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Discovery of a new species of troglobitic eel loach from southern India

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Abstract

A new species of troglobitic eel loach *Pangio juhuae*, is described from Kerala, India. *Pangio juhuae* sp. nov. is distinguished from its subterranean congeners by the presence of a dorsal fin; four pectoral rays and five segmented anal fin rays. Genetic analyses using mitochondrial cytochrome C oxidase 1 gene revealed that *P. juhuae* is closely related to *P. bhujia*, being separated by a genetic distance of 1.5%. However, the current species is conspicuously distinct from *P. bhujia* by the presence of a dorsal fin, among other features. A low genetic distance and significant morphological difference between these two *Pangio* species suggest that they have diverged from an immediate common ancestor and have evolved distinct adaptations to intricate subterranean niches of the region. Compared to *P. bhujia* and *P. pathala, less evolved troglobitic traits* reflect that *P. juhuae* can also be a connecting species in the evolutionary ladder of terrestrial loaches to subterranean ones. Hence, this could also be an instance of possible subterranean speciation of fishes in underground habitats.

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Introduction

Troglobitic (or stygobitic) fishes are found in subterranean habitats worldwide except Antarctica (Borowsky, 2018). They differ from troglophiles in exhibiting troglomorphism, or adaptations to life in caves and underground cavities (Zhao and Zhang, 2006; 2011). These adaptations often include the reduction or loss of eyes, pigmentation, scales and swim bladders, as well as the modification of appendages (Romero and Green, 2005). The evolution of troglomorphic traits can even occur through accidental entrapment, active colonisation, or regressive evolution (Barr, 1968; Romero, 1984; Wilkens and Stecker, 2017). The discovery of new subterranean fish species has surged in recent years. The global count reached 309 cave and groundwater species, 53 interstitial species and 55 non-subterranean species exhibiting troglomorphic traits (Proudlove, 2024). China, Brazil, Mexico and India are recognised as hotspots for this underground ichthyofaunal diversity.

In India, research on subterranean fauna has been limited, with significant gaps in our understanding of faunal structure, distribution and ecological roles within groundwater ecosystems. However, Kerala, located in south-western India, is emerging as a potential hotspot for subterranean fish diversity. This region boasts 12 described species, representing four families across four orders (Menon, 1951; Talwar and Jhingran, 1991; Bailey and Gans, 1998; Gopi, 2002; Babu and Nayar, 2004; Vincent and Thomas, 2011; Babu, 2012; Britz *et al*., 2019; Anoop *et al*., 2019; Kumar *et al.*, 2019; Sundar *et al*., 2022). Notably, the order Cypriniformes, particularly the suborders Cyprinoidei and Cobitoidei, exhibits the highest number of documented troglobitic species.

Globally, the Cobitidae family and more specifically the genus *Pangio* (commonly known as eel-loaches), is less frequently encountered in subterranean habitats compared to the Cyprinidae family. Within India, the *Pangio* genus encompasses eight recognised species, with five documented in Peninsular India and the remaining three distributed across the eastern and north-eastern regions of the country (Menon, 1999; Britz *et al.,* 2012; Anoop *et al.,* 2019; Sundar *et al*., 2022). To date, only two subterranean eel-loach species, *P. bhujia* (Anoop *et al.*, 2019) and *P. pathala* (Sundar *et al*., 2022), have been formally described. The present study focuses on description of a new species of subterranean eel-loach from Kerala, South India.

Materials and methods

Five specimens of a subterranean eel-loach were collected on 18 December 2021 from a dugout well in Naduvannur, Kozhikode, located in northern Kerala. Careful examination of these specimens revealed distinct characteristics that differentiate them from the two previously described species of subterranean eel-loach in the genus *Pangio,* warranting further investigation and potential classification as a new species.

Four specimens were fixed in 10% formalin and then preserved in 70% ethanol. These were used for morpho-meristic analysis. One specimen was preserved in absolute ethanol and used for molecular analysis. Morphometric data were recorded using digital callipers to the nearest tenth of a millimetre. Vertebrae was counted from the microCT image and fin rays were counted against transmitted light.

