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Biogeography and population diversity in a host-parasite system:

ectoparasites of Galápagos doves

Bachelor thesis

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Abstract [in Czech]

Tato studie je zaměřena na společnou evoluční historii hrdličky galapážské (*Zenaida galapagoensis*) a jejích ektoparazitů, rodu *Physconelloides* a *Columbicola*, na 4 galapážských ostrovech (Genovesa, Wolf, Darwin, Pinta). Úroveň populační struktury a genetické diverzity parazitů a hostitele byla porovnána pomocí homologní oblasti 1000 bp mtDNA genu pro cytochrom oxidázu I (COI).

Klíčová slova: co-speciace, Galapágy, hostitel, ektoparazit, *Zenaida galapagoensis*, *Columbicola macrourae*, *Physconelloides galapagensis*, populační struktura, genetická diverzita

Abstract

This study is focused on common evolutionary history of the Galápagos dove (*Zenaida galapagoensis*) and its ectoparasites, species *Physconelloides* and *Columbicola*, on the 4 Galápagos Islands (Genovesa, Wolf, Darwin, Pinta). The level of population structure and genetic diversity of parasites and host were compared using a homologous 1000 bp region of the cytochrome oxidase I (COI) mtDNA gene.

Keywords: co-speciation, Galápagos, host, ectoparasite, *Zenaida galapagoensis*, *Columbicola macrourae*, *Physconelloides galapagensis*, population structure, genetic diversity

Declaration [in Czech]

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České Budějovice, 7. 12. 2019

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Stanislava Wolfová

Acknowledgements [in Czech]

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Contents

1. Introduction

The parasitic way of life is one of the most widespread life strategies in nature (Price 1980). It may seem that parasites are rare but that is because most of them live covertly. Free-living organisms frequently host multiple lineages of closely related parasites. In the view of the fact that parasitism is one of the most successful life strategies, a question about the evolutionary processes that are responsible for parasite diversification is of high importance (Poulin and Morand 2000). One of the often proposed mechanisms is co-speciation.

Co-speciation (or co-diversification at the population level) is a process when the hosts and parasites go through synchronous process of speciation. The two species head down an evolutionary pathway together when the changes in genetic compositions of the species reciprocally affect each other's evolutionary histories (Eichler 1948; Hafner and Nadler 1988). Parasites, particularly the host specific ones, are evolutionary hitchhikers and the evolutionary histories of each lineage often run in parallel. This process, when independent phylogenetic trees of host and parasitic species show mirror image branching patterns, is called Farhenholz rule (Eichler 1948).

1.1. Co-evolution

The co-speciation is thought to be one of the mechanisms that could assist the diversification of parasites. It is hypothesized to ensue from allopatric co-divergence of host-parasite populations (Koop et al. 2014). The host and the parasite often exert selective pressure on each other. The host and the parasite are antagonists – they have opposite interests in their development (Combes 2000). The parasite genotype is constantly evolving, which is influenced by the host genotype due to close interaction via host defence (e.g. behavioural or immunological). This constant arms race causes the counterparts to co-evolve.

Population genetics can be used to understand the ecology and evolution of single species by acknowledging the impact of host population genetic structure on parasites (McCoy et al. 2005). Parasites can also be a self-contained source of information when the host evolutionary data are not sufficient (Nieberding and Olivieri 2007). Generation time for most parasites is

much shorter compared to their hosts. It leads to the accumulation of multiple mutations over a period of time (Page and Charleston 1998; Huyse et al. 2005). Due to the higher mutation rate, the parasite genes allow better reconstruction of recent history. Because the parasite may accurately reflect the history of its host, parasite information can then be used to reconstruct the evolutionary and demographic history of the host organism (Nieberding and Olivieri 2007).

Evolutionary parallel histories of hosts and parasites can be found under different conditions. Congruence between host and parasite population genetic structure depends on three main parasite characteristics: the degree of host specificity; the presence or absence of an intermediate host or wild developmental stage (Clayton and Johnson 2003c) as well as geographical distribution and many ecological factors (Johnson et al. 2002; McCoy et al. 2003). Short generation times and small effective population sizes, Ne (Nadler et al. 1990; Huyse et al. 2005; Nieberding and Olivieri 2007) then translate into faster mutation rates, as can be seen in many host-parasite interactions, like in the seabirds and their tick ectoparasite (McCoy et al. 2005) or butterflies and their specialist parasitoids (Anton et al. 2007).

Even very tight ecological links, such as those between a host and its monoxeneous parasite, do not guarantee strict co-diversification. The findings from population genetic analyses of hosts and parasites are very variable, many host-parasite systems show disagreeing lineage evolutions (Paterson et al. 2000; Johnson et al. 2002). There are several reasons why lineages may not have similar evolutionary histories. The parasites can for example switch the hosts, speciate within a host, fail to speciate, miss the boat or extinct, all of which erodes the congruence (Johnson et al. 2003; Clayton et al. 2016).

1.2. Co-phylogenetic dynamics

Many lice are host specific and they possess close association with the host in microevolutionary time as well as in macroevolutionary time (Clayton et al. 2003a). That is why they serve as a good model for work at co-evolutionary time scales. This is one of the least understood aspects of evolutionary biology, how co-evolution creates the genotypic and phenotypic diversity over time. The parasite dispersal is usually connected with host dispersal.

When there is a barrier to host movement it also influences the movement of their parasites. It is especially common between permanent parasites, which finish their entire life cycle on the body of the host. If the barriers participate on lineage diversification of both – host and parasite, then they will co-diversify. If the process runs with the same timing they may undergo co-speciation (Clayton et al. 2016).

Microevolution factors that are driven by ecological factors could be also responsible for long-term macroevolutionary events in defiance of the enormous expanse of time involved (Clayton et al. 2003b).

The co-evolution of the parasite and the host is affected by many important factors. One of them is the parasite transmission. Vertical transmission of parasites from generation to generation is believed to lead to complete congruence of phylogenies (Johnson and Clayton 2004; Wirth et al. 2004; Whiteman and Parker 2005). It is associated with host specificity of parasites and local adaptations preventing the transmission of parasites to new lines of hosts (Clayton and Johnson 2003c; Prugnolle et al. 2005). But it is not necessary to have strict vertical transmission, when parasites are transmitted horizontally only within and not between diverging host lines. Horizontal transmission can also lead to the parasite and host codivergence (Wirth et al. 2004; Whiteman and Parker 2005).

General scheme of the parasite-host speciation distinguishes several different scenarios (Fig. 1). It is necessary to have as many empirical studies as possible, and to look at the population genetic structure of both the parasite and the host to distinguish between them. Except for co-speciation (a) other macroevolutionary events may confuse the phylogenetic signal, such as incomplete host switching (f), when the parasite colonizes a new host, but maintains gene flow with parasites on the original host; host switching with speciation (h) and host switching in which parasite colonizes a new host and becomes extinct on the original one (g). Other events are represented by duplication of parasites on one host (c). Co-speciation can be succeeded with extinction of one parasite (d). Another event is called “missing the boat“ (e), when the parasite fails to colonize one of the two diverging host lineages. And the last event is the parasite cohesion when the parasite fails to speciate and maintains gene flow

between the diverging host populations (b; Johnson et al. 2003; Clayton et al. 2016).

