MOLECULAR TAXONOMY OF THE FISHES OF MEENACHIL RIVER, KERALA

MINOR RESEARCH PROJECT

(No. of. MRP/12th Plan/ 14 – 15/ KLMG035UGC-SWRO dated 10th December 2014)

Submitted to

University Grants Commission

SOUTH WESTERN REGIONAL OFFICE

Bangalore

PRINCIPAL INVESTIGATOR

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Letha P Cheriyan

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Post Graduate and Research Department of Zoology

MAR THOMA COLLEGE

(Re-accredited with A grade by NACC;affiliated to Mahatma Gandhi University)

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December 2018

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CERTIFICATE

This is to certify that this research project work entitled "Molecular Taxonomy of the Fishes of Meenachil River, Kerala" is an authentic and original report of the research work carried out by me for the UGC Minor Research Project, funded as per order No.F. MRP/12th Plan/ 14 – 15/ KLMG035/ UGC-SWRO dated 10th December 2014 by the Southern Western Regional Office, University Grants Commission, Bangalore

LETHA P CHERIYAN

Principal Investigator

Tiruvalla

30/12/2018

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LETHA P CHERIYAN

CONTENTS

Executive Summary

The Meenachil river, originating from Western Ghats is a degrading riverine ecosystem in Central Kerala of India, may be the only river in Kerala which is characterised by the presence of human settlement right from the source of the river till it confluences at Vembanad lake, India's second largest wetland ecosystem. Even though the rain fed river is only 78kilometers in length, as it originates from the world's biodiversity hotspot, it is a home to a wide variety of fish with many rare and endemic taxa. The drastic deterioration of riverine health due to indiscriminate human activities, resulted in devastating effects on riverine biodiversity as a whole. Accurate fish species identification and the measurement of the fish diversity along with the assessment of biological and physico-chemical parameters offers an obvious and relevant indicator of riverine health .Eight different sampling stations were identified from the upstream, mid-stream and downstream areas of river, and studies were conducted during Pre-Monsoon, Monsoon and Post-Monsoon seasons. Physico-chemical parameters of sampling sites were determined and diversity indices have been calculated. Diversity of fishes have been assessed using 16s mitochondrial sequence as molecular marker. Among the 44 species identified, 16s sequence of 25 fishes have been generated and the phylogenetic tree of the species observed in the study has been constructed. The reference database of 16s sequences of fishes identified from Meenachil is a first attempt to better know the molecular diversity of fresh water fish species of Meenachil River, providing a basis for future studies of this fauna, extending the ability to identify these species from all life stages and even from fragmentary remains for a better understanding of species interactions, diversity and the pattern of distribution which offers the implementation of appropriate conservation and river rehabilitation measures.

INTRODUCTION

Knowledge of species inhabiting natural freshwater ecosystems is a fundamental requirement for the management of any Riverine ecosystem. Many of the Indian rivers experiencing serious gaps in the biodiversity composition due to the lack of robust documentation efforts as reflected in the minor contributions to the international literature on conservation and freshwater biology. Declines in the biodiversity are far greater in fresh water other than the most affected terrestrial ecosystems. (Sala et al., 2000). Over 10,000 fish species live in fresh water (Lundberg et al., 2000); approximately 40% of global fish biodiversity and one quarter of global vertebrate biodiversity. The unreliability of estimates of species richness in individual rivers make it virtually certain that regional national inventories, museum collections and taxonomic knowledge in many parts of the tropics are inadequate to document extinctions and thus widespread undetected extinctions of inconspicuous species have already taken place (Harrison and Stiassny 2000).

Identification and species discovery can be substantially improved and democratized by global application and development of DNA barcoding along with all the traditional morphological ways to recognize a species. DNA barcoding significantly augment morphology- based identification in many sectors and endeavours will emerge more and more as a species discovery tool, and will become routine for the scientific community (e.g. Kankare et al., 2005; Kuhlmann et al., 2007; Ficetola et al., 2008; Jurado-Rivera et al., 2009; King et al., 2008;Valentini et al. 2009; White et al. 2008; Aliabadian et al., 2009; Ferri et al., 2009, Becker et al 2011, Kottelet 2013, Steinke et al 2016, Steinke et al 2017) as well as the lay community.

The Meenachil River originating from Western Ghats is a degrading riverine ecosystem in Central Kerala of India, may be the only river in Kerala which is characterized by the presence of human settlement right from the source of the river till it confluences at Vembanad Lake, India's second largest wetland ecosystem. Even though the rain fed river is only 78kilometers in length, as it originates from the world's biodiversity hotspot, it is a home to a wide variety of fish with many rare and endemic taxa. The drastic deterioration of riverine health is due to rampant sand mining, diversion of water from the catchment area, loss of vegetation from the banks in upper reaches, unscientific construction of check dams, pollution from pesticide, chemical discharge from the rubber plantation and factories, and other solid wastes resulted in devastating effects on the riverine biodiversity as a whole. Measurement of fish diversity offers an obvious and relevant indicator for riverine health. A related point is a need to assess the physicochemical parameters and other biological aspects of the river and its watershed area. It may be noted that only limited studies have been conducted on the physico-chemical and biological aspects of the Meenachil River and its watershed area. But the recent research trends are quite promising.

The importance of the river systems, including the Meenachil River, in protecting the Kuttanad wetland area has been delineated well by Thampatti and Padmakumar (1999). Padmini et al. (2000) studied the water balance of the Meenanthara watershed of Meenachil River basin and computed the water deficit during the non-monsoon periods. The importance of the physical components and their influence on the watershed health of the Vazhikadavu dam has been well narrated by Ajaykumar (2003). Thapanjith et al. (2004) studied the distribution of ichthyofauna of the lower reaches of the Meenachil River

Review of literature

Fish species identification is traditionally based on external morphological features. Yet in some cases morphological features are of limited value for identification and differentiation purposes even with whole specimen because they can show either considerable intraspecific variation or small differences between species. Furthermore; identification of early life stages (eggs and larvae) is even more complicated than adult individuals (Strauss and Bond, 1990). DNA based micro genomic identification methods offer an analytically powerful addition or even an alternative. Among the largest ongoing efforts to catalogue biodiversity are those predicated on DNA bar-coding (Ratnasingham and Hebert, 2007), which relies on sequencing and comparison of a standardized portion of the genome- most often cytochrome c oxidase subunit 1 (COI) region of mtDNA (Hebert et al. 2003a)- for species delineation and identification. Mitochondrial genes such as Cyt b and 16S rRNA are common fish species identification markers that have been widely utilized in sea food control (Quinteiro et al. 1998; Sotelo et al. 2001 Santa Clara, 2006), and species delineation (Kochzius et al., 2003; Lemer et al., 2007).Molecular methods also reveal cryptic species indistinguishable by morphological characters(Semina and Polyakova.2007). However such a molecular database is totally lacking in Indian rivers. The aim of this project is to increase our understanding of the biodiversity and processes generating it on one hand, and to produce information useful for delimiting management and conservation units, including possible identification of yet unknown (i.e. cryptic) species found in Meenachil river, on the other.

.We lack robust data on the population status on the vast majority of rare or economically important fresh water species. For example even landing statistics for wild- caught river fish in Asia are in extremely short supply (FAO,1999). It follows, therefore that we need additional surveys. There are no published works on the fish diversity of many rivers of Kerala. (Bijukumar et al., 2010) Notable studies on the freshwater fish fauna of Kerala was reviewed by Kurup et al., (2004). They confirmed the presence of 175 freshwater fishes from

41 west flowing and 3 east flowing river systems of Kerala (Kurup et al., 2004). These can be grouped under 106 ornamental and 67 food fishes. The biodiversity status of these fishes was assessed according to IUCN criteria. The results showed that populations of the majority of fish species showed drastic reduction over the past five decades. Thirty-three fish species were found to be endemic to the rivers of Kerala. The distributions of the species were found to vary within and between the river systems and some of the species exhibited a high degree of habitat specificity.

The diversity and abundance of the species generally showed an inverse relationship with altitude. The serious threats faced by the freshwater fishes of Kerala are mostly in the form of human interventions and habitat alterations. Conservation plans have to be immediately implemented for the protection and preservation of the unique and rare fish biodiversity of Kerala. However, new species are still being described from this region (Abraham, 2011; Baby et al. 2011; Plamoottil et al., 2013, 2014a, b). Apart from new descriptions, several taxonomic changes have taken place including both synonymization of species, as well as resurrection from synonymy (Dahanukar and Raghavan, 2013).

A data base on fish biodiversity is essential as a decision making tool for conservation and management of fish germplasm, declaration of part of the rivers as aquatic sanctuaries, protection and preservation of endangered species and mitigation of anthropogenic activities so as to fulfil India's obligations under conventions on biological diversity with special reference to Articles 6 and 8 of UNEP (1992). Even though some notable surveys were conducted on the major rivers of Kerala, like Pamba, Manimala and Achancovil (Thomas and George, 2010), only a very few attempts were made to document the fish diversity of dying rivers like Meenachil, in Kerala, which is in need of immediate restoration measures.

GENERAL GEOGRAPHY OF MEENACHIL

Meenachil River, one of the major rivers of Kerala, originates from Araikunnumudi at an elevation of 1097 m above MSL. Its watershed extend from 90 25' to 90 55' N latitudes and 760 20' to 760 55' E longitudes (Figure 1). The river has a length of 78 km. Meenachil River Basin has an area of 1272 km2, falling within 57 panchayats and 3 municipal towns in Kottayam as well as in Vaikom, Kanjirappally, Meenachil and Changanacherry Taluks. The entire river basin falls within the district of Kottayam. The main municipalities such as Kottayam and Pala and Panchayats like Erattupetta, Bharananaganam, Kidangoor, Ayarkunnam, Kumaranallor, Aymanam, Thiruvarppu, Vijayapuram, Ettumannor, Thidanadu, Thalappalam, Meenachil and Mutholy are situated near the bank of the river (Watershed Atlas, 1996). The major tributaries of Meenachil River are Kurusumalai, Thikovil, Chattappuzha, Kadapuzha, Kalathukadavuar, Poonjar, Chittar, Lalamthodu, Pannaganthodu and Meenadomar (Anonymous, 1970). The KadapuzhaAr originates from Annakunnumudi at an elevation of +922 m and Pazhavattikudi at an elevation of +1117 m above MSL. TikovilAr originates from Kurusumala at an elelvation of 1050 m and Marmala at an elevation of 1011 m above MSL. The Poonjarriver originates from Kolahalamedu at an elevation of +1156 m and the Chit Ar originates from Anangampadi at an elevation of +530 m above MSL. The river finally flows to the Vembanadlake. The general elevation ranges from 77 m to 1156 m in the upper region, 8 m to 68 m in the middle region and less than 2 m in the lower region. The main river originates in the north-eastern part of the watershed. The Kadapuzhariver flows in a southerly direction and is joined by the Konipadthodu to form the Kalathudavu Ar. The ThikovilAr also joins it at Cheripad. The southerly direction of flow is continued till Erattupetta. Here it receives the Poonjar river and flows in a westerly direction.

At Erattupetta the main river takes a sharp turn and flows towards West till Kondur. At Kondur it is joined by the Chit Ar. Then the river flows in a westerly direction and reaches Pala. At Pala, another important tributary, the Payapparathodu joins the main river. Then the river traces a south-westerly course till it reaches Kottayam. Then the river splits up into numerous inter-connecting water courses and finally joins the Vembanadlake through a series of criss-crossing channels.

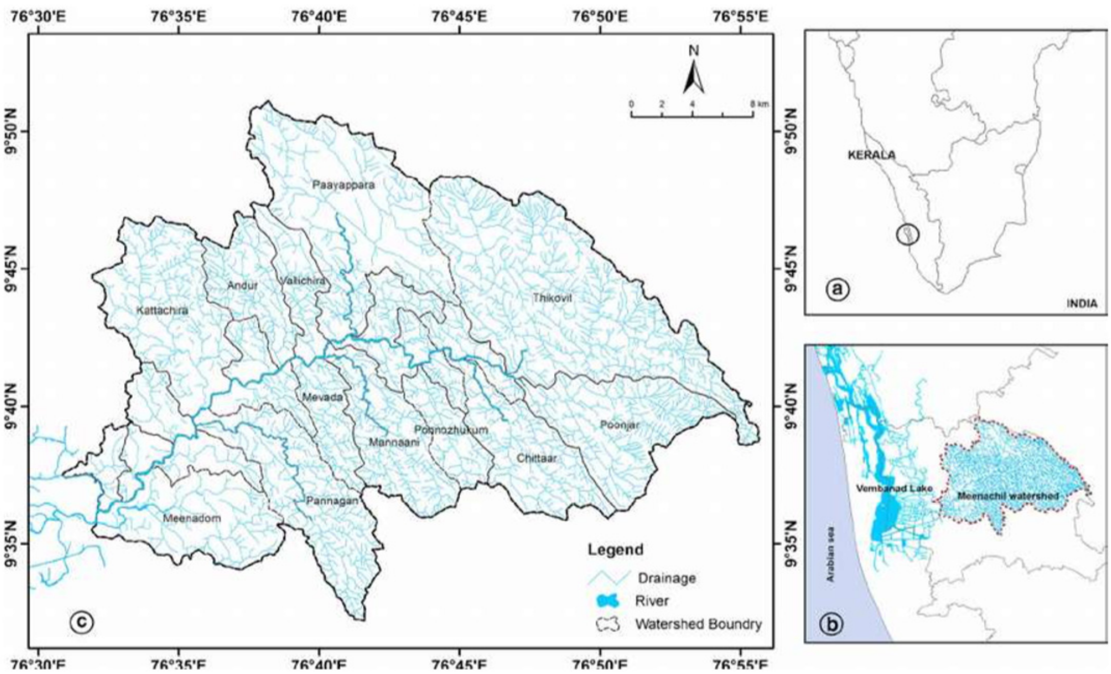


Figure 1.Meenachil river

METHODOLOGY

SAMPLING SITES AND METHODS

Sampling sites were selected from the upstream midstream and downstream areas of the river including the four major tributaries like Teekoy River , Illikkal River, Poonjar and Chittar which falls in the upstream area and other sites from the mid and down streams.

SAMPLING STATIONS

Upstream Midstream Downstream

Station Code		Station Code		Station Code	
Me /1	Teekoy	Me/2	Poonjar	Me/3	Erattupetta
Me/4	Bharananganam	Me/5	Pala Mutholi	Me/6	Cherpunkal
Me/7	Nagampadam	Me/8	Kumarakom		

METHODS

Three seasons were identified in a year: South west monsoon –June to September, North east monsoon or the post monsoon – October to February. The pre –monsoon ranging from March to May. Fish diversity assessment and physicochemical parameters monitoring were conducted during these seasons in the above mentioned stations.

Fishes were collected using a variety of active and passive gears such as scoop nets, drag nets, cast nets, gill nets and specially designed and fabricated net made of mosquito nets. Random surveys were also carried out in the major markets and landing centres along all the major tributaries. Voucher specimens were preserved in 70% ethyl alcohol and tissue samples were preserved in 95% ethanol for DNA analysis.

FISH DIVERSITY ASSESMENT

The fishes were caught by cast nets (8 muzham) and gill nets (2-2.5 cm mesh size). Drag nets, sweeping nets, towing nets and bag nets were also employed for suitable places like stagnant water. The cast nets were operated 10 times, covering a distance of about 200m in the sampling area. The number of fish species collected during each sampling was recorded. Before beginning the cast net operations, the gill net was set across the river. Morphological and morphometric identification of fish species were done according to the fish identification manual by Day (1875 – 78; 1889).

The collected fishes were released into the river after counting and photography. Minimum numbers of specimens were retained for the purpose of identification.

Taxonomy: Initial fish identification is done by the traditional methods of considering morphological and meristic characters. Thirteen morphological attributes were taken into consideration. They were Total Length (TL), Standard Length (SL), Snout Length (SNL), Head Length (HL), Pre Pectoral Length (POL), Pre Dorsal Length (PDL), Pre Ventral Length (PVL), Distance between Rayed dorsal fin and Adipose Dorsal fin (Inter dorsal Di), Length of the adipose Dorsal fin base (ADIPOSe Basel), Length of Caudal Peduncle (LCPD), Length of Ventral Fin (LVF), Depth of Caudal Peduncle (Dcpd) and Depth of Body (DOB).

Fishes were identified by comparing measurements with the voucher specimens, with those of the type/type series and/or as mentioned in the original description.

Fish diversity assessment, density assessment as abundance index was studied as per River Fish Monitoring programme – Manual of Methodology by Bijukumar et al. (2010).

ASSESMENT OF WATER QUALITY PARAMETERS

PHYSICO-CHEMICAL PARAMETERS

Water samples were collected from different sampling stations of Meenachil river as mentioned in the table during the post-monsoon, pre-monsoon , and monsoon seasons Water quality parameters like PH, change in temperature , Total Dissolved Solids (TDS),Electrical Conductivity (EC), salinity ,Dissolved Oxygen content (DO)were periodically analysed from the selected sampling sites using digital water quality analyser .Turbidity and colour were studied .

DNA Barcoding

Tissue samples were preserved in absolute alcohol upon collection of the fishes from the above sampling sites. Genomic DNA was isolated using Qiagen Dneasy kit. The quantity and quality of the extracted DNA were determined through spectrophotometer and electrophoresis. PCR amplification of the 16S with appropriate primers were performed in a in a thermal cycler and sequenced.DNA barcodes generated can be used for the accurate identification of the fish species collected including any cryptic species.

RESULTS & DISCUSSION

Water Quality Analysis

Table 1. The summary of statistics of the Water quality parameters of 2015-16

	pH	ATC	COND	TDS	DO
Min	5.58	25.3	25.9	12.5	2.9
Max	6.93	31.1	815	385.6	6.98
Mean	6.330	27.53	140.48	66.308	5.577
Stand. Dev	0.3820	1.50	245.60	113.135	1.0282

The Table 1. Illustrates the results of the analyses of the five water quality parameters tested during the monsoon to post monsoon period the year 2015-16 from 8 locations. The pH values have not shown any variation between the locations since the standard deviation is very low and the mean value (6.33) is in between minimum (5.58) and maximum (6.93).

The atmospheric temperature (ATC) showed a marked difference between the stations as the std deviation showed a higher value (1.50). The minimum temperature recorded was 25.3 and the maximum was 31.1. Cherpungal (28.1), Nagambadam (30.2) and Kumarakom (30.1) were the location where the maximum temperatures recorded. The mean temperature of the all the eight locations were 27.53^{o}C.

The conductivity showed a marked difference from station to station. The minimum value of the conductivity (25.9) was recorded from Theekoy during the monsoon period of 2015. However, a high value of (815) was recorded from Kumarkom during the premonsoon period of 2015. There was a marked variation between the stations in the conductivity of the water (Std deviation 245.60).

