

Morphometric analysis on ecomorphologically equivalent cichlid species from Lakes Malawi and Tanganyika

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Abstract

Landmark-based geometric morphometric techniques were used to test the hypothesis that *Petrochromis* spp. from Lake Tanganyika are ecomorphologically equivalent to *Petrotilapia* spp. from Lake Malawi. Both genera are epilithic algal feeders and inhabit the rocky shores of their respective lakes. We investigated the morphological component of the ecomorphology hypothesis by investigating body shape, using landmark-based morphometric techniques. A MANOVA revealed significant differences among species and an ordination of all species along the first two CV axes showed clear separation of the two genera in the morphospace with *Petrochromis fasciolatus* as an intermediate. A thin-plate spline analysis revealed that *Petrochromis* spp. had a deeper, broader anterior body, larger gape, shorter anal fin base and narrower caudal peduncle than *Petrotilapia* spp. Basically, differences between lakes were found, but there were no similarities or clusters of presumptive ecomorphs. Based on such results, we reject the hypothesis of morphological equivalence between these two genera. However, considering the non-significant difference in body shape revealed between *P. fasciolatus*, *Petrotilapia genalutea* and *Petrotilapia* 'mumbo blue', we conclude that these three species represent morphological equivalence and hence display a best example of convergent evolution.

Key words: ecomorphological equivalence, epilithic algal feeders, *Petrochromis*, *Petrotilapia*, geometric morphometrics

INTRODUCTION

The cichlid fishes of the Great Lakes of Africa are well known for their adaptive radiation in feeding habits and trophic morphology as well as explosive speciation (Fryer & Iles, 1972; Greenwood, 1974, 1981; Barel *et al.*, 1977; Witte, 1981). The three Great Lakes of Africa, Lakes Victoria, Tanganyika and Malawi, are the world's third, seventh and ninth largest inland water bodies. However, to ecological, behavioural and evolutionary biologists, the most intriguing aspect of these three lakes does not lie in their size but rather that these lakes tend to be inhabited largely by endemic species. It is estimated that Lakes Victoria, Malawi and Tanganyika, have 500, 800 and 250 cichlid species, respectively, of which 99% in each of Lakes Malawi and Victoria are endemic while 98% is endemic in Lake Tanganyika (Snoeks, 2000). Even though Lakes Malawi and Victoria possess a greater number of cichlid species than Lake Tanganyika, it is

believed that the latter has cichlids that are both morphologically and behaviourally more diverse (Fryer & Iles, 1972). Perhaps their relative ages may partly explain such diversity difference with Lake Tanganyika being the oldest, estimated to be between 9 and 12 million years old (Cohen, Soreghan & Scholz, 1993) whereas Lakes Malawi and Victoria are the more recent: < 1 million years old (see Meyer *et al.*, 1990; Johnson, Scholz & Talbot, 1996). Such a wide gap in the ages clearly shows that speciation in Lakes Malawi and Victoria has been rapid.

In each of these lakes, the cichlids seem to have a similar preference for certain habitats in which they apparently exploit similar resources. A prime example is the rock-dwelling cichlids of Lake Malawi, which consist of a group of closely related species, locally known as Mbuna. In Lake Tanganyika, rock dwellers are referred to as Ndongo. The apparent exploitation of similar resources led Fryer & Iles (1972) to hypothesize that some genera or species between the two lakes are ecologically and morphologically equivalent. One of the most famous examples of the equivalence is *Petrotilapia* spp. from Lake Malawi and *Petrochromis* spp. from Lake Tanganyika

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(Fryer & Iles, 1972; Ribbink *et al.*, 1983), both of which are epilithic algal grazers. Fryer & Iles (1972) concluded that the two genera indicate convergent evolution. Yamaoka (1997) reported that not all *Petrochromis* spp. are morphologically and ecologically similar to *Petrotilapia* spp., but rather there is only one species, *Petrochromis fasciolatus*, that resembles members of the later genus. In light of this disagreement, we conducted a morphological study of these two genera, focusing on overall body shape. Geometric morphometrics (GM), which uses the locations of homologous points (landmarks) to represent body shape, was used for the quantification of morphological variation (see Methods).