DNA extraction and amplification of the cytochrome C oxidase 1 gene sequence

A standard phenol/chloroform extraction protocol was followed for DNA extraction. A 650 bp region of the cytochrome C oxidase 1 (CO1) gene was amplified using the universal primer (Folmer *et al*., 1994) using a Biorad T100 thermocycler (Biorad, USA). The PCR products were purified, sequenced, and the 650 bp region of COI gene sequence was deposited in GenBank with accession no: ON509664. Cytochrome C oxidase 1 sequence of *Pangio juhuae* sp. nov. was aligned with sequences retrieved from GenBank of other valid species of *Pangio*; *P. bhujia, P. ammophila, P. anguillaris, P. pangia, P. kuhlii, P. pathala* and *P. oblonga* using Clustal W in MEGA X. A phylogenetic tree was constructed using the maximum likelihood method with 1000 bootstraps. Tree topology was also tested using maximum parsimony, UPGMA and neighbor-joining methods. The tree was then rooted with CO1 sequences of *Etroplus suratensis* retrieved from GenBank. Genetic divergence between all the species was analysed using kimura 2 p distance values and uncorrected p values in MEGA X.

Results

Pangio juhuae New species

Class: *Teleostei*

Order: *Cypriniformes*

Family: *Cobitidae* Swainson, 1838

Genus: *Pangio* Blyth, 1860

Species: *Pangio juhuae* sp. nov. Sreenath *et al*.

Proposed common name: Juhu's pigmy eel-loach

Material examined

Holotype: CMFRI DNR GB 22.4.1.1 Collected on 18 December 2021 from Naduvannur, Kozhikode, Kerala.

Paratypes: (I) CMFRI DNR GB 22.4.1.1.1, (II) CMFRI DNR GB 22.4.1.1.2. Collected on 18 December 2021 from Naduvannur, Kozhikode, Kerala.

Diagnosis

Pangio juhuae is morphologically distincti by the presence of a prominent dorsal fin, which is absent in its hypogeal congeners *P. bhujia* and *P. pathala*. In addition, the eye diameter of *P. juhuae* (7.4-8.8% HL) is relatively larger than the latter two species [*P. bhujia* (5.7-6.7); *P. pathala* (5.2-7.3)]. It is further distinguished from *P. bhujia* in having four rays in the pectoral fins (*vs*. three) and five in the anal fin (*vs*. six). The troglomorphic traits such as a slender body, lack of pigmentation, lack of scales and diminutive eyes make them different from all other non-subterranean congeners.

Description

General appearance as in Fig. 1 (Preserved specimen in Fig. 2). Morphometric data are given in Table 1. The body is thin, slender, elongated and anguilliform and its depth is 9.2-11% standard length (SL). The pre-dorsal region is oval in cross-section, body width of 2.3-4.3% SL; the caudal region, from immediately after the dorsal fin, is laterally compressed, its width 0.8-1.4% SL. Pre-caudal adipose keels are well developed, extending more than half the distance dorsally and ventrally to the dorsal and anal fins, respectively. Scales absent. Head rounded, small, head length (HL) 9.72-11.3% SL. Mouth subterminal. Eyes small (7.4-8.8% HL), located approximately in the middle of the head, snout length 50-52% HL. Barbels in 4 pairs. The nasal barbel is slightly longer

Fig. 1. *P. juhuae*; Holotype: CMFRI DNR GB 22.4.1.1; Type Locality: Naduvannur, Kozhikode District, Kerala, India

Fig. 2. *P. juhuae*; Holotype: CMFRI DNR GB 22.4.1.1 after preservation in formalin