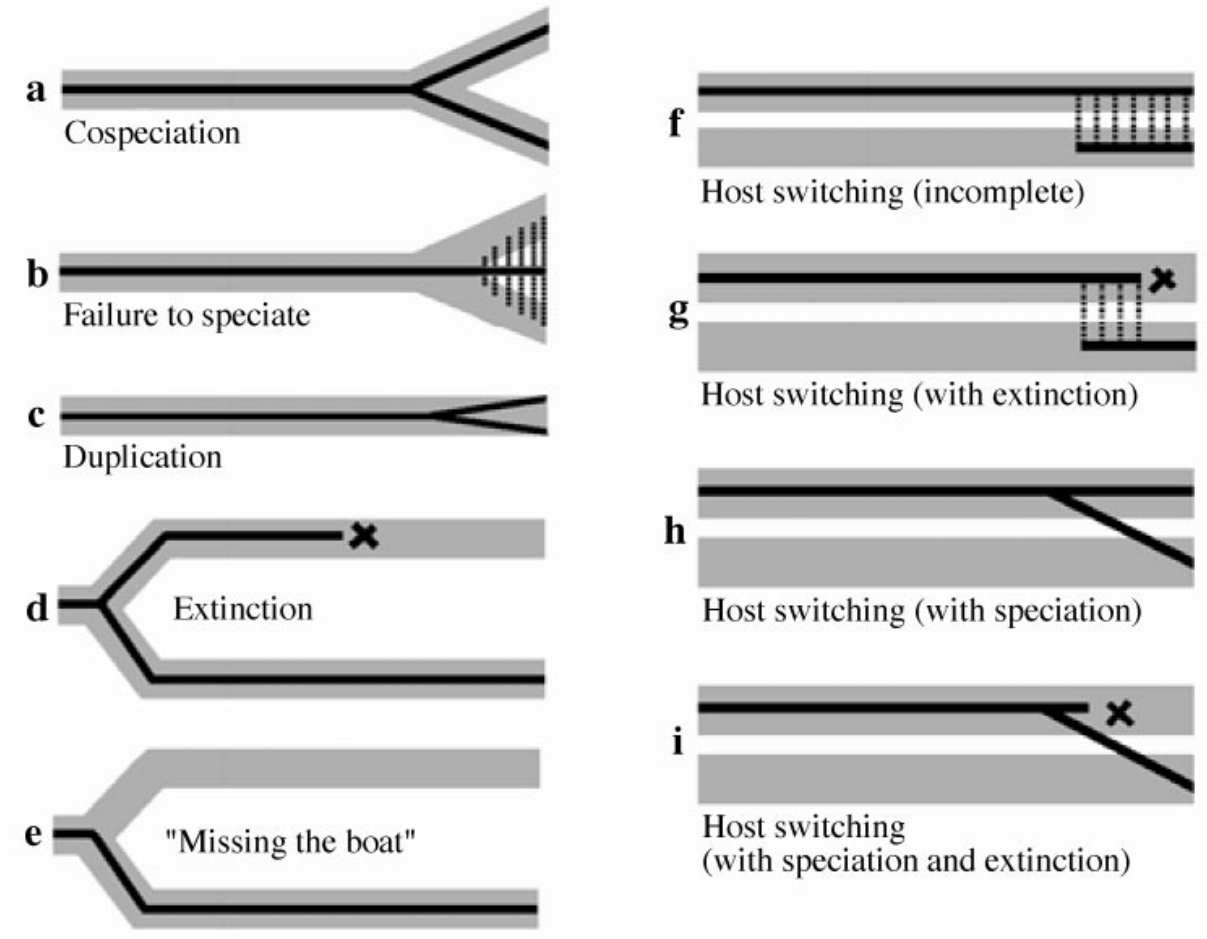

Fig. 1: Macroevolutionary events in host-parasite co-evolutionary histories. Host lineages are represented by pipes, parasite lineages are black lines (adopted from Johnson et al. 2003).

The dispersal ability of a louse species to switch from the typical host to an atypical one has been called as host transfer (Kethley and Johnston 1975), straggling (Ròzsa 1993) and secondary interspecific infestation (Clay 1949). Straggling and host-switching is assumed as a powerful force in phthirapteran evolution (Clay 1949; Tompkins and Clayton 1999; Clayton and Johnson 2003c). Straggling is the precursor of host switching (Ròzsa 1993). Higher tendency to co-speciate are supposed to have the louse species that tend to maintain loyalty to their host, while the taxa prone to straggling are supposed to show less signal of co-speciation (Clayton et al. 2003a).

Whiteman et al. (2004) sampled *Zenaida galapagoensis* and *Buteo galapagoensis* (Galápagos Hawk) for ectoparasites. They found *Columbicola macrourae* and *Physconelloides*

galapagensis on both species, but they confirmed with DNA barcoding that individuals infecting hawks are stragglers from the Galápagos dove, because the hawks feeds on them. Nevertheless, no study has documented straggling rates inside these ectoparasite lineages (Whiteman et al. 2004).

1.3. Host and its ectoparasite

Congruent evolutionary histories are common between birds and their lice. Single birds serve as islands, which restricts the free movement of genes between parasites from individual birds in a manner similar to that observed in geographical island populations (Koop et al. 2014).

Congruent evolutionary histories between birds and their ectoparasites were shown in a study by Štefka et al. (2011). They studied co-evolutionary patterns between populations of mockingbirds *Mimus* spp. and three ectoparasite species (*Analges* sp., *Myrsidea nesomimi and Brueelia galapagensis*). Štefka at al. used mitochondrial DNA sequences, which were complemented with nuclear EF1α sequences in selected samples of parasites and with information from microsatellite loci in the mockingbirds. Reconstructed phylogeographic analyses have shown that the population structure between the *Mimus* spp. and the lines of their parasites is very similar (in spite of varying levels of genetic variability between species) and their diversification is organized according to the geological age of each island. Mockingbirds are relatively good fliers, but they avoid flying long distances (over the sea), so the population of mockingbirds and their parasites are highly structured and they represent evolutionarily independent units on most islands (Štefka et al. 2011).

Compared to that, Whiteman et al. (2007) studied population genetic structures of the endemic Galápagos hawk and its three ectoparasitic insect species - amblyceran, ischnoceran louse (Insecta: Phthiraptera) and hippoboscid fly (Insecta: Diptera). The host is a recently arrived lineage on the Galápagos Islands. The level of population structure and genetic diversity of the parasites and their host were analyzed using a homologous region of the cytochrome oxidase I (COI) mtDNA gene. Whiteman et al. found one haplotype for the hawk on a vast majority of the islands, but the population structure of its ectoparasites was much more delineated due to the higher mutation rate (Whiteman et al. 2007).

