



***Channa royi* (Teleostei: Channidae): a new species of snakehead from Andaman Islands, India**

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ABSTRACT

A new species of snakehead fish *Channa royi* sp. nov., has been described based on 21 specimens collected from the South, Middle and North Andaman Islands, India. It is distinguished from all its congeners by a greenish-grey dorsum, pale brown to black pectoral fin with 2-3 inconspicuous semicircular bands, a series of 7-9 obliquely-arranged, saddle-like, dark olive to grey oblique streaks on green background on upper half of the body, 42-45 pored lateral-line scales, 12-13 branched caudal rays, 6-7 pre-dorsal scales, 43 vertebrae, two rows of teeth on the lower jaw, an outer row of numerous minute slender, pointed teeth and single inner row of large uniform sized teeth without any large canine like teeth on the anterior fourth of the lower jaw. Phylogenetically *C. royi* sp. nov. is closely related to *C. harcourtbutleri*, with a genetic distance (K2-P) of 2.4-2.8%, but morphologically differs in having greater inter-orbital width, fewer pelvic-fin rays (5 vs. 5-7, mode 6); fewer caudal-fin rays (ii- i, 10-12, i-ii vs. ii, 15, ii); more pre-dorsal scales (6-7 vs. 4) and fewer mid row lateral-line scales (9-13 vs. 15-16). Though *Channa royi* sp. nov. is a part of *C. gachua* species-group, it differs from the topotypic *C. gachua* from Bengal with higher pair-wise sequence distance of 20.7-22.8%.

Keywords: Andaman Islands, *Channa gachua*, *Channa limbata*, *Channa royi* sp. nov., Channidae, Snakeheads

Introduction

Snakeheads of the family Channidae are mostly distributed in tropical Africa, parts of the Middle East and Asia. These are found to exist in a wide variety of freshwater habitats ranging from hill streams to derelict swamps (Courtenay and Williams 2004; Geetakumari and Vishwanath, 2011). At present, the family Channidae consists of 44 valid species. There are 17 valid species of *Channa* reported from India; 24 species from South-east Asia and three species under the genus *Parachanna* from Africa (Conte-Grand *et al.*, 2017; Fricke *et al.*, 2018; Praveenraj *et al.*, 2018a, b; Adamson and Britz, 2018). The species-level diversity of the family Channidae is still not fully known (Britz, 2013), especially for species groups like that of *C. gachua*, which was proposed by Britz (2008) based on the molecular studies of Li *et al.* (2005). The species in this group have a body colouration from grey to greyish-brown, ventral region being white or dirty white and the anal, caudal and dorsal fins are iridescent green or blue with an orange margin (Ng and Lim, 1990; Lee and Ng, 1994), pectoral fins with a varying number of dark and light semicircular bands (Britz, 2008) and two large cycloid scales on each side of lower jaw (Vishwanath and

Geetakumari, 2009). The present study describes a new species of *Channa* belonging to *C. gachua* species-group, collected from the South, Middle and North Andamans.

Materials and methods

Study area and sampling

Specimens were collected from four locations in the Andaman group of islands, India *viz.*, streams in Mannarghat (11°45'43.45"N; 92°43'2.03"E), Garacharma (11°36'53.93"N; 92°43'5.00"E) and Goalghar (11°39'45.49"N; 92°44'5.15"E) of South Andaman District, Nimbudera (12°43'21.99"N; 92°53'11.00"E) in Middle Andaman District. A few specimens were also collected from Diglipur fish market, North Andaman District. Topotypes of the comparative specimens were purchased through the aquarium traders and a few others were also collected with the help of collaborating institutes. The specimens were fixed in 10% formalin and subsequently preserved in 70% alcohol for further studies.

Abbreviations: ZSI/ANRC-Zoological Survey of India, Andaman and Nicobar Regional Centre, India; ZSI FF-Zoological Survey of India, Freshwater Fish collections, Kolkatta, India; CIARI/FF-ICAR-Central

Island Agriculture Research Institute, Freshwater Fish collections, India; MNHN-Museum National d'Histoire Naturelle, Paris; MCSNG-Museo Civico di Storia Naturale "Giacomo Doria", Italy; MKC-Marcus Knight Collections, Chennai, India.

Meristics, morphometry and osteology

Methods for taking counts and measurements followed Musikasinthorn (1998) and Britz (2008). Measurements were made with digital calipers to the nearest 0.1 mm. Subunits of the body parts are presented as percent of standard length (SL) and subunits of the head are presented as percent of head length (HL). Numbers in parenthesis after a count denotes the frequency of that count. Data from Ng *et al.* (1999), Musikasinthorn (2000) and Britz (2008) were used for comparison. The original descriptions of various synonyms of *C. gachua* and counts taken from type specimen photographs were used in the study. Osteological preparations were made following staining procedure of Taylor and Van Dyke (1985) and osteological nomenclature from Murray (2012). Vertebrae were counted from one specimen of the new species which was cleared and stained for osteology. Teeth and ceratobranchial were cleared and stained from a single specimen each of *C. stewartii*, *C. melanostigma*, *C. gachua* and *C. limbata*. Photographs of the stained bones were taken with an Olympus SP570 UZ digital camera using super-macro mode.

Genetic analysis

Clippings from right side pectoral fin and muscle tissue dissected from the right side of the body were collected from 13 sampled specimens: CIARI/FF-10-11 from Goalghar, South Andaman; CIARI/FF-13-15 from Garacharma, South Andaman; CIARI/FF-17-18 from North Andaman; CIARI/FF-19-20 from Middle Andaman; CIARI/FF-21-24 from Mannarghat, South Andaman. Two topotype specimens of *C. gachua* from Bengal (CIARI/FF-25-27) and one topotype of *C. limbata* from West Java Bogor and Indonesia (CIARI/FF-34) were also barcoded for comparison. DNA was extracted using Qiagen blood and tissue kit from fin clippings and muscle tissues. DNA amplification of the cytochrome C oxidase subunit I (cox1) gene was done using the species specific primer (COX Forward-CTCGACTAATCACAAAGACATCG and COX Reverse-GGT GCCCAAAGAATCAGAAT) designed using the PRIMER3 software (Koressaar and Remm, 2007; Untergasser *et al.*, 2012). PCR amplification reaction was performed in a final volume of 50 µl PCR mix consisting of 5 µl of PCR buffer, 5 µl of 2 mM dNTPs, 4 µl of MgCl₂, 2 µl each of 10 pmol forward and reverse primers, 2 µl of dimethyl sulphoxide (DMSO), 2 µl of 5 units µl⁻¹ Taq polymerase and 26 µl nuclease free water.

PCR reaction was carried out in a thermocycler (Bio-Rad, USA) following the conditions as described by Lakra *et al.* (2010). The amplified PCR products were sequenced using COX-F and COX-R primers in ABI 3500 DNA analyser (ShrimpeX Biotech Pvt. Ltd., Chennai). The homology of the generated sequences were analysed using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1990). The sequences were trimmed by CLC sequence viewer ver. 8 (QIAGEN) and submitted in the National Center for Biotechnology Information (NCBI) GenBank database.