No study so far has attempted to morphologically compare cichlid species between these two lakes using a GM approach. Our approach seems feasible for several reasons. First, focusing on the whole body allows us to address generally whether there are morphological similarities between genera. This is a logical approach to the study of morphological equivalence in this system, since previous authors have not explicitly stated what specific features are similar between *Petrotilapia* and *Petrochromis*. Second, by using the GM approach, our findings can be statistically evaluated, unlike the classical descriptive approach that has been used previously to compare the two genera qualitatively. Additionally, the GM approach has several merits over the traditional morphometrics of simple linear measurements. For instance, GM is more effective at capturing information about the shape of an organism, since the geometry of the organism is preserved throughout the analysis. GM also identifies subtle variation in shape, and shows this shape variation graphically, which eases the interpretation of results (Rohlf & Marcus, 1993).

Hence the objective of this study was to test the following two hypotheses: (1) *Petrochromis* spp. and *Petrotilapia* spp. are not morphologically different (Fryer & Iles, 1972); (2) *Petrochromis fasciolatus* is the only species not morphologically different from *Petrotilapia* spp. (Yamaoka, 1997).

MATERIALS AND METHODS

Sample collection

In April 2001, the following *Petrotilapia* species were collected from West Thumbi Island in the Cape Maclear region of Lake Malawi (14°00'S, 34°50'E): *P. genalutea* (hereafter referred to as PTG), *P. nigra* (PTN) and an undescribed taxon *P.* 'mumbo blue' (PTMB) ($n = 30$ for each species). For the *Petrochromis* species, specimens were used which were collected in 1996 from Kasenga Bay (Zambia): *P. polyodon* (PCP), *P. fasciolatus* (PCFS), *P. famula* (PCFA), and *P. trewavasae* (PCT) ($n = 30$ for each species). Fishes were captured by SCUBA divers using hand nets and gill nets. Just after capture, fishes were killed and placed in a 10% formalin solution. Each specimen also received an injection of formalin solution

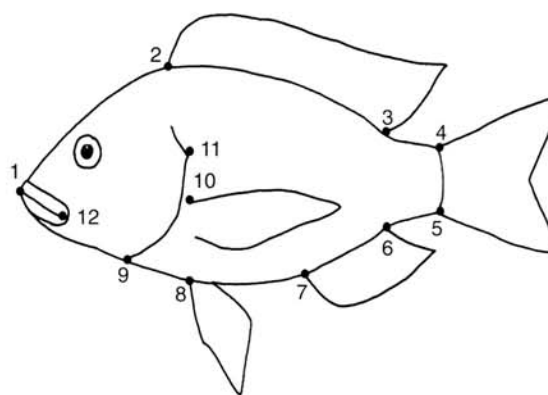


Fig. 1. Landmarks collected from the left side of each cichlid specimen: 1, tip of the premaxilla; 2, 3, anterior and posterior insertion of the dorsal fin; 4, 5, upper and lower insertion of caudal fin; 6, 7, posterior and anterior insertion of the anal fin; 8, insertion of the pelvic fin; 9, insertion of the operculum on the body profile; 10, upper insertion of the pectoral fin; 11, posterior extremity of the operculum; 12, posterior extremity of the gape.

into the body cavity. Specimens were later transferred to 70% ethanol, and stored until examination.

Geometric morphometric analysis

Cichlid body shape was quantified using landmark-based GM methods (Rohlf & Marcus, 1993). An OLYMPUS digital camera with a resolution of 3.3 megapixels was used to take images of all 210 specimens. The x,y coordinates of 12 homologous landmarks (Fig. 1) were digitized from the left side of each individual using TPSDIG32 (Rohlf, 2001). These landmarks were chosen for their capacity to capture overall body shape. Unfortunately, direct analysis of the landmark coordinates is not possible, as they contain components of both shape and non-shape variation. To obtain shape variables, non-shape variation in the landmark coordinates was removed through the generalized Procrustes analysis (GPA) (Rohlf & Slice, 1990). GPA removes non-shape variation by scaling all specimens to unit size, translating them to a common location, and rotating them so that their corresponding landmarks line up as closely as possible. From the aligned specimens, one means of obtaining shape variables is through generating partial warp scores and uniform components using the thin-plate spline and standard uniform equations (Bookstein, 1989, 1991, 1996). Together, the uniform and non-uniform components are treated as a set of shape variables for statistical comparisons of shape variation within and among groups (see e.g. Caldecutt & Adams, 1998; Adams & Rohlf, 2000; Rüber & Adams, 2001). The above procedures were implemented in TPSRELW (Rohlf, 1999a).

