Passeriformes colonization and related ectoparasites in insular and mainland populations

"Documento Definitivo"

Doutoramento em Biologia e Ecologia das Alterações Globais

Especialidade em Ecologia e Biodiversidade Funcional

André Filipe Ventura Tomás

Tese orientada por:

Professora Doutora Maria Teresa Ferreira Ramos Nabais de Oliveira Rebelo (FCUL–UL)

Professora Doutora Isabel Maria Soares Pereira da Fonseca de Sampaio (FMV–UL)

Documento especialmente elaborado para a obtenção do grau de doutor

2021

UNIVERSIDADE DE LISBOA   UNIVERSIDADE DE AVEIRO

FACULDADE DE CIÊNCIAS   DEPARTAMENTO DE BIOLOGIA

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Nota prévia

A presente tese apresenta artigos científicos já publicados ou submetidos para publicação (capítulos 2 a 4), de acordo com o previsto no nº 2 do artigo 25º do Regulamento de Estudos de Pós-Graduação da Universidade de Lisboa, publicado no Diário da República, 2.ª série — N.º 57 — 23 de março de 2015. Uma vez que estes trabalhos foram realizados em colaboração o candidato esclarece que participou integralmente na conceção dos trabalhos, obtenção dos dados, análise e discussão dos resultados, bem como na redação dos manuscritos.

Lisboa, 8 fevereiro de 2022

André Filipe Ventura Tomás

Acknowledgments

First, I would like to thank my advisors, Professor Maria Teresa Rebelo and Professor Isabel Pereira da Fonseca. Thank you for always being available to help. For your guidance, motivation and knowledge shared. Your support and encouragement were invaluable during this PhD.

A sincere thank you to Thijs Valkenburg, for all the friendship. For having been with me in the field. And always being optimist, especially on those days in the pouring rain on Terceira Island and that bumpy flight to the Azores.

I also want to thank all the Professors and colleagues that I had the pleasure of known during the PhD. I prefer not to specify so as not to forget anyone, but your support was what several times helped keep me motivated.

I would like to thank to my friends, who with their support, friendship and patience, they made these years easier.

Finally, I would like to thank my mother, father, brothers and girlfriend for your endless support, patience and comprehension. To my parents, without whom these words would not be being written, I will be eternally grateful for providing me with all the necessary conditions to get where I am. Thank you for always believing in me. To my brothers, thank you for every moment they asked me joking questions about parasites, they allowed me to learn how to respond to adults with a 6 year old's mentality (just kidding). To Margarida, my girlfriend and best friend, thank you for all moment of happiness but above all, for being my main support in every moment of frustration and sadness. You were my pillar of sanity.

Resumo

As Ilhas, especialmente as Ilhas Oceânicas, têm sido foco de inúmeros estudos biogeográficos, permitindo não só documentar formas de vida únicas, como também estudar os mecanismos evolutivos subjacentes à expansão, distribuição e adaptação de espécies. A síndrome insular, manifestada como todas as diferenças demográficas, reprodutivas, comportamentais e morfológicas em vertebrados, é um dos efeitos mais assináveis do isolamento populacional. Embora seja um tema emergente, os padrões biogeográficos das populações de parasitas insulares têm sido alvo de uma série de estudos, contribuindo para um melhor conhecimento dos padrões de colonização e do efeito da insularidade nas populações de parasitas. Como observado nas populações de vertebrados, os parasitas capazes de persistir nas Ilhas, frequentemente desenvolvem alterações decorrentes da insularidade, denominadas síndromes insulares de parasitas, que se manifestam sobretudo por alterações na riqueza de espécies, na especificidade de hospedeiro e na prevalência dos parasitas. Estes estudos têm-se focado sobretudo em grupos específicos de parasitas de hospedeiros endémicos, hospedeiros recém-colonizadores ou colonizadores de longo-prazo. De forma a melhorar o conhecimento do efeito da insularidade sobre os parasitas, a inclusão de mais do que um grupo de parasitas, de hospedeiros com diferentes escalas de isolamento em Ilhas Oceânicas, reveste-se de uma importância acrescida, pois permite aprofundar a nossa compreensão sobre a evolução das características típicas das populações de parasitas insulares, quais os fatores que poderão estar envolvidos no desenvolvimento de síndromes insulares e se estes evoluem de igual modo, independentemente do período de isolamento dos hospedeiros.

Na presente Tese, foram estudadas as populações de ectoparasitas de quatro espécies de Passeriformes, *Turdus merula*, *Sylvia atricapilla*, *Fringilla coelebs* e *Erithacus rubecula*, de Portugal continental e três Ilhas do Arquipélago dos Açores, especificamente São Miguel, Terceira e Flores. Apesar de estas espécies, em termos evolutivos, serem consideradas recém-colonizadores dos Açores, apresentam eventos de isolamento com diferentes escalas temporais. O Arquipélago dos Açores, geograficamente localizado a Oeste do Continente Europeu e abrangido pela região da Macaronésia, amplamente reconhecida como um *hotspot* de biodiversidade, representa assim, uma importante área para o estudo dos padrões de evolução nas Ilhas. As aves foram aleatoriamente capturadas com recurso a redes de neblina e amostradas para a presença de ectoparasitas utilizando o método tradicional de câmara de fumigação modificada, onde os corpos das aves são expostos a um agente fumigante, neste caso clorofórmio. Os ectoparasitas, especificamente as moscas hipoboscídeas, pulgas e piolhos

mastigadores foram devidamente identificados microscopicamente. A partir deste conjunto de dados, as populações de ectoparasitas insulares foram comparadas com as populações continentais focando essencialmente três características das síndromes insulares de parasitas, ou seja, riqueza de espécies, prevalência do parasitismo e a especificidade do hospedeiro. Além disso, informações referentes à área das Ilhas e a sua distância ao continente foram utilizados de forma a avaliar a sua correlação com a riqueza de espécies de ectoparasitas, observada em cada Ilha dos Açores.

No que diz respeito às moscas hiboboscídeas, na generalidade a riqueza de espécies foi semelhante entre as duas áreas geográficas em estudo. No entanto, avaliando a riqueza parasitária em cada espécie de hospedeiro, foi possível verificar que especialmente os melros, apresentavam maior diversidade de espécies na região insular (mais uma espécie), revelando que este grupo parasitário não tende a falhar a colonização de Ilhas Oceânicas, ou seja, as associações parasita-hospedeiro não se encontram comprometidas nas Ilhas. Este resultado muito provavelmente decorre da pouca especificidade de hospedeiro comum às moscas hiboboscídeas observada nas duas áreas em estudo. Todavia, apesar da pouca riqueza parasitária no Arquipélago dos Açores e em Portugal continental, este trabalho permitiu aumentar a fauna de moscas hiboboscídeas em ambos os locais, com a identificação de *Ornithoica turdi* na Ilha das Flores e Terceira, *Ornithomya fringillina* nas três Ilhas dos Açores e *Icosta minor* em Portugal continental. O resultado mais interessante do estudo das síndromes insulares associadas a moscas hiboboscídeas, foi a evidência da elevada prevalência de parasitas no Arquipélago dos Açores, sobretudo no caso dos melros. Várias hipóteses foram avançadas para explicar esta descoberta, tais como as elevadas densidades de hospedeiros nas Ilhas, maior tamanho corporal das aves, saneamento deficitário do ninho e as melhores condições ambientais para os parasitas nas Ilhas.

Em relação ao grupo parasitário das pulgas, apenas foram observadas espécies no Arquipélago dos Açores. Embora este resultado indique que as pulgas estão bem estabelecidas nas Ilhas, considerando as associações parasita-hospedeiro previamente documentadas em Portugal continental, seria um erro, classificar esta característica dos parasitas insulares como uma síndrome. As espécies identificadas nas Ilhas são conhecidas pela sua pouca especificidade de hospedeiro. O resultado mais intrigante deste estudo, e muito provavelmente acidental, foi a ocorrência de *Ctenocephalides felis felis* num melro insular. No entanto, considerando que as espécies de pulgas primariamente parasitavam mamíferos, deixamos em aberto a hipótese de a pulga do gato ter encontrado nas Ilhas, as condições favoráveis para começar a parasitar aves.

Utilizando como termo de comparação a prevalência de *Dasypsyllus gallinulae* registada em outros países europeus, é de salientar a maior prevalência de pulgas nas Ilhas. Várias hipóteses foram formuladas para suportar este resultado, mas sabendo da sazonalidade das pulgas nidícolas, menos comuns no corpo das aves após a nidificação e a maior longevidade, na ausência de hospedeiros, quando expostas a baixa temperatura e alta humidade, é de considerar que a humidade típica das Ilhas dos Açores, tende a beneficiar as pulgas nidícolas.

No que respeita aos piolhos mastigadores, globalmente a riqueza de espécies foi superior nas Ilhas dos Açores, observando mais 3 espécies do que no continente. Este resultado demonstra que os piolhos não falharam a colonização das Ilhas, tal como verificado para os outros grupos parasitários, não comprometendo as associações parasita-hospedeiro. Ainda que nenhuma nova associação parasita–hospedeiro tenha sido reconhecida, este trabalho permitiu aumentar exponencialmente a fauna de piolhos mastigadores, especialmente da Ilha das Flores, Terceira e Portugal continental, com todas as espécies a serem registadas pela primeira vez. Ao analisar a riqueza de piolhos nas quatro espécies de ave foi possível verificar que apenas os melros, exibiram maior diversidade de piolhos nos Açores, com mais 3 espécies do que no continente. Várias hipóteses foram avançadas para a elevada riqueza de piolhos em melros insulares, das quais se destaca os eventos fundadores dos parasitas associados aos dois eventos de colonização do Arquipélago dos Açores por parte dos melros. O conjunto de piolhos observado nas Ilhas manteve a mesma característica do continente, exibindo uma elevada especificidade de hospedeiro (a maioria ao nível da espécie e uma espécie mais generalista, a ocorrer numa ampla gama de Passeriformes). A análise da prevalência de piolhos demonstrou que na generalidade esta é superior na região insular, sendo especialmente notável no caso do tentilhão e da toutinegra-de-barrete-preto. Esta observação poderá ter resultado das elevadas densidades de hospedeiros e do maior tamanho corporal das aves nas Ilhas, mas as condições ambientais apresentam-se como fator determinante. Prova disso, foram as diferentes prevalências identificadas em algumas espécies de piolhos, muito provavelmente decorrentes da maior aptidão dessas espécies para tolerar ambientes húmidos ou áridos.

O conjunto de dados referentes aos três grupos parasitários permitiu constatar que os eventos de colonização e a sua escala temporal poderão ser fundamentais na evolução das comunidades insulares. As síndromes insulares de parasitas foram mais evidentes em melro, espécie com pelo menos dois eventos de colonização das Ilhas dos Açores, um mais antigo (0,47 milhões de anos) e outro mais recente (0,09 milhões de anos), seguindo de tentilhão e toutinegra-de-barrete-preto, espécies com um único evento de isolamento (1,5 e 0,1 milhões de anos,

respetivamente). Nenhuma síndrome insular foi observada em pisco-de-peito-ruivo, espécie com um único evento de colonização bastante recente (0,08 milhões de anos). Estes resultados representaram uma nova perspetiva na compreensão das síndromes insulares associadas a parasitas, e poderão ter um potencial impacto em estudos futuros.

Por fim, o presente estudo permitiu identificar a ausência de correlação da riqueza de espécies insulares com a área da Ilha e a sua distância ao continente, sugerindo que estas características insulares raramente regulam as populações de ectoparasitas insulares. Caso isto ocorra, possivelmente resultará da pressão exercida pela área e distância da Ilha, sobre os hospedeiros. Contudo foi ainda observada uma prevalência de ectoparasitas ligeiramente superior na Ilha Terceira, em comparação com as outras duas Ilhas dos Açores, provavelmente em resultado da elevada humidade que caracterizou as áreas de estudo na Ilha Terceira, localizadas em zonas de elevada altitude no sudoeste e centro da Ilha.

Palavras-chave: Ectoparasitas; Síndromes insulares de parasitas; Passeriformes; Ilhas dos Açores; Portugal.

Abstract

The isolation process of Island parasite populations has revealed a remarkable set of changes in their characteristics, termed parasite island syndromes. Improving knowledge of the effect of insularity on parasite communities from hosts with different time scales of isolation, is essential for understanding the processes involved in the evolution of parasitism and how the period of host isolation, manifests itself on parasite island syndromes.

This thesis addresses the ectoparasite communities of *Turdus merula*, *Sylvia atricapilla*, *Fringilla coelebs* and *Erithacus rubecula*, from mainland Portugal and three Islands of the Azores Archipelago, specifically São Miguel, Terceira and Flores. The Azores Archipelago, geographically positioned as the Northernmost Archipelago of Macaronesia and composed of nine Oceanic Islands recognized as biodiversity hotspots, make it an important area to study the patterns of insular evolution. Live birds were captured with mist nets and sampled for presence of ectoparasites using the modified fumigation chamber method. Traditional methods were used for microscopic identification of hippoboscid flies, fleas and chewing lice. Insular ectoparasite communities of host species were compared with continental communities based on parasite island syndromes (species richness, prevalence and host specificity). Additionally, insular species richness was compared between the area of the Islands and their distance from the mainland.

The present study showed that ectoparasites do not failed to establish to the Azores Islands, and furthermore, the diversity of the ectoparasites communities, especially to chewing lice, was clearly enriched on the Azores Islands, with more 3 species than mainland birds. The results also revealed a high insular prevalence of ectoparasites. In regards to host specificity, insular ectoparasites have retained the characteristic specificity of the mainland. Even though island syndromes were not correlated with Island area and distance from the mainland, the overall findings indicate that characteristics associated with the Islands, specifically ambient humidity, the parasites, mainly ability to tolerate ambient humidity, and the hosts, particularly population densities, body size, and nest sanitation, influence the structuring of insular ectoparasite assemblages. Parasite island syndromes were especially notable in *T. merula*, a bird with two colonization events on the Azores Islands, which suggest that the host isolation on Islands may influence the evolution of insular parasite communities.

Keywords: Ectoparasites; Parasite island syndromes; Passerine birds; Azores Islands; Portugal.

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CI – Confidence interval

Km – Kilometer

Km^2 – Square kilometer

My – Million years

MP – Megapixel

n – Number of sampled birds

N – North

NW – Northwest

Sc – Sample score

Sest – Asymptotic species richness

Sobs – Observed species richness

SD – Standard deviation

SE – Southeast

W – West

mm – Millimeter

μm – Micrometer

% – Percentage

χ^2 – Chi-square test

p – *p-value*

…it is not too much to say that when we have mastered the difficulties presented by the peculiarities of Island life we shall find it comparatively easy to deal with the more complex and less clearly defined problems of continental distribution…

Alfred Russel Wallace, "Island life", 1892

Chapter 1

General Introduction

1.1. Island as natural laboratories of evolutionary experimentation

When in December 1831, Charles Darwin, a 23-year-old inexperienced naturalist, embarked on his five-year odyssey in Her Majesty's Ship Beagle, he was far from imagining that he would become one of the rising scientific stars. Darwin's observations on this expedition became known in 1839 through his diary's publication, "Journal of Researches into the Geology and Natural History of the Various Countries Visited by H. M. S. Beagle", where "every observable detail of the animals, birds and plants" as well as, "descriptions and even sketches of the movements and habits of hitherto unfamiliar species" were revealed (Darwin, 2009). After returning to London, Darwin obtained a Treasury Grant, with which he employed five experts in vertebrates who helped him to publish a splendid work, made up of five-volume parts with fascinating and detailed results of "The Zoology of The Voyage of H.M.S. Beagle", between 1838 and 1842. These researches, especially the observations of five weeks he spent in Galapagos Islands, shaped his thinking about evolutionary process, as revealed by some of his notes about the famous Darwin's finches: "The remaining land-birds form a most singular group of finches, related to each other in the structure of their beaks, short tails, form of body and plumage: there are thirteen species, which Mr. Gould has divided into four subgroups. All these species are peculiar to this Archipelago. (…) Seeing this gradation and diversity of structure in one small, intimately related group of birds, one might really fancy that from an original paucity of birds in this Archipelago, one species had been taken and modified for different ends" (Darwin, 2008a).

Finally, two decades after starting work on his scientific theory of the evolution of species by natural selection, Charles Darwin published his most celebrated book "On the Origin of Species" in 1859. Although Darwin's observations shocked religious society at the time, sparked interest in the scientific community providing the foundation for modern evolutionary and ecological studies, which happens until today (Darwin, 2008b). Since Darwin's findings, researchers have understood the scientific potential of Islands, especially Oceanic Islands, for study of the evolutionary processes and have come to regard Islands as natural laboratories of evolutionary experimentation. So, almost two centuries after Darwin's voyage, the knowledge of insular organisms, especially fauna and flora, have been greatly studied on Islands throughout the world, allowing to document incredible life organisms and the underlying evolutionary mechanisms of species formation and adaptive radiation (Losos and Ricklefs, 2009; Whittaker and Fernández-Palacios, 2007).

But what is so special about Oceanic Islands, to make them focal points for evolutionary studies? Oceanic Islands are originate from volcanic activity, have clearly defined boundaries and never been connected to continental landmasses, *i.e.* represents an empty land space for living forms to colonize, proliferate and diversify (Losos and Ricklefs, 2009). Additionally, Alfred Russel Wallace, the British naturalist best known as the "co-originator", with Charles Darwin, of the Theory of Evolution by Natural Selection, clearly identified "the extreme remoteness and isolation of these Islands, their small area and comparatively recent origin" in his 1881 book "Island life", as properties of great "importance in those cases where the evidence as to the exact origin of the fauna and flora of an Island is less clear", *i.e.* these peculiar attributes make the Islands a remarkable place to observe and interpret patterns of evolution (Wallace, 1892).

"…The scarcity of kinds – the richness in endemic forms in particular classes or sections of classes, – the absence of whole groups, as of batrachians, and of terrestrial mammals notwithstanding the presence of aerial bats…" revealed by Charles Darwin in 1859, characterize many of the insular environments that we know (Darwin, 2008b). In global terms, Islands are rich in endemic organisms, *i.e.* species found nowhere else; thus, the out of proportion number of endemism's per unit area makes the Islands a biodiversity hotspot. In contrast, Islands are typically species poorer per unit area than the mainland. Moreover, smallest, more remote and low-lying Islands tend to have greater species impoverishment (Whittaker and Fernández-Palacios, 2007).

Inspired by two of these patterns of species richness, specifically species–area (positive relationship between the area of habitat and the number of species) and species–distance (negative relationship between the distance from the source of colonization and the number of species) relationships, as well as the species turnover, Robert H. MacArthur and Edward O. Wilson's, in the early 1960s in their Zoogeographic Model and later in a general model, in his 1967 book "The Theory of Island Biogeography", proposed that Island species diversity tend towards dynamic equilibrium by balance between immigration and extinction, termed Equilibrium Model of Island Biogeography (MacArthur and Wilson, 1963, 1967).

Using the assumptions that immigration rate declines exponentially and extinction rate increases exponentially as the number of species on the Island increases, since "as more species become established, fewer immigrants will belong to new species" and as "more species there are present the more there are to become extinct" respectively, the famous graphical model of Equilibrium of Island Biogeography, postulates that near Islands from the source of

colonization, will have higher immigration rates than far Islands and small Islands will have higher extinction rates than large Islands (MacArthur and Wilson, 1967). Even half a century since its publication in "The Theory of Island Biogeography" book, the basic principle of Equilibrium Model of Island Biogeography, has been used as a basis for the study of many insular taxa and thereby is considered essential to debate Island ecology (Whittaker and Fernández-Palacios, 2007).

1.2. Macaronesia: Azores Islands

The term Macaronesia, firstly used by the British botanist Philip Barker-Webb, in 1845, to refer the Archipelagos of Canaries and Madeira, was later extended to include the biogeographical region of all the North-West Atlantic Archipelagoes of the Europe and North Africa, *i.e.* Madeira, Selvagens, Azores, Canaries and the Cape Verde Islands. Although the concept of Macaronesia has been used with different meanings, in general it is a phytogeographical term. However based on the recent molecular phylogenetic analyses of terrestrial flora, some authors question the validity of this region (Whittaker and Fernández-Palacios, 2007). The floristic affinities of Azores, Madeira and Canaries, and Cape Verde Islands with continental regions of the Eurosiberian–Atlantic, Mediterranean and Saharan–Sudanian, respectively, led Kunkel (1999) to suggest the hierarchical division of Macaronesia in Central Macaronesia (including Canaries and Madeira inlands), Lauri Macaronesia (including Central Macaronesia, Azores Islands and a part of the Iberian peninsula) and Great Macaronesia (including Lauri Macaronesia, Cape Verde Islands, and a part of the Africa). In turn, Lobin's proposes dropping the concept Macaronesia altogether to define a phytogeographical unit and suggests that can be used strictly in the geographical sense (Lobin, 1982). Regardless, the Macaronesia Islands have long been recognized as one of the world's biodiversity hotspots, *i.e.* are the Atlantic equivalent of Hawaii and the Galapagos Islands.

Geographically positioned as the Northernmost Archipelago of Macaronesia, Azores are consisted of nine volcanic Islands and several islets, located about 1.500 Km West of continental Europe, between latitudes 36º55'–39º43'N and longitudes 24º46'–31º16'W. The oceanic distribution of these Islands along a general NW–SE direction, define three geographical groups: the Western group (Flores and Corvo), Central group (Faial, Pico, São Jorge, Terceira and Graciosa) and Eastern group (São Miguel and Santa Maria). These Islands have recently emerged above sea level in the so-called Azores Plateau, 0.3 to 8 Million years

(My) old, situated in a convergence zone where the North American, Eurasian and African lithospheric plates meet, resulting into the existence of important tectonic structures, namely the Mid-Atlantic ridge, North Azores fracture zone, West Azores fracture zone, East Azores fracture zone, Terceira Rift and Gloria Fault, responsible for the intense seismic and volcanic activity in region. The 31 destructive earthquakes and 28 volcanic eruptions, since the settlement of the Archipelago, at the beginning of the fifteenth century, attest the active nature of these volcanic systems (França *et al*., 2003; Gaspar *et al*., 2015).

"…Described by the first settlers as Islands covered by dense native vegetation with thousands of birds", the Azores Archipelago, like others Macaronesia Islands, were a stopover point for many naturalists during their transatlantic voyage (Rodrigues and Cunha, 2011). At that time, the Archipelago was considered a bird paradise; while Darwin (2009), on the Beagle's return journey to London just observed "old English friends amongst (...) the birds, the starling, water-wag tail, chaffinch, and blackbird" in Terceira Island, in turn Wallace (1892), using the Frederick du Cane Godman work of the "Fauna and Flora of the Azores", referred abundant "flying creatures", reporting "fifty-three species of birds" with "larger proportion (...) either aquatic or waders" and including a "peculiar species", the Azores bullfinch *Pyrrhula murina* Godman, 1866. Today, it is known that the Azorean avifauna comprises at least 40 regularly breeding species, of which 2 endemic species, Azores bullfinch and Monteiro's storm-petrel *Oceanodroma monteiroi* (Bolton *et al*. 2008) and 11 endemic subspecies, of which are included several seabirds' species of great importance in a European context (Rodrigues and Cunha, 2011). Unfortunately, others endemic birds species have gone extinct after human colonization, namely one owl species (*Otus frutuosoi* Rando, Alcover, Olson & Pieper, 2013), three rails species (*Rallus montivagorum* Alcover *et al*., 2015, *Rallus carvaoensis* Alcover *et al*., 2015 and *Rallus minutus* Alcover *et al*., 2015) and the large bullfinch from Graciosa Island (*Pyrrhula crassa* Rando *et al*., 2017), likely related with the human perturbations, introduction of predators as well as to the habitat degradation or destruction (Alcover *et al*., 2015; Rando *et al*., 2013, 2017).

1.2.1. Bird species and colonization events

The geographical position of the nine Islands of the Azores Archipelago makes them a place of scale for more than 370 species of Palearctic and Nearctic origin (Pereira and Melo, 2016). Early, the presence of rare or accidental species of birds aroused Wallace's interest and shaped

his thinking about the origin of the breeding birds of the Azores Archipelago. According to Wallace (1892) "the fact that birds are most numerous in the eastern group, and diminish as we go westward (…) it is strictly in accordance with the view that they are all stragglers from Europe, Africa, or the other Atlantic Islands".

Recent molecular studies showed that Wallace's reasoning was right. The common chaffinch *Fringilla coelebs* Linnaeus, 1758, a species widely distributed throughout the Western Palearctic, including Portugal and the Macaronesian archipelagos (except Selvagens and Cape Verde), represents an interesting model to study speciation processes on oceanic islands (Recuerda *et al*., 2021). Portuguese chaffinch populations are mainly sedentary, however populations in mainland increase significantly during autumn by the arrival of wintering individuals, while in the Azores only move between the islands of the archipelago (Catry *et al*., 2010; Recuerda *et al*., 2021). In one of the first studies on the origin of the Macaronesian chaffinch, Grant (1980) proposed an independent colonization of each archipelago from its nearest mainland. In contrast, in more recent studies, Rodrigues *et al*. (2014) suggested "that the Macaronesian populations constitute a monophyletic group, consistent with a single colonization event (…) around 1.5 My ago", while Recuerda *et al*. (2021) revealed "a circuitous colonization pathway in Macaronesia, from the mainland to the Azores, followed by Madeira, and finally the Canary Islands", suggesting that the first differentiation of the chaffinch occurred about 0.83 My ago. These authors go further, proposing that the "common chaffinch be divided into five different species, corresponding to Eurasia (*Fringilla coelebs*), North Africa (*Fringilla spodiogenys/africana*), Azores (*Fringilla moreletti*), Madeira (*Fringilla maderensis*) and the Canary Islands (*Fringilla canariensis*)" (Recuerda *et al*., 2021).

The blackbird *Turdus merula* Linnaeus, 1758, one of the most common and widespread bird species in the Western Palearctic, including the Macaronesian archipelagos, is mainly sedentary in mainland Portugal and the Azores archipelago (Catry *et al*., 2010). Recent studies suggested the existence of at least two consecutive isolation events of *T. merula* in the Azores, a first isolation event about 0.47 My ago, and a more recent event, approximately 0.09 My ago (Rodrigues *et al*., 2016). Although it is difficult to ascertain the origin of the first Azores blackbird isolation event, *i.e.* whether the birds diverged in a continental environments before colonizing the Azores and Madeira or whether they diverged under island conditions, the sharing of all blackbird haplotypes from Madeira with their Azorean counterparts led Rodrigues *et al*. (2016) to suggested "that Madeira was probably the point of origin for a second blackbird colonization of the Azores".

The blackcap *Sylvia atricapilla* (Linnaeus, 1758) is a widespread forest passerine in Palearctic, including the Atlantic islands of Macaronesia. Even though this species is sedentary in the southern Mediterranean areas and Macaronesia, it is also the species with the "greatest diversity of migratory behaviors described so far within a species" (Pérez-Tris *et al.*, 2004). Thus, during autumn and winter season this species occurs in greater abundance in mainland Portugal, as a result of the regular annual influx of migrating and overwintering European blackcaps (Catry *et al.*, 2010). Although poorly studied, these migrating European blackcaps also appear to occur on the two easternmost islands of the Canary archipelago (Shirihai *et al.*, 2001). Although Azores blackcaps differ morphometrically within the Azores and among the other Macaronesian islands, recent molecular studies have revealed that blackcaps from Azores are genetically very similar to other Macaronesian populations and the majority of the other populations (Dietzen *et al.*, 2008; Pérez-Tris *et al.*, 2004; Rodrigues *et al.*, 2018). These results support a relatively recent expansion of blackcaps into Macaronesia, estimating that this expansion occurred about 4.000 to 13.000 years ago (Pérez-Tris *et al.*, 2004) or 4.000 to 40.000 years ago (Dietzen *et al.*, 2008). More recently Rodrigues *et al.* (2018) indicated a single colonization event of the Azores, within the last 0.1 My ago.

The robin *Erithacus rubecula* (Linnaeus, 1758), a bird distributed throughout the Palearctic (including Macaronesia), is a common resident in mainland Portugal and all the islands of the Azores, except the two islands of the western group, Flores and Corvo, where it does not occur. In mainland Portugal, it is especially abundant in winter with the arrival of wintering birds (Catry *et al.*, 2010; Dietzen *et al.*, 2003). Even though previously the robins of the Azores, Madeira and Western Canary Islands were classified as subspecies *E. r. microrhynchos* Reichenow (1906), currently the robins of the Azores are retained within the nominate form *E. r. rubecula* (Hounsome, 1993; Rodrigues *et al.*, 2013). Morphologically, Azorean robins exhibited differences among islands, and genetically revealed a low genetic diversity, sharing their most common haplotype with Madeira and the Continental Western Palearctic robins, consistent with a recent founder event (Rodrigues *et al.*, 2013). These authors suggested "one first dispersal event from the Continental Western Palearctic to the Canary Islands and/or Madeira, from which they colonized the Azores, in the last 80.000 years" as the most probable hypothesis for the recent colonization of the Azores by robins (Rodrigues *et al.*, 2013).

1.3. Evolution of Island Populations

During initial steps of Island colonization, mostly taking as an example the true Oceanic Islands, when a new population of settlers establishes on an Island after a long-distance dispersal, it brings with them only a very small proportion of the genetic variability of the parent population immediately providing a bias in the genetic diversity of the Island population, termed founder effect (Mayr, 1954). Foundation events therefore represent a form of population bottleneck, where a temporary reduction in population size influences its genetic variability (Mayr, 1963). The colonists genetic changes produced by the bottlenecks, have been usually associated with events of loss of genetic diversity (heterozygosity), but in some circumstances these changes generate an increase of genetic variability (Carson, 1992; Mayr, 1954). These effects may occur at other points in the lifespan of species, as verified with Hawaiian *Drosophila*, whose studies indicate that they occurred not only in a first event of colonization of the Archipelago, but also in repeated founder events on each Island and as result of catastrophic habitat disturbances by volcanic eruptions (Carson, 1983; Carson *et al*., 1990). Usually, the genetic effects of the population depends of the size of the founder event, rate of the population growth following the founder event, Island size and their distance from the continent rates, migration and dispersion and natural selection (Frankham, 1997). Thus, the "genetic revolutions" (Mayr term in his book "Evolution as a Process") generated by population bottlenecks, seems to contribute to the rapid evolutionary divergence in Island populations (Whittaker and Fernández-Palacios, 2007).

Following the founding events, in response to the new biotic and abiotic features of the Islands, the settlers undergo a wide range of niche shifts. These changes do not necessarily imply speciation generating the endemism characteristic of the Islands, but they frequently result in remarkable changes in morphology, behavior and life-history of Island populations, termed island syndrome (Adler and Levins, 1994) (Subchapter 1.3.1. Island Syndrome). The term island syndrome may be replaced throughout the present work by the term insular syndrome (Thiollay, 1993). Both terms will be used to define all population differences related to insularity. To better explore these ecological–evolutionary changes it is essential to first understand the two general responses to differential occupation of the Islands, specifically, the ecological release and density compensation (Blondel, 2000).

Ecological release has frequently been used to refer the niche expansion by Island-colonizing species, as response to insular conditions in which some particular competitors, such as predators, are lacking (Lomolino, 1984). This phenomenon mainly take two forms of response: i) loss of "unnecessary" features – in the absence of close competitors, Island species tend to

gradually lose some features, such as, flying ability and defensive traits. Diamond (1991) argued that "on Oceanic Islands free of mammalian predators, reduction of flight muscle brings great energy savings with little penalty" to flightless rail endemic to Solomon Islands, *Hypotaenidia rovianae* (Diamond, 1991). Stamps and Buechner (1985) suggested that a decrease in aggressiveness may occur as a result of exaggerated defense costs, because of high-density populations on Islands (see below); ii) increase in variation of morphological features – the absence of close competitors, allows to Island species to occupy a different and wider array of niches, resulting in the variation of morphological features. A classic example of this response is provided by the morphological differences of Island finches. Schluter (1988) argued that the greatest morphological divergence of the beak size of the finch species on remote Islands such as Hawaii and the Galápagos, compared with finches on continent, result from an absence of competitor taxa on the Islands.

Density compensation refers to higher population densities on the Island than the mainland's conspecific populations, though the species richness on the Islands is generally lower. This ecological response was first recognized by Crowell (1962) in a comparative study of birds from Bermuda and North American mainland, and suggested that "the density which populations of individual species may attain therefore depends on the number of competing species". When this population density compensation occurs in apparently excessive degree, is termed density overcompensation (Wright, 1980). In addition to the low pressure of competitor species on the Islands, MacArthur *et al*. (1972) considered that density compensation on Islands, may occur due greater availability of resources and more stable environments on Islands, with less oscillation in the level of resources. They argued that the increase of population density on Islands, due to the presence of fewer competing species and greater availability of resources, "may occur without any niche shift (...) continuing to feed in the same place in the same way and simply finding more food" or accompanied by "niche shifts, such that a species utilizes more space (...) or employs a wider range of foraging techniques, or broadens its diet" (MacArthur *et al*., 1972). Although it might be considered an inaccuracy to generalize for all the populations of the Islands, here we show a combination of factors involved in the ecological responses of species to the singular conditions of the Islands.

1.3.1. Island Syndrome

The term island syndrome, was firstly used by Adler and Levins (1994) to refer the "systematic differences in demography, reproduction, behavior, and morphology" of Island rodent populations when compared to mainland population. In this extensive work, the authors synthetized results from a range of empirical studies on Island rodent populations (Table 1.1).

Adler and Levins (1994) noted that populations of rodents isolated on Island tend to evolve "higher and more stable densities, better survival, increased body mass, and reduced aggressiveness, reproductive output, and dispersal", suggesting that these micro-evolutionary changes oftentimes evolve together, and not only individually, and may be the result of both short-term and long-term responses. For instance, reproductive, body size and behavioral changes may be a short-term response to higher insular densities. The authors argued that "higher densities lead to reduced reproductive output, which then leads to greater body size"; moreover, initial behavioral changes may be "owing to better survival and reduced dispersal, which result in less population turnover, greater neighbor familiarity, and less aggression". In turn, they attributed different origins to long-term responses: reduced dispersal in response to directional selection against dispersers, "increased body size in response to increased intraspecific competition, reduced reproductive output (...) in response to reduced mortality schedules, and reduced aggressiveness" (Adler and Levins, 1994). Thus, although the Adler and Levins' work have been developed to populations of insular rodents, they suggested that conclusions may be applicable to other Islands life organisms that show evidence of island syndrome, provided that "characteristics such as body size, vagility and community composition are considered".

Table 1.1 – Short-term and long-term changes in Island rodents and proposed explanations. Adapted from Adler and Levins (1994).

Island trait	Proposed explanation
Reduced dispersal	Immediate constraint (short-term response) and natural selection against dispersers (long-term response).
Reduced aggression	Initially, reduced population turnover, greater familiarity with neighbors, and kin recognition. Long-term directional selection for reduced aggression.
Crowding effect	Isolation ("fence effect" resulting from reduced dispersal) and reduced number of mortality agents such as predation, both of which result in crowding of individuals and consequently higher population densities.
Greater individual body size	Initially, a norm of reaction as a response to higher density. Long-term directional selection for increased body size in response to increased intraspecific competition.
Lower reproductive output per individual	Initially, a reaction norm as a response to increased density. Long-term directional selection in response to decreased mortality.
Greater life expectancy (higher survival probabilities for individuals)	Reduced number of mortality agents such as predation.

Patterns of island syndrome have been observed at a wide variety of insular taxa, including insect and plants species, but are vertebrates that present the most striking general patterns. One of the most emergent pattern of insular syndrome, was termed the island rule by Van Valen (1973), to refer to the "regular evolution of mammalian body size on Islands". Previously, Foster (1964) had already described a set of different trends in non-flying insular mammals, towards dwarfing in carnivores, lagomorphs and artiodactyls while murine rodents generally tend to gigantism. Lomolino (1985) reinterpreted the pattern of island rule as a graded trend of size increase in small vertebrates (gigantism), and size reduction in large vertebrates (dwarfism). Moreover, Lomolino (2005) demonstrated that the island rule may be more general, applying to others vertebrates, such as bats, passerine birds, snakes and turtles. At a general level, the island rule seems to favor the evolution of an optimal, or fundamental, body size, most advantageous energetically, for a particular bauplan and ecological strategy (McNab, 2002).

The hypothetical factors in the generation of the island rule in insular vertebrates, were summarized by Lomolino (2005), which arguing that this results form a combination of convergent forces whose influences vary among species of different size. For instance, the absence of large predators and competitors on the Islands, where communities are less diverse but more dense, the "advantages of remaining small will be lessened and the advantages of being larger and hence dominating conspecifics increases, thus promoting increased size or gigantism in the otherwise small vertebrates" (Lomolino, 2005). In turn, the relative resource limitation on Islands and specialization for insular niches, amplified by high population densities, "confer higher fitness to smaller individuals because they require less energy" and so, the tendency toward insular dwarfism should be most common for largest species that require more energy; moreover, in the absence of predators, the adaptive advantage of being larger on the continent tends to be released on Island (ecological release), releasing the energy used to "outgrow, outrun or outfly predators on the mainland, to adapt more efficiently to insular environments and their ecologically simple communities" (Lomolino, 2005). However, instead of a rule, Meiri *et al*. (2008) considered that there are just a "few clade-specific patterns".

Regardless of the last consideration, the island rule, as Lomolino (2005) wrote, is considered an emerging pattern in different taxa and involving a combination of several particular mechanisms in a range of spatial and temporal scales. Proof of this phenomenon was the recent discovery of a fossil of a small hominid, *Homo floresiensis* Brown *et al*., 2004, which inhabited the Island of Flores, in Indonesia, about 18.000 years ago (Brown *et al*., 2004). Brown and collaborators interpreted the hobbit-like appearance of *H. floresiensis* as an example of human dwarfism, in result of long-term isolation on a comparatively small Island and as response to the low calorific environment for hominins, recognized to tropical rainforests; so, they suggested that "the genus *Homo* is morphologically more varied and flexible in its adaptive responses than previously thought".