Gene sequences were aligned using MUSCLE (Multiple Sequence Alignment with High Accuracy and High Throughput) (Edgar, 2004). The genetic distance between the *Channa* spp. sequences was determined by the Kimura 2-parameter (K2-P) model (Kimura, 1980) in the software program MEGA7 (Molecular Evolutionary Genetics Analysis) (Kumar *et al.*, 2016). The best fit nucleotide substitution model was selected from 24 models, based on the one with the lowest BIC scores (Bayesian Information Criterion), which was considered to describe the best substitution pattern (Nei and Kumar, 2000). The phylogenetic tree was constructed based on the maximum likelihood fits in MEGA7. Reliability of the phylogenetic tree was estimated using bootstrap values run for 1000 iterations.

Results

Channa royi, sp. nov. Praveenraj & Knight (Fig. 1, 2, 3)

Common name: Andaman emerald snakehead

Holotype: ZSI/ANRC-12467, 1 ex., 108.8 mm SL (Fig. 1, 2; Table 1), Mannarghat, South Andaman, India (11°45'43.45"N; 92°43'2.03"E), J. Praveenraj, 02 May 2015,

Paratypes: ZSI/ANRC-12468, 1 ex., 44.6 mm SL, same data as holotype; ZSI FF 7662, 2 ex., 160.3-170.9 mm SL, Diglipur fish market, North Andaman, J. Praveenraj, 17 February 2017; ZSI FF 7663, 1 ex., 138.3 mm SL, pond located near a stream, Nimbudera, Middle Andaman, (12°43'21.99"N; 92°53'11.00"E), J. Praveenraj, 17 February 2017; CIARI/FF-08-12, 5 ex., 121.7-160.0 mm SL (139.3 and 145.3 mm SL, GenBank Acc. No. KY214142, KY214136), a stream draining into a municipal canal, Goalghar, South Andaman (11°39'45.49"N; 92°44'5.15"E), J. Praveenraj, 25 January 2016; CIARI/FF-13-15, 3 ex., 108.3-118.4 mm SL, GenBank Acc. No. KY214138, KY562577, KY562578), a stream passing through ICAR-CIARI, Garacharma, South Andaman (11°36'53.93"N; 92°43'5.00"E), J. Praveenraj, 21 January 2017; CIARI/FF-17-18, 2 ex., 170.2-180.9 mm SL (GenBank Acc. No.



Fig. 1. *Channa royi* sp. nov. holotype, ZSI/ANRC-12467, 108.8 mm SL, South Andaman (Photo credit: Dr. J.D.M. Knight)

KY863530, KY863529), same data as North Andaman specimens; CIARI/FF-19-20, 2 ex., 163.5-190.3 mm SL (GenBank Acc. No. KY863528, KY863527), same data as Middle Andaman specimens; CIARI/FF-21-23, 3 ex., 128.5-142.2 mm SL (128.5, 141.5 and 142.2 mm SL, GenBank Acc. No. KY214137, KY562575, KY562574), Mannarghat hill stream, South Andaman (11°45'43.45"N; 92°43'2.03"E), J. Praveenraj, 1 February 2017. CIARI/FF-24, 1 ex., 99.2 mm SL, same data as holotype (cleared and stained for osteology, GenBank Acc. No. KY562576).

Diagnosis: *Channa royi* sp. nov. is distinguished from all other species of the genus *Channa* belonging to the *Channa gachua* species-group by a combination of following characters: unique greenish-grey dorsum; upper half of body with a series of 7-9 obliquely-arranged, saddle-like, dark olive to grey oblique streaks on green background; throat with marbled pattern; 34-38 dorsal-fin rays; 22-25 anal-fin rays; 42-45 pored scales on body; 1-3 scales on caudal fin base; 3½ scales above lateral line and 6½ scales below lateral line; 6-7 pre-dorsal scales; 43 vertebrae;

two rows of teeth on the lower jaw, single outer row of numerous minute slender pointed teeth and single inner row of enlarged uniform teeth without any large canine like teeth on anterior-fourth of the lower jaw; two rows of teeth on palatine; outer row with numerous minute sharp slender teeth and inner row with 4 to 6 large canine like teeth and pectoral fins pale brown to black in adults with 2-3 pale grey to black inconspicuous semicircular bands.

Description: Body elongate and round in cross section anteriorly, its width a little less than its depth, gradually becoming laterally compressed towards caudal peduncle. Dorsal profile of head gently curved anteriorly, ventral profile almost straight. Caudal peduncle deep, its depth greater than its length. Head large, length 3.0-3.6 times in SL, dorso-ventrally flattened, its widest portion between eye and opercle. Eyes small, located anteriorly on head, closer to tip of snout than to distal opercular margin. Mouth large, oblique, angle of gape extending beyond the posterior margin of the eye. Lower jaw projecting slightly beyond upper jaw, lips fleshy. Fifth ceratobranchial

Table 1. Morphometric data of *Channa royi* sp. nov. (n = 21) including those of the holotype

Parameters	Holotype	Range	Mean±SD
Standard length, SL (mm)	108.8	44.6-190.3	139.2±33.2
In % SL			
Head length	32.0	27.5-34.1	30.3±1.7
Head depth	14.7	13.0-18.5	15.6±1.1
Head width	19.3	17.3-20.8	18.7±1.0
Body depth	16.5	14.3-19.5	17.3±1.2
Body width	15.2	10.7-15.7	13.5±1.4
Pre-dorsal length	36.9	32.7-39.0	34.9±1.7
Pre-anal length	51.1	49.2-55.4	52.4±1.8
Pre-pectoral length	32.2	28.5-34.8	31.9±1.6
Pre-pelvic length	35.3	32.4-37.8	35.1±1.4
Dorsal fin base length	56.8	56.3-66.8	60.6±2.6
Anal fin base length	36.6	33.8-44.0	39.5±2.8
Pectoral fin length	20.8	17.3-22.0	19.5±1.5
Pelvic fin length	10.2	7.8-11.0	9.3±0.9
Caudal peduncle length	8.6	5.1-9.2	7.8±1.0
Caudal peduncle depth	9.8	9.8-13.0	11.5±0.8
Snout length	7.0	5.3-7.5	6.3±0.5
Eye diameter	5.1	3.7-8.1	4.6±0.9
Pre-orbital depth	8.1	5.9-10.1	8.1±1.0
Post-orbital depth	11.4	8.4-12.6	10.7±1.0
Post-orbital length	20.8	18.0-22.1	20.5±1.0
Inter-orbital width	8.8	8.3-9.6	8.9±0.4
Upper jaw length	13.1	9.2-13.8	11.5±1.2
In % head length			
Snout length	46.1	17.7-23.5	20.8±1.3
Eye diameter	16.0	12.6-23.7	15.0±2.2
Head depth	46.1	38.2-63.1	51.7±5.2
Head width	60.4	52.0-69.1	61.9±4.6
Pre-orbital depth	25.2	20.2-32.6	26.7±3.7
Post-orbital depth	35.8	30.1-43.6	35.4±3.1
Post-orbital length	65.0	63.2-71.7	67.8±2.0
Inter-orbital width	27.5	26.5-34.1	29.5±1.9
Upper jaw length	40.9	33.3-42.6	37.9±2.9

slender, with numerous long sharp inward pointing teeth (Fig. 4a). Both jaws with multiple rows of sharp and pointed teeth. Two rows of teeth on the palatine, outer row with numerous minute sharp slender teeth and inner row with 4 to 6 large canine like teeth (Fig. 4b). Two rows of teeth on lower jaw, an outer row of numerous minute slender pointed teeth and single inner row of enlarged uniform teeth without any large canine like teeth on anterior-fourth of lower jaw (Fig. 4c). Vomer with 6 small sharp, pointed teeth and 4 canine-like teeth.

