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An investigation of maternal origin of morphometrically defined Tilapia species established in Sri Lanka

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ABSTRACT

Three species of tilapia *viz; Oreochromis mossambicus, Oreochromis niloticus* and *Tilapia rendalli,* are well established in Sri Lanka and significant in fresh water fishery. Introgressive hybridisation is common between certain tilapia species producing hybrids that can obscure the morphological boundaries between species and also have numerous negative effects on long-term fishery. Therefore an easy index for identification of tilapia species and their hybrids in the field, which is based on morphometry of tilapia fish is essential. However, such a morphological index substantiated by DNA based study is not available. The present investigation reports the most important morphometric characters for the identification of tilapia species in Sri Lanka. The maternal origin of selected individuals of such groups was determined using heteroduplex formation of PCR products of a part of control region of mitochondrial DNA (mtDNA) with the PCR product of the same region of an authentic *O. mossambicus.* The majority of individuals tested had maternal origin conforming to the present morphologically defined species, whereas a few (03) *O. niloticus* individuals had mossambicus maternal origin indicating hybridisation.

Key words: Tilapia, introgressive hybridisation, maternal origin, morphometry, *Oreochromis*

INTRODUCTION

Introduced cichlids, commonly known as tilapia, contribute significantly to the reservoir fishery in Sri Lanka. Among those, *Oreochromis mossambicus* and *Oreochromis niloticus* are well established and constitute a major part of the commercial catches. Three other cichlid species *viz., Tilapia rendalli, Tilapia hornorum* and *Tilapia zilli* have also been introduced, of which, only *Tilapia rendalli* is recorded to be established (De Silva 1988). Morphological similarities among many tilapia species make their visual identification difficult (Trewavas 1983), particularly due to possible intermediate morphologies of hybrids resulting from introgressive hybridisation which is common among several species of tilapia (Macaranas *et al.* 1986; Pante *et al.* 1988).

O. mossambicus and *O. niloticus* known to interbreed easily resulting in male dominant populations (Hickling 1971; Wohlfarth and Hulata 1983). Amarasinghe and De Silva (1996) also have shown that hybridisation of Ram. *O. niloticus* and *O. mossambicus* in Sri Lanka has influenced the sex ratio with male dominance and decline in fecundity. On the other hand, sustainability of the Sri Lankan reservoir fishery depends upon the high reproductive capacity of *O. mossambicus* (De Silva and Chandrasoma 1980; De Silva 1986) which forms over 70% of the total landings (De Silva 1988). Consequently, a decline in this trait would adversely affect long-term fishery. Further, their growth performances and mortality are also important parameters in evaluating the effects of hybridisation of tilapia on fresh water fishery.

Therefore, it is an important requisite to determine the species composition of tilapia and their hybrids in reservoirs in order to appraise their influence on the fresh water fishery and its management. Consequently, an investigation aimed at identification of important morphometric characters in the development of a morphological index, to differentiate tilapia species and their hybrids is needed. The genetic basis of the groups separated by those parameters must be validated using biochemical or more appropriately DNA based techniques. Present study is a preliminary step in that direction.

The control region of mtDNA is known to have a high rate of evolution compared to rest of the mitochondrial genome (Aquadro and Greenberg 1983) and, has been used for genetic differentiation since this reveals the maternal origin of the species and hybrids. In the present attempt, tilapia fish were clustered according to their morphological variations. The maternal origin of a number of individuals representing each cluster was

Abbreviations: PCR - Polymerase chain Reaction; TGGE - Temperature gradient gel electrophoresis

determined. For this, heteroduplex formation of PCR product of a part of the control region of mtDNA with the same of an authentic *O. mossambicus* was employed.

MATERIALS AND METHODS

Samples of fish were collected from several reservoirs/waterbodies (Table 1) in the Southern, Western and North-western regions of Sri Lanka. Fish were caught by means of gill nets of mesh size 6-12 cm as well as hook and line. Live specimens were brought to the laboratory and were kept in fibreglass tanks until investigation.

Table 1. Number of fish collected from different reservoirs/water-bodies and their distribution into species as defined by the morphometric characters.

Reservoier/waterbody	Sample size	Morphological cluster		
		Niloticus	Mossam	Rendalli
Chaudrikawewa (C)	15 (6)	02	01	12
Udawalawa (U)	17 (7)	03	06	08
Seguwantive (P)	19 (7)	02	17	00
Univ. Pond (Ru)	05 (3)	03	02	00
Univ. Canal (M)	03 (3)	00	03	00
Badagiriya (B)	36 (2)	24	02	00
Negambo lagoon (N)	08 (6)	01	07	00
Total	103(34)	45	38	20

* Sample size for mt.DNA analysis is given in parenthesis.

