



# The world's largest cave fish from Meghalaya, Northeast India, is a new species, *Neolissochilus pnar* (Cyprinidae, Torinae)

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## Abstract

The world's largest subterranean fish was discovered in 2019, and was tentatively identified as a troglomorphic form of the golden mahseer, *Tor putitora*. Detailed analyses of its morphometric and meristic data, and results from molecular analyses now reveal that it is a new species of the genus *Neolissochilus*, the sister taxon of *Tor*. We formally describe the new species as *Neolissochilus pnar*, honouring the tribal communities of East Jaintia hills in Meghalaya, Northeast India, from where it was discovered. *Neolissochilus pnar* possesses a number of characters unique among species of *Neolissochilus*, with the exception of the similarly subterranean *N. subterraneus* from Thailand. The unique characters that diagnose *N. pnar* from all epigeal congeners comprise highly reduced eye size to complete absence of externally visible eyes, complete lack of pigmentation, long maxillary barbels, long pectoral-fin rays, and scalation pattern. *Neolissochilus pnar* is distinguished from the hypogean *N. subterraneus*, the type locality of which is a limestone cave ~2000 kms away in Central Thailand, by a lesser pre-pelvic length (47.8–49.4 vs. 50.5–55.3 %SL), a shorter caudal peduncle (16.1–16.8 vs. 17.8–23.7 %SL), and shorter dorsal fin (17.4–20.8 vs. 21.5–26.3 %SL). In addition, *Neolissochilus pnar* is also genetically and morphologically distinct from its close congeners with a raw genetic divergence of 1.1–2.7% in the COI gene with putative topotype of *N. hexastichus* and 2.1–2.6% with putative topotype of *N. hexagonolepis*.

## Keywords

Eastern Himalaya, limestone cave, mahseer, new species, subterranean fishes

## Introduction

Roughly 1.6% (293 species) of all known (~18,000) freshwater fish species live their whole lives either in caves, or in groundwater aquifers (Proudlove 2023). These 'troglo-

biotic' or 'stygiobiotic' fishes occur in 36 countries across six continents, with China harbouring close to one-third (96 species) of the global diversity, followed by Brazil

**Table 1.** List of subterranean fish species with adult sizes in excess of 200 mm standard length (SL).

Family/Species	Country	Maximum SL (mm)
<b>Cyprinidae</b>		
<i>Sinocyclocheilus guanyangensis</i>	China	202
<i>Sinocyclocheilus hugeibarbus</i>	China	217
<i>Neolissochilus subterraneus</i>	Thailand	217
<b>Heptapteridae</b>		
<i>Rhamdia enfunada</i>	Brazil	218
<b>Synbranchidae</b>		
<i>Typhlosynbranchus laticolus</i>	Cameroon	209
<i>Rakthamichthys digressus</i>	India	242
<i>Ophisternon infernale</i>	Mexico	325
<i>Ophisternon candidum</i>	Australia	356

(43 species), Mexico and India (18 species each) (Proudlove 2023). A number of these species are evolutionary relics of an ancient fauna, often with long-term isolation in these high-stress environments (Gibert and Deharveng 2002). Such evolutionarily relictual lineages include among others, the Aenigmachannidae – a “living fossil” with a putative Jurassic origin (Britz et al. 2020), the enigmatic Kryptoglanidae (Britz et al. 2014), the cistern catfish *Phreatobius* (Muriel-Cunha and de Pinna 2005), and the blind aquifer-dwelling *Horaglanis* (Raghavan et al. 2023). However, the large majority of cavefishes represent “young” lineages that have much more recently invaded subterranean habitats – examples include the European cave loach, *Barbatula* sp., and the Mexican blind swamp eel, *Ophisternon infernale* (Behrmann-Godel et al. 2017; Mar-Silva et al. 2022).

Most subterranean fishes have evolved a small-sized body plan to meet the limitations in food resources, light availability and space in underground habitats. The mean size of subterranean fish species is 85.5 mm, with most species below 130 mm (Harries et al. 2019), with at least eight species reaching sizes in excess of 200 mm (Table 1). The largest-known subterranean fish until now is the Australian blind cave eel *Ophisternon candidum* Mees (family Synbranchidae) which measures 385 mm in total length (Moore et al. 2018).

Stories of a ‘white cavefish’ from the Siju Caves in the Garo Hills of Meghalaya, Northeast India have been documented for 100 years, but were suggested to be slightly decolorized specimens of *Neolissochilus hexastichus* (M’Clelland) that appeared almost white when observed inside the water, under the light of a torch (Kemp and Chopra 1924). Only in the 1990s, a large, pale, cyprinid fish was observed in the limestone caves of the Jaintia Hills of Meghalaya (Harries et al. 2008), individuals of which were eventually photographed and collected in 2019 (Harries et al. 2019), and made available for detailed scientific studies. The largest individual observed in the cave exceeded 400 mm in standard length, which makes it the largest known individual of any subterranean fish in the world (Harries et al. 2019). Preliminary morphological studies based on two, medium-sized specimens, revealed morphometric and meristic data similar with the golden mahseer, *Tor putitora* Hamilton (mem-

ber of the cyprinid sub-family Torinae). However, they also showed significantly different characters including a complete lack of pigmentation and a reduction of the eye, which is small in juveniles, and completely invisible externally in adults (Harries et al. 2019).

The availability of additional fresh specimens of this unique cyprinid fish has now enabled us to study its morphological characters in more detail and to include it in a molecular genetic analysis. This combined evidence reveals that the world’s largest cavefish is an undescribed species of the cyprinid genus *Neolissochilus*, for which we make a name available below.

## Materials and methods

### Specimen collection

The specimens of our study were collected from the Krem Um Ladaw and the Krem Chympe caves in Meghalaya, Northeast India in 2019 and 2020. All the specimens were fixed in 4% formaldehyde, after preserving pectoral fin-clips in absolute ethanol for DNA analysis. Specimens are deposited in the museum collection of the Kerala University of Fisheries and Ocean Studies (KUFOS), Kochi, India.

