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RESEARCH PAPER

A Pilot Study on The Population Genetic Structure of the Selected Fish Species from Meenachil River, India

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Abstract

Meenachil River is the only river originating from Southern Western Ghats of India having human settlement right from the headwaters to the mouth. The river is subjected to human-induced stressors to varying extents, resulting in a drastic decline in riverine health and fish diversity. The population genetic structure of two species commonly distributed in different geographical zones of Meenachil River, *Dawkinsia filamentosa* and *Pseudetroplus maculatus* were studied using the 16S mitochondrial ribosomal RNA gene sequences. Haplotype clustering and the presence of unique haplotypes were identified by phylogenetic and network analysis. Six haplotypes were identified for *D. filamentosa* and ten for *P. maculatus*. The neutrality estimations of the upstream and midstream populations of the species revealed the presence of an excess of common alleles with neutral mutations indicating a population expansion after bottleneck and stabilizing selection. The downstream population revealed the presence of an excess of rare alleles with non-neutral mutations indicating a positive directional selection and population sub-division. Population expansion demonstrated star-like phylogenies with rare haplotypes separated by mutational steps from the ancestral haplotypes. The private haplotypes indicated the existence of selection pressure in the downstream zone of the river.

Introduction

Fishes can rapidly adapt to newly invaded or captive environments (Makinen *et al.,* 2015; Christie *et al.,* 2016) and highly polluted habitats. But all the fish populations cannot adapt to the alterations in habitats through genetic variations. A small population of fish species may arise due to habitat destructions and fragmentations, invasive species, and over-exploitation of the species, along with drastic climatic shifts (Mhemmed *et al.,* 2008). These can affect the demographic dynamics of fishes and, finally, the genetic diversity, which is crucial for the persistence of a species in the environment (Barasa *et al.*, 2016). In a small population, the loss of genetic diversity can be rapid. The genetic drift can erode genetic variations in a few generations by developing highly differentiated private haplotypes with limited gene flow (Frankham *et al.,* 2002).

Analysis using molecular markers offers the most effective method to quantify the levels of genetic diversity and the impact of human-induced disturbances and biotic interactions on fish populations (Leitwein *et al*., 2019). Fast amplification of specific sequences from the samples was made possible by universal primers with broad phylogenetic utility (Meyer, 2003). It is possible to correctly infer the genetic structure and demographic history of freshwater fishes by using slow evolving 16S ribosomal genes (Tabata *et al*., 2016; Joseph *et al.,*2019).

Data on the genetic population structure of the fish fauna of a river is crucial for optimizing the identification of stock enhancements, breeding programs, and also management for sustainable yields and the preservation of genetic diversity (Dinesh *et al.,* 1993). The knowledge about the population genetic structure of riverine fishes is limited. However, such information is crucial for the delineation of fisheries management units, fishery management interaction, and the conservation of genetic variations on a longer time scale (Allendorf *et al.,* 1987; Avise, 1994).

Meenachil River originating from Southern Western Ghats, India is a degrading riverine system of Kerala. The entire stretch of this short river (78kms) from headwaters to mouth is subjected to human-induced stressors in varying extents, resulting in a drastic decline in riverine health and fish diversity. Despite all the multiple stressors, the river still homes many unique and endemic species of fishes.

One of the fish species is *Dawkinsia filamentosa* belonging to Cyprinidae, the most diverse fish family and the largest vertebrate family in general. *D filamentosa* (Valenciennes, 1844) – the most common Cyprinid of the southernmost state of the Indian peninsula and Sri Lanka form a vital part of the local food and ornamental fishery (Pethiyagoda, 1991; Talwar & Jhingran, 1991). They are distributed from upstream to downstream zone in the Meenachil River and have shown a drastic decline in abundance (Letha & Manu, 2020) during the past few years which require further investigation.

Another fish species is *Pseudetroplus maculatus,* the orange chromid*. P. maculatus* is a euryhaline species of cichlid fish endemic to Southern India and Sri Lanka, inhabiting the fresh waters, brackish waters, lagoons, and estuaries (Jayaram, 1999). It is reported to survive in waters close to 21% salinity after gradual acclimatization (Shilta *et al.,* 2016). They can tolerate wide salinity variations due to structural adaptations in their gills and kidneys. Suresh *et. al.* (2022) esfigished *P. maculatus* as a potential euryhaline fish model to assess the climate change adaptation in fishes.

The fish species still existing in the river ensure the need to implement strict conservation measures for the dying river and its fauna. There is only limited information about the genetic population structure of any of the fishes of the Meenachil River. Such information is critical for the conservation and management of the deteriorating river and its dwindling fish resources. The baseline information on genetic variation and population substructure generated from this study would help to plan effective strategies for the conservation and rehabilitation of declining fish fauna of the Meenachil River.

