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Divergent parasite infections in sympatric cichlid species in Lake Victoria

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Abstract

Parasitism has been proposed as a factor in host speciation, as an agent affecting coexistence of host species in species rich communities, and as a driver of post-speciation diversification. Young adaptive radiations of closely related host species of varying ecological and genomic differentiation provide interesting opportunities to explore interactions between patterns of parasitism, divergence and coexistence of sympatric host species. Here, we explored patterns in ectoparasitism in a community of 16 fully sympatric cichlid species at Makobe Island in Lake Victoria, a model system of vertebrate adaptive radiation. We asked if host niche, host abundance or host genetic differentiation explain variation in infection patterns. We found significant differences in infections, the magnitude of which was weakly correlated with the extent of genomic divergence between the host species, but more strongly with the main ecological gradient, water depth. These effects were most evident with infections of *Cichlidogyrus monogeneans*, while the only host species with a strictly crevice-dwelling niche, *Pundamilia pundamilia*, deviated from the general negative relationship between

depth and parasitism. In accordance with the Janzen-Connell hypothesis, we also found that host abundance tended to be positively associated with infections in some parasite taxa. Data on the *Pundamilia* sister species-pairs from three other islands with variable degrees of habitat (crevice) specialization suggested that the lower parasite abundance of *P. pundamilia* at Makobe could result from both habitat specialization and the evolution of specific resistance. Our results support influences of host genetic differentiation and host ecology in determining infections in this diverse community of sympatric cichlid species.

Keywords: Adaptive radiation, genomic differentiation, host-parasite interactions, incipient species, Janzen-Connell mechanism, sympatric speciation.

Introduction

Parasitism is one possible source of divergent selection between populations during speciation and adaptive radiation of host organisms (Eizaguirre *et al.*, 2009; Karvonen & Seehausen, 2012). Parasitism is also a potentially important factor affecting coexistence between host species in species rich ecological communities via the Janzen-Connell hypothesis (Janzen, 1970; Connell, 1971; Connell, 1978). Under Janzen-Connell mechanisms, coexistence is facilitated because specialized natural enemies decrease survival rates of locally abundant species, leading to fitness benefits for rare species, and thereby acting as a stabilizing force in coexistence. Further, parasitism is likely a common source of divergent selection after speciation (Wills *et al.*, 2016). Despite strong potential for interactive effects of community ecological mechanisms and evolutionary dynamics of host divergence, interactions between these areas remain largely unexplored (Ricklefs, 2015). Young sympatric species radiations where many potential host species with different levels of genomic and ecological differentiation coexist within single communities are ideal places to study all of these

aspects of the evolutionary ecology of host-parasite systems in the same communities. Here we take an evolutionary community ecology perspective, investigating patterns of parasitism in a sympatric species community within the young adaptive radiation of Lake Victoria cichlid fishes.

Recent experimental studies have provided empirical support for individual components of the theory of parasite-driven host speciation, for example, by demonstrating parasite-mediated divergent selection on immunogenes that are pleiotropically associated with mate choice (Eizaguirre *et al.*, 2009; Eizaguirre *et al.*, 2012a; Eizaguirre *et al.*, 2012b). The first prerequisite of parasite-mediated divergent selection in natural populations is that infections differ between diverging host populations. Currently, there is a growing body of literature describing differentiated parasite infections in ecotypes or closely related species particularly in freshwater fishes (Knudsen *et al.*, 1997; Knudsen *et al.*, 2003; MacColl, 2009; Eizaguirre *et al.*, 2011; Natsopoulou *et al.*, 2012; Karvonen *et al.*, 2013a; Karvonen *et al.*, 2013b; Karvonen *et al.*, 2015) including cichlid fishes (Blais *et al.*, 2007; Maan *et al.*, 2008; Raeymaekers *et al.*, 2013; Hablutzet *et al.*, 2016; Hablutzet *et al.*, 2017). Overall, these studies suggest that conditions for parasite-mediated divergent selection between ecological niches are not uncommon. So far, most studies have compared infections either in systems with little or no genetic differentiation between the host populations, or in well-defined species-pairs that are genetically strongly divergent and have already diverged in many different traits. To infer a possible role of parasitism in the initiation or early facilitation of host speciation, we should seek to know at what point in the speciation process infections become differentiated (Karvonen & Seehausen, 2012). This is important because diverging parasite infections are typically associated with other (ecological) sources of divergent selection that could also initiate and/or facilitate speciation. In such situations, parasitism may complement the process initiated by other ecological factors, but it may be difficult to isolate its contribution to host divergence.

Systems that include pairs of host taxa at differing degree of divergence provide opportunities to tackle the above questions. By including incipient species pairs with very low degrees of ecological and genetic differentiation, and extending comparisons to pairs with complete reproductive isolation and strong ecological differentiation, it is possible to compare if differentiation in infections precedes genetic differentiation of the host populations or follows it, relative to other ecological differentiation of the hosts. Such analyses could help to improve our understanding of the role of parasitism in initiation, facilitation and/or maintenance of speciation, but suitable host systems may be rare.

Community ecologists too have a longstanding interest in the role of parasites in mediating the coexistence of host species that compete for similar resources. Recent meta-analyses and reviews (Comita *et al.*, 2014; Bever *et al.*, 2015), as well as experimental manipulations (Bagchi *et al.*, 2014) have found broad support for the importance of parasites in mediating coexistence. While originally proposed in the context of pathogens and herbivores in forest tree communities, the Janzen-Connell mechanism has been shown to apply to animal communities too (Marhaver *et al.*, 2013). The general idea is that parasites may mediate host species coexistence through trade-offs between host competitive ability and resistance and through parasite specialization. The hypothesis predicts that parasites specialize on relatively more abundant species, which may lead to density-dependent regulation of more abundant species and prevent them from driving rare species to extinction. Under this hypothesis, abundant host species are predicted to be more heavily infected.

Alternatively, rare species may be more infected if abundant host species can evolve resistance, but rare species could be constrained in their ability to evolve resistance and are cross-infected from the abundant host species around them. In this situation, parasites may constrain host species diversity.

A third scenario is a hump-shaped relationship between host abundance and parasite damage. In this case, rare host species are attacked primarily by generalist parasites and abundant hosts by

specialized parasites, but hosts of intermediate abundance are attacked by both generalist and specialist parasites leading those species of intermediate abundance to be hit the hardest of all (Bachelot *et al.*, 2016).

Much work on the influence of pathogens and parasites in community ecology has focused on species rich communities, such as tropical forests (Gilbert, 2002; Freckleton & Lewis, 2006). In contrast, most work on parasite-mediated speciation has been done in low diversity systems such as fish in postglacial lakes (Knudsen *et al.*, 1997; Andreou *et al.*, 2017). A comprehensive understanding of the role of parasites in the diversification of host communities will require the integration of evolutionary and ecological parasite-mediated dynamics and how these dynamics play out in species-rich systems.

