DR. ALEXANDER HAYWARD (Orcid ID : 0000-0001-7413-718X)

DR. HANNE LOVLIE (Orcid ID : 0000-0003-4352-6275)

Received Date : 02-Dec-2016

Revised Date : 27-Jan-2017

Accepted Date : 07-Feb-2017

Article type : Research Papers

Evolutionary associations between host traits and parasite load: insights from Lake Tanganyika cichlids

Alexander Hayward[a], Masahito Tsuboi[b], Christian Owusu[b], Alexander Kotrschal[c], Séverine D. Buechel[c], Josefina Zidar[d], Charlie K. Cornwallis[e], Hanne Løvlie[d], Niclas Kolm[c]

[a] Centre for Ecology and Conservation, University of Exeter, UK

[b] Department of Animal Ecology, Uppsala University, Sweden

[c] Department of Zoology, Stockholm University, Sweden

[d] IFM Biology, Linköping University, Sweden

[e] Department of Ecology, Lund University, Sweden

Running title: *Host traits and parasite load*

Manuscript type: Research Article

Corresponding authors:

*Dr Alexander Hayward, Centre for Ecology and Conservation, Biosciences, College of Life and Environmental Sciences, University of Exeter, Penryn Campus, Treliever Road, Penryn, Cornwall, TR10 9FE. Tel: +44 (0)1326 259147, Fax: +44 (0)1326 371859, Email: alex.hayward@exeter.ac.uk

*Professor Niclas Kolm, Stockholms universitet, Zoologiska institutionen: Etologi, 106 91 Stockholm, Tel: +46 (0)8 164050, Fax: +46 (0)8 159522, Email: niclas.kolm@zoologi.su.se

Acknowledgements

We are very grateful to: Sebastian Scott for providing valuable assistance in Zambia; the staff of the Department of Fisheries, Mpulungu, Zambia, for their outstanding help and support; Professor Mats Björklund at the Department of Animal Ecology, Uppsala University, for very kind support with housing and processing of parasite material; Anton Lamboj for confirming several cichlid specimen identifications; Björn Rogell and two anonymous referees for useful comments on the manuscript. This study was financially supported through funds from the Davis Expedition Fund (University of Edinburgh), the Helge Ax:son Johnsons Stiftlese, and the Swedish Royal Academy of Sciences (KVA) Stiftelsen Hierta-Retzius stipendiefond to A.H., the Japanese Student Services Organization (JASSO) to M.T., the Zoologiska foundation to M.T. and A.K., an Austrian Science Fund grant to A.K. (J 3304-B24), and a Swedish Research Council grant to N.K.

Abstract

Parasite diversity and abundance (parasite load) vary greatly among host species. However, the influence of host traits on variation in parasitism remains poorly understood. Comparative studies of parasite load have largely examined measures of parasite species richness, and are predominantly based on records obtained from published data. Consequently, little is known about the relationships between host traits and other aspects of parasite load, such as parasite abundance, prevalence, and aggregation. Meanwhile, understanding of parasite species richness may be clouded by limitations associated with data collation from multiple independent sources. We conducted a

field study of Lake Tanganyika cichlid fishes and their helminth parasites. Using a Bayesian phylogenetic comparative framework, we tested evolutionary associations between five key host traits (body size, gut length, diet breadth, habitat complexity, number of sympatric hosts) predicted to influence parasitism, together with multiple measures of parasite load. We find that the number of host species that a particular host may encounter due to its habitat preferences emerges as a factor of general importance for parasite diversity, abundance, and prevalence, but not parasite aggregation. In contrast, body size and gut size are positively related to aspects of parasite load within, but not between species. The influence of host phylogeny varies considerably among measures of parasite load, with the greatest influence exerted on parasite diversity. These results reveal that both host morphology and biotic interactions are key determinants of host-parasite associations, and that consideration of multiple aspects of parasite load is required to fully understand patterns in parasitism.

Keywords: endoparasite, evolution, fish, helminth, parasitic worm

Introduction

Parasites are diverse and abundant components of all ecosystems with important and varied impacts on their hosts, including decreasing host fitness (Little *et al.*, 2010), promoting host phenotypic innovation (Hamilton & Zuk, 1982; Preston *et al.*, 2009; Feschotte & Gilbert, 2012), and driving host diversification (Buckling & Rainey, 2002). Indeed, parasite-mediated selection is postulated to be an important evolutionary force (Bell, 1982; Hamilton & Zuk, 1982; Koskella & Lively, 2009), and consequently there is a need to determine how aspects of host biology influence host-parasite interactions (Lively *et al.*, 2014).

To date, comparative phylogenetic analyses of the influence of host traits on parasitism remain largely restricted to considerations of parasite species richness (PSR) (e.g. Table S1). This is because most studies are literature-based, and PSR is often the only measure obtainable from published records. Consequently, the relationship between host biology and additional measures that more fully characterise parasite load, such as parasite abundance, prevalence, and aggregation, have been poorly explored in an evolutionary context.

