1 **Parasite escape through trophic specialization in a species flock**

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23

24 **Abstract**

25 In adaptive radiations species diversify rapidly to occupy an array of ecological niches. In these

26 different niches, species might be exposed to parasites through different routes and at different

27 levels. If this is the case, adaptive radiations should be accompanied by a turnover in parasite

28 communities. How the adaptive radiation of host species might be entangled with such a turnover of

29 parasite communities is poorly documented in nature. In the present study, we examined the

30 intestinal parasite faunas of eleven species belonging to the tribe Tropheini, one of several adaptive

31 radiations of cichlid fishes in Lake Tanganyika. The most parsimonious ancestral foraging strategy

32 among Tropheini is relatively unselective substrate ingestion by browsing of aufwuchs. Certain

33 lineages however evolved more specialized foraging strategies, such as selective combing of

34 microscopic diatoms or picking of macro-invertebrates. We found that representatives of such

35 specialized lineages bear reduced infection with intestinal acanthocephalan helminths. Possibly, the

36 evolution of selective foraging strategies entailed reduced ingestion of intermediate invertebrate

37 hosts of these food-web transmitted parasites. In Tropheini, trophic specialization is therefore

38 intertwined with divergence in parasite infection. We conclude that the study of parasite

39 communities could improve our understanding of host evolution, ecological speciation and the origin

40 of adaptive radiations.

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Introduction

A popular approach to speciation research is to study adaptive radiations during which a lineage evolves rapidly to specialize into an array of distinct ecological niches. Exposure to parasite infection is for a large part determined by the hosts' niche and ecological divergence can invoke shifts in parasite communities (Knudsen *et al.*, 1996; MacColl, 2009a). How parasite communities shift when their host diversifies is an important question in evolutionary ecology that received increased attention in recent years (Eizaguirre *et al.*, 2009; MacColl, 2009b; Karvonen & Seehausen, 2012; Vanhove *et al.*, 2016). Parasite turnover (e.g. loss of or 'escape' from, but also gain of certain parasite taxa) is often considered in a spatial context, where hosts can for example avoid parasites while they invade areas that do not harbour their native parasite communities ('enemy release hypothesis' (Keane & Crawley, 2002)). Less documented is ecological or evolutionary escape where changes in host traits lead to parasite escape *in situ* (Chew & Courtney, 1991). In analogy, host species can also become infected with new parasites, e.g. when they adopt a predatory life-style and start feeding on intermediate hosts (Bell & Burt, 1991). Finally, parasite turnover might also be unrelated to diet, but rather follow demographic or phylogenetic divergence of their hosts (Wagner & McCune, 2009; Koblmüller *et al.*, 2010; Grégoir *et al.*, 2015; Vanhove *et al.*, 2015). In the context of adaptive radiations, differentiated parasite communities of incipient host species could impose divergent selection pressures that add up to frequently recognized drivers of speciation such as mate choice or resource competition. Parasites could thereby potentially accelerate and stabilize host divergence (Eizaguirre *et al.*, 2009; MacColl, 2009b; Karvonen & Seehausen, 2012). Parasites are ubiquitous in nature, likely comprising more than half of global animal diversity (Windsor, 1998). They are known to exert strong selection on hosts and can be highly host specific (Morand, 2015). Insights in changes of parasite infection upon host diversification are therefore of general relevance and could significantly improve our understanding of adaptive radiations.

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69 The likelihood of parasite infection is, aside from parasite infectivity and host susceptibility, often

70 determined by exposure risk related to habitat use and trophic position. Many parasites have

71 intermediate larval stages infecting prey of their definite (or secondary intermediate or paratenic)

72 hosts (Williams & Jones, 1994). Food-web transmission is therefore a prime infection route,

73 especially for intestinal helminths. At the micro-evolutionary scale, trophic divergence of has been

74 shown to lead to predictable differences in parasite infection among recently diverged species pairs

75 (Karvonen & Seehausen, 2012) or specialized trophic phenotypes (Stutz *et al.*, 2014). Similarly, diet

76 has long been recognized as an important predictor of parasite infection at the macro-evolutionary

77 scale (e.g. Drobney *et al.*, 1983; Bell & Burt, 1991; Vitone *et al.*, 2004; Valtonen *et al.*, 2010).

78 Diversification along dietary gradients occurs in many adaptive radiations including the iconic

79 examples of sticklebacks (Schluter, 1996), lake whitefish (Kahilainen *et al.*, 2011) and cichlids

80 (Muschick *et al.*, 2012). Adaptive radiations should therefore be expected to be accompanied by a

81 predictable turnover in parasite communities. For example, trophic specialization may lead to the

82 avoidance of intermediate hosts as prey items and as such breaking infection routes. The opposite,

83 acquiring new parasite species through new intermediate host prey or other niche-related features

84 (e.g. interactions with other hosts species), is possible too. While it is widely recognized that such

85 turnovers could accelerate and stabilize the process of host species divergence (Eizaguirre *et al.*,

86 2009; MacColl, 2009b), there is a remarkable shortage of empirical research on the interaction

87 between host evolution and parasite infection in adaptive radiations.