### Morphometric data collection and analysis

Characterization and analysis of morphometric and meristic information was carried out in line with previous studies on members of the subfamily Torinae (Pinder et al. 2018; Lalramliana et al. 2019). Numbers in parentheses after the count indicate number of specimens. Morphometric data for *Neolissochilus subterraneus*, the only other known species of cave dwelling Torinae, were taken from its original description (Vidthayanon and Kottelat 2003). Size-corrected multivariate morphometric data, expressed as percentages of standard length (SL), were visualized using Principal Component Analysis (PCA) to

**Table 2.** GenBank accession numbers and GenSeq nomenclature for sequences generated in the current study.

Species	Locality	Voucher	COI	cytb	16S	GenSeq
<i>Neolissochilus pnar</i>	Krem Um Ladaw	KUFOS.F.2022.701	OQ351360	OQ349705	OQ357607	genseq-1 COI, cytb, 16S
<i>Neolissochilus pnar</i>	Krem Um Ladaw	KUFOS.F.2022.702	OQ351361	OQ349706	OQ357608	genseq-2 COI, cytb, 16S
<i>Neolissochilus pnar</i>	Krem Chympe	KUFOS.F.2022.703	OQ351362	OQ349707	OQ357609	genseq-2 COI, cytb, 16S

check whether *N. pnar* and *N. subterraneus* formed distinct clusters. PCA was performed on correlation matrix to account for scale difference. The null hypothesis, i.e., that there was no significant morphometric difference between the two species, was tested using PERMANOVA (Anderson 2001). Both PCA and PERMANOVA were performed in the freeware PAST 4.12 (Hammer et al. 2001).

## Genetic analysis

DNA was extracted from alcohol preserved fin-clips of the three specimens in the type series using QIAamp® DNA Mini Kit (Qiagen, Germany) following manufacturer's protocol. Three mitochondrial genes, i.e., cytochrome oxidase subunit 1 (COI), cytochrome b (cytb) and large subunit ribosomal ribonucleic acid (16S) were amplified, purified and sequenced following published protocols (Ali et al. 2013; Dahanukar et al. 2013; Verma et al. 2019). Chromatograms of DNA sequences were checked for the quality of base calls in FinchTV 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; <http://www.geospiza.com>).

A total of nine sequences were generated for the three genes (COI, cytb and 16S) from the holotype and two paratypes. GenBank accession numbers and GenSeq nomenclature (Chakrabarty et al. 2013) for sequences generated in the current study are provided in Table 2. Additional sequence data for other species of *Neolissochilus*, and the two closely related taxa *Tor* and *Naziritor* were retrieved from GenBank (Supplementary informations, Table S1), to understand the phylogenetic position of the proposed species. Sequences were aligned separately for each gene using MUSCLE 3.8.31 (Edgar 2004) implemented in MEGA 11 (Tamura et al. 2021) and then concatenated using SEAVIEW 5.0.5 (Gouy et al. 2021). Data were partitioned into three genes (COI, cytb and 16S) and the respective three codon positions for COI and cytb genes. Partition analysis (Chernomor et al. 2016) and ModelFinder (Kalyaanamoorthy et al. 2017) were used to identify the best partitioning scheme, and nucleotide substitution model for the partition scheme based on the minimum Bayesian Information Criterion (BIC) (Schwarz 1978). Maximum likelihood (ML) analysis was performed in IQ-TREE 2.2.0 (Minh et al. 2020) with the best partition scheme and nucleotide substitution model (Table S2). Ultrafast bootstrap support (Hoang et al. 2018) for clades was estimated based on 1000 iterations. The maximum likelihood tree was edited in Fig-Tree v1.4.4 (Rambaut 2018).

Because COI sequences were available for a larger dataset of *Neolissochilus*, we performed a separate ML

analysis (as described above) including all available COI sequences of *Neolissochilus* in GenBank (Table S3). The best partition scheme and nucleotide substitution analysis for COI dataset is provided in Table S4. We performed molecular species delimitation using Assemble Species by Automatic Partitioning (ASAP), employing uncorrected genetic distances, for barcode gap analysis and species delimitation (Puillandre et al. 2021).

## Results

### *Neolissochilus pnar* sp. nov.

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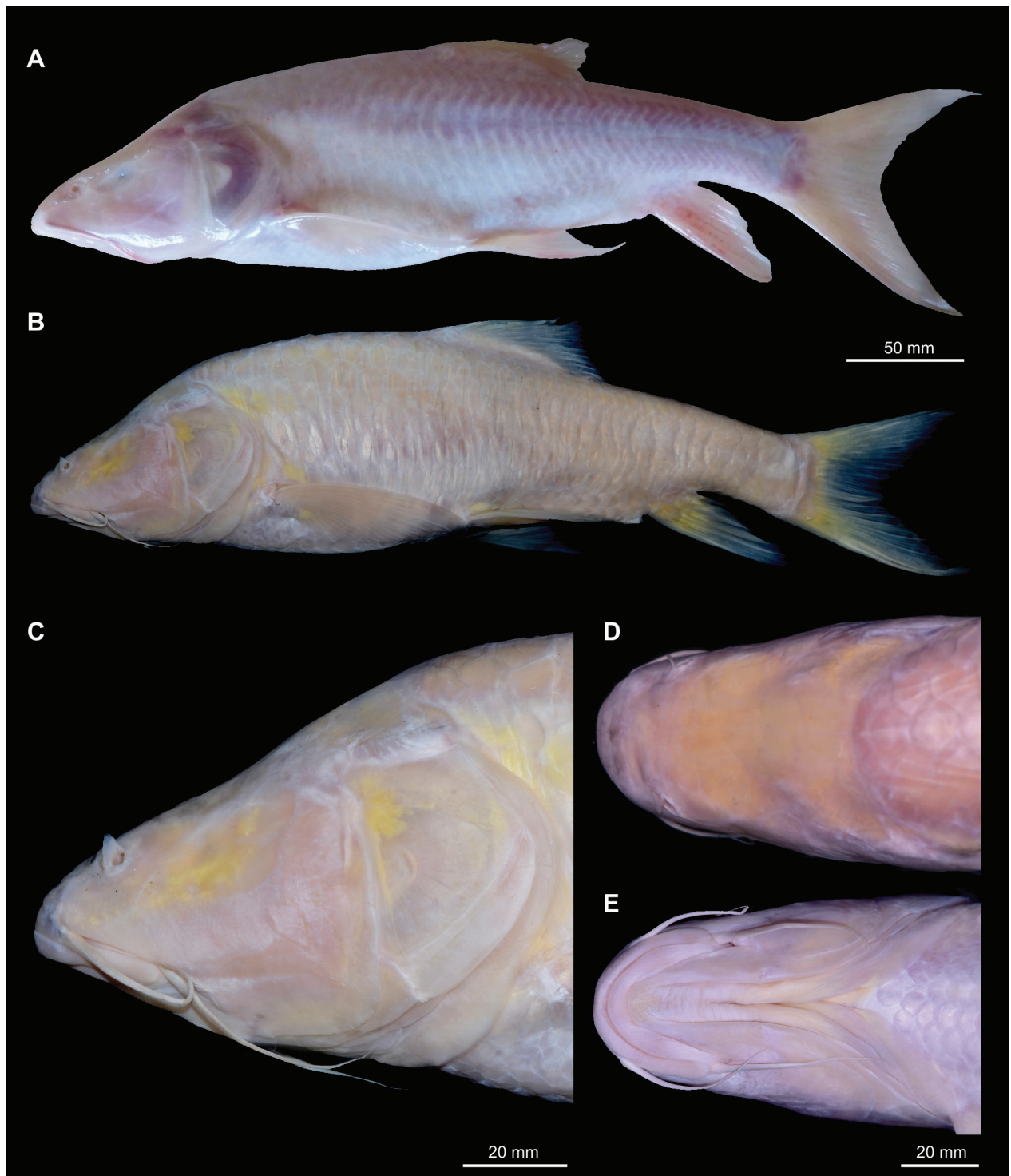
Fig. 1

