



Habitat use and its implications to functional morphology: niche partitioning and the evolution of locomotory morphology in Lake Tanganyikan cichlids (Perciformes: Cichlidae)

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Animal locomotory morphology, i.e. morphological features involved in locomotion, is under the influence of a diverse set of ecological and behavioral factors. In teleost fish, habitat choice and foraging strategy are major determinants of locomotory morphology. In this study, we assess the influence of habitat use and foraging strategy on important locomotory traits, namely the size of the pectoral and caudal fins and the weight of the pectoral fin muscles, as applied to one of the most astonishing cases of adaptive radiation: the species flock of cichlid fishes in East African Lake Tanganyika. We also examine the course of niche partitioning along two main habitat axes, the benthic vs. limnetic and the sandy vs. rocky substrate axis. The results are then compared with available data on the cichlid adaptive radiation of neighbouring Lake Malawi. We find that pectoral fin size and muscle weight correlate with habitat use within the water column, as well as with substrate composition and foraging strategies. Niche partitioning along the benthic–limnetic axis in Lake Tanganyikan cichlids seems to follow a similar course as in Lake Malawi, while the course of habitat use with respect to substrate composition appears to differ between the cichlid assemblages of these two lakes. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **118**, 536–550.

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INTRODUCTION

Locomotion and related morphological features (i.e. ‘locomotory morphologies’) occur in nearly all animal taxa. Vertebrates display a compelling diversity of locomotion strategies that involve a variety of body parts such as limbs, fins or, as seen for example in snakes or eels, the entire body. Locomotion and locomotory morphologies often correlate with the habitat in which a given species lives and forages. A classic textbook example for this correlation is lizards of the genus *Anolis*, in which limb lengths correlate with twig diameters (Losos, 1990; Irschick & Losos, 1999; Mattingly & Jayne, 2004; Vanhooydonck, Herrel & Irschick, 2006). Moreover, the same set of forms showing a strong correspondence between limb

lengths and twig diameters evolved repeatedly and convergently on different islands of the Caribbean (Losos, 1990; Losos *et al.*, 1998; Mahler *et al.*, 2013). This phenomenon is generally regarded as a strong indicator for the importance of natural selection in shaping this correlation. Other vertebrate taxa rely on different body parts to generate movement, yet show similar correlations between morphology and habitat. Wing length in birds, for example, is often correlated with habitat structure, with species living in habitats characterized by dense vegetation exhibiting shorter wings than species living in open areas [reviewed by Hamilton (1961)]. Fish, conversely, often show a phenotype–environment correlation between fin morphology and benthic or limnetic habitat use [e.g. Malmquist (1992) and Dynes *et al.* (1999) for Arctic and brook charr,

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Hulsey *et al.* (2013) for cichlids and Robinson, Wilson & Margosian (2000) for pumpkinseed sunfish].

Most available studies in fish have investigated intraspecific variation, while studies linking divergence along a benthic–limnetic axis with repeated changes in locomotory morphology in more complex multi-species systems remain scarce. Notable exceptions are a study on selection towards different adaptive optima in locomotor phenotypes in neotropical geophagine cichlids (Astudillo-Clavijo, Arbour & Lopez-Fernandez, 2015) and the study by Hulsey *et al.* (2013), who examined the evolution of locomotory morphology in 24 species of Lake Malawi cichlids. The latter study found that benthic species exhibit larger pectoral fins and more massive, i.e. heavier, pectoral muscles compared with limnetic ones. Interestingly, the repeated shift between limnetic and benthic lifestyles in Lake Malawi cichlids seems to have been accompanied by convergent modifications in locomotory morphology (Hulsey *et al.*, 2013). Overall, however, habitat shifts along the benthic to limnetic axis, and the associated adaptations in locomotory morphology, have gained relatively limited attention in the study of East African Great Lake cichlids, although such habitat shifts have played an important role in shaping cichlid diversity in all three Great Lakes (Cooper *et al.*, 2010; Muschick, Indermaur & Salzburger, 2012; Hulsey *et al.*, 2013; Muschick *et al.*, 2014 and reviewed in Burrell, 2015) as well as in various small crater lakes in Africa and Central America (Schliewen, Tautz & Paabo, 1994; Barluenga *et al.*, 2006). The species assemblages in Lake Victoria, Lake Malawi and Lake Tanganyika, which collectively are the most species-rich adaptive radiations in vertebrates (Salzburger, Van Bocxlaer & Cohen, 2014), contain extreme forms adapted to benthic or limnetic lifestyles (Cooper *et al.*, 2010). These mirror benthic–limnetic shifts that have occurred in a wide range of other fish groups including sunfishes, whitefishes, perch, charr and stickleback (Malmquist, 1992; Dynes *et al.*, 1999; Robinson *et al.*, 2000; Rundle *et al.*, 2000; Hjelm *et al.*, 2001; Gillespie & Fox, 2003; Amundsen *et al.*, 2004; Ostbye *et al.*, 2006).

Teleost fish, which constitute at least 50% of all known vertebrate species, exhibit a variety of swimming modes, often coupled with distinct body and fin morphologies. These locomotion strategies range, in the form of a continuum, from anguilliform to sub-carangiform, carangiform and thunniform swimming modes (Webb, 1984a, b; Blake, 2004). The anguilliform swimming mode usually involves more than two lateral flexures present along the fish's body at a time. Moving over sub-carangiform and carangiform swimming, the number of bends decreases continuously until, in thunniform swimming, the caudal fin

and peduncle remain the only body parts involved in generating thrust (McDowall, 2003). In addition, there are some highly specialized swimming modes, such as ostraciform, which are found in few specialized groups only (i.e. Tetraodontiformes). On the basis of the observation that benthic species exhibit larger pectoral fins and muscles in many fish taxa (Malmquist, 1992; Dynes *et al.*, 1999; Robinson *et al.*, 2000; Hulsey *et al.*, 2013), it has been hypothesized that limnetic species may continuously make use of other locomotory structures, for example their caudal fin, to generate thrust (Hulsey *et al.*, 2013).

Specialization according to macro-habitat use is generally interpreted as the first step in the so-called 'stages model' of adaptive radiation, which was first developed for Lake Malawi cichlids (Danley & Kocher, 2001) and later generalized for vertebrates (Streelman & Danley, 2003). The second stage after macro-habitat specialization would be divergence according to trophic morphology, followed by diversification with respect to communication and coloration traits (stage 3) [reviewed in Gavrillets & Losos (2009)].

Another connection exists between locomotory morphology and feeding strategy in many animal taxa (Irschick & Losos, 1998; Domenici, 2001; Dean & Lannoo, 2003; Higham, 2007b). In fish, precise maneuvering while feeding is an important aspect of prey acquisition. In suction feeding species, for example, accurate positioning of the mouth relative to the prey item is essential, and pectoral fins play a crucial role in deceleration while maintaining approach stability. Therefore, fish that feature limited suction feeding abilities with respect to the water volume ingested often feature larger pectoral fins, thereby increasing their maneuverability and ability to correctly focus their attack on a prey item (Higham, 2007b).

