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## ***Cabdio crassus*, a new species of cyprinid fish (Teleostei: Cyprinidae) from the Kaladan River of Mizoram, India**

LALRAMLIANA\*<sup>1</sup>, SAMUEL LALRONUNGA<sup>2</sup> & MAHENDER SINGH<sup>3</sup>

<sup>1</sup>Department of Zoology, Pachhunga University College, Aizawl-Mizoram, India.

Email: [lrl\\_zoo@yahoo.co.in](mailto:lrl_zoo@yahoo.co.in)

<sup>2</sup>Department of Environmental Science, Pachhunga University College, Aizawl-Mizoram, India.

<sup>3</sup>ICAR-National Bureau of Fish Genetic Resources, Lucknow, India.

\*Corresponding author

### **Abstract**

*Cabdio crassus*, a new fish species, is described from the Kaladan River in Mizoram, India. The new species is distinguished from all its congeners by having a ventral keel extending from the middle of the chest, between the posterior base of the pectoral fin and along the abdomen up to the anus (vs. more or less keeled median scales from mid-point of abdomen between posterior base of pelvic fin up to anus in all other *Cabdio*) and 11½–12½ branched anal-fin rays (vs. 7 in *C. jaya* and 9 in both *C. morar* and *C. ukhrulensis*). It is further distinguished from *C. morar* and *C. ukhrulensis* by possessing more lateral-line scales (45–51 vs. 38–42 in *C. morar* and 35–37 in *C. ukhrulensis*), more predorsal scales (20–23 vs. 17–18 in *C. morar* and 14 in *C. ukhrulensis*) and more lateral transverse scales (½7/1/3½ vs. 5/1/2 in both *C. morar* and *C. ukhrulensis*). It also differs from *C. jaya* in having fewer lateral-line scales (45–51 vs. 52–60), more lateral transverse scales (½7/1/3½ vs. 5/1/3) and more pharyngeal tooth-rows (3 vs. 2). Furthermore, the *cytochrome c oxidase sub unit I (coi)* gene sequence separates *Cabdio crassus* from all other *Cabdio* species (interspecies distance ranges from 7.8–12.3%). The anomalies observed among the GenBank sequences of the genus *Cabdio* are discussed and resolved.

**Keywords:** Taxonomy, DNA barcode, freshwater fish, Indo-Burma Biodiversity hotspot

### **Introduction**

Hamilton (1822) described *Cyprinus morar* and *C. jaya* under the divisions *Chela* and *Cabdio* respectively. Heckel (1848) erected the genus *Aspidoparia* giving *A. sardina* as type species. This was subsequently acknowledged by Günther (1868: 285–286), who tentatively included *A. morar* and *A. jaya* in the genus. Day (1878), however, considered *Aspidoparia sardina* to be a synonym of *A. morar* and included only two species under the genus, viz., *A. jaya* and *A. morar*. *Aspidoparia* was regarded as a valid genus by subsequent authors (Talwar & Jhingran 1991; Selim & Vishwanath 2001) until recent studies (Tang *et al.* 2010; Liao *et al.* 2011; Kottelat 2013) showed it to be a junior synonym of *Cabdio*. There are currently three valid species of *Cabdio*, viz., *C. jaya* (Hamilton 1822), *C. morar* (Hamilton 1822) and *C. ukhrulensis* (Selim & Vishwanath 2001).

The genus *Cabdio morar* (type locality ‘Yamuna and Tista Rivers, India’) has been reported from Iran, Pakistan, India, Nepal, Bangladesh, Myanmar and Thailand. However, the identity of the species reported as *C. morar* from Iran, Myanmar, Pakistan and Thailand needs to be confirmed (Chaudhury 2010a). *Cabdio jaya* (type locality ‘Northern Behar, India’) occurs over a narrow range along the Ganges-Brahmaputra basin in India, Nepal and Bangladesh (Chaudhury 2010b); while *C. ukhrulensis* (type locality ‘Chatrickong River, Ukhrul District, Manipur, India’) is restricted to the Chindwin-Ayeyarwaddy basin.

Recent collections of fishes in the Kaladan River included a species of *Cabdio* that differs from the three other species of the genus already reported. A comparison of the morphology and *cytochrome c oxidase sub unit I (coi)* sequences of the species with congeners revealed it to be an unnamed species, which is described herein as *Cabdio crassus*, sp. nov.

## Material and methods

**Morphological analysis.** Specimens were fixed in 10% formalin and subsequently preserved in 70% alcohol. Counts and measurements were made on the left side of specimens following Kottelat & Freyhof (2007) (Table 1). Measurements were made point to point with digital callipers to the nearest 0.1 mm. Measurements, except standard length, are given as proportions of standard length (SL). Sub-units of the head are presented as proportions of head length. Fin rays were counted under a stereo zoom microscope. For vertebral counts, two specimens were cleared and stained in alizarin. Numbers in parentheses after a meristic value indicate the frequency of that value. Abbreviation of institutions: PUCMF for Pachhunga University College Museum of Fishes, Mizoram, India; MUMF for Manipur University Museum of Fishes, Manipur, India; ZSI/ERS for Eastern Regional Station, Zoological Survey of India, Shillong, Meghalaya, India; and ZSI for Zoological Survey of India, Kolkata. SD is standard deviation.

**TABLE 1.** Morphometric data for *Cabdio crassus* (n=15). (ZSI FF 8218, FF 8219; PUCMF 17017, 17018). Ranges include values of holotype.

