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43/2 Varadarajulu Nagar, 5th Street West, Ganapathy, Coimbatore, Tamil Nadu 641006, India
Registered Office: 3A2 Varadarajulu Nagar, FCI Road, Ganapathy, Coimbatore, Tamil Nadu 641006, India
Ph: +91 9385339863 | www.threatenedtaxa.org
Email: sanjay@threatenedtaxa.org

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Cover: Green Bee-eater with colour pencils and watercolor wash by Elakshi Mahika Molur.

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COMMUNICATION

Genetic evidence on the occurrence of *Channa harcourtbutleri* (Annandale, 1918) in Eastern Ghats, India: first report from mainland India

Boni Amin Laskar [1], Harikumar Adimalla [2], Shantanu Kundu [3], Deepa Jaiswal [4] & Kailash Chandra [5]

[1] High Altitude Regional Centre of Zoological Survey of India, Solan, Himachal Pradesh 173211, India.
[1,4] Freshwater Biology Regional Centre, Zoological Survey of India, Attapur, Hyderabad, Telangana 500032, India.
[2] House No. 2-60, Village Turkapalle, Nalgonda, Telangana 508266, India.
[3,5] Zoological Survey of India, New Alipore, Kolkata, West Bengal 700052, India.
[1] boniamin.laskar@gmail.com (corresponding author), [2] harikumaradimalla92@gmail.com, [3] shantanu1984@gmail.com,
[4] deepajzsi@gmail.com, [5] kailash616@gmail.com

Abstract: *Channa harcourtbutleri* (Annandale) was described from Inle Lake (Southern Shan State) in Myanmar, and is currently considered as a valid species in the *Channa gachua* species-group. Notwithstanding several detailed studies on *Channa* from India in the recent, none has mentioned the occurrence of *C. harcourtbutleri* in the Indian mainland. In continuation to the faunal diversity exploration in Eastern Ghats, India, a few specimens in the *C. gachua* species-group were collected from the river Sabri sub-basin of the river Godavari basin in the East Godavari District of Andhra Pradesh which was identified as *C. harcourtbutleri* through DNA barcoding. This is a first report on occurrence of the species in the wild in the Eastern Ghats, India.

Keywords: Channidae, DNA barcoding, Godavari basin, Inle Lake, phylogeny, taxonomy.

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Author details: Boni Amin Laskar is currently working as scientist-E in High Altitude Regional Centre of Zoological Survey of India, Solan. His field of research is molecular studies and taxonomy of freshwater fishes. He has over 20 years of research experience including field surveys throughout various biogeographic zones in India. Harikumar Adimalla is a budding researcher in the field of molecular studies of freshwater fishes from Deccan peninsular biogeographic zone. Shantanu Kundu is a molecular biologist with over 15 years of experience including field surveys and molecular studies of Indian fauna, especially the Himalayan and northeastern Indian region. He is currently working as a post-doctoral fellow in the Department of Marine Biology, Pukyong National University, Busan, South Korea. Deepa Jaiswal is working as scientist-E in Freshwater Biology Regional Centre of Zoological Survey of India, Hyderabad. Her field of specialization is taxonomy of aquatic Insects. Kailash Chandra is the former director of Zoological Survey of India. He is a renowned taxonomist in India and is a recipient of E.K. Janaki Ammal National Award on taxonomy.

Author contributions: BAL & HA did field surveys and collected the specimens. BAL studied the morphology and meristics of the specimens for taxonomic identification. HA generated the DNA data. BAL & SK did the molecular analysis. BAL, SK & DJ wrote the article. BAL & KC reviewed the article.

