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Full Length Research Paper

Genetic and morphological comparism of stocks from different rivers in Malawi

***Tamika O. chihana, Bingu Bakili and Mapanje Banda**

Aquaculture and Fisheries Science Department, Lilongwe University of Agriculture and Natural Resources (LUANAR), Bunda Campus, P. O. Box 219, Lilongwe, Malawi.

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Fisheries management continues to be a nightmare due to over exploitation of fish stocks and various anthropogenic activities resulting in a reduction of genetic resources. *Opsaridium microlepis*, a commercially exploited fish species from Lake Malawi, is no exception, hence it is listed as endangered. *Opsaridium microlepis* stocks from four different rivers were analyzed using 13 geometric morphometric landmarks and 20 microsatellite loci, to determine if the stocks were morphologically and/or genetically different. AMOVA performed on DNA data revealed a significant (*P* < 0.001) genetic differentiation with 16.4% of the total genetic variance ascribed to differences among populations, and 83.6% due to differences within population. This finding was supported by higher pairwise FST values (FST = 0.17). MANOVA of morphological data showed significant body shape variation among the stocks (Wilk's λ = 0.0913; *P* < 0.0001). Pairwise comparisons using both methods indicated that all pairs were significantly different, except morphologically for Bua and Linthipe (*P*=0.3311). The morphological differences consisted of shorter gape and shorter head of the Bua/Linthipe stock was seen in the North Rukuru and Dwangwa stocks. The morpho-genetic differentiation revealed in this study implies that the populations are distinct and should be considered as separate management and conservation units.

Key words: Lake Malawi, Mpasa, procrustes distance, genetic differentiation, endangered species, fish stocks, conservation.

INTRODUCTION

Lake Malawi, a global biodiversity asset, has attracted worldwide attention amongst evolutionary biologists, due to the fastest large-scale adaptive radiation ever recorded in evolutionary history (Ribbink, 2001). It is estimated that

*Corresponding Author. Email: dr.tamika49@yahoo.com

the lake harbors over 800 species of cichlids, of which 99% are endemic (Snoeks, 2000), as well as about 50 other fish species, of which 50% are endemic (Ribbink, 1994). The lake's fish population also supports a multi-species fishery (Weyl et al., 2004, 2005), being both a source of income to the local people and a basis of national food security (Ribbink 2001; Weyl et al., 2005). In the multi-species fishery, *Opsaridium microlepis* (Günther, 1864) is one of the major species that is commercially exploited in this lake. It is endemic to Lake Malawi and its affluents where it is commonly known as lake salmon and locally known as Mpasa. Mpasa is commonly caught in the northern and central regions of Malawi, where it ascends rivers to breed. It has been known that catches have been on the decline (Tweddle, 1981; GoM, 2016). During their spawning migrations, Mpasa are heavily exploited by gillnets set near river mouths, and numerous fishing methods in the rivers themselves. Though, Mpasa is known to inhabit the Lake Malawi and its in-flows, it has disappeared in some in-flows where it used to be abundant due to ecosystem degradation, overfishing during migration (Ndamala, 2006), and a surge in human population density in the areas surrounding the in-flow rivers (Kingdon et al., 1999). Mpasa stocks have drastically declined by not less than 50% such that the species was listed in IUCN 2006 as endangered species (Kazembe et al., 2005), and still remains in that status until the time this study was conducted.The fact that this species remains endangered is a valid reason for immediate action to ensure the survival of this important commercial/food resource. Management procedures must be put in place in order to conserve the species; however, such management procedures require information on whether different affluent rivers inhabit different stocks or one panmictic population in the lake whose individual fish are free to go up into any river. Tweddle (1981) suggested that each river has got its own Mpasa stock, with little or no intermingling, however, such conclusion can only be confirmed through some quantitative analysis. Population studies need to address both genetic and phenotypic divergence because population structure can result in restricted gene flow, color variation and local adaptation in particular morphological features that indicate population divergence. Geometric morphometrics, a landmark-based approach for investigating body shape changes, has been shown to detect fine-scale morphological differences (Kassam et al., 2003a, b; Adams et al., 2004; Maderbacher et al., 2008). In combination with neutral genetic markers, such as microsatellites, changes in the phenotype of an organism can be used to assess the intensity and direction of natural selection (Raeymaekers et al., 2007; Maquia et al., 2013). Hence, this study aimed at unraveling whether stocks from different rivers were morphologically and/or genetically similar or not, since such information is crucial in the management of stocks/species.