A recent meta-analysis that tested the role of host body size, geographical range size, population density, and latitude on PSR across a large sample of studies found that the first three of these traits act as universal predictors (Kamiya *et al*., 2014a). This finding provides strong evidence that host traits have an important influence on parasitism. Thus, further studies are necessary to extend consideration to a wider suite of host traits, and to elucidate if similar relationships exist for additional measures of parasite load.

Here, we collect data on host and parasite traits simultaneously and consistently across host species by conducting a large-scale field study, for analysis within a phylogenetic comparative framework. Importantly, we extend our analysis beyond the typical focus on PSR, to perform a more complete dissection of the evolutionary relationships between host traits and parasite load. We focus on Lake Tanganyika cichlid fishes (Cichlidae: Pseudocrenilabrinae) as a model host system. While this spectacular vertebrate adaptive radiation has been heavily utilised for evolutionary research (Koblmüller *et al.*, 2009; Muschick *et al.*, 2012), and despite their great acknowledged promise for evolutionary parasitology, Lake Tanganyika cichlid parasites remain poorly explored (Vanhove *et al.*, 2016). The Lake Tanganyika cichlid system provides particular potential to examine the influence of host biology on parasite load, since it offers extreme diversity in host traits among closely-related host species, with repeated trait gains and losses across phylogeny (Muschick *et al.*,

2012). This allows the influence of specific host traits to be teased apart without the confounding effect of long divergence times between hosts. Additionally, co-existence of many hosts at the same locality avoids confounding effects arising as a consequence of geographical separation.

We focus on patterns for gastrointestinal helminths (acanthocephalans, cestodes, nematodes, trematodes), which are a diverse and important group of endoparasitic taxa that can exert varied effects on their hosts (Chowdhury & Aguirre, 2001; Moore, 2002; Poulin, 2007). Specifically, we test how different measures of parasite load (parasite diversity, abundance, prevalence, and aggregation) respond to variation in host species traits predicted to exert a positive effect on parasitism. Host traits examined are body size, host gut length, ecological niche as determined by dietary breadth and complexity of the surrounding habitat, and coexistence with other host species (see Table 1 for details of the hypotheses examined and predicted relationships).

Materials and Methods

Specimen collection

Thirty-seven species from across Lake Tanganyika cichlid diversity were selected for sampling to capture variation across the host radiation (Harvey & Pagel, 1991). Sampling was conducted in 2012, at the southern tip of Lake Tanganyika near Kalambo Falls in Zambia (-8°37'24.36" 31°12'3.28", 781m.a.s.l.), in compliance with local legislation, during August-September, which corresponds to the end of the dry season. Cichlids were collected while snorkeling or scuba diving using hand nets and monofilament gill nets, with the assistance of local fishermen. In total, 502 individuals were sampled from the target host species (see Figure 1 for species names). After collection, fish were housed in single-species lakeside tanks without access to food for ~24h to reduce gut contents and facilitate sorting of parasite material. Samples were processed in a field laboratory adjacent to the collection site. Fish were euthanised in small batches in the field using an overdose of benzocaine,

after which they were immediately photographed, weighed (precision = 1mg), and measured (standard length in mm; from the tip of the snout to the end of the caudal peduncle). The gastrointestinal tract was excised from oesophagus to anus, uncoiled along a millimetre ruler to determine length, and placed in a petri dish containing saline solution (0.9% NaCl). The gastrointestinal tract was opened longitudinally to release contents, which were fixed together with the opened gut in 95% ethanol following a standard gut wash protocol (Justine *et al.*, 2012). All researchers measuring guts (AH, AK, SDB, JZ) processed individuals of all species to avoid observer bias. Gut material was stored at 4°C until sorting, wherein parasite specimens were separated under a dissecting microscope according to higher helminth taxa (Acanthocephala, Cestoda, Digenea, Nematoda) following Paperna (1996).

Parasite measures

We focussed on helminth endoparasites in our analysis, which are a common group to examine when considering broad-scale patterns in host-parasite interactions (Table S1). Representatives from the four main gut endoparasite groups (Acanthocephala, Cestoda, Digenea, Nematoda) were found in our samples, and all cichlid species examined except one (*Limnochromis staneri*) harboured parasites (raw data are provided in Supplementary Table 2). Four measures of parasite load were investigated: parasite diversity, abundance, prevalence, and aggregation (described below). While these measures are not fully independent, each considers a distinct aspect of parasite load (see below).

Parasite diversity was calculated at the level of higher helminth taxa (Acanthocephala, Cestoda, Digenea, Nematoda) in our study. Consequently, we refer to this measure, which is a simple count of the number of higher helminth taxa identified in a host, as 'higher taxa richness'. Higher taxa richness is a coarser measure of parasite diversity compared to parasite species richness (PSR), which is a count of the number of parasite species recorded for a

given host species, and the measure typically employed in comparative analyses of parasitism. This difference should be considered when comparing our results with those of studies that employ PSR, since species-level counts typically lead to higher estimates of diversity than those captured by our measure. We were constrained to consider higher taxa richness by the poor current taxonomic understanding of Lake Tanganyika cichlid helminth communities. However, the level of diversity we adopt considers classical parasitic helminth groups separated by fundamental ecological and evolutionary differences (Paperna, 1996; Goater *et al*., 2013).

