The role of parasites in host speciation

Gobbin, Tiziana P.

DOI:
10.33612/diss.168426043

7

General synthesis

Tiziana P Gobbin

Mechanisms of speciation have been investigated in many animal and plant taxa by countless studies. Many of them focused on ecological speciation, namely on the role of resource competition and interspecific interactions (as prey-predator and host-parasite) in driving species divergence (Nosil, 2012). Parasites may be an important source of ecological selection, as they can have strong effects on their hosts (e.g. negatively affecting host growth, reproduction and/or behaviour). Locally different parasite infection may drive adaptive host population divergence that may promote reproductive isolation between host populations and ultimately speciation. Studies on the role of parasites in host diversification have begun to accumulate (Greischar & Koskella, 2007; Eizaguirre et al., 2011; Eizaguirre et al., 2012a; Stutz et al., 2014; Feulner et al., 2015; Karvonen et al., 2015). However, it is still unclear at what stage of the speciation process parasite-mediated divergent selection acts; and to what extent it actually contributes to speciation, especially in the context of adaptive radiation (Vanhove & Huyse, 2015; El Nagar & MacColl, 2016). In this thesis, I investigated these aspects by studying the role of parasites in the diversification of cichlid species from Lake Victoria.

In the following pages, I summarise the main findings of previous chapters of this thesis (**Table 7.1**) and discuss their implications for the understanding of parasite-mediated divergence and speciation in natural hosts and particularly in African cichlids. Since results differed according to the parasite infection level analysed – between parasites of higher taxonomic levels (hereafter referred to as parasite higher taxon level) and between species of *Cichlidogyrus* (hereafter referred to as *Cichlidogyrus* species level) – I present them separately. I also suggest directions for future research, for the cichlid model system and beyond.

7.1. THE ROLE OF PARASITES (AT HIGHER TAXON LEVEL) IN HOST DIVERSIFICATION

Temporally consistent differences in infection between host species – I tested two prerequisites for parasite-mediated speciation – namely species differences in infection and temporal consistency in the direction of such differences. To this end, I analysed parasite infection patterns (parasite prevalence, abundance and parasite community composition; see **Box I** for definitions) of closely related cichlid species living in sympatry in southern Lake Victoria. The number and diversity of parasites differed between reproductively isolated cichlid species (**chapters 2 and 3**). These contrasting infection patterns among host species were not unexpected, given the ecological diversity of Lake Victoria cichlids (Greenwood, 1981; Witte & van Oijen, 1990; Seehausen, 1996b; Bouton et al., 1997; Bouton et al., 1999.), but they were remarkable given the young age of the Lake Victoria radiation (14'600 years old; Johnson et al., 1996). Such species differences in infection patterns remained consistent after a period of four years. These findings suggest that parasite-mediated selection is indeed divergent in cichlids and

that its direction is maintained over time, possibly facilitating a role of parasites in host divergence.

In addition to species variation in parasite numbers and diversity, I also found another axis of species divergence in infection: parasite microhabitat distribution over the fish gills (based on the number of parasites found on each of 36 spatial subdivisions of the gills; **chapter 5**). Most parasite taxa had non-random microhabitat distributions on gills, which also differed between parasite species. In addition, microhabitat distribution patterns of *Cichlidogyrus* spp. and *L. monodi* were more distinct between host species of different genera than within the same genus. This host genus effect suggests that parasites differences accumulate after speciation. The non-random microhabitat distribution did not differ between host species (**chapter 5**), suggesting it is unrelated to parasite interspecific relationships, but may rather result from other factors, such as egg spreading, tissue accessibility or enhancement of mating opportunities. In particular, mating facilitation seem to be important in monogeneans – as they are hermaphrodites with obligate cross-fertilization that reproduce in the host – whereas egg spreading seems to be important in copepods – as they mainly position themselves in the distal parts of the gills, exposing the eggs to water flow. Together, these findings may indicate host species-specificity in parasite niche selection and consequently in the host-parasite relationship, consistent with a role of parasites in host differentiation. Microhabitat distribution patterns of *Cichlidogyrus* spp. were largely based on many parasite individuals not identified at species level. Hence these patterns could result from host species harbouring different species of *Cichlidogyrus*, some of which have different microhabitat distributions (e.g. microhabitat distribution in *P. pundamilia* could be driven by a higher abundance of *Cichlidogyrus furu* than other species). To address this, it would be necessary to identify more specimens of the collected *Cichlidogyrus*.

In many copepod species, females have egg clutches appended to their body, allowing me to investigate whether their reproductive activity constitutes another axis of infection variation between host species. The proportion of female copepods carrying egg clutches was similar among all sampled wild host species (**chapter 5**) and among the two lab-reared species of *Pundamilia* and their interspecific hybrids (**chapter 4**). This suggests that reproductive activity of copepods is not influenced by host species identity nor by host ecology, hence it does not constitute an infection trait under divergent selection.

