DNA barcoding revealed a new species of Neolissochilus Rainboth, 1985 from the Kaladan River of Mizoram, North East India

Article *in* Mitochondrial DNA Part A · April 2018

DOI: 10.1080/24701394.2018.1450398

CITATIONS
11

READS
1,042

4 authors, including:

Samuel Lalronunga
Mizoram University
61 PUBLICATIONS **263** CITATIONS

SEE PROFILE

Mahender Singh
ICAR-National Bureau of Fish Genetic Resources
132 PUBLICATIONS **1,300** CITATIONS

SEE PROFILE

Taylor & Francis
Taylor & Francis Group

Mitochondrial DNA Part A

DNA Mapping, Sequencing, and Analysis

ISSN: 2470-1394 (Print) 2470-1408 (Online) Journal homepage: http://www.tandfonline.com/loi/imdn21

DNA barcoding revealed a new species of Neolissochilus Rainboth, 1985 from the Kaladan River of Mizoram, North East India

Lalramliana, Samuel Lalronunga, Sumit Kumar & Mahender Singh

To cite this article: Lalramliana, Samuel Lalronunga, Sumit Kumar & Mahender Singh (2018): DNA barcoding revealed a new species of Neolissochilus Rainboth, 1985 from the Kaladan River of Mizoram, North East India, Mitochondrial DNA Part A, DOI: 10.1080/24701394.2018.1450398

To link to this article: https://doi.org/10.1080/24701394.2018.1450398

MITOCHONDRIAL DNA PART A, 2018
https://doi.org/10.1080/24701394.2018.1450398

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

DNA barcoding revealed a new species of *Neolissochilus* Rainboth, 1985 from the Kaladan River of Mizoram, North East India

Lalramliana[a], Samuel Lalronunga[a], Sumit Kumar[b] and Mahender Singh[b]

[a]Department of Zoology, Pachhunga University College, Aizawl, India; [b]ICAR-National Bureau of Fish Genetic Resources, Lucknow, India

ABSTRACT

Neolissochilus kaladanensis sp. nov., a new cyprinid species, is described from the Kaladan River drainage of Mizoram. It differs from all other valid *Neolissochilus* species in having higher number of gill rakers on the lower arm of the first gill arch (13–14 vs. 12 or below in all the species). The analysis of mitochondrial gene cytochrome c oxidase subunit I (COI) sequences separated *N. kaladanensis* sp. nov. from all other *Neolissochilus* and *Tor* species with an average genetic distance of 6.0%. It is further separated from the morphologically most similar species *N. hendersoni* and *N. soroides* by a genetic distance of 6.7% and 6.8%, respectively. Based on the lowest BIC and AICc scores, best fit model for COI dataset was TN93 + G + I, out of 24 different nucleotide substitution models tested. The maximum-likelihood (ML) phylogenetic tree was constructed using the COI sequences of representative *Neolissochilus* and *Tor* species. The anomalies observed among the GenBank sequences of the genera *Tor* and *Neolissochilus* are also discussed.

ARTICLE HISTORY
Received 10 November 2017
Accepted 6 March 2018

KEYWORDS
Cytochrome oxidase I; DNA barcode; genetic distance; *Neolissochilus kaladanensis* sp. nov.; sequence divergence

Introduction

The freshwater cyprinid fishes of the genus *Neolissochilus* Rainboth, 1985, also known as Mahseer, are medium to large fish, widely distributed throughout the southern and south eastern Asia (Rainboth 1991). The genus is characterized by having dorsal fin with four unbranched and 8/9 branched rays, last unbranched ray not serrated; pectoral fin with one unbranched and 13–17 branched rays, pelvic fin with one unbranched and 7/8 branched rays, anal fin with three unbranched and five branched rays, caudal fin forked with convex distal margin on each lobe, 20–29 + 2 or three lateral-line scales on the body, 6–10 predorsal scales; 16–20 circumferential scales; 12 circumpeduncular scales; scales large and heavy; lips thick, but not hypertrophied; pairs of maxillary and rostral barbels present; cheeks with numerous tubercles; gill rakers long, slender, with 2–6 rakers on epibranchial and 7–12 rakers on ceratobranchial; pharyngeal teeth with three rows (5,3,2) (Rainboth 1985).

Currently, there are 28 species of the genus *Neolissochilus* recognized as valid (Eschmeyer et al. 2017), and the identification to the species level is confusing due to overlapping morphological and meristic characters resulting in complicated taxonomy. The cryptic nature of the genus as a whole requires concerted efforts of taxonomists and molecular biologists to generate molecular signatures of morphologically well studied specimens. DNA barcoding, relying on approximately 650 nucleotides of the mitochondrial gene cytochrome c oxidase subunit I (COI), focuses on the setting up of DNA barcode sequence libraries of known species for unambiguous species identification in future (Hebert et al. 2003). The efficiency of DNA barcoding in fish species identification has been demonstrated by Ward et al. (2005), Hubert et al. (2008) and Lakra et al. (2016), as well as in mahseer species by Laskar et al. (2013).

Recent investigation of the Kaladan River and its tributaries in Mizoram, northeastern India, found a new species of *Neolissochilus*. Detailed morphomeristic comparisons of this material with congeners and analysis of their COI gene sequences revealed it as an unnamed species. The description of this material as *Neolissochilus kaladanensis* sp. nov. formed the basis of this study.

Materials and methods

Sample collection and morphological analysis

A total of 14 fish specimens were collected, 13 from the Kaladan River in the vicinity of Kawlchaw Village (22°25′52″N 92°56′29″E) and one from the Kaladan River in the vicinity of Darzokai Village (22°54′09″N 92°55′60″E). The specimens were fixed in 10% formalin and then transferred to 70% ethanol for longer preservation. Prior to fixation, small muscle tissues were excised from the right side of some specimens for DNA extraction. The meristic counts and morphological measurements followed Hubbs and Lagler (1964) and Rainboth (1985). For vertebral counts and other osteological studies, specimens were cleared and stained in alizarin. Vertebral counts included the first four vertebrae of the Weberian apparatus. Fin rays were counted using a stereomicroscope

CONTACT Mahender Singh dr.mahendersingh29@gmail.com ICAR-National Bureau of Fish Genetic Resources, Lucknow, India

Supplemental data for this article can be accessed here.

and were confirmed through cleared and stained specimens. The small posteriormost ray of the dorsal and anal fins, articulating with the same pterygiophore as the preceding ray, were counted as ½. Numbers in parentheses after a meristic value indicate the frequency of that value. Type specimens were deposited at the Zoological Survey of India (ZSI), Kolkata, India (Registration No. ZSI FF 7605) and Pachhunga University College Museum of Fishes (PUCMF), Mizoram, India.

