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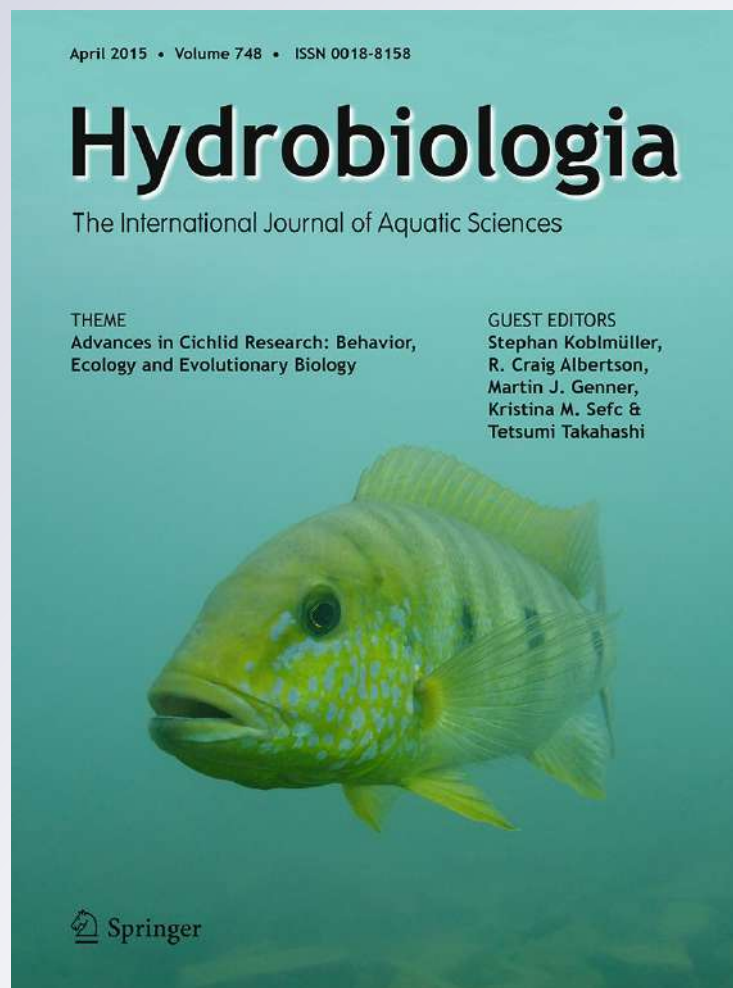
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Geometric morphometrics reveals surprising diversity in the Lake Malawi cichlid genus *Labeotropheus*

Michael J. Pauers · Scott A. McMillan

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Abstract The Lake Malawi cichlid genus *Labeotropheus* has been a source of confusion among biologists and taxonomists. Although unique populations of both *L. fuelleborni* and *L. trewavasae* exist throughout the lake, these populations have not been elevated to species, despite taxonomists doing so for populations within other Lake Malawi cichlids. One reason for this oversight is the supposed consistent differences in morphology between *Labeotropheus* species; since, where they co-occur, *L. fuelleborni* is always deeper-bodied than *L. trewavasae*, it is thought that all deeper-bodied populations of *Labeotropheus* are *L. fuelleborni*, and the slender ones are *L. trewavasae*. Using geometric morphometrics, we analyze 18 populations of *Labeotropheus* and show that body shape varies

among populations, and does not always fall into a deep-body/slender-body dichotomy. These differences in body shape are not related to geographical distance among populations, but are possibly related to the type of habitat in which the populations are found. Further, head shape is extremely variable among populations, and we find two locations where there is convergence in head shape between sympatric *L. fuelleborni* and *L. trewavasae*. Our results suggest that the morphological criteria applied to the *Labeotropheus* are not accurate, and hamper the recognition of *Labeotropheus* biodiversity.

Keywords *Labeotropheus* · Morphology · Geometric morphometrics · Head shape · Habitat type · Species concepts · Species criteria · Lake Malawi · Cichlid

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Introduction

The cichlid fishes of Lake Malawi represent both a treasure trove for evolutionary biologists and a potential headache for systematic ichthyologists. Their unusually rapid and extensive bouts of speciation fascinate those interested in discovering the factors underlying their remarkable evolution (Barlow, 2000; Turner, 2000; Stauffer et al., 2007), while their astounding diversity can be daunting to those attempting to delimit and catalog these species (Stauffer & McKaye, 2001;

Barlow, 2002). Evolutionary biologists are fascinated by the trophic, morphological, reproductive, coloration, and habitat preference differences (e.g., Arnegard et al., 1999; Genner et al., 2007; Albertson, 2008; Martin & Genner, 2009; Pauers & McKinnon, 2012) among species and/or populations, but cichlid taxonomists often struggle with how to use this information, or even determining which of these characteristics are most important, when attempting to describe new species (Stauffer & McKaye, 2001; Pauers, 2010). This is especially true of the rock-dwelling haplochromines known collectively as the “mbuna,” which can display considerable overlap in these characteristics, even among long-established “good” species (Stauffer and McKaye, 2001; Barlow, 2002; Pauers, 2010).

The genus *Labeotropheus* has been challenging to those interested in delimiting and describing species of mbuna. First described in 1927 by Ahl (1927), the genus contains two species, *L. fuelleborni* Ahl 1927 and *L. trewavasae* Fryer 1956. Fryer (1956) distinguished *L. trewavasae* from *L. fuelleborni* primarily on the basis of body shape; *L. trewavasae* is a distinctly more slender-bodied species than *L. fuelleborni*. Later work on this genus suggested that these differences in body shape may contribute to ecological differences between the species as well. Ribbink et al. (1983a, b), based on extensive surveys of Lake Malawi, found that *L. fuelleborni* was more common in shallower water than *L. trewavasae*, and that *L. fuelleborni* was observed scraping algae from the tops of large rocks, while *L. trewavasae* most often scraped algae from the sides and bottoms of smaller rocks; further, while feeding, *L. trewavasae* keeps its head at a much smaller angle (ca. 35° above the horizontal) than does *L. fuelleborni* (ca. 44°–48° above the horizontal; Stauffer & Posner, 2006). Ribbink et al. (1983a, b) suggested that *L. trewavasae*'s slender profile allowed it to feed more efficiently from such surfaces, while Konings (2007) suggested that *L. fuelleborni*'s deeper and somewhat wider body may help it maintain both position and equilibrium in the high energy habitats in which it is most commonly found. Further, these same authors (Ribbink et al., 1983a, b; Konings, 2007) claim to have never found more than two “forms” of *Labeotropheus* at any location, which suggests, to some authors, that *L. fuelleborni* and *L. trewavasae* are the only possible species in this genus (Turner, 1999, 2000; Turner et al., 2001).

Despite these consistent morphological and ecological differences between the species of *Labeotropheus*, Ribbink et al. (1983a, b) also noted extensive divergence of male nuptial coloration among populations of both species, which they suggested was strong evidence for reproductive isolation among populations, and that these populations may actually represent distinct species of *Labeotropheus*. Thus, while these authors only recognized the two formally described species in their publications, Ribbink et al. (1983a, b) did say that their decision to do so was based only on simplicity and expediency. They further advised that future researchers should not similarly confine themselves to this taxonomic shorthand, and should explore the possibility that there may be more than two species of *Labeotropheus*. Unfortunately, this proviso has been long ignored, and much contemporary writing (e.g., Turner, 1999, 2000; Turner et al., 2001; Konings, 2007) posits that, especially because of the apparently consistent morphological differences between the “forms” of *Labeotropheus* throughout Lake Malawi, there are only two species in this genus.

