VERTEBRATE ZOOLOGY

68 (2): 165–175
15.8.2018

SENCKENBERG

Integrative taxonomy reveals a new species of snakehead fish, *Channa stiktos* (Teleostei: Channidae), from Mizoram, North Eastern India

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Accepted July 09, 2018.
Published online at www.senckenberg.de/vertebrate-zoology on July 27, 2018.

Editor in charge: Uwe Fritz

Abstract

Channa stiktos, a new species of snakehead fish, is described from the River Kaladan and its tributaries, Mizoram, North Eastern India based on comparison of morphological and molecular features with closely related species. *Channa stiktos* is morphologically similar to *C. ornatipinnis* described from the Rakhine State of Myanmar, however, differs from it in having black spots on dorsal and ventral sides of the head (vs. no spots on dorsal and ventral sides of the head, but rather spots restricted to the post-orbital lateral region of the head), and lacking dark spot on the anal fin of juveniles (vs. presence of series of upto 10 dark spots). The molecular analysis, based on cytochrome c oxidase subunit I (COI) sequences, shows that *C. stiktos* is distinct from other close *Channa* species, except clade 3 of *C. ornatipinnis*, available in the GenBank (interspecies distance ranges from 8.24–25.33%). *Channa stiktos* clustered cohesively with clade 3 of *C. ornatipinnis* (only 1.43% genetic distance) indicating that they are conspecific. The genetic distance between *Channa stiktos* and *C. ornatipinnis* (from the type locality and another locality in the Ayeyarwaddy basin) are 8.24–8.59%, and between *C. stiktos* and *C. pulchra* is 12.92%, supporting the conclusion that they are different species.

Key words

Channa ornatipinnis, C. pulchra, COI, Taxonomy, Freshwater fish, River Kaladan, Indo-Burma Biodiversity hotspot.

Introduction

Fishes of the family Channidae are widely distributed in tropical Africa, parts of the Middle East, and Asia. They are known to inhabit a wide variety of freshwater habitats ranging from hill streams to swamps (Geetakumari & Vishwanath, 2011), and are able to survive out of water for short periods as they possess accessory air breathing organs (Britz, 2013). Although Channidae is a small family of fishes, the knowledge about their species-level diversity is far from complete, and the taxonomy of some species group is still complicated (Britz, 2013). Species of the genus *Channa* are characterized by an elongated cylindrical body, a large mouth with well-developed teeth on the jaws, vomer and palatines, long dorsal- and anal-fin bases with soft rays, and accessory air breathing organs (modified epibranchial 1) situated in the suprabranchial cavity in the head (Musikasinthorn, 1998; Courtenay & Williams, 2004). Currently there are 40 valid species under the genus *Channa* (Eschmeyer *et al*., 2017), some of these were recently discovered from the Eastern Himalayan region (BRITZ, 2008 & 2013; Geetakumari & Vishwanath, 2011; Knight, 2016; Lalhlimpuia *et al*., 2016).

Many of the snakehead fish species currently recognized were discovered before the beginning of the 20th century, and the last two decades have witnessed quite a few discoveries (BRITZ, 2013). However, due to the existence of species complexes among some species group viz. *C. gachua, C. marulius, C. punctata* and *C. striata* (COURTENAY & WILLIAMS, 2004; RAINBOTH, 1996; ADAMSON *et al.* 2010; SERRAO *et al*., 2014; CONTE-GRAND *et al*., 2017), accurate identification to the species level using morphological approach alone is sometimes challenging. Therefore, a morphological approach when dealing with these species complexes likely benefits from supporting molecular barcoding analyses. Although the molecular approach, also known as DNA barcoding, which employs the conserved short stretch sequences of cytochrome c oxidase sub-unit I gene of the mitochondria (HEBERT *et al*., 2003), has some limitations and pitfalls (MORITZ & CICERO, 2004; RUBINOFF *et al.*, 2006; DUDU *et al.*, 2016), it has proven to be useful in species identification of fishes (WARD *et al*., 2005; HUBERT *et al*., 2008), particularly of *Channa* species (ZHU *et al*., 2013; SERRAO *et al*., 2014; CONTE-GRAND *et al*., 2017).

During field surveys in the River Kaladan and its tributaries, Mizoram, North Eastern India, specimens very similar to *Channa ornatipinnis* and *C. pulchra* were collected. These specimens were previously identified as *Channa ornatipinnis* due to lack of conspicuous morphological differences between the two species. However, detailed morphological examination of the collected specimens and comparisons with similar species, as well as molecular (COI) comparison with the results of recent studies (SERRAO *et al*., 2014; CONTE-GRAND *et al*., 2017) revealed that these specimens were an undescribed species, which we herein describe as *Channa stiktos* sp. nov.

Materials and methods

Collected specimens were fixed in 10% formalin and subsequently preserved in 70% alcohol. Tissue samples were taken from the specimens prior to formalin fixation. The specimens examined in this study are registered in Zoological Survey of India (ZSI), Kolkata, India and the Pachhunga University College Museum of Fishes (PUCMF), Mizoram, India. Methods for taking counts and measurements follow MUSIKASINTHORN (1998). Measurements were taken with digital calipers to the nearest 0.1 mm. Numbers in parentheses after a count denote the frequency of that count. Osteological preparations were made following the method of TAYLOR & VAN DYKE (1985). Osteological nomenclature follows BRITZ (2008). Vertebrae were counted from two cleared and stained specimens (PUCMF 16010). Data from the original descriptions of HAMILTON (1822) for *Ophiocephalus aurantiacus*; CUVIER, (1829) for *Ophicephalus marginatus*, CUVIER & VALENCIENNES (1831) for *O. fuscus*; MCCLELLAND (1842) for *O. montanus*; GÜNTHER, A. (1861) for *O. kelaarti*; MUSIKASINTHORN (2000) for *Channa aurantimaculata*; BRITZ, (2008) for *C. ornatipinnis* and *C. pulchra*; and BRITZ, (2013) for *C. andrao* were used for comparison.