Comparative materials

Neolissochilus (Barbus) compressus: ZSI F5513, 1, 142.8 mm SL; *Neolissochilus (Barbus) blythii*: ZSI F5553, 1, 45.8 mm SL; *Neolissochilus (Barbus) dukai*: ZSI F 2388, 1, 104.1 mm SL; *Neolissochilus hexagonolepis*: PUCMF 17016, 2, 102.3–150.1 mm SL; *Neolissochilus (Barbus) stracheyi*: ZSI F 2175, 1, 277.0 mm SL.

The published information used for comparisons were: McClelland (1839) for *N. hexagonolepis* and *N. hexastichus*; McClelland (1845) for *N. spinulosus*; Day (1869) for *N. blythii* and *N. compressus*; Day (1870) for *N. stevensonii*; Day (1871) for *N. stracheyi*; Day (1873), Ali et al. (2014) and Arunachalam et al. (2017) for *N. wynaadensis*; Day (1878) for *N. dukai*; Herre (1940) and Khaironizam et al. (2015) for *N. hendersoni* and *N. soroides*; Smith (1945) for *N. paucisquamatus*; Yinrui and Xinluo (1985) for *Tor hemispinus* and *T. qiaojiensis*; Chen et al. (1999) for *N. baoshanensis* and *N. heterostomus*; Vidthayanon and Kottelat (2003) for *N. subterraneus*; Arunachalam et al. (2017) for *N. acutirostris*, *N. capudelphinus*, *N. micropthalmus*, *N. minimus* and *N. tamiraparaniensis*.

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was isolated from 25 mg (approx.) of fish muscle tissue following standard phenol:chloroform:isoamyl alcohol method (Sambrook and Russell 2001). The DNA, after precipitation, was dissolved in TE buffer (10 mM Tris–HCl, 0.1 mM EDTA, pH 8), and Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA) was used for determining the concentration and quality. The fish universal primer pair Fish F1: 5′-TCAACCAACCACAAAGACATTGGCAC-3′ and FishR1: 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′ (Ward et al. 2005) was used to amplify the partial COI gene. The 15 µl PCR reaction contained 1× buffer, 100 µM dNTPs, 2 mM $MgCl_2$, 5 pm of each primer, 2 U Taq DNA polymerase and 100 ng template DNA. The amplification was done using Veriti 96 PCR machine (Applied Biosystems, Inc., Foster City, CA) and the DNA sequencing was performed following the dideoxynucleotide chain termination method (Sanger et al. 1977), using an automated DNA sequencer ABI 3500 (Applied Biosystems, Inc., Foster City, CA). All the amplifications and sequencing conditions followed Singh et al. (2017).

DNA sequence analysis

In the present study, COI gene sequences of three individuals of *N. kaladanensis* sp. nov. (NCBI Accession Nos. MG518431–MG518433) and three sequences of *Tor barakae* (NCBI Accession Nos. MG518434–MG518436) were generated. A total of 106 sequences of *Neolissochilus* and *Tor* species along with *Puntius chelynoides* as out group, were included from GenBank to make a comprehensive analysis and elucidate phylogenetic relationship (Supplementary Electronic Table 1). Sequences were aligned using CLUSTALW integrated in MEGA 7 (Molecular Evolutionary Genetics Analysis) software (Kumar et al. 2016). The generated sequences were blasted in NCBI (http://www.ncbi.nlm.nih.gov) for the nearest matches. The genetic distance between the groups was calculated by averaging pair wise comparisons of sequence across all individuals (Supplementary Electronic Table 2). The genetic distance of *N. kaladanensis* sp. nov. was plotted against 24 *Neolissochilus* and *Tor* species (Figure 1). Based on the lowest BIC (Bayesian Information Criterion) and AICc scores (Akaike Information Criterion, corrected), best fit nucleotide substitution model for present COI dataset was TN93 + G + I given by Tamura and Nei 1993 (Tamura–Nei + Gamma distribution with five rate categories + certain fraction of sites are evolutionarily invariable). The maximum-likelihood (ML) phylogenetic tree was constructed with 500 bootstraps using one representative COI

Table 1. Morphometric data for *Neolissochilus kaladanensis* sp. nov. ($n = 14$).