|                             | holotype | Range      | Mean ± SD |
|-----------------------------|----------|------------|-----------|
| Standard length (SL) in mm  | 88.1     | 85.8–111.4 |           |
| <b>% of SL</b>              |          |            |           |
| Body depth at dorsal        | 28.9     | 25.2–30.4  | 27.6±1.4  |
| Body depth at anal          | 22.4     | 20.2–23.4  | 21.7±0.8  |
| Maximum body depth          | 29.2     | 25.8–30.9  | 28.2±1.3  |
| Predorsal length            | 60.3     | 56.8–61    | 59.3±1.2  |
| Prepectoral length          | 25.7     | 23.3–26.1  | 25.0±0.7  |
| Prepelvic length            | 54.1     | 50.9–54.2  | 52.9±0.8  |
| Preal anal length           | 77.8     | 73.8–78.7  | 76.3±1.2  |
| Anal to pectoral            | 54.1     | 50.7–55.3  | 53.4±1.2  |
| Anal to pelvic              | 25.5     | 22–26.8    | 25.1±1.2  |
| Dorsal to hypural           | 45.3     | 42.8–46.1  | 44.3±0.9  |
| Caudal-peduncle depth       | 10.3     | 10.3–11.3  | 10.6±0.3  |
| Caudal-peduncle length      | 10       | 10–13.5    | 12.1±0.9  |
| Dorsal-fin height           | 21       | 19.1–22.1  | 20.5±0.9  |
| Anal-fin length             | 14.2     | 12.6–15.6  | 13.8±0.8  |
| Pectoral-fin length         | 25       | 23.2–26.5  | 24.7±1.0  |
| Pelvic-fin length           | 15.8     | 15.4–17.2  | 16.0±0.5  |
| Head length                 | 23.2     | 21.1–23.6  | 22.4±0.7  |
| <b>% of HL</b>              |          |            |           |
| Snout length                | 31.9     | 28.5–31.9  | 30.1±1.1  |
| Eye diameter                | 25.5     | 23.7–28.6  | 25.5±1.1  |
| Internarial                 | 20.1     | 19–22.2    | 20.8±0.8  |
| Interorbital width          | 36.8     | 34.5–37.6  | 36.3±1.0  |
| Head width at eye           | 46.6     | 40.4–46.9  | 44.0±2.3  |
| Head width at opercle       | 51       | 47–53.1    | 49.8±1.7  |
| Head depth at eye           | 57.8     | 55.8–62.1  | 59.8±1.7  |
| Head depth posterior to eye | 69.6     | 64.6–71.6  | 69.6±1.7  |
| Head depth at occiput       | 80.4     | 76.5–83.7  | 81.2±1.9  |

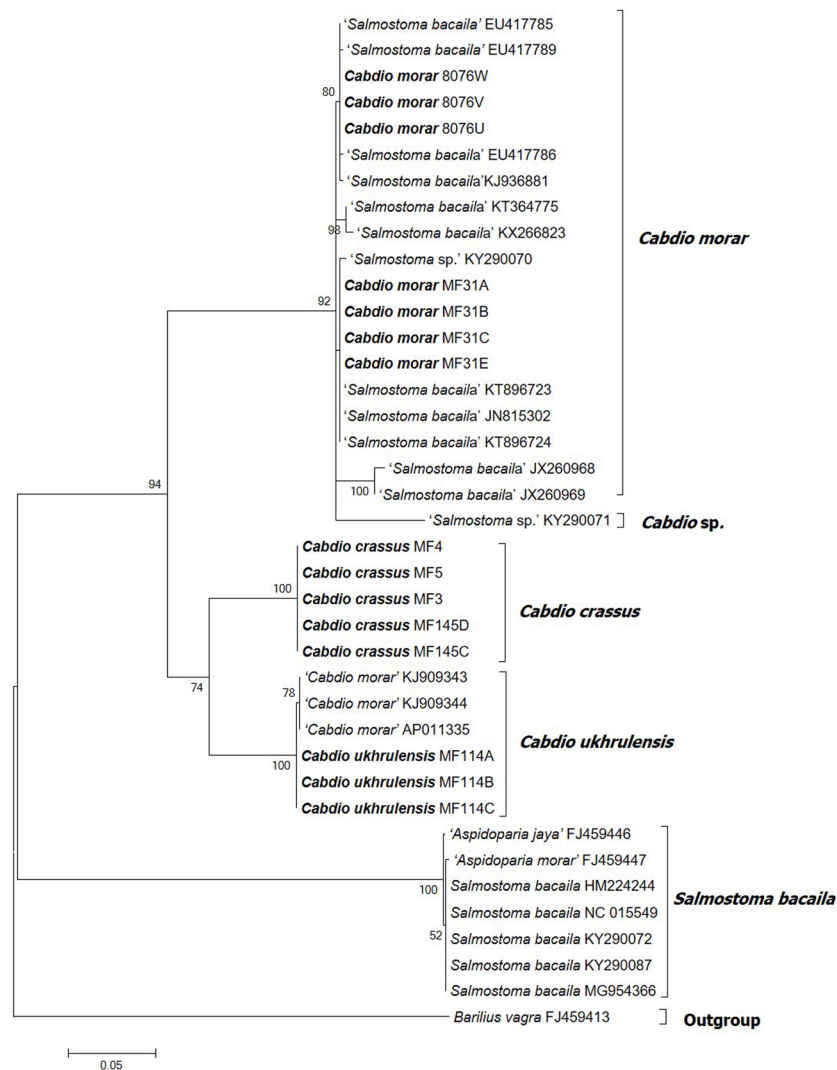
**DNA extraction, PCR amplification and DNA sequencing.** Approximately 40 mg of muscle tissue samples (5 paratype specimens for *Cabdio crassus*, 7 for *C. morar* and 3 for *C. ukhrulensis*) were extracted from the voucher specimens and preserved in 100% ethanol prior to fixation of the specimens in formalin. DNA extraction follows Sambrook & Russel (2001). Amplification of the *coi* gene were performed using the primers Fish-F1 and Fish-R1 (Ward *et al.* 2005) in Veriti 96 fast thermal cycler (Applied Biosystems, Inc., USA). A total of 25 µl PCR volume

containing 1X buffer, 100  $\mu$ M dNTPs, 2 mM MgCl<sub>2</sub>, 5 pmol of each primer, 2U Taq DNA polymerase and 100 ng template DNA were prepared. The PCR conditions are: initial denaturation of 3 min at 94°C, followed by 35 cycles of denaturation at 94°C for 50 sec, annealing at 54°C for 30 sec., extension at 72°C for 80 sec. with final extension of 10 min at 72°C. Sequencing was performed in forward direction only using an automated ABI 3500 Genetic Analyzer (Applied Biosystems, Inc, USA).