Finally, fin size and morphology (including pigmentation patterns) can also be under the influence of sexual selection in fish. In many cichlids, for example, males, but not females, show enlarged or elongated and often elaborately colored paired (e.g. pectoral) or unpaired (e.g. anal, caudal) fins (Konings, 2015).

Against this background we investigate the cichlid assemblage of Lake Tanganyika to test whether or not pectoral and caudal fin sizes correlate with: (1) habitat use along a benthic–limnetic axis as found in other fish species and assemblages, (2) sandy–rocky habitat use, and (3) foraging mode. Furthermore, we use a wider sample of Lake Tanganyikan cichlids and a direct characterization of habitat use per species to examine benthic–limnetic and sandy–rocky habitat use through time and then compare it to patterns previously found in other teleost adaptive

radiations. Specifically, we test for evidence for an early divergence in habitat use leading to distinct lineages adapted to live on particular substrates as proposed by the radiation in stages model. It has previously been suggested that the three East African cichlid radiations depict, to some extent, replicated radiation events (Kocher *et al.*, 1993; Santos & Salzburger, 2012). Demonstrating temporal similarities or discrepancies in the process of adaptation and speciation between these three cichlid flocks should thus be interesting in the light of the ongoing quest to answer the question on whether there are general temporal patterns emerging in the course of adaptive radiations.

MATERIAL AND METHODS

In 2013 and 2014, we collected a total of 546 mature specimens representing 28 Lake Tanganyikan cichlid species in the southern part of Lake Tanganyika, Zambia. The samples include a phylogenetically and ecologically diverse set of species from 11 out of the 14 described Lake Tanganyikan cichlid tribes (Muschick *et al.*, 2012) (see Supporting Information, Table S1). Fish were caught using gill nets or, for some deep-water species, obtained from local fishermen. After euthanasia with clove oil, the sex of each specimen was determined, specimens were measured (standard and total length and weight were recorded) and photographed in a standardized way laying flat on the right side. We then dissected each specimen in the field and extracted all four pectoral adductor muscles (*arrector dorsalis*, *adductor radialis*, *adductor medialis* and *adductor superficialis*) of both pectoral fins. The four pectoral adductor muscles function together to pull the fin posteriorly. We refrained from examining the four pectoral abductor muscles that function to pull the fin anteriorly, as it has previously been shown that the forces of these two sets of muscles likely counterbalance each other and show fairly similar weights (Thorsen & Westneat, 2005; Hulsey *et al.*, 2013). All four muscles were measured together, but separately for each pectoral fin. Each set of muscles was measured twice and the mean of both measurements was taken for further analyses to increase measurement robustness. Concurrently, both pectoral fins and the caudal fin were separated from the fish's body, cleaned from dirt and mucus, and dyed with Indian ink to increase contrast. Each set of fins per specimen was then placed on a Styrofoam plate covered with an individual piece of white paper together with a premeasured reference plate of known area. Fins were spread using pins in a naturally erect position, i.e. in a maximal expanded position without over-expanding/damaging the fins.

Each set of fins was then photographed using a Nikon D5000 digital camera (Nikon Corporation, Tokyo, Japan). Later on, each digital image was analyzed using the software FinPix, specifically written for this purpose (available under <http://www.salzburgerlab.org/publications/software>). More precisely, this software calculates the area of each fin (mm^2) by comparing the number of pixels constituting each fin with the number of pixels constituting a reference plate of known size. To do so, the program subdivides the picture into three sectors: (1) the upper half of the sheet in landscape orientation containing the left and right pectoral fins and the caudal fin, in that order, (2) the lower left quarter containing the reference area and (3) the lower right quarter, which is ignored by the program but may be used to add for example the specimen number or further annotations. First, the program searches for the reference area in the lower left quarter using the contrast between the white paper sheet and the black reference plate and subsequently counts the number of pixels that constitute this reference area. Next, the program consecutively searches, from left to right, the individual fins in the upper half of the sheet using the same method. Again, the number of pixels constituting each fin is counted and finally, by comparing the number of pixels constituting each individual fin and the number of pixels constituting the reference area of known size, the program calculates the area of each fin and provides a table containing the individual measurements. Given the high resolution of the digital images and the sharp contrast between ink-dyed fins and the white background, this method allows a highly accurate measurement of fin area. In addition, the program provides pictures with the pixels that were actually counted. The areas counted are highlighted in red giving the user the opportunity to cross-check whether the measurements had been performed correctly.

All trait measurements were screened for potential methodical problems (e.g. not fully expanded fins, imperfectly dissected muscles) or apparent measurement errors. If methodical problems or measurement errors were detected, individual trait measurements were excluded from further analyses. After this procedure, our dataset consisted of 530–536 individual values per trait and 8–23 specimens per species (see Supporting Information, Table S1). After this initial quality check, the average of the right and left pectoral muscle mass, the average of the right and left pectoral fin area and the caudal fin area were recorded for each specimen separately. Fifteen pectoral fin area and 15 pectoral muscle mass measurements were solely based on the left or right fin apparatus, respectively, as trait values were only available for one side.

To compute phylogenetically size-corrected values of traits (Revell, 2009) we used a modified version of the 'phyl.resid' function in the phytools package (Revell, 2012) in R (R Development Core Team, 2008) that allows for multiple individuals per species (Lopez-Fernandez *et al.*, 2014). We used the weight of an individual specimen to size-correct the muscle mass or centroid size (which is, in this case, essentially a measurement of the body area, see Supporting Information, Fig. S1) to size-correct the fin areas. Centroid size was calculated using tpsDig (Rohlf, 2010) and MorphoJ (Klingenberg, 2011), on the basis of nine landmarks distributed over the fish's body (Supporting Information, Fig. S1). Species were later individually tested for sexual dimorphism in all traits using a *t*-test in PRISM v.6.0e (GraphPad Software, La Jolla, CA, USA, www.graphpad.com). Furthermore, we used stable isotope data for the rare isotopes of Nitrogen and Carbon from Muschick *et al.* (2012) to further assess each species' position along the benthic-limnetic axis (Carbon) as well as each species' position within the food web (Nitrogen) [e.g. DeNiro & Epstein (1978); Hobson, Piatt & Pitocchelli (1994); Post (2002)]. As stable isotope data were not available for *Bathybates leo* and *Hemibates stenosoma*, analyses incorporating stable isotope values could only be conducted in 26 out of the 28 species. We further used data on intestinal tract length from Muschick *et al.* (2014) that we size-corrected using standard length as size measurement following the same procedure as described above. Again, data were not available for all species in our dataset, which reduced the species number to 24 when incorporating intestinal tract lengths (data were missing for *Bathybates graueri*, *Gnathochromis permaxillaris*, *Hemibates stenosoma* and *Trematocara marginatum*).

