

Biometric and DNA Barcoding of rosy barb *Puntius conchoni* (Hamilton, 1822) along Lower Anicut and Mananjeri, Tamil Nadu

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Abstract

Analysis on morphometrics (expressed cytochrome C Oxidase subunit I gene as % standard length and % head length), meristic characters and mitochondria sequence of the freshwater rosy barb *Puntius conchoni* have made to confirm the species identity. The investigations on phylogenetic relationship for between species of this family have been made by constructing neighbor-joining tree to fulfill the gap on taxonomical validation.

Keywords: Cyprinidae - *Puntius conchoni* - COI-Phylogeny-Neighbor-joining.

Introduction

Fishes are the most ancient and most numerous forms of species among the vertebrates. In Indian, region out of 2,500 species, the 1,570 are marine and 930 are freshwater inhabitants belong to 326 genera, 99 families and 20 orders (Talwar and Jhingran, 1991) [23]. The genus *Puntius* represents the largest number of cyprinid genera in Asian tropics. Furthermore, *Puntius conchoni* was found in north eastern India (Taki *et al.* 1977) [21], Kashmir and also found in Dal and Mansbal Lake (Kullander *et al.*, 1999) [12]. *Puntius* has long been recognized as a “catch all” genus for a variety of small tropical Asian cyprinids whose inter-relationships are poorly understood (Kottelat and Pithyagoda, 1989; Kullander, 2008) [13]. Despite, the revision of this genus by *Puntius* the taxonomical view continues to be ambiguous. There is general consensus that when fully revised many species will be placed into new or different genera. The genus *Puntius* comprise some 120 valid species of small to medium sized barbs mostly inhabits and breed in common water-bodies; therefore chances of interbreeding and hybridization are higher. Many species of this are considered as weed fishes while some of them are of ornamental.

The small-sized south Asian cyprinid species contained in the *Puntius conchoni* group, diagnosed by Taki *et al.* (1978) [22], are popular ornamental fishes with about 10 species regularly available. One of these species is generally known as the “Odessa barb” or “ruby barb” and was first introduced in the aquarium hobby in the early 1970s. It was said to have first appeared in pet fish enthusiasts’ circles in Odessa, Ukraine, from where it got the name, and was treated later variously as an undescribed species of unknown origin (Sterba, 1988), as a subspecies of *P. ticto* (Stallknecht, 1973) [20], or as *P. conchoni* (Axelrod *et al.* 1980) [1].

Molecular techniques have become a major tool for systematic ichthyologists and may also be useful to fishery biologists for ratification of taxonomic problems at species level (Ciftci *et al.*, 2002, Okumus *et al.*, 2003 and Povh *et al.*, 2008) [2, 14, 16].

Modern taxonomic work regularly employs an internal anatomy, physiology, behavior, genes, isozymes, geography and morphological characters which remain the cornerstone of taxonomic treatments (Ward *et al.*, 2009) [25]. However, there are some difficulties in relying primarily on morphology when attempting to identify fishes during various stages of their development. Even when intact adult specimens are available, the morphological characters used to discern species can be subtle that identification is difficult, even for trained taxonomists (Ward *et al.* 2009) [25]. It is well recognised that DNA-based identification system or commonly known as DNA barcoding (Hebert *et al.* 2003) [6], can aid the resolution of the vast diversity of life with its millions of species (Hebert *et al.* 2003a and b and Tautz *et al.*, 2003) [5, 24]. In addition, DNA barcodes have been obtained for more than 8000 species of fish and COI sequences deposited in the Barcode of Life Data Systems (BOLD) online workbench and repository (Ragnasingham and Hebert 2007) [17]. So far, the present study aims to describe the taxonomic identity of rosy barb *Puntius conchoni* from Lower Anicut (Kollidam River) and Mananjeri Sluice.

