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

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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 and 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.

Introduction

Parasites are diverse and abundant components of all ecosystems with important and varied impacts on their

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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 characterize parasite load, such as parasite abundance, prevalence and aggregation, has 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 whether 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. Although this spectacular vertebrate adaptive radiation has been heavily utilized 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, because 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, coexistence 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″, 781 m.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 Fig. 1 for species names). After collection, fish were housed in single-species lakeside tanks without access to food for ~24 h to reduce gut contents and facilitate sorting of parasite material. Samples were processed in a field laboratory adjacent to the collection site. Fish were euthanized in small batches in the field using an overdose of benzocaine, after which they were immediately photographed, weighed (precision = 1 mg) 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 Table S2). Four measures of parasite load were investigated: parasite diversity, abundance, prevalence and aggregation (described below). Although these measures are not fully independent, each considers a distinct aspect of parasite load.

Parasite diversity was calculated at the level of higher helminth taxa (Acanthocephala, Cestoda, Digenea,

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 & 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, because 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, because 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 among hosts leading to higher rates of parasite prevalence and the support of more abundant and diverse parasite communities (e.g. Anderson & May, 1979; May & Anderson, 1979; Krasnov *et al.*, 2004; Young *et al.*, 2013)

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, because 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: (1) we examined individual host parasite higher taxa richness data in Bayesian phylogenetic mixed model analyses (BPMM), (2) we examined mean parasite higher taxa richness per host species using phylogenetic generalized least squares analysis (PGLS), (3) 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 favourable 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 ($I_D = s^2(n-1)/\bar{x}$), 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, although 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: 7–1586 g; gut length:

11–961 mm). 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 were also taken from existing data sets (Seehausen et al., 1999; Gonzalez-Voyer et al., 2008, 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 whether 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 utilizes just sandy habitat can only come into contact with cichlid species that also utilize the sandy habitat. In contrast, a cichlid species that utilizes 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 utilize all habitats (Fig. S1).
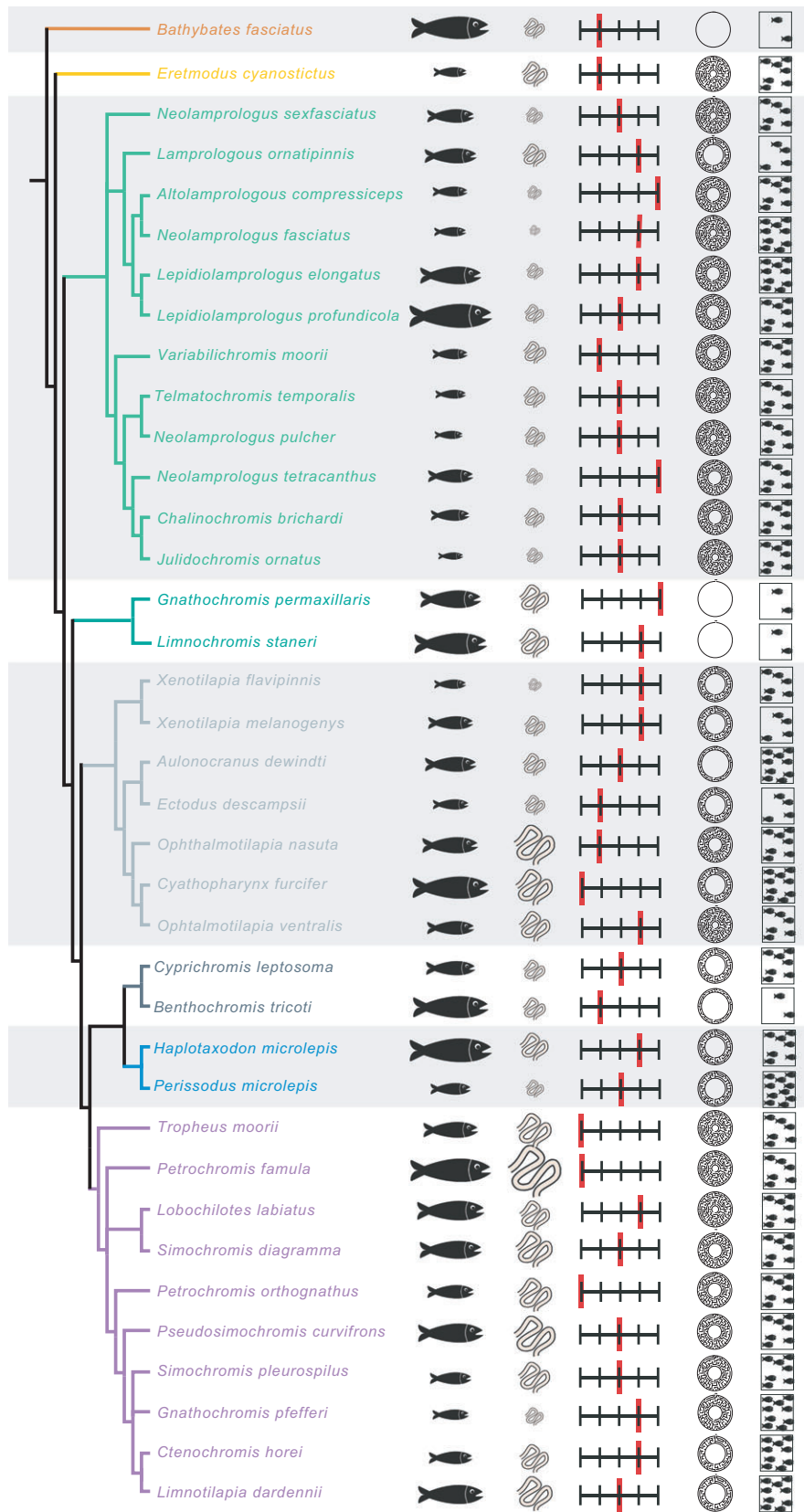
Comparative analyses