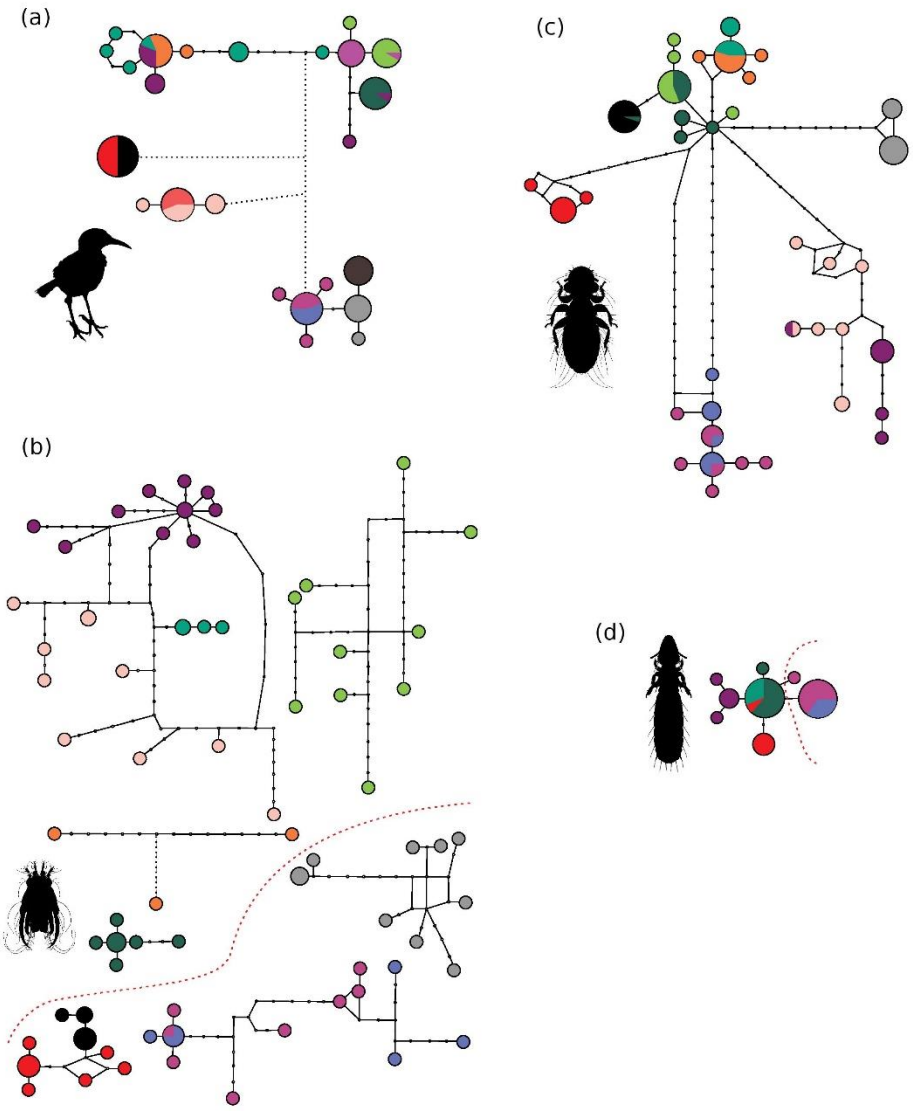

Fig. 2: MtDNA haplotype networks of populations generated with TCS. (a) Mimus, (b) Analges, (c) Myrsidea and (d) Brueelia (adopted from Štefka et al. 2011).

Levin et Parker (2013) studied genetic structure of two ectoparasitic flies (*Olfersia spinifera, Olfersia aenescens*) and its host frigatebirds (*Fregata minor*) and Nazca boobies (*Sula granti*) on Galápagos islands. The flies are highly specialized obligate parasites and usually spend all of their adult life on the host. But most of the species of these hippoboscid flies have functional wings and they can fly between individual hosts (Harbison et al. 2009; Harbison and Clayton 2011). Levin et Parker used mitochondrial DNA sequence data and found out very high level of gene flow in both fly species, despite significant differences in the genetic structure of the

bird host. The discrepancy could be caused by movements of the host (juveniles, non-breeding), which has been shown as a key factor in parasite gene flow, and there is also a possible explanation which involves transmission on closely related species of birds (McCoy et al. 2012; Gómez-Díaz et al. 2012; Levin and Parker 2013).

As a model species of host-parasite co-diversification, I chose to study patterns of genetic diversity in the populations of *Zenaida galapagoensis* and its louse ectoparasites of the genera *Physconelloides* and *Columbicola* within and between four Galápagos islands (Darwin, Wolf, Pinta and Genovesa).

1.3.1. *Zenaida galapagoensis*

Galápagos dove *Zenaida galapagoensis* is the ony member of the order Columbiformes which is found on the archipelago. It occurs on all the major islands and the northern isolated islands Darwin and Wolf (Santiago-Alarcon and Parker 2007). Columbiformes are good pilots and are able to move long distances (Baptista et al. 1997).

Gifford (1913) suggested that doves inhabiting the northern-most islands (Wolf, formerly Wenman) and Darwin, formerly Culpepper) are larger than those located within the main cluster of islands. Because of this, Galápagos dove populations were classified as two subspecies: *Z. g. exsul* (on Wolf and Darwin) and *Z. g. Galapagoensis* (on main islands; Gifford 1913; Swarth 1931; Baptista et al. 1997).

Santiago-Alarcon et al. (2006) studied genetic population structure and morphological differentiation between island populations of the Galápagos dove. Their study was focused on the populations of *Z. g. Galapagoensis* (the southern subspecies). Islands selected for this study were - Santiago, Santa Cruz, Santa Fe, Genovesa and Española. These islands were chosen because of geographic isolation between populations (Española versus Genovesa) and widest (east-west and north-south), but still, those are islands close to the main islands. Because of the closeness of many islands in the archipelago, they expected high gene flow among populations of doves and no morphological differentiation (Santiago-Alarcon et al. 2006).

The level of population structure, genetic diversity, gene flow and effective population sizes were analyzed using five microsatellite markers. They found out that the populations with

larger geographical isolation were not more genetically distinct than those which are closer to one another. No significant differences in allelic richness and gene diversity was found between populations. On the other hand, they found out a significant difference in overall body size between populations of Santa Fe and Santa Cruz islands (both males and females) and among Española and Santa Fe islands, where there were significant differences between males only). Santiago-Alarcon et al. (2006) suggest that variance in body size among populations with high rates of gene flow testify that differentiation may be thanks to phenotypic plasticity or ecotypic differentiation.

They also found out that Genovesa, which is the smallest island which was sampled, displays the largest number of migrants from the other islands. The highest numbers of migrants coming to Genovesa are from Española, which has the largest geographic isolation among the islands, which were sampled. These two islands have also the largest genetic diversities and highest rates of gene flow according to this study. The travel routes might be influenced by environmental factors like wind currents, but it is probably not the main reason. Doves have strong flight skills and distance between some of the islands are relatively short. Doves can simply move between islands to look for food resources and appropriate environmental conditions.

In a later study (Santiago-Alarcon and Parker 2007) the authors added to the analyses samples from the Wolf island, which is one of the two islands inhabited by the subspecies *Z. g. exsul*. It is the first study, where statistically significant morphological evidence supporting the separation of the Galápagos dove into two subspecies was provided. These results suggested that the populations on Wolf and Darwin islands might be somewhat isolated from the rest of the archipelago.

Among the members of the New World dove genus *Zenaida* we can find great variation in the size of their geographical ranges. *Z. macroure* (the Mourning dove) and *Z. auriculata* (Eared dove) are distributed over North and South America, the Galápagos dove (*Z. galapagoensis*) and Socorro dove (*Z. graysoni*) inhabit small islands. The White-winged dove (*Z. asiatica*) is distributed in southern North and Central America and Pacific dove (*Z. meloda*) is spread along the west coast of South America, and the Zenaida dove (*Z. aurita*) in the Caribbean. According to molecular studies, the genus *Zenaida* is most closely related to the New World doves of the genera *Leptotila* and *Greotrygon* (Johnson and Clayton 2000). Johnson et Clayton

(2000) reconstructed a phylogeny for seven species of the genus *Zenaida*. They combined analysis of mitochondrial (NADH dehydrogenase subunit 2 and cytochrome b) and nuclear DNA sequences (fibrinogen intron 7). The colonization of the Galápagos islands by *Z. galapagoensis* precedes the split between *Z. macroure* and *Z. auriculata* of over 2 million years ago. It is proposed that it colonized the archipelago between 2.5 and 3 million years ago, which means that it occurred a substantial amount of time after the geologic formation of the Galápagos.