Table 7.1
Key findings presented in this thesis, at parasite higher taxon level (P) and at *Cichlidogyrus* species level (C), and whether they support or not a role of parasites in host diversification (* indicates support for sympatric diversification, NA indicates that the finding alone is not relevant in the context of parasite-mediated diversification).

Chapter	**Study system**, key findings		**Support**
2	**Community of sympatric cichlid species, belonging to three lineages (radiation, *A. alluaudi*, *Ps. multicolor*) and differing in ecological specialisations**		
	P:	Host species of a large community show temporally consistent infection differences.	yes
	C:	Infection pattern of species of *Cichlidogyrus* differs between host lineages, but not between closely related species within the radiating lineage.	no
	P, C:	Variation in infection between host species is not fully explained by differences in host ecology. Instead, the best predictor of infection was species identity.	yes
	P, C:	Lack of geographic pattern in infection profiles.	yes*
3	**Four pairs of closely related species/forms of *Pundamilia***		
	P:	Temporally consistent infection differences between already differentiated pairs of closely related host species.	yes
	P:	Infection divergence accumulates as host genetic differentiation increases.	yes
	C:	Infection divergence is unrelated to host genetic differentiation.	no
	P, C:	Variation in infection between forms is not fully explained by differences in host ecology alone. Instead, the best predictor of infection was population identity.	yes
	P, C:	Lack of geographic pattern in infection profiles.	yes*
4	**Wild and lab-bred representatives of two closely related species of *Pundamilia* and their lab-bred hybrids**		
	P:	Species differences in infection in the wild are not maintained in laboratory conditions.	no
	P:	No hybrid disadvantage in infection levels.	no
	-	Reproductive activity of *L. monodi* does no differ between host species.	no
5	**Community of sympatric cichlid species, belonging to the radiating lineage and differing in ecological specialisations**		
	P, C:	Non-random spatial distribution in the host gills.	partial
	P:	Parasite taxa differ in gill microhabitat distribution.	partial
	C:	Species of *Cichlidogyrus* have overlapping gill microhabitats.	NA
	P, C:	Parasite spatial distribution on gills differs between host species.	yes
	P, C:	Directions of parasite-parasite abundance relationships differ between taxonomical level (positive between parasite genera, negative within *Cichlidogyrus*), but do not differ between host species.	no
	-	Reproductive activity of *L. monodi* does no differ between host species.	no
6	**Species of *Cichlidogyrus* infecting cichlids of southern Lake Victoria**		
	C:	Formal description of four new species of *Cichlidogyrus* and re-description of other two species.	NA

What stage of the speciation process do parasites contribute? – The role of parasites in host speciation (if any) may range between two extremes. On one extreme, parasites may be crucial for the evolution of host reproductive isolation and ultimately for host divergence (driver role). On the other extreme, parasites may be one of the many factors differentiating host populations, thus they may contribute to host reproductive isolation by accelerating or strengthening it (contributor role). To disentangle whether parasites drive or contribute to host divergence, I also analysed infection patterns in replicates of host species pairs (blue and red forms of *Pundamilia* spp.) varying in the age of speciation and extent of genetic differentiation (**chapter 3**). In sympatric and allopatric pairs of *Pundamilia*, divergence in infection profiles increased with host genetic differentiation. This positive relationship was stronger among allopatric pairs than within sympatric pairs, suggesting that parasites may contribute to host divergence in allopatry rather than in sympatry, or alternatively that the time since geographical isolation contributes to both parasite dissimilarity and genetic distance. Within sympatric pairs, infection differences were significant for the old and most genetically differentiated pair (blue and red forms at Makobe), but not for host pairs with low genetic differentiation (except 2 cases). These findings suggest that infection differences only accumulate after host genetic differentiation: a certain amount of genetic differentiation (driven by other factors) may be needed for parasite-mediated divergent selection to act and to lead to significant species differences in infection. This supports a contributor role of parasites in host divergence rather than a driver role, consistent with observations in Lake Tanganyika. There, differences in infection were detected between allopatric populations of *Tropheus moorii* at early stages of diversification (although 15-75'000 years older than populations of *Pundamilia*; Koblmüller et al., 2011), but these did not increase with host neutral genetic differentiation (Hablützel et al., 2016). This supports the hypothesis of a threshold in (neutral) genetic differentiation required for parasite-mediated selection to act.