Parasite diversity is a complicated variable that can be estimated and analysed in various ways, and we employed several approaches to ensure robust conclusions: (i) we examined individual host parasite higher taxa richness data in Bayesian phylogenetic mixed model analyses (BPMM, see below), (ii) we examined mean parasite higher taxa richness per host species using phylogenetic generalised least squares analysis (PGLS, see below), (iii) we examined cumulative parasite higher taxa richness using both BPMM and PGLS analyses. Many literature-based studies on PSR (Table S1) are constrained to consider cumulative parasite diversity per host species. However, cumulative parasite diversity assumes that low and high incidence parasites contribute equally to parasite diversity, and so alternative measures may be favorable where individual-level data are available.

Parasite abundance was calculated as the total number of parasite specimens recovered per individual host. We considered individual host-level parasite abundance data in BPMM analyses, and mean abundance per host species using PGLS. Parasite prevalence was calculated as the proportion of infected hosts per species. Parasite aggregation, the degree of evenness in parasite abundance within a sample, was calculated for each species using the index of dispersion ($I_D = s^2(n\text{-}1)/\bar{x}$), by multiplying the variance-to-mean ratio by the number of hosts sampled minus 1 (Elliott, 1971). In each case, these measures combine parasites

across all four major helminth groups. This provides the potential to explore general patterns in parasitism, particularly with respect to epidemiological aspects of overall infection. However, as for any measure of parasitism, there are potential drawbacks associated with this approach that should be considered when interpreting our results. For example, while we have no *a priori* reason to suspect differences in host virulence among the helminths we recovered, and no extreme variation was observed in helminth size, two hosts with the same parasite abundance may potentially experience drastically different impacts from parasitism, given variation in biomass and virulence among the helminth species that infect them.

Host biology

Values for body mass and gut length were calculated directly from our samples, and varied considerably among species (body mass: 7-1586g; gut length: 11-961mm). An alternative measure of body size, standard length, is sometimes used instead of body mass. However, body mass and length are strongly correlated in our data ($R_S = 0.95$, $n = 502$, $p < 0.001$), and so we focus on body mass. We control for variation in body mass in our analyses, as it is known to be correlated with many other traits. Diet breadth was calculated as the number of taxonomically distinct animal prey groups that can act as intermediate hosts for helminths (1-4: molluscs, insects, crustaceans, and/or fish) that cichlid species feed on, as determined from published studies (Gonzalez-Voyer *et al.*, 2009; Muschick *et al.*, 2012). Data on habitat complexity was also taken from existing datasets (Seehausen *et al.*, 1999; Gonzalez-Voyer *et al.*, 2008; Gonzalez-Voyer *et al.*, 2009). Habitat complexity was scored as a continuous measure according to the physical properties of the environment (particularly rugosity), in replicate quadrats as determined by Pollen *et al.* (2007), and weighted according to preferred host habitat in order of increasing complexity: (1) benthopelagic, (2) semi-pelagic, (3) sandy or shallow vegetated, (4) rocky or rubble, (5) rock (Gonzalez-Voyer *et al.*, 2009). To

investigate if parasite load is influenced by surrounding host diversity, we included a measure of 'host environment species richness', which describes the number of cichlid species that a host is exposed to as a consequence of its habitat preferences and geographic distribution in Lake Tanganyika. Host environment species richness was calculated with reference to the cichlid literature, across the following discrete and well-established habitat categories, which are distributed around the entire shoreline of Lake Tanganyika: deep, sandy, intermediate (rocky or rubble), rock (Pollen *et al.*, 2007; Gonzalez-Voyer *et al.*, 2009; Shumway, 2010). For example, a cichlid species that utilises just sandy habitat can only come into contact with cichlid species that also utilise the sandy habitat. In contrast, a cichlid species that utilises sandy, intermediate, and rock habitats can come into contact with cichlid species present in each of these habitats. Host environment species richness varies from 29 species for cichlids that only inhabit the deep habitat to 126 species for those that utilise all habitats (Figure S1).

Comparative analyses

To test the influence of host species traits on the different measures of parasite load, while accounting for the non-independence of data arising due to shared ancestry between host species, we used phylogenetic comparative analyses (Harvey & Pagel, 1991). To model the evolutionary relationships between species we used a previously published phylogeny of Tanganyika cichlids estimated using mitochondrial sequences (Amcoff *et al*., 2013), that we pruned to match sampled species.

To explore and control for both within- and between-species variation in the traits of interest, we used Bayesian phylogenetic mixed models (BPMMs) with Markov chain Monte Carlo (MCMC) estimation performed in the R package MCMCglmm (Hadfield, 2010). For models examining parasite abundance and higher taxa richness of parasites, one value per

individual was used and repeated measures per species were taken into account by fitting 'species' as a random effect. We modelled the non-independence of data arising from the phylogenetic relationships between host species by fitting a phylogenetic covariance matrix as a random effect (Hadfield, 2010; Hadfield & Nakagawa, 2010). Both parasite higher taxa richness and abundance were count data and we therefore modelled these variables using a Poisson error distribution. As some data were missing for gut length or body mass for individual fish, a reduced data set of 475 individuals was utilised for parasite higher taxa richness and abundance. Aggregation and prevalence data were expressed as a total value per species. Thus, only a phylogenetic covariance matrix was fitted, since a random effect was included and species averages for body mass and gut length were fitted in models as fixed effects. We modelled parasite prevalence using a binary error distribution, and parasite aggregation using a Gaussian error distribution. Measures of parasite prevalence and aggregation we derived from the full dataset of 502 individuals.

