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mtDNA Diversity among Remnant Populations of Two *Epiplatys* Species in Kainji Lake Basin Inferred from D-loop and 16SrRNA Gene Sequences

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Abstract: In this study, partial sequences of D-loop and 16S rRNA mitochondrial genes were analyzed to genetically characterise 2 *Epiplatys* species discovered in riparian streams in Kainji Lake Nigeria. Sequence analyses of both genes show Jebba population to be more distantly separated from the Monai and Shagwa populations of *Epiplatys bifasciatus*. Based on 16S rRNA gene sequences, Kimura 2-parameter distance between *E. biifasciatus* and *E. spilargyrieus* the ranged from 0.0921-0.0946. Kimura 2 parameter also showed that *Epiplatys roloffi* (U73241) was genetically closer to *E. spilargyrieus* (K2P = 0.074) than to *E. bifasciatus*. The Neighbour-Joining tree showed that the *Epiplatys bifasciatus* from each stream clustered together except for those of Monai and shagwa, which formed a single clade.

Key words: *Epiplatys bifasciatus*, *Epiplatys spilargyrieus*, 16S rRNA gene, genetic variation, Lake Kainji

INTRODUCTION

Before the impoundment of the River Niger at Kainji, Nigeria, 3 species of the African killifish were recorded in the area, which included *Epiplatys spilargyreius*, *Epiplatys bifasciatus* and *Poropanchax normani* (Daget, 1962; Bank *et al*., 1965). These species soon disappeared due to the disappearance of associating grassy habitats at the margin of new lacustrine environment. Recently, remnant populations of the two species, *E. bifasciatus* and *E. spilargyrieus* localized in Monai Stream (9°53 45'N and longitude 04°33 14'E); a grassy stream about 1.9 km in length, which flows into the lower Western portion of the lake were rediscovered. Interestingly, the two species are absent in similar streams in this study of the lake and farther North, only *E. bifasciastus* was found localized in two riparian streams, Shagwa and Auna (Fig. 1). Alterations to the physical habitat with the possibilities of reduced dispersal and limits to the realization of biological cycle could threaten animal populations through a possible erosion of their genetic variability by bottlenecks, genetic drift or inbreeding leading to extinction (Gaines *et al*., 1997).

The Lake Kainji under the management of the Nigeria Institute of Freshwater Fisheries Research had concentrated efforts on the studies of biology and spatial

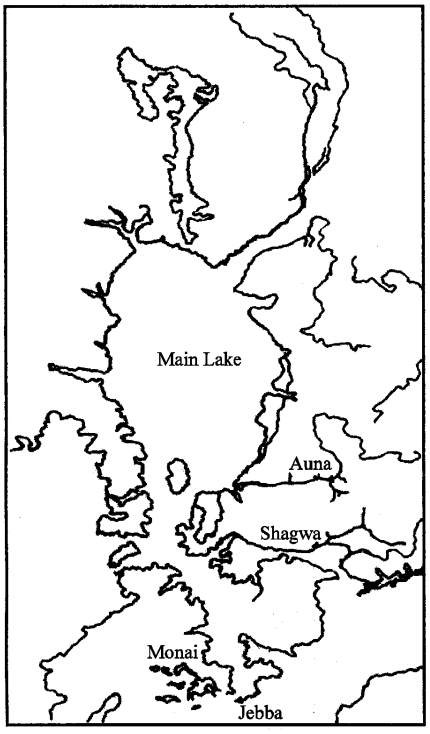

Fig. 1: Map of study area sampling sites (make obvious markes for sample collection sites)

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distribution of species of commercial interest to fishermen. However, attempts are being made toward documenting genetic diversities. Analysis of genetic diversity of any fishery resource provides information for management, identification of the resource and size estimation. Information on genetic diversity of non-commercial fish of Lake Kainji is scarce.

In this study, we used mitochondrial 16S rRNA gene and control region sequences to examine genetic variations in *E. spilargyreius* and *E. bifasciatus* and among patchy populations of *E. bifasciatus* rediscovered around Kainji Lake in Nigeria.

The aim of this study was to provide baseline genetic information on remnant population of *E. spilargyreius* and *E. bifasciatus* of Lake Kainji.