Total pored scales 42 (7)* (numbers in parenthesis after a count denotes the frequency of that count and asterisk denotes count for holotype), 43 (12), 44 (1) and 45 (1); mid row pored scales 12 (5) or 13 (16)*; lower

row pored scales 29 (3)*, 30 (16), 31 (1), 33 (1); scales on caudal fin base 1 (12)*, 2 (6), 3 (3); transverse scales 3½ /1/ 6½ (21); pre-dorsal scales 6 (1), 6½ (2), 7 (18)*; circumpeduncular scales 24 (8), 26 (13)*; pre-pelvic scales 10 (18)*, 10½ (3). Fin rays: dorsal 34 (2), 35 (7)*, 36 (6), 37 (2), 38 (4); anal 22 (2), 23 (6), 24 (7)*, 25 (6); pectoral 14 (2), 15 (19)*; caudal 12 (1), 13 (20)*; pelvic 5 (21). Vertebrae 43 (39 abdominal + 4 caudal) (1).

Colouration: Live specimens (Figs. 2a, 3) appears greenish-grey, top of head and opercular region dark green. Dorsum greenish, gradually becoming pale grey towards the abdomen, upper half of body with series of 7-9 obliquely-arranged, saddle-like dark olive to grey oblique streaks on green background. Throat region marbled with white blotches on grey background. Dorsal fin is bluish green in colour with thin orange outer margin. Anal fin bright-blue in base and darker towards margin with a pale-orange colour towards distal region. Caudal fin membrane bluish-green with bright-green rays and a thin orange border. Pectoral fins pale brown to black in mature adults (> 150 mm SL) with 2-3 inconspicuous black to pale grey semicircular bands (Fig. 3 a, b). Pelvic fins grey with a white margin. Sclera on the eyes red with a black patch below orbit. Juvenile specimen (< 50 mm SL) pale greyish cream in colour with numerous black spots scattered along the head, opercle and body. Dorsal and anal fins hyaline with numerous large, dark-grey elongated blotches. Caudal fin hyaline with 5-6 vertical pale grey bars. Pectoral fins hyaline, with two inconspicuous creamy white semicircular bands (Fig. 2b).

In alcohol preserved specimens (Fig. 1), dorsum, sides of head and body uniformly dark greyish-brown gradually becoming lighter towards the throat and abdomen. Dorsal, anal and caudal fins dark-grey with a thin white margin. Pectoral fins dark-grey with two white semicircular bands. Pelvic fins grey with a white margin.

Etymology: The species is named after Dr. S. Dam Roy, in appreciation for his immense encouragement and support for the exploration of the freshwater fishes of Andaman and Nicobar Islands. The species name is formed as a noun in the masculine genitive singular. The common name 'Andaman emerald snakehead' is given considering its unique greenish dorsum.

Distribution: *Channa royi* sp. nov. is currently known only from South, Middle and North Andaman Islands, India and is abundant in ditches, ponds and hill streams of the sampled locations. The holotype was collected from clear, slow flowing water with cobble substrate without much aquatic vegetation.

Genetic analysis: All partial COX1 sequences derived were submitted in the National Center for Biotechnology

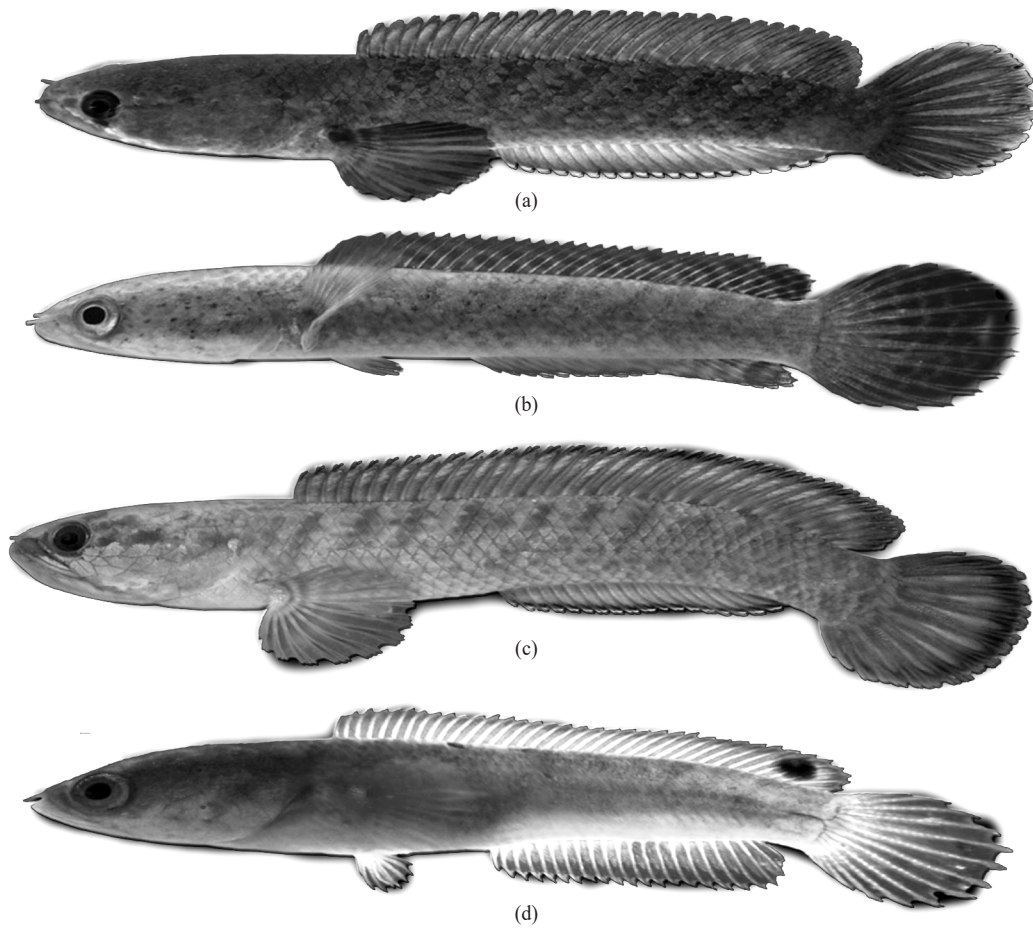


Fig. 2. (a). *Channa royi* sp.nov. holotype prior to preservation (ZSI/ANRC-12467), (b). Juvenile specimen of *C. royi*, uncatalogued, 44.6 mm SL, (c). *Channa gachua* (CIARI/FF-27), 94.33 mm SL, (d). Juvenile specimen of *Channa gachua* (MKC 191), 46.2 mm SL. (Photo credits: Dr. J. D. M. Knight)