In all models, body mass, gut length, diet breadth, habitat complexity, and host environment species richness were fitted as fixed effects. Prior to analysis, the continuous fixed effects body mass and gut length were log transformed to normalise the data. An inverse gamma prior was specified in final models for all R and G-side random effects ($V = 1$, $v = 0.002$). For models with binary error distributions we set a prior of $mu = 0$, $V = 1+pi^2/3$ for each fitted effect, which improved chain mixing and reduced autocorrelation (Hadfield, 2010). During exploratory analyses, models were run using a variety of alternative priors, including parameter-expanded priors, and results were not found to be sensitive to prior specification. Mixed models allow for uneven sample sizes across random effects, however, for parasite aggregation, where species means were required (since I_D is a measure of parasite aggregation across a sample), data points for each species were weighted by the number of

individuals used to calculate the mean. This was accomplished using the mev term (1/(n-3)) (Hadfield & Nakagawa, 2010), thereby taking into account variation in sample size across species.

In all cases, MCMC chains were run for 8 million iterations, with a burn-in of 500,000 and a thinning interval of 2,500 to generate 3,000 posterior samples. Chains were examined to ensure good mixing and convergence tests were applied (Plummer *et al.*, 2006). Each analysis was run three times and the Gelman-Rubin statistic (potential scale reduction factor, PSRF) was used to compare within- and between-chain variance (Gelman & Rubin, 1992). Plots of MCMC traces from separate runs were examined for overlap, and PSRF was < 1.01, where convergence is indicated by a value < 1.1. Stationarity was assessed using the Heidelberg-Welch test (Heidelberger & Welch, 1983). Autocorrelation was examined using effective sample size estimates and chain lag values, and was low between successive samples of the posterior distribution (< 0.1). Posterior samples were used to calculate posterior means, 95% confidence intervals (lower and upper CI), and pMCMC (the number of simulated cases that are > 0 or < 0, corrected for a finite number of MCMC samples). Terms were considered statistically significant when 95% CIs did not span 0 and pMCMC values were less than 0.05 (Hadfield, 2010). Lynch's phylogenetic heritability ($\lambda = \sigma^2_P/(\sigma^2_P + \sigma^2_R)$) (Lynch, 1991), which is equivalent to Pagel's λ, was used as an estimator of phylogenetic signal (see 'phylogenetic variance', Figure 2) (Freckleton *et al.*, 2002; Hadfield & Nakagawa, 2010).

In addition to BPMMs, phylogenetic generalised least squares analyses (PGLS) (Martins & Hansen, 1997), were performed in the R package Caper (Orme *et al.*, 2011). BPMM analyses offer statistical flexibility and the ability to take into account and control for both within and between species variation in a Bayesian statistical framework. However, the approach is relatively new and few previous studies have been conducted using the method. Thus, inclusion of PGLS analyses provides a more direct comparison with previous work, while also allowing the robustness of our findings to be evaluated independently across methods. For analyses of parasite higher taxa richness, abundance, and prevalence, response variables were included as species averages, since it

is not possible to include multiple measurements per species within the PGLS framework. Additionally, parasite abundance and parasite aggregation (I_D) were $\log_{10}$ transformed, and parasite prevalence was logit transformed, to normalize data, as non-Gaussian error distributions cannot be analysed using PGLS. To permit comparisons among included variables, each explanatory variable was scaled to have a mean of zero and a standard deviation of one. During PGLS analyses, Pagel's λ was estimated using maximum likelihood based on the amount of phylogenetic signal observed in the model residuals. In all cases, homogeneity of variance was visually examined using a Q-Q plot.

Results

Effect of host species richness on parasite load

We found that host environment species richness had a significant positive effect on parasite higher taxa richness (BPMM: β = 1.01, CI = 1.00-1.02, pMCMC = 0.003, Table 2). This suggests that hosts that overlap in habitat with a greater diversity of other host species are more likely to support a higher diversity of parasite taxa. Host environment species richness also shared a significant positive relationship with parasite abundance (BPMM: β = 1.03, CI = 1.01-1.05, pMCMC = 0.013, Table 2), and parasite prevalence (BPMM: β = 5.09, CI = 5.00-5.17, pMCMC = 0.032, Table 2), suggesting that high host diversity also leads to a greater likelihood of infection, and infection with a greater number of parasites. No relationship between parasite aggregation and host environment species richness was recovered, this implies that the trait acts evenly across individuals within a species. Collectively these results provide support for the importance of the transmission hypothesis in determining parasite load (Table 1). The results of the phylogenetic generalised least squares (PGLS) analyses indicate that host environment species richness was the only host factor that was significantly related to parasite load, with a significant relationship to parasite higher taxa richness, abundance, and prevalence (Table 3).