Additionally to this first dataset, we generated a second dataset containing 159 Lake Tanganyikan cichlid species grouped into categories according to their position on a benthic to limnetic axis and to whether a species prefers sandy or rocky habitats (see Fig. 1 and Supporting Information, Table S2). Information on habitat use was compiled from several literature sources as well as our own transect data. The species were then categorized into four discrete categories according to benthic to limnetic habitat use (benthic, semi-benthic, semi-limnetic, limnetic) and two categories (sandy or rocky) according to their substrate preference by one of the authors (AI) (Coulter, 1991; Hori *et al.*, 1993; Muschick *et al.*, 2012; Konings, 2015). Species categorized as semi-benthic or semi-limnetic, respectively, are species that are mainly associated with one macro-habitat but can occasionally also be encountered in the other macro-habitat.

In a next step, we created a new phylogenetic hypothesis for East African cichlids (196 taxa) on the basis of mitochondrial and nuclear sequence data obtained from GenBank (see Supporting Information, Fig. S2; Tables S5 and S6). To this end, we used nuclear sequence data from 42 genes (Meyer & Salzburger, 2012; Meyer, Matschiner & Salzburger, 2015) as backbone, and combined it with sequences of the mitochondrial NADH dehydrogenase subunit 2 (ND2) of 195 taxa, leading to a concatenated dataset of 18 592 bp in length and the most comprehensive phylogeny of cichlid fishes for Lake Tanganyika to date. As the nuclear data were only available for 45 taxa, we ended up with a proportion of gaps and undetermined positions of 72.85%. However, it has previously been shown that such a large proportion of missing data can still lead to reliable phylogenetic estimates (Wiens & Morrill, 2011). Model choice and data partitioning was done with PartitionFinder (Lanfear *et al.*, 2012). The resulting 18 partitions and models were subsequently used in the program GARLI version 2.0 (Zwickl, 2006) on the CIPRES Science Gateway (Miller, Pfeiffer & Schwartz, 2010) to perform a phylogenetic inference. The optimal tree was searched in 50 replicates, and 339 nonparametric bootstrap runs were conducted for confidence assessment, both using *Tylochromis polylepis* as out-group (see Salzburger *et al.*, 2002). Models of the 18 partitions were allowed to differ and rates of subsets to change proportionally to one another (link models = 0; subset specific rates = 1). The resultant tree was then trimmed using ape (Paradis, Claude & Strimmer, 2004) in R to match the species for which trait data were available. Note that, for this study, we were not primarily interested in the phylogenetic hypothesis *per se*, but instead, used it to correct for phylogenetic signal and to reconstruct habitat use through time (see below).

We then applied correlational analyses in R on the dataset consisting of 28 species, once using a classical linear model and once using phylogenetic generalized least squares (PGLS) to correct for phylogenetic dependence of trait values. PGLS analyses were done using the R package caper (Orme, 2012) and a phylogeny trimmed to match the species sample of the trait dataset. *P*-values were subsequently corrected for multiple comparisons using a Bonferroni correction. We tested all species for sexual dimorphism in trait values as this could influence our correlational analyses. Significant sexual dimorphism regarding fin sizes and/or muscle mass was detected in only one out of 28 studied species: in *Enantiopus melanogenys*, females exhibited significantly larger pectoral fins than their male conspecifics ($P < 0.0028$). To account for this dimorphism, we created a secondary dataset excluding

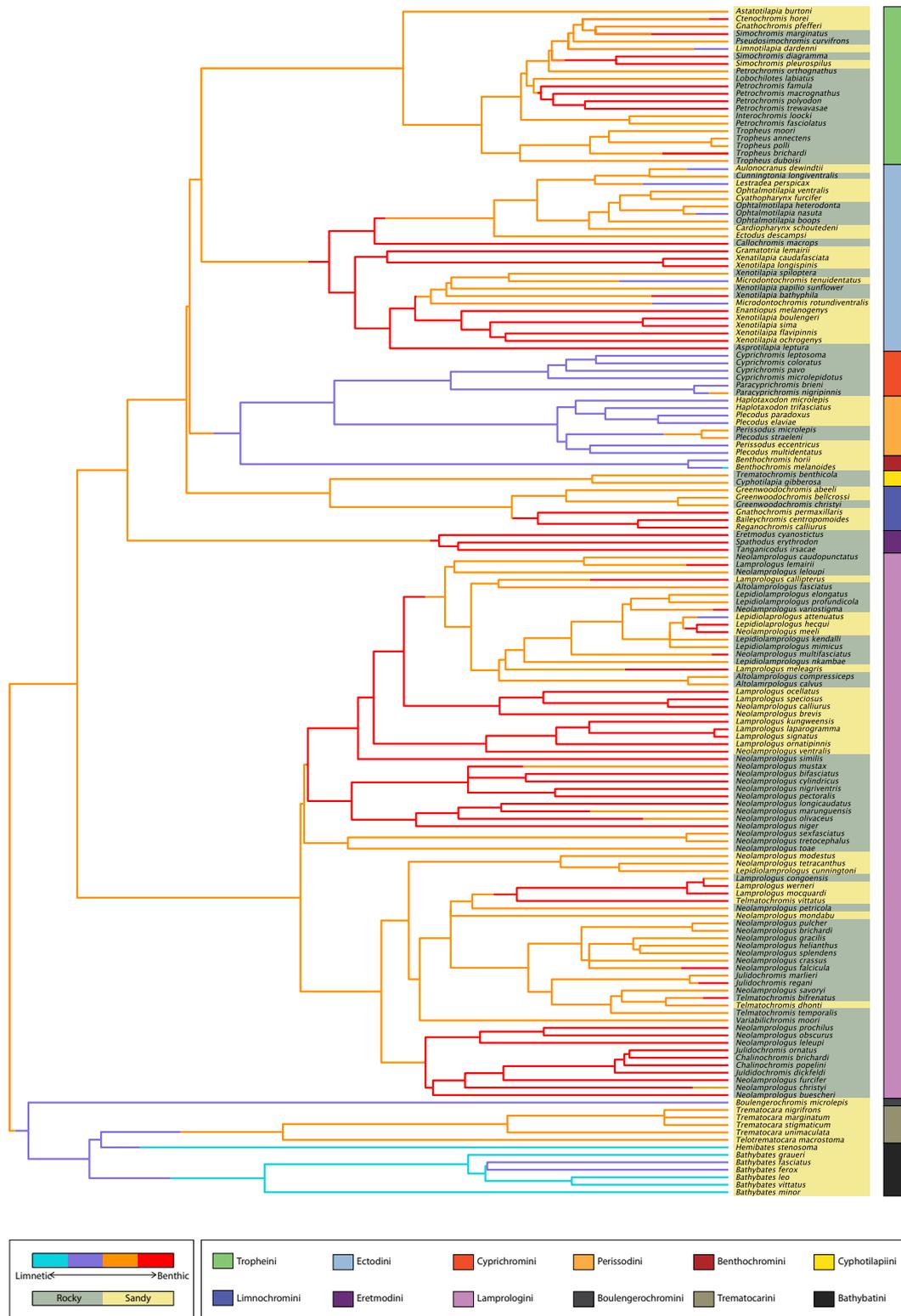


Figure 1. Ancestral character state reconstruction of 159 Lake Tanganyikan cichlid species according to a species' position along the benthic–limnetic axis (four categories). Preferences for rocky or sandy habitats are highlighted by grey and yellow boxes, respectively. The phylogenetic hypothesis presented here is inferred with a maximum likelihood approach using GARLI and is based mainly on mitochondrial ND2 sequences for all species and 42 nuclear markers where available.