In this paper, we explore parasite infections in the community of closely related, sympatric cichlid species of Makobe Island in Lake Victoria, an offshore rocky reef ca. 5 km from the mainland shore and surrounded by deep water (Seehausen & Bouton, 1997). Cichlids of the Great Lakes of Eastern Africa represent one of the most species-rich and best-studied examples of adaptive radiation (Kocher, 2004; Seehausen, 2006). The rocky-shore cichlid community at Makobe Island consists of well over 30 fully sympatric species. These include incipient and sister species with low genetic differentiation and recent or ongoing hybridization (Magalhaes *et al.*, 2012; Keller *et al.*, 2013; Meier *et al.*, 2017b) extending to older species pairs that no longer exchange genes and belong to different morphologically defined genera (Wagner *et al.*, 2013) all of which have evolved within the past 15'000 years (Meier *et al.*, 2017a). All of the species coexist within a relatively narrow depth range (ca. 0.5-10 m) defined by the available rocky habitat, but most species show species-specific microdistribution with restricted depth ranges within the habitat (Seehausen & Bouton, 1997).

Species also exhibit specialized feeding ecologies and associated morphologies and can be assigned to several distinct trophic groups including algivores, insectivores, planktivores, molluscivores and piscivores (Witte & van Oijen, 1990; Bouton *et al.*, 1997; Seehausen & Bouton, 1998). The depth distributions of species tend to be correlated with their trophic groups (e.g. algivores are found mainly in shallow water whereas planktivorous species tend to occupy deeper water) although all species are capable of movement across the full depth range of the site, facilitated by the small spatial distance between depths.

We investigated 16 species from the Makobe cichlid community (Fig. 1) for ectoparasitic infections that are known to influence fitness-related traits of the host (Maan *et al.*, 2008).

We included the eight most abundant species in the community that together account for 95.4% of the individuals in this cichlid community. In addition, we included eight species with lower abundance: six of these species together account for 2.7% of the remaining individuals, and two very rare species account for 0.08% and 0.03%. All of these species are clearly genetically differentiated from each other and form reciprocally monophyletic groups in phylogenetic analyses (Wagner *et al.*, 2013). Here, we studied the same individuals that has been used for phylogenomic work earlier and calculated pairwise genetic differentiation between all species using high-resolution genome-wide SNP data.

We then analysed the relationship between pairwise species genetic distance and the magnitude of differences in parasite infections. With regard to the effects of parasites on speciation and post-speciation species divergence, we made the following predictions:

1. If divergent infections were involved in the initiation of speciation, then infection divergence is expected to occur ahead of genetic divergence among host populations. This is because under this hypothesis, parasite-mediated divergent selection is required for the evolution of reproductive isolation, and reproductive isolation can only be detected in allele frequency differences sometime after it has evolved.

2. If divergent infections were not an important component of the divergent selection forces that initiate speciation, infection divergence might only increase after speciation and associated genetic differentiation, if post-speciation species divergence was associated with divergence in infections, or infection divergence might be entirely unrelated to genetic divergence between species.

We tested these predictions by analysing the relationship between differences in parasite communities of all pairwise comparisons of the 16 sympatric cichlid species from Makobe Island. Additionally, we extended this analysis to a previously studied “speciation continuum” involving replicate cases of sympatric red and blue sister species in the genus *Pundamilia* from three other islands in order to ask if patterns of infections in replicate sister species-pairs were repeatable and predictable from their degrees of genetic and habitat differentiation, which vary among islands (Seehausen *et al.*, 2008; Meier *et al.*, 2017b). For testing the Janzen-Connell hypothesis of parasite-mediated coexistence, we investigated the relationship between cichlid species abundance, and parasite diversity and load.

Materials and methods

Fish sampling and examination

Fish specimens were collected during a sampling campaign conducted on the rocky shore cichlid community in the Makobe Island, Lake Victoria, Tanzania, in 2010. The sampling targeted 16 cichlid species of different abundance in this community, representing a variety of ecologically and morphologically specialized species (Wagner *et al.*, 2013). In short, the species included highly specialized epilithic algae scrapers *Neochromis rufocaudalis*, *N. gigas*, *N. omnicaeruleus* and *Paralabidochromis* sp. 'short snout scraper' as well as morphologically more generalized algae scrapers *Mbipia mbipi* and *M. lutea*. *Paralabidochromis chilotes* and *Pundamilia pundamilia* are specialized insect larvae feeders, and the latter species, unlike any of the others, is found exclusively in rock crevices. Samples also included zooplanktivorous *Pundamilia nyererei* and *Pundamilia* sp. 'pink anal fin', *Labrochromis* sp. 'stone', which has distinctive pharyngeal bones and enlarged molariform teeth specialized in crushing snails, the large piscivore *Harpagochromis* cf. *vonlinnei*, the egg and fry eating (paedophagous) *Lipochromis melanopterus*, and '*Haplochromis*' *cyaneus*, a species that feeds on chironomid midges. The two remaining species were the morphologically generalized omnivore *Paralabidochromis sauvagei* and an 'ecotype' or incipient species of *Neochromis* (Seehausen, 2006; Magalhaes *et al.*, 2012) *Neochromis* sp. 'unicuspid scraper' Wagner *et al.* (2013). The main ecological characteristics of each species are summarized in Table 1. All of these are described in detail in Seehausen (1996). To see if the patterns of infection were repeatable between cases of sympatric sister species at different islands, blue and red *Pundamilia* were collected from three neighbouring islands, Kissenda, Python and Luanso, situated approximately 23-37 km from Makobe and 7 to 17 km from each other (Supporting Information, Fig. S1). At Kissenda and Python, these species are *P. sp.* "pundamilia-like" and *P. sp.* "nyererei-like", and at Luanso it is a single genetically panmictic population with polymorphic male coloration (Meier *et al.* 2017).

Fish were caught by angling or with gillnets of variable size set in different depths to cover the entire depth range of all the fish species. Capture depth of each fish was recorded. All fish were sacrificed with a solution of MS222, fin-clipped and preserved in 4% formalin after capture, and thereafter transferred into alcohol for long-term storage. Specimens selected for parasitological examination from Makobe included 8-10 mature males from each of the 16 species covering the entire depth range of each species. Samples were also retrieved from several net sets on different dates. Because females and sub-adults of these species can often not be identified to species level reliably, we only used adult males. The same sampling approach was used also at the other islands. Sample sizes were based on previous studies, i.e. parasitological and genetic analyses at Makobe (see below) were conducted on the same individuals used in Wagner *et al.* (2013). Further, similar sample sizes were used for all the species regardless of their abundance, common or rare. We focused only on ectoparasites of the skin and the gills of the fish, which are known to comprise important components of the parasite community in the previously studied species *Pundamilia pundamilia* and *P. nyererei* (Maan *et al.*, 2008). Each fish was first measured for standard length and examined for infections on the skin and fins under a microscope. Gill arches were then removed from the right side of each fish and examined under a microscope for macroparasite infections. All parasites were counted and identified to the level of species or genus according to Paperna (1996), and prevalence (% fish infected) and mean abundance (mean number of parasites per host) were calculated for each parasite taxa.