**TABLE 2.** List of sequences (n=39) analysed in this study.

| SI No. | Species                          | NCBI Accn. No. | Country                         | Reference               |
|--------|----------------------------------|----------------|---------------------------------|-------------------------|
| 1.     | <i>Cabdio crassus</i> MF145C     | MN065162       | Mizoram, India                  | This study              |
| 2.     | <i>Cabdio crassus</i> MF145D     | MN065163       | Mizoram, India                  | This study              |
| 3.     | <i>Cabdio crassus</i> MF3        | MN065164       | Mizoram, India                  | This study              |
| 4.     | <i>Cabdio crassus</i> MF4        | MN065165       | Mizoram, India                  | This study              |
| 5.     | <i>Cabdio crassus</i> MF5        | MN065166       | Mizoram, India                  | This study              |
| 6.     | <i>Cabdio morar</i> 8076U        | MN065167       | Lucknow, India                  | This study              |
| 7.     | <i>Cabdio morar</i> 8076V        | MN065168       | Lucknow, India                  | This study              |
| 8.     | <i>Cabdio morar</i> 8076W        | MN065169       | Lucknow, India                  | This study              |
| 9.     | <i>Cabdio morar</i> MF31A        | MN065170       | Mizoram, India                  | This study              |
| 10.    | <i>Cabdio morar</i> MF31B        | MN065171       | Mizoram, India                  | This study              |
| 11.    | <i>Cabdio morar</i> MF31C        | MN065172       | Mizoram, India                  | This study              |
| 12.    | <i>Cabdio morar</i> MF31E        | MN065173       | Mizoram, India                  | This study              |
| 13.    | <i>Cabdio ukhrulensis</i> MF114A | MN065174       | Myanmar (Fish Market)           | This study              |
| 14.    | <i>Cabdio ukhrulensis</i> MF114B | MN065175       | Myanmar (Fish Market)           | This study              |
| 15.    | <i>Cabdio ukhrulensis</i> MF114C | MN065176       | Myanmar (Fish Market)           | This study              |
| 16.    | <i>Salmostoma bacaila</i>        | EU417785       | India                           | GenBank                 |
| 17.    | <i>Salmostoma bacaila</i>        | EU417789       | India                           | GenBank                 |
| 18.    | <i>Salmophasia bacaila</i>       | KJ936881       | Meghalaya, India                | GenBank                 |
| 19.    | <i>Salmostoma bacaila</i>        | EU417786       | India                           | GenBank                 |
| 20.    | <i>Salmophasia bacaila</i>       | KT364775       | Tanguar Haor, Bangladesh        | GenBank                 |
| 21.    | <i>Salmophasia bacaila</i>       | KX266823       | South India                     | GenBank                 |
| 22.    | <i>Salmophasia bacaila</i>       | KT896724       | Manipur, India                  | GenBank                 |
| 23.    | <i>Salmophasia bacaila</i>       | JN815302       | Assam, India                    | GenBank                 |
| 24.    | <i>Salmophasia bacaila</i>       | KT896723       | Manipur, India                  | GenBank                 |
| 25.    | <i>Salmostoma bacaila</i>        | JX260969       | Andhra Pradesh, India           | GenBank                 |
| 26.    | <i>Salmostoma bacaila</i>        | JX260968       | Andhra Pradesh, India           | GenBank                 |
| 27.    | <i>Salmostoma</i> sp.            | KY290070       | Assam, India                    | GenBank                 |
| 28.    | <i>Salmostoma</i> sp.            | KY290071       | Assam, India                    | GenBank                 |
| 29.    | <i>Cabdio morar</i>              | KJ909343       | Manipur, India                  | GenBank                 |
| 30.    | <i>Cabdio morar</i>              | KJ909344       | Manipur, India                  | GenBank                 |
| 31.    | <i>Cabdio morar</i>              | AP011335       | Aquarium trade (unknown source) | Tang <i>et al.</i> 2010 |
| 32.    | <i>Aspidoparia jaya</i>          | FJ459446       | India                           | GenBank                 |
| 33.    | <i>Aspidoparia morar</i>         | FJ459447       | Assam, India                    | GenBank                 |
| 34.    | <i>Salmostoma bacalia</i>        | HM224244       | Bangladesh                      | Tang <i>et al.</i> 2010 |
| 35.    | <i>Salmostoma bacalia</i>        | MG954366       | West Bengal, India              | GenBank                 |
| 36.    | <i>Salmostoma bacalia</i>        | NC015549       | West Bengal, India              | Tang <i>et al.</i> 2010 |
| 37.    | <i>Salmostoma bacalia</i>        | KY290087       | Assam, India                    | GenBank                 |
| 38.    | <i>Salmostoma bacalia</i>        | KY290072       | Assam, India                    | GenBank                 |
| 39.    | <i>Barilius vagra</i>            | FJ459413       | Uttarakhand, India              | GenBank                 |

**DNA sequence analysis.** The partial *coi* genes of fifteen individuals of three species of the genus *Cabdio* (5 individuals of *C. crassus*, 7 of *C. morar* and 3 of *C. ukhrulensis*) were sequenced. In addition, 23 sequences comprising *Cabdio* and *Salmostoma bacaila* species along with one outgroup, *Barilius vagra*, were included from GenBank for comparative analysis (Table 2). The sequences were aligned using CLUSTAL\_W, blasted in NCBI (<http://www.ncbi.nlm.nih.gov>) for the nearest matches and submitted to NCBI GenBank (Accession Nos. MN065162–MN065176). The maximum likelihood (ML) and Bayesian phylogenetic tree (using the *coi* dataset), implemented in MEGA7 (Kumar *et al.* 2016) and BEAST (Bouckaert *et al.* 2014) respectively, resulted into well-supported trees with similar topologies. Since both the resultant trees exhibited similar topology, only the ML tree is presented herein (Fig. 1). The genetic distance was calculated by averaging pairwise comparisons of sequences across close relatives of *Cabdio* by the Kimura 2 parameter in MEGA7. Based on the lowest BIC (Bayesian Information Criterion), the best fit nucleotide substitution model (out of 24 models) for present *coi* data set was K2+G+I given by Nei & Kumar (2000).



**FIGURE 1.** Maximum Likelihood (ML) tree of *coi* dataset of 39 sequences using K2+G+I nucleotide substitution model with 1000 bootstraps

## Results

### *Cabdio crassus*, sp. nov.

(Fig. 2)

**Holotype:** ZSI FF 8218, 88.1 mm SL; India; Mizoram, Kaladan River, in the vicinity of Kawlchaw village; 23°28'29"N; 92°23'27"E; Lalramliana *et al.*, 24 Nov. 2015.