Material and Methods

Sample Collection and DNA Extraction

Two species commonly distributed in the different geographical zones-the high lands (upstream), midlands (midstream) and the low lands (downstream) of Meenachil River were selected, namely *Dawkinsia filamentosa* and *Pseudetroplus maculatus*. For sample collections, two sites each from the upstream, midstream, and downstream geographical zones of the river (Figure 1) were taken to get a good representation of ecological and habitat variations to find out any genetic variants. The locality, longitude, latitude, and altitude of the collection points were given in Table 1. Cast nets and bag nets were used to collect fish with the help of fishing expertise. The caudal fin clips from species were collected and preserved in 95% ethanol for DNA extraction and analysis.

Total genomic DNA was isolated using the Qiagen D Neasy Blood and Tissue Kit (QIAGEN, INDIA). A partial fragment of 16S rRNA was amplified through polymerase chain reaction using universal primers. The primers used were 16Sar-L F1-5'-CGCCTGTTTATCAAAAACAT-3' and 16Sbr-H R1-5'-CCGGTCTGAACTCAGATCACGT-3' (Palumbi *et al*., 1991). PCR amplification was performed with a thermal cycler (Eppendorf) in a total volume of 10µL containing 2µL of 5X PCR buffer, 0.2µL of dNTP (2Mm), 0.5 µL of each primer (10mM),0.2µL of Phire Taq DNA polymerase (Applied Bio systems, Foster City, CA), 5.5µL of ddH_2O and 1 - 3µL of template DNA (10-20 ng). The cycling conditions as follows 95°Cfor 2 minutes followed by 40 cycles of 95°C for 30s, 55°C for 40s, 72°C for 90s followed by final extension step at 72°C for 5mins. There was negative control for each round of PCR to check for contamination. PCR products were purified using ExoSap IT (USB Corporation, Cleveland, OH) and sequenced in an ABI 3730 capillary sequencer using Big Dye Terminator V.3.1 Cycle sequencing kit (Applied Biosystems). Sequences of haplotypes were submitted to GenBank. (Acc. No MW399093 to MW399107, MW398904, MW399126 to MW399131).

Data Analysis

The 16S rRNA mtDNA sequences of 60 individuals were generated, 30 for each species, *D. filamentosa* and *P. maculatus* from the three different geographical zones (Figure 1) of Meenachil River. Population diversity indices such as numbers of segregating site (S), haplotype number (h), haplotype diversity (Hd), and nucleotide diversity (π) and average number pairwise

nucleotide differences within the population (K) were estimated using DnaSP 5.10. Neighbour-Joining (NJ) analysis was done using haplotypes of *D. filamentosa* and *P. maculatus* with 16S rRNA (501bp) sequences using K2 (Kimura2 parameter) as the best nucleotide substitution model in MEGA X software (Kumar *et al.,* 2018). *D. chelynoides* (JQ435839) and *D. sophore* (HE681796) for *D. filamentosa*; *P. suratensis* (GU566027) and *P. canarensis* (KC835202) were used as out groups. Branch evaluation was performed with 1,000 bootstrap replicates (Felsenstein, 1985). Uncorrected *p*-values for 16S alignments were calculated using MEGA X with a pairwise delete option. Medium joining network (Bandelt *et al*., 1999) was constructed in PopART to visualize haplotype distributions of the two species. The population demographic expansion indices Tajima's D (Tajima, 1989) and Fu's F (Fu, 1997), the neutrality estimators were generated for each population by calculating Wright's F-statistics, and gene flow (Nm) was calculated using Arlequin v 3.5x (Excoffer & Lisher, 2010). Population expansion and bottlenecks were evaluated based on Tajima's *D* test and Fu's F statistics using Arlequin.

Results

Population Structure and Demographic History

Dawkinsia filamentosa

In the study, 501 bp from the 16S rRNA sequences were amplified from the three different geographical populations of *D. filamentosa* from the upstream, midstream, and downstream zones of the Meenachil River. A total of 20 polymorphic sites, and 49 parsimony informative sites were detected with eight transitions, 14 transversions, 20 insertions, and eight deletions. Each nucleotide composition was as follows: C 25.4%, G 22.1%, T 22.8%, A 29.7%. A total of six haplotypes were identified from the 501 sequences, out of which one was widely distributed. Genetic variation was found to be low in all the geographical populations studied. The lowest haplotype diversity (H) 0.29±0.12 and nucleotide diversity (π) 0.001±0.00 (Table 2) were noticed in the midstream population.