		Paratypes	
	Holotype	Range	Mean ± SD
Standard length (SL) in mm	126.5	69.8–400.2	
In percentage (%) of standard length			
Head length	25.0	23.9–28.7	26.1 ± 1.4
Body depth	36.9	33.4–37.8	35.9 ± 1.4
Body width at dorsal-fin origin	21.3	12.4–21.4	16.7 ± 2.7
Dorsal-fin base length	18.7	15.9–19.4	18.2 ± 0.9
Dorsal-fin height	31.4	24.2–34.6	31.5 ± 2.6
Dorsal spinous height	27.4	22.2–27.7	25.6 ± 1.8
Anal-fin base length	8.5	7.4–9.1	8.1 ± 0.5
Anal-fin height	18.7	20.2–22.6	21.3 ± 0.7
Pectoral-fin length	22.4	21.3–24.6	23.1 ± 0.9
Pelvic-fin length	20.3	20.2–27.4	21.8 ± 1.8
Pre-dorsal length	52.2	52.3–59.8	54.7 ± 1.8
Pre-pectoral length	25.8	23.6–28.9	25.9 ± 1.5
Pre-pelvic length	52.6	50.6–58.3	53.5 ± 2.0
Pre-anal length	77.2	76.3–86.3	78.6 ± 2.6
Post-anal length	26.9	24.5–27.8	26.0 ± 0.9
Pre anus length	74.9	73.8–83.3	76.5 ± 2.4
Post-dorsal length	51.7	32.5–57.1	52.6 ± 6.1
Post-pectoral length	55.5	72.9–83.5	77.0 ± 2.9
Post-pelvic length	52.1	48.3–56.3	50.7 ± 2.2
Caudal-peduncle length	18.4	13.7–18.3	17.2 ± 1.2
Caudal-peduncle depth	14.3	12.7–17.4	13.9 ± 1.1
Distance from pectoral fin to pelvic fin	28.5	27.2–32.8	29.0 ± 1.5
Distance from pectoral fin to vent	52.2	28.6–55.7	50.0 ± 6.5
Distance from pectoral fin to anal fin	54.6	52.5–65.1	55.1 ± 3.3
Distance from pelvic fin to vent	24.6	22.1–28.8	24.0 ± 1.9
Distance from pelvic fin to anal fin	27.3	24.3–31.7	26.6 ± 2.0
Anal to vent	2.2	1.6–2.3	2.0 ± 0.2
In percentage (%) of head length			
Head depth	87.0	73.3–82.1	76.7 ± 2.2
Head depth at pupil	61.4	56.3–85.5	60.4 ± 7.4
Head width	66.8	55.4–74.0	61.6 ± 5.8
Snout length	39.9	30.9–39.4	35.1 ± 2.6
Pre-nostril length	25.0	19.0–23.9	21.5 ± 1.2
Eye diameter/length	24.7	16.4–32.5	26.7 ± 4.3
Pre-occipital length	84.8	76.1–84.7	79.9 ± 2.3
Interorbital	44.9	29.0–50.7	38.4 ± 5.5
Internarial	24.7	18.8–28.5	21.9 ± 2.4
Upper jaw length	26.9	20.9–26.8	23.2 ± 1.5
Lower jaw length	21.8	15.7–20.3	4.8 ± 0.4

Table 2. Meristic comparison of *Neolissochilus kaladanensis* sp. nov. with closely related species of genus *Neolissochilus*.

Species	Lateral-line scales	Gill rakers on the lower arm of the gill arch	Predorsal scales	Transverse scale rows (lateral line to dorsal-fin origin)	Transverse scale rows (lateral line to pelvic-fin origin)	Circumferential scale rows	Circumpeduncular scale rows
N. kaladanensis sp. nov.	20–22	13–15	8–10	3½	2½–3	17–18	12
N. hexastichus	26	9	9–10	4½	3½	18	12
N. hexagonolepis	28–29	8	9–10	4½	3	19–20	12
N. wynaadensis	26–29	9	10	4½	2½–3	18–19	12
N. stracheyi	26	–	7	4½	4	18	12
N. compressus	28	≤11[a]	9	4	3½	18	12
N. blythii	25	≤11[a]	6	3½	2½	17	11
N. dukai	28–29	–	9–10	3½	2½	16	12
N. capudelphinus	30–32	9	11–12	6	5	24	14
N. minimus	28–31	7	11–13	5–6	6	23–24	14
N. micropthalmus	28–29	8	8–9	4–5	5	18	12
N. acutirostris	29–32	7	10	5	5–6	20	12
N. tamiraparaniensis	28–30	9	11–12	5–6	5–6	20–2	12–14
N. spinulosus	32	–	–	–	–	–	–

Other data sources are mentioned in comparative materials.
[a]Data from Rainboth (1985).

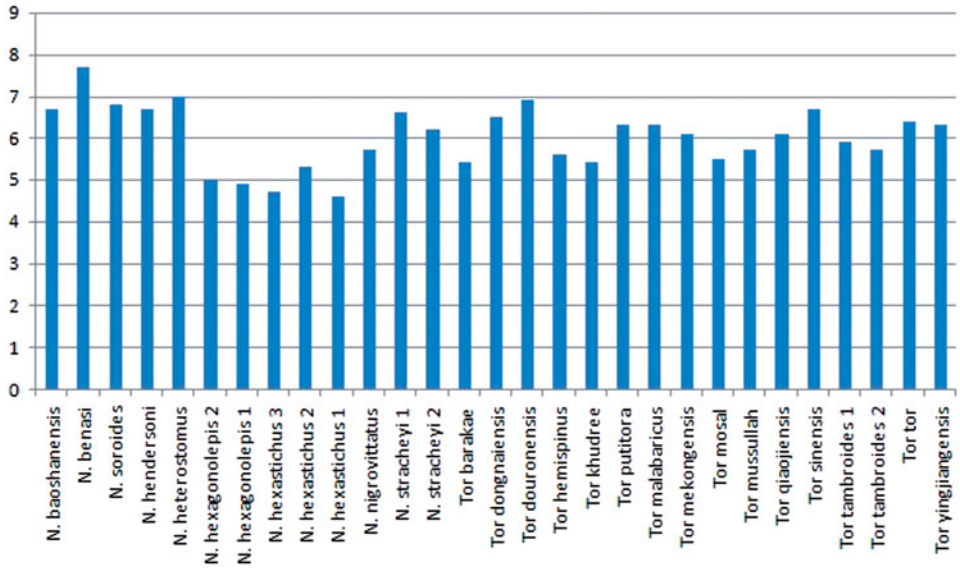

Figure 1. Percent genetic distance (*Y*-axis) of *Neolissochilus kaladanensis* sp. nov. calculated against 24 species of *Neolissochilus* and *Tor* genera (*X*-axis).

sequence of 25 *Neolissochilus* and *Tor* species (Figure 2), as well as multiple COI sequences of same species (Supplementary Electronic Figure 1).