Pairwise genetic distance

Pairwise genetic distances for all host species pairs in the dataset were calculated from the genomic data of Wagner *et al.* (2013). Reads from RAD sequencing (see Wagner *et al.* (2013) for library preparation and sequencing methods) were aligned to the *P. nyererei* reference genome (Brawand *et al.*, 2014) using Bowtie (Langmead *et al.*, 2009), allowing two

mismatches from the reference (v 2), including reads with only a single possible alignment (m 1), and using the default mapping quality for each read (mapq 60). For variant calling we used UnifiedGenotyper from GATK (McKenna *et al.*, 2010) setting the sequencing quality threshold to 20 (mbq 20, stand_call_confidence 20). After genotyping, we removed invariant sites and filtered the dataset to include only sites where the minor allele was observed at least three times, and sites with data for at least four individuals per species in the 16 species dataset. After this filtering, we were left with a set of 1591 SNPs for 156 individuals.

Pairwise F_{ST} values were calculated in R in the package ‘adegenet’ using Nei’s algorithm (Nei, 1973). Pairwise F_{ST} values for the four sympatric *Pundamilia* species and morph pairs of Luanso, Kissenda, Python and Makobe Island (the “speciation continuum”) were taken from Meier *et al.* (2017b). Note that the value for the pair of Makobe Island is different from the one used in the Makobe community dataset (different samples and different SNP filtering).

Statistical analysis

Effect of cichlid ecology (water depth, trophic group, abundance) on infections

To analyse the population-level effect of water depth on the infections, the mean capture depth was calculated for each of the 16 cichlid species and contrasted with the total abundance of parasites, as well as abundance of parasites with different modes of transmission: copepods, monogeneans and benthic parasites. Copepods have a fully limnetic and direct life cycle, and they undergo several planktonic stages during their development with the last stages attaching and maturing on the gills of fish. Benthic parasites (here including trematodes and parasitic larvae of molluscs), on the other hand, are transmitted to fish through benthic invertebrates. Dactylogyridean monogeneans (here members of the genus *Cichlidogyrus*) represent an intermediate of the two former as their eggs

released from fish by the adult worms hatch to free-swimming larvae that probably can inhabit a large sector of the water column.

Effect of depth distance between any two of the 16 cichlid species on the difference between their parasite communities was analysed by first constructing a matrix of pairwise mean capture depth distances including all possible cichlid species-pairs (n=120). Two corresponding matrices of difference in parasite infections between the paired species were then constructed by calculating Bray-Curtis dissimilarities for parasite species composition (occurrence of species) and for the mean parasite abundances. Effects of depth distance on differences in infection were then analysed using partial Mantel tests (controlling for pairwise genetic distance among the fish species, see below) based on Pearson's product-moment correlation with 9999 permutations of the data. Bray-Curtis dissimilarity was also calculated separately for mean abundances of copepods, monogeneans and benthic parasites, and the relationship between depth distance and difference in infections was analysed as above.

Effect of trophic group was tested by categorizing the 16 cichlid species into five groups according to their feeding specialization: algivores (4 species), algivores/omnivores (3 species), insectivores (4 species), planktivores (2 species), and others (3 species). The last group included the specialized egg and fry eater *Lipochromis melanopterus*, the molluscivore *Labrochromis* sp. 'stone' and the piscivorous *Harpagochromis* cf. *vonlinnei* (Table 1). The data on total parasite abundance were analysed using generalized linear models (GLM) with negative binomial probability distribution and log link function. Trophic group and fish species nested within the trophic group were used as fixed factors, and fish standard length as a covariate. The GLM was also run separately for abundance of copepods, monogeneans and benthic parasites. Further, differences in proportions of parasites

belonging to different groups were analysed among the host trophic groups using Kruskal-Wallis test.

To test relationships between host abundance and parasite infections, the relative abundance of each cichlid species in the community was calculated using all catch data of O. Seehausen from the period 1991 to 1996 (total n = 7519 fish). The relationships were tested between the host abundance, and the number of parasite species and the total abundance of parasites, as well as the abundance of parasites with different modes of transmission (copepods, monogeneans and benthic parasites).

Finally, we used an AIC-based model averaging approach to analyse the relative importance of water depth, host abundance, host size and host trophic group (for algivores and planktivores with higher replication of species) for total parasite abundance and that of copepods, monogeneans and benthic parasites. To assess the impact of the predictor variables (relative-impact value) in the model we used an AIC-based model (Burnham & Anderson, 2002). The full set of additive models was generated and the relative importance of each fixed effect was calculated. This relative importance can vary on a scale from 0 to 1, and is calculated as the sum of the Akaike weights of the models in which the variable appears. Models with higher likelihood have larger Akaike weights and thus variables that contribute positively to model fit will have higher relative-importance values (closer to one).

Effect of cichlid genetic distance on infection divergence

Effect of genetic distance on the difference in parasite communities was analysed by constructing a matrix of pairwise F_{ST} distances (see above) among the 120 possible cichlid species-pairs of the Makobe community and plotting this against the corresponding matrices of difference in parasite species composition and abundance. The analyses were also conducted separately for copepods, monogeneans and benthic parasites. Relationships were analysed using partial Mantel tests (controlling for pairwise differences in the mean capture depth among the fish species) as described above. Mantel tests were performed in R version 2.15.2 using the package 'permute'.

It should be noted that although phylogenies can be constructed from these data, we have purposefully avoided using phylogenetic comparative methods here because of uncertainty about whether phylogenetic relationships in this case reflect phylogenetic history, or other processes, which may overwhelm the signal of ancestry (e.g. hybrid speciation, post-speciation gene flow, selection; Keller *et al.* (2013); Wagner *et al.* (2013); Meier *et al.* (2017b)). Because pairwise F_{ST} values rely on fewer assumptions than phylogenetic comparative methods, in this situation these represent a better choice for asking about how relatedness influences parasite infections among these species. However, phylogenetic analyses focused on these species, using the same genetic dataset used here, have previously been conducted (Keller *et al.*, 2013; Wagner *et al.*, 2013; Meier *et al.*, 2017b).

Results

Parasite fauna

We found six macroscopically differentiated ectoparasite taxa in the Makobe cichlid community (Table 2). These included the copepods *Lamproglena monodi*, *Ergasilus lamellifer* and an unidentified species of *Ergasilus* (referred here to as *Ergasilus* sp.) on the gills of fish. *Lamproglena monodi* was found on all host species with a prevalence ranging between 20-100% depending on the host species, while the two *Ergasilus* taxa were encountered more infrequently. Monogeneans of the genus *Cichlidogyrus* were the most abundant parasites and were found on the gills of 13 of the 16 cichlid species, with prevalence ranging between 12.5-100%. Trematodes of the genus *Neascus* were found on skin and fins of all cichlid species in low abundance. Glochidia larvae of bivalves were encountered very infrequently and in low abundance on four cichlid species (Table 2).