Species differences in infection can result from host ecology (thereby exposure to parasites), host immune response and interactions between them (Wolinska & King, 2009). In the context of parasite-mediated diversification, species differences in infection profiles are assumed to be initiated by variation in ecological exposure and then maintained by differences in immunological traits. In **chapters 2 and 3**, I found that host ecology (i.e. water depth, diet) played a role in infection heterogeneity but did not fully explain it. Host species identity was the best predictor of infection levels, suggesting that intrinsic factors (e.g. resistance, tolerance) are more important than extrinsic factors of infection variation. For one of the young and weakly differentiated *Pundamilia* pairs (at Python Island), I found that infection differences as in the wild are not maintained in uniform parasite exposure conditions (**chapter 4**). This suggests that the contribution of parasite exposure to infection variation is larger than that of species immunity in recently diverged host species, and it may initiate the species differentiation in infection in the wild. This constitutes no evidence for a contribution of parasites to divergence in *Pundamilia*. However, this does not mean that parasites are not an important dimension of ecological species differentiation: the genetic differentiation between these incipient species is very low and thus

it is not surprising that they may not have diverged in resistance or tolerance yet. Results from similar pairs at more advanced stages of speciation (i.e. *Pundamilia* at Makobe Island) suggest that divergence in resistance/tolerance may become evident as infection differences accumulate (**chapter 3**).

If parasites contribute to the reproductive isolation of *Pundamilia*, then hybrids were expected to be disadvantaged, as a hybrid disadvantage would contribute to reproductive isolation between parental populations. This was not observed in young and closely related *Pundamilia* species from Python Island: first-generation laboratory-bred hybrids did not differ in infection profiles from either parental species (**chapter 4**). Even though hybrids did not have an intrinsic disadvantage in the laboratory, they are rare in the field, likely because of species-assortative mating rather than because of fitness reduction (van der Sluijs et al., 2008a; van der Sluijs et al., 2008b). This suggests that parasites do not contribute to the rarity of hybrids that is observed in the wild and hence additional (ecological) factors may drive assortative mating. This implies that parasites do not drive or strengthen reproductive isolation in *Pundamilia*.

Geographically consistent species differences in infection profiles – Exposure to parasites may depend not only on host ecology, but also on the geographical location where the host occurs. Indeed, chances of getting infected and the number of parasites infecting conspecific host populations varied between locations **(chapters 2 and 3**). For example, the prevalence and abundance of copepods were higher at Makobe than at Kissenda and Python islands (**chapter 3**). However, geographical variation did not seem to affect differences in infection patterns within sympatric host forms. For example, the direction of the infection difference of copepod between blue and red forms was maintained at all sampled locations: red forms of *Pundamilia* tended to harbour more copepods than the blue forms (although this difference was significant only at one location, Makobe). A similar pattern was observed in two Tropheini species co-occurring at several locations in Lake Tanganyika: the direction of species differences in monogenean infection abundance and intensity was maintained despite geographical variation in infection (Grégoir et al., 2015). In addition, differences in parasite community composition were not associated with increasing geographic distance among allopatric pairs of *Pundamilia* (**chapter 3**). The maintenance of the direction of differences in infection patterns within sympatric blue-red forms despite geographical variation in infection suggests that those differences arise because of species-specific host traits (e.g. resistance, host ecology), consistent with parasite-mediated diversification.

7.2. THE ROLE OF *CICHLIDOGYRUS* IN HOST DIVERSIFICATION

The gill parasite *Cichlidogyrus* was the best candidate for testing parasite-mediated speciation, because: *i) Cichlidogyrus* is a species-rich genus, with high morphological diversity, *ii)* species often display high host specificity, infecting only one or few cichlid species, *iii)* it has radiated in at least one other African lake, where it co-evolved with cichlids (Vanhove et al., 2015). To investigate its potential role in Lake Victoria cichlid diversification, I morphologically identified species of *Cichlidogyrus* (described in **chapter 6**). I analysed infection patterns of *Cichlidogyrus* at the level of the species community in each host (hereafter reffered to as *Cichlidogyurs* species level). Results at *Cichlidogyrus* species level are compared to those of higher taxonomic groups (e.g. *Lamproglena*, nematodes), as they often differ.

Divergence in infection of *Cichlidogyrus* between ancient cichlid genera but not between species of the radiation – The first prerequisite for *Cichlidogyrus*-mediated diversification – namely host species differences in infection – was partially met. Similarly to what I observed at parasite higher taxon level, the abundance and community composition of species of *Cichlidogyrus* differed between two sympatric and reproductively isolated species of *Pundamilia* (at Makobe Island, **chapter 3**). However, when considering additional sympatric cichlid species of the adaptive radiation occurring at the same location previously considered (Makobe Island), the community composition of *Cichlidogyrus* species did not differ between cichlid species of the radiation, contrary to the species differences in infection observed at parasite higher taxon level (**chapter 2**). This homogenous infection pattern within the cichlid radiation does not support a role of *Cichlidogyrus* in host diversification, as recently diverged radiation members were expected to evolve species-specific resistance leading to infection divergence. Differentiation in *Cichlidogyrus* infection may take longer time and be visible only between strongly genetically differentiated host species. This hypothesis is supported by two observations. First, *Cichlidogyrus* infection differs between cichlid genera – that diverged more than 5 million years ago – but not between species – that diverged in the past 15'000 years (**chapter 2**). Second, *Cichlidogyrus* infection differs between host species of the nearly 3-4 million years older Tropheini of Lake Tanganyika (Vanhove et al., 2015).

