Garra mini, a new small species of rheophilic cyprinid fish (Teleostei: Cyprinidae) from southeastern hilly areas of Bangladesh

Md. Mizanur Rahman*, Abdur Rob Mollah*, Michael Norén** and Sven O. Kullander**

Garra mini, new species, is described from the Shuvolong, Shailopropat and Chingthong waterfalls in the Karnafuli and Sangu River drainages. The largest specimens recorded is 46.8 mm SL and specimens over 40 mm SL have reached reproductive size. Alongside G. ethelwynnae (28 mm SL) and G. poecilura (44.5 mm SL), G. mini is one of the smallest species in the genus. Garra mini is diagnosed by morphological and meristic characters in combination, particularly the numerous small predorsal scales and the presence of a contrasted dark stripe along the middle of the side, and also by the DNA barcode sequence (cytochrome oxidase subunit I, COI) with three unique substitutions.

Introduction

The cyprinid fish genus Garra Hamilton (1822) contains about 127 species with a combined distribution extending from southern China, across Southeast Asia, India and the Middle East to northern and central Africa (Froese & Pauly, 2015; Kottelat, 2013; Thoni & Mayden, 2015). They are benthic fishes adapted to fast flowing rocky streams by the depressed shape, adhesive pads on paired fins and highly modified mouth for suction. The smallest species recorded is G. ethelwynnae Menon (1958) from Eritrea, with a recorded maximum length of 28 mm SL (Menon, 1964), and the largest species is G. imberba Garman (1912) from southern China, reaching about 300 mm SL (Menon, 1964). Species of Garra share a conservative body plan, and show relatively little variation in basic colour pattern and meristic characters. Adults of many species, however, are notable for more or less conspicuous projections and enlarged tubercles on the head (Menon, 1964; Nebeshwar & Vishwanath, 2013). Species determination in Garra is difficult not only because of the general similarity among species, but also uncertainties about the identification of species described in the 19th Century. The latest revision (Menon, 1964) recognized 38 species of Garra. Only over the last 15 years (2000–2015), 52 new species were described. In the absence of an evidently much needed major revision of the species of Garra, it is a challenge to introduce one more species.

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Extensive collecting in the mountains of southeastern Bangladesh has yielded several species of *Garra* from waterfalls, rapids and jhiris. One of those is an undescribed species remarkable for its small size. It is a significant addition also to the fish fauna of Bangladesh and deserves recognition because it seems limited to fast flowing sections of mountain streams. Incomplete information on other species of *Garra* and difficult access to type material of all 127 species would severely impede attempts to describe this species based on morphology alone, but addition of the DNA barcode provides a solution to the dilemma.

**Material and methods**

Specimens are kept in the following collections: DU, Zoology Department, University of Dhaka, Dhaka; NRM, Swedish Museum of Natural History, Stockholm. Measurements were taken with digital callipers to a precision of 0.1 mm. Counts and measurements were made according to Kullander & Fang (2004), except that lateral line scales are recorded as the number on the body plus the number on the base of the caudal fin to enable comparisons with data including scales on the caudal fin (e.g., Menon, 1964; Nebeshwar & Vishwanath, 2013). Fin-ray and vertebral counts were taken from X-radiographs made with a Philips MG-105 low voltage X-ray unit and Kodak EDR2 plates. Abdominal vertebrae counts include the Weberian apparatus (assumed to contain four centra). Terminology of mouth parts follows Kullander & Fang (2004). Meristic data from the holotype are marked with an asterisk (*).