Results

Morphological analysis and comparison

The measurements of morphomeristic parameters for holotype and average values for all paratypes of *N. kaladanensis* sp. nov. are given in Table 1. Measurements are given as percentage of standard length (SL) and subunits of the head are expressed in proportions of head length (HL) (see Figure 3 for morphology and general appearance). *Neolissochilus kaladanensis* sp. nov. is markedly different from all other species of the genus in having higher number of gill rakers on the lower arm of the first gill arch (13–14 vs. below 12 in all other species) and, with the exception of *N. hendersonii, N. paucisquamatus* and *N. soroides*, in having fewer scales on the lateral line (20–22 scales in *N. kaladanensis* sp. nov. vs. 24–32 in other species). The *N. kaladanensis* sp. nov. is compared with *N. acutirostris*, *N. blythii*, *N. capudelphinus*, *N. compressus*, *N. dukai*, *N. hexagonolepis*, *N. hexastichus*, *N. micropthalmus*, *N. minimus*, *N. spinulosus*, *N. stracheyi*, *N. tamiraparaniensis* and *N. wynaadensis*. The comparisons of important diagnostic characters with these closely related species are shown in Table 2.

Genetic distances and phylogenetic analysis

The COI sequence separate *N. kaladanensis* sp. nov. from all other *Neolissochilus* and *Tor* species with an average genetic distance of 6.0%. It is further separated from the closely related species available in GenBank, *N. hendersoni* and *N. soroides*, by a genetic distance of 6.7% and 6.8%, respectively. *Neolissochilus kaladanensis* sp. nov. is genetically closer to *N. hexagonolepis* and *N. hexastichus* and together form a monophyletic group in the ML tree of COI dataset (Figure 2 and Supplementary Electronic Figure 1), however, separated by a distance of 4.9% from both species (Supplementary Electronic Table 2). The genetic distance of *N. kaladanensis* sp. nov. is compared with all the 24 *Neolissochilus* and *Tor*

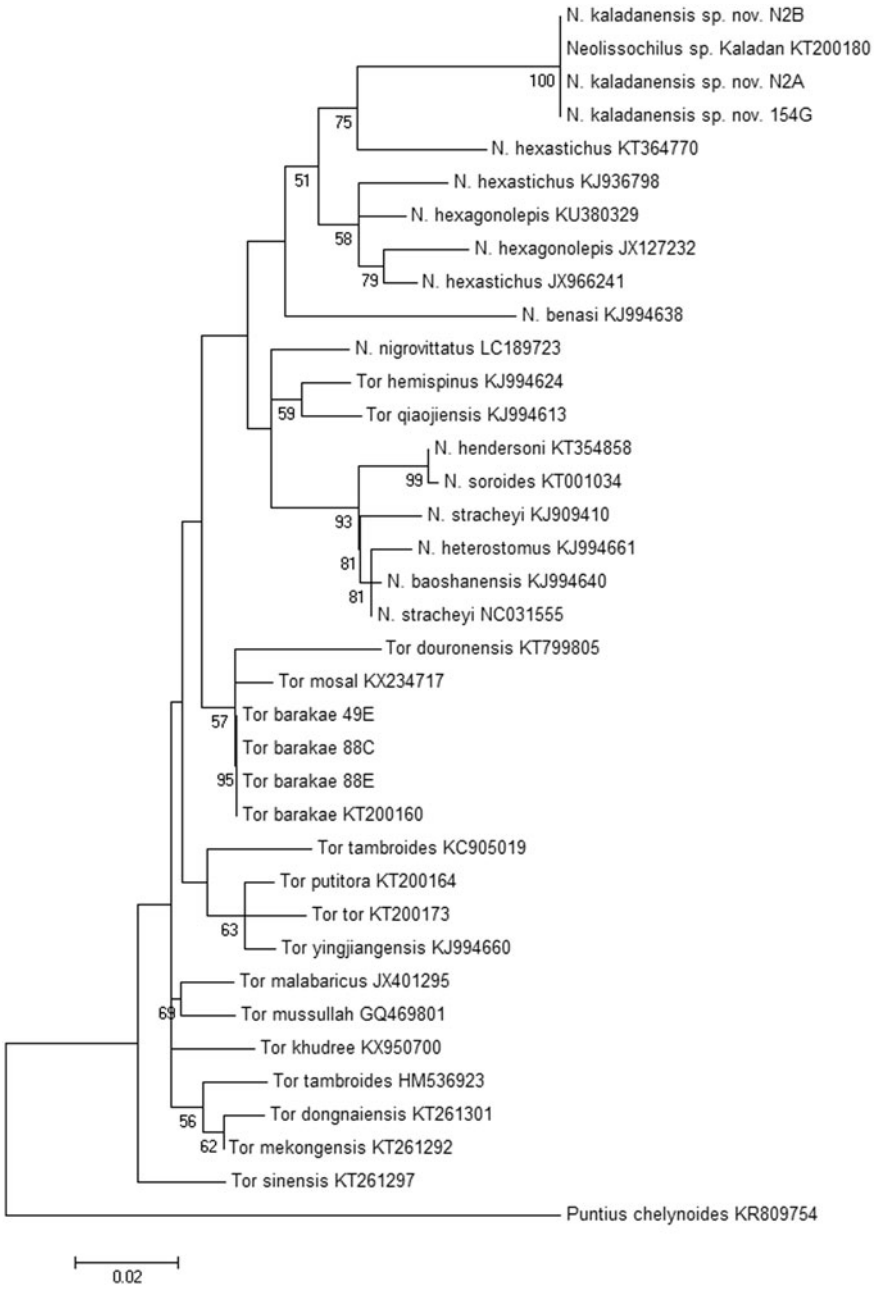

Figure 2. Molecular phylogenetic analysis by maximum-likelihood method including 36 COI sequences of 25 species and *Puntius chelynoides* as outgroup.

species, for which COI sequence is publically available (Figure 1). The interesting observation is more than one clades formation by the subgroups of same species, interspersed by other species in phylogenetic tree and large genetic distance among them. Based on this result, *N. hexastichus* is divided into three subgroups with average genetic distance of 4.2% among subgroups. Similarly, *N. hexagonolepis*, *T. tambroides*, and *N. stracheyi* are divided into two subgroups each, with genetic distance of 2.7%, 2.6% and 1.3% between the related subgroups, respectively. Conversely, very low genetic distance is also observed between valid species, viz., *N. hendersoni* (KT354858) exhibit 0% and 0.2% genetic distance with *N. soroides* (AP011314) and *N. soroides* (KT001034), respectively. Similarly, *N. baoshanensis* (KJ994640) show only 0.2% genetic distance with *N. stracheyi* subgroup 1 (KT261305, AP011252, NC031555, KT261302–KT261304, Supplementary Electronic Table 2).