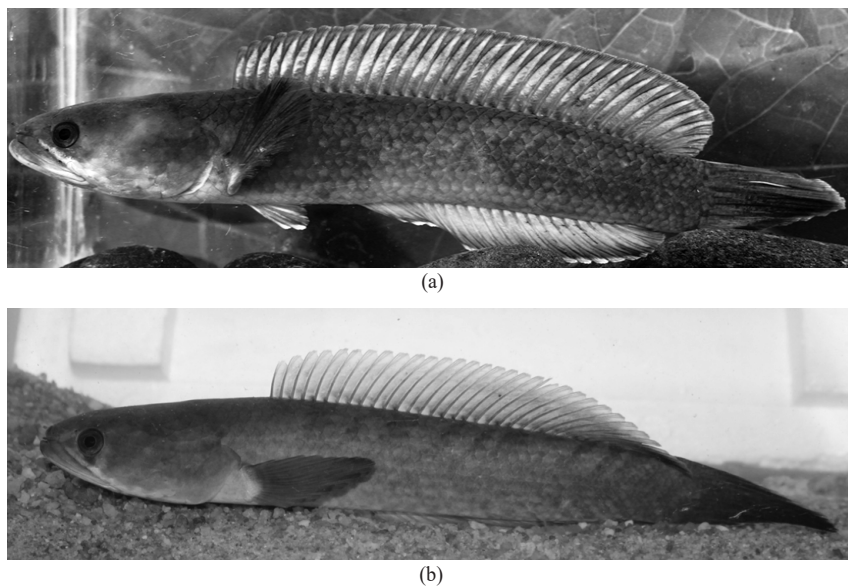


Fig. 3. *Channa royi* sp. nov. (a) Adult, 190.5 mm SL, showing black pectoral fin, uncatalogued, (b) Adult, 165.1 mm, showing pale grey pectoral fin, uncatalogued. (both maintained in aquarium) (Photo credits: J. Praveenraj)

Information (NCBI) GenBank database with accession numbers: *Channa royi* - KY214136-KY214138, KY214142, KY562574-KY562578 and KY863527-KY863530; *Channa limbata*, topotype = KY563772 and *Channa gachua*, topotype - KY214140-KY214143. Additional DNA sequences for the phylogenetic trees were retrieved from the NCBI Channidae barcode library generated by Conte-Grand *et al.* (2017) (Table 2)

Model test suggested the best fit nucleotide substitution model to be the Tamura Nei model (TN93) with gamma distribution and assumption that a certain fraction of sites are evolutionarily invariable (+I) [(G+I), AICc = 11301.56274, lnL = -5544.373986, (+I) = 0.569402471, (+G) = 1.790251869]. The maximum likelihood tree generated, suggests that, the closest genetic congener of *C. royi* is *C. harcourtbutleri* (Fig. 5) (MF496804-MF496808), from which it differs by genetic distance (K2-P distance) of 2.4-2.8%. Though *C. royi* belongs to the *C. gachua* species-group, it differs from topotypic *C. gachua* (KY214140, KY214143) from Bengal by a genetic distance of 20.7-22.8%. Genetic distance between the new species and members of the *C. gachua* species-group and other congeners are provided in the Table 3.

Discussion

Based on the results of molecular analyses by Li *et al.* (2005), Britz (2008) proposed the *Channa gachua* species-group. All species under this group have their pectoral fins with varying number of dark and light semicircular bands. In addition to *C. orientalis*, *C. gachua*, *C. burmanica*, *C. bleheri* and *C. barca*, Britz (2008) included *C. stewartii*, *C. aurantimaculata*, *C. ornatipinnis* and *C. pulchra* also to the *C. gachua* species-group. Other recently described species, *i.e.*, *C. melanostigma*, *C. andrao*, *C. pardalis*, *C. aurantipectoralis*, *C. pomanensis*, *C. stiktos*, *C. quinquefasciata* and *C. bipuli* also belong to the *C. gachua* species-group. *Channa royi* unquestionably belongs to this group of species as its pectoral fins have the characteristic semicircular bands and two large cycloid scales on each side of lower jaw. In the present work, these characters are strongly supported by the phylogenetic analysis, depicting three major clades separating *C. gachua*, *C. marulius* and *Parachanna* groups. *Channa royi* sp. nov. shares close relatedness with *C. harcourtbutleri* (Annandale, 1918), forming a sister clade in the phylogenetic tree. It shares similar morphometry in most aspects but, differs in having a greater inter-orbital width 26.5-34.1% (*vs.* 25.9-26.5% HL) of *C. harcourtbutleri*. *C. royi* sp. nov. further differs from *C. harcourtbutleri* in having pelvic fin rays 5 (*vs.* 5-7, mode 6); branched caudal rays, i-ii, 12-13, i-ii (*vs.* ii, 15, ii); pre-dorsal scales from base of first dorsal

ray to the first fused cephalic scales 6-7 (*vs.* 4); lateral line scales, dropping one scale row at scale 12-13 (*vs.* 15-6); pectoral fin pale brown to black with 2-3 inconspicuous pale grey to black semicircular bands (*vs.* pectoral fins greyish, with 2-5 semi-concentric black rings); dorsum greenish-grey (*vs.* black to purplish-black); dropping one scale row 12-13 (*vs.* 15-16) and dorsal fin bluish-green, anal fin bright-blue (*vs.* dorsal and anal fins purplish-black to dark-grey); throat region marbled with whitish blotches on grey background (*vs.* throat marbled with bluish spots and blotches on dark-grey background).

Though *C. royi* sp. nov. forms part of the *C. gachua* species-group, it can be distinguished from topotypic *C. gachua* (Hamilton, 1822) by the presence (*vs.* absence) of large canine like teeth on the palatine (Fig. 4 b, k); 43 (*vs.* 42) vertebrae; 42-45 + 1-3 (*vs.* 39-40 + 1) lateral line scales and absence (*vs.* presence) of an ocellus at the posterior rays of the dorsal fin in juvenile fish (Fig. 2 d). The adults of *C. gachua* can be easily distinguished from *C. royi* by its bulged cheek and head appearing more convex when viewed laterally (Fig. 2c), whereas *C. royi* sp. nov. possess a uniform cheek and head with flat ventral profile.