**Paratypes:** ZSI FF 8219, (2), 91.2–94.4 mm SL; PUCMF 17017 (5), 85.8–111.4 mm SL; PUCMF 17018 (3), 90.0–95.5 mm SL; (dissected and preserved in glycerin with thymol) data as holotype.

**Diagnosis.** *Cabdio crassus* markedly differs from all other valid species of *Cabdio* (Fig. 3) by having the venter keeled from mid-point of chest, between the posterior base of the pectoral fin and along the abdomen up to the anus, and possessing  $11\frac{1}{2}$ – $12\frac{1}{2}$  (vs. 7–9) branched anal-fin rays. It is further distinguished from *C. morar* and *C. ukhrulensis* by possessing more lateral-line scales (45–51 vs. 38–42 in *C. morar* and 35–37 in *C. ukhrulensis*), more predorsal scales (20–23 vs. 17–18 in *C. morar* and 14 in *C. ukhrulensis*) and more lateral transverse scales ( $\frac{1}{2}7/1/3\frac{1}{2}$  vs.  $5/1/2$  in both *C. morar* and *C. ukhrulensis*). It also differs from *C. jaya* in having fewer lateral-line scales (45–51 vs. 52–60), more lateral transverse scales ( $\frac{1}{2}7/1/3\frac{1}{2}$  vs.  $5/1/3$ ) and more pharyngeal tooth-rows (3 vs. 2).



**FIGURE 2.** *Cabdio crassus* ZSI FF 8218, holotype, 88.1 mm SL; India, Mizoram, Kaladan River

**Description.** See Table 1 for morphometric data and Figure 2 for general appearance. Body elongate, laterally compressed, body depth greatest at dorsal-fin origin. Dorsal profile rising from tip of snout to dorsal-fin origin, then gradually sloping towards caudal peduncle. Ventral profile convex up to caudal peduncle; dorsal and ventral profile of caudal peduncle slightly concave. Chest and abdomen keeled from mid-point of chest between posterior base of pectoral fin through abdomen till anus. Head compressed along entire length; depth greater than width.

Eye ovoid, located in anterior half of head, visible in both dorsal and ventral view. Snout rounded with cluster of tubercles; its length equal to eye diameter. Mouth subterminal, rictus not reaching vertical through anterior margin of orbit. Barbel absent.

Dorsal fin with 2 (15) unbranched and  $6\frac{1}{2}$  (1) or  $7\frac{1}{2}$  (14) branched rays, its origin at vertical through posterior base of pelvic fin, posterior margin slightly concave. Pectoral fin with 1 unbranched and 12 (3), 13 (9) or 14 (3) branched rays, adpressed fin tip reaching two-thirds distance between its origin and pelvic-fin origin, axillary scale present at its base. Pelvic fin with 1 unbranched and 7 (15) branched rays, adpressed fin tip reaching midway between its origin and anal-fin origin, axillary scale present at its base. Anus closer to anal-fin origin than to base of last pelvic-fin ray. Anal fin with 3 unbranched and  $11\frac{1}{2}$  (2) or  $12\frac{1}{2}$  (13) branched rays. Caudal fin forked, with 10 + 9 (15) principal caudal-fin rays, its lobes more or less equal.

Lateral line complete, prominent, curved, with 45 (2), 46 (6), 47 (3), 49 (1), 50 (2), 51 (1) on body plus 1 or 2 scales on caudal-fin base;  $\frac{1}{2}7/1/3$  (4) or  $\frac{1}{2}7/1/3\frac{1}{2}$  (11) scales in transverse line from dorsal-fin origin to pelvic-fin origin. Circumpeduncular scales 16 (15), predorsal scales 20 (3), 21 (3), 22 (7) or 23 (2), pre-pelvic and pre-anal region scaled. Five infraorbital bones (IO1–5); IO3 largest, deep, partly overlapping preoperculum; post cleithrum absent; 3 rows of pharyngeal teeth. Vertebrae (abdominal + caudal): 24 + 16 = 40 (1) or 25 + 15 = 40 (1).





**FIGURE 3.** Image of other *Cabdio* spp. A) *Cabdio jaya*, ZSI FF 5607, 74.3 mm SL; India, Arunachal Pradesh. B) *C. morar*, ZSI FF 4968, 70.6 mm SL; India, West Bengal, Ganga River. C) *C. ukhrulensis*, PUCMF 15054, 72.7 mm SL.

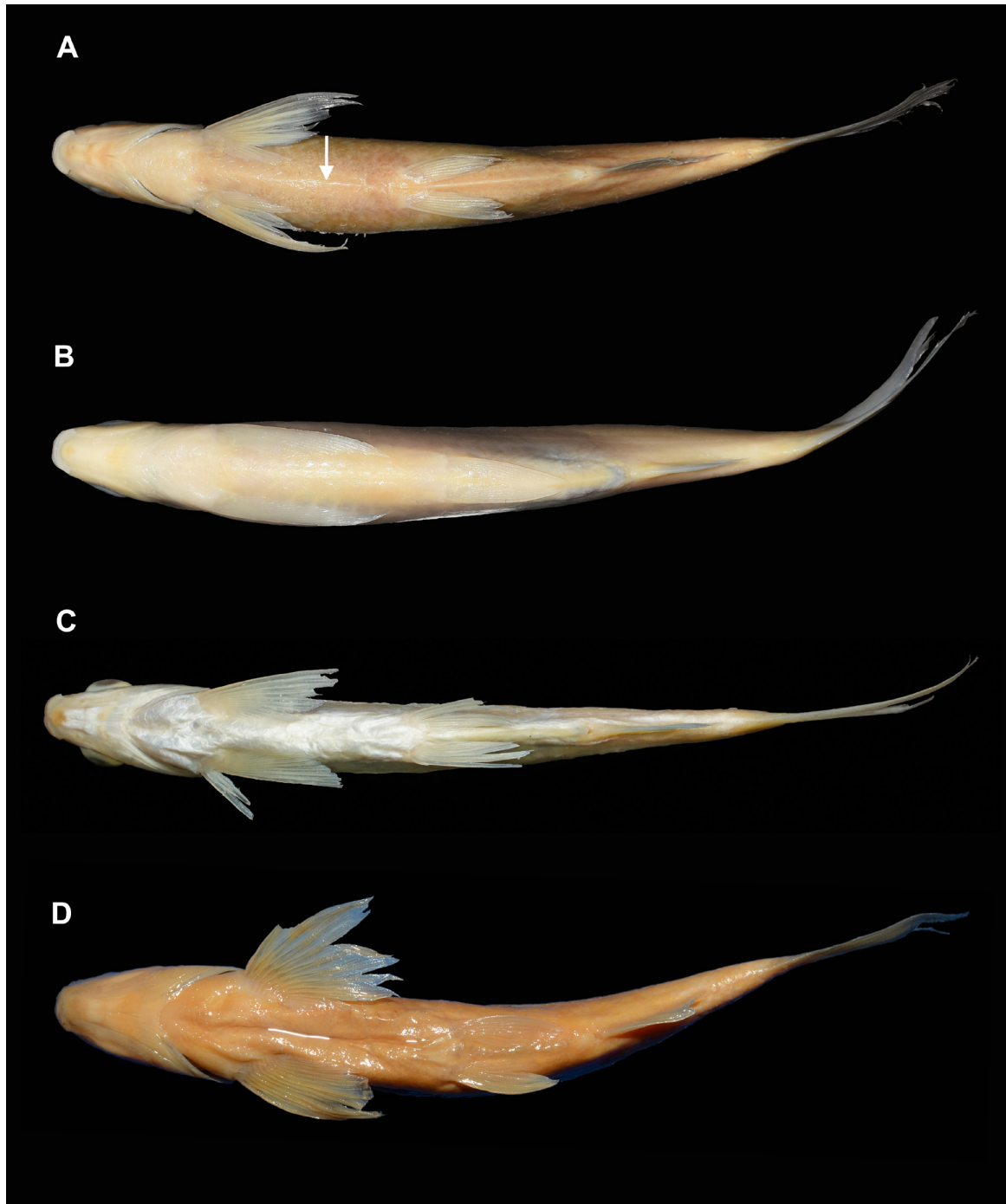
**Colouration.** (Fig. 2) Dorsum brownish, lighter towards flank. Lower half of body and belly silvery or pale white. Caudal fin hyaline with black pigment distally. Other fins hyaline without markings.