Instead of within-radiation differences, the community composition of *Cichlidogyrus* differed between the three ancient host lineages – the radiating lineage and the two older lineages represented by *Astatoreochromis alluaudi* and *Pseudocrenilabrus multicolor* – indicating a deeper phylogenetic signature. This infection pattern of within-radiation homogeneity and between-lineages differences may result from two alternative scenarios (see **7.3** for further discussion). In the first scenario, *Cichlidogyrus* species sorted among host species during the radiation. In the second scenario, *Cichlidogyrus* species specialised on the radiation lineage as a whole (i.e. all newly evolved cichlid species represent one resource) and subsequently evolved

in the lake. A third scenario, namely ancestral *Cichlidogyrus* species co-evolved with each radiation member, can be excluded because it would result in radiation members differing in infection (which I do not observe). Despite full sympatry of the cichlid hosts investigated, species of *Cichlidogyrus* infecting one lineage rarely infected another lineage, suggesting an opportunity for host specialisation. However, this cannot be linked to parasite-mediated speciation, as the three lineages considered are too distantly related to be informative in such context.

Just like at parasite higher taxon level, I found variation in parasite microhabitat distribution over gills for the two most common species of *Cichlidogyrus*, within and between host species (**chapter 5**). This supports the species-specificity of the cichlid-*Cichlidogyrus* relationship and seems to contrast with the uniform infection pattern found within the radiation. This highlights the importance of exploring more axes of variation in infection (i.e. spatial niche) as it may reveal more differences than canonical measures (i.e. parasite counts).

No contribution of *Cichlidogyrus* to species divergence in *Pundamilia* – As I did for parasites at higher taxon level, I analysed infection patterns in replicate speciation events of blue-red forms of *Pundamilia* to investigate at what stage of host speciation *Cichlidogyrus* species contributed to host divergence (**chapter 3**). Contrary to findings at parasite higher taxon level, infection differences in the community of *Cichlidogyrus* species did not increase with host genetic differentiation within sympatric and allopatric pairs of *Pundamilia*. Among sympatric blue-red forms, only the oldest and most genetically differentiated pair (at Makobe) differed in infection parameters of *Cichlidogyrus* (as found at parasite higher taxon level). A lack of correlation between differentiation in *Cichlidogyrus* infection and host genetic differentiation was also observed at early stages of diversification in Lake Tanganyika (i.e. between allopatric populations of the same cichlid species), although older than *Pundamilia* populations (Grégoir et al., 2015). This indicates that *Cichlidogyrus* infection diverges only when host species have already strongly diverged genetically, whereas at earlier stages of speciation *Cichlidogyrus*-mediated selection does not differ between *Pundamilia* forms. This suggests that species of *Cichlidogyrus* do not contribute to host divergence.

Young blue-red pairs with nearly zero genetic differentiation (at Luanso) and with intermediate levels of genetic differentiation (at Kissenda and Python) did not significantly differ in parasite community composition. Only when host populations reach a certain threshold of genetic differentiation (driven by other factors, mainly ecology-related) they start to diverge in parasite infection. Thus, the extent of differences in infection patterns, thereby their contribution to host divergence, may depend on both host genetic differentiation and ecological divergence.

Geographically consistent species differences in *Cichlidogyrus* infection profiles – Similar to what I observed at parasite higher taxon level, geographical locations differed in the abundance of some species of *Cichlidogyrus* (**chapters 2 and 3**). For example, one host species (*A. alluaudi*)

had higher numbers of *Cichlidogyrus longipenis* at Makobe than at Sweya (**chapter 2**). However, this did not generate differences in the community composition of *Cichlidogyrus* between allopatric host populations of *A. alluaudi*, as proportions between the different species of *Cichlidogyrus* infecting them did not change. In addition, variation in community composition of *Cichlidogyrus* was not associated with geographic distance (**chapter 3**). A similar pattern was observed in allopatric populations of two Tanganyikan cichlids, a strong and a weak disperser (Grégoir et al., 2015). Since infection differentiation between host populations was stronger in the weak disperser than in the strong disperser species, Grégoir et al. (2015) suggested that low host dispersal enhances infection differentiation. In *Pundamilia*, I found indications for the opposite pattern: blue forms, which show isolation by distance (Seehausen et al., 2008; Meier et al., 2017b), have lower inter-population infection differences than red forms. This suggests that infection profiles are species-specific, rather than simply determined by geographic variation in exposure or by connectivity among host populations, in line with parasite-mediated diversification.

7.3. DIRECTIONS FOR FUTURE RESEARCH

Based on the findings presented in this thesis, I propose several directions for future research.