C. royi sp. nov. can be readily distinguished from both *C. stewartii* (Playfair, 1867) and *C. melanostigma* Geetakumari and Vishwanath, 2011 by the absence (*vs.* presence) of numerous black spots along the side of the body; 6½ (*vs.* 7½) scales below lateral line at anal fin origin; 43 (*vs.* 48 and 50) vertebrae respectively; a more slender (*vs.* deeper) fifth ceratobranchial (Fig. 4 d, g) and the absence (*vs.* presence) of large canine like teeth on the anterior fourth of the lower jaw (Fig. 4C, F, I). *Channa royi* can further be distinguished from *C. stewartii* by the presence of 34-38 (*vs.* 39-40) dorsal fin rays and 42+6 + 1-3 (*vs.* 47-48 + 2) lateral-line scales. It can be distinguished from *C. melanostigma* by the absence (*vs.* presence) of 14-15 distinct zigzag cross bars on the caudal fin. Further, *C. royi* sp. nov. can be distinguished from *C. aurantimaculata* Musikasinthorn 2000 and *C. barca* (Hamilton, 1822) by its fewer dorsal-fin rays 34-38 (*vs.* 45-52) and fewer lateral line scales 42-45 + 1-3 (*vs.* > 50).

C. royi sp. nov. differs from *C. orientalis* Bloch & Schneider, 1801, *C. burmanica* Chaudhuri 1919, *C. bleheri* Vierke 1991 and *C. andrao* Britz 2013, due to the presence (*vs.* absence) of pelvic fins. It can be distinguished from both *C. ornatipinnis* Britz 2008 and *C. pulchra* Britz 2008, by the absence (*vs.* presence) of spots on the post-orbital region and flanks, 6½ (*vs.* 7½) scales below lateral line at anal fin origin; absence (*vs.* presence) of black

Table 2. GenBank accession numbers from NCBI, locations and voucher numbers for COI gene sequences of various species of *Channa*

Species	Location	Voucher	GenBank Accession No.
<i>Channa andrao</i>	India	—	MF496661
<i>Channa aurantimaculata</i>	India	CA1001	KJ847157
<i>Channa aurantipectoralis</i>	Mizoram, India	—	MH559819
<i>Channa barca</i>	—	—	MF496699
<i>Channa bipuli</i>	Garbhanga, Assam, India	ZSI FF 7650	MG825616
<i>Channa bleheri</i>	Alipurduar, India	—	MF496702
<i>Channa burmanica</i>	Putao, Myanmar	—	MF496706
<i>Channa gachua</i>	Java, Bogor, Indonesia	—	MF496735
<i>Channa gachua</i>	Seletar, Singapore	—	MF496730
<i>Channa gachua</i>	Bagayat, Maharashtra, Western India	CIARI/FF-50	KY863544
<i>Channa gachua</i>	Ponneri, Tamil Nadu, South India	CIARI/FF-51	MG989241
<i>Channa gachua</i>	Indonesia	—	MF496767
<i>Channa gachua</i>	Laos	—	MF496766
<i>Channa gachua</i> (Topotype)	Ganrapota, North 24 Parganas, West Bengal, India	CIARI/FF-25	KY214140
<i>Channa gachua</i> (Topotype)	Ganrapota, North 24 Parganas, West Bengal, India	CIARI/FF-27	KY214143
<i>Channa harcourtbutleri</i>	—	—	MF496809
<i>Channa harcourtbutleri</i>	Inn Khaung-Chaung between Aung Ban and Heho Chaung, Myanmar	—	MF496804
<i>Channa harcourtbutleri</i>	—	—	MF496805
<i>Channa harcourtbutleri</i>	—	—	MF496806
<i>Channa harcourtbutleri</i>	—	—	MF496807
<i>Channa harcourtbutleri</i>	—	—	MF496808
<i>Channa limbata</i>	West Java, Bogor, Indonesia,	CIARI/FF-34	KY563772
<i>Channa lucius</i>	Ketapang fish market, Borneo, Indonesia	—	MF496832
<i>Channa marulius</i>	Bengalen aquarium trade, India; Aquarium Glaser, Frankfurt, Germany	—	MF496849
<i>Channa ornatipinnis</i>	—	—	MF496874
<i>Channa pardalis</i>	—	—	MF496879
<i>Channa pseudomarulius</i>	Kerala aquarium trade, India	—	MF496884
<i>Channa pulchra</i>	—	—	MF496885
<i>Channa punctata</i>	Golbazar, India,	WILD-17-PIS-330	MF462273
<i>Channa quinquefasciata</i>	Torsa River, Howlong bridge, near Bhutan foothills, North Bengal, India	ZSI FF 7906	MH627036
<i>Channa royi</i>	Goalghar ditch, South Andaman, India	CIARI/FF-10	KY214142
<i>Channa royi</i>	Goalghar ditch, South Andaman, India	CIARI/FF-11	KY214136
<i>Channa royi</i>	Garacharama stream, South Andaman, India	CIARI/FF-13	KY214138
<i>Channa royi</i>	Garacharama stream, South Andaman, India	CIARI/FF-14	KY562577
<i>Channa royi</i>	Garacharama stream, South Andaman, India	CIARI/FF-15	KY562578
<i>Channa royi</i>	Diglipur, North Andaman, India	CIARI/FF-17	KY863530
<i>Channa royi</i>	Diglipur, North Andaman, India	CIARI/FF-18	KY863529
<i>Channa royi</i>	Nimbudera, Middle Andaman, India	CIARI/FF-19	KY863528
<i>Channa royi</i>	Nimbudera, Middle Andaman, India	CIARI/FF-20	KY863527
<i>Channa royi</i>	Mannarghat, South Andaman, India	CIARI/FF-21	KY214137
<i>Channa royi</i>	Mannarghat, South Andaman, India	CIARI/FF-22	KY562575
<i>Channa royi</i>	Mannarghat, South Andaman, India	CIARI/FF-23	KY562574
<i>Channa royi</i>	Mannarghat, South Andaman, India	CIARI/FF-24	KY562576
<i>Channa stewartii</i>	—	—	MF496931
<i>Channa stiktos</i>	Mizoram, India,	—	MH559824
<i>Channa striata</i>	Aquarium trade, Guahati, Assam, India, Aquarium Glaser, Frankfurt, Germany	—	MF496935
<i>Parachanna africana</i>	—	—	MF496973

'—' Denotes lack of data

Table 3. K2-P genetic distance (%) between *Channa royi* sp. nov. and its congeners