The neutrality indices for the upstream and midstream geographical populations showed negative values for Tajima's D and downstream population

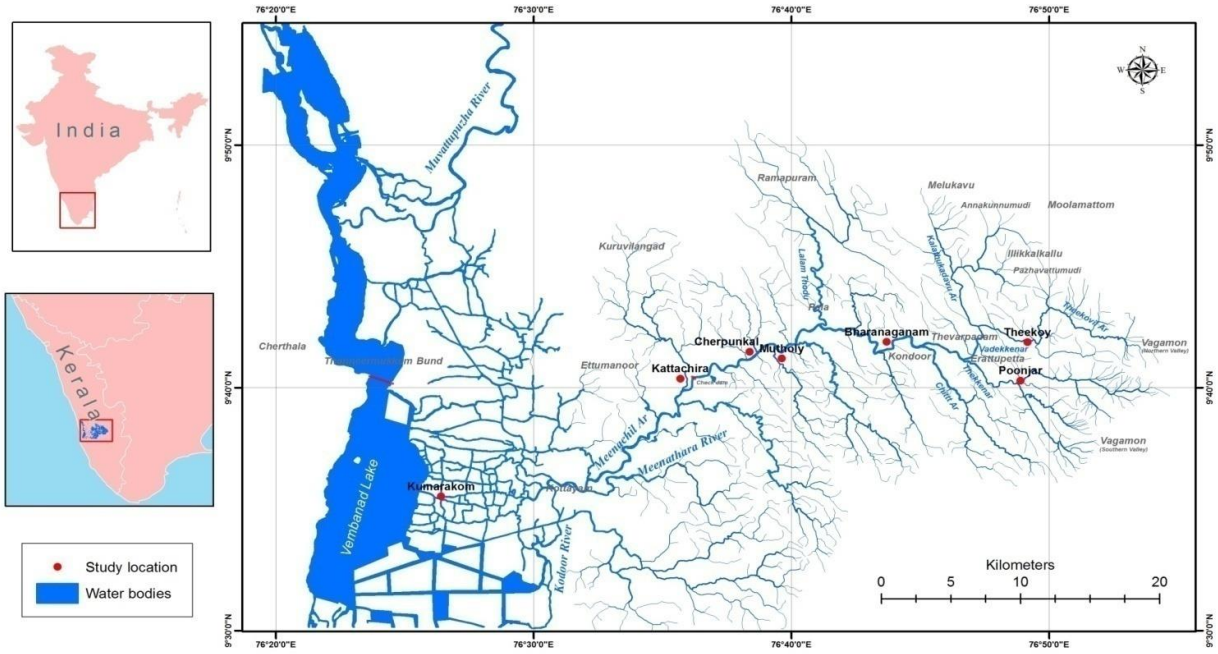

Figure 1. River map of Meenachil River basin showing the sampling sites

Table1. Sampling locations for *D. filamentosa* and *P. maculatus*

Geographical Zone	Locality	Latitude	Longitude	Altitude	No.of individuals (n)
Upstream	THEEKOY	9.7028°N	76.8115°N	274m (898.95ft)	10
	BHARANAGAANAM	9.6994°N	76.7250°E	35m (114.83ft)	10
Midstream	PALA MUTHOLI	9.6923°N	76.6430°E	31m (102.00ft)	10
	CHERPUNKAL	9.6852°N	76.6384°E	22m (72.18ft)	10
Downstream	KATTACHIRA	9.6898°N	76.6363°E	19m (62.33ft)	10
	KUMARAKOM	9.3534°N	76°26′07′′E	9m (30.00ft)	10

showed a significant positive value for Tajima's D. Negative Fu's F values for midstream and downstream evidenced an excess number of alleles indicating a recent population expansion and significant positive values were obtained, for the upstream population (Table 3).

The pairwise comparison for population differentiation index F_{ST} values for the mid and downstream was negative. Weak genetic differentiation with limited gene flow was observed between upstream and midstream, and midstream and downstream populations (Table 3).

Among all three populations midstream, upstream and downstream a weak genetic differentiation was observed with low pairwise F_{ST} values (Table 3). A high gene flow between upstream and downstream and moderate between upstream and midstream and very low between midstream and downstream was detected.

Haplotypes identified from 16S sequences of *D. filamentosa* in the present study, along with reference sequence of the *D. filamentosa* retrieved from the GenBank, was used for phylogenetic analysis. The sampled population's monophyletic nature was inferred from the Neighbour-Joining (NJ) tree obtained in the study (Figure 2). Pairwise uncorrected *p*-distance between haplotypes was found to be 0.2-0.8% (Table 4). A median- joining network was constructed to visualize the relation among haplotypes of *D. filamentosa* (Figure 3). Robust haplotype linkages were seen with all the sampling locations. Unique haplotypes were seen upstream (Hap1) and downstream (Hap5). Haplotype 2 was the founder haplotype with the largest number of individuals distributed in all three geographical populations.

Pseudetroplus maculatus

Thirty sequences of the mitochondrial 16S region were generated from the three geographical populations of *P. maculatus*. The final alignment had 432 bases with seven polymorphic sites and 30 parsimony informative sites. Each nucleotide composition was as follows C 25.4%, G 22.1%, T 22.8%, and A 29.8%. Of the 30 sequences, ten haplotypes were identified (Table 5).

Significant negative Tajima D values for midstream and -1.75 upstream with $p<0.02$ and a significant positive Tajima D value of 1.375 downstream were recorded. Negative Fu's F value -7.07 and -1.80 was noticed in the upstream and downstream populations of *P. maculatus,* respectively. Ten haplotypes were detected among the 30 sequences with a high level of divergence between haplotypes (Figure 4). Each geographical zone of the river has its own specific haplotype. The maximum number of haplotypes was observed in the upstream geographical population with high haplotype diversity and (hd=0.86±0.14; π=0.180±0.10) (Table 5).