Species delimitation was investigated using 655 basepairs from the 5’ end of the mitochondrial cytochrome oxidase subunit I (COI, also known as mt-COI or COX1) gene of five specimens of *Garra mini*. This is the standard barcoding fragment suggested by the Barcode of Life consortium (Steinke & Hanner, 2011), and corresponds to position 51–705 of the COI gene (reference sequence: *Garra flavatra*, GenBank accession number NC_022953). DNA was extracted using a Thermo Scientific™ KingFisher™ Duo (Thermo Fisher Scientific) fully automated liquid-handling instrument, with the Thermo Scientific KingFisher Cell and Tissue DNA Kit (Thermo Fisher Scientific) and recommended protocol. The COI fragment was amplified using the fish barcoding primers Fish-F1 and Fish-R1 (Ward et al. 2005). PCR were performed with the puReTaq Ready-To-Go PCR kit (Amersham Biosciences AB, Uppsala, Sweden). PCR cycling: 94 °C 4 min; 35 * (94 °C 30 s; 52 °C 30 s; 72 °C 30 s); 72 °C 8 min. PCR products were checked on agarose gel, and purified by adding 5 µl of a mix consisting of 20 % Exonuclease I and 80 % FastAP Thermosensitive Alkaline Phosphatase (Fermentas/Thermo Fischer Scientific, Gothenburg, Sweden) to each 25 µl PCR reaction, incubated at 37 °C for 30 minutes, then heated to 80 °C for 15 minutes. Sequencing of both strands was carried out using the same primers, by Macrogen Europe (Amstelveen, The Netherlands). All sequences were proofread and assembled using the software Geneious R8 (Kearse et al., 2012).

The uncorrected p-distance is the proportion (p) of nucleotide sites at which two homologous DNA fragments differ. It is calculated by dividing the number of nucleotide differences by the total number of nucleotides in the DNA fragment.

The new barcode sequences generated for this study have the following GenBank accession and voucher collection accession numbers:

- KT223104, DU 0401169
- KT223105, DU 1001055
- KT223106, NRM 66745
- KT223107, NRM 66748
- KT272021, DU 6240

**Garra mini, new species**

(†-2)

**Holotype.** DU 70001, 44.4 mm SL; Bangladesh: Chittagong Division: Rangamati District, Borokal: Karnafuli River drainage: Kaptai Lake: pool at bottom of Shuvolong waterfall, 22°42’51” N 92°14’52” E; M. M. Rahman, S. O. Kullander, M. Norén, A. Obaida, M. Hossain & M. Rashid, 28 Nov 2014.

**Paratypes.** All from Bangladesh, Chittagong Division. DU 6002, 1, 43.3 mm SL; DU 6240, 1, 44.9 mm SL; Bandarban District: Thanchi: Shailo-propat waterfall, 22°09’06” N 92°14’52” E; S. Mandal & M. Rashid, 15 May 2015. – DU 80001, 39, 25.1–30.2 mm SL; NRM 67299, 30, 23.9–31.7 mm SL; NRM 67300, 10, 28.9–46.8 mm SL; NRM 66744, 1, 35.9 mm SL; NRM 66745, 29.4 mm SL; NRM 66746, 1, 33.7 mm SL; NRM 66747, 1, 30.8 mm SL; NRM 66748, 31.9 mm SL; same data as holotype. – DU 1001055, 1, 38.4 mm SL; NRM 66226, 6, 34.1–44.2 mm SL; same locality as holotype;
Diagnosis. *Garra mini* is a small species of *Garra*, maximum size recorded less than 50 mm SL, diagnosed by characters in combination: smoothly rounded snout tip; very slight transverse frontal groove; lateral lobe present; proboscis absent; scales absent from chest; scales present on abdomen, two pairs of barbels, lateral line scales on body 30–31, total lateral line scales 31–33, predorsal scales very small, deeply embedded, 14–16 along predorsal midline; circumpeduncular scales 16; black spots absent from base of dorsal fin; transverse dark bars absent from caudal fin; contrasting dark band from head to caudal-fin base.

Uniquely distinguished from all 318 sequences of the corresponding fragment of the mitochondrial cytochrome oxidase subunit 1 (COI) gene, representing 74 species of *Garra*, which were available in GenBank on 20 June 2015, in having thymine in position 139 (vs. cytosine in all other species), adenosine in position 141 (vs. cytosine in all other species), and guanine in position 207 (vs. adenosine in all other species).