MATERIALS AND METHODS

Sampling

For geometric morphometrics, the four major rivers, three in the central region {Bua (n = 49), Linthipe (n = 40) and Dwangwa (n = 20)}, and one in the northern region (North Rukuru, n = 30) were sampled. The samples were preserved in 10% formalin, and transported to Lilongwe University of Agriculture and Natural Resources (LUANAR) Bunda Campus where image acquisition was done within the same month since long preservation affects shape of any fish. For genetic analysis, 50 fish from each of the four rivers were sampled in February and April, 2010. Tissue samples (fin clips and muscle) were extracted from individual fish and preserved in vials with 95% ethanol and later kept at 4°C in the laboratory at Chancellor College, University of Malawi.

DNA extraction and amplification

Genomic DNA was extracted following a standard SDS- proteinase K/phenol-chloroform procedure (Hillis et al., 1996). Twenty polymorphic microsatellite loci (Table 1) (Changadeya et al., 2013) were used for genotyping. The polymerase chain reaction (PCR) was carried out in a Mastercycler gradient 5331 Eppendorf (Version 2.30.31-09) with the following PCR conditions: initial denaturing at 94°C for 2 min, then 30 amplification cycles of denaturing at 94°C for 30 s, annealing at an optimal temperature for a specific primer pair for 15 s and elongation at 72°C for 30 s. The final extension was at 72°C for 20 min followed by a soaking temperature of 4°C. Finally, the amplified products of PCR were run on 6% polyacrylamide gel in BIORAD Sequi-Gen® GT Nucleic Acid Electrophoresis Cell where pGem DNA marker (Promega, 2000 USA) and ϕ X174 DNA/*Hinf* 1 (Promega, 2000 (USA) were used as band size standard markers.

Analysis of molecular data

Genetic differentiation among populations was assessed by analysis of molecular variance (AMOVA, in ARLEQUIN version 3.1, Excoffier et al., 2006) followed by a computation of pair wise F_{ST} values using GENEPOP (Raymond and Rousset, 1995). Mantel' test was performed to test if a correlation existed between the genetic and geographical distances, genetic and morphological distances and finally morphological and geographical distances. Unweighted Paired Group with Arithmetic Average (UPGMA) algorithm, based on Nei's (1973) genetic distance, was used to analyze population clustering in NTSYS (Rohlf, 1998).

Geometric morphometrics

TPSDIG32 program (Rohlf, 2004) was used to digitize 13 landmarks (Figure 1) on all specimens. Procrustes superimposition was performed in CoordGen (IMP 6 package, Sheets, 2004). The superimposition translates all specimens to a common location and rotates them so that corresponding landmarks align as close to each other as possible and separates the two components of form, namely, size and shape. Separation of shape and size enables one to analyze each component separately depending on questions addressed (Bruner and Manzi, 2004; Kassam et al., 2004). The weight matrix of partial warp scores, representing shape variables, was generated from the overall procrustes superimposition and subsequent conventional statistics was applied to this weight matrix.

Table 1. Total number of alleles (A), allele size range (SR) in base pairs and microsatellite primer polymorphic information content (PIC).

