

Comparative scale morphology in the adaptive radiation of cichlid fishes (Perciformes: Cichlidae) from Lake Tanganyika

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The morphology of fish scales has been investigated for > 200 years, but research on evolutionary patterns of scale morphology is scarce. Here, we study scale morphology and its evolution in the adaptive radiation of cichlid fishes from Lake Tanganyika, which are known for their exceptional diversity in habitat use, feeding ecology and morphology. Based on a geometric morphometric approach on eight scales per specimen (covering different body regions), we quantify scale types and morphology across nearly all ~240 species of the cichlid adaptive radiation in Lake Tanganyika. We first show that scale type, shape and ctenii coverage vary along the body, which is probably attributable to adaptations to different functional demands on the respective scales. Our comparative analyses reveal that flank scale size is tightly linked to phylogeny, whereas scale shape and ctenii coverage can be explained only in part by phylogenetic history and/or our proxy for ecology (stable isotopes and body shape), suggesting an additional adaptive component. We also show that our measured scale characteristics can help to assign an individual scale to a taxonomic group or ecotype. Thus, our data may serve as a valuable resource for taxonomic studies and to interpret fossil finds.

ADDITIONAL KEYWORDS: ctenii coverage – ecology – geometric morphometric – morphological delineation – scale shape – scale size.

INTRODUCTION

Scales (ossified platelets covering the skin) are a vital phenotypic trait of most bony fishes. Swiss zoologist Louis Agassiz (1807–1873) was among the first to use scale characteristics as a tool for taxonomic assignments, grouping fishes into four categories according to their scale morphology: Placoidei, Ganoidei, Ctenoidei and Cycloidei (Agassiz, 1834). Although it soon became clear that this clustering reflects evolutionary relationships only in part, the morphological classification of fish scales established by Agassiz has remained in place ever since (Roberts, 1993).

Teleost fishes, which make up ~96% of all extant fish species, feature two types of scales, ‘cycloid’ and ‘spined’ scales. Both scale types can vary in shape and thickness (Garduño-Paz *et al.*, 2010; Masood *et al.*, 2015; Wainwright & Lauder, 2016; Bräger *et al.*, 2017)

and in the position of their ‘focus’, i.e. the central part of the scale, which is also the first part to appear during ontogeny (Lagler, 1947; see Fig. 1). Cycloid scales have smooth posterior edges (Fig. 1A, centre), whereas spined scales have spine-like features on their posterior side, by which they can be divided further into three subtypes (Helfman *et al.* 2009): ‘crenate’ scales (with indentations in the scale margin), ‘spinoid’ scales (with spine-like processes on the posterior scale surface that are part of the scale ossification) and ‘ctenoid’ scales (with tooth-like spines called ‘ctenii’ that are separately ossified structures; Fig. 1A, top) that vary in the degree of ctenii coverage (Wainwright & Lauder, 2016).

Fish scales serve multiple purposes. Important functions of scales include physical protection against environmental influences, parasites and predators (Vernerey & Barthelat, 2014; Wainwright & Lauder, 2017). For example, the mechanics and structure of fish scales make it harder for predators to penetrate the skin of a potential prey with their teeth (Chen *et al.*, 2012; Yang *et al.*, 2013; Zhu *et al.*, 2013). In

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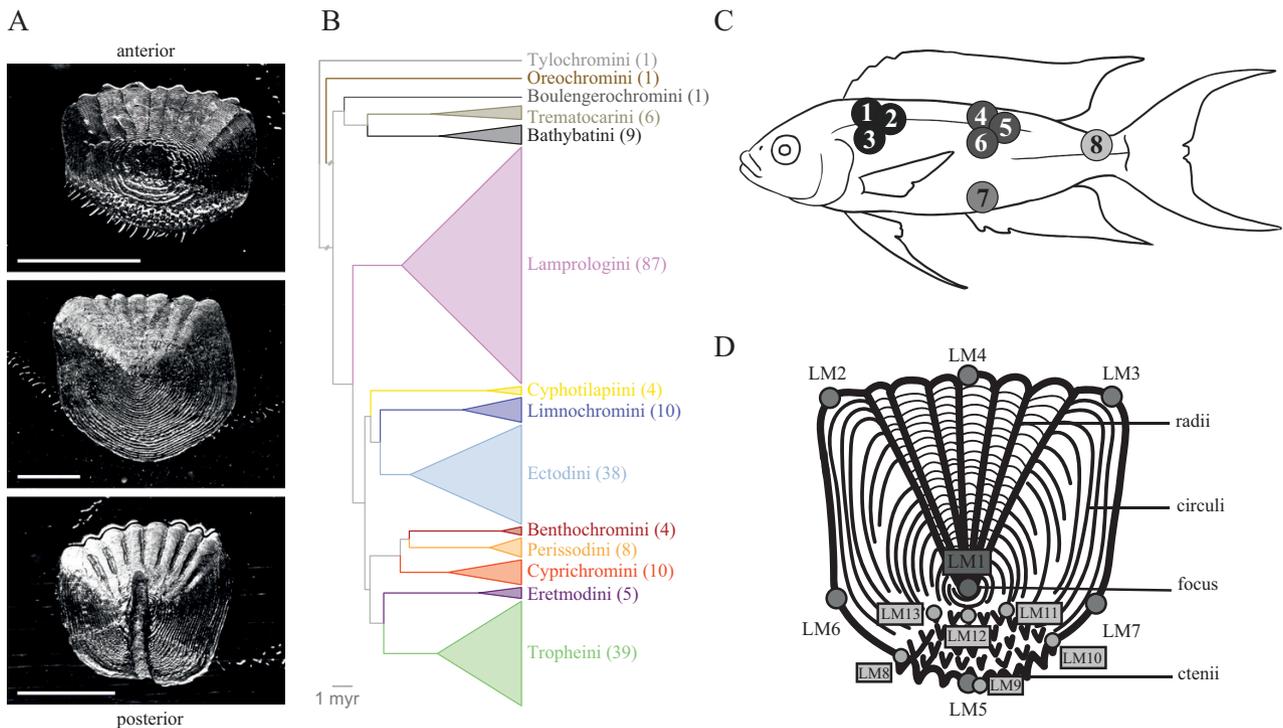


Figure 1. A, scale types in cichlid fishes from African Lake Tanganyika: ctenoid (top), cycloid (centre) and lateral line scales (bottom). Scale bars: 1 mm. B, phylogenetic tree of the cichlid radiation of African Lake Tanganyika (data from Ronco *et al.*, 2021). The different colours denote the cichlid tribes, with the number of species indicated in parentheses. Note that the clade ages for Oreochromini and Tylochromini are not drawn to scale. C, locations of the scales sampled from four different body regions used in this study, following a colour code: anterior flank area (scales 1–3), central flank area (scales 4–6), ventral area near the anal fin (scale 7) and the caudal peduncle area (scale 8). D, schematic drawing of a ctenoid scale, with scale characteristics and the selected landmarks (LMs) for morphometric analyses: LM1 is the focus; LM2–LM7 describe the overall scale shape; and LM8–LM13 define the ctenii area.

sharks, it was found that densely aligned small scales (in this case dermal denticles, a functional analogue of ossified scales) on the ventral part of the fish protect their internal organs (Sudo *et al.*, 2002). For African cichlid fishes, it has also been suggested that large and thick scales provide protection against scale-eating cichlids, because these scales are harder to pluck (Mushagalusa *et al.*, 2019). Scales also play an important role in locomotion. Ctenii (together with other scale features, such as circuli, radii and scale curvature) influence the bending and stiffness range of scales, which in turn influences the force needed to bend the body while swimming and thus the general swimming ability (Wainwright & Lauder, 2016, 2017). Additionally, the scale surface has been hypothesized to have hydrodynamic functions; for example, the smooth surface of cycloid scales reduces friction in laminar boundary layers, and the rough surface of ctenoid scales is likely to control turbulences in turbulent boundary layers, which reduces drag by delaying layer separation (Aleyev, 1977; Burdak, 1986; Wainwright & Lauder, 2016). However, ctenii

also facilitate the attachment of epidermal and mucous layers on the scales, thereby reducing surface friction (Wainwright & Lauder, 2017). Variation in scale morphology across fish species can thus reflect ecological adaptations (Ibáñez *et al.*, 2009; Garduño-Paz *et al.*, 2010). On the other hand, the morphology of fish scales has been shown to mirror phylogenetic relationships (Ibáñez *et al.*, 2007; Masood *et al.*, 2015).