Materials and methods

The Lower Anicut (Kollidam River) inhabits ornamental and edible fishes like *Puntius* species. Species were collected from Lower Anicut 11° 15'N latitude and 79° 30' E longitude (Figure-1) and another sampling were collected from Mananjeri Sluice. Measurements were taken point to point with digital calipers measuring to 0.01 mm, rounded to nearest 0.1 mm. *Puntius conchoni* specimens were captured by cast nets (2-4 cm squares size) and were brought to the laboratory in live conditions. Specimens were mopped by filter paper to remove excess of water from their body surface, thereafter recorded to the biometric characters. *Puntius* individuals were identified at species level following by Smith *et al.*, (1945) [19]. Morphometric and meristic characters were followed by Hubbs *et al.*, (1964) [7], Dwivedi *et al.*, (1974) [4], Jayaram, 1984 and

1999. Body measurements were expressed as percentage of standard length (% SL); head length (% HL). In the present study, 50 - morphometrics (Standard length = $Nbc/SL*100$) and Head length = $HLC/HL*100$) and 20-meristics characters of the genus *Puntius*. The various statistical values were made by using SPSS 16.0 version.

After photography, collected specimens were preserved in 7% aqueous formaldehyde solution. Separated jar with label was used for preserving individuals. Furthermore, the specimens were identified by Jayaram (2013) [10].

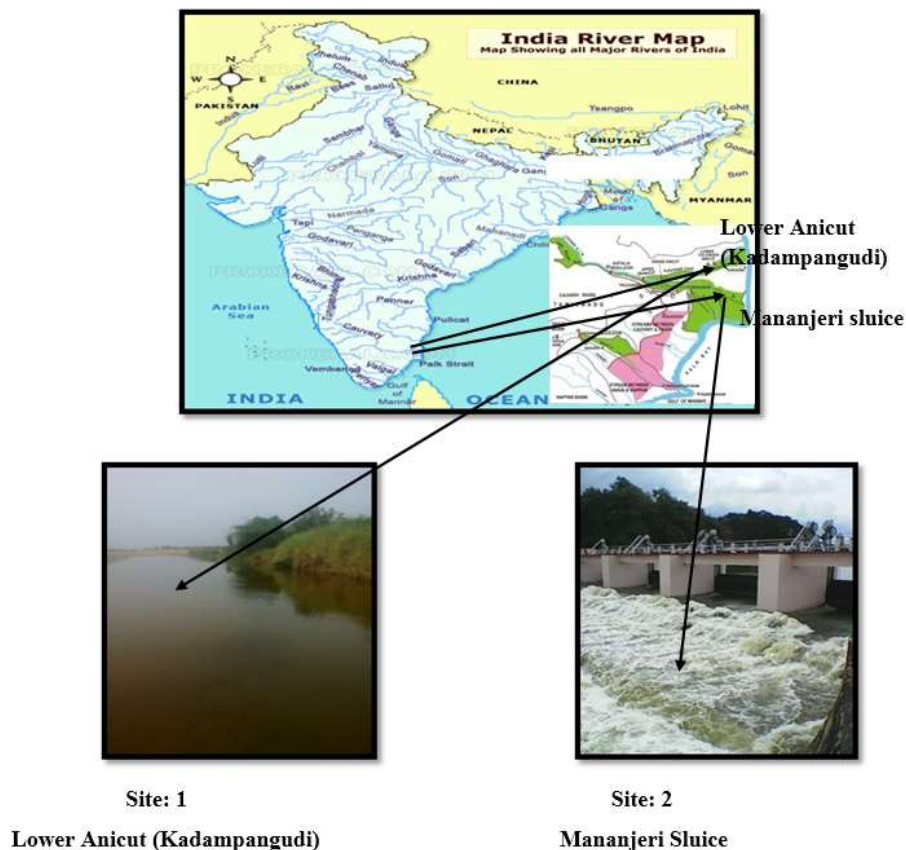


Fig 1: Sample collection sites

Systematic Classification

Kingdom : Animalia
 Phylum : Actinopterygii
 Order : Cypriniformes
 Family : Cyprinidae
 Genus : *Puntius*
 Species : *Puntius conchoni* (Hamilton, 1822)
 Common Name: Rosy barb or Red barb
 Vernacular Name: Patta salli and Mullu salli (in Tamil)

Synonyms

Cyprinus conchoni (Hamilton, 1822)
Systemus conchoni (McClelland, 1839) and (Bleeker, 1853)
Systemus pyropterus (McClelland, 1839) and (Bleeker, 1853)
Barbus pyropterus (Gunther, 1868)
Barbus conchoni (Day, 1878)
Barbus (Puntius) conchoni (Hora & Law, 1941)
Puntius conchoni (Chauhan & Ramakrishana, 1953)
Puntius conchoni khagariansis (DattaMunshi & Srivastava, 1988)
Pethia conchoni (Pethiyagoda, Meegaskumbura & Maduwage, 2012)