**Geographical distribution.** Known from the main channel of the Kaladan River drainage of Mizoram, India (Fig. 5).

**Etymology.** The specific name *crassus* is a Latin adjective meaning stout or thick, referring to the stout or thick body in comparison with other species.

**Molecular analysis.** In the *coi* tree, *Cabdio crassus* is nested within *Cabdio* (interspecies distance ranges from 7.8 to 12.3%) (Fig. 1). The genetic distance between *Cabdio crassus* and *C. morar* is 11.7–12.3%, and between it and *C. ukhrulensis* is 7.8–8.0%. The inability to collect fresh specimens results in non-availability of tissue sample in the case of *C. jaya*.

**Identity of *Cabdio* reported in GenBank.** Analysis of the generated *Cabdio* sequences with close species available in the GenBank revealed several potentially misidentified specimens (Table 3). Our 7 developed sequences of *Cabdio morar* (morphologically identified through a thorough comparison of specimens from ZSI, Kolkata and ZSI, Shillong: see Comparative Material, below) clustered in the ML tree with GenBank sequences labelled *Salmostoma bacaila* (downloaded from the NCBI KT364775, KX266823, EU417785, EU417786, EU417789, KJ936881, KJ896723, KJ896724 and JN815302) with 0.0–1.2 % K2P distance, indicating that these GenBank sequences in fact represent *Cabdio morar*. Further, the calculated genetic distance between *Cabdio morar* and the species labelled as *Salmostoma* sp. (JX 260968, JX 260969), which is 2.0–3.0%, and between *C. morar* and *Salmostoma* sp. (KY 290071), which is 4.4%, suggested that the latter two may represent species of *Cabdio*, rather than to *Salmostoma*. Our sequences of *Cabdio ukhrulensis* show a very low genetic distance (0.0–0.2%) and cluster together in the ML tree with GenBank sequences labelled *C. morar* (AP011335, KJ909343 and KJ909344) indicating that they too, are conspecific. Furthermore, the GenBank sequences labelled as *Aspidoparia morar* (FJ459447) and *A. jaya* (FJ459446) clustered together with *Salmostoma bacaila* (HM224244, MG954366, NC015549, KY290072, KY290087) with a low genetic distance of 0.3–0.7 % indicating that they represent the latter species.



**FIGURE 4.** Ventral view of A) *Cabdio crassus*, ZSI FF 8218, holotype, 88.1 mm SL (arrow showing keeled chest); B) *C. ukh-rulensis*, PUCMF 15054, 72.7 mm SL; C) *C. morar*, PUCMF 2094, 64.3 mm SL; D) *C. jaya*, ZSI FF 5607, 74.3 mm SL.

## Discussion

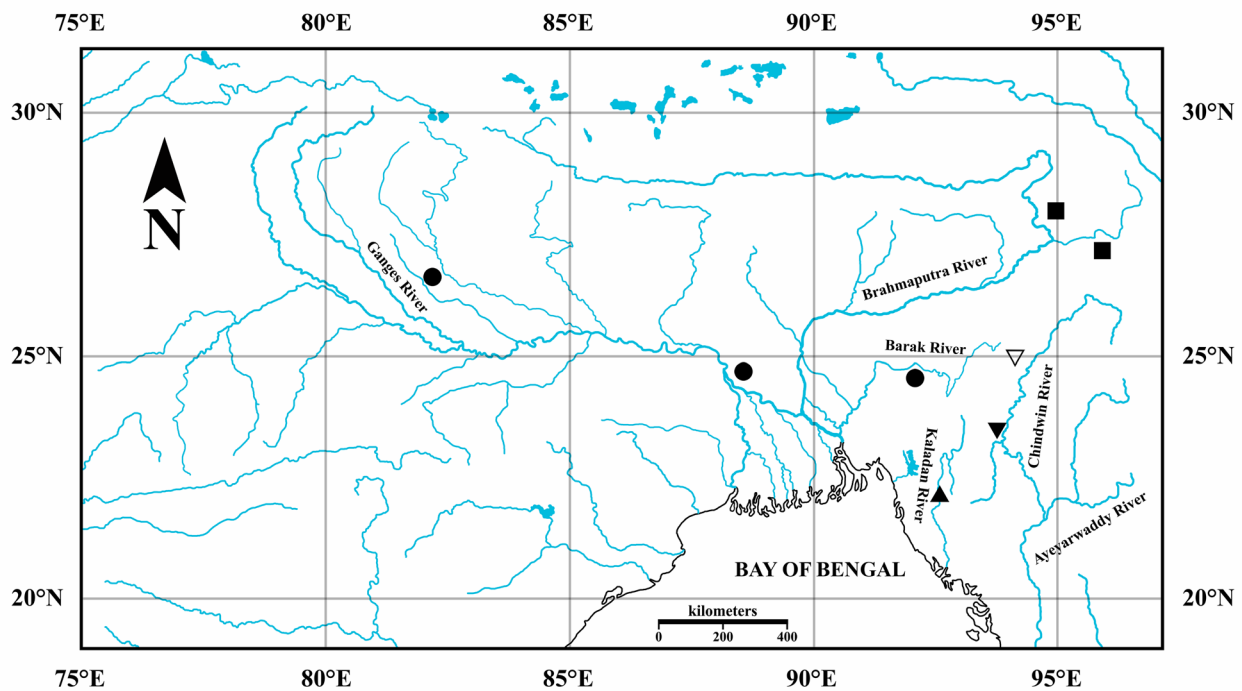
*Cabdio crassus* conforms to the conception of the genus (as *Aspidoparia*) by Heckel (1848), Day (1878), Talwar & Jhingran (1991) and Vishwanath *et al.* (2007) except that it possesses a keeled ventral surface from the mid-point of the chest between the posterior base of the pectoral fin and along the abdomen, up to the anus, and a higher number of branched anal-fin rays (12–13 vs. 7–9 in all other *Cabdio* species).