Other adaptive radiations – In this thesis, I investigated the adaptive radiation of haplochromines in Lake Victoria, which is 14'600 years old (Johnson et al., 2000; Stager & Johnson, 2008; Wagner et al., 2013; Meier et al., 2017a) and two older lineages, which are 10 million years old (Meier et al., 2017a; Schedel et al., 2019). Many other examples of adaptive radiations exist and could be investigated for parasite-mediated speciation. However, old radiations would be not informative in this context, as it is not possible to discriminate between species differences that arose at the onset of the divergence – possibly driving it – and the many others that accumulated *after* speciation. As presented in **chapters 2 and 3**, the time elapsed since divergence is an important factor determining infection divergence and its detectability. Detectability of infection differences depended also on the taxonomic resolution of parasite identification. At parasite higher taxon level, major infection differences are observed already between species of the radiation (i.e. not later than 14'600 years ago) with low levels of host genetic differentiation (i.e. low F_{ST} values), consistently with a role of parasites in host diversification. On the other hand, at *Cichlidogyrus* species level, infection differences are not observed within the radiation, inconsistently with parasite-mediated speciation (**chapter 2**). Since only young (about 14'600 years old in Victorian cichlids) and old (3-5 million years old in Victorian cichlids, **chapter 2**; and 10 million years in Tanganyikan cichlids, Vanhove et al., 2015) cichlid species were investigated for their divergence in infection, we need to study infection patterns of species with an estimated divergence time that would fill this temporal gap. Since

infection differentiation takes place at different time scales, we need to study infection patterns along a wide range of host divergence time (e.g. from incipient species to species younger than one million years) in order to estimate at what speciation stage infection starts to diverge.

Additional non-speciating lineages – Comparison of infection profiles of *Cichlidogyrus* species between cichlid species revealed a deep phylogenetic split between ancient host genera (**chapter 2**). Three ancient cichlid lineages were considered here: the species-rich radiation lineage and two single-species lineages that never speciated in the Lake Victoria region. Although the latter category is under-represented by definition in terms of species, in Lake Victoria two other species failed to radiate: *Oreochromis variabilis* and *O. esculentus*. Parasitological analysis of these may support the observed pattern if they harbour a set of species of *Cichlidogyrus* that differ from the other lineages. However, both species of *Oreochromis* are critically endangered and it would not be justified to sacrifice individuals solely for this purpose. Individuals of *Oreochromis* kept and bred in cages within the lake may be an option to study their natural infection without impacting on the wild population.

Other species pairs – Beside *Pundamilia*, other species pairs have replicates that vary in the extent of genetic differentiation, ranging from ongoing to recently completed speciation. These offer the opportunity to investigate whether parasites drive or contribute to host differentiation. Examples of speciation replicates from the animal kingdom are: normal-size benthic and dwarf limnetic forms of whitefish (*Coregous clupeaformis*) and wing morphotypes of the *Heliconius* butterflies. Among cichlids, suitable species pairs could be the species of *Neochromis* from Lake Victoria (Magalhaes et al., 2012) and gold/dark colour forms of Midas *Amphilophus citrinellus* from Nicaraguan crater lakes (Kusche et al., 2015). In particular, I find species pairs of *Neochromis* the most promising ones. The genetic differentiation range of *Neochromis* pairs is slightly lower than that of *Pundamilia* pairs (F_{ST} 0.001-0.019 vs. 0.003-0.101), but *Neochromis* are more differentiated in their ecology (especially trophic morphology) than *Pundamilia* (Magalhaes et al., 2012; van Rijssel et al., 2018a). Speciation of *Neochromis* was proposed to be mainly driven by ecological divergence (van Rijssel et al., 2018a). Since ecological differences may be associated with different parasite threats (**chapter 2**; Hablützel et al., 2017), *Neochromis* are expected to respond to parasite-mediated selection.

More precise ecological factors – Species-specific depth distributions and diet play a role in species variation in infection, although these effects depended on the extent of genetic differentiation and age of the host species considered (i.e. outweighed by other intrinsic species traits in reproductively isolated sympatric hosts, but not in younger ones; **chapters 2, 3 and 4**). However, in this thesis I considered only two species-specific ecological traits: water depth and host diet. Since the infection differentiation could depend on every factor that differentiates a host species, it may be worthy to include more and increasingly more specific ecological traits (e.g. in **chapter 2** an increase in resolution of depth categorization lead to larger differences).

Trophic guilds could be split into narrower categories of foraging behaviour (e.g. snail crusher and snail sheller molluscivores) and/or items eaten (e.g. ostracods or bivalves for molluscivores, fly larvae or caddis-fly larvae for insectivores). Feeding ecology could also be assessed through analysis of stable isotope ratios (Muschick et al., 2012). Additional parameters that may explain interspecific variation in infection could include (but are not limited to): host habitat type and spatial range, host population size and density, parasite population size and density.

Assessment of parasite ecological data – In previous studies, ecological data reported concerning parasites are mainly limited to host species. In **chapter 5**, I showed that microhabitat distribution of parasites over gills may represent an important axis of divergence in infection between host species, whereas the parasite reproductive activity is not. Therefore, I recommend to integrate the location of parasites on the host in future studies. Other ecological aspects of parasites that may be relevant for infection variation and host specificity that may be considered in future studies are: aggregation of conspecific parasites, adult body size, rate of infection success.