Pauers (2004) did find significant morphological differences among populations of both *L. fuelleborni* and *L. trewavasae*, suggesting that differences in body shape between these species might not be as consistent as once thought. Further, Arnegard et al. (1999) found significant genetic differentiation among populations of *L. fuelleborni* in the southern portion of the lake, which does give some support to Ribbink et al.'s (1983a, b) contention that geographically isolated populations of *Labeotropheus* are likely reproductively isolated. Thus, it would seem that a re-evaluation of the species-status of populations of *Labeotropheus* should begin with a re-evaluation of the criteria that have been cited as evidence that these populations do not represent separate species. Here, we evaluate the morphological criteria that have been used to distinguish *L. fuelleborni* and *L. trewavasae* by comparing body shape both between species and among populations within a species. If these morphological criteria have been accurately applied to the *Labeotropheus*, then populations within a species should show the consistent, stereotypical body shape differences that authors have used as evidence to claim that separate populations of *Labeotropheus* cannot be separate species. Further, we explored possible sources of morphological variation, including differences related to feeding (i.e., head shape), the different habitat types at which these

populations were found, and geographical distance among populations.

Materials and methods

Specimens

Specimens of both recognized species of *Labeotropheus* were obtained from several institutions for morphological analysis. 160 individuals from ten populations of *L. fuelleborni* were obtained, as were 67 from 9 populations of *L. trewavasae*; see Table 1 for a full accounting of sample sizes for each population. Most of these specimens were preserved in a manner that did not distort their bodies in any noticeable way; i.e., they were stored in appropriately sized vessels so that their bodies were not curved or their tails were not bent. While there were some individuals bent in some way, these specimens were rare, and, as pointed out by Kerschbaumer & Sturmbauer (2011), an advantage of geometric morphometrics is the ability to limit, if not entirely remove, the effects of such distortions on the results of the dataset; these authors further demonstrate how these techniques can control for allometric effects as well. While we did not explicitly control for allometric

effects, all specimens used were sexually mature adults; i.e., sex could be easily determined externally. Supplementary Fig. 1 shows the photographs of some of the specimens used in the analyses described below.

Problematic specimens

That we used museum specimens in these analyses raises questions about the effects of chemical preservation on body shape. Preservatives have measurable effects on both length and mass in fishes, and different methods of preservation (i.e., freezing vs chemical preservation; type(s) of chemical used; concentrations of the chemical) have varying effects on these characteristics (Fox, 1996; Fey & Hare, 2005; Santos et al., 2009). While we do not argue that the body shapes of the specimens we used are indistinguishable from freshly caught specimens (see Martinez et al., 2013 for an analysis of the effects of preservation on geometric morphometrics), we wish to point out some details that seem to ensure that the patterns of differentiation among the populations in our study should be consistent no matter the age of specimen used. All the specimens we used are kept in the same preservative, 70% ethanol, and have been for years, so the effects on body shape should be the same among all individuals. Secondly, there is an asymptotic effect of preservative on changes

Table 1 Sample sizes of *Labeotropheus* species from each location, and habitat type present at the location

Location	Habitat	<i>L. fuelleborni</i>	<i>L. trewavasae</i>
Chidunga	Rock/Reef	31 (85.3–112.9)	8 (55.1–90.1)
Katale ^a	Island/Reef	19 (71.9–113.5)	4 (94.5–110.4)
Lumbila	Mainland	5 (65.7–100.8)	–
Maleri	Island	28 (63.2–105.9)	7 (68.9–93.2)
Masinje	Island	4 (87.2–94.8)	17 (70.6–100.1)
Messule ^b	Mainland	24 (71.8–94.4)	–
Mitande	Rock/Reef	2 (92.6–94.6)	6 (61.3–87.6)
Mumbo	Island	24 (40.1–114.9)	3 (48.4–62.8)
Nakantenga	Island	15 (71.3–114.4)	5 (71.8–93.1)
Nkhata Bay	Mainland	–	12 (63.1–89.3)
Thumbi West	Island	13 (36.9–87.7)	–
Total		165	62

Numbers in parentheses following sample sizes represent the size range of specimens in mm SL

^a At Katale Island, *L. fuelleborni* was found at the island itself, while *L. trewavasae* was found at a nearby submerged reef (Stuart Grant Ltd., pers. comm.)

^b The number of *L. fuelleborni* specimens from Messule includes five incorrectly identified individuals of *L. trewavasae*; see text, Fig. 1 and Table 2 for details

in length and mass; after 60–120 days in preservative, the effects of that preservative eventually reach a maximum (see graphs in Fox, 1996; Fey & Hare, 2005; Santos et al., 2009); i.e., there is only so much change that the preservative can affect. Third, the Principal Components Analysis performed by Martinez et al. (2013) on their geometric morphometric data demonstrates a considerable amount of overlap between fresh and preserved individuals. Thus, this analytical technique, commonly used with geometric morphometric data, may not unequivocally distinguish between fresh and preserved specimens.

Further, our preliminary analyses detected some potential issues with misidentification of specimens from two locations. At Katala Island, specimens initially identified as *L. fuelleborni* have relatively slender bodies that somewhat resemble those generally found in specimens of *L. trewavasae*. At Messule, on the Mozambique coast of Lake Malawi, specimens labeled as *L. trewavasae* were found to have a deep, robust morphology more typical to that of *L. fuelleborni*. To test for possible misidentification of these populations, we used Fryer's (1956) body depth–body length (n.b.: Fryer used total length; here we use SL) criterion for distinguishing *L. trewavasae* from *L. fuelleborni*. We found that the body depth–SL ratio of the *L. fuelleborni* Katala population is significantly different from the *L. fuelleborni* type series (Lumbila population), the *L. trewavasae* type series (Nkhata Bay population), as well as *L. trewavasae* from Katala Reef (Fig. 1; Table 2). The *L. trewavasae* Messule population, on the other hand, was found to have a body depth–SL ratio that was significantly different from the *L. trewavasae* type series, but was not significantly different from the *L. fuelleborni* type series or the *L. fuelleborni* specimens from Messule (Fig. 1; Table 2). Thus, while it appears that the specimens labeled as *L. trewavasae* Messule are actually misidentified as *L. fuelleborni*, the *Labeotropheus* from Katala Island, both *L. fuelleborni* and *L. trewavasae*, were correctly identified.