Effect of host morphology (gut length and body mass) on parasite load

Gut length was found to be significantly positively associated with parasite higher taxa richness (BPMM: $\beta = 2.1$, CI = 1.06-3.84, pMCMC = 0.02, Table 2), and parasite abundance (BPMM: $\beta = 3.15$, CI = 1.36-7.1, pMCMC = 0.006, Table 2). This suggests that host species with longer guts harbour more parasites and a greater parasite higher taxa richness, but are not more likely to become initially infected. Body mass was significantly positively associated with parasite abundance (BPMM: $\beta = 3.16$, CI = 1.59-6.21, pMCMC = 0.002, Table 2), suggesting that larger hosts support a larger number of parasites, but are not more likely to harbour a higher diversity of parasite taxa or become initially infected.

In contrast to the BPMM models, we found no significant effect of gut length or body mass on any measure of parasite load using PGLS. This is potentially because in contrast to BPMMs, PGLS models do not consider within species variation. To test this hypothesis explicitly, we repeated BPMM analyses for parasite higher taxa richness and parasite abundance, including a fixed effect of species mean, which models variation between species, and a fixed effect of the mean-centered value per individual (e.g. individual body mass minus the species mean, divided by the standard deviation of body mass for that species), which models within species variation, for both body mass and gut length. This revealed that the observed significant results for body mass and gut length occurred only for mean-centered values and not for species means (Parasite higher taxa richness: mean-centered body size BPMM: $\beta = 0.01$, pMCMC = 0.9, species mean body size BPMM: $\beta = 0.65$, pMCMC = 0.164, mean-centered gut length BPMM: $\beta = 0.13$, pMCMC = 0.017, species mean gut length BPMM: $\beta = 0.62$, pMCMC = 0.317. Parasite abundance: mean-centered body size BPMM: $\beta = 0.21$, pMCMC = 0.001, species mean body size BPMM: $\beta = 1.27$, pMCMC = 0.254, mean-centered gut length BPMM: $\beta = 0.18$, pMCMC = 0.004, species mean gut length BPMM: $\beta = 0.322$, pMCMC = 0.824). Together these analyses confirm that the observed relationships between parasite abundance and body mass and gut length, and parasite higher taxa richness and body mass and gut length, represent within-species, microevolutionary effects, rather than macroevolutionary patterns.

Effect of ecological niche on parasite load

Habitat complexity and diet breadth were not significantly related to any of the measures of parasite load examined. The lack of any effects of habitat complexity and diet breadth on parasite load were consistent across both BPMM and PGLS analyses (see Table 2 and Table 3).

Cumulative parasite higher taxa richness

The results of analyses of parasite higher taxa richness based on cumulative higher taxa richness per species (see Parasite Variables, Material and Methods) (Table S3), were similar to those conducted with BPMMs (Table 2) and PGLS (Table 3). However, gut length was no longer significant in the BPMM analysis of cumulative higher taxa richness, presumably since signal is lost when not accounting for inter-individual variation using this measure of parasite higher taxa richness.

Host phylogenetic effects on parasite load

The amount of variance (%) explained by phylogeny varies considerably across measures of parasite load. Parasite higher taxa richness was the most evolutionarily conserved among the measures of parasite load considered, while parasite prevalence was the least evolutionarily conserved (Figure 2). High phylogenetic signature for parasite higher taxa richness suggests that closely related cichlid species harbour a more similar level of parasite diversity than more distantly related cichlid species, suggesting a co-evolutionary signature. Meanwhile, the low phylogenetic signature observed for parasite prevalence suggests that either the mechanisms regulating parasite prevalence may evolve rapidly, depleting phylogenetic signal, or that the initial likelihood of becoming infected with parasites versus remaining uninfected is predominantly influenced by epidemiological parameters such as force of infection, transmission rates, and mode of infection. The phylogenetic signature for parasite abundance was between that observed for parasite higher taxa richness and parasite prevalence, with slightly greater variation explained by species differences over and above

the influence of phylogeny. This suggests that phylogeny exerts an important influence on patterns of parasite abundance, but that stochastic and non-phylogenetic influences also play a considerable role.

Discussion

Our results suggest that a strong determinant of multiple aspects of parasite load is overlap in habitat among host species (host environment species richness). The relationship is positive in each case, such that individuals of a species whose habitat is occupied by many other host species are more likely to be infected in the first instance, and by a higher abundance and diversity of parasites. The effect of host environment species richness across multiple measures of parasitism implies that certain host traits can exert a general influence over distinct aspects of parasite load. However, we find that two other host traits, body mass and gut length, share a less general relationship with specific measures of parasite load (body mass, abundance only; gut length, higher taxa richness and abundance), and only at the within-species level. Consideration of the influence of host traits on estimates of parasite load in addition to PSR is poorly explored, and we suggest this should be investigated further in future studies.