Discussion

The taxonomic ambiguities of the genus *Neolissochilus* has been discussed earlier (Khaironizam et al. 2015; Zheng et al. 2016; Arunachalam et al. 2017). The genus closely resembles *Tor* in morphological appearance and, further, both the genera occupy the same distributional range (Rainboth 1985). Besides, the existence of polymorphism (*Tor* like morph) has been reported in *N. soroides* (Roberts and Khaironizam 2008) and *N. stracheyi* (Hoang et al. 2015). The two genera are said to be distinguished by the presence of a continuous labial groove (Laskar et al. 2013; Hoang et al. 2015), sometimes developed into a fleshy lobe, and 10–14 gill rakers on the lower arm of the first gill arch in *Tor*, whereas an interrupted groove and 6–9 gill rakers on the lower arm of the first gill arch in *Neolissochilus*. This statement seems incomplete as

Figure 3. *Neolissochilus kaladanensis* sp. nov., PUCMF 17011, holotype, 126.5 mm SL; India: Mizoram: Kaladan River.

Rainboth (1985) himself clearly stated the existence of variation on the lower arm of gill rakers (first gill arch) depending upon their geographical distribution in *Neolissochilus* and *Tor*, viz., 6–9 vs. 10–14 from peninsular India and Sri Lanka, 7–11 vs. 12–16 from Myanmar and 7–12 vs. 12–17 from Thailand and Malaya. Nevertheless, due to the overlapping character like presence of mental lobe in *N. hexastichus*, Laskar et al. (2013) suggested to emphasize the number of gill rakers alone for generic distinction between the two genera. It is apparent that the genus *Tor* (except *T. hemispinus* and *T. qiaojiensis*) possess higher gill rakers as compared to *Neolissochilus* in general, and the suggestion of Laskar et al. (2013) is almost acceptable, but, may still be chaotic due to high total gill rakers count reported in *N. baoshanensis* (13–14), *N. heteterostomus* (13–19) and *N. soroides* (15–17) overlapping with those of the genus *Tor*. Therefore, depending upon their particular habitat, we do not reject the use of gill rakers' count to distinguish the two genera at first hand; however, we suggest that the use of molecular markers is a better approach to resolve the ambiguity because of overlapping morphological characters between the two genera. Despite having high gill rakers, the COI analysis is evident that *N. kaladanensis* sp. nov. is well nested inside the clade of the genus *Neolissochilus* and therefore undoubtedly belonging to the genus.

Zheng et al. (2016) suggested that *T. hemispinus* and *T. qiaojiensis* should be grouped under the genus *Neolissochilus* and *N. benasi* should be given another genus name. It is apparent that *Tor hemispinus* and *T. qaiojiensis* were placed under the genus *Tor* due to the presence of well-developed median lobe at their lower lips. *Tor qiaojiensis sensu stricto* was placed under the genus *Neolissochilus* by Rainboth (1985), however treated as *T. qiaojiensis* by Eschmeyer et al. (2017). We strongly agree with the suggestion of Zheng et al. (2016), placing the two species under *Neolissochilus* but decline the suggestion of placement of *N. benasi* to other genus since the three species were well nested within the *Neolissochilus* clade based on the COI sequences analysis. Furthermore, the gill rakers count (11–12 and 12, respectively) of *T. hemispinus* and *T. qaiojiensis* are unexpectantly lower in comparison to other species belonging to the genus *Tor*. *Neolissochilus kaladanensis* sp. nov. differs from both the species in having lower lateral line scales and higher count of gill rakers.

Delineation of species using DNA barcoding depends on the intra- and inter-species distance where the sequences are divergent enough to enable recognition of the species (Hebert et al. 2003). However, the DNA barcoding will succeed in recognition and demarcation of species only if the barcoded specimens have been identified precisely prior to submission of the sequence in public databases like NCBI GenBank. It has been observed that there are lot of inconsistencies among the COI gene sequences of *Tor* and *Neolissochilus* available in the GenBank. This type of inconsistency leads to more complications in the identification of species and must be resolved by linking morphological data and DNA sequences. The anomalies may be attributed to misidentification of the species, synonymy, cryptic nature of the species, species complex within single species or recently evolved species with lesser divergence in DNA sequences. The Peninsular Malaysian species of *N. hendersoni* and *N. soroides* are morphologically distinct from each other (Khaironizam et al. 2015); however the genetic distance does not support this. Similar is the case of *N. baoshanensis* and *N. stracheyi* subgroup 1. The question is whether or not the two species are a case of synonym or the result of species misidentification or limitation of DNA barcoding. Conversely, *N. hexastichus* was divided into three subgroups with average genetic distance of 4.2% among subgroups. Similarly, *N. hexagonolepis*, *T. tambroides* and *N. stracheyi* were divided into two subgroups each, with genetic distance of 2.7%, 2.6% and 1.3%, respectively. The existence of subgroups in a species, with high genetic distance indicates the presence of species complex and requires concerted efforts of molecular biologists and traditional taxonomists.

In conclusion, we agreed with the use of DNA barcoding as a successful tool for identification and delineation of species, the anomalies observed among the submitted sequences are otherwise an indication of the existence of cryptic species and calls for the taxonomic revision of the two genera *Neolissochilus* and *Tor*.

Taxonomy

Neolissochilus kaladanensis sp. nov. (Figure 3).

Type specimens

Holotype. PUCMF 17011, 126.5 mm SL, 22°25′52″N 92°56′29″E, Kaladan River in the vicinity of Kawlchaw Village, Lalramliana and Lalronunga S, March 3 2017.