Material examined

Labeotropheus fuelleborni

MRAC 91-54-P-58-65 (8 alcohol specimens), Chidunga Rocks, Malawi; MRAC 99-041-P-1594-1595 (7 alcohol specimens), Chidunga Rocks, Malawi;

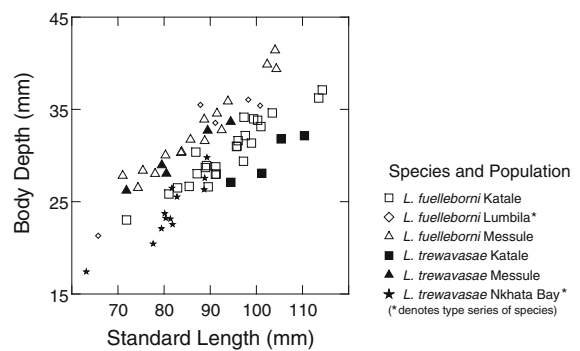


Fig. 1 Scatterplot demonstrating the relationship between body depth and SL for the Katala and Messule populations of *Labeotropheus*, including the type series of both *L. fuelleborni* and *L. trewavasae*, for comparison. While the *L. fuelleborni* from Katala Island have a more slender morphology than that of the other *L. fuelleborni* populations displayed in this figure, it is still a deeper body than that displayed by the *L. trewavasae* morphology found in the nearby Katala Reef. At Messule, on the other hand, the five specimens currently identified in the MRAC collection as “*L. trewavasae*” have the same body profile as the *L. fuelleborni* from this location; thus these specimens are considered to be *L. fuelleborni* in all subsequent analyses presented herein

MPM 50037 (1 alcohol specimen), Chidunga Rocks, Malawi; MPM 50038 (13 alcohol specimens), Chidunga Rocks, Malawi; MPM 50041 (1 alcohol specimen), Chidunga Rocks, Malawi; MPM 50042 (2 alcohol specimens), Chidunga Rocks, Malawi; MPM 50035 (1 alcohol specimen), Katala Island, Malawi; MPM 50036 (10 alcohol specimens), Katala Island, Malawi; MPM 50039 (1 alcohol specimen), Katala Island, Malawi; MPM 50040 (7 alcohol specimens), Katala Island, Malawi; ZMB 22707 (1 alcohol lectotype), Alt Langenburg (Lumbila), Tanzania; ZMB 23922 (1 alcohol paralectotype), Alt Langenburg (Lumbila) Tanzania; ZMB 23923 (1 alcohol paralectotype), Alt Langenburg (Lumbila) Tanzania; ZMB 33818 (1 alcohol paralectotype), Alt Langenburg (Lumbila) Tanzania; ZMB 33819 (1 alcohol paralectotype), Alt Langenburg (Lumbila) Tanzania; MRAC 91-54-P-46-57 (12 alcohol specimens), Maleri Island, Malawi; MRAC 99-041-P-1656-1659 (4 alcohol specimens), Maleri Island, Malawi; MRAC 99-041-P-1660-1662 (3 alcohol specimens), Maleri Island, Malawi; MRAC 99-041-P-1663-1666 (4 alcohol specimens), Maleri Island, Malawi; MRAC 99-041-P-1667-1671 (5 alcohol specimens), Maleri Island, Malawi; MRAC 91-54-P-66-69 (4 alcohol specimens), Masinje, Malawi;

Table 2 ANOVA of body depth–SL ratio for the Katala and Messule populations of *Labeotropheus*, including the type series of both *L. fuelleborni* and *L. trewavasae*

(A) Analysis of variance					
Variable	Sum-of-squares	dF	Mean-square	<i>F</i>	<i>P</i>
Population (species)	0.061	5	0.012	47.385	0.000
Error	0.016	61	0.000		
(B) Pairwise comparisons (pairwise mean differences)					
	<i>L. fuelleborni</i> Katala	<i>L. fuelleborni</i> Lumbila	<i>L. fuelleborni</i> Messule	<i>L. trewavasae</i> Katala	" <i>L. trewavasae</i> " Messule
<i>L. fuelleborni</i> Lumbila	0.040***				
<i>L. fuelleborni</i> Messule	0.050***	0.010 ^{ns}			
<i>L. trewavasae</i> Katala	−0.034**	−0.074***	−0.084***		
" <i>L. trewavasae</i> " Messule	0.037***	−0.003 ^{ns}	−0.013 ^{ns}	0.071***	
<i>L. trewavasae</i> Nkhata Bay	−0.028***	−0.068***	−0.078***	0.005 ^{ns}	−0.065***

Note that the five Messule "*L. trewavasae*" individuals are actually *L. fuelleborni*, and are considered as such in all subsequent analyses presented herein. All *P* values are Bonferroni corrected

ns non-significant

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

MRAC 79-40-P-89-110 (22 alcohol specimens), Messule, Mozambique; MRAC 99-041-P-1610-1612 (3 alcohol specimens), Messule, Mozambique; UMMZ 238326 (2 alcohol specimens), Mitande Rocks, Malawi; MCZ 157254 (2 alcohol specimens), Mumbo Island, Malawi; UMMZ 238339 (14 alcohol specimens), Mumbo Island, Malawi; USNM 261886 (9 alcohol specimens), Mumbo Island, Malawi; MRAC 91-54-P-70-72 (3 alcohol specimens), Nakantenga Island, Malawi; UMMZ 237733 (12 alcohol specimens), Nakantenga Island, Malawi; AMNH 92810 (4 alcohol specimens), Thumbi West Island, Malawi; USNM 261919 (9 alcohol specimens), Thumbi West Island, Malawi.

Labeotropheus trewavasae

MRAC 91-54-P-73-78 (6 alcohol specimens), Chidunga Rocks, Malawi; MRAC 99-041-P-1598-1599 (2 alcohol specimens), Chidunga Rocks, Malawi; MPM uncatalogued (4 alcohol specimens), Katala Reef, Malawi; MRAC 91-54-P-86-88 (3 alcohol specimens), Maleri Island, Malawi; MRAC 99-041-P-1644-1647 (4 alcohol specimens), Maleri Island, Malawi; MRAC 91-54-P-84-85 (2 alcohol specimens), Masinje, Malawi; MRAC 91-54-P-89-103 (15 alcohol specimens), Masinje, Malawi; MRAC 99-041-P-1635-1639 (5 alcohol specimens), Messule, Mozambique—these

specimens are currently labeled as *L. trewavasae* in the MRAC collection, so we include them as such in this list, but are actually *L. fuelleborni*; UMMZ 237728 (1 alcohol specimen), Mitande Rocks, Malawi; USNM 270447 (5 alcohol specimens), Mitande Rocks, Malawi; MCZ 98593 (2 alcohol specimens), Mumbo Island, Malawi; USNM 261913 (7 alcohol specimens; only one of these seven was used in the analyses presented herein), Mumbo Island, Malawi; MRAC 91-54-P-79-83 (5 alcohol specimens), Nakantenga Island, Malawi; BMNH 1965.11.2.1 (1 alcohol holotype) Nkhata Bay, Malawi; BMNH 1965.11.2-12 (11 alcohol paratypes), Nkhata Bay, Malawi.