In this study, we examine the extent to which body scale morphology is correlated with phylogeny vs. ecology in the massive adaptive radiation of cichlid fishes in African Lake Tanganyika, through the examination of an almost complete collection of Tanganyikan cichlids. The cichlid fish fauna of Lake Tanganyika is the product of the oldest cichlid adaptive radiation in any of the African Great Lakes, with an estimated age of ~10 Myr (Ronco *et al.*, 2021), and represents the ecologically, morphologically and behaviourally most diverse cichlid species flock (Fryer & Iles, 1972; Greenwood, 1984; Salzburger *et al.*, 2014). The ~240 cichlid species occurring in Lake Tanganyika differ substantially in body shape and

trophic morphology, reflecting adaptations to a variety of niches provided by the lake (Muschick *et al.*, 2012, 2014; Ronco *et al.*, 2021). The cichlid species of Lake Tanganyika cover multiple trophic levels, a wide range of habitat preferences along the benthic–pelagic axis and various swimming modes related to habitat use and foraging (Muschick *et al.*, 2012; Wilson *et al.*, 2015; Colombo *et al.*, 2016; Ronco *et al.*, 2021). Recently, it has been shown that ecomorphological diversification did not happen gradually over the course of the adaptive radiation of cichlid fishes in Lake Tanganyika, but instead occurred in trait-specific pulses of accelerated phenotypic evolution (Ronco *et al.*, 2021).

Although scale characteristics and other lepidological traits, including scale shape and squamation patterns, have been investigated before in cichlids (Lippitsch, 1991, 1998, 2001), they have not yet been examined in a larger phylogenetic and taxonomic framework in Lake Tanganyika, nor in the context of the evolutionary process of adaptive radiation. Here, we make use of a recently available genome-wide phylogeny and ecological assessment through stable isotope signatures of virtually all species of the cichlid adaptive radiation in Lake Tanganyika (Ronco *et al.*, 2021) to explore and disentangle potential associations between body scale morphology and phylogeny, ecology and overall body measurements. The taxonomic sampling of the present study comprised eight scales each of, typically, two specimens of 223 species of cichlid fishes from Lake Tanganyika, belonging to 14 subclades (i.e. tribes; Fig. 1B). By taking a geometric morphometric approach, we quantified scale measurements, such as scale size (centroid size), ctenii coverage (ctenii area/scale area) and scale shape. These characteristics are frequently used to identify and classify teleost genera or even species (Roberts, 1993; Sire & Arnulf, 2000; Jawad, 2005; Ibáñez *et al.*, 2007, 2011, 2012; Garduño-Paz *et al.*, 2010; Masood *et al.*, 2015; Wainwright & Lauder, 2016; Bräger *et al.*, 2017). Based on this comprehensive dataset, we initially characterized the diversity of scale features across the entire cichlid species flock in Lake Tanganyika and quantified differences and similarities among scales sampled from different body regions and among the subclades of the radiation. Furthermore, we integrated our data into a phylogenetic framework and investigated the association of different scale measurements with niche use of the respective species (approximated via stable carbon and nitrogen isotope signatures) and with overall body morphology (body size and shape). Finally, we assessed whether scale morphology can potentially be used for taxonomic or ecotype assignment in Lake Tanganyika cichlids, which might become highly relevant for the prospective scientific drilling project in Lake Tanganyika (Russell *et al.*, 2020).

MATERIAL AND METHODS

SAMPLE COLLECTION

Scale samples were obtained from preserved specimens from the Lake Tanganyika cichlid collection of the Zoological Institute, University of Basel, Switzerland. In total, we sampled scales from 223 cichlid species from Lake Tanganyika, covering ~93% of all cichlid species occurring in that lake and 14 of the 15 tribes (all but Coptodonini, of which only a single species occurs in riverine estuaries around Lake Tanganyika) (Ronco *et al.*, 2020). Two specimens were sampled per species, except for *Bathybates hornii* Steindachner, 1911 (tribe: Bathybatini), for which only one specimen was available. All sampled specimens were adults, except for *Oreochromis tanganyicae* (Günther, 1894) (tribe: Oreochromini), for which scales were taken from subadults. Further details on the specimens used for this study, such as sampling locations and dates, in addition to individual body size and weight information, are provided as Supporting Information (Table S1). From each specimen, eight scales were plucked from the right side of the body, from four different body regions (Fig. 1C): the anterior flank area (scales 1–3), the central flank area (scales 4–6), the ventral area near the anal fin (scale 7) and the caudal peduncle area (scale 8). Both the anterior and the central flank areas included one lateral line scale each (Fig. 1A, bottom). The specialized scales on the lateral line are characterized by canals harbouring canal neuromasts, which function as mechanoreceptors. With these receptors, fish can determine the source of hydrodynamic fluctuations, which is important for navigation, prey and predator detection and communication (Kasumyan, 2003; Webb & Ramsay, 2017).

Whenever possible, we sampled the scales from the same positions in each specimen. In the case of missing scales or regenerating (hence, not fully grown) scales, we sampled the neighbouring scale. In total, we collected 3552 scales from 444 specimens.

Upon removal from the specimens, scales were soaked for 20 min in washing detergent (handymatic supreme, concentration: 1 g in 10 mL H₂O) to detach residual mucus and skin. Remaining mucus and/or skin was carefully removed with a Q-tip. The scales were then rinsed with 70% ethanol and distilled water. Clean scales were mounted on a glass slide with double-sided adhesive tape (all eight scales of a given specimen were arranged on a single slide). The glass slide was immediately covered with a second slide to avoid curling of the scales and sealed with glue. Each scale was subsequently photographed using a Leica stereomicroscope (Leica M205 FA) with a mounted digital camera (Leica DFC310 FX) and the LEICA APPLICATION SUITE, v.3.7.0 (build: 681). A scale bar was included on each digital image.

DATA COLLECTION

To obtain the size, shape and ctenii area of the investigated cichlid scales, we placed 13 landmarks (Fig. 1D) on each digital image of the scales using the software TPSDIG2 (Rohlf, 2008; v.2.31). The image scale information was extracted from the image metadata file and included in the tps file to retain size information of the landmark coordinates. One landmark (LM1) represented the position of the focus in the centre of the scale. Six landmarks (LM2–LM7) were defined to describe the overall shape of the scales; another six landmarks (LM8–LM13) defined the area covered with ctenii. Whenever this area was missing, which was the case for cycloid scales, these landmarks were recorded as missing data (NA). All subsequent statistical analyses were performed in RSTUDIO (RStudio Team, 2018; v.1.1.447).

The following measurements were calculated from the raw landmark coordinates under consideration of the scale bar information: centroid size, scale area (LM2–LM7), ctenii area (LM8–LM13) and ctenii coverage (ctenii area/scale area). Areas were calculated using the `areapl` function in the R package `splanx` (Rowlingson & Diggle, 2017; v.2.01-40). Note that, hereafter, we refer to centroid size as scale size. Scale area was used only to calculate ctenii coverage (see “Characterization of scale type and morphology”) and in the discriminant analyses (see “Discriminant analyses based on scale morphology”).

CHARACTERIZATION OF SCALE TYPE AND MORPHOLOGY

To assess intraspecific variation in scale type, we scored, per species, the scales from the different body regions as either ctenoid (both individuals ctenoid), mixed (one individual ctenoid and one individual cycloid) or cycloid (both individuals cycloid). In an additional step, we classified each species as ctenoid, mixed or cycloid on the basis of all eight scales investigated per specimen and summarized the results per tribe. Differences among scales sampled from body regions or tribes, respectively, were assessed using pairwise Fisher’s tests, implemented in the R package `reporttools` (Rufibach, 2009; v.1.1.2), applying a Benjamini–Hochberg correction to adjust for multiple testing.