Species Authority: *Puntius conchoni* (Hamilton, 1822)

Refers to www.fishbase.com, Sanjay Molur *et al.*, 1998, and www.wikipedia.co.in

Colour

The body colour in black-olive back; with present in yellowish olive appeared of belly. Conspicuous black spot near the base of the anal fin. Tip of dorsal fin and anal fin side spread in black and reddish – brown colour appeared. Caudal near side one black spot surrounding of the side appeared in Greenish-yellow colour and dorsal fin, anal fin tip of side black colour spread.

Description

Body deeply compressed with large and reflective scales; Concave type of anal fin and no barbels. The spot located in each side of 17-20th lateral line scales, dorsal fin spine serrated and Incomplete lateral line.

Distribution

Bangladesh, Afghanistan, India (Ganga, Brahmaputra, Mahandi and Cauvery river systems), Nepal, and Pakistan (Indus river drainger) (Talwar and Jhingran, 1991) [23].

Biometric (Morphometric and Meristic) analyses

Minimum and maximum ranges of body and Head characters

The values of the two sampling sites (Lower Anicut and Mananjeri Sluice) 10 *Puntius conchoni* were analysed in different body and head characters are represented in Table 1.

1. Standard length, 2. Snout to urocentrum, 3. Pre anal length, 4. Pre dorsal length, 5. Pre pelvic length, 6. Pre pectoral length, 7. Occipital length, 8. Peduncle length, 9. Dorsal origin / Pelvic insert, 10. Dorsal spinous height, 11. Anal fin height, 12. Peduncle depth, 13. Caudal fin length, 14. Dorsal fin height, 15. Pectoral fin length, 16. Pelvic fin length, 17. Pelvic auxiliary scale Length, 18. Occiput to dorsal origin, 19. Occiput to Pectoral insert, 20. Occiput to Pelvic insertion, 21. Dorsal insert to Pelvic insert, 22. Dorsal origin \ Pectoral insert, 23. Dorsal

origin to anal org, 24. Dorsal insertion / Caudal, 25. Dorsal insertion / anal org, 26. Dorsal insert \ anal insert, 27. Dorsal fin base Length, 28. Anal fin base Length, 29. Pectoral insert / Pelvic insert, 30. Pectoral insert \ anal origin, 31. Pelvic insert to anal origin, 32. Post-dorsal length, 33. Body depth, 34. Distance b/w pectfin / vent, 35. Distance b/w pelvic fin / vent, 36. Head length, 37. Snout to opercle, 38. Upper jaw length, 39. Snout length, 40. Pre nasal length, 41. Orbit width, 42. Inter orbital width, 43. Inter nasal width, 44. Head width, 45. Gape width, 46. Lower jaw to isthmus, 47. Head depth at nostril, 48. Head depth at pupil, 49. Head depth at occiput, 50. Total length.

Table 1: Morphometric characters of *Puntius conchoni* from lower Anicut and Mananjeri Sluice