The same six parasite taxa were found also in our samples from the three other islands, infecting *P. sp.* “pundamilia-like and *P. sp.* “nyererei-like” at Kissenda and Python and *P. sp.* “Luanso” at Luanso (Table 2). Similar to the Makobe community, *Cichlidogyrus* monogeneans were the most abundant taxa, followed by *Neascus* sp. trematodes. Overall, total parasite abundance was higher in *Pundamilia* from these other islands compared to *P. pundamilia* and *P. nyererei* at Makobe (ANOVA on log-transformed data: $F_{3,62} = 22.401$, $p < 0.001$ (population), $F_{1,62} = 12.585$, $p < 0.001$ (species), $F_{3,62} = 1.793$, $p = 0.158$ (population*species)), particularly because of higher abundances of *Ergasilus* sp., *Cichlidogyrus* and *Neascus*.

Effects of host habitat, trophic group and abundance

There was a strong and significant negative relationship between the mean capture depth of a cichlid species and its mean parasite abundance in the Makobe community (Pearson correlation: $r = -0.678$, $n = 16$, $p = 0.004$; Fig. 1). Individuals of shallow-dwelling cichlid species carried many more parasites than those of deep-dwelling species. The crevice-dwelling species *Pundamilia pundamilia*, however, deviated from this pattern, showing very low levels of infection despite living in shallow water (Fig. 1). The relationship with water depth was stronger when confined to the species living outside the rocky interstices ($r = -0.823$, $n = 15$, $p < 0.001$). The negative correlation between mean parasite abundance and mean depth of cichlid species was strongest in the monogeneans *Cichlidogyrus* ($r = -0.563$, $n = 16$, $p = 0.023$) while the negative relationships between depth and abundance of the limnetic copepods ($r = -0.368$) and benthic parasite species ($r = -0.414$) were not statistically significant on their own ($p > 0.1$). Exclusion of *P. pundamilia* did not change these results.

Mean capture depth of a cichlid species and the mean number of parasite species found on individual fish were also negatively correlated, but this was not statistically significant ($r = -0.409$, $n = 16$, $p = 0.116$). Similarly, parasite abundance, but not parasite species composition, became more strongly differentiated with increasing difference in the mean water depth occupation between cichlid species in pairwise comparisons (Fig. 2, Table 3). On the level of individual fish within species, however, capture depth did not correlate negatively with parasite abundance in any of the 16 species ($p > 0.1$ for all). This indicates that the individual capture depth, unlike the mean depth of a species, did not influence parasite abundance.

Trophic group of the cichlids had a significant effect on the total parasite abundance and on the abundance of the three functional groups of parasites (Table 4). These patterns were caused mainly by the higher parasite abundance in algivores and algivores/omnivores (Fig. 3), while the parasite abundance was very low in the deep-water planktivores and piscivores. Importantly, there were also

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significant differences in parasite abundance among the cichlid species within the trophic groups, except for the benthic parasites (Table 4). The exclusion of the crevice-dwelling *P. pundamilia* did not qualitatively change results of the generalized linear models. As expected, limnetic copepods comprised the highest proportion of the parasite community in the planktivorous cichlids and the lowest in algivores, although this was not significant at five percent level (Kruskal-Wallis test: $H' = 7.852$, $p = 0.097$; Fig. 3). On the other hand, algivores harboured the highest proportion of monogeneans ($H' = 21.632$, $p < 0.001$), whereas the communities of piscivores were dominated by benthic parasites ($H' = 9.585$, $p = 0.048$; Fig. 3). The lowest proportion of benthic parasites was observed in the planktivore group. Overall, parasite abundances of the cichlid trophic groups tended to be negatively correlated with the mean water depth occupation (Pearson correlation: $r = -0.838$, $n = 5$, $p = 0.076$) with the most heavily infected algivores found in the shallow water. Thus, differences in parasitism among the different trophic groups also strongly reflect the overall trend of decrease in infections with water depth occupation (Fig. 1).

Host abundance tended to correlate positively with the total parasite abundance (ln-transformed data; $r = 0.340$, $p = 0.198$) and this pattern was most evident in *Cichlidogyrus* monogeneans ($r = 0.458$, $p = 0.074$; Table 5; Fig. 4). A similarly strong positive trend was detected for the relationship between host abundance and the number of parasite taxa per cichlid species ($r = 0.453$, $p = 0.078$). While these results are not statistically significant at five percent level, they suggest that host abundance may matter for infection diversity and abundance. However, in the copepod infections the relationship resembled a hump-shaped with parasite abundances peaking at intermediate host abundances, while there was a very weak, non-significant positive trend for the benthic parasites ($r = 0.150$, $p = 0.580$) (Table 5; Fig. 4). Model averaging indicated that the relative importance of host abundance for variation in parasitism among the cichlid species was low (0.14-0.19), whereas the importance of water depth was higher (0.652; Table 5). Interestingly, the algivore trophic status was

clearly the most important determinant of monogenean infections (0.924), exceeding that of water depth (0.161; Table 5). This result may derive from algivores all living in shallow water, where the *Cichlidogyrus* infection is also highest.

Effect of genetic distance

Pairwise genetic distance among the Makobe cichlid species did not correlate with differences in parasite abundance at the level of the whole parasite community (Fig. 2, Table 3). However, monogenean infections tended to become more differentiated with increasing genetic distance among the host species and this relationship became stronger and significant when the only strictly crevice-dwelling species, *P. pundamilia* was excluded from the analysis (Fig. 2, Table 3). Differences in parasite species composition, on the other hand, did not correlate with the genetic distance between host species (Fig. 2, Table 3).

To see if the lack of a relationship between genetic differentiation and parasite infections was due to convergence in depth habitat and associated parasite infections in more distantly related cichlid species, we analysed a subset of the data from seven pairs of morphological sister species. These were *Mbipia lutea*-*M.mbipi*, *Neochromis omnicaeruleus*-*N.gigas*, *N.omnicaeruleus*-*N. sp. 'unicuspid scaper'*, *Paralabidochromis sp. 'short snout scraper'*-*P. sp. sauvagei*, *Pundamilia pundamilia*-*P. nyererei*, *P. pundamilia*-*Pundamilia sp. 'pink anal fin'*, and *P. nyererei*-*Pundamilia sp. 'pink anal fin'*, following earlier work (Seehausen, 1996; Seehausen *et al.*, 2008; Magalhaes *et al.*, 2012; Keller *et al.*, 2013; Wagner *et al.*, 2013; Brawand *et al.*, 2014). Contrary to our expectation of positive relationships between F_{ST} and residual distance in parasite infections after taking depth differences into account, the correlation with parasite species composition tended to be negative (Fig. 2; $r = -0.793$) and there was no strong trend for parasite abundance (we do not report p-values because

some of these pairwise comparisons are statistically non-independent and do not form a full matrix).