Host detection by parasites – Pathways of transmission are important in understanding infection patterns. Most ectoparasites studied here actively search for a suitable host in the water column. To recognize a suitable host, parasites are assumed to exploit specific host signals, such as visual or chemical cues. Aquatic parasites with low visual sensitivity are likely to use chemical cues (e.g. substances in skin or gill mucus) to locate their host (Whittington, 1997). Identification of these substances may help to explain differences in parasite abundances observed between host species and differences in infection profiles of *Cichlidogyrus* observed between cichlid lineages. For example, all radiation members may secrete the same set of chemicals (supporting the hypothesis that they are all perceived as one suitable host by the *Cichlidogyrus* species infecting radiation members) but in different quantities (explaining variation in infection levels). Chemicals secreted by radiation members might differ from those of the two non-radiating lineages, explaining the *Cichlidogyrus* infection differences between host lineages. If such chemical cues are species-specific (i.e. attractive only for the *Cichlidogyrus* species actually infecting these hosts), this would constitute support for host-parasite coevolution.

Phylogenetics of *Cichlidogyrus* – The *Cichlidogyrus* community was shared within the Lake Victoria cichlid radiation, but it differed between the radiation and two distantly related cichlid species that did not radiate in the lake (*Astatoreochromis alluaudi* and *Pseudocrenilabrus multicolor*; **chapter 2**). This pattern may result from different mechanisms. It is necessary to perform genetic analysis to date the origin of the species of *Cichlidogyrus* relative to the origin of the Lake Victoria cichlids, in order to understand whether *Cichlidogyrus* species were introduced into the lake with the ancestors of the cichlid radiation (14’600 years ago; Seehausen et al., 2003; Meier et al., 2017a) and then *i)* sorted among cichlid lineages, or *ii)* diversified between cichlid lineages. Since the ancestors of the Lake Victoria cichlid radiation are more

closely related to each other than they are to *A. alluaudi* and *Ps. multicolor*, both scenarios predict that species of *Cichlidogyrus* infecting radiation members are more closely related to each other than those infecting *A. alluaudi* and *Ps. multicolor*. The first scenario also predicts that radiation-infecting species of *Cichlidogyrus* are as young as or younger than the hybrid swarm that gave rise to the cichlid radiation itself (i.e. 14'600 years old or younger). The second scenario predicts that radiation-infecting species of *Cichlidogyrus* are older than the cichlid radiation but younger than the Lake Victoria cichlid superflock (i.e. between 14'600 and 100'000 years old). Alternatively, *Cichlidogyrus* species may have been introduced into the region by the founders of the Lake Victoria Region Superflock (LVRS, about 100'000 years ago; Verheyen et al., 2003; Seehausen, 2006) and then *iii)* sorted over the emerging cichlid species, or *iv)* evolved with the cichlids. The third scenario implies that species of *Cichlidogyrus* are as young as or younger than the origin of the LVRS but older than the radiation (i.e. between 14'600 and 100'000 years old), whereas the fourth scenario predicts that *Cichlidogyrus* species are older than the LVRS (i.e. older than 100'000 years). In addition, genetic analyses may disclose the presence of more *Cichlidogyrus* haplotypes/species than that currently identified with morphological methods, which may have higher host-specificity (both previously observed in monogeneans by Pouyaud et al., 2006 and in trematodes by Jousson et al., 2000; Donald et al., 2004). If such cryptic species are sorted among host species, this would imply that radiation members actually differ in infection profiles of *Cichlidogyrus*, possibly supporting parasite-mediated speciation.

Extrinsic vs. intrinsic traits – My findings suggest that variation in parasite exposure contributed to variation in infection among host species (**chapters 2, 3 and 4**), while host immunity-related intrinsic traits did not (at least between young host species; **chapter 4**). Anyway, I cannot exclude that older host species may have intrinsic differences (i.e. host immunity). This can be assessed by comparing species differences in infection profiles between wild and laboratory-bred fish of species pairs with low, intermediate and high levels of genetic differentiation (similar to what I did for the incipient species of *Pundamilia* from Python Island). Maintenance of species differences in infection under uniform exposure would indicate that differences in resistance (or other immune-related traits) have evolved. This would support a role of parasites in strengthening host divergence if immunity differences arise before the completion of reproductive isolation. In addition, MHC genotyping can reveal whether species within a pair differ in resistance (see below).