Locus	A	Gene Bank accession	T_A	Allele Size Range (SR)	Repeat Motif	PIC
CypG49	12	AY349167	53.6	170-192	$(TA)_{11-21}$	0.74
CypG3	11	AY349122	56.4	150-202	$(CAGA)_2$	0.76
CypG13	13	AY349132	53.4	140-172	$(TAGA)_{10}$	0.73
Ca3	10	AF277575	54.5	162-182		0.78
CypG5	12	AY349124	54.3	114-172	$(TAGA)_{12}$	0.76
CypG4	17	AY349123	54.3	134-202	$(TAGA)_{12}$	0.79
CypG30	22	AY349148	54.3	118-182	$(TAGA)_7$	0.77
Lid1	17		55.3	140-194	(TTTC)7	0.75
CypG48	16	AY349166	55.3	126-158		0.80
MFW	11	EF144124	55.3	106-126		0.76
CypG22	12	AY349140	55.7	202-224		0.70
CypG6	12	AY349125	52.5	192-218	$(TAGA)_7$	0.71
CypG8	18	AY349127	52.5	132-166	$(CAGA)_6$	0.73
CypG21	14	AY349139	52.7	158-184	$(CAGA)_6$ $(TAGA)_7$	0.75
CypG27	14	AY349145	52.7	104-188	$(TAGA)_8$	0.76
Lid11	16		53.7	200-228	(TTTG)8	0.77
AP1	13	AJ428582	53.4	158-182	$(TA)_{11-21}$	0.74
AP2	18	AJ428583	55.0	110-188	$(AC)_{18-20}$	0.78
Ru2	17		53.6	142-174		0.75
CypG15	20	AY349134	53.8	116-158		0.82
Mean	14.75					**0.76**

T_A annealing temperature.

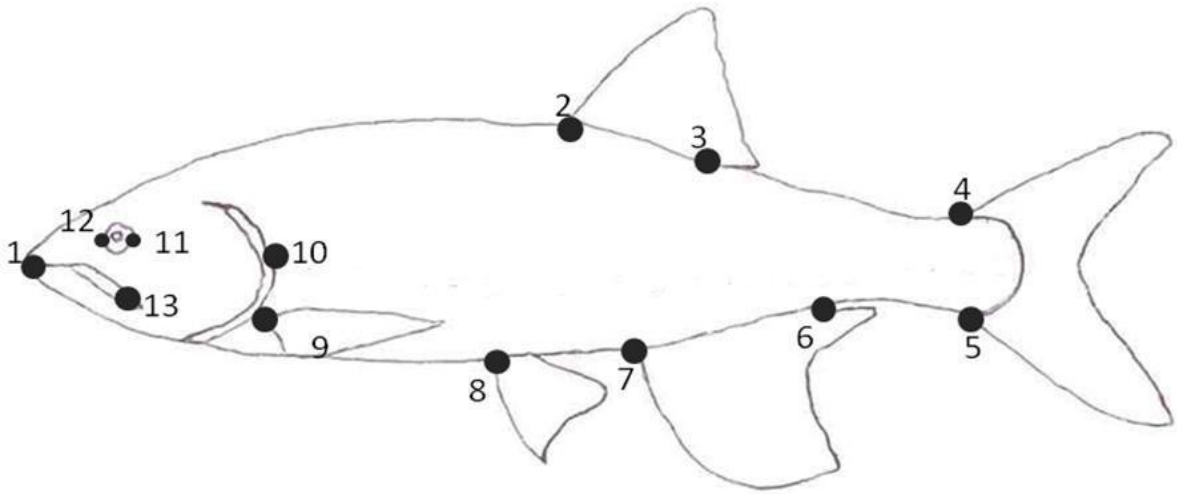

Figure 1. Landmarks digitized on every Mpasa specimen. 1: snout tip; 2-3:anterior and posterior insertion of dorsal fin; 4-5: caudal fin base; 6-7: posterior and anterior insertion of anal fin; 8: insertion of pelvic fin; 9: insertion of pectoral fin; 10: end tip of operculum: 11-12: eye diameter; 13: posterior end of gape.

Analysis of morphometry data

A canonical variate analysis (CVA) was performed on the weight matrix in order to determine differences in body shape among stocks through multivariate analysis of variance (MANOVA). If MANOVA revealed significant differences among stocks, pairwise multiple comparisons using Goodall's F-test on Procrustes Distance was performed to determine which groups differ from one another.

Table 2. Pairwise comparison among all the four populations of Mpasa on morphological and genetic parameters.