DNA extraction, PCR amplification and DNA sequencing

The extraction of DNA was performed from approximately 25 mg of caudal muscle tissue following SAMBROOK & RUSSELL (2001). The partial COI gene was amplified using the universal fish primers Fish-F1 and Fish-R1 (WARD *et al*. 2005). The PCR reaction was carried out in 25 µl volume containing 1X buffer, 100 µM dNTPs, 2 mM $MgCl_2$, 5 pmol of each primer, 2U Taq DNA polymerase and 100 ng template DNA. Amplifications were performed in Veriti 96 fast thermal cycler (Applied Biosystems, Inc., USA) and the 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 sequencer (Applied Biosystems, Inc, USA).

DNA sequence analysis

The cytochrome c oxidase subunit-I (COI) gene of seven individuals of *Channa* (5 *C. aurantipectoralis* and 2 *C. stiktos*) were sequenced. Altogether, the 7 developed sequences and another 44 sequences of species belonging to *Channa gachua* species group alongwith one outgroup, *Parachanna obscura* (KJ937424), from GenBank were included to make a comparative analysis. The sequences were aligned using CLUSTAL_W integrated in MEGA 7 (Molecular Evolutionary Genetics Analysis) software (KUMAR *et al.*, 2016). The sequences were blasted in NCBI (http://www.ncbi.nlm.nih.gov) for the nearest matches and submitted to NCBI GenBank (NCBI Accession Nos. MH559824 & MH559825 for *Channa stiktos* and MH559819–MH559823 for *C. aurantipectoralis*). The genetic distance was calculated by averaging pairwise comparisons of 51 sequences across close relatives of *Channa stiktos* by the Kimura 2 parameter in MEGA 7 (Table 3). The maximum likelihood (ML) phylogenetic tree was constructed using COI dataset of 41 sequences of *Channa* species, including seven sequences generated in this study and one sequence of *Parachanna obscura* (KJ937424) as outgroup. Based on the lowest BIC (Bayesian Information Criterion), the best fit nucleotide substitution model (out of 24 models) for present COI dataset was TN93+G+I given by TAMURA & NEI (1993) (Tamura-Nei + Gamma distribution with 5 rate categories + certain fraction of sites are evolutionarily invariable).

Results

Channa stiktos sp. nov.

Figs. 1 and 2

Holotype: ZSI FF 7727, 188.3 mm SL; India, Mizoram, Tiau River in the vicinity of Zokhawthar Village, Kaladan River drainage, 23°22′28″N 93°23′13″E, Lalramliana, 16 November 2010.

Paratypes: PUCMF 16007, 1 specimen, 83.3 mm SL; India, Mizoram, Kawlchaw River in the vicinity of Kawlchaw Village, Kaladan River drainage, 22°23′53″N 92°58′09″E, Lalramliana, 12 March 2012; PUCMF 16008, 2 specimens, 73.6–89.8 mm SL; India, Mizoram, Ianava River in the vicinity of Lungbun village, Kaladan River drainage, 22°28′01″N 93°06′15″E, Lalramliana, 20 December 2013; PUCMF 16009, 2 specimens, 75.3–95.6 mm SL; India, Mizoram, Tiau river in the vicinity of Farkawn Village, Kaladan River drainage, 23°04′20″N 93°20′18″E, Lalhlimpuia, 21 April 2016; PUCMF 16010, 3 specimens (two specimens 73.9 mm SL and 74.5 mm SL were cleared and stained for osteology), 69.9–74.5 mm SL; India, Mizoram, Niawh River, in the vicinity of Niawhtlang Village, Kaladan River drainage, 22°29′34″N 93°05′56″E, Lalramliana, 20 December 2013.

Comparative materials

Channa aurantipectoralis: ZSI FF 5634, holotype, 165 mm SL; India: Mizoram: Mamit District: Keisalam River, a tributary of Karnaphuli River, in the vicinity of Phuldungsei; — FF 5635, 3 ex., paratypes, 103–151 mm SL; PUCMF 16004, 7 ex., paratypes, 89.3–160 mm SL; — 16006, 5 ex., paratypes, 77.0–125 mm SL; India: Mizoram: Mamit District: Seling River, a tributary of Karnaphuli River, in the vicinity of Damparengpui.

Channa gachua: ZSI F 2705/1, 1 ex., 246.0 mm SL, Bangladesh: Bulagunj, Sylhet; PUCMF 15026, 3 ex., 85.6–112.5 mm SL; India: Mizoram: Barak River drainage: Tlawng River. —15038, 1 ex., 90.2 mm SL; India: Mizoram: Barak River drainage: Tuivai River; MKC 191, 10 ex. (two specimens 78.9 and 90.9 mm SL were cleared and stained), 56.6–98.2 mm SL; India: West Bengal: Ganrapota, North 24 Parganas. — 182, 1 ex., 52.1 mm SL; India: Meghalaya. — 430, 1 ex., 49.7 mm SL; India: Tamil Nadu: Kaveripattanam; — 431, 6 ex., 56.3–97.9 mm SL; India: Visakhapatnam.

Channa harcourtbutleri: ZSI F9439/1, holotype, 46.8 mm SL; Myanmar: southern Shan State, Inle Lake. Additional data from NG *et al*. (1999)

Channa marulius: PUCMF 11068, 1 ex., 137.7 mm SL; India: Mizoram: Barak River drainage: Serlui River. — 13118, 1 ex., 94.9 mm SL; India: Mizoram: Kaladan River drainage: Kaladan River.

Channa melanostigma: MKC 012, 4 ex. (two specimens 120.2 and 122.9 mm SL were cleared and stained), 66.2–122.9 mm SL; India: Assam: Tinsukia, Brahmaputra River.

Channa pardalis: BNHS FWF 181, holotype, 127.5mm SL; — FWF 182, paratype, 141.1mm SL; ZSI/SRC F 8954, paratype, 139.3mm SL. Additional Materials: MKC 429, 102.1 mm SL; — 437, 114.2 mm SL. All specimens from streams in Nongstoin, West Khasi Hills, Meghalaya, India.