The conductivity and the Total Dissolved Solids (TDS) are positively correlated parameters and the variations followed in the same pattern. The minimum value of 12.5 was recorded from Theekkoy during monsoon and the maximum value was recorded from Kumarakom.

Dissolved Oxygen showed a high variation between locations (standard deviation 5.577). The Dissolved oxygen is very at Kumarakom and had a high value of 6.98 at Bharanaganam.

Table 2. The summary of statistics of the Water quality parameters of 2016-17

	ph	ATC	COND	TDS	DO
Min	5.34	25.2	26.73	13.3	3.8
Max	6.9	29	450.2	225.1	6
Mean	6.18	27.20	101.74	51.125	4.951
Stand. Dev	0.351	1.25	126.02	64.30	0.654

The Table 2. Illustrates the results of the analyses of the five water quality parameters tested during the monsoon to post monsoon period the year 2016-17 from 8 locations. The pH values has not shown any variation between the locations since the standard deviation is very low and the mean value (6.18) is in between minimum (5.34) and maximum (6.18).

The atmospheric temperature (ATC) showed a marked difference between the stations as the std deviation showed a higher value (1.25). The minimum temperature recorded was 25.2

and the maximum was 31.1. Kumarakom (25.20) and Bharanganam (29.00) were location where the minimum and maximum temperatures recorded respectively. The mean temperature of the all the eight locations were 27.20^{o}C.

The conductivity showed a marked difference from station to station. The minimum value of the conductivity (26.73) was recorded from Poonjar during the monsoon period of 2016. However, a high value of (450) was recorded from Kumarkom during the premonsoon period of 2016-17. There was a marked variation between the stations in the conductivity of the water (Std deviation 126.02).

The conductivity and the Total Dissolved Solids (TDS) is positively correlated parameters and the variations followed in the same pattern. The minimum value of 13.3 was recorded from Theekkoy during monsoon and the maximum value was recorded from Kumarakom.

Dissolved Oxygen showed a high variation between locations (standard deviation 5.577). The Dissolved oxygen is very at Nagambadam during post monsoon and had a high value of 6..00 at Poonjar during pre monsoon period.

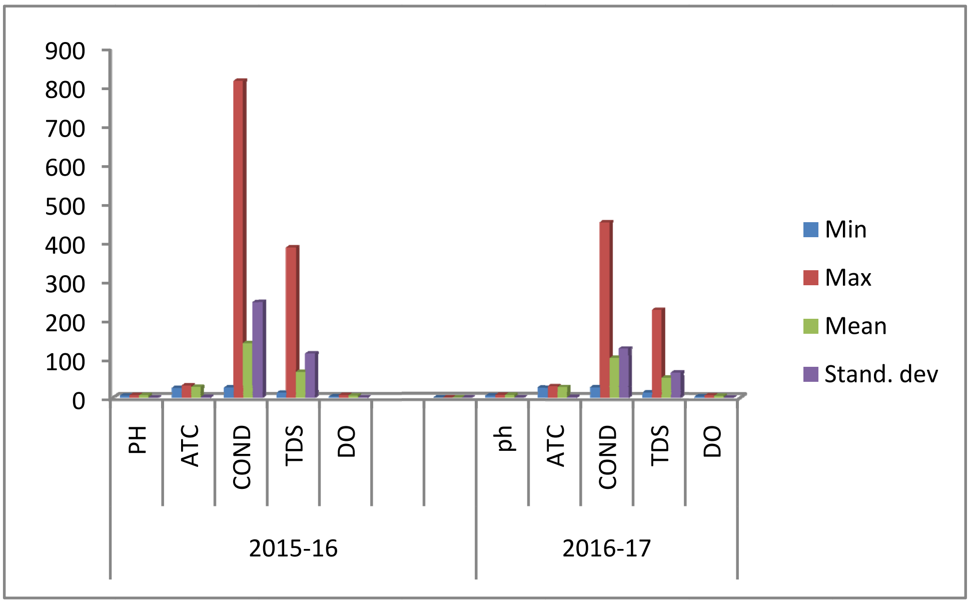


Figure 1. Minimum, Maximum, Mean and std Deviation of the water quality parameters studied in Meenachil river

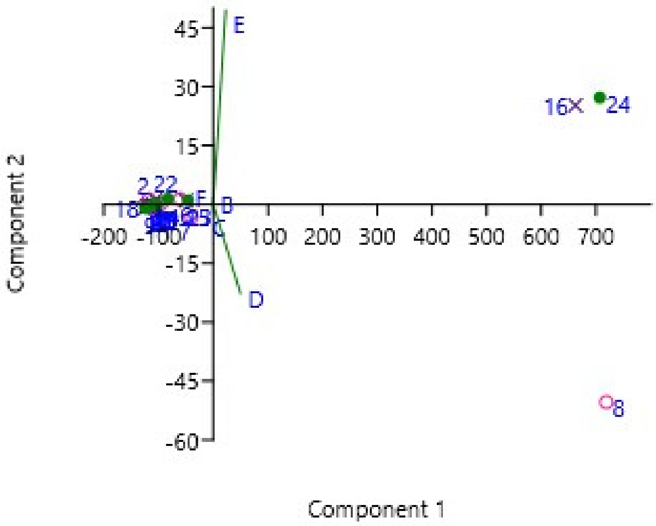


Figure 2. PCA scatter diagram showing the principal factor of five parameters (2015-16)

1-24 8 location in three season (Pre monsoon, monsoon and Post monsoon) locations

Post monsoon; X Monsoon; 0-Premonsoon; B-F-pH; ATC (C); Cond (µsm/cm);TDS(ppm);DO ppm

The principal components of the water quality parameters of 2015-2016 is the total dissolved solids.

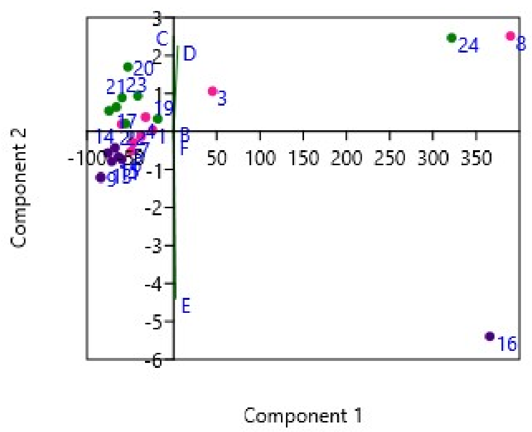


Figure 3. PCA scatter diagram showing the principal factor of five parameters

Post monsoon; X Monsoon; 0-Premonsoon; B-F-pH; ATC (C); Cond (µsm/cm);TDS(ppm);DO ppm

1-24 8 location in three season (Pre monsoon, monsoon and Post monsoon) locations (2016-17).

The Conductivity is found to be the principal factor among the five parameters studied in eight locations during the 2016-17 period. The Conductivity value during the post monsoon period and premonsoon period is found to be the principal $_{\text{factor}}$. The total dissolved solids (TDS) is the other principal factor.

FISH DIVERSITY

The fishes recorded from the Meenachil river during the study period from 2015-2017 is given in the Table 1.

Table 1. Fishes recorded from the Meenachil river

Fish Species	**IUCN status**	**Endemism**
ORDER: ANGUILLIFORMES		
Family: Anguillidae (Freshwater eels)		
1. *Anguilla bengalensis* (Gray, 1831)	NT	
2. *Anguilla bicolor McClelland*, 184I	NT	
ORDER: CYPRINIFORMES		
Family: Cyprinidae (Carplet)		
3.*Amblypharyngodonmelettinus* (Val, 1844)	LC	KL
4. *Devariomalabaricus*(Jerdon, 1849)	LC	WG
5. *Salmophasia acinaces* (Val, 1844)	LC	
6. *Salmophasia boopis* (Day, 1874)	LC	
7. *Barilius bakeri* (Day, 1865)	LC	
8. *Barilius gatensis* (Val, 1844)	LC	

9. *Hyposelobarbus curmuca* (Hamilton, 1807)	EN	
10. *Labeo dussumieri* (Val, 1842)	LC	
11. *Dawkinsia filamentosa* (Val, 1844)	VU	WG
12. *Puntius mahecola* (Val, 1844)	DD	KL
13. *Pethia punctata* (Day, 1865)	LC	WG
14. *Pethia vittatus* (Day, 1865)	LC	
15. *Systomus subnasutus* (Val, 1842)	NE	WG
16. *Rasbora dandia* (Val, 1844)	NE	
17. *Horadandia brittani* (Rem &Menon, 1992)	LC	
18. *Garra mullya* (Sykes, 1839)	LC	

ORDER SILURIFORMES

Family: Bagridae (river catfshes)

19. *Horabagru sbrachysoma* (Günther, 1864)	VU	WG
20. *Mystus oculatus* (Val, 1840)	LC	WG
21. *Mystus montanus* (Jerdon,1849)	LC	

Family: Siluridae (buttrcatfshes)

22. *Ompok bimaculatus* (Bloch, 1794)	NT	
23.*Wallago attu* (Bloch & Schneider, 1801)	NT	

Family: Heteropneustiae (stigingcatfshes)

24. *Heteropneustes fossilis* (Bloch, 1794) LC

ORDER: CYPRINODONTIFORMES

Family: Aplocheilidae (panchax)

25. *Aplocheilus lineatus* (Val, 1846) LC

ORDER: BELONIFORMES

Family: Belonidae (Needlefihes)

26. *Xenentodon cancila* (Hamilton, 1822)

Family: Hemiramphidae (Halfbeaks)

27. *Hyporhamphu slimbatus* (Val, 1847) LC

ORDER: SYNBRANCHIFORMES

Family: Mastacembelidae (spiny eels)

28. *Mastacembelus armatus* (Lacepède, 1800) LC

29. *Macrognathus guentheri* (Day, 1865) LC

ORDER PERCIFORMES

Family: Ambassidae (Asiatic glassfihes/perchlets)

30. *Parambassis ranga* (Hamilton, 1822) LC

31. *Parambassis dayi* (Bleeker, 1874) LC

32. *Parambassis thomassi*(Day) LC

Family: Cichlidae (pearlspot)

33. *Etroplus suratensis* (Bloch, 1790) LC

34. *Pseudetroplus maculatus* (Bloch, 1795) LC

Family: Gobiidae (gobies)

35. *Glossogobius giuris* (Hamilton, 1822) LC

Family: Nandidae (Leaf fishes)

36 .*Nandus nandus*(Hamilton, 1822) DD

37. *Pristolepis malabaricus* (Jerdon, 1849) DD

Family: Anabantiae (climbing perch)

38. *Anabas testudineus* (Bloch, 1792) DD

Family: Osphronemidae (paradise fish)

39. *Pseudosphromenus cupanus* (Cuvier, 1831) LC

Family: Channidae (snakeheads)

40. *Channa gachua* (Hamilton, 1822) LC

41. *Channa marulius* (Hamilton, 1822) LC

42. *Channa striata* (Bloch, 1793) LC

43. *Channa diplogramma* (Day, 1865) VU

ORDER: TETRAODONTIFORMES

Family: Tetraodontiae (puffr fish)

44. *Carinotetraodon travancoricus*(Hora and Nair)   VU   WG

A total of 44 species belonging to 6 orders were represented in the sampling of belonging to 6 orders were represented in the sampling of Meenachil river till January 2017.

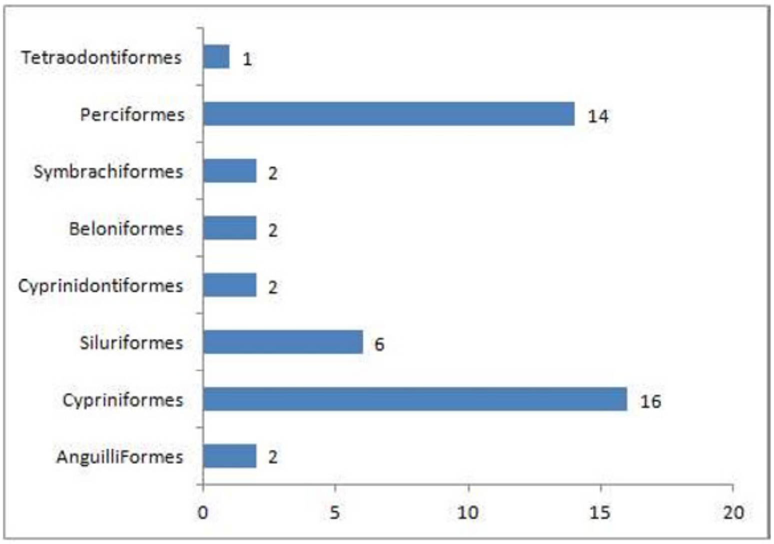


Figure 2. Fishes belonging to different orders

Table 3. Fishes belonging to different IUCN category

Category	No. species
DD	4
EN	1
NE	2
LC	28
NT	4
VU	4

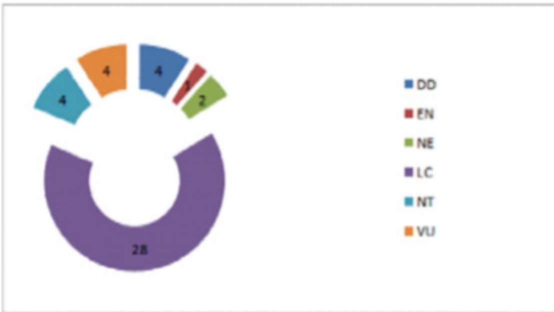


Figure 3. Fishes belonging to different IUCN categories

The order Cypriniformes outnumbered in the group with 16 species which was followed by Perciformes (14) species. The Tetraodontiformes accommodated only one species regarding the endemism, two species are endemic to Kerala, viz, Amblypharyngodon melettinus (Val, 1844) and Puntiusmahecola (Valenciennes, 1844). Seven species are endemic to the Western Ghats. Devario malabaricus (Jerdon, 1849), Dawkinsia filamentosa (Valenciennes, 1844), Pethiapunctata (Day, 1865),

Systomussubn asutus (Valenciennes,1842), Horabagrus brachysoma (Günther, 1864), Mystusoculatus (Valenciennes, 1840) and Carinotetraodon travancoricus (Hora& Nair, 1941) are known only from the Western Ghats (Figure 2).

Regarding the IUCN status of the fish species from Meenachil river. One endangered species (Hypselobarbuscurmuca), four nearly threatened species and 28 species are Least Concern species (Table 3 and Figure 3).

DIVERSITY, DOMINANCE, EVENNESS CHANGES

The study sites are given in the Table 2

Table 2. The study sites

Sl. No	Location	Land use pattern
1	Pala Mutholi	Agricultural land
2	Pala Cherpumkal	Agricultural land
3	Bharnaganam	Agricultural land
4	Thekkoy	Agricultural land
5	Poonjar	Agricultural land
6	Kumarokam	Agricultural land

Table 3. The diversity indices, dominances indices and evenness indices of Pala Mutholi during 2015-16.

Indices	**PMon**	**Mon**	**PT Mon**
Dominance_D	0.1357	0.1223	0.2971
Simpson_1-D	0.9058	0.9175	0.8087
Shannon_H	2.474	2.637	1.992
Evenness_e^H/S	0.8482	0.8259	0.5858
Brillouin	2.203	2.375	1.816
Menhinick	1.606	1.709	1.269
Margalef	3.002	3.482	2.578
Equitability_J	0.9376	0.932	0.7859
Fisher_alpha	5.041	5.91	3.906
Berger-Parker	0.25	0.2424	0.4667

Table 4. The diversity indices, dominances indices and evenness indices of Pala Mutholi during 2016-17.

Indices	PMon	Mon	PTMon
Dominance_D	0.1617	0.1323	0.3331
Simpson_1-D	0.8979	0.9179	0.7864
Shannon_H	2.37	2.643	1.804
Evenness_e^H/S	0.8918	0.8355	0.6745
Brillouin	2.066	2.328	1.645
Menhinick	1.618	1.95	0.9762
Margalef	2.745	3.695	1.801
Equitability_J	0.9539	0.9358	0.8208
Fisher_alpha	4.733	6.802	2.543
Berger-Parker	0.3091	0.2763	0.5059

Table 5.The diversity indices, dominances indices and evenness indices of Pala Cherpumkal during 2015-16.

Indices	**Pmon**	**Mon**	**PT Mon**
Dominance_D	0.1474	0.1291	0.1651
Simpson_1-D	0.9075	0.8709	0.9001
Shannon_H	2.572	2.465	2.621
Evenness_e^H/S	0.7779	0.5603	0.6604
Brillouin	2.315	2.214	2.36
Menhinick	1.717	1.958	1.958
Margalef	3.49	4.215	4.215
Equitability_J	0.9107	0.8097	0.8631
Fisher_alpha	5.94	7.527	7.527
Berger-Parker	0.3061	0.2609	0.3391
Chao-1	23.5	21.75	34

Table 6.The diversity indices, dominances indices and evenness indices of Pala Cherpumkal during 2016-17.

Indices	PMon	Mon	PT Mon
Dominance_D	0.1335	0.1375	0.1651
Simpson_1-D	0.907	0.9079	0.9005
Shannon_H	2.48	2.519	2.531
Evenness_e^H/S	0.8542	0.8279	0.7422
Brillouin	2.196	2.202	2.215
Menhinick	1.661	1.861	2.003
Margalef	3.05	3.354	3.741
Equitability_J	0.9403	0.9303	0.8946
Fisher_alpha	5.223	6.113	7.023
Berger-Parker	0.2535	0.2615	0.3056
Chao-1	20	25.5	31

Table 7. The diversity, dominances and evenness indices at Bharanaganam 2015-16

Indices	PMon	Mon	PT Mon
Individuals	446	368	0.125
Dominance_D	0.1597	0.1136	0.895
Simpson_1-D	0.878	0.9092	2.471

Shannon_H	2.415	2.591	0.6397
Evenness_e^H/S	0.6308	0.7154	2.403
Brillouin	2.334	2.489	0.7829
Menhinick	0.8523	0.9904	2.822
Margalef	2.787	3.047	0.8455
Equitability_J	0.8391	0.8847	3.753
Fisher_alpha	3.763	4.247	0.2445
Berger-Parker	0.3296	0.2255	22

Table 8. The diversity, dominances and evenness indices at Bharanaganam 2016-17

Indices	PMon	Mon	PT Mon
Dominance_D	0.2322	0.7891	0.7889
Simpson_1-D	0.8402	1.666	1.666
Shannon_H	1.884	0.8822	0.8817
Evenness_e^H/S	0.9399	1.487	1.437
Brillouin	1.669	0.866	1.029
Menhinick	1.021	1.292	1.418
Margalef	1.558	0.93	0.9298
Equitability_J	0.9681	1.81	2.114
Fisher_alpha	2.277	0.5833	0.6176
Berger-Parker	0.383	6	7

Table 9.The diversity indices, dominances indices and evenness indices at Poojar during 2015-16.