pectoral fin area measurements of *Enantiopus melanogenys* and applied the same correlational analyses as on the original dataset (see Supporting Information, Tables S3 and S4). As the results were fairly similar, we here primarily rely on the results of the complete dataset for further interpretation (but see Discussion and Supporting Information). An ancestral character state reconstruction according to a species position along the benthic to limnetic axis in four categories (Fig. 1) was done with SIMMAP version 1.5.2 (Bollback, 2006) with an empirical prior and a linear ordering of states, setting the rate parameter to 'branch length prior'. The resultant figure was later modified in Adobe Illustrator CS 4 version 14.0.0 (Adobe Systems Inc., San José, CA, USA).

On the first dataset, consisting of 28 species, we again used PRISM to compare the four groups according to a species' position on the benthic to limnetic axis using an ordinary one-way ANOVA and Tukey's multiple comparisons test on pectoral fin area, caudal fin area and muscle mass, respectively, to compare benthic and limnetic groups. We further performed a *t*-test to contrast the group categorized as preferring sandy habitats with the group characterized as preferring rocky habitats.

Finally, we conducted two separate disparity through time (DTT) analyses using 159 species' grouping according to benthic/limnetic and sandy/rocky habitat use following Harmon *et al.* (2003). To this end, we used GEIGER (Harmon *et al.*, 2008) in R with the number of unique character states ('num.-states', currently the only option for discrete character data) as disparity index. We computed 1000 Brownian motion simulations of trait disparity over the phylogeny and compared it with our actual habitat use data. We then calculated the morphological disparity index (MDI) over the first 75% of the relative timeline to correct for tip over-dispersion due to incomplete taxon sampling.

RESULTS

PHYLOGENETIC ANALYSIS

With respect to the relationships between tribes, the phylogenetic hypothesis presented here (Fig. 1; Supporting Information, Fig. S2 for the complete phylogeny including bootstrap values) largely agrees with a recent multilocus nuclear phylogenetic hypothesis for Lake Tanganyika cichlids (Meyer *et al.*, 2015), which is not unexpected given that we used the nuclear data from this study. As in Meyer *et al.* (2015), the Boulengerochromini, Bathybatini and Trematocarini form a basal clade, a sister group to the Lamprologini and all remaining tribes that exclusively consist of mouthbrooding lineages.

Within these, the Eretmodini branched off first [see Fig. 2B and discussion in Meyer *et al.* (2015)], followed by the Limnchromini and Cyphotilapiini, a clade formed by the Perissodini and Cyprichromini, the Ectodini and the Tropheini (as part of the Haplochromini). The internal branches, especially between the mouthbrooding tribes, are rather short suggesting a rapid period of lineage formation. This result is congruent with all previous analyses [e.g. Salzburger *et al.* (2002); Clabaut, Salzburger & Meyer (2005); Day, Cotton & Barraclough (2008)]. Regarding the placement of taxa within the tribes, our phylogeny is consistent with earlier studies based on mitochondrial DNA markers (Salzburger *et al.*, 2002; Day *et al.*, 2008; Sturmbauer *et al.*, 2010), which is also not unexpected, given that we largely relied on data from these studies for the mitochondrial DNA part of the concatenated sequence alignment. Overall, we feel confident about using our new phylogenetic hypothesis to correct for phylogenetic signal in the trait data and for the DTT analyses.

CORRELATIONAL ANALYSES

Both correlational analyses, PGLS and the classical linear model, revealed a significant positive correlation between pectoral muscle mass and fin area. Both analyses also revealed a strong positive correlation between pectoral and caudal fin area and, to a lesser extent, between pectoral muscle mass and caudal fin area (Tables 1 and 2). Pectoral fin area also correlated positively with intestinal tract length in both analyses, whereas we observed a negative correlation between pectoral fin area and $\delta^{15}\text{N}$ stable isotope measurements in the linear model; however, this correlation disappears in the PGLS analysis [yet is still evident in both the linear model and PGLS when excluding the sexually dimorphic *E. melanogenys* (Supporting Information, Tables S3 and S4)]. Pectoral muscle mass, correlating with pectoral fin area, showed a similar pattern: we also found a negative correlation with $\delta^{15}\text{N}$ and a positive one with intestinal tract length with the difference that the correlation also holds in the PGLS analysis of the complete dataset. Caudal fin area, which correlated with pectoral fin area as well as pectoral muscle mass, showed positive correlations with intestinal tract length in both analyses.

HABITAT USE

Characterization of habitat use in 159 Lake Tanganyikan cichlid species led to six species being characterized as limnetic, 22 as semi-limnetic, 67 as semi-benthic and 64 as benthic. From the same pool