General morphometric analyses

We took photos of each specimen with a digital camera (Sony CyberShot 7.2 megapixel) under fluorescent room lighting. The photographs were imported into ImageJ, and 19 landmarks were digitized from each specimen; see Fig. 2 for a picture and description of landmarks. First, using the Integrated Morphometrics Package program CoordGen6f, the landmark coordinates were superimposed using Generalized Procrustes Analysis superimposition, but standardized to a centroid size of one (Kassam et al., 2007; Kerschbaumer & Sturmbauer, 2011). After this standardization, we imported the superimposed

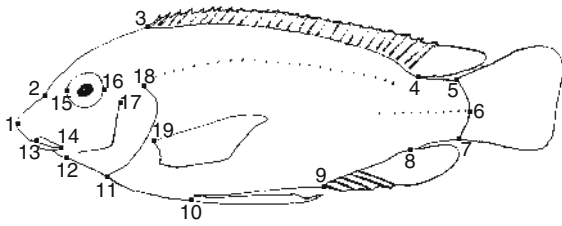


Fig. 2 Landmarks used in morphometric analyses. (1) Anterior tip of snout; (2) dorsal tip of premaxillary pedicel; (3) origin of dorsal fin; (4) insertion of dorsal fin; (5) dorsal insertion of caudal fin; (6) caudal border of hypural plate, aligned with lower lateral line; (7) ventral insertion of caudal fin; (8) insertion of anal fin; (9) origin of anal fin; (10) base of pelvic fin spine; (11) a point on posterior margin of opercular membrane meeting ventral margin of head; (12) posterior end of dentary symphysis; (13) anteriormost margin of upper jaw soft tissue; (14) posterior end of jaws; (15) anterior margin of midline through the eye; (16) posterior margin of midline through the eye; (17) dorsal end of preopercle; (18) dorsalmost end of opercule; (19) origin of pectoral fin. Note that for analyses of head shape, only landmarks 1–3 and 11–19 were used. Landmarks adapted from Chakrabarty (2005)

coordinates into the TPS program TPSRelW to calculate relative warp (RW) scores. We then used these RW scores to address four specific questions: (1) Do populations within a species vary morphologically? (2) Does head shape vary among populations, and is this variation congruent with the variation in body shape? (3) Is morphology related to distance among populations within a species? (4) Are morphological differences related to habitat, regardless of species?

Morphological differences among populations

To examine how morphology varies among populations within a species, we obtained a between-groups F matrix based on the RW scores from the Discriminant Function Analysis module in Systat 10.2. The pairwise differences encoded in this F matrix are proportional to Mahalanobis distances (Cohen & Wilkinson, 2000); we imported this matrix into PHYLIP 3.69 and used the NEIGHBOR algorithm to construct a UPGMA phenogram representative of morphological similarity among populations, and not phylogenetic relationships (Kassam et al., 2007).

In order to specifically examine variation in head morphology among populations, which would give some indication of ecological divergence among populations (Chakrabarty, 2005), the above procedure

was repeated, but used only the coordinates of landmarks found on the heads of the fish (Fig. 2).

Geographic distance and morphology

In order to determine whether differences in morphology are proportional to geographical distances among populations, we first measured straight-line distances among populations using Google Earth, creating two pairwise matrices of linear distances among these populations, one for each species of *Labeotropheus* (Fig. 3). We then constructed, using the previously calculated F matrix based on the RW scores of all individuals in the sample, a pairwise between-groups F matrix for the populations within each species. We then compared the between-groups F matrix to the matrix of pairwise geographical distances among populations using a Mantel test of matrix correspondence in XLStat. In consideration of the fact that, at least in the present-day Lake Malawi basin, *Labeotropheus* are highly unlikely to travel the vast straight-line distances we measured among populations, we performed an additional Mantel test comparing only the specimens from six populations (Chidunga Rocks, Maleri Island,

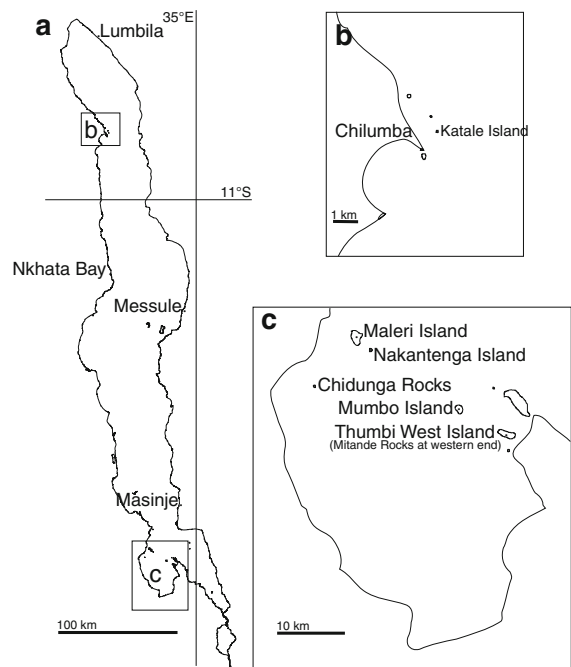


Fig. 3 Map of Lake Malawi showing *Labeotropheus* populations used in this study. **a** Entire lake, **b** islands surrounding Luromo peninsula in the northwest portion of the lake, and **c** southwestern arm of Lake Malawi

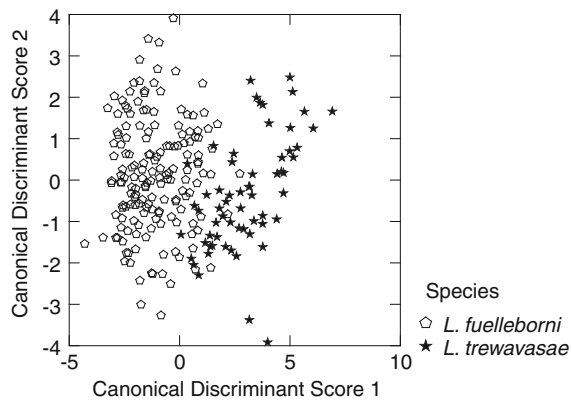


Fig. 4 A plot of Canonical Discriminant Scores 1 and 2 resulting from a discriminant analysis in which species was used as a grouping variable

Nakantenga Island, Mumbo Island, Thumbi West Island, and Mitande Rock) found within close proximity to each other in the southwestern area of the lake.

Habitat-specific morphology

In order to determine whether or not morphology is related to gross habitat type, we first classified each population where *Labeotropheus* specimens were collected as Mainland, Island, or Rock/Reef habitat, following Ribbink et al. (1983b) and Pauers (2011); refer back to Table 1 for these categories. While the characteristics of each of these habitats may not vary (i.e., amount of rock vs sand cover, sizes and shapes of rocks present, etc.), the depths at which *Labeotropheus* occur within each of these gross habitat types does differ, with Mainland populations found at the shallowest depths and Rock/Reef populations found at the deepest (Ribbink et al., 1983b). Using the uniform component of the RW scores as a surrogate for overall shape differences among populations (Bookstein, 1996), we performed a MANOVA on the uniform component, using species, habitat type, and the crossed effect of species and habitat type as independent variables.

Results

Whole-body morphology among populations

The discriminant analysis performed using the RW scores of all 18 populations of *Labeotropheus* was robust

and significant (Wilks' $\lambda = 0.282$; $F_{10,216} = 54.866$; $P \leq 0.0001$). Due to the number of populations in the analysis, a plot of the canonical discriminant scores of each population is somewhat impractical, but we include a plot (Fig. 4) of these scores for both species. The analysis correctly classified 96% of both species (161 of 165 *L. fuelleborni* and 58 of 62 *L. trewavasae*; jackknifed classification), though there is some overlap between *L. fuelleborni* and *L. trewavasae*.