This suggests that, if anything, the more closely related species had more divergent infections in these species-pairs.

Variation along the *Pundamilia* speciation continuum

In the Makobe community, the crevice-dwelling species *P. pundamilia* significantly deviated from the other species showing low infection in shallow water (Fig. 1). To test if this was repeatable and if parasitism correlated with crevice-dwelling in *Pundamilia*, but also to test for more general repeatability of parasite trends in similar cases of species divergence, we compared infections between sympatric red/blue pairs of *Pundamilia* at Makobe and three other islands with phenotypically similar species or morph pairs (Seehausen *et al.*, 2008; Meier *et al.*, 2017b). We found that the total parasite abundance was strongly correlated between sympatric *Pundamilia* spp. across different islands ($r = 0.999$) with all red forms (*P. nyererei*, *P. sp.* “nyererei-like”, and red morph of *P. sp.* “Luanso”) showing higher parasite abundances than the blue forms (*P. pundamilia*, *P. sp.* “pundamilia-like”, and blue morph of *P. sp.* “Luanso”, Fig. A1) (Fig. 5). This suggests that the overall level of infections in the red and blue forms is mainly driven by the specific level of parasitism at each island, but also that the red forms generally have somewhat higher infections. The pattern of overall infection level was similar for *Cichlidogyrus* monogeneans ($r = 0.978$) and benthic parasites ($r = 0.937$). However, no such relationship was observed in the copepod infections ($r = -0.163$) particularly because of low abundances in *P. sp.* “pundamilia-like” at Python and *P. Pundamilia* at Makobe despite higher abundances of their sympatric red sister species (Fig. 5).

The total parasite abundance also tended to be negatively correlated with the water transparency at the islands (Secchi values taken from Seehausen *et al.* (2008)) both in red forms ($r = -0.840$) and blue forms of *Pundamilia* (-0.855), suggesting a general increase in infections with the magnitude of eutrophication. The pattern was most evident in benthic parasites ($r = -0.993$ and $r = -0.894$ for red and blue forms, respectively).

Correlation between residual difference in parasite abundance (after depth was taken into account) and F_{ST} of sympatric *Pundamilia* species tended to be positive ($r = 0.459$) and, similarly to the trends within the complete Makobe community, this was strongest in the monogenean infections ($r = 0.437$). Vice versa, correlation between residual difference in parasite abundance (after F_{ST} was taken into account) and distance in mean capture depth was positive too ($r = 0.645$).

Discussion

Parasitism is a potent source of selection on host populations and may play a role in both speciation and in the coexistence of host species, as well as in species divergence after speciation. Rapid speciation, coexistence of many sympatric species, and rapid post-speciation adaptive divergence are hallmarks of adaptive radiation. Parasites could play an important role in all of these processes, and hence be a key factor shaping the diversity of species in adaptive radiations. There is published evidence for the existence of all three above-listed effects of parasites on host diversity, but few studies connect these frequently disparate avenues of research. Studies of parasitism in replicate morphs or species pairs at different stages of differentiation embedded in the wider community of closely related species are also needed to address fundamental questions such as at what stage in the speciation process parasite-mediated divergent selection becomes important, and what form parasite-mediated selection takes in communities of older sympatric species. Here, we present

results connecting all these areas in a study of parasite and host communities in the rapid Lake Victoria cichlid adaptive radiation.

We explored ectoparasite infections in a community of 16 sympatric cichlid species at Makobe Island. Some of these species are only weakly genetically differentiated and incompletely reproductively isolated (Magalhaes *et al.*, 2012; Keller *et al.*, 2013), while others are fully isolated sister-species or more distant relatives that have not exchanged genes for a long time (Bezault *et al.*, 2011; Keller *et al.*, 2013; Wagner *et al.*, 2013). Our results indicated no association between divergence in total parasite load and genetic distance among the cichlid species, neither among all cichlid species nor among the subset of sister species at Makobe. No relationships were found between genetic relatedness and parasite species composition either, when all Makobe species were analysed, but there was a negative trend for the morphological sister species pairs. However, when examining infections separately for parasite groups with different modes of transmission, differences in the monogenean infection levels showed a weak, but significant positive relationship with the magnitude of host genetic differentiation, suggesting that growing infection divergence may add to divergent selection between host species after the onset of speciation (Wills *et al.*, 2016). This pattern was driven mainly by the deep-living cichlid species harbouring few or no monogenean infections. In addition, we found a stronger effect of host population mean depth occupation on the infections such that infections became fewer with depth. This led to more differentiated infections with increasing water depth difference among the cichlid species. Overall, this suggests that ecological factors, and to some extent also evolutionary factors, explain the observed differences in parasitism in the Makobe cichlid community. Similarly, in the *Pundamilia* speciation continuum, infection differences, particularly those of monogeneans, tended to be positively correlated with the genetic differentiation between the sympatric species-pairs. These results confirm those from the Makobe community and suggest repeatability in these patterns.

We also found a weak positive, albeit statistically non-significant, relationship between the abundance of a cichlid species, and its number of parasite species and total parasite load. This is consistent with the Janzen-Connell hypothesis and with a potential parasite mediated advantage for rare cichlid species (Fig. 4), which could facilitate species coexistence. When analysed at the level of different transmission groups of parasites, positive effects of abundance on parasite load were observed for *Cichlidogyrus* monogeneans, but a hump-shaped relationship emerged for the gill copepods *Lamproglena* and *Ergasilus*. While these relationships were not statistically significant, hump-shaped relationships would be consistent with the simultaneous presence of host generalists and host specialists amongst the parasitic copepods, whereas the positive relationships between parasite load and host abundance point to a predominance of host specialists among the monogeneans (Bachelot *et al.*, 2016). These are promising leads for further investigations into host-parasite coevolution in this system.

Over the recent years, studies have begun to explore differentiation in parasitism among morphs and incipient species of freshwater fish, mainly in species poor communities (Knudsen *et al.*, 1997; Knudsen *et al.*, 2003; MacColl, 2009; Eizaguirre *et al.*, 2011; Natsopoulou *et al.*, 2012; Karvonen *et al.*, 2013a; Karvonen *et al.*, 2013b; Raeymaekers *et al.*, 2013; Karvonen *et al.*, 2015; Anaya-Rojas *et al.*, 2016; Siwertsson *et al.*, 2016) (but see Blais *et al.* (2007); Raeymaekers *et al.* (2013); Hablutzet *et al.* (2016) for examples from species rich communities). However, there are no comprehensive studies yet of parasitism in young adaptive radiations with replicated pairs of closely related sympatric species of variable degree of differentiation within the same evolutionary lineage of hosts (e.g. a host speciation continuum). In addition, several studies have dealt with pathogens as agents of intraspecific density-dependence of host populations in plants (Comita *et al.*, 2014) and there is increasingly strong evidence that these interactions can facilitate coexistence in species rich communities of tropical forest trees (Bagchi *et al.*, 2014). There is also at least one study that

revealed evidence for a role of pathogens in post-speciation adaptive diversification of tree species in two species rich tropical forests (Wills *et al.*, 2016). Our present results significantly add to this earlier work by showing that parasites could act as agents of divergent selection after speciation or in advanced stages of speciation in closely related cichlids, as well as potentially contribute to coexistence in species-rich vertebrate communities.