88 The Tropheini tribe comprises one of several adaptive radiations of cichlid fishes from Lake

89 Tanganyika, one of the Great East African Lakes. It currently includes 23 nominal species which occur

90 mostly in sympatry on rocky outcrops in the littoral zone throughout the lake. Phylogenetic

91 relationships among tropheine species are well resolved (Koblmüller *et al.* 2010; Fig. 1A) and the

92 trophic and behavioural ecology of most species has been studied extensively (Kawanabe, Hori &

93 Nagoshi 1997). Tropheini diversified in foraging behaviour and four trophic ecomorphs have been

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94   recognized among them: pickers (preying on arthropods), suckers (molluscs), combers (diatoms

95   attached to aufwuchs) and browsers (aufwuchs, mostly consisting of filamentous algae; Yamaoka

96   1997; Muschick *et al.* 2012; Tada *et al. in press*). Considering the most complete phylogenetic tree of

97   Tropheini (Koblmüller *et al.*, 2010), opportunistic browsing of aufwuchs is the most parsimonious

98   ancestral state for the Tropheini radiation from which more specialized foraging strategies have

99   evolved. Browsers are also the most heterogeneous ecomorph regarding their trophic ecology,

100   comprising both specialized aufwuchs-feeders as well as more generalist species supplementing their

101   algae-diet with insects, crustaceans, fish and fish eggs (Muschick *et al.*, 2012). Foraging strategies in

102   Tropheini have direct effects on many axes of diversification including morphology and the feeding

103   apparatus (Kawanabe *et al.*, 1997; Muschick *et al.*, 2012), intestine length (Sturmbauer *et al.*, 1992;

104   Wagner *et al.*, 2009; Tada *et al.*, *in press*), territorial behaviour (Kawanabe *et al.*, 1997) or dispersal

105   capacity (Wagner & McCune, 2009; Koblmüller *et al.*, 2010; Grégoir *et al.*, 2015; Vanhove *et al.*,

106   2015).

107   Acanthocephala are the most abundant intestinal parasites of Tropheini (Raeymaekers *et al.*, 2013;

108   Hablützel *et al.*, 2016). They are typically generalistic parasites with relatively low species diversity

109   and wide host ranges (Vanhove *et al.*, 2016). We reported in an earlier study that Acanthocephala

110   infection levels in sympatric *Simochromis diagramma* (Günther 1894) and *Tropheus moorii* Boulenger

111   1898 co-varied across sites (Hablützel *et al.*, 2016), indicating that these hosts (and potentially all

112   Tropheini species) might be infected by the same Acanthocephala species. Acanthocephala exhibit a

113   complex life-cycle with arthropods (commonly amphipods, ostracods or copepods (Williams & Jones,

114   1994)) serving as intermediate hosts. Infection occurs upon ingestion of the intermediate host

115   (Williams & Jones, 1994), which remains unidentified to date for Lake Tanganyika. We repeatedly

116   observed ostracods in the intestines of several Tropheini species (pers. obs.), making them candidate

117   vectors of Acanthocephala intermediate stages.

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118   During the Tropheini adaptive radiation, evolutionary versatility of the feeding apparatus and novel

119   ecological opportunities allowed species to conquer new positions in the food-web. We therefore

120   hypothesize that trophic specialization within the Tropheini radiation is accompanied by predictable

121   shifts in the intestinal parasite communities. Specifically, we expect that species with little

122   discrimination for the ingested particles (browsers and suckers) more often accidentally swallow

123   intermediate invertebrate hosts than the more specialized pickers and combers. We will test this

124   hypothesis by relating intestine lengths (serving as a univariate proxy for diet) with the abundance of

125   metazoan parasites in eleven species of Tropheini, covering all genera and ecomorphs of this species

126   flock.

127

128   **Material & Methods**

129   *Sampling and parasitological screening*

130   Fish were collected in September 2011 and August 2012 at three locations (Cape Kanwankoko

131   (2012): 8° 27' 8.0'' S, 30° 27' 20.0'' E, Muzumwa (2011): 8° 42' 5.7'' S, 31° 11' 59.8'' E and Toby's Place

132   (2012): 8° 37' 18.9'' S, 31° 11' 59.9'' E) at the Zambian shoreline of Lake Tanganyika at a depth of

133   about 0.5 - 3 m (Fig. 1B). Eleven species of Tropheini cichlids encompassing all nine genera were

134   sampled at one, two or three locations respectively (sample sizes in Table 1). At all sites, the

135   collected species occupy the same habitat and are commonly caught in the same net. Between

136   capture and dissection, fish were kept in tanks filled with lake water for at least one night to empty

137   their intestine (which allows for a more reliable parasite count). Keeping fish up to three days in such

138   tanks has little (slight increase for *Gyrodactylus*) or no effect (other parasite taxa) on parasite counts

139   (Raeymaekers et al., 2013). Immediately before dissection, fish were euthanized with an overdose of

140   MS222 and measured to the nearest 0.1 mm (standard length). Intestines were screened for

141   metazoan macro-parasites in the field using a stereomicroscope. The dissection of each fish started