Pairwise F_{ST} indicates weak genetic differentiation a high gene flow (Nm) between the upstream and midstream populations. A moderate gene flow (Nm) was observed between the upstream and downstream, when compared to the limited gene flow between the midstream and downstream, (Table 6).

Haplotypes identified from 16S sequences of *P. maculatus*, along with reference sequence of the *P. maculatus* retrieved from the GenBank, was used for phylogenetic analysis. The sampled population's shows monophyletic nature was inferred from the Neighbour-Joining (NJ) tree obtained in the study (Figure 5). Pairwise uncorrected *p*-distance between haplotypes was found to be 0.0-1% (Table 7). A median- joining network was constructed to visualize the relation among haplotypes of *P. maculatus* (Figure 4). Haplotype sharing was seen with all the sampling locations. Unique haplotypes were seen upstream (Hap 1) and downstream (Hap 9). Haplotype 2 was the founder haplotype with the largest number of individuals distributed in all three geographical populations.

Table 2. Diversity and neutrality indices of *D. filamentosa* population calculated from the nucleotide sequence of mitochondrial 16S gene.

Population	Hd±SD	π ± S.D	Tajima' D	Fu's Fs	Fu's Li's D	Fu's Li's F
Upstream	0.47±0.20	0.003±0.00				
Midstream	0.29±0.12	0.001±0.00	-1.01^{ns}	-0.09	-1.05 ^{ns}	-1.10
Down stream	0.38±0.18	0.002±0.00	1.79*	-0.84	-2.08*	-2.26*
Total	0.35±0.11	0.002±0.00	-2.34**	-1.66	-3.56**	3.73**

*P<0.05, **P<0.01, **P<0.02

Table 3. Pairwise genetic distance (F_{ST} in lower diagonal) and gene flow (Nm in upper diagonal between different populations of *D. filamentosa* calculated from nucleotide sequences of mitochondrial 16S gene.

	Upstream	Midstream	Down stream
Upstream		28.50	59.00
Midstream	0.02^{ns}		13.00
Down stream	-0.009^{ns}	-0.04^{ns}	

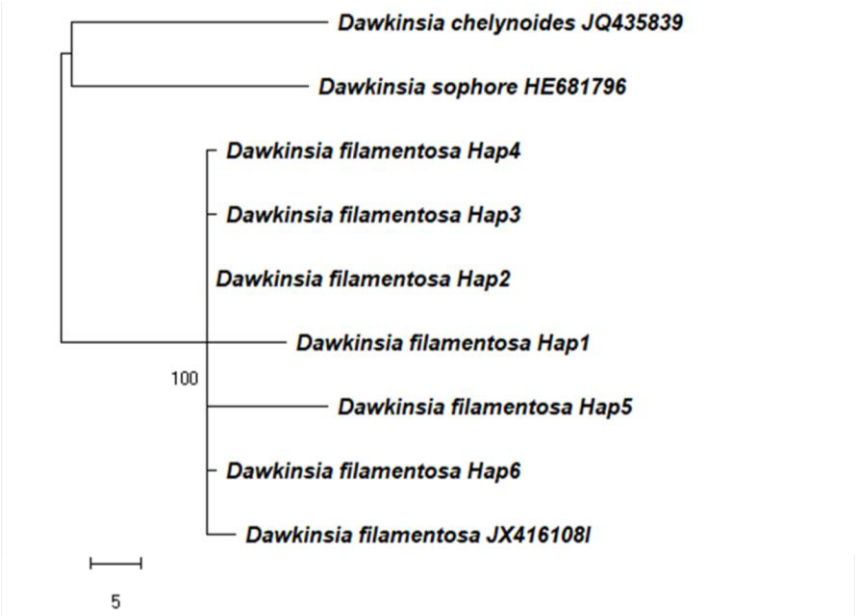

Figure 2. Neighbor-Joining (NJ) phylogenetic tree constructed using 16S showing haplotype diversity of *D. filamentosa.*

Table 4. Genetic distance matrix of all haplotypes of *D. filamentosa* calculated using Kimura 2-parameter

	Species name	1	2	3	4	5	6	7
1	*DawkinsiafilamentosusJX416108*	0.000						
2	*Dawkinsiafilamentosa Hap1*	0.002	0.000					
3	*Dawkinsiafilamentosa Hap2*	0.006	0.016	0.000				
4	*Dawkinsiafilamentosa Hap3*	0.008	0.018	0.002	0.000			
5	*Dawkinsiafilamentosa Hap4*	0.008	0.018	0.002	0.004	0.000		
6	*Dawkinsiafilamentosa Hap5*	0.003	0.041	0.024	0.027	0.027	0.000	
7	*Dawkinsiafilamentosa Hap6*	0.008	0.018	0.002	0.004	0.004	0.027	0.000