**Holotype.** KUFOS.F.2022.701, 329.2 mm SL, 92 m below the surface in Krem Um Ladaw, Meghalaya, India; collected 7 Jan 2020.

**Paratypes (n = 2).** KUFOS.F.2022.702, 179.7 mm, same locality as holotype, collected 21 Feb 2019; KUFOS.F.2022.703, 208.9 mm SL, Krem Chympe cave, Meghalaya, India, collected 7 Jan 2020.

**Etymology.** The species name *pnar*, honours the 'pnar', the sub-tribal group of the Khasi people in the state of Meghalaya, India.

**Diagnosis.** *Neolissochilus pnar* is distinguished from all its congeners by mandibular barbel long, reaching anterior margin of opercle (vs. short, not reaching margin of opercle). It is further distinguished from all epigeic congeners by atrophied eyes, highly reduced in size in juveniles and small-adults and absence of externally visible eyes in adults (vs. presence of well-developed eyes in all life-stages); complete absence of pigmentation (vs. presence); long pectoral-fin reaching anterior base of pelvic fin (vs. short, not reaching anterior base of pelvic fin); and distinct scalation pattern with 28+2 (2) or 31+1 (1) lateral line scales, 8 scales in transverse series with 4 above the lateral line and 3 below the lateral line. *Neolissochilus pnar* is distinguished from the only other subterranean congener, *N. subterraneus* by shorter pre-pelvic length (47.8–49.4 vs. 50.5–55.3 %SL), shorter caudal-peduncle length (16.1–16.8 vs. 17.8–23.7 %SL) and shorter dorsal-fin length (17.4–20.8 vs. 21.5–26.3 %SL).



**Figure 1.** Holotype of *Neolissochilus pnar* (KUFOS.F.2022.701, 329.2 mm SL). **A** Immediately after capture. **B** In preservation. **C** Details of head in lateral view. **D** Details of head in dorsal view. **E** Details of head in ventral view. Yellow patches on head, body, and bases of fins represent fat deposits.

**Description.** General appearance as in Fig. 1 and selected morphological characters presented in Table 3. Body elongate, laterally compressed. Dorsal profile sharply rising from tip of snout to nape, posteriorly gently decreasing up to end of caudal peduncle. Ventral profile sloping, almost straight convex. Head large, slightly more than a quarter of standard length. Eyes tiny and highly reduced in size to a black spot or externally invisible in adults, slightly larger, but still reduced in size compared

to epigeal congeners in juveniles; eyes when present situated dorso-laterally, nearer to tip of snout than to posterior margin of opercle. Mouth subterminal, lips thick. Two pairs of barbels. Rostral barbel reaching midlength of maxillary barbel. Maxillary barbel long, reaching anterior margin of opercle.

Dorsal fin with 13 soft rays (iv+9), its origin almost midway between tip of snout and end of caudal peduncle, or slightly in advance. Posterior margin of adpressed



**Table 3.** Morphometric and meristic data of the holotype and two paratypes of *Neolissochilus pnar*.

	Holotype KUFOS.F.2022.701	Paratype KUFOS.F.2022.702	Paratype KUFOS.F.2022.703
<b>Morphometric information</b>			
Total length (TL, mm)	409.9	216.1	255.2
Standard length (SL, mm)	329.2	179.7	208.9
Head length (HL, mm)	98.9	47.8	60.8
<b>% SL</b>			
Head Length	30.0	26.6	29.1
Snout Length	12.6	10.6	11.1
Pre-dorsal length	49.8	47.4	48.6
Pre-pectoral length	30.8	26.6	29.3
Pre-pelvic length	49.4	47.8	48.9
Pre-anal length	73.5	71.2	75.1
Caudal peduncle length	16.6	16.1	16.8
Dorsal-fin length	17.4	20.8	19.4
Dorsal-fin base length	16.1	15.8	16.4
Pectoral-fin length	22.9	22.2	23.4
Pectoral-fin base length	5.3	4.6	5.8
Pelvic-fin length	19.8	18.0	16.4
Pelvic-fin base length	4.7	5.3	5.0
Anal-fin length	19.5	15.8	16.5
Anal-fin base length	6.5	8.5	7.7
Caudal-fin length	24.7	25.9	23.6
Caudal-fin base length	10.7	12.5	11.6
Body depth at dorsal fin	25.1	24.3	23.9
Body depth at anal fin	13.9	15.7	16.4
Body width at dorsal fin	12.6	13.5	14.3
Body width at anal fin	7.3	8.3	7.2
Caudal-peduncle depth	9.0	10.1	9.6
<b>% HL</b>			
Snout length	42.0	39.9	38.3
Inter-orbital length	38.5	36.8	37.7
Maxillary barbel length	45.9	43.6	40.1
Rostral barbel length	44.5	44.7	43.6
<b>Meristic information</b>			
Dorsal-fin rays	iv, 9	iv, 9	iv, 9
Pectoral-fin rays	i, 15	i, 15	i, 15
Pelvic-fin rays	i, 8	i, 8	i, 8
Anal-fin rays	iii, 5	iii, 5	iii, 5
Principal caudal-fin rays	19	19	19
Perforated lateral-line scales	31+1	28+2	28+2

dorsal fin reaching anal-fin origin. Pectoral fin with 16 rays (i+15), its length shorter than head length. Adpressed pectoral fin reaching vertical at dorsal-fin origin, and almost reaching pelvic-fin origin. Pelvic-fin with 9 rays (i+8), its origin slightly posterior to vertical at dorsal-fin origin. Anal fin with 8 rays (iii+5). Caudal fin forked with 19 principal caudal rays. Caudal peduncle 2–2.3 times as long as deep.