Description. Elongate, predorsal contour little ascending to slightly anterior to dorsal-fin base, posteriorly straight, slightly sloping. Caudal peduncle not much shallower than rest of body. Ventrally flattened on head, chest and belly. Head wide, rounded in dorsal aspect, depressed, with marked decurved upper contour; shallow transverse depression in snout immediately anterior to nostrils in some specimens only. Orbit in middle of head length. Snout rounded, smooth, decurved, with short triangular lateral projection residing in shallow lateral depression. Lateral lobe with row of 1–3 short sharp tubercles in specimens over 40 mm SL. Anterior maxillary barbel emerging from anterior part of lateral depression, short, not reaching margin of rostral cap. Posterior barbel at rictus, similar in length to anterior barbel or shorter. Rostral cap covering upper jaw, distal margin with wide band of papillae, margin entire (Fig. 2). Upper lip absent. Lower jaw laterally attached to papillose fold at rictus; lower jaw posteriorly margined by wide papillose lower lip (Fig. 2). Central pad smooth, slightly wider than long, posterior margin slightly crenate in adults (Fig. 2); in small specimens rounded, covered with minute papillae, and posterior margin smooth.

Dorsal-fin rays iii.7½* (18). Dorsal fin inserted slightly anterior to mid-body and slightly anterior to vertical from pelvic-fin origin. Pelvic fin with short subacuminate tip, first and second branched rays longest, extending slightly beyond last ray; posterior margin slightly concave, posterior-most ray not extending to vertical from anal-fin origin. Anal-fin rays iii.4½* (19). Anal fin short, with rounded anterior corner and straight distal margin, first branched ray longest, tip barely reaching caudal-fin base or shorter. Caudal-fin rays 9+9* (6), 10+9 (13). Caudal fin emarginate, lobes equally long, lobe tips subacuminate, 10th ray shortest. Pectoral-fin rays i.12 (9), i.13* (10). Pectoral fin elongate, tip broadly rounded, 6th–7th branched rays longest, not reaching to base of pelvic fin; anterior six rays with long thick adhesive pads in specimens over 40 mm SL, pads on fewer rays or absent in smaller specimens. Pelvic-fin rays i.7* (6), i.8 (12). Pelvic fin elongate, tip rounded, 3rd or 4th branched ray longest, not extending to base of anal fin; elongate adhesive pads present on three anterior rays in specimens over 40 mm SL, fewer or absent in smaller specimens.

Table 1. Proportional measurements of *Garra mini*. Standard length (SL) in millimetres, other measurements expressed as percents of SL. Holotype (HT) values listed separately and included in sample range. SD, standard deviation.

<table>
<thead>
<tr>
<th>Measure</th>
<th>HT</th>
<th>N</th>
<th>range</th>
<th>mean</th>
<th>SD</th>
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<tr>
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<td>28.9–46.8</td>
<td>36.7</td>
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<tr>
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<td>24.3</td>
<td>19</td>
<td>22.0–27.3</td>
<td>24.5</td>
<td>1.4</td>
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<td>Snout length</td>
<td>10.4</td>
<td>19</td>
<td>8.7–10.9</td>
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<tr>
<td>Orbital diameter</td>
<td>6.5</td>
<td>19</td>
<td>6.2–8.5</td>
<td>7.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Interorbital width</td>
<td>9.0</td>
<td>19</td>
<td>9.0–10.3</td>
<td>9.7</td>
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<tr>
<td>Head width</td>
<td>17.1</td>
<td>19</td>
<td>16.4–19.4</td>
<td>17.6</td>
<td>0.9</td>
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<tr>
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<td>12.0–16.3</td>
<td>14.1</td>
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<td>0.9</td>
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<td>1.4</td>
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<td>19</td>
<td>20.8–26.9</td>
<td>22.6</td>
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</table>
Lateral line scales 30 + 1(9), 30 + 2*(5), 31 + 1(3), 31 + 2(2). Scales in transverse row 4½/1/3½*(19); circumpeduncular scale rows 16*(19). Scales absent from chest to level of end of base of pectoral fin; scales present on abdomen, minute, thin, partly non-imbricated, covered by skin; scales imbricated and clearly visible posterior to pelvic-fin insertion, with about 5–6 small elongate scales in midline between pelvic-fin insertion and vent. On predorsal midline about 14–16 very small scales embedded in skin, without free margin.

Dissected specimens NRM 66226, 43.7 mm SL, and 41.6 mm SL, a female with large eggs, 0.5 mm in diameter, and a male with swollen testes, respectively. Several specimens < 40 mm SL dissected with undifferentiated gonads only. No external sexual dimorphism observed.