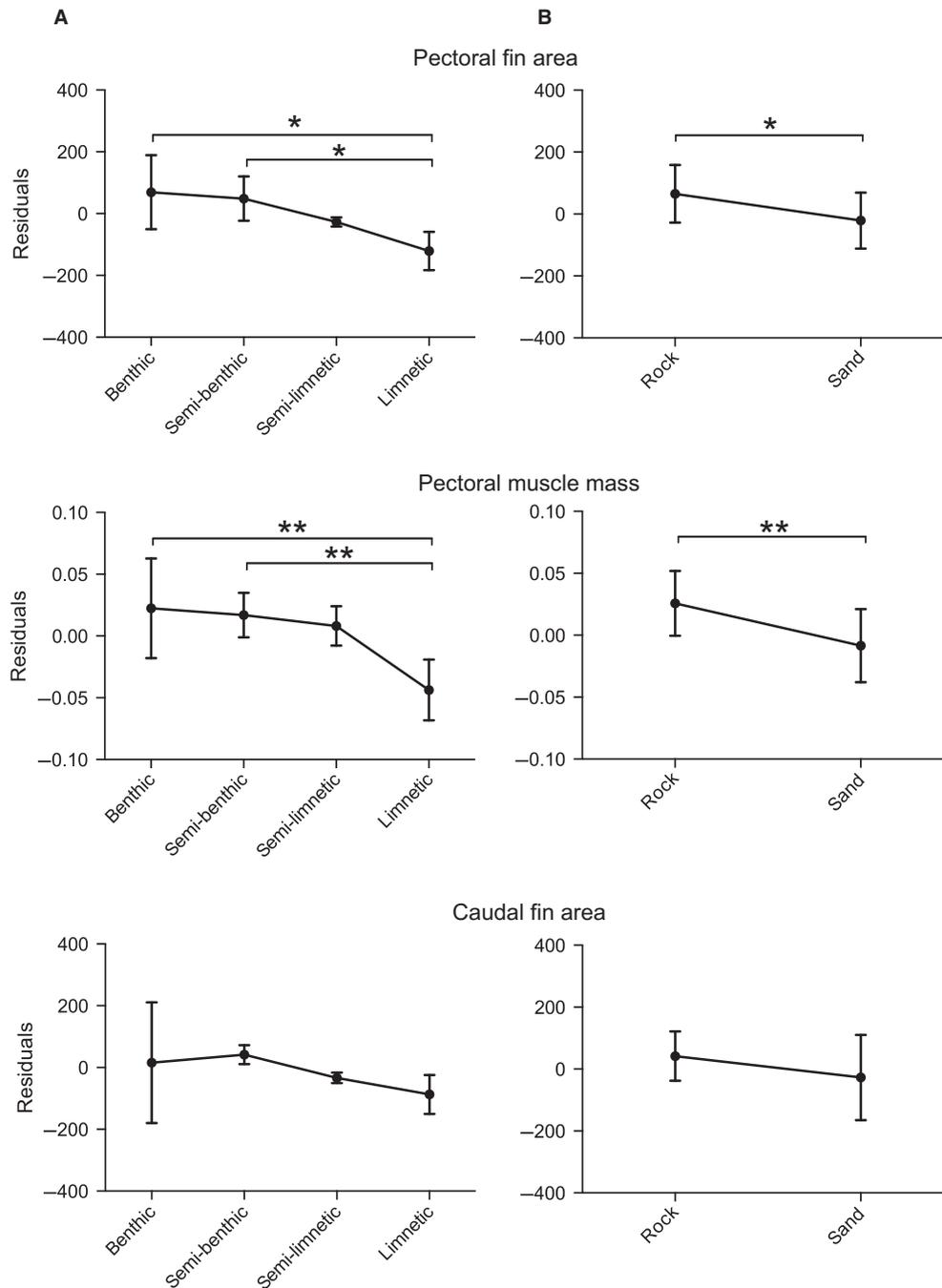


Figure 2. Comparison between species grouped according to habitat preferences. A, One-way ANOVA with grouping according to a species' position along a benthic to limnetic axis, revealing a gradient towards smaller pectoral fins and muscles with an increasingly limnetic habitat use. Significant differences were detected in pectoral fin area and muscle mass between the benthic and semi-benthic groups and the limnetic group. B, Student's *t*-test between species grouped according to either sandy or rocky habitat use, revealed significantly smaller pectoral fins and lighter muscles in species preferring sandy habitats. * $P < 0.05$, ** $P < 0.01$.

of species, 90 can be regarded as exhibiting an association with rocky substrate and 69 as exhibiting a lifestyle connected to sandy substrate (Fig. 1; Supporting Information Table S2).

Tukey's multiple comparisons test following a one-way ANOVA between our four groups according to a species' position along a benthic to limnetic axis revealed increasingly smaller pectoral fins and

Table 1. Results of a correlation analysis according to a classic linear model

| | <i>t</i> -Value | Adjusted <i>P</i> -value | <i>R</i> ² | <i>N</i> _{species} |
|-------------------------|-----------------|-----------------------------|-----------------------|-----------------------------|
| Pectoral fin area | | | | |
| Pectoral muscle mass | 7.431 | 0.000 | 0.680 | 28 |
| Caudal fin area | 6.121 | 0.000 | 0.590 | 28 |
| $\delta^{15}\text{N}$ | -2.865 | 0.043 | 0.255 | 26 |
| $\delta^{13}\text{C}$ | 2.705 | 0.062 | 0.234 | 26 |
| Intestinal tract length | 5.063 | 0.000 | 0.538 | 24 |
| Caudal fin area | | | | |
| Pectoral muscle mass | 3.502 | 0.008 | 0.321 | 28 |
| Pectoral fin area | 6.121 | 0.000 | 0.590 | 28 |
| $\delta^{15}\text{N}$ | -1.226 | 1 | 0.059 | 26 |
| $\delta^{13}\text{C}$ | 1.787 | 0.433 | 0.118 | 26 |
| Intestinal tract length | 3.908 | 0.004 | 0.410 | 24 |
| Pectoral muscle mass | | | | |
| Pectoral fin area | 7.431 | 0.000 | 0.680 | 28 |
| Caudal fin area | 3.502 | 0.008 | 0.321 | 28 |
| $\delta^{15}\text{N}$ | -3.029 | 0.029 | 0.277 | 26 |
| $\delta^{13}\text{C}$ | 2.730 | 0.058 | 0.237 | 26 |
| Intestinal tract length | 4.426 | 0.001 | 0.471 | 24 |

P-values were corrected for multiple comparisons using a Bonferroni correction. Bold values indicate *P*-values < 0.05 after Bonferroni correction.

lighter pectoral fin muscles towards a more limnetic lifestyle. For both traits, we detected significant differences between both the group exhibiting a benthic lifestyle and the group exhibiting a semi-benthic lifestyle when compared to the group exhibiting a limnetic lifestyle (Fig. 2; Table 3A). We found similar results when comparing species grouped according to their habitat use (rocky vs. sandy): both pectoral fin area and muscle mass showed significantly smaller values for species exhibiting a lifestyle connected to sandy habitats (Fig. 2; Table 3B).

Plotting benthic to limnetic habitat use over the most inclusive molecular phylogeny for Lake Tanganyikan cichlid species available revealed a rather disparate habitat use distribution (Fig. 1). There were only a few tribes featuring only benthic or limnetic living species, respectively. However, the Bathybatini exclusively consist of limnetic or semi-limnetic species, while, conversely, the Eretmodini and Limnchromini feature only benthic or semi-benthic species. Only Cyphotilapiini, Eretmodini, Boulengerochromini and Trematocarini, which were

Table 2. Results of a correlation analysis corrected for phylogenetic dependence of trait values using PGLS

| | <i>t</i> -Value | Adjusted <i>P</i> -value | <i>R</i> ² | λ | <i>N</i> _{species} |
|-------------------------|-----------------|-----------------------------|-----------------------|-----------|-----------------------------|
| Pectoral fin area | | | | | |
| Pectoral muscle mass | 6.184 | 0.000 | 0.595 | 0.975 | 28 |
| Caudal fin area | 5.055 | 0.000 | 0.496 | 1 | 28 |
| $\delta^{15}\text{N}$ | -2.325 | 0.144 | 0.184 | 1 | 26 |
| $\delta^{13}\text{C}$ | 0.856 | 1 | 0.030 | 1 | 26 |
| Intestinal tract length | 4.118 | 0.002 | 0.435 | 1 | 24 |
| Caudal fin area | | | | | |
| Pectoral muscle mass | 3.502 | 0.008 | 0.321 | 0 | 28 |
| Pectoral fin area | 6.121 | 0.000 | 0.590 | 0 | 28 |
| $\delta^{15}\text{N}$ | -1.226 | 1 | 0.059 | 0 | 26 |
| $\delta^{13}\text{C}$ | 1.787 | 0.433 | 0.118 | 0 | 26 |
| Intestinal tract length | 3.908 | 0.004 | 0.410 | 0 | 24 |
| Pectoral muscle mass | | | | | |
| Pectoral fin area | 6.365 | 0.000 | 0.609 | 0.838 | 28 |
| Caudal fin area | 2.782 | 0.050 | 0.229 | 0.773 | 28 |
| $\delta^{15}\text{N}$ | -2.914 | 0.038 | 0.261 | 0.639 | 26 |
| $\delta^{13}\text{C}$ | 2.730 | 0.058 | 0.237 | 0 | 26 |
| Intestinal tract length | 4.196 | 0.002 | 0.445 | 1 | 24 |