	Characters	Lower Anicut n=05		Mananjeri Sluice n=05	
		Range	Mean±STD	Range	Mean±STD
Body Characters (%)					
1	Standard Length	74.92-80.99	78.08±2.80	73.99-76.38	75.24±1.00
2	Snout to urocentrum	93.44-95.52	94.82±0.89	93.00-95.49	94.62±1.03
3	Pre anal length	72.63-75.66	74.01±1.43	71.96-77.22	73.56±2.14
4	Pre dorsal length	51.36-57.72	54.03±2.67	53.68-57.73	55.65±1.88
5	Pre pelvic length	50.20-52.98	51.39±1.16	50.20-51.59	50.96±0.55
6	Pre pectoral length	26.38-28.37	26.95±0.82	27.07-28.28	27.66±0.48
7	Pre occipital length	19.24-23.41	21.32±1.79	21.54-23.38	22.26±0.73
8	Peduncle length	12.08-10.13	10.99±0.86	10.94-13.96	12.30±1.31
9	Dorsal origin / Pelvic insert	41.47-45.33	42.88±1.57	40.07-45.28	42.84±2.30
10	Dorsal spinous height	17.69-25.65	21.24±3.37	20.04-25.42	23.80±2.14
11	Anal fin height	14.71-16.50	15.32±0.70	14.97-16.95	15.89±0.79
12	Peduncle depth	15.45-16.58	15.99±0.47	14.70-16.39	15.24±0.65
13	Caudal fin length	29.88-34.05	31.95±1.66	30.31-34.03	31.54±1.53
14	Dorsal fin height	16.56-23.79	19.58±2.67	23.76-29.45	27.81±2.31
15	Pectoral fin length	19.54-24.81	22.33±2.38	22.21-24.78	22.94±1.09
16	Pelvic fin length	18.64-23.10	20.66±1.66	19.69-20.77	20.34±0.43
17	Pelvic auxiliary scale Length	6.88-9.70	8.51±1.19	6.01-9.08	7.30±1.11
18	Occiput to dorsal origin	29.49-36.86	32.80±2.93	33.26-36.85	35.34±1.63
19	Occiput to Pectoral insert	27.42-27.79	27.59±0.15	24.14-27.37	26.14±1.43
20	Occiput to Pelvic insertion	43.88-48.96	47.26±1.99	45.14-47.81	46.68±1.05
21	Dorsal insert to Pelvic insert	36.25-41.45	39.01±2.36	35.99-40.57	38.76±2.29
22	Dorsal origin \ Pectoral insert	38.80-43.20	40.74±1.98	37.5-41.97	40.07±2.04
23	Dorsal origin to anal org	41.97-48.18	44.89±2.46	42.20-45.36	43.89±1.16
24	Dorsal insertion / Caudal	26.35-28.96	27.90±1.20	25.30-30.93	29.22±2.31
25	Dorsal insertion / anal org	31.25-34.17	32.94±1.36	29.48-34.14	31.58±1.67
26	Dorsal insert \ anal insert	25.64-29.39	27.86±1.54	26.07-29.01	27.54±1.05
27	Dorsal fin base Length	20.18-21.15	20.65±0.42	19.81-22.48	20.79±1.04
28	Anal fin base Length	12.48-16.01	14.33±1.57	10.57-15.97	12.63±2.01
29	Pectoral insert / Pelvic insert	25.46-27.38	26.30±0.70	25.28-27.79	26.36±1.04
30	Pectoral insert \ anal origin	40.41-44.02	41.88±1.69	41.16-43.96	42.55±1.04
31	Pelvic insert to anal origin	12.79-20.67	17.20±2.89	16.78-19.70	18.30±1.08
32	Post-dorsal length	48.31-52.94	50.06±1.81	45.56-49.77	47.53±1.98
33	Body depth	39.07-44.85	42.70±2.51	42.05-43.98	43.33±0.76
34	Distance b/w pect fin / vent	43.35-47.93	45.49±1.89	43.31-45.85	44.74±1.29
35	Distance b/w pelc fin / vent	19.39-22.99	20.89±1.30	19.93-21.87	20.79±0.69
36	Head Length	26.82-29.87	28.48±1.42	27.17-29.87	28.63±1.14
Head Characters (%)					
37	Snout to opercle	66.53-75.42	70.82±3.47	68.30-77.36	73.64±3.49
38	Upper jaw length	18.29-27.14	23.36±3.29	22.36-25.22	23.23±1.24
39	Snout length	25.87-29.40	27.58±1.48	23.87-28.22	26.11±1.57
40	Pre nasal length	18.06-21.04	20.12±1.19	18.70-22.39	20.84±1.39
41	Orbit width	34.61-37.61	35.87±1.16	33.88-40.43	38.01±2.44
42	Inter orbital width	39.77-45.63	42.28±2.89	39.66-42.80	40.97±1.25
43	Inter nasal width	18.59-25.63	21.02±2.80	17.94-22.01	20.59±1.64
44	Head width	40.23-45.94	42.69±2.29	43.52-53.17	48.64±3.48
45	Gape width	24.18-27.93	26.42±1.43	23.93-26.44	25.70±1.08
46	Lower jaw to isthmus	65.70-67.81	67.10±0.85	64.18-73.85	68.73±3.50