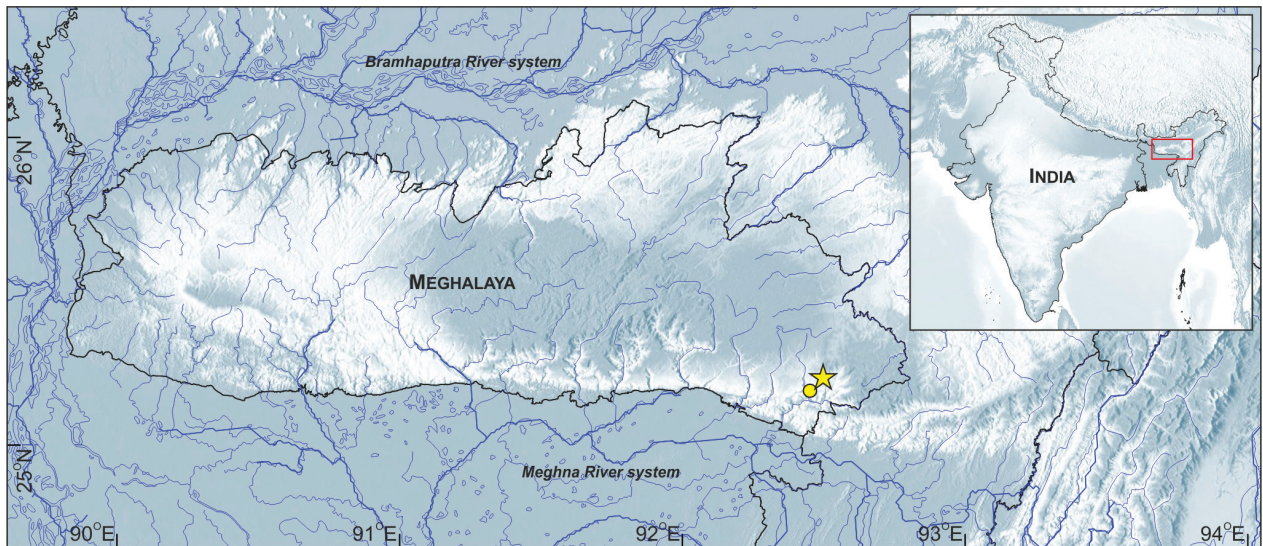
Body lateral line continuous, with 28–31 perforated scales, and an additional 1–2 on caudal-fin base. Transverse series with 8 scale rows, 4 scale rows between dorsal-fin origin and row of lateral line scales, 3 scale rows between row of lateral line scales and pelvic-fin origin. Pre-dorsal scales 9.

**Coloration.** In life (Fig. 1A), body white, pinkish without melanophore pigmentation. All fins hyaline. After preser-

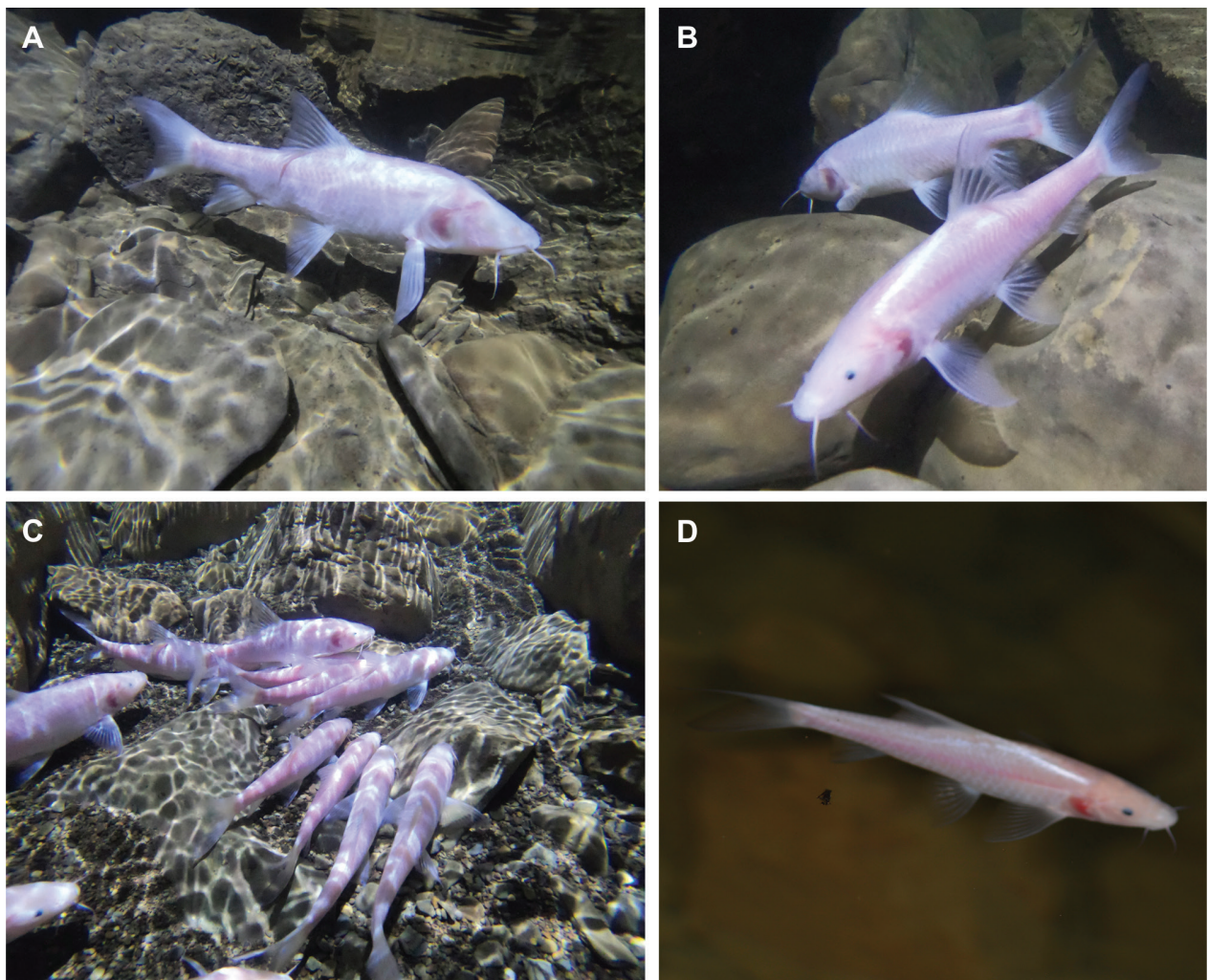
vation (Fig. 1B), body beige with slight yellowish tinge. Eye, if present, visible as a black spot, larger eyes in juveniles with black iris. Some areas on the head and body of the fish appear yellow in the preserved specimens, likely due to fat deposition.