As a next step, we focused on the ctenoid scales and quantified variation in ctenii coverage. We therefore calculated the mean ctenii coverage of the scales per species, once for each scale sampled from the different body regions and once over all eight scales (summarized per tribe). We then tested for differences in ctenii coverages among scales from different body regions and among tribes using an ANOVA and performed pairwise comparisons using

the function `TukeyHSD` to account for multiple testing.

To analyse overall scale shape, we used the landmarks present in all scales (LM1–LM7) and performed a generalized Procrustes analysis (GPA) to scale, rotate and align the raw coordinates using the R package `geomorph` (Adams and Otárola-Castillo, 2013; v.3.0.6). We then used these size-independent shape variables to perform a principal components analysis (PCA). This was done for all eight scales together and for scales from the different body regions separately. To visualize the changes in shape associated with the principal component (PC) axes, we plotted a deformation grid for the minimum and maximum score per PC axis based on the mean shape of all scales using the R package `geomorph`.

COMPARATIVE ANALYSES OF FLANK SCALE MORPHOLOGY

Following previous studies (Ibáñez *et al.*, 2009; Ibáñez & O’Higgins, 2011; Bräger & Moritz, 2016; Bräger *et al.*, 2017), we focused on the morphology of the central flank scales for phylogenetic comparative analyses. It has become apparent that flank scales are the most suitable for comparative analyses in fish, and these scales are also the most common ones to be found in sediments (Shackleton, 1988). Given that scale 5 is a lateral line scale, we selected scale 6 as a representative flank scale across all specimens; however, we repeated the analyses using scale 4, which led to similar results. To analyse the evolution of scale morphology across the cichlid adaptive radiation in Lake Tanganyika, we used species means of ctenii coverage and scale shape (PC1–PC2; see previous subsection). Furthermore, we obtained the centroid size of the scales, hereafter referred to as scale size. Given that scale size depends strongly on body size ($R^2 = 0.46$, $P < 0.005$), we size-corrected the scales for downstream analyses using the ratio of scale size to body centroid size (hereafter referred to as relative scale size); data on body centroid sizes of the same specimens were obtained from Ronco *et al.* (2021). The phylogenetic hypothesis (based on genome-wide single nucleotide polymorphism data) was taken from Ronco *et al.* (2021) and pruned to our taxon sampling. Given that *O. tanganyicae*, *Tylochromis polylepis* (Boulenger, 1900) and *Xenotilapia ornatipinnis* (south) Boulenger, 1901, were missing in the phylogeny, we excluded these species for all phylogenetic comparative analyses ($N = 220$ species).

To determine the phylogenetic signal in the flank scale measurements (scale 6), we used the function `phylosig` from the R package `phytools` (Revell, 2012; v.0.7.47). Two methods were applied to estimate the strength of the phylogenetic signal: λ (Pagel’s

lambda; Pagel, 1999) and K (Blomberg's K ; Blomberg *et al.*, 2003), whereby λ can range from zero (no phylogenetic signal) to one (trait values match the phylogeny, assuming Brownian motion) and K can reach values above one, indicating greater phylogenetic signal than expected under a Brownian motion model of evolution.

To investigate the relationships between scale measurements and ecology and between scale measurements and overall body morphology (body size and shape), we used available data derived from the same specimens (Ronco *et al.*, 2021). As a proxy for niche use, we used stable nitrogen and carbon isotope signatures ($\delta^{15}\text{N}$ values and $\delta^{13}\text{C}$ values). Thereby, $\delta^{15}\text{N}$ informs about the relative trophic position of species, and $\delta^{13}\text{C}$ can be used to estimate the ecology of species along the littoral–pelagic trajectory (Ronco *et al.*, 2021). For *Ctenochromis benthicola* (Matthes, 1962), no stable isotope data were available; hence, we excluded this species from analyses that included stable isotope data. Data on body measurements (centroid size and body shape) were extracted from landmark data (Ronco *et al.*, 2021). We used PC1 of body shape, which represents mainly body elongation (low PC1 scores for deep-bodied species and high PC1 scores for elongated species), because this is the main axis of body shape disparity across the cichlid adaptive radiation in Lake Tanganyika (Muschick *et al.*, 2012; Ronco *et al.*, 2021). For *Xenotilapia caudafasciata* Poll, 1951, *Julidochromis marksmithi* Burgess, 2014, *Baileychromis centropomoides* (Bailey & Stewart, 1977), *Limnotilapia dardennii* (Boulenger, 1899) and *Petrochromis* sp. ‘giant’, measurements were not available for the same specimens from which the scales were taken. Therefore, we excluded these five species from analyses that required body measurements.

To test for an association between scale characteristics and ecology ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values) and overall body morphology, we performed phylogenetic regression analyses using phylolm (Ho & Ané, 2014), applying the ‘lambda’ model. Furthermore, we tested whether there is an association between scale shape (PC1 and PC2) and scale size and ctenii coverage, respectively. To assess the variance of scale measurements (scale size, ctenii coverage and scale shape) explained by phylogeny, ecology and overall body morphology, we calculated partial R^2 values for each explanatory variable using the function R^2 from the rr2 package (Ives, 2019). This function compares the variance explained by a full model (scale measurements and all the covariates) with a reduced model and returns the lost variance of the dropped variable. Specifically, we chose the R^2_{lik} method to assess the importance of our different parameters for variation in scale characteristics.

DISCRIMINANT ANALYSES BASED ON SCALE MORPHOLOGY

To examine the possibility of drawing conclusions about the taxonomy, ecology (e.g. habitat and foraging strategy) and/or body size or shape of a species from information obtained from scale data alone, we next focused on the raw scale measurements (without applying any size correction).

To compute the probability of assigning a specimen to its correct taxonomic or ecological group, we performed a series of linear discriminant analyses (LDAs) using the `lda` function implemented in the R package MASS (Venables & Ripley, 2002; v.7.3-51.6) and applying a leave-one-out cross-validation. As explanatory variables in the models, we used the flank scale parameters scale area, ctenii area and scale shape (PC1–PC6) from both specimens per species. In the first LDA, we tested for taxonomic delineation by using tribe as a grouping factor. To test for grouping by ecotype, we used available ecological categories of the species (Ronco *et al.*, 2021). Habitat was represented by the five categories ‘deep’, ‘intermediate’, ‘littoral’, ‘pelagic’ and ‘shallow’. Feeding ecology was represented by 11 different dietary preferences ranging from eating invertebrates, plants, plankton, other fish and fry to scales and mixed diets. We divided the species into further categories according to size (small, medium or large body size) and shape (deep, intermediate or elongated body) and tested whether it is possible to predict the size or shape of a specimen given its scale measurements.