47	Head depth at nostril	34.29-41.40	37.10±2.85	32.64-39.63	35.34±3.05
48	Head depth at pupil	58.11-65.60	60.33±3.02	60-64.79	62.44±1.89
49	Head depth at occiput	83.57-89.50	86.86±2.28	77.57-84.79	81.13±3.14

Meristic analysis of *Puntius conchoni*

Previously investigation reported that the number of Dorsal, Anal, Pelvic and Pectoral fin spines and rays were distinguished characters in these fishes by Francis Day, 1958, Talwar & Jhingran, 1991 and K.C. Jayaram, 1991.

Present investigations of 20 meristic characters were analyzed in 10 *Puntius conchoni* specimens and its counts were given in Table 2. The number of black spots, position and their dorsal fin spine and rays were distinguishing character in these fishes.

Table 2: Meristic characters of Remarks and Present investigation in *Puntius conchoni*

Sl. No.	Meristic counts	Remarks			Present Investigation	
		Francis Day (1958)	Talwar & Jhingran (1991)	K.C.Jayaram (1991)	Lower Anicut	Mananjeri Sluice
1.	Unbranched dorsal fin spine	iii	iii	III	IV	IV
2.	Branched dorsal fin rays	8	7-8	8	9	9
3.	Unbranched anal fin spine	ii	ii-iii	II	III	III
4.	Branched anal fin rays	5	5	5	6	6
5.	Unbranched pelvic fin spine	-	i	I	II	II
6.	Branched pelvic fin rays	9	8	8	7-8	8
7.	Unbranched pectoral fin spine	-	i	-	I	I
8.	Branched pectoral fin rays	11	18	11	13-14	13-14
9.	Caudal fin upper lobe		-	19	10	10
10.	Caudal fin lower lobe		-		9	9
11.	Upper transverse rows	5½/6½	-		5½	5½
12.	Lower transverse rows		-		5½	5½
13.	Lateral line to pelvic scale rows		-		5½	5½
14.	Lateral line scales	24-26	-	24-26	25-26	25-26
15.	Pre dorsal scales		-		9	9
16.	Circumpeduncular scales		-		12	12
17.	Circumferential scales		-		26	26
18.	Transverse breast rows		-		7	6
19.	Anal scale rows		-		1	1
20.	Pre anal scales		-		20-21	20-21

Molecular Identification and Phylogenetic Relationship

In the present study, Standard DNA barcode region of animal system 5' end of the mitochondrial Cytochrome oxidase subunit I gene (COI) was sequenced for molecular identification of *P. conchoni*. Totally 582 bp was sequenced in both individuals and were conceptually translated into proteins using vertebrate mitochondrial genetic code. Based on the BLAST analysis, >99% similarity and 100% sequence coverage with *Pethia conchoni* (AB863607) in the Gen bank nucleotide database, the present studied species was confirmed as *Puntius conchoni*.

In addition, phylogenetic analyses were performed using COI sequences among the *Puntius conchoni* individuals (Present study and JQ667569, JX260948, KF511543) and *P. khugae*

(KF511543), *P. terio* (JX260958) used as ancestors. The multiple sequence alignment showed no insertion and deletions and found eight variable sites within the *P. conchoni*, 17 variable sites found between *P. conchoni* and ancestor species. But no variations were found at the protein level. It is suggested that mutations in the nucleotides could not alter the proteins. The present studied species showed close relationship with other *P. conchoni* individuals and formed a single cluster. The genetic distance was very high between the ancestors as seen in Fig. 2. At same time, minimal genetic distance was observed between the individuals of *P. conchoni*. From all the molecular and phylogenetic analyses it was confirmed that the present studied species belonging to *P. conchoni*.