Channa punctata: PUCMF 1055, 2, 134.1–144.9 mm SL; India: Mizoram: Barak River drainage: Serlui River. — 15025, 1, 53.0 mm SL; India: Mizoram: Barak River drainage: Teirei River.

Channa stewartii: ZSI/ERS/V/F 3443, 1, 105.0 mm SL; India: Assam: Bagha (North Cachar Hills); MKC 100, 2 ex. (128.6 mm SL specimen was cleared and stained), 109.2–128.6 mm SL; India: Assam: Cachar, near the border with Meghalaya; — 192, 5 ex. (121.2 mm SL specimen was cleared and stained), 78.1–121.2 mm SL; India: West Bengal: Jalpaiguri district: Jaigaon.

Diagnosis. *Channa stiktos* is distinguished from all other species of *Channa*, except *C. ornatipinnis*, *C. pulchra*, *C. pardalis*, *C. melanostigma* and *C. stewartii*, by a unique colour pattern consisting of numerous large, black spots on the head and the body. *Channa stiktos* is distinguished from *C. ornatipinnis* in having black spots on dorsal and ventral sides of the head (vs. no spots on dorsal and ventral sides of the head, but rather spots restricted to the post-orbital lateral region of the head), and absence of dark spot on the anal fin of juveniles (vs. presence of series of up to 10 dark spots).

It can be distinguished from *Channa pulchra* in having black spots on the body well distributed above and below the lateral line (vs. sparsely distributed or absent on the body below the lateral line); from *C. pardalis* in having black spots all over the head (vs. spots restricted to post-orbital region of the head); and from both *C. stewartii* and *C. melanostigma* in having well defined black spots on the head (vs. spots on the head absent, and restricted to the body).

Description. See Table 1 for morphological data and Figs. 1 and 2 for general appearance. Body elongate, round in cross section anteriorly, its width a little less than its depth; gradually becoming compressed laterally towards caudal peduncle. Dorsal profile of head gently curved anteriorly; ventral profile almost straight. Caudal peduncle deep, its depth slightly greater than its length. Head large, dorsoventrally flattened, about one-third standard length, its widest portion between eye and opercle. Eyes small, located anteriorly on head, diameter less than snout length.

Mouth large, oblique; lips thick, angle of gape vertical through beyond posterior margin of eye. Lower jaw projecting slightly beyond upper jaw. Scales on cheeks in 5 horizontal rows. Both jaws with multiple rows of sharp teeth. Vomer with small sharp, pointed teeth. Premaxilla with 4–5 rows of numerous minute inward curved teeth. Palatine with 2 or 3 stout inward curved canine teeth and a single row of minute sharp teeth. Fifth ceratobranchial slender, with numerous long sharp, inward curved, pointed teeth. Dentary with numerous minute, slender, inward curved, pointed teeth and an inner row of inward curved large teeth.

Lateral line with 47 (1), 48 (5) or 49 (3) pored scales (plus 1–2 scales on caudal-fin base), extending from shoulder girdle in a horizontal line, dropping one scale row at scale 14 (4), 15 (3) or 16 (2), then continuing horizontally to caudal peduncle; 4½ scales above lateral line and 7½ scales below lateral line at anal-fin origin. Pre-dorsal scales 13 (2), 14 (7). Scales absent on gular region. One or two large scales on ventral surface of lower jaw. Dorsal-fin rays 34 (3), 35 (3) or 36 (3); anal-fin rays 24 (8) or 25 (1); pectoral-fin rays 16 (9); pelvic-fin rays 6 (9); principle caudal-fin rays 6+6 (1) or 6+7 (8); circumpeduncular scales 28; vertebrae 45 (39 abdominal + 6 caudal) (2).

Colouration. In alcohol (Fig. 1), dorsum, side of head and body uniformly dark grey, gradually becoming

Fig. 1. *Channa stiktos* sp. nov., holotype, ZSI FF 7727, 188.3 mm SL, Tiau River, Mizoram, India.

lighter towards throat and abdomen. A series of 10–12 cream-coloured chevron markings present on body along anal-fin base. Well-defined black spots, smaller than size of pupil present on head and body. Spots on head larger on post-orbital region, gradually becoming smaller on cranial region of head. Black spots on opercle and ventral surface of head. Black spots on flanks numerous, scattered above and below lateral line, smaller than spots on post-orbital region. Dorsal and anal fins dark grey with narrow white margin. Dorsal fin with 5–6 black blotches on mid-dorsal fin region between rays 1–22 in juveniles < 60 mm SL, either absent or persist as single blotch in specimens > 80 mm SL. Pectoral fin with 4–7 (usually 4) alternating dark brown and white semi-circular bands. Pelvic fin creamy white. Caudal fin with series of black blotches irregularly arranged in two or three semicircular rows.

In life (Fig. 2), body uniformly greenish grey or brown with well-defined numerous black spots as in preserved specimens; black spots more conspicuous and set apart from body background colour. Lip light grey. Iris greyish with orange rim around pupil. Body with alternating dark and light chevron marks sometimes tinged with orange or red. Pectoral fin with 4–7 (usually 4) alternating dark brown and white semi-circular bands as in preserved specimens. Dorsal fin greenish grey in adults with 1–3 black blotches, absent sometime; anal fin pale bluish grey, both fins with faint white margin. Caudal fin colour pattern as in preserved specimens.