Morphometrics

The morphological and meristic characters based on standard taxonomic features given by Trewavas (1983) for this group of fishes were recorded (Table 2). All length measurements were converted to proportions to give some degree of independence from individual body size and were used in the analysis (items 8-13 in Table 2). The data were analysed by hierarchical cluster analysis using SPSS/PC statistical package.

Table 2. List ofmorphomeric characters used in the study.

Character	
Morphological	Meristic
1. Standard length (SL)	1. No. of stripes present on the body
2. Maximum depth (MD)	2. No. of caudal fin bars
3. Head length (HL)	3. No. of dorsal fin rays
4. Depth of the caudal peduncal (CPD)	4. No. of dorsal spines
5. Length of the caudal peduncal (CPL)	5. No. of anal spines and rays
6. Pre-dorsal distance (PDD)	6. No. of gill rakers
7. Pre-anal distance (PAD)	7. No. of lateral line scales
8. MD/SL	
9. HL/SL	
10. CPD/CPL	
11.CPD/SL	
12. PDD/SL	
13.PAD/SL	

Mitochondrial DNA analysis

Total genomic DNA was extracted as described by Hills and Mortiz (1990) from 100mg of white muscle tissue of 34 selected individuals representing different morphological clusters. Four hundred base pair (bp) region of DNA on the left side of the control region of the mtDNA was amplified using primers 98H (CCTGAAGTAGGAACCAGATG) and L 19 (CCACTAGCTCCCAAAGCTA) created by GIBCOBRL. Each PCR reaction was set up in a volume of 20 µl and composed of 0.5 mM of each primer, 0.25 mM each of dNTPs mixture, 1 X PCR buffer, 3 mM $MgCl_2$ and 0.44 units of t^{th} polymerase. Approximately 100-200 ng of total genomic DNA in 1 X TE buffer was added to this. The following amplification cycles were performed in minicycler (MJ Research Inc.).
(1) 94° C for 5 min. (2) 94° C for 30 min. (3) 55° C for 1 min. (4) 72° C for 2 min. (5) Cycle to step 2, 39 more times (6) 68° C for 8 min. (7) 4° C for overnight storage.

A horizontal Temperature Gradient Gel Electrophoresis (TGGE) system was used for heteroduplex analysis of the amplified tilapia DNA samples with a single reference of pure *O. mossambicus* (TGGE hand book 1993). Amplified PCR products of authentic *O. niloticus* : *O. niloticus* "Chitralda" from Fiji and *O. niloticus* "Israel" from Israel and *O. mossambicus* (Agustin 1999) were also included in TGGE to identify the banding pattern given by niloticus and mossambicus mtDNA PCR products. Optimum temperature gradient was set according to Augustin (1998). Electrophoretic runs were of four-hour duration, using parallel temperature gradient of 13°C to 47°C. DNA was visualised using silver staining (TGGE Hand Book 1993).

RESULTS

Morphometrics

Table 1 shows the number of individuals used for cluster analysis from each reservoir. According to the hierarchical cluster analysis, fish from all the reservoirs grouped into following three main clusters: niloticus-like, mossambicus-like and rendalli-like. Figure 1 gives the results of clustering of individuals of various morphological and meristic characters. The morphological characters (items 8-13) and all meristic characters (Table 2) were used in this cluster analysis. Nevertheless, the meristic characters 2-7 in the Table 2 were identified as the most important characters in the