P-values were corrected for multiple comparisons using a Bonferroni correction. Bold values indicate *P*-values < 0.05 after Bonferroni correction.

represented by one to five species per tribe in our phylogeny, exhibited a uniform habitat use with all species falling into the same habitat category. Of these tribes, only the Eretmodini showed a strictly benthic habitat use, while the species of the other 'uniform' tribes all fell into intermediate categories. All other tribes are non-uniform and show within-tribe diversity related to habitat use with species falling into two to three categories within a tribe. Nevertheless, no tribe was found to feature all four habitat categories. We observed a similar pattern associated with habitat use according to sandy or rocky substrate: The species-rich tribes feature species from both categories and only the rather species-poor tribes feature species restricted to either rocky or sandy substrate, i.e. the Cyprichromini, Benthochromini, Cyphotilapiini, Eretmodini, Boulengerochromini, Trematocarini and Bathybatini.

Table 3. Test statistics corresponding to the comparison between species grouped according to habitat preferences (Fig. 2). (A) Tukey's multiple comparisons test following a one-way ANOVA with grouping according to a species' position along a benthic to limnetic axis. (B) Student's *t*-test between species grouped according to either sandy or rocky habitat preference

| A | | | | B | | | |
|--------------------------------|--------------------------|---------|--------------------------|---------------|--------------------------|---------|-----------------|
| Comparison | Difference between means | Summary | Adjusted <i>P</i> -value | Comparison | Difference between means | Summary | <i>P</i> -value |
| Pectoral fin area | | | | | | | |
| Benthic vs. semi-benthic | 20.60 | ns | 0.947 | Sand vs. rock | -86.39 | * | 0.021 |
| Benthic vs. semi-limnetic | 96.04 | ns | 0.366 | | | | |
| Benthic vs. limnetic | 190.10 | * | 0.016 | | | | |
| Semi-benthic vs. semi-limnetic | 75.45 | ns | 0.519 | | | | |
| Semi-benthic vs. limnetic | 169.50 | * | 0.023 | | | | |
| Semi-limnetic vs. limnetic | 94.05 | ns | 0.543 | | | | |
| Pectoral muscle mass | | | | | | | |
| Benthic vs. semi-benthic | 0.0055 | ns | 0.967 | Sand vs. rock | -0.03415 | * | 0.015 |
| Benthic vs. semi-limnetic | 0.0143 | ns | 0.861 | | | | |
| Benthic vs. limnetic | 0.0661 | ** | 0.007 | | | | |
| Semi-benthic vs. semi-limnetic | 0.0088 | ns | 0.955 | | | | |
| Semi-benthic vs. limnetic | 0.0606 | ** | 0.008 | | | | |
| Semi-limnetic vs. limnetic | 0.0518 | ns | 0.112 | | | | |
| Caudal fin area | | | | | | | |
| Benthic vs. semi-benthic | -26.03 | ns | 0.950 | Sand vs. rock | -69.05 | ns | 0.560 |
| Benthic vs. semi-limnetic | 48.97 | ns | 0.911 | | | | |
| Benthic vs. limnetic | 102.90 | ns | 0.520 | | | | |
| Semi-benthic vs. semi-limnetic | 75.00 | ns | 0.708 | | | | |
| Semi-benthic vs. limnetic | 129.00 | ns | 0.276 | | | | |
| Semi-limnetic vs. limnetic | 53.98 | ns | 0.930 | | | | |

ns, non-significant, * $P < 0.05$, ** $P < 0.01$. Significant *P*-values are depicted in bold.

DISPARITY THROUGH TIME

DTT analyses of habitat use (rocky vs. sandy and benthic vs. limnetic) both showed no signs of an early burst (Fig. 3), with MDI statistics for both analyses being positive (rocky vs. sandy, MDI = 0.1734; benthic vs. limnetic, MDI = 0.0316). Nevertheless, we detected periods where average subclade disparity remains lower than predicted by Brownian motion simulations: just at the onset of the radiation for rocky vs. sandy habitat use and around 0.2 in relative time for benthic vs. limnetic habitat use. However, following these valleys, average subclade disparity consistently remains higher than predicted, indicating elevated disparity within subclades.

DISCUSSION

In the present study, we analyzed pectoral and caudal fin size and pectoral fin muscle weight in the species flock of cichlid fishes from Lake Tanganyika, and correlated it with ecological and behavioral

traits to test hypotheses regarding phenotype-environment correlations, previously established in other, mostly species-poor fish assemblages. Further, we tested hypotheses on habitat use and its diversification through time. Namely, that habitat use according to sandy and rocky habitat use represents the first axis of divergence while habitat use along the benthic-limnetic axis diverged over a prolonged time span. These scenarios were previously discussed for example in Lake Malawi cichlids. If habitat use indeed represents the first axis of divergence in Lake Tanganyikan cichlids, an 'early burst'-like pattern should be visible in our DTT plots.

Correlation between pectoral fin area and muscle mass in Lake Tanganyikan cichlids was shown to be significant and comparable with the outcome of a similar study in Lake Malawi cichlids (Hulsey *et al.*, 2013). Both the classical linear model and PGLS analyses revealed correlations between the area of the pectoral fins and the mass of the muscles that are used to move the respective fins through the water (Tables 1 and 2). This correlation becomes even stronger when excluding the sexually dimorphic