A keeled chest and abdomen as in *Cabdio crassus* is present also in species of the cyprinid genera *Securicula* and *Salmostoma*; this apparently apomorphic character has never yet been reported in other species of *Cabdio*. However, *C. crassus* differs from both *Securicula* and *Salmostoma* in having the lower jaw shorter than the upper



jaw (i.e. a sub-terminal mouth) and lacking a symphyseal process (vs. lower jaw longer or jaws subequal, a symphyseal process present). Moreover, the species is clearly nested within the clade comprising species of *Cabdio* in the *coi* inferred ML tree (Fig. 1). It is perhaps worth mentioning that, although it has not been reported by previous authors, *C. ukhrulensis*, *C. morar* and *C. jaya* exhibit an abdomen in which the median scales more or less form a keel (see Fig 4).

DNA barcoding represents a rapid, accurate and automatable species-identification method using short and standardized gene regions (Hebert & Gregory 2005). However, inaccurate identification of the barcoded species in the GenBank register retards the utility of identification using barcodes and leads to confusion (Conte-Grand *et al.* 2017; Lalramliana *et al.* 2018; Lalramliana *et al.* 2019). For instance, the genetic distance between the seven sequences of *Cabdio morar* developed here and species labelled as *Salmostoma bacaila* (KT364775, KX266823, EU417785, EU417786, EU417789, KJ936881, KJ896723, KJ896724 and JN815302) in GenBank is very low (99% identity with 0.0–0.8 % K2P distance) indicating that they belong to the same species.



**FIGURE 5.** Drainage Map showing the locality of *Cabdio crassus* sp. nov. (▲) and the distribution of the genus *Cabdio* examined in this study.

▲ *Cabdio crassus* ■ *Cabdio jaya* ● *Cabdio morar* ▼ *Cabdio ukhrulensis* (▽ holotype)

The examples of *Cabdio morar* studied here were collected from the Barak River Drainage (Brahmaputra basin) of Mizoram and Ganges River near Lucknow, India, and identified morphologically through comparison with specimens from the Ganga basin present in ZSI, Kolkata, India. *Cabdio morar* is characterized by having 2 simple and 7½ branched dorsal-fin rays, 1 simple and 7½ branched pelvic-fin rays, 1 simple and 14 branched pectoral-fin rays, 3 simple and 9½ branched anal-fin rays, a complete lateral line with 38–42 + 1–2 scales, ½4/1½2–3 scales in transverse line from dorsal-fin origin to pelvic-fin origin and 3 pharyngeal teeth rows (Hamilton 1822; Day 1878; Selim & Vishwanath 2001; ZSI FF 4968; ZSI/V/ERS 8354; PUCMF 15051–53). It therefore appears that the species identified as *Salmostoma bacaila* (mentioned above) in the NCBI Genbank in fact represents *Cabdio morar*. Similarly, the same anomaly is observed between the three developed sequences of *Cabdio ukhrulensis* and species labelled as *C. morar* (AP011335, KJ909343 and KJ909344). The *Cabdio ukhrulensis* analysed in this study were collected from the local fish market in Tahan, Myanmar, presumably from the nearby Myittha River, a tributary of Chindwin River (as per communication with the fishermen). Examination of, and comparison with the type specimens revealed these specimens to be *C. ukhrulensis* (type locality: Chattrickong River, Chindwin drainage, Manipur). Surprisingly, the 3 developed sequences of *Cabdio ukhrulensis* shared 99% identity (0.2% K2P distance) with the three GenBank sequences of *C. morar* (AP011335, KJ909343 and KJ909344).

**TABLE 3.** Summary of synonymized species/probable misidentification and resolution of the genus *Cabdio* and *Salmostoma bacaila* in the NCBI GenBank, accession numbers and habitat locality