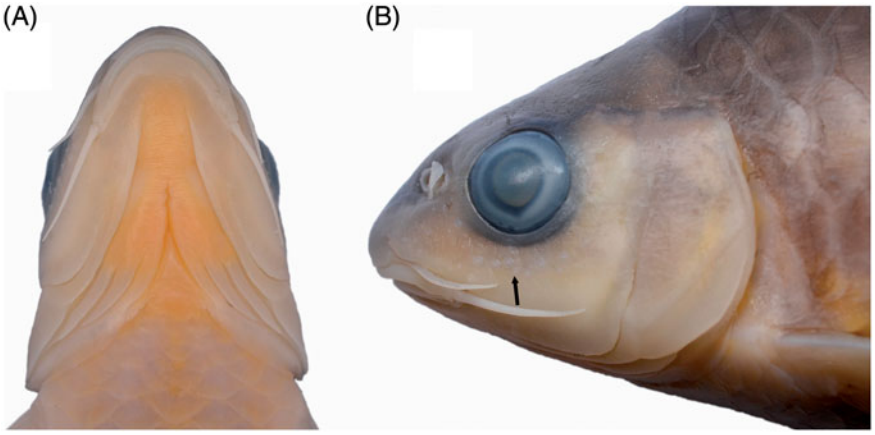

Figure 4. *Neolissochilus kaladanensis* sp. nov., PUCMF 17011, holotype, 126.5 mm SL. (A) Lower lip region. (B) Lateral view of head showing tubercles.

Paratypes. ZSI FF 7605, 3, 88.7–142.0 mm SL; PUCMF 17012,2, 88.6–152.5 mm SL; same as holotype; PUCMF 17013, 4, 69.8–94.8 SL;22°25′52″N 92°56′29″E, Kaladan River in the vicinity of Kawlchaw Village, Lalramliana and Lalronunga S, November 24 2015; PUCMF 17014, 3 (two specimens were cleared and stained in alizarin), 79.7–92.2 mm SL, 22°25′52″N 92°56′29″E, Kaladan River in the vicinity of Kawlchaw Village, Lalramliana and Lalronunga S, November 24 2015; PUCMF 17015, 1, 415.2 mm SL, 22°54′09″N 92°55′60″E, Kaladan River in the vicinity of Darzokai Village, acquired from Department of Fisheries, Government of Mizoram.

Morphological diagnosis

Neolissochilus kaladanensis sp. nov. is distinguished from all other congeners in having higher number of gill rakers on the lower arm of the first gill arch (13–14 vs. below 12 in all other species). It is further distinguished in having the following combination of characters: 20–22 scales on the lateral line; 1/2 3/1/2 1/2–3 scales in transverse line from dorsal-fin origin to pelvic-fin origin; 8–10 predorsal scales; 17–18 circumferential scales; 12 circumpeduncular scales and 35 vertebrae.

Description

Head short, deep, its length is about one-fourth of the SL, its depth is slightly more than three-fourths of its length. Body elongate, laterally compressed, maximum depth is slightly less than half of the SL. Dorsal profile rising from tip of snout to origin of dorsal fin, then sloping gently from dorsal-fin base to caudal-fin base; body depth greatest at dorsal-fin base. Ventral profile slightly curved to chest; abdomen straight up to anal-fin origin; caudal peduncle profile slightly concave, its least depth is slightly less than its length.

Eye ovoid, moderately large, located in anterior half of head, its diameter is about one-third of the HL. Snout rounded, its length is more than or equal to eye diameter, cheek with irregular rows of tubercles (Figure 4(B)). Mouth is subterminal and oblique, rictus is not reaching vertical through anterior margin of orbit. Lips are fleshy, median lobe of lower lip is not developed (Figure 4(A)); pair of rostral and maxillary barbels are present.

Dorsal fin with four (12) unbranched and 9 1/2 branched rays (12), its length slightly greater than HL, its origin above 7th lateral-line scale and slightly anterior to vertical through pelvic-fin origin; last unbranched ray ossified, strong and smooth; first branched ray longest; posterior margin concave; dorsal-fin base covered with scale sheath. Pectoral fin with one unbranched and 14 (4), 15 (7) or 16 (1) branched rays, first branched ray longest, adpressed fin tip reaching two-thirds distance between pectoral and pelvic-fin origin. Pelvic fin with one unbranched and eight (12) branched rays, first branched ray longest, adpressed fin tip not reaching base of anal fin, axillary scale present at its base. Vent closer to anal-fin origin than to base of last pelvic-fin ray. Anal fin with three (12) unbranched and 5 1/2 (12) branched rays, first branched ray longest, adpressed fin tip almost reaching base of caudal fin; anal-fin base covered with scale sheath. Caudal fin forked, lobe tips rounded, principal caudal rays 10 + 9 (12), 10th ray shortest, lobes more or less equal.

Body covered with large scales; breast covered with smaller scales. Lateral line curved, prominent and complete with 20(2) or 21 (7) or 22 (3) scales on the body plus 1 (1) or 2 (10) scales on the caudal-fin base; 1/2 3/1/2 1/2 (10) or 1/2 3/1/3 (2) scales in transverse line from dorsal-fin origin to pelvic-fin origin; 8 (3), 9 (8) or 10 (1) predorsal scales; 17 (3) or 18 (9) circumferential scales; 12 (12) circumpeduncular scales. Total gill rakers on the first gill arch 17 (1), 18 (1) or 20 (1); 4 (1), 5 (1) or 6 (1) on epibranchial, 1 (3) at angle and 12 (2) or 13 (1) on ceratobranchial. Pharyngeal teeth three rows with 5, 3, 2 (3) formula. Vertebrae (abdominal + caudal): 21 + 14 = 35 (2).

Colouration

Dorsum dark brownish; sides becoming gradually lighter towards abdomen with yellowish to orange hue; inconspicuous dark brownish spots on the caudal peduncle; cheek, gill cover and ventral portion of head pale brownish. All fins hyaline without any marking. Distal part of dorsal and caudal fins is black edged.

Etymology

The species is named after the Kaladan River, from where it was collected (an adjective).