Indices	PMon	Mon	PT Mon
Individuals	139	61	0.1741
Dominance_D	0.2161	0.1836	0.8631
Simpson_1-D	0.8653	0.8734	2.138
Shannon_H	2.29	2.125	0.7712
Evenness_e^H/S	0.6659	0.9304	2.044
Brillouin	2.119	1.901	0.7301
Menhinick	1.272	1.152	1.843
Margalef	2.837	1.946	0.8917
Equitability_J	0.8485	0.9672	2.416
Fisher_alpha	4.27	2.915	0.2996
Berger-Parker	0.4101	0.3115	11.5

Table 10.The diversity indices, dominances indices and evenness indices at Poonjar during 2016-17.

Indices	PMon	Mon	PT Mon
Individuals	3	112	76
Dominance_D	1	0.224	0.2884
Simpson_1-D	0.4444	0.8409	0.8161
Shannon_H	0.6365	1.973	1.878
Evenness_e^H/S	1	0.7994	0.7394
Brillouin	0.3662	1.837	1.704
Menhinick	1.155	0.8504	1.032
Margalef	0.9102	1.695	1.847
Equitability_J	0.9183	0.8981	0.8596
Fisher_alpha	2.622	2.306	2.656
Berger-Parker	1	0.3661	0.4342

Table 11.The diversity indices, dominances indices and evenness indices at Theekoy during 2015-16.

Indices	PMon	Mon	PT Mon
Dominance_D	0.2477	0.2704	0.2177
Simpson_1-D	0.826	0.8434	0.8452
Shannon_H	2.001	2.047	2.074
Evenness_e^H/S	0.6731	0.7752	0.6652
Brillouin	1.869	1.828	1.92
Menhinick	0.9297	1.231	1.086
Margalef	2.024	2.148	2.29
Equitability_J	0.8348	0.8892	0.8353
Fisher_alpha	2.797	3.278	3.299
Berger-Parker	0.3929	0.4545	0.3525

Table 12.The diversity indices, dominances indices and evenness indices at Theekoy during 2016-17.

Indices	PMon	Mon	PT Mon
Individuals	113	194	162
Dominance_D	0.1456	0.1186	0.1377
Simpson_1-D	0.8986	0.9082	0.8975
Shannon_H	2.432	2.51	2.413
Evenness_e^H/S	0.8156	0.8202	0.798
Brillouin	2.231	2.367	2.261
Menhinick	1.317	1.077	1.1
Margalef	2.75	2.658	2.555
Equitability_J	0.9227	0.9268	0.9145
Fisher_alpha	4.208	3.794	3.676
Berger-Parker	0.2832	0.2216	0.2593

Table 13.The diversity indices, dominances indices and evenness indices at Kumarakom during 2015-16.

Indices	PMon	Mon	PT Mon
Dominance_D	0.1179	0.1318	0.1508
Simpson_1-D	0.8865	0.8734	0.8553
Shannon_H	2.347	2.276	2.176
Evenness_e^H/S	0.4979	0.4639	0.4407
Brillouin	2.339	2.27	2.17
Menhinick	0.237	0.2169	0.2005
Margalef	2.23	2.187	2.064
Equitability_J	0.7709	0.7477	0.7265
Fisher_alpha	2.624	2.559	2.401
Berger-Parker	0.1721	0.2057	0.2624

Table 14.The diversity indices, dominances indices and evenness indices at Kumarakom during 2016-17.

Indices	PMon	Mon	PT Mon
Dominance_D	0.1097	0.1204	0.1634
Simpson_1-D	0.896	0.8864	0.8503
Shannon_H	2.397	2.334	2.172
Evenness_e^H/S	0.5495	0.5734	0.5487
Brillouin	2.384	2.323	2.157
Menhinick	0.3042	0.2684	0.3012
Margalef	2.27	2.021	1.888
Equitability_J	0.8001	0.8076	0.7835
Fisher_alpha	2.712	2.387	2.241
Berger-Parker	0.1966	0.2233	0.3001

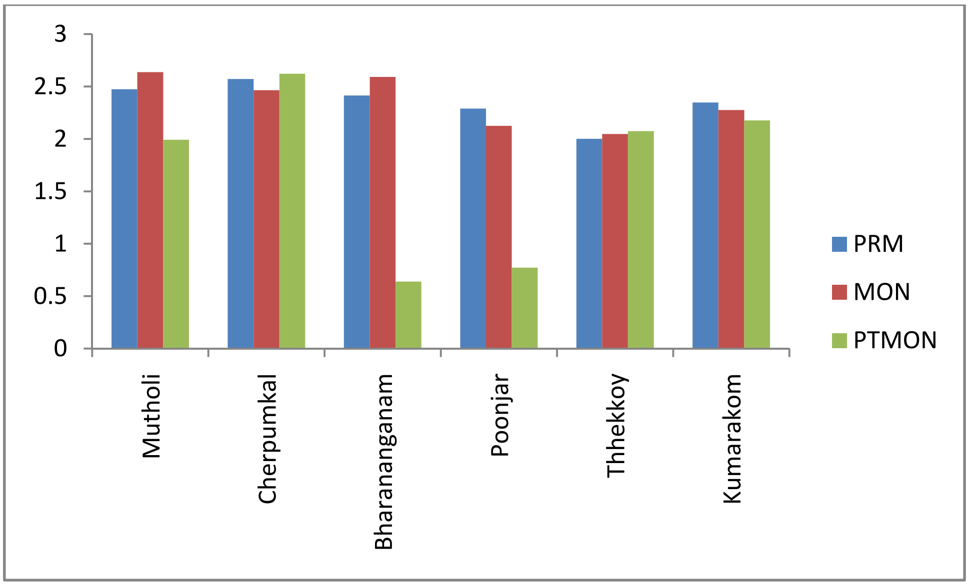


Figure. Shanon Index of diversity changes in three different seasons of 2015-16 period

During the pre monsoon sampling, the high diversity was more or less maintained a uniform pattern in all the six locations. However, Pala Cherpumkal and Pala Mutholi had the highest number of species and individuals. During the Monsoon season, Bharanaganam had the highest species diversity and Theekkoy has recorded for the least number of species. The post monsoon season the diversity changes are apparent. Pala cherpumkal maintained a high diversity and assemblage of species whereas Bharanganam and Poonjar has the least.

Pala Cherpumkal is the location which maintained a uniform diversity in all the season of 2015-16. Poonjar and Bharanganam has showed a changes in the diversity, where the former has recorded a high diversity during Monsoon period and very low species diversity during Post monsoon period.

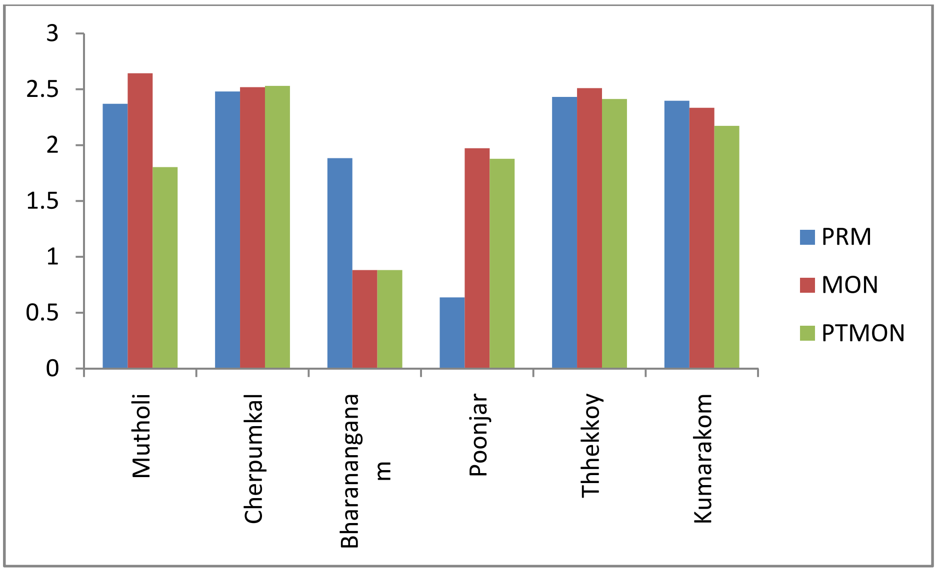


Figure. Shanon Index of diversity changes in three different seasons of 2016- 17 period

During 2016-17 period, the diversity of fishes was more or less in a uniform pattern during the pre monsoon season in all the locations except Bharanaganam and Poonjar. Bharanaganam was noted for the low diversity of fishes during the Monsoon season and post monsoon season.

Pala Cherpumkal, Theekkoy and Bharanganam and Kumarakom had a high assemblage of species during the post monsoon season. The post monsoon period followed the same pattern of 2015-16 in Bharanganam and Poonjar.

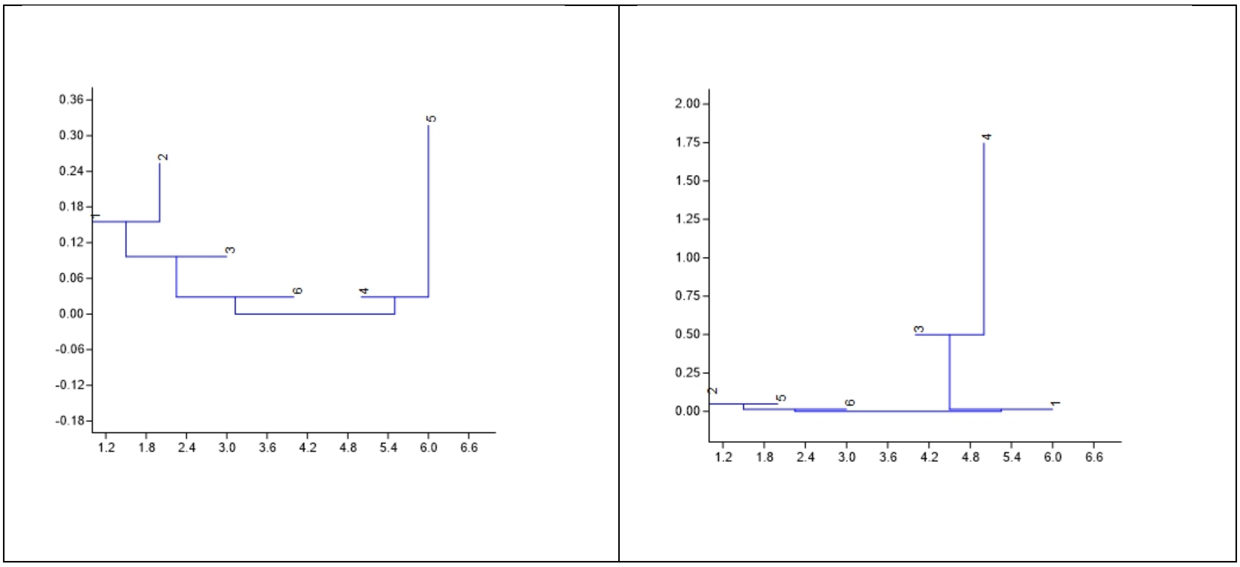


Figure Neighbourhood joining clustering based on the Shanon Index of diversity in the six location of the Meenachil river during Pre Monsoon (1.Mutholi; 2-Cherpumkal; 3-Bharananganam; 4-Poonjar; 5-Thhekkoy; 6-Kumarakom)

From the Figure, it is obvious that during the pre monsoon of 2015-16, Theekoy has been outgrouped from the cluster due to the poor diversity compared to other locations. However, during the 2016-17, Poonjar was outgrouped from the cluster due to the poor representation of the diversity.

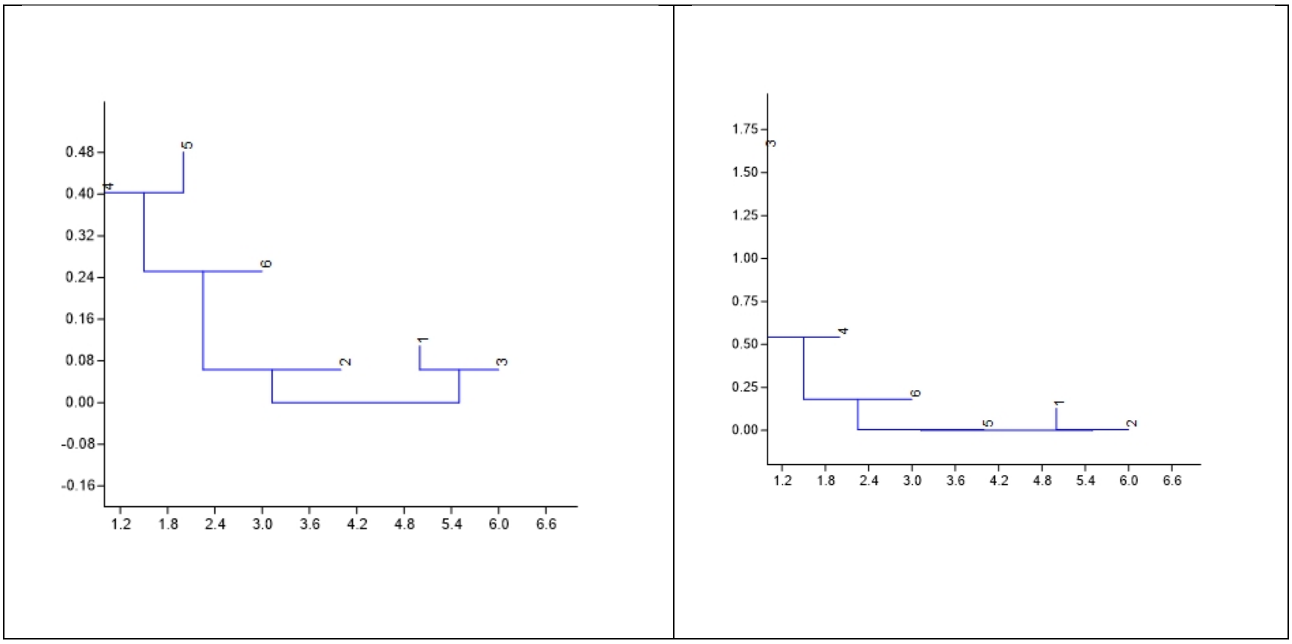


Figure Neighbourhood joining clustering based on the Shanon Index of diversity in the six location of the Meenachil river during Monsoon (1.Mutholi; 2-Cherpumkal; 3-Bharananganam; 4-Poonjar; 5-Thhekkoy; 6-Kumarakom).

The neighbouring joining clustering clearly indicates that during 2015 monsoon period, there was no drastic decline or rise in the species diversity. However, the monsoon 2016 showed high decline in the diversity of fished Bharanganam and Poonjar.

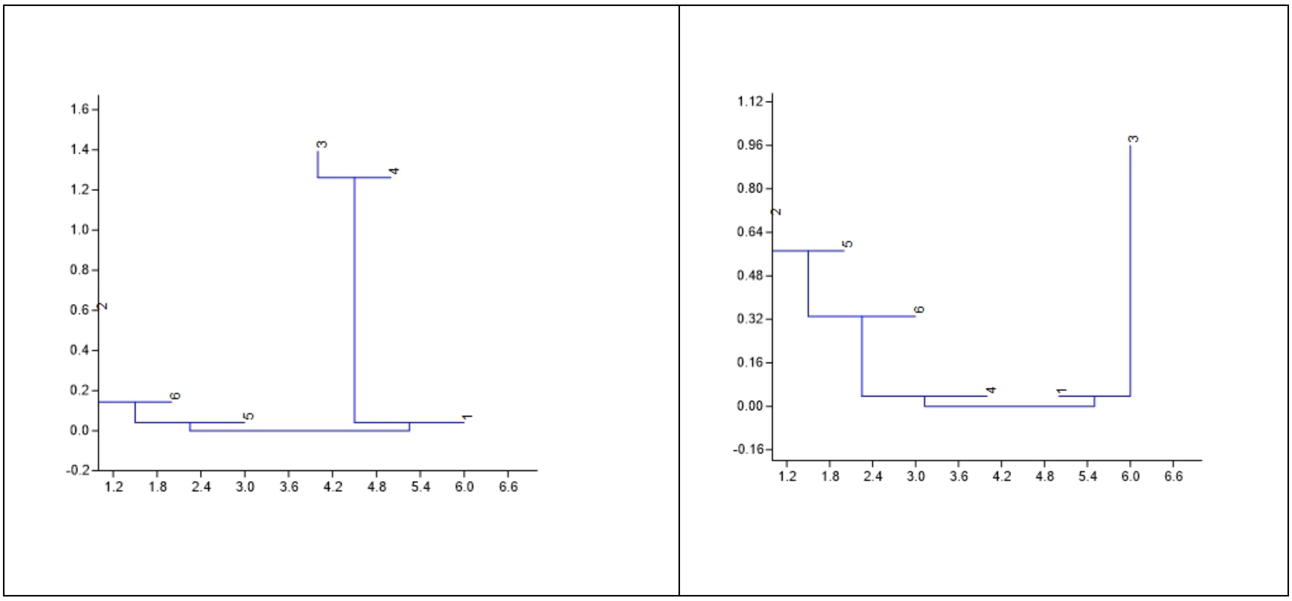


Figure Neighbourhood joining clustering based on the Shanon Index of diversity in the six location of the Meenachil river during Post Monsoon (1.Mutholi; 2-Cherpumkal; 3-Bharananganam; 4-Poonjar; 5-Thhekkoy; 6-Kumarakom).

The neighbouring joining clustering clearly indicates that during 2015 post monsoon period, The Bharanganam and Poonjar maked for very low species representation in the sampling. The species diversity was poor in Bharanganam during the post monsoon period of 2016.

Evenness expresses how evenly the individuals in the community are distributed over the different species.

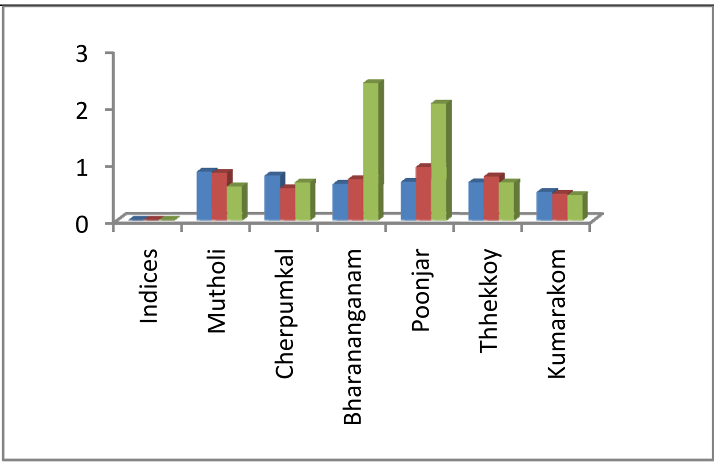


Figure. Evenness indices three different seasons of 2015- 16 period

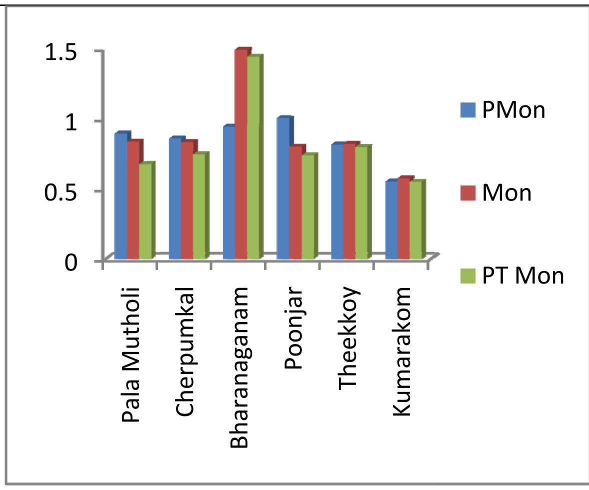


Figure. Evenness indices in three different seasons of 2016- 17 period

Evenness indices of the six locations indicates that during 2015-16, a high value has been recorded at Bharanganam and Poonjar during the post monsoon season and in monsoon and post monsoon season in 2016-17. This could be due to the outnumbering of certain species such as *Haludaria fasicatus* and *Dawkinisia filamentosa* Bharanganam.