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142   with the screening of its integument for monogeneans and crustaceans (copepods, branchiurans,

143   isopods) and any kind of helminthic cyst. The mouth cavity was then inspected for parasitic isopods

144   and branchiurans. Fish were inspected for gill parasites including branchiurans, copepods, bivalves,

145   monogeneans, and any kind of helminthic cyst. To do so, the gills were immediately dissected and

146   stored in 100% ethanol for later processing. Regarding endoparasites, fish were screened for

147   monogeneans, digeneans, acanthocephalans, nematodes, and any kind of helminthic cysts. To do so,

148   stomach, intestines, gall and urinary bladder were dissected immediately after euthanization of the

149   host and inspected in a Petri dish with lake water. Since most host specimen were dissected after

150   they emptied their intestine, the abundance of ostracodes could not be recorded systematically.

151   Processed fish were wrapped in cheese cloth, preserved on formalin and deposited at the Royal

152   Museum for Central Africa (Tervuren, Belgium) as vouchers (samples 2011: collection MRAC B1.23;

153   samples 2012: collection MRAC B2.38).

154

155   *Intestine length as an univariate proxy for diet*

156   Specialization in foraging ecology among Tropheini species has repeatedly and strongly been

157   associated with evolutionary changes in intestine length (Sturmbauer *et al.*, 1992; Wagner *et al.*,

158   2009; Tada *et al.*, *in press*). Pickers feed on readily digestible prey and, in line with the costly tissue

159   hypothesis (Tsuboi *et al.*, 2016), evolved short intestines. In contrast, combers and some specialized

160   browsers (both species of *Pseudosimochromis* Nelissen 1977 represented in our sampling) have long

161   intestines that can digest low quality diet such as diatoms or filamentous algae. Mean intestine

162   length (as a proportion of host standard length) per species was obtained from Tada *et al.* (*in press*).

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165   *Statistical analyses*

166   We used a generalized linear model (GLM) to describe the effects of host species, sampling site, host

167   size (standard length) and host sex (male, female or immature) on abundance (count of parasite

168   specimens per host individual) of acanthocephalan helminths, and the gill-infecting ectoparasite

169   genera *Cichlidogyrus* Paperna 1960 (Monogenea, Ancyrocephalidae) and *Ergasilus* von Nordmann

170   1832 (Copepoda, Ergasilidae). Other parasites (Nematoda, Digenea and unidentified helminthic cysts)

171   were found in the intestines in low numbers (overall prevalence < 0.05), preventing the application

172   of statistical models. The effect of sampling year was confounded with sampling site and was not

173   included in the model. We have shown earlier that parasite infection was relatively stable between

174   the two sampling years in one host species (*T. moorii*; Raeymaekers *et al.*, 2013). Since we were

175   interested in how far the species effect varies among sites, we ran the model a second time after

176   adding a species x site interaction effect. Abundance was fit on a GLM assuming a Poisson

177   distribution of parasite counts. Analysis of variance was conducted using type II sums of squares.

178   The association between species-level variation of intestine length and median abundance of

179   acanthocephalans, *Cichlidogyrus* or *Ergasilus* per host species and per site was analysed in a second-

180   order polynomial regression model. Sampling site was included as a random effect. Since the

181   distribution of parasite counts was not normal (few host individuals had many parasites) and the

182   relationship between Acanthocephala counts and intestine was right-tailed (Fig. 1D), both response

183   and predictor variables were log-transformed prior to statistical analyses. We repeated the analysis

184   with a reduced dataset from which all host species with less than 10 specimens (*Interochromis lookii*

185   (Poll 1949), *Limnotilapia dardennii* (Boulenger 1899) and *Pseudosimochromis curvifrons* (Poll 1942))

186   were excluded in order to assess whether our analysis was sensitive for the limited sample size of

187   some of the host species. To test the hypothesis that parasites replace each other across host taxa,

188   we conducted statistical tests for Pearsons's product-moment correlations among median

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189   abundances of the three most abundant parasite groups. All analyses were conducted in R v.3.3.0 (R

190   Development Core Team, 2011).

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Results

Parasites infecting every species included intestinal acanthocephalans, the ancyrocephalid monogenean *Cichlidogyrus* and the copepod *Ergasilus* on the gills. Parasites which were not present on every single host species included the gyrodactylid monogenean *Gyrodactylus* on skin and fins, intestinal nematodes, the monogenean *Urogyrus* in the urinary bladder, branchiurans in the gill cavity or on the opercula, intestinal digeneans, and a number of unidentified helminthic cysts in skin, fin or gill tissue. Acanthocephalans (found in all host species; median abundance: 0-6.5; Fig. 1D) dominated the intestinal parasite fauna while nematodes (7 host species; median abundance: 0), digeneans (2 host species; median abundance: 0) and helminthic cysts (3 host species; median abundance: 0) were observed sporadically and in low numbers (Appendix S1 Table S1). Host species was the strongest predictor of abundance of all parasite groups, while sampling sites, host size, host sex and the interaction between species and site had minor, although significant (except for host size on Acanthocephala and host sex on *Ergasilus*), effects (Table 2).