Native Galápagos bird species display different colonization histories. The lineages represent a broad age distribution and diverse geographic origins. The time at which the species arrived on the islands varies greatly. Arbogast et al. (2006) studied the origin and diversification of Galápagos mockingbirds and found out that the complete radiation seems to be fast and relatively recent, with the start within the last 0.6– 5.5 million years (Arbogast et al. 2006). Darwin´s finches were studied by Sato et al. (2001). It was estimated that finches colonized Galápagos about 2.3 million years ago (Sato et al. 2001). Compared to that, Bollmer et al. (2005) studied mtDNA haplotypes of the Galápagos hawk and its closest relative – the Swanson´s hawk. The data shows that the former´s ancestors arrived to the islands about 300 000 years ago. That makes them the most recent native species incomer known (Bollmer et al. 2005).

Some of these species colonized Galápagos even before the youngest of the current islands formed. That means that they had to arrive to the eastern, geologically oldest islands. Although the specific origin of colonizer's lineages cannot always be resolved, all native land birds were derived from the New World, where their closest living sister taxa breed. For the seabirds the closest related taxa are found in other places in the Pacific ocean (Sari and Bollmer 2018).

Galápagos species often differ between themselves in the way their diversification patterns proceeded after colonization, depending on life-history traits, island geology and trade winds. The mockingbirds and Darwin´s finches radiated into multifold species. Compared to that, other species have not radiated, likely because of high rates of gene flow – (e.g. doves), or deficient time since colonization (e.g. hawks, warblers; Sari and Bollmer 2018).

1.3.2. *Columbicola macrourae, Physconelloides galapagensis*

Lice (Phthiraptera) are the most species-rich lineage of ectoparasites, which is why understanding the ecological processes driving their evolution is of general interest to evolutionary biologists (Marshall 1981; Clayton et al. 2003a, b). Lice are wingless insects and complete the whole life cycle on the body of the host, meaning they are permanent parasites possessing specific adaptations. Lice have sensory organs in their mouths and on their antennae (Clay 1970; Crespo and Vickers 2012). Their eggs are attached to the fur or feathers with glandular cement (Marshall 1981). Lice are sensitive to the temperature, which helps them to orient on the host´s body. They need warm, humid environment near the host´s skin to live (Harbison and Boughton 2014).

Lice are traditionally split in two orders – Anoplura, which are sucking lice and are the parasites of placental mammals; and Mallophaga, which are called chewing lice and are more ecologically and evolutionarily diverse. Mallophaga are parasites of birds and placental and marsupial mammals (Durden 2002; Price et al. 2003).

The endemic Galápagos dove (*Z. galapagoensis*) is the only typical host for the lice *Columbicola macrourae* and *Physconelloides galapagensis* and they are also native to the archipelago (Whiteman et al. 2004).

C. macrourae is known as "wing" lice and *P. galapagensis* is known as "body" lice. The two genera are somewhat related (Cruickshank et al. 2001), they belong to the family Philopteridae (order Phthiraptera, suborder Ischnocera) containig 100 genera and 1500 species.

Physconelloides lice spend most of their life on the host´s abdominal feathers, where they also lay their eggs. That helps them also against the host defense. When the host is preening, they can burrow into the bottom section of the feathers (Clayton et al. 1999). Compared to that, *Columbicola* spend most of their time on feathers of host´s wings and tail. They can resist host defense thanks their oblong shape so they can insert themselves in the space between barbs of flight feathers (Clayton et al. 1999).

The chewing lice consume feathers, skin debris and secretions. Some species also suck blood (Marshall 1981; Lehane 1991). The feathers are nutritionally incomplete so the chewing lice have endosymbiotic bacteria, which provides lice dietary additives like vitamin B (Perotti et

al. 2008). Ischnocera are so specialized that they do not leave the body even if it is dead. Ischnoceran lice on birds are in many cases called feather lice, because they spend almost all of their life on feathers (Clayton and Johnson 2003a).

Earlier works found out that dove body lice are more host specific and have greater population genetic structure than dove wing lice (Johnson et al. 2002). They are usually transmitted to new hosts via straight contact between host individuals. It can be during mating or breeding (Clayton and Tompkins 1994).

Clayton and Johnson (2003) studied 13 species of doves from a diversity of localities in the New World (United States, Mexico, Peru, Brazil) and compared the macroevolutionary histories of two genera of feather lice - *Columbicola* and *Physconelloides*. They found out that *Physconelloides* demonstrate strong evidence of co-speciation whereas, conversely, *Columbicola* do not (Clayton and Johnson 2003b). That can be thanks to the fact that *Physconelloides* are more host-specific than *Columbicola*, meaning that *Physconelloides* shows significantly higher level of population structure. Body lice tend to show population genetic structure between geographic localities considering populations on the same host species. *Columbicola* had an independent evolutionary history, while *Physconelloides* tightly tracked the evolutionary history of the dove. The differences are probably based on ecological factors, such as different dispersal abilities of the lice (Johnson et al. 2002), particularly phoresis (hitch-hiking) of *Columbicola* on hippoboscid vectors, for which many records of phoresis were registered unlike records for *Physconelloides*, which are fairly rare (Keirans 1975).

Abundance of parasites can be explained by the study Santiago-Alarcon (2012) who analyzed the associations of three different parasites (*C. macrourae, P. galapagensis,* and the blood parasite *Haemoproteus multipigmentatus*) with the body condition of the Galápagos dove (Santiago-Alarcon et al. 2012). On a general level genetic diversity is positively related with fitness of individuals (Reed and Frankham 2003). Correlational studies on birds and vertebrates demonstrated that individuals with higher genetic diversity have lower number of parasite individuals (Whiteman et al. 2006; Acevedo-Whitehouse et al. 2006). Santiago-Alarcon (2012) showed that individual doves with higher genetic diversity had better body condition and lower abundances of all three parasite species. Path analysis acknowledged the

relationship between genetic diversity and body condition, but it was not possible to confirm directionality between body condition and parasite abundance.

2. Study area

Oceanic archipelagos are natural evolutionary laboratories because of their isolation from mainland biota, low probability of multiple colonization events and simplified fauna (Barton 1996). They are a good model to study co-phylogenetic patterns in hosts and parasites.

The Galápagos Islands are a part of the Republic of Ecuador. It is an archipelago of volcanic islands which are spread on both sides of the equator in the Pacific Ocean. It consists of ten major islands and a lot of small islands and rocks. It is one of the most important ecological spots in the planet for its unique biodiversity, active geology, and relatively well-preserved ecosystems (Orellana and Smith 2016). It is well known for its endemic biodiversity like giant tortoise, marine iguana, lava lizards as well as Galápagos penguin, Galápagos hawk and the flightless cormorant. Native terrestrial birds show high level of endemism 84%, and 59% of all vertebrates are endemic. Endemism is much lower in the seabirds (26%) and shorebirds (23%; Tye et al. 2002).

Endemism is strongly affected by the geographical isolation of the islands. The isolation of the archipelago combined with a large number of individual islands allowed independent procession of evolution, which made these islands famous for and, until recently, it saved the archipelago from major human impact that affected the vast majority of the Earth´s surface (Orellana and Smith 2016).