Dominance

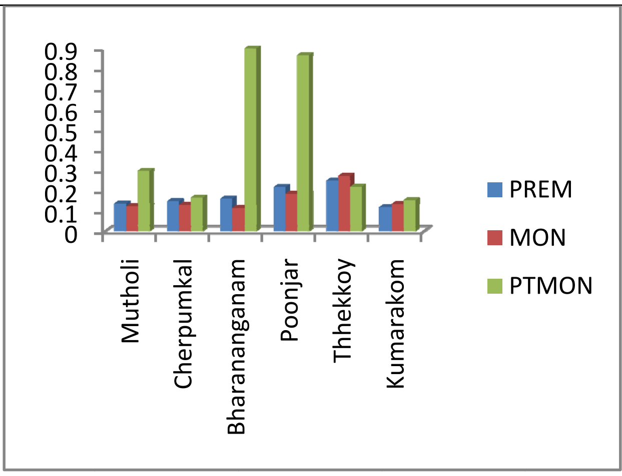


Figure. Dominance indices three different seasons of 2015- 16 period

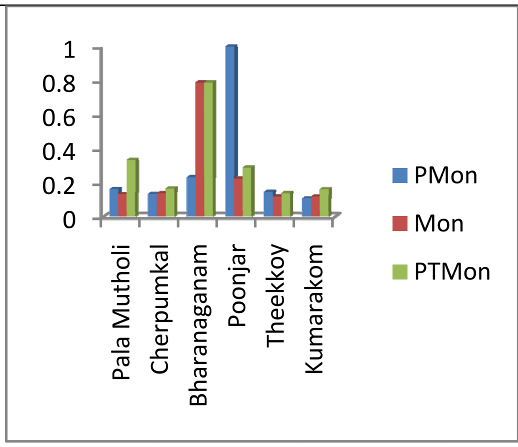


Figure. Dominance indices three different seasons of 2016- 17 period

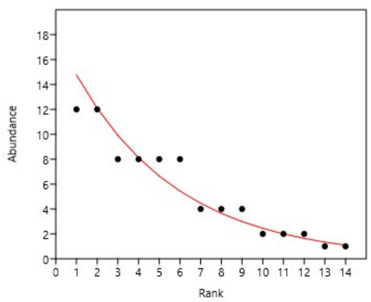
Dominance indices are weighted toward the abundance of the commonest species. The analyses of the sampling data clearly indicate outnumbering of the some species in the samples. All the locations showed a uniform abundance value during the pre monsoon and Monsoon period. However, Bharanganam and Poonjar showed high dominace alue during 2015-16 during the post monsoon season. In Bharanganam, during 2015-16, *Dawkinsia filamentosa* outnumbered in the samples.

During 2016-17, a high dominance index was recorded in Poonjar during the pre monsoon season. This was due to the outnumbering of Garra mullya in the sampling.

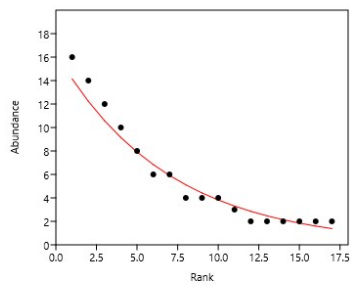
During the monsoon season, species like Parambassis thomassi dominated in the samples. This could be the reason for the high dominance value in the Bharanganam during monsoon season. During the post monsoon period witnessed a high abundance of Dawkinsia filamentosa in Bharanganam.

Abundance

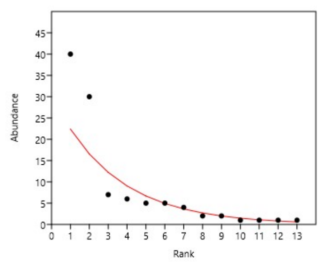
The rank abundance curve visually depicts both species richness and species evenness. Species richness can be viewed as the number of different species on the chart i.e., how many species were ranked. Species evenness is reflected in the slope of the line that fits the graph (assuming a linear, i.e. logarithmic series, relationship). A steep gradient indicates low evenness as the high-ranking species have much higher abundances than the low-ranking species. A shallow gradient indicates high evenness as the abundances of different species are similar.



Pre Monsoon



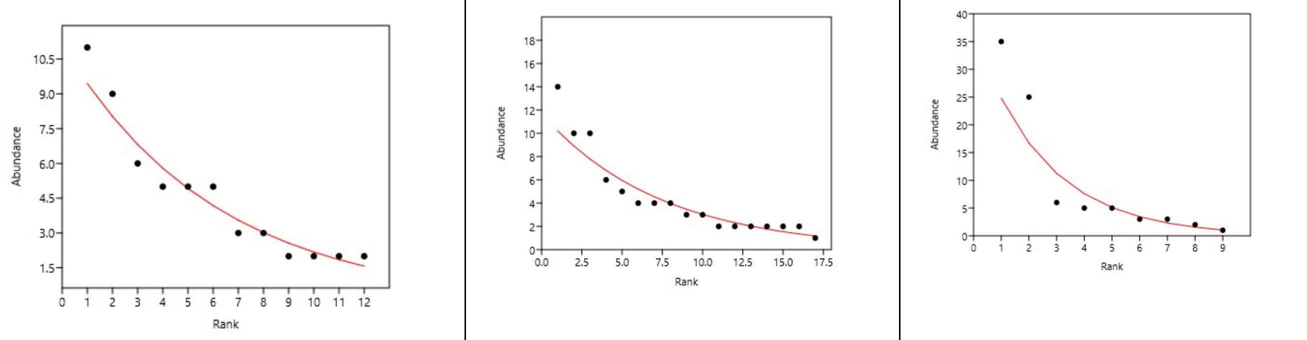
Monsoon



Post Monsoon

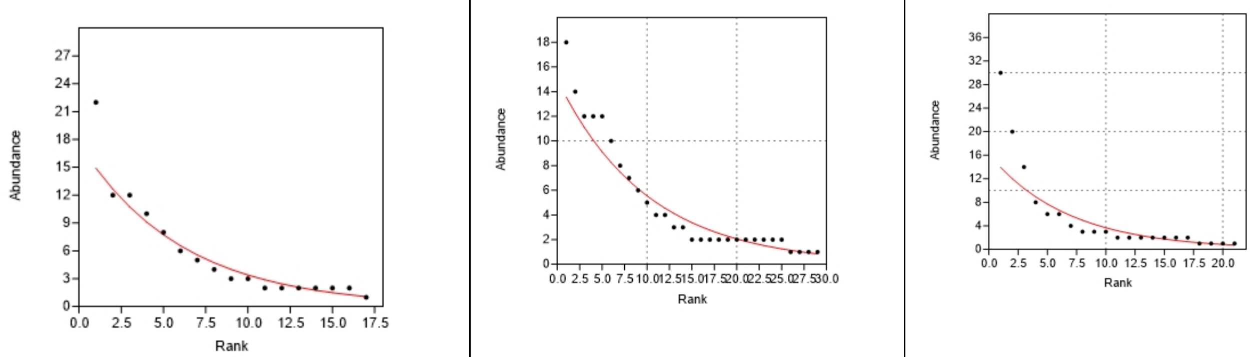
Figure Rank abundance curve-2015-16 Pala Mutholi

During the monsoon period of 2015-16, the abundance of the species was not uniform and two species dominated in the samples of Pala Mutholi. The empirical data suggests the over abundance of *Devario malabarica* and *Dawkinsia filamentosa* in the samples. During the post monsoon season, *Pseudoetroplus maculata* and *Rasbora dandia* outnumbered in the samples.



Pre Monsoon | Monsoon | Post Monsoon

Figure Rank abundance curve-2016-17 Pala Mutholi



Pre Monsoon | Monsoon | Post Monsoon

Figure Rank abundance curve-2015-16 Pala Cherpumkal

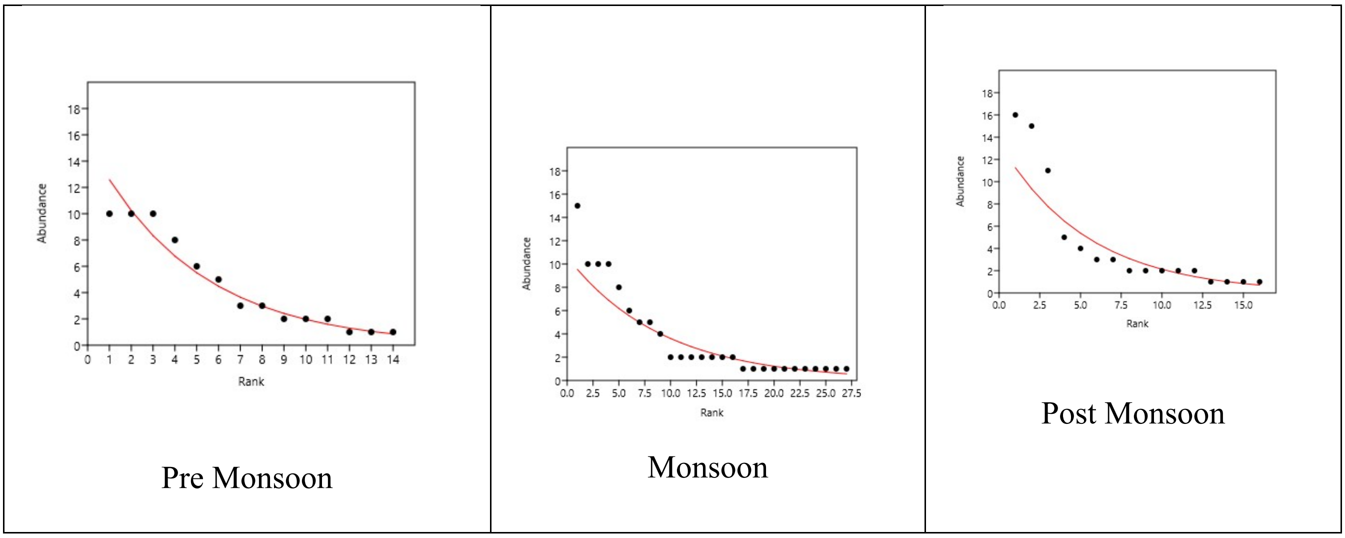


Figure Rank abundance curve-2016-17 Pala Cherpumkal

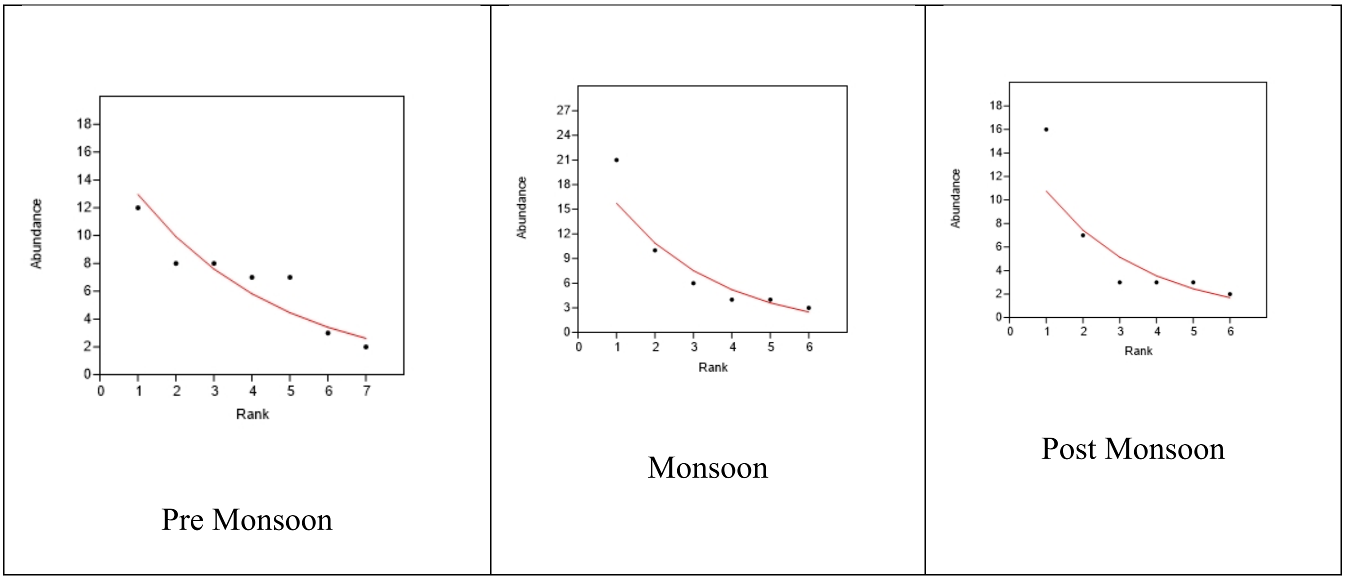
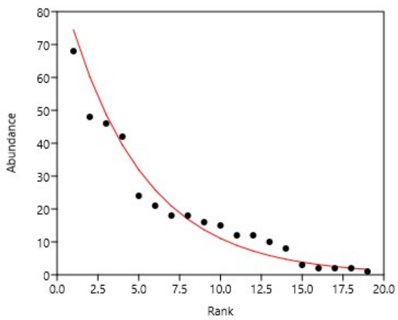
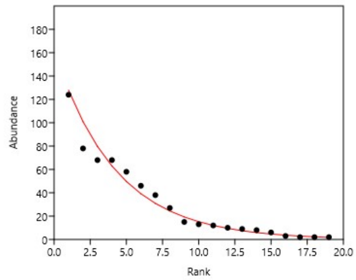


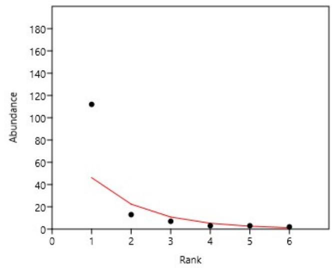
Figure Rank abundance curve-2015-16 Bharanganam



Pre Monsoon

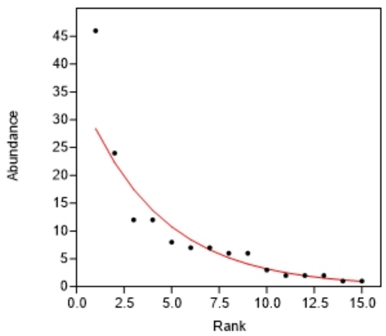


Monsoon

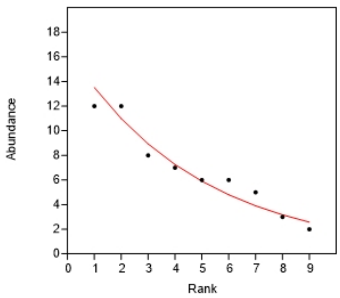


Post Monsoon

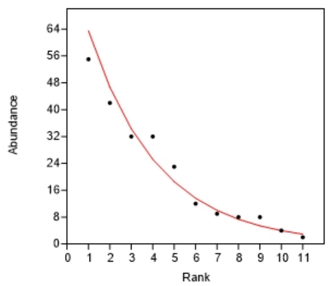
Figure Rank abundance curve-2016-17 Bharanganam



Pre Monsoon

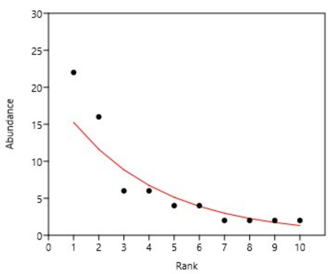


Monsoon



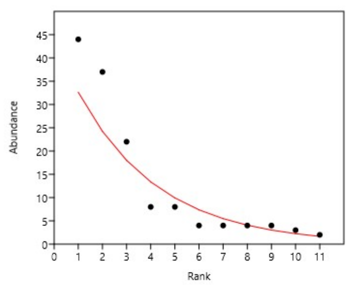
Post Monsoon

Figure Rank abundance curve-2015-16 Poonjar

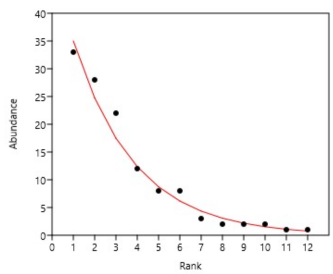


Pre

Monsoon

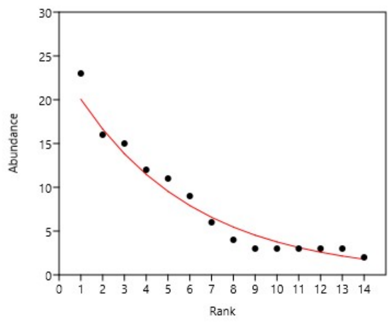


Monsoon

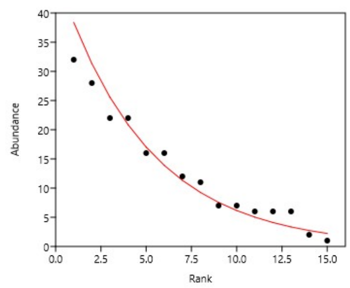


Post Monsoon

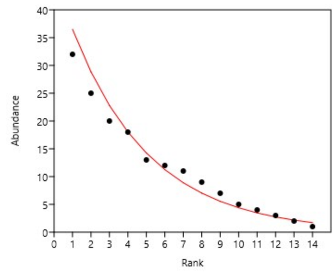
Figure Rank abundance curve-2015-16 Theekkoy