Species	<i>Channa royi</i> sp. nov.
<i>Channa harcourtbutleri</i>	2.4-2.8
<i>Channa gachua</i> (Laos)	5.8-6.3
<i>Channa gachua</i> (Indonesia)	5.6-6.0
<i>Channa limbata</i>	5.8-6.3
<i>Channa gachua</i> (Java Bogor)	5.8-6.2
<i>Channa gachua</i> (Singapore Seletor)	5.8-6.5
<i>Channa andrao</i>	17.8-18.8
<i>Channa aurantimaculata</i>	14-14.8
<i>Channa barca</i>	18.7-19.9
<i>Channa pardalis</i>	17.7-18.4
<i>Channa bipuli</i>	15.2-15.7
<i>Channa bleheri</i>	18.4-18.7
<i>Channa stewartii</i>	16.5-17.4
<i>Channa quinquefasciata</i>	13.2-13.9
<i>Channa burmanica</i>	18.1-18.7
<i>Channa aurantipectoralis</i>	20.2-21.2
<i>Channa gachua</i> (Western India)	19.2-20.2
<i>Channa orientalis</i>	21.2-21.9
<i>Channa gachua</i> (South India)	19.5-20.8
<i>Channa gachua</i> (Topotype)	20.7-22.8
<i>Channa pulchra</i>	27-28.2
<i>Channa stiktos</i>	26.6-27.7
<i>Channa ornatipinnis</i>	27.6-28
<i>Channa punctata</i>	26.8-28
<i>Channa striata</i>	25.4-26.9
<i>Channa panaw</i>	28.1-28.9
<i>Channa lucius</i>	33.4-34.3
<i>Channa pseudomarulius</i>	28.3-29.9
<i>Channa marulius</i>	31.2-32.6
<i>Parachanna africana</i> (outgroup)	33.5-34.3

blotches on the anterior third of the dorsal fin and absence (vs. presence) of parallel, oblique, linear white marks at anal fin base.

Six species viz., *Channa pardalis* Knight, 2016, *C. aurantipectoralis* Lalhlimpua *et al.*, 2016, *C. pomanensis* Gurumayum and Tamang, 2016 and *C. stiktos* Lalramliana *et al.*, 2018, *C. quinquefasciata* Praveenraj *et al.*, 2018a and *C. bipuli* Praveenraj *et al.*, 2018b have been described recently from north-eastern India. *Channa royi* sp. nov. can be distinguished from *C. pardalis* and *C. bipuli* by the absence (vs. presence) of numerous large black spots on the post-orbital region of the head, opercle and body; transverse scale $3\frac{1}{2}$ (vs. $4\frac{1}{2}$) above lateral line and 43 (vs. 45) vertebrae. It can be distinguished from *C. aurantipectoralis*, by presence of fewer lateral line scales (42-45 vs. 51-64); 14-15 pectoral fin rays (vs. 13-14); pectoral fin pale brown to black with 2-3 semicircular bands (vs. orange pectoral fin devoid of any bands) and transverse scale rows $3\frac{1}{2}/1/6\frac{1}{2}$

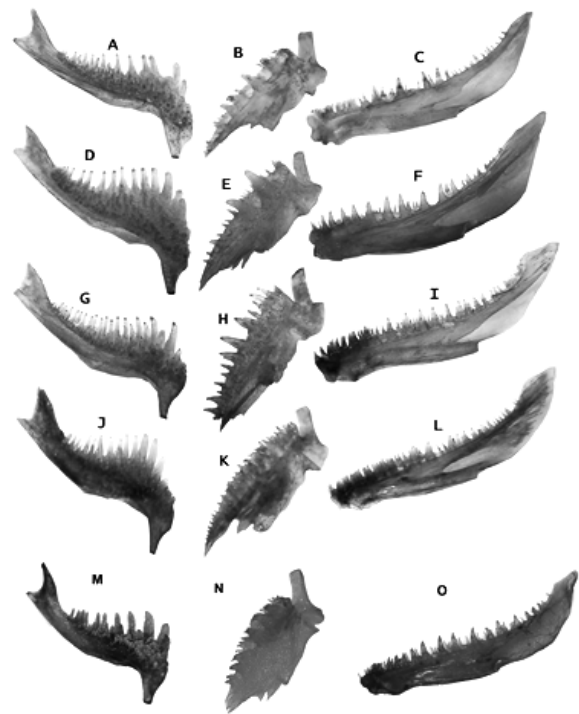


Fig. 4. Left side bones of *Channa* species: row 1- *C. royi*; row 2 - *C. stewartii*; row 3 - *C. melanostigma*; row 4 - *C. gachua*; row 5 - *C. limbata*. Column 1 - fifth ceratobranchial; column 2 - palatine; column 3 - inner view of dentary. (Photo credits: Dr. J. D. M. Knight and Dr. J. Praveenraj)

(vs. $5\frac{1}{2}$ - $6\frac{1}{2}$ / $1/7\frac{1}{2}$ - $8\frac{1}{2}$). *Channa royi* sp. nov. differs from *Channa pomanensis* in having fewer rows of transverse scales ($3\frac{1}{2}$ / $1/6\frac{1}{2}$ vs. $4\frac{1}{2}$ - $5\frac{1}{2}$ / $1/7\frac{1}{2}$ - $8\frac{1}{2}$), fewer caudal fin rays (i-ii, 10-12, i-ii vs. i-ii, 11-14, ii-iii) and varies in colouration of outer margin of dorsal and anal fin with thin orange border (vs. white margin). *Channa royi* sp. nov. also differs from *C. quinquefasciata* by the absence of stripes on the body (vs. presence of five stripes), shorter snout length (17.7-23.5 vs. 18.2-26.0% HL) and more circumpeduncular scales (12-13 vs. 9-10).

It is relevant to note that quite a few descriptions were synonymised with *C. gachua*. Of these *Ophiocephalus aurantiacus*, Hamilton 1822 (type locality: Goyalpara on the north-east frontier of Bengal), *Ophicephalus marginatus*, Cuvier, 1829 (type locality: Vizagapattam, Kottelat, 2000) and *O. fuscus* Cuvier, 1831 (type locality: Bengal/Maissour) have been reported from India. We were able to examine specimens collected from North-east India, West Bengal, Visakhapatnam (=Vizagapattam) and the River Cauvery, which flows through Mysore (=Maissour). In case of *O. fuscus*, photograph of the syntype (MNHN A.398, A.623) was examined. *C. royi* sp. nov. can be distinguished