RESULTS

CHARACTERIZATION OF SCALE TYPES FROM DIFFERENT BODY REGIONS AND ACROSS TRIBES

The comparison of the eight scales per specimen sampled from different body regions reveals that the majority of cichlid species in Lake Tanganyika feature exclusively ctenoid scales on the caudal peduncle (86.1%), in the central flank area (scale 4, 76.7%; scale 5, 84.8%; scale 6, 85.7%; for examples of the scale diversity of scale 6, see Fig. 2A) and in the ventral–anal area (scale 7, 71.3%; Fig. 2B). Most of the variation in scale types, both within and among species, is detected in the anterior flank area, where only 35.9% (scale 1), 37.7% (scale 2) and 57.9% (scale 3) of the species have exclusively ctenoid scales. Furthermore, it appears that lateral line scales (scales 2 and 5) do not seem to have a tendency to be either cycloid or ctenoid. A comparison of scale types among the Tanganyikan cichlid tribes, irrespective of the body region from which these were sampled, reveals that the members of most tribes, especially of the more species-rich

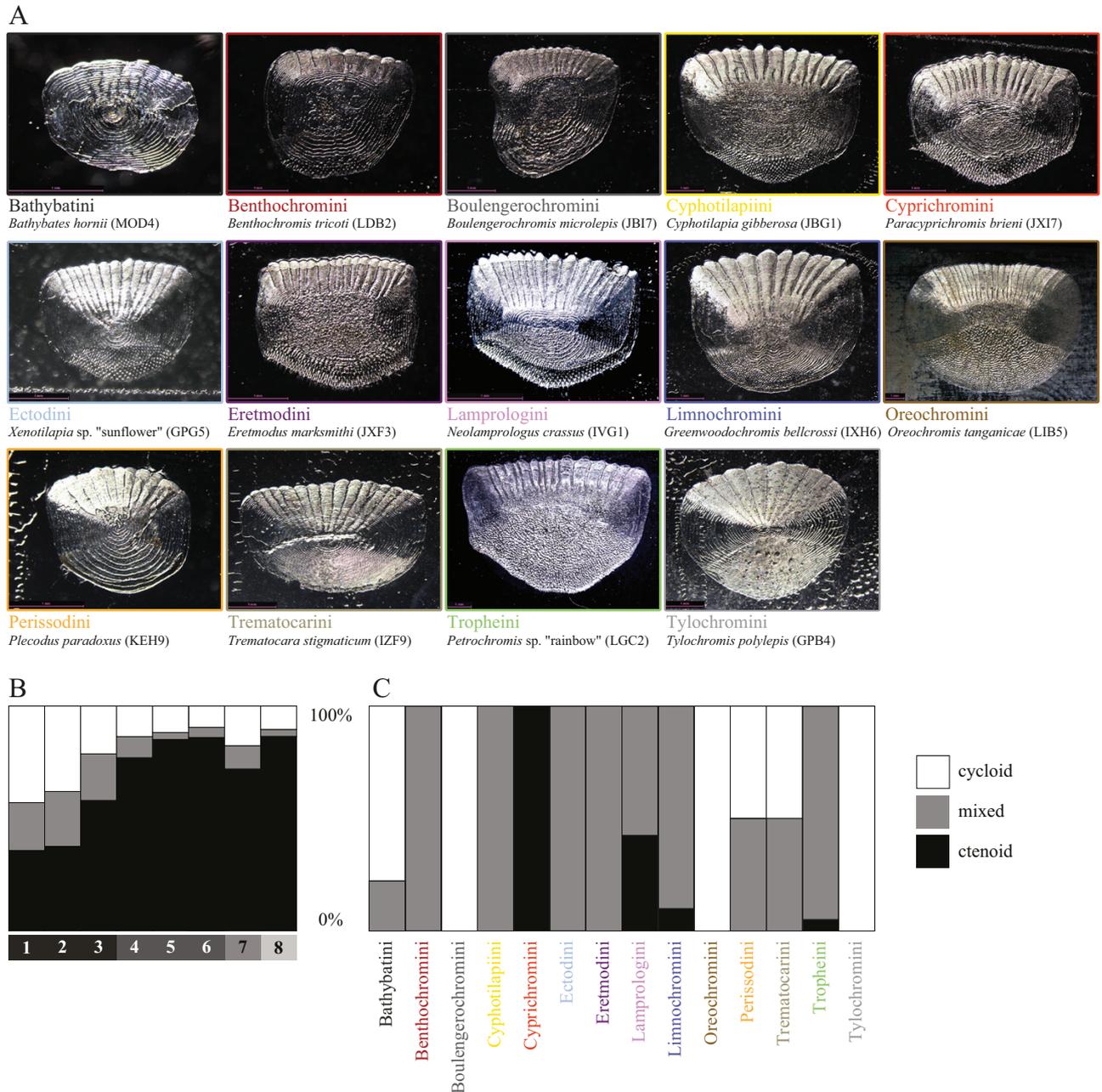


Figure 2. Overview of scale diversity in cichlids from Lake Tanganyika. A, photographs showing scale variation in flank scales (scale 6). For each tribe, we show the scale closest to the mean shape of the tribe. B, C, to show variation in scale type in the species flock, both individuals per species were combined to classify the species as ‘cycloid’, ‘mixed’ and ‘ctenoid’ in scales sampled from different regions of the fish’s body (B; for body regions, see Fig. 1) and across all body regions, summarized by the cichlid tribes of Lake Tanganyika (C). For each category (body region or tribe), the bar plot shows the percentage of species with all scales being cycloid (white), all scales being ctenoid (black) or with both scale types present (grey). Note that the tribes vary substantially in their number of species, as indicated in Figure 1A.

tribes (Ectodini, Lamprologini and Tropheini), possess both scale types (Fig. 2C). In contrast, the three cichlid tribes that are represented by a single species in Lake Tanganyika (Boulengerochromini,

Oreochromini and Tylochromini) turn out to have cycloid scales exclusively. Furthermore, one of the most ancestral tribes of the cichlid adaptive radiation in Lake Tanganyika, the Bathybatini (nine species),

have predominantly cycloid scales, with the exception of *Bathybates vittatus* Boulenger, 1914, and *Hemibates koningsi* Schedel & Schliewen, 2017. In case of the Cyprichromini (ten species), all species have ctenoid scales on all sampled body regions. Interestingly, the species in the tribes Perissodini and Benthochromini, which are closely related to the Cyprichromini (see Fig. 1B), have both scale types, and not a single species in these tribes possesses exclusively ctenoid scales.

The proportions of the scale area covered with ctenii (ctenii coverage) differs among scales sampled from different body regions (Fig. 3A; ANOVA_{body region}: $F = 31.4$, $P < 0.0001$) and among tribes (Fig. 3B; ANOVA_{tribe}: $F = 45.54$, $P < 0.0001$). Tukey's HSD test reveals significant differences in ctenii coverage between two anterior flank scales (scales 1 and 3) and the central flank scales (scales 4–6). Among the scales of the anterior flank, there is no difference in ctenii coverage (Fig. 3A). Also, the scales on the central flank (scales 4–6) are similar in ctenii coverage. Ctenii coverage of scale 7 does not differ from scales 2, 4 and 6. In scale 8, the ctenii coverage is comparable to the ctenii

coverage of the scales on the central flank area. Across all scales, the tribes Eretmodini and Lamprologini have a significantly greater ctenii coverage than the Bathybatini, Benthochromini, Limnochromini and Trematocarini [Fig. 3B; for pairwise comparisons in scale type and ctenii coverage analyses (tribes and scales from different body regions), see Supporting Information, Tables S2 and S3]. With a ctenii coverage of 35.8%, *Altolamprologus compressiceps* (Boulenger, 1898) is the Tanganyikan cichlid species with the highest ctenii coverage.

SCALE SHAPE DIVERSITY

The PCA of landmark data across all species and scales sampled from the different body regions shows that the axes of most variance (PC1, explaining 56% of the total variance) mainly describes the aspect ratio of the scales (Fig. 4). Principal component 2 (explaining 17% of the total variance) is associated with changes in shape involving the width of the anterior vs. the