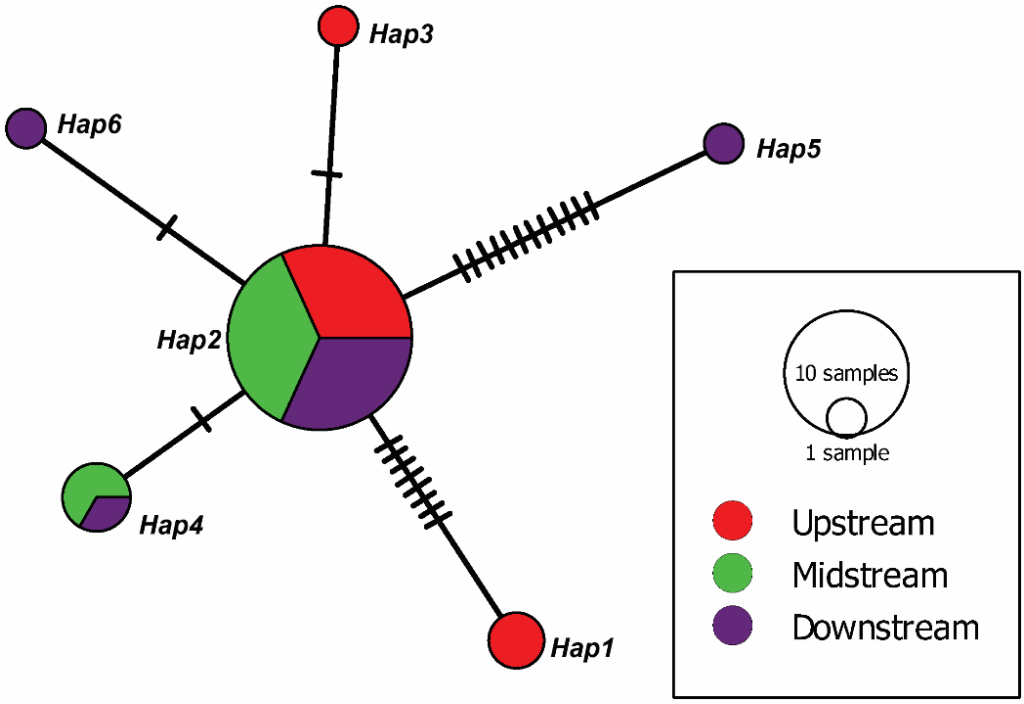

Figure 3. Median joining haplotype network (MJN) constructed in Pop ART depicting relationships among haplotypes represented by sampled populations of *D. filamentosa.*

Discussion

Dawkinsia filamentosa

Genetic variation within the population decreases through genetic drifts or bottlenecks (Chang *et al*., 2012). Significant negative Tajima D values were observed for the upstream and midstream populations of *D. filamentosa* from Meenachil River, indicating an excess of low-frequency polymorphism (Table 2) in the corresponding populations. The population had undergone an expansion after a bottleneck, which reduced the effective population size (Grant & Bowen, 1998; Avise, 2000). The lowest haplotype and nucleotide diversity noticed in the midstream population of *D. filamentosa* indicated a periodic region-wise bottleneck with strong selection or recent population expansion, which could have resulted in a meta population structure (Fauvelot *et al*., 2003). The downstream population showed significant positive Tajima's D (Table 2), which indicates low levels of both low and high-frequency polymorphism showing a balancing selection or population subdivision (Rand *et al.,* 2002). Negative Fu's F values of midstream and downstream evidenced an excess number of alleles indicating a recent population expansion. (Table 3) A significant positive Fu's F value was obtained for the downstream population of *D. filamentosa* indicating deficiency of alleles from a recent bottleneck or an over dominant selection (Fu & Li, 1993).

Table 5. Diversity and neutrality indices of *P. maculatus* population calculated from nucleotide sequence of mitochondrial 16S gene

Population	Hd±SD	π ± S.D	Tajima' D	Fu's Fs	Fu's Li's D	Fu's Li's F
Upstream	0.86±0.14	0.180±0.10	-1.75**	-7.07^{ns}	-1.83**	-2.01**,
Midstream	0.80±0.17	0.013±0.01	-1.17^{ns}	1.66^{ns}	-1.17^{ns}	-1.27^{ns}
Down stream	0.71±0.18	0.002±0.00	1.35^{ns}	-1.80^{ns}	-1.43^{ns}	-1.52^{ns}
Total	0.76±0.10	0.068±0.01		7.81**	-4.11**	4.28**