In addition to the island rule, remarkable patterns of island syndrome have been described in a wide variety of insular organisms, manifesting themselves as:

i. Loss of dispersal powers – although the extinct dodo, *Raphus cucullatus* (Linnaeus, 1758) in Mauritius, is the most emblematic Island form of flightlessness, many others insular birds exhibit this characteristic. Roff (1994) studied the evolution of flightlessness, seeing that, at general level, there is an insular tendency to reduced flight ability in birds; however, this feature only was formally showed for the rails group, "in

which each flightless species probably represents a separate evolutionary transition". Diamond (1991) argued that the selective force for flightlessness of insular rails "is surely the energetic burden of flight muscle", *i.e.*, in Oceanic Islands free of predators it is energetically more advantageous to "release" the fly ability. Additionally, McCall *et al*. (1998) highlighted that 8 out of 11 avian families in which flightlessness has evolved independently, contain both flightless and flighted species and, these volante species tend to be shorter wing lengths. They showed "that birds with relatively short wings pay a high energetic cost of flight" and so, their findings support the energetic costs hypothesis.

ii. Change in fecundity patterns – vertebrates colonizing Islands also frequently display changes in fecundity patterns. Lack (1947) observed that insular birds have a "tendency for a reduced average clutch-size" than their continental conspecifics. The same pattern has been recorded for Island lizards and mammals, and is often associated with shorter breeding seasons (Stamps and Buechner, 1985). Moreover, others fecundity trends have been reported for insular vertebrates, for instance, marked habitat changes in nesting sites and a significant increase in egg size (Lack, 1942; Wiggins *et al*., 1998). Several hypotheses have been proposed to explain the patterns of fecundity of insular birds, of which highlighting: "The resource predictability hypothesis (Ashmole's hypothesis)" – which predicts that clutch size is directly proportional to the degree of seasonal fluctuation of resources. Thus, in stable insular environments, it is expectable a less seasonal fluctuation of resources resulting in a smaller the average clutch size (Ashmole, 1963); and "The energy reallocation hypothesis (Cody's hypothesis)" – this hypothesis is based on the concept of optimization of reproductive effort considering the life expectancy. So, the greater environmental predictability on Islands will lower mortality, resulting in lower population fluctuations and therefore, a "lower the clutch size and reinvest the energy thereby saved into other components of fitness such as better quality of young and/or increased longevity for the parents through better foraging efficiency, predator avoidance and competition in saturated environments", was suggested as the best adaptive strategy (Blondel, 1985).

iii. Change in behavior traits – Stamps and Buechner (1985) demonstrated that insular vertebrates (lizards, birds, and mammals) often exhibit reduced situation-specific aggression toward conspecifics, and this pattern can be expressed as: "(1) reduced territory sizes, (2) increased territory overlap with neighbors, (3) acceptance of

subordinates on the territory, (4) reduced aggressiveness to certain classes of conspecifics, or (5) abandonment of territorial defense". Two nonexclusive hypotheses have been proposed to explain these behavioral traits: "The resource hypothesis" – which predicts that territorial behavior is primarily adjusted to resource densities, *i.e.*, on Islands where competitors are generally absent, resources are more abundant. Therefore, in response to higher resource density, insular organisms tend "to reduce their territory sizes" and/or "to increase the degree of territory overlap" (Stamps and Buechner, 1985); and "The defense hypothesis" – supports that the costs of defense against both territorial intruders and contenders for vacant territories are higher on Islands. Thus, in response to the higher defense costs, it is expectable that insular life forms select reduced territories, increased territorial overlap and/or accept subordinates (Stamps and Buechner, 1985).

Although the insular environments present unique species, many of which endemics and many others with only some syndrome, each of these trends presented above requires careful evaluation before the potential effect of the Island would be accepted. However, in general, these insular organisms are especially vulnerable to stress factors, inherent to the natural dynamics of Island environments or related directly or indirectly with human activity. Consequently, since only anthropogenic threats can be controlled, is crucial to understand insular evolutionary–ecological mechanisms, to prevent extinction events of Island species, as occurred with the Azorean rail species.

1.3.2. Parasite Island Syndromes

Considering the widely known effects of insularity in populations of vertebrates, some of which have been described above, over the last two decades, the studies on the biogeographical patterns of insular parasite populations have been multiplied, to understand the fundamental ecological processes involved. Know these mechanisms, is especially important to understand and predict the risks and the patterns of parasites introduction in new areas, and possibly mitigate their spread and impact (MacLeod *et al*., 2010). Numerous examples of invasive parasite-driven outbreaks and extinctions in insular fauna were listed by Wikelski *et al*. (2004) for Galápagos birds.

Little is known about which mechanisms are important in colonizing success of parasites in new regions following host introduction or colonization (MacLeod *et al*., 2010). However, it is

known that during the hosts range expansion, parasites often are lost, by one of two independent events: i) "missing the boat" – individuals hosts of founder population may not be infected with the parasite, as resulted of the patchy distribution of parasite throughout the range of the host population or some other stochastic event (Paterson *et al.*, 1999; Torchin *et al.*, 2003); and ii) "drowning on arrival" – parasites do arrive with the founder hosts, but fail the establishment, as result of "sinking with the boat" when infected hosts arrive but fail to establish, or "lost overboard" when parasite establishment fail for other reasons (MacLeod *et al.*, 2010). Several factors associated to host or parasites traits have been suggested as contributing to insufficient parasite transmission and consequent "lost overboard". Proposed host factors include small founding populations, high mortality rates, low levels of social interaction and small host body size (Paterson *et al.*, 1999; Rózsa *et al.*, 1996); while, parasite factors include low vagility, high host-specificity and complex life cycle, *i.e.*, requirement of one or more host species, or vectors, to complete the life cycle, that may be absent in the new area (Clayton *et al.*, 2003; Ishtiaq *et al.*, 2010; Paterson *et al.*, 2003; Torchin *et al.*, 2003). Additionally, the number of individual parasites introduced and the distribution among founder hosts may influence the parasite persistence; low numbers of parasites or highly aggregated parasites among hosts have more likely to be "lost overboard" through stochastic events (Lockwood *et al.*, 2005; Poulin, 2007).

Such occurs with insular vertebrates' populations that often undergo a series of evolutionary changes (morphometric, life-history, behavioral, physiological and genetic) as result of isolation process, the parasite species capable persist in a new region, and apparently, they can also develop changes resulting from the insularity, termed parasite island syndromes. The term parasite island syndromes was firstly used by Pérez-Rodríguez *et al.* (2013) to refer to changes in the characteristics of haemoparasite assemblages of blackcap *Sylvia atricapilla* (Linnaeus, 1758) in the Macaronesia. They reported an impoverishment of haemosporidians assemblage of blackcap, with an approximate 90% loss of parasite richness known on mainland, absence of host specificity for the insular parasites and lower parasites prevalence in the insular populations. However, to our knowledge, the first empirical evidence of the use of the island syndrome concept to refer changes in a parasite species, was provided by Nieberding *et al.* (2006), when describing the loss of genetic diversity in the insular populations of *Heligmosomoides polygyrus* (Dujardin, 1845), a specific nematode of micromammals of the *Apodemus* genus. Moreover, Nieberding and collaborators also observed the ecological niche enlargement in Corsica, describing them on *Mus musculus* Linnaeus, 1758.

In addition to the evidences for the existence of some components of the island syndrome in nematode and blood parasite, respectively described by Nieberding *et al.* (2006) and Pérez-Rodríguez *et al.* (2013), other studies have shown proofs of the effect of insularity on parasite populations, although these differences were not always mentioned as syndrome.

Among the few existing studies, De Bellocq *et al.* (2002) reported a loss of helminth species richness in *Apodemus sylvaticus* (Linnaeus, 1758) of the Mediterranean Islands and significantly decrease of host specificity of helminth parasites infecting insular *A. sylvaticus*, *M. musculus* and *Clethrionomys glareolus* (Schreber, 1780), whereas no change in host specificity was recorded for the parasites infecting *Rattus rattus* (Linnaeus, 1758). Mas-Coma and Feliu (1984) and Mas-Coma *et al.* (1998, 2000) described a considerable impoverishment in the number of helminth species in micromammals on the Balearic Archipelago. Moreover, Mas-Coma and Feliu (1984) also showed that the host specificity of helminth parasites in some micromammals species tends to decrease on Balearic Islands compared with that on the nearby mainland. Fromont *et al.* (2001) found less parasite richness in feral cats that lived on Kerguelen Island, where only *Toxocara cati* (Schrank, 1788) was found, and its prevalence was higher on the Islands. In the case of haemoparasites, Ewen *et al.* (2012) and Barrientos *et al.* (2014) described similar results to those of Pérez-Rodríguez *et al.* (2013), namely, presence of globally generalist avian malaria parasites in the remote Island Archipelago of New Zealand, and loss of species richness and lower haemoparasites prevalence to *Bucanetes githagineus* (Lichtenstein, MHC, 1823) in the Canary Islands than mainland populations, respectively. Regarding ectoparasites, Wiggins *et al.* (1998) discovered that the level of fleas infestation in nests of *Parus major* Linnaeus, 1758 was highest on two Danish Islands and lowest on the mainland; and Barrientos *et al.* (2014) reported a higher prevalence of mites to *B. githagineus* in the Canary Islands than mainland (Iberian Peninsula and North Africa). More recently, Literák *et al.* (2015) presented results to chewing lice populations of blackcap from Azores Islands, describing the loss of chewing lice species richness in Islands, high host specificity of insular parasites and significantly higher parasites prevalence in the insular region.

Several hypotheses have been proposed to understand parasite island syndromes. The low genetic diversity in insular populations was explained by Nieberding *et al.* (2006) as a founder effect, *i.e.*, Island colonization is usually carried out by a small number of individuals and thus, provide a loss of part of the continental genetic pool (Frankham, 1997). From the same point of view, Fromont *et al.* (2001) argued that the absence of parasites species in the founder group or the species' inability to persist in Islands, due to low density of hosts at the beginning of

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colonization may explain in part, the loss of species richness in Islands. Additionally, they suggested that the life cycle complexity, such as the absence of intermediate hosts needed to complete the life cycle or unfavorable temperature, for parasites that need soil maturation, are hypotheses for the low number of parasite species in Islands.

The complexity of the life cycle has also been suggested by other authors to explain the altered prevalence patterns of parasites. For instance, the lower prevalence of haemoparasites in insular populations were attributed in part to the reduced availability of appropriate haemoparasites vectors on Islands (Barrientos *et al*., 2014; Pérez-Rodríguez *et al*., 2013). Pérez-Rodríguez *et al*. (2013) further argued that this may be particularly relevant to the many specific haplotypes known from continental population usually associated to vector specific, often absent from Islands, and thus, this would explain the absence of these specific parasites there. In the case of ectoparasites (mites and chewing lice), direct transmission is a highly effective way of dispersal of these arthropods. Thus, Literák *et al*. (2015) proposed that the differences of chewing lice prevalence patterns between Island and mainland populations may be correlated with ecological traits. For instance, the risk of parasite infestation usually host-density dependent tend to increase on the Islands (Dobson, 1988; Fromont *et al*., 2001). In addition to the host density, Fromont *et al*. (2001) suggested other hypothesis for this syndrome in helminths populations: i) the low parasite species richness on Islands should result in low competition between species and, thus, allow parasites that persist on the Islands to be more abundant; and ii) the loss of heterozygosity on Islands, as the result of the founder effect, make the inbred populations particularly more susceptible to parasite infestations (Meagher, 1999).

In general, chewing lice are considered host-specific parasites and the presence of not-specific chewing lice usually are associated to host-switching events between unrelated hosts (Johnson and Clayton, 2003; Paterson *et al*., 2003). Thus, Literák *et al*. (2015) questioned themselves whether it was mainland birds to acquire unspecific species or if insular birds lost these species through a "missing the boat event". In the case of the ecological niche enlargement of *H. polygyrus* in Corsica, although Nieberding *et al*. (2006) does not provided an explanation for this event, since the infestation of other rodent species by these nematode is extremely rare on the mainland, they argued that *H. polygyrus* underwent an enlargement of its ecological niche during the Turkish *A. sylvaticus* colonization of Corsica.

In this way, it is evident that normal mainland host–parasite associations are compromised on Islands and that this insular syndrome can assume different patterns (species richness, prevalence and host specificity) always dependent on the group of parasites. Whereas these

patterns have been observed in hosts with and without long-term isolation, Pérez-Rodríguez *et al*. (2013) argued that parasite island syndromes "evolve even before insular host populations become completely isolated from their mainland counterparts".

1.4. Theory of Island Biogeography: key-factor to island syndrome?

The Island area and isolation are two Islands characteristics, referred by MacArthur and Wilson (1967) in their book "Theory of Island Biogeography", as two key-factors for the species richness found on Islands, as previously mentioned (Subchapter 1.1. Island as natural laboratories of evolutionary experimentation). More recently, these two Islands' characteristics, have been proposed as determining features in the evolutionary differentiation verified between insular and continental populations, as occurs in the island syndrome of vertebrate and parasite populations.

Adler and Levins (1994) in their synthetic work on Island rodent populations developed a conceptual model, using Island area and isolation, to explain the insular syndrome described to rodents, namely, higher and more stable densities, better survival, increased body mass, and reduced aggressiveness, reproductive output, and dispersal (Figure 1.1).
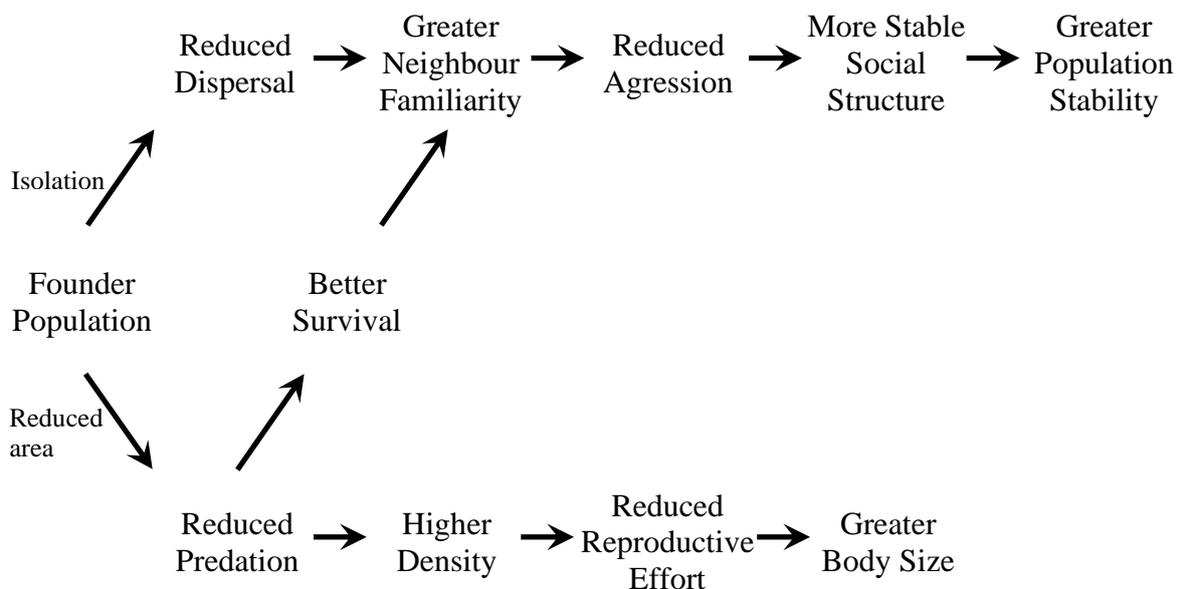
Figure 1.1 – Schematic diagram showing the initial short-term effects of Island isolation and area on rodent populations. These short-term effects may be reaction norms in an environment of increased density, as well as initial micro-evolutionary responses to novel selection pressures. Long-term effects of insularity are directional selection for increased body size, reduced reproductive output, and reduced aggression. Redrawn from Adler and Levins (1994).

According to this model, the rodent population density and other manifestations of the island syndrome are predicted, in general, to increase with Island isolation and to decrease with increase of Island area, and may even disappear altogether if Islands are relatively large *i.e.* mainland-like. Adler and Levins (1994) argued that "the effect of isolation is direct by limiting dispersal, while the effect of area is less direct", suggesting that density-depressing factors, such as, predators, competitors, and habitat complexity, are primarily responsible for the effect of area, since, as the area increases, these factors also increase.

Following this reflection, given that the "effects of immigrant selection should vary with isolation, and that those of ecological release and resource limitation should vary with area, isolation and diversity of insular communities", Lomolino (2005), suggested that "body size of insular populations of a particular species should be correlated with area and isolation of the Islands it inhabits". His hypothesis was supported by the results of several studies, where such correlations have been reported for insular vertebrates – see Table 3 from Lomolino (2005). For instance, body size of the Asian tri-colored squirrel *Callosciurus prevostii* (Desmarest, 1822) increases with Island area up to about 10.000 Km^2. On Islands larger than this, more closely resembling a mainland area, body size decreases significantly (Heaney, 1978). This author developed a model with the major factors which may affect the body size of insular mammals (predation, competition, food limitation, and physiological efficiency) and stated that interspecific competition is most important on large Islands, while food limitation is most important on small Islands. This finding is consistent with McNab (2002) results, where the basal metabolic rates are lowest for populations from smaller Islands, *i.e.* where resources should be more limited. Contrary, body size of the Asian tri-colored squirrel was negatively correlated with distance to mainland or nearest large Island, *i.e.*, body size decreases with increasing isolation (Heaney, 1978). This result was interpreted by the author as an indirect effect of the Island area, since in general, more distant Islands tend to be smaller in area, "due to sloping off the continental shelf". Moreover, Anderson and Handley (2002) predicted that the higher immigration rates occur on closer Islands to the mainland, an idea postulated by the Theory of Island Biogeography of MacArthur and Wilson (1963), and may regulate the rates of evolution of body size "by differentially diluting in situ evolution on closer Islands with colonists from the mainland".

The area and isolation of Islands have been associated with other syndromes of insular vertebrates, such as change in fecundity patterns. The change in fecundity patterns that is mainly manifested by small clutch-size, according to the study of Higuchi (1976), the clutch-size of

Sittiparus varius (Temminck & Schlegel, 1845) decrease with increasing isolation and with decreasing Island area. Although the author did not present a clear explanation for the effect of area and isolation of Islands, he suggested that "the combined effects of the density factor and the size factor (and unknown factors)" might be responsible for reduction of the clutch-size.

More recently, the area and isolation of Islands, one of the mainstays of insular ecological studies, have been incorporated into parasite island syndromes analysis. The reduced haemoparasite species richness in the Macaronesia previously mentioned (Subchapter 1.3.2. Parasite Island Syndromes), was negatively correlated with Island distance from the continent (Pérez-Rodríguez *et al.*, 2013). These authors proposed three mechanisms, not mutually exclusive, that can explain this correlation: i) sequential population bottlenecks in the haemoparasites associated with the blackcaps colonization, *i.e.*, initial colonization of the Eastern Islands (closer to the mainland) and then spreading to the Western Islands, or multiple colonization events in the Eastern Islands more frequent; ii) the seasonal flow of migratory blackcaps on the Islands closest to the mainland, favors the recurrent parasite colonization; or iii) the pattern of colonization of the Islands by generalist parasites transported from African continent, apparently follows the east-west direction. Nieberding *et al.* (2006) also described a negative correlation between isolation of Islands and nematode *H. polygyrus* haplotype diversity. According to the authors, this result was surprising due the colonization pattern not natural of Mediterranean Islands by *H. polygyrus* and its host, *i.e.*, it had anthropogenic influence. Moreover, De Bellocq *et al.* (2002) showed similar correlation between parasite species richness of *A. sylvaticus* and degree of insularity. Regardless of the mechanisms involved, these correlations supports the basic prediction of Theory of Island Biogeography that the equilibrium between extinction and immigration rates, favors greater species richness on Islands located near the putative source (MacArthur and Wilson, 1967).

In contrast, no effect of the Island isolation were recorded by Ishtiaq *et al.* (2010) and Literák *et al.* (2015) in the insular species richness of avian haematozoan parasites and chewing lice, respectively. Although these results do not support the tenet of Theory of Island Biogeography previously mentioned, Ishtiaq *et al.* (2010) suggested that could be a result to the immigration rates of haemoparasites, which are closely dependent on the presence of vertebrates and invertebrate hosts.

Regarding the effect of the Island area on the parasite species richness, Ishtiaq *et al.* (2010) described a positive correlation between *Plasmodium* lineage richness and Island size. This species-area relationship does not fit the predictions that specialist parasites have a more

restricted geographical distribution relative to generalists, based on the idea that they rare host-switching. No significant correlation between species richness of parasites and Island area was observed to avian haemaparasites, chewing-lice and nematodes from Macaronesian (Literák *et al*., 2015; Nieberding *et al*., 2006; Pérez-Rodríguez *et al*., 2013).

Whatever the mechanisms involved and although the studies presented did not show fully concordant results, the importance of the area and the degree of isolation of the Islands in the development of parasite insular syndromes, such as parasite species richness, became evident.

1.5. Ectoparasites behavior

Parasites, by definition, are smaller organisms that live in an intimate relationship with larger organisms called the hosts. According to Price (1980) "parasites form a large proportion of the diversity of life on earth". Among them, a relatively small number of arthropod species of the classes Arachnida (mites and ticks) and Insecta (Diptera, fleas and lice), described as ectoparasites of major veterinary importance, developed the ability to parasitize other life forms, such as birds, typically living on or burrowing "into the surface of their host's epidermis" (Wall and Shearer, 2001). Although it is unknown when the host parasite relationship evolved, it is known that parasites and hosts developed co-adaptive behavioral, physiological and morphological characteristics (Price, 1980). This co-adaptation allowed ectoparasites to: i) specialize in certain locations of the host, on the basis of morphology and the way they avoid the host grooming (Johnson and Clayton, 2003); and ii) synchronize the timing of the development, in the blood-feeding lice with timing of birds breeding, and thus, potentiate the transmission of parasites to the offspring (Forster, 1969).

On the other hand, avian hosts have developed reciprocal strategies in order to directly reduce the impact of ectoparasites on their bodies, namely: i) feathers molt may help reduce arthropod ectoparasites loads (Ash, 1960); ii) melanin rich feathers apparently are more resistant to mechanical abrasion, limiting the damage by feather feeding ectoparasites (Bonser, 1995; Kose *et al*., 1999); iii) toxins, specially batrachotoxins, present in the plumage and muscle tissue of some bird species repels and kills ectoparasites due high toxicity and make these feathers less attractive for ectoparasites to feed (Dumbacher, 1999; Dumbacher and Pruett-Jones, 1996); iv) uropygial oil, known to help maintain the strength and flexibility of the birds' feathers, have been proposed to help in the combat against ectoparasites by reducing their mobility, repelling or killing them (Dumbacher and Pruett-Jones, 1996; Moyer *et al*., 2003); v) self-preening, the

most common feather cleaning behavior in birds is extremely relevant in the control of ectoparasites (Clayton, 1991). For instance, birds with deformed mandibles have a reduced efficiency of self-preening resulting in high ectoparasites infestations (Clayton *et al*., 1999); vi) allopreening and scratching, which consists of plumage preen one another and self-scratch with the feet, respectively, allows birds to control ectoparasites on the body regions that are impossible to self-preen, such as the head and neck (Clayton, 1991; Murray, 1990); vii) sunning is thought to help control ectoparasites, by killing or making them more vulnerable to preening (Moyer and Wagenbach, 1995). Moreover, bathing and dusting are also thought to control ectoparasites, although no direct effect of this behavior on ectoparasites has ever been tested (Clayton *et al*., 2010); viii) anting behavior, in which birds allow ants to crawl through their feathers or birds themselves crush and smear ants on their plumage, lead to secretion of acid formic by ants and, thus, may kill or deter ectoparasites (Clayton and Wolfe, 1993); ix) cosmetic coloration, which consists of self-application of substances in the bodies of birds, such as, soil stained with iron oxide, which have been suggested to help combat ectoparasites due the oxidative properties of these substances (Negro *et al*., 1999).

In addition to the grooming behavior of their bodies, birds have developed nest maintenance behaviors that may help control ectoparasites (especially, fleas, flies, true bugs, mites and ticks), such as: i) territoriality behavior, choose parasite-free nest and nesting in smaller colonies, may be beneficial for the ectoparasites control, because their transmission occurs mainly during the nesting season (Brown and Brown, 1986; Møller *et al*., 1993); ii) nest sanitation may represent an adaptive advantage in the control of ectoparasites, by removing nest material (Clayton *et al*., 2010); iii) nest fumigation with aromatic vegetation, may act as insecticidal agent, due to the volatile chemical components present in plants (Clark and Russell Mason, 1988); iv) heterospecific cleaning, which consists of nest cleaning interactions between different species, allows some species of birds to tolerate and benefit from the "adopted" species by them feed on their ectoparasites (Smith, 1968). When all nest maintenance strategies fail, birds can simply assume a more drastic strategy, namely the desertion of parasitized nest, which can occur on a large scale (Brown and Brown, 1986; Duffy, 1983).

1.5.1. Chewing lice

Chewing lice (Phthiraptera) are small and permanent ectoparasites of birds and some mammals, belonging to three suborders: Amblycera and Ischnocera – most species are parasites of birds;

and Rhynchophthirina – parasites of mammals. The Amblycera and Ischnocera suborders are distinguished morphologically and by the composition of their diet. Morphologically they are distinguished by: i) antennal segments – 4 segments protected in lateral grooves, where 3 are pedunculated in the case of Amblycera and 3-5 fully exposed filiform segments in Ischnocera species; ii) maxillary palps – only present in Amblycera species; iii) movement of the mouthparts – vertical move in Amblycera species and horizontal in Ischnocera species; and iv) thoracic segments – mesothorax and metathorax well divided in Amblycera, while Ischnocera have mesothorax and metathorax fused forming a pterothorax. Regarding the composition of their diet, Amblycera species feeds mainly on skin, blood and feather tip, while Ischnocera is based on feathers and dead skin (Clayton *et al.*, 2008; Price *et al.*, 2003).

Bird lice are well documented and comprise about 4.000 species. Chewing lice have the ability to complete their entire life cycle in the body of the host and most lice are highly bird' host specific. These characteristics condition the geographical distribution of the lice, which often corresponds to the distribution of the hosts (Price *et al.*, 2003).

Transmission of chewing lice is facilitated by physical direct contact between hosts, mainly between copulating birds and between parents and their offspring (Hillgarth, 1996; Tompkins *et al.*, 1996). In addition, according to Keirans (1975) some ischnoceran species are capable transported in phoretic association with hippoboscid flies, due to the horizontal orientation of their mouthparts. However, according to Clayton *et al*. (2004), the less host-specific of some ischnoceran lice can be explained in part by the phoretic association with hippoboscid flies.

1.5.2. Hippoboscid flies

Members of the family Hippoboscidae known as hippoboscid flies, louse flies or keds are obligatory blood-sucking ectoparasites of mammals and birds. Morphologically, adult flies are dorsoventrally flattened, with a depressed head, a strongly sclerotized proboscis and a hind pair of wings, which have considerable flight capacity. However some louse fly species lack wings or have vestigial wings, making them unable to fly (Reeves and Lloyd, 2019). Hippoboscid flies are larviparous, with larval development occurring in uterus of female. When the larvae are fully developed, pre-pupae are deposited or dropped in the proximity to the hosts, especially in or around the birds' nest and mammals' hair, where the cycle is completed (Hutson, 1984; Maa and Peterson, 1987).

To date, there are about 213 known louse flies, of which 75% of species are parasites of birds (Dick, 2006). Hippoposcid fly fauna have a worldwide distribution but is more rich in the tropical and subtropical regions (Maa and Peterson, 1987). Contrary to the high host specificity of most chewing lice, the host specificity of louse flies varies among different species, which include hippoboscid species restricted to a single host species, species restricted to closely related species and species more generalists to the host (Hutson, 1984; Reeves and Lloyd, 2019).

Transmission of louse flies is facilitated by the flying ability of most species. However, for those species of flies where flightlessness is a common feature, transmission occurs mostly at nest sites (Hutson, 1984). This evidence becomes clearer at colonial nesting sites, where according to Hutson (1981), *Cretaerina* species do not need wings to find and parasitize swifts.

1.5.3. Fleas

Fleas (Siphonaptera) are obligate hematophagous ectoparasites of birds and mammals. Adult fleas are morphologically small, laterally flattened, strongly chitinized, wingless and with strongly developed hind legs, highly specialized for jumping. As typical holometabolous insects, the flea life cycle typically consists of an egg deposited in the fur or the surroundings of the host, such as in the nest, where they hatch into a larval (typically undergoes three larval molts) and a pupal stage. The life cycle is completed when the adult fleas emerge from the cocoon being immediately ready for a blood meal (Krasnov, 2008; Marshall, 1981).

Worldwide, approximately 2.500 flea species belonging to 238 genera are known. Most fleas have been described as parasitic on mammals, while only 6% of the total numbers of flea species are ornithophilic, suggesting that birds are alternative flea hosts (Durden and Hinkle, 2019). This idea was corroborated by recent work of Whiting *et al*. (2008), who observed that mammals were primary hosts of fleas and only later in flea evolution developed the ability to parasitize birds. The geographical distribution of fleas extends to all continents and most Oceanic Islands (Krasnov, 2008).

Although fleas can be contact-transmitted, they are mainly parasites active in host-seeking. As a result, most flea species are rarely host-species specific, exploring a wide range of hosts (Krasnov, 2008). However, some flea species exhibit specificity for particular species or host groups (Bitam *et al*., 2010). In the case of bird fleas, according to Tripet *et al*. (2002) colonial

birds with highly aggregated nests not only tend to harbor more flea species, but fleas are also more specialized than those infesting nests of territorial birds.

1.6. Thesis objectives and structure

1.6.1. Objectives

So far, the study of parasite island syndromes associated to parasites of birds has mainly focused on haemoparasites of hosts species of the Macaronesian Islands. Improving knowledge regarding parasite island syndromes is important to understanding the processes involved in the evolution of insular parasites populations and so, understand and possibly predict the ecological risks of the parasites introduction in new areas.

Thus, the general objectives of this work were:

i) to evaluate the ectoparasite diversity of four Passeriformes species with dissimilar colonization times of Macaronesian Islands, Eurasian blackbird *Turdus merula* Linnaeus, 1758, Eurasian blackcap, *Sylvia atricapilla* (Linnaeus, 1758), common chaffinch *Fringilla coelebs* Linnaeus, 1758 and European robin *Erithacus rubecula* (Linnaeus, 1758), from mainland Portugal and three Islands of Azores Archipelago (São Miguel, Terceira and Flores);

ii) to understand whether insular communities of ectoparasites vary geographically, testing predictions derived from parasite island syndromes, specifically.

The specific objectives were:

a) evaluate whether island host populations have lower parasite richness than the mainland one;

b) evaluate whether insular birds' populations have higher ectoparasites prevalence than the mainland one;

c) determine which is the host specificity pattern of the parasites found on the Islands;

d) lastly, aiming at understanding and apply the Theory of Island Biogeography to avian ectoparasites, the correlation of parasite richness with the Islands area and their proximity to the continent was assessed.

1.6.2. Thesis Structure

In addition to the general introduction chapter to topics focused on scientific communications (Chapter 1), this thesis compiles three articles (one published and two submitted) each corresponding to a chapter (Chapter 2-4), where the parasite island syndromes of different groups of ectoparasites, are studied. Additionally, in Chapter 5 a general discussion is presented, where the most relevant results of the work are integrated and discusses; a brief note is also provided where the future focus of parasite island syndromes research is envisioned.

The specific objectives of Chapters 2 to 4 were as follow:

Chapter 2

Tomás, A., Fonseca, I.P. da, Valkenburg, T., Rebelo, M.T. (2021) Louse flies in Azorean and mainland populations of four Passeriformes species: a new perspective to parasite island syndromes. *International Journal for Parasitology: Parasites and Wildlife*, 14: 33-40

In this chapter, the louse fly populations of Eurasian blackbird, Eurasian blackcap, common chaffinch and European robin from Azores Islands and mainland Portugal were identified, to understand the main patterns of island syndrome (species richness, prevalence and host specificity) of these highly mobile and obligate blood-sucking parasites and to assess the effect of Island area and isolation degree on these syndromes, especially on species richness.

Chapter 3

Tomás, A., Fonseca, I.P. da, Valkenburg, T., Rebelo, M.T. Parasite island syndromes in the context of nidicolous ectoparasites: fleas (Insecta: Siphonaptera) in wild passerine birds from Azores Archipelago. *Submitted to Parasitology International*

The flea communities of Eurasian blackbird, Eurasian blackcap, common chaffinch and European robin from Azores Islands and mainland Portugal were assessed, to understand the patterns of island syndrome (species richness, prevalence and host specificity) of these nidicolous and obligate hematophagous parasites and to assess the effect of island area and isolation on these syndromes, especially on species richness.

Chapter 4

Tomás, A., Fonseca, I.P. da, Valkenburg, T., Rebelo, M.T. Parasite island syndromes in the context of ectoparasites highly host specific: chewing lice (Phthiraptera: Ischnocera and Amblycera) in wild Azorean and Portugal mainland populations of four passerine species. *Submitted to Medical and Veterinary Entomology*

In this chapter, the chewing louse species of Eurasian blackbird, Eurasian blackcap, common chaffinch and European robin from Azores Islands and mainland Portugal were studied, to understand the main patterns of island syndrome (species richness, prevalence and host specificity) of these permanent and obligate parasites and to evaluate the effect of island area and isolation degree on these syndromes.

References

Adler, G.H., Levins, R., 1994. The Island Syndrome in Rodent Populations. Q. Rev. Biol. 69, 473–490. doi:10.1086/418744

Alcover, J.A., Pieper, H., Pereira, F., Rando, J.C., 2015. Five new extinct species of rails (Aves: Gruiformes: Rallidae) from the Macaronesian Islands (North Atlantic Ocean). Zootaxa 4057, 151–190. doi:10.11646/zootaxa.4057.2.1

Anderson, R.P., Handley, C.O., 2002. Dwarfism in insular sloths: Biogeography, selection, and evolutionary rate. Evolution (N. Y). 56, 1045–1058. doi:10.1111/j.0014-3820.2002.tb01415.x

Ash, J.S., 1960. A study of the Mallophaga of birds with particular reference to their ecology. Ibis (Lond. 1859). 102, 93–110. doi:10.1111/j.1474-919X.1960.tb05095.x

Ashmole, N.P., 1963. The Regulation of Numbers of Tropical Oceanic Birds. Ibis (Lond. 1859). 103 b, 458–473. doi:10.1111/j.1474-919X.1963.tb06766.x

Barrientos, R., Valera, F., Barbosa, A., Carrillo, C.M., Moreno, E., 2014. Biogeography of haemo- and ectoparasites of an arid-land bird, the Trumpeter finch. J. Arid Environ. 106, 11–17. doi:10.1016/j.jaridenv.2014.03.005

Bitam, I., Dittmar, K., Parola, P., Whiting, M.F., Raoult, D., 2010. Fleas and flea-borne diseases. Int. J. Infect. Dis. 14, 667–676. doi:10.1016/j.ijid.2009.11.011

Blondel, J., 1985. Breeding Strategies of the Blue Tit and Coal Tit (*Parus*) in Mainland and Island Mediterranean Habitats: A Comparison. J. Anim. Ecol. 54, 531. doi:10.2307/4497

Blondel, J., 2000. Evolution and ecology of birds on islands: Trends and prospects. Vie Milieu 50, 205–220.

Bonser, R., 1995. Melanin and the Abrasion Resistance of Feathers. Condor 97, 590–591. doi:10.2307/1369048

Brown, C.R., Brown, M.B., 1986. Ectoparasitism as a Cost of Coloniality in Cliff Swallows

(*Hirundo Pyrrhonota*). Ecology 67, 1206–1218. doi:10.2307/1938676

Brown, P., Sutikna, T., Morwood, M.J., Soejono, R.P., Jatmiko, Saptomo, E.W., Due, R.A., 2004. A new small-bodied hominin from the Late Pleistocene of Flores, Indonesia. Nature 431, 1055–1061. doi:10.1038/nature02999

Carson, H.L., 1983. Chromosomal Sequences and Interisland Colonizations in Hawaiian *Drosophila*. Genetics 103, 465–482. doi:10.1093/genetics/103.3.465

Carson, H.L., 1992. Genetic change after colonization. GeoJournal 28, 297–302. doi:10.1007/BF00177245

Carson, H.L., Lockwood, J.P., Craddock, E.M., 1990. Extinction and recolonization of local populations on a growing shield volcano. Proc. Natl. Acad. Sci. U. S. A. 87, 7055–7057. doi:10.1073/pnas.87.18.7055

Catry, P., Costa, H., Elias, G., Matias, R., 2010. Aves de Portugal. Ornitologia do território continental, 1st Ed. Assírio & Alvim, Lisboa. 944 pp. ISBN: 978-972-37-1494-4

Clark, L., Russell Mason, J., 1988. Effect of biologically active plants used as nets material and the derived benefit to starling nestlings. Oecologia 77, 174–180. doi:10.1007/BF00379183

Clayton, D., Lee, P., Tompkins, D., Brodie III, E.D., 1999. Reciprocal Natural Selection on Host-Parasite Phenotypes. Am. Nat. 154, 261–270. doi:10.1086/303237

Clayton, D.H., 1991. Coevolution of avian grooming and ectoparasite avoidance. In: Zuk, M., Loye, J.E. (Eds.), Bird-Parasite Interactions: Ecology, Evolution, and Behaviour. Oxford University Press, Oxford, pp. 258–289. ISBN: 978-0198577386

Clayton, D.H., Adams, R.J., Bush, S.E., 2008. Phthiraptera, the Chewing Lice. In: Thomas, N.J., Hunter, D.B., Atkinson, C.T. (Eds.), Parasitic Diseases of Wild Birds. Wiley-Blackwell, Iowa, pp. 515–526. ISBN: 978-0-8138-2081-1

Clayton, D.H., Al-Tamimi, S., Johnson, K.P., 2003. The ecological basis of coevolutionary history. In: Page, R.D.M. (Ed.), Tangled Trees: Phylogeny, Cospeciation and Coevolution. The University of Chicago Press, Chicago, pp. 310–341. ISBN: 0-226-64467-7

Clayton, D.H., Bush, S.E., Johnson, K.J., 2004. Ecology of Congruence: Past Meets Present. Syst. Biol. 53, 165–173. doi:10.1080/10635150490265102

Clayton, D.H., Koop, J.A.H., Harbison, C.W., Moyer, B.R., Bush, S.E., 2010. How Birds Combat Ectoparasites. Open Ornithol. J. 3, 41–71. doi:10.2174/1874453201003010041

Clayton, D.H., Wolfe, N.D., 1993. The adaptive significance of self-medication. Trends Ecol. Evol. 8, 60–63. doi:10.1016/0169-5347(93)90160-Q

Crowell, K.L., 1962. Reduced Interspecific Competition among the Birds of Bermuda. Ecology 43, 75–88. doi:10.2307/1932042

Darwin, C., 2008a. The Voyage of the Beagle, Reprint Ed. Dodo Press, Gloucester. 468 pp. ISBN: 9781406520019

Darwin, C., 2008b. On the Origin of Species, Revised Ed. Oxford University Press, New York. 432 pp. ISBN: 978-0-19-921922-3

Darwin, C., 2009. Journal of Researches into the Geology and Natural History of the Various Countries Visited by H. M. S. Beagle, Reprint Ed. Cambridge University Press, Cambridge. 637 pp. ISBN: 9780511693342. doi:10.1017/CBO9780511693342

De Bellocq, J.G., Morand, S., Feliu, C., 2002. Patterns of parasite species richness of Western Palaeartic micro-mammals: Island effects. Ecography (Cop.). 25, 173–183. doi:10.1034/j.1600-0587.2002.250205.x

Diamond, J., 1991. A New Species of Rail from the Solomon Islands and Convergent Evolution of Insular Flightlessness. Auk 108, 461–470. doi:10.2307/4088088

Dick, C., 2006. Checklist of world Hippoboscidae (Diptera: Hippoboscoidea). Dep. Zool. F. Museum Nat. Hist. Chicago.