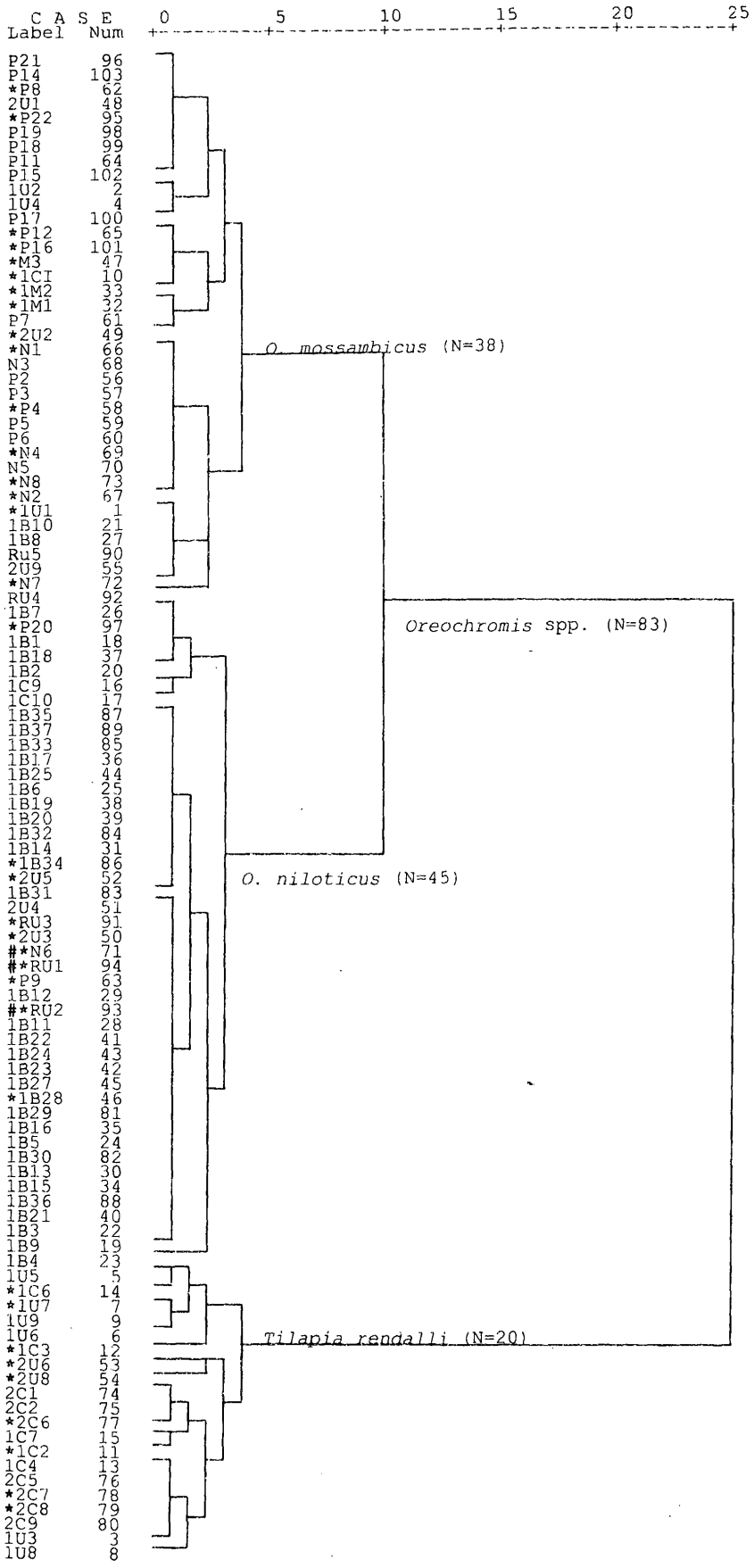

Fig. 1. Clustering of tilapia specimens according to their morphometry.

U - udawalawa, B - Badagiriya, C - Chandrikawewa, N - Negambo, P - Seguwantive, M - University canal, RU - University pond:
*** - Individuals sampled for mtDNA analysis;**
 # - Individuals with different maternal origin to their species.

differentiation of the three tilapia species. Using only those morphometric characters a very similar dendrogram (not shown) could be generated to separate the three species. However, use of all characters clustered individuals into smaller groups.

mtDNA analysis

Results of the TGGE (Figure 2) show three different banding patterns. One banding pattern is identical to the banding pattern (labelled as mossambicus pattern) of the reference, which was a pure *0. mossambicus* (R, Lane 1) and also to *0. mossambicus* OM_{26} (Lane 26).

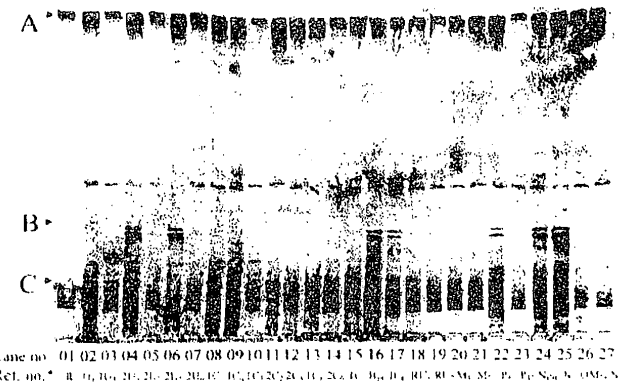

Fig.2. A sectionof a silver stained polyacrylamide TGGE gel showing the different haplotypes of mtDNA of tilapia specimens investigated.
*** R Reference *O. Mossambicus* N_{CH} Pure *O. Niloticus*: *O. niloticus* "Chitralda" U, C, B, P, Ru, As in Table 1.**
A - *O. mossambicus* haplotype, B - *O. niloticus* haplotype, C *T. rendalli* haplotype.