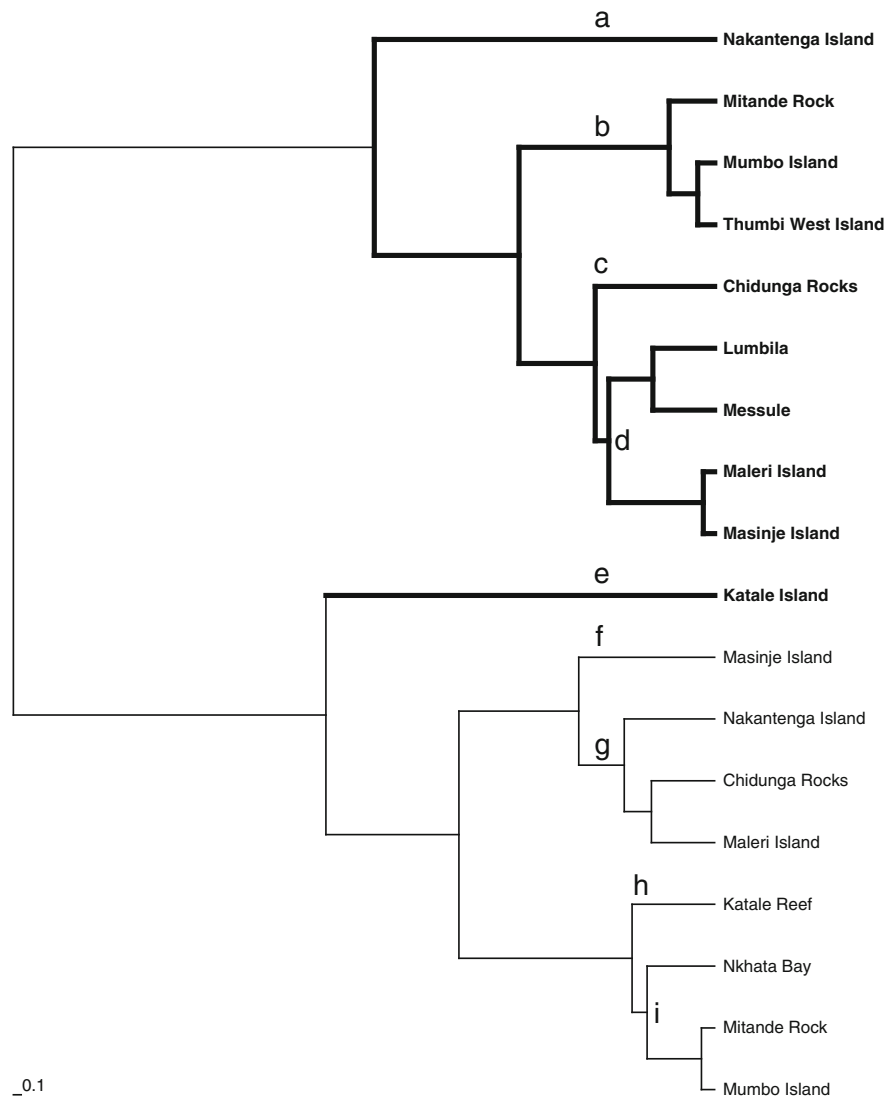
Figure 5 displays the UPGMA phenogram based on the between-groups *F* matrix obtained from the discriminant function analysis. This phenogram appears to identify nine distinct morphotypes among the 18 sampled populations of *Labeotropheus*, five within *L. fuelleborni* and four within *L. trewavasae*. The most surprising results of this phenogram are the nesting of a population of *L. fuelleborni* within the “morphoclade” of *L. trewavasae*. The Katala Island population of *L. fuelleborni* is found within the *L. trewavasae* cluster, though its morphology is still distinct from *L. trewavasae*, especially in terms of head depth, body depth, and lower jaw length (see further discussion below).

The deformation grids derived from the RW analysis are shown in Fig. 6. As might be expected, most *L. fuelleborni* populations have the deeper, more robust body shape typical of their own species (Fig. 5a–e), though the relatively more slender body of the *L. fuelleborni* found at Katala Island (Fig. 5e), when compared to other *L. fuelleborni* populations, is likely what makes it similar to *L. trewavasae*. Similarly, most *L. trewavasae* populations display the long, slender body characteristic of this species (see Fig. 5f–i). It is interesting to note the slightly more posterior positioning of posterior dentary symphysis in the populations from Nkhata Bay, Mumbo Island, and Mitande Rock (Fig. 5i), perhaps giving them a longer lower jaw than other *L. trewavasae* populations. Along these lines, a comparison of Fig. 5e and h reveals that the *L. fuelleborni* Katala Island population has a much deeper body and head, and a longer lower jaw, than the *L. trewavasae* population found at Katala Reef. The *L. fuelleborni* found at Nakantenga Island seem to have a shorter lower jaw, as indicated by the relatively more anterior positioning of the posterior dentary symphysis (Fig. 5a).

Head morphology among populations

The UPGMA phenogram based on the between-groups *F* matrix is shown in Fig. 7; head shape was

Fig. 5 UPGMA phenogram based on whole-body morphometric analyses demonstrating overall morphological similarity among *Labeotropheus* populations. Bold branches and labels *L. fuelleborni*, fine branches and labels *L. trewavasae*

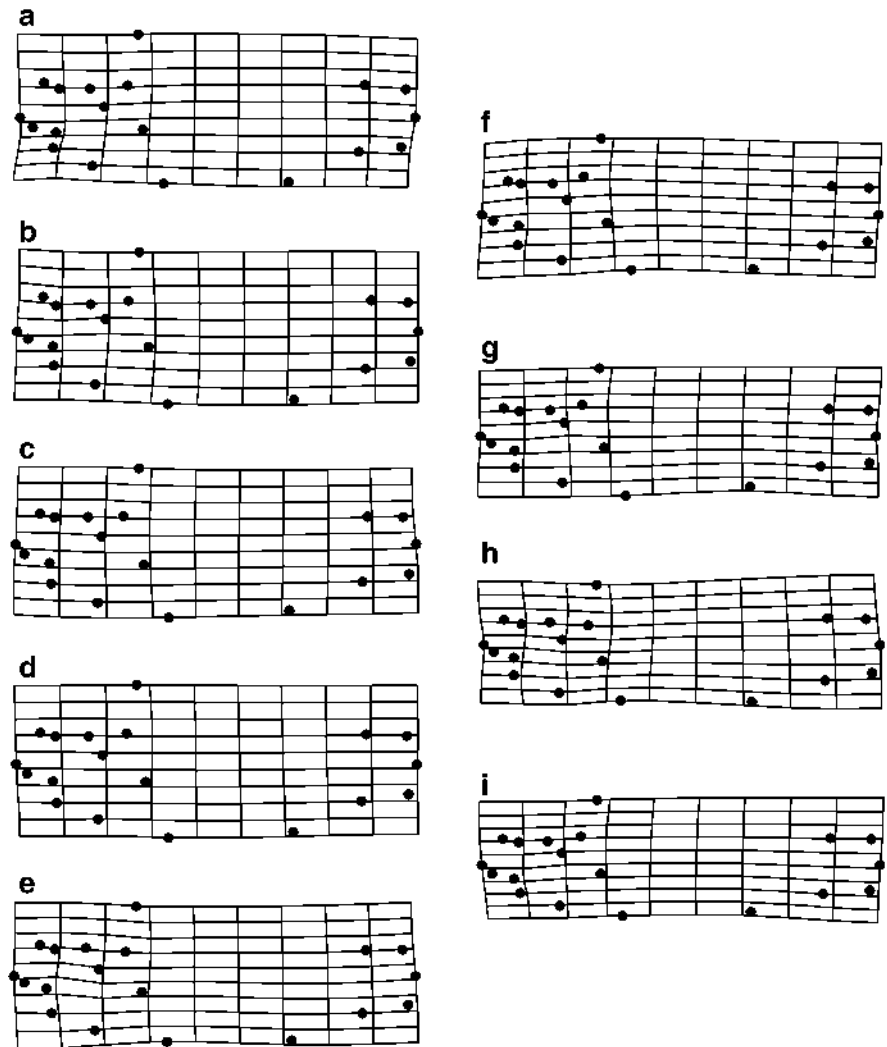


extremely variable among species and populations, and very few clear patterns or cephalic morphotypes can be identified from the phenogram. The Chidunga Rocks and Nkhata Bay populations of *L. trewavasae* and the Nakantenga population of *L. fuelleborni* have distinct head morphologies, and are widely separated from each other and the remaining populations. The rest of the phenogram consists of two larger morphoclares, one primarily consisting of *L. trewavasae*, though the Katale Island population of *L. fuelleborni* is nested within this clade, and the other consisting of the remaining *L. fuelleborni* populations.