Hybrid disadvantage – In the context of parasite-mediated divergence, hybrids are expected to be more infected than either parental species (Schluter, 2001), contributing to reproductive isolation. In the field, hybrids of blue and red *Pundamilia* are rare, suggesting some selection against them. However, such hybrid disadvantage was not observed in laboratory conditions (**chapter 4**), suggesting that selection against hybrids is not exerted by parasites. Since the frequency of hybrids varies across wild populations of *Pundamilia* (Seehausen et al., 2008), I propose to explore whether the extent of hybridisation in wild host populations (as a measure

of reproductive isolation) is associated with the infection level of hybrids. I expect that the greater the genetic/phenotype differentiation between host species, the lower the fitness in hybrids and consequently the stronger the assortative mating (Stelkens & Seehausen, 2009). Hybrids of reproductively isolated *Pundamilia* (e.g. at Makobe Island) do not occur in the field, but they can be obtained by housing heterospecific together in semi-natural conditions (as done in **chapter 4** for Python populations, in Lake Tanganyika cichlids by Rajkov et al., 2018). However, it is possible that most pairs of *Pundamilia* are too closely related to reveal an association between hybridisation and infection. Differences in infection, resistance and hybrid disadvantage may become evident after a certain threshold of host divergence (as suggested in **chapter 2**). This would be consistent with parasites contributing to – but not driving – host divergence.

Fitness costs of parasite infection – Throughout this thesis, I have assumed that parasites impose a fitness cost on hosts. Although this is widely assumed, experimental evidence quantifying the parasite impact of each species in single- or multiple species infection is still scarce. Future research would need to address whether and how parasites exert selection on cichlids and to what extent infection is costly. Since natural populations often harbour more than one parasite species, it is also important to investigate multi-species infections. Moreover, single-species infections may be not costly in itself, but may increase vulnerability to other infections or may become more/less costly in the presence of another parasite. Infection cost can be estimated by assessing local damage (i.e. histopathological responses at the attachment site; Reda & El-Naggar, 2003; Arafa et al., 2009; Igeh & Avenant-Oldewage, 2020) and indirect costs on life-history traits (Barker et al., 2002; Bollache, 2015). The latter can be estimated by comparing survival, growth, male nuptial coloration and reproductive output between parasite-free fish and conspecifics with infection at increasing intensities. A complementary approach would also address *how* hosts respond to parasitic infection and if this differs between host species. This can be done by analysing blood (e.g. cytokines, serum protein, immunoglobulins), mucus production or even expression of immune-related genes of experimentally infected fish (as recently done in Nile tilapia by Zhi et al., 2018; Chen et al., 2019).

Host divergence in immunity – Parasites could drive genetic adaptations in host immune resistance, such as the Major Histocompatibility Complex (MHC) (Haldane, 1949; Klein et al., 1994). MHC genes can be subject to divergent selection by local parasites (in sticklebacks, Eizaguirre et al., 2012a, b; in Lake Tanganyika cichlids, Hablützel et al., 2016). The gene pool of MHC varies between cichlid species of Lake Malawi (Klein et al., 1993; Ono et al., 1993; Blais et al., 2007), Lake Tanganyika (Hablützel et al., 2013; Hablützel et al., 2016) and of Nicaraguan lakes (Hofmann et al., 2017). On the other hand, a large sharing of MHC alleles and polymorphisms are observed in cichlids of Lake Victoria (Nagl et al., 1998; Klein et al., 2007). Assessing the MHC diversity of our sampled cichlids could reveal whether species differences in resistance alleles are associated with species differences in infection. Immunogenetic differentiation is particularly

relevant in early stages of host divergence: in the context of parasite-mediated divergence, MHC differentiation is expected to precede neutral genetic differentiation. This pattern was observed in allopatric populations of a Tanganyikan cichlid (Hablützel et al., 2016), in sympatric closely related cichlids in Lake Malawi (Blais et al., 2007) and in sympatric limnetic/benthic populations of lake sticklebacks (Matthews et al., 2010).

Individual variation in parasite resistance – Throughout this thesis, I investigated infection patterns at interspecific level. Future work may address infection differences at host individual level to investigate the heritability of parasite defence strategies – which is necessary for parasites to contribute to host divergence. The heritability of parasite resistance has been tested in only few vertebrate organisms (soay sheep Smith *et al.* 1999, kittiwakes Boulinier et al., 1997, barn swallow Moller 1990), including fish (beaked dace cyprind; Mazé-Guilmo et al., 2014) but not in cichlids. To experimentally test the heritability of resistance, researchers should perform intraspecific crosses between individuals with high and low resistance. The offspring resistance is expected to be determined by the parental resistance. If this is the case, researchers could take a step further and investigate whether such heritable variation in resistance is linked to mate selection based on resistance. Host individuals are expected to mate with the most resistant partners, in order to generate a resistant offspring. Such link between female mate choice and parasite load was observed only indirectly in *Pundamilia*: mate choice is based on male red coloration, which is associated with parasite load (Maan et al., 2006b; Maan et al., 2008) and with antibody response (Dijkstra et al., 2007). If different signals become associated with heritable immunity in different subpopulations, then mate choice might be mediated by parasite resistance, which could contribute to parasite-mediated divergence.

7.4. CONCLUSION

In this thesis, I investigated parasite infection patterns of Lake Victoria cichlids, in order to contribute to the understanding of ecological speciation. My findings allow the following general conclusions.