Table 1. Morphometric measurements of *Pangio juhuae*

Morphometric measurements (mm)	GB 22.4.1.1	GB 22.4.1.2	GB 22.4.1.1.1	GB 22.4.1.1.2	Mean	SD
Total length (mm)	28.7	31.2	26.1	28.56	28.6	2.1
Standard length (mm)	26.2	30	25	26.53	26.9	2.1
% Standard length						
Pre-dorsal length	69.1	67.7	73.6	63.89	68.6	4.0
Pre-anal length	72.1	73.0	73.2	69.05	71.8	1.9
Body depth	9.9	11.0	9.2	9.6	9.9	0.8
Body width	2.3	4.3	3.6	3.9	3.5	0.9
Caudal peduncle length	23.7	22.3	25.2	22.24	23.4	1.4
Caudal peduncle depth	3.4	4.3	3.2	3.6	3.6	0.50
Caudal peduncle width	0.8	1.0	1.2	1.4	1.1	0.3
Head length	9.9	11.3	10.0	9.72	10.2	0.70
% Head length						
Snout length	50.0	50.0	52.0	51.9	51.0	1.1
Eye diameter	7.7	8.8	8.0	7.4	8.0	0.6

than the maxillary and mandibular. Four branchiostegal rays. The dorsal fin is placed in the posterior half of the body (pre-dorsal length 63.89-73.6% SL), bearing 5 rays; the first and last are smaller than the middle three, which are equal in length. The pectoral fin is slender, bearing 4 rays, all segmented, the third one the longest. Pelvic fin absent. Anal fin short with a rounded margin, its origin behind the dorsal fin (pre-anal length 69.05-73.2% SL); bearing 5 rays, almost equal in length, all unbranched and segmented. Caudal fin lanceolate; Caudal fin rays 14+6 (principal rays + procurrent rays), all segmented. Vertebrae numbers counted using micro-CT (Webberian + 57 + Hypural) (Fig. 5) were also found to be lesser than that of *P. bhujia* (62-63) and *P. pathala* (67).

Colouration

In life, the body is translucent and devoid of melanin, with the vertebrae visible. The colour varies from burnt red in the abdominal region due to the apparent internal organs and blood vessels to reddish brown on the dorsal side. The caudal peduncle is transparent tan towards both the dorsal and ventral margins, with the caudal artery visible as a red streak in the centre. The diminutive eyes appear as small black specks. Uniformly beige in preservative, the eyes visible as dark spots.

Distribution

Currently, the species is known only from its type locality, Naduvannur, a village in Kozhikode District, Kerala State, India.

Habitat

The fish was first observed in a water tank filled with water drawn from a dug-out well (Fig. 3) on a sloping terrain (150 m asl). The well is supplied with underwater springs.

Etymology

The species is named after a four-year-old girl, Juhu, who first noticed it.

Fig. 3. The well from which the samples were collected

Genetic analyses

The phylogenetic tree constructed using 13 sequences of CO1 of the eight *Pangio* species showed distinct clustering among species (Fig. 4) with significant bootstrap values. *Pangio juhuae* sp. nov. formed a sub-cluster of *Pangio bhujia* with significant bootstrap value. *P. pathala* formed a sister species to *P. juhuae* and *P. bhujia*. Similar tree topology was also obtained when phylogenetic analyses were conducted using maximum likelihood, maximum parsimony and neighbour-joining methods. Kimura 2 p (K2P) distance percentage values (Table 2) between species showed 1.5, 9.95, 23, 23.3, 18.9, 18.3, 22.13% divergence between the new species and *P. bhujia*, *P. pathala*, *P. kuhlii*, *P. oblonga, P. anguillaris*, *P. ammophila* and *P. pangia* respectively. So genetically, the new species is closest to *P. bhujia* followed by *P. pathala.* Concordant results were obtained with uncorrected p percentage values also, as the divergence of *P. juhuae* from *P. bhujia*, *P. pathala*, *P. kuhlii*, *P. oblonga, P. anguillaris*, *P. ammophila* and *P. pangia,* were recorded as 1.46, 9.15, 19.68, 19.68, 16.58, 16.06 and 18.97% respectively.