Intestine length significantly predicted median Acanthocephala abundance (Table 3). The association was curvilinear with species with short or long intestines bearing the lowest number of Acanthocephala (Table 3; Fig. 1D). The polynomial regression term remained significant after removing three host species with low sample sizes (p = 0.004). Pickers with short intestines had zero median abundance, although Acanthocephala could occasionally be observed in all host species (Appendix S1 Fig. S1). Suckers and three genera (*Limnotilapia* Regan 1920, *Simochromis* Boulenger 1898 and *Tropheus* Boulenger 1898) of browsers with intermediate intestine length showed the highest Acanthocephala infection (median abundance: 4-6.5). The two browser species of the genus *Pseudosimochromis* with rather long intestines were infected with relatively low numbers of Acanthocephalans (median abundance: 0.5-3). Finally, combers also showed low Acanthocephala abundance (median abundance: 0.5-1). Intestine length was not significantly correlated with *Cichlidogyrus* and *Ergasilus* counts (Table 3). The relationships did not change upon removal of three

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217   host species with low sample sizes (p = 0.579 and p = 0.184). We further found that parasite groups

218   did not replace each other across host taxa (Acanthocephala vs. *Cichlidogyrus*: correlation coefficient

219   = -0.143, p-value = 0.676; Acanthocephala vs. *Ergasilus*: correlation coefficient = -0.340, p-value =

220   0.307; *Cichlidogyrus* vs. *Ergasilus*: correlation coefficient = 0.407, p-value = 0.214).

221

222   **Discussion**

223   We hypothesized that diversification in foraging ecology could be accompanied by shifts in

224   (intestinal) parasite communities as found in several sympatric species pairs (Knudsen *et al.*, 1997;

225   MacColl, 2009a). Using the adaptive radiation of the Lake Tanganyika cichlid tribe Tropheini as a

226   model, we found that the abundance of trophically transmitted acanthocephalan helminths was

227   predicted by inter-specific variation in intestine length, which itself is strongly correlated with

228   differentiation in foraging strategy and diet (Sturmbauer *et al.*, 1992; Wagner *et al.*, 2009; Tada *et al.*,

229   *in press*). This observation was not paralleled by ectoparasites. We discuss to which extent trophic

230   diversification and parasite infection are intertwined and how this interplay might affect the hosts'

231   adaptive radiation.

232

233   *Foraging ecology predicts parasite infection*

234   Tropheini species could escape their acanthocephalan parasites twice by evolving specialized feeding

235   strategies (although escape is incomplete, since both pickers and combers may be infested with low

236   numbers of Acanthocephala; Figs 1C and 1D). Browsers (the most parsimonious ancestral ecomorph)

237   shear filamentous algae *in toto* from the substrate (Yamaoka, 1997), along with the associated micro-

238   invertebrate fauna (thus including the putative intermediate host of acanthocephalans). The grazing

239   species *Petrochromis* spp. and *Interochromis loocki*, in contrast, are specialized diatom feeders who

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240   comb their food from filamentous algae (Yamaoka, 1997). They are therefore able to selectively

241   ingest tiny particles (thus excluding the putative intermediate host, which measures around 1 mm,

242   while diatoms range from about 0.002-0.2 mm). The picker-lineage encompassing *'Ctenochromis'*

243   *horei* (Günther 1894) and *'Gnathochromis' pfefferi* (Boulenger 1898) evolved into selective predators

244   of insect larvae and larger crustaceans (e.g. shrimps (Muschick *et al.*, 2012)) that probably do not

245   carry Acanthocephala larval stages. The sucker *Lobochilotes labiatus* (Boulenger 1898) also preys on

246   macro-invertebrates (mainly molluscs (Colombo *et al.*, 2013), which are not known as hosts of

247   Acanthocephala (Williams & Jones, 1994)). However, due to its sucking feeding behaviour, the

248   species is (similar to browser species) relatively indiscriminate about the ingested items (Muschick *et*

249   *al.*, 2012). Indeed, we found, on average, high infection with acanthocephalan parasites in *L. labiatus*.

250   The relationship between parasite infection and host trophic ecology might be confounded by

251   geographic variation in parasite abundance and host-parasite co-evolutionary interactions (Bell &

252   Burt, 1991; Stutz *et al.*, 2014). Acanthocephala infections do indeed vary across the study area

253   (Raeymaekers *et al.*, 2013; Hablützel *et al.*, 2016; this study) but we found the confounding effect of

254   geography to be of little importance compared to the main host species effect. Ultimately, parasite

255   load will not only be influenced by ecological (exposure to propagules) but also evolutionary

256   (parasite virulence and host susceptibility) factors. Acanthocephalans are known to interact with the

257   immune system, although pathological effects are typically only observed upon massive infection

258   (Paperna, 1996). Laboratory experiments provided empirical evidence for heritable variation in

259   susceptibility to Acanthocephala infection in sticklebacks (Mazzi & Bakker, 2003), indicating that

260   different degrees of resistance could explain variation in infection intensities among host species.

261   Resistance to parasite infection might come at an immunological cost (Råberg *et al.*, 2009) that

262   trades off against the parasite burden, favouring tolerance towards the parasite if its virulence is low.