| S No. | I | Specimen ID in NCBI        | Specimen ID corrected     | NCBI No. | Accn. | Country                         | Reference               |
|-------|---|----------------------------|---------------------------|----------|-------|---------------------------------|-------------------------|
| 1.    |   | <i>Salmostoma bacaila</i>  | <i>Cabdio morar</i>       | EU417785 |       | India                           | GenBank                 |
| 2.    |   | <i>Salmostoma bacaila</i>  | <i>Cabdio morar</i>       | EU417789 |       | India                           | GenBank                 |
| 3.    |   | <i>Salmophasia bacaila</i> | <i>Cabdio morar</i>       | KJ936881 |       | Meghalaya, India                | GenBank                 |
| 4.    |   | <i>Salmostoma bacaila</i>  | <i>Cabdio morar</i>       | EU417786 |       | India                           | GenBank                 |
| 5.    |   | <i>Salmophasia bacaila</i> | <i>Cabdio morar</i>       | KT364775 |       | Tanguar Haor, Bangladesh        | GenBank                 |
| 6.    |   | <i>Salmophasia bacaila</i> | <i>Cabdio morar</i>       | KX266823 |       | South India                     | GenBank                 |
| 7.    |   | <i>Salmophasia bacaila</i> | <i>Cabdio morar</i>       | KT896724 |       | Manipur, India                  | GenBank                 |
| 8.    |   | <i>Salmophasia bacaila</i> | <i>Cabdio morar</i>       | JN815302 |       | Assam, India                    | GenBank                 |
| 9.    |   | <i>Salmophasia bacaila</i> | <i>Cabdio morar</i>       | KT896723 |       | Manipur, India                  | GenBank                 |
| 10.   |   | <i>Salmostoma bacaila</i>  | <i>Cabdio morar</i> (?)   | JX260969 |       | Andhra Pradesh, India           | GenBank                 |
| 11.   |   | <i>Salmostoma bacaila</i>  | <i>Cabdio morar</i> (?)   | JX260968 |       | Andhra Pradesh, India           | GenBank                 |
| 12.   |   | <i>Salmostoma</i> sp.      | <i>Cabdio morar</i>       | KY290070 |       | Assam, India                    | GenBank                 |
| 13.   |   | <i>Salmostoma</i> sp.      | <i>Cabdio</i> sp.         | KY290071 |       | Assam, India                    | GenBank                 |
| 14.   |   | <i>Cabdio morar</i>        | <i>Cabdio ukhrulensis</i> | KJ909343 |       | Manipur, India                  | GenBank                 |
| 15.   |   | <i>Cabdio morar</i>        | <i>Cabdio ukhrulensis</i> | KJ909344 |       | Manipur, India                  | GenBank                 |
| 16.   |   | <i>Cabdio morar</i>        | <i>Cabdio ukhrulensis</i> | AP011335 |       | Aquarium trade (unknown source) | Tang <i>et al.</i> 2010 |
| 17.   |   | <i>Aspidoparia jaya</i>    | <i>Salmostoma bacalia</i> | FJ459446 |       | India                           | GenBank                 |
| 18.   |   | <i>Aspidoparia morar</i>   | <i>Salmostoma bacalia</i> | FJ459447 |       | Assam, India                    | GenBank                 |

It is interesting that *Cabdio morar* and *C. ukhrulensis* are superficially similar. *Cabdio morar* differs from the latter, however, in having more lateral line scales (38–42 vs. 35–37), more predorsal scales (17–18 vs. 14) and more pharyngeal tooth-rows (3 vs. 2). Further, analysis of the three *C. morar* sequences in NCBI revealed the provenance of the two specimens (KJ909343 and KJ909344) as the Chindwin drainage of Manipur (as per data from NCBI), while AP011335 is from aquarium trade, with unknown locality (Tang *et al.* 2010). It appears therefore that the sequences of *Cabdio morar* previously submitted in NCBI have been misidentified: they belong to *C. ukhrulensis* rather than to *C. morar*. Furthermore, the available sequences of *Cabdio jaya* (as *Aspidoparia jaya*, FJ459446) and *C. morar* (as *Aspidoparia morar*, FJ459447) also appear to cases of misidentification. These two sequences appear to be conspecific and represent *Salmostoma*, based on the low genetic distance and further consensus with *Salmostoma bacaila* in the ML tree.

*Cabdio jaya* is a species described by Hamilton (1822) from the rivers of northern Bihar (River Ganges) without any extant type material. Its original description did not include the lateral line scales count. However, the species was characterized by subsequent workers (Day 1878; Talwar & Jhingran 1991; Selim & Vishwanath 2001; Vishwanath *et al.* 2007), including the lateral-line scales count, and we have relied on these data to identify this species. Our many attempts to obtain fresh material of *C. jaya* from the type locality, unfortunately, failed. The examined specimens of *C. jaya* (labelled as *Aspidoparia jaya*) from ZSI Kolkata are from Brahmaputra River of Arunachal Pradesh, India. Though, the examined specimens are not from the exact type locality of *C. jaya*, data from literature suggest that this identification is correct.

Kar & Sen (2007) listed *Cabdio morar* (as *Aspidoparia morar*) from the Kaladan River of Mizoram without providing a description or illustration, and not mentioning where their specimens are deposited. It is likely that the specimens they reported as *C. morar* were in fact *C. crassus*.

In conclusion, the discovery of *Cabdio crassus* from the Kaladan raises the number of *Cabdio* species to four. Despite the stated weakness in using GenBank sequences for comparison, the present study demonstrates that the *coi* gene fragment is an effective tool for identification of species of *Cabdio*.

## Comparative material

- Cabdio jaya*: ZSI FF 5607, 11 ex., 56.0–74.5 mm SL; India: Arunachal Pradesh: Namdapha National Park & Tiger Reserve.—ZSI/V/F/ERS 222; 11 ex., 46.4–64.2 mm SL; India: Arunachal Pradesh: D. Ering Sanctuary, E. Siang.
- Cabdio morar*: ZSI FF 4968, 2 ex., 67.4–70.6 mm SL; India: West Bengal: Ganga River in the vicinity of Palaghat, Panchanan-dapur.—ZSI/V/ERS 8354; 9 ex., 46.4–81.1 mm SL; India: Assam: River at Goalpara, Goalpara District.—PUCMF 2094, 1, 64.3 mm SL; India: Mizoram: Terei River, a tributary of the Tlawng river (Barak River drainage) in the vicinity of Saikhawthlir village.—PUCMF 15051, 2, 52.7–63.2 mm SL; India: Mizoram: Terei River, a tributary of the Tlawng river (Barak River drainage) in the vicinity of Chuhvel village. —PUCMF 15052–53, 2, 61.5–64.3 mm SL; India: Mizoram: Mar River, a tributary of the Karnaphuli River in the vicinity of Phuldungsei village.
- Cabdio ukhrulensis*: MUMF 1025, 1, holotype, 75.1 mm SL; India: Manipur: Ukhrul District, Chatrickong River (Chindwin drainage).—PUCMF 15054, 2, 49.0–72.7 mm SL; Myanmar: Tahan, Tahan Market (presumably from the nearby Myittha River, a tributary of Chindwin River).—PUCMF 15055, 2, 45.3–54.2 mm SL; data same as PUCMF 15054 (cleared and stained).
- Salmostoma bacaila*: ZSI/V/F/ERS 794, 1 ex., 72.3 mm SL; India: Assam: Banawh River, 4 km East of Bijni, Bongaigaon District.
- Salmostoma phulo*: ZSI/V/F/ERS 942, 2 ex., 48.7–50.3 mm SL; India: Assam: Bortari, Kamrup District.—ZOO NEHU 49, 2 ex., 51.6–53.9 mm SL; India: Meghalaya: Bugi River.