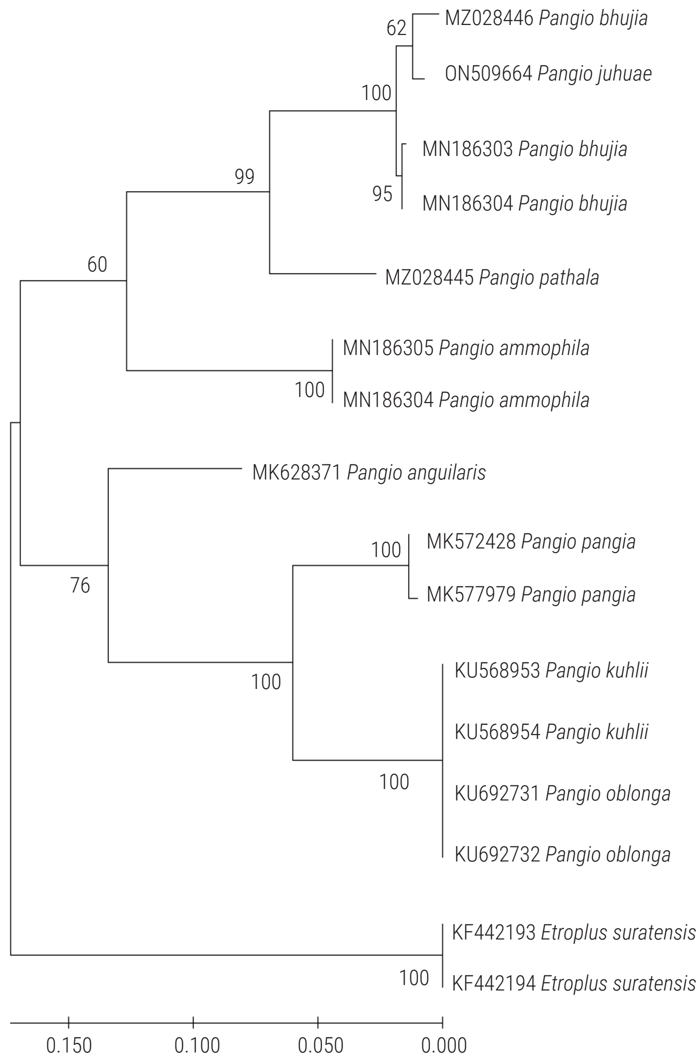

Fig. 4. The phylogenetic tree constructed by the Maximum Likelihood method using COI sequences of *Pangio* species

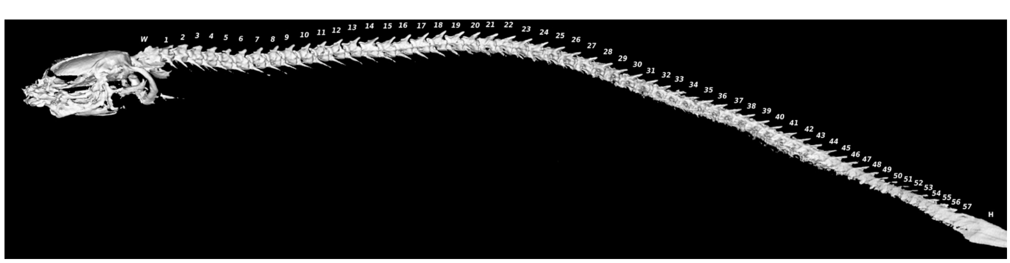

Fig 5. MicroCT image of *P. juhuae.* W: Weberian apparatus, Numbered vertebrae (57), H: Hypural

Table 2. K2P (below diagonal) and uncorrected p distance (above diagonal) percentage values between species

Species	*P. juhuae*	*P. bhujia*	*P. pathala*	*P. kuhlii*	*P. oblonga*	*P. pangia*	*P. anguillaris*	*P. ammophila*
P. juhuae	--	1.46	9.15	19.68	19.68	18.97	16.58	16.06
P. bhujia	1.50	--	9.06	19.08	19.08	19.35	15.80	15.80
P. pathala	9.95	9.85	--	19.17	19.17	19.05	16.58	14.68
P. kuhlii	23.34	22.46	22.61	--	1.12	10.12	15.19	20.72
P. oblonga	23.34	22.46	22.61	1.12	--	10.12	15.19	20.72
P. pangia	22.13	22.67	22.31	11.06	11.06	--	15.11	19.13
P. anguillaris	18.96	17.91	18.97	17.28	17.28	17.17	--	16.75
P. ammophila	18.33	17.98	16.54	24.81	24.81	22.58	19.33	--