Juveniles (Fig. 2A) brown with large black spot above pectoral-fin origin and on caudal-fin base. A faint orange or yellow band running between these two spots, pigmentation below band being darker than above. Black spots on head and body smaller and inconspicuous. Anal

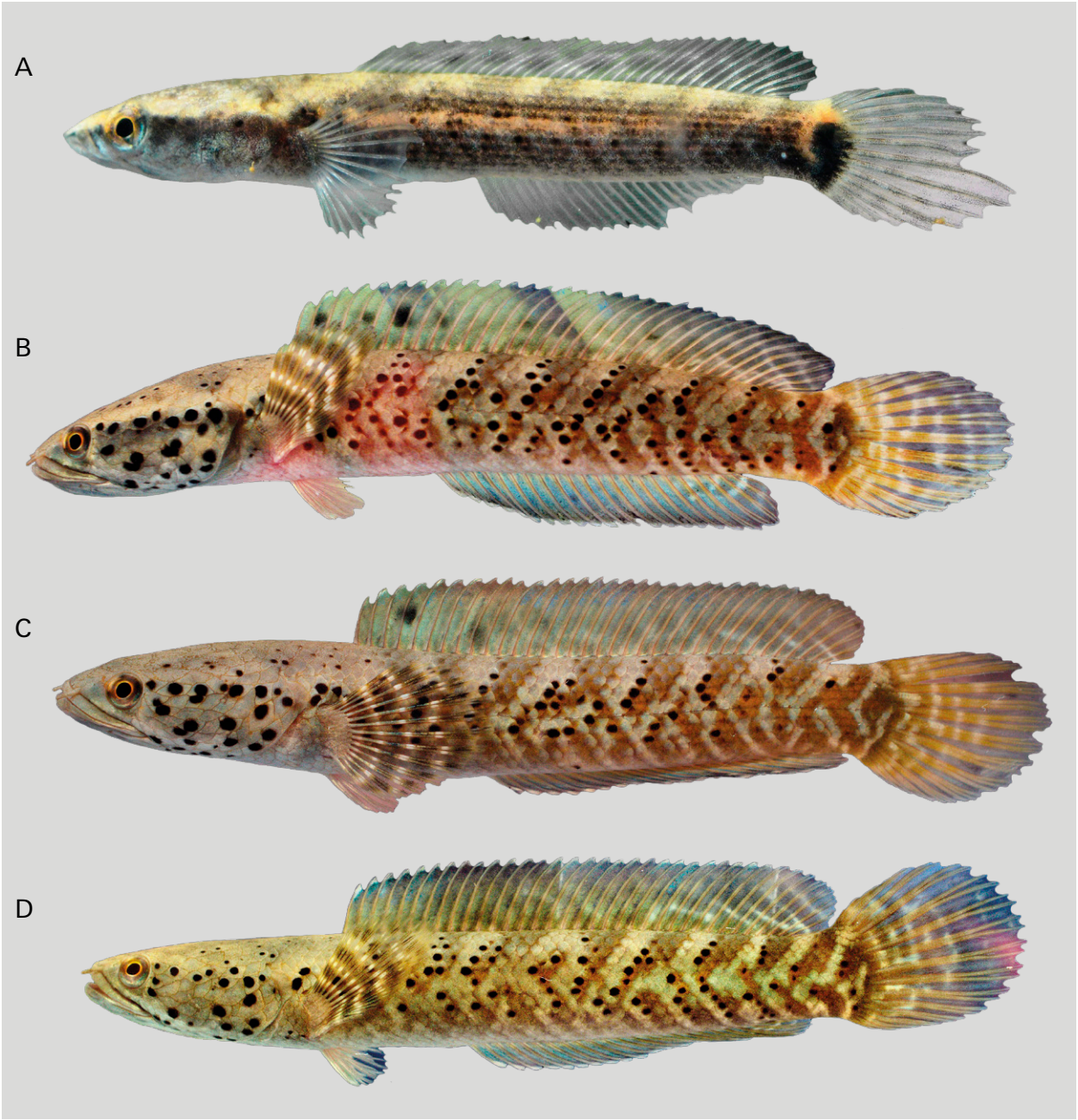

Fig. 2. *Channa stiktos* sp. nov. live specimens from Tiau River, Mizoram, India. **A**: ca. 35 mm SL; **B**: ca. 90 mm SL; **C**: ca. 110 mm SL; **D**: ca. 120 mm SL.

fin hyaline without any marking or blotch. Dorsal fin with 5–6 black blotches on mid-dorsal fin region in specimens < 60 mm SL, either disappear or reduce to 1–3 blotches in adult. Other fins either hyaline or pale grey.

Etymology. The specific epithet '*stiktos*' meaning 'spotted' in Greek, referring to the numerous conspicuous spots present on the body. It is use as an adjective.

Distribution and habitat. *Channa stiktos* was collected from the Ianava, Niawh and Tiau Rivers of the Kaladan River drainage, Mizoram, North Eastern India (Fig. 3). The streams were clear, slow flowing with cobble substrate and no aquatic vegetation (Fig. 4).

Molecular analysis

The two COI gene sequences of *Channa stiktos* generated in this study are distinct from all other *Channa* species available in GenBank (interspecies distance ranges from 8.24–25.33%), with the exception of clade 3 of *C. ornatipinnis*, the samples of which originated from India (KU667357–KU667361 & KJ847162–KJ847166). Our samples of *Channa stiktos* clustered together with samples of clade 3 of *C. ornatipinnis* (only 1.43% genetic distance) indicating that both are conspecific. The genetic distance (K2P distance) between *Channa stiktos* and its morphologically closest species, clade 1 of *C. ornatipinnis* (*C. ornatipinnis* from the type locality