Morphology with respect to geographic distance

For *L. fuelleborni*, there is no relationship between morphology, expressed as *F* matrix distances between pairs of populations, and geographical distance between populations (Table 3A). This is true for both comparisons we made on these data; the results from the entire dataset and those populations located in the southwest arm are not significant. For *L. trewavasae*, on the other hand, there is a strong relationship between geographical distance and *F* matrix distance for the southwestern arm populations ($r_{AB} = 0.660$; $P = 0.044$), indicating that neighboring populations

Fig. 6 RW deformation grids of the morphologies indicated on the phenogram in Fig. 5; each grid **a–i** represents the average shape of the corresponding group in Fig. 5



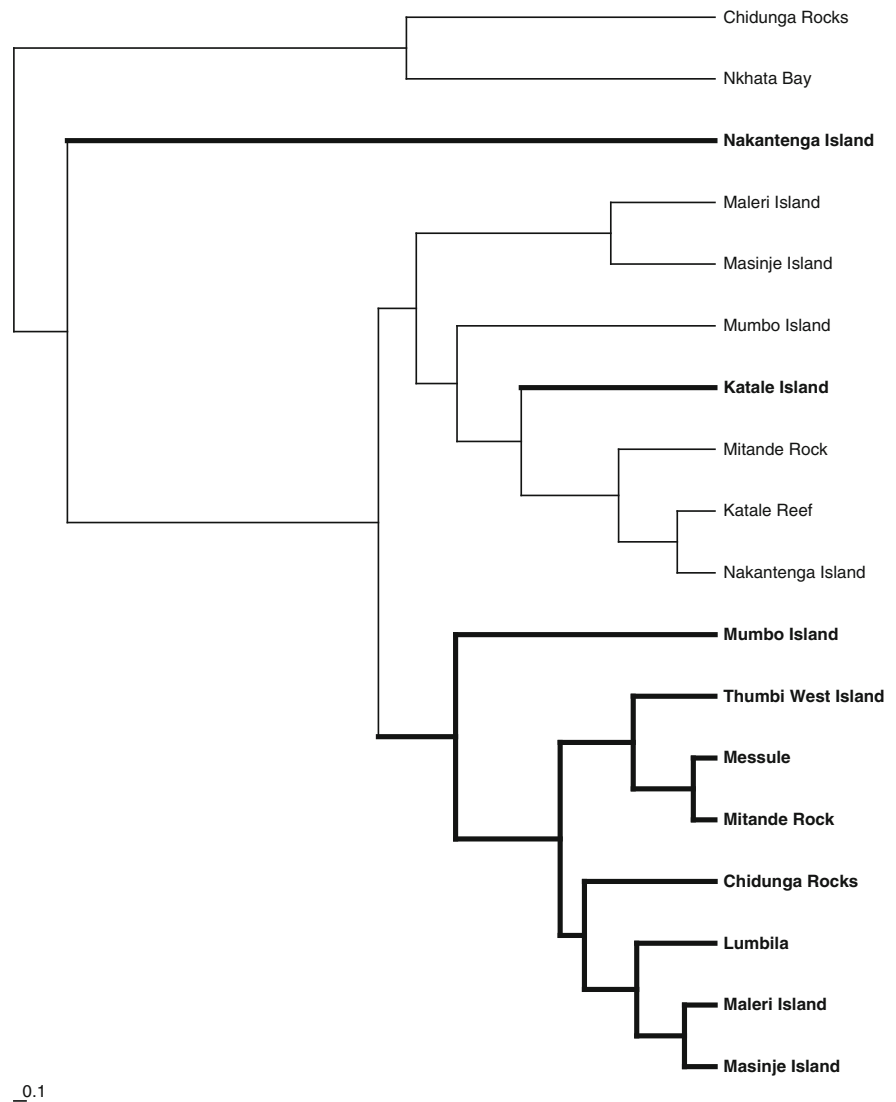
have more similar morphologies than to distant populations. The comparison made using the entire dataset of *L. trewasae* indicates no relationship between morphological similarity/disparity and geographic distance (Table 3B).

Morphology with respect to habitat

The results of the MANOVA on the effects of species and habitat type on the uniform component of body shape are listed in Table 4. Both factors influenced the same dimension of the uniform component. The species effect was robust (Wilks' $\lambda = 0.369$; $P \leq 0.001$), but only had a significant effect on the x uniform component ($P \leq 0.001$). The effect of habitat type was also robust

(Wilks' $\lambda = 0.904$; $P \leq 0.001$), and also had a significant effect on the x uniform component ($P = 0.039$). The crossed effect of species and habitat type was both robust (Wilks' $\lambda = 0.939$; $P = 0.007$) and a significant contributor to the variation observed in the x uniform component, suggesting that both species and habitat type act together to alter *Labeotropheus* body shape. To explore this relationship further, we performed another discriminant analysis on the RW scores, using species and habitat type as grouping variables. This analysis successfully discriminated among all six species–habitat combinations, though not perfectly (64% of all classifications were made correctly in a jack-knifed classification), but what is striking about these results is that habitat, especially the Rock/Reef habitat, seems to

Fig. 7 UPGMA phenogram based solely on morphometric analysis of head landmarks. *Bold branches and labels* *L. fuelleborni*, *fine branches and labels* *L. trewavasae*



be influencing the morphology of both species *Labeotropheus* in a similar way (Table 5; Fig. 8).