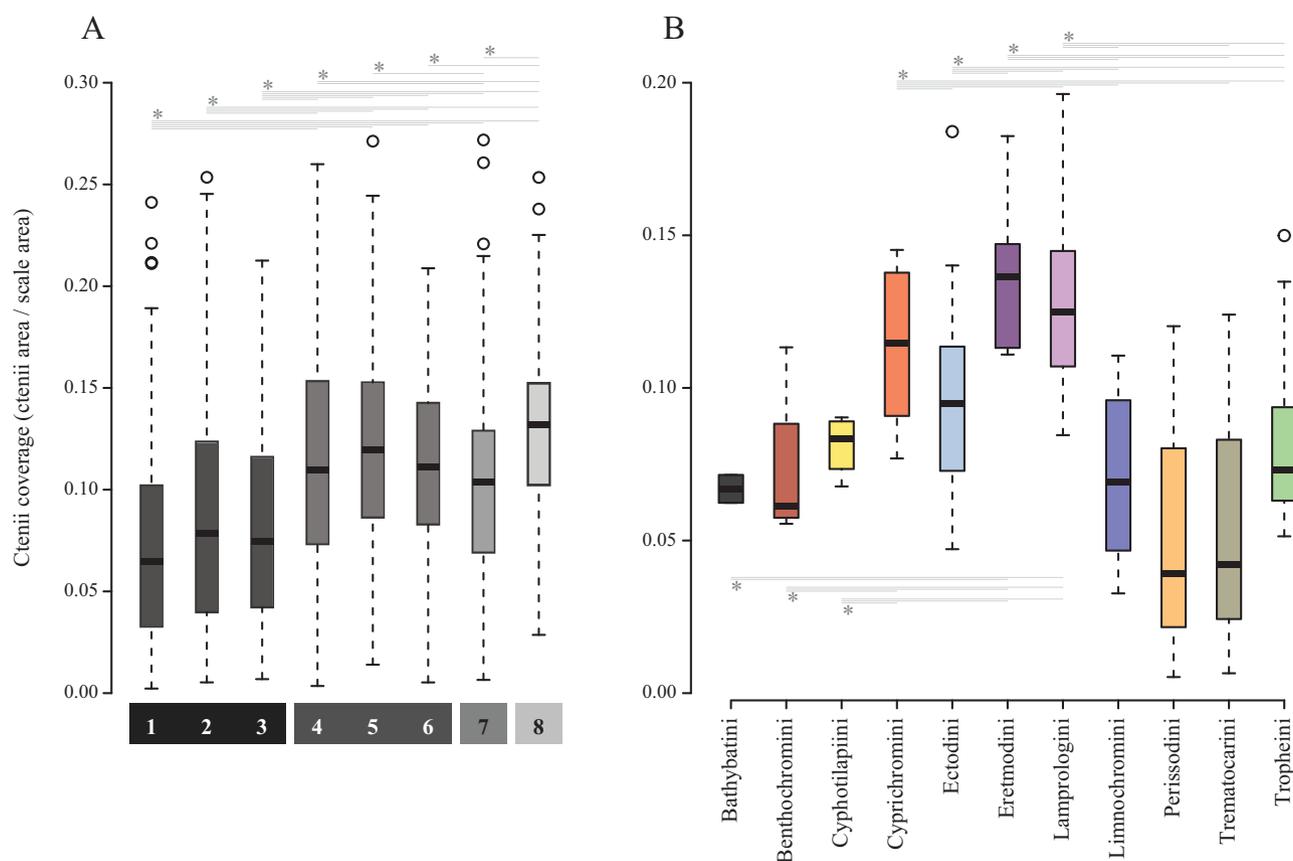


Figure 3. A, ctenii coverage (species means) of scales sampled from different regions of the body (see Fig. 1C; scales 1–3 on the anterior flank area, scales 4–6 on the central flank area, scale 7 on the ventral area, and scale 8 on the caudal peduncle area). B, ctenii coverage (species means) grouped per cichlid tribe of Lake Tanganyika (for the number of species per tribe, see Fig. 1B). *Significant differences between groups (for P -values, see Supporting Information, Table S3).

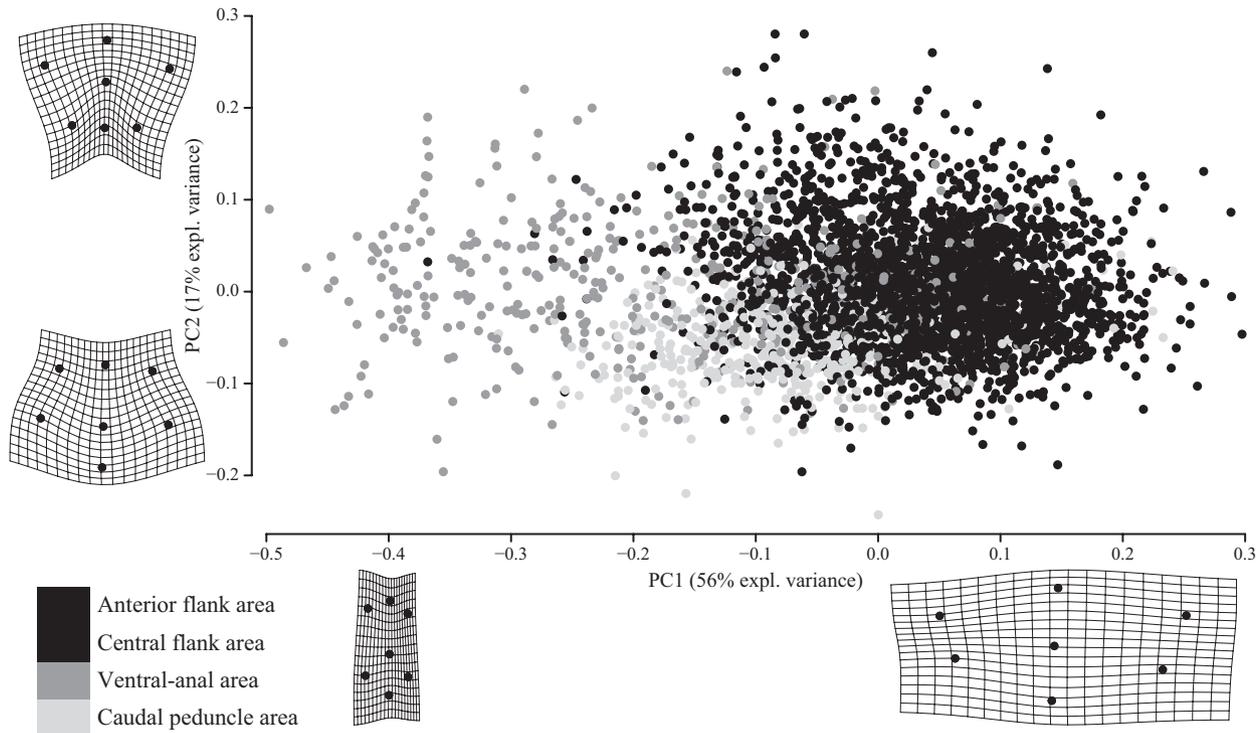


Figure 4. Variation in scale shape on different body regions of Lake Tanganyika cichlids. Scatterplot of principal component (PC) 1 (56% explained variance) against PC2 (17% explained variance) of a principal components analysis carried out on seven landmarks (LM1–LM7). Each data point represents a scale of a specimen (eight scales per specimen). The deformation grids shown next to the axes represent predictions when deforming the average landmark configuration of all specimens to the extreme points of PC1 and PC2, respectively.

posterior end of the scale in combination with a shift of the focus.

Principal component 1 mainly separates the scales sampled from the ventral–anal region of the body (scale 7) from all other scales. The highly elongated shape in its anterior–posterior length and a short width in its dorsoventral axis makes scale 7 the most differentiated scale regarding shape. Scales from both flank areas (scales 1–6) tend to group with a minor shift in their PC2 scores. Scales in the anterior flank area (scales 1–3) tend to have a wider anterior edge compared with their posterior side. In contrast, scales on the central flank area (scales 4–6) show a wider posterior edge compared with their anterior end. Scales from the caudal peduncle area (scale 8), which has a nearly pentagonal shape, have the lowest PC2 scores, associated with a wider posterior edge compared with the anterior end. Interestingly, the lateral line scales do not cluster separately in the PCA, suggesting that these do not differ in their overall shape captured by the selected set of LMs.

Overall, the PCA shows that the scales tend to cluster in morphospace according to the four body regions from which they were sampled, rather than

according to phylogenetic grouping (for the PCA coloured by cichlid tribe, see [Supporting Information, Fig. S1](#); for PCAs per body region, see [Supporting Information, Fig. S2](#)).