We also examine phylogenetic signal across multiple aspects of parasite load, which has been poorly investigated thus far. Several studies have considered the relationship between host phylogeny and parasite taxonomic diversity, but results are variable. Little evidence of any phylogenetic signal in PSR for helminths and microparasites was found in a large sample of carnivores (Lindenfors *et al.*, 2007), or in carnivores, primates (exluding macroparasites where Pagel's $\lambda = 0.692$), and ungulates (Cooper *et al.*, 2012). In contrast, ectoparasite taxonomic richness was found to be phylogenetically conserved in seabirds (Hughes & Page, 2007). Our results suggest that the influence of phylogeny varies considerably among different measures of parasite load, with the greatest influence exerted on parasite higher taxa richness, and the least influence exerted on

parasite prevalence. As an accumulation of further studies consider the influence of phylogeny on measures of parasite load across host-parasite systems, it will be possible to explore the generality of these patterns and the potential mechanisms underlying them.

The positive association of host environment species richness with parasite higher taxa richness, abundance and prevalence, suggests that cichlid species that share habitats with a high diversity of other potential hosts have a greater parasite load. For parasite higher taxa richness, this finding is in line with ecological and evolutionary predictions shown to be of general importance in a recent meta-analysis (Kamiya *et al.*, 2014b). Specifically, from an ecological perspective, richer habitat heterogeneity (in this case a more diverse host community) is predicted to support richer species diversity (e.g. Kerr & Packer, 1997). From an evolutionary perspective, following Eichler's Rule (Eichler, 1942), richer host clades may support richer parasite diversity as a consequence of coevolutionary processes (e.g. Hawkins & Lawton, 1987). In the case of Lake Tangyika cichlids, these mechanisms presumably act in concert due to the sympatric nature of the adaptive radiation, making host environment species richness a particularly important parameter in this system.

For parasite prevalence and abundance, positive associations with host environment species richness may be driven by an increased likelihood of parasite transmission, as predicted by models of disease spread (Anderson & May, 1979; May & Anderson, 1979). Our results show that high host environment species richness is correlated with an increased, rather than reduced, risk of parasitism in Lake Tanganyika cichlids. This argues against any form of dilution effect acting among alternative cichlid hosts, whereby there is a negative relationship between disease risk and host diversity (Ostfeld & Keesing, 2000). Rather, our results support a form of amplification effect, where increased host diversity leads to a greater risk of parasitism (Keesing *et al.*, 2006). This may arise as a consequence of the close genetic relationship shared among Lake Tanganyika cichlids, despite their great ecological diversity.

Measures of host species richness are not frequently included in comparative studies that investigate the relationship between host factors and parasite load. However, Krasnov *et al.* (2004) reported a positive relationship between flea PSR and the number of sympatric host species belonging to the same subfamily for rodents. Similarly, Young *et al.* (2013) found that helminth PSR was positively associated with primate geographic range overlap, and that malaria prevalence in chimpanzees was positively associated with mammal species richness. Consequently, host environment species richness may represent an important variable for explaining variation in patterns of parasite load. This result has important evolutionary implications, as it implies that host traits directly related to parasitism, for example immune function, may not evolve simply in response to parasites, but also in response to the influence of surrounding host species, and how these coevolve with parasites.

An alternative explanation for the observed relationship between host environment species richness and parasite load is that host density is the major determinant of parasitism. However, if density were the dominant factor in this study, we would expect the following: (i) a significant result for habitat complexity, which is strongly associated with density in Lake Tanganyika cichlids (Shumway, 2010), and (ii) low variation in parasite load among hosts from the same habitat, since cichlid density in these habitats would be the major driver of parasite load. Neither of these patterns were observed.

The gut is the focal interface for interactions between hosts and gut helminth parasites. We found that gut length emerges as a predictor of parasite load, sharing a positive relationship with parasite higher taxa richness and abundance, but only at the within-species level. Presumably this result arises as a consequence of the species-area effect, whereby larger areas support a greater number and diversity of parasites (MacArthur & Wilson, 1967; Holmes, 1990; Poulin, 2007).

Differential investment in the gut is much greater across than within species, and if energetic trade-offs were the causal factor, a significant between-species effect would be expected (Tsuboi *et al.*, 2015).

Interestingly, diet breadth and habitat complexity did not influence any aspect of parasite load, suggesting that trophic exposure and habitat do not exert strong effects on parasitism, at least in the focal host system. For diet breadth, behavioural adaptations or additional immune investment could offset exposure risks (Daly & Johnson, 2011; Boots *et al.*, 2013). Alternatively, individuals from different host species may all consume sufficient quantities of small invertebrates to overshadow an effect of variation in diet. For example, supposedly strict vegetarian hosts may ingest considerable quantities of small invertebrates together with plant matter, as is apparently also the case for herbivorous sea chub and cleaner fish examples from the Great Barrier Reef (Jones *et al.*, 2004; Huston *et al.*, 2016). Indeed, individuals of *Tropheus moorii,* a specialist algal feeder, and *Perissodus microlepis*, which feeds predominantly on the scales of other fish, both displayed reliatively high parasite loads.