**Colouration in preservative.** Top of head grey; predorsal midline grey, sides of head pale brown. Sides dull yellowish grey dorsally, lighter ventrally, to yellow on ventral surfaces. Small black spot on cleithrum immediately posterior to dorsal end of gill opening. Dark grey or black band

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Fig. 1. *Garra mini*, holotype, DU 70001, 44.4 mm SL; Bangladesh: Shuvolong Falls.

Fig. 2. *Garra mini*, DU 70001, holotype, 44.4 mm SL; Bangladesh: Shuvolong Falls; ventral aspect of mouth.
from cleithral spot to end of caudal peduncle, anteriorly 1½ scales wide, posteriorly narrower to one scale wide. Middle membranes of caudal fin basally darker than rest of fin which sparsely pigmented. Other fins with sparse dark pigment, but no markings formed.

**Geographical distribution and habitat.** *Garra mini* is so far known only from three localities, viz., the Shuvolong Falls in Kaptai Lake in the Karnafuli River system, and the Chingthong Jhiri and Shailpropat Falls in the Sangu River drainage (Fig. 3). The Shuvolong habitat, a shallow pool at the foot of an approximately 100 m tall fall, was described by Kullander et al. (2015). At this locality, which is very restricted in extension, *G. mini* was collected in numbers together with only one other fish species, *Danio annulosus* Kullander, Rahman, Norén & Mollah (2015). The Shailpropat Falls, traversing a wide exposed sandstone cliff, include one major drop of about ten meters and downstream rapids over gravel and large boulders. Syntopic species at Shailpropat include two other species of *Garra* similar to *G. dampaensis* and *G. lamta*, respectively. The Chingthong habitat is a small pool, about 1 m deep, where water flows from very shallow hillstream riffles before entering the Sangu River. The pool setting is rocky with big boulders and formed in crevices of the hills. Sampling was made in the rainy season, when the water was fast flowing and relatively cold, estimated to be close to 18–20 °C. The pool is bordered by algal vegetation, in a setting of shrubs and trees in the surrounding hills. Associated species were the same species of *Garra* as in Shailpropat, and also a species of *Neolissochilus*. At the Shailpropat and Chingthong localities only few specimens were obtained despite intensive efforts.

**Etymology.** The species epithet is a universal contraction of forms of the Latin adjective minimus, meaning small or short, and refers to the small size of the species. It is here used as a noun in apposition.

**DNA Barcode.** The COI sequences (barcodes) of three specimens of *G. mini* from the Shuvolong Falls in the Karnafuli River drainage (GenBank accession number KT223105–KT22307) are identical, but differ from two specimens from the Sangu River at Chingthong Jhiri (KT223104) and Shailpropat Falls (KT272021) in 4 nucleotide positions (uncorrected intraspecific pairwise p-distance = 0.6 %).

A BLAST (Altschul et al., 1990) search of the GenBank non-redundant nucleotide database (*nr*) on 20 June 2015 found no exact matches to the *G. mini* barcodes. The closest matches among 318 sequences from 74 putative species of *Garra* were identified as *Garra* sp. ‘Tuirivang’ and *Garra* sp. ‘Kolodyne’ (12 % uncorrected pairwise p-distance).

The following three positions are unique for *G. mini* among the *Garra* sequences in GenBank: thymine in position 139 of the COI gene (cytosine in all other *Garra* species), adenosine in position 141 (cytosine or thymine in all other *Garra*), and guanine in position 207 (adenosine in all other *Garra*).

Entering the *Garra mini* barcodes into the identification tool at the Barcode of Life database (BOLD) portal (www.boldsystems.org) and searching for a match at the “species” level found no matches. A search of “all barcode records on BOLD” found that the three closest matches in BOLD were *Garra* sp. ‘Tuiral’ (12 % uncorrected pairwise p-distance), the South American characid *Pseudocorynopoma doriae* (14.4 %), and *Garra arupi* (14.4 %).
Discussion