Its isolation and the fact that major part of the archipelago (79% of the land surface) is dedicated as a protected National park, could not prevent recent pressure on the development of the islands, leading to a rapid increase in sea and air traffic between the islands, interconnection with mainland South America and the expansion of permitted visitors' places and activities (Grenier 2007). This led to an increased risk of biological invasions, effects on animal behaviour, physical disruption and degradation of the wilderness (Watkins et al. 2007).

Oceanic islands, such as Galápagos, due to their relative isolation from continental biota, limited area and simplified fauna, provide a suitable model for the study of cophylogenetic patterns in hosts and parasites. There is also a low probability of multiple colonization events, and founding populations of island colonists are usually small in size and have limited number of gene alleles, which leads to rapid coalescence in host and parasite lineages. The effects of selection and genetic drift quickly lead to genetic differentiation and the formation of new

species (Barton 1996). Islands in general are places where the most dramatic morphological and genetic differentiations have happened (Grant 1998, 2001).

The Galápagos are at the interface of two lithospheric earthboards, where geological and volcanic activity occur. Cooled magma gives rise to individual islands. The archipelago is geologically quite young. Scientific studies have shown the Galápagos to be a complex system in which climate, ocean currents, biology and geology profoundly affect one another. This allows a detailed examination of the impact of geographical isolation on the formation of population structure and speciation in the habitat of Galápagos (Harpp et al. 2014)

For at least twenty million years there has been almost continuous volcanism as a result of a mantle plume beneath the east-ward moving Nazca Plate, which has given rise to a 3 kilometre thick platform under the island chain and seamounts (Harpp et al. 2014).

The Galápagos and its northern „alternative“, the Hawai´i, differ in their morphology, chemical composition and structural evolution (Harpp et al. 2014), but both of them arose in the form of a successive chain of volcanic islands. The phenomenon sometimes called the progression rule suggests that the pattern of speciation by endemics follow the successional origin of islands in the chain (Funk and Wagner 1995).

Geological evidence shows a northwest to southeast gradient in the age of the Galápagos islands (Fig.3). The volcanoes at the west end are in general younger and have well-developed calderas (big hollows that form shortly after the emptying of a magma reservoir in a volcanic eruption) and those on the east are shorter, older and have a more diverse composition (Harpp et al. 2014).

The north-western edge of the archipelago is the youngest. The last eruptions are up to several thousand years old. Fernandina on the west is for example just 0.05 million years old and it is, therefore, the most active Galápagos volcano. To the south and southeast, we can find geologically older islands up to 3 million years old (Harpp et al. 2014).

There are also some islands which are drowned due to erosion. Radiometric ages for them range from 5 to 9 million years (Christie et al. 1992). That indicates that organisms on Galápagos islands may have had much longer time for speciation.

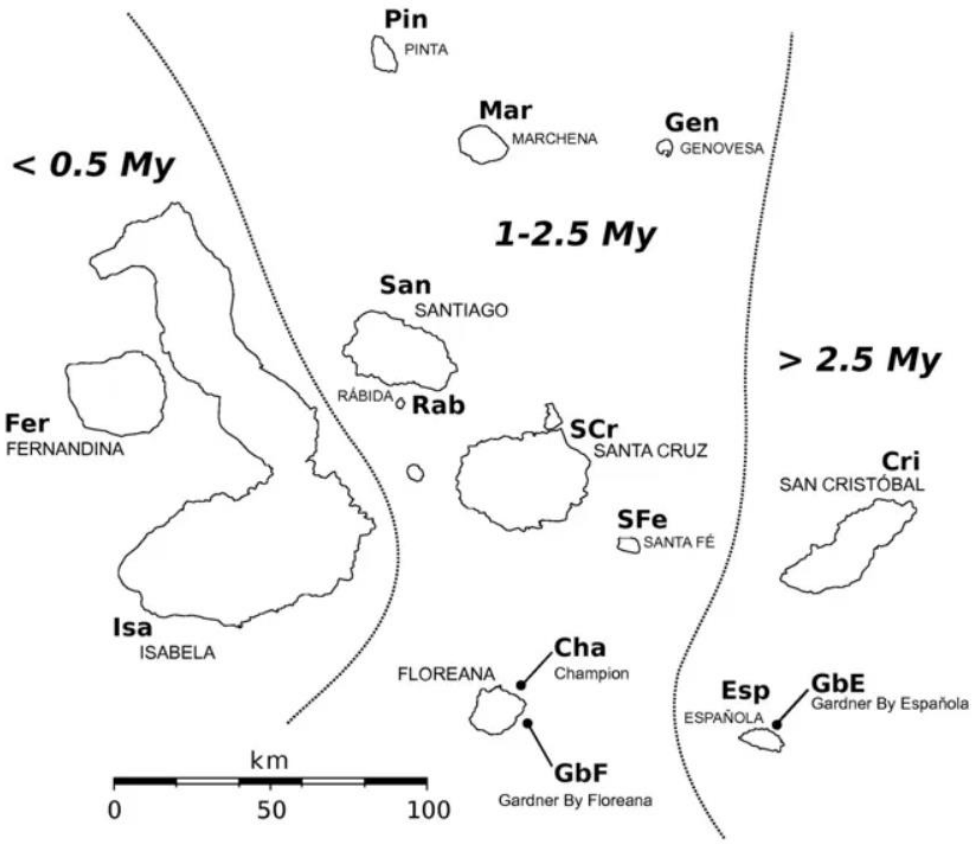

Fig. 3: Map of the Galápagos islands. Estimated geological age of the archipelago (My = million years). It is based on literature data (White et al. 1993; adopted from Štefka et al. 2011).

Island species, especially endemic species, tend to have lower genetic diversity compared to their continental couterparts (Frankham 1996, 1997). The small populations together with the low genetic variability have a straight effect on the evolutionary potential of organisms and the way they handle changing environments (Hedrick 2001; Petit et al. 2008).

Radiations on Galápagos islands are comparatively rare. Although many taxa have speciated from their former ancestors from the mainland, they often did not have enough time to speciate compared to older archipelagos (Tye et al. 2002).

3. Objectives

The aim of the project was to study the joint evolutionary history of the Galapagoensis dove (*Zenaida galapagoensis*) and its ectoparasites, *Physconelloides* and *Columbicola*, on four Galápagos Islands (Genovesa, Wolf, Darwin, Pinta). The level of population structure and genetic diversity of parasites and host were compared using mitochondrial DNA to answer the following questions.

1) Does the pattern of population structure differ between islands, some of which show a high degree of isolation (migration barrier in the form of up to 150 km of the open sea)?

2) Do parasites have a deeper population structure than their host due to faster molecular evolution?

3) Do the host and its parasites undergo synchronous co-diversification? If so, is it related to the two suggested host subspecies (*Z. g. galapagoensis* and *Z. g. exsul*)?

4. Methods

4.1. Material collection

Collection of the host and parasite material was held in 2014 by my supervisor and his collaborators within their previous research. Dove blood samples were obtained from 4 Galápagos islands – Genovesa, Pinta, Darwin and Wolf. The birds were examined for ectoparasites on all the islands. *Columbicola macrourae* were found on all the islands in contrast with *Physconelloides galapagensis* which was not found on birds caught on Genovesa. Ectoparasites were stored in alcohol. Birds were caught using mist nets or potter traps. The blood sample was taken from a small puncture on the wing vein. Ectoparasites were collected from the feather of the birds using dust ruffling (Clayton and Drown 2001) and preserved in ethanol. Birds were ringed to prevent resampling and released immediately after sample collection (Štefka et al. 2011).