Dietzen, C., Garcia-Del-Rey, E., Castro, G.D., Wink, M., 2008. Phylogenetic differentiation of *Sylvia* species (Aves: Passeriformes) of the Atlantic islands (Macaronesia) based on mitochondrial DNA sequence data and morphometrics. Biol. J. Linn. Soc. 95, 157–174. doi:10.1111/j.1095-8312.2008.01005.x

Dietzen, C., Witt, H.-H., Wink, M., 2003. The phylogeographic differentiation of the European robin *Erithacus rubecula* on the Canary Islands revealed by mitochondrial DNA sequence data and morphometrics: evidence for a new robin taxon on Gran Canaria? Avian Sci. 3, 115–132.

Dobson, A.P., 1988. Restoring Island Ecosystems: The Potential of Parasites to Control Introduced Mammals. Conserv. Biol. 2, 31–39.

Duffy, D.C., 1983. The ecology of tick parasitism on densely nesting Peruvian seabirds. Ecology 64, 110–119. doi:10.2307/1937334

Dumbacher, J.P., 1999. Evolution of toxicity in pitohuis: I. Effects of homobatrachotoxin on

chewing lice (order Phthiraptera). Auk 116, 957–963. doi:10.2307/4089675

Dumbacher, J.P., Pruett-Jones, S., 1996. Avian Chemical Defense. In: Nolan, V., Ketterson, E.D. (Eds.), Current Ornithology. Springer, Boston, MA, pp. 137–174. ISBN: 978-1-4615-5881-1. doi:10.1007/978-1-4615-5881-1_4

Durden, L.A., Hinkle, N.C., 2019. Fleas (Siphonaptera). In: Mullen, G.R., Durden, L.A. (Eds.), Medical and Veterinary Entomology. Elsevier, London, pp. 145–169. ISBN: 978-0-12-814043-7. doi:10.1016/b978-0-12-814043-7.00010-8

Ewen, J.G., Bensch, S., Blackburn, T.M., Bonneaud, C., Brown, R., Cassey, P., Clarke, R.H., Pérez-Tris, J., 2012. Establishment of exotic parasites: The origins and characteristics of an avian malaria community in an isolated island avifauna. Ecol. Lett. 15, 1112–1119. doi:10.1111/j.1461-0248.2012.01833.x

Forster, M.S., 1969. Synchronized Life Cycles in the Orange-Crowned Warbler and Its Mallophagan Parasites. Ecology. doi:10.2307/1934858

Foster, J.B., 1964. Evolution of Mammals on Islands. Nature 202, 234–235. doi:10.1038/202234a0

França, Z., Cruz, J.V., Nunes, J.C., Forjaz, V.H., 2003. Geologia Dos Açores: Uma Perspectiva Actual. Açoreana 10, 11–140.

Frankham, R., 1997. Do island populations have less genetic variation than mainland populations? Heredity. 78, 311–327. doi:10.1038/hdy.1997.46

Fromont, E., Morvilliers, L., Artois, M., Pontier, D., 2001. Parasite richness and abundance in insular and mainland feral cats: Insularity or density? Parasitology 123, 143–151. doi:10.1017/S0031182001008277

Gaspar, J.L., Queiroz, G., Ferreira, T., Medeiros, A.R., Goulart, C., Medeiros, J., 2015. Earthquakes and volcanic eruptions in the Azores region: geodynamic implications from major historical events and instrumental seismicity. In: Gaspar, J.L., Guest, J.E., Duncan, A.M., Barriga, F.J.A.S., Chester, D.K. (Eds.), Volcanic Geology of São Miguel Island (Azores Archipelago). Geological Society, London, Memoirs, pp. 33–49. doi:10.1144/M44.4

Grant, P.R., 1980. Colonization of Atlantic islands by chaffinches (*Fringilla* sp.). Bonner Zool. Beiträge. 31, 311-317

Heaney, L.R., 1978. Island area and body size of insular mammals: evidence from the tri-

colored squirrel (*Calliosciurus prevosti*) of Southwest Africa. Evolution (N. Y.). 32, 29–44. doi:10.1111/j.1558-5646.1978.tb01096.x

Higuchi, H., 1976. Comparative study on the breeding of mainland and island subspecies of the varied tit, *Parus varius*. Tori 25, 11–20.

Hillgarth, N., 1996. Ectoparasite Transfer during Mating in Ring-Necked Pheasants *Phasianus colchicus*. J. Avian Biol. 27, 260. doi:10.2307/3677232

Hounsome, M. V., 1993. Biometrics and origins of some atlantic island birds. Bol. do Mus. Munic. do Funchal. 2, pp. 107-129

Hutson, A.M., 1981. The population of the louse-fly, *Crataerina pallida* (Diptera, Hippoboscidae) on the European swift, *Apus apus* (Aves, Apodidae). J. Zool. 194, 305–316. doi:10.1111/j.1469-7998.1981.tb04583.x

Hutson, A.M., 1984. Keds, Flat-flies and Bat-Flies: Diptera, Hippoboscidae and Nycteribiidae. Handbooks Identif. Br. Insects 10, 1–40.

Ishtiaq, F., Clegg, S.M., Phillimore, A.B., Black, R.A., Owens, I.P.F., Sheldon, B.C., 2010. Biogeographical patterns of blood parasite lineage diversity in avian hosts from southern Melanesian islands. J. Biogeogr. 37, 120–132. doi:10.1111/j.1365-2699.2009.02189.x

Johnson, K.P., Clayton, D.H., 2003. The biology, ecology, and evolution of chewing lice. In: Price, R.D., Hellenthal, R.A., Palma, R.L., Johnson, K.P., Clayton, D.H. (Eds.), The Chewing Lice: World Checklist and Biological Overview. Illinois Natural History Survey Special Publication, Illinois, pp. 449–476. ISBN: 1-882932-08-0

Keirans, J.E., 1975. A review of the phoretic relationship between Mallophaga (Phthiraptera: Insecta) and Hippoboscidae (Diptera: Insecta). J. Med. Entomol. 12, 71–76. doi:10.1093/jmedent/12.1.71

Kose, M., Mänd, R., Møller, A., 1999. Sexual selection for white tail spots in the barn swallow in relation to habitat choice by feather lice. Anim. Behav. 58, 1201–1205. doi:10.1006/anbe.1999.1249

Krasnov, B.R., 2008. Functional and evolutionary ecology of fleas: A model for ecological parasitology, 1st Ed. Cambridge University Press, Cambridge. 593 pp. ISBN: 978-0-521-88277-4. doi:10.1017/CBO9780511542688

Kunkel, G., 1999. Die Kanarischen Inseln und ihre Pflanzenwelt (In German), 3rd Ed.

Spektrum Akademischer Verlag. 239 pp. ISBN: 9783437303111

Lack, D., 1942. Ecological Features of the Bird Faunas of British Small Islands. J. Anim. Ecol. 11, 9. doi:10.2307/1298

Lack, D., 1947. The Significance of Clutch-size. Ibis (Lond. 1859). 89, 302–352. doi:10.1111/j.1474-919X.1947.tb04155.x

Literák, I., Sychra, O., Resendes, R., Rodrígues, P., 2015. Chewing Lice in Azorean Blackcaps (*Sylvia atricapilla*): A Contribution to Parasite Island Syndromes. J. Parasitol. 101, 252–254. doi:10.1645/14-601.1

Lobin, W., 1982. Untersuchung über Flora, Vegetation und biogeographische Beziehungen der Kapverdischen Inseln (In German). Cour. Forschungsinstitut Senckenb. 53, 1–112.

Lockwood, J.L., Cassey, P., Blackburn, T., 2005. The role of propagule pressure in explaining species invasions. Trends Ecol. Evol. 20, 223–228. doi:10.1016/j.tree.2005.02.004

Lomolino, M.V., 1984. Immigrant selection, predation, and the distributions of *Microtus pennsylvanicus* and *Blarina brevicauda* on islands. Am. Nat. 123, 468–483. doi:10.1086/284217

Lomolino, M.V, 1985. Body Size of Mammals on Islands: The Island Rule Reexamined. Am. Nat. 125, 310–316. doi:10.1086/284343

Lomolino, M.V., 2005. Body size evolution in insular vertebrates: Generality of the island rule. J. Biogeogr. 32, 1683–1699. doi:10.1111/j.1365-2699.2005.01314.x

Losos, J.B., Ricklefs, R.E., 2009. Adaptation and diversification on islands. Nature 457, 830–836. doi:10.1038/nature07893

Maa, T., Peterson, B., 1987. Hippoboscidae. In: McAlpine, J. (Ed.), Manual of Neartic Diptera - Volume 2. Agriculture Canada Monograph No. 28, pp. 1271–1281.

MacArthur, R.H., Diamond, J.M., Karr, J.R., 1972. Density Compensation in Island Faunas. Ecology 53, 330–342. doi:10.2307/1934090

MacArthur, R.H., Wilson, E.O., 1963. An Equilibrium Theory of Insular Zoogeography. Evolution (N. Y). 17, 373. doi:10.2307/2407089

MacArthur, R.H., Wilson, E.O., 1967. The Theory of Island Biogeography, 1st Ed. Princeton University Press, Princeton. 215 pp. ISBN: 9780691080505

MacLeod, C.J., Paterson, A.M., Tompkins, D.M., Duncan, R.P., 2010. Parasites lost - do

invaders miss the boat or drown on arrival? Ecol. Lett. 13, 516–527. doi:10.1111/j.1461-0248.2010.01446.x

Marshall, A., 1981. The Ecology of Ectoparasitic Insects. Academic Press, London. 459 pp. ISBN: 0124740804.

Mas-Coma, S., Esteban, J.G., Fuentes, M.V., Bargues, M.D., Valero, M.A., Galan-Puchades, M.T., 1998. Helminth parasites of small mammals (insectivores and rodents) on the Pityusic Island of Formentera (Balearic Archipelago). Res. Rev. Parasitol. 58, 125–133.

Mas-Coma, S., Esteban, J.G., Fuentes, M.V., Bargues, M.D., Valero, M.A., Galan-Puchades, M.T., 2000. Helminth parasites of small mammals (insectivores and rodents) on the Pityusic Island of Eivissa (Balearic Archipelago). Res. Rev. Parasitol. 60, 41–49.

Mas-Coma, S., Feliu, C., 1984. Helminthfauna from small mammals (insectivores and rodents) on the Pityusic Islands. In: Kuhbièr, H., Alcover, J.A., Guerau d'Arellano Tur, C. (Eds.), Biogeography and Ecology of the Pityusic Islands. Springer, Dordrecht, pp. 469–525. ISBN: 978-94-009-6539-3. doi:10.1007/978-94-009-6539-3_23

Mayr, E., 1954. Change of genetic environment and evolution. In: Huxley, J.S., Hardy, A.C., Ford, E.B. (Eds.), Evolution as a Process. Allen & Unwin, London, pp. 157–180.

Mayr, E., 1963. Animal Species and Evolution, 1st Ed. Harvard University Press, Cambridge. 797 pp. ISBN: 9780674865327; doi:10.4159/harvard.9780674865327

McCall, R.A., Nee, S., Harvey, P.H., 1998. The role of wing length in the evolution of avian flightlessness. Evol. Ecol. 12, 569–580. doi:10.1023/A:1006508826501

McNab, B.K., 2002. Minimizing energy expenditure facilitates vertebrate persistence on oceanic islands. Ecol. Lett. 5, 693–704. doi:10.1046/j.1461-0248.2002.00365.x

Meagher, S., 1999. Genetic diversity and *Capillaria hepatica* (Nematoda) prevalence in Michigan deer mouse populations. Evolution (N. Y). 53, 1318–1324. doi:10.1111/j.1558-5646.1999.tb04547.x

Meiri, S., Cooper, N., Purvis, A., 2008. The island rule: Made to be broken? Proc. R. Soc. B Biol. Sci. 275, 141–148. doi:10.1098/rspb.2007.1056

Møller, A.P., Dufval, R., Allander, K., 1993. Parasites and the Evolution of Host Social Behavior. In: Slater, P.J.B., Rosenblatt, J.S., Snowdon, C.T., Milinski, M. (Eds.), Advances in the Study of Behavior. Academic Press, pp. 65–102. doi:10.1016/S0065-3454(08)60405-2

Moyer, B.R, Rock, A.N., Clayton, D.H., 2003. Experimental Test of the Importance of Preen Oil in Rock Doves (C*olumba livia*). Auk 120, 490–496. doi:10.1093/auk/120.2.490

Moyer, B.R., Wagenbach, G.E., 1995. Sunning by Black Noddies (*Anous minutus*) May Kill Chewing Lice (*Quadraceps hopkinsi*). Auk 112, 1073–1077. doi:10.2307/4089047

Murray, M.D., 1990. Influence of host behaviour on some ectoparasites of birds and mammals. In: Banard, C., Behnke, J. (Eds.), Parasitism and Host Behaviour. Taylor & Francis, New York, pp. 290–315. ISBN: 0-203-48995-0

Negro, J.J., Margalida, A., Hiraldo, F., Heredia, R., 1999. The function of the cosmetic coloration of bearded vultures: when art imitates life. Anim. Behav. 58, F14–F17. doi:10.1006/anbe.1999.1251

Nieberding, C., Morand, S., Libois, R., Michaux, J.R., 2006. Parasites and the island syndrome: The colonization of the western Mediterranean islands by *Heligmosomoides polygyrus* (Dujardin, 1845). J. Biogeogr. 33, 1212–1222. doi:10.1111/j.1365-2699.2006.01503.x

Paterson, A.M., Palma, R.L., Gray, R.D., 1999. How Frequently Do Avian Lice Miss the Boat? Implications for Coevolutionary Studies. Syst. Biol. 48, 214–223. doi:10.1080/106351599260544

Paterson, A.M., Palma, R.L., Gray, R.D., 2003. Drowning on arrival, missing the boat, and x-events: How likely are sorting events. In: Page, R.D.M. (Ed.), Tangled Trees: Phylogeny, Cospeciation, and Coevolution. The University of Chicago Press, Chicago, pp. 287–307. ISBN: 0-226-64467-7

Pereira, C., Melo, C., 2016. As Aves do Cabo da Praia: Um tesouro a descobrir, um património a preservar. Letras Lavadas, Ponta Delgada. 130 pp. ISBN: 978-989-20-6867-1

Pérez-Rodríguez, A., Ramírez, Á., Richardson, D.S., Pérez-Tris, J., 2013. Evolution of parasite island syndromes without long-term host population isolation: parasite dynamics in Macaronesian blackcaps *Sylvia atricapilla*. Glob. Ecol. Biogeogr. 22, 1272–1281. doi:10.1111/geb.12084

Pérez-Tris, J., Bensch, S., Carbonell, R., Helbig, A.J., Tellería, J.L., 2004. Historical diversification of migration patterns in a passerine bird. Evolution (N. Y). 58, 1819–1832. doi:10.1111/j.0014-3820.2004.tb00464.x

Poulin, R., 2007. Evolutionary Ecology of Parasites, 2nd Ed. Princeton University Press. Princeton, 360 pp. ISBN: 9781400840809. doi:10.1515/9781400840809

Price, P.W., 1980. Evolutionary biology of parasites., Monographs in population biology, Volume 15. Princeton University Press, Princeton. doi:10.2307/4157

Price, R.D., Hellenthal, R.A., Palma, R.L., 2003. World checklist of chewing lice with host associations and keys to families and genera. In: Price, R.D., Hellenthal, R.A., Palma, R.L., Johnson, K.P., Clayton, D.H. (Eds.), The Chewing Lice: World Checklist and Biological Overview. Illinois Natural History Survey Special Publication, Illinois, pp. 1–448. ISBN: 1-882932-08-0

Rando, J.C., Alcover, J.A., Olson, S.L., Pieper, H., 2013. A new species of extinct scops owl (Aves: Strigiformes: Strigidae: *Otus*) from São Miguel island (Azores archipelago, north Atlantic ocean). Zootaxa 3647, 343–357. doi:10.11646/zootaxa.3647.2.6

Rando, J.C., Pieper, H., Olson, S.L., Pereira, F., Alcover, J.A., 2017. A new extinct species of large bullfinch (Aves: Fringillidae: *Pyrrhula*) from Graciosa Island (Azores, North Atlantic Ocean). Zootaxa 4282, 567–583. doi:10.11646/zootaxa.4282.3.9

Recuerda, M., Illera, J.C., Blanco, G., Zardoya, R., Milá, B., 2021. Sequential colonization of oceanic archipelagos led to a species-level radiation in the common chaffinch complex (Aves: *Fringilla coelebs*). Mol. Phylogenet. Evol. 164, 1–13. doi:10.1016/j.ympev.2021.107291

Reeves, W.K., Lloyd, J.E., 2019. Louse Flies, Keds, and Bat Flies (Hippoboscoidea). In: Mullen, G.R., Durden, L.A. (Eds.), Medical and Veterinary Entomology. Elsevier, London, pp. 421–438. ISBN: 978-0-12-814043-7. doi:10.1016/B978-0-12-814043-7.00020-0.

Rodrigues, P., Cunha, R.T., 2011. Azorean Birds - A natural Heritage. Açoreana 319–330.

Rodrigues, P., Lopes, R.J., Drovetski, S. V., Reis, S., Ramos, J.A., Tristão da Cunha, R., 2013. Phylogeography and genetic diversity of the Robin (*Erithacus rubecula*) in the Azores Islands: Evidence of a recent colonisation. J. Ornithol. 154, 889–900. doi:10.1007/s10336-013-0953-4

Rodrigues, P., Lopes, R.J., Reis, S., Resendes, R., Ramos, J.A., Tristão da Cunha, R., 2014. Genetic diversity and morphological variation of the common chaffinch *Fringilla coelebs* in the Azores. J. Avian Biol. 45, 167–178. doi:10.1111/j.1600-048X.2013.00229.x

Rodrigues, P., Lopes, R.J., Resendes, R., Ramos, J.A., Tristão da Cunha, R., 2016. Genetic Diversity of the Azores Blackbirds *Turdus merula* Reveals Multiple Founder Events. Acta Ornithol. 51, 221–234. doi:10.3161/00016454AO2016.51.2.008

Rodrigues, P., Micael, J., Resendes, R., Lopes, R.J., Ramos, J.A., Tristão da Cunha, R., 2018. Genetic and morphometric variation of the Blackcap (*Sylvia atricapilla*) on the Azores

Archipelago reveals a recent range expansion. J. Nat. Hist. 52, 2413–2435. doi:10.1080/00222933.2018.1539194

Roff, D.A., 1994. The evolution of flightlessness: Is history important? Evol. Ecol. 8, 639–657. doi:10.1007/BF01237847

Rózsa, L., Rékási, J., Reiczigel, J., 1996. Relationship of host coloniality to the population ecology of avian lice (Insecta: Phthiraptera). J. Anim. Ecol. 65, 242–248. doi:10.2307/5727

Schluter, D., 1988. Character displacement and the adaptive divergence of finches on islands and continents. Am. Nat. 131, 799–824. doi:10.1086/284823

Shirihai, H., Gargallo, G., Helbig, A.J., Kirwan, G.M., Svensson, L., 2001. Sylvia Warblers: Identification, taxonomy and phylogeny of the Genus Sylvia, 1st Ed., Helm Identification Guides. Bloomsbury Publishing, London. 576 pp. ISBN: 9780713639841

Smith, N.G., 1968. The Advantage of being Parasitized. Nature 219, 690–694. doi:10.1038/219690a0

Stamps, J.A., Buechner, M., 1985. The Territorial Defense Hypothesis and the Ecology of Insular Vertebrates. Q. Rev. Biol. 60, 155–181. doi:10.1086/414314

Thiollay, J.-M., 1993. Habitat segregation and the insular syndrome in two congeneric raptors in New Caledonia, the White-bellied Goshawk *Accipiter haplochrous* and the Brown Goshawk *A. fasciatus*. Ibis (Lond. 1859). 135, 237–246. doi:10.1111/j.1474-919X.1993.tb02840.x

Tompkins, D.M., Jones, T., Clayton, D.H., 1996. Effect of vertically transmitted ectoparasites on the reproductive success of swifts (*Apus apus*). Funct. Ecol. 10, 733. doi:10.2307/2390508

Torchin, M.E., Lafferty, K.D., Dobson, A.P., McKenzie, V.J., Kuris, A.M., 2003. Introduced species and their missing parasites. Nature 421, 628–630. doi:10.1038/nature01346

Tripet, F., Christe, P., Møller, A.P., 2002. The importance of host spatial distribution for parasite specialization and speciation: A comparative study of bird fleas (Siphonaptera: Ceratophyllidae). J. Anim. Ecol. 71, 735–748. doi:10.1046/j.1365-2656.2002.00639.x

Van Valen, L., 1973. Pattern and the balance of nature. Evol. Theory 1, 31–49.

Wall, R., Shearer, D., 2001. The Importance and Diversity of Arthropod Ectoparasites. In: Veterinary Ectoparasites: Biology, Pathology and Control. Wiley-Blackwell Science, Oxford, pp. 1–22. ISBN: 0-632-05618-5

Wallace, A.R., 1892. Island life; or, the phenomena and causes of insular faunas and floras,

including a revision and attempted solution of the problem of geological climates, 2nd Ed. Macmillan, London. 563 pp.

Whiting, M.F., Whiting, A.S., Hastriter, M.W., Dittmar, K., 2008. A molecular phylogeny of fleas (Insecta: Siphonaptera): Origins and host associations. Cladistics 24, 677–707. doi:10.1111/j.1096-0031.2008.00211.x

Whittaker, R.J., Fernández-Palacios, J.M., 2007. Island Biogeography - Ecology, Evolution, and Conservation, 2nd Ed. Oxford University Press, New York. 401 pp. ISBN: 978-0-19-856611-3

Wiggins, D.A., Møller, A.P., Sørensen, M.F.L., Brand, L.A., 1998. Island biogeography and the reproductive ecology of great tits *Parus major*. Oecologia 115, 478–482. doi:10.1007/s004420050544

Wikelski, M., Foufopoulos, J., Vargas, H., Snell, H., 2004. Galápagos birds and diseases: Invasive pathogens as threats for island species. Ecol. Soc. 9. doi:10.5751/ES-00605-090105

Wright, S.J., 1980. Density compensation in island avifaunas. Oecologia 45, 385–389. doi:10.1007/BF00540211

Chapter 2

Louse flies in Azorean and mainland populations of four Passeriformes species: a new perspective to parasite island syndromes

Louse flies in Azorean and mainland populations of four Passeriformes species: a new perspective to parasite island syndromes

Abstract

Hippoboscid flies, also known as louse flies, are obligate blood-feeders ectoparasites of birds and mammals. By studying louse fly parasites of four Passeriformes species, Eurasian blackbird (*Turdus merula*), Eurasian blackcap (*Sylvia atricapilla*), common chaffinch (*Fringilla coelebs*) and European robin (*Erithacus rubecula*), with dissimilar time of colonization of Azores Islands, we tested whether: i) Island host populations have lower parasite richness than the mainland one; ii) insular host populations undergo higher parasite prevalence, mean intensities and mean abundance than the mainland one; iii) Island parasite diversity are composed exclusively by specific parasites and iv) parasite richness is positively correlated with the Island area and proximity to the continent. For these purposes, 775 birds were sampled for presence of louse flies, by modified fumigation chamber method, from Azores Islands (São Miguel, Terceira and Flores) and mainland Portugal. Insular and mainland parasite assemblages were statistically compared. We record for the first time to Azores, *Ornithomya fringillina and Icosta minor* from mainland Portugal. Louse flies had highest prevalence and abundance from Azores Islands compared to those observed in mainland birds, especially blackbirds. The insular parasite diversity of Azores blackbirds, blackcaps and chaffinches was richer than the one observed in mainland population. None of the hippoboscid flies observed on the Islands and mainland were host-specific. Thus, our findings provide an upgrade of parasite island syndromes knowledge, in the context of the ectoparasites, namely to the hippoboscid flies case.

Keywords: Hippoboscid flies; Louse flies; Wild birds; Ectoparasite assemblage; Azores; Portugal.

Introduction

Over the last two decades, the insular diversity of parasites have been the focus of biogeographical studies, to understand which factors are involved in species' range expansion (Losos and Ricklefs, 2010; Moyer *et al*., 2002; Poulin, 2004). Studies on parasite traits found that the parasite ability of establishment during the host expansion is crucial. Parasites may

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have a successful establishment, or instead be absence from the new area by "missing the boat" (parasites do not present from the founding hosts that colonize a new region) or "drowning on arrival" (parasites do arrive with hosts, but fail the establishment) (MacLeod *et al.*, 2010; Paterson *et al.*, 2003). Not least are the ecological features of the host, such as: population size, geographic range and migration events. For example, seabirds with trans-oceanic dispersal movements, larger population and geographical range, may explain the high ectoparasites diversity (Gómez-Díaz *et al.*, 2012; Hughes and Page, 2007). Additionally, environmental parameters can determine ectoparasite species distributions. Birds in arid regions have fewer ectoparasitic lice than birds in humid regions (Moyer *et al.*, 2002), but arid conditions provide a climatic refuge from the competitively superior species (Malenke *et al.*, 2011).

The insular vertebrates populations often undergo a series of changes (morphometric, life-history, behavioral, physiological and genetic) as result of isolation, phenomenon known as insular syndrome (Blondel, 2000). This concept has been adapted to insular communities of parasites, originating the parasite island syndromes. Nieberding *et al.* (2006) studied the colonization patterns of the Mediterranean Islands by *Heligmosomoides polygyrus* (Dujardin, 1845), a specific nematode of rodents and recorded a significant loss of genetic diversity and an ecological niche enlargement following colonization, as result of founder effect. Additionally, Pérez-Rodríguez *et al.* (2013) studied the haemoparasites in the Macaronesia and reported: i) impoverishment of insular haemosporidians assemblage; ii) lower prevalence of parasites in the Island populations compared with mainland and iii) reduced host specialization on Islands. The authors attributed these results to: reduced availability of appropriate vectors on Islands, sequential founder population bottlenecks and migratory traits of birds. Recently, the parasite island syndromes were studied to Azorean communities of ectoparasites, namely to chewing lice by Literák *et al.* (2015) and found: i) fewer chewing lice species in the Azores birds; ii) higher louse prevalence from insular birds and iii) only chewing lice host specific in the Azores. These authors suggested that the findings can be correlated with migratory and ecological traits of birds and chewing lice features.

Island area and his distance from the mainland source population are key factors to the island syndrome (Blondel, 2000; Losos and Ricklefs, 2010). However, these factors shown contradictory results to parasite island syndromes; while Nieberding *et al.* (2006) and Pérez-Rodríguez *et al.* (2013) reported a decreasing parasite richness with increasing Island distance to the continent, Ishtiaq *et al.* (2010) and Literák *et al.* (2015) did not record this effect.

Hipboscid flies, known as louse flies or keds, are obligate blood-feeders ectoparasites of domestic and wild birds and mammals. The members of the Hipboscidae family are larviparous, with larval development occurring in uterus, where they are nourished by milk glands; when fully developed, pre-pupae are deposited or released in proximity to the host, such as birds' roost, nests or the hair of mammals, and immediately begin to darken and form the *puparium*, *i.e.*, the last larval instar (Hutson, 1984; Maa and Peterson, 1987). Adult louse fly are dorsoventrally flattened, with a depressed head and a hind pair of wings, although few species having vestigial or no wings (Reeves and Lloyd, 2019).

Louse flies are known to act as vectors of infectious agents, including arbovirus, bacteria, avian and mammalian trypanosomes, haemosporidians, blood protozoa and helminths (Baker, 1967; Halos *et al*., 2004; Rani *et al*., 2011), and serve as disseminators of lice and mites, which have with them a phoretic relationship (Hill *et al*., 1967; Keirans, 1975). Additionally, Gancz *et al*. (2004) and Farajollahi *et al*. (2005) suspected the vector competence of louse fly in the transmission of West Nile Virus.

Worldwide, approximately 213 louse fly species are known, of which 30 have been recorded in Europe (Dick, 2006; Pape *et al*., 2015). The Portuguese hipboscid fauna is composed of 10 species on the mainland territory, 4 species from Azores Islands and 3 from Madeira Island (Carles–Tolrá and Báez, 2002; Oslejskova *et al*., 2020; Smit, 2008, 2010). However, considering the few studies directed to louse fly species research, we believe that this list is still far from complete.

The main goal of this study was to characterize the diversity of hipboscid flies infesting four species of passerines, Eurasian blackbird *Turdus merula* Linnaeus, 1758, Eurasian blackcap, *Sylvia atricapilla* (Linnaeus, 1758), common chaffinch *Fringilla coelebs* Linnaeus, 1758 and European robin *Erithacus rubecula* (Linnaeus, 1758) from Azores Islands, and compare it with the diversity found on the same species in mainland Portugal. A comparison of louse flies in hosts originating from the mainland and Islands allowed to test the predictions derived from parasite island syndromes and Theory of Island Biogeography: i) whether Island host populations have lower parasite richness than the mainland one; ii) whether Island host populations have higher parasite prevalence, mean intensities and mean abundance than the mainland one; iii) whether Island parasite diversity are composed exclusively by specific parasites; and iv) whether parasite richness are positively correlated with the Island area and proximity to the continent.

Material and Methods

<u>Study Area</u>

Portugal is situated in the Southwest of the European continent, covers 92.090 Km^2 and comprises a continental part and two Macaronesian Archipelagos constituted by volcanic Islands and islets located in North Atlantic, Azores Archipelago and Madeira Archipelago. Azores (36º55' and 39º43'N and 25º01' and 31º07'W) is located approximately about 1.600 Km from Europe and 1.900 Km from North America and consists of nine Islands geographically clustered into three groups: Eastern group, with São Miguel (area: 747 Km^2; distance to mainland: 1.364 Km) and Santa Maria Islands; Central group, constituted by Faial, Pico, São Jorge, Terceira (area: 403 Km^2; distance to mainland: 1.519 Km) and Graciosa Islands and Western group, with Flores (area: 141 Km^2; distance to mainland: 1.839 Km) and Corvo Islands (França *et al*., 2003; Pacheco *et al*., 2013).

This study took place in the South mainland Portugal, specifically at Silves and Olhão, and in three Islands of the Azores Archipelago, namely São Miguel, Terceira and Flores (Figure 2.1). The selection of the places was determined by the occurrence of the studied bird species, and hence, the three Islands representing locations with different areas and distance to mainland.

Figure 2.1 – Map of the mainland Portugal and Azores Islands with the geographic distribution of the study areas (Silves, Olhão, São Miguel Island, Terceira Island and Flores Island).

Field Sampling

Live birds of the species *T. merula* (subspecies: *T. m. merula* Linnaeus, 1758 from mainland and *T. m. azorensis* Hartert, 1905 from Azores), *S. atricapilla* (subspecies: *S. a. atricapilla* (Linnaeus, 1758) from mainland and *S. a. gularis* Alexander, 1898 from Azores), *F. coelebs* (subspecies: *F. c. balearica* von Jordans, 1923 from mainland and *F. c. moreletti* Pucheran, 1859 from Azores) and *E. rubecula* were captured randomly with mist nets, during October-December of two consecutive years (2018-2019). The time of the year was determined by the studied bird species abundance, namely in the case of robin and chaffinch that are more common in mainland Portugal during winter migration. Due to the patchy distribution and abundance of sampled bird species, individuals were captured at 2-3 sites on each Island, to increase capture rates and to avoid repeated captures of the same individuals. Each bird was individualized with metal ring, sexed and aged (juveniles: <1 year old; adults: >1 year old), based on plumage features (Demongin.L, 2016).

Birds were sampled for presence of louse flies using a modified fumigation chamber method from Clayton and Drown (2001), where birds' bodies were exposed to chloroform, for 5 minutes and bird's heads underwent visual examination (Visnak and Dumbacher, 1999). This is a standard practice performed by numerous bird banders throughout the world, especially for the study of avian chewing lice (Sychra *et al*., 2008; Literák *et al*., 2015). All birds were released after examination at the site of capture.

Arthropods Collection

The hippoboscid flies were placed individually into small tubes containing 70% ethanol, until further processing at Entomology Laboratory at Faculty of Sciences, University of Lisbon. Each louse fly was examined under a Stereo Microscope Olympus SZX7, and identified using the following dichotomous keys: Hill (1962), Hutson (1984), Maa (1966, 1969), Maa and Peterson (1987) and Petersen *et al*. (2007). The systematics and nomenclature rules followed Maa and Peterson (1987). Chewing lice and mites found in phoretic association with louse flies, were identified using specific identification keys (Gustafsson and Bush, 2017; Mironov *et al*., 2005). Images were acquired on Zeiss Stereo LUMAR stereoscope V12, equipped with a Zeiss Axiocam 503 colour 3MP, controlled with the Axiovision 4.9.1 software and digitally processed using ImageJ 1.52p software (Schindelin *et al*., 2012).

Observed louse fly species richness was compared with estimated species using a rarefaction analyses with 100 randomization models, which were extrapolated to a total of 500 samples using bias-corrected formula for Chao1 and Chao2 which included the upper limit to be considered as a rare or infrequent species (R=2). Chao2 estimator, an asymptotic species richness Chao (1987), was chosen as the best estimator. Additionally, the Shannon index of diversity was used to estimate diversity. These analysis were performed using EstimateS 9.1.0 software (Colwell, 2013).

Hippoboscid fly species shared between Islands and mainland populations were compared to test different prevalence, mean abundance and intensity. Firstly, prevalence, abundance and intensity of each louse fly was estimated. Due to the low prevalence of louse flies on chaffinch, robin and blackcap, these birds were excluded from the others statistical analyses. Statistical differences in the geographical patterns of louse fly species were assessed with Fisher's Exact Test and bootstrap 2-sample t-test with 1000 replications, to detect associations in louse fly prevalence (%), and in intensity and abundance, respectively. Blackbird's age, gender and year of fieldwork were used as co-factors that could produce variation in louse fly prevalence between Islands and mainland. Posteriorly, a Chi-square test adjusted using a post hoc test, Bonferroni correction, and Kruskal-Wallis with Dunn's post hoc test (adjusted using the Bonferroni correction) was used to detect the statistical differences of louse fly prevalence only among Islands populations of blackbirds.

Sample size varied depending on the variable in analyses, since not all data from all individuals were collected.

General statistical analyses were done with the software Quantitative Parasitology 3.0 (Reiczigel *et al*., 2019). The analysis of post hoc tests was done using IBM®SPSS®Statistics Version 26 (IBM Corp., 2019).