** P<0.10, ** P<0.02

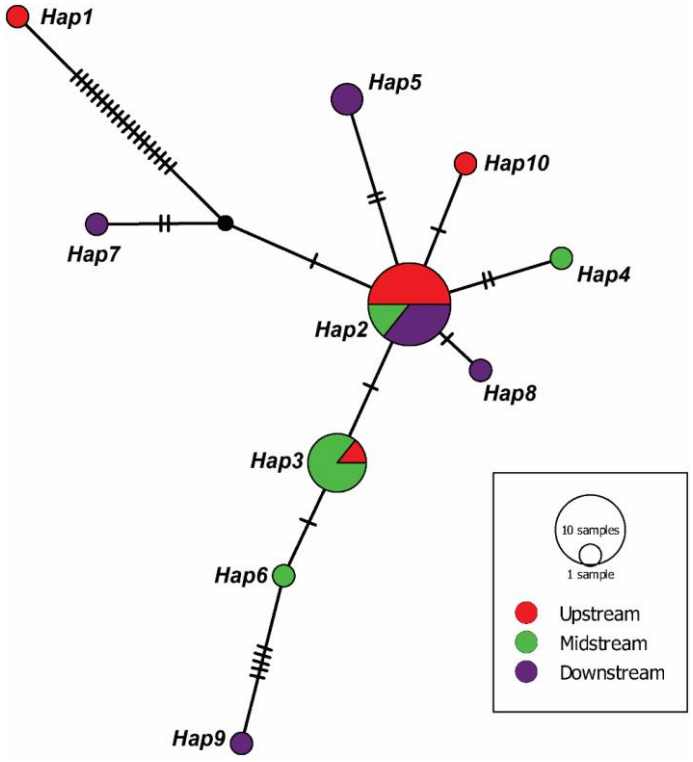

Figure 4. Median joining haplotype network (MJN) constructed in Pop ART depicting relationships among haplotypes represented by sampled populations of *P. maculatus.*

Table 6. Pairwise genetic distance (F_{ST} in lower diagonal) and gene flow (N_m in upper diagonal between different populations of *P. maculatus* calculated from nucleotide sequences of mitochondrial 16S gene.

	Upstream	Midstream	Downstream
Upstream		178.61	73.27
Midstream	0.003^{ns}		21.17
Down stream	0.007^{ns}	0.023^{ns}	

ns=P> 0.10; *P<0.05

Haplotype network analysis of *D. filamentosa* showed that haplotype 2 was the most abundant and all other haplotypes connected to it with mutational steps were private, indicating local adaptations (Sjostrand *et al*., 2014) resulting from selective local pressures. The pairwise comparison for population differentiation index (F_{ST}) values for the mid and downstream was negative (Table 3) which showed that the within-population variation was higher than between populations, which was also reported by Liu et.al. (2019). A weak genetic differentiation with high gene flow was noticed between the upstream and midstream populations. The rapid population expansion of *D. filamentosa* followed by a period of low effective population size accompanied by new mutations was clearly reflected in the present study (Figure 3). The ancestral haplotype may be dispersed over a wide area, but the more recent ones with the mutation were confirmed to specific regions (Bermingham & Avise, 1986).

Pseudetroplus maculatus

Significant negative Tajima D of the upstream population represented low-frequency polymorphism indicating population expansion after a bottleneck (Grant& Bowen, 1998; Avise, 2000). Low differentiation among populations of *P. maculatus* indicated the high genetic connectivity between these populations, which was in line with the population genetic structure analysis done in the Belonid, *Hyporhamphus sajori* by Yu *et. al.* (2016). Negative Fu's F values upstream and downstream respectively showed an excess number of alleles, indicating a recent population expansion. A significant positive Tajima D value of the downstream indicates low levels of high and low-frequency polymorphism, showing a decrease in population size or balancing selection under selective pressures. A significant positive value for Fu's F, in midstream, suggested a deficiency of alleles from a recent bottleneck phenomenon (Joelin *et al*., 2019; Fu & Li, 1993). Ten haplotypes were detected with a high level of divergence between haplotypes and each geographical zone of the river had its own specific haplotype. Both haplotype and nucleotide diversities of the two species studied showed variations.

P. maculatus had high haplotype diversity and lowest nucleotide diversity and *D. filamentosa* had low haplotype diversity. High haplotype and low nucleotide diversity are indicators of rapid population expansion resulting in the accumulation of new haplotypes without large sequence differences (Grant & Bowen 1998; Avise,

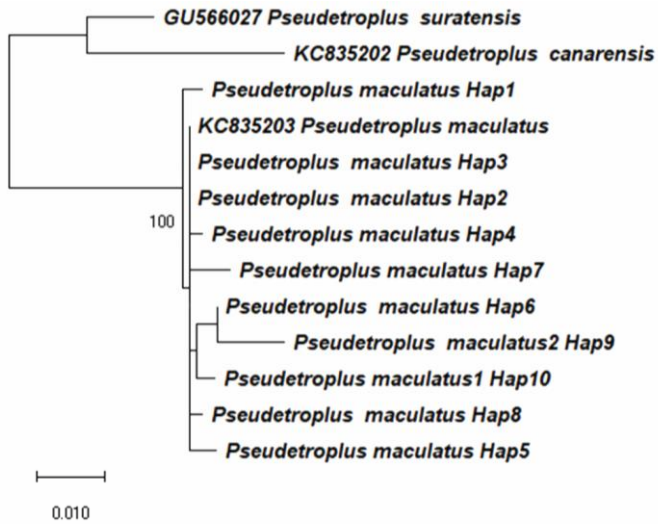

Figure 4. Median joining haplotype network (MJN) constructed in Pop ART depicting relationships among haplotypes represented by sampled populations of *P. maculatus.*