MATERIALS AND METHODS

Genetic analysis was conducted on *E. spilargyreius* and *E. bifasciatus* obtained from Auna, Monai, Shagwa and Jebba (Fig. 1 and Table 1). *E. spilargyreius* was found only in Monai stream. Fish tissues were preserved in 95% ethanol and the standard phenol-chloroform-isoamyl alcohol procedure was used to extract genomic DNA after digestion with proteinase K at 50°C. Fragments of 16S rRNA gene were amplified on a 2004 Eppendorf MasterCycler gradient (Applied Biosystems, USA) under the following conditions: 94°C, 3 min; then 40 cycles of 94°C, 45 sec; 50°C, 45 sec and 72°C, 45 sec followed by 72°C, 10 min. The primers used were L2510 (5'-CGC CTG TTT AAC AAA AAC AT-3') and H3059 (5'-CCG GTC TGA ACT CAG ATC ATGT-3') (Inoue *et al*., 2001). Primers used for the amplification of the D-loop region were ACCCCTAGCTCCCAAAGCTA (forward) and CCTGAAGTAGGAACCAGATG (reverse) (Agnèse *et al*., 2006) at 35 cycles beginning with 3 min at 93°C for initial denaturation followed by cycles of 30 sec at 93°C, 30 sec at 52°C for annealing and 30 sec at 72° for extension, with a final 5 min extension step at 72°C.

Amplified DNA was resolved on 1.5% agarose gel, recovered using Gel Extraction Minikit (Watson's Biotechnologies Inc, Shanghai) and sequenced on an ABI prism 377 DNA sequencer (Applied Biosystems) from both sides using the ABI Prism BigDye Terminator cycle Sequencing Kit (Applied Biosystems). Sequences were edited using Seqman software and aligned with CLUSTAL X (Thompson *et al*., 1997). Complete alignment was manually inspected for misalignment and consequently modified.

Sequence comparisons and identification of haplotypes were performed using MEGA version 3.1 (Kumar *et al*., 2004). The values of sequence distance, number of polymorphic Sites (S), haplotype diversity (hd) and nucleotide diversity (π) were carried out by DnaSP version DNASP 4.0 (Rozas *et al*., 2003). The phylogenetic tree was constructed with Neighbour-Joining method and tree reliability was tested using 500 bootstrap replicates. A hierarchical AMOVA was performed on *E. bifasciatus* D-loop haplotypes using Arlequin 3.1 to partition variance components in order to evaluate patterns of spatial genetic structure. AMOVA was conducted using two groups, Jebba and Auna specimens as a group and Monai and Shagwa specimens as the second.

RESULTS

The size of amplified sequence of mitochondrial 16S rRNA gene fragment of two species of *Epiplatys* was 494 base pairs in length. The sequences were aligned with GenBank sequences for *E. boulengeri* (U73239), *E. chaperi* (U73240) and *E. roloffi* (U73241) (Murphy and Collier, 1997). The nucleotide contents for the two species were 26.6% (T), 21.6% (C), 32.2% (A), 19.6% (G) for *E. bifasciatus* and 27.3% (T), 20.5% (C), 32.9% (A), 19.4% (G) for *E. spilargyreius*.

A total of 45 sites (9.11%) of the gene fragment were variable between the two species. About 54% of these substitutions were transitions. When the GenBank sequences were included, 107 positions were variable, of which, 68 were phylogenetically informative. Three haplotypes were recorded in 13 *E. bifasciatus* individuals from the 4 sampling localities (h±SE = 0.705±0.064) and 1 haplotype in 4 individuals of *E. spilargyreius*. One haplotype was common to Monai and

Table 1: Genetic diversity in *E. bifasciatus* and *E. spilarygreius* based on Control Region (CR) and 16SrRNA gene sequences

	16SrRNA					CR				
Species/population	N	S	h	hd	π	N	Ss	h	hd	π
E. bifasciatus										
Monai	3	0	1	0	0	5	2	3	0.700	0.00193
Shagwai	2	0	1	0	0	4	7	3	0.833	0.00964
Auna	5	0	1	0	0	3	8	3	1.000	0.01285
Jebba	3	0	1	0	0	6	2	2	0.333	0.00160
Total	13	0	3	0	0	18	19	9	0.863	0.01068
E. spilarygreius	4	0	1	0	0	6	35	3	0.600	0.02974