Parasite distribution frequently varies in time and space (Poulin, 1998), and the composition of our parasite samples may have differed had we considered alternative sampling sites, or the same site at a different time points. However, the observed strong and consistent associations between our measures of parasite load and particular host traits observed here suggest that between-species patterns were not obscured by within-species stochasticity in parasite sampling.

The findings of this study demonstrate that aspects of host biology can play a key role in structuring parasite communities, above and beyond an effect on parasite species richness alone. Thus, comparative analyses of host biology that include multiple measures of parasite load offer the potential to provide valuable insights into the macro- and micro-evolutionary dynamics of host-parasite interactions, and should be investigated further in future studies.

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Figure legends

Figure 1. Variation in host traits across sampled Lake Tanganyika cichlid fish phylogeny. Branch colors on the phylogenetic tree represent cichlid tribes (orange, Bathybatini; yellow, Eretmodini; light green, Lamprologini; dark green, Limnochromini; light grey, Ectodini; dark grey, Cyprichromini; blue, Perissodini; purple, Tropheini). Host trait icons represent: body mass, gut length, diet breadth, habitat complexity, host environment species richness. Symbols for body mass and gut length are scaled by area, and both traits are log transformed. Diet breadth is represented by a scale from 0 to 4, indicating the number of taxonomically distinct prey groups consumed by each host species that may act as intermediate hosts (i.e. molluscs, insects, crustaceans, and/or fish). Habitat complexity is illustrated in 5 increasing increments from benthopelagic (represented by a circle outline) to rock habitat (full circular maze). Host environment species richness is represented by an increasing number of fish symbols from 2 to 10 (10 representing the greatest density).

Figure 2. Bar chart of percentage variance against the source of variance (phylogeny, species, error), as determined for each measure of Lake Tanganyika cichlid parasite load examined using Bayesian phylogenetic mixed models.

Table legends

Table 1. Predicted relationships between parasite load and the host traits examined in this study.

Table 2. Output statistics from Bayesian phylogenetic mixed model multivariate analyses for each measure of gut helminth parasite load and host traits examined across 37 species of Lake Tanganyika cichlid. Boldface type indicates significance, with level of significance denoted using asterisks as follows: * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

Table 3. Output statistics from phylogenetic generalized least squares multivariate analyses for each measure of gut helminth parasite load and host traits examined across 37 species of Lake Tanganyika cichlid. Boldface type indicates significance, with level of significance denoted using asterisks as follows: * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

Supporting Information

Supplementary Figure 1. Euler plot of the number of cichlid species per habitat type.

Supplementary Table 1. Overview of comparative studies investigating the influence of host traits on variation in parasite load. All studies used independent contrasts (IC) for analyses, except Cooper et al. (2012) who used phylogenetic generalized least squares (PGLS), and Bordes et al. (2011) who used PGLS and IC. Significance is reported in bold for host traits that were significant without controlling for additional traits (excluding body mass). Direction of correlation is indicated as positive (+) or negative (-). Abbreviations are: Sp., species; Endo, endoparasites; Ecto, ectoparasites; Micro, microparasites; PSR, parasite species richness; Tax. dist., taxonomic distinctness; Var., variance; Abun., abundance; Prev., prevalence; Shan. ind., Shannon index; BMR, basal metabolic rate.

Supplementary Table 2. Raw dataset of Lake Tanganyika cichlid host traits and parasite load.

Supplementary Table 3. Output statistics from Bayesian phylogenetic mixed model and phylogenetic generalized least squares analyses for parasite diversity considering maximum diversity and host traits examined across 37 species of Lake Tanganyika cichlid. Boldface type indicates significance, with level of significance denoted using asterisks as follows: * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

Supplementary Table 4. Output statistics from Phylogenetic Generalized Least Squares (PGLS) analysis for parasite higher taxa richness considering maximum parasite higher taxa richness and host trait examined across 37 species of Lake Tanganyika cichlid. Boldface type indicates significance, with level of significance denoted using asterisks as follows: * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

Table 1. Predicted relationships between parasite load and the host traits examined in this study

Host trait	Predicted relationship with measures of parasite load	Hypotheses
Gut size: gut length	Diversity: positive Abundance: positive Prevalence: positive Aggregation: positive	**Species-area:** evolution of a larger gut offers a greater area for colonization with more niches, leading to higher parasite abundance and diversity in hosts with a larger gut (e.g. Holmes 1990; Poulin 2007). **Costly-tissue:** investment into production and maintenance of costly tissue such as the gut may limit investment in defence against parasites, leading to higher parasite prevalence, abundance, and diversity in hosts with a larger gut (e.g. Sheldon and Verhulst 1996; Rauw 2012).
Dietary breadth: diversity of animal groups in the diet	Diversity: positive Abundance: none Prevalence: none Aggregation: none	**Trophic exposure:** hosts that evolve a wider dietary range may be exposed to a greater diversity of trophically transmitted parasites, since parasite species may vary in use of intermediate hosts and egg laying sites (e.g. Poulin 1995; Marcogliese 2002).
Habitat complexity: structural complexity of the habitat	Diversity: positive Abundance: none Prevalence: none Aggregation: none	**Habitat:** hosts in complex habitats may be infected with a greater diversity of parasites, since a wider variety of niches are available to support host-parasite coevolution involving a wider range of intermediate hosts (e.g. Combes 2005; Poulin 2007).
Host environment species richness: number of sympatric host species that overlap in habitat	Diversity: positive Abundance: positive Prevalence: positive Aggregation: none	**Transmission:** coevolution with other susceptible hosts can increase transmission rates of parasites amongst hosts leading to higher rates of parasite prevalence, and the support of more abundant and diverse parasite communities (e.g. May & Anderson 1979; Anderson & May 1979; Krasnov et al. 2004; Young et al. 2013).

