1	*Crossodonthina*
K03432.1	*Homo*
NR_046237.3	*Rattus*
XR_010350767.1	*Passer*
L23799.1	*Nucleospora*
KJ736741.1	*Gregarina*
LR814087.1	*Gregarina*
AY117418.1	*Jakoba*
AY117417.1	*Reclinomonas*
KU664396.1	*Gregarina*
M19172.1	*Plasmodium*
MK650492.1	*Plasmodium*
MK650552.1	*Plasmodium*
U07367.1	*Plasmodium*
MK650858.1	*Leucocytozoon*

Table S2. Parasites identified in 168 silvereye individuals comprising 53 Tasmanian residents, 56 Tasmanian migrants and 59 mainland residents with information about their previously recorded hosts and life cycle complexity.

Genus	Host	Life cycle	Source
Eimeria	Vertebrates	Simple	López-Osorio *et al.* (2020)
Isospora	Vertebrates	Simple	Fayer (1980); Box (1981); Parsa *et al.* (2023)
Atoxoplasma	Vertebrates	Simple	Levine (1982)
Haemoproteus	Vertebrates	Complex	Valkiunas (2004)
Plasmodium	Vertebrates	Complex	Valkiunas (2004)
Leucocytozoon	Vertebrates	Complex	Valkiunas (2004)
Lankesterella	Vertebrates	Complex	Keckeisen *et al.* (2024)
Hepatozoon	Vertebrates	Complex	Bennett *et al.* (1992)

Table S3. Pairwise variance inflation factors (VIFs) calculated for each predictor variable considered for inclusion in the GLMs.

Term	VIF
Model: GLM testing sampling state impact on parasite community of residents and Tasmanian migrants	
Population	1.06
Sex	1.06
Sampling Date	4.28
State	4.16
Model: GLM testing life cycle complexity impact on parasite sharing	
Population	1.06
Life Cycle Complexity	1.00
Sex	1.00
Sampling Date	1.06
Model: GLM testing body condition impact on parasite community	
Body condition	1.35
Population	1.47
Sex	1.04
Sampling Date	1.13