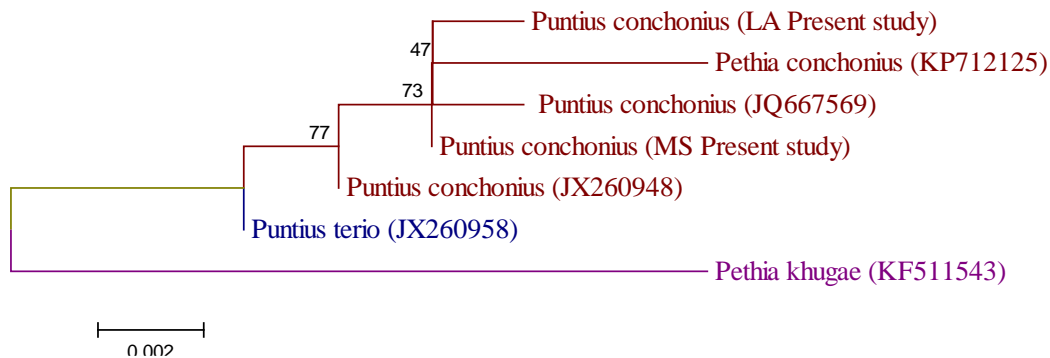


Fig 2: Neighbor – Joining phylogenetic tree of *Puntius* species based on the COI gene sequence data. The figures on the nodes indicate the bootstrap values and scale indicates the genetic distance.

Table 3: Kimura 2 Parameter (K2P) genetic distance in *Puntius conchonioides*

Species	<i>P. conchonioides</i> (MS Present study)	<i>P. conchonioides</i> (LA Present study)	<i>P. conchonioides</i> (JQ667569)	<i>P. conchonioides</i> (JX260948)	<i>P. conchonioides</i> (KP712125)	<i>P. khugae</i> (KF511543)	<i>P. terio</i> (JX260958)
<i>P. conchonioides</i> (MS Present study)	*****						
<i>P. conchonioides</i> (LA Present study)	0.002	*****					
<i>P. conchonioides</i> (JQ667569)	0.002	0.003	*****				
<i>P. conchonioides</i> (JX260948)	0.002	0.003	0.003	*****			
<i>P. conchonioides</i> (KP712125)	0.005	0.007	0.007	0.007	*****		
<i>P. khugae</i> (KF511543)	0.021	0.023	0.023	0.019	0.026	*****	
<i>P. terio</i> (JX260958)	0.003	0.005	0.005	0.002	0.009	0.017	*****

Discussion

Cypriniformes is the most dominant group throughout the River (53 species). *Danio rerio*, *Puntius conchonioides* and *Puntius sophore* were the most common species in the Beki River recorded by Gaurab Jyoti Kalita *et al.*, 2015.

Most of the morphometric characters of fishes are similar and often overlap within the population. This morphometric data are not enough to support the established genetic structure of the population often that leads to taxonomic uncertainty (Daniel, 1997; Ponniah and (Gopalakrishnan, 2000) [15].

DNA barcoding has become a promising tool for rapid and accurate identification of various taxa and it has been used to reveal unrecognized species in several animal groups. Animal DNA barcodes (600-800 base-pair segments) of the mitochondrial cytochrome oxidase I (COI) gene have been proposed as a means to quantify global biodiversity. DNA barcoding has the potential to improve the way the world relates to wild biodiversity (Janzen *et al.* 2005). Moreover, the introduction of DNA barcoding has highlighted the expanding use of the COI as genetic marker for species identification (Dawnay *et al.* 2007).

Puntius padamya is similar in proportions to other species of the *P. conchonioides* group, *P. didi*, *P. tiantian*, *P. macrogramma*, *P. thelys*, *P. nankyweensis*, and *P. erythromycter* (Kullander, 2008) [13], but shares a relatively small eye only with *P. tiantian*.

The multiple sequence alignment showed no insertion and deletions and found eight variable sites within the *P. conchonioides*, 17 variable sites found between *P. conchonioides* and ancestor species. But no variations were found at the protein level. It is suggested that mutations in the nucleotides could not alter the proteins. The present studied species showed close

relationship with other *P. conchonioides* individuals and formed a single cluster.

The results of the multivariate cluster analysis of the present study have been summarized in Table 3.

The present studied species showed close relationship with other *P. conchonioides* individuals and formed a single cluster. The genetic distance was very high between the ancestors. At same time, minimal genetic distance was observed between the individuals of *P. conchonioides*. From all the molecular and phylogenetic analyses it was confirmed that the present studied species belonging to *P. conchonioides*.

Among the population, sample from Lower Anicut and Mananjeri Sluice were genetically different among the other population and stands apart largely owing the high genetic diversity.

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