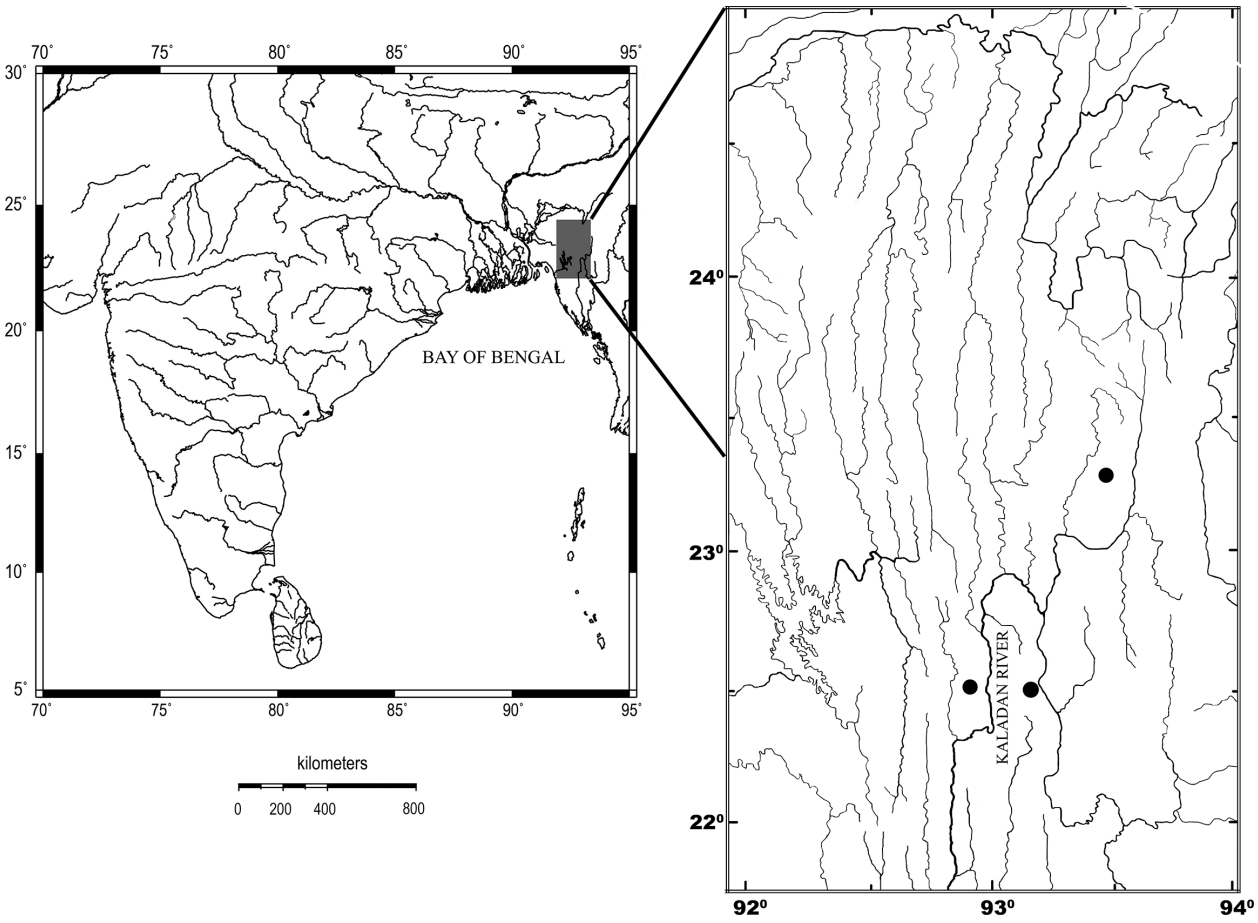

Fig. 3. Map showing collection locality of *Channa stiktos* sp. nov. in Mizoram, India.

Fig. 4. Collection locality of *Channa stiktos* sp. nov. at Ianava River, in the vicinity of Lungbun village, Kaladan River drainage, Mizoram, India.

Table 1. Morphometric data of *Channa stiktos* sp. nov. (n = 9) (ZSI FF 7727 & PUCMF 16007–16010). Ranges include values of holotype.

Morphometrics	**Holotype**	**Range**	**Mean** (SD)
Standard length in mm	188.3	69.9–188.3	
In percent SL			
Head length	29.9	26.5–29.9	28.2±1.0
Head depth	13.0	10.3–13.3	11.9±0.9
Head width	20.8	15.9–20.8	17.5±1.3
Body depth	15.5	13.1–16.6	14.8±1.2
Body width	12.5	10.0–12.5	11.3±0.8
Pre–dorsal length	33.9	34.2–36.9	35.6±1.0
Pre–anal length	52.6	50.7–53.3	52.0±0.9
Pre–pelvic length	33.7	30.7–34.9	32.6±1.3
Dorsal fin length	56.5	54.4–59.2	57.1±1.4
Anal fin length	38.1	37.4–40.4	39.0±0.9
Pectoral fin length	19.9	19.0–20.9	20.0±0.5
Pelvic fin length	10.1	9.6–11.0	10.4±0.4
Caudal peduncle length	10.5	7.0–10.6	9.2±1.3
Caudal peduncle depth	11.3	8.9–11.3	10.3±0.8
Snout length	6.7	4.8–6.7	5.7±0.5
Eye diameter	5.2	4.3–5.5	5.0±0.3
Pre–orbital depth	6.8	6.7–7.9	7.2±0.4
Post–orbital depth	10.9	8.8–10.9	9.7±0.5
Post–orbital length	20.2	17.0–20.4	18.1±1.1
Inter–orbital width	8.4	7.3–8.7	8.0±0.4
Upper jaw length	12.3	9.4–12.3	10.7±0.8
In percent HL			
Snout length	22.6	17.6–22.8	20.3±1.9
Eye diameter	18.3	15.2–20.4	18.1±1.5
Head depth	46.2	37.7–47.1	42.5±3.4
Head width	67.5	58.3–67.5	62.0±2.6
Pre–orbital depth	25.8	24.4–28.9	26.0±1.3
Post–orbital depth	35.8	30.8–35.8	34.4±1.6
Post–orbital length	67.4	60.3–70.7	64.3±3.0
Inter–orbital width	31.7	26.7–31.7	28.9±1.7
Upper jaw length	42.4	35.2–42.4	38.2±2.4

in Myanmar) and clade 2 of *C. ornatipinnis* (collected from 70 km southeast of its type locality), are 8.24% and 8.59% respectively and further, with *C. pulchra* is 12.92%. The deep divergence in the sequence indicates that they are genetically different species. *Channa stiktos* further differs from *C. pardalis* and *C. stewartii* in having deep interspecies distance of 21.04 and 22.09% respectively. The *Channa stiktos* clustered with *C. ornatipinnis* (KJ847162-KJ847166, KJ937428, KU667357–KU667361, MF694873–75) and *C. pulchra* (KJ937349, KJ937434, KJ937442, MF496885, MF496886) in the same clade, indicating that they are genetically close species.