To test the influence of host species traits on the different measures of parasite load, while accounting for the nonindependence 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 nonindependence 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 utilized 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, because 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 data set 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 normalize the data. An inverse gamma prior was specified in final models for all R and G-side random effects ($V = 1$, $\nu = 0.002$). 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 (as 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 2500 to generate 3000 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 <0.05 (Hadfield, 2010). Lynch's phylogenetic heritability ($\lambda = \sigma_P^2 / (\sigma_P^2 + \sigma_R^2)$) (Lynch, 1991), which is equivalent to Pagel's λ, was used as an estimator of phylogenetic signal (see 'phylogenetic variance', Fig. 2) (Freckleton *et al.*, 2002; Hadfield & Nakagawa, 2010).

In addition to BPMMs, phylogenetic generalized 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, because 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: $\beta = 1.01$, 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: $\beta = 1.03$, CI = 1.01–1.05, pMCMC = 0.013, Table 2), and parasite prevalence (BPMM: $\beta = 5.09$, 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 generalized 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).
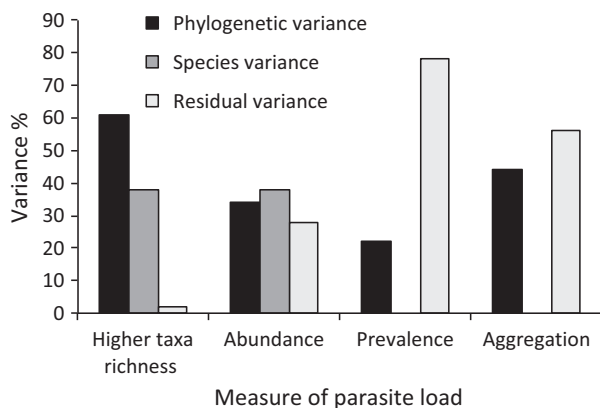
Fig. 2 Bar chart of percentage variance against the source of variance (phylogeny, species, error), as determined for each measure of Lake Tanganyika cichlid parasite load examined using Bayesian phylogenetic mixed models.

Table 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.

	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

Boldface type indicates significance, with level of significance denoted using asterisks as follows: *$P \leq 0.05$, **$P \leq 0.01$.

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

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.

	$\beta \pm SE$	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	

Boldface type indicates significance, with level of significance denoted using asterisks as follows: *$P \leq 0.05$.

effect of species mean, which models variation between species, and a fixed effect of the mean-centred 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-centred values and not for species means (parasite higher taxa richness: mean-centred body size BPMM: $\beta = 0.01$, pMCMC = 0.9, species mean body size BPMM: $\beta = 0.65$, pMCMC = 0.164, mean-centred gut length BPMM: $\beta = 0.13$, pMCMC = 0.017, species mean gut length BPMM: $\beta = 0.62$, pMCMC = 0.317; parasite abundance: mean-centred body size BPMM: $\beta = 0.21$, pMCMC = 0.001, species mean body size BPMM: $\beta = 1.27$, pMCMC = 0.254, mean-centred 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 was consistent across both BPMM and PGLS analyses (see Tables 2 and 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, Materials and Methods section) (Table S3 and S4) 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 because signal is lost when not accounting for interindividual 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, whereas parasite prevalence was the least evolutionarily conserved (Fig. 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 coevolutionary 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 vs. 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 nonphylogenetic 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 (excluding 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: (1) a significant result for habitat complexity, which is strongly associated with density in Lake Tanganyika cichlids (Shumway, 2010), and (2) low variation in parasite load among hosts from the same habitat, because 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 relatively 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 microevolutionary dynamics of host–parasite interactions and should be investigated further in future studies.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Euler plot of the number of cichlid species per habitat type.

Table S1. Overview of comparative studies investigating the influence of host traits on variation in parasite load.

Table S2. Raw data set of Lake Tanganyika cichlid host traits and parasite load.

Table S3. 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.

Table S4. 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.