263   In at least one species of Tropheini cichlids (*T. moorii*), Acanthocephala infection has little or no

264   effect on host body condition, indicating some degree of tolerance evolution (Hablützel *et al.*, 2014).

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265   The selective pressure for tolerance or resistance evolution might be expected to be itself related to

266   exposure risk. Species suffering from high exposure should therefore experience the strongest

267   selection pressure to become resistant (or tolerant), a hypothesis that is not unlikely, but cannot be

268   tested with the current data.

269

270   *Parasite infection: an understudied dimension of adaptive radiations*

271   Specialization in foraging ecology is one of the most prominent processes in adaptive radiations.

272   Species divergence in this context is often considered a consequence of character displacement due

273   to resource competition (Schluter, 1994). The observation that parasite infection is inherently

274   intertwined with trophic diversification adds an understudied dimension to this process. Speciation

275   models and field studies suggest that trophic niche partitioning might be plastic at first and becomes

276   heritable upon genetic divergence of the incipient species (Pfennig *et al.*, 2010). Evolutionary escape

277   from parasites might accelerate and stabilize this process in two ways. First, the cost of adaptation to

278   new food sources might be compensated by parasite escape. Second, immunity gene pools might

279   diverge among incipient host species (Eizaguirre *et al.*, 2012) under both resistance or tolerance

280   scenarios. Dietary versatility through phenotypic plasticity might become costly upon immunogenetic

281   divergence, since neither of the diverging host lineages will be immunogenetically adapted to the

282   parasite community that is associated with the alternative foraging strategy. Certainly, the strong co-

283   variance between parasite community variation and niche divergence of their hosts highlights an

284   understudied component of adaptive radiations.

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298 **References**

299 Bell, G. & Burt, a. 1991. The Comparative Biology of Parasite Species-Diversity - Internal Helminths of
300   Fresh-Water Fish. *J. Anim. Ecol.* **60**: 1047–1063.

301 Chew, F.S. & Courtney, S.P. 1991. Plant apparency and evolutionary escape from insect herbivory.
302   *Am. Nat.* **138**: 729–750.

303 Colombo, M., Diepeveen, E.T.E., Muschick, M., Santos, M.E., Indermaur, A., Boileau, N., *et al.* 2013.
304   The ecological and genetic basis of convergent thick-lipped phenotypes in cichlid fishes. *Mol.*
305   *Ecol.* **22**: 670–84.

306 Drobney, R.D., Train, C.T. & Fredrickson, L.H. 1983. Dynamics of the platyhelminth fauna of wood
307   ducks in relation to food habits and reproductive state. *J. Parasitol.* **69**: 375–380.

308 Eizaguirre, C., Lenz, T.L., Kalbe, M. & Milinski, M. 2012. Rapid and adaptive evolution of MHC genes
309   under parasite selection in experimental vertebrate populations. *Nat. Commun.* **3**.

310 Eizaguirre, C., Lenz, T.L., Traulsen, A. & Milinski, M. 2009. Speciation accelerated and stabilized by
311   pleiotropic major histocompatibility complex immunogenes. *Ecol. Lett.* **12**: 5–12.

312 Grégoir, A.F., Hablützel, P.I., Vanhove, M.P.M., Pariselle, A., Volckaert, F. & Raeymaekers, J.A.M.
313   2015. A link between host dispersal and parasite diversity in two sympatric cichlid fishes of Lake
314   Tanganyika. *Freshw. Biol.* **60**: 323–335.

315 Hablützel, P.I., Grégoir, A.F., Vanhove, M.P.M., Volckaert, F.A.M. & Raeymaekers, J.A.M. 2016. Weak
316   link between dispersal and parasite community differentiation or immunogenetic divergence in
317   two sympatric cichlid fishes. *Mol. Ecol.* **25**: 5451-5466.

318 Hablützel, P.I., Vanhove, M.P.M., Gregoir, A.F., Hellemans, B., Volckaert, F.A.M. & Raeymaekers,
319   J.A.M. 2014. Intermediate number of major histocompatibility complex class IIB length variants
320   relates to enlarged perivisceral fat deposits in the blunt-head cichlid *Tropheus moorii*. *J. Evol.*
321   *Biol.* **27**: 2177–2190.

322 Kahilainen, K.K., Siwertsson, A., Gjelland, K., Knudsen, R., Bøhn, T. & Amundsen, P.A. 2011. The role
323   of gill raker number variability in adaptive radiation of coregonid fish. *Evol. Ecol.* **25**: 573–588.

324 Karvonen, A. & Seehausen, O. 2012. The role of parasitism in adaptive radiations – when might
325   parasites promote and when might they constrain ecological speciation? *Int. J. Ecol.* **2012**: 1–20.

326 Kawanabe, H., Hori, M. & Nagoshi, M. 1997. *Fish communities in Lake Tanganyika*. Kyoto University
327   Press, Tokyo.

328 Keane, R.M. & Crawley, M.J. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends*
329   *Ecol. Evol.* **17**: 164-170.