Results

Louse fly richness

Three species of louse fly were found: *Ornithoica turdi* (Olivier, 1811) (Figure 2.2 a–b), *Ornithomya fringillina* Curtis, 1836 (Figure 2.2 c–d) and *Icosta minor* (Bigot, 1858) (Figure 2.2 e–f).
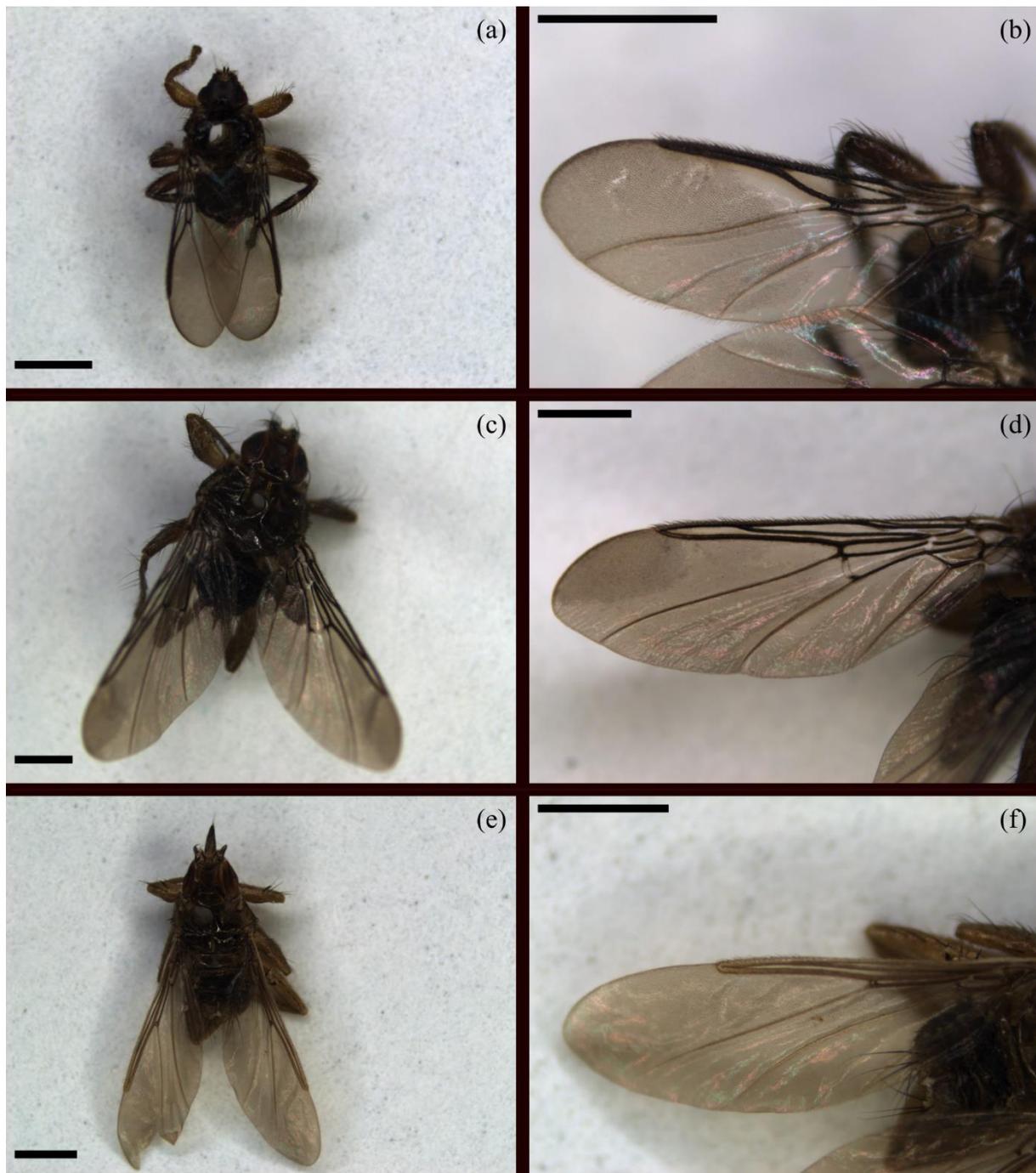
Figure 2.2 – Photos of three species of hippoboscid fly and their wings collected from Passeriformes species: (a–b) *Ornithoica turdi*, (c–d) *Ornithomya fringillina* and (e–f) *Icosta minor*. Scale bar: 1 mm.

Considering the four host species together, 2 louse fly species were recorded in Azores Islands, *O. turdi* and *O. fringillina* and 2 in mainland Portugal, *O. turdi* and *I. minor*. The observed richness of louse flies for each host species was: 2 – *O. turdi* and *O. fringillina* – and 1 – *O. turdi* – species in blackbirds from Azores Islands and mainland Portugal, respectively; 2 – *O. turdi* and *O. fringillina* – in blackcap and chaffinch from Azores Islands; and 1 species in robin from Azores Islands – *O. turdi* – and mainland Portugal – *I. minor*.

The results of the rarefaction analyses and Shannon index of diversity were summarized in Table 2.1. The observed richness (Sobs) coincided with asymptotic species richness (Sest) for all analyses. Asymptotic species richness reaching the asymptote in the sample numbers of: 26 and 49 for blackbirds from Azores and mainland, respectively; 119 for blackcap from Azores; 117 for chaffinch from Azores; 23 and 29 for robin from Azores and mainland, respectively; 88 and 209 birds of any species from Azores and mainland, respectively.

Table 2.1 – Number of birds analyzed (*n*) and respective observed richness of species (Sobs), asymptotic richness of species (Sest), the best estimator of richness (Chao2) and Shannon index of diversity for the louse fly assemblages of each bird species from Azores and mainland Portugal.

	n	Sobs	Sest	Chao2 (CI 95%)	Shannon index ± SD
Blackbird					
Azores	180	2	2	2.00 (2.00-2.32)	0.4 ± 0.11
Mainland	60	1	1	1.00 (1.00-1.80)	0
Blackcap					
Azores	181	2	2	2.00 (2.00-2.66)	0.65 ± 0.06
Chaffinch					
Azores	180	2	2	2.00 (2.00-2.6)	0.63 ± 0.07
Robin					
Azores	25	1	1	1.00 (1.00-2.15)	0
Mainland	29	1	1	1.00 (1.00-3.60)	0
Total					
Azores	566	2	2	2.00 (2.00-3.14)	0.46 ± 0.1
Mainland	209	2	2	2.00 (2.00-3.84)	0.5 ± 0

The highest species diversity (±SD) was observed in the community of Azorean blackbirds (0.4±0.11) compared with mainland; Independently of the species, mainland birds (0.5±0) shown high louse fly diversity than Azores (0.46±0.1).

Observed parasite richness was not correlated with the Island area and their distance to mainland.

Prevalence of louse flies

Overall, 19.4% of the 775 individuals analyzed for this study were infected by at least one hippoboscid fly species. The highest prevalence of flies was found in blackbirds (50.8%), followed by robins (5.6%), chaffinches (5.4%), and blackcaps (5.0%) (Figure 2.3; see Supplementary material, Table S2.1, for detailed data of louse flies prevalence). *O. turdi* was the most representative species (16.0%), followed by *O. fringillina* (5.8%) and *I. minor* founded only in a bird species (0.1%). The highest prevalence of infestation by *O. turdi* was found in blackbirds (45.4%), followed by robins (3.7%), chaffinches (3.3%) and blackcaps (2.1%). For the case of *O. fringillina*, the highest prevalence was found in blackbirds (13.8%), followed by blackcaps (2.9%) and chaffinches (2.1%). Finally, *I. minor* was only found in a robin (1.9%). Among the infested birds per species, 102 (42.5%) blackbirds were infested by only one fly species, whereas 20 (8.3%) carried a double infestation (*O. turdi* and *O. fringillina*), therefore only single infestations were recorded in blackcaps, chaffinches and robins.
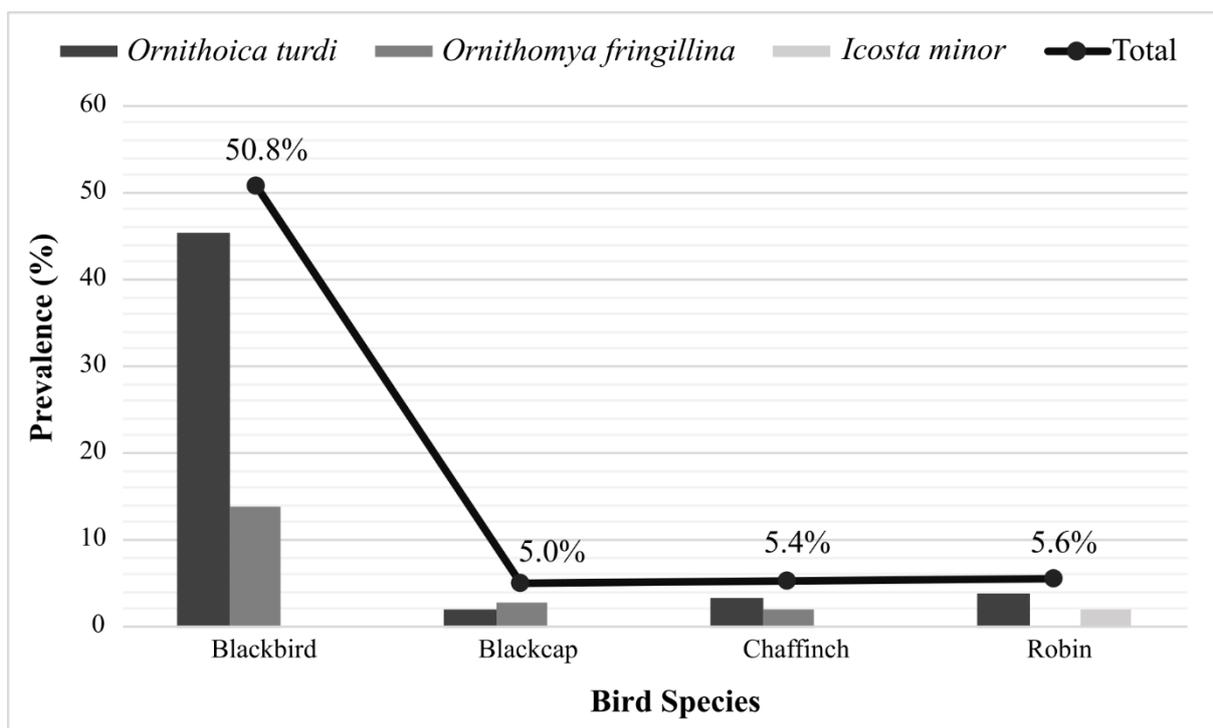
Figure 2.3 – Prevalence (%) of hippoboscid fly species found on blackbirds, blackcaps, chaffinches and robins from the Azores Islands and mainland Portugal.

<u>Louse fly infestation and location</u>

Louse fly prevalence changed according location of fieldwork (Figure 2.4; see Supplementary material, Table S2.2, for detailed data of louse flies prevalence). Overall louse flies prevalence was much higher in Azorean blackbirds, blackcap and chaffinch (66.1%, 6.6% and 7.2%, respectively) than mainland specimens (5.0%, 0.0% and 0.0%) (Fisher test; blackbirds: $p \leq 0.001$, blackcap and chaffinch: $p \leq 0.05$).

Considering each louse fly species, only blackbirds exhibit prevalence differences between Azores Islands and mainland. *Ornithoica turdi* were found on 58.3%, 65%, 53.3% and 5% of blackbirds from Flores, Terceira, São Miguel and mainland, respectively. These differences in the prevalence differ statistically between each Azores Islands and mainland (Fisher test; $p \leq 0.001$). For the case of *O. fringillina*, were only found in Flores, Terceira and São Miguel blackbirds, 5.0%, 26.7% and 23.3%, respectively. A single *Icosta minor* was recorded from mainland robin.

When comparing the prevalence of *O. turdi* and *O. fringillina* only among Islands, infestation rates revealed not to be statistically different to *O. turdi* ($\chi^2 = 1.698$; $p \geq 0.05$), while *O. fringillina* prevalence was statistically different among Azores Islands ($\chi^2 = 10.909$; $p \leq 0.01$). *Ornithomya fringillina* of blackbirs from Flores Island differ statistically to Terceira (Kruskal-Wallis test: $p \leq 0.01$) and São Miguel Island (Kruskal-Wallis test: $p \leq 0.05$).

Overall, the mean abundances of hippoboscid flies were higher in the Azores birds than on the mainland (see Table S2.2 in Supplementary material). *Ornithoica turdi*, the only shared species among blackbird populations, was statistically more abundance in Islands than mainland.
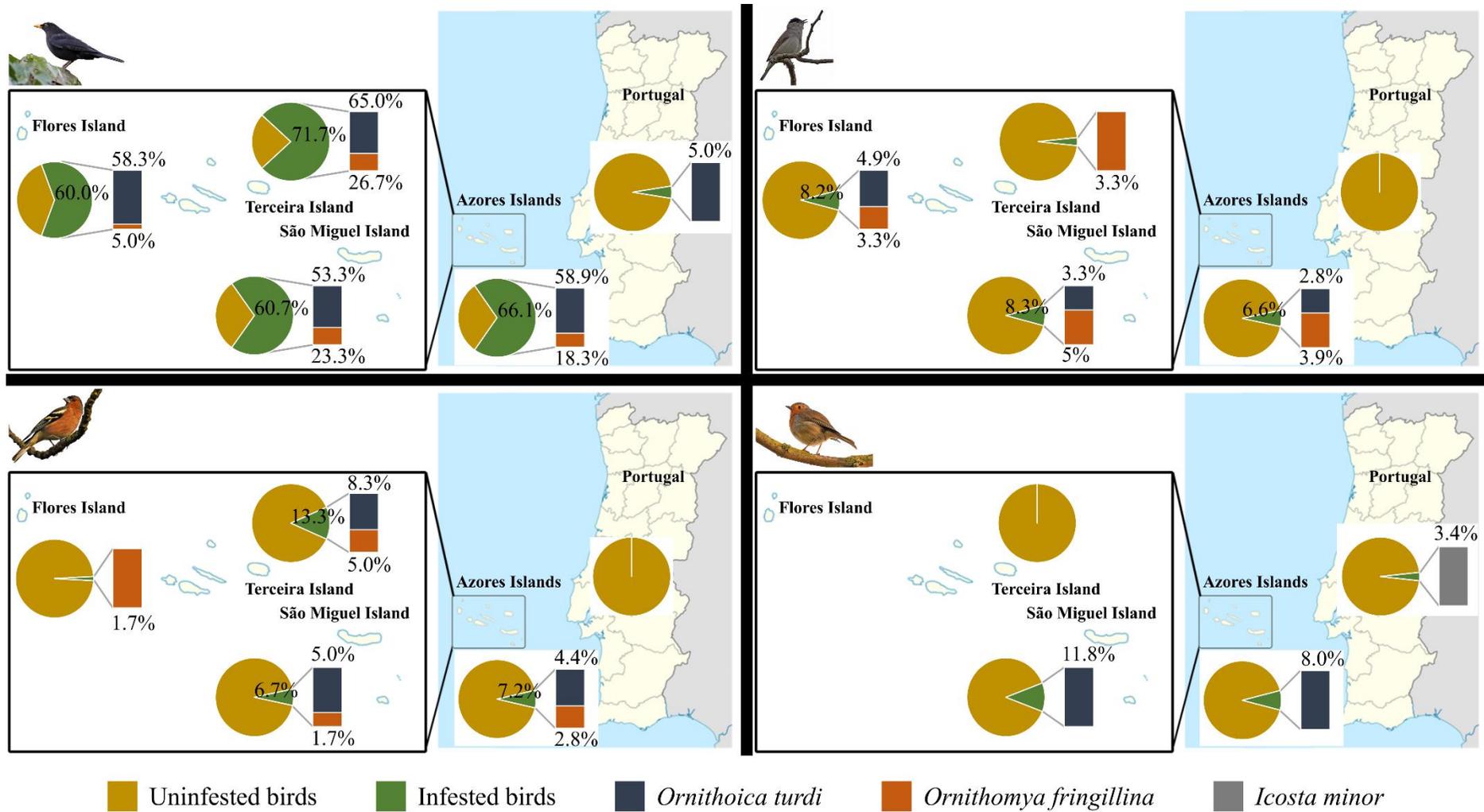
Figure 2.4 – Map showing the prevalence (represented by different color; yellow color represent the prevalence of uninfested birds) of hippoboscid fly species in blackbirds, blackcaps, chaffinches and robins in the each of the sampled Azorean Islands (São Miguel, Terceira and Flores) and the mainland Portugal.

Mainland prevalence's of louse fly species of the blackbird, blackcap, chaffinch and robin from different European countries were summarized in (Table 2.2). Overall, birds from European countries showed a low prevalence of hippoboscid flies.

Table 2.2 – Reports of Hippoboscidae species in wild Passeriformes species from Europe.

Location	Host Species	Louse fly	n	Prevalence (%)	Literature source
Czech Republic	*T. merula*	-	12	-	Sychra *et al*. (2008)
	S. atricapilla	*O. fringillina*	78	3.8	
	E. rubecula	*O. fringillina*	15	33.3	
	T. merula	-	21	-	Sychra *et al*. (2011)
	S. atricapilla	-	114	-	
	E. rubecula	-	281	-	
	F. coelebs	-	31	-	
Germany	*T. merula*	-	42	-	Labitzke and Jentzsch (2019)
	S. atricapilla	*O. fringillina*	1595	0.2	
	F. coelebs	-	4	-	
	E. rubecula	-	235	-	
Slovakia	*T. merula*	-	2	-	Bush *et al*. (2018)
	S. atricapilla	-	1	-	
	F. coelebs	-	4	-	
	E. rubecula	-	2	-	
Finland	*F. coelebs*	*O. fringillina*	162	3.1	Sorjonen (1971)
	E. rubecula	*O. fringillina*	19	10.5	

Phoresy

Overall, 4 (1.4%) and 47 (16.4%) of 286 louse fly carried chewing lice and mites, respectively. Summarized in Table 2.3, 51 phoretic cases, involve hippoboscid flies of the species *O. fringillina* (10) and *O. turdi* (41).

Table 2.3 - Prevalence of phoresy of chewing lice and mites on louse flies from blackbirds and blackcap.

	Bird–Hippoboscid fly associations		
	Blackbird *O. turdi*	Blackbird *O. fringillina*	Blackcap *O. fringillina*
Number of flies	218	37	7
% chewing lice on hippoboscid flies	0	10.8	0
% mites on hippoboscid flies	18.8	13.5	14.3

Ornithomya fringillina bearing phoretic lice of the *Guimaraesiella amsel* (Figure 2.5 a) were only collected on blackbirds species (10.8%); Mites of the family Epidermoptidae were found attached to *O. turdi* on blackbirds (Figure 2.5 b) (18.8%) and *O. fringillina* on blackbirds (13.5%) and a blackcap (14.3%).
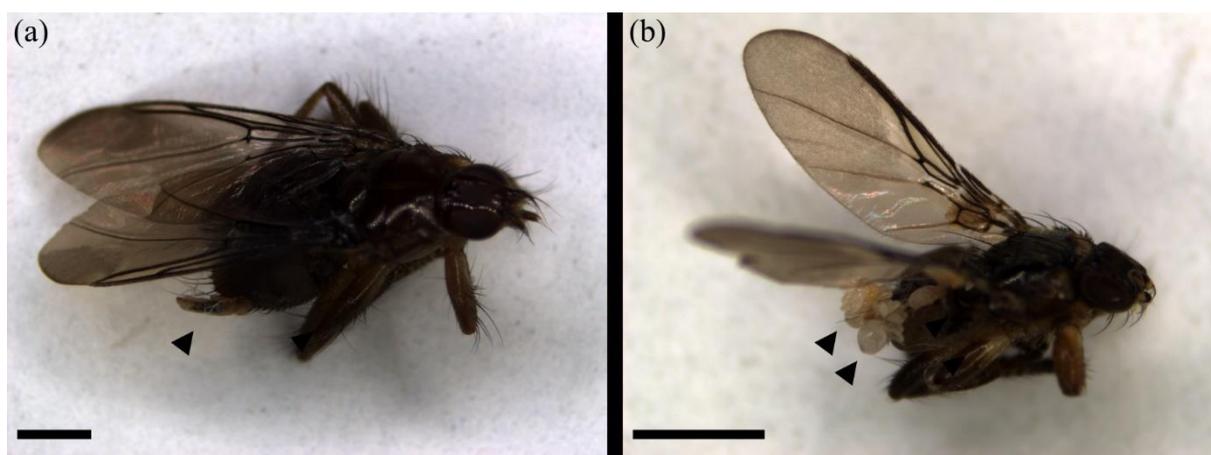
Figure 2.5 – Photos of phoretic association of (a) *Guimaraesiella amsel* on *O. fringillina* and (b) Epidermoptidae mites on *O. turdi*. Scale bar: 1 mm.

Discussion

Considering the records published by Carles–Tolrá and Báez (2002) and Smit (2010) our work contributed for this topic by reporting the following new records for the Azores Islands and mainland Portugal: i) *Ornithoica turdi* from Flores and Terceira Island; ii) *Ornithomya fringillina* from Flores, Terceira and São Miguel Island; and iii) *Icosta minor* from mainland Portugal.

The diversity of hippoboscid flies found in Azores blackbirds, blackcap and chaffinch was richer than the one observed in mainland population of the same species. In the case of blackcap

and chaffinch, the mainland diversity was clearly impoverished, without presence of ectoparasites. Contrariwise, when consider all hosts species together, the richness of louse flies was similar among Azores and mainland, with two species at each location. Both results do not support the one of the assumptions of the parasite island syndromes postulated by Paterson *et al*. (2003) that host–parasite associations are compromised on Islands, resulting in lower numbers of species, as recorded from Macaronesia on: blackcap chewing lice (Literák *et al*., 2015); and blackcap and trumpeter finches haemosporidians (Barrientos *et al*., 2014; Pérez-Rodríguez *et al*., 2013). Our result suggest that hipboscid species do not fail the establishment to the new area by "missing the boat" or "drowning on arrival" (MacLeod *et al*., 2010; Paterson *et al*., 1999), but instead had a successful establishment on Azores Islands. The louse fly species identified by us are generalist parasites, parasitizing mainly small birds, including various Passeriformes species, as observed by Oboňa *et al*. (2019) and can rely on other host species to colonize and thrive in the Islands, this result was to be expected. Although the parasites infesting mainland birds are also not host-specific, *I. minor* was only found in the mainland. According to the hosts geographical range, we suspected that the European robin, acquired *I. minor* through host-switching events between unrelated hosts as previously suggested by Paterson *et al*. (2003).

Overall, louse flies do not display host-specificity and so, we could not confirm one of the parasite island syndromes hypothesis advanced by Pérez-Rodríguez *et al*. (2013), that the Island parasite was not host-specific. Furthermore, we found an uncorrelation among parasites richness and Island area and their distance from the mainland. These uncorrelation do not support the basic principle of the Theory of Island Biogeography, that biggest Island and Islands that are located near the putative source of colonizers, have greater species richness, but may be attributed to do not host-specificity of louse flies (Losos and Ricklefs, 2010).

The general observed louse fly prevalence in Islands was significantly higher compared with mainland birds; Azorean blackbirds showed prevalence and mean abundance significantly higher than to those observed in mainland Portugal populations; blackcaps and chaffinches only presented hipboscid flies in the Azores; no statistical differences were observed for the robins. Mainland prevalence's of louse fly species were very similar with findings from other European countries (Table 2.2). *Ornithoica turdi* and *I. minor* were not found in blackbirds, blackcap, chaffinch and robin from Czech Republic, Germany, Slovakia and Finland. Furthermore, *O. fringillina* was reported in: 3.8% and 0.2% blackcaps from Czech Republic

and Germany, respectively; 3.1% chaffinches from Finland; and 33.3% and 10% robins from Czech Republic and Finland, respectively.

The statistically differences verified between mainland and Azorean birds are in accordance with the results of blackcap chewing lice (Literák *et al*., 2015). Conversely, Pérez-Rodríguez *et al*. (2013) reported lower prevalence of haemoparasites in the Island populations and explain this, with the absence or reduced availability of appropriate vectors. In the case of ectoparasites, such as louse flies, due to direct transmission routes, without any interference of vectors, the transmission is more efficient (Sychra *et al*., 2008). Thus, some hypotheses may account for the scarcity of avian hippoboscid flies in mainland and high prevalence in Azores Islands such as: i) insular birds, where predation risk is either absent or negligible, takes less time in the nest sanitation and consequently could be exposed to more louse flies, attracted by fecal volatile components (Ibáñez-Álamo *et al*., 2016). Nest sanitation has been considered a behavioral adaptation to arthropod control and reduction of predator attraction, mainly by rejection of feces over the side of the nest, removal of the fecal sacs of the young and frequent renovation of the nest lining material (Bucher, 1988; Petit *et al*., 1989); ii) the risk of parasite infestation seems to be host density dependent (Begon *et al*., 1996). High host densities in Islands could account for the high parasite prevalence in Islands (Dobson, 1988). Although Lynch and Baker (1993) report a chaffinch density in Azores Islands (5-10 birds/ha) fairly higher than that from mainland (1 bird/ha) and our observations over the last two years of fieldwork (unpublished data) are in agree with this observations, we do not have recent data on birds density in Azores Islands and mainland, to confirm these hypothesis; iii) Abiotic factors, namely the absolute minimum temperature (ºC) and/or total precipitation (mm) showed to be more favorable in Azores Islands (Table S2.3 in Supplementary material) for the high parasites' prevalence. Senar *et al*. (1994) found that the best time of the year for collecting adult louse flies is during the period May – October, partly due to abiotic factors; iv) the large flow of migratory bird specimens in mainland at the time of year when the sampling was carried out, could represent a low prevalence of parasites. Birds migration offers an adaptive advantage "in terms of reduced risks of parasitism by moving to areas that harbor lower densities of conspecifics" (Sychra *et al*., 2011); while, Cork *et al*. (2001) suggested that birds with higher parasite loads may die in the early stages of the migration, never reach to the wintering and/or nesting site. Moreover, Sychra *et al*. (2008, 2011) and Hutson (1981) only found hippoboscid flies post-breeding migration from Czech Republic and Britain birds, respectively.

Our data are partially in accordance with the results of Barrientos *et al*. (2014) and Literák *et al*. (2015), for chewing lice in the Canary Islands and Azores Islands, respectively, who observed that parasitological parameters do not have significant differences among Island populations. However, our results for *O. fringillina* in blackbirds show a lower prevalence on the Flores Island compared to the other two Islands. Considering the low host-specificity of this louse fly species, mainly found in Passeriformes, but also in other bird orders, this result was unexpected (Oboňa *et al*., 2019).

Looking at Azores birds, we report a general fairly high prevalence of louse flies from blackbirds compared with blackcap, chaffinch and robins. Using the premise of the classical Theory of Island Biogeography and the analogy postulated by Kuris *et al*. (1980), which predict that hosts, can be viewed as Islands for parasite colonization. Thus, it will be expectable that larger host species, provide more space for parasites. Although our results did not show highest parasite richness on Azorean blackbirds, the larger-bodied species of our study, we believe that the same principle could be apply to the prevalence of parasites, as had been shown by Corbet (1956). Moreover, as louse flies hide between the feathers to escape the preening activity of birds, Tella *et al*. (1998) suggested that louse flies may exhibit a positive correlation with feathers size.

Furthermore, louse flies are known to provide a ride on another less mobile organism, such as mites and chewing lice. Our results corroborate the Keirans (1975) and Philips and Fain (1991) findings, that phoresy is more common for Ischnoceran lice and skin mites (Epidermoptidae) and appears to be exceptionally rare amongst Amblyceran lice and feather mites, respectively. *Guimaraesiella amsel* and Epidermoptidae mites represent new records to the Azores fauna. However, phoretic association with louse flies, *O. fringillina* and *O. turdi*, have already been recorded by Bartlow *et al*. (2016) and Philips and Fain (1991). Thus, despite the phoresy being a non-transversal behavior to all mites and chewing lice, some species can use this behavior as a dispersal mechanism, shaping their distribution and abundance.

Our findings resulted in the recording of 1 species of louse fly new to the fauna of Azores: *Ornithomya fringillina*; and a new species to the fauna of mainland Portugal, *Icosta minor*. Our results point out that Macaronesian birds, especially blackbirds, have higher louse flies load and mean abundance when compared with their mainland counterparts. Moreover, these results do not support the idea of parasite island syndromes (low richness, frequent host-switching and reduced specialization) as for the blackcaps haemosporidians from Macaronesia. The parasite parameters changes between Island and mainland bird species may be partially interpreted as

the likely outcome of host and abiotic factors. Considering that hippoboscid flies are obligates blood-feeders ectoparasites of birds and do not depend of any vectors, our study adds a new host-parasite interaction perspective to the parasite island syndromes concept. Thus, we are aware that to better understand the parasite island syndromes on the Azores Islands is still necessary to proceed with an extensive acquisition of knowledge on bird–parasite interactions.

References

Baker, J.R., 1967. A Review of the Role Played by the Hippoboscidae (Diptera) as Vectors of Endoparasites. J. Parasitol. 53, 412–418. doi:10.2307/3276603

Barrientos, R., Valera, F., Barbosa, A., Carrillo, C.M., Moreno, E., 2014. Biogeography of haemo- and ectoparasites of an arid-land bird, the Trumpeter finch. J. Arid Environ. 106, 11–17. doi:10.1016/j.jaridenv.2014.03.005

Bartlow, A.W., Villa, S.M., Thompson, M.W., Bush, S.E., 2016. Walk or ride? Phoretic behaviour of amblyceran and ischnoceran lice. Int. J. Parasitol. 46, 221–227. doi:10.1016/j.ijpara.2016.01.003

Begon, M., Harper, J.L., Townsend, C.R., 1996. Ecology: Individuals, populations and communities, 3rd Ed. Blackwell Scientific Publications, Sunderland. 1068 pp. ISBN: 0865421110

Blondel, J., 2000. Evolution and ecology of birds on islands: Trends and prospects. Vie Milieu 50, 205–220.

Bucher, E.H., 1988. Do birds use biological control against nest parasites? Parasitol. Today 4, 1–3. doi:10.1016/0169-4758(88)90045-2

Bush, S.E., Gustafsson, D.R., Clayton, D.H., 2018. New records of ectoparasites from passerine birds in the High Tatras of Slovakia. Oecologia Mont. 27, 43–45.

Carles–Tolrá, M., Báez, M., 2002. Hippoboscidae. In: Carles-Tolrá, M. (Ed.), Catálogo de Los Díptera de España, Portugal y Andorra (Insecta). Sociedad Entomológica Aragonesa (SEA), Zaragoza, p. 208. ISBN: 84-932807-0-4

Chao, A., 1987. Estimating the Population Size for Capture-Recapture Data with Unequal Catchability. Biometrics 43, 783. doi:10.2307/2531532

Clayton, D.H., Drown, D.M., 2001. Critical evaluation of five methods for quantifying chewing

lice (Insecta: Phthiraptera). J. Parasitol. 87, 1291–1300. doi:10.1645/0022-3395(2001)087[1291:CEOFMF]2.0.CO;2

Colwell, R.K., 2013. EstimateS: Statistical estimation of species richness and shared species from samples. Version 9.1.

Corbet, G.B., 1956. The Life-History and Host-Relations of a Hippoboscid Fly *Ornithomyia fringillina* Curtis. J. Anim. Ecol. 25, 403–420. doi:10.2307/1934

Cork, S.C., Csörgö, T., Scebba, S., Lövei, G., 2001. The prevalence of nematodes parasites in transcontinental songbirds. In: Association of Veterinary Teachers and Research Workers. Current Topics in Veterinary Science. 55th Annual Conference, Scarborough, UK, 9th-12th April, 2001. Abstracts. Research in Veterinary Science, 2001, 70 (Suppl A), 20, pp. 1–53.

Demongin, L., 2016. Identification guide to birds in the hand, Beauregard-Vendon. Laurent Demongin. 392 pp. ISBN: 978-2-9555019-0-0.

Dick, C., 2006. Checklist of world Hippoboscidae (Diptera: Hippoboscoidea). Dep. Zool. F. Museum Nat. Hist. Chicago.

Dobson, A.P., 1988. Restoring Island Ecosystems: The Potential of Parasites to Control Introduced Mammals. Conserv. Biol. 2, 31–39.

Farajollahi, A., Crans, W.J., Nickerson, D., Bryant, P., Wolf, B., Glaser, A., Andreadis, T.G., 2005. Detection of West Nile virus RNA from the louse fly *Icosta americana* (Diptera: Hippoboscidae). J. Am. Mosq. Control Assoc. 21, 474–476. doi:10.2987/8756-971x(2006)21[474:downvr]2.0.co;2

França, Z., Cruz, J.V., Nunes, J.C., Forjaz, V.H., 2003. Geologia Dos Açores: Uma Perspectiva Actual. Açoreana 10, 11–140.

Gancz, A.Y., Barker, I.K., Lindsay, R., Dibernardo, A., McKeever, K., Hunter, B., 2004. West Nile virus outbreak in North American owls, Ontario, 2002. Emerg. Infect. Dis. 10, 2135–2142. doi:10.3201/eid1012.040167

Gómez-Díaz, E., Morris-Pocock, J.A., González-Solís, J., McCoy, K.D., 2012. Trans-oceanic host dispersal explains high seabird tick diversity on Cape Verde islands. Biol. Lett. 8, 616–619. doi:10.1098/rsbl.2012.0179

Gustafsson, D.R., Bush, S.E., 2017. Morphological revision of the hyperdiverse *Brueelia*-complex (Insecta: Phthiraptera: Ischnocera: Philopteridae) with new taxa, checklists and

generic key. Zootaxa 4313, 1–443. doi:10.11646/zootaxa.4313.1.1

Halos, L., Jamal, T., Maillard, R., Girard, B., Guillot, J., Chomel, B., Vayssier-Taussat, M., Boulouis, H.-J., 2004. Role of Hippoboscidae Flies as Potential Vectors of *Bartonella* spp. Infecting Wild and Domestic Ruminants. Appl. Environ. Microbiol. 70, 6302–6305. doi:10.1128/AEM.70.10.6302-6305.2004

Hill, D.S., 1962. Revision of the British species of *Ornithomyia* Latreille (Diptera: Hippoboscidae). Proc. R. Ent. Soc. Lond. (B) 31, 11–18. doi:10.1111/j.1365-3113.1962.tb01163.x

Hill, D.S., Wilson, N., Corbet, G.B., 1967. Mites associated with British species of *Ornithomya* (Diptera: Hippoboscidae). J. Med. Entomol. 4, 102–122. doi:10.1093/jmedent/4.2.102

Hughes, J., Page, R.D.M., 2007. Comparative tests of ectoparasite species richness in seabirds. BMC Evol. Biol. 7, 1–21. doi:10.1186/1471-2148-7-227

Hutson, A.M., 1981. The population of the louse-fly, *Crataerina pallida* (Diptera, Hippoboscidae) on the European swift, *Apus apus* (Aves, Apodidae). J. Zool. 194, 305–316. doi:10.1111/j.1469-7998.1981.tb04583.x

Hutson, A.M., 1984. Keds, Flat-flies and Bat-Flies: Diptera, Hippoboscidae and Nycteribiidae. Handbooks Identif. Br. Insects 10, 1–40.

Ibáñez-Álamo, J.D., Ruiz-Raya, F., Rodríguez, L., Soler, M., 2016. Fecal sacs attract insects to the nest and provoke an activation of the immune system of nestlings. Front. Zool. 13, 1–9. doi:10.1186/s12983-016-0135-3

IBM Corp., 2019. IBM®SPSS®Statistics for Windows. Version 26.

Ishtiaq, F., Clegg, S.M., Phillimore, A.B., Black, R.A., Owens, I.P.F., Sheldon, B.C., 2010. Biogeographical patterns of blood parasite lineage diversity in avian hosts from southern Melanesian islands. J. Biogeogr. 37, 120–132. doi:10.1111/j.1365-2699.2009.02189.x

Keirans, J.E., 1975. A review of the phoretic relationship between Mallophaga (Phthiraptera: Insecta) and Hippoboscidae (Diptera: Insecta). J. Med. Entomol. 12, 71–76. doi:10.1093/jmedent/12.1.71

Kuris, A.M., Blaustein, A.R., Alio, J.J., 1980. Hosts as Islands. Am. Nat. 116, 570–586.

Labitzke, V., Jentzsch, M., 2019. Louse fly records during bird ringing at the Helmestausee Berga-Kelbra (Diptera, Hippoboscidae). Vogelwarte 57, 81–89.

Literák, I., Sychra, O., Resendes, R., Rodrígues, P., 2015. Chewing Lice in Azorean Blackcaps (*Sylvia atricapilla*): A Contribution to Parasite Island Syndromes. J. Parasitol. 101, 252–254. doi:10.1645/14-601.1

Losos, J.B., Ricklefs, R.E., 2010. The Theory of Island Biogeography Revisited, Revised Ed. Princeton University Press. 496 pp. ISBN: 978-0-691-13653-0

Lynch, A., Baker, A.J., 1993. A Population Memetics Approach to Cultural Evolution in Chaffinch Song: Meme Diversity Within Populations. Am. Nat. 141, 597–620. doi:10.1086/285493

Maa, T., 1966. The genus *Ornithoica* Rondani (Diptera: Hippoboscidae). Pacific Insects Monogr. 10, 10–124.

Maa, T., 1969. Revision of *Icosta* (= *Lynchia* Auctt .) With Erection of a Related Genus *Phthona* (Diptera: Hippoboscidae). Pacific Insects Monogr. 20, 25–203.