To determine the degree of morphometric differentiation among species, a canonical variate analysis (CVA) was performed on the partial warp scores including the

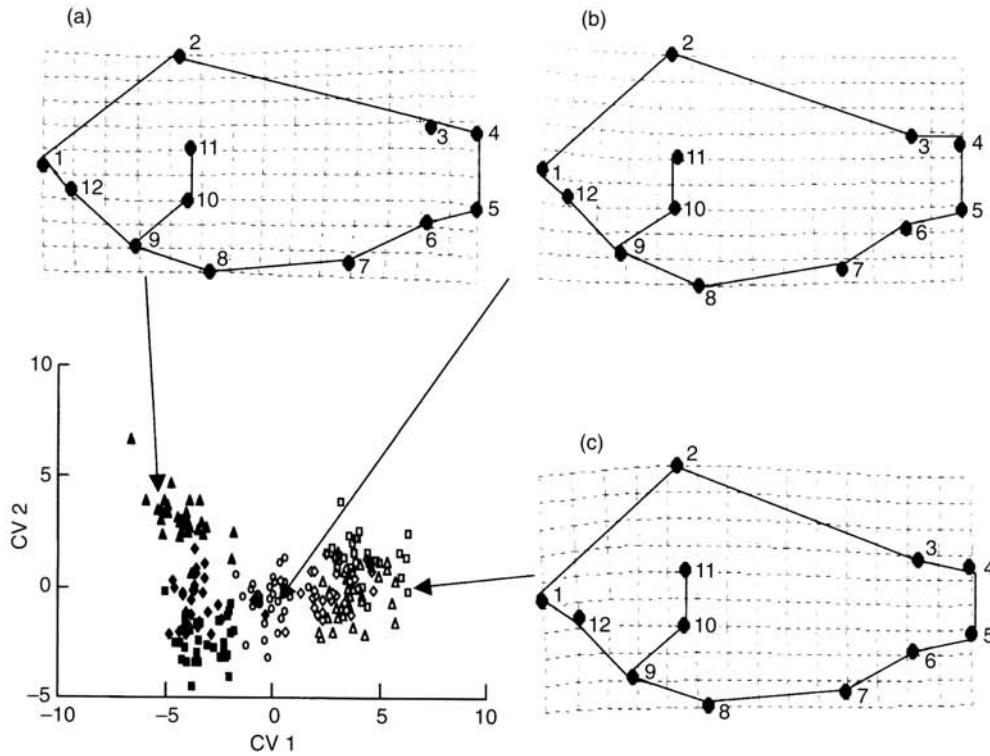


Fig. 2. Ordination of all cichlid specimens along the first two canonical axes together with spline grids showing shape deformations. Graph: closed symbols, *Petrotilapia* spp.: triangle, PTN; diamond, PTG; square, PTMB; open symbols, *Petrochromis* spp.: circle, PCFS; square, PCT; triangle, PCFA; diamond, PCP. Spline: (a) *Petrotilapia* spp.; (b) PCFS; (c) *Petrochromis* spp.

uniform component of shape. The CVA also provides an ordination of all the specimens in a morphological space. Pairwise multiple comparisons were performed to determine which species (if any) significantly differed from one another. These were based on generalized Mahalanobis distance (D^2) from a CVA. The critical α for these tests was adjusted using the Bonferroni procedure, where the original $\alpha = 0.05$ is divided by the number of comparisons (21). This yielded a critical $\alpha = 0.00238$, for an experimental error rate of $\alpha = 0.05$. CVA was performed in NTSYS-PC version 2.1 (Rohlf, 2000). Shape differences along the first CV axis were described using thin-plate spline deformation grids, generated in TPSREGR (Rohlf, 1999b). Using the generalized Mahalanobis distances, a dendrogram was generated (by UPGMA) to show overall similarity in body shape among species and not phylogenetic relationships.