COMPARATIVE ANALYSES OF FLANK SCALE MORPHOLOGY (SCALE 6)

Scale shape diversity among tribes

In the separate PCA of the central flank scale (scale 6; [Fig. 5](#)), only a few tribes cluster separately in morphospace. Species of the tribes Bathybatini and Benthochromini occupy the most extreme positions along PC1, whereby the scales of the Bathybatini are the widest along the dorsoventral axis and scales from the Benthochromini are longer than wide in anterior–posterior axis. In PC2, the Bathybatini and Benthochromini are distinguishable from other tribes by a wider anterior margin of the scale and a focus that is placed around the centre of the scale or anterior to it. Overall, however, there is no clear clustering in the shape of scale 6 according to tribe. The most species-rich tribe, the Lamprologini, features very diverse scale shapes and overlaps with members of almost all

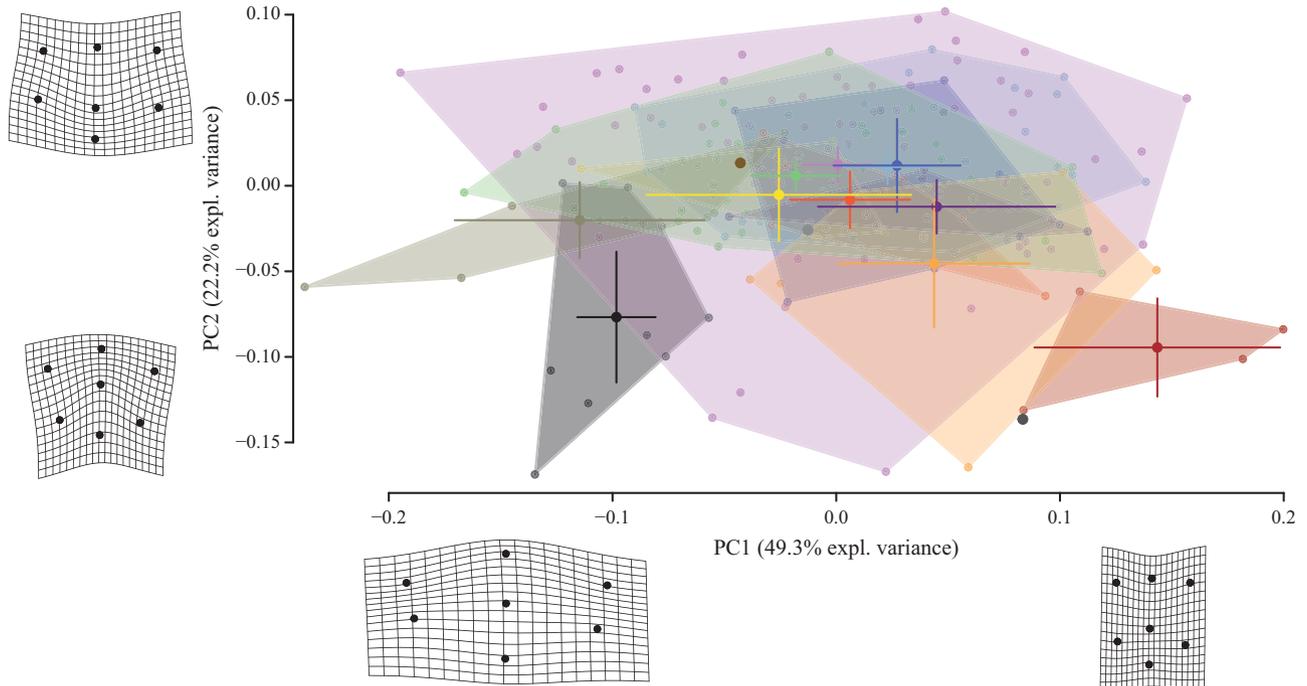


Figure 5. Variation in scale shape of the central flank scale (scale 6) among Lake Tanganyika cichlids. Scatterplot of principal component (PC) 1 (49.3% explained variance) against PC2 (22.2% explained variance) of a principal components analysis carried out on seven landmarks (LM1–LM7) coloured according to the species' cichlid tribe of Lake Tanganyika (for colour key and number of species per tribe, see Fig. 1B). Each data point represents a species mean. The larger coloured dots show the tribe means with 95% confidence intervals of the mean (note that three tribes are represented by one species and therefore have no confidence interval). The deformation grids shown next to the axes represent predictions when deforming the average landmark configuration of all specimens to the extreme points of PC1 and PC2, respectively.

the other tribes (Fig. 5). The PCA of the second flank scale (scale 4) reveals very similar results (Supporting Information, Fig. S2).

Phylogenetic signals in flank scale characteristics

When testing for phylogenetic signals in all examined scale characteristics of the central flank scale, we find evidence for a strong phylogenetic signal in relative scale size, with $K > 1$ ($\lambda = 0.99$, $K = 1.06$). Furthermore, we observe phylogenetic signals in ctenii coverage ($\lambda = 0.75$, $K = 0.66$) and scale shape (PC1: $\lambda = 0.77$, $K = 0.38$; PC2: $\lambda = 0.77$, $K = 0.43$) that are weaker than expected under a Brownian motion model of trait evolution. All observed phylogenetic signals pass the 0.05 significance threshold (Table 1; Supporting Information, Table S4).

Association between scale characteristics and covariates

A phylogenetic linear regression analysis to test for an association between each of the scale parameter [relative scale size (corrected for body size), ctenii coverage and scale shape (PC1 and PC2)] and ecology and overall body morphology (while accounting for

phylogenetic dependence of data points), reveals that relative scale size scales weakly with body shape ($R^2 = 0.06$, $P < 0.0002$). Thus, there is a tendency for fish with deeper bodies to have larger scales, whereas more elongated fish have smaller scales. No association is found between relative scale size and stable isotope signatures ($\delta^{13}\text{C}$: $R^2 = -0.0045$, $P = 0.84$; $\delta^{15}\text{N}$: $R^2 = -0.0004$, $P = 0.34$). Ctenii coverage shows only a very weak association with body size ($R^2 = 0.023$, $P = 0.016$) and stable isotope signatures ($\delta^{13}\text{C}$: $R^2 = 0.018$, $P = 0.029$; $\delta^{15}\text{N}$: $R^2 = 0.012$, $P = 0.08$), but none with body shape ($R^2 = -0.004$, $P = 0.65$). Likewise, there is no association between scale shape (PC1 and PC2) and overall body morphology or between scale shape and our proxy for ecology (see Table 1). Nevertheless, we find that scale shape is linked to relative scale size (PC1: $R^2 = 0.013$, $P = 0.055$; PC2: $R^2 = 0.24$, $P < 0.005$) and ctenii coverage (PC1: $R^2 = 0.03$, $P = 0.006$; PC2: $R^2 = 0.13$, $P < 0.0001$; see Table 2). The separation of data points along PC1 suggests that wide scales are typically larger and not as much covered with ctenii as are the more narrow and smaller scales. Principal component 2 shows that scales with their focus posterior to the centre and with

Table 1. Phylogenetic signal inferred for each scale parameter and results of the phylogenetic regression analyses testing for an association of scale parameters [relative scale size, ctenii coverage and shape (PC1 and PC2)] with ecology or body measurements. The inferred phylogenetic signals are supported by P-values below 0.05.

Ecology and body	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$			Body size (centroid size)			Body shape (PC1)			Phylogenetic signal	
	R^2	λ	P-value	R^2	λ	P-value	R^2	λ	P-value	R^2	λ	P-value	λ	K
Relative scale size	-0.005	1.00	0.843	0.000	1.00	0.339	0.061	1.00	0.000	0.057	1.00	0.000	0.999	1.062
Ctenii coverage	0.018	0.78	0.029	0.012	0.76	0.082	0.023	0.74	0.016	-0.004	0.77	0.654	0.749	0.771
Shape (PC1)	-0.002	0.78	0.411	-0.001	0.79	0.363	-0.003	0.77	0.594	-0.004	0.78	0.691	0.772	0.384
Shape (PC2)	-0.005	0.77	0.838	-0.004	0.77	0.614	-0.002	0.79	0.449	0.000	0.74	0.345	0.771	0.436

their ventral and dorsal sides parallel to each other tend to be larger in relative size. These scales also feature a higher ctenii coverage than scales with the focus closer to the anterior side (more negative PC2 values).