Population pair	Parameter					
	Geographical distance (km)	Procrustes distance	*P -values*	Nm	F_{ST}	Genic and genotypic differentiation *P -values*
NR& BWA	335.9	0.0225	0.0044	0.67	0.16	0.0000
NR & LTP	451.9	0.0234	0.0056	0.49	0.16	0.0000
BWA& LTP	122.11	0.0123	0.3311	0.70	0.14	0.0000
NR & DWA	294.24	0.0302	0.0011	0.38	0.19	0.0000
BWA& DWA	43.98	0.0287	0.0031	0.56	0.16	0.0000
LTP & DWA	158.85	0.0321	0.0022	0.45	0.19	0.0000

NR, North Rukuru river ; BWA, Bua river; DWA, Dwangwa river ; LTP; Linthipe river; Nm, gene flow; $F_{ST,}$ population fixation index

These statistical analyses were done in IMP software and CVA analysis was executed by CVAGen6, while pairwise comparisons were done by TwoGroup6 (Sheets, 2004). To determine population clustering due to morphological similarity, a UPGMA algorithm, based on Procrustes distance, was done using NTSYS (Rohlf, 1998).

RESULTS

Genetic population differentiation

AMOVA revealed a significant ($P < 0.001$) genetic variation with 16.4% of the total genetic variance attributed to differences among populations and 83.6% was due to differences within population. In this study, the average number of migrants per generation was *Nm* = 0.91, and the highest number of migrants were between the Linthipe and Bua (*Nm* = 0.70), while the lowest was between North Rukuru and Dwangwa (*Nm* = 0.38) populations (Table 2). The overall genetic differentiation (F_{ST} = 0.17) signified high genetic variation among populations with the highest variation between North Rukuru and Dwangwa, Linthipe and Dwangwa population pairs (F_{ST} = 0.19), while the lowest was between Linthipe and Bua population pair (F_{ST} = 0.14) (Table 2).

Morphological differentiation

MANOVA revealed significant body shape differences among stocks (Wilk's λ = 0.0913; $p< 0.0001$). Pairwise comparisons indicated that all pairs were different except the stock from Bua which was not significantly different from Linthipe stock (Table 2). From the deformation grids generated (Figure 2), the subtle morphological differences observed consisted of shorter gape of the Bua/Linthipe stocks as evidenced by the posterior and anterior displacements of landmarks 1 and 13, respectively, as opposed to the other 2 stocks. The anterior displacement of landmark 10 against posterior displacement of landmark 1 signified that the Bua/Linthipe stock had a shorter head than the North Rukuru and Dwangwa stocks. The Dwangwa stock had wider caudal peduncle than the North Rukuru stock as evidenced by displacements of landmarks 4 and 5.

Morpho-genetic cluster analysis among populations

Both dendrograms (Figures 2 and 3) indicated that Bua stock was closely related (morpho-genetically) to Linthipe stock, despite Bua is geographically closer to Dwangwa than Linthipe (Table 2). However, the morphological dendrogram (Figure 2) unlike the genetic dendrogram (Figure 3) correlates with geographical distance differences by depicting a Bua-Linthipe-Dwangwa cluster that is clearly delineated from North Rukuru cluster. Mantel' tests revealed that there were no significant correlations between geographical distance and genetic distance (r = 0.18; *p* = 0.6369), geographical distance and morphological distance (r = -0.01900; *p* = 0.4856) and also between genetic and morphological distances (r = 0.72; *p* = 0.9328) among the stocks. This indicates that the morphological and genetic structuring observed among these stocks, is not necessarily due to geographical isolation.

DISCUSSION

Genetic differentiation

Pair-wise comparison amongst the four populations revealed significant genetic differentiation in *O. microlepis*. This suggests partitioning of the breeding population, limitation in migration between different areas and the existence of a distinct stock structure among populations. The high overall value of F_{ST} (0.17) of microsatellite loci in *O. microlepis* was significantly different from zero ($P < 0.001$) suggesting great genetic differentiation among the populations. Wright (1978)