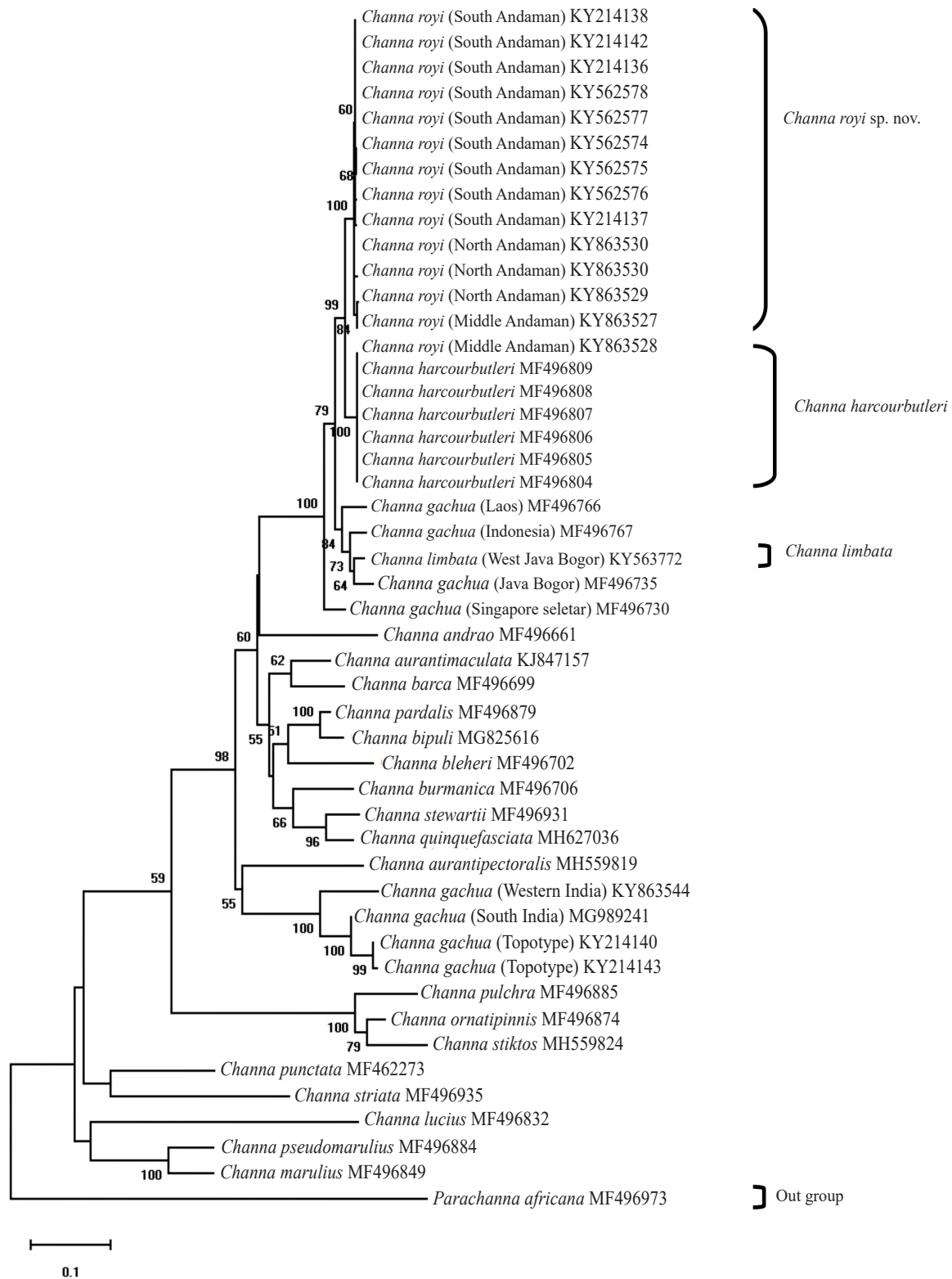


Fig. 5. Phylogenetic position of *Channa royi* sp. nov. based on maximum likelihood analysis (Values along the nodes are percent bootstraps for 1000 iterations; *Parachanna africana* is used as out-group)

using specimen photographs and the topotypes, which were similar to *C. gachua* in having more than (*vs.* less than) 42 lateral line pored scales. *C. royi* sp. nov. can be easily distinguished from its topotypes by the difference in pectoral fins which are pale brown to black with 2-3 pale grey to black inconspicuous semicircular bands (*vs.* pectoral fin orange with 4-5 semicircular, narrow black bands) and absence (*vs.* presence) of an ocellus at the posterior rays of the dorsal fin in juvenile fish in specimens from West Bengal, Visakhapatnam and the River Cauvery.

Ophicephalus limbatus Cuvier, 1831 (type locality: Bogor, West Java, Indonesia, see Kottelat, 2000) is shown only on a figure (Cuvier and Valenciennes, 1831: pl. 201) based on a specimen from Java. Kottelat (2000) concluded that the MNHN A.396 [115 mm SL, 142 mm TL] is the model of the plate 201 figured by Cuvier, in Cuvier and Valenciennes, 1831 for *O. limbatus*

(=*C. limbata*). Photographs of MNHN A.396 were examined, which is clearly differentiated from *Channa royi* sp. nov. in possessing a uniform, depressed head and body; equal head depth and body depth; mid row pored scale 10 (*vs.* 12-13); pre-dorsal scales from base of first dorsal ray (scale on which it is attached, not counted) to first fused cephalic scale 8 (*vs.* 6½-7 in *C. royi* sp. nov.). In addition, from the photograph it is very evident that the MNHN A.396 specimen has a series of canine-like teeth in the lower jaw, which is absent in *C. royi* sp. nov. Topotypes representing *C. limbata* (Fig. 6 a,b) from Bogor, West Java, Indonesia matched the scale count, teeth pattern and general appearance of the holotype (MNHN A.396). *Channa royi* sp. nov. differs from the topotypic *C. limbata* in possessing more lateral line pored scales (>40) *vs.* 39-40 and total vertebrate 43 (*vs.* 42). Further, the topotypic *Channa limbata* can be distinguished from *C. royi* sp. nov. by its teeth pattern, it has two rows of



Fig. 6. (a). *Channa limbata* topotype, West Java, Bogor, Indonesia, CIARI/FF-36, 112.0 mm, (b). *Channa limbata* topotype, West Java, Bogor, Indonesia 141.67 mm SL, (c). *Channa* sp. from Ciliwung River, West Java, Indonesia, CIARI/FF-33, 117.12 mm SL (Photo credit: Dr. J. Praveenraj)

teeth on the lower jaw with an outer row of long, slender, pointed canine teeth in the anterior fourth ceratobranchial (vs. presence of small minute numerous needle like teeth); 9-10 large canine teeth in the anterior fourth of the lower jaw (vs. absent in *C. royi* sp. nov.); 13 (vs. 7) blunt inward facing teeth in the fifth ceratobranchial; palatine with 2 rows of teeth and 7-8 (vs. 4-6) canine-like teeth (Fig 4. m, n, o). Morphologically *C. royi* sp. nov. differs from the topotypic *C. limbata* by possessing a longer head length (27.5-34.1 vs. 24.3-25.7% SL); greater body depth (13.0-18.5 vs. 10.54-12.34% SL). *Channa royi* sp. nov. varies from the topotypic *C. limbata* sequence (KY563772) by a pair-wise sequence distance of 5.8-6.3%. Phylogenetic analysis also grouped the topotypic *Channa limbata* sequence (KY563772) with other GenBank sequence, *C. gachua* from Bogor, Java (MF496735), Indonesia (MF496767), Java, Bogor (MF496730) under a single clade, which indicates distinctiveness of the Indonesian *Channa* clade including *C. limbata* (Conte-Grand *et al.*, 2017).