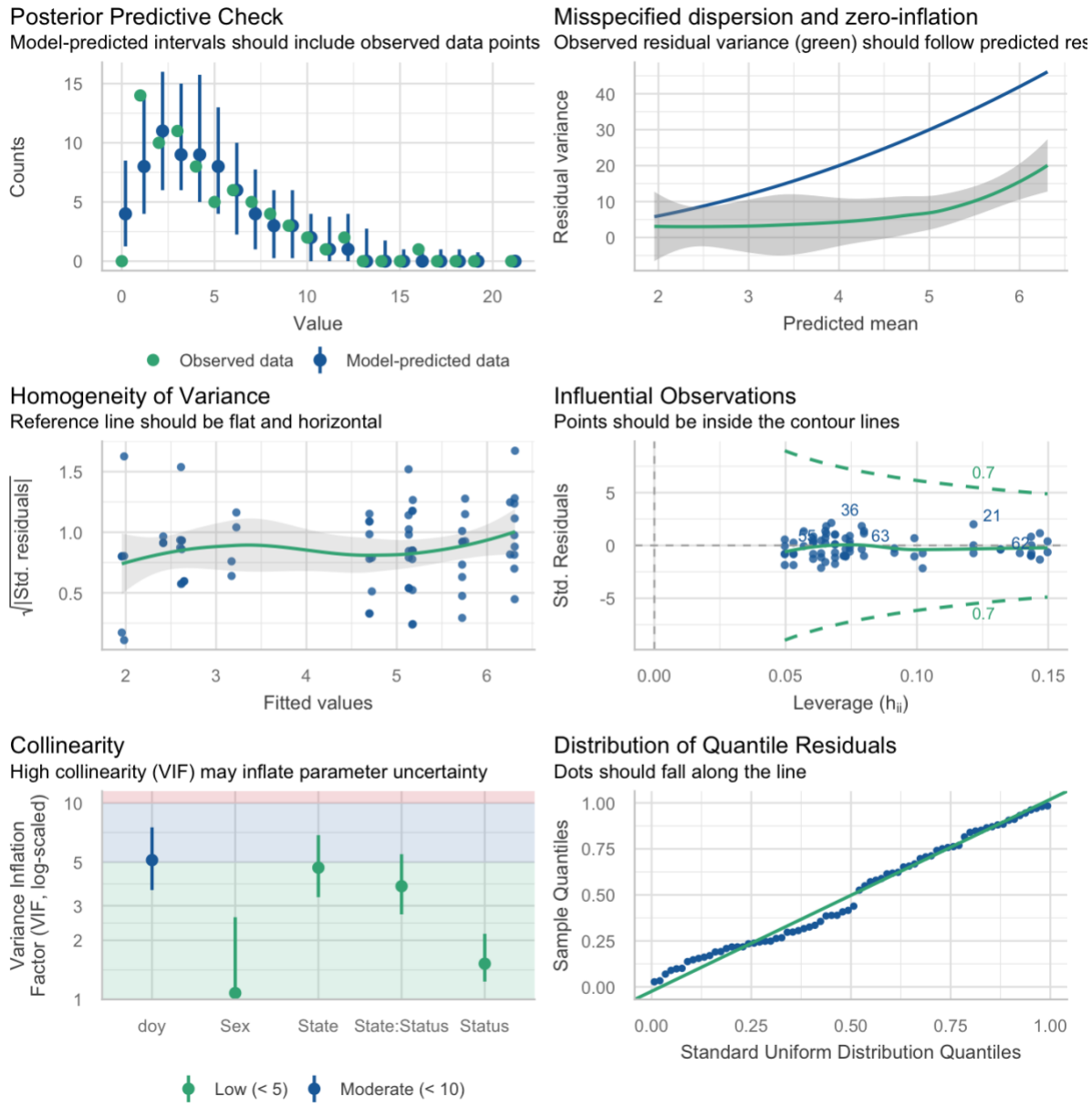

Figure S2. Model fit diagnostic panel produced using the *performance* package (Lüdecke *et al.*, 2021) for GLM testing Chao1 richness according to population, state, sex and date of sampling in Tasmanian migrants and mainland residents