The very small size, up to 46.8 mm SL recorded, makes *G. mini* the shortest or second shortest species of *Garra* in Asia. The smallest species in the genus may be *G. ethelwynnae* with the largest specimen 28 mm SL (Menon, 1958, 1964), but this species has not been recorded after the original description and Menon (1958, 1964) actually did not conclude that his specimens represented adults, although it is implied in his remark that *G. ethelwynnae* is a “dwarf cognate” of *G. tibonica* Trewavas (1941). The smallest Asian species is *G. poecilura* Kullander & Fang (2004) from the Ayeyarwady basin in Myanmar, but it is known only from two specimens, 32.7–44.5 mm SL (Kullander & Fang, 2004), however, and they may be subadult. *Garra poecilura* differs from *G. mini* in colour pattern, having a dark stripe on the dorsal fin, black spots forming two narrow wavy vertical stripes on the caudal fin, lacking a distinct lateral band, and in fewer lateral line scales (29 vs. 30–31 on body) (cf. Kullander & Fang, 2004). The largest specimen of *G. minima* from Arunachal Pradesh in India was listed by Arunachalam et al. (2013) as 53.1 mm SL. Data in the original description of *G. minima* suggest that it is different from *G. mini* in scale counts (35–37 vs. 31–33 lateral line scales including scales on the caudal fin; 12 vs. 16 circumpeduncular scales), 8–9 branched pelvic-fin rays (vs. 7), 14–16 branched pectoral-fin rays (vs. 12–13), fully scaled abdomen, and absence of a lateral band. *Garra minima* was originally described as *G. minimus*. The Latin minimus, however, is an adjective, and because *Garra* is feminine the correct form is *G. minima*.

In the combination of characters *G. mini* fits with couplet 26 in Menon’s (1964: 243–245) key to *Garra*, similar to *G. annandalei* (26b). This position separates *G. mini* and *G. annandalei* from the African and Western Asian species of *Garra*, the *G. lissorhynchus* group characterized by dark markings on the dorsal and caudal fin (reviewed by Lalronunga et al., 2013), species with a distinct frontal groove, and species with a marked proboscis (reviewed by Nebeshwar & Vishwanath, 2013). *Garra mini* differs from *G. annandalei* in having the vent closer to the anal-fin origin, the space between the vent and the anal fin fitting 5 times in the distance between the pelvic and anal fins, vs. 3 times in *G. annandalei* according to Menon (1964). A dark lateral band is present in several Asian species of *Garra* without proboscis or dark band on the caudal fin, e. g., *G. hughi* Silas (1955), and *G. mullya* (Sykes,1839) from peninsular India. Those species and most other *Garra* have smaller scales than *G. mini*, about 36 and more lateral line scales.


Except for *G. nigricollis* Kullander & Fang (2004) with 33 lateral line scales, the species of *Garra* from Rakhine State in Myanmar described by Kullander & Fang (2004) all have low numbers of lateral line scales (27–31 + 1–2), but differ from

Rahman et al.: *Garra mini*
Garra mini in colour pattern, and none of them has a conspicuous dark band along the side.

Three species of Garra have been reported from Bangladesh, viz., G. annandalei by Mohsin (2007), G. gotyla by Rahman & Ruma (2007) and Ahmed et al. (2013), and G. orientalis Nichols (1925) by Ahmed et al. (2013). Garra gotyla has long been a catch-all name for species of Garra with pronounced probasic. Nebeshwar & Vishwanath (2013) designated a neotype for G. gotyla from the Tista River in Sikkim, India, and gave the type locality as the total distribution. It is likely that a species of Garra with long probasic occurs in Bangladesh, but the species identification needs to be revised in view of the neotype designation for G. gotyla. Garra orientalis is restricted to China (Zhang et al., 2000).

Garra annandalei was described from Darjeeling and Assam, India (Hora, 1921), and potentially may occur in Bangladesh, but there is no recent revision of this species. Menon (1964) redescribed G. annandalei based on the holotype and specimens from the Kosi River and Darjeeling district. Menon counted 33–34 scales in the lateral line, and 9–10 predorsal scales. He mentions a dark midlateral band in young specimens, which might resemble the condition in G. mini. Besides differences in scale counts and relative position of the vent, G. annandalei is a larger species than G. mini as reported by Hora (1921; up to 118 mm SL) and Menon (1964; up to 146 mm SL).

Garra mini is similar in meristics and presence of a lateral band to G. vittatula Kullander & Fang (2004) from western Rakhine Yoma. In that species, however, the lateral band is much wider and less distinct. Garra vittatula has 11–12 partly exposed predorsal scales, (vs. about 14–16 embedded scales in G. mini), and the abdomen is fully scaled and the scales are well visible, not embedded as in G. mini (cf. Kullander & Fang, 2004).