**Distribution.** The species is known from the caves at Krem Um Ladaw, and the adjacent Krem Chympe in Jaintia Hills, Meghalaya, India, which drain into the Meghna River System (Fig. 2).

**Habitat.** The entrance to the cave in Krem Um Ladaw is in the form of a large open pitch head, lies in a large, rocky, seasonally dry streambed within a forest. The entrance series is predominantly vertical with some short (<20 m) horizontal to steeply sloping sections. After descending for just over 100 m, the entrance series drops



**Figure 2.** Collecting localities of *Neolissochilus pnar* in Jaintia Hills, Meghalaya, North East India. Star indicates the type locality Krem Um Ladaw, and circle indicates Krem Chympe, where one of the paratypes was collected.



**Figure 3.** Live images of *Neolissochilus pnar* in their habitat from Krem Um Ladaw (Photos A, B, C: Uros Aksamovic, D: Dan Harries).

into a horizontal and relatively narrow (3–4 m) streamway, the floor of which has several pools of standing water. The cave floor is predominantly rocky with areas of

bedrock, boulders and coarse gravel (Fig. 3). The floor of the boulder passage is mostly elevated well above water level although there are pools in places along the left wall



and in lower floor sections. Debris consisting of forest vegetation is strewn along the floor indicating this area of the cave is seasonally flooded.

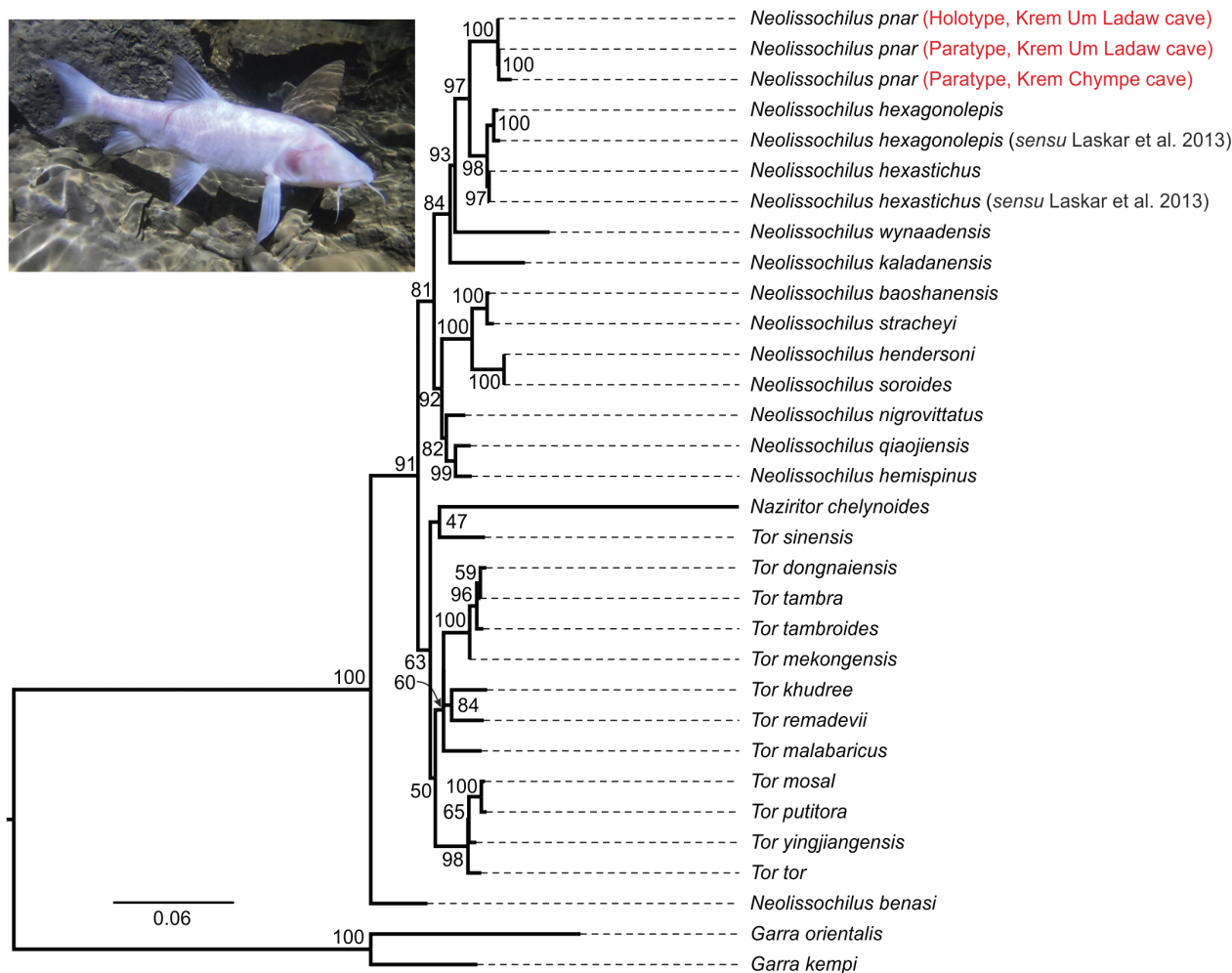
The fish reside in small-sized (~3m x 4m) to large (>10m x 10m) pools. Although the invertebrate community in the cave is plentiful, it is not noticeably more abundant than that of many caves in Meghalaya. Amongst the terrestrial invertebrates were brown crickets (*Eutachyines* sp.), cellar spiders (Pholcidae) and fungus gnat larvae (Keroplatidae). Isopods were also frequently encountered including *Cubaris* sp. and *Philoscia* sp. Aquatic invertebrates included shrimp (*Macrobrachium* cf. *cavernicola*), snails (*Paludomus* sp.), pond skaters (Geridae), and a few tadpoles. No significant bat roosts were encountered, and therefore no guano deposits or other obvious sources of nutrients were observed within the cave. It is conceivable that seasonal flood debris (bamboo, tree branches and leaf litter) carried into the cave from the surrounding forest provides the primary food source for the fish population. There is no plant growth in the caves and in the absence of bat guano, there is probably no other primary energy source in the habitat.