N: Sample size; S: Number of polymorphic sites, h: Number of haplotype, hd: haplotype, π: Nucleotide diversity

[	1111111	2222222222	2222222223	3333333344	44444]
[	1162448999	0011112222	2233445580	0224688900	34458]
[	8967280368	5813592367	8908022833	9591967423	03580]
ES2	TTTCGATTAC	ATACTAATGA	TCTACGAAGA	ATTATAGTCG	TCCAT
ES9	.....	.....	.....	.....	.....
ES10	.....	.....	.....	.....	.....
ES12	.....	.....	.....	.....	.....
M10	ACCATTCCCT	CGTAATCACT	ATCTTAGG.C	GCCGAGAAAA	CTTGC
M11	ACCATTCCCT	CGTAATCACT	ATCTTAGG.C	GCCGAGAAAA	CTTGC
M12	ACCATTCCCT	CGTAATCACT	ATCTTAGG.C	GCCGAGAAAA	CTTGC
S11	ACCATTCCCT	CGTAATCACT	ATCTTAGG.C	GCCGAGAAAA	CTTGC
S13	ACCATTCCCT	CGTAATCACT	ATCTTAGG.C	GCCGAGAAAA	CTTGC
A13	ACCATT.CCT	CGTAATCACT	ATCTTAGG.C	GCCGAGAAAA	CTTGC
A12	ACCATT.CCT	CGTAATCACT	ATCTTAGG.C	GCCGAGAAAA	CTTGC
A9	ACCATT.CCT	CGTAATCACT	ATCTTAGG.C	GCCGAGAAAA	CTTGC
A7	ACCATT.CCT	CGTAATCACT	ATCTTAGG.C	GCCGAGAAAA	CTTGC
A1	ACCATT.CCT	CGTAATCACT	ATCTTAGG.C	GCCGAGAAAA	CTTGC
J3	ACCATT.CCT	CGTAATCACT	ATCTTAGGCC	GCCGAGAAAA	CTTGC
J13	ACCATT.CCT	CGTAATCACT	ATCTTAGGCC	GCCGAGAAAA	CTTGC
J14	ACCATT.CCT	CGTAATCACT	ATCTTAGGCC	GCCGAGAAAA	CTTGC

Fig. 2: 16S rRNA gene Nucleotide variations in sequences of *E. bifaciastus* and *E. spilargyreius*

Shagwa specimens of *E. bifasciatus* (Fig. 2). The genetic distance (Kimura 2-parameter) between *Epiplatys bifasciatus* and *E. spilargyreius* ranged between 0.0921 and 0.0946. The average nucleotide difference between the two species was 43.62, while the average number of nucleotide substitution per site between them was 0.0903. Nucleotide difference within *E. bifasciatus* was between 0 and 2 resulting in low nucleotide diversity (Table 1).

Sequencing a segment of the D-loop produced 415-416 nucleotide base pairs in *E. bifasciatus* and 399-400 bp in *E. spilarygereius*. Multiple alignment resulted in a consensus length of 420 base pairs. Only 26 indels were observed which were associated with extra bases in the *Epiplatys bifasciatus* sequences. The nucleotide composition for the two species were 31% (T), 15.9% (C), 37.1% (A), 16% (G) for *E. bifasciatus* and 30% (T), 19.6% (C), 37.4% (A), 13% (G) for *E. spilargyreius*.

A total of 199 nucleotide polymorphisms were detected between the species defining a total of 12 different haplotypes, leading to a high estimate of diversity (h±SE = 0.902±0.038). Within *E. bifasciatus*, 9 haplotypes defined by 17 nucleotide polymorphisms were detected (h±SE = 0.863±0.061), one haplotype was shared between Monai and Shagwa and another between Auna and Jebba (Fig. 3). Within population haplotype diversity