Maa, T., Peterson, B., 1987. Hippoboscidae. In: McAlpine, J. (Ed.), Manual of Neartic Diptera - Volume 2. Agriculture Canada Monograph No. 28, pp. 1271–1281. ISBN: 0-660-12125-5

MacLeod, C.J., Paterson, A.M., Tompkins, D.M., Duncan, R.P., 2010. Parasites lost - do invaders miss the boat or drown on arrival? Ecol. Lett. 13, 516–527. doi:10.1111/j.1461-0248.2010.01446.x

Malenke, J.R., Newbold, N., Clayton, D.H., 2011. Condition-specific competition governs the geographic distribution and diversity of ectoparasites. Am. Nat. 177, 522–534. doi:10.1086/658176

Mironov, S. V., Bochkov, A. V., Fain, A., 2005. Phylogeny and evolution of parasitism in feather mites of the families Epidermoptidae and Dermationidae (Acari: Analgoidea). Zool. Anz. 243, 155–179. doi:10.1016/j.jcz.2004.10.001

Moyer, B.R., Drown, D.M., Clayton, D.H., 2002. Low humidity reduces ectoparasite pressure: implications for host life history evolution. Oikos 97, 223–228. doi:10.1034/j.1600-0706.2002.970208.x

Nieberding, C., Morand, S., Libois, R., Michaux, J.R., 2006. Parasites and the island syndrome: The colonization of the western Mediterranean islands by *Heligmosomoides polygyrus* (Dujardin, 1845). J. Biogeogr. 33, 1212–1222. doi:10.1111/j.1365-2699.2006.01503.x

Oboňa, J., Sychra, O., Greš, S., Heřman, P., Manko, P., Roháček, J., Šestáková, A., Šlapák, J.,

Hromada, M., 2019. A revised annotated checklist of louse flies (diptera, hippoboscidae) from Slovakia. Zookeys 2019, 129–152. doi:10.3897/zookeys.862.25992

Oslejskova, L., Kounkova, S., Gustafsson, D.R., Resendes, R., Rodrigues, P., Literak, I., Sychra, O., 2020. Insect ectoparasites from wild passerine birds in the Azores Islands. Parasite 27, 64. doi:10.1051/parasite/2020063

Pacheco, J.M., Ferreira, T., Queiroz, G., Wallenstein, N., Coutinho, R., Cruz, J. V., Pimentel, A., Silva, R., Gaspar, J.L., Goulart, C., 2013. Notas sobre a geologia do arquipélago dos Açores. In: Dias, R., Araújo, A., Terrinha, P., Kullberg, J.C. (Eds.), Geologia de Portugal, Vol. 2. Escolar, pp. 596–690. ISBN: 978-972-592-364-1

Pape, T., Beuk, P., Pont, A., Shatalkin, A., Ozerov, A., Woźnica, A., Merz, B., Bystrowski, C., Raper, C., Bergström, C., Kehlmaier, C., Clements, D., Greathead, D., Kameneva, E., Nartshuk, E., Petersen, F., Weber, G., Bächli, G., Geller-Grimm, F., Van de Weyer, G., Tschorsnig, H.-P., de Jong, H., van Zuijlen, J.-W., Vaňhara, J., Roháček, J., Ziegler, J., Majer, J., Hůrka, K., Holston, K., Rognes, K., Greve-Jensen, L., Munari, L., de Meyer, M., Pollet, M., Speight, M., Ebejer, M., Martinez, M., Carles-Tolrá, M., Földvári, M., Chvála, M., Barták, M., Evenhuis, N., Chandler, P., Cerretti, P., Meier, R., Rozkosny, R., Prescher, S., Gaimari, S., Zatwarnicki, T., Zeegers, T., Dikow, T., Korneyev, V., Richter, V., Michelsen, V., Tanasijtshuk, V., Mathis, W., Hubenov, Z., de Jong, Y., 2015. Fauna Europaea: Diptera – Brachycera. Biodivers. Data J. 3, e4187. doi:10.3897/BDJ.3.e4187

Paterson, A.M., Palma, R.L., Gray, R.D., 1999. How Frequently Do Avian Lice Miss the Boat? Implications for Coevolutionary Studies. Syst. Biol. 48, 214–223. doi:10.1080/106351599260544

Paterson, A.M., Palma, R.L., Gray, R.D., 2003. Drowning on arrival, missing the boat, and x-events: How likely are sorting events. In: Page, R.D.M. (Ed.), Tangled Trees: Phylogeny, Cospeciation, and Coevolution. The University of Chicago Press, Chicago, pp. 287–307. ISBN: 0-226-64467-7

Pérez-Rodríguez, A., Ramírez, Á., Richardson, D.S., Pérez-Tris, J., 2013. Evolution of parasite island syndromes without long-term host population isolation: parasite dynamics in Macaronesian blackcaps *Sylvia atricapilla*. Glob. Ecol. Biogeogr. 22, 1272–1281. doi:10.1111/geb.12084

Petersen, F.T., Damgaard, J., Meier, R., 2007. DNA taxonomy: How many DNA Sequences are needed for solving a Taxonomic Problem? The Case of two Parapatric Species of Louse

Flies (Diptera : Hippoboscidae : *Ornithomya* Latreille, 1802). Arthropod Syst. Phylogeny 65, 119–125.

Petit, K.E., Petit, L.J., Petit, D.R., 1989. Fecal Sac Removal: Do the Pattern and Distance of Dispersal Affect the Chance of Nest Predation? Condor 91, 479–482. doi:10.2307/1368331

Philips, J., Fain, A., 1991. Acarine symbionts of louseflies (Diptera : Hippoboscidae). Acarologia 32, 377–384.

Poulin, R., 2004. Macroecological patterns of species richness in parasite assemblages. Basic Appl. Ecol. 5, 423–434. doi:10.1016/j.baae.2004.08.003

Rani, P.A.M.A., Coleman, G.T., Irwin, P.J., Traub, R.J., 2011. *Hippobosca longipennis* - a potential intermediate host of a species of *Acanthocheilonema* in dogs in northern India. Parasit. Vectors 4, 1–7. doi:10.1186/1756-3305-4-143

Reeves, W.K., Lloyd, J.E., 2019. Louse Flies, Keds, and Bat Flies (Hippoboscoidea). In: Mullen, G.R., Durden, L.A. (Eds.), Medical and Veterinary Entomology. Elsevier, London, pp. 421–438. ISBN: 978-0-12-814043-7. doi:10.1016/B978-0-12-814043-7.00020-0

Reiczigel, J., Marozzi, M., Fábián, I., Rózsa, L., 2019. Biostatistics for Parasitologists – A Primer to Quantitative Parasitology. Trends Parasitol. 35, 277–281. doi:10.1016/j.pt.2019.01.003

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682. doi:10.1038/nmeth.2019

Senar, J.C., Copete, J.L., Domenech, J., von Walter, G., 1994. Prevalence of louse-flies Diptera, Hippoboscidae parasiting a cardueline finch and its effect on body condition. Ardea 82, 157–160.

Smit, J.T., 2008. Diptera (Scenopinidae, Xylomyidae, Syrphidae, Tephritidae, Hippoboscidae, Oestridae, Tachinidae). In: Borges, P.A.V, Abreu, C., Aguiar, A.M.F., Carvalho, P., Jardim, R., Melo, I., Oliveira, P., Sérgio, C., Serrano, A.R.M. (Eds.), A List of the Terrestrial Fungi, Flora and Fauna of Madeira and Selvagens Archipelagos. Direcção Regional do Ambiente da Madeira and Universidade dos Açores, Funchal and Angra do Heroísmo, pp. 328–339. ISBN: 978-989-95790-0-2

Smit, J.T., 2010. Diptera (Hippoboscidae, Scenopinidae, Syrphidae, Tachinidae, Tephritidae).

In: Borges, P.A.V., Costa, A., Cunha, R., Gabriel, R., Gonçalves, V., Martins, A., Melo, I., Parente, M., Raposeiro, P., Rodrigues, P., Santos, R.S., Silva, L., Vieira, P., Vieira, V. (Eds.), A List of the Terrestrial and Marine Biota from the Azores. Princípia, Cascais, pp. 233–240. ISBN: 978-989-8131-75-1.

Sorjonen, J., 1971. Occurrence of three species of *Ornithomya* Diptera, Hippoboscidae on birds in two areas of southern Finland. Ann. Zool. Fennici 8, 442–445.

Sychra, O., Literák, I., Podzemný, P., Benedikt, V., 2008. Insect ectoparasites from wild passerine birds in Czech Republic. Parasite 15, 599–604. doi:10.1051/parasite/2008154599

Sychra, O., Literák, I., Podzemný, P., Harmat, P., Hrabák, R., 2011. Insect ectoparasites on wild birds in the Czech Republic during the pre-breeding period. Parasite 18, 13–19. doi:10.1051/parasite/2011181013

Tella, J.L., Gajon, A., Gortazar, C., Osacar, J.J., 1998. High Host Specificity of *Crataerina melbae* (Diptera: Hippoboscidae) in a Mixed Colony of Birds. J. Parasitol. 84, 198–200. doi:10.2307/3284563

Visnak, R.M., Dumbacher, J.P., 1999. Comparison of four fumigants for removing avian lice. J. F. Ornithol. 70, 42–48.

Supplementary material

Table S2.1– Louse fly species in their host species with respectively prevalence and confidence intervals (95%) in square bracket. As there were no differences within any population regarding host sex or age, year of fieldwork we pooled data to obtain a single value per population

Host species	Louse fly species		P% [CI 95%]
Turdus merula	*Ornithoica turdi*		45.4 [39.0-51.9]
	Ornithomya fringillina		13.8 [9.7-18.8]
		Total	50.8 [44.3-57.3]
Sylvia atricapilla	*Ornithoica turdi*		2.1 [0.7-4.8]
	Ornithomya fringillina		2.9 [1.2-5.9]
		Total	5.0 [2.6-8.5]
Fringilla coelebs	*Ornithoica turdi*		3.3[1.4-6.5]
	Ornithomya fringillina		2.1 [0.7-4.8]
		Total	5.4 [2.9-9.1]
Erithacus rubecula	*Ornithoica turdi*		3.7 [0.5-12.7]
	Icosta minor		1.9 [0-9.9]
		Total	5.6 [1.2-15.4]

Table S2.2 – Prevalence, confidence intervals (95%) in square brackets, intensity and abundance of louse flies species on blackbirds, blackcaps, chaffinches and robins from the Azores Islands (Flores, Terceira and São Miguel) and mainland Portugal. As there were no differences within any population regarding host sex or age, year of fieldwork we pooled data to obtain a single value per population. Statistical differences in the geographical patterns of louse fly were compared among each Island and continent and three Azores Islands together and mainland. Sample sizes (n) are shown in brackets.

Louse fly species	Characteristics of louse flies	Blackbird Flores (n=60)	Blackbird Terceira (n=60)	Blackbird São Miguel (n=60)	Blackbird Total (n=180)	Blackbird Mainland (n=60)	Blackcap Flores (n=61)	Blackcap Terceira (n=60)	Blackcap São Miguel (n=60)	Blackcap Total (n=180)	Blackcap Mainland (n=60)	Chaffinch Flores (n=60)	Chaffinch Terceira (n=60)	Chaffinch São Miguel (n=60)	Chaffinch Total (n=180)	Chaffinch Mainland (n=60)	Robin Terceira (n=8)	Robin São Miguel (n=17)	Robin Total (n=25)	Robin Mainland (n=29)
Ornithoica turdi	Prevalence (%)	58.3*	65.0*	53.3*	58.9*	5.0	4.9	0.0	3.3	2.8	0.0	0.0	8.3	5.0	4.4	0.0	0.0	11.8	8.0	0.0
	[CI 95%]	[44.9-70.9]	[51.6-76.9]	[40.0-66.3]	[51.3-66.2]	[1.0-13.9]	[0.1-13.7]		[0.4-11.5]	[0.9-6.3]			[2.8-18.4]	[1.0-13.9]	[1.9-8.6]			[1.5-36.4]	[1.0-26.0]	
	Intensity (mean±SD)	2.3 ± 0.3	1.8 ± 0.2	2.0 ± 0.3	2.0 ± 0.2	1.3 ± 0.3	1.0 ± 0.0	0	1.0 ± 0.0	1.0 ± 0.0	0	0	1.4 ± 0.2	1.0 ± 0.0	1.3 ± 0.2	0	0	1.0 ± 0.0	1.0 ± 0.0	0
	Intensity range	1 - 8	1 - 5	1 - 7	1 - 8	1 - 2	1	0	1	1	0	0	1 - 2	1	1 - 2	0	0	1	1	0
	Abundance (mean±SD)	1.3 ± 0.2*	1.2 ± 0.2*	1.1 ± 0.2**	1.2 ± 0.1*	0.1 ± 0.04	0.05 ± 0.03	0	0.03 ± 0.02	0.03 ± 0.01***	0	0	0.1 ± 0.05	0.05 ± 0.03	0.06 ± 0.02***	0	0	0.1 ± 0.08	0.08 ± 0.06	0
Ornithomya fringillina	Prevalence (%)	5.0	26.7*	23.3*	18.3*	0.0	3.3	3.3	5.0	3.9	0.0	1.7	5.0	1.7	2.8	0.0	0.0	0.0	0.0	0.0
	[CI 95%]	[1.0-13.9]	[16.1-39.7]	[13.4-36.0]	[13.0-24.8]	0.0	[0.4-11.3]	[0.4-11.5]	[0.1-13.9]	[1.6-7.8]		[0.0-8.9]	[1.0-13.9]	[0.0-8.9]	[0.9-6.4]		0.0	0.0	0.0	0.0
	Intensity (mean±SD)	1.0 ± 0.0	1.1 ± 0.3	1.1 ± 0.4	1.1 ± 0.3	0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	0	1.0	1.0 ± 0.0	1.0	1.2 ± 0.4	0	0	0	0	0
	Intensity range	1	1 - 2	1 - 2	1 - 2	0	1	1	1	1	0	1	1	2	1 - 2	0	0	0	0	0
	Abundance (mean±SD)	0.1 ± 0.03	0.3 ± 0.07**	0.3 ± 0.07**	0.2 ± 0.03*	0	0.03 ± 0.02	0.03 ± 0.02	0.05 ± 0.03	0.04 ± 0.01***	0	0.02 ± 0.02	0.05 ± 0.03	0.03 ± 0.03	0.03 ± 0.02	0	0	0	0	0
Icosta minor	Prevalence (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.4
	[CI 95%]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	[0.1-17.8]
	Intensity (mean±SD)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0
	Intensity range	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Abundance (mean±SD)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.03 ± 0.03
Total	Prevalence (%)	60.0*	71.7*	66.7*	66.1*	5.0	8.2	3.3	8.3	6.6***	0.0	1.7	13.3**	6.7	7.2***	0.0	0.0	11.8	8.0	3.4
	[CI 95%]	[46.5-72.4]	[58.6-82.5]	[53.3-78.3]	[58.7-73.0]	[1.0-13.9]	[2.7-18.1]	[0.4-11.5]	[2.8-18.4]	[3.5-11.3]	0.0	[0.0-8.9]	[5.9-24.6]	[1.8-16.2]	[3.9-12.0]	0.0	0.0	[1.5-36.4]	[1.0-26.0]	[1.5-36.4]
	Intensity (mean±SD)	2.3 ± 0.3	2.0 ± 0.2	2.0 ± 0.2	2.1 ± 0.1	1.3 ± 0.3	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	0	1.0	1.3 ± 0.2	1.3 ± 0.3	1.2 ± 0.1	0	0	1.0 ± 0.0	1.0 ± 0.0	1.0
	Intensity range	1 - 8	1 - 5	1 - 7	1 - 8	1 - 2	1	1	1	1	0	1	1 - 2	1 - 2	1 - 2	0	0	1	1	1
	Abundance (mean±SD)	1.4 ± 0.2*	1.5 ± 0.2*	1.3 ± 0.2*	1.4 ± 0.1*	0.07 ± 0.04	0.08 ± 0.04***	0.03 ± 0.02	0.08 ± 0.04	0.07 ± 0.02**	0	0.02 ± 0.02	0.2 ± 0.06***	0.08 ± 0.04	0.09 ± 0.03***	0	0	0.1 ± 0.08	0.08 ± 0.06	0.03 ± 0.03

Statistically significant: * $p \leq 0.001$; ** $p \leq 0.01$; *** $p \leq 0.05$

Table S2.3– Average of absolute maximum and minimum temperature and total precipitation of the previous month of fieldwork*.

	Tº min	Tº max	Precipitation
Flores			
14 Out '18 - 19 Out '18	17.3	28.5	66.6
9 Out '19 - 13 Out '19	16.5	27.1	54.0
Terceira			
22 Out '18 - 29 Out '18	17.4	26.5	56.2
18 Out '19 - 20 Out '19	16.4	26.0	56.0
São Miguel			
31 Out '18 - 5 Nov '18	13.5	25.1	301.9
28 Out '19 - 3 Nov '19	13.9	26.5	299.6
Mainland Portugal			
25 Nov '18 - 11 Dez '18	7.4	22.1	67.4
14 Nov '19 - 23 Nov '19	10.6	29.7	23.8

Tº min – Average of absolute minimum temperature (degrees Celsius)

Tº max – Average of absolute maximum temperature (degrees Celsius)

Precipitation – Average of total precipitation (millimeters)

* Data collected from the Monthly Climatological Bulletin of the Instituto Português do Mar e da Atmosfera

Chapter 3

Parasite island syndromes in the context of nidicolous ectoparasites: fleas (Insecta: Siphonaptera) in wild passerine birds from Azores Archipelago

Parasite island syndromes in the context of nidicolous ectoparasites: fleas (Insecta: Siphonaptera) in wild passerine birds from Azores Archipelago

Abstract

Island syndrome, previously established for isolation process of insular vertebrates' populations, have been adapted to insular parasites communities, termed parasite island syndromes. In this work, were studied for the first time the insular syndromes for nidicolous ectoparasites of the bird species, *Turdus merula*, *Sylvia atricapilla*, *Fringilla coelebs* and *Erithacus rubecula* from Azores and the mainland Portugal. Flea species were only recorded on Azorean birds, namely *Dasypsyllus gallinulae* and *Ctenocephalides felis felis*, known as not host-specific parasites. In the absence of shared flea species between mainland and Islands birds, a comparison among our fleas prevalence to Azores Islands and mainland fleas prevalence, recorded to others European studies, showed that Azorean host populations undergo higher prevalence than the mainland one. This result was consistent with parasite island syndromes predictions recorded to ectoparasites, hippoboscid flies and chewing lice, that fleas have higher prevalence on the Azores Islands compared to mainland Portugal. However, our results provide a new perspective to parasite island syndromes assumptions, namely in the context of nidicolous ectoparasites that spend only brief periods on the hosts' body.

Keywords: Fleas; Nidicolous ectoparasites; Parasite island syndromes; Wild birds; Azores Islands; Portugal.

Introduction

Isolation processes of vertebrate populations on Islands, and in some groups of insects and plants as well, frequently involve an ecological and life-history shifts (morphometric, behavioral, physiological and genetic), comparatively to related mainland forms. These patterns together are known as the island syndrome (Adler and Levins, 1994; Baeckens and Van Damme, 2020; Burns, 2019). Over the last two decades, the biogeographical patters of insular parasite populations have also been studied to understand the fundamental ecological and evolutionary processes involved. In Macaronesia, for instance, these studies included patters for different avian parasites groups, including: i) haemoparasites – haemoparasite assemblages

of blackcap *Sylvia atricapilla* (Linnaeus, 1758) in the Macaronesia was impoverished, with low prevalence, frequent host-switching and reduced host specialization (Pérez-Rodríguez *et al*., 2013). Likewise, Barrientos *et al*. (2014) described the loss of species richness and lower prevalence to *Bucanetes githagineus* (Lichtenstein, MHC, 1823) in the Canary Islands. In turn, Illera *et al*. (2015) did not find an impoverishment of parasite diversity and lower parasite prevalence in the *Sylvia conspicillata* (Temminck, 1820) in Macaronesia; ii) chewing lice – Azorean blackcaps had fewer parasite richness, higher chewing lice prevalence and host specialization (Literák *et al*., 2015); iii) hippoboscid flies – insular passerine birds had highest parasite diversity, prevalence and abundance (Tomás *et al*., 2021); iv) mites – lower species richness in Azorean birds than their continental relatives (Rodrigues *et al*., 2015). Additionally, Barrientos *et al*. (2014) recorded higher prevalence of mites in *Bucanetes githagineus* (Lichtenstein, MHC, 1823) from Islands; and v) coccidian parasite – higher linage diversity on the Macaronesian population of *S. conspicillata* than on the continental areas (Illera *et al*., 2015). On others European islands, particularly Danish islands, insular nests of *Parus major* Linnaeus, 1758 showed a higher level of fleas infestation than mainland (Wiggins *et al*., 1998). Even though not considered a parasite, avian poxvirus was more prevalent on the Canary Islands in *Calandrella rufescens* (Vieillot, 1819) in relation to their mainland counterparts (Carrete *et al*., 2009). These changes in the insular communities of parasites were termed parasite island syndromes (Pérez-Rodríguez *et al*., 2013).

Even though little is known about which mechanisms are important in colonizing success of parasites in new regions, it is known that during the hosts range expansion, parasites often are lost, by one of two independent events: i) "missing the boat" – individuals hosts of founder population may not be infected with the parasite (Paterson *et al*., 1999; Torchin *et al*., 2003); and ii) "drowning on arrival" – parasites do arrive with the founder hosts, but "sinking with the boat" when infected hosts arrive but fail to establish, or "lost overboard" when parasite establishment fail for other reasons (MacLeod *et al*., 2010). Several factors, associated with parasites – low vagility, high host-specificity and life cycle complexity (Ishtiaq *et al*., 2010; Paterson *et al*., 2003; Torchin *et al*., 2003); and hosts – small founding populations, high mortality, low social interaction and small body size (Paterson *et al*., 1999), have been suggested to explain parasites "lost overboard".

The elementary assumptions of Theory of Island Biogeography, that the area of the Island and the distance of the Island from the mainland source population are determinant factors to the insular vertebrates differentiation, have also been incorporated in parasite island syndromes

analysis (Adler and Levins, 1994; MacArthur and Wilson, 1967). Pérez-Rodríguez *et al.* (2013) described a negative correlation between haemoparasites species richness and the Island distance from the mainland. Moreover, Wiggins *et al.* (1998) described a positive correlation between level of ectoparasite infestation in nests and Island isolation. Regarding the effect of the Island size, Ishtiaq *et al.* (2010) found a positive correlation between *Plasmodium* lineage richness and Island area. In turn, Spurgin *et al.* (2012) observed that *Anthus berthelotii* Bolle, 1862, an endemic Macaronesian avian species, harbored fewer bloodborne pathogens (avian malaria, poxvirus and trypanosomes) on smaller and more isolated Islands than larger and less isolated Islands. No significant correlation between species richness of parasites and Island size and isolation degree have been recorded in other studies (Literák *et al.*, 2015; Pérez-Rodríguez *et al.*, 2013).

Fleas (Insecta: Siphonaptera) are highly specialized holometabolous insects, small, wingless, laterally flattened, heavily chitinized and with strongly developed hind legs, adapted for jumping. As obligate blood-sucking parasites of mammals and birds, immediately when adult fleas emerge from the cocoon, they seek a host to find a blood meal (Krasnov, 2008; Marshall, 1981). Of the over 2.500 species worldwide currently described, only 6% of the species are ornithophilic (Durden and Hinkle, 2019). Throughout the world, flea-borne diseases with medical and veterinary importance such as plague (caused by *Yersinia pestis*), murine typhus (caused by *Rickettsia typhi*) and cat-scratch disease (being *Bartonella henselae* its etiological agent) cause significant morbidity and mortality (Eisen and Gage, 2012).

The main goal of this study was characterize the flea populations on Eurasian blackbird *Turdus merula* Linnaeus, 1758, Eurasian blackcap *Sylvia atricapilla*, common chaffinch *Fringilla coelebs* Linnaeus, 1758 and European robin *Erithacus rubecula* (Linnaeus, 1758), on the Azores Islands and the mainland Portugal, to test the three assumptions of the parasite island syndromes: i) the parasite diversity on the islands should be very similar to that observed on the mainland. Because parasites can "missing the boat" or "drowning on arrival" during colonization events, we expect parasite richness in island birds to be equal to or less than birds on the mainland (MacLeod *et al.*, 2010; Paterson *et al.*, 1999; Torchin *et al.*, 2003); ii) parasites prevalence should be different between island and mainland birds. Parasites are subject to different conditions in island environments, usually showing to be favorable to ectoparasites (Literák *et al.*, 2015; Tomás *et al.*, 2021). Therefore, we expect the fleas to be more prevalent in island regions; and iii) generalist parasites should be more likely to occur on the islands. Since generalist parasites can use diverse hosts to thrive on the islands, we predict these

parasites to be more frequent on both islands and the mainland (MacLeod *et al*., 2010). Furthermore, parasite diversity on the islands should not be positively correlated with their area and proximity to the mainland. Since ectoparasites depend on birds to thrive on the islands, and birds are well established on Azores Archipelago, we expect that fleas' populations should not be structured according to the assumptions of Theory of Island Biogeography.

Material and Methods

Field sampling

Fieldwork took place in three Islands of the Azores Archipelago, namely São Miguel (37°46'49.48"N, 25°29'49.369"W), Terceira (38°43'17.908"N, 27°13'14.077"W) and Flores Island (39°26'50.896"N, 31°11'38.202"W), and South mainland Portugal, specifically at Silves (37°11'20.393"N, 8°26'28.338"W) and Olhão (37°1'33.751"N, 7°50'32.464"W), during the post-breeding seasons (October-December) of 2018-2019. Live birds of the species *T. merula* (n_{total}= 240; n=60 to each Azores Island (and mainland Portugal), *S. atricapilla* (n_{total}=241; n=61 to Flores Island and n=60 to Terceira Island, São Miguel Island and mainland Portugal), *F. coelebs* (n_{total} =240; n=60 to each Azores Island and mainland Portugal) and *E. rubecula* (n_{total} =54; n=8 to Terceira Island, n=17 to São Miguel Island and n=29 to mainland Portugal) were randomly captured with mist nets at 2–3 sites on each Island, due to the patchy distribution and abundance of sampled species. Except European robin, the remaining bird species are endemic taxa in the Azores Archipelago and the following subspecies are recognized, *T. m. azorensis* Hartert, 1905, *S. a. gularis* Alexander, 1898, *F. c. moreletti* Pucheran, 1859. Robins, as one of the species that most recently colonized the Azores Islands, are absent from the Western group and therefore were not sampled on Flores Island.

Each bird was individualized with metal ring and identified and sampled for fleas presence using standard practice performed by numerous bird banders for the avian ectoparasites study, the modified fumigation chamber method. During this procedure hosts bodies were exposed to chloroform, for 5 minutes and bird's heads underwent visual examination (Visnak and Dumbacher, 1999). All birds were released after examination.

The collected specimens were placed individually into small tubes containing 70% ethanol. The fleas were examined microscopically at Faculty of Sciences, University of Lisbon, and identified according to identification keys on the Whitaker (2007). Images were acquired on Olympus BX51 microscope, equipped with TIS DFK 1.9MP Sony CCD color camera, controlled with the μManager 2.0-gamma software and digitally processing using ImageJ 1.52p software (Schindelin *et al*., 2012).

Statistical data analyses

Observed fleas species richness was recorded for the four bird species from each location. Prevalence, as the rate of birds infested, with confidence intervals (95%) of each flea species was estimated; the absence of shared flea species between Islands and mainland did not allow exploring differences in prevalence. Furthermore, a Chi-square test adjusted using Bonferroni correction, as *post hoc* test, was used to detect the statistical differences of fleas prevalence between Islands populations. General statistical analyses and analysis of *post hoc* tests were done with the software Quantitative Parasitology 3.0 (Reiczigel *et al*., 2019) and IBM®SPSS®Statistics Version 26 (IBM Corp., 2019). A *p*-value of 0.05 or less was considered significant.

Results and Discussion

Two fleas species were found in wild birds from Azores Islands, namely moorhen flea *Dasypsyllus gallinulae* (Dale, 1878) (Figure 3.1a) and cat flea *Ctenocephalides felis felis* (Bouché, 1835) (Figure 3.1b); no fleas species were found in the mainland Portugal birds. Moorhen flea specimens were observed in blackbirds and chaffinches in three Azores Islands (Flores, Terceira and São Miguel), while only robins and blackcaps from Terceira Island showed this flea species. A single individual of cat flea was identified in blackbird from Flores Islands. These two fleas species are well-described in insect fauna of the Azores (Borges, 2010). Moreover, Oslejskova *et al*. (2020) identified the *D*. *gallinulae*-hosts associations in the São Miguel Island and also the occurrence of fleas of the genus *Ceratophyllus*. Our data showed no significant differences of moorhen flea prevalence among insular populations of blackbirds (χ^2 = 2.212; $p \geq 0.05$) and chaffinches ($\chi^2 = 5.581$; $p \geq 0.05$). Still, a slightly higher prevalence of

fleas was observed on Terceira Island.
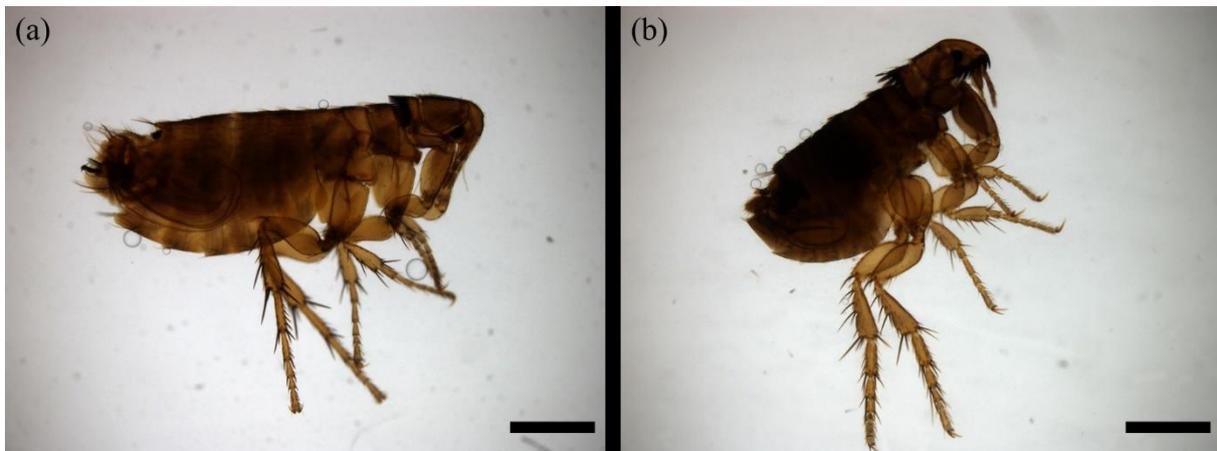
Figure 3.1 – Lateral view of two flea species collected in the Azorean *Turdus merula* Linnaeus, 1758 from Flores Island: (a) *Dasypsyllus gallinulae* (Dale, 1878) and (b) *Ctenocephalides felis felis* (Bouché, 1835). Scale bar: 500 µm.

Despite the low number of fleas species found, the richness was higher from Azorean birds than mainland one. Similar species richness was recorded by us, for hippoboscid flies species, which like fleas, immature stages occur in the bird' nests (Tomás *et al*., 2021); and for other parasite groups, ecologically quite different from fleas, such as haemoparasites and coccidian parasites (Illera *et al*., 2015). This finding to fleas species richness do not support the hypothesis that host–parasite associations are compromised on Islands, as result of "missing the boat" or "drowning on arrival" events (MacLeod *et al*., 2010). However, knowing the Portuguese bird flea fauna (Ribeiro, 2007), the absence of fleas observed on the mainland was somewhat surprising. Thus, would be mistaken to assume that this higher species richness on the Azores Islands is an insular syndrome. The absence of flea species on mainland birds may result from the season where birds were sampled. Moorhen flea, the most common species in our study, as well as most species of bird fleas are nidicolous, *i.e.*, living essentially in the nest, spending only brief periods on the host to feed (Durden and Hinkle, 2019).

The population of nidicolous fleas seems to be seasonal, with the peak infestation in the early spring, as resulted of increased nesting activity by the birds (Fowler *et al*., 1983; Sychra *et al*., 2011); and during post-breeding session, a marked decreases in the incidence of adult fleas, as resulted of the breeding nest abandonment (Fowler *et al*., 1983; Sychra *et al*., 2008). Seasonality of *D. gallinulae* was also reported by Cyprich *et al*. (2006) in Slovakia, by observing three peaks of infestation in January, July and October; and four peaks of low abundance in March,

June, September and December. Like other ectoparasite species, the distribution of fleas seems to be affected by abiotic conditions. The immature stages of the moorhen flea are sensitive to air temperature and humidity, having limited development in dry climates and more extreme temperatures (Rothschild, 1952). Moreover, in the absence of available hosts, the adult fleas appear to have a greater longevity when exposed to low temperatures and high humidity, such as during winter in temperate regions (Durden and Hinkle, 2019). Thus, considering the time of year of fieldwork (October – December), it was expectable the lower species richness on the birds body and it is possible that Azores fleas might have benefited from the typical high humidity and mild temperature of the Azores Islands.

Our work, report what is to our knowledge, the first record of blackbird as host for *C. felis felis*. We believe that this single individual of *C. felis felis*, a mammal flea mainly associated with cats and dogs was clearly a casual parasite on a blackbird. Considering the well-established feral cat populations in the Azores Islands, often associated to birds feeding areas, it is very possible that cats were the source of the cat flea. However knowing that *C. felis felis* was previously described in a Brazilian owl (Linardi and Santos, 2012), and that fleas primary parasitized mammals and only later acquired the ability to parasitize birds, suggesting that birds are alternative hosts for fleas (Durden and Hinkle, 2019; Whiting *et al.*, 2008), we leave here the following hypothesis: did the cat flea find the favorable conditions on the Islands to start parasitizing birds? Even though unlikely, this hypothesis should be considered in future studies.

The other flea species observed on all host species examined, moorhen flea, retained the host unspecificity that characterizes it in mainland regions. Despite the host unspecificity, this result does not preclude the possibility that moorhen flea on the Islands exhibits intraspecific genetic variation, as reported to other flea species (Gómez-Díaz *et al.*, 2007).

The results of fleas' prevalence on blackbirds, blackcaps, chaffinches and robins from the Azores Islands were summarized in Figure 3.2. The high prevalence of fleas observed in the Azores Archipelago was consistent with the results of Oslejskova *et al.* (2020), who found an even higher prevalence of moorhen flea on São Miguel Island, 10.0% for *E. rubecula*, 11.1% for *F. coelebs*, 18.2% for *T. merula* and 23.5% for *S. atricapilla*. Besides the prevalence, the total mean intensity (±SD) observed on the Azores Islands was 1 flea on *E. rubecula*, *F. coelebs* and *S. atricapilla* and 1.2 (±0.4) on *T. merula*, which was very similar to the results of Sychra *et al.* (2011) and Oslejskova *et al.* (2020), supporting the idea that nidicolous fleas are found in low numbers on the body of birds examined outside their nest (Durden and Hinkle, 2019).
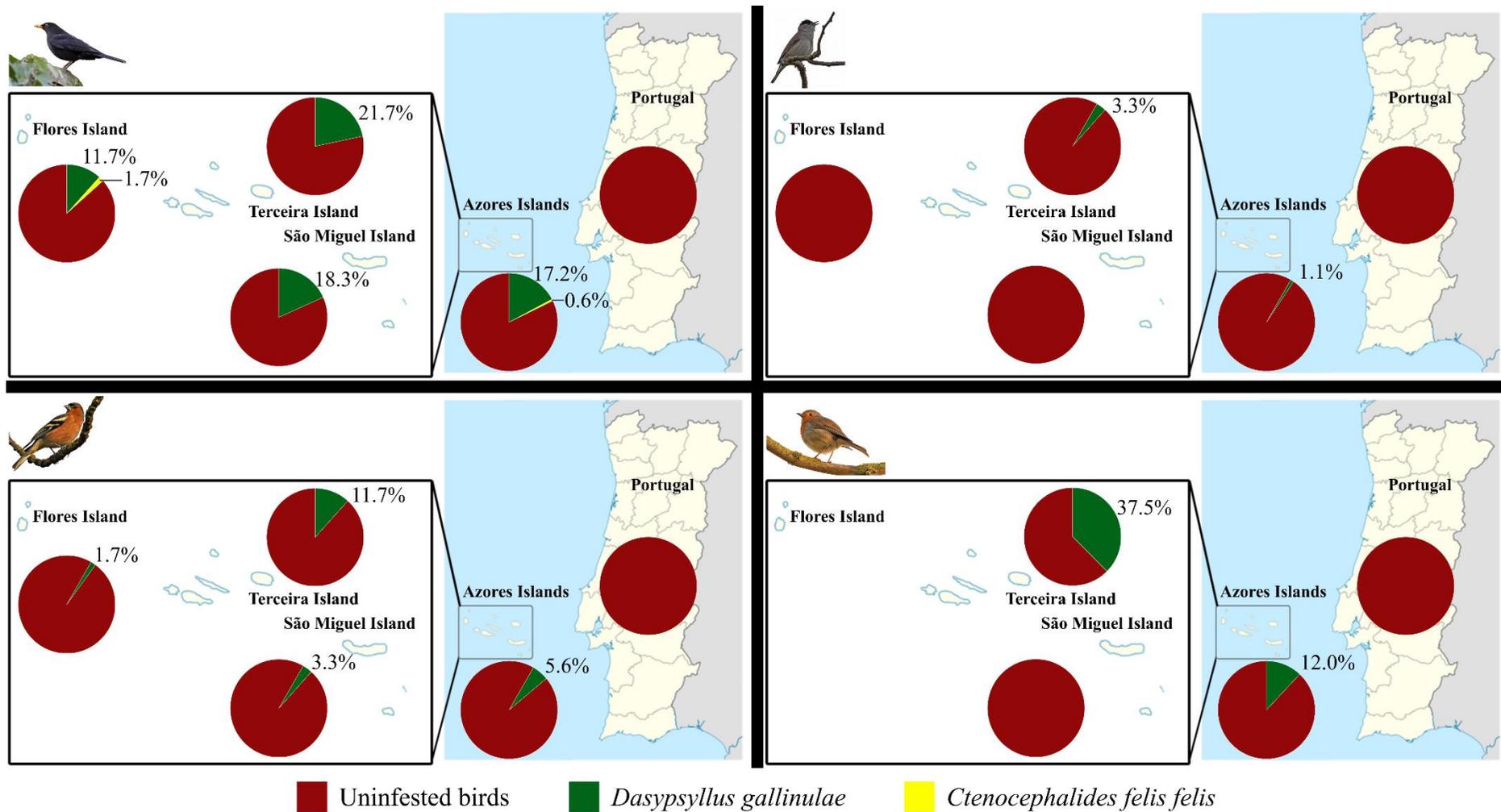
Figure 3.2 – Map showing the geographical patterns of fleas' prevalence recorded from four Passeriformes species. The pie charts represent the prevalence of fleas species, represented by different color: red color - prevalence of uninfested birds; green color - birds infested by *Dasypsyllus gallinulae* (Dale, 1878); blue color – birds infested by *Ctenocephalides felis felis* (Bouché, 1835). Passeriformes species represented by: blackbirds (n=60, each Azores Island; n=60, mainland Portugal), blackcaps (n=61, Flores Island; n=60, Terceira and São Miguel Islands; n=60, mainland Portugal), chaffinches (n=60, each Azores Island; n=60, mainland Portugal) and robins (n=8, Terceira Island; n=17, São Miguel Island; n=29, mainland Portugal).