A phylogenetic linear regression analysis testing the association between each scale parameter (relative scale size, ctenii coverage and the PC1 and PC2 of scale shape) and all covariates jointly reveals that the full model (scale characteristic ~ body size + body shape + $\delta^{13}\text{C}$ + $\delta^{15}\text{N}$, given the phylogeny) explains 75.9% of the variation in relative scale size, 54.6% in ctenii coverage, 36.6% in scale shape PC1 and 25.2% in scale shape PC2.

Calculating partial R^2 values for each covariate shows that 49.9% of the variation of relative scale size is explained by phylogeny, 7.5% by body shape and 8.2% by body size. Ecology does not appear to be a parameter that influences scale size. Likewise, variation in ctenii coverage is mostly explained by phylogeny (37.9%). However, in this case, ecology explains 3.1% ($\delta^{13}\text{C}$, 1.78% and $\delta^{15}\text{N}$, 1.9%) of the variation in scale size, and overall body morphology explains very little (body shape, ~0.003% and body size, 1.4%). In PC1 of scale shape, the phylogeny explains 35.6% of the variation, and in PC2 13.2%. The other parameters explain < 1% of the variation in scale shape.

The detailed results of the phylogenetic signal analysis, the phylogenetic linear regression analysis and the partial R^2 are shown in the [Supporting Information \(Table S4, for scales 6 and 4\)](#).

DISCRIMINANT ANALYSES BASED ON SCALE MORPHOLOGY

The cross-validated LDA of the flank scale measurements [scale area, ctenii coverage and scale shape (PC1–PC6)] show that 62.1% of the specimens can be assigned correctly into their respective tribe. However, the members of the tribes Boulengerochromini, Cyphotilapiini and Cyprichromini cannot be assigned to their tribes. Oreochromini, which are represented by a single species (two individuals), can be assigned correctly (100%). Lamprologini species are correctly assigned for 90.8% of the specimens, followed by Trematocarini with 75% and Tropheini with 64.1%.

The LDA with habitat as the grouping factor assigns 64.2% of the specimens correctly; 41.6% are correctly assigned according to feeding ecology, where the invertebrate feeding species are classified correctly with almost 70%, and herbivore species with 65%. When we test whether scale measurements are able to inform about the body morphology, the results show a correct assignment of 81% to body size and 62.6% to body shape (all results of the LDA are presented in the [Supporting Information, Table S5](#)).

Table 2. Results of the phylogenetic regression analyses testing for an association between scale shape (PC1 and PC2) and relative scale size as well as ctenii coverage

Within scale	Relative scale size			Ctenii coverage		
	R^2	λ	P -value	R^2	λ	P -value
Shape (PC1)	0.013	0.76	0.055	0.031	0.79	0.006
Shape (PC2)	0.238	0.48	< 0.0001	0.130	0.74	< 0.0001

DISCUSSION

SCALE DIVERSITY IN LAKE TANGANYIKA CICHLIDS

Our examination of 233 cichlid species from Lake Tanganyika reveals the presence of both scale types (cycloid and ctenoid) in this species flock, with variation with respect to tribe and to the body region from where the scales were taken (Fig. 2). The diversity in general scale type is comparable to what has been reported before from Lake Malawi cichlids (Kuusipalo, 1998). We find that cycloid scales are more frequent in the anterior flank area (scales 1–3) than in the other, more caudal body regions investigated. This is in agreement with a previous study by Lippitsch (1990), who found predominantly cycloid scales on the heads of 105 cichlid species from Lakes Victoria, Malawi and Tanganyika. Our results also show that the ctenoid scales on the central flank area vary in their ctenii coverage and that the scales on the caudal peduncle (scale 8) have the highest ctenii coverage. Similar results were reported for *Lepomis macrochirus* Rafinesque, 1810 (Wainwright & Lauder, 2016). Thus, the Tanganyikan cichlids appear to exemplify a general trend in fish, with cycloid scales being more commonly found on the anterior part of the body and ctenoid scales on the caudal part. This appears also to be reflected in ctenii coverage of ctenoid scales, where low ctenii coverage is observed on the anterior part of the body, and the coverage is highest close to the caudal peduncle. This increase of ctenii (presence and coverage) toward the posterior parts of the body can potentially be explained by different scale functions in these body regions. It has been hypothesized that cycloid scales are beneficial to reduce friction drag in the anterior part of the body, where laminar boundary layers should prevail. In contrast, in the posterior part of the body ctenoid scales help to control turbulences in the turbulent boundary layers, inhibiting separation of the layers, which in turn reduces drag (Aleyev, 1977; Burdak, 1986; Wainwright & Lauder, 2016).

Next to their function in increasing swimming performance, the presence of ctenii and, accordingly, also ctenii coverage might facilitate the attachment of epidermis and mucus on the respective scales

(Wainwright & Lauder, 2016, 2017). In this context, it is interesting to note that the Tanganyikan cichlid species possessing the flank scales with the highest ctenii coverage, *Altolamprologus compressiceps*, has previously been suggested to feature specific morphological traits in its scales that protect this species from predation by the scale-eating cichlid *Perissodus microlepis* Boulenger, 1898 (Mushagalusa *et al.*, 2019). For example, the scales of *A. compressiceps* are large, thick and difficult to tear off (Mushagalusa *et al.*, 2019), and this species, albeit being rather common in the shallow, rocky habitat of Lake Tanganyika, is indeed only rarely predated by scale eaters (Kovac *et al.*, 2019). Perhaps the outstandingly high ctenii coverage in this species reflects yet another defence mechanism against scale predators; for example, by facilitating epidermal embedding of the scale (Wainwright & Lauder, 2016, 2017).

Apart from scale surface modifications such as ctenii, scale shape is also likely to influence swimming performance. Ibáñez *et al.* (2009), for example, suggested that scales that are long in the anterior–posterior axis diminish thrust and water pressure generated by swimming. On the contrary, scales that are wide in the dorsoventral axis but short in the anterior–posterior axis should be beneficial in a subcarangiform swimming style. In the cichlid species from Lake Tanganyika, we predominantly find scales with the latter shape in the anterior and central flank scales (scales 1–6). However, in the absence of detailed information about the swimming style and performance in Lake Tanganyika cichlids, we cannot test for a potential association between scale shape and swimming style. The caudal peduncle scale (scale 8) is clearly distinct from the flank scales (scales 1–6) with respect to its size and shape. The characteristic pentagonal shape of scale 8 and its elongation along the anterior–posterior axis suggest its involvement in increasing swimming efficiency by controlling thrust and water pressure (Ibáñez *et al.*, 2009).

The observed variation in scale shape and characteristics among scales from different body regions (Figs 2B, 3A, 4) is likely to be the result of different functional demands depending on the

scales in different body region, as has been suggested in multiple studies before (e.g. [Ibáñez *et al.*, 2009](#), [2009](#); [Albutra *et al.*, 2014](#); [Wainwright & Lauder, 2016](#)). However, our knowledge of the hydrodynamic implications of scale morphology and the prevailing boundary layers in swimming fish is limited, and related hypotheses remain to be tested formally.

EVOLUTIONARY PATTERNS OF FLANK SCALE MORPHOLOGY

For an evolutionary analysis of scale morphology, we focus on a central flank scale above the lateral line (scale 6), because previous studies in several teleost species have shown that flank scales are the most suitable ones for the purpose of species identification ([Ibáñez *et al.*, 2009](#); [Ibáñez & O'Higgins, 2011](#); [Bräger & Moritz, 2016](#); [Bräger *et al.*, 2017](#)). More specifically, we test, in a comparative framework, for an association of scale characteristics (relative scale size, scale shape and ctenii coverage) with ecology and body morphology (size and shape) across the cichlid species from Lake Tanganyika.