RESULTS

A multivariate analysis of variance (MANOVA) revealed significant differences among species (Wilks' $\Lambda = 0.00201$, $F_{(120,1070,9)} = 17.199$, $P < 0.0001$). Assignment of individual specimens to the species correctly classified 196 of 210 (93.3%) specimens, showing that the species were well separated in morphospace. Using the generalized Mahalanobis distances, 12 of the 21 comparisons indicated significant differentiation among

Table 1. Pairwise comparisons based on generalized Mahalanobis distances among all cichlid species. *, significantly different pairs. For abbreviations see Materials and methods

Group	PTG	PTMB	PTN	PCFA	PCFS	PCP	PCT
PTG	0						
PTMB	3.70	0					
PTN	4.52	5.82*	0				
PCFA	7.61*	7.43*	8.87*	0			
PCFS	4.95	5.54	6.55*	5.65	0		
PCP	6.75*	6.53*	7.86*	3.17	4.83	0	
PCT	8.26*	8.29*	8.77*	3.23	5.81*	3.06	0

species (Table 1). From this it is clear that *Petrotilapia* spp. are different from *Petrochromis* spp., with the exception of one species (PCFS), which is intermediate between the two genera. This difference among genera is clearly illustrated in Figs 2 & 3, where the different genera can be identified as distinct clusters. There are two main observations that can be made from Table 1 and Fig. 2. First, *Petrotilapia* spp. are clearly different from *Petrochromis* spp. as is seen by their clear separation in morphospace. Second, PCFS seems to be intermediate between the two genera, and its individuals overlap with individuals from both genera. Some intrageneric variation was also detected, e.g. PTN is significantly different from PTMB while PCFS is different from PCT (Table 1).

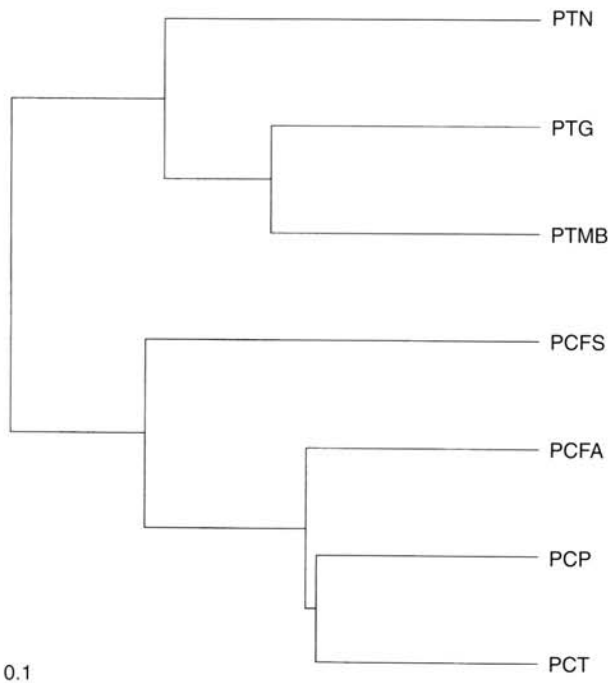


Fig. 3. UPGMA dendrogram showing overall similarity in body shape among cichlid species generated using the generalized Mahalanobis distances from CVA.

To visualize shape differences between groups, spline deformation grids were generated for each group (genus) mean and for PCFS, the intermediate taxon, relative to the overall consensus. From the deformation grids (Fig. 2a,c), *Petrochromis* spp. was found to have a deeper, broader anterior body and larger gape than *Petrotilapia* spp. The mouth in *Petrotilapia* spp. is terminal, whereas in *Petrochromis* spp. it is slightly inferior sub-terminal. Another difference exists in the caudal peduncle region, where *Petrotilapia* spp. has a broader caudal peduncle region including the caudal fin base and also longer anal fin base than *Petrochromis* spp. Thus, using the GM analysis and associated graphics (thin-plate spline deformation grids), it is clear why PCFS is considered to have an intermediate morphology (Fig. 2b).