Table 2. Output statistics from Bayesian Phylogenetic Mixed Model (BPMM) multivariate analyses for each measure of gut helminth parasite load and host trait examined across 37 species of Lake Tanganyika cichlid. Boldface type indicates significance, with level of significance denoted using asterisks as follows: * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

	Posterior mean	Lower CI	Upper CI	pMCMC	% variance
Parasite higher taxa richness:					
Fixed effects					
Body mass	1.281	0.758	2.112	0.344	
Gut length	**2.100**	**1.064**	**3.842**	**0.020 ***	
Diet breadth	1.067	0.897	1.285	0.461	
Habitat complexity	1.159	0.941	1.390	0.145	
Host environment species richness	**1.013**	**1.004**	**1.022**	**0.003 ****	
Random effects					
Phylogenetic variance	0.220	0.000	0.613		0.607
Individual variance	0.090	0.000	0.282		0.375
Residual variance	0.004	0.000	0.013		0.018
Parasite abundance:					
Fixed effects					
Body mass	**3.163**	**1.589**	**6.214**	**0.002 ****	
Gut length	**3.152**	**1.364**	**7.102**	**0.006 ****	
Diet breadth	0.873	0.557	1.313	0.522	
Habitat complexity	1.189	0.766	1.943	0.456	
Host environment species richness	**1.028**	**1.007**	**1.052**	**0.013 ***	
Random effects					
Phylogenetic variance	1.437	0.000	4.889		0.342
Individual variance	1.107	0.000	2.409		0.375
Residual variance	0.874	0.681	1.069		0.282
Parasite prevalence:					
Fixed effects					
Body mass	0.650	0.159	0.939	0.565	
Gut length	0.496	0.075	0.913	0.994	
Diet breadth	0.368	0.227	0.532	0.103	
Habitat complexity	0.532	0.342	0.683	0.722	
Host environment species richness	**0.509**	**0.500**	**0.517**	**0.032 ***	
Random effects					
Phylogenetic variance	1.476	0.000	8.446		0.216
Residual variance	3.215	0.000	6.441		0.784
Parasite aggregation:					
Fixed effects					
Body mass	0.155	-0.586	0.950	0.689	
Gut length	-0.015	-0.971	0.919	0.977	
Diet breadth	-0.062	-0.242	0.121	0.495	
Habitat complexity	-0.014	-0.194	0.181	0.873	

Host environment species richness	0.005	-0.002	0.014	0.190	
Random effects					
Phylogenetic variance	0.105	0.000	0.369		0.443
Residual variance	0.092	0.000	0.225		0.557

Table 3. Output statistics from Phylogenetic Generalized Least Squares (PGLS) multivariate analyses for each measure of gut helminth parasite load and host trait examined across 37 species of Lake Tanganyika cichlid. Boldface type indicates significance, with level of significance denoted using asterisks as follows: * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

	β ± s.e.	*P*	
Parasite higher taxa richness:			
Fixed effects			
Body mass	0.39 ± 0.19	0.063	
Diet breadth	-0.03 ± 0.16	0.849	
Habitat complexity	0.31 ± 0.18	0.090	
Host environment species richness	**0.41 ± 0.16**	**0.017**	*
Gut length	0.08 ± 0.20	0.682	
Total R^2:	0.41		
Parasite abundance:			
Fixed effects			
Body mass	0.26 ± 0.21	0.214	
Diet breadth	-0.16 ± 0.15	0.314	
Habitat complexity	0.24 ± 0.20	0.223	
Host environment species richness	**0.46 ± 0.17**	**0.012**	*
Gut length	-0.07 ± 0.27	0.793	
Total R^2:	0.31		
Parasite prevalence:			
Fixed effects			
Body mass	0.31 ± 0.21	0.137	
Diet breadth	-0.18 ± 0.16	0.277	
Habitat complexity	0.26 ± 0.18	0.158	
Host environment species richness	**0.40 ± 0.17**	**0.024**	*
Gut length	-0.05 ± 0.22	0.803	
Total R^2:	0.40		
Parasite aggregation:			
Fixed effects			
Body mass	0.20 ± 0.24	0.409	
Diet breadth	-0.20 ± 0.17	0.259	
Habitat complexity	0.08 ± 0.21	0.691	
Host environment species richness	0.29 ± 0.18	0.125	
Gut length	-0.19 ± 0.31	0.542	
Total R^2:	0.16