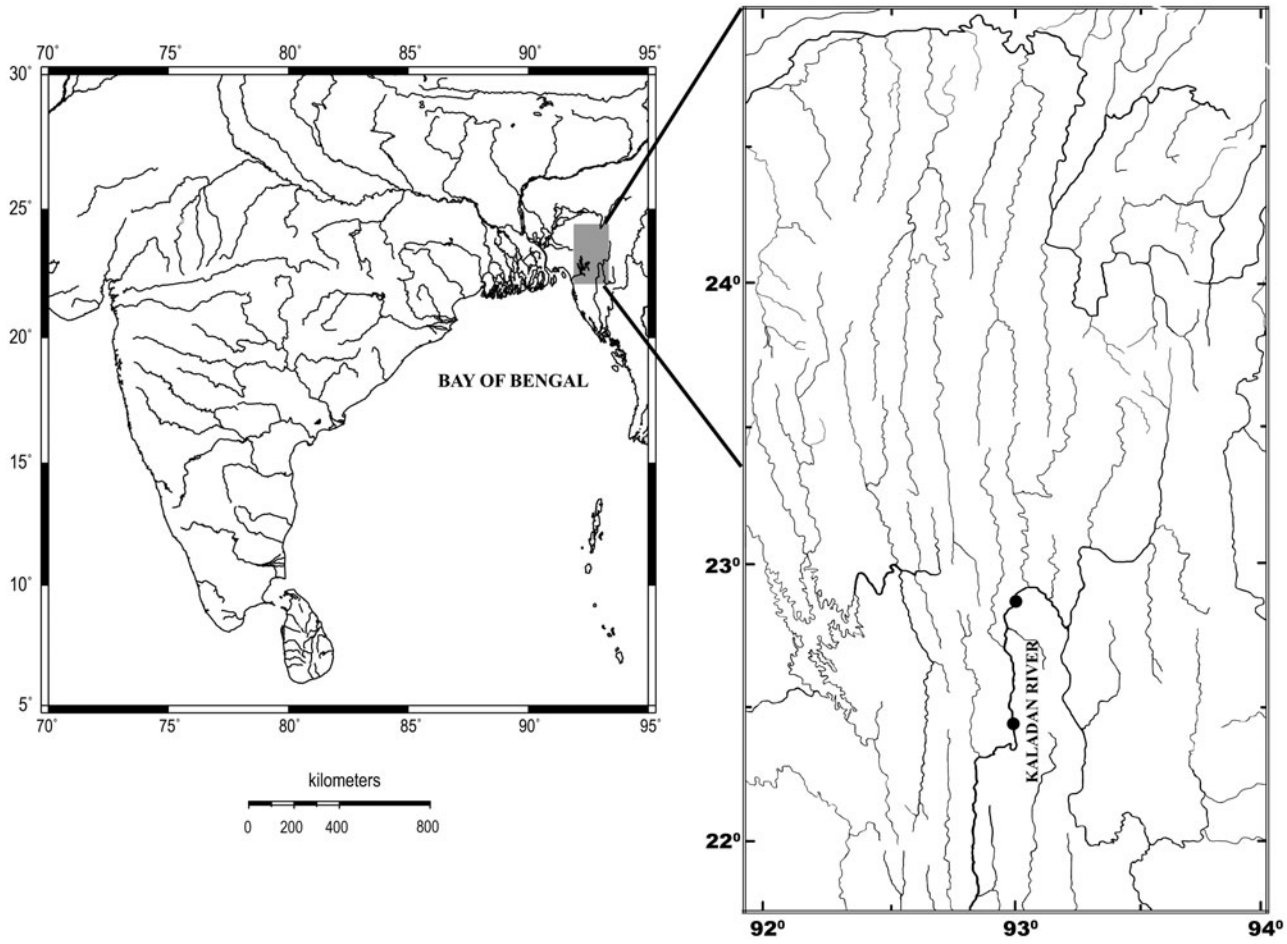

Figure 5. Drainage map showing collection spot (●) of *Neolissochilus kaladanensis* sp. nov.

Distribution

Presently known from the Kaladan River (Kaladan River drainage) at Kawlchaw and Darzokai villages (Figure 5).

Remarks

Neolissochilus kaladanensis sp. nov. shared characters with *N. hendersonii*, *N. paucisquamatus* and *N. soroides*, in having fewer scales on the lateral line, a notable character which distinguishes it from all other valid congeners, other than above-mentioned species (20–22 scales in *N. kaladanensis* sp. nov. vs. 24–32 in other species). It is worth mentioning here that the examination of the ZSI specimens revealed that the lateral-line scales of *N. blythii* and *N. compressus* are 25 and 28 respectively rather than 22 scales as reported in the original description by Day (1870). *Neolissochilus kaladanensis* sp. nov. markedly differs from *N. blythii* and *N. compressus* in having fewer lateral-line scales (20–22 vs. 25 and 28) and higher (13–15 vs. $\leq$11) gill rakers on the lower arm of the gill arch. Furthermore, it also differs from *N. stevensonii*, the Akyab species, in having fewer lateral-line scales (20–22 vs. 27). According to Rainboth (1985), grouping of species based on their geographical distributional range, *N. kaladanensis* sp. nov. is morphologically similar to species from Thailand and Malaysia in having high gill rakers count. However, it differs from all of them, with the exception of *N. hendersoni*, *N. paucisquamatus* and *N. soroides*, in having lower lateral-line scales (20–22 vs. 24–29 in all other species). It further differs from *N. hendersoni* in having more predorsal scales (8–10 vs. 6–8) and more gill rakers on the lower arm of the first gill arch (14–16 vs. 9–10); from *N. paucisquamatus* in having more predorsal scales (8–10 vs. 7), dorsal-fin origin over 7th scale (vs. 6th scale) of lateral line, adpressed pectoral fin not reaching (vs. reaching) base of pelvic fin and adpressed pelvic fin not reaching (vs. reaching) anus; and from *N. soroides* in having more gill rakers on the lower arm of the first gill arch (14–16 vs. 9–10) and fewer scales at anal-fin base (3–4 vs. 4–6).

Acknowledgements

We are grateful to Director, Zoological Survey of India, Kolkata and Laishram Kosygin (ZSI) for permission to access materials under their care; Vanlalmalsawma (Sawmtea) for field assistance; Dr Tawnenga, Principal, Pachhunga University College and Director, ICAR-National Bureau of Fish Genetic Resources, Lucknow for providing laboratory facilities.