The high prevalence of fleas on the Azores Islands, may result from several, not mutually exclusive processes: i) higher host densities on the Islands may increase the risk of flea transmission, manifesting as higher prevalence and intensity (Krasnov *et al*., 2002). Although direct contact is an alternative route in flea transmission, this may be especially relevant in the host breeding period, where birds are in close contact in nests (Tripet *et al*., 2002); and ii) the larger body size of Island populations (unpublished data, but considered a trend in insular birds (Andrade *et al*., 2015; Lomolino, 2005)), offers to ectoparasites, more space for colonization and refuge to avoid host defenses (Kuris *et al*., 1980; Rózsa, 1997). Even though nesting fleas spend brief moments on the host body outside the nest, we believe that host body mass is positively correlated with the prevalence and intensity of flea parasitism, as recorded by Young *et al*. (2015) in small mammals. Some studies have identified changes in the immune system of populations in island environments, especially acquired (humoral) immunity, as a result of reduced species richness on islands (Lobato *et al*., 2017). Because host-parasite associations evolved long before the colonization of the Azores islands and we have not observed a reduction in parasite pressure on the islands, we are convinced that either the immune system is a minor factor in the evolution of island flea populations, or evolution/reorganization in the birds' immune system simply has not occurred (Matson, 2006).

Despite the influence of humidity and temperature on flea community structuring, it appears that flea intensity and prevalence in nests are not affected by these abiotic factors (Harper *et al*., 1992; Heeb *et al*., 2000). However, knowing that relative humidity increases the longevity of adult fleas, we are convicted that *D. gallinulae* on the Islands exhibit the same active host-seeking behavior outside the nests, on the grass and plants, throughout the year, as recorded by Rosický (1957), during spring. Thus, we expect a higher prevalence on Islands with relatively constant abiotic conditions during all seasons.

Our observations on flea species richness and prevalence showed an apparent temporal stability of *D. gallinulae* between the years 2018 (6.0%) and 2019 (5.8%), which may indicate that insular fleas, are very well stabilized on the Azores Islands. This result support predictions that more generalist parasites have a wider geographic distribution because they can rely on other host species to thrive on Islands (MacLeod *et al*., 2010). Despite the apparent uncorrelation found between species richness and Island area and distance to the mainland, the low flea richness observed on the Azores Islands did not allow us to effectively test the Equilibrium Model of Island Biogeography. According to this graphical model, the balance between immigration and extinction rate favors greater species richness on large Islands and Islands near

from the putative source of colonization (MacArthur and Wilson, 1967). Previously, the Island isolation have been negatively correlated with haemoparasites (Pérez-Rodríguez *et al*., 2013) and nematode richness (De Bellocq *et al*., 2002; Nieberding *et al*., 2006) and area positively correlated with haemoparasite richness (Ishtiaq *et al*., 2010). Although the distribution of ectoparasites does not appear to be related to these Island characteristics, the occurrence of fleas of the genus *Ceratophyllus* only in the Eastern group of the Azores Archipelago observed by Oslejskova *et al*. (2020) and not found by us on any other Island, suggests that specimens of this genus only became established on the Islands closer to the mainland or have undergone extinction events on the central and western islands..

The distribution of *D. gallinulae* provided evidence that the timing of colonization of their avian hosts may be important in the evolution of insular ectoparasite communities. Blackbirds with two colonization events in the Azores (0.47 My and 0.09 My ago) and chaffinches with a longer one (1.5 My ago), exhibited fleas on all three Islands under study (Rodrigues *et al*., 2014, 2016). On the other hand, blackcaps and robins, two of the most recent colonizers of the Azores Archipelago (0.1 My and 0.08 My ago, respectively), only exhibited fleas on Terceira Island (Rodrigues *et al*., 2013, 2018). This result suggests that bird species with the oldest colonization events and, perhaps, higher dispersal among islands tend to exhibit, very well stabilized ectoparasite communities on the islands, than those species with recent waves of colonization, even if we are dealing with generalist parasites..

Even though we did not correlate the effect of insularity on ectoparasite richness, overall, Azorean birds showed higher flea infestation rates than their mainland European counterparts, supporting part of the predictions postulated to parasite island syndromes. However, further studies are needed, especially during or immediately to post-breeding bird, in order to have a more reliable sample of common ectoparasites, which will allow for a better understanding of the parasite island syndromes in the context of nidicolous ectoparasites.

References

Adler, G.H., Levins, R., 1994. The Island Syndrome in Rodent Populations. Q. Rev. Biol. 69, 473–490. doi:10.1086/418744

Andrade, P., Rodrigues, P., Lopes, R.J., Ramos, J.A., Cunha, R.T. Da, Gonçalves, D., 2015.

Ecomorphological patterns in the Blackcap *Sylvia atricapilla*: Insular versus mainland populations. Bird Study 62, 498–507. doi:10.1080/00063657.2015.1077780

Baeckens, S., Van Damme, R., 2020. The island syndrome. Curr. Biol. 30, R338–R339. doi:10.1016/j.cub.2020.03.029

Barrientos, R., Valera, F., Barbosa, A., Carrillo, C.M., Moreno, E., 2014. Biogeography of haemo- and ectoparasites of an arid-land bird, the Trumpeter finch. J. Arid Environ. 106, 11–17. doi:10.1016/j.jaridenv.2014.03.005

Borges, P.A.V., 2010. Strepsiptera, Trichoptera, Siphonaptera. In: Borges, P.A.V., Costa, A., Cunha, R., Gabriel, R., Gonçalves, V., Martins, A., Melo, I., Parente, M., Raposeiro, P., Rodrigues, P., Santos, R.S., Silva, L., Vieira, P., Vieira, V. (Eds.), A List of the Terrestrial and Marine Biota from the Azores. Princípia, Cascais, pp. 232–240. ISBN: 978-989-8131-75-1

Burns, K.C., 2019. Evolution in isolation: The search for an island syndrome in plants. Cambridge University Press, Cambridge. 226 pp. ISBN: 9781108379953.

Carrete, M., Serrano, D., Illera, J.C., López, G., Vögeli, M., Delgado, A., Tella, J.L., 2009. Goats, birds, and emergent diseases: Apparent and hidden effects of exotic species in an island environment. Ecol. Appl. 19, 840–853. doi:10.1890/07-2134.1

Cyprich, D., Krumpál, M., Mancošová, Ľ., 2006. Catalogue of fleas (Siphonaptera: Insecta) on birds (Aves) of Slovakia. NOI, Bratislava.

De Bellocq, J.G., Morand, S., Feliu, C., 2002. Patterns of parasite species richness of Western Palaeartic micro-mammals: Island effects. Ecography (Cop.). 25, 173–183. doi:10.1034/j.1600-0587.2002.250205.x

Durden, L.A., Hinkle, N.C., 2019. Fleas (Siphonaptera). In: Mullen, G.R., Durden, L.A. (Eds.), Medical and Veterinary Entomology. Elsevier, London, pp. 145–169. doi:10.1016/b978-0-12-814043-7.00010-8

Eisen, R.J., Gage, K.L., 2012. Transmission of Flea-Borne Zoonotic Agents. Annu. Rev. Entomol. 57, 61–82. doi:10.1146/annurev-ento-120710-100717

Fowler, J.A., Cohen, S., Greenwood, M.T., 1983. Seasonal variation in the infestation of blackbirds by fleas. Bird Study 30, 240–242. doi:10.1080/00063658309476804

Gómez-Díaz, E., González-Solís, J., Peinado, M.A., Page, R.D.M., 2007. Lack of host-dependent genetic structure in ectoparasites of *Calonectris* shearwaters. Mol. Ecol. 16, 5204–

5215. doi:10.1111/j.1365-294X.2007.03539.x

Harper, G.H., Marchant, A., Boddington, D.G., 1992. The Ecology of the Hen Flea *Ceratophyllus gallinae* and the Moorhen Flea *Dasypsyllus gallinulae* in Nestboxes. J. Anim. Ecol. 61, 317. doi:10.2307/5324

Heeb, P., Kölliker, M., Richner, H., 2000. Bird-ectoparasite interactions, nest humidity, and ectoparasite community structure. Ecology 81, 958–968. doi:10.1890/0012-9658(2000)081[0958:BEINHA]2.0.CO;2

IBM Corp., 2019. IBM®SPSS®Statistics for Windows. Version 26.

Illera, J.C., Fernández-Álvarez, Á., Hernández-Flores, C.N., Foronda, P., 2015. Unforeseen biogeographical patterns in a multiple parasite system in Macaronesia. J. Biogeogr. 42, 1858–1870. doi:10.1111/jbi.12548

Ishtiaq, F., Clegg, S.M., Phillimore, A.B., Black, R.A., Owens, I.P.F., Sheldon, B.C., 2010. Biogeographical patterns of blood parasite lineage diversity in avian hosts from southern Melanesian islands. J. Biogeogr. 37, 120–132. doi:10.1111/j.1365-2699.2009.02189.x

Krasnov, B., Khokhlova, I., Shenbrot, G., 2002. The effect of host density on ectoparasite distribution: An example of a rodent parasitized by fleas. Ecology 83, 164–175. doi:10.1890/0012-9658(2002)083[0164:TEOHDO]2.0.CO;2

Krasnov, B.R., 2008. Functional and evolutionary ecology of fleas: A model for ecological parasitology. Cambridge University Press, Cambridge. 593 pp. ISBN: 978-0-521-88277-4. doi:10.1017/CBO9780511542688

Kuris, A.M., Blaustein, A.R., Alio, J.J., 1980. Hosts as Islands. Am. Nat. 116, 570–586.

Linardi, P.M., Santos, J.L.C., 2012. *Ctenocephalides felis felis* vs. *Ctenocephalides canis* (Siphonaptera: Pulicidae): some issues in correctly identify these species. Rev. Bras. Parasitol. Veterinária 21, 345–354. doi:10.1590/S1984-29612012000400002

Literák, I., Sychra, O., Resendes, R., Rodrígues, P., 2015. Chewing Lice in Azorean Blackcaps (*Sylvia atricapilla*): A Contribution to Parasite Island Syndromes. J. Parasitol. 101, 252–254. doi:10.1645/14-601.1

Lobato, E., Doutrelant, C., Melo, M., Reis, S., Covas, R., 2017. Insularity effects on bird immune parameters: A comparison between island and mainland populations in West Africa. Ecol. Evol. 7, 3645–3656. doi:10.1002/ece3.2788

Lomolino, M. V., 2005. Body size evolution in insular vertebrates: Generality of the island rule. J. Biogeogr. 32, 1683–1699. doi:10.1111/j.1365-2699.2005.01314.x

MacArthur, R.H., Wilson, E.O., 1967. The Theory of Island Biogeography, 1st Ed. Princeton University Press, Princeton. 215 pp. ISBN: 9780691080505

MacLeod, C.J., Paterson, A.M., Tompkins, D.M., Duncan, R.P., 2010. Parasites lost - do invaders miss the boat or drown on arrival? Ecol. Lett. 13, 516–527. doi:10.1111/j.1461-0248.2010.01446.x

Marshall, A., 1981. The Ecology of Ectoparasitic Insects. Academic Press, London. 459 pp. ISBN: 0124740804

Matson, K.D., 2006. Are there differences in immune function between continental and insular birds? Proc. R. Soc. B Biol. Sci. 273, 2267–2274. doi:10.1098/rspb.2006.3590

Nieberding, C., Morand, S., Libois, R., Michaux, J.R., 2006. Parasites and the island syndrome: The colonization of the western Mediterranean islands by *Heligmosomoides polygyrus* (Dujardin, 1845). J. Biogeogr. 33, 1212–1222. doi:10.1111/j.1365-2699.2006.01503.x

Oslejskova, L., Kounkova, S., Gustafsson, D.R., Resendes, R., Rodrigues, P., Literak, I., Sychra, O., 2020. Insect ectoparasites from wild passerine birds in the Azores Islands. Parasite 27, 64. doi:10.1051/parasite/2020063

Paterson, A.M., Palma, R.L., Gray, R.D., 2003. Drowning on arrival, missing the boat, and x-events: How likely are sorting events, in: Page, R.D.M. (Ed.), Tangled Trees: Phylogeny, Cospeciation, and Coevolution. The University of Chicago Press, Chicago, pp. 287–307.

Paterson, A.M., Palma, R.L., Gray, R.D., 1999. How Frequently Do Avian Lice Miss the Boat? Implications for Coevolutionary Studies. Syst. Biol. 48, 214–223. doi:10.1080/106351599260544

Pérez-Rodríguez, A., Ramírez, Á., Richardson, D.S., Pérez-Tris, J., 2013. Evolution of parasite island syndromes without long-term host population isolation: parasite dynamics in Macaronesian blackcaps *Sylvia atricapilla*. Glob. Ecol. Biogeogr. 22, 1272–1281. doi:10.1111/geb.12084

Reiczigel, J., Marozzi, M., Fábián, I., Rózsa, L., 2019. Biostatistics for Parasitologists – A Primer to Quantitative Parasitology. Trends Parasitol. 35, 277–281. doi:10.1016/j.pt.2019.01.003

Ribeiro, H., 2007. Lista anotada das pulgas de Portugal (Continente, Açores e Madeira) (Insecta: Siphonaptera). Fauna entomológica de Portugal, 4, Lisboa. 50 pp. ISBN: 0873-5417.

Rodrigues, P., Lopes, R.J., Drovetski, S. V., Reis, S., Ramos, J.A., Tristão da Cunha, R., 2013. Phylogeography and genetic diversity of the Robin (*Erithacus rubecula*) in the Azores Islands: Evidence of a recent colonisation. J. Ornithol. 154, 889–900. doi:10.1007/s10336-013-0953-4

Rodrigues, P., Lopes, R.J., Reis, S., Resendes, R., Ramos, J.A., Tristão da Cunha, R., 2014. Genetic diversity and morphological variation of the common chaffinch *Fringilla coelebs* in the Azores. J. Avian Biol. 45, 167–178. doi:10.1111/j.1600-048X.2013.00229.x

Rodrigues, P., Lopes, R.J., Resendes, R., Ramos, J.A., Tristão da Cunha, R., 2016. Genetic Diversity of the Azores Blackbirds *Turdus merula* Reveals Multiple Founder Events. Acta Ornithol. 51, 221–234. doi:10.3161/00016454AO2016.51.2.008

Rodrigues, P., Mironov, S., Sychra, O., Resendes, R., Literak, I., 2015. Feather mites (Acari, Astigmata) from Azorean passerines (Aves, Passeriformes): lower species richness compared to European mainland. Parasite 22. doi:10.1051/parasite/2015009

Rodrigues, P., Micael, J., Resendes, R., Lopes, R.J., Ramos, J.A., Tristão da Cunha, R., 2018. Genetic and morphometric variation of the Blackcap (*Sylvia atricapilla*) on the Azores Archipelago reveals a recent range expansion. J. Nat. Hist. 52, 2413–2435. doi:10.1080/00222933.2018.1539194

Rosický, B., 1957. Fleas – Aphaniptera. In: Fauna ČSR 10 (In Czech). Nakladatelsví ČAV, Prague, p. 446.

Rothschild, M., 1952. A collection of fleas from the bodies of British birds. Bull. Br. Museum (Natural Hist.) Entomol. 2, pp. 185–232.

Rózsa, L., 1997. Patterns in the abundance of avian lice (Phthiraptera: Amblycera, Ischnocera). J. Avian Biol. 28, 249–254. doi:10.2307/3676976

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682. doi:10.1038/nmeth.2019

Spurgin, L.G., Illera, J.C., Padilla, D.P., Richardson, D.S., 2012. Biogeographical patterns and co-occurrence of pathogenic infection across island populations of Berthelot's pipit (*Anthus*

berthelotii). Oecologia 168, 691–701. doi:10.1007/s00442-011-2149-z

Sychra, O., Literák, I., Podzemný, P., Benedikt, V., 2008. Insect ectoparasites from wild passerine birds in Czech Republic. Parasite 15, 599–604. doi:10.1051/parasite/2008154599

Sychra, O., Literák, I., Podzemný, P., Harmat, P., Hrabák, R., 2011. Insect ectoparasites on wild birds in the Czech Republic during the pre-breeding period. Parasite 18, 13–19. doi:10.1051/parasite/2011181013

Tomás, A., Pereira da Fonseca, I., Valkenburg, T., Rebelo, M.T., 2021. Louse flies in Azorean and mainland populations of four Passeriformes species: A new perspective to parasite Island syndromes. Int. J. Parasitol. Parasites Wildl. 14, 33–40. doi:10.1016/j.ijppaw.2020.12.004

Torchin, M.E., Lafferty, K.D., Dobson, A.P., McKenzie, V.J., Kuris, A.M., 2003. Introduced species and their missing parasites. Nature 421, 628–630. doi:10.1038/nature01346

Tripet, F., Christe, P., Møller, A.P., 2002. The importance of host spatial distribution for parasite specialization and speciation: A comparative study of bird fleas (Siphonaptera: Ceratophyllidae). J. Anim. Ecol. 71, 735–748. doi:10.1046/j.1365-2656.2002.00639.x

Visnak, R.M., Dumbacher, J.P., 1999. Comparison of four fumigants for removing avian lice. J. F. Ornithol. 70, 42–48.

Whitaker, A.P., 2007. RES Handbooks for the Identification of British Insects Vol 1. Part 16: Fleas (Siphonaptera), 2nd Ed. Field Studies Council. 178 pp. ISBN: 9780901546852.

Whiting, M.F., Whiting, A.S., Hastriter, M.W., Dittmar, K., 2008. A molecular phylogeny of fleas (Insecta: Siphonaptera): Origins and host associations. Cladistics 24, 677–707. doi:10.1111/j.1096-0031.2008.00211.x

Wiggins, D.A., Møller, A.P., Sørensen, M.F.L., Brand, L.A., 1998. Island biogeography and the reproductive ecology of great tits *Parus major*. Oecologia 115, 478–482. doi:10.1007/s004420050544

Young, H.S., Dirzo, R., McCauley, D.J., Agwanda, B., Cattaneo, L., Dittmar, K., Eckerlin, R.P., Fleischer, R.C., Helgen, L.E., Hintz, A., Montinieri, J., Zhao, S., Helgen, K.M., 2015. Drivers of intensity and prevalence of flea parasitism on small mammals in East African savanna ecosystems. J. Parasitol. 101, 327–335. doi:10.1645/14-684.1

Chapter 4

Parasite island syndromes in the context of ectoparasites highly host specific: chewing lice (Phthiraptera: Ischnocera and Amblycera) in wild Azorean and Portugal mainland populations of four passerine species

Parasite island syndromes in the context of ectoparasites highly host specific: chewing lice (Phthiraptera: Ischnocera and Amblycera) in wild Azorean and Portugal mainland populations of four passerine species

Abstract

Isolation process of insular vertebrates' populations have been thoroughly debated, and recently adapted to insular communities of parasites, termed parasite island syndromes. In this work, we studied variation in parasitological parameters – diversity, prevalence and host specificity – of avian chewing lice between mainland Portugal and Azores Islands. In addition to parasitological patterns, we explored mechanisms behind geographical variation of ectoparasites. Chewing lice (Phthiraptera) of *Turdus merula*, *Sylvia atricapilla*, *Fringilla coelebs* and *Erithacus rubecula* from Mainland Portugal and Azores Archipelago was sampled, using the modified fumigation chamber method. Parasite species richness, prevalence and host specificity between mainland Portugal and Azores Islands were compared. Finally, the correlation between ectoparasites richness and Islands isolation and area was evaluated. In all, 9 chewing lice species were identified in passerines from Azores Islands and 6 in the mainland Portugal. Most chewing lice found on the Islands and mainland have proven to be host specific; the exception to host specificity were *M. eurysternus,* a generalist louse, and *Sturnidoecus* sp., an uncommon louse of blackbirds. Moreover, the prevalence of some parasites shared between insular and mainland birds differ statistically, which in general manifested by higher lice prevalence in the Island populations. Parasite richness was not correlated with isolation and Islands area. Our results show a geographical variation in avian lice patterns (species richness, prevalence and host specificity) and relate them with combined characteristics of the hosts, species of parasites and climatic conditions. These results are thus relevant to understand that patters of the insular chewing lice evolve even in association with birds' species that have recently colonized Oceanic Islands.

Introduction

Early the peculiar characteristics of some life forms that inhabit the Oceanic Islands caught the attention of researchers. One of the first to report evidence of insularity was Charles Darwin, with his famous Galapagos finches (Darwin, 2008b). Since then, Island populations, especially vertebrates, have been the focus of multiple researches, allowing not only understanding the processes involved in species expansion, as well as the effects of population isolation. Adler and Levins (1994) in an extensive work of synthesis termed the "systematic differences in demography, reproduction, behavior and morphology" of insular rodent populations, as island syndrome. Over the last two decades, the parasites have been the focus of several studies on the biogeographical patterns of insular parasite populations, allowing studied the patterns of Island colonization and the insularity effect on parasite (Literák *et al*., 2015; Nieberding *et al*., 2006; Pérez-Rodríguez *et al*., 2013).

Although the fundamental mechanisms in colonizing success of parasites in new regions are unknown, it is recognized that during the hosts range expansion, parasites often lost, being absent from the new area by: i) "missing the boat" – founder population may not be infected with some parasite species, as resulted of the patchy distribution of parasites or some other stochastic event (Paterson *et al*., 1999; Torchin *et al*., 2003); and ii) "drowning on arrival" – parasites do arrive with the founder population, but fail the establishment, as result of "sinking with the boat" when infected hosts fail to establish in the new area, or "lost overboard" when parasite fail the establishment for other reasons (MacLeod *et al*., 2010). These factors have been associated to parasites traits – low numbers of parasites, low vagility, high host-specificity and complex life cycle (Lockwood *et al*., 2005; Paterson *et al*., 2003; Torchin *et al*., 2003); and host traits – small founding populations, high mortality rates, low social interaction and small host body size (Paterson *et al*., 1999; Rózsa *et al*., 1996).

Regardless of the patterns of parasites colonization, parasites apparently can also develop changes resulting from the insularity, like occurs with insular vertebrates' populations. Termed parasite island syndromes by Pérez-Rodríguez *et al*. (2013) to describe the changes in the characteristics of haemoparasite assemblages (impoverishment of haemosporidians assemblage, absence of parasites host-specific and lower parasites prevalence) of blackcap *Sylvia atricapilla* (Linnaeus, 1758) in the Macaronesia, the effect of insularity have also been observed in others groups of parasites, such as helminths and ectoparasites. Regarding ectoparasites, Barrientos *et al*. (2014) reported a higher prevalence of mites of *Bucanetes githagineus* (Lichtenstein, MHC, 1823) in the Canary Islands than Iberian Peninsula and North

Africa; Wiggins *et al*. (1998) recorded highest fleas infestation in nests of *Parus major* Linnaeus, 1758 on two Danish Islands than the mainland and Tomás *et al*. (personal communication – Chapter 3) only found fleas species on Azorean birds; Tomás *et al*. (2021) observed high richness and prevalence of hippoboscid flies on Azorean birds and absence of host specificity. In the only study of chewing lice, (Literák *et al*., 2015) described the loss of chewing lice species richness in insular populations of blackcap, high host specificity to insular parasites and significantly higher parasites prevalence in the Azores Islands. Several hypotheses associated to ecological traits of birds and parasites have been proposed to explain parasite island syndromes.

Chewing lice (Phthiraptera: Ischnocera and Amblycera) are permanent and obligatory ectoparasites of bird, completing their entire life cycle on the host body, where feed mainly on feathers and dermal debris, although some species feed on blood (Johnson and Clayton, 2003). Usually, chewing lice are benign parasites, however in severe infestations, the lice promote feather damage, which can influence major avian life history traits, namely flight performance, thermoregulatory capacity, host body condition, sexual selection and survival (Booth *et al*., 1993; Clayton *et al*., 2008; Kose *et al*., 1999).

To date, there are about 4.000 known lice species of birds. Most chewing lice species are highly bird' host specific, behaviorally adapted for particular microhabitats on the hosts, and consequently, the geographic distribution of these ectoparasites frequently corresponds to the distribution of the hosts (Johnson and Clayton, 2003). The lice fauna have been well documented on Europe mainland birds (Price *et al*., 2003); on the contrary, considering the few studies on Azores Islands directed to lice research, we believe that Azorean lice fauna is still far from complete (Literák *et al*., 2015; Oslejskova *et al*., 2020; Palma, 2010).

Eurasian blackbird *Turdus merula* Linnaeus, 1758, Eurasian blackcap *Sylvia atricapilla* (Linnaeus, 1758), common chaffinch *Fringilla coelebs* Linnaeus, 1758 and European robin *Erithacus rubecula* (Linnaeus, 1758) are common and widespread bird species in the Western Palearctic. In mainland Portugal, these bird species are resident, which coexist with wintering populations, especially in the case of blackcaps, chaffinches and robins (Catry *et al*., 2010). The four bird species are recent colonizers of the Azorean Archipelago, living in all Azores Islands except the robins that not occur in Western group (Flores and Corvo) (Rodrigues *et al*., 2013, 2014, 2016, 2018).

Regarding the mainland and insular ectoparasites patterns previously recorded, the main goal of this study was to characterize the chewing lice infestations of Eurasian blackbird, Eurasian blackcap, common chaffinch and European robin on three Azores Islands and mainland Portugal and to test four predictions derived from parasite island syndromes and Theory of Island Biogeography whether: i) insular birds populations will have lower chewing lice diversity than the mainland one; ii) specific parasites will be more likely to occur on Islands; iii) parasites that are shared between insular and mainland birds populations will be more prevalent on Azores Islands; and iv) parasite diversity on Azores Islands will be positively correlated with the Island area and proximity to the mainland Portugal.

Material and Methods

<u>Study areas</u>

Portugal covers 92.090 Km^2 and comprises a mainland part and two North Atlantic Archipelagos constituted by volcanic Islands and several islets, Azores Archipelago and Madeira Archipelago. Azores Archipelago is located about 1.500 Km west of continental Europe, between latitudes 36º55'–39º43'N and longitudes 24º46'–31º16'W, and is consisted of nine Islands and some islets geographically defined into three groups: Eastern group – Santa Maria and São Miguel – Central group – Faial, Pico, São Jorge, Terceira and Graciosa – and Western group – Flores and Corvo (França *et al*., 2003; Gaspar *et al*., 2015). Fieldwork was carried out in the Southwestern part of the European continent, specifically in two localities of South mainland Portugal, Silves and Olhão, and in three Islands of the Azores Archipelago, namely São Miguel, Terceira and Flores Islands (Figure 4.1). The selection of field work sites was determined not only by the occurrence and abundance of the studied bird species, as well as because the three Islands of the Azores represent places with different areas and distance from the continent.
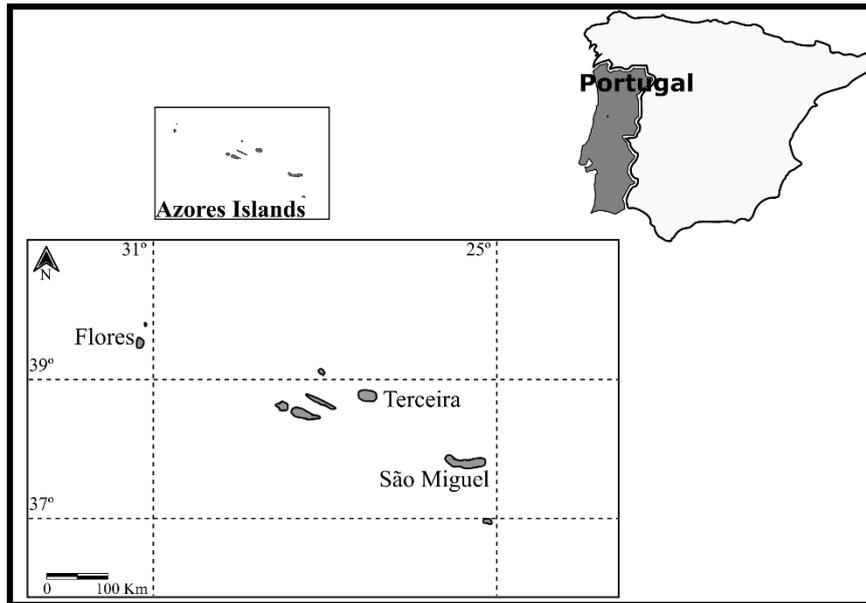
Figure 4.1 – Map of the mainland Portugal and Azores Archipelago (Author's Original).

Field sampling

During October to December of two consecutive years of 2018 and 2019, 775 live birds of the species *Turdus merula* Linnaeus, 1758, *Sylvia atricapilla* (Linnaeus, 1758), *Fringilla coelebs* Linnaeus, 1758 and *Erithacus rubecula* (Linnaeus, 1758) were randomly captured with mist nets. The time of the year was determined by the studied bird species abundance, namely robins and chaffinches that are more common in mainland Portugal during winter migration. Due to the patchy distribution of sampled bird species, individuals were captured at 2–3 sites on each locality, to increase capture rates and to avoid repeated captures. Birds were individualized with a metal ring, sexed and aged (juveniles: <1 year old; adults: >1 year old), based on plumage characteristics (Demongin.L, 2016).

Chewing lice samples were collected by a standard practice performed by numerous bird banders, the modified fumigation chamber method from Clayton and Drown (2001) where birds' bodies were exposed to chloroform, for 5 minutes and bird's heads underwent visual examination (Visnak and Dumbacher, 1999). All birds were released alive after sampling at the site of capture.

Collected lice were stored individually in 70% ethanol at room temperature, until further processing at Entomology Laboratory at Faculty of Sciences, University of Lisbon. Chewing lice were subsequently slide-mounted following to the Canada balsam technique of (Palma, 1978), for proper identification. Each louse was examined under a Stereo Microscope Olympus

SZX7, with a appropriated magnification, and identified firstly using the key to genera of (Price *et al*., 2003) and posteriorly the specific keys: Clay (1966), Gustafsson and Bush (2017), Price (1977), Sychra *et al*. (2016) and Złotorzycka (1964). The systematics and nomenclature of the chewing lice followed Price *et al*. (2003), Gustafsson and Bush (2017) and Sychra and Literák, (2008).

Statistical analysis

The following parameters were analyzed to evaluate parasite island syndromes: (i) species richness – number of lice species on an avian hosts; (ii) host specificity – range of avian hosts infested by a given louse species; (iii) prevalence – percentage of hosts infested with ectoparasites; (iv) mean abundance – total number of parasites of a particular species among all members of the host population; and (v) intensity – total number of parasites of a particular species among infected members of the host population (Bush *et al*., 1997).

Simple comparisons were performed between the richness of chewing lice species found in the Azorean blackbirds, blackcaps, chaffinches and robins with mainland counterparts. In order to control the effect of different sampling effort between Azores Islands and mainland Portugal, curves of cumulative lice richness were constructed using EstimateS 9.1.0 software (Colwell, 2013). From these cumulative curves, parasite richness was comparable between Island and mainland populations in different infestations scores (Sc_{100}: four bird species together; Sc_{40}: blackbirds, blackcaps and chaffinches; Sc_{20}: robins). Moreover, for purposes to test the correlation between observed number of chewing lice per Island and (i) the Island distance to mainland and (ii) Island area, were performed Pearson's correlations coefficient.

Chewing lice species shared between insular and mainland populations of blackbirds, blackcaps, chaffinches and robins were compared to test whether prevalence, mean abundance and intensity differ geographically. Firstly, prevalence, abundance and intensity of each lice species were estimated. Given that the prevalence, and intensity and abundance of some particular chewing lice differ geographically, was assessed statistical significance using Fisher's Exact Test and bootstrap 2- sample t-test with 1000 replications, respectively. These statistical analyses were done with the software Quantitative Parasitology 3.0 (Reiczigel *et al*., 2019)

Moreover, also assessed the statistical differences of overall parasites prevalence only among Islands populations, using a Chi-square test adjusted to a post hoc test, Bonferroni correction,

and Kruskal-Wallis with Dunn's post hoc test (adjusted using the Bonferroni correction). This test was done using IBM®SPSS®Statistics Version 26 (IBM Corp., 2019). A *p*-value of 0.05 or less was considered significant.

Results

Nine different chewing lice species were found in four passerine birds' species from Azores and the mainland Portugal (Figure 4.2). Insular parasites comprise six species also observed among mainland birds – *Menacanthus eurysternus* (Burmeister, 1838), *Myrsidea thoracica* (Giebel, 1874), *Myrsidea sylviae* Sychra & Literak, 2008, *Guimaraesiella amsel* (Eichler, 1951), *Guimaraesiella tovornikae* (Balát, 1981), *Philopterus fortunatus* (Zlotorzycka, 1964) – as well as other three species of chewing lice – *Philopterus turdi* (Denny, 1842), *Turdinirmus merulensis* (Denny, 1842) and *Sturnidoecus* sp..
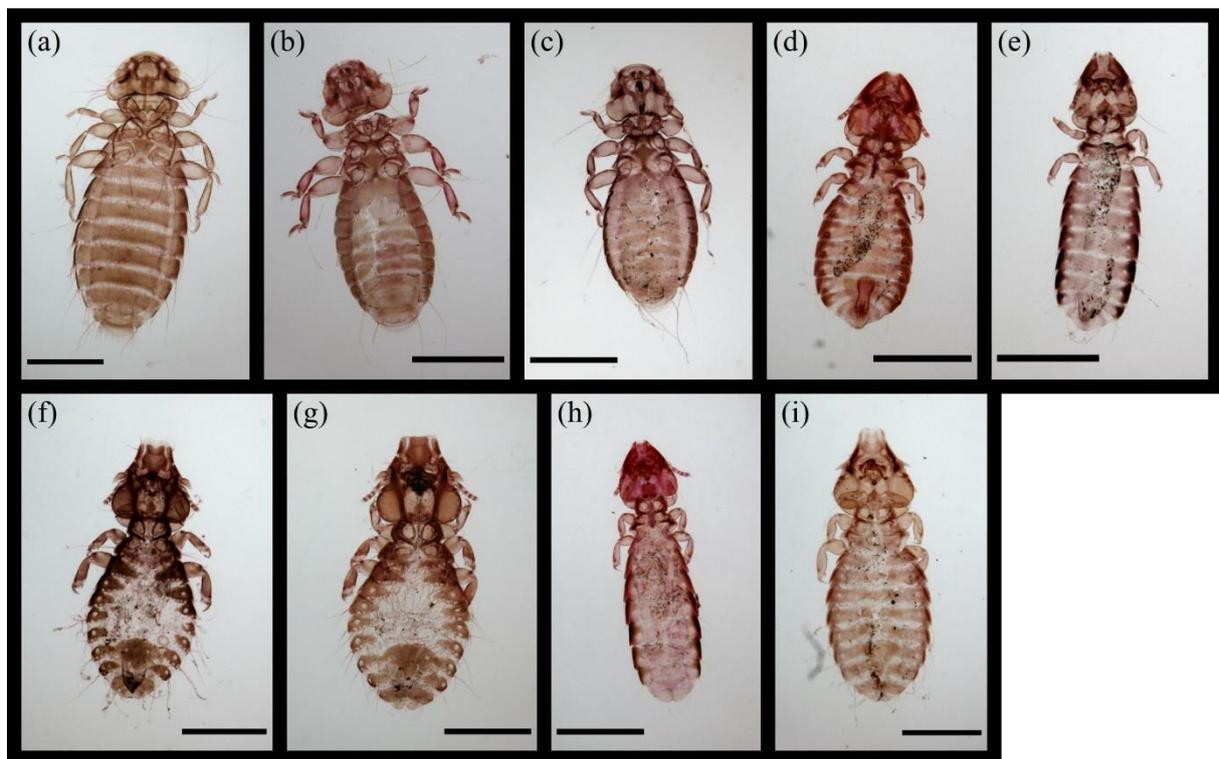
Figure 4.2 – Original pictures of chewing lice species observed on the mainland and Azorean passerine birds: (a) *Menacanthus eurysternus*, female (from *T. merula*), (b) *Myrsidea thoracica*, female (from *T. merula*), (c) *Myrsidea sylviae*, female (from *S. atricapilla*), (d) *Guimaraesiella amsel*, male (from *T. merula*), (e) *Guimaraesiella tovornikae*, male (from *S. atricapilla*), (f) *Philopterus fortunatus*, female (from *F. coelebs*), (g) *Philopterus turdi*, female (from *T. merula*), (h) *Turdinirmus merulensis*, female (from *T. merula*) and (i) *Sturnidoecus* sp., female (from *T. merula*). Scale bar: 500 µm.

A total of twelve louse-host associations were found in Azores Islands and nine in mainland Portugal (Figure 4.3). Azorean blackbirds were parasitized by six species (*M. eurysternus*, *M. thoracica*, *G. amsel*, *T. merulensis*, *P. turdi* and *Sturnidoecus* sp.); whereas mainland blackbirds were hosts of three species (*M. eurysternus*, *M. thoracica* and *G. amsel*). The observed richness of chewing lice in blackcaps, chaffinches and robins was similar between Azorean and mainland birds: three species in blackcaps (*M. eurysternus*, *M. sylviae* and *G. tovornikae*), two species in chaffinches (*M. eurysternus* and *P. fortunatus*), and one species in robins (*M. eurysternus*).