Unlike Um Ladaw, the Krem Chympe, where one of the paratypes were collected, is a broadly horizontal river

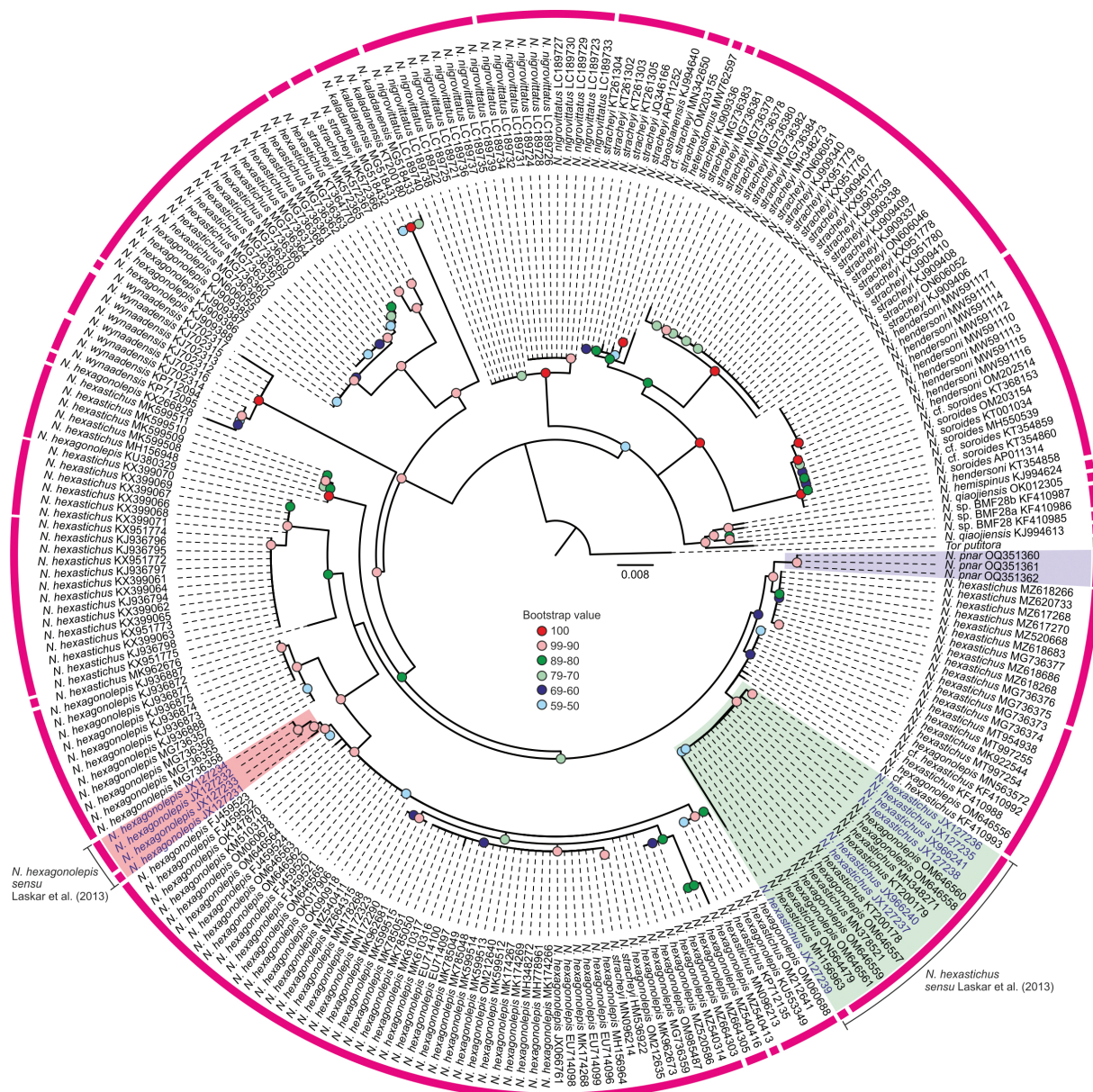
cave, with a massive tunnel of deep water, and various small waterfalls/dams inside. *Neolissochilus pnar* occurs here in pools in a side passage. The biodiversity in this cave comprises of fish (*Garra* sp.), shrimps (*Macrobrachium* sp.), and tadpoles. Further details and photographs of both Um Ladaw and Chympe caves are available from Candade (2022a, b).

### Phylogenetic position of *Neolissochilus pnar* and molecular species delimitation

Phylogenetic analysis based on ML analysis revealed that the new species forms a distinct clade, and the sister taxon to a clade containing two other species of *Neolissochilus*, namely *N. hexagonolepis* (M'Clelland) and *N. hexastichus*, both from the Brahmaputra River system of northeast India (Fig. 4). Maximum likelihood analysis of all available COI sequences of *Neolissochilus* (Fig. 5) and barcode gap analysis (Table S5) revealed that the species diversity within this genus maybe severely underestimated with multiple undescribed species. *Neolissochilus pnar* forms a reciprocally monophyletic clade that is also



**Figure 4.** Maximum likelihood analysis of *Neolissochilus*, *Tor* and *Naziritor* based on concatenated mitochondrial COI, cytb and 16S sequences. Bootstrap values based on 1000 iterations are shown along the nodes. *Garra* species are used as outgroup. Live specimen of *Neolissochilus pnar* is shown in inset (specimen not collected) (Photo: Uros Aksamovic).