Discussion

Britz (2008) proposed the '*Channa gachua* species group' based on the diagnostic feature of having a varying number of dark and light semicircular bands on the pectoral fin. *Channa stiktos* shares this character indicating its inclusion into the *C. gachua* species group. *Channa stiktos* closely resembles *C. ornatipinnis* and *C. pulchra* in having prominent spots on the head and the body.

Channa ornatipinnis was reported from the Tuivawl River (Barak River drainage) of Mizoram, India by MUTHUKUMAR *et al*. (2017). The snakehead figured in MUTHUKUMAR *et al*. (2017) is very similar to *C. stiktos* in morphological appearance and meristic counts, however, the numerous black spots on the body are sparsely distributed or absent below the lateral line (Fig. 1 of MUTHUKUMAR *et al*. 2017). Inaccessibility of the specimens reported in MUTHUKUMAR *et al*. (2017) for further study and absence of any DNA-sequence data from these specimens do not allow us to comment further on the taxonomic identity and potential conspecificity of the *C. ornatipinnis*-like snakehead from Tuivawl River with *C. stiktos*.

Recently, CONTE-GRAND *et al.* (2017) identified three significantly different clades when analyzing the COI sequences of *Channa ornatipinnis*. They observed high intraspecific divergence between *C. ornatipinnis* (of clade 1) from the type locality (Waloun Chaung, northern Rakhine State, Myanmar) and *C. ornatipinnis* (of clade 2) from another tributary of the Ayeyarwaddy (ca. 70 km southeast of the type locality). A number of additional COI sequences deposited in GenBank as *C. ornatipinnis* from India, but without precise locality information, however, were even more divergent and considered to belong to a different species by CONTE-GRAND *et al.* (2017), who recommended a detailed taxonomic study of *C. ornatipinnis* specimens other than from the type locality. The COI analysis of our *Channa stiktos* and comparison with other *Channa* COI sequences available in GenBank revealed that it is significantly different from all of them, except CONTE-GRAND *et al.*'s (2017) clade 3 of *C. ornatipinnis*, the Indian samples. The sequence difference in the COI gene between specimens of CONTE-GRAND *et al.*'s (2017) clade 3 of *C. ornatipinnis* and *C. stiktos* is only 1.43% indicating that the two are conspecific. In contrast, the deep interspecies distance (8.24%) between *C. stiktos* with *C. ornatipinnis* from the type locality (clade 1 of *C. ornatipinnis*) and CONTE-GRAND *et al.*'s (2017) clade 2 of *C. ornatipinnis* (8.59%) supports the idea that *C. stiktos* and *C. ornatipinnis* are two different species.

In addition to differences in the COI sequence and the distinguishing characters mentioned in the diagnosis section, adults of *Channa stiktos* can be further distinguished from *C. ornatipinnis* in having the lips and lachrymal region greyish green (vs. golden orange or reddish). *Channa stiktos* is distinguished from *C. pulchra*, another Rakhine species, in having more pored scales (47–49 vs. 43–46) in the lateral line, a shorter (26.5–29.9 vs. 31.0–32.0% SL) and shallower (10.3–13.3 vs. 13.8–14.7% SL) head, and by the absence (vs. presence) of reddish blotches on the sides of the body. It can be distinguished from *C. pardalis* by the presence (vs. absence) of blotches on the mid dorsal fin region in juveniles and adults, by the pectoral

Table 2. Detail of sequences (n=52) used in this study and NCBI GenBank accession numbers.

Sl No.	*Channa* Species	NCBI Accn. No.	No. of sequences	Country	Reference
1.	*C. andrao*	MF496660–MF496661	2	India	Conte-Grand *et al.*, 2017
2.	*C. aurantimaculata*	MF496670, MF496671	2	India	Conte-Grand *et al.*, 2017
3.	***C. aurantipectoralis***	**MH559819–MH559823**	**5**	**India**	**This study**
4.	*C. barca*	MF496698, MF496699	2	India	Conte-Grand *et al.*, 2017
5.	*C. bleheri*	EU924636	1	India	Lakra *et al*., 2016
6.	*C. bleheri*	MF496700	1	India	Conte-Grand *et al.*, 2017
7.	*C. burmanica*	KJ937381	1	Myanmar	Serrao *et al.*, 2014
8.	*C. burmanica*	MF496706	1	Myanmar	Conte-Grand *et al.*, 2017
9.	*C.* cf. *melanostigma*	KF511545	1	India	Dhar & Ghosh, 2015
10.	*C. gachua*	MF462263, MF462271	2	Myanmar	Conte-Grand *et al.*, 2017
11.	*C. harcourtbutleri*	MF496801–MF496802	2	Myanmar	Conte-Grand *et al.*, 2017
12.	*C. limbata*	LC190111–LC190112	2	Myanmar	Kano *et al.*, 2016
13.	*C. orientalis*	MF496868–MF496869	2	Sri Lanka	Conte-Grand *et al.*, 2017
14.	*C. ornatipinnis*	KJ847162–KJ847166	5	India	Barman *et al.*, 2014 (unpublished)
15.	*C. ornatipinnis*	KJ937428	1	—	Serrao *et al.*, 2014
16.	*C. ornatipinnis*	KU667357–KU667361	5	India	Pandey *et al.*, 2016 (unpublished)
17.	*C. ornatipinnis*	MF496871–MF496872	2	—	Conte-Grand *et al.*, 2017
18.	*C. ornatipinnis*	MF496873–MF496875	3	—	Conte-Grand *et al.*, 2017
19.	*C. pardalis*	MF496879, MF496880	2	—	Conte-Grand *et al.*, 2017
20.	*C. pulchra*	KJ937349, KJ937434, KJ937442	3		Serrao *et al.*, 2014
21.	*C. pulchra*	MF496885, MF496886	2	Myanmar	Conte-Grand *et al.*, 2017
22.	*C. stewartii*	MF496930–MF496931	2	India	Conte-Grand *et al.*, 2017
23.	***C. stiktos* sp. nov.**	**MH559824–MH559825**	**2**	**India**	**This study**
24.	*P. obscura*	KJ937424	1	Senegal	Serrao *et al.*, 2014

Table 3. The percent K2P genetic distance among *Channa* species, morphologically and genetically close to *Channa stiktos* sp. nov.