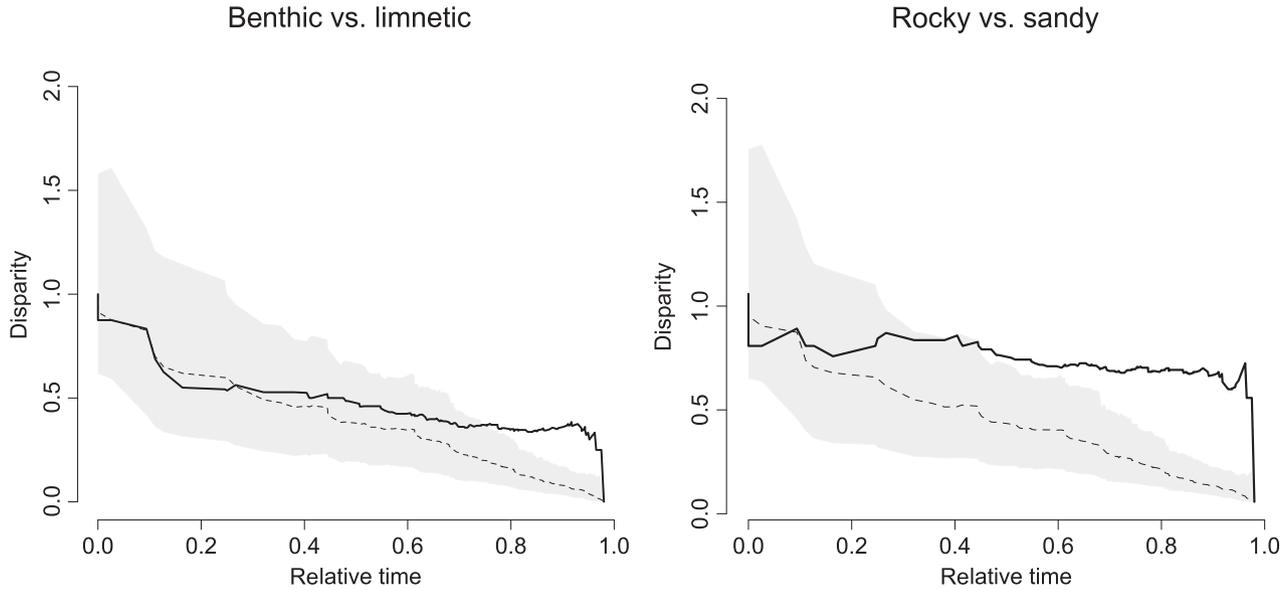


Figure 3. Disparity through time plots according to habitat preference along the benthic–limnetic axis (left, four categories, MDI = 0.0316) and rocky vs. sandy habitat preference (right, two categories, MDI = 0.1734). Average subclade disparity remains generally higher than the mean of 1000 Brownian motion simulations, indicating elevated disparity within subclades.

E. melanogenys (Supporting Information, Tables S3 and S4). Moreover, pectoral fin area and muscle mass showed very similar correlations with ecological factors and habitat use (discussed below). Larger pectoral fins coupled with heavier pectoral fin muscles should directly lead to increased maneuverability and a more efficient deceleration, also during prey capture (Higham, 2007a, b). To decelerate, fish commonly use their extended pectoral, caudal and median fins to increase drag (Drucker & Lauder, 2002; Rice & Westneat, 2005; Higham, 2007a). Conversely, a more powerful pectoral apparatus should lead to increased locomotory performance, when used to generate thrust.

Of 159 characterized Lake Tanganyikan cichlid species, 28 species exhibited a limnetic or semi-limnetic habitat association, whereas 131 species had a benthic or semi-benthic habitat association. This suggests that the structured and diverse benthic macro-habitat provides far more niches for species diversification than the rather uniform limnetic macro-habitat. A more equal distribution is found regarding substrate preference, for which 90 species can be regarded as being associated with rocky substrate and 69 species with sandy substrate. Again, the higher number of species preferring rocky substrates indicates that the densely structured rocky habitat likely provides more niches for species to forage in than the more uniform sandy habitat.

The most pronounced axis of divergence between closely related fish taxa often coincides with

adaptations to a benthic vs. a limnetic lifestyle (Schluter, 1993; Rundle *et al.*, 2000; Barluenga *et al.*, 2006; Machado-Schiaffino *et al.*, 2015). Most of these shifts are accompanied by alterations of the feeding apparatus and the general body shape including fin morphology. In Arctic (*Salvelinus alpinus*) and brook charr (*Salvelinus fontinalis*), for example, pronounced differences exist between limnetic and benthic morphs, *inter alia* involving locomotory morphology (Malmquist, 1992; Dynes *et al.*, 1999). Limnetic morphs exhibit shorter pectoral fins and a more fusiform body, whereas benthic forms feature longer pectoral fins and a deeper body. Moreover, it has been shown that the limnetic morph feeds more effectively on plankton, suggesting that the beforehand mentioned morphological differences are adaptive to a planktivorous diet. In addition, morphologically distinct benthic and limnetic morphs are thought to have mediated reproductive isolation in Arctic charr (Jonsson & Jonsson, 2001), as well as in threespine stickleback (Rundle *et al.*, 2000; Boughman, 2001), thus providing a possible mechanism for speciation along this major ecological axis. In perch (*Perca fluviatilis*), benthic and limnetic morphs differ in body depth, with the benthic morph showing a deeper body than its limnetic conspecific (Hjelm *et al.*, 2001). The same was found for pumpkinseed sunfish (*Lepomis gibbosus*), in which some populations additionally show divergence in pectoral fin size: benthic morphs have larger fins in some populations (Robinson *et al.*, 2000) but not in others

(Gillespie & Fox, 2003). Similarly, neotropical geophagine cichlids exhibit an early divergence in locomotor phenotypes towards two distinct adaptive peaks: one that includes deep-bodied, predominantly benthically feeding fish and one including mostly ram-feeding species with streamlined bodies (Astudillo-Clavijo *et al.*, 2015).

We found a gradient towards smaller pectoral fins and lighter muscles with increasingly limnetic lifestyle, with significant differences in pectoral fin area and muscle mass between benthic and semi-benthic species and the limnetic species group (Fig. 2A and Table 3). Differences concerning pectoral fin area and muscle mass seem to be partially explained by differences in benthic vs. limnetic habitat use. A similar pattern has been documented for Lake Malawi cichlids (Hulsey *et al.*, 2013). More generally, morphological differences influencing locomotion connected with benthic vs. limnetic lifestyles have been demonstrated for various temperate water species (Malmquist, 1992; Schluter, 1993; Dynes *et al.*, 1999; Robinson *et al.*, 2000; Svanback & Eklov, 2004).

In this study, we provide evidence that foraging strategy influences fin morphology in Lake Tanganyikan cichlids. Both $\delta^{15}\text{N}$ values (a measure for a species' position within the food web) and intestinal tract length (with longer intestinal tract length pointing towards a more herbivorous diet) correlated strongly with pectoral fin area and muscle mass (although the correlation between pectoral fin area and $\delta^{15}\text{N}$ appeared weaker in the PGLS analysis of the complete dataset). A similar correlation was found between intestinal tract length and caudal fin area, further emphasizing the association between feeding and locomotion. Species ranking lower in the food web exhibit larger pectoral and caudal fins.

Pectoral fins play a crucial role in maneuvering in fish and are essential in turning, fine correction, rapid acceleration, deceleration, backward swimming and stationary hovering (Webb, 2006). Herbivores require a more precise maneuvering during foraging to feed effectively along substrata with varying topologies and at varying angles (Webb, 1984a; Rice & Westneat, 2005) and thus likely require larger fins to meet the demands of this foraging strategy. Furthermore, efficient deceleration, mainly relying on movements of the pectoral fins, is crucial when feeding from substrate as it prevents collisions that could otherwise harm the fish (Rice & Westneat, 2005; Higham, 2007a). The correlation between foraging strategy and locomotory morphology is probably connected with the correlation between locomotory morphology and benthic vs. limnetic habitat use (see above), as herbivorous species seem to be more common in benthic habitats due to higher availability of

suitable food items (Hori *et al.*, 1993; Muschick *et al.*, 2012).