In laboratory individual tubes with ectoparasite samples were sorted, ectoparasites identified and remaining pyrethroid dust removed.

4.2. DNA extraction

A single parasite specimen was analysed for each parasite taxon per host individual, with a few exceptions where there were not enough sampled host individuals.

DNA extraction of parasite samples was performed using MicroDNA extraction kit (Qiagen), which is recommended for purification of genomic and mitochondrial DNA from small samples. Each louse individual was cut it between body and head to allow more efficient lysis by proteinase. Host DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen). DNA extraction was performed according to the manufacturer's instructions.

4.3. mtDNA sequencing, PCR, gel electrophoresis

The level of population structure and genetic diversity of the parasites and their host were analyzed using a homologous region of the cytochrome oxidase I (COI) mtDNA gene. For doves, cytochrome B could be a more variable marker, but for a direct comparison of genetic diversity with the parasites, I decided to use homologous gene sequences. The metazoan mitochondrial genome is one of the largest ones and it has bigger adaptability than the other mitochondrial genes. It is also one of the most important molecular markers used for molecular

taxonomy and systematics of living things and microorganisms (Hebert et al. 2003; Karimian et al. 2014).

The fragments of approximately 1000 bp were sequenced using a combination of the previously described universal primers and newly designed primers based on sequences of related taxa available from GenBank (Table I).

The PCR contained 12 µl: 1 µl of the extracted DNA sample, 1 µl of each primer, 6.25 µl of PCR mix (Qiagen Mastermix) and 2.75 µl of PCR water.

Primers H7005 and F1490 (Tab. I) were used to amplify *Columbicola* DNA. In order to improve the quality of obtained reads, internal sequencing primers (Int_Fw_C and Int_Rev_C) were designed in the Geneious program, using sequences obtained with PCR primers. Primers COIHP and COILP were used to amplify *Physconelloides* DNA. The PCR profile was for both as follows: 15 minutes at 95° C followed by repeated cycles (30x) for 30 seconds at 94° C, 30 seconds at 48° C and 45 seconds at 72° C. The final elongation step was performed for 5 minutes at 72° C.

To amplify mtDNA of the host a BirdF1 and a modified reverse primer were used. Then internal primers Zen_int_R and Zen_int_F were designed based on the sequences obtained. The PCR profile was as follows: 15 minutes at 95 ° C followed by repeated cycles (30x) for 30 seconds at 94 ° C, 30 seconds at 55 ° C and 45 seconds at 72 ° C. The final elongation step was performed for 5 minutes at 72 ° C.

Tab. I: PCR primers for amplification of the COI gene.

Species	Primer name	Direction	Primer sequence	Reference
Zenaida galapagoensis	BirdF1_Zen	F	TCCACCAACCACAAAGACATTGGCAC	Modified from BirdF1 (Kerr et al. 2007)
	COIH_Zen	R	AGTGGGCAACTACGTAGTATGTGTCATG	Modified from COI H7005 (Hafner et al. 1994)
	Zen_int_F	F	CTTCAGACCGAAACCTAAA	This study
	Zen_int_R	R	TAACATGGCCCATACCATTCCTATGTA	This study
Columbicola macrourae	H7005	R	CCGGATCCACNACRTARTANGTRTCRTG	(Hafner et al. 1994)
	F1490	F	GGTCAACAAATCATAAAGATATTGG	(Folmer et al. 1994)
	Int_Fw_C	F	TAGGGACAGGGTGGACAGTT	This study
	Int_Rev_C	R	CTGGTAAAGAATTGGGTCCCCA	This study
Physconelloides galapagensis	COIHP	R	AATGAGCAACNACATARTAWGTRTCRTG	This study
	COILP	F	GGYTTTTTTCTTCTAATCAYAARGATATTGG	This study

The results of the PCR reactions were visualized by agarose gel electrophoresis at 100 V using GelRed (Biotium), Gel Loading Dye (6X; Thermo Fisher) and 1 kb GeneRuler Ladder (Thermofisher). I prepared a 1% gel by mixing 0.2 g of agarose (Thermo Fisher) with 20 ml of TAE buffer (for 8 samples; Merck).

I visualized it with gel imaging device and Image lab system. I purified well-visualized PCR products with 0.5 µl Exo I enzyme (New England Biolabs) and 2 µl FastAP (Thermosensitive alkaline phosphatase; Thermo Fisher), and sent them for sequencing.

Sequencing was carried out with the commercial company Seqme (CZ). Contigs were assembled and sequences aligned in the software Geneious.

4.4. Population genetic analysis of sequence data

The data were exported for statistical and haplotype analysis to compare the diversity and relationships of Galápagos populations. Genetic diversity of each species and their populations were summarized in the DNASP 6.0 program. First, measures of population diversity were calculated (haplotype diversity, nucleotide diversity). Then a test of population differentiation (GammaST, Snn test) and tests of neutrality (Fu's F_S and Tajima's D) were calculated. Finally, tests of population size changes were performed (Raggedness test, Ramos-Onsins and Rozas' R_2 statistic). Statistical significance of the tests was checked using 10.000 coalescent simulations.

Haplotype networks were reconstructed using TCS algorithm in PopArt to visualize population genealogies.

5. Results

In this study, I present molecular analyses of a total of 25 *Columbicola*, 18 *Physconelloides* and 34 *Zenaida galapagoensis* individuals.

The level of genetic diversity was overall low. However, statistical analysis of populations surprisingly showed that a higher level of diversity was preserved in the host than in its parasites. Generally, *Z. galapagoensis* showed higher nucleotide diversity (Tab. II) than its ectoparasites, which is reflected also in the haplotype network showing up to three clusters separated by multiple mutations in the host (Fig. 4). Nucleotide diversity (Pi) is defined as the average number of nucleotide differences per site in pairwise comparisons among DNA sequences (Nei 1987). While haplotype diversity (also known as gene diversity) represents the probability that two randomly sampled alleles are different (Nei 1987), which was preserved the highest in *Columbicola*.

As expected, the lowest value of haplotype diversity for doves was found on the smallest and most isolated island of Darwin (Hd = 0.46429). On the contrary, values of Pi for doves did not differ much between the islands.

Tab. II: Genetic diversity of populations and species (number of sequences, number of haplotypes, nucleotide diversity, haplotype diversity). Abbreviations: *Columbicola macrourae* (C), *Physconelloides galapagensis* (P), *Zenaida galapagoensis* (D).

	Number of sequences			**Number of haplotypes**			**Pi (nucleotide diversity)**			**Hd (Haplotype diversity)**		
	C	**P**	**D**	**C**	**P**	**D**	**C**	**P**	**D**	**C**	**P**	**D**
Darwin	6	5	8	4	3	3	0,00131	0,00120	0,01472	0,86667	0,80000	0,46429
Pinta	9	8	9	7	2	4	0,00323	0,00090	0,01312	0,94444	0,25000	0,58333
Genovesa	5	0	9	4	0	5	0,00281	-	0,01695	0,90000	-	0,86111
Wolf	5	5	8	4	2	4	0,00244	0,00072	0,01964	0,90000	0,60000	0,75000
Total	25	18	34	12	4	10	0,00264	0,00100	0,01502	0,90700	0,54200	0,68100

In terms of nucleotide diversity, parasite populations showed much less genetic diversity compared to their host. The lowest value of haplotype diversity was found on Pinta Island for *Physconelloides* (Hd = 0.25000). *Physconelloides* had a haplotype and nucleotide diversity lower everywhere, which indicates an overall low level of intra-specific diversity.