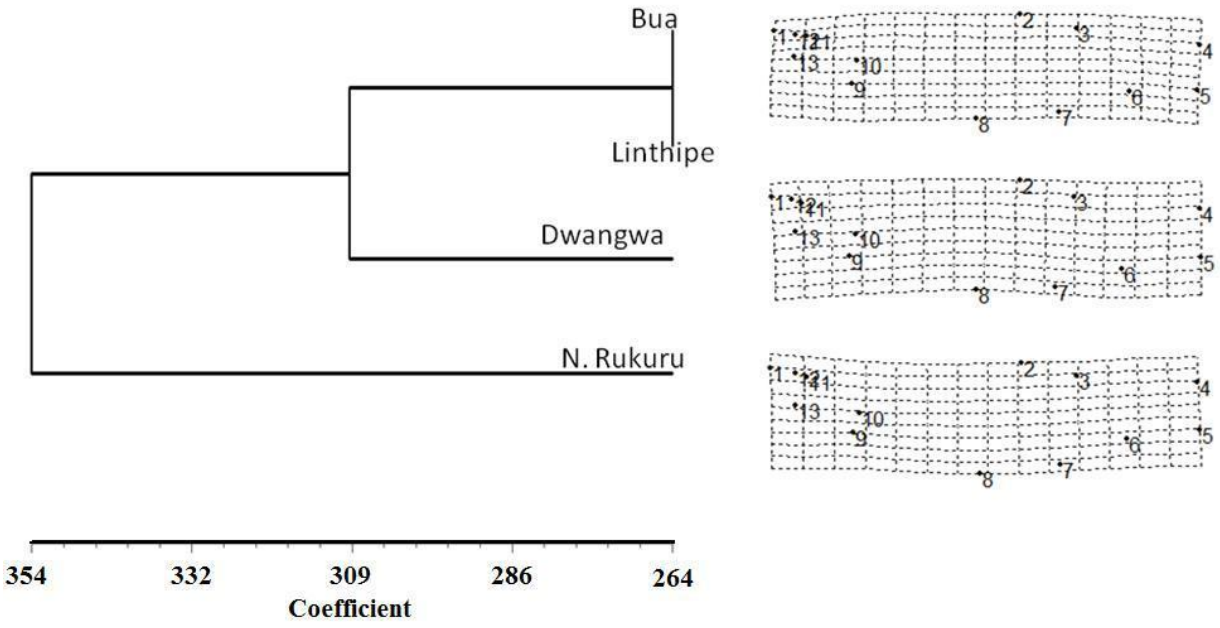

Figure 2. UPGMA phenogram based on Procrustes distances for the four populations of *Opsaridium mucrolepis* with consensus configurations representing the stocks at the tips of the phenogram.

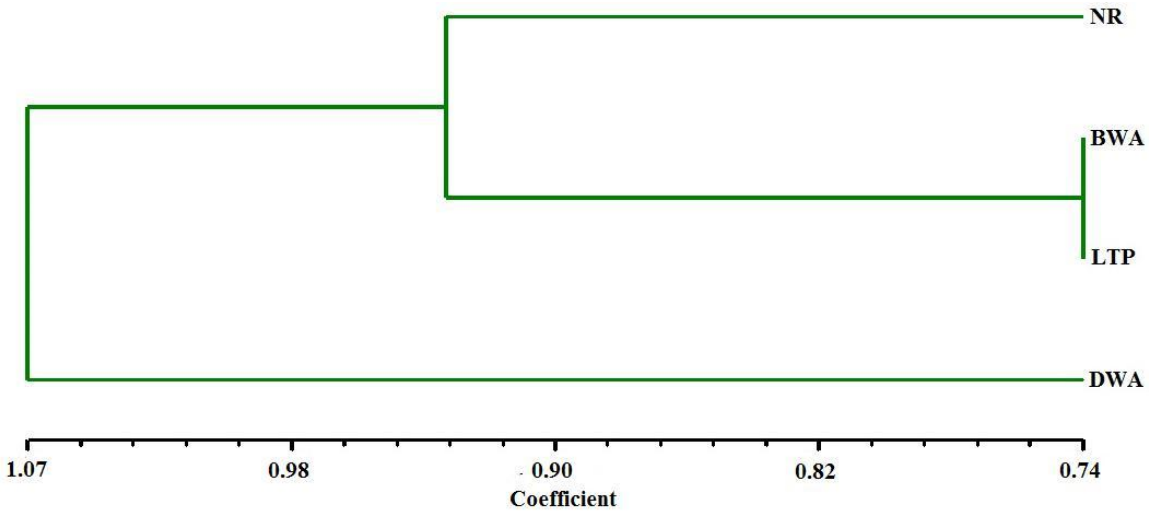

Figure 3. UPGMA phenogram based on Nei's genetic distances for the four populations of *Opsaridium mucrolepis*.