330 Knudsen, R., Klemetsen, a & Staldvik, F. 1996. Parasites as indicators of individual feeding
331   specialization in Arctic charr during winter in northern Norway. *J. Fish Biol.* **48**: 1256–1265.

15

332   Knudsen, R., Kristoffersen, R. & Amundsen, P.A. 1997. Parasite communities in two sympatric morphs
333   of Arctic charr, Salvelinus alpinus (L.), in northern Norway. *Can. J. Zool. Can. Zool.* **75**: 2003–
334   2009.

335   Koblmüller, S., Egger, B., Sturmbauer, C. & Sefc, K.M. 2010. Rapid radiation, ancient incomplete
336   lineage sorting and ancient hybridization in the endemic Lake Tanganyika cichlid tribe
337   Tropheini. *Mol. Phylogenet. Evol.* **55**: 318–334.

338   MacColl, A.D.C. 2009a. Parasite burdens differ between sympatric three-spined stickleback species.
339   *Ecography (Cop.).* **32**: 153–160.

340   MacColl, A.D.C. 2009b. Parasites may contribute to "magic trait" evolution in the adaptive radiation
341   of three-spined sticklebacks, Gasterosteus aculeatus (Gasterosteiformes: Gasterosteidae). *Biol.*
342   *J. Linn. Soc.* **96**: 425–433.

343   Mazzi, D. & Bakker, T.C.M. 2003. A predator's dilemma: prey choice and parasite susceptibility in
344   three-spined sticklebacks. *Parasitology* **126**: 339–347.

345   Morand, S. 2015. (macro-) Evolutionary ecology of parasite diversity: From determinants of parasite
346   species richness to host diversification. *Int. J. Parasitol. Parasites Wildl.* **4**: 80–87.

347   Muschick, M., Indermaur, A. & Salzburger, W. 2012. Convergent evolution within an adaptive
348   radiation of cichlid fishes. *Curr. Biol.* **22**: 2362–8.

349   Paperna, I. 1996. *Parasites, infections and diseases of fishes in Africa, an update.* Food and
350   Agriculture Organization of the United Nations, Rome.

351   Pfennig, D.W., Wund, M.A., Snell-Rood, E.C., Cruickshank, T., Schlichting, C.D. & Moczek, A.P. 2010.
352   Phenotypic plasticity's impacts on diversification and speciation. *Trends Ecol. Evol.* **25**: 459-467.

353   R Development Core Team. 2011. R: A language and environment for statistical computing. R
354   Foundation for Statistical Computing, Vienna, Austria.

355   Råberg, L., Graham, A.L. & Read, A.F. 2009. Decomposing health: tolerance and resistance to
356   parasites in animals. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **364**: 37–49.

357   Raeymaekers, J.A.M., Hablützel, P.I., Grégoir, A.F., Bamps, J., Roose, A.K., Vanhove, M.P.M., *et al.*
358   2013. Contrasting parasite communities among allopatric colour morphs of the Lake Tanganyika
359   cichlid *Tropheus*. *BMC Evol. Biol.* **13**: 41.

360   Schluter, D. 1996. Ecological Causes of Adaptive Radiation. *Am. Nat.* **148**: S40–S64.

361   Schluter, D. 1994. Experimental evidence that competition promotes divergence in adaptive
362   radiation. *Science (80-.).* **266**: 798–801.

363   Sturmbauer, C., Mark, W. & Dallinger, R. 1992. Ecophysiology of Aufwuchs-eating cichlids in Lake
364   Tanganyika: niche separation by trophic specialization. *Environ. Biol. Fishes* **35**: 283–290.

365   Stutz, W.E., Lau, O.L. & Bolnick, D.I. 2014. Contrasting patterns of phenotype-dependent parasitism
366   within and among populations of threespine stickleback. *Am. Nat.* **183**: 810–825.

367   Tada, S., Hori, M., Yamaoka, K. & Hata, H. *in press*. Diversification of functional morphology in
368   herbivorous cichlids (Perciformes: Cichlidae) of the tribe Tropheini in Lake Tanganyika.
369   *Hydrobiologia* 1–19.

370   Tsuboi, M., Kotrschal, A., Hayward, A., Buechel, S.D., Zidar, J., Løvlie, H., *et al.* 2016. Evolution of
371   brain–body allometry in Lake Tanganyika cichlids. *Evolution (N. Y).* **70**: 1559–1568.

372   Valtonen, E.T., Marcogliese, D.J. & Julkunen, M. 2010. Vertebrate diets derived from trophically
373   transmitted fish parasites in the Bothnian Bay. *Oecologia* **162**: 139–152.

374   Vanhove, M.P.M., Hablützel, P.I., Pariselle, A., Šimková, A., Huyse, T. & Raeymaekers, J.A.M. 2016.
375   Cichlids: a host of opportunities for evolutionary parasitology. *Trends Parasitol.* **32**: 820–832.

376   Vanhove, M.P.M., Pariselle, A., Van Steenberge, M., Raeymaekers, J.A.M., Hablützel, P.I., Gillardin, C.,
377   *et al.* 2015. Hidden biodiversity in an ancient lake: phylogenetic congruence between Lake
378   Tanganyika tropheine cichlids and their monogenean flatworm parasites. *Sci. Rep.* **5**: 13669.