Discussion

The Cobitidae family, a prominent component of the Eurasian ichthyofauna with around 31 extant genera ranging from Portugal to Japan and Siberia to Java (Kottelat, 2012; Fricke *et al*., 2022), presents a captivating subject for biogeographic studies. The genus *Pangio* Blyth, 1860, the largest within the southern lineages of Cobitidae, now encompasses 35 recognised species (Froese and Pauly, 2023), further amplifying its significance in biogeographic research. The recent discovery of subterranean species within this genus adds another layer of intrigue.

The identification of several subterranean ichthyofauna in both the Ghats and lowland regions of Kerala has shed light on the complex underground habitats and their associated biodiversity. It is noteworthy that the genus *Pangio* in India now exhibits both epigean (surface-dwelling) species such as *P. goaensis* and *P. ammophila* and hypogean (subterranean) varieties such as *P. bhujia, P. pathala*, and *P. juhuae*. These hypogean species are exclusively known from Kerala, which is likely attributed to the region's extensive lateritic geology, which fosters the formation of subterranean caverns, tunnels and aquiferous features. This geological context aligns with previous phylogeographic studies on *Pangio* (Bohlen *et al.*, 2011; Slechtova *et al.*, 2021), suggesting that multiple vicariance and dispersal events from the Sundaland have shaped their current distribution. Considering the established Gondwanian connection of the peninsular Indian landmass, the discovery of relict populations and their evolved successors adapted to subterranean conditions is not unexpected (Vincent, 2012).

Comparative morphology confirms that all three hypogean species have developed regressive evolutionary troglomorphic traits, such as reduced eyes, lack of pigmentation and absence of scales, as adaptations to their underground habitats. *Pangio juhuae* stands out for its intermediate troglomorphic features, notably its rudimentary eyes, which are more prominent than those of the other two hypogean congeners yet less developed than the eyes of epigean species such as *P. ammophila* or *P. goaensis*. Additionally, the presence of a dorsal fin, which is absent in *P. bhujia* and *P. pathala*, suggests that *P. juhuae* might still be in the process of evolving towards a fully hypogean life.

The phylogenetic distance of 1.5% between *P. juhuae* and *P. bhujia* exceeds the lowest inter-species variation of 1.12% among congeners (*P. oblonga vs P. kuhlii*; Table 2). While both species inhabit the same geographic area, with the holotype of *P. bhujia* recorded only 35 km away but at a lower altitude, this does not preclude speciation. Speciation within overlapping ranges is documented, with niche partitioning within caves potentially driving the segregation of body types (troglobiomorphisms) in other taxa (Langille *et al.*, 2021). Despite a modest genetic distance, the substantial morphological difference between the two *Pangio* species suggests recent divergence from a common ancestor and adaptation to distinct subterranean niches.

The comparatively less evolved subterranean characteristics of *P. juhuae* compared to *P. bhujia* and *P. pathala* indicate its potential as a transitional species, offering insights into subterranean speciation processes. The discovery of this species, along with others, underscores the need for technological advancements in exploring subterranean habitats. These unique life forms face mounting pressures due to changing monsoon patterns and land use, which place increasing demands on groundwater. It is crucial to consider whether the recent surge in discoveries of hypogean fish species in the region reflects heightened awareness, increased precipitation and aquifer flushing, or intensified groundwater extraction. By understanding the evolutionary history and ecological context of these subterranean species, we can better appreciate the biodiversity hidden beneath our feet and implement effective conservation strategies to protect these fragile ecosystems.

In conclusion, this discovery could serve as a valuable model for understanding subterranean speciation and the adaptive mechanisms underlying the colonisation of these unique habitats. Further research into this loach has the potential to shed light on broader evolutionary patterns and the remarkable biodiversity thriving in the hidden world beneath our feet.

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