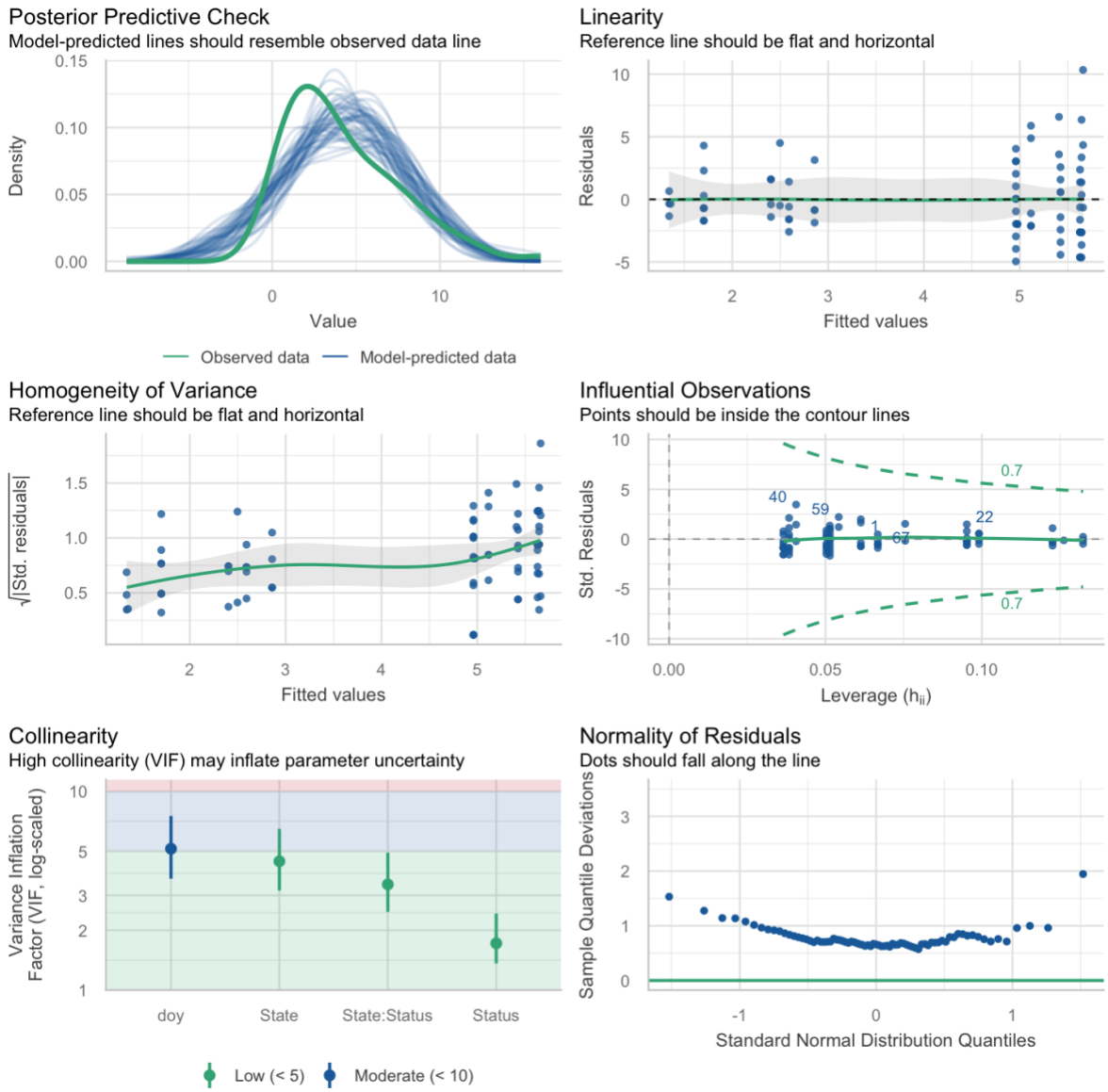

Figure S3. Model fit diagnostic panel produced using the *performance* package (Lüdecke *et al.*, 2021) for GLM testing observed richness according to population, state, sex and date of sampling in Tasmanian migrants and mainland residents.