Pre Monsoon

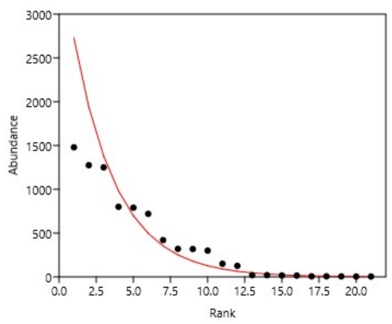


Monsoon

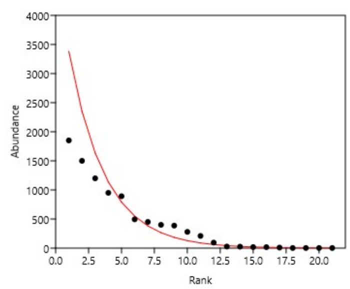


Post Monsoon

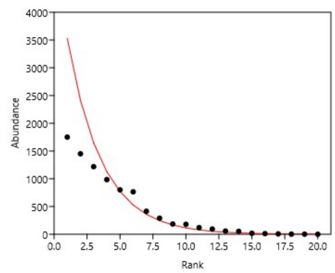
Figure Rank abundance curve-2016-17 Theekkoy



Pre Monsoon

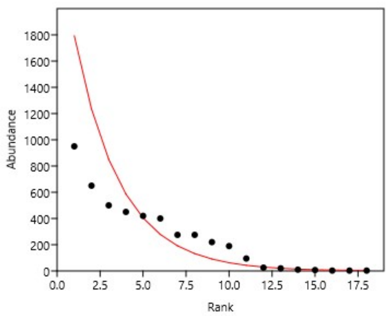


Monsoon

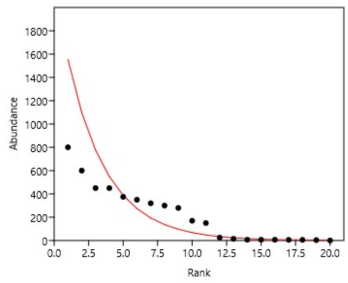


Post Monsoon

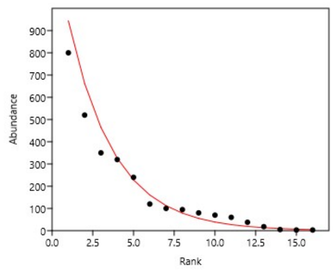
Figure Rank abundance curve-2015-16 Kumarakom



Pre Monsoon



Monsoon



Post Monsoon

Figure Rank abundance curve-2016-17 Kumarakom

16 S sequences of the following fish species were obtained.

Species	16S sequence
Ompokmalabaricus	TGACAGCAGGCGGACCGTGCGAAGGTAGCGCAATCACTTGTCTTTTAAAT CGATATTAATTGACAGGATCGGCGGTTTAACACAAATCACTTTTTTTTT GAAGACCTGTATGAATGGCAAAACGAGGGCTTAACTGTCTCCCTTCTCCA GTCAATGAAATTGATCTGCCCGTGCAGAAGCGGACATACAAATACCTCGA CGAGAAGACCCTTTGGAGCTTAAGATAACAAATCAACTATGTCAAGAACC TAAAAAACAGGTCAAACTAAATAGCAACTGATTCCTATCTTCAGTTGGGG CGACTGCGGGAGAAAACAAAGCTCCCACGAGGATTGGGACATACATCCTA AAACCAAGAAAGACACTTCTAAGTAACAGAACATCTGACCTTTTAAGATC CGGCCACCAGCCGATCAACGGACCAAGTTACCCTAGGGATAACAGCGCAA TCCCCTTTCAGAGTCCATATCGACAAGGGGGTTTACGACCTCGATGTTGG ATCAGGACATCCTAATGGTGCAGCCGCTATTAAGGTTTCGTTTGTTCAAC GATTAAAGTCCTACGTGATCTGATTTCAAAACGGGAAGCGCTGTT
Salmostomabacalia	CCGACCAGGAAATTTTATACGGAGGTCGTTGTGAAGGATCCGCTTTTGCC CCAAATACGTCCTGTTTTTGAGAAACCGGCGACCGGGGGAAGCCCGTAAT CCTCGTCCTTTGAATGGGGTCAAGTATGAAAGGCTAGACGAGGGTTCTAA CTGTCTCCCTTTTCCAGTCAGTGAAATTGATCTATCCGTGCAGAAGCGGG TATAAAACTACCTTCTCGAGAAGACCCTTTGGAGCTTTAGGTACAGGACT TAACCACGTCAAGCAAGTATATAAAAACTTAAACCCCGTGGCACATAAGA CCCTACCTTGGGTTGGGGCGACCATGGAGAAAAAATAATCCTCCGAGTGG ACTGGGGACACCCCTAGAACCAAGAGACACACCTCTAAGTCACAGAACTT CTGACCAAAAATGATCCGGACATAAGACCGATCAACGAACCAAGTTACCC TAGGGATAACAGCGCAATCCCCTCCCAGAGCCCATATCGACGAGGGGGTT TACGACCTCGATGTTGGATCACGACATCCTAATGGTGCAGCCGCTATTAA GGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTTCAGACC GGG
Etroplusmaculatus	CTTTTTTCTCGACGGATCGGGTCTGACTCAAATTAGTATAGGTTTTTGAT CGCAGGGGTTTGACCGTGCAAAGGTAGCGCAATCACTTGTCTTTTAAATG AAGACCTGTATGAATGGCATAACGAGGGCTTAACTGTCTCCCTTTTCCAG TCAATGAAATTGATCTCCCCGTGCAGAAGCGGGGATTAAAACATAAAACG AGAAAACCCTATGGAGCTTTAAACAAAGAGCAAACCATGTTAAACACACC TAAATAAAGGACAAAACCAATTGGCCCCTGCCCTTATGTCTTTGGTTGGG GCGACCGCGGGGAAACAAAAAACCCCCATGTGGACTGAAAACATTCCTCT TCACAACCAAAAGCCACAGCTCTAAGCAACAGAACATCTGACCAACAAAA TCCGGCACTAACCCGATCAACGGACCGAGTTTACCCTAGGGATAACAGCC GCAATCCTCCTTTTAAAGCCCCCATCGACAAGGAGGTTTACGACCTCAAT GTTGAACCAGGACATCCAATGGTGGAACCCGCTTTTAAGGGTTCGTTTGG TTAACGATAAAGTCCTACGTGATCTGAGTTCAAACGGGATCTCTCTGTGT GTACAAAACTAAATATGTGTCTGATTTTCAAGAGGGGCGCTGTGGTAAAA AAAAAAGAAAG

Salmostomabacalia	GGGAAATTATTAGAACGGATCCCAGGCTGACTAAAATCATTAATTTGTTT TTGCGAAATTGGGACCGTGCGAAGGTAGCGTAATCACTCGTCCTTTAAAT GGGGTCAAGTATGAAAGGCTAGACGAGGGTTTAACTGTCTCCCTTTTCCA GTCAGTGAAATTGATCTATCCGTGCAGAAGCGGGTATAAAACTACAAGAC GAGAAGACCCTTTGGAGCTTTAGGTACAGGACTTAACCACGTCAAGCAAG TATATAAAAACTTAAACCCCGTGGCACATAAGACCCTACCTTGGGTTGGG GCGACCATGGAGAAAAAATAATCCTCCGAGTGGACTGGGGACACCCCTAG AACCAAGAGACACACCTCTAAGTCACAGAACTTCTGACCAAAAATGATCC GGACATAAGACCGATCAACGAACCAAGTTTACCCTAGGGATAACAGCGCA ATCCCCCTCCCAGAGCCCATATCGACGAGGGGGTTTACGACCCTCGATGT TGGATCAGGACATCCCTATGGTGCAGCCGCTATTAAGGGTTCGTTTTGTT CAACGATTAAAGTCCTACGTGATCTGAGTTCAAACCGGATGTTGTCTTGT TTACAAAAACTAATCGTGATCTGATTTCAACGGGGTGCGATATATATCA AAAACTA
Dawkinsiafilamentosus	CGGGGAAAAAAAACAGATAAAATACGCTATTAATGGATCAGGGTTTAAAA AAAAACGTACCGGTTTTTGAAAAAGGGGGTTTGGTGTGCCCCGGTAGCGC AATCACTTGGCTTTTAAATAAAGACCCGTATGAATGGTTAAACGAGGGCT TAACTGTCTCCCATTTCCAGTCAGTGAAATTGATCTACCCGTGCAGAAGC GGGTATAAATATACAAGACGAGAAGACCCTTTGGAGCTTAAGGTACAAAA CTCAACCACGTCAATCAACTCAATAAAAAGCAACTTTTTACTAAACCTTA TGACAAATGAGACTATACCTTCGGTTGGGGCGACCAAGGAGGAAAAGAAA GCCTCCAAGTGGACTGGGTCAACACCCTAAAACTAAGAGAGACATCTCTA AGCCACAGAACATCTGACCATAAATGATCCGGTCAATTAAGACCGATCAA CGAACCAAGTTACCCTAGGGATAACAGCGCAATCCCCTCCCAGAGTCCAT ATCGACGAGGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGG TGCAGCCGCTATTAAGGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGA TCTGATTTCAAGACCGGATGGTCGCCTGT
Rasborarasbora	AGTTCGGAATTAGACCGGATCGGCGTCTGTGCTAAAATCACTTTATGTTT TTGATAAAAAGGGGAGCCCCGTGCAAAGGTAGCGCAATCACTCGTCCTTT AAATGGGGACCTGTATGAATGGTTAGACGAGGGCTTAACTGTCTCCCTTT TCCAGTCAGTGAAATTGATCTATCCGTGCAGAAGCGGGTATAACAATACA AGACGAGAAGACCCTTTGGAGCTTTAGGTACAAGTCTCACCCATGTTAAG CAAACTCCAACAAGTCCAAAACCCAATGAAAAATGAGACCCTACCTTCGG TTGGGGCGACCACGGAGGAAAAAACAGCCTCCAAGTGGATTGGATATTTT CCAAAACCAAGAACAACATTTCTAAGTAACAGAACATCTGACCTTACATG ACCCGGCAATTCTAAACCGATCAACGAACCAAGTTACCCAAGGGATAACA GCGCAATCCTCTTCCAGAGTTCATATCGACGAGGGGGTTTACGACCTCGA TGTTGGATCAGGACATCCTAATGGTGCAGCCGCTATTAAGGGTTCGTTTG TTCAACGATTAAAGTCCTACGTGATCTGAGTTCAGACCCGGATGTCGCCT GTTTATCAAAAACATATACGTGATCTGATTTCAAAACCGGAGGCGCTTTT TAAAAAAATAAAATCG
Opsariusbakeri	AGAAATGAATTAGACAGGCATCGCCGGTTAACCCAAAGATATATAGTTTT TGGACACAGGGGTCTGACCGTGCAAAGGTAGCGTAATCACTCGTCCTTTA AATGGGGTCAAGTATGAAAGGCTAAACGAGGGCTTAACTGTCTCCCCCCC CAGGTCAGTGAAATTGATCTATCCGTGCAGAAGCGGGTATAAACATTCAA GACGAGAAGACCCTTTGGAGCTTTAGGTACAAGACTAAACCACGTTTAAC AAGTCAATGAAAACACAAACCCCGTGGAAACTTAAGACTTTACCTTCGGT TGGGGCGACCACGGAGTAAAATAAAACCTCCAAGTGGACTGGGGAAACCC CTAAAACTAAGAGAACCACCTCTAAGTCACAGAAATTCTGACCAAAATGA TCCGGACACTTAACCGATTAACGAACCAAGTTACCCTAGGGATAACAGCG CAATCCTCTCCCAGAGCCCATATCGACGAGGGGGTTTACGACCTCGATGT TGGATCAGGACATCCTAATGGTGCAGCCGCTATTAAGGGTTCGTTTGTTC AACGATAAAGTCCTACGTGATCTGAGTTTCAAAACGGGACCTCGCCTGTT TATAAAAAAACCTTAGCGTGGTGTGTATTTCACAACCAGGGTGCAATTGTC AGCAAAAAGTATAAA

Labeodussumieri	TCGGAGAAAAAAATTTAAGGAGTGCGCTTCTTACGGATCCGGGTTTTAAC CCAAATATGTATAGGTTTTTGAGCAGAGGGGGTTTGACTGTGCCCGGTAG CGCAATCACTTGTCTTTTAAATAGAGACCTGTATGAATGGCTAAACGAGG GCTTAACTGTCTCCCCTTTCCAGTCAGTGAAATTGATCTGCCCGTGCAGA AGCGGACATAAAAATACAAGACGAGAAGACCCTTTGGAGCTTAAGGTACA AAACTCAACCACGTCAAGCAACTCAATAAAAAGCAAAAACTTTGTGGAAT ATGAGATTTTACCTTCGGTTGGGGCGACCACGGAGGAAAACAAAGCCTCC AAGTGGATTGGGAAAACCTCCTAAAACCAAGAGAGACATCTCTAAGCCAC AGAACATCTGACCAAACATGATCCGGCTACCCGAAGCCGATCAACGAACC AAGTTACCCTAGGGATAACAGCGCAATCCTCTCCCAGAGTCCATATCGAC GAGAGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCAGC CGCTATAACGGTTCGTTTGTTCAACGATTAAAGTCTACGTGATCTGAGTT CAGAACCAGGATGTCGGCTGTTTATCAAAAAACATATGACTGAGTTCTGT GTTTCAAACAGAGCTG
Strongylurakrefftii	ACACCGATATAAAGCGTGGGTCGAACGAAGGATCCGGGGTGACAAAAACA TATTATGTTTTTGAGAACAGGCGACCGTGCAAGGTCGCGCAATCACTTGT CTTTTAAATGAAGACCTGTATGAATGGCATAACGAGGGCTTAACTGTCTC CTTTTTCCAGTCAATGAAATTGATCTCCCCGTGCAGAAGCGGGGATAATT ACATAAGACGAGAAGACCCTATGGAGCTTTAGATAAGAGATGGACCATGT TAATAAATCTATTTAATAAGAACAAACTTATTGGTCACCATTTTAATATC TTTGGTTGGGGCGACCGCGGGGTAATAATTAACCCCCAAGTGGAAAAAGA GAACCTCTCTCATAACCTAGACTGACAAATCCAAGTATCAGAAAATCTGA CCAAAAATGACCCGGCAAAGCCGATCAACGAACCGAGTTACCCTAGGGAT AACAGCGCAATCCTCTCCTAGAGCCCATATCGACAAGAGGGTTTACGACC TCGATGTTGGATCAGGACATCCTAATGGTGCAGCCGCTATTAAGGGTTCG TTTGTTCAACGATTAAAGTCCTACGTGATCTGATTTCAGAACGGGAGACT CTCCTGTTTATCAAAAACATATACGTTGATTTTGAGTTCACAACGGGGG
Strongylura krefftii	CCCTACCTTAATTAGACGGTATCCCGCCTGCACTGTGACACTAGTTTAAC GGCCGCGGTATTTTGACCGTGCAAAGGTAGCGCAATCACTTGTCTTTTAA ATGAAGACCTGTATGAATGGCATAACGAGGGCTTAACTGTCTCCTTTTTC CAGTCAATGAAATTGATCTCCCCGTGCAGAAGCGGGGATAATTACATAAG ACGAGAAGACCCTATGGAGCTTTAGATAAGAGATGGACCATGTTAATAAA TCTATTTAATAAGAACAAACTTATTGGTCACCATTTTAATATCTTTGGTT GGGGCGACCGCGGGGTAATAATTAACCCCCAAGTGGAAAAAGAGAACCTC TCTCATAACCTAGACTGACAAATCCAAGTATCAGAAAATCTGACCAAAAA TGACCCGGCAAAGCCGATCAACGAACCGAGTTACCCTAGGGATAACAGCG CAATCCTCTCCTAGAGCCCATATCGACAGAGGGTTTACGACCTCGATGTT GGATCAGGACATCCTAATGGTGCAGCCGCTATTAGGGGTTCGTTTGTTCA ACGATTAAAGTCCTACGTGATCTGATTCAAAACCGGGAGTTCCTCTGTTT ATTAAAAAAAAAAATGGGTGGTGCTGATTCCAACAGCGGGTTTTTTTTTT TTATATACTTGCTTT
Puntius sp.	CGGAAAAGAAAATTAAGGGCACCAGACCGACGGACCCCGGCTGACGCAAA TACATATAGGTTTTTGGTCGCGGGGGCTTGACCGTGCACGGTAGCGCAAT CACTTGTCTTTTAAATAAAGACCTGTATGAAAGGTTAGACGAAGGCTTAA CTGTCTCCCATTTCCAGTCAGTGAAATTGATCTACCCGTGCAGAAGCGGG TATAATAATACAAGACGAGAAGACCCTTTGGAGCTTTAGGTGAAAACTCA ACCATGTCAAGCAACTCAACAAAAAGCAACTATTAATAATTTATTCAAAC CTATAGTAAACTGAGAATACACCTTCGGTTGGGGCGACCATGGAGAAAAA AAGAGCCTCCAAGTGGACCGGAATAATATTCCAAAACCAAGAAAGACATT TCCAAGCCACAGAACATCTGACCATAAATGACCCGGCAAACAATGACCGA TCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCTCTCCCAGAGC CCATATCGACAAGAGGGTTTACGACCTCGATGTTGGATCAGGACATCCTA ATGGTGCAGCCGCTATTAAGGGTTCGTTTGTTCAACGATTAAAGTCCTAC GTGATCTGATTTCAGACCCGGATCGCCGCCTGTCA