In *Columbicola* we can see several island specific haplotypes (Fig. 4), for example, on Pinta. But most other *Columbicola* hapotypes (and of the other species) are geographically mixed, which supports the assumption that doves often migrate between islands.

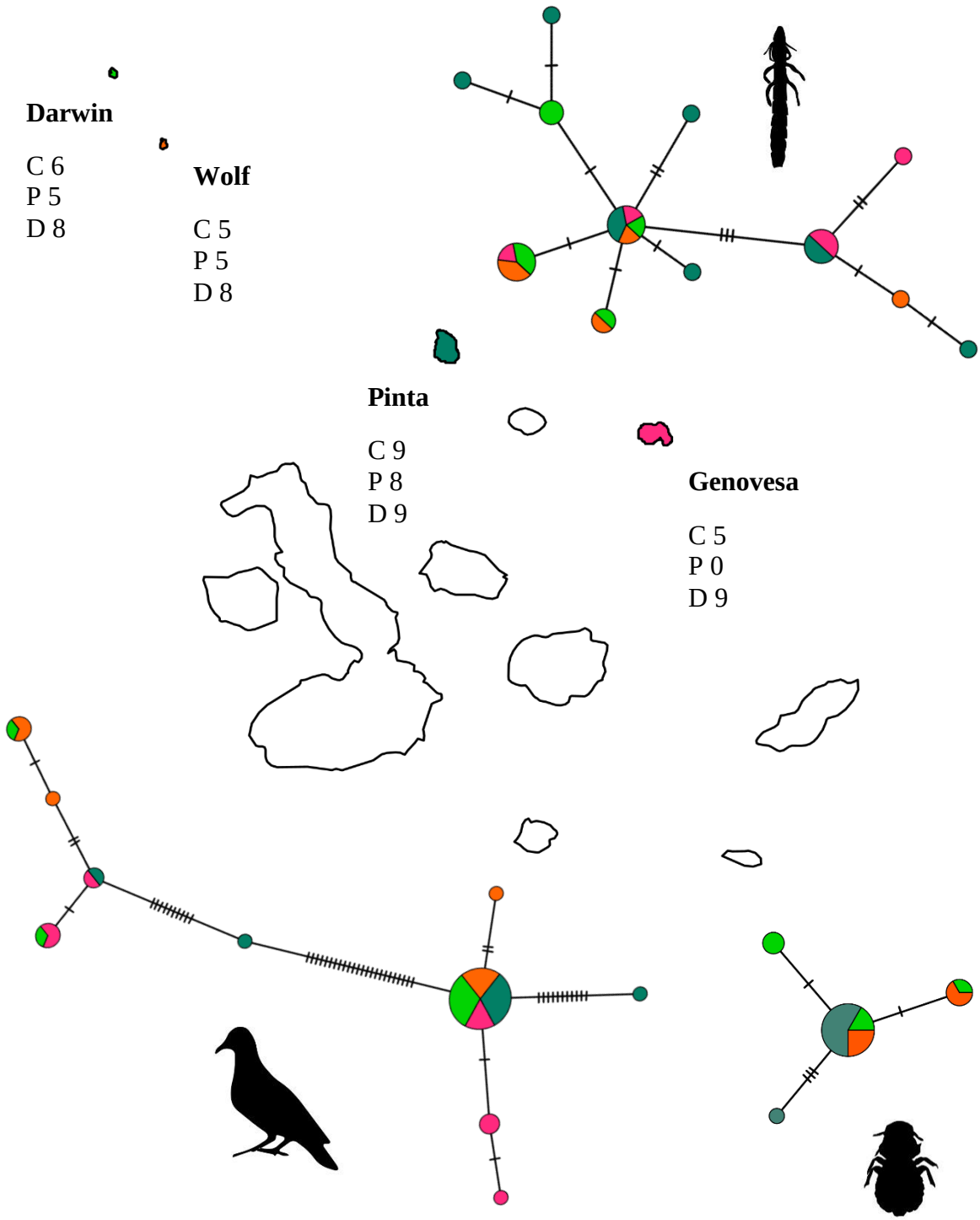

Fig. 4: mtDNA haplotype network showing relationships and geographic origin of haplotypes for the three organisms (*C. macrourae* – top right, *Z. galapagoensis* – bottom left and *P. galapagensis* – bottom right).

We performed two statistical tests (Tab. III) for detecting genetic differentiation of these populations. These statistic tests can be used when the genetic data are collected on individuals sampled from two or more localities.

Tab. III: Statistical tests of geographical differentiation. Population pairs showing significant values of the Snn test are marked by an asterisk ($P < 0.05$).

		GammaSt		
Population 1	**Population 2**	**C**	**P**	**D**
Darwin	**Genovesa**	0.27431	-	0.01360
Darwin	**Pinta**	0.10632	0.14107	0.00274
Darwin	**Wolf**	0.07981	0.13514	0.02367
Genovesa	**Pinta**	0.06456	-	0.02152
Genovesa	**Wolf**	0.13178	-	0.01850*
Pinta	**Wolf**	0.06117	0.14267	0.03515

GammaSt indicates the level of population structure among the populations. Doves always showed lower values compared to their parasites. In the Snn test, which is based on haplotype sharing, the value for the Wolf x Genovesa islands (Snn = 0.0140) was significant, which means that they share haplotypes less than randomly. For the other islands, we did not find statistically significant signal for population structure in the Snn test. Lack of significance in the pairs showing increased GammaST values (e.g. in parasites) may be affected by a lower number of sequenced individuals.

Tab. IV: Neutrality tests (Tajima's D, TD; Fu's F_S) and tests of population size changes (Ramos-Onsins and Rozas´s R_2; Raggedness, r) Significant values are marked by an asterisk * ($0.05>P>0.001$).

Zenaida galapagoensis	**Total**	**Darwin**	**Wolf**	**Pinta**	**Genovesa**
Tajima's D, TD	1.954370*	0.326517	1.838640	0.096933	1.600630*
Fu's F_S	7.850300*	8.404110*	6.738860*	6.101900*	4.861790*
Ramos-Onsins and Rozas's R_2	0.186519*	0.194435	0.253316*	0.179200	0.236393*
Raggedness, r	0.088926*	0.473214*	0.167092	0.326389*	0.107353

Columbicola macrourae	Total	Darwin	Wolf	Pinta	Genovesa
Tajima's D, TD	-0.837220	0.338389	-0.668229	-0.700056	0.286384
Fu's F_S	-4.332970*	-1.159580	-0.567000	-2.165460	-0.331576
Ramos-Onsins and Rozas's R_2	0.088742	0.216880	0.262996	0.111684*	0.200693
Raggedness, r	0.019333*	0.364440	0.230000	0.108025	0.030000*

Physconelloides galapagensis	Total	Darwin	Wolf	Pinta	Genovesa
Tajima's D, TD	-1.343630	0.243139	1.224750	-1.447510	-
Fu's F_S	-0.525145	-0.475000	0.626000	1.415000	-
Ramos-Onsins and Rozas's R_2	0.146362	0.250000	0.300000	0.330719	-
Raggedness, r	0.133538	0.360000	0.400000	0.687500	-

Neutrality test statistics were conducted to infer historical evolution of the populations. Tajima´s test values larger than 0 suggest either a recent population bottleneck or some form of balancing selection. Values smaller than 0 suggests either population expansion or purifying selection. The results for total populations are below zero for the parasites, but they are not statistically significant. The highest value of Tajima´s test, which was positive and significant, was for *Z. galapagoensis* in a total (TD = 1.954370*). Significant positive value for doves was also found on Genovesa (TD = 1.600630*).