Two other names, *Philypnoides surakartensis* Bleeker, 1849 and *Ophiocephalus apus* Canestrini, 1861, which are two Indonesian species and synonymous to *C. gachua* (Kottelat, 2013) also represent *C. limbata* group (Conte-Grand *et al.*, 2017). We have also examined specimens from Indonesia and nearby localities (Fig. 6C) and the photograph of *Ophiocephalus apus* lectotype (MCSNG 39373) and found that *Channa royi* sp. nov. differs from the lectotype (MCSNG 39373) by possessing 12-13 (vs. 8) mid row pored scales; presence (vs. absence) of pelvic fin. *Channa royi* sp. nov. differs from the fresh specimens by 42-45 (vs. 29-34) lateral line pored scales; pectoral fin pale brown to black with 2-3 pale grey to black inconspicuous semicircular bands (vs. pectoral fin radiating from pale orange to bright orange with 2-4 broad semicircular bands).

Ophiocephalus montanus M'Clelland and Griffith 1842 (type locality: Afghanistan ?), is currently a synonym of *C. gachua* (Kottelat, 2013). *Channa royi* sp. nov. can be distinguished from *O. montanus* by presence of 34-38 (vs. 32) dorsal fin rays and 22-25 (vs. 17) anal fin rays.

Another synonym of *C. gachua* is *Ophiocephalus gachua* var. *basalis* Gunther, 1861 (type locality: East Indies). It is most probably *C. gachua* (Kottelat, 2013), as Gunther mentions that this species has a black ocellus edged with white on the hindmost part of the dorsal fin when young, which is absent in *C. royi* sp. nov.

Ophiocephalus guachua var. *malaccensis* Peters, 1868 (type locality: Singapore), which was previously considered the synonym of *C. gachua* and now represent

the population of eastern lineage for which the oldest name was *C. limbata* (Conte-Grand *et al.*, 2017). *Channa royi* sp. nov. can be distinguished from this species by its 34-38 (vs. 34) dorsal-fin rays, and by the presence (vs. absence) of large canine teeth in the palatine. We have also examined photographs and meristic data of *Channa* sp. from Singapore which are distinct from *C. royi* sp. nov. by its pectoral fin possessing 4 black semicircular bands with orange interspaces (vs. pale brown to black with 2-3 pale grey to black inconspicuous bands), mid row pored scales 11 (vs. 12-13) and transverse scales 3½/1/ 7½ (vs. 3½/1/6½). *Channa royi* sp. nov. genetically differs from *C. gachua* from Singapore (MF496730) reported by Conte-Grand *et al.* (2017) by a pair-wise sequence distance of 5.8-6.5%.

Channa longistomata Nguyen, Nguyen and Nguyen, 2012, a recently described *Channa* species from Vietnam is tentatively considered as a synonym of *C. gachua* (Kottelat, 2013) probably because the original description in Vietnamese does not clearly differentiate the species from *C. gachua*. The image of *C. longistomata* in Nguyen *et al.* (2012) (159, Fig. 1) shows a species with a short rounded head and light coloured pectoral fins with three to five dark semicircular bands. *Channa royi* sp. nov. can be distinguished from *C. longistomata* in having pale brown to black pectoral fin with 2-3 pale grey to black inconspicuous bands.

Comparative materials

Channa harcourtbutleri: ZSI F9439/1, 1 ex., holotype, 46.8 mm SL; Myanmar: southern Shan State, Inle Lake; ZSI F9451 /1, 2 ex., 39.1-53.0 mm SL; Myanmar: southern Shan State, Thumakam (Hsamongkam); N. Annandale; Additional data from Ng *et al.* (1999).

Channa gachua: CIARI/FF-25-27, 3 ex., 95.1-94.3 mm SL (95.1 and 95.2 mm SL, GenBank Acc. No. KY214140, KY214143), Ganrapota, North 24 Parganas, West Bengal, India, October 2016, Soutrik Gosh; ZSI FF 7654, 2 ex., 34.2-67.0 mm SL (67.0 mm SL, GenBank Acc. No. MG989241), Arani River, Pazhaverkadu, Tamil Nadu; CIARI/FF-46, 109.0 mm SL, Cauvery River, Erode, Salem District, Tamil Nadu; CIARI/FF-48, 112.0 mm SL, Dhahagoan, Dhapoli District, Maharashtra; CIARI/FF-51, 1 ex., 109.4 mm SL, Ponneri, Tiruvallur District, Southern India, J. Praveenraj and team; MKC191, 11 ex., 46.2-98.2 mm SL, Ganrapota, North 24 Parganas, West Bengal, India, March 2009, Andrew Rao (Two specimens 78.9 and 90.9 mm SL were cleared and stained for osteology); MKC 182, 1 ex., 52.1 mm SL, Meghalaya, India, March 2009, A. Rao. MKC 430, 1 ex., 49.7 mm SL, Kaveripattanam, Tamil Nadu, India, July 2015, Nikhil

Sood; MKC 431, 6 ex., 56.3-97.9 mm SL, Visakhapatnam, India, July 2015, Pranay Kumar.

Channa limbata: CIARI/FF-28-33, 6 ex., 86.9-117.1 mm SL, Ciliwung River, West Java, Indonesia (6°39'32.9"S 106°53'01.0"E), August 2014, Beni Halalludin; CIARI/FF-34-37, 4 ex., 78.0-121.0 mm SL, Bogor, West Java, Indonesia (6°32'35.8"S 106°49'23.0"E), September 2014, Silukoki Thariq (78.0 mm SL, GenBank Acc. No. KY563772) (two specimens 121.0 and 101.0 mm SL, were cleared and stained for osteology); CIARI/FF-52-53, 2 ex., 120.7-141.7 mm SL, Bogor, West Java, Indonesia, (6°32'35.8"S 106°49'23.0"E), January 2014, Heiko Bleher.

Channa stewartii: CIARI/FF-38, 1 ex., 118.5 mm SL, Jaigaon, Jalpaiguri District, West Bengal, April 2015, Soutrik Gosh; MKC 100.0, 2 ex., 109.2-128.6 mm SL, Cachar, near Meghalaya border, Assam December 2010, A. Rao (128.6 mm SL, specimen was cleared and stained for osteology); MKC 192, 5 ex., 78.1-121.2 mm SL, Jaigaon, Jalpaiguri District, West Bengal, March 2009, Andrew Rao (121.2 mm SL, specimen was cleared and stained for osteology).

Channa melanostigma: MKC 012, 4 ex., 66.2-122.9 mm SL, Brahmaputra River, Tinsukia, Assam, November 2008, Andrew Rao (two specimens 120.2 and 122.9 mm SL, were cleared and stained for osteology).

Channa pardalis: ZSI/FF 7652, 123.7 mm SL; CIARI/FF-43, 114.5 mm SL (GenBank Acc. No. MG825618), West Khasi Hills, Meghalaya, India.

Photographs examined

Ophicephalus limbata: holotype-MNHN A.396

Ophicephalus fuscus: syntypes-MNHN A.398, A. 623

Ophiocephalus apus lectotype: MCSNG 39373

Channa sp. from Singapore provided by Hung Tsun Cheng

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