Labeodussumieri	GGGGCAAACAATCCAAAAGAACGTAGACTATACGGATCCGTGCTCACGCA TATTGACTACGGGTTTTACGGCCGCGGTGTTTTGACCGTGCAAAGGTAGC GCAATCACTTGTCTTTTAAATAGAGACCTGTATGAATGGCTAAACGAGGG CTTAACTGTCTCCCCTTTCCAGTCAGTGAAATTGATCTGCCCGTGCAGAA GCGGACATAAAAATACAAGACGAGAAGACCCTTTGGAGCTTAAGGTACAA AACTCAACCACGTCAAGCAACTCAATAAAAAGCAAAAACTTTGTGGAATA TGAGATTTTACCTTCGGTTGGGGCGACCACGGAGGAAAACAAAGCCTCCA AGTGGATTGGGAAAACCTCCTAAAACCAAGAGAGACATCTCTAAGCCACA GAACATCTGACCAAACATGATCCGGCTACCCGAAGCCGATCAACGAACCA AGTTACCCTAGGGATAACAGCGCAATCCTCTCCCAGAGTCCATATCGACG AGAGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCAGCC GCTATTAAGGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGATT CAGACCGGACGGTCGCCTGTTTATCAAAAACATATACGTGATCTGAGTTC AGAACAGGAGGG
Puntiusmahecola	CGAAAAAAATAAAAATAAAAATGCCTACCGACGGACCCCGTTTAAATAAA ATAAGCATATGTTTTTGATACACAGGGGACTGACCGTGCCAAGGAAGCGC AATCACTTGTCTTTTAAATAAAGACCTGTATGAATGGCCAAACGAGGGCT TAACTGTCTCCCCTTTCAAGTCAGTGAAATTGATCTGCCCGTGCAGAAGC GGACATAATAATACAAGACGAGAAGACCCTATGGAGCTTAAGGCATAAAA CTCAACCACGCCAAAAACTCCGCAAAAGTTAACAAGCCCAGTGATAAATG AGACCATGCCTTCGGTTGGGGCGACCATGGAGGAAAATAAAGCCTCCAGG AGGAATGGGGTTAAACCCTTAAACCAAGAGACACATCTCTAAGCTTCAGA ACATCTGACCATAAATGATCCGGCCAACCAAGACCGATCAACGAACCAAG TTACCCTAGGGATAACAGCGCAATCCTCTCTGAGAGTTCGTATCGACGAG GGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCAGCAGC TATTAAGGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTC AGACCCGGAGGGTCGCCTGTTTATCAAAGAACATATACGTTGATCTGAGT TTTAGACCGGGTGTG
Puntiusmahecola	CCGCCTTCGGAATTAGATGGCATCCGTCCTGCCTGCCCAATGACCACAAG TTCAACGGCCGCGGTACCCTGACCGTGCAAAGGTAGCGCAATCACTTGTC TTTTAAATAAAGACCTGTATGAATGGCCAAACGAGGGCTTAACTGTCTCC CCTTTCAAGTCAGTGAAATTGATCTGCCCGTGCAGAAGCGGACATAATAA TACAAGACGAGAAGACCCTATGGAGCTTAAGGCATAAAACTCAACCACGC CAAAAACTCCGCAAAAGTTAACAAGCCCAGTGATAAATGAGACCATGCCT TCGGTTGGGGCGACCATGGAGGAAAATAAAGCCTCCAGGAGGAATGGGGT TAAACCCTTAAACCAAGAGACACATCTCTAAGCTTCAGAACATCTGACCA TAAATGATCCGGCCAACCAAGACCGATCAACGAACCAAGTTACCCTAGGG ATAACAGCGCAATCCTCTCTGAGAGTTCGTATCGACGAGGGGGTTTACGA CCTCGATGTTGGATCACGACATCCTAATGGTGCAGCAGCTATAAGGGGTT CGTTTGTTCAACGATTAAAGTCCTACGTGATCTGATTTCAGACCAGGATT GTTCTCTGTTTATCAAAACATATTACGTGATCCTGAAGTTACGAACAGGG GGGGGTGTTTACCAGACAGTG
Aplocheilus sp. **(Aplocheiluslineatus)**	GCGCCTGCCTAGACGGATCGCCTGCCTGCCCTGTGACTTAAGTTTAACGG CCGCGGTATTCTAACCGTGCAAAGGTAGCGCAATCACTTGTTTTTTAAAT GAAGACCTGTATGAATGGCATAACGAGGGCTAAACTGTCTCCTTCCCCCA GTCAATGAAATTGATCCCCCCGTGCAGAAGCGGGGATAAATACATAAGCC GAGAAGACCCTATGGAGCTTAAAACGAAAGAATAGAACATGTTATATCCC TTTAATTAAAAAATCAAACTAAATGCTTCCTATTCTAGTGTTTTCGGTTG GGGCGACCACGGAGCACAACAAAACCCCCGTGAGGAAAAAATAAACAACA TTTTAAATTAAGAGTGACAACTCTAGATAACAGAAACTCTGACCCTAATA GATCCGGCCAAGCCGATCAACGGACCGAGTTACCCTAGGGATAACAGCCG CAATCCCCTTTTAGAGCCCACATCGAACAGGGGGGTTTACGACCTCGATG TTGGATCAGGACATCCTAATGGTGCAGCGCTATTAAGGGTTCGTTTGTTC AACGATTAAGGTCTACGTGATCTGAGTTCAAACCGGATCCCGCTTGGTGT ATAAAAAACATATACGTGTCTTATTTAGACCGGG

Schisturascaturigina	GGGGGGCAAAGTTGAGGCTGACGATTGACCGACGGACCCGGTCTTACAAA ATACGTTTTGTTTTTGGCCGCAGGGGTTTGACCGTGCAAAGGTAGCGCAA TCACTTGTCTTTTAAATGAAGACCTGTATGAATGGCCAAACGAGGGCTTA ACTGTCTCCCCCATCAAGTCAGTGAAATTGATCTATCCGTGCAGAAGCGG GTATTAATATACAAGACGAGAAGACCCTTTGGAGCTTAAGGTACAAACCC ACCTACGTTAAACAGCTTATTAAACAAGCGCAAACCTAATAGAATATGGT ATTTTACCTTCGGTTGGGGCGACCACGGAGAACAAAAAATCCTCCTAGTG GACTGGGAACTTCCCTAAAACCAAGAAAGACATTTCCAAGTCACAGAAAA TCTGACCAAACATGATCCGGCCATCCCGGCCGATCAACGAACCAAGTTAC CCTAGGGATAACAGCGCAATCCCCTCCAAGAGTCCATATCGACGAGAGGG TTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCAGCCGCTATT AAGGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTCAGAC AGAATGTTCGCCTTGTTTATTAAAAAAACATAATCGTTGATCTTGTTTTC ACAAACGGGGACT
Labeodussumieri	GGGAGCGAAATTAAGCGAAAGGAGACTAACGGATCGGGGTGACCCAAAAA CGTATATGTTTTTGATAGAGGGGGAAAGACCGTGCCAGGTAGCGCAATCA CTTGTCTTTTAAATAGAGACCTGTATGAATGGCTAAACGAGGGCTTAACT GTCTCCCCTTTCCAGTCAGTGAAATTGATCTGCCCGTGCAGAAGCGGACA TAAAAATACAAGACGAGAAGACCCTTTGGAGCTTAAGGTACAAAACTCAA CCACGTCAAGCAACTCAATAAAAAGCAAAAACTTTGTGGAATATGAGATT TTACCTTCGGTTGGGGCGACCACGGAGGAAAACAAAGCCTCCAAGTGGAT TGGGAAAACCTCCTAAAACCAAGAGAGACATCTCTAAGCCACAGAACATC TGACCAAACATGATCCGGCTACCCGAAGCCGATCAACGAACCAAGTTACC CTAGGGATAACAGCGCAATCCTCTCCCAGAGTCCATATCGACGAGAGGGT TTACGACCTCGATGTTGGATCAGACATCCTAATGGTGCAGCCGCTATTAA GGGTTCGTTTGTTCAACGATTAAGTCCTACGTGATCTGAGTTCAGAACCA GGATGTCCGCCTGTTTTTTCAAAAACATAATACCTCGTCTTTGAGTTCAC AACCGTGTC
Horabagrusbrachysoma	ACGGAAAAATTACGAGAAAACAACGAATAGACGGATCGCGTGTAAACACA AATCACGTATATGTTTTTGATAAACAGGGGGACAGTGCTAACGTATAGAA ATCACTTGTCTTTTAAATGAAGACCTGTATGAATGGTGGAACGAGGGCTT AACTGTCTCCCCTTTCAAGTCAATGAAATTGATCTGCCCGTGCAGAAGCG GACATACAAATACAAGACGAGAAGACCCTTTGGAGCTTAAGATACAGAAT CAACTATGTCAAGAACCCTAAAATAAAGTTAAACTAAATAGCAACTGATC CCTATCTTCGGTTGGGGCGACTGCGGGAGAAAATAAAGCTCCCATGCGGA CTGGGGCCACCCCTAAAGCTAAGAGAGACATCTCTAAGTCACAGAACATC TGACCACAAAGATCCGGCCAATGGCCGACCAACGGACCAAGTTACCCTAG GGATAACAGCGCAATCCCCTTCCAGAGTCCATATCGACAAGGGGGTTTAC GACCTCGATGTTGGATCAGGACATCCTAATGGTGCAGCCGCTATTAAGGG TTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTCAAACCAGA
Opsariusbakeri	TAATAAGGTATTTGACGGAATCCGCGGCTTTGCTCAAATCACGTATGGGT TTTTGACAAGAGGGGCTGCCCGTGCAACGGTAACGTAATCACTCGTCCTT TAAATGGGGTCAAGTATGAAAGGCTAAACGAGGGCTTAACTGTCTCCCCC CCCAGGTCAGTGAAATTGATCTATCCGTGCAGAAGCGGGTATAAACATAC AAGACGAGAAGACCCTTTGGAGCTTTAGGTACAAGACTAAACCACGTTTA ACAAGTCAATGAAAACACAAACCCCGTGGAAACTTAAGACTTTACCTTCG GTTGGGGCGACCACGGAGTAAAATAAAACCTCCAAGTGGACTGGGGAAAC CCCTAAAACTAAGAGAACCACCTCTAAGTCACAGAAATTCTGACCAAAAT GATCCGGACACTTAACCGATTAACGAACCAAGTTACCCTAGGGATAACAG CGCAATCCTCTCCCAGAGCCCATATCGACGAGGGGGTTTACGACCTCGAT GTTGGATCAGGACATCCTAATGGTGCAGCCGCTATTAAGGGTTCGTTTGT TCAACGATAAAAGTCCTACGTGATCTGAGTTCAGACCGGATCTTCGCCTG TTTTTCAAAAACATAAACGTGTTCTGAGTTCAGACGGGTGT

Opsariusbakeri	CGTTTTGATTGGAACGGGGATCGTCCGGCTTATTCAGATATATTATTTCT TTGGTCACGACGCGCTGACCGTGCAAGGTAGCGTAATCACTCGTCCTTTA AATGGGGTCAAGTATGAAAGGCTAAACGAGGGCTTAACTGTCTCCCCCCC CAGGTCAGTGAAATTGATCTATCCGTGCAGAAGCGGGTATAAACATACAA GACGAGAAGACCCTTTGGAGCTTTAGGTACAAGACTAAACCACGTTTAAC AAGTCAATGAAAACACAAACCCCGTGGAAACTTAAGACTTTACCTTCGGT TGGGGCGACCACGGAGTAAAATAAAACCTCCAAGTGGACTGGGGAAACCC CTAAAACTAAGAGAACCACCTCTAAGTCACAGAAATTCTGACCAAAATGA TCCGGACACTTAACCGATTAACGAACCAAGTTACCCTAGGGATAACAGCG CAATCCTCTCCCAGAGCCCATATCGACGAGGGGGTTTACGACCTCGATGT TGGATCACGACATCCTAATGGTGCAGCCGCTATTAAGGGTTCGTTTGTTC AACGATAAAAGTCCTACGTGATCTGAGTTCAAGACCGGACTCCGGCTGTT TATCAAAAACATAATACGGTGATTCTTGAGTTTCAGAACAGAATG
Danioaequipinnatus	GCATATTGATTAGACGGATCCGGTCTGACTCAGATATGTATATGTTTTTG ATAAAAAGGGGGTGCGGGTTTAAATAAAAATTTTTTTTGTTTTTGATAAA ACGGGGTATGACTGTTTAAACGAGGGCTTAACTGTCTCCCCCTTCAGGTC AGTGAAATTGATCTATCCGTGCAGAAGCGGATATAAGAATACAAGACGAG AAGACCCTTTGGAGCTTTAGGTACAAGGCTTACTTACGTCAAATAATCTA ATCAAGGCGCAAAACTTAGTAAAACATAAGACTTTACCTTCGGTTGGGGC GACCATGGAGGAAAAAATAGCCTCCAAGTGGATTGGACACATCCCAAAAA CCAAGAGAAACATCTCCACGTCACAGAACATCTGACCAATTATGATCCGG TTACAAAACCGATCAACGGACCAAGTTACCCTAGGGATAACAGCGCAATC CTCTCCAAGAGTCCATATCGACGAGGGGGTTTACGACCTCGATGTTGGAT CACGACATCCTAATGGTGCAGCCGCTATTAAGGGGTTCGTTTGTTCAACG ATTATAGTCCTACGTGATCTGAGTTCAGAACCAGGA
Etroplusmaculatus	TGGTTTCGCGTTTAAACGGCATCGCCGGCTGAATAAAATATTTATGGGTT TGGGAGCGGAAGTGTGACCGTGCAAAGGTAGCGCAATCACTTGTCTTTTA AATGAAGACCTGTATGAATGGCATAACGAGGGCTTAACTGTCTCCCTTTT CCAGTCAATGAAATTGATCTCCCCGTGCAGAAGCGGGGATTAAAACATAA GACGAGAAGACCCTATGGAGCTTTAGACAAAGAGCAGACCATGTTAAACA CACCTAAATAAAGGACAAAACCAATTGGCCCCTGCCCTTATGTCTTTGGT TGGGGCGACCGCGGGGAAACAAAAAACCCCCATGTGGACTGAAGACACTC CTCTTCACAACCAAGAGCCACAGCTCTAAGCAACAGAACATCTGACCAAC AAGATCCGGCACTAAGCCGATCAACGGACCGAGTTACCCTAGGGATAACA GCGCAATCTCCTTTTAGAGCCCCCATCGACAAGGAGGTTTACGACCTCGA TGTTGGATCAGGACATCCTAATGGTGCAGCCGCTATTAAGGGTTCGTTTG TTCAACGATTAAAGTCCTACGTGATCTGAGTTTCAGACCGAGATTTCTCC TGTTTATCAAAAAATAAACGTGATCTGTTTTCAGAGCCGGGTTTTCTTTT TTTTTATATACATTATTGT
Etroplusmaculatus	AAACCATAAACCAGCAACTTTCACTAACGGATCTCGCCTGACCAGAAACC TGAGTTTATTGGCCGCGGTATTTTGACCGTGCAAAGGTAGCGCAATCACT TGTCTTTTAAATGAAGACCTGTATGAATGGCATAACGAGGGCTTAACTGT CTCCCTTTTCCAGTCAATGAAATTGATCTCCCCGTGCAGAAGCGGGGATT AAAACATCTAGACGAGAAGACCCTATGGAGCTTTAGACAAAGAGCAGACC ATGTTAAACACACCTAAATAAAGGACAAAACCAATTGGCCCCTGCCCTTA TGTCTTTGGTTGGGGCGACCGCGGGGAAACAAAAAACCCCCATGTGGACT GAAGACATTCCTCTTCACAACCAAGAGCCACAGCTCTAGCAACAGAACAT CTGACCAACAAGATCCGGCACTAAGCCGATCAACGGACCGAGTTACCCTA GGGAATAACAGCCGCATCCTCCTTTTAGAGCCCCCATCGACAGGAAGGTT TACGACCTCGATGTTGGGATCAAGAACATCCCTAATGGTGCAGCCGCTAT TAAGGGGTCCGTTTGTTCAACGAATAAAGTTCCTACGTGATCTGAGTTCC AACCCGGAACCTCTCCTGTTTTTCAAAACATAGTCCGGATCTTGTTTCAA ACGGTGTG