Zeng et al. (2006) point out that there are important aspects of the data that Tajima's D does not take in to account. That is why the Fu's simulations propose that F_S is a more sensitive indicator of population expansion and genetic hitchhiking (Zeng et al. 2006).

The negative values of Fu's F_S test, which is based on the distribution of haplotypes, indicates an excess of rare haplotypes over what would be expected under neutrality. It would be presumed from a recent population expansion or from genetic hitchhiking. A positive value of F_S is evidence for a lack of alleles, as would be expected from a recent population bottleneck or from overdominant selection. We can find positive significant values for doves on all the islands, the highest one on Darwin (F_S = 8.404110*). We can find positive values also for *P. galapagensis* on Wolf (F_S = 0.626000) and on Pinta (F_S = 1.415000), but in both cases the values are only moderately increased and non-significant. In parasites, negative values of Fu´s

F_S test can be seen on most islands. For *C. macroure* the highest negative value was found on Pinta (F_S = -2.165460).

I have also used Ramos-Onsins and Rozas' (2002) R_2 statistic. This test is based on the difference between the number of singleton mutations and the average number of nucleotide differences. Lower values of R_2 are expected under a scenario of recent population growth, higher values are supposed to reflect larger populations or populations thet experienced bottlenecks. Higher and significant values can be seen for *Z. galapagoensis* on Wolf island (R_2 = 0.253316*) and on Genovesa (R_2 = 0.236393*).

Ramos-Onsins and Julio Rozas (2002) found in several cases that Fu's F_S test and R_2 test are the most powerful tests for detecting population growth. R_2 test is better for small sample sizes, while F_S is better for large sample sizes (Ramos-Onsins and Rozas 2002).

The 'raggedness' index was the highest (and significant) for the population of *Z. galapagoensis* (r = 0.473214*) on Darwin island.

6. Discusion

In this study, I present evidence that correspondingly to the high mobility of doves, very few specific haplotypes for any of the islands in any of the three taxa can be found. Also other molecular analyses (GammaST, Snn, Haplotype networks) did not reveal any clearly differentiated lineages.

Geographically non-specific haplotypes support the assumption that doves are good fliers and probably no less than 150 km of the open sea is a migration barrier for them. Available data also suggest that certain level of diversity was preserved in doves after bottleneck.

Particularly, parasite data suggest a sharp decline in the number of individuals in the population followed by sudden population growth (low statistically significant values of R_2 and r and negative values of F_S in Table IV) and that the decline in genetic diversity has not yet been fully restored.

Contrarily to the parasites, it is possible that the doves have retained some of the genetic diversity that their ancestral population possessed before the Galápagos colonization. This is shown by the haplotype network containing up to three clusters separated by multiple mutations, by the higher nucleotide diversity compared to the ectoparasites, and results of the statistic tests - positive value of Tajima´s test, positive value of Fu's F_S test, high values of Raggedness and Ramos-Onsins and Rozas's R_2 test, which are all statistically significant.

It is possible that novel singleton mutations (haplotypes) seen in the network originated recently during population growth following the colonization. This is visible especially in *Columbicola*, where higher mutation rate known for parasites (Hafner et al. 1994; Štefka et al. 2011) allowed faster acquisition of new mutations compared to the host.

In the mtDNA dataset analysed here, I found no support for the two morphologically defined subspecies of the *Z. galapagoensis* (*Z. g. exsul* on Wolf and Darwin and *Z. g. Galapagoensis* on main islands; Gifford 1913) as provided by Santiago-Alarcon study from 2007. It was the first study, where statistically significant morphological evidence that supports the separation of the Galápagos dove into two subspecies was provided. My study does not support the

suggestion that populations on Wolf and Darwin islands might be significantly isolated from the other islands.

In contrast to the doves, open sea represents a strong migration barrier in other Galápagos birds, such as mockingbirds, *Mimus* spp. who are not good long-distance pilots. Previous studies (e.g. Štefka et al. 2011) have shown specific haplotypes for islands, corroborated by microsatellite-based assignment patterns. Reconstructed phylogeographic analyses have shown that the population structure between the *Mimus* and the lineages of their parasites are very similar and their diversification reflects geological age of each island. After colonization mockingbirds formed evolutionarily independent units on each island, which cannot be said about the doves.

The fact that we found almost no specific haplotypes for any of the islands can be compared to the hawks, which are also good pilots. But, contrarily to the doves, hawks have much lower population densities, with only a few pairs living on most islands. In the result of Whiteman (2007) we can see one haplotype on the vast majority of islands, although their ectoparasites showed much deeper population structure, which cannot be seen in dove´s ectoparasites. The population structure of hawk´s ectoparasites is much more detailed due to the higher mutation rate, in spite of the fact that hawks colonized Galápagos very recently. The data shows that hawk's ancestors arrived to the islands only about 300 000 years ago (Bollmer et al. 2005). In comparison, doves are estimated to have arrived between 2.5 and 3 million years ago (Johnson et Clayton 2000), which means that it has occurred a substantial amount of time after the geological formation of Galápagos.

Parasites of *Zenaida galapagoensis* are likely to have experienced a much larger bottleneck as their diversity is much more limited. This can easily happen because they do not have a 100% prevalence on the host, ectoparasite populations are usually more fragmented (Koop et al 2014) and thus possess lower Ne. Colonization was probably carried out with a small number of host individuals (moreover, probaly not each of them was infected) and the parasites were often related so that their genetic diversity was much smaller than that of the doves.

There are also differences between the two species of parasites. *Columbicola* showed higher level of population diversity than *Physconelloides*, correspondingly with the differences in their prevalence. *Physconelloides* populations probably possess fewer individuals, because

they infect fewer hosts, and generally have a lower intensity of infection. Only a few haplotypes were found across the sampled islands and on Genovesa we did not find any *Physconelloides* parasites. Either it occurs on this island with a very low density, or it is missing completely, either due to local extinction, or it did not colonize the island with its host (so called "missing the boat" phenomenon). Compared to this, Johnson et al. (2002b) sampled 13 species of doves from a diversity of localities in the New World (United States, Mexico, Peru, Brazil) and found out that *Physconelloides* are more host specific than *Columbicola*, which had an independent evolutionary history, while *Physconelloides* highly track evolutionary history of the dove and showed significantly more population structure. *Physconelloides* even showed population genetic structure between geographic localities of the same host. The differences are probably based on an ecological factors such as different dispersal abilities of the lice (Johnson et al. 2002b) resulting in lower Ne in *Physconelloides*.

Similar genetic structure as *P. galapagoensis* in our study (extremely low genetic diversity, lack of island specific haplotypes) can be seen also in the study by Štefka et al. (2011), particularly in the haplotype network of *Brueelia galapagensis* (Fig. 2). However, a different process then here (low prevalence combined with host-based dispersal) was assumed responsible for the pattern. *Brueelia* was an exception in the study, the two other investigated parasites (*Myrsidea nesomimi* and *Analges* mites) showed allopatric co-divergence with their host. Thus, it was suggested that occasional phoresis to a non-specific host migrating between the islands (e.g. Darwin finches) allowed dispersal in *Brueelia*.

In conclusion, the overall low level of genetic diversity seen in all three organisms suggests that doves and their parasites experienced a strong bottleneck, and their genetic diversity has not been fully restored yet. The data also showed lack of population structure formation, probably due to high migration capabilities of the doves.

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