We also found a gradient towards smaller pectoral fins and lighter pectoral muscles in species living on sandy substrate as compared to species living in rocky habitats, with the former exhibiting significantly smaller pectoral fins and lighter pectoral muscles (Fig. 2B and Table 3). This discrepancy likely evolved due to increased demands on maneuverability when foraging in a complex, rocky environment, both in terms of precise swimming in-between rocks and cavities, as well as braking to prevent collisions with sharp-edged rocks when feeding from the substrate.

Given the apparent correlation between benthic habitat use and enlarged pectoral fins in various fish species (Malmquist, 1992; Dynes *et al.*, 1999; Robinson *et al.*, 2000; Hulsey *et al.*, 2013) one could expect that limnetic living species make increased use of other locomotory features such as the caudal fin. If this is the case in Lake Tanganyika cichlids, it is not reflected by caudal fin size as we did not find any evidence for limnetic species having larger caudal fins (Fig. 2A and Table 3A). Moreover, we found a strong correlation between pectoral and caudal fin area (and pectoral muscle mass), which might be due to a constraint in fin size evolution, i.e. that the evolution of larger pectoral fins positively influences the size of the caudal fin or *vice versa*. This result would mean that pectoral and caudal fin sizes are regulated in common, possibly by the same set of genes.

There was only one species with significant sexual dimorphism concerning pectoral fin size, namely *Enantiopus melanogenys*. This species is found predominantly on open sand plains and hence has little need for enhanced maneuverability. It is also a lek forming species with males competing in large and dense aggregations. Sexual dimorphism is hence relatively pronounced in coloration, body size and also the patterning and size of the unpaired fins. Combined with a relative low ecological selection pressure on pectoral (paired) fins and maneuverability this might account for the intersexual differences in this species.

We did not find any evidence for an early burst of diversification, defined as a rapid initial diversification followed by a drop in evolutionary rate as ecological space becomes filled (Schluter, 2000; Harmon *et al.*, 2003, 2010) in Lake Tanganyikan cichlids in terms of habitat use – neither according to habitat use towards rocky vs. sandy substrate nor along the benthic to limnetic axis. Early divergence with respect to macro-habitat use would be expected under the 'radiation in stages' model, and would have led to a persistent deep split in the phylogeny according to habitat use. This is because the

available niches would have been filled during the initial phases of divergence, leaving little opportunity for subsequent habitat changes within subclades. Such a persistent split, for example into rock dwelling and sand dwelling lineages, as found in Lake Malawi cichlids (Danley & Kocher, 2001; Streelman & Danley, 2003), is not visible in the Lake Tanganyikan cichlid assemblage. DTT analyses and MDI statistics of sandy vs. rocky habitat use show no sign of an early, continuous split according to these categories (Fig. 3).

Another pattern becomes evident when inspecting sandy vs. rocky habitat use plotted onto the phylogeny (Fig. 1): There is little clustering of habitat use according to phylogenetic relationships. We therefore conclude that discrepancies in habitat use between Lake Tanganyika cichlid species are not the result of an early burst at the onset of the radiation but, contrary to the pattern discussed for Lake Malawi, evolved over a prolonged timespan with habitat shifts recurrently occurring within subclades. This discrepancy in the timing of niche partitioning might be explained by differences in the origin and history of these two cichlid assemblages. In contrast to the quasi-monophyletic Lake Malawian cichlid species flock, the Lake Tanganyika assemblage was presumably seeded by several cichlid lineages and diversified into a variety of tribes (Salzburger *et al.*, 2002), possibly facilitating niche sharing and niche co-occupation by phylogenetically distinct species (Muschick *et al.*, 2012). A similar pattern of recurrent habitat shifts was found for habitat use along the benthic–limnetic axis: We did not find any signs of an early divergence leading to distinct lineages along this axis, but rather a pattern of recurrent shifts in habitat use within subclades. This is in accordance with findings concerning the Lake Malawi cichlid species flock (Hulsey *et al.*, 2013). Similarly, Muschick *et al.* (2014) found no evidence for a temporal ordering of trait evolution according to the ‘radiation in stages’ model in Lake Tanganyikan cichlids. Compared with traits associated with foraging, macro-habitat-related traits show less phylogenetic signal and a more accelerated rate of trait evolution across the radiation, indicating that traits associated with feeding actually diverged earlier than macro-habitat-related traits. Other studies did not recover an ‘early burst’ in two components of trophic morphology in Lake Tanganyika cichlids, the shape of the lower pharyngeal jaw (Muschick *et al.*, 2012) and operculum shape (Wilson *et al.*, 2015).

Taken together, we show that specializations in habitat use, both with respect to rocky vs. sandy and benthic vs. limnetic, occurred repeatedly within the cichlid species flock of Lake Tanganyika, and that habitat use shows little phylogenetic constraints.

Furthermore, these shifts in habitat use are accompanied by convergent modification of the locomotory system with species preferring benthic and rocky habitats exhibiting larger pectoral fins and heavier muscles. This could mainly be explained by increased demands regarding maneuverability required for foraging in these habitats and/or feeding and grazing between rocks. In addition to this correlation with habitat use, and probably connected to it, locomotory morphology of Lake Tanganyikan cichlids was shown to be influenced by foraging strategies with herbivorous species ranking lower in the food web, exhibiting larger pectoral fins and muscles.

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SUPPORTING INFORMATION

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

Figure S1. Position of nine landmarks over the fish's body used to assess centroid size on each specimen. The picture shows *Gnathochromis permaxillaris*.

Figure S2. New phylogenetic hypothesis including 196 East African cichlid species. Bootstrap support values are indicated at nodes. The phylogeny is based on mitochondrial (ND2) sequences obtained from GenBank (see Supporting Information, Table S5) and nuclear sequence data (42 genes) obtained from Meyer *et al.* (2015); Meyer & Salzburger (2012) and GenBank (Supporting Information, Table S6).

Table S1. Sample sizes per species for fin area and muscle weight measurements.

Table S2. Characterization of 159 Lake Tanganyikan cichlid species according to benthic–limnetic and sandy–rocky habitat use.

Table S3. Results of a correlation analysis according to a classic linear model excluding the sexually dimorphic *Enantiopus melanogenys*.

Table S4. Results of a correlation analysis corrected for phylogenetic dependence of trait values using PGLS excluding the sexually dimorphic *Enantiopus melanogenys*.

Table S5. Listed are the used ND2 sequences with species name and accession number: first are the species for which nuclear markers are also available [from Meyer & Salzburger (2012) and Meyer *et al.* (2015)]; then followed by other available species (alphabetically ordered) with ND2. It is indicated if the species was used for further analyses.

Table S6. Additional sequences for nuclear loci downloaded from GenBank.