DISCUSSION

The GM approach clearly shows that body shape in *Petrotilapia* spp. is different from *Petrochromis* spp. Our finding that *Petrochromis* spp. consists of species with a deeper and broader anterior body concurs with Brichard's (1989) description of the genus. Yamaoka's (1997) hypothesis seems to be partially correct, but the resemblance of PCFS to all members of *Petrotilapia* spp. may have been overstated, since we found that PTN is clearly different from PCFS. Hence, we partly agree with Yamaoka (1997), in that some members (PTMB and PTG) resemble PCFS, but not that all members of *Petrotilapia* spp. do.

The equivalence between PTG and PCFS seems not only to lie on morphological aspect, but on feeding ecology as well. Through the analysis of two feeding parameters, i.e. grazing speed and number of bites per bout, K. Yamaoka (pers. obs.) observed that mean value for number of bites per bout for PTG was larger than any other *Petrochromis* spp. but closer to PCFS (since PCFS shows largest mean value for both parameters among its congeners), while the mean value for grazing speed was similar to *Petrochromis orthognathus*. He concluded that PTG must be sharing trophic behavioural features with PCFS and *P. orthognathus*. Based on such observations, perhaps it is reasonable to conclude that PCFS and PTG are both ecologically and morphologically equivalent. The morphological similarity of PCFS, PTG and PTMB leads us to conclude that, strictly speaking, these three species display an apparent example of convergent evolution, but, unlike the suggestion of Fryer & Iles (1972), not all species of both genera are convergent in morphology.

The intrageneric variation revealed herein calls for a further detailed study at the intrageneric level with the aim of understanding coexistence mechanism among congeners. For instance, Yamaoka (1983) found that the difference in grazing speed among *Petrochromis* spp. leads to differential exploitation of food resources and hence coexistence. No detailed study, however, has been done to investigate if such variation in grazing speed is also reflected in morphological adaptation. The body shape variations reported in this study within *Petrotilapia* spp. seem to be consistent with findings by D. D. Kassam (pers. obs.) that PTN was different from its congeners, mainly on the gape size. He inferred that such gape size variation may be attributed to differences in feeding strategy in such a way that the smaller gape in PTN is very efficient in combing the diatoms from the algae strands while the larger gape for PTG and PTMB are suitable for pulling/pull-scraping the algae from rocks. Such variation in feeding technique is suggested to play a significant role in enhancing the coexistence among these three *Petrotilapia* spp. However, underwater observation is required to shed more light on the biological significance of gape size variation among *Petrotilapia* spp.

Yamaoka (1997) was forced to conclude that the morphological diversity in *Petrotilapia* spp. is very low as it could be equated to PCFS alone, a conclusion that is in line with the common belief that cichlids from Lake Tanganyika are both morphologically and behaviourally more diverse than those from Lake Malawi. However, the significant variation revealed in this study between PTN and PCFS calls Yamaoka's (1997) conclusion into question. Considering that other undescribed taxa belonging to *Petrotilapia* spp. were not included in this study, inclusion of such taxa might show even more variation than anticipated, hence the conclusion of low morphological diversity in the genus *Petrotilapia* would seem to be inappropriate at this level of comparison. The conclusion by some researchers that cichlids from Lake Malawi are less diverse than those from Lake Tanganyika may be partially because many biologists have explored Lake Tanganyika in more detail than Lake Malawi, and

thus, less information has been gathered on cichlids from Lake Malawi.

Our approach to the study of body shape using geometric morphometrics has proven to be more robust than traditional approaches, as it allowed us to clearly show that the two genera are not morphologically similar. Use of such methods will enable researchers to understand more about shape variation and body shape similarities among African cichlids, and allow the discrimination of morphotypes based on subtle morphological differences which are otherwise not detected using traditional approaches.

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