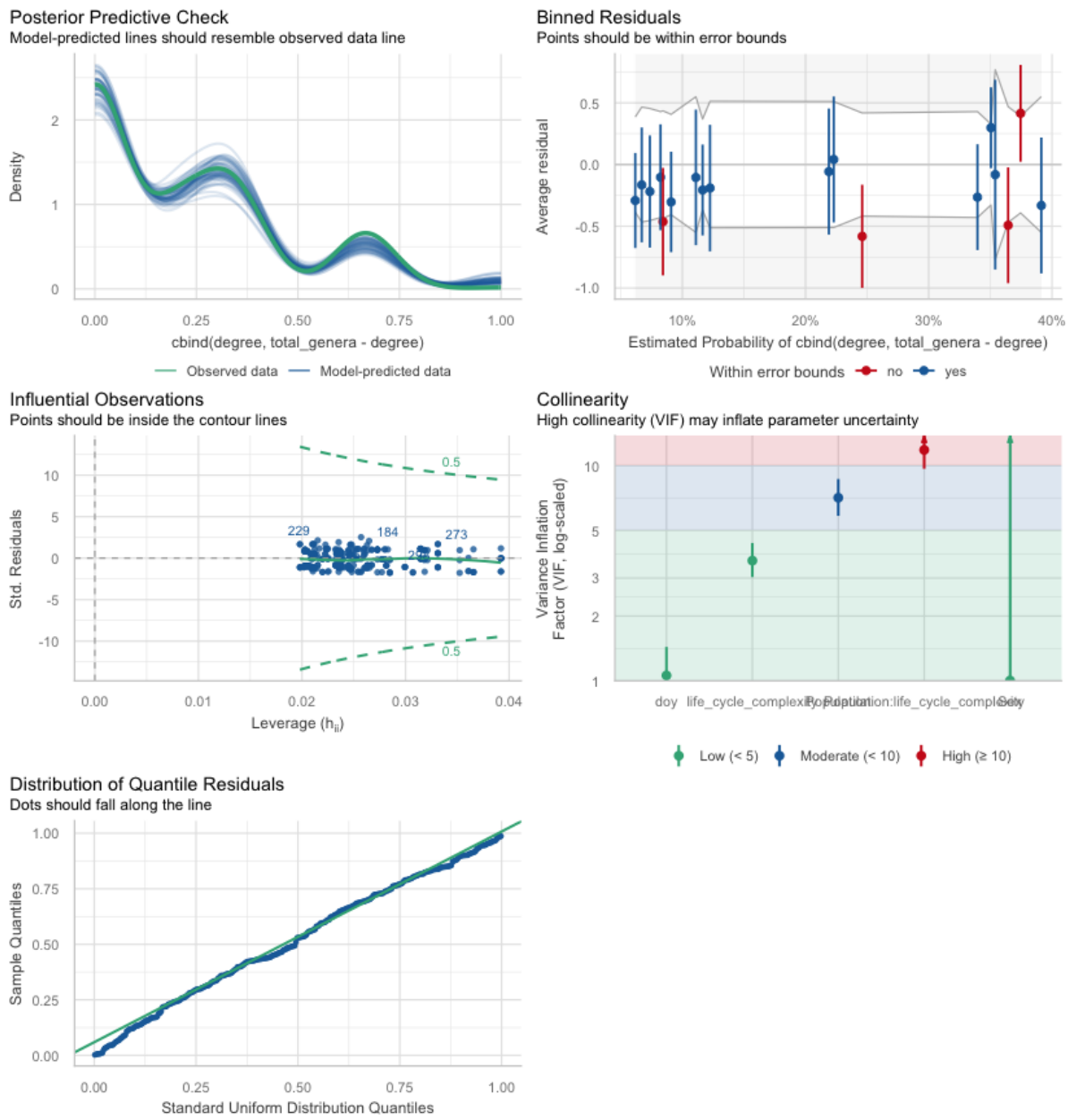

Figure S4. Model fit diagnostic panel produced using the *performance* package (Lüdecke *et al.*, 2021) for GLM predicting scaled degrees extracted from a bipartite network (i.e. the number of links to each parasite genera with simple and complex life cycles per host individual, standardised for total parasite genera).