C. andrao																	
C. aurantimaculata	15.69																
C. aurantipectoralis	18.60	14.86															
C. barca	15.71	8.86	15.93														
C. bleheri	17.13	14.47	17.56	14.54													
C. burmanica	17.21	11.73	18.62	13.12	15.56												
C. gachua	19.19	16.62	17.00	18.15	18.69	18.20											
C. harcourtbutleri	15.50	12.89	16.44	16.83	17.31	16.01	16.87										
C. limbata	15.58	12.99	17.29	16.24	16.49	15.08	17.16	2.91									
C. orientalis	20.02	16.04	18.06	17.87	17.51	16.45	9.59	16.45	18.09								
*C. ornatipinnis*_3	24.72	21.68	24.44	21.41	21.09	23.40	22.72	23.82	23.35	20.04							
*C. ornatipinnis*_1	21.24	21.96	23.05	20.29	20.67	24.19	23.05	22.40	23.11	20.36	8.54						
*C. ornatipinnis*_2	22.99	21.18	22.68	20.00	20.60	24.86	23.36	21.38	21.82	20.86	9.07	4.65					
C. pardalis	14.59	10.56	16.95	10.89	12.62	13.37	14.05	15.70	15.63	15.05	20.60	20.40	20.57				
C. pulchra	23.25	23.21	24.10	21.72	22.88	24.99	23.54	22.55	21.98	21.57	12.68	8.84	10.56	21.51			
C. stewartii	15.52	12.61	17.03	13.32	15.12	11.98	16.85	14.19	14.53	17.11	21.03	22.35	23.51	12.55	21.25		
C. stiktos sp. nov.	25.33	22.16	23.25	21.40	21.08	23.19	22.28	24.13	23.17	21.16	1.43	8.24	8.59	21.04	12.92	22.09	
C. cf. *melanostigma*	15.08	7.60	17.83	10.28	15.12	10.56	17.30	14.70	16.21	17.01	23.68	23.95	23.61	11.58	24.54	9.95	23.50

fin having 4–7 alternating dark brown and white bands (vs. 3–4 grey bands fading towards the distal margin), the dorsal and anal fins with a narrow (vs. broad) white margin, and more pored scales (47–49 vs. 46–47) in the lateral line. *Channa stiktos* differs from *C. stewartii* by having 34–36 (vs. 39–40) dorsal-fin rays; and from *C. melanostigma* by the absence (vs. presence) of 14–15 distinct zigzag cross bars on the caudal fin.

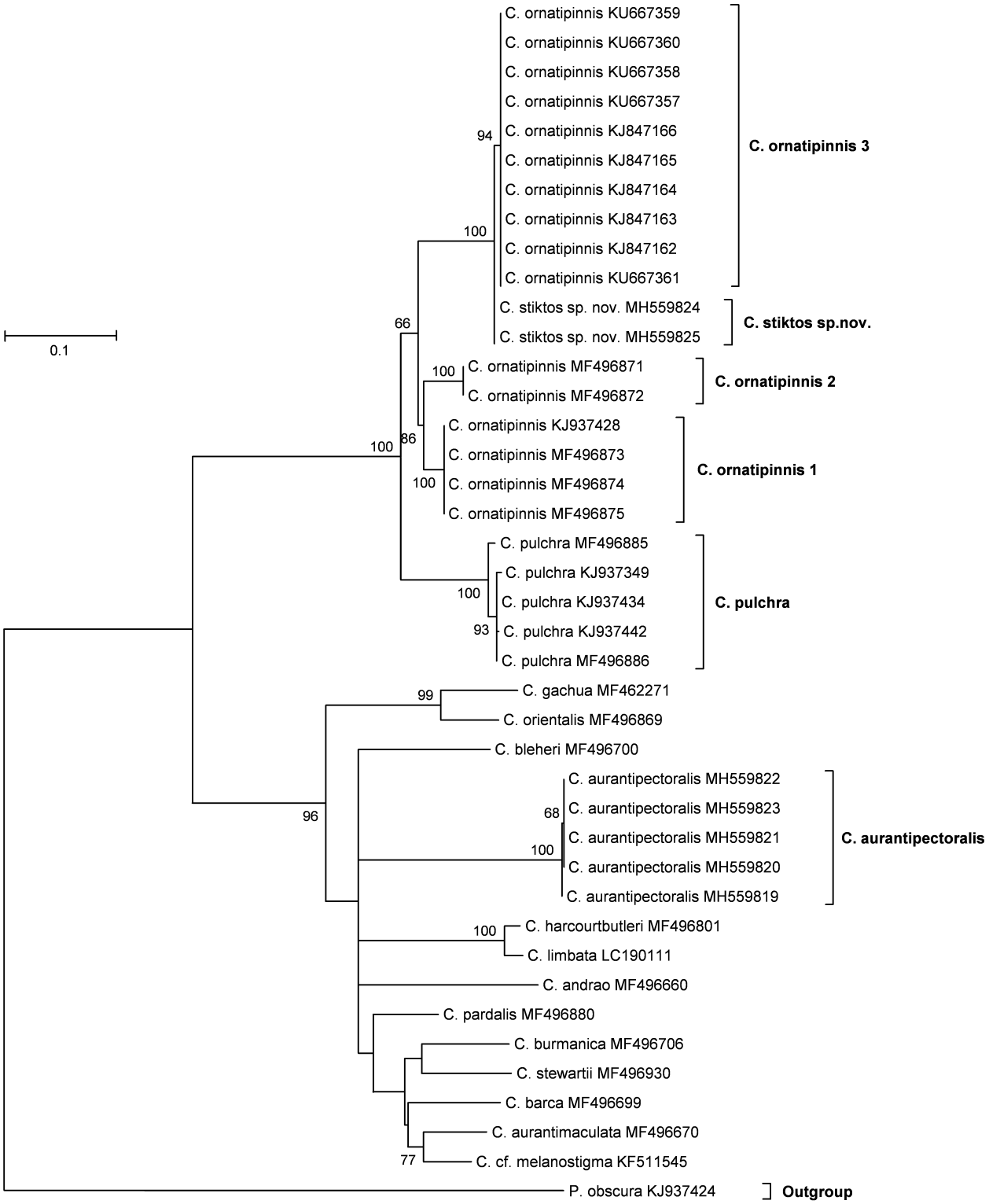

Fig. 5. Maximum Likelihood (ML) tree constructed by using TN93+G+I nucleotide substitution model with 1000 bootstraps for COI dataset of 41 sequences using MEGA7 software.