Hora (1921) provided a detailed description of specimens from the Manipur Valley identified by him as G. rupeculus (currently G. rupicola (M’Clelland, 1839); Kottelat, 2013). In the text, p. 675, Hora refers to females with eggs “none more than 2 inches in length”, but in the table on p. 676 he reports six specimens 29.7–34.4 mm total length, what at most can be rounded to 1.5 inches. The lateral line scale count is given as 32–34 and the transverse scale count as 9. In the colour description is referred to specimens that are black dorsally and light ventrally as well as to specimens with a black longitudinal band on the lateral line margined with light. The caudal fin is said to have a “wavy band in the middle”. Garra rupicola was described from the Mishmi Hills in Arunachal Pradesh. M’Clelland (1839) gives a precise type locality, but the description and figure are very superficial, without mention of the colour pattern. Menon (1964) synonymized G. abhoyai, described from Ukhrul, with G. rupicola based on Hora’s Manipur specimens. Nebeshwar et al. (2009) resurrected G. abhoyai, considering it to be distinct from G. rupicola but obviously doing so only because of the distribution in different drainages. It seems nevertheless reasonable to assume that G. rupicola, with type locality in the Brahmaputra drainage in northern India, is not present in the Chindwin River, and the small specimens described by Hora from Ukhrul may indeed be G. abhoyai, sharing with the type material of that species the w-shaped band in the caudal fin. Nebeshwar et al. (2009) reported specimens of G. abhoyai 45.0–54.9 mm SL, and the syntypes measured by Hora (1921) were up to 65 mm SL, thus larger than the largest G. mini.

Garra lamta (Hamilton, 1822) is the only of four originally included species that has remained in the genus. It was described from the Rapti River in Gorakhpur district (Uttar Pradesh) and Bihar in northern India, but has been reported from a wider area (e.g., Hora, 1921; Menon, 1964). The original description (Hamilton, 1822) mentions a dark spot in advance of the caudal fin, which excludes the possibility that it may be the same species as G. mini.

The proportional measurements of G. mini are similar to those of G. flavatra, G. vittatula, G. propulvinus, G. rakhinica, and G. poecilura, all described by Kullander & Fang (2004), representing a spectrum of small to medium-sized species of Garra, but the comparison is limited by the small size of G. mini and limited availability of juvenile or young of other species of Garra. A considerable amount of morphometric data in recent papers on Asian species of Garra cannot be used because it does not take into account size allometry, and mostly does not include juveniles.

Genetic distance has been used as an aid in delimiting putative species in numerous studies. Hebert et al. (2003) and Ward (2009) empirically found that if the corrected pairwise p-distance between two individuals of fish is ≥ 2 % they are likely (≥ 95 %) to be of different species, while if their pairwise p-distance is less than 0.4 % they
are likely (>89 %) to be conspecific. The genetic distance between *G. mini* and the closest other *Garra* (12 %) supports species status.

The surprising similarity to the characiform *Pseudocorynopoma doriae* in the BOLD database is an artefact caused by the low quality of the *P. doriae* sequence: almost 15 % of the positions consist of ambiguities or gaps, resulting in chance similarity to a wide range of taxa.

The limited material suggests that the populations of the Sangu and Karnafuli Rivers represent the same species but different populations, separated by 0.6 % *p*-distance. Although geographically adjacent in Bangladesh, with mouths in the Bay of Bengal close to Chittagong, the Karnafuli and Sangu rivers have partly widely different sources, the former in the Mizo Hills in Mizoram, the latter in the Rakhine Yoma in Myanmar, but the smaller Raikkhyang Khal River parallels the upper Sangu River in Bangladesh, and is a tributary of the Kaptai Lake. The shared presence of *G. mini* in the Karnafuli and Sangu Rivers indicates that there has been at least one stream capture event from one to the other river sufficiently long ago for genetic divergence. The Chittagong and Arakan Hills Tracts are seismically active areas of uplift and rivers in the region are likely to have shifted course with earthquakes. The Karnafuli and Sangu Rivers are connected by the Murari Khal canal close to the coast, but it is unlikely to be a dispersal route for a rheophilic fish like *G. mini*.

**Comparative material.** Specimens listed in Kullander & Fang (2004).

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