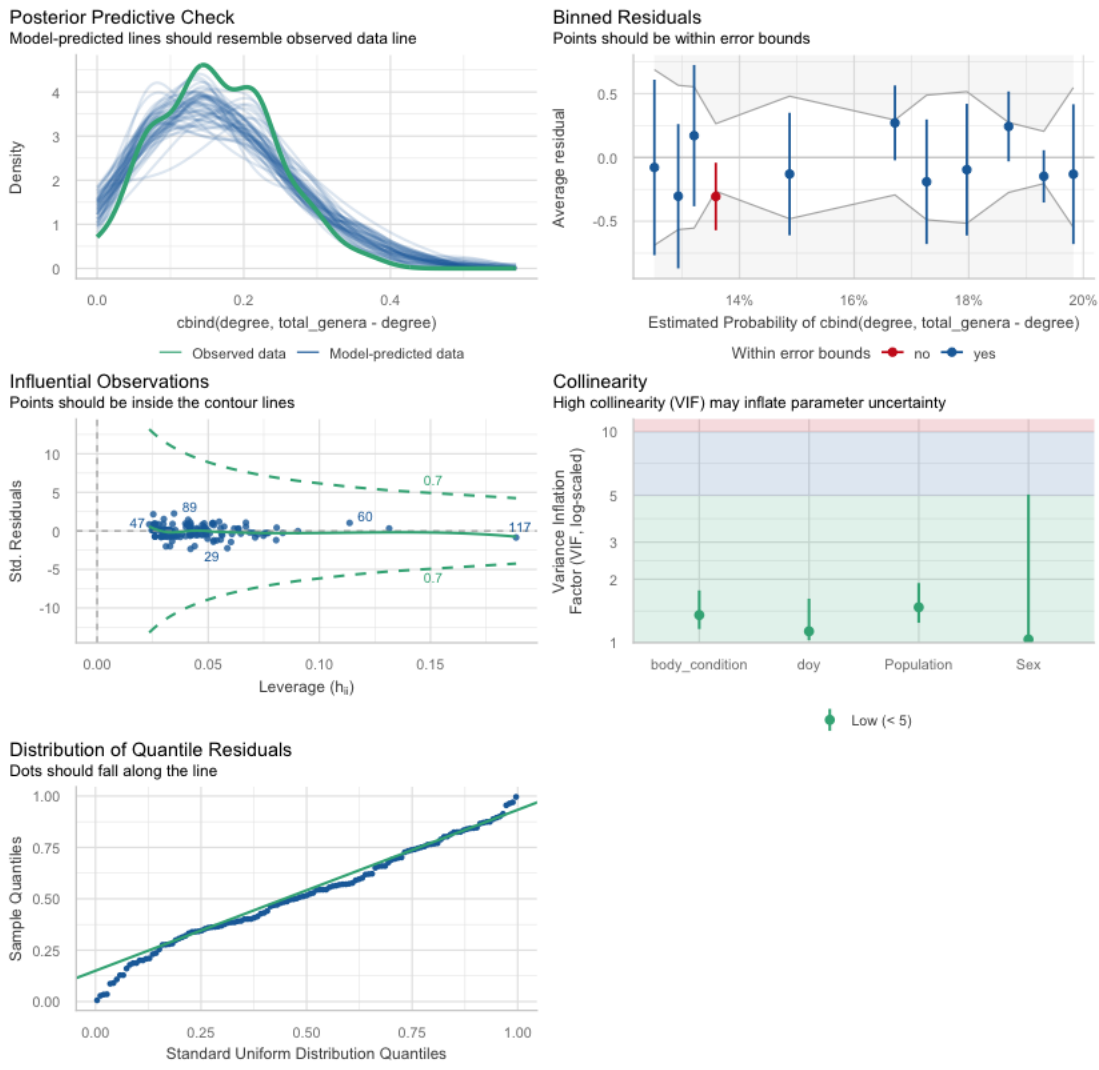

Figure S5. Model fit diagnostic panel produced using the *performance* package (Lüdecke *et al.*, 2021) for GLM with a binomial error distribution predicting scaled degrees (extracted from a bipartite network, representing parasite infections standardised according to total parasites detected) according to body condition, population, sex and date of sampling.

Table S4. Model selection results for GLM predicting Chao1 richness per hosts for mainland residents and Tasmanian migrants sampled in New South Wales and Queensland. Each row represents an individual model and includes parameter estimates (intercept and coefficients), degrees of freedom (df), log-likelihood (logLik), Akaike Information Criterion corrected for small sample size (AICc), delta AICc (ΔAICc), and Akaike weights. NA values indicate that the parameter was not included in the model.

	(Intercept)	Date of Sampling	Sex	State	Status	State x Status	df	logLik	AICc	delta	weight
5	1.70	NA	NA	+	NA	NA	3	-167.68	341.71	0.00	0.26
7	1.80	NA	+	+	NA	NA	4	-166.94	342.49	0.78	0.18
13	1.63	NA	NA	+	+	NA	4	-167.45	343.49	1.79	0.11
6	2.00	0.00	NA	+	NA	NA	4	-167.65	343.90	2.19	0.09
15	1.72	NA	+	+	+	NA	5	-166.57	344.04	2.33	0.08
8	1.79	0.00	+	+	NA	NA	5	-166.94	344.80	3.09	0.06
29	1.66	NA	NA	+	+	+	5	-167.27	345.46	3.75	0.04
2	4.17	-0.02	NA	NA	NA	NA	3	-169.55	345.46	3.75	0.04
14	1.81	0.00	NA	+	+	NA	5	-167.44	345.79	4.08	0.03
31	1.75	NA	+	+	+	+	6	-166.43	346.16	4.45	0.03
16	1.50	0.00	+	+	+	NA	6	-166.55	346.40	4.69	0.03
4	4.12	-0.02	+	NA	NA	NA	4	-169.13	346.85	5.15	0.02
10	4.11	-0.02	NA	NA	+	NA	4	-169.48	347.55	5.84	0.01
30	2.25	0.00	NA	+	+	+	6	-167.19	347.68	5.97	0.01
32	1.83	0.00	+	+	+	+	7	-166.43	348.62	6.91	0.01
12	4.04	-0.02	+	NA	+	NA	5	-168.97	348.86	7.15	0.01
3	1.66	NA	+	NA	NA	NA	3	-175.10	356.55	14.84	0.00
1	1.51	NA	NA	NA	NA	NA	2	-176.30	356.78	15.07	0.00
11	1.58	NA	+	NA	+	NA	4	-174.77	358.13	16.42	0.00
9	1.45	NA	NA	NA	+	NA	3	-176.14	358.64	16.93	0.00

Table S5. Model selection results for GLM predicting observed richness per hosts for mainland residents and Tasmanian migrants sampled in New South Wales and Queensland. Each row represents an individual model and includes parameter estimates (intercept and coefficients), degrees of freedom (df), log-likelihood (logLik), Akaike Information Criterion corrected for small sample size (AICc), delta AICc (ΔAICc), and Akaike weights. NA values indicate that the parameter was not included in the model.