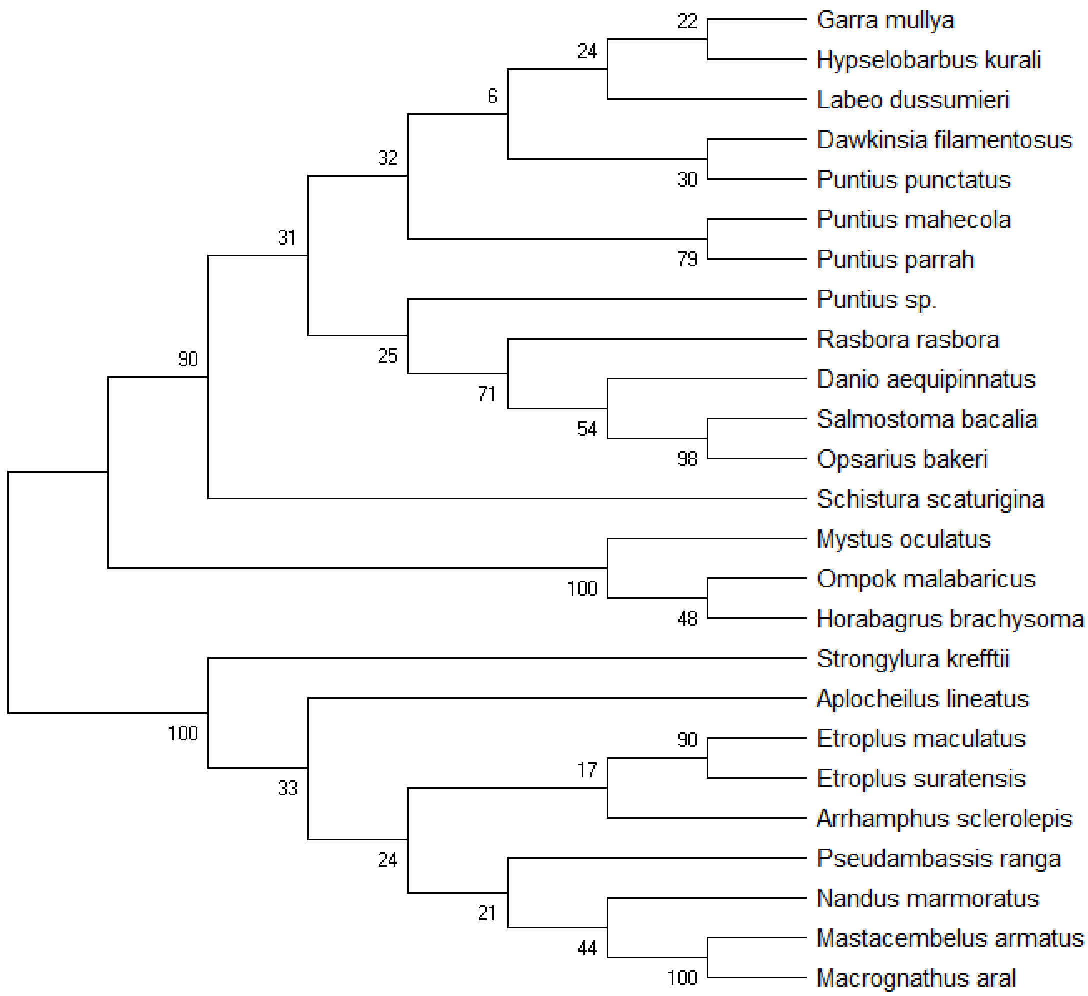
Etroplussuratensis	GCGGCGCCGTAATTAAACAACATGGTCGTTTATAGAAGGACCCACGTTTT AACAAAAATATGTCATGTTTTTGACAGAGGCGACCGGGGTTACCTCTACA ATCCTTGTCTTTTGAAGAAGACCCGTATGAATGGCATAACGAGGGCTTAA CTGTCTCCCTTTTCCAGTCAATGAAATTGATCTCCCCGTGCAGAAGCGGG GATCCCCACATAAGACGAGAAGACCCTATGGAGCTTTAGACAAAAGACAG CCCATGTCAAACACCCCTAAACAAAGGACAAAACTAATTGGCCCCTGTCC TAATGTCTTTGGTTGGGGCGACCGCGGGGAAACAAAAAACCCCCATGTGG ACTGAAGGCACCCTTCTTCACAACCAAGAGCCACAGCTCTAAGTAACAGA ACATCTGACCAACAAGATCCGGAATAATAACCGATCAACGGACCGAGTTA CCCTAGGGATAACAGCGCAATCTCCTTCTAGAGCCCATATCGACAAGGAG GTTTACGACCTCGATGTTGGATCAGACATCCTAATGGTGCAGCCGCTATT AAGGGTTCGTTTGTTCAACGATTAAGTCCTACGTGATCTGAGTTCAGACC GGAACCTTCGCCCTGCTTTATCAAAAAATATAATCACGTTTTGATTTTAG ACAGGGTCGCGATT
Garramullya	AAAAAGGAAGTTTTACACAGGGAACGAATAAAGGACCCGGGCTGACACAA ATCACGTATGGGTTTTTGATAAAAAGGGGATGAGAGGCAAGGTATCGCAA TCACTTGTCTTTTAAATAGAGACCTGTATGAATGGCCAAACGAAGGCTTA ACTGTCTCCCCTTTCAAGTCAGTGAAATTGATCTACCCGTGCAGAAGCGA GTATAATCATACAAGACGAGAAGACCCTTTGGAGCTTAAGGTACAAAATT CAACCACGTCAAACAACTCAATAAAAAGCAAAAACTTTGCGGAACATGAA CTTTTACCTTCGGTTGGGGCGACCACGGAGAAAAACAAAACCTCCAAGTG GATTGGGATAATTTTCCTAAAACCAAGAGAGACATCTCTAAGCCTCAGAA TATCTGACCAAAAATGATCCGGTCACCAAGACCGATCAACGAACCAAGTT ACCCTAGGGATAACAGCGCATTCCTCTCCCAGAGTTCGTATCAACGAAGA GGGTTTACGACCTCGATGTTGGATCAGGACATCCTAAATGGTGCAGCCCG CTAATTAGGGGTCCGTTTTGTTTCAACGATAAAGGTCCTACGTGATCTGA GTTTCAAAACCCGGAGCTCGCCTGTTTTCAAAAACATAAAACTGGATCTT AGGTTTCAAACGGGGGG
Horabagrusbrachysoma	ACTGCTTTAATAGGACGGATCGCCTTTTTACCCAGATGATTTTGATTTGC CGCAGAACGGGGACCGTGCGAAGGTCGCGCAATCACTTGTCTTTTAAATG AAGACCTGTATGAATGGTGGAACGAGGGCTTAACTGTCTCCCCTTTCAAG TCAATGAAATTGATCTGCCCGTGCAGAAGCGGACATACAAATACATCACG AGAAGACCCTTTGGAGCTTAAGATACAGAATCAACTATGTCAAGAACCCT AAAATAAAGTTAAACTAAATAGCAACTGATCCCTATCTTCGGTTGGGGCG ACTGCGGGAGAAAATAAAGCTCCCATGCGGACTGGGGCCACCCCTAAAAC TAAGAGAGACATCTCTAAGTCACAGAACATCTGACCACAAAGATCCGGCC AATGGCCGACCAACGGACCAAGTTACCCTAGGGATAACAGCGCAATCCCC TTCCAGAGTCCATATCGACAGGGGGTTTACGACCTCGATGTTGGATCAGG ACATCCTAATGGTGCAGCCGCTATTAAGGGTTCGTTTGTTCAACGATTAA AGTCCTACGTGATCTGATTTCAAAAACCGGATTTCGCCTGTTTTTATTAA AAAACATGAGCGTGAATCTGATTTCAAAAACAGATTAG
Hypselobarbuskurali	CAAAATTCTTGACCAAAAAAACATTAGTGACGGATGAGGTGCTGCCCGAA AAAGACTATGATTTCAACGGCCGCGGTATTTTGACCGTGCAAAGGTAGCG CAATCACTTGTCTTTTAAATAGAGACCTGTATGAATGGCCAAACGAGGGC TTAACTGTCTCCCTTTTCAAGTCAGTGAAATTGATCTACCCGTGCAGAAG CGGGTATAATAATACAAGACGAGAAGACCCTATGGAGCTTAAGGTACAAA ACTCAACCACGTCAAGCAACTCAATGAAAAGAAAAAACTTTGTGAAACAT GAGATTTTACCTTCGGTTGGGGCGACCACGGAGGAAAAAAAAGCCTCCAA GTGGAATGGAACAAATTCCTAAAACCAAGAGAGACATCTCTAAGCCACAG AACATCTGACCAAACATGATCCGGCCCAAAAAAGCCGATCAACGAACCAA GTTACCCTAGGGATAACAGCGCAATCCCCTCCCAGAGTCCATATCGACGA GGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCAGCCG CTATTATGGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTT TCAGACCAGGACTCGGCCTGTTTTATCAAAAAACAAATACGTCGAGCTGA GTTCAGAGCAGGGACCGGCGTTC

Arrhamphussclerolepis	CCCACACGATTTTACAAGGGACGAGAGTAGACGGATCGCTGTGAAACATA TCACGTATGCGTTTTTGAGAACAGGCGATCCGTTTTGAACCGCAATCACT TGTCTTTTAAAAGAAGACCCGTATGAATGGCTCCACGAGGGCTTAACTGT CTCTTTCCCCCAGTCAATGAAATTGATCTCCCCGTGCAGAAGCGGGGATC CTCACATAAGACGAGAAGACCCTTTGGAGCTTTAGACAAAAAGTAGACCA TGTCCCCACCCCCAAATAAAAGGAATGAACTAATTGGTCCCTACCCTTCG TCTTTGGTTGGGGCGACCGCGGGGAAACAAAAAACCCCCATGTGGAACGG AAGACTATCTTCTATAACCAAGACCCACAGCTCTAAGTTTCAGAATTTCT GACCAAAAAGATCCGGCACCGCCGATCAACGGACCGAGTTACCCTAGGGA TAACAGCGCAATCCCCTTTTAGAGCCCATATCGACAAGGGGGTTTACGAC CTCGATGTTGGATCAGGACATCCTAATGGTGCAGCCGCTATTAAGGGTTC GTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTCAGACCGGATGTC GCCTGTTTATCAAAAACATATACGTGATCTGATTTCAAACCGGGGGGCTC GTGACTAAATATGATCCT
Mastacembelusarmatus	GCGGAAAAAATTGAACGGATCCGCGGCTGACAAAAAAACGTCCGGGTTTT TGAGAAGACGCGAGGACCGTTTAAGGTAGCGCAATCACTTGTCTTTTAAA TGAAGACCTGTATGAATGGCATAACGAAGGCTTAACTGTCTCCTTTCTCT AGTCAATGAAATTGATCTCCCCGTGCAGAAGCGGGGATAATCCCATAAGA CGAGAAGACCCTGTGGAGCTTTAGACACAAAGCAGATCTGGCAAACCCAC ACCCCCCCAAGCTAAAAGAACCCTGCTCTAATGTCTTCGGTTGGGGCGAC CACGGGGAAACAAACAACCCCCACGCAGAATAAGAGAACACCCTCTTAAA AACGAGAGCCACCACTCCAAAAAACAGAACTTCTGACTACAAGATCCGGA AACACCGATCAACGAACCAAGTTACCCCAGGGATAACAGCGCAATCCTCT TCAAGAGCCCATATCGACAGGGGGTTTACGACCTCGATGTTGGATCAGGA CATCCTAATGGTGCAGCCGCTATTAAGGGTTCGTTTGTTCAACGATTAAA GTCCTACGTGATCTGAGTTCAGACCGGATCCCCGCCTGTTTATCAAAGAA ATATACGTGAGTTGAGTTTACAGAGGATCCGGCGGTTTTGCAAAAAAATT GTT
Macrognathusaral	GAAACAAAAATGTTAAGACGGCGAGAGCGACGGATCCGGTCTGACAAATA AACGTACTGTTTTTGAAAACAGGCGACCGGGTGCACGTATTAAGCACTTG GCTTTTAAATGAAGCCCGTATGAATGGCATAACGAAGGCTTAACTGTCTC CTTTTTCAAGTCAATGAAATTGATCTCCCCGTGCAGAAGCGGGGATATAT ACATAATACGAGAAGACCCTGTGGAGCTTTAGACACAAAGCAGATCTGGC CAAAACCCACACAACCCAAGCTAAAAGAATCCTGCTCCCATGTCTTCGGT TGGGGCGACCACGGGGAAACAAATAACCCCCACGCAGAACAAGAGCACAC CCTCTTAAAAACGAGAGTCACCACTCCAAAAAACAGAACCTCTGACTATA AGATCCGGTAATACCGATCAACGGACCAAGTTACCCCAGGGATAACAGCG CAATCCCCTTTAAGAGCCCATATCGACAGGGGTTTACGACCTCGATGTTG GATCAGACATCCTAATGCTGCAGCCGCTATTATGGTTCGTTTGTTCAACG ATTAAAGTCCTACGTGATCTGAGTCAAACCAGAACT
Mystusoculatus	GAATATATAATTCGGACGGGACCGGCTTTGTCTGCAAAGATGACTAGTTA ACGGCCGCGGTATTTTGACCGTGCGAAGGTCGCGCAATCACTTGTCTTTT AAATGAAGACCTGTATGAATGGTGGAACGAGGGCTTAACTGTCTCCCTCT TCAAGTCAATGAAATTGATCTGCCCGTGCAGAAGCGGGCATACCCATATT TCGACAGGAAGACCCTTTGGAGCTTAAGATGTAAGATCAACTATATCAAG AATTACCAAAATTAATTAAACTAAATAGCAACTGATCCCTATCTTCGGTT GGGGCGACCACGGGAGAAAACAAAGCTCCCACGCGGACTGGGGACACCCC CTAAAACTAAAAAAAGACATCTCTAAGTCACAGAACATCTGACCACAAAG ATCCGGCCGCCGGCCGATCAACGGACCAAGTTACCCTAGGGAATAACAGC GCATTCCTCTTCCCAGAGTCCATATCGACAGAGGGTTTACGACCTCGATG TTGAATCAGGACATCCTATGGTGCAGCCGCTATTAGGGTTCGTTTTGTTC AACGATAAGTTCTACGTGATCTGATTCCAACCGGATCGTCCTGTTTTTAA ATCATATTCTGATCTGATTTCACACGGGTGG

Nandusmarmoratus	CCGAATTTAAATAAAACGAGATAGGCTTTATACAAAAATTGTATGGGTTT TAGACAAAAAGGGGAGACCGTGCAAAGGTATCGCAATCACTTGTCTTTTA AATGAAGACCTGTATGAATGGCATAACGAGGGCTAAGCTGTCTCCTTTTT CCAGTCAATGAAATTGATCTCCCCGTGCAGAAGCGGGGATGGCCCCATAA GAGAGAGAAGACCCTATGGAGCTTTAGACACCAAACAGATTTTGTAAAAC CTTCCCCCCTCACACGGGAACGAACAAAAAACACCTGTCCTAATGTCTTT GGTTGGGGCGACCGCGGGGAAACAAAAAACCCCCACGTGGAACGGAGACA CCTTCCCCTGAAATAAGAGTTACAACTCTAACTAGCAGAACTTCTGACCT TATGATCCGGCATACGCCGATCAACGAACCGAGTTACCCTAGGGATACAG CGCAATCCTCTTTTAGAGCCCATATCGACAGAGGGTTTACGACCTCGATG TTGGATCAGGACATCCCTAATGGTGCAGCCGCTATTAAGGGTTCGTTTGG TCAACGATTAAGTCTACGTGATCTGATTCAACCGGGACTCTGCCTGTTTA AATACATCACGGTATCTGATTACAATCGGAAAAATATATAACCTCATA
Puntiusparrah	GGTTCGAGAGTTAGGCAGGACCAGTTTGCGGTTGTCAAGTGATACTAGAT TTAAAGGCCGCGGTATTTTGACCGTGCAAAGGTAGCGCAATCACTTGTCT TTTAAATAAAGACCTGTATGAATGGCTAAACGAGGGCCTAACTGTCTCCC TTTTCAAGTCAGTGAAATTGATCTGCCCGTGCAGAAGCGGACATAACCAT ACAAGACGAGAAGACCCTATGGAGCTTAAGGCATAAAATTCAACCACGTC AAACAACTCTATAAAAAGCCACCAAACCTTGTGGTAAATGAAACCGTGCC TTCGGTTGGGGCGACCATGGAGGAAAGAAAAGCCTCCAAGTGGGCTGGGA TTACATTCCTAAAACCAAGAAAGACATTTCTAAGCCACAGAACATCTGAC CAATAATGATCCGGCCCATGTGGCCGATCAACGAACCAAGTTACCCAAGG GATAACAGCGCAATCCTCTCCCAGAGTCCATATCGACGAGGGGGTTTACG ACCTCGATGTTGGATCAGGACATCCTAATGGTGCAGCCGCTATTAAGGGT TCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGATTTCAGACCAGGAT CTTCGCCTGTTTTATCAAAAACATATACGTGATCTGTAGTTCAAGAGCAG GAGCCG
Puntiuspunctatus	AAAAGGAGCAAGAATGCGTGACTAGAAGGAAAGGCTGTATGCCAAATGAC CATGGGTTTTTGGCCGCGGTATTTTGACCGTGCACAGGTAGCGCAATCAC TTGTCTTTTAAATAAAGACCTGTATGAATGGCTAAACGAGGGCTTAACTG TCTCCCATCTCTAGTCAGTGAAATTGATCTCCCCGTGCAGAAGCGGGCAT AAAAATCCAGAGACGAGAAGACCCTTTGGAGCTTAAGGTACAAAACTCAA CCACGTTAAACAACTCTATAAAAAGCAGCTTAAACCTTGTGGATACTGAG ACCATACCTTCGGTTGGGGCGACCACGGAGGAAAATAAAGCCTCCAAGTG GACTAGGGCCAACCCCCTAAAACTAAGAGAGACATCTCTAAGCAACAGAC ATCTGACCATACATGATCCCGGCTAACCCAGCCGATCAACGAACCAAGTT ACCCTAGGGATACCAGCGCATCCCCTCCCAGAGTCCATATCGACGAGGGG GGTTTACGACCTCGATTGTCGCATCAGGACATCCTAATGTTGCAGCCGCT TATAAGGATTCGATTTGTTCCACCGATTAAAGTCCTTACCGTGGATCTGA GTTCACAACACGAGA
Pseudambassisranga	GGACTTAACGCAAAGAACGTTTATAGACGGACCCGGTCTGAAAAAAAAAA GCATATGTTTTTGATAAACGGCGTGACTTTTTAACTTAAAGCAATCACTT GTCTTTTAATAGAGACCTGTATGAATGGCATAACGAGGGCTTGACTGTCT CCTTTTTCCAGTCAATGAAATTGATCTCCCCGTGCAGAAGCGGGGATAAT AACATAAGACGAGAAGACCCTGTGAAGCTTTAAGACAATAAGCAGACCAA ACTAACCACCCCGAACAAAAGGACTAAAGCACAGGACCCTGCCCTAATGT CTTCGGTTGGGGCGACCACGGAGAAACAAAAAACCCCCATGTGGACCAGG AGCACCCTTACTTCCCCTCCCCCTCCTACAACCAAGAGTTACAACTCAAA GTACCAAAACCCTTGACCAAACATGATCCGGCAACGCCGATTAACGAACC AAGTTACTCCAGGGATAACAGCGCAATCCCCTTCTAGAGCCCATATCGAC AAGGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCAGC CGCTATTAAGGGCTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAG TTCAAACAAAGATTCCCGCCTGTT

Strongylurakrefftii	CCAAATTTATTTAAACAGGACCGCGGGTTTACTGTGATATATTTTGTTTT TGCGAAATTGGGACCGTGCAAGGTAGCGCAATCACTTGTCTTTTAAATGA AGACCTGTATGAATGGCATAACGAGGGCTTAACTGTCTCCTTTTTCCAGT CAATGAAATTGATCTCCCCGTGCAGAAGCGGGGATAATTACATAAGACGA GAAGACCCTATGGAGCTTTAGATAAGAGATGGACCATGTTAATAAATCTA TTTAATAAGAACAAACTTATTGGTCACCATTTTAATATCTTTGGTTGGGG CGACCGCGGGGTAATAATTAACCCCCAAGTGGAAAAAGAGAACCTCTCTC ATAACCTAGACTGACAAATCCAAGTATCAGAAAATCTGACCAAAAATGAC CCGGCAAAGCCGATCAACGAACCGAGTTACCCTAGGGATAACAGCGCAAT CCTCTCCTAGAGCCCATATCGACAGAGGGTTTACGACCTCGATGTTGGAT CAGGACATCCTAATGGTGCAGCCGCTATTAAGGGTTCGTTTGTTCAACGA TTAAAGTCCTACGTGATCTGATTCAAAAACGGGAGCTCCTCTGGTTTATA AAAAAAAAAAAACGTGATCTGAGTTCAACAACCCGGTTGTTTTTTTTTAA AAAAAAATATTTG

NJ Tree based on 16 s sequences generated in the present study (Neighbour joining)



K2P Genetic distance based om 16 s sequences

Garra_mullya																								
Hypselobarbus_kurali	0.072																							
Labeo_dussumieri	0.069	0.052																						
Dawkinsia_filamentosus	0.087	0.057	0.059																					
Puntius_punctatus	0.118	0.093	0.077	0.082																				
Puntius_mahecola	0.110	0.092	0.092	0.105	0.116																			
Puntius_parrah	0.104	0.079	0.071	0.079	0.098	0.084																		
Puntius_sp.	0.105	0.082	0.086	0.081	0.124	0.129	0.097																	
Rasbora_rasbora	0.151	0.121	0.121	0.127	0.154	0.154	0.129	0.113																
Danio_aequipinnatus	0.183	0.148	0.160	0.152	0.199	0.186	0.186	0.172	0.163															
Salmostoma_bacalia	0.159	0.124	0.134	0.119	0.152	0.170	0.151	0.139	0.135	0.177														
Opsarius_bakeri	0.126	0.118	0.123	0.127	0.144	0.171	0.151	0.157	0.144	0.154	0.084													
Schistura_scaturigina	0.110	0.097	0.094	0.105	0.122	0.148	0.117	0.124	0.148	0.172	0.157	0.137												
Mystus_oculatus	0.175	0.165	0.150	0.162	0.174	0.173	0.150	0.185	0.189	0.239	0.206	0.203	0.170											
Ompok_malabaricus	0.206	0.169	0.148	0.177	0.181	0.189	0.174	0.191	0.193	0.246	0.220	0.238	0.187	0.142										
Horabagrus_brachysoma	0.155	0.120	0.109	0.115	0.132	0.134	0.133	0.147	0.165	0.198	0.162	0.176	0.145	0.094	0.102									
Strongylura_krefftii	0.224	0.191	0.210	0.213	0.226	0.234	0.211	0.189	0.224	0.296	0.220	0.247	0.207	0.240	0.236	0.203								
Aplocheilus_lineatus	0.249	0.215	0.226	0.223	0.227	0.226	0.238	0.252	0.251	0.286	0.253	0.226	0.217	0.237	0.235	0.223	0.173							
Etroplus_maculatus	0.207	0.172	0.183	0.189	0.195	0.184	0.192	0.178	0.201	0.259	0.207	0.210	0.194	0.217	0.217	0.179	0.163	0.140						
Etroplus_suratensis	0.217	0.182	0.202	0.194	0.208	0.203	0.190	0.190	0.194	0.256	0.212	0.212	0.201	0.215	0.222	0.187	0.149	0.160	0.049					
Arrhamphus_sclerolepis	0.231	0.210	0.216	0.225	0.230	0.222	0.231	0.205	0.235	0.295	0.235	0.221	0.215	0.231	0.244	0.216	0.151	0.158	0.121	0.110				
Pseudambassis_ranga	0.232	0.191	0.205	0.218	0.215	0.218	0.214	0.209	0.226	0.302	0.227	0.224	0.209	0.252	0.257	0.227	0.163	0.187	0.137	0.132	0.142			
Nandus_marmoratus	0.271	0.224	0.246	0.250	0.252	0.243	0.244	0.249	0.244	0.325	0.269	0.275	0.243	0.262	0.253	0.239	0.172	0.179	0.140	0.116	0.152	0.163		
Mastacembelus_armatus	0.227	0.209	0.224	0.230	0.226	0.221	0.223	0.221	0.235	0.293	0.238	0.236	0.228	0.251	0.245	0.229	0.168	0.178	0.168	0.151	0.174	0.144	0.142	
Macrognathus_aral	0.243	0.218	0.240	0.216	0.238	0.225	0.230	0.231	0.252	0.287	0.246	0.252	0.247	0.252	0.253	0.223	0.194	0.185	0.172	0.167	0.189	0.165	0.169	0.059

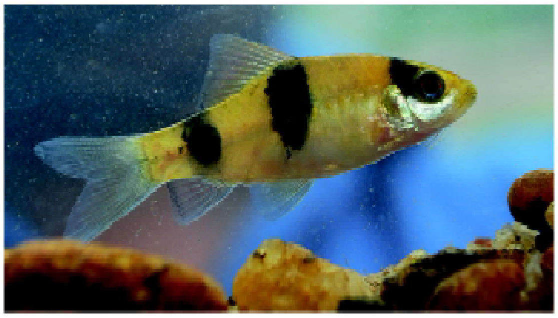
CONCLUSION

DNA based microgenomic species identification methods is analytically a powerful technique for the valid identification of fishes even from the early life stages like eggs and larvae. The integration of research areas for species identification can bring great benefits in biodiversity characterisation in regions where such information is lacking, avoiding the loss of endemic species that may perhaps occur in these regions, enabling the creation of conservation areas.