Disclosure statement

We declare that there are no known conflicts of interest associated with this publication. We undertake that the work is our original work and the

manuscript have not been published elsewhere or simultaneously submitted to other journals.

Funding

We are grateful to Department of Biotechnology, Government of India, for financial support (No. BT/388/NE/TBP/2012).

ORCID

Mahender Singh http://orcid.org/0000-0001-5335-5460

References

Ali A, Dahanukar N, Philip S, Krishnakumar K, Raghavan R. 2014. Distribution, threats and conservation status of the Wayanad Mahseer, *Neolissochilus wynaadensis* (Day, 1873) (Teleostei: Cyprinidae): an endemic large barb of the Western Ghats, India. J Threat Taxa. 6:5686–5699.

Arunachalam M, Sivakumar P, Murugan M. 2017. Descriptions of five new species of *Neolissochilus* Rainboth, 1985 (Cypriniformes: Cyprinidae) from streams/rivers of the Western Ghats, Peninsular India. Fish Taxa. 2:1–27.

Chen XY, Yang JX, Chen YR. 1999. A review of the cyprinoid fish genus *Barbodes* Bleeker, 1859, from Yunnan, China, with descriptions of two new species. Zool Stud. 38:82–88.

Day F. 1869. Remarks on some of fishes in the Calcutta museum: on freshwater fishes of Burma. Part I. Proc Zool Soc Lond. 37:548–560.

Day F. 1870. On the freshwater fishes of Burmah. Part II. Proc Zool Soc Lond. 1870:99–101.

Day F. 1871. Monograph of Indian Cyprinidae. J R Asiat Soc Bengal. 40:277–336.

Day F. 1873. On some new fishes of India. Zool J Linn Soc. 11:524–530.

Day F. 1878. The fishes of India: being natural history of the fishes known to inhabit the sea and freshwaters of India, Burma and Ceylon. London: William Dawson & Sons; p. 564.

Eschmeyer WN, Fricke R, van der Laan R. 2017. Catalogue of fishes: genera, species, references; [accessed 2017 Oct 1]. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp.

Hebert PDN, Cywinska A, Ball SL, de Waard JR. 2003. Biological identifications through DNA barcodes. Proc Biol Sci. 270:313–321.

Herre AWCT. 1940. New species of fishes from the Malay Peninsula and Borneo. Bull Raffles Mus. 16:5–26.

Hoang HD, Pham HM, Durand JD, Tran NT, Phan PD. 2015. Mahseers genera *Tor* and *Neolissochilus* (Teleostei: Cyprinidae) from southern Vietnam. Zootaxa. 4006:551–568.

Hubbs CL, Lagler KF. 1964. Fishes of the Great Lakes Region. Ann Arbor (USA): University of Michigan Press.

Hubert N, Hanner R, Holm E, Mandrak NE, Taylor E, Burridge M, Watkinson D, Dumont P, Curry SA, Bentzen P, et al. 2008. Identifying Canadian Freshwater Fishes through DNA barcodes. PLoS One. 3:e2490.

Khaironizam MZ, Zakaria-Ismail M, Armbruster JW. 2015. Cyprinid fishes of the genus *Neolissochilus* in Peninsular Malaysia. Zootaxa. 3962:139–157.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol. 33:1870–1874.

Lakra WS, Singh M, Goswami M, Gopalakrishnan A, Lal KK, Mohindra V, Sarkar UK, Punia PP, Singh KV, Bhatt JP, et al. 2016. DNA barcoding Indian freshwater fishes. Mitochondrial DNA A. 27:4510–4517.

Laskar BA, Bhattacharjee MJ, Dhar B, Mahadani P, Kundu S, Ghosh SK. 2013. The species dilemma of northeast Indian mahseer (Actinopterygii: Cyprinidae): DNA barcoding in clarifying the riddle. PLoS One. 8:e53704.

McClelland J. 1839. Indian Cyprinidae. Asiat Res. 19:217–471.

McClelland J. 1845. Apodal fishes of Bengal. Calcutta J Nat Hist. 5:151–226.

Rainboth WJ. 1985. *Neolissochilus*, a new genus of south Asian Cyprinid fishes. Beaufortia. 35:25–35.

Rainboth WJ. 1991. Cyprinids of South East Asia. In: Winfield IJ, Nelson JS, editors. Cyprinid fishes: systematics, biology, and exploitation. London (UK): Chapman and Hall; p. 156–210.

Roberts TR, Khaironizam MZ. 2008. Trophic polymorphism in the Malaysian fish *Neolissochilus soroides* and the old world Barbs (Teleostei, Cyprinidae). Nat Hist Bull Siam Soc. 56:25–53.

Sambrook J, Russell DW. 2001. Molecular cloning – a laboratory manual. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory (CSH).

Sanger F, Nicklen S, Coulson AR. 1977. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA. 74:5463–5467.

Singh M, Verma R, Yumnam R, Vishwanath W. 2017. Molecular phylogenetic analysis of genus *Osteobrama* Heckel, 1843 and discovery of *Osteobrama serrata* sp. nov. from North East India. Mitochondrial DNA A. DOI: 10.1080/24701394.2017.1285289

Smith HM. 1945. The freshwater fishes of Siam or Thailand. Bull US Natl Mus. 188:1–622.

Tamura K, Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol. 10:512–526.

Vidthayanon C, Kottelat M. 2003. Three new species of fishes from Tham Phra Daeng and Tham Phra Sai Ngam caves in the northern Thailand. Ichthyol Explor Freshw. 14:159–174.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. 2005. DNA barcoding Australia's fish species. Philos Trans R Soc Lond B Biol Sci. 360:1847–1857.

Yinrui C, Xinluo C. 1985. Systematic study of the genus *Tor* (Pisces: Cyprinidae) with description of a new species. Zool Res. 6:79–86.

Zheng LP, Yang JX, Chen XY. 2016. Molecular phylogeny and systematics of the Barbinae (Teleostei: Cyprinidae) in China inferred from mitochondrial DNA sequences. Biochem Syst Ecol. 68:250–259.