	(Intercept)	Date of Sampling	Sex	State	Status	State x Status	df	logLik	AICc	delta	weight
5	5.34	NA	NA	+	NA	NA	3.00	-193.16	392.64	0.00	0.24
13	4.90	NA	NA	+	+	NA	4.00	-192.60	393.75	1.10	0.14
7	5.76	NA	+	+	NA	NA	4.00	-192.61	393.77	1.12	0.14
15	5.32	NA	+	+	+	NA	5.00	-191.89	394.62	1.97	0.09
6	6.78	-0.01	NA	+	NA	NA	4.00	-193.12	394.80	2.16	0.08
29	5.00	NA	NA	+	+	+	5.00	-192.55	395.94	3.29	0.05
14	6.03	-0.01	NA	+	+	NA	5.00	-192.57	395.99	3.35	0.05
8	6.00	0.00	+	+	NA	NA	5.00	-192.60	396.05	3.41	0.04
2	15.91	-0.08	NA	NA	NA	NA	3.00	-194.98	396.29	3.65	0.04
31	5.41	NA	+	+	+	+	6.00	-191.84	396.89	4.24	0.03
16	5.02	0.00	+	+	+	NA	6.00	-191.88	396.97	4.32	0.03
10	15.33	-0.08	NA	NA	+	NA	4.00	-194.51	397.58	4.94	0.02
4	15.91	-0.07	+	NA	NA	NA	4.00	-194.76	398.07	5.43	0.02
30	7.28	-0.02	NA	+	+	+	6.00	-192.48	398.15	5.51	0.02
12	15.27	-0.07	+	NA	+	NA	5.00	-194.19	399.22	6.58	0.01
32	5.89	0.00	+	+	+	+	7.00	-191.84	399.30	6.66	0.01
1	4.22	NA	NA	NA	NA	NA	2.00	-202.13	408.42	15.78	0.00
3	4.74	NA	+	NA	NA	NA	3.00	-201.49	409.32	16.67	0.00
9	3.72	NA	NA	NA	+	NA	3.00	-201.51	409.34	16.70	0.00
11	4.24	NA	+	NA	+	NA	4.00	-200.69	409.94	17.30	0.00

Table S6. Model selection results for GLM predicting scaled degrees from simple and complex life cycle parasites in a bipartite network. Each row represents an individual model and includes parameter estimates (intercept and coefficients), degrees of freedom (df), log-likelihood (logLik), Akaike Information Criterion corrected for small sample size (AICc), delta AICc (ΔAICc), and Akaike weights. NA values indicate that the parameter was not included in the model.