Discussion

The results of our analyses present a somewhat clouded picture of patterns of *Labeotropheus* morphology and morphological divergence both between the two recognized species and among populations within a species. The analyses of whole-body morphology demonstrated that the supposedly characteristic body shape of each species, and the differences between them, are not as absolute as sometimes presented in the

literature. Among populations within a species, we discovered distinct differences in shape, and there was an interesting case of overlap, with the Katala Island population of *L. fuelleborni* having a body shape more similar to that found among populations *L. trewavasae*. Our analyses of head shape show some distinction between species, but some populations within both species also possess divergent head morphologies. Further, most of the observed morphological divergence among populations is not related to geographic distance, though we did find some evidence for clinal variation in morphology in southwestern populations of *L. trewavasae*. Gross habitat type, and more specifically the depth at which *Labeotropheus* populations occur at

Table 3 Combined geographical/*F* ratio distance matrices, and results of two-tailed Mantel tests of matrix correspondence

(A) <i>Labetropheus fuelleborni</i> : All specimens, $r_{AB} = -0.006$; $P = 0.976$; Southwestern populations (sw), $r_{AB} = 0.199$; $P = 0.472$									
	Chidunga ^{sw}	Katale	Lumbila	Maleri ^{sw}	Masinje	Messule	Mitande ^{sw}	Mumbo ^{sw}	Nakantenga ^{sw}
Katale	388.29/10.744								
Lumbila	490.32/4.185	102.90/3.535							
Maleri ^{sw}	9.57/6.08^{sw}	382.95/15.094	485.15/3.093						
Masinje	53.12/3.669	350.44/6.464	448.14/2.099	43.90/0.883					
Messule	227.52/4.666	176.37/6.767	277.40/1.800	220.57/3.697	182.25/2.006				
Mitande ^{sw}	29.89/3.448^{sw}	399.26/2.149	501.96/1.540	25.55/2.264^{sw}	50.18/2.232	232.38/1.575			
Mumbo ^{sw}	21.82/9.446^{sw}	394.37/8.472	496.89/3.348	17.23/9.749^{sw}	47.45/5.031	228.97/5.866	8.37/1.245^{sw}		
Nakantenga ^{sw}	10.16/10.068^{sw}	385.03/7.511	487.02/2.606	2.66/5.956^{sw}	44.51/2.512	222.25/3.446	22.86/1.074^{sw}	14.58/3.114^{sw}	
Thumbi ^{sw}	28.99/12.344^{sw}	398.54/10.900	501.25/6.044	24.18/12.337^{sw}	49.62/5.001	231.78/7.249	1.05/2.042^{sw}	7.45/8.384^{sw}	21.71/5.230^{sw}
(B) <i>Labetropheus trewavasae</i> : All specimens, $r_{AB} = -0.182$; $P = 0.371$; Southwestern populations (sw), $r_{AB} = 0.660$; $P = 0.044$									
	Chidunga ^{sw}	Katale	Maleri ^{sw}	Masinje	Mitande ^{sw}	Mumbo ^{sw}	Nakantenga		
Katale	388.29/10.744								
Maleri ^{sw}	9.57/1.94^{sw}	382.95/5.075							
Masinje	53.12/17.691	350.44/9.278	43.90/8.802						
Mitande ^{sw}	29.89/10.646^{sw}	399.26/3.214	25.55/9.083^{sw}	50.18/27.172					
Mumbo ^{sw}	21.82/14.082^{sw}	394.37/7.963	17.23/15.251^{sw}	47.45/29.842	8.37/5.774^{sw}				
Nakantenga ^{sw}	10.16/4.713^{sw}	385.03/4.104	2.66/2.129^{sw}	44.51/7.798	22.86/6.934^{sw}	14.58/9.360^{sw}			
Nkhata Bay	248.56/9.277	140.13/5.302	243.63/9.157	213.24/37.823	260.94/7.851	255.69/5.693	245.72/5.383		

Bold numbers indicates geographical distance (km); italic numbers indicates *F* ratio. r_{AB} , correlation between geographical distance matrix and *F* ratio distance matrix

Table 4 MANOVA of habitat type and species effects on the uniform component of morphology

Variable	Sum-of-squares	dF	Mean-square	<i>F</i>	<i>P</i>
Constant (Wilks' $\lambda = 0.693$; $F_{2,220} = 48.812$; $P \leq 0.001$)					
Uniform component (<i>x</i>)	0.016	1	0.016	95.619	0.000
Error	0.036	221	0.000		
Uniform component (<i>y</i>)	0.000	1	0.000	0.668	0.414
Error	0.018	221	0.000		
Species (Wilks' $\lambda = 0.369$; $F_{2,220} = 187.968$; $P \leq 0.001$)					
Uniform component (<i>x</i>)	0.061	1	0.061	374.516	0.000
Error	0.036	221	0.000		
Uniform component (<i>y</i>)	0.000	1	0.000	0.086	0.769
Error	0.018	221	0.000		
Habitat type (Wilks' $\lambda = 0.904$; $F_{4,440} = 5.716$; $P \leq 0.001$)					
Uniform component (<i>x</i>)	0.003	2	0.001	9.007	0.000
Error	0.036	221	0.000		
Uniform component (<i>y</i>)	0.000	2	0.000	2.263	0.106
Error	0.018	221	0.000		
Species \times habitat type (Wilks' $\lambda = 0.939$; $F_{4,440} = 3.542$; $P = 0.007$)					
Uniform component (<i>x</i>)	0.002	2	0.001	6.901	0.001
Error	0.036	221	0.000		
Uniform component (<i>y</i>)	0.000	2	0.000	0.590	0.555
Error	0.018	221	0.000		

these habitats, does appear to have a slight influence on body shape, as represented by the uniform component of the RW; interestingly, it seems that there is some convergence in the morphology of both species at Rock/Reef habitats. Our findings, then, seem to refute the many claims that the respective morphologies of *L. fuelleborni* and *L. trewavasae* are distinct and unvarying throughout Lake Malawi.

With respect to whole-body morphology, populations within both species tended to cluster together on the UPGMA phenogram, though individual clusters of populations were not always found closest to conspecific clusters. Interestingly, the branch lengths separating the intraspecific populations on the phenogram were, in some cases, nearly as long as those between the species, indicating a greater degree of morphological diversity within each species than previously reported or even considered possible (Ribbink et al., 1983a, b; Turner, 1999, 2000; Konings, 2007). The Katala *Labeotropheus* in particular present a strong challenge to the long-standing thoughts regarding *Labeotropheus* body shape. While the *L. trewavasae* population at Katala Reef has the slender morphology

characteristic of the species, the *L. fuelleborni* at Katala Island also has a slender body that is atypical of the species. Thus, the long-held deep-body/slender-body dichotomy between these species is not as constant or consistent as previously believed. While at least one other author claims to have found a population of *L. trewavasae* morphologically similar to *L. fuelleborni* (at Chirwa Island; Konings, 2007), our results are the first empirical confirmation that there is morphological overlap between these species.

Head shape appears to be even more variable than body shape in *Labeotropheus*. While individual intra-specific clusters appear scattered throughout the phenogram, there does not appear to be a single distinct head morphotype for either species. Given the ecomorphological demands placed on the heads of fishes, especially among the cichlids (Bouton et al., 2002; Chakrabarty, 2005), this is not terribly surprising, and may represent adaptations to local habitat structure, food sources, or turbulence. Interestingly, there appears to be some degree of similarity between the head shapes of *L. fuelleborni* and *L. trewavasae* at the Katala Island/Reef location; even though the reef is

Table 5 Canonical scores of group means from a discriminant analysis on species and habitat type

	CDS 1	CDS 2
<i>L. fuelleborni</i> Island	1.050	0.366
<i>L. fuelleborni</i> Mainland	0.813	0.037
<i>L. fuelleborni</i> Rock/Reef	0.563	-1.407
<i>L. trewavasae</i> Island	-1.862	0.177
<i>L. trewavasae</i> Mainland	-2.364	0.558
<i>L. trewavasae</i> Rock/Reef	-2.581	-0.405