All species of chewing lice were observed on only one host species with the exception of the *M. eurysternus*, which was recorded on the four bird species analyzed. Although most species of chewing lice found in the mainland Portugal and Azores were hosts specific, the specimens of *Sturnidoecus* sp. found in insular blackbirds represents a not specific host-parasite associations.
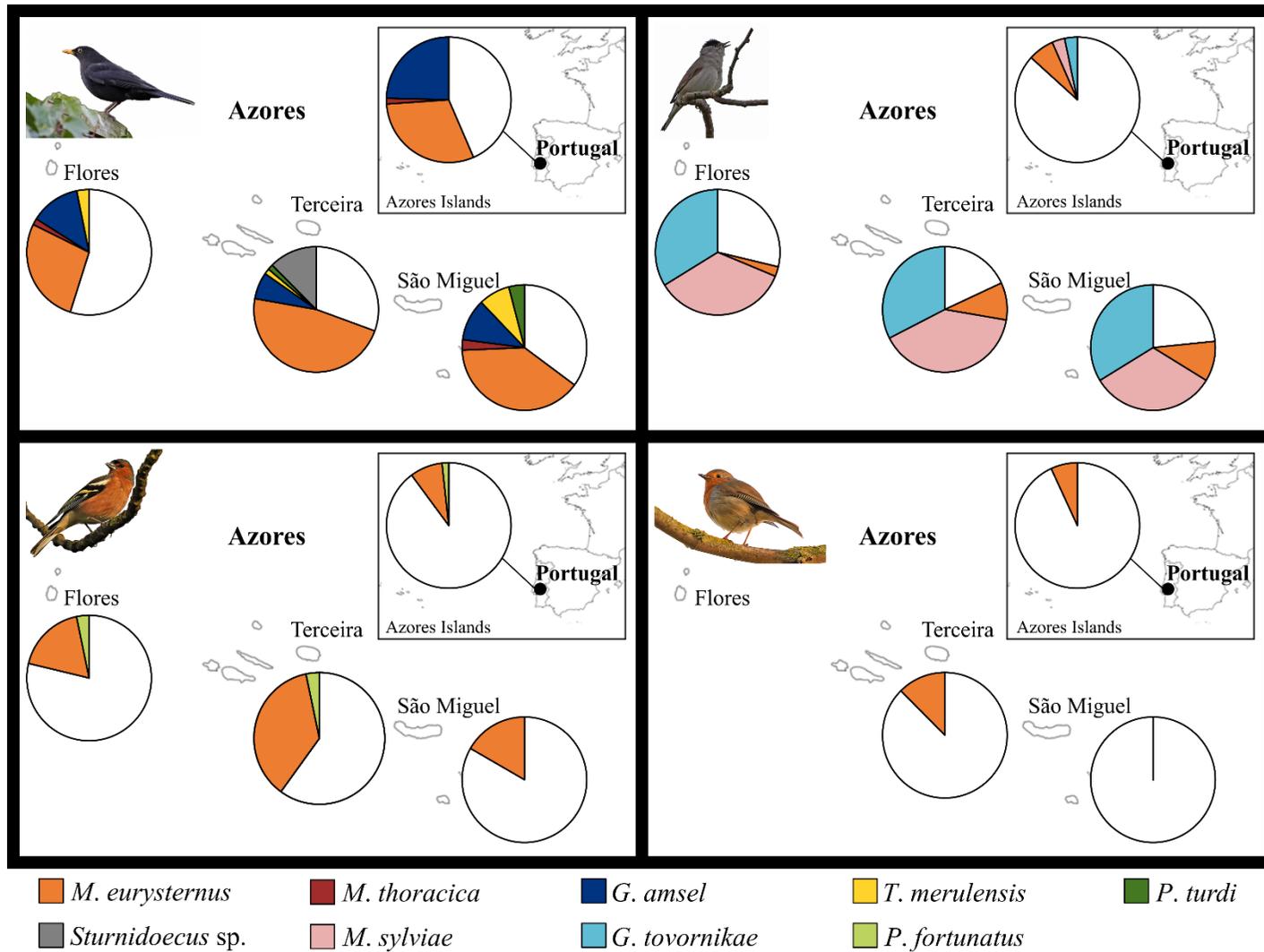
Figure 4.3 – Geographic distribution of the chewing lice richness found in each of the four sampled birds (blackbirds, blackcaps, chaffinches and robins). The pie charts represent the prevalence of each chewing lice species (symbolized by different colors; white color shows the percentage of uninfested birds) in Azores Islands (Flores, Terceira and São Miguel) and in the mainland Portugal.

The high species richness of insular parasites observed to the four bird species together, and more specifically to blackbirds, compared with that on mainland counterparts, was supported by the differences observed in chewing lice richness when controlling for sampling effort (mean ± standard deviation; Insular populations: Sc_{100}: 7.17 ± 0.65, mainland populations: Sc_{100}: 4.42 ± 0.86; t=13.413; $p \leq 0.001$; Figure 4.4a. Insular blackbirds: Sc_{40}: 4.97 ± 0.44, mainland blackbirds: Sc_{40}: 2.67 ± 0.27; t=7.465; $p \leq 0.001$; Figure 4.4b). Regarding to lice of blackcaps, chaffinches and robins, there was no difference between observed and estimated richness.
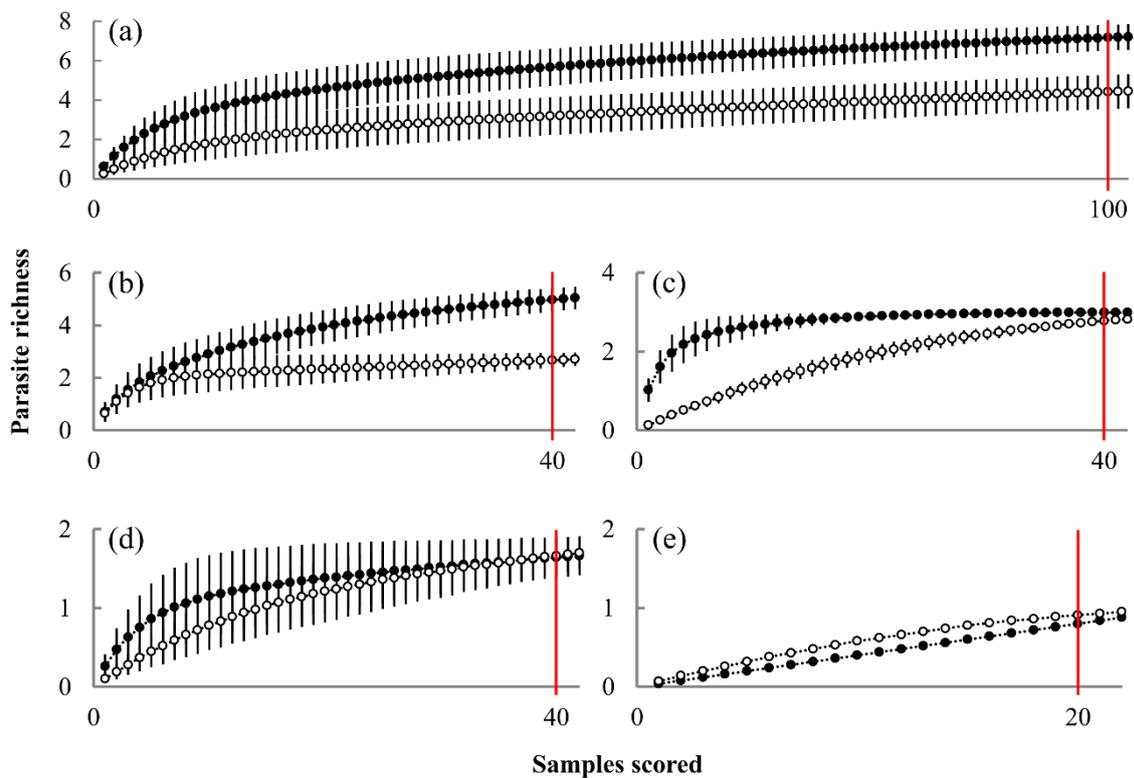
Figure 4.4 – Cumulative chewing lice richness in insular (black dots) and mainland (open circles) populations of four passerine species together (a) and individual populations of blackbirds (b), blackcaps (c), chaffinches (d) and robins (e). The curves represent mean richness (±SD) with increasing number of samples. The vertical red lines represent the sampling effort at which parasite richness was compared between Islands and mainland.

Chewing lice richness was not significantly correlated with Islands distance to the mainland Portugal (Pearson's r=-0.947, p=0.207; n=3) and Island area (Pearson's r=0.824, p=0.383; n=3).

A total of 316 (40.8%) of the 775 birds analyzed for this study were parasitized by at least one chewing lice species. The highest prevalence of chewing lice was found in blackcaps (55.2%),

followed by blackbirds (53.3%), chaffinches (21.7%), and robins (5.6%). Most parasitized birds were infested with a single species of chewing louse (71.8%), whereas co-occurrence of two or more species of lice was recorded on 89 birds (28.2%). Among the infested birds per species, 94 (73.4%) blackbirds, 80 (60.2%) blackcaps, 50 (96.2%) chaffinches were parasitized with only one species of chewing lice, but the co-occurrence of two or more species of lice was recorded on 34 (26.6%) blackbirds, 53 (39.8%) blackcaps, 2 (3.8%) chaffinches; only single infestations were recorded in robins.

Two hundred and seventy (47.7%) out 566 birds were found to be parasitized in the Azores Islands; while forty six (22.0%) of the 209 mainland birds were infested by at least one chewing lice (Table 4.1). The observed average chewing lice prevalence was statistically much higher on the Islands compared with that observed in mainland populations (Fisher test: $p \leq 0.001$). Global parasite prevalence according to bird species was higher in Azorean blackbird (54.4%), blackcap (69.1%) and chaffinch (25.6%), and lower in robin (4.0%) than mainland counterparts (50.0%; 13.3%; 10.0% and 6.9%, respectively). These different prevalence varied significantly to blackcap and chaffinch (Fisher test: blackcap: $p \leq 0.001$; chaffinch: $p \leq 0.05$). Overall chewing lice prevalence also varied significantly among Islands to the four bird species together ($\chi^2=10.818$; $p \leq 0.01$) and to chaffinch ($\chi^2=10.045$; $p \leq 0.01$); in both cases, the parasite prevalence of birds from Terceira Island (57.4% and 40%, respectively) differ statistically to Flores (42% and 20%, respectively; Kruskal-Wallis test: total populations and chaffinch: $p \leq 0.01$) and São Miguel Island (43.7% and 16.7%, respectively; Kruskal-Wallis test: total populations: $p \leq 0.01$; chaffinch: $p \leq 0.05$).

Table 4.1 – Prevalence (mean ± SD) of the chewing lice identified in populations of blackbirds, blackcaps, chaffinches, robins and the four species together from Azores (Flores, Terceira and São Miguel Island) and mainland Portugal. Statistical differences in the geographical patterns were compared between the three Azores Islands together and mainland Portugal and only among the three Islands of the Azores Archipelago.

	Chewing lice prevalence (mean ± SD)				
	Azores	**Mainland**	**Flores**	**Terceira**	**São Miguel**
Blackbird	54.4 ± 0.037	50.0 ± 0.065	44.3 ± 0.065	63.3 ± 0.063	56.7 ± 0.065
Blackcap	69.1 ± 0.034*	13.3 ± 0.044	62.3 ± 0.063	75.0 ± 0.056	70.0 ± 0.060
Chaffinch	25.6 ± 0.033***	10.0 ± 0.181	20.0 ± 0.052	40.0 ± 0.064**	16.7 ± 0.049
Robin	4.0 ± 0.040	6.9 ± 0.048	-	12.5 ± 0.125	0.0
All Population	47.7 ± 0.021*	22.0 ± 0.029	42.0 ± 0.037	57.4 ± 0.036**	43.7 ± 0.035

Statistically significant: *$p \leq 0.001$; **$p \leq 0.01$; ***$p \leq 0.05$

Mainland prevalence's of chewing lice species of the blackbird, blackcap, chaffinch and robin from different European countries were summarized in Table 4.2. Overall, birds from European countries showed a low prevalence of chewing lice.

Table 4.2 –Reports of chewing louse species in wild Passeriformes (blackbird, blackcap, chaffinch and robin) from European countries.

Location	Host Species	*n*	Chewing lice	Prevalence (%)	Literature source
Czech Republic	Blackbird	12	*M. eurysternus*	25.0	Sychra *et al*. (2008)
	Blackcap	78	*M. eurysternus*	3.8	
			M. sylviae	2.6	
			G. tovornikae	1.3	
	Robin	15	-	-	
	Blackbird	21	*M. eurysternus*	14.3	Sychra *et al*. (2011)
			G. amsel	9.5	
	Blackcap	114	*M. sylviae*	0.9	
	Robin	281	*M. eurysternus*	1.7	
	Chaffinch	31	*M. eurysternus*	3.2	
Slovakia	Blackbird	2	*G. amsel*	50.0	Bush *et al*. (2018)
	Blackcap	1	-	-	
	Chaffinch	4	*P. fortunatus*	75.0	
	Robin	2	-	-	
Greece	Blackbird	10	*M. eurysternus*	10.0	Diakou *et al*. (2017)
			G. amsel	30.0	
	Blackcap	79	-	-	
	Chaffinch	9	*M. eurysternus*	11.1	
			P. fortunatus	22.2	
	Robin	19	-	-	
Turkey	Blackbird	17	*M. eurysternus*	52.9	Dik *et al*. (2017)
	Blackcap	60	-	-	
	Chaffinch	2	-	-	
	Robin	15	-	-	
	Blackbird	1	-	-	Dik *et al*. (2011)
	Robin	6	*M. eurysternus*	16.7	

Considering each chewing lice species, the prevalence of some parasites shared between insular and mainland birds differ statistically (Figure 4.5). Overall, these differences were manifested by higher prevalence in the Island populations: *M. sylviae and G. tovornikae* from blackcaps were 44.2 and 40.9% more prevalent on the Islands than mainland ($p \leq 0.001$); *M. eurysternus* from chaffinches were 15.6% more prevalent on the Islands than mainland ($p \leq 0.01$). Only one parasite shared between Azorean and mainland blackbirds, *G. amsel*, had lower prevalence in the Islands (11.7%) than mainland (28.3%; $p \leq 0.01$).
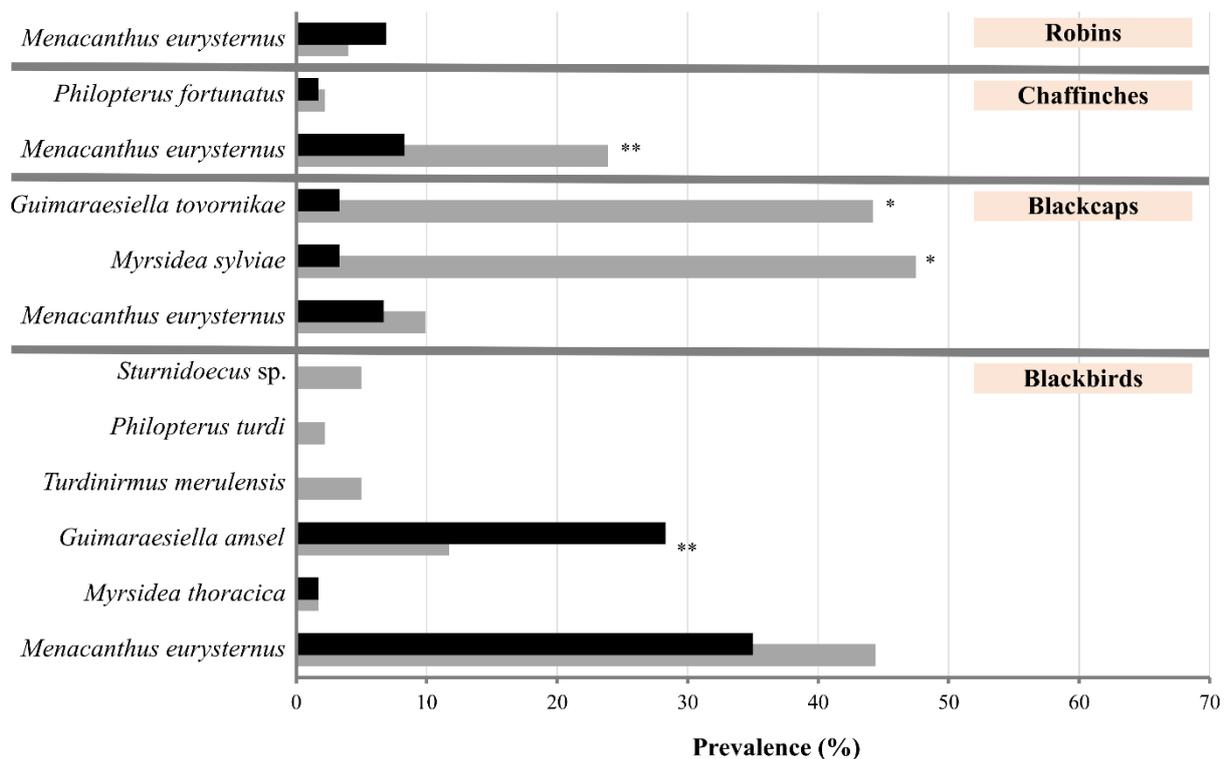
Figure 4.5 – Prevalence of chewing lice parasitizing blackbirds, blackcaps, chaffinches, robins and the four birds species together, in the mainland Portugal populations (black bars) and on Azores Islands (grey bars). Statistical differences in the geographical patterns of chewing lice prevalence were compared using Fisher's exact test. Statistically significant: *$p \leq 0.001$; **$p \leq 0.01$.

Overall, the mean abundance of species of chewing lice shared among insular and mainland populations was higher in the Azores birds (Table S4.1 in supplementary material). These differences were statistically significant in some parasite species. No difference was observed concerning parasite intensity. Additionally, no differences were recorded between the two years of fieldwork and according the sex of the birds. Although a higher prevalence of chewing lice was found on young birds (blackbirds and chaffinches) from Azores Islands than adult birds (Table S4.2 in supplementary material).

Discussion

The assemblage of chewing lice species found in Azorean Passeriformes birds was very similar from the one observed in mainland Portugal populations, sharing six parasite species. Although the species of lice recorded on the Azores Islands and mainland Portugal correspond to a portion of the known diversity of louse-host associations for each of the bird species, collectively the insular parasite assemblage was clearly enriched than the parasite diversity known for mainland. This result was related with parasite richness of blackbirds, the bird species with the highest diversity of louse recorded in our study, 6 species on the Azores Islands, which was 50% higher than the diversity of parasites observed on the mainland Portugal (3 species).

Contrariwise, in the specific case of insular blackcaps, chaffinches and robins, they shared the same three species of parasites with their counterparts in mainland Portugal. These differences between the Azorean chewing lice and that of the mainland do not support the assumption of the parasite island syndromes who argues that parasite–host associations are compromised on Islands as reported by Pérez-Rodríguez *et al*. (2013) to haemoparasites of Macaronesian blackcaps, and corroborated by Literák *et al*. (2015) to blackcap lice from Azores Islands. However, this result was similar to others ectoparasites works published by us, namely to fleas and louse flies Tomás *et al*. (2021), suggesting that also chewing lice species do not fail the establishment to the Azores Islands by "missing the boat", "drowning on arrival" or "lost overboard" (MacLeod *et al*., 2010; Paterson *et al*., 1999). The chewing lice richness differences on insular blackbirds may result from various, not mutually exclusive processes: (i) multiple founder events in the chewing lice associated with the *T. merula*, which according to Rodrigues *et al*. (2016) colonized the Azores Islands at least two consecutive isolation events, first approximately 0.47 My ago and then more recently, around 0.09 My ago; (ii) migratory behavior of the mainland birds may be an adaptive advantage since hosts, usually prior to migration, exhibit better body condition, controlling parasites more effectively (Johnson and Clayton, 2003; Marshall, 1981), and moving to areas with lower densities of conspecifics, reducing the risk of infestation (Begon *et al*., 1996). Thus, the migratory behavior of mainland birds can result in changes in host-parasite dynamics; or (iii) climate-related differences, especially the ambient humidity, considered an important factor in the composition of the chewing louse fauna, may play a role in the geographic variation of community structure of lice fauna (Bush *et al*., 2009; Moyer *et al*., 2002).

Whatever the mechanisms, our results to species richness apparently do not support the basic tenet of Theory of Island Biogeography that the equilibrium between immigration and

extinction rate favors greater species richness on large Islands and Islands near from the putative source of colonization (MacArthur and Wilson, 1967). However, considering the high host-specificity of most insular parasite species and bird species well established in the Azores Islands, this result was to be expected (Johnson and Clayton, 2003). In addition, it is important to note that the smaller number of islands sampled, may condition the effectiveness of this evaluation.

The exceptions of the host specificity were: (i) *M. eurysternus*, a generalist euryxenous parasite, which may colonize and thrive on the Azores Islands through other bird species (Martinů *et al*., 2015); and (ii) *Sturnidoecus* sp., a uncommon parasite to *T. merula*, firstly and only observed on the blackbirds on the Easternmost Island of Azores Archipelago, Santa Maria Island (Oslejskova *et al*., 2020). This species, as well as other species of the *Brueelia*-complex, may use phoresy on louse flies to colonize new hosts and thus spread through the Islands of the Archipelago (Bartlow *et al*., 2016).

Together with the observed increase of chewing lice richness on the Azores Archipelago, overall louse prevalence also rise up from 22.0% on the mainland Portugal to 47.7% on the Azores Islands. This difference cannot be explained by the absence on the mainland Portugal of the some chewing lice species, because: (i) only insular blackbirds showed high species richness compared mainland and the overall parasite prevalence on this host do not differ among mainland and Islands; and (ii) most chewing lice species that were shared between insular and mainland birds showed higher prevalence on Islands. The geographic difference was related to the high prevalence of parasites in blackcaps and chaffinches, which increased from 13.3% and 10.0% in mainland Portugal to 69.1% and 25.6% in the Azores Islands, respectively. The low prevalence of chewing lice on the mainland blackcaps and chaffinches was also found in other European countries. It might indicate that regardless of the host, Passeriformes of the mainland naturally have a low prevalence of chewing lice when they are in good health. This idea is supported by the previously mentioned assumption that the migratory traits of the mainland birds may be an advantage in parasite control (Begon *et al*., 1996; Marshall, 1981). Additionally, our results to Azorean blackcaps and chaffinches were very similar with Literák *et al*. (2015) and Barrientos *et al*. (2014) observations, who also identified a high prevalence of ectoparasites on the Islands. Apart from being more prevalent, the insular parasites demonstrated apparent temporal stability between the years 2018 and 2019 in the populations of chaffinches and blackcaps on the Azores Islands, which may indicate that the insular parasites are very well stabilized.

The high chewing lice prevalence, as well as other parasite parameters, on the Azorean birds can be explained from various, not mutually exclusive hypothesis: (i) the higher population densities on the Island than the mainland's conspecific populations – referred as "density compensation" by Crowell (1962) – may increase the risk of infestation by parasites, since direct contact between hosts is know the main route of lice transmission (Begon *et al*., 1996). This is especially relevant in the host' breeding period, where the transfer of lice occurs not only among adult birds, but also from adult birds to young, generally resulting in an increased prevalence of chewing lice in young birds during this period (Johnson and Clayton, 2003). According to Sychra *et al*. (2011), the differences in chewing lice prevalence on young and adult birds continue for some time post-breeding, which was corroborated by our observations in the Azores Islands; (ii) the greater body size on the insular populations – referred as an "island syndrome" by Adler and Levins (1994) – provide more space for parasites colonization, more refuge to avoid host defenses and greater longevity, providing a larger window of opportunity for infestation by louse (Kuris *et al*., 1980; Rózsa, 1997). The host body size was previously correlated with parasite species richness, abundance and prevalence (Chu *et al*., 2019; Marshall, 1981; Rózsa, 1997); or (iii) abiotic factors, especially the humidity conditions, beyond structuring the lice community, can influence parasitic load (Moyer *et al*., 2002). Thereby, it is expected that birds in humid environments, such as the Azores Islands, are under higher ectoparasitic pressure.

The air humidity may be particularly relevant to explain the altered geographic patterns of the some chewing lice species. Usually, amblyceran lice are more affected by adverse conditions of the arid environments than ischnoceran lice due to physiological traits related to inability to uptake water vapor (Rudolph, 1983). The most prevalent amblyceran species on Azorean blackcaps was *Myrsidea sylviae*, differing statistically from dries habitats on the mainland Portugal. Similarly, Bush *et al*. (2009) found more *Myrsidea* prevalence on *Aphelocoma californica* (Vigors, 1839) from humid areas than dry areas in the American Southwest; while, in dry areas on the African continent, Halajian *et al*. (2012) and Najer *et al*. (2012) reported few *Myrsidea* prevalence. Thereby, although lice of the genus *Myrsidea* have a worldwide distribution, they appear to be adapted to more humid habitats.

The other Amblycera louse with different prevalence among the humid habitats of the Azores Islands and the dry ones of the mainland Portugal was *Menacanthus eurysternus* in chaffinches. This species, a known parasite of wide range of hosts from diverse environments, makes this result surprising. However, it is possible that *M. eurysternus* from the Islands and the continent

belong to two different subgroups, which according to Takano *et al*. (2019) "it is possible that different subgroups of *M. eurysternus* are adapted to different humidity levels, which may be so recent that this has not yet translated into distinct morphologies".

Lice of the *Brueelia*-complex, contains both genera that are adapted to arid environments and humid environments (Bush *et al*., 2009; Carrillo *et al*., 2007). Specifically, the genus *Guimaraesiella*, the only ischnoceran with different prevalence patterns among insular and continental populations, supposedly is adapted to more humid areas (Takano *et al*., 2019). However, it is interesting to note that *G. tovornikae* was more prevalent in the Azores Islands (wet areas), while *G. amsel* was more prevalent in mainland Portugal (dry areas), so it is possible that different species of *Guimaraesiella* are tolerant to different humidity levels. In addition to tolerance/adaptation to more or less humid environments, Carrillo *et al*. (2007) suspected that chewing lice can adapt their life cycle to environmental conditions, overcoming the most critical periods of low humidity in arid regions and, thus, not necessarily being under lower ectoparasitic pressure than in humid regions.

Our results showed that the birds of the Azores have not only not lost the parasite species usually found on their mainland counterparts, as they have also exhibited a richer parasitic fauna; this was especially notorious in blackbirds, with the observation of three more species of chewing lice, including an unusual louse, *Sturnidoecus* sp.. Together with the enrichment the Island community of parasites compared to mainland populations, the Azorean birds, namely blackcaps and chaffinches, showed a higher chewing lice prevalence than their mainland counterparts. We are aware that although the observed parasite patterns do not fully support the ideas of parasite island syndromes to chewing lice (low richness, high prevalence and high host specialization) suggested by Literák *et al*. (2015), they may be interpreted as the likely outcome of many factors (abiotic, hosts and parasites). Thereby, regardless of its causes, our study adds new data to the existing literature on parasite island syndromes, making clear the importance of knowing the bird-parasite interaction patterns in different geographic locations.

References

Adler, G.H., Levins, R., 1994. The Island Syndrome in Rodent Populations. Q. Rev. Biol. 69, 473–490. doi:10.1086/418744

Barrientos, R., Valera, F., Barbosa, A., Carrillo, C.M., Moreno, E., 2014. Biogeography of haemo- and ectoparasites of an arid-land bird, the Trumpeter finch. J. Arid Environ. 106, 11–

17. doi:10.1016/j.jaridenv.2014.03.005

Bartlow, A.W., Villa, S.M., Thompson, M.W., Bush, S.E., 2016. Walk or ride? Phoretic behaviour of amblyceran and ischnoceran lice. Int. J. Parasitol. 46, 221–227. doi:10.1016/j.ijpara.2016.01.003

Begon, M., Harper, J.L., Townsend, C.R., 1996. Ecology: Individuals, populations and communities, 3rd Ed. Blackwell Scientific Publications, Sunderland. 1068 pp. ISBN: 0865421110

Booth, D.T., Clayton, D.H., Block, B.A., 1993. Experimental Demonstration of the Energetic Cost of Parasitism in Free-Ranging Hosts. Proc. R. Soc. B Biol. Sci. 253, 125–129. doi:10.1098/rspb.1993.0091

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis *et al*. revisited. J. Parasitol. 83, 575–583. doi:10.2307/3284227

Bush, S.E., Gustafsson, D.R., Clayton, D.H., 2018. New records of ectoparasites from passerine birds in the High Tatras of Slovakia. Oecologia Mont. 27, 43–45.

Bush, S.E., Harbison, C.W., Slager, D.L., Peterson, A.T., Price, R.D., Clayton, D.H., 2009. Geographic variation in the community structure of lice on western scrub-jays. J. Parasitol. 95, 10–13. doi:10.1645/GE-1591.1

Carrillo, C.M., Valera, F., Barbosa, A., Moreno, E., 2007. Thriving in an arid environment: High prevalence of avian lice in low humidity conditions. Ecoscience 14, 241–249. doi:10.2980/1195-6860(2007)14[241:TIAAEH]2.0.CO;2

Catry, P., Costa, H., Elias, G., Matias, R., 2010. Aves de Portugal. Ornitologia do território continental, 1st Ed. Assírio & Alvim, Lisboa. 944 pp. ISBN: 978-972-37-1494-4

Chu, X., Dik, B., Gustafsson, D.R., Che, X., Zhang, Q., Zou, F., 2019. The influence of host body size and food guild on prevalence and mean intensity of chewing lice (Phthiraptera) on birds in southern China. J. Parasitol. 105, 334–344. doi:10.1645/17-137

Clay, T., 1966. Contributions towards a revision of *Myrsidea* Waterston. I. (Menoponidae: Mallophaga). Bull. Br. Museum (Natural Hist). 17, 327–395. doi:10.5962/bhl.part.14816

Clayton, D.H., Adams, R.J., Bush, S.E., 2008. Phthiraptera, the Chewing Lice. In: Thomas, N.J., Hunter, D.B., Atkinson, C.T. (Eds.), Parasitic Diseases of Wild Birds. Wiley-Blackwell, Iowa, pp. 515–526. ISBN: 978-0-8138-2081-1

Clayton, D.H., Drown, D.M., 2001. Critical evaluation of five methods for quantifying chewing lice (Insecta: Phthiraptera). J. Parasitol. 87, 1291–1300. doi:10.1645/0022-3395(2001)087[1291:CEOFMF]2.0.CO;2

Colwell, R.K., 2013. EstimateS: Statistical estimation of species richness and shared species from samples.

Crowell, K.L., 1962. Reduced Interspecific Competition among the Birds of Bermuda. Ecology 43, 75–88. doi:10.2307/1932042

Darwin, C., 2008. On the Origin of Species, Revised Ed. Oxford University Press, New York. 432 pp. ISBN: 978-0-19-921922-3

Demongin, L., 2016. Identification guide to birds in the hand, Beauregard-Vendon. Laurent Demongin. 392 pp. ISBN: 978-2-9555019-0-0

Diakou, A., Pedroso Couto Soares, J.B., Alivizatos, H., Panagiotopoulou, M., Kazantzidis, S., Literák, I., Sychra, O., 2017. Chewing lice from wild birds in northern Greece. Parasitol. Int. 66, 699–706. doi:10.1016/j.parint.2017.07.003

Dik, B., Erciyas-Yavuz, K., Per, E., 2017. Chewing lice (Phthiraptera: Amblycera, Ischnocera) on birds in the Kizilirmak delta, Turkey. Rev. Med. Vet. 167, 53–62.

Dik, B., Sekercioglu, C.H., Kirpik, M.A., 2011. Chewing Lice (Phthiraptera) Species Found On Birds Along the Aras River, Iğdır, Eastern Turkey Bilal. Kafkas Univ Vet Fak Derg 17, 567–573.

França, Z., Cruz, J.V., Nunes, J.C., Forjaz, V.H., 2003. Geologia Dos Açores: Uma Perspectiva Actual. Açoreana 10, 11–140.

Gaspar, J.L., Queiroz, G., Ferreira, T., Medeiros, A.R., Goulart, C., Medeiros, J., 2015. Chapter 4 Earthquakes and volcanic eruptions in the Azores region: geodynamic implications from major historical events and instrumental seismicity. In: Gaspar, J.L., Guest, J.E., Duncan, A.M., Barriga, F.J.A.S., Chester, D.K. (Eds.), Volcanic Geology of São Miguel Island (Azores Archipelago). Geological Society, London, Memoirs, pp. 33–49. doi:10.1144/M44.4

Gustafsson, D.R., Bush, S.E., 2017. Morphological revision of the hyperdiverse *Brueelia*-complex (Insecta: Phthiraptera: Ischnocera: Philopteridae) with new taxa, checklists and generic key. Zootaxa 4313, 1–443. doi:10.11646/zootaxa.4313.1.1

Halajian, A., Sychra, O., Luus-Powell, W., Engelbrecht, D., 2012. Chewing lice of the genus

Myrsidea (Phthiraptera: Amblycera: Menoponidae) from passerines (Aves: Passeriformes) in South Africa, with descriptions of three new species. Zootaxa 68, 58–68.

IBM Corp., 2019. IBM®SPSS®Statistics for Windows. Version 26.

Johnson, K.P., Clayton, D.H., 2003. The biology, ecology, and evolution of chewing lice. In: Price, R.D., Hellenthal, R.A., Palma, R.L., Johnson, K.P., Clayton, D.H. (Eds.), The Chewing Lice: World Checklist and Biological Overview. Illinois Natural History Survey Special Publication, Illinois, pp. 449–476. ISBN: 1-882932-08-0

Kose, M., Mänd, R., Møller, A., 1999. Sexual selection for white tail spots in the barn swallow in relation to habitat choice by feather lice. Anim. Behav. 58, 1201–1205. doi:10.1006/anbe.1999.1249

Kuris, A.M., Blaustein, A.R., Alio, J.J., 1980. Hosts as Islands. Am. Nat. 116, 570–586.

Literák, I., Sychra, O., Resendes, R., Rodrígues, P., 2015. Chewing Lice in Azorean Blackcaps (*Sylvia atricapilla*): A Contribution to Parasite Island Syndromes. J. Parasitol. 101, 252–254. doi:10.1645/14-601.1

Lockwood, J.L., Cassey, P., Blackburn, T., 2005. The role of propagule pressure in explaining species invasions. Trends Ecol. Evol. 20, 223–228. doi:10.1016/j.tree.2005.02.004

MacArthur, R.H., Wilson, E.O., 1967. The Theory of Island Biogeography, 1st Ed. Princeton University Press, Princeton. 215 pp. ISBN: 9780691080505

MacLeod, C.J., Paterson, A.M., Tompkins, D.M., Duncan, R.P., 2010. Parasites lost - do invaders miss the boat or drown on arrival? Ecol. Lett. 13, 516–527. doi:10.1111/j.1461-0248.2010.01446.x

Marshall, A., 1981. The Ecology of Ectoparasitic Insects. Academic Press, London. 459 pp. ISBN: 0124740804

Martinů, J., Sychra, O., Literák, I., Čapek, M., Gustafsson, D.L., Štefka, J., 2015. Host generalists and specialists emerging side by side: An analysis of evolutionary patterns in the cosmopolitan chewing louse genus *Menacanthus*. Int. J. Parasitol. 45, 63–73. doi:10.1016/j.ijpara.2014.09.001

Moyer, B.R., Drown, D.M., Clayton, D.H., 2002. Low humidity reduces ectoparasite pressure: implications for host life history evolution. Oikos 97, 223–228. doi:10.1034/j.1600-0706.2002.970208.x

Najer, T., Sychra, O., Literák, I., Procházka, P., Čapek, M., Koubek, P., 2012. Chewing lice (Phthiraptera) from wild birds in Senegal, with descriptions of three new species of the genera *Brueelia* and *Philopteroides*. Acta Parasitol. 57, 90–98. doi:10.2478/s11686-012-0005-x

Nieberding, C., Morand, S., Libois, R., Michaux, J.R., 2006. Parasites and the island syndrome: The colonization of the western Mediterranean islands by *Heligmosomoides polygyrus* (Dujardin, 1845). J. Biogeogr. 33, 1212–1222. doi:10.1111/j.1365-2699.2006.01503.x

Oslejskova, L., Kounkova, S., Gustafsson, D.R., Resendes, R., Rodrigues, P., Literak, I., Sychra, O., 2020. Insect ectoparasites from wild passerine birds in the Azores Islands. Parasite 27, 64. doi:10.1051/parasite/2020063

Palma, R.L., 1978. Slide-mounting of lice: a detailed description of the Canada balsam technique. New Zeal. Entomol. 6, 432–436. doi:10.1080/00779962.1978.9722313

Palma, R.L., 2010. Phthiraptera. In: Borges, P.A.V., Costa, A., Cunha, R., Gabriel, R., Gonçalves, V., Martins, A., Melo, I., Parente, M., Raposeiro, P., Rodrigues, P., Santos, R.S., Silva, L., Vieira, P., Vieira, V. (Eds.), A List of the Terrestrial and Marine Biota from the Azores. Princípia, Cascais, pp. 214–215. ISBN: 978-989-8131-75-1

Paterson, A.M., Palma, R.L., Gray, R.D., 1999. How Frequently Do Avian Lice Miss the Boat? Implications for Coevolutionary Studies. Syst. Biol. 48, 214–223. doi:10.1080/106351599260544

Paterson, A.M., Palma, R.L., Gray, R.D., 2003. Drowning on arrival, missing the boat, and x-events: How likely are sorting events. In: Page, R.D.M. (Ed.), Tangled Trees: Phylogeny, Cospeciation, and Coevolution. The University of Chicago Press, Chicago, pp. 287–307. ISBN: 0-226-64467-7

Pérez-Rodríguez, A., Ramírez, Á., Richardson, D.S., Pérez-Tris, J., 2013. Evolution of parasite island syndromes without long-term host population isolation: parasite dynamics in Macaronesian blackcaps *Sylvia atricapilla*. Glob. Ecol. Biogeogr. 22, 1272–1281. doi:10.1111/geb.12084

Price, R.D., 1977. The *Menacanthus* (Mallophaga: Menoponidae) of the Passeriformes (Aves). J. Med. Entomol. 14, 207–220.