Furthermore, *Channa stiktos* is markedly different from other species of the *C. gachua* species group viz. *C. orientalis*, *C. burmanica*, *C. bleheri* and *C. andrao* by the presence (vs. absence) of pelvic fins. It can be distinguished from *C. gachua* by the presence (vs. absence) of numerous large black spots on the head and the body, pectoral fin with 4–7 alternating dark brown and white bands (vs. 3–4 grey bands fading towards the distal margin), presence (vs. absence) of blotches in the mid dorsal fin region in juveniles and adults, and more (28 vs. 24) circumpeduncular scales; from *C. aurantimaculata* and *C. barca* in having fewer (34–36 vs. 45–52) dorsal-fin rays and fewer pored scales (47–49 vs. 51–54 in *C. aurantimaculata* and 60–64 in *C. barca*) in the lateral line;

from *C. harcourtbutleri* in having more pored scales (47–49 vs. 43–46) in the lateral line and by the presence (vs. absence) of black spots on the body; and from *C. aurantipectoralis* in having striped (vs. plain) pectoral fins, fewer pored scales (47–49 vs. 51–64) in the lateral line and fewer scale rows (4½ vs. 5½–6½) above the lateral line.

The taxonomic confusion between *Channa gachua* and *C. limbata* has been addressed by CONTE-GRAND *et al.* (2017). Using COI sequence data, they identified that a western lineage of *gachua*, the true *Channa gachua* (lineage 1 with specimens from west of the Indo-Burman ranges covering Sri Lanka, India, Nepal, Bangladesh, and the Rakhine area of Myanmar) differs from an eastern lineage of *gachua*, for which they applied the oldest available name *C. limbata* (lineage 2 with specimens from east of the Indo-Burman ranges from Myanmar reaching east to Vietnam and southern China and south to Indonesia and Malaysia). *Channa stiktos* markedly differs from *C. limbata* by the presence (vs. absence) of numerous large black spots on the head and the body.

There are some names still buried in the synonymy of *Channa gachua*. Of these, *Ophiocephalus aurantiacus* HAMILTON 1822 (type locality: Goyalpara on the North East frontier of Bengal), *Ophicephalus marginatus* CUVIER 1829 (type locality: Vizagapattam, see KOTTELAT, 2000) and *O. fuscus* CUVIER 1831 (type locality: Bengal) are species reported from India. *Channa stiktos* can be distinguished from *O. aurantiacus, O. fuscus* and *O. marginatus* (which are similar to *C. gachua*), by the presence (vs. absence) of well-defined black spots on the head and the body. *Channa stiktos* is further distinguished from *C. fuscus* and *C. marginatus* in having more than (vs. fewer than) 40 pored scales in the lateral line. Moreover, two other names, *Ophicephalus montanus* MCCLELLAND, 1842 (type locality: Afghanistan?) and *O. kelaarti* GÜNTHER, 1861 (type locality: Sri Lanka) are also currently in the synonymy of *C. gachua* (KOTTELAT, 2013). *Channa stiktos* can be distinguished from *O. montanus* by having more (34–36 vs. 32) dorsal-fin rays and more (24–25 vs. 17) anal-fin rays. Further, it can be distinguished from *O. kelaarti* by having more pored scales (47–49 vs. 38) in the lateral line and more (24–25 vs. 22) anal-fin rays. Another similar species, *Ophiocephalus gachua* var. *basalis* GÜNTHER, 1861 (type locality: East Indies), is most probably a synonym of *C. gachua* (KOTTELAT, 2013) due to having a black ocellus with white edge on the hindmost part of the dorsal fin, a typical character of juvenile *C. gachua*. *Channa stiktos* can be distinguished from *O. gachua* var. *basalis* in having more (24–25 vs. 21–23) anal-fin rays, more scales (4½ vs. 3) above lateral line, and juveniles with blotches on the mid dorsal fin region, between rays (vs. a black ocellus with white edge on the hindmost part of the dorsal fin).

The discovery of *Channa stiktos* from the Kaladan River Basin of Mizoram, part of the Indo-Burma biodiversity hotspot region, highlights north-east India and north western Myanmar as hotspots of species level diversity for *Channa* as recognized previously (BRITZ, 2008 & 2013; KNIGHT, 2016; LALHLIMPUIA *et al*., 2016; VISHWANATH & GEETAKUMARI, 2009; CONTE-GRAND *et al.* 2017), and the need for continued exploration of this area to properly document its fish diversity.

Acknowledgements

We gratefully acknowledge the support provided by the Director, Zoological Survey of India, Kolkata; K. Ilango (Officer-in-charge) and Jayasree Thilak (Scientist-D), of the Southern Regional Centre, Zoological Survey of India, Chennai. We thank Andrew Rao, Nikhil Sood, Beta Mahatvaraj, Pranay Kumar and Praveen Raj for helping us obtain the comparative materials used in this study. We are grateful to Prachya Musikasinthorn and Ralf Britz for providing valuable literatures; Vanlalmalsawma (Sawmtea), Lalhminghlua and Vanlalhlimpuia for their field assistance; Henry Lalmawizuala and K. Lalchhandama for language editing; the two anonymous reviewers for invaluable suggestions and critical reviewing of the manuscript; Tawnenga, Principal, PachhungaUniversity College; and Director, ICAR-National Bureau of Fish Genetic Resources, Lucknow, Uttar Pradesh, India for providing laboratory facilities. The financial support from Department of Biotechnology, Government of India (No. BT/388/NE/TBP/2012 dated 11-12-2014 under DBT's Twinning Programme) is thankfully acknowledged.

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