First, parasites are non-randomly distributed across host species, despite their host full sympatry (**chapters 2 and 3**). This is a requirement for a role of parasites in host differentiation (Karvonen & Seehausen, 2012) and is consistent with parasite specialisation. Also within hosts, I observed non-random distributions of parasites: parasites were more frequent in certain gill microhabitats and this niche distribution differed across host species in some cases (**chapter 5**).

Second, species differences in infection profiles were temporally consistent (**chapters 2 and 3**). This indicates that another prerequisite for parasite-mediated selection is met: parasite-mediated divergent selection maintains its direction over time.

Third, when host species start to be genetically differentiated, they also begin to accumulate differences in parasite communities (**chapters 2, 3, 4**). This implies that differentiation in parasite infection arise during the divergence process, but is not driving it. In young host species, that hardly differ genetically, differences in infection as observed in the wild disappear when equalizing exposure (**chapter 4**), indicating that exposure may drive the onset of infection differentiation, rather than defence-related species factors (e.g. immunological traits).

Fourth, infection profiles of old species strongly differ from those of young species. This deep phylogenetic signature in infection is consistent with parasite specialisation, but not with parasite-mediated speciation.

Fifth, patterns observed at parasite higher taxon level differ from those at within-genus level in *i)* infection profiles across hosts, *ii)* gill niche distribution and *iii)* parasite-parasite interspecific interactions within hosts. Infection profiles differed between host species of the radiation when considering parasite genera, whereas they did not when considering species of *Cichlidogyrus*. Niches in the gills differed between parasite genera, whereas niches overlapped among species of *Cichlidogyrus*. Interspecific interactions were synergistic among parasite genera, whereas they were antagonistic among species of *Cichlidogyrus*. This may be explained by the higher similarity at within-genus level than between higher taxon level.

Based on previous studies, I considered *Cichlidogyrus* the main candidate for driving parasite-mediated speciation. In this thesis, I did not observe such pattern, as infection differences of *Cichlidogyrus* only become evident between distantly related species, after differences in infection of other parasite taxa. This may have three explanations. First, the fitness cost of *Cichlidogyrus* may be too low at natural infection levels to exert selection for specialised resistance in the host, inconsistent with parasite-mediated diversification. Alternatively, the imposed fitness cost may be so high to kill highly infected hosts before they can be sampled, hampering any conclusion from field studies. Second, although a young radiation is a good model to study mechanisms of host speciation, the cichlid radiation in Lake Victoria may be too young to allow detection of patterns of cichlid speciation mediated by monogeneans. Third, the diversity of *Cichlidogyrus* may have been underestimated by morphological identification. Molecular investigations may actually reveal an infection pattern that is consistent with parasite-mediated diversification. Since most observed species of *Cichlidogyrus* were new to science and genetic data are currently lacking, I cannot estimate the age of these species. However, I can speculate that these species of *Cichlidogyrus* are endemic to the Lake Victoria basin (or even to the lake itself) (**chapter 6**). The potential endemism of *Cichlidogyrus* species of Lake Victoria suggests that these species may have evolved in the basin or even in the lake (contrary to the globally distributed copepod species observed there). Thus, comparing infection patterns of Victoria cichlids with *Cichlidogyrus* species and those with copepod species can help us to distinguish recent from ancient eco-evolutionary mechanisms in the host-parasite interactions.

Although most evidence supporting parasite-mediated speciation currently comes from sticklebacks (e.g. Milinski & Bakker, 1990; Wegner et al., 2003; Eizaguirre et al., 2009a; MacColl, 2009b; Matthews et al., 2010; Eizaguirre et al., 2012a), African cichlids have the potential to diverge in response to parasite, as they harbour a high richness of parasite species (this thesis, Raeymaekers et al., 2013; Vanhove et al., 2015) and they have a diverse MHC that allows them to rapidly adapt to local parasite threats (Blais et al., 2007). Cichlids also have the advantage to provide many cases of young closely related species along with species that never diversified, allowing the study of early stages of host divergence and comparison with "diversification failures".

Although the evidence for a role of parasites in driving or contributing to host differentiation is increasing (this thesis; Eizaguirre et al., 2009a; Raeymaekers et al., 2013), we are still scratching the surface of the potential evolutionary impact of parasites on hosts and further research is much needed. African cichlids are a promising model system in the context of parasite-mediated speciation but also for other researches linked to parasites. For example, the parasite fauna of Lakes Victoria and Malawi is largely unknown and even in Lake Tanganyika (the most investigated of the three Great Lakes for parasitology) studies on parasite ecology, life history and genomics are needed. Additional future research may also focus on factors determining the host-parasite interaction, on host (divergent) adaptations in response to (different) infections, on the interplay of parasites and other (ecological) factors contributing to host divergence, and on mechanisms of host reproductive isolation driven by parasite-mediated selection (especially those mediated by immune traits).