Price, R.D., Hellenthal, R.A., Palma, R.L., 2003. World checklist of chewing lice with host associations and keys to families and genera. In: Price, R.D., Hellenthal, R.A., Palma, R.L., Johnson, K.P., Clayton, D.H. (Eds.), The Chewing Lice: World Checklist and Biological

Overview. Illinois Natural History Survey Special Publication, Illinois, pp. 1–448. ISBN: 1-882932-08-0

Reiczigel, J., Marozzi, M., Fábián, I., Rózsa, L., 2019. Biostatistics for Parasitologists – A Primer to Quantitative Parasitology. Trends Parasitol. 35, 277–281. doi:10.1016/j.pt.2019.01.003

Rodrigues, P., Lopes, R.J., Drovetski, S. V., Reis, S., Ramos, J.A., Tristão da Cunha, R., 2013. Phylogeography and genetic diversity of the Robin (*Erithacus rubecula*) in the Azores Islands: Evidence of a recent colonisation. J. Ornithol. 154, 889–900. doi:10.1007/s10336-013-0953-4

Rodrigues, P., Lopes, R.J., Reis, S., Resendes, R., Ramos, J.A., Tristão da Cunha, R., 2014. Genetic diversity and morphological variation of the common chaffinch *Fringilla coelebs* in the Azores. J. Avian Biol. 45, 167–178. doi:10.1111/j.1600-048X.2013.00229.x

Rodrigues, P., Lopes, R.J., Resendes, R., Ramos, J.A., Tristão da Cunha, R., 2016. Genetic Diversity of the Azores Blackbirds *Turdus merula* Reveals Multiple Founder Events. Acta Ornithol. 51, 221–234. doi:10.3161/00016454AO2016.51.2.008

Rodrigues, P., Micael, J., Resendes, R., Lopes, R.J., Ramos, J.A., Tristão da Cunha, R., 2018. Genetic and morphometric variation of the Blackcap (*Sylvia atricapilla*) on the Azores Archipelago reveals a recent range expansion. J. Nat. Hist. 52, 2413–2435. doi:10.1080/00222933.2018.1539194

Rózsa, L., 1997. Patterns in the abundance of avian lice (Phthiraptera: Amblycera, Ischnocera). J. Avian Biol. 28, 249–254. doi:10.2307/3676976

Rózsa, L., Rékási, J., Reiczigel, J., 1996. Relationship of host coloniality to the population ecology of avian lice (Insecta: Phthiraptera). J. Anim. Ecol. 65, 242–248. doi:10.2307/5727

Rudolph, D., 1983. The water-vapour uptake system of the Phthiraptera. J. Insect Physiol. 29, 15–25. doi:10.1016/0022-1910(83)90101-4

Sychra, O., Kolencik, S., Palma, R.L., 2016. Three new species of *Myrsidea* (Phthiraptera: Menoponidae) from New Zealand passerines (Aves: Passeriformes). Zootaxa 4126, 397–410. doi:10.11646/zootaxa.4126.3.5

Sychra, O., Literák, I., 2008. *Myrsidea sylviae* (Phthiraptera, Menoponidae), a new species of chewing louse from *Sylvia atricapilla* (Passeriformes, Sylviidae). Dtsch. Entomol. Zeitschrift 55, 241–243. doi:10.1002/mmnd.200800024

Sychra, O., Literák, I., Podzemný, P., Benedikt, V., 2008. Insect ectoparasites from wild passerine birds in Czech Republic. Parasite 15, 599–604. doi:10.1051/parasite/2008154599

Sychra, O., Literák, I., Podzemný, P., Harmat, P., Hrabák, R., 2011. Insect ectoparasites on wild birds in the Czech Republic during the pre-breeding period. Parasite 18, 13–19. doi:10.1051/parasite/2011181013

Takano, O.M., Voelker, G., Gustafsson, D.R., Light, J.E., 2019. Molecular phylogeny and novel host associations of avian chewing lice (Insecta: Phthiraptera) from South Africa. Syst. Entomol. 44, 289–304. doi:10.1111/syen.12319

Tomás, A., Pereira da Fonseca, I., Valkenburg, T., Rebelo, M.T., 2021. Louse flies in Azorean and mainland populations of four Passeriformes species: A new perspective to parasite Island syndromes. Int. J. Parasitol. Parasites Wildl. 14, 33–40. doi:10.1016/j.ijppaw.2020.12.004

Torchin, M.E., Lafferty, K.D., Dobson, A.P., McKenzie, V.J., Kuris, A.M., 2003. Introduced species and their missing parasites. Nature 421, 628–630. doi:10.1038/nature01346

Visnak, R.M., Dumbacher, J.P., 1999. Comparison of four fumigants for removing avian lice. J. F. Ornithol. 70, 42–48.

Wiggins, D.A., Møller, A.P., Sørensen, M.F.L., Brand, L.A., 1998. Island biogeography and the reproductive ecology of great tits *Parus major*. Oecologia 115, 478–482. doi:10.1007/s004420050544

Złotorzycka, J., 1964. Mallophaga parasitizing Passeriformes and Pici III. Philopterinae. Acta Parasitol. Pol. 12, 401–430.

Supplementary material

Table S4.1 – Prevalence (%), confidence intervals (95%) in square brackets, intensity and abundance of chewing lice species on blackbirds, blackcaps, chaffinches and robins from the Azores Islands (Flores, Terceira and São Miguel) and mainland Portugal. As there were no differences within any population regarding host sex or age, year of fieldwork we pooled data to obtain a single value per population. Statistical differences in the geographical patterns were compared among each Island and continent and three Azores Islands together and mainland. Sample sizes (*n*) are shown in brackets.

Species	Characteristics	Blackbird Azores Flores (n=60)	Blackbird Azores Terceira (n=60)	Blackbird Azores São Miguel (n=60)	Blackbird Azores Total (n=180)	Blackbird Mainland (n=60)	Blackcap Azores Flores (n=61)	Blackcap Azores Terceira (n=60)	Blackcap Azores São Miguel (n=60)	Blackcap Azores Total (n=181)	Blackcap Mainland (n=60)	Chaffinch Azores Flores (n=60)	Chaffinch Azores Terceira (n=60)	Chaffinch Azores São Miguel (n=60)	Chaffinch Azores Total (n=180)	Chaffinch Mainland (n=60)	Robin Azores Terceira (n=8)	Robin Azores São Miguel (n=17)	Robin Azores Total (n=25)	Robin Mainland (n=29)
Menacanthus eurysternus	Prevalence (%)	28.3	56.7***	48.3	44.4	35.0	3.3	13.3	13.3	9.9	6.7	18.3	36.7*	16.7	23.9**	8.3	12.5	0.0	4.0	6.9
	[CI 95%]	[17.5 – 41.4]	[43.2 – 69.4]	[35.2 – 61.6]	[37.1 – 52.0]	[23.1 – 48.4]	[0.4 – 11.3]	[5.9 – 24.6]	[5.9 – 24.6]	[6.0 – 15.3]	[1.8 – 16.2]	[9.5 – 30.4]	[24.6 – 50.1]	[8.3 – 28.5]	[17.9 – 30.8]	[2.8 – 18.4]	[0.3 – 52.7]	-	[0.1 – 20.4]	[0.8 – 22.8]
	Intensity (mean±SD)	5.82 ± 1.79	13.79 ± 6.60	8.52 ± 1.93	10.19 ± 2.91	6.62 ± 2.27	1.00 ± 0.00	1.88 ± 0.35	2.13 ± 0.88	1.89 ± 0.41	1.00 ± 0.00	12.36 ± 6.80	20.32 ± 11.94	12.6 ± 7.49	16.49 ± 6.52	11.20 ± 9.46	2.00 ± 0.00	0	2.00 ± 0.00	2.00 ± 1.00
	Intensity range	1 – 29	1 – 224	1 – 52	1 – 224	1 – 49	1	1 – 4	1 – 8	1 – 8	1	1 – 77	1 – 217	1 – 78	1 – 217	1 – 49	2	0	2	1 – 3
	Abundance (mean±SD)	1.65 ± 0.60	7.82 ± 3.82	4.12 ± 1.08	4.53 ± 1.34	2.32 ± 0.88	0.03 ± 0.02	0.25 ± 0.09	0.28 ± 0.14	0.19 ± 0.06	0.07 ± 0.04	2.27 ± 1.35	7.45 ± 4.50	2.10 ± 1.34	3.94 ± 1.63	0.93 ± 0.82	0.25 ± 0.25	0	0.08 ± 0.08	0.14 ± 0.11
Myrsidea thoracica	Prevalence (%)	1.7	0.0	3.3	1.7	1.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	[CI 95%]	[0.0 – 8.9]		[0.4 – 11.6]	[0.3 – 4.8]	[0.0 – 8.9]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Intensity (mean±SD)	1.00 ± 0.00	0	2.00 ± 1.00	1.67 ± 0.67	1.00 ± 0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Intensity range	1	0	1 – 3	1 – 3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Abundance (mean±SD)	0.02 ± 0.02	0	0.07 ± 0.05	0.03 ± 0.02	0.02 ± 0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Guimaraesiella amsel	Prevalence (%)	13.3	8.3	13.3	11.7	28.3**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	[CI 95%]	[5.9 – 24.6]	[2.8 – 18.4]	[5.9 – 24.6]	[7.4 – 17.3]	[17.5 – 41.4]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Intensity (mean±SD)	1.88 ± 0.52	1.40 ± 0.40	2.00 ± 0.86	1.81 ± 0.38	2.53 ± 0.52	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Intensity range	1 – 5	1 – 3	1 – 8	1 – 8	1 – 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Abundance (mean±SD)	0.25 ± 0.11***	0.12 ± 0.06***	0.27 ± 0.14	0.21 ± 0.06	0.72 ± 0.21***	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turdinirmus merulensis	Prevalence (%)	3.3	1.7	10.0	5.0	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	[CI 95%]	[0.4 – 11.5]	[0.0 – 8.9]	[3.8 – 20.5]	[2.3 – 9.3]		-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Intensity (mean±SD)	1.00 ± 0.00	1.00 ± 0.00	1.17 ± 0.17	1.11 ± 0.11	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Intensity range	1	1	1 – 2	1 – 2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Abundance (mean±SD)	0.03 ± 0.02	0.02 ± 0.02	0.12 ± 0.05	0.06 ± 0.02	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Philopterus turdi	Prevalence (%)	0.0	1.7	5.0	2.2	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	[CI 95%]		[0.0 – 8.9]	[1.0 – 13.9]	[0.6 – 5.6]		-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Intensity (mean±SD)	0	1.00 ± 0.00	1.33 ± 0.33	1.25 ± 0.25	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Intensity range	0	1	1 – 2	1 – 2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Abundance (mean±SD)	0	0.02 ± 0.02	0.07 ± 0.04	0.03 ± 0.01	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sturnidoecus sp.	Prevalence (%)	0.0	15.0	0.0	5.0	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	[CI 95%]		[7.1 – 26.6]		[2.3 – 9.3]		-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Intensity (mean±SD)	0	1.33 ± 0.24	0	1.33 ± 0.24	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Intensity range	0	1 – 3	0	1 – 3	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Abundance (mean±SD)	0	0.20 ± 0.07	0	0.07 ± 0.02	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Myrsidea sylviae	Prevalence (%)	-	-	-	-	-	45.9*	55.0*	41.7*	47.5*	3.3	-	-	-	-	-	-	-	-	-
	[CI 95%]	-	-	-	-	-	[33.1 – 59.2]	[41.6 – 67.9]	[29.1 – 55.1]	[40.1 – 55.1]	[0.4 – 11.5]	-	-	-	-	-	-	-	-	-
	Intensity (mean±SD)	-	-	-	-	-	3.25 ± 0.40	4.73 ± 1.18	3.92 ± 1.09	4.01 ± 0.57	7.50 ± 2.50	-	-	-	-	-	-	-	-	-
	Intensity range	-	-	-	-	-	1 – 9	1 – 40	1 – 26	1 – 40	5 – 10	-	-	-	-	-	-	-	-	-
	Abundance (mean±SD)	-	-	-	-	-	1.49 ± 0.28*	2.60 ± 0.71***	1.63 ± 0.51***	1.91 ± 0.31*	0.25 ± 0.19	-	-	-	-	-	-	-	-	-
Guimaraesiella tovornikae	Prevalence (%)	-	-	-	-	-	44.3*	45.0*	43.3*	44.2*	3.3	-	-	-	-	-	-	-	-	-
	[CI 95%]	-	-	-	-	-	[31.5 – 57.6]	[32.1 – 58.4]	[30.6 – 56.8]	[36.8 – 51.8]	[0.4 – 11.5]	-	-	-	-	-	-	-	-	-
	Intensity (mean±SD)	-	-	-	-	-	3.30 ± 0.84	2.96 ± 0.80	3.77 ± 0.58*	3.34 ± 0.43**	1.00 ± 0.00	-	-	-	-	-	-	-	-	-
	Intensity range	-	-	-	-	-	1 – 23	1 – 22	1 – 10	1 – 23	1	-	-	-	-	-	-	-	-	-
	Abundance (mean±SD)	-	-	-	-	-	1.46 ± 0.42***	1.33 ± 0.4***	1.63 ± 0.35*	1.48 ± 0.23*	0.03 ± 0.02	-	-	-	-	-	-	-	-	-
Philopterus fortunatus	Prevalence (%)	-	-	-	-	-	-	-	-	-	-	3.3	3.3	0.0	2.2	1.7	-	-	-	-
	[CI 95%]	-	-	-	-	-	-	-	-	-	-	[0.4 – 11.5]	[0.4 – 11.5]		[0.6 – 5.6]	[0.0 – 8.9]	-	-	-	-
	Intensity (mean±SD)	-	-	-	-	-	-	-	-	-	-	1.00 ± 0.00	1.50 ± 0.50	0	1.25 ± 0.25	1.00 ± 0.00	-	-	-	-
	Intensity range	-	-	-	-	-	-	-	-	-	-	1	1 – 2	0	1 – 2	1	-	-	-	-
	Abundance (mean±SD)	-	-	-	-	-	-	-	-	-	-	0.03 ± 0.02	0.05 ± 0.04	0	0.03 ± 0.01	0.02 ± 0.02	-	-	-	-
Total	Prevalence (%)	43.3	63.3	56.7	54.4	50.0	62.3*	75.0*	70.0*	69.1*	13.3	20.0	40.0*	16.7	25.6***	10.0	12.5	0.0	4.0	6.9
	[CI 95%]	[30.6 – 56.8]	[49.9 – 75.4]	[43.2 – 69.4]	[46.9 – 61.9]	[36.8 – 63.2]	[49.0 – 74.4]	[62.1 – 85.3]	[56.8 – 81.2]	[61.8 – 75.7]	[5.9 – 24.6]	[10.8 – 32.3]	[27.6 – 53.5]	[8.3 – 28.5]	[19.4 – 32.6]	[3.8 – 20.5]	[0.3 – 52.7]	-	[0.1 – 20.4]	[0.8 – 22.8]
	Intensity (mean±SD)	4.50 ± 1.26	12.89 ± 5.93	8.18 ± 1.67	9.03 ± 2.40	6.10 ± 1.65	4.79 ± 0.82	5.58 ± 1.15	5.07 ± 0.91	5.17 ± 0.57	2.63 ± 1.16	11.50 ± 6.29	18.75 ± 10.98	12.60 ± 7.49	15.52 ± 6.11	9.50 ± 7.91	2.00 ± 0.00	0	2.00 ± 0.00	2.00 ± 1.00
	Intensity range	1 – 30	1 – 224	1 – 52	1 – 224	1 – 49	1 – 30	1 – 43	1 – 27	1 – 43	1 – 10	1 – 77	1 – 217	1 – 78	1 – 217	1 – 49	2	0	2	1 – 3
	Abundance (mean±SD)	1.95 ± 0.61	8.17 ± 3.82	4.63 ± 1.08	4.92 ± 1.35	3.05 ± 0.91	2.98 ± 0.59**	4.18 ± 0.92***	3.55 ± 0.71**	3.57 ± 0.4*	0.35 ± 0.19	2.30 ± 1.35	7.50 ± 4.50	2.10 ± 1.34	3.97 ± 1.63	0.95 ± 0.82	0.25 ± 0.25	0	0.08 ± 0.08	0.14 ± 0.11

Statistically significant: *$p \leq 0.001$; **$p \leq 0.01$; ***$p \leq 0.05$

Table S4.2 – Overall prevalence (%) of chewing lice on blackbirds, blackcaps, chaffinches and robins from the Azores Islands and mainland Portugal, according host Age and Sex and year of Fieldwork.

	Azores Islands						Mainland Portugal					
	Sex of birds		Age of birds		Fieldwork Age		Sex of birds		Age of birds		Fieldwork Age	
	Male	Female	Juvenile	Adult	2018	2019	Male	Female	Juvenile	Adult	2018	2019
Blackbird	51.5	59.7	67.3*	32.8	51.1	57.8	51.4	47.8	59.0	33.3	46.7	53.3
Blackcap	72.6	61.9	72.6	62.5	69.2	68.9	12.9	13.8	14.3	11.1	13.3	13.3
Chaffinch	26.7	24.0	30.6***	15.3	26.7	24.4	19.2	2.9	9.3	11.8	6.7	13.3
Robin	-	-	5.3	0.0	0.0	10	-	-	8.3	0.0	4.5	14.3
Total	51.1	47.6	53.8*	36.2	46.5	48.9	29.8	18.6	23.6	18.0	18.8	25.8

Statistically significant: *$p \leq 0.001$; **$p \leq 0.01$; ***$p \leq 0.05$

Chapter 5

General Discussion

General Discussion

Understanding island parasite communities provides important insight into the effect of insularity on parasites and thus the typical evolution of parasitism on islands. In the scope of this PhD thesis, it was possible to verify that: (i) island syndromes are manifested in all three parasite groups, hippoboscid flies, fleas and chewing lice, with greater evidence in the latter group (Subchapter Island syndrome and ectoparasites group); (ii) the characteristics of the Azores islands tend to benefit ectoparasite communities for the evolution of syndromes (Subchapter Parasite insular syndromes and Island features); and (iii) the host colonization, namely the time scales and the total of colonization movements, may be an important factor in the evolution of parasite populations (Subchapter Parasite island syndromes and host colonization).

Island syndrome and ectoparasites group

In our study areas, all three groups of ectoparasites were identified, namely hippoboscid flies, fleas and chewing lice, the latter being the most common ectoparasites in both the Azores Islands and mainland Portugal. Fleas were the only ectoparasite group absent from mainland Portugal.

The composition of the ectoparasite assemblages identified on the Azores Islands and mainland Portugal correspond to portion of the known diversity of parasite-host associations (Carles–Tolrá and Báez, 2002; Price *et al.*, 2003; Ribeiro, 2007). Although this composition of the insular ectoparasite communities was very similar to that observed on the mainland, collectively these ectoparasite assemblages were clearly enriched on the Azores Islands, which do not support the hypothesis that host-parasite associations are compromised in insular environments, as observed to other parasite groups, such as blood parasites (Barrientos *et al.*, 2014; Pérez-Rodríguez *et al.*, 2013) and chewing lice (Literák *et al.*, 2015). Above all, these data indicated a successful colonization of fleas, lice, and louse flies, suggesting that these ectoparasite groups do not failed the establishment to Macaronesia. This was particularly relevant in the case of chewing lice due to their high host specificity, as according to MacLeod *et al.* (2010), this characteristic may disturb the colonization of new area. Analyzing each ectoparasite group separately, it was possible to observe that the species richness of louse flies remained identical between the two areas (Chapter 2), while the high species richness on the Azores Islands was especially

remarkable for fleas and chewing lice (Chapter 3, 4). In the case of fleas this result was essentially due to the absence of species identified in birds from mainland Portugal. However, knowing the Portuguese flea fauna (Ribeiro, 2007), it is not accurate to assume that this higher species richness on the Islands is an insular syndrome (Chapter 3). In addition to the parasite-host associations already described in the literature, we added the association of the flea *Ctenocephalides felis felis* in *Turdus merula*, even though this may have been an accidental association (Chapter 3).

While investigating the structure of louse communities in each bird species, the high overall richness on the Islands was associated with the high parasite richness of Azorean blackbirds (Chapter 4), not corroborating the observations of Literák *et al*. (2015). These chewing lice richness differences between mainland and insular blackbirds may be related with multiple founder events in the chewing lice associated with the Azorean blackbirds (Rodrigues *et al*., 2016), changes in host-parasite dynamics on the mainland as a result of the migratory behavior of some specimens (Begon *et al*., 1996; Marshall, 1981) and variation of community structure of lice fauna as a consequence of ambient humidity (Moyer *et al*., 2002). This type of data on chewing lice provides a clearly evidence of evolution of insular ectoparasite characteristics, representing a good example of the parasite island syndrome.

Another interesting feature of the composition of the ectoparasite communities on the Islands concerns host specificity. Insular louse fly and flea species (Chapter 2, 3) maintained the reduced host specificity that characterizes them on the mainland (Hutson, 1984); whereas most chewing louse (Chapter 4) evidenced high specificity at both sites (Johnson and Clayton, 2003; Krasnov, 2008). These results indicate that host specificity of Island ectoparasites did not change, probably as a result of their ecological and behavioral characteristics, contradicting the assumption that typical insular parasite assemblages exhibit changes in host specificity (Pérez-Rodríguez *et al*., 2013). Literák *et al*. (2015) had already documented the preservation of high host specificity of chewing lice, which leads us to think that host specificity may not be the best indicator of insular syndrome associated with avian ectoparasites.

In addition to the high richness that characterized the composition of the insular ectoparasite communities, the overall ectoparasite prevalence varied between birds from the Azores Archipelago and the mainland Portugal. Louse flies, fleas and chewing lice showed a high prevalence on the Islands (Chapter 2, 3, 4), corroborating the observations

of Barrientos *et al.* (2014) and Literák *et al.* (2015) for mites and chewing lice, respectively. This characteristic of insular ectoparasites probably arose in response to some hosts characteristics on Islands, namely higher population densities (Begon *et al.*, 1996; Crowell, 1962), larger body size (Adler and Levins, 1994; Kuris *et al.*, 1980) and poorer nest sanitation (Clayton *et al.*, 2010; Ibáñez-Álamo *et al.*, 2016); and characteristics on the Islands themselves, specifically abiotic factors (Subchapter Parasite insular syndromes and Island features). This finding, which is probably the most remarkable insular syndrome of ectoparasite evolution on the Islands, also allowed us to observe that this evolution occurred not only across all ectoparasite species, but also within the same parasite group, and was particularly evident in louse species. Even though only a few species of chewing louse became more prevalent on the Islands (Chapter 4), the suitability of these species for more humid environments supported these geographical patterns (Bush *et al.*, 2009; Takano *et al.*, 2019).

These findings, in addition to improving our knowledge of insular populations of louse fly, flea and louse species, allowed us to realize that some of the ideas previously advanced for insular syndromes associated with ectoparasites apply equally to these parasite groups. Even though chewing lice were the ectoparasites with the most striking island syndromes, several mechanisms that may help understand the evolution of ectoparasite communities on the Islands were presented.

Parasite insular syndromes and Island features

Detailed information about ectoparasite communities on different Islands of an Archipelago may provide insights into the importance of Island area and distance to the mainland, in the development of island syndromes, specifically species richness. In this work, we tried to establish a relationship between the area and distance to the mainland of the Islands of São Miguel, Terceira and Flores, and the ectoparasite species richness. However, a causal link of Island characteristics on the evolution of parasite island syndrome was not identified (Chapter 2, 3, 4). These data did not corroborate the results of other parasite groups, namely concerning haemoparasites and helminths, in which a positive correlation was observed between species richness and Island area, and negative with distance from the Island to the mainland (De Bellocq *et al.*, 2002; Ishtiaq *et al.*, 2010; Nieberding *et al.*, 2006; Pérez-Rodríguez *et al.*, 2013).

Based on these results and considering the direct transmission of the ectoparasites studied, it is expected that Island area and distance of the Island from the mainland source populations rarely regulate insular ectoparasite communities, but if it does occur this should arise indirectly, by pressure exerted directly on hosts. This should be most evident in highly host-specific parasites, such as chewing lice, while more generalist parasites can rely on other host species to thrive on Islands (MacLeod *et al*., 2010).

Even though no association was observed between Island area and distance, and species richness, a slightly higher prevalence of ectoparasites was noted on Terceira Island, in comparison with the other two Azores Islands. These data corroborated the work of Pérez-Rodríguez *et al*. (2013), where they suggested that the different prevalence of haemoparasites probably resulted from the lower abundance of competent vectors on some Islands. Regarding ectoparasites, the direct transmission route of lice did not seem to control their prevalence on the Islands, as observed by Literák *et al*. (2015).

An obvious candidate to explain the altered prevalence among Islands might be the abiotic conditions, particularly the humidity. Relative humidity is a determining factor in the structuring of ectoparasite communities, in their seasonality, as well as in their prevalence in host populations (Fowler *et al*., 1983; Moyer *et al*., 2002; Senar *et al*., 1994). Thus, the high relative humidity that characterizes the climate of the Azores, which tends to a significant asymmetry in the interior of each Island, higher on the coast to the South of the Islands and in places of high altitude, may exert a disparate pressure on the insular communities of parasites (Borges *et al*., 2009). Therefore, the study areas in Terceira Island located in high-altitude zones in the Southwest and center of the Island (proximity of the Reserva Florestal de Recreio da Serreta and Reserva Natural Geológica do Algar do Carvão, respectively) offer a favorable microclimate for ectoparasites when compared with the study areas on the São Miguel Island (high-altitude zones in the Northeast of the Island - proximity of Serra da Tronqueira - and low-altitude zones in the Northwest of the Island - proximity of Lagoa das Sete Cidades) and on the Flores Island (low-altitude zones in the Northwest - Fajã Grande).

Although these results suggested that Island area and distance from the mainland do not affect ectoparasite species richness, it was evidenced here that other Island characteristics, such as relative humidity, may play an important role in the structuring, seasonality, and prevalence of ectoparasite communities.

Parasite island syndromes and host colonization

This thesis provided evidence that the time scales of hosts' colonization may be important in the evolution of insular communities of ectoparasites. Blackbirds with two events of the Azores Islands colonization, an older one 0.47 My ago and a more recent one 0.09 My ago, showed evident island syndromes, namely, higher richness of lice and louse flies and high prevalence of flies (Rodrigues *et al*., 2016). At the opposite extreme, robins, a bird absent from the Westernmost group of Azores and with a single recent founding event (about 0.08 My ago), exhibited no insular syndromes (Rodrigues *et al*., 2013). The other two bird species, the chaffinch with colonization about 1.5 My ago and the blackcap with a more recent colonization 0.1 My ago, showed a single syndrome, specifically, high prevalence of chewing lice (Rodrigues *et al*., 2014, 2018). Although this was the first study to include more than one parasite group of host species with different scales of Island colonization, in the specific case of ectoparasites, previous studies give some support to our finding. For example, Barrientos *et al*. (2014), in a long-established population of *B. githagineus* from Canary Islands only observed an island syndrome, high mite prevalence; in turn, Literák *et al*. (2015) in short-term population of blackcaps from the Azores Islands, in addition to higher lice prevalence, reported lower species richness.

Notwithstanding the other hypotheses previously advanced for parasite island syndromes, these results suggested that: (i) the higher prevalence of ectoparasites on Islands appears to be a well-defined island syndrome for hosts with different time scales of Island colonization, with the exception of fairly recent colonizing hosts, probably as a result of the also recent establishment of the parasites, as observed in robins; and (ii) species richness should be more related to the number of host colonization events, possibly the repeated founding events should result in a species richness increase, as observed in blackbirds. Thus, these data represent a new perspective for understanding Island syndromes associated with ectoparasites, in which the importance of host colonization in the evolution of insular communities and the respective typical parasite island syndromes was observed.

Study limitations

Like any scientific study, this PhD work has some limitations, which will be listed below:

   i)   Despite the apparent no relationship between parasite species richness and

sampling effort, the lower sampling of birds in the mainland region may have given rise to the apparent higher parasite diversity in the islands. This limitation could be easily overcome by sampling similar number of birds at both places.

ii) Despite the apparent no relationship between parasite richness and island area and distance from the mainland, the low number of sampled islands did not allow us to effectively test the theory of the biogeography of islands associated with ectoparasites. This limitation could be easily overcome by sampling on more Macaronesian islands.

iii) Throughout the thesis work, several hypotheses were presented for the discussion of the chapters, and some of them could have been proven with own data. For example: a) the density of birds that tends to be higher in the islands, could have been easily evaluated, using the birds populations captured by territorial coverage (square meters) of the sampling mist nets (Dunn and Ralph, 2004); b) even though our preliminary results indicated that the body size of birds tends to be higher on the Azores Islands, we preferred not to include this variable in the work, because it lacked a more detailed treatment of the data.

These limitations come not only from the lack of funds for a larger sampling, but essentially from the scarcity of human resources to carry out all the tasks, since in general this work (field work, laboratory work and statistical analysis) was achieved by the author.

Future research

The extended monitoring of avian ectoparasite communities on the Azores Islands would be particularly relevant in helping to understand whether the island syndromes seen here are temporary, or on the contrary, will be maintained over long periods of time. This data on the insular syndromes of parasites would provide relevant information to help understand the long-term dynamics of parasite populations and predict the risk of introducing parasites into new areas. Furthermore, detailed information on the ectoparasite populations of the Azores Islands and mainland Portugal are imperative to know the species of avian parasites that are particularly poorly documented there.

In regards to parasites, expanding the study to other parasite groups, such as haemoparasites and helminths, would contribute not only to increase the knowledge of

these organisms, which to date are poorly studied in the Azores Islands, but also improve the understanding of their evolution on Oceanic Islands and respective island syndromes involved. Even though the evolution of blood parasites assemblages in some Macaronesia Islands is already known, it would be interesting to understand if the insular syndromes manifest themselves in the same way in the Easternmost Archipelago of Macaronesia. Additionally, loss of genetic diversity has been reported as an island syndrome associated with haemoparasites and helminths. Therefore, including genetic analysis even in the case of ectoparasites would be extremely useful, as it would allow testing whether this insular syndrome occurs equally in ectoparasites and whether this variation also occurs among Islands.

Furthermore, extending the study to other geographical areas of mainland Portugal would allow increase the number of samples collected in order to control a possible data bias, which will allow information to be obtained on the possible geographical variation of mainland ectoparasite populations and relate this to environmental conditions, as these are fundamental in structuring ectoparasite communities. Similarly, obtaining data from more Macaronesia Islands would also be of the utmost importance to understand if insular parasite syndromes are equally evident and possibly relate them to the hosts' movement in the colonization of Macaronesia. Furthermore, more data on Island populations would provide robustness to the test of the relationship between species richness and Island area and distance from the nearest continent, since these insular characteristics have previously been correlated with parasite species richness.

In general, the composition of ectoparasite assemblages may be related to the environmental conditions of the site where the hosts reside. Therefore, including information on ectoparasite communities from two seasons of the year with distinct environmental conditions (*e.g.*, bird breeding and post-breeding seasons) would provide better insight into the relationship between environmental conditions, namely humidity and temperature, and their potential effect on the structuring of parasite communities.

References

Adler, G.H., Levins, R., 1994. The Island Syndrome in Rodent Populations. Q. Rev. Biol. 69, 473–490. doi:10.1086/418744

Barrientos, R., Valera, F., Barbosa, A., Carrillo, C.M., Moreno, E., 2014. Biogeography

of haemo- and ectoparasites of an arid-land bird, the Trumpeter finch. J. Arid Environ. 106, 11–17. doi:10.1016/j.jaridenv.2014.03.005

Begon, M., Harper, J.L., Townsend, C.R., 1996. Ecology: Individuals, populations and communities, 3rd Ed. Blackwell Scientific Publications, Sunderland, 1068 pp. ISBN: 0865421110

Borges, P.A.V., Azevedo, E.B., Borba, A., Dinis, F.O., Gabriel, R., Silva, E., 2009. Ilhas Oceânicas. In: Pereira, H.M., Domingos, T., Vicente, L., Proença, V. (Eds.), Ecossistemas e Bem-Estar Humano: Avaliação Para Portugal Do Millennium Ecosystem Assessment. Escolar Editora, Lisboa, pp. 463–510. ISBN: 978-972-592-274-3

Bush, S.E., Harbison, C.W., Slager, D.L., Peterson, A.T., Price, R.D., Clayton, D.H., 2009. Geographic variation in the community structure of lice on western scrub-jays. J. Parasitol. 95, 10–13. doi:10.1645/GE-1591.1

Carles–Tolrá, M., Báez, M., 2002. Hippoboscidae. In: Carles-Tolrá, M. (Ed.), Catálogo de Los Díptera de España, Portugal y Andorra (Insecta). Sociedad Entomológica Aragonesa (SEA), Zaragoza, p. 208. ISBN: 84-932807-0-4

Clayton, D.H., Koop, J.A.H., Harbison, C.W., Moyer, B.R., Bush, S.E., 2010. How Birds Combat Ectoparasites. Open Ornithol. J. 3, 41–71. doi:10.2174/1874453201003010041

Crowell, K.L., 1962. Reduced Interspecific Competition among the Birds of Bermuda. Ecology 43, 75–88. doi:10.2307/1932042

De Bellocq, J.G., Morand, S., Feliu, C., 2002. Patterns of parasite species richness of Western Palaeartic micro-mammals: Island effects. Ecography (Cop.). 25, 173–183. doi:10.1034/j.1600-0587.2002.250205.x

Dunn, E.H., Ralph, C.J., 2004. Use of mist nets as a tool for bird population monitoring. Stud. Avian Biol. 29, 1–6.

Fowler, J.A., Cohen, S., Greenwood, M.T., 1983. Seasonal variation in the infestation of blackbirds by fleas. Bird Study 30, 240–242. doi:10.1080/00063658309476804

Hutson, A.M., 1984. Keds, Flat-flies and Bat-Flies: Diptera, Hippoboscidae and Nycteribiidae. Handbooks Identif. Br. Insects 10, 1–40.

Ibáñez-Álamo, J.D., Ruiz-Raya, F., Rodríguez, L., Soler, M., 2016. Fecal sacs attract insects to the nest and provoke an activation of the immune system of nestlings. Front.

Zool. 13, 1–9. doi:10.1186/s12983-016-0135-3

Ishtiaq, F., Clegg, S.M., Phillimore, A.B., Black, R.A., Owens, I.P.F., Sheldon, B.C., 2010. Biogeographical patterns of blood parasite lineage diversity in avian hosts from southern Melanesian islands. J. Biogeogr. 37, 120–132. doi:10.1111/j.1365-2699.2009.02189.x

Johnson, K.P., Clayton, D.H., 2003. The biology, ecology, and evolution of chewing lice. In: Price, R.D., Hellenthal, R.A., Palma, R.L., Johnson, K.P., Clayton, D.H. (Eds.), The Chewing Lice: World Checklist and Biological Overview. Illinois Natural History Survey Special Publication, Illinois, pp. 449–476. ISBN: 1-882932-08-0

Krasnov, B.R., 2008. Functional and evolutionary ecology of fleas: A model for ecological parasitology. Cambridge University Press, Cambridge, 593 pp. ISBN: 978-0-521-88277-4. doi:10.1017/CBO9780511542688

Kuris, A.M., Blaustein, A.R., Alio, J.J., 1980. Hosts as Islands. Am. Nat. 116, 570–586.

Literák, I., Sychra, O., Resendes, R., Rodrígues, P., 2015. Chewing Lice in Azorean Blackcaps (*Sylvia atricapilla*): A Contribution to Parasite Island Syndromes. J. Parasitol. 101, 252–254. doi:10.1645/14-601.1

MacLeod, C.J., Paterson, A.M., Tompkins, D.M., Duncan, R.P., 2010. Parasites lost - do invaders miss the boat or drown on arrival? Ecol. Lett. 13, 516–527. doi:10.1111/j.1461-0248.2010.01446.x

Marshall, A., 1981. The Ecology of Ectoparasitic Insects. Academic Press, London. 459 pp. ISBN: 0124740804

Moyer, B.R., Drown, D.M., Clayton, D.H., 2002. Low humidity reduces ectoparasite pressure: implications for host life history evolution. Oikos 97, 223–228. doi:10.1034/j.1600-0706.2002.970208.x

Nieberding, C., Morand, S., Libois, R., Michaux, J.R., 2006. Parasites and the island syndrome: The colonization of the western Mediterranean islands by *Heligmosomoides polygyrus* (Dujardin, 1845). J. Biogeogr. 33, 1212–1222. doi:10.1111/j.1365-2699.2006.01503.x

Pérez-Rodríguez, A., Ramírez, Á., Richardson, D.S., Pérez-Tris, J., 2013. Evolution of parasite island syndromes without long-term host population isolation: parasite dynamics in Macaronesian blackcaps *Sylvia atricapilla*. Glob. Ecol. Biogeogr. 22, 1272–1281.

doi:10.1111/geb.12084

Price, R.D., Hellenthal, R.A., Palma, R.L., 2003. World checklist of chewing lice with host associations and keys to families and genera. In: Price, R.D., Hellenthal, R.A., Palma, R.L., Johnson, K.P., Clayton, D.H. (Eds.), The Chewing Lice: World Checklist and Biological Overview. Illinois Natural History Survey Special Publication, Illinois, pp. 1–448. ISBN: 1-882932-08-0

Ribeiro, H., 2007. Lista anotada das pulgas de Portugal (Continente, Açores e Madeira) (Insecta: Siphonaptera). Fauna entomológica de Portugal, 4, Lisboa. 50 pp. ISBN: 0873-5417

Rodrigues, P., Lopes, R.J., Drovetski, S. V., Reis, S., Ramos, J.A., Tristão da Cunha, R., 2013. Phylogeography and genetic diversity of the Robin (*Erithacus rubecula*) in the Azores Islands: Evidence of a recent colonisation. J. Ornithol. 154, 889–900. doi:10.1007/s10336-013-0953-4

Rodrigues, P., Lopes, R.J., Reis, S., Resendes, R., Ramos, J.A., Tristão da Cunha, R., 2014. Genetic diversity and morphological variation of the common chaffinch *Fringilla coelebs* in the Azores. J. Avian Biol. 45, 167–178. doi:10.1111/j.1600-048X.2013.00229.x

Rodrigues, P., Lopes, R.J., Resendes, R., Ramos, J.A., Tristão da Cunha, R., 2016. Genetic Diversity of the Azores Blackbirds *Turdus merula* Reveals Multiple Founder Events. Acta Ornithol. 51, 221–234. doi:10.3161/00016454AO2016.51.2.008

Rodrigues, P., Micael, J., Resendes, R., Lopes, R.J., Ramos, J.A., Tristão da Cunha, R., 2018. Genetic and morphometric variation of the Blackcap (*Sylvia atricapilla*) on the Azores Archipelago reveals a recent range expansion. J. Nat. Hist. 52, 2413–2435. doi:10.1080/00222933.2018.1539194

Senar, J.C., Copete, J.L., Domenech, J., von Walter, G., 1994. Prevalence of louse-flies Diptera, Hippoboscidae parasiting a cardueline finch and its effect on body condition. Ardea 82, 157–160.

Takano, O.M., Voelker, G., Gustafsson, D.R., Light, J.E., 2019. Molecular phylogeny and novel host associations of avian chewing lice (Insecta: Phthiraptera) from South Africa. Syst. Entomol. 44, 289–304. doi:10.1111/syen.12319