The similarity of banding patterns with niloticus "Chitralda" (N_{CH}, Lane 24) and niloticus "Israel" (Ni, Lane 25) concluded niloticus maternal origin of the individuals. Remaining banding pattern (labelled as rendalli pattern) showed different migration from *O. niloticus* and *O. mossambicus* patterns. This pattern was assigned to *T. rendalli*, as those individuals were identified as *T. rendalli* considering their morphometric features.

All the individuals sampled from mossambicus-like and rendalli-like clusters had the same banding pattern characteristic to the species. However, there were three individuals (two from University pond stock - Ru_1 and Ru_2 and one from Negambo lagoon - N_6 - Lanes 18, 19 and 27) showing DNA migration pattern characteristic to *O. mossambicus* while having niloticus morphometric characters.

DISCUSSION

For the morphometric data analysis, 103 individuals from different reservoirs were used and only 34 individuals were used for mtDNA analysis. As shown in Figure 1 two genera *(Tilapia* and *Oreochromis)* were differentiated at the first level (distant coefficient 100%) and the two species of *Oreochromis (0. mossambicus* and *0. niloticus)* were separated at the second level, which further divided into smaller clusters.

Figure 1 reveals the distribution of tilapia species in different reservoirs/waterbodies. Accordingly, all the individuals except three from Chandrikawewa and most of the individuals from Udawalawa grouped into rendalli like cluster. None of the individuals from the other reservoirs and waterbodies were compatible with the rendalli-like cluster. Three individuals from the laboratory stock and most of the individuals from Badagiriya were grouped into niloticus-like cluster. De Silva (1988) has shown that the fishery of Badagiriya reservoir almost totally depended on *0. mossambicus*. According to our results, *0. niloticus* are more abundant now, indicating a shift in the dominant species from *0. mossambicus* towards *0. niloticus* within a decade. Puttlam and Negambo had mainly mossambicus-like individuals and the three individuals from University canal also belonged to this group.

Although, morphological characters 8-13 and meristic characters 1-7 in the Table 2 contributed towards the dendrogram in Fig. 1, different combinations of the morphometric characters were also tried to identify the most important characters useful in differentiating tilapia species. In this process, meristic characters 2-7 in the Table 2 by themselves also gave a similar clustering pattern as Dendrogram in Figure 2 with same individuals in each major cluster.

This indicates that morphological characters 8-13 in Table 2 and the first meristic character have lesser value in differentiating species. Furthermore, morphological features alone are not sufficient to cluster the individuals into respective species. Nevertheless, hierarchical clustering of the individuals using the morphometric characters could not separate the hybrids identified by mtDNA analysis.

The morphological clustering of all individuals of *T. Rendalli, O. mossambicus* and most of the individuals of *O. niloticus* were compatible with their maternal origin. However, one individual from Negambo lagoon (N_6) and two individuals from the University pond (Ru_1 and Ru_2) were exceptional in that they showed mossambicus maternal origin, despite their niloticus morphology. De Silva and Ranasinghe (1989) have indicated the gene introgression in this stock and the present results clearly show the hybridisation had taken place in their ancestry. These results revealed that certain

individuals with morphology of a particular *Oreochromis* species could have the mtDNA from the other species. This demonstrated that *Oreochromis* spp. in reservoirs of Sri Lanka can have a maternal origin which is different from the species defined by their morphology, which indicates hybridisation between two species. On the other hand, *T. rendalli* present in sampled reservoirs have not been hybridised with a related species during their ancestry. Although, this mtDNA technique is not a direct method for identification of hybrids, it has appreciably helped to achieve the aim of identification of maternal origin of tilapia species at the level of DNA. In a recent investigation, microsatellites have shown a promising genetic marker for identification of hybrids (De Silva *et al.* 1999). Present results showed that at least some hybrids cannot be recognised using the frequently used morphometric characters. Future work should concentrate on the development of a morphological index to identify hybrids by combining molecular and morphometric data. Additional morphometric data and their derivatives may be required for this purpose. This index will be used in the identification of hybrids easily in the field, which will enable to evaluate the effect of hybridisation on the reservoir fishery of Sri Lanka.

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