379   Vitone, N.D., Altizer, S. & Nunn, C.L. 2004. Body size, diet and sociality influence the species richness
380   of parasitic worms in anthropoid primates. *Evol. Ecol. Res.* **6**: 183–199.

381   Wagner, C.E. & McCune, A.R. 2009. Contrasting patterns of spatial genetic structure in sympatric
382   rock-dwelling cichlid fishes. *Evolution (N. Y).* **63**: 1312–1–326.

383   Wagner, C.E., McIntyre, P.B., Buels, K.S., Gilbert, D.M. & Michel, E. 2009. Diet predicts intestine
384   length in Lake Tanganyika's cichlid fishes. *Funct. Ecol.* **23**: 1122–1131.

385   Williams, H. & Jones, A. 1994. *Parasitic Worms of Fish*. Taylor & Francis, London, UK.

386   Windsor, D.A. 1998. Most of the species on Earth are parasites. *Int. J. Parasitol.* **28**: 1939–1941.

387   Yamaoka, K. 1997. Trophic Ecomorphology of Tanganyika Cichlids. In: *Fish Communities in Lake
388   Tanganyika* (H. Kawanabe, M. Horic, & M. Nagoshi, eds), pp. 25–56. Kyoto University Press,
389   Kyoto.

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391

392   Table 1: Sampling sizes and median Acanthocephala abundance for eleven Tropheini species. Dashes

393   at Cape Kanwankoko and Muzumwa indicate that the species was very rare (and not necessarily

394   absent) at the respective sampling site. Dashes at Toby's place reflect limited sampling efforts due to

395   different initial study aims. Full names of species can be found in Fig. 1A.

| | **Sample size** | | | | **Median Acanthocephala abundance** | | | |
Species	Cape Kanwankoko	Muzumwa	Toby's Place	all sites	Cape Kanwankoko	Muzumwa	Toby's Place	all sites
Ctho	5	6	10	21	0	0	0	0
Gnpf	5	5	10	20	0	0	0	0
Lida	0	6	0	6	–	6.5	–	6.5
Lola	5	6	10	21	0	7	9	4
Inlo	0	6	0	6	–	1	–	1
Pefam	5	7	0	12	0	1	–	0.5
Pefas	5	6	0	11	1	1.5	–	1
Psba	5	5	0	10	4	1	–	3
Pscu	6	0	0	6	0.5	–	–	0.5
Sidi	5	31	35	71	3	4	4	4
Trmo	5	89	86	180	7	5	4	4
Total	46	167	151	364				

396

397   Table 2: Results of the generalized linear model for host species-level variation in abundance of

398   Acanthocephala, *Cichlidogyrus* and *Ergasilus* accounting for confounding effects of sampling site,

399   host standard length and host sex.

Acanthocephala

Effect	LR X^2	Num DF	Den DF	Pr(> X^2)
Species	308.89	10	149	< 0.001
Site	37.44	2	149	< 0.001
SL	1.54	1	149	0.214
Sex	16.89	2	149	< 0.001

Effect	LR X^2	Num DF	Den DF	Pr(> X^2)
Species	308.89	10	149	< 0.001
Site	37.44	2	149	< 0.001
SL	0.57	1	149	0.449
Sex	29.93	2	149	< 0.001
Species x Site	81.76	11	149	< 0.001

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Cichlidogyrus

Effect	LR X^2	Num DF	Den DF	Pr($> X^2$)
Species	5841.70	10	134	< 0.001
Site	394.91	2	134	< 0.001
SL	944.42	1	134	< 0.001
Sex	67.00	2	134	< 0.001

Effect	LR X^2	Num DF	Den DF	Pr($> X^2$)
Species	5841.70	10	134	< 0.001
Site	394.91	2	134	< 0.001
SL	592.77	1	134	< 0.001
Sex	30.55	2	134	< 0.001
Species x Site	356.72	11	134	< 0.001

Ergasilus

Effect	LR X^2	Num DF	Den DF	Pr($> X^2$)
Species	612.24	10	133	< 0.001
Site	108.71	2	133	< 0.001
SL	164.02	1	133	< 0.001
Sex	2.20	2	133	0.333

Effect	LR X^2	Num DF	Den DF	Pr($> X^2$)
Species	612.24	10	133	< 0.001
Site	108.71	2	133	< 0.001
SL	110.1	1	133	< 0.001
Sex	1.22	2	133	0.543
Species x Site	90.91	11	133	< 0.001

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Table 3: Results of the general linear mixed-effect model for the relationship between intestine length (predictor) and median parasite count (response). Sampling site was included as a random effect. Wald X^2-tests were used to assess the statistical significance of the linear model fit or of the improvement of the application of a second-order polynomial function, respectively.