One of the prerequisites of parasite-mediated divergent selection is that parasites should impose fitness consequences on their hosts. In cichlids, some of the gill parasite taxa are known to cause injuries to gill epithelium (review in Paperna (1996)). Further, studies on the two sister species *P. pundamilia* and *P. nyererei* in the Makobe community have shown that parasite infection levels comparable to those we reported here are negatively associated with brightness of the male breeding coloration and male territory size (Maan *et al.*, 2006; Maan *et al.*, 2008). These data are consistent with the hypothesis of parasite-mediated selection in this system. On the other hand, temporal consistency in infection differences, another prerequisite of parasite-mediated selection, is largely unknown in Lake Victoria. In principle, environmental dynamics such as fluctuations in temperature, precipitation, and lake level (Bouton *et al.*, 1997; Raeymaekers *et al.*, 2013) could cause variation in parasitism. On the other hand, studies on Lake Tanganyika cichlids support temporal stability in parasite assemblages even on a greatly larger geographical scale than ours (Raeymaekers *et al.*, 2013). The weak association we observed between parasitism and pairwise genetic distance among cichlid species nevertheless suggests that the overall genetic differentiation would be more strongly driven by factors other than parasitism. However, it is possible that more notable signatures of parasite-mediated selection could be seen at loci directly linked to parasite defence, such as those of the immune system (Blais *et al.*, 2007; Eizaguirre *et al.*, 2012b). Moreover, while we found a positive relationship between cichlid species genetic differentiation and distance in *Cichlidogyrus* infections, it is possible that variation in host specificity among lineages within the

Cichlidogyrus (Vanhove *et al.*, 2015) could lead to larger infection differences among the cichlid species than what we have estimated here based on parasite load resolved only at coarse taxonomic levels. These are aspects for future research.

Water depth represented the main abiotic gradient for infections with the mean capture depth explaining a very high proportion of variation in mean parasite abundance among the 16 sympatric cichlid species. Infections clearly became fewer at greater depth and this was also tightly connected with the variation in infections among and supported by those within the cichlid trophic groups. Such a relationship could be related to factors like habitat-specific feedbacks in parasite transmission. For example, algivores had the highest abundances and proportions of *Cichlidogyrus* parasites, which may be related to higher host densities in shallow waters providing favourable conditions for parasite transmission. The more even distribution of the copepod parasites among all trophic groups, on the other hand, may be related to their limnetic life cycle enabling wider dispersal through active swimming and aid of water currents. Overall, trophic ecology of the cichlids and water depth together explain much of the interspecific variation in parasite infections. Unlike the species mean capture depth, however, individual capture depth was not negatively correlated with parasite abundance within individual cichlid species. This might imply that although individuals are clearly moving along the depth gradient, such movement may be limited and the variation in individual mean depth occupation may be reduced compared to the variation in individual capture depth in our data set (we sampled with individual depth overdispersion on purpose, see methods), such that species mean depth is a better predictor of parasite abundance than individual capture depth. Our result also suggests at least some degree of consistency in infections over time so that infections do not change with short-term movements of fish. Overall, this supports earlier findings on the fine-scale micro-distribution and habitat specialization of the cichlids in the Makobe community (Seehausen & Bouton, 1997; van Rijssel *et al.*, 2018).

Despite the strong negative correlation between the mean water depth occupation and infection abundance in the Makobe community, one species, *P. pundamilia*, deviated from this patterns showing very low abundance of infection in shallow water. The difference of this species to all others at Makobe is that it lives exclusively in the interstices between rock boulders, which could reduce the risk of acquiring infections. Comparison with the ecologically similar species pairs *P. sp. "pundamilia-like"* and *P. sp. "nyererei-like"* at Python and Kissenda islands, with varying degrees of crevice-dwelling habitat specialization in the *P. sp. pundamilia-like* (Seehausen, 1996; Seehausen *et al.*, 2008), indicated that infection differences between islands were strongly correlated between the species. This pattern was true for all parasites except copepods, where the crevice-dwelling *P. pundamilia* from Makobe and *P. sp. "pundamilia-like"* from Python had lower abundances than in other islands predicted from the abundance of copepods in the sympatric sister species *P. nyererei* and *P. sp. "nyererei-like"*. This may be related to the habitat specialization of the former, being confined to crevices in both islands, which may provide a refuge from infections (see also Hablutzel *et al.* (2017)). Alternatively, it could be related to the more insectivorous feeding habits, which could limit exposure to limnetic parasites compared to the more planktivorous *P. nyererei* and *P. sp. "nyererei-like"*. On the other hand, the low abundance of *Cichlidogyrus* infections in *P. pundamilia* of the Makobe population (one fish individual infected with one parasite specimen; other cichlid species in the same depth range with prevalence of 80-100%) does not seem to be explained just by living in crevices as similar lack of *Cichlidogyrus* was not observed at Python Island. Alternatively, this could suggest evolution of specific resistance against *Cichlidogyrus* in *P. pundamilia* at Makobe. While suggestive, this would need to be verified experimentally.

We also observed that parasite abundances were equal, or in most cases higher, among the males of red forms of *Pundamilia* compared to the males or the blue forms, which is consistent with earlier results from Makobe (Maan *et al.*, 2008). Interestingly, the same pattern was found regardless of the

magnitude of genetic differentiation of the species at different islands. This consistency could point towards interactions with sexual selection on carotenoid-derived red male colouration (Fig. A1), which may compromise immune defence under carotenoid scarcity (Lozano 1994). However, studies have demonstrated intra- and intersexual selection advantages of the red males (Maan *et al.*, 2006; Dijkstra *et al.*, 2007), which might compensate their parasite-driven fitness loss. How this operates at different islands with varying intensities of male nuptial red colouration, is currently unknown.