Table 7. Genetic distance matrix of all haplotypes of *P. maculatus* calculated using Kimura 2-parameter

	Species name	1	2	3	4	5	6	7	8	9	10	11
1	*Pseudetroplus maculatus KC835203*	0.000										
2	*Pseudetroplus maculatus Hap1*	0.004	0.000									
3	*Pseudetroplus maculatus Hap2*	0.000	0.004	0.000								
4	*Pseudetroplus maculatus Hap3*	0.000	0.004	0.000	0.000							
5	*Pseudetroplus maculatus Hap4*	0.002	0.006	0.002	0.002	0.000						
6	*Pseudetroplus maculatus Hap5*	0.004	0.008	0.004	0.004	0.006	0.000					
7	*Pseudetroplus maculatus Hap6*	0.004	0.008	0.004	0.004	0.006	0.008	0.000				
8	*Pseudetroplus maculatus Hap7*	0.006	0.010	0.006	0.006	0.008	0.010	0.010	0.000			
9	*Pseudetroplus maculatus Hap8*	0.002	0.006	0.002	0.002	0.004	0.006	0.006	0.008	0.000		
10	*Pseudetroplus maculatus Hap9*	0.014	0.018	0.014	0.014	0.016	0.018	0.010	0.020	0.016	0.000	
11	*Pseudetroplus maculatus Hap10*	0.004	0.008	0.004	0.004	0.006	0.008	0.008	0.010	0.006	0.014	0.000

2000). Negative Tajima's D for the upstream and midstream geographical populations, in general, implies an excess of low-frequency polymorphisms indicating a sudden population expansion. Positive Tajima's D for the downstream population of the two species denoted low levels of both high and low-frequency polymorphism, indicating a directional/non-random balancing selection of population subdivision (Tajima, 1989; Rand *et al*., 2002). Negative values obtained for Fu's analysis for the downstream populations indicate the presence of non-neutral mutations under selection pressure (Fu & Li, 1993).

The star-like haplotype network also supports the recent population expansion hypothesis in accordance with the observation by Yodsiri *et. al.* (2017) in twisted jaw fish populations. Haplotype network analysis indicated recent population expansion with founder haplotypes 2 for *D. filamentosa* and *P. maculatus*, which were commonly distributed in different geographical populations of the two species. All the other haplotypes observed were private (Sjostrand *et al*., 2014) and might have resulted from the local environmental pressures. The mitochondrial genealogy indicated no evidence of divergent lineages for *D. filamentosa* and *P. maculatus* from different geographical zones, which revealed that the genetic differentiation observed between the downstream and upstream populations, was likely to be a recent event. The sharing of mtDNA haplotypes between these fish populations indicated some gene flow has occurred between the populations. Mitochondrial sequences originated from only a single haplotype suggesting that all the populations were founded on individuals from a common ancestor (Ayres *et al*., 2010).

Although populations from downstream possess considerable genetic diversity, the relatively low haplotype diversity in the population of *D. filamentosa* compared to others contributed to the significant pairwise F_{ST} values indicating genetic drift, which has played a role in their genetic differentiation. The negative Nm values represented significant divergence, resulting from the restricted gene flow between the geographical populations related to habitat fragmentation and other anthropogenic stressors. This is in line with the studies conducted by Barasa *et. al.* (2016), on the African catfish, *Clarias gariepinus* populations in the two satellite lakes of Lake Victoria, which was reported with high genetic diversity, restricted gene flow, and genetic drift in the two geographically separated populations.

The neutrality tests suggested that the upstream population has deviated significantly from the neutral, and might have experienced a recent population expansion event, while the expansion of other populations was not detected. The upstream population, whose population dynamics was more stable which was located near the source of the Meenachil River, where habitat protection was far better than the rest of the river. Midstream geographical zone was also comparatively less polluted than the most affected downstream stretch of the river. Low differentiation among the upstream and midstream populations supported the high genetic connectivity between these populations. Lack of obstacles or open habitats in the riverine environment often reduces the genetic heterogeneity among populations making it difficult to differentiate discrete regional populations (Han *et al*., 2008).

Human activities have disrupted the normal development and natural selection of the downstream population of the two species studied from the Meenachil River. Declining fish diversity and abundance in the downstream estuarine zone of the river deviating from the increasing trend along the longitudinal gradient from upstream to the lower stretches was recorded (Letha & Manu, 2020) Genetic variation level determines the risk of extinction in certain species and populations (Saad, 2019).