The reference library generated from 16s sequences, the diversity assessment and pattern of distribution of fishes along with the water quality analysis data, generated from the present study, offers as a base line data for launching a long term study on the deteriorating river and its fauna in order to plan and implement effective conservation measures for restoring Meenachil river, the life line of Central Kerala

PLATES

Amblypharyngodon melettinus



Haluduria fasciatus

Barilius bakeri

Barilius gatensis

Danio malabaricus

Dawkinsia filamentosa

Systomus subnasutus

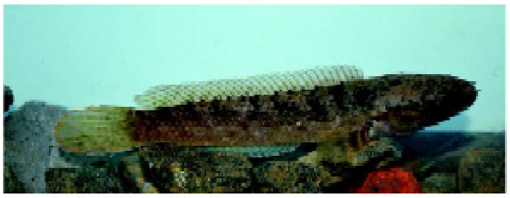
Pethiapunctata

Puntius mahecola

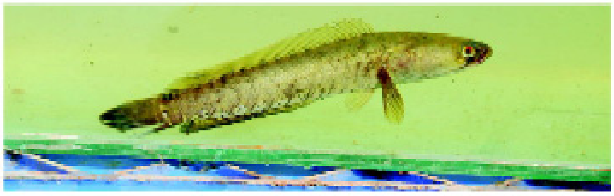
Pethia vittatus

Mesonoemacheilus triangularis

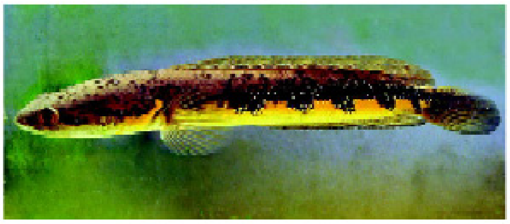
Lepidocephalicthys thermalis



Channa gachua



Channa striata

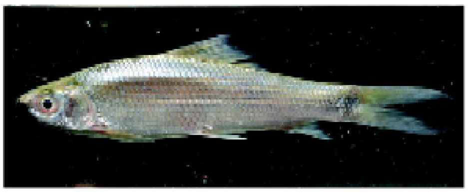


Channa marulius

Glossogobius giuris

Rasbora dandia

Salmophasia acinaces



Labeo dussumieri

Hyposelobarbus curmuca

Tor hudree

Garra mullya

Etroplus maculatus

Etroplus suratensis

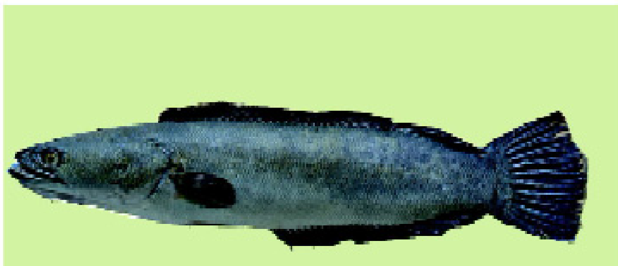
Heteropneustes fossilis

Horabagrus barchysoma

Aplocheilus lineatus

Horadandia atukorali

Anabas testudineus



Channa diplogramma

Teekoy headwaters- the river source

Marmala waterfalls stream

Pebble and cobbles substratum upstream

Collection from upstream

Poonjar tributary

Upstream tributary poonjar - kadaladimattom

Boulders in the upstream

Sampling at Bharananganam

Analysis of physico-chemical parameters of Meenachil river.

Sampling at Erattupetta

Post-monsoon fish sampling during winter at Pala, Mutholy

Monsoon sampling at Pala Mutholy

Trap net operation at Pala,Cherpunkal

Checkdams in Meenachil

Fishing near checkdams

Etroplus suratensis caught from Pala, Mutholy

Meandering of river

Fishing operations from Bamboo raft downstream

Sampling station at Nagampadom ,Kottayam.

Sampling at Kumarakom, gill nets

Interconnecting water courses of Meenachil river joining the Vembanadu lake

Meenachil river confluencing at India's second largest wetland ecosystem , the Ramsar site, Vembnadu lake

PUBLICATION

Paper: letha 2015. Riverine barcoding : a proposed DNA barcoding study on the fresh water fish species of Meenachil River of Kerala, India. Genome, 2015, 58(5): **163 – 303, 10.1139/gen – 2015 – 0087**

PAPERS UNDER PREPARATION

1. Indigenous fishing methods used in Meenachil river
2. Effect of hospital discharge on fishes inhabiting lower stretch of Meenachil river
3. Influence of checkdams on the pattern of distribution of some selected species of fishes in Meenachil river
4. Impact of houseboat tourism on the fish diversity of lower stretch of Meenachil river

References

1. Abraham, R.K., Kelkar, N. and Kumar, A.B. 2011. Freshwater fish fauna of the Ashambu Hills landscape, Southern Western Ghats, India, with notes on some range extensions. Journal of Threatened Taxa3: 1585-1593

2. Ajaykumar, B. 2003. The impact of land deterioration on the watershed health – a study on the physical components of the catchment area of the Vazhikadavu Dam of Meenachil River. Proceedings of the national seminar on river conservation and management, Thrissur organized by Limnological Association of Kerala: 31-41

3. Aliabadian M, Kaboli M, Nijman V, Vences, M (2009) Molecular identification of birds: performance of distance-based DNA barcoding in three genes to delimit parapatric species. PLOS One, 4(1): e4119. doc10.1371/journal.pone.0004119.

4. Biju C.R., Thomas R. & Ajithkumar C.R., 1999. Fishes of Parambikulam Wildlife Sanctuary, Palakkad District, Kerala. *J. Bombay. Nat. Hist. Soc*. 96(1): 82-87.

5. CLARK, R. & KING, J. (2004). The Water Atlas: Mapping the World's Most Critical Resource. Earthscan/James & James, London,U.K.

6. Clesceri L.S., Greenberg,A.E. & Trussell, R.R. 1989. *Standard methods for the examination of water and waste water, 17th edition*. APHA-AWWAWPCF, USA.

7. Dahanukar, N., R. Raghavan, A. Ali, R. Abraham & C.P. Shaji (2011). The status and distribution of freshwater fishes of the Western Ghats, pp. 21–48. In: Molur, S., K.G. Smith, B.A. Daniel & W.R.T. Darwall (compilers). The Status of Freshwater Biodiversity

in The Western Ghats, India. International Union for Conservation of Nature (IUCN) Gland, Switzerland & Zoo Outreach Organization (ZOO) Coimbatore, India, 116pp.

8. **Eschmeyer, W.N. (ed.)**, 2004. Catalog of fishes. Updated database version of January 2004. Catalog databases as made available to Fish Base in January 2004.
9. Ferri E, Barbuto M, Bain O et al. Integrated taxonomy. Traditional approach and DNA barcoding for the identification of filaroid worms and related parasites (Nematoda). Frontiers in Zoology, 6, 1–27.
10. Ficetola GF, Miaud C, Pompanon F, Taberlet P (2008) Species detection using environmental DNA from water samples. Biology Letters, 4, 423–425.
11. HARRISON, I. J. & STIASSNY, M. L. J. (1999). The quiet crisis. A preliminary listing of the freshwater fishes of the world that are extinct or 'missing in action'. In Extinctions in Near Time(ed. R. D. E. MacPhee), pp. 271–331. Kluwer Academic/Plenum Publishers, New York, U.S.A.
12. Hebert PDN, deWaard JR, Landry JF (2009) DNA barcodes for 1/1000 of the animal kingdom. Biology Letters 6: 359–362. doi: 10.1098/rsbl.2009.0848
13. JACKSON, R. B., CARPENTER, S. R., DAHM, C. N., MCKNIGHT,D. M., NAIMAN, R. J., POSTEL, S. L. & RUNNING, S. W.(2001). Water in a changing world. Ecological Applications 11,1027–1045.
14. Jayaram K.C. 1981. *Fresh water fishes of India handbook. Zoological survey of India*. Culcutta.
15. Jurado-Rivera JA, Vogler AP, Reid CAM, Petitpierre E, Gome Zurita J (2009) DNA barcoding insect-host plant associations. Proceedings of the Royal Society B: Biological Sciences, 276(1567), 639–648.

16. Kankare M, Stefanescu C, Van Houhuys S, Shaw MR (2005)Molecular phylogeny of Cotesia Cameron. parasitoids associated withMelitaeini butterflies (Insecta: Lepidoptera: Nymphalidae:Melitaeini). Journal of the Linnean Society, 86, 45–65.
17. King RA, Tibble AL, Symondson WOc (2008) Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms. Molecular Ecology, 17, 4684–4698.
18. Kochzius M (2009) Trends in fishery genetics. In: Beamish RJ, Rothschild BJ, editors. The future of fisheries science in North America. Dordrecht, The Netherlands: Fish & Fisheries Series 31, Springer. pp. 453–493.
19. KOTTELAT, M. & WHITTEN, T. (1996). Freshwater biodiversity in Asia with special reference to fish. World Bank Technical Paper 343, 1–59.
20. Kuhlmann M, Else GR, Dawson A, Quicke DLj(2007) Molecular,biogeographical and phenological evidence for the existence of three western European sibling species in the Colletes succinctus group (Hymenoptera: Apidae). Organisms, Diversity and Evolution , 7 155–165.,
21. Kurup B.M. 1994. An account on threatened fishes of river systems flowing through Kerala. *In Proc. Nat. Sem. Endangered Fish India*. pp. 129-140
22. LUNDBERG, G., KOTTELAT, M., SMITH, G. R., STIASSNY, M. L. J. &GILL, A. C. (2000). So many fishes, so little time: an overview of recent ichthyological discovery in continental waters. Annalsof the Missouri Botanical Gardens 87, 26–62.
23. Menon A.G.K. 1993. Rare and endangered fishes of Malabar, Seminar on the conservation of endangered fauna of Malabar paper 1, Abstracts, p. 1.NBFGR 1998. *Conservation assessment and management plan C.A.M.P Workshop Report*. India, Zoo Outreach Organisation/CBSG. pp. 1-156.

24. Padmini, V., Jjidheshini, K., Malini, T. G. and James, E. J. 2000. Water Balance study of the Meenanthara watershed of Meenachil River Basin of Kerala. Proceedings of the Twelfth Kerala Science Congress: 41-45.

25. **Plamoottil, M. and N.P. Abraham**, 2014. *Puntius viridis* (Cypriniformes, Cyprinidae), a new fish species from Kerala, India. J. Res. Bio. 3(7):1093-1104.

26. POSTEL, S. & RICHTER, B. (2003). Rivers for Life: Managing Water for People and Nature. Island Press, Washington D.C., U.S.A

27. Quinteiro J, Sotelo CG, Rehbein H, Pryde SE, Medina I, et al. (1998) Use of mtDNA direct polymerase chain reaction (PCR) sequencing and PCR-restriction fragment length polymorphism methodologies in species identification of canned tuna. J Agric Food Chem 46: 1662–1669.

28. Raju Thomas, K. and John George, M. 2010. Primary Hillstream Fishes of Muvattupuzha and Meenachil rivers, Central Kerala, India. Millenium Zoology, 11(1): 17 -19

29. SALA, O. E., CHAPIN, F. S., ARMESTO, J. J., BERLOW, R.,BLOOMFIELD, J., DIRZO, R., HUBER-SANWALD, E., HUENNEKE,L. F., JACKSON, R. B., KINZIG, A., LEEMANS, R., LODGE, D.,MOONEY, H. A., OESTERHELD, M., POFF, N. L., SYKES, M. T.,WALKER, B. H., WALKER, M. & WALL, D. H. (2000).Global biodiversity scenarios for the year 2100. Science 287, 1770–1774.

30. Santaclara FJ, Cabado AG, Vieites JM (2006) Development of a method for genetic identification of four species of anchovies: *E. encrasicolus*, *E. anchoita*, *E. ringens* and*E. japonicus*. Eur Food Res Technol 223: 609–614.

31. Sotelo CG, Calo-Mata P, Chapela MJ, Pérez-Martín RI, Rehbein H, et al. (2001) Identification of flatfish (Pleuronectiforme) species using DNA-based techniques. J Agric Food Chem 49: 4562–4569.

32. Strauss RE, Bond CE (1990) Taxonomic methods: morphology.

33. Talwar P.K. & Jhingran A.G. 1991. *Inland fishes of India and adjacent countries*. New Delhi, Oxford & IBH Publishing Co.

34. Teletchea F (2009) Molecular identification methods of fish species: reassessment and possible applications. Rev Fish Biol Fish 19: 265–293.

35. Thampatti, K. C. M. and Padmakumar, K. G. (1999). Rice bowl in turmoil: The Kuttanad wetland. Resonance, 4(3): 62-70.

36. Thapanjith, T., Narayanan, S. P., and Thomas, A. P. 2004. A study on the ichthyofauna of Aymanam panchayath in Vembanad wetland ecosystem, Kerala. Zoo's Print, 20(9): 1980-1982.

Annexure - III

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE IN RESPECT OF MINOR RESEARCH PROJECT

1. Name of Principal Investigator : Letha P.Cheriyan

2. Dept. of PI : Zoology

   Name of College : Mar Thoma College, Tiruvalla

3. UGC approval Letter No. and Date : (No. of. MRP/12th Plan/ 14 – 15/ KLMG035 SWRO dated 10th December 2014)

4. Title of the Research Project : Molecular Taxonomy of the Fishes Of Meenachil River, Kerala

5. Effective date of starting the project : 08.03.15

6. a. Period of Expenditure : From 08.03.15 to 12.12.17

   b. Details of Expenditure

S.No.	Item	Amount Approved (Rs.)	Expenditure Incurred (Rs.)
I	Books & Journals	10,000	14,734
Ii	Equipment	2,00,000	1,95,287
iii.	Contingency	40,000	41,000
iv.	Field Work/Travel(Give details in the proforma)	40,000	40,500
V	Special needs	30,000	30,245
vi.	Chemicals & Glassware	25,000	25,775

7. if as a result of check or audit objection some irregularly is noticed at later date, action will be taken to refund, adjust or regularize the objected amounts.

8. It is certified that the grant of Rs.2,77,500 (Rupees. Two lakhs seventy seven thousand five hundred only) received from the University Grants Commission under the scheme of support for Minor Research Project entitled Molecular Taxonomy of the Fishes Of Meenachil River, Kerala vide UGC letter No. F. MRP/12t Plan/ 14 – 15/ KLMG035 SWRO dated 10th December 2014 has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

SIGNATURE OF PRINCIPAL INVESTIGATOR PRINCIPAL

Annexure -VI

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002.

Annual/Final Report of the work done on the Minor Research Project.

1. Project report No. 1st /Final` : Final

2. UGC Reference No.F dated : (No. of. MRP/12th Plan/ 14 – 15/ KLMG035SWRO 10th December 2014)

3. Period of report : from: 08.03.2015 to 12.12.2017

4. Title of research project : Molecular Taxonomy of the Fishes Of Meenachil River, Kerala

5. (a) Name of the Principal Investigator : Letha P.Cheriyan

   (b) Deptt. : Zoology

   (c) College where work has progressed : Mar Thoma College, Tiruvalla

6. Effective date of starting of the project : 08.03.2015

7. Grant approved and expenditure incurred during the period of the report:

   a. Total amount approved. :3,45,000

   b. Total expenditure : 3,56,708

   c. Report of the work done : Report attached

i Brief objective of the project:

To increase our understanding of taxonomic and genetic biodiversity of the fish faun of Kerala by
a) Barcoding different fish species of Meenachil River and to access their population and distribution status, including endangered or threatened species by generating 16s mitochondrial gene sequences

b) Identifying the areas where considerably large population of these species is available and Thereby identifying the hotspots having rich diversity of threatened species
c) assessment of water quality parameters which can affect the distribution of fishes

ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication : Paper published in the International journal, Genome

Cheriyan 2015. (Riverine barcoding : a proposed DNA barcoding study on the fresh water fish species of Meenachil River of Kerala, India .) Genome, 2015, 58(5): **163 – 303, 10.1139/gen – 2015 – 0087**

iii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons : Yes

iv. please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to the concerned Regional Office of the UGC. : Bound copy of final report along with summary is attached

v. Any other information

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

PRINCIPAL

(Seal)