The strong correlation across islands in total parasite abundance between the sympatric *Pundamilia* species-pairs, as well as those in *Cichlidogyrus* and benthic parasites, suggests that variation in the overall level of parasitism among the islands is primarily environmentally driven. Infections were more abundant at the three other islands compared to Makobe and tended to be negatively correlated with water transparency so that infections became more abundant with magnitude of eutrophication. In general, the connection between eutrophication and parasitism is well-described, showing both positive and negative correlations (Marcogliese, 2001; Blonar *et al.*, 2009; Karvonen *et al.*, 2013b) and is typically related to the distribution and abundance of parasite intermediate and definitive hosts. For example, we found the strongest associations in benthic parasites, which may be connected to presumably higher abundances of molluscs in waters of the more eutrophic islands. Increase of parasitism because of human-induced eutrophication may significantly contribute to changes in the selective landscape experienced by cichlid species in the wake of the environmental changes in Lake Victoria. While eutrophication is known to influence sexual selection and genetic species differentiation in this system (Seehausen *et al.*, 1997; Seehausen *et al.*, 2008), the importance of relationships between eutrophication and parasitism for such processes remains to be explored.

To conclude, our results indicated a strong and dominant effect of host ecology on interspecific variation in ectoparasite infections in the Makobe Island cichlid community. Interestingly, there was only a weak association between differences in the overall magnitude of parasitism and genetic differentiation of cichlid species, with no signs of a significant increase in infection distance at any point of the continuum of genetic differentiation that would indicate the onset of parasite-mediated divergent selection. While this suggests a stronger effect of other (environmental) factors, the result does not exclude the possibility of parasite-mediated divergent selection on loci directly involved with defence against parasites. Either way, the positive relationship between post-speciation genetic differentiation of hosts and the magnitude of their distance in *Cichlidogyrus* infections, where the most closely related cichlid species have more similar infections than expected given their habitat differences (negative residuals from a regression against depth difference), suggests that signatures of parasite-mediated selection may pick up only after speciation has progressed to the stage of genome-wide genetic differentiation. On the other hand, the tendency for a positive association between abundance of cichlid species and that of their parasites suggests that infections could facilitate coexistence by favouring rare host species. This provides interesting avenues for future research on coexistence of species in systems outside plant-pathogen interactions where these concepts were originally developed. Recent investigations into adaptive radiations of cichlids have also explored the question of why some lineages show marked diversification while other closely related taxa, even in the same habitat, do not (Wagner *et al.*, 2012). Such a question is intriguing also in terms of parasite-mediated divergent selection. Adaptive radiations could play a key role in such approaches because of the opportunities they provide to study many closely related species at different stages of speciation in sympatry, particularly if they can be used to detect the onset of parasite-mediated divergent selection.

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Table 1. Ecological characteristics of the 16 cichlid species sampled from Makobe Island and the *Pundamilia* morph and species-pairs from islands Luanso, Python and Kissenda in Lake Victoria. The species nomenclature follows that in Seehausen (1996) (see also Wagner *et al.* (2013)). Capture depth (m) and standard length (mm) indicate mean (range) for each cichlid species. The relative abundance of cichlid species is the relative abundance among all cichlids collected and identified by O. Seehausen at Makobe Island between 1991 and 1998 (n = 7519), including 15 rare species not included in this study.

Island	Species	Trophic group	Main habitat	Relative abundance	Capture depth	Standard length
Makobe	<i>Mbipia lutea</i>	Algivore	Benthic	0.0045	0.67 (0.5-1.0)	124 (98-161)
	<i>Neochromis gigas</i>	Algivore	Benthic	0.0094	0.78 (0.50-1.0)	120 (105-130)
	<i>Neochromis rufocaudalis</i>	Algivore	Benthic	0.0892	2.27 (1.0-4.25)	89 (84-93)
	<i>Neochromis omnicaruleus</i>	Algivore	Benthic	0.1959	3.33 (0.75-5.0)	92 (76-102)
	<i>Mbipia mbipi</i>	Algivore / omnivore	Benthic	0.2559	1.73 (0.75-5.0)	97 (85-109)
	<i>Paralabidochromis</i> sp. 'short snout scraper'	Algivore / omnivore	Benthic	0.0033	3.03 (1.0-6.0)	100 (87-111)
	<i>Neochromis</i> 'unicuspid scraper'	Algivore / omnivore	Benthic	0.0020	11.03 (1.75-17.0)	89 (75-110)
	<i>Pundamilia pundamilia</i>	Insectivore	Crevice-dweller	0.0173	1.29 (0.75-2.5)	90 (69-105)
	' <i>Haplochromis</i> ' <i>cyaneus</i>	Insectivore	Benthic	0.0145	2.83 (0.75-5.0)	103 (95-109)
	<i>Paralabidochromis</i> sp. <i>sauvagei</i>	Insectivore	Benthic	0.0116	4.27 (1.65-8.5)	103 (88-112)
	<i>Paralabidochromis</i> sp. <i>chilotes</i>	Insectivore	Partial crevice-dweller	0.0040	5.83 (1.0-11.0)	115 (91-134)
	<i>Pundamilia nyererei</i>	Planktivore	Bentholimnetic	0.3116	8.13 (2.0-17.15)	76 (70-86)
	<i>Pundamilia</i> sp. 'pink anal fin'	Planktivore	Bentholimnetic	0.0580	10.32 (4.5-14.25)	76 (70-85)
	<i>Lipochromis melanopterus</i>	Egg and fry eater	Benthic	0.0037	8.48 (1.65-18.0)	89 (74-110)
	<i>Labrochromis</i> sp. 'stone'	Molluscivore	Benthic	0.0008	13.83 (3.0-18.0)	120 (99-145)
	<i>Haplogochromis</i> cf. <i>vonlinnei</i>	Piscivore	Benthic	0.0003	12.34 (8.5-17.5)	130 (107-140)
Luanso	<i>Pundamilia</i> sp. blue males	Insectivore	Crevice-dweller /		1.64 (0.75-2.75)	79 (65-96)

			bentholimnetic			
	<i>Pundamilia sp. red males</i>	Insectivore	Bentholimnetic		3.41 (2.5-4.4)	85 (72-97)
Python	<i>Pundamilia pundamilia</i>	Insectivore	Crevice-dweller		2.08 (1.0-4.4)	97 (80-112)
	<i>Pundamilia nyererei</i>	Insectivore	Bentholimnetic		3.81 (1.0-7.15)	75 (61-86)
Kissenda	<i>Pundamilia pundamilia</i>	Insectivore	Crevice-dweller		2.83 (1.0-6.75)	95 (82-107)
	<i>Pundamilia nyererei</i>	Insectivore	Bentholimnetic		3.16 (1.0-6.75)	85 (73-97)

Table 2. Prevalence (% fish infected) of the six ectoparasite taxa observed in the community of 16 cichlid species sampled from Makobe Island, and the *Pundamilia* morph and species pairs from islands Luanso, Python and Kissenda in Lake Victoria. Full species names are given in Table 1.