**Figure 5.** Maximum likelihood analysis of available COI sequences for *Neolissochilus* with *Tor putitora* as outgroup. Species delimitation based on ASAP is shown as a dashed circle surrounding the phylogenetic tree. Clade containing *Neolissochilus pnar* is highlighted in blue. Clades containing morphologically identified putative topotypes of *Neolissochilus hexagonolepis* and *N. hexastichus sensu* Laskar et al. (2013) are highlighted in red and green respectively, with sequences generated in their original paper highlighted in blue. *Neolissochilus benasi* is excluded from the analysis as it does not group with remaining species of *Neolissochilus* (see Fig. 3). Bootstrap values based on 1000 iterations are shown along the nodes. *Tor putitora* is used as an outgroup.

delimited as a distinct species in ASAP (Fig. 5). Though multiple species have been misidentified in the literature (and in GenBank) as either *N. hexagonolepis* or *N. hexastichus*, morphologically matching putative topotypes of the two nominal species (*sensu* Laskar et al. 2013), form clades distinct from *N. pnar* (Fig. 5). Raw genetic distance in the COI gene between *N. pnar* and *N. hexagonolepis* is 2.1 to 2.6%, and 1.1 to 2.7% between *N. pnar* and *N. hexastichus*. *Neolissochilus pnar* was recovered as the sister group to a clade within the '*N. hexastichus* complex' comprising sequences from Assam (MZ520668) and Nagaland (MZ617268, MZ617270, MZ618266, MZ618268, MZ618683, MZ618686, MZ620733) – the northeast Indian states neighbouring Meghalaya. Between members

of this clade and *N. pnar* there is a genetic divergence of 0.5 to 0.8%.

## Discussion

The limestone caves of Meghalaya, in northeastern India harbour a remarkable diversity of subterranean taxa (Haries et al. 2008), including several enigmatic fish species. The region is one of the two hotspots of subterranean fish diversity and endemism on the Indian subcontinent, the other being the lateritic aquifers of Kerala (Ragha-



van et al. 2021). At least three species of subterranean nemacheilid loaches, *Schistura sijuensis* Menon, *S. papulifera* Kottelat, Harries & Proudlove, and *S. larketensis* Choudhury, Mukhim, Basumatary, Warbah & Sarma are already known from this region (Proudlove 2022). *Neolissochilus pnar*, the largest cave fish described until now, is a remarkable addition to this cave ichthyofauna of the Eastern Himalayan region.

The genus *Neolissochilus* represents a poorly-known group of medium- to large-sized cyprinids, with currently 31 species, distributed across South and Southeast Asia (Fricke et al. 2023). Despite its cultural and commercial importance, there have been no comprehensive studies on the taxonomy or systematics of this group since the description of the genus (Rainboth 1985). As a result, the identity and distribution of the majority of *Neolissochilus* species remains unclear. Even the identity of commercially-valuable species of Northeast India, such as *N. hexastichus* and *N. hexagonolepis*, on which much research has been carried out, have been considered to be confusing (Rainboth 1985). The advent of molecular taxonomy, has nevertheless resulted in the proliferation of large numbers of genetic sequences representing various species of *Neolissochilus*, but only very few sequences are linked to morphological data and/or voucher specimens. Our phylogenetic analysis reveals clearly the extent of this chaos related to the misidentifications of *Neolissochilus* species in GenBank, with currently available sequences forming distinct monophyletic clades, despite being identified and lodged under the same name (Fig. 5).

Laskar et al. (2013) clarified the identity of both *N. hexagonolepis* and *N. hexastichus* using an integrative taxonomic approach using topotypic specimens. For the sake of the present study, we consider the clades that includes sequences used by Laskar et al. (2013) for *N. hexagonolepis* and *N. hexastichus* to represent these species (marked as sensu Laskar et al. 2013 in Figs 4 and 5). *Neolissochilus pnar*, and the putative topotypes of *N. hexastichus* sensu Laskar et al. (2013) show very low genetic divergence – a raw genetic distance of 1.1–2.7% in the COI gene. However, the two species are clearly, morphologically distinct. Based on the original description (M'Clelland 1839, p. 269, pl. 39, fig. 2), *N. pnar* differs from *N. hexastichus* in having more lateral line scales (30–32 vs. 25), more dorsal-fin rays (13 vs. 11), more transverse scale rows (8 vs. 6) and a longer maxillary barbel that reaches the anterior margin of the opercle (vs. shorter, not reaching anterior margin of opercle). Similarly, *Neolissochilus pnar* and putative topotypes of *N. hexagonolepis* sensu Laskar et al. (2013) are separated by a raw genetic distance of 2.1 to 2.6% in the COI gene. Based on the original description of *N. hexagonolepis* (M'Clelland 1839, p. 270, pl. 41, fig. 3), *N. pnar* is distinct in having more lateral line scales (30–32 vs. 27) and anal-fin rays (8 vs. 7).

The sister taxon of *Neolissochilus pnar* (Fig. 5) is likely to be an epigeic congener that is currently misidentified both in the literature, and in GenBank as '*N. hexastichus*'. This sister group includes specimens from the Brahmaputra River basin in the neighboring states