	(Intercept)	Sampling Date	Life cycle complexity	Population	Sex	Life cycle complexity ×Population	df	logLik	AICc	delta	weight
23	-2.64	NA	+	+	NA	+	6	-308.59	629.44	0.00	0.35
24	-2.39	0.00	+	+	NA	+	7	-308.43	631.22	1.77	0.14
7	-2.27	NA	+	+	NA	NA	4	-311.57	631.27	1.82	0.14
31	-2.60	NA	+	+	+	+	7	-308.50	631.35	1.91	0.13
8	-2.03	0.00	+	+	NA	NA	5	-311.42	633.02	3.58	0.06
32	-2.35	0.00	+	+	+	+	8	-308.34	633.14	3.70	0.05
15	-2.23	NA	+	+	+	NA	5	-311.48	633.15	3.70	0.05
3	-2.31	NA	+	NA	NA	NA	2	-315.43	634.89	5.45	0.02
16	-1.99	0.00	+	+	+	NA	6	-311.33	634.92	5.48	0.02
11	-2.26	NA	+	NA	+	NA	3	-315.28	636.64	7.20	0.01
4	-2.20	0.00	+	NA	NA	NA	3	-315.39	636.85	7.41	0.01
12	-2.16	0.00	+	NA	+	NA	4	-315.25	638.63	9.19	0.00
5	-1.51	NA	NA	+	NA	NA	3	-363.75	733.57	104.13	0.00
6	-1.28	0.00	NA	+	NA	NA	4	-363.61	735.34	105.89	0.00
13	-1.47	NA	NA	+	+	NA	4	-363.66	735.45	106.01	0.00
1	-1.55	NA	NA	NA	NA	NA	1	-367.29	736.59	107.14	0.00
14	-1.25	0.00	NA	+	+	NA	5	-363.53	737.24	107.80	0.00
9	-1.51	NA	NA	NA	+	NA	2	-367.16	738.35	108.91	0.00
2	-1.45	0.00	NA	NA	NA	NA	2	-367.25	738.54	109.10	0.00
10	-1.41	0.00	NA	NA	+	NA	3	-367.13	740.33	110.89	0.00
23	-2.64	NA	+	+	NA	+	6	-308.59	629.44	0.00	0.35
24	-2.39	0.00	+	+	NA	+	7	-308.43	631.22	1.77	0.14
7	-2.27	NA	+	+	NA	NA	4	-311.57	631.27	1.82	0.14
31	-2.60	NA	+	+	+	+	7	-308.50	631.35	1.91	0.13
8	-2.03	0.00	+	+	NA	NA	5	-311.42	633.02	3.58	0.06
32	-2.35	0.00	+	+	+	+	8	-308.34	633.14	3.70	0.05
15	-2.23	NA	+	+	+	NA	5	-311.48	633.15	3.70	0.05
3	-2.31	NA	+	NA	NA	NA	2	-315.43	634.89	5.45	0.02
16	-1.99	0.00	+	+	+	NA	6	-311.33	634.92	5.48	0.02
11	-2.26	NA	+	NA	+	NA	3	-315.28	636.64	7.20	0.01
4	-2.20	0.00	+	NA	NA	NA	3	-315.39	636.85	7.41	0.01
12	-2.16	0.00	+	NA	+	NA	4	-315.25	638.63	9.19	0.00
5	-1.51	NA	NA	+	NA	NA	3	-363.75	733.57	104.13	0.00
6	-1.28	0.00	NA	+	NA	NA	4	-363.61	735.34	105.89	0.00
13	-1.47	NA	NA	+	+	NA	4	-363.66	735.45	106.01	0.00

1	-1.55	NA	NA	NA	NA	NA	1	-367.29	736.59	107.14	0.00
14	-1.25	0.00	NA	+	+	NA	5	-363.53	737.24	107.80	0.00
9	-1.51	NA	NA	NA	+	NA	2	-367.16	738.35	108.91	0.00
2	-1.45	0.00	NA	NA	NA	NA	2	-367.25	738.54	109.10	0.00
10	-1.41	0.00	NA	NA	+	NA	3	-367.13	740.33	110.89	0.00

Table S7. Model selection results for GLM predicting scaled degrees extracted from a bipartite network (i.e. the number of links to each parasite genera per host individual, standardised for total parasite genera). Each row represents an individual model and includes parameter estimates (intercept and coefficients), degrees of freedom (df), log-likelihood (logLik), Akaike Information Criterion corrected for small sample size (AICc), delta AICc (ΔAICc), and Akaike weights. NA values indicate that the parameter was not included in the model.

	(Intercept)	Body Condition	Sampling Date	Population	Sex	df	logLik	AICc	delta	weight
5	-1.46	NA	NA	+	NA	3	-196.35	398.89	0.00	0.27
6	-1.49	-0.06	NA	+	NA	4	-196.13	400.58	1.69	0.11
7	-1.09	NA	0.00	+	NA	4	-196.19	400.69	1.81	0.11
13	-1.47	NA	NA	+	+	4	-196.34	401.00	2.12	0.09
2	-1.65	-0.15	NA	NA	NA	2	-198.56	401.21	2.33	0.08
4	-0.90	-0.14	0.00	NA	NA	3	-197.88	401.95	3.07	0.06
8	-1.13	-0.06	0.00	+	NA	5	-195.98	402.44	3.56	0.05
14	-1.50	-0.06	NA	+	+	5	-196.11	402.71	3.82	0.04
15	-1.09	NA	0.00	+	+	5	-196.17	402.83	3.94	0.04
3	-0.67	NA	-0.01	NA	NA	2	-199.48	403.06	4.18	0.03
10	-1.68	-0.16	NA	NA	+	3	-198.50	403.20	4.32	0.03
1	-1.65	NA	NA	NA	NA	1	-200.62	403.26	4.38	0.03
12	-0.91	-0.14	0.00	NA	+	4	-197.79	403.89	5.01	0.02
16	-1.14	-0.06	0.00	+	+	6	-195.94	404.57	5.69	0.02
11	-0.67	NA	-0.01	NA	+	3	-199.44	405.07	6.19	0.01
9	-1.66	NA	NA	NA	+	2	-200.61	405.31	6.42	0.01

Table S8. Pairwise Dunn's tests for each sample type and metric calculated using ASV sequences extracted from faecal and blood samples of 168 silvereye, group 1 and 2 indicate pairwise comparison. LCBD = Local Contribution to Beta Diversity. P values adjusted with Holm correction for multiple comparisons.