Island	Species	n (fish)	<i>Lamproglena monodi</i>	<i>Ergasilus lamellifer</i>	<i>Ergasilus</i> sp.	<i>Cichlidogyrus</i> spp.	<i>Neascus</i> sp.	Glochidia
Makobe	<i>M. lutea</i>	10	90.0	10.0	0	80.0	50.0	20.0
	<i>N. gigas</i>	10	100	0	0	90.0	20.0	0
	<i>N. rufocaudalis</i>	9	33.3	11.1	0	100	33.3	0
	<i>N. omnicaruleus</i>	9	44.4	0	0	88.9	44.4	0
	<i>M. mbipi</i>	10	70.0	0	10.0	100	60.0	0
	<i>Pa. 'short snout'</i>	9	77.8	33.3	0	0	66.7	0
	<i>N. 'unicuspid'</i>	8	75.0	37.5	0	62.5	50.0	12.5
	<i>P. pundamilia</i>	8	50.0	0	0	12.5	37.5	12.5
	<i>H. cyaneus</i>	9	88.9	22.2	0	100	55.6	0
	<i>Pa. sauvagei</i>	10	80.0	10.0	0	0	80.0	0
	<i>Pa. chilotes</i>	8	37.5	37.5	0	50.0	37.5	0
	<i>P. nyererei</i>	8	87.5	25.0	0	100	25.0	12.5
	<i>P. 'pink'</i>	10	50.0	0	10.0	30.0	10.0	0
	<i>Li. melanopterus</i>	8	37.5	0	0	75.0	62.5	0
	<i>La. 'stone'</i>	9	55.6	0	0	66.7	33.3	0
	<i>Ha. vonlinnei</i>	10	20.0	0	0	0	30.0	0
Luanso	<i>P. pundamilia</i>	8	0	0	37.5	100	100	12.5
	<i>P. nyererei</i>	9	0	0	33.3	44.4	88.9	11.1
Python	<i>P. pundamilia</i>	10	0	10.0	20.0	100	90.0	0
	<i>P. nyererei</i>	10	80.0	60.0	20.0	100	100	20.0
Kissenda	<i>P. pundamilia</i>	10	70.0	20.0	50.0	100	90.0	10.0
	<i>P. nyererei</i>	10	70.0	30.0	70.0	100	100	20.0

Table 3. Results of partial Mantel test on the relationships between depth distance and genetic distance, and difference in abundance of all parasites taxa and the three functional groups of copepods, monogeneans and benthic parasites in the community of 16 cichlid species at Makobe island. In each analysis, the effect of depth distance is controlled for the genetic distance, and vice versa. Difference in parasite abundance was calculated as Bray-Curtis dissimilarity (see methods). Genetic distance is pairwise F_{ST} between the fish species.

Test	All fish species		<i>P. pundamilia</i> excluded	
	Depth distance	Genetic distance	Depth distance	Genetic distance
Species composition:				
All parasites	r=-0.034, p=0.584	r=0.076, p=0.305	r=-0.008, p=0.488	r=0.061, p=0.347
Abundance:				
All parasites	r=0.240, p=0.032	r=0.084, p=0.321	r=0.381, p=0.002	r=0.142, p=0.246
Copepods	r=0.053, p=0.293	r=0.090, p=0.310	r=0.092, p=0.219	r=0.077, p=0.350
Monogeneans	r=0.169, p=0.090	r=0.298, p=0.065	r=0.249, p=0.039	r=0.377, p=0.031
Benthic parasites	r=0.070, p=0.257	r=-0.247, p=0.895	r=0.116, p=0.164	r=-0.290, p=0.929

Table 4. Results of GLM analyses on effect of fish trophic group on the abundance of all parasites, limnetic copepods, *Cichlidogyrus* monogeneans and benthic parasite species. Trophic group and fish species nested within trophic group were used as fixed factors, and fish standard length as a covariate.

Source	All parasites		Copepods		Monogeneans		Benthic	
	Wald	p	Wald	p	Wald	p	Wald	p
Trophic group	33.950	<0.001	18.216	0.001	22.528	<0.001	9.639	0.047
Species(Trophic group)	31.319	0.001	30.916	0.001	30.436	<0.001	12.470	0.329
Length	6.007	0.014	4.012	0.045	7.171	0.007	0.308	0.579

Table 5. Result of the model averaging analysis on the relative importance of variables explaining variation in parasite abundance among the 16 cichlid species in Lake Victoria. Analysis was performed separately for abundance of all parasites and that of copepods, monogeneans and benthic parasites.

Variable	All parasites	Copepods	Monogeneans	Benthic parasites
Mean capture depth	0.652	0.380	0.161	0.259
Host abundance	0.181	0.145	0.195	0.168
Host size	0.144	0.143	0.150	0.152
Algivore	0.370	0.212	0.924	0.219
Insectivore	0.197	0.323	0.161	0.237

Figure captions

Fig. 1. Relationship between mean capture depth and mean number of parasites in the 16 cichlid species in the Makobe community. The deviating point near the origin is the crevice-dwelling species *Pundamilia pundamilia*. The fitted line is linear regression. Colours of the dots correspond to those in Wagner *et al.* (2013) presenting phylogeny of the species.

Fig. 2. Relationships between the distance in parasite species composition, abundance of all parasites and abundance of monogeneans, and the difference in mean capture depth and degree of genomic differentiation measured as pairwise F_{ST} in the community of 16 sympatric cichlid species at Makobe Island, Lake Victoria. Infection distances plotted against depth difference are residuals from F_{ST} , and vice versa. Panels in A show the relationships with all 16 cichlid species included, and those in B when the crevice-dwelling species *Pundamilia pundamilia* was excluded. Red dots indicate pairwise comparisons among the seven sister species-pairs (see the main text for details). Lines are linear regressions to indicate the direction of statistically significant relationships (see Table 3).

Fig. 3. Mean abundance (\pm SE) of all parasites, limnetic copepods, *Cichlidogyrus* monogeneans, and benthic parasite species on individual cichlids assigned to major trophic groups among 16 cichlid species (data combined for individuals of all species within each trophic group) in the Makobe community. "Others" include snail and fish eaters and paedophages. Dots indicate mean proportion (\pm SE) of limnetic copepods, monogeneans and benthic parasites within each cichlid trophic group.

Fig. 4. Relationships between host abundance (log scale), and abundance of all parasites, copepods, monogeneans and benthic parasites among the 16 cichlid species in the Makobe community. The fitted line is linear regression. Colours of the dots correspond to those in Fig. 1 and in Wagner *et al.* (2013) presenting phylogeny of the species.

Fig. 5. Relationship between mean abundance (\pm SE) of all parasites, copepods, monogeneans and benthic parasites in *Pundamilia pundamilia* and *P. nyererei* at three islands, Makobe (M), Python (P) and Kissenda (K), and in blue and red morphs of *Pundamilia sp.* "Luanso" at Luanso Island (L). Dashed lines are linear regressions to indicate the direction of the relationships. Solid lines represent 1:1 relationships in parasite abundances between the cichlid species or morphs.