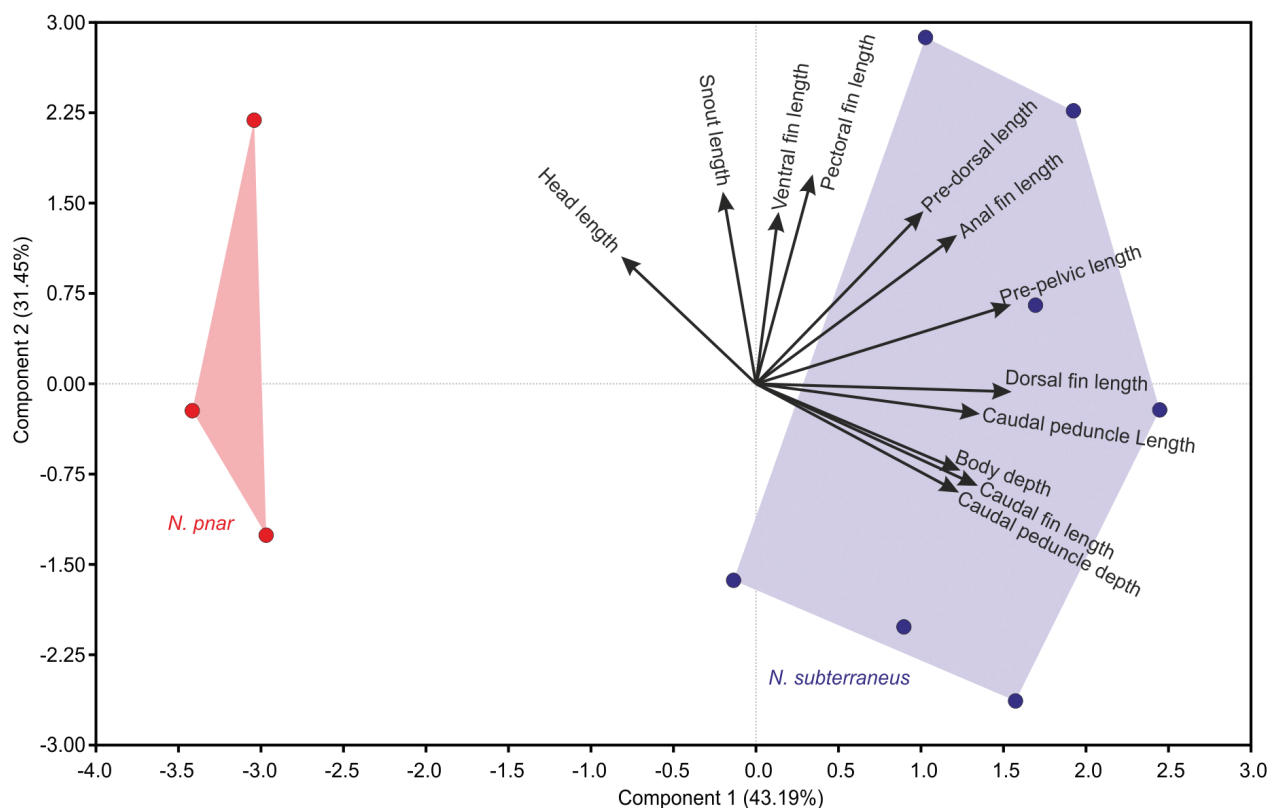
of Assam and Nagaland, as opposed to the type locality of *N. pnar* that drains into the Meghna River basin. The fish identified as *N. hexastichus* in the Siju Cave, Garo Hills, Meghalaya (Hora 1924; Kemp and Chopra 1924), and the unidentified pale cyprinids in the same cave in February 2019 (Harries et al. 2020) could also likely belong to this group, but this needs to be confirmed. Though *N. pnar* forms a reciprocally monophyletic clade distinct from this sister taxon, and supported additionally by the ASAP-based species delimitation, the two groups are separated by a low raw genetic distance of 0.5–0.8% in the COI gene. The low genetic divergence between the valid species of *Neolissochilus* have also been documented previously (Laskar et al. 2013; Lalramliana et al. 2019). However, further studies using multiple genes, supported by morphological and skeletal anatomical observations are required to conclusively understand this interesting sister-taxon relationship, and also the genetic diversity and its correlation with morphological diversity in members of the genus *Neolissochilus*.

One of the paratypes (KUFOS.F.2022.703) was collected from about 1.2 km inside the adjacent Krem Chympe cave, had a slightly different appearance including distinctly larger eyes and scalation pattern than fish of a similar size from those in the Krem Um Ladaw. Although the paratype of *Neolissochilus pnar* from the Krem Chympe cave was identical to the COI barcoding region and partial 16S genes of holotype and paratype from Krem Um Ladaw, there is a 1.4% raw genetic distance in the *cyt b* gene between the two populations.

The eye size in individuals of the Krem Um Ladaw population reduces as fish size increases. The smallest individuals have distinct, but atrophied eyes, which then become less distinct in larger individuals, and appear to be entirely absent in the largest individuals. This pattern appears consistent over all individuals photographed and videoed in the Krem Um Ladaw.

Genetic data for *Neolissochilus subterraneus*, the only other known subterranean species of *Neolissochilus* described from Tham Phra Wang Daeng cave in Thailand (Vidthayanon and Kottelat 2003), are not available. However, *N. pnar* is morphologically distinct from *N. subterraneus*, and the two species form distinct clusters (PERMANOVA, 9999 permutations,  $F = 7.572$ ,  $p = 0.0084$ ) in multivariate morphometric space (Fig. 6). It is also highly unlikely that these two species inhabiting two distinct biogeographic regions – *N. pnar* in the Eastern Himalaya and *N. subterraneus* in Indo Burma, separated by the 950km long Arakan Mountains, are conspecific.

*Neolissochilus pnar* is the largest known troglotic species by a considerable margin. There has been the view that troglotic adaptations are a consequence of the limited food availability in cave habitats. The need to locate sparse food reserves is thought to drive the development of the enhanced chemosensory capabilities typical of troglotes (Wilkens and Strecker 2017: 91–94). Among troglotic fish, the limited food availability is also thought to constrain the body size of fish that can develop in the cave environment (Volkoff 2016). So, the occurrence of *N. pnar*, a cave fish with both a relatively



**Figure 6.** Principal component analysis biplot of factor scores and factor loadings of morphometric data of the two subterranean species of *Neolissochilus*. Factor scores are shown as scatter of points and factor loadings are shown as arrows. Percentage variation, out of total variation in the data, explained by each principal component, are provided in parenthesis.

large size and striking troglomorphy from the Um Ladaw requires further study.

Despite the ichthyofaunal richness in aquifers and caves on the Indian subcontinent, there is only a limited number of studies dealing with their diversity and distribution. Recent descriptions of not only new species (Choudhury et al. 2017; Anoop et al. 2019; Britz et al. 2019; Sundar et al. 2022; Raghavan et al. 2023), but also new genera (Vincent and Thomas 2011; Britz et al. 2019, 2021) and even family level taxa (Britz et al. 2014; 2020) of freshwater fishes from the subterranean waters of India suggests major knowledge gaps in our understanding of these largely inaccessible habitats of the Indian subcontinent. Given that these habitats are also the most vulnerable to a number of anthropogenic activities (Raghavan et al. 2021), there is an immediate need to explore and understand the hidden diversity of subterranean realms in the region. Description of the world's largest subterranean fish *Neolissochilus pnar* is therefore likely to drive further explorations and understanding of this unique habitat and its remarkable fauna.

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## Supplementary material 1

### Supplementary informations

**Authors:** Dahanukar N, Sundar RL, Rangad D, Proudlove G, Raghavan R (2023)

**Data type:** .docx

**Explanation notes:** **Tables S1.** GenBank details for sequences used for Figure 3. — **Table S2.** Statistics for partition scheme and substitutional model analysis for maximum likelihood analysis provided in Figure 3. — **Table S3.** GenBank details for COI sequences used for Figure 4. — **Table S4.** Statistics for partition scheme and substitutional model analysis for maximum likelihood analysis provided in Figure 4. — **Table S5.** Statistics of barcode gap analysis using ASAP. Best partition is shown in the first row highlighted in grey.

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