explained that any genetic differentiation ≥15% signifies high genetic differentiation and is associated with very low gene flow among populations. Level of genetic differentiation demonstrated by Mpasa in this study is higher than values seen in Pacific herring (F_{ST} = 0.023), Atlantic herring (F_{ST} = 0.035) and widespread anadromous fish like Atlantic salmon (F_{ST} = 0.054) (McConnell et al., 1995). The plausible reasons for the observed high genetic differentiation among the populations are low gene flow among populations (Nm=0.91) and an absence of recent genetic bottlenecks as indicated by Bottleneck tests entailing that despite heavy exploitation, the populations are outbreed due to possible presence of large numbers of fish. The high F_{ST} obtained in this study hence signify that the populations are distinct requiring independent conservation management for each river system. Mills and Allendorf (1996) concluded that the rate of migration of *Nm*≥1

leads to considerable homogeneity among populations but population divergence and structuring occurs when *Nm*≤ 1. The populations in the present study have an overall migration rate of *Nm*≤ 1 rendering them to structuring and divergence.

Morphological diversity

Geometric morphometrics as used in this study has proved more robust than the traditional morphometric approach which could not clearly distinguish these stocks according to Chigamba et al. (2012). The morphological differences though subtle, are important because they clearly indicated that stocks from different rivers are not the same in body shape. These findings confirm Tweddle (1981) suggestion that the stocks of Mpasa are specific to each river system with little or no intermingling. Additionally, Chigamba et al. (2012) found that water quality in these rivers was also different, revealing different usage and status of environmental degradation of the rivers' water and air sheds. Therefore, the management and conservation measures of each fish population and its specific river should be independent and targeted because loss of one river' fish stock would mean extermination of a morphologically unique fish stock.

Kassam et al. (2003a) found a strong link in gape size with the feeding habit of some cichlid species. Gape size of a predator and body depth of the prey are the main factors determining whether a gape-limited piscivore can ingest a potential prey. Consequently, gape size governs predator-prey relationships. Magnhagen and Hiebo (2001) reported that a smaller Pike with relatively bigger gapes was observed surrounded by lowest prey availability, while a larger Pike with smaller gapes had the highest prey density. On the other hand, size of caudal peduncle has been related to sex and homing behavior. Beachan (1984) found that in Chum salmon, males had larger caudal peduncles than females, while Chum salmon from larger rivers had larger peduncles than those from smaller rivers. From the above, it seems probable than the differences in body shapes between the different stocks of Mpasa could be a reflection of many factors including size of the rivers, feeding behavior and availability of prey. More studies are required to determine the factors that have led to such differences in body shape. Nevertheless, the differences in body shapes as detected by this study infer that these stocks are different, and should be considered as separate entities in every aspect conservation and management.

In the present investigation, Mantel' tests showed no significant correlation between geographical, morphological and molecular distances among the populations studied. Low association between genetic and morphological distances is due to the fact that SSR loci are non-coding DNA which is not expressed hence not subjected to the same forces of selection which shape morphological characters (Kjaer et al., 2004; Vieira et al., 2007). Therefore, morphological and molecular differences observed among the populations may be due to local selection pressures imposed on the stocks since there is no evidence of isolation-by-distance effect. This revelation concurs with other studies of *Lenthrinops* species flock (Duponchelle et al., 1999; Changadeya et al., 2001), which reported fish flocks not fitting the isolation by distance model though in those studies, the fish populations exhibited high gene flow.

Conclusions

The genetic and morphological differentiation revealed in this study underscores the need for separate monitoring and conservation strategies for each of the four populations. The study revealed closely comparable results between geometric morphometric and genetic analysis; therefore, geometric morphometric techniques can reliably be used in similar studies where DNA analysis is not possible due to high running costs and lack of specialized equipment. Thus, this study recommends that the management and conservation measures of each fish population and its specific river should be independent and targeted because loss of one river' fish stock would mean extermination of a morphologically unique fish stock.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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