Our analyses reveal a strong phylogenetic signal in the relative scale size data ([Table 1](#)), indicating that closely related species tend to be more similar in relative scale size to one other than when compared with more distantly related species. The phylogenetic regression analysis and the partial R^2 analysis show that relative scale size tends to scale negatively with body elongation (PC1 of body shape); that is, more elongated fish tend to have relatively smaller scales in comparison to high-bodied species. Smaller scale sizes have previously been associated with a faster swimming speed in fish ([Albutra *et al.*, 2014](#)). One might, therefore, hypothesize that the relatively smaller scales of elongated Tanganyikan cichlids are attributable to adaptations for a faster swimming speed in predatory and/or pelagic species, which tend to be more elongated ([Ronco *et al.*, 2021](#)). However, our integrative analyses do not support an association between relative scale size and our proxy for ecology, the stable isotope signatures. This is most probably because, in Lake Tanganyika cichlids, elongated body forms are not restricted to predatory and/or pelagic species, as illustrated by the most elongated cichlid species in Lake Tanganyika by means of the PC1 of body shape (*Enantiopus melanogenys*), which is a sand-dwelling invertebrate feeder.

In contrast to relative scale size, the shape of flank scales does not differ substantially among the cichlid tribes in Lake Tanganyika, with PC1 and PC2 of scale shape showing only a rather weak phylogenetic signal. Instead, we find substantial variation in scale shape within tribes and, with the exception of the Bathybatini

and Benthochromini, a lot of overlap between tribes in scale shape morphospace ([Fig. 5](#)). The phylogenetic linear models and the partial R^2 calculations reveal that variation in scale shape is evolutionarily not associated with body size and shape, nor with the ecology of the species. Also, phylogenetic relationships explain the observed differences in scale shape across species only in part. Given that neutral evolution of scale shape is thus an unlikely explanation for these findings, it is possible that ecological or functional associations are at play with respect to the evolution of scale shape that were not captured by our proxies for ecology (the stable isotope signatures) and for swimming mode and performance (body shape) ([Long *et al.*, 1996](#); [Ibáñez *et al.*, 2009](#)). For example, scale shape could potentially be influenced by (scale) predation pressure ([Nshombo *et al.*, 1985](#); [Kovac *et al.*, 2019](#); [Mushagalusa *et al.*, 2019](#)) or depend on the composition and quantity of food or compensatory growth during development ([Ibáñez *et al.*, 2012](#); [Staszny *et al.*, 2012](#)). Furthermore, using morphological traits related to foraging and food processing could potentially capture ecological trajectories missed by our stable isotope data.

The phylogenetic linear regression analysis shows that PC2 of scale shape is associated with relative scale size and with ctenii coverage. This implies that the shape of the anterior/posterior edge of the scale and the position of the focus is linked to scale size and ctenii coverage. These correlations within scales could potentially reflect correlated selection; however, developmental or genetic constraints could lead to similar relationships ([Powder *et al.*, 2015](#); [Albertson *et al.*, 2018](#)).

Similar to scale shape, ctenii coverage shows less phylogenetic signal than expected under a Brownian motion model of trait evolution. In addition, phylogenetic relationships can explain the observed variation between species only in part, suggesting an adaptive component of ctenii coverage; for example, to interact with the water flow, thereby influencing swimming performance ([Burdak, 1986](#); [Wainwright & Lauder, 2016](#)). Nonetheless, variation in ctenii coverage is not evolutionarily associated with body shape and can be explained only to a small extent by the stable isotope signatures. This again suggests that our proxy for ecology might not capture the relevant ecological trajectory.

SCALE CHARACTERISTICS AND TAXONOMY

Previous studies have shown that scale characteristics such as scale size and shape can be used taxonomically to assign specimens in Mugilidae ([Ibáñez *et al.*, 2007](#)), Centropomidae ([Ibáñez & O'Higgins, 2011](#)), Salmonidae ([Garduño-Paz *et al.*, 2010](#)), Nemipteridae ([Renjith *et al.*, 2014](#)), Cyprinidae ([Staszny *et al.*, 2012](#))

and Clupeidae (Bräger *et al.*, 2017). Also, the presence or absence, coverage and shape of ctenii has been used to delineate species (Ibáñez & Gallardo-Cabello, 2005; Ibáñez *et al.*, 2011). In our case of the cichlid fauna of African Lake Tanganyika, we are able to assign 62.1% of the flank scales to the correct tribe based on a discriminant analysis including scale area, ctenii area and scale shape (PC1–PC6) and to assign 64.2% of the flank scales to the correct ecotype of the respective species. In the more species-rich tribes, assignment is more accurate (see Supporting Information, Table S5), which might reflect, in part, a higher probability of a correct assignment by chance.

Overall, our results show that using a few scale characteristics can provide valuable information on tribe or ecotype in cichlids from Lake Tanganyika. Nonetheless, it is likely that the assignment can be improved by the addition of more scale measurements and characteristics, such as scale thickness and flexibility, number of circuli and radii (see Fig. 1), or patterns of the canals on the lateral line scales (Webb, 1990). Obtaining taxonomic or ecological information from single scales could potentially be of great value, in particular in the case of fossils obtained from sediments (Cockerell, 1919; Bieńkowska-Wasiluk *et al.*, 2015; Martín-Abad, 2017; Antczak & Bodzioch, 2018). A reliable taxonomic assignment of fossil scales can help in understanding the compilation of fish populations (Davidson *et al.*, 2003), speciation and extinction dynamics through time, e.g. through fossil-based time calibrations of phylogenetic trees (Heath *et al.*, 2014; Gunter *et al.*, 2016; Zhang *et al.*, 2016; Matschiner *et al.*, 2020), whereas an ecological interpretation of fossil scales might help in understanding eco-evolutionary dynamics (Shackleton, 1987; Antczak & Bodzioch, 2018). Thus, our study provides a valuable baseline dataset for taxonomic, ecological and evolutionary studies and will also become highly relevant for the future scientific drilling campaign in the oldest lake in Africa (Russell *et al.*, 2020).

CONCLUSION

In this study, we provide a broad-scale analysis of the diversity of scales across the entire adaptive radiation of cichlid fishes from Lake Tanganyika, on the basis of eight scales per specimen taken from four different body regions and 233 cichlid species in total. We report a great diversity with respect to scale type, size and shape, and we show that some of these characteristics are tightly linked to phylogeny. Furthermore, scale measurements vary not only among tribes or species, but also among body regions, which can be explained by different functional requirements of the scales (Ibáñez *et al.*, 2009; Garduño-Paz *et al.*, 2010; Tomiyama, 2013; Spinner *et al.*, 2016; Albertson *et al.*,

2018). Focusing on one flank scale per specimen, we find only weak support for an evolutionary association with ecology by means of our ecological proxies, stable C and N isotope values or overall body size and shape. However, we also find that phylogenetic relationships are not sufficient to explain the observed variation in scale shape and scale features, suggesting an additional adaptive component. Furthermore, we show that a small set of scale characters from an isolated scale alone is, to some extent, informative to assign a specimen into its taxonomic group or ecotype. In combination with other traits or an extended set of characters, our dataset is likely to serve as a valuable reference dataset to help in the interpretation of fossil finds.

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DATA AVAILABILITY

All data generated for this study are provided in the Supporting Information (Appendices S1 and S2).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Principal components analysis of scale shape across the adaptive radiation of cichlid fishes in Lake Tanganyika, coloured according to tribe.

Figure S2. Principal components analyses of scale shape for each scale sampled from different body regions.

Table S1. Taxon sampling of the study, including sampling locations of each specimen.

Table S2. *P*-values of the pairwise comparisons (Fisher's test) of scale types among scales sampled from different body regions (A) and among tribes (B).

Table S3. Pairwise comparisons (Tukey's HSD test) of ctenii coverage among scales sampled from different body regions (A) and among tribes (B).

Table S4. Phylogenetic signals, phylogenetic regression analyses and partial R^2 calculations.

Table S5. Discriminant function analyses.

Appendix S1. tps file with raw landmark data of the scales.

Appendix S2. Table with scale type assignment (cycloid or ctenoid) for each scale.