Wilks' $\lambda = 0.143$; $F_{50,970} = 10.335$; $P \leq 0.001$

CDS canonical discriminant score

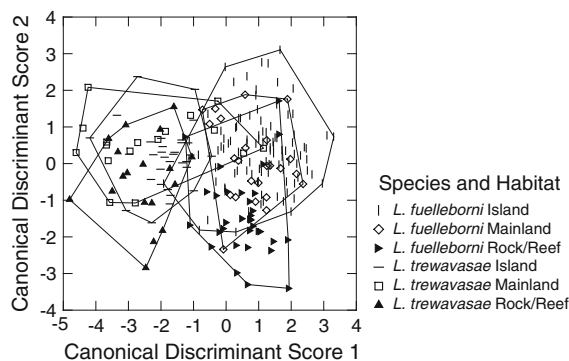


Fig. 8 A plot of Canonical Discriminant Scores 1 and 2 resulting from a discriminant analysis in which species and habitat type were used as grouping variables. Note that while Canonical Discriminant Score 1 largely separates *L. fuelleborni* and *L. trewavasae*, Canonical Discriminant Score 2 especially distinguishes Rock/Reef habitats from Island and Mainland habitats

separate from the island, there may be some ecological commonality present in this geographical location that may play a role in the development of head morphology. For example, Stauffer and van Snik Gray (2004) demonstrated that individuals of *L. fuelleborni* fed different diets develop strikingly different head morphologies. It is possible, then, that differences in food availability among locations where *Labeotropheus* populations are found, or how that food is acquired (Stauffer & Posner, 2006), could lead to differing head morphologies; the differences in lower jaw length we recorded among morphotypes could result from such circumstances. Further, such localized similarities in head morphology could make a phylogeographic analysis of *Labeotropheus* populations an extremely powerful test of hypotheses regarding the relative

timing of ecological and reproductive divergence in adaptive radiation (Albertson et al., 1999; Strelman & Danley, 2003; Choat et al., 2012).

The patterns of variation between body shape and geographic distance are equivocal. While most of our comparisons of body shape differences and geographic distance revealed no significant relationships, the *L. trewavasae* populations in the southwest arm of Lake Malawi did demonstrate such a relationship; populations that were closer to one another had more similar morphologies. The lack of clinal variation in body shape among most *Labeotropheus* populations could be due to the effects of habitat and/or other ecological factors, like food resources (Stauffer & van Snik Gray, 2004). Habitat type might influence morphology via depth, since the depth at which *Labeotropheus* populations are found varies among the three gross habitat types used in this analysis (Ribbink et al., 1983a, b; Pauers, 2011); further, the southwestern population of *L. trewavasae* all occur at islands or rock reefs, giving additional support to our hypothesis that convergent morphologies could be adaptations to habitat type. Interestingly, genetic distance among populations of *L. fuelleborni* in southern Lake Malawi is related to geographical distance (Arnegard et al., 1999). Similarly, Arnegard et al. (1999) found that male nuptial coloration did not have a clear relationship with genetic differentiation; these authors suggested that genetically diverged populations could have similar color patterns if they lived in similar habitats. Considering our findings with respect to morphology and distance in southwestern *L. trewavasae* populations, a similar pattern is likely occurring with respect to morphology and habitat.

Given that our results indicate that gross habitat type has at least some influence on both head and whole-body morphology in the *Labeotropheus*, an important next step would be a detailed examination of exactly how habitat influences morphology in this genus. For example, a recent study by Albertson (2008) found that at two populations in Lake Malawi, species of the genus *Tropheops* displayed significant differences in jaw morphology based on five variables: the depth at which the population was found, the amount of sediment present in the habitat, the surface area of the feeding surface (i.e., the length \times width of the rocks on which specimens were foraging), the degree to which the feeding surface was sheltered, and the slope of the feeding surface. Ribbink et al. (1983a, b) found that, in

the populations they surveyed, the habitats in which they found *L. fuelleborni* and *L. trewavasae* differ dramatically in these (or similar) variables; thus, these differences may contribute to the stereotypical morphological differences between these species. Since our results indicate that these morphological differences are not as consistent as previously thought, it is critical to evaluate them not just between the recognized species of *Labeotropheus*, but among populations within both species (Albertson, 2008).

Conclusions

Our results demonstrate a lack of any clear pattern of morphological divergence among *Labeotropheus* populations. While these results certainly challenge the deep body-shallow water/slender body-deep water dichotomy typically used to describe *L. fuelleborni* and *L. trewavasae*, respectively, they also indicate some exciting directions for future research on *Labeotropheus* evolution. A first hypothesis that stems from our results could be that morphological similarity is congruent to genetic relatedness. At first blush, this may seem unlikely, given that there is a geographic component to relatedness in *Labeotropheus* (Arnegard et al., 1999; Mims et al., 2010), and that our results indicate that geographic relationships to morphological similarity are equivocal. Nonetheless, there are some branches on our UPGMA tree that consist of geographically proximate populations (e.g., the Masinje Island-Nakantenga Island-Chidunga Rocks-Maleri Island branch of *L. trewavasae*, and the Mitande Rock-Mumbo Island-Thumbi West Island branch of *L. fuelleborni*), not to mention the significant Mantel results of the southwestern *L. trewavasae* populations. Evaluating the genetic relatedness of these populations would obviously be crucial to testing this hypothesis.

Perhaps a more interesting approach would be to attempt to analyze the roles both habitat and environment play in the evolution of *Labeotropheus* morphology. Since the type of food consumed has a distinct effect on head shape in captive *L. fuelleborni* (Stauffer & van Snik Gray, 2004) an assessment of both gut contents and food availability among *Labeotropheus* populations in nature would reveal whether or not the effect extends to wild populations, as well as the magnitude of the effect. Further, Konings' (2007)

assertion that turbulence due to wave action is the likely force behind the evolution of the deep, stocky body of *L. fuelleborni* is testable in the lab. If this hypothesis is correct, broods of *L. fuelleborni* raised in different turbulence regimes should develop different morphologies, with fish raised in turbulent conditions developing the deep, wide morphology thought to be characteristic of *L. fuelleborni*; it would be worthwhile to examine whether and how turbulence affects *L. trewavasae* morphology, as well.

Interestingly, Stauffer and van Snik Gray (2004) point out that the effects they observed of diet on head morphology would have an influence on how we might delineate cichlid species. Given that measurements on the head are often used in the descriptions and diagnoses of African cichlids, the effects of diet and environment may have greater and more practical or applied effects than simply measuring whole-body or trunk-based morphological differences among populations.

The description of *L. trewavasae* (Fryer, 1956) unintentionally established a morphological dichotomy within *Labeotropheus*: *L. fuelleborni* was shown to be a robust, deep-bodied fish, while *L. trewavasae* was much more slender. While this dichotomy certainly holds true for the specimens Fryer examined for his description (n.b.: Fryer does not specifically mention the sources of the individuals of *L. fuelleborni* he used for his comparison, though he does note that he did not examine the type series of *L. fuelleborni*; Fryer, 1956), our analyses demonstrate that this is by no means an invariable relationship. Since we used specimens from populations unavailable to Fryer at the time of his description, our results reveal a broader picture of both morphological diversity and diversification within this genus than he could have anticipated with his description of a single new species of *Labeotropheus*. It is thus important to contemporary investigations of Lake Malawi biodiversity to finally take Ribbink et al.'s (1983a, b) advice and to begin investigating the *Labeotropheus* without a priori constraints regarding the number of species in the genus.

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