Sample Type	Metric	Group 1	Group 2	Statistic	*p.adj*
Blood	Chao1	Tasmania Resident	Migrant	0.210	1.000
Blood	Chao1	Tasmania Resident	Mainland Resident	-0.425	1.000
Blood	Chao1	Migrant	Mainland Resident	-0.629	1.000
Blood	LCBD	Tasmania Resident	Migrant	-0.029	1.000
Blood	LCBD	Tasmania Resident	Mainland Resident	-0.417	1.000
Blood	LCBD	Migrant	Mainland Resident	-0.368	1.000
Blood	Observed Richness	Tasmania Resident	Migrant	-0.817	0.828
Blood	Observed Richness	Tasmania Resident	Mainland Resident	0.488	0.828
Blood	Observed Richness	Migrant	Mainland Resident	1.341	0.540
Combined	Chao1	Tasmania Resident	Migrant	2.434	0.045
Combined	Chao1	Tasmania Resident	Mainland Resident	2.404	0.045
Combined	Chao1	Migrant	Mainland Resident	-0.112	0.911
Combined	LCBD	Tasmania Resident	Migrant	-2.398	0.049
Combined	LCBD	Tasmania Resident	Mainland Resident	-1.251	0.422
Combined	LCBD	Migrant	Mainland Resident	1.235	0.422
Combined	Observed Richness	Tasmania Resident	Migrant	1.713	0.174
Combined	Observed Richness	Tasmania Resident	Mainland Resident	2.398	0.050
Combined	Observed Richness	Migrant	Mainland Resident	0.673	0.501
Faecal	Chao1	Tasmania Resident	Migrant	2.626	0.026
Faecal	Chao1	Tasmania Resident	Mainland Resident	1.648	0.199
Faecal	Chao1	Migrant	Mainland Resident	-1.154	0.249
Faecal	LCBD	Tasmania Resident	Migrant	-2.761	0.017
Faecal	LCBD	Tasmania Resident	Mainland Resident	-1.143	0.253
Faecal	LCBD	Migrant	Mainland Resident	1.856	0.127
Faecal	Observed Richness	Tasmania Resident	Migrant	3.090	0.006
Faecal	Observed Richness	Tasmania Resident	Mainland Resident	3.033	0.006
Faecal	Observed Richness	Migrant	Mainland Resident	-0.109	0.913

Table S9. Model-averaged estimates for GLM predicting scaled degrees (proportion of degrees linking parasite genera to host individuals extracted from bipartite network). Coefficient estimates (β), standard errors (SE), 95% cluster robust confidence intervals (95% CI) and weighted importance scores (WIP) are based on model averaging across the top six models (ΔAICc < 2). The parameters included in each model are indicated by black dots. The intercept represents the predicted scaled degrees when all predictor variables are equal to zero or their reference (baseline) for categorical variables. For each model in the top set, we also present degrees of freedom (df), Log-likelihood (logLik), small-sample corrected Akaike Information Criterion (AICc), the difference in AICc from the top model (delta) and the Akaike weight, which reflects the relative support for each model.

Parameter	1	2	3	β	SE	95% CI	WIP
(Intercept)	•	•	•	-1.384	0.359	(-2.087, -0.680)	NA
Population (Tasmanian Migrant)	•	•	•	-0.124	0.165	(-0.447, 0.199)	1.00
Population (Tasmanian Resident)	•	•	•	-0.408	0.160	(-0.722, -0.094)	1.00
Body Condition		•		-0.058	0.089	(-0.232, 0.116)	0.23
Sampling Date			•	-0.002	0.004	(-0.011, 0.006)	0.22
df	3.00	4.00	4.00				
logLik	-196.35	-196.13	-196.19				
AICc	398.89	400.58	400.69				
delta	0.00	1.69	1.81				
weight	0.55	0.23	0.22				

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