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## Literature cited

- Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.H., Xie, D., Suchard, M.A., Rambaut, A. & Drummond, A.J. (2014) BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. *PLoS Computational Biology*, 10, 1–7. <https://doi.org/10.1371/journal.pcbi.1003537>
- Chaudhury, S. (2010a) *Cabdio morar*. The IUCN Red List of Threatened Species 2010: e.T166469A6215983. <https://doi.org/10.2305/IUCN.UK.2010-4.RLTS.T166469A6215983.en>
- Chaudhury, S. (2010b) *Aspidoparia jaya*. The IUCN Red List of Threatened Species 2010: e.T166469A6215983. <https://doi.org/10.2305/IUCN.UK.2010-4.RLTS.T166469A6215983.en>
- Conte-Grand, C., Britz, R., Dahanukar, N., Raghavan, R., Pethiyagoda, R., Tan, H.H., Hadiaty, R.K., Yaakob, N.S. & Rüber, L. (2017) Barcoding snakeheads (Teleostei, Channidae) revisited: Discovering greater species diversity and resolving perpetuated taxonomic confusions. *PLoS ONE*, 12, e0184017. <https://doi.org/10.1371/journal.pone.0184017>
- Day, F. (1878) *The fishes of India: Being a natural history of the fishes known to inhabit the seas and freshwaters of India, Burma and Ceylon*. Bernard Quaritch, London, i-xx + 778pp. <https://doi.org/10.5962/bhl.title.55567>
- Günther, A. (1868) *Catalogue of the fishes in the British Museum. Vol. 7*. Order of the Trustees, London, 512 pp.
- Hamilton, F. (1822) *An account of the fishes of River Ganges and its branches*. George Ramsay and Co., London, 405 pp. <https://doi.org/10.5962/bhl.title.59540>
- Hebert, P.D.N. & Gregory, T.R. (2005) The promise of DNA barcoding for taxonomy. *Systematic Biology*, 54, 852–859. <https://doi.org/10.1080/10635150500354886>
- Heckel, J.J. (1848) Nachtrag zur Charakteristik und Classification der Cyprineen-Gattungen. In: Russeger, J. (Ed.), *Reisen in Europa, Asien und Afrika mit besonderer Rücksicht auf die naturwissenschaftlichen Verhältnisse der betreffenden Länder, unternommen in den Jahren 1835 bis 1841. Zweiter Band. Reise in Egypten, Nubien und Ost-Sudan. Dritter Theil. Zweite Reise durch Nubien und Egypten*. Naturhistorischen Anhang, Schweizerbart, Stuttgart, pp. 273–290. [1846–1849]
- Kar, D. & Sen, N. (2007) Systematic list and distribution of fishes in Mizoram, Tripura and Barak drainage of northeastern India. *Zoos' Print Journal*, 22, 2599–2607. <https://doi.org/10.11609/JoTT.ZPJ.1571a.2599-607>
- Kottelat, M. (2013) The fishes of the inland waters of Southeast Asia: A catalogue and core bibliography of the fishes known to

- occur in freshwaters, mangroves and estuaries. *The Raffles Bulletin of Zoology (Supplement)*, 27, 1–663.
- Kottelat, M. & Freyhof, J. (2007) *Handbook of European freshwater fishes*. Publications Kottelat, Cornol and Freyhof, Berlin, 646 pp.
- Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870–1874.  
<https://doi.org/10.1093/molbev/msw054>
- Lalramliana., Knight, J.D.M., Lalhlimpuia, D.V. & Singh, M. (2018) Integrative taxonomy reveals a new species of snakehead fish, *Channa stiktos* (Teleostei: Channidae), from Mizoram, North Eastern India. *Vertebrate Zoology*, 68 (2), 165–175.
- Lalramliana., Lalronunga, S., Kumar, S. & Singh, M. (2019) DNA barcoding revealed a new species of *Neolissochilus* Rainboth, 1985 from the Kaladan River of Mizoram, North East India. *Mitochondrial DNA Part A*, 30 (1), 52–59.  
<https://doi.org/10.1080/24701394.2018.1450398>
- Liao, T.Y., Ünlü, E. & Kullander, S.E. (2011) Western boundary of the subfamily Danioninae in Asia (Teleostei, Cyprinidae): derived from the systematic position of *Barilius mesopotamicus* based on molecular and morphological data. *Zootaxa*, 2880, 31–40.  
<https://doi.org/10.11646/zootaxa.2880.1.3>
- Nei, M. & Kumar, S. (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press, New York, 333 pp.
- Sambrook, J. & Russell, D.W. (2001) *Molecular cloning: a laboratory manual*. 3<sup>rd</sup> edition, Cold Spring Harbor Laboratory Press, New York, 2100 pp.
- Selim, K. & Vishwanath, W. (2001) A new freshwater cyprinid fish *Aspidoparia* from the Chatrickong River, Manipur, Indian. *Journal of Bombay Natural Historical Society*, 98 (2), 254–257.
- Talwar, P.K. & Jhingran, A.G. (1991) *Inland fishes of India and adjacent countries. Vol. 1*. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, 541 pp.
- Tang, K.L., Agnew, M.K., Hirt, M.V., Sado, T., Schneider, L.M., Freyhof, J., Sulaiman, Z., Swartz, E., Vidthayanon, C., Miya, M., Saitoh, K., Simons, A.M., Wood, R.M. & Mayden, R.L. (2010) Systematics of the subfamily Danioninae (Teleostei: Cypriniformes: Cyprinidae). *Molecular Phylogenetics and Evolution*, 57, 189–214.  
<https://doi.org/10.1016/j.ympev.2010.05.021>
- Vishwanath, W., Lakra, W.S. & Sarkar, U.K. (2007) *Fishes of Northeast India*. National Bureau of Fish Genetic Resources, Lucknow, India, 264 pp.
- Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. & Hebert, P.D.N. (2005) DNA Barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B*, 360, 1847–1857.  
<https://doi.org/10.1098/rstb.2005.1716>