Parasite	Model	Df	AIC	BIC	logLik	Deviance	Test	X^2	X Df	$Pr(>X^2)$
Acanthocephala	linear	6	128.0	135.0	-58.0	116.0	linear	0.25	1	0.615
	polynomial	10	117.6	129.4	-48.8	97.6	linear vs. polynomial	18.3	4	0.001
Cichlidogyrus	linear	6	225.8	232.8	-106.9	213.8	linear	0.74	1	0.390
	polynomial	10	231.0	242.8	-105.5	211.0	linear vs. polynomial	2.7	4	0.602
Ergasilus	linear	6	152.8	159.8	-70.4	140.8	linear	0.53	1	0.467
	polynomial	10	154.0	165.8	-67.0	134.0	linear vs. polynomial	6.7	4	0.150

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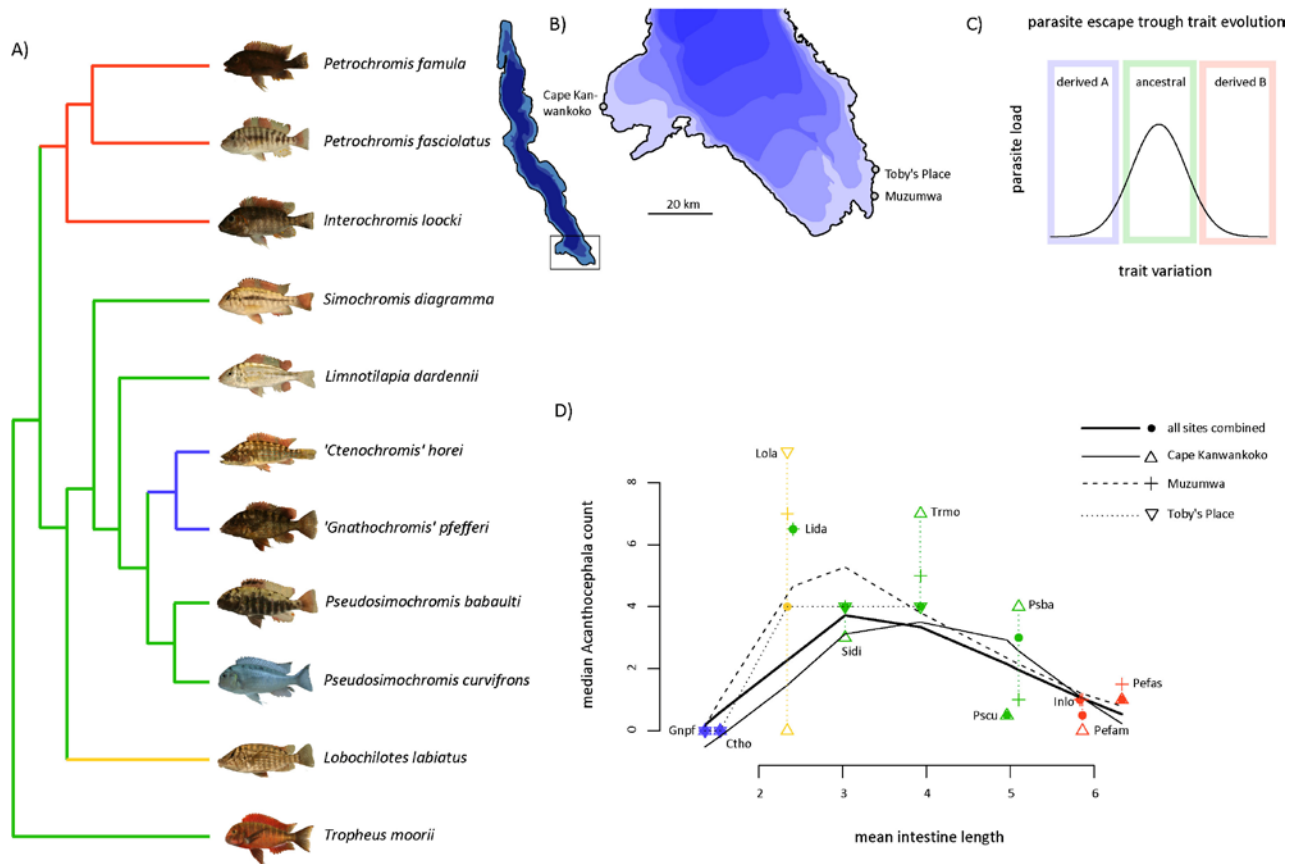
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411 Fig. 1: **A)** Cladogram reflecting phylogenetic relationships following Koblmüller *et al.* (2010). Host

412 ecomorph is coded as follows: green = browsers (most parsimonious ancestral state), yellow =

413 suckers, blue = pickers, red = combers. **B)** Sampling sites at the southern shore of Lake Tanganyika in

414 Zambia. **C)** Illustration of theoretical framework of parasite escape through specialization. Hosts with

415 ancestral trait state suffer from high parasite infection. Upon specialization, hosts are less exposed to

416 parasite infection. In the present example, the trait is foraging behaviour with browsing as ancestral

417 and sucking, picking or combing as derived states. **D)** The relative length of intestine (which directly

418 relates on diet and hence foraging ecology) is highly predictive for the abundance of

419 acanthocephalan helminths. Lines are lowess-functions. Species abbreviations include the first two

420 (three) letters of the genus names and species epithets, respectively.

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