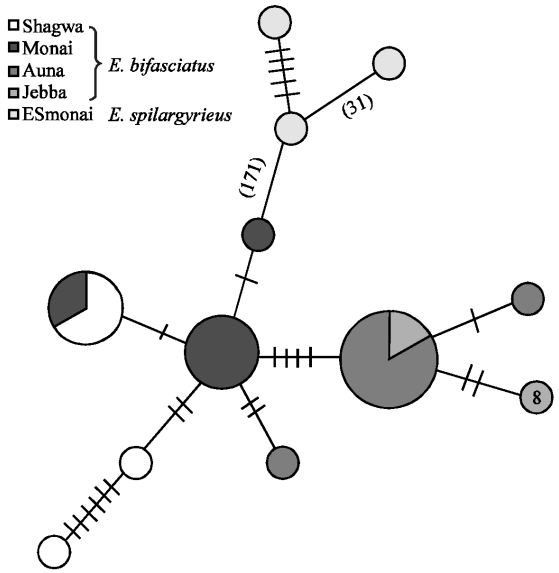

Fig. 3: Minimum spanning haplotype netwotk

was high to low (Table 1). The Jebba population had the lowest estimate of diversity, with 2 haplotypes from 5 specimens. Three haplotypes characterized the 6 specimens of *E. spilargyreius*. The number of variable sites and other measure of genetic diversity within species are given in Table 1. The genetic distance between *Epiplatys bifasciatus* and *E. spilargyreius* ranged between 0.66 and 0.75 with an average of 0.27. Inferences of the Kimura 2-parameter genetic distances based on nucleotide divergence within *E. bifasciatus*, showed that the nucleotide divergence values varied from 0.00 (distance between identical nucleotide sequences) to 0.03 and 0.00-0.09 among *E. spilargyreius*.

AMOVA results for the D-loop show that genetic variation among *E. bifasciatus* populations accounts for 61.02% of total variation, whereas within population variation was responsible for 32.84%.

The NJ tree inferred from 16S rRNA gene and D-loop showed two clusters of *E. bifasciatus*: Monai and Shagwa grouping together. There was therefore no sequence variation among those individual from Shagwa and Monai.

DISCUSSION

The analysis of mtDNA haplotypes represents an important tool for the characterization of distinct populations, for the evaluation of the genetic variability and for the identification of genetic markers, which could be extremely useful in conservation.

The A + T content observed in the 16S rRNA sequence was similar to those of other fish in this region

of the mtDNA sequence (Guo *et al*., 2007). A high A+T could be imposing a constraint on the 16S rRNA sequence. Furthermore, the D-loop nucleotide compositions indicated that this region within both species was AT-rich and similar observations were found in many other vertebrates (Brown *et al*., 1986; Perdices and Doadrio, 2001; Batista and Alves-Gomes, 2006).

From this study, the 16S rRNA gene showed little variation among *E. bifasciatus* obtained from Auna, Monai, Shagwa and Jebba streams. Each stream was represented by a single haplotype with the exception of Monai and Shagwa, which shared one haplotype. For both the D-loop and 16S rRNA, haplotype genetic distances within the populations falls under species divergence (Billington and Hebert, 1991). However, the control region exhibited high level of haplotype diversity, which is consistent with theoretically high evolution rate in the region, despite the small sample size. High levels of genetic variability in the D-loop nucleotide sequence have been commonly identified in wild populations of other fish species (Iguchi *et al*., 1999; Martins *et al*., 2003). The lower 16S rRNA sequence genetic diversity indices and divergences when compared with D-loop may reflect the slow evolutionary rate and the constraints on 16S rRNA (Xiong and Kocher, 1993; Cheng *et al*., 2008).

The analysis of the NJ tree of the D-loop and 16S rRNA gene sequences of *E. bifascaitus* showed no variation in Monai and Shagwa populations. However, the Jebba population appears genetically distinct from those of other streams, which suggests a probable discrete population.

These supports suggestions that management of riverine populations could be conducted at stream reach scales as small as 2 km (Vokoun and Rabeni, 2005). The variation is attributable to genetic drift, which was expected to have resulted from discrete habitats created by meandering river course. The closure of the Niger in 1968 and severe drought of the 1970s was implicated in the extirpation of some species or severe reduction in the population of others (Adeniji, 1975; Lelek and El-Zarka, 1973). These events restricted these remnant populations to isolated narrow niches.

CONCLUSION

In this study, evaluation of genetic variation was based on few individuals. Although, the markers proved useful for differentiating the two species and revealed significant structuring, there is the need to further assess the diversity using more variable nuclear markers and more specimens.

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