Community composition studies done on the fish fauna of Meenachil River showed a decline in abundance of *D. filamentosa* towards the lower stretches of the river. This indicates specific physical and environmental stressors acting on the downstream inhabitants of the river. There can be two possible reasons for the genetic differentiation between the geographical populations upstream and lower stretches of the Meenachil River. Limited gene flow among the populations of upstream and midstream/downstream could be attributed to the presence of numerous check dams constructed across the river without any fish passages, which might have probably restricted the movement of fish from one geographical zone to the other. Many previous studies have focused on the relationship between habitat fragmentation and loss of genetic diversity among populations (Mhemmad *et al*., 2008; Zhang *et al*., 2009; Wu *et al*., 2010; Sanchez-Rodriguez & Gebauer, 2012). Another serious issue is the temporary retention of water in the downstream stretch of the Meenachil River due to the closure of Thanneermukkam Bund which was constructed in 1974, in the confluence zone of Meenachil River across Vembanad Lake and the Arabian Sea for preventing saltwater intrusion into the low lying paddy fields associated with the Vembanad wetland ecosystem. Closing of the bund for six months every year for agricultural purposes causes retention of water in the Vembanadu Lake, prevents the easy discharge of waters from the rivers that confluence the lake before emptying to the Arabian Sea. Retention of waters in the interconnected channels of Meenachil River downstream with all the washed-out pollutants leached out fertilizers and pesticides from the plantations and paddy fields, midstream and downstream had seriously affected the water quality and the inhabiting fish fauna downstream. Due to the opening and closure of T. bund at the confluence zone every six months a year, the downstream fish fauna gets regularly exposed to fluctuating environmental attributes, nitrates, nitrites,

phosphates, and sulfates above the permissible levels were reported in the Vembanadu Lake during the months from December to May when the bund is closed. Organic pollution of the lake due to the closure of the bund and the drastic decline of the fish populations in Vembanad backwaters and associated water bodies were documented by Padamakumar *et. al*. (2002).

Even though species adapt to environmental changes, the adaptive potential varies within species and populations according to the generation time, population size, and population structure (Caroll *et al.,* 2014).

Based on the variations in haplotype and nucleotide diversity values, Grant and Bowen (1998) classified fishes into four categories. In accordance with Grant and Bowen's classification, *D. filamentosa* belongs to the first category designed by them with their very low values for haplotype and nucleotide diversities, which indicated a population bottleneck or founder event involving a single or a few mitochondrial linkages might have occurred recently (Chandrasekar *et al*., 2019). Although *D. filamentosa* at present is categorized by IUCN under the "Least Concern" category, the data analysis of the present study indicated that the *D. filamentosa* population of Meenachil River suffers a low genetic diversity with weak genetic differentiation and is critical for implementing immediate conservation measures *P. maculatus* falls into the fourth category (high Hd & π)specifying a secondary contact between previously distributed allopatric lineages for the former and population expansion after a low effective population size.

Conclusion

The preliminary investigation of the genetic population structure of two selected species of fishes *D. filamentosa and P. maculatus* distributed commonly in the three different geographical zones of the Meenachil River were conducted using their mitochondrial 16S rRNA sequences. Neutrality tests, Tajima's D (Tajima, 1989), and Fu's F statistics (Fu, 1997) were carried out to examine the genetic variability among the populations. The neutrality estimations of the upstream and midstream populations of the two species studied revealed the presence of an excess of common alleles with neutral mutations indicating a population expansion after bottleneck and stabilizing selection. The downstream population of the two species revealed the presence of an excess of rare alleles with non-neutral mutations indicating a positive directional selection and population sub-division downstream. Population expansion of the two species studied was demonstrated by star-like phylogenies, where the rare haplotypes were separated by the mutational steps from the ancestral haplotypes. Haplotype network analysis showed different mutational steps with a founder ancestral haplotype commonly distributed in all the geographical populations. The private haplotypes indicated the existence of selection pressure in the downstream zone of the river. The check dam fragmenting the river resulted in the spatial separation of the populations with limited interaction, which was evidenced by the limited gene flow between the upper and lower stretches of the river. Human interventions have disrupted the normal development and natural selection of the fishes of the Meenachil River. The genetic population structure generated by the present study may provide insights to develop species-specific conservation tools for the fish fauna of Meenachil River, which integrate genetic and environmental variables and their interactions. The present study conducted was a pilot study attempted to understand the genetic population structure of the selected fishes of the Meenachil River. The limitation of 16S markers can be overcome with the use of more mitochondrial markers such as, Cytb, 12S, and the nuclear markers such as 18S, 28S and Recombination activating gene 1 (Rag-1) validating the results of the pilot study. A comparative study of the population genetic structure of the commonly distributed fishes of Meenachil River with higher number species from other rivers that confluence to Vembanad Lake using multiple genetic markers, including microsatellites, can better elucidate the population demographics of the riverine fish fauna of Kerala.

Ethical Statement

Ethics not applicable.

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Author Contribution

Letha P Cheriyan-Conceptualization, Investigation, Data Curation, Writing - Original Draft.

Sanil George-Methodology, Review & Editing, Visualization, Formal analysis.

Manu Oommen-Validation, Supervision and project administration.

Anoop Vasudevan Sheeja- Visualization, Formal analysis

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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