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INTRODUCTION

Snakehead fishes belong to the family Channidae, are most popular in ornamental fish trade. The species in the genus *Channa* Scopoli, 1777 are currently distributed in southern, eastern, and southeastern Asia while their allied species in the genus *Parachanna* Teugels & Daget, 1984 are endemic in Africa. Snakehead fishes are broadly categorized into two major groups based on the presence of gular scales. The first group having gular scales comprises of all the African species of the genus *Parachanna* and four Asian species of the genus *Channa* while the second group lacking gular scales comprises of all the rest of the channid species (Musikasinthorn & Taki 2001; Zhang et al. 2002). Further, the *Channa gachua* species group (sensu Britz 2008) was characterized by a varying number of dark and light semi-circular bands on the pectoral-fin, which has been subsequently followed by other ichthyologists (Britz et al. 2019; Praveenraj et al. 2019). The presence of gular scales is regarded as a plesiomorphic state within the Channidae (Li et al. 2006). As mentioned in Li et al. (2006), the African taxa of *Parachanna* differ from the Asian taxa of *Channa* by the absence of supporting 'lamellas' or process of the first epibranchial and hyomandibular in the suprabranchial organs (Senna 1924; Bonou & Teugels 1985). Further, the study has shown that the species lacking pelvic fins clade with the species having the pelvic fins, and has explained that the loss of the pelvic fins occurred at least three times independently during the evolution of the taxa in Channidae (Bonou & Teugels 1985). The gular region with scales or without scale is one of the key characters in channid taxonomy (Talwar & Jhingran 1991; Li et al. 2006), and the shape of isthmus was also shown to be a key feature in differentiating *marulius* and *gachua* species-groups (Vishwanath & Geetakumari 2009). Among the currently reported 24 channid species in India, 18 species (15 with pelvic fins and 3 without pelvic fins) are included in the *C. gachua* species-group (sensu Britz 2008). Britz et al. (2019) mentioned that snakehead fishes have a centre of diversity in the eastern part of the Himalaya Biodiversity Hotspot (Conte-Grand et al. 2017; Rüber et al. 2019). In the recent two decades, quite a good number of new species, mostly in the *C. gachua* species-group, have been described. However, a few of the recent descriptions have been retained in synonymy by Britz et al. (2019).

Channa harcourtbutleri (Annandale) was described from Inle Lake (southern Shan State) in Myanmar (Annandale, 1918), but it was placed in synonymy with *C. gachua* (Hamilton) by Hora & Mukerji (1934). However, since the latter species is younger than the former, this synonymy at its first instance appears incorrect. Ng et al. (1999) resurrected the species and discussed the differences between them. The taxonomy of *C. gachua* has been a complex problem (Ng et al. 1999), but the recent phylogenetic study suggested two distinct lineages within the *C. gachua* species-complex (Conte-Grand et al. 2017). The true *C. gachua* as referred in Conte-Grand et al. (2017) is restricted to the area west of the Indo-Burman ranges (i.e., Rakhine Yoma and Chin Hills) and covers Sri Lanka, India, Nepal, Bangladesh, and the Rakhine area of Myanmar. The taxon previously recorded as *C. gachua* from Sri Lanka has been revalidated as *C. kelaartii* (Gunther), and has its population also distributed in southern peninsular India (Sudasinghe et al. 2020). The eastern lineage of the *C. gachua* species-complex, nominally referred to as *C. limbata*, is distributed to the east of the Indo-Burman ranges from Myanmar reaching east to Vietnam and southern China and south to Indonesia and Malaysia (Conte-Grand et al. 2017). Notwithstanding several detailed studies on channid taxa from India recently, none has mentioned the occurrence of *C. harcourtbutleri* in mainland India (Conte-grand et al. 2017; Britz et al. 2019; Sudasinghe et al. 2020).

In continuation to the faunal diversity exploration in Eastern Ghats, India, several specimens of the genus *Channa* were collected from various localities. Among the examined specimens, a few specimens collected from the river Sabri sub-basin of the river Godavari basin in the East Godavari District of Andhra Pradesh, were morphologically identified as similar to *C. harcourtbutleri* and were confirmed through DNA barcoding. *C. harcourtbutleri* is a Burmese species, hitherto not recorded from mainland India.

MATERIALS AND METHODS

The study incorporates several specimens of the genus *Channa* from various localities within India. However, this study is specifically aimed to resolve the identity of the specimens in the *Channa gachua* species-group collected from the northern Eastern Ghats, within a range of around 30 km to the north-east of Papikonda National Park.

DNA isolation, PCR and DNA sequence

DNA isolation followed basic methods after partial modification (Sambrook & Russell 2001; Laskar et al. 2018). Partial segment of the mitochondrial cytochrome oxidase C subunit I (COI) gene was amplified using the primer pairs FishF1-FishR1 (Ward et al. 2005). A total of 28 COI sequences for nine channid species from India were generated in this study. All the examined specimens were registered in the National Zoological Collections of Zoological Survey of India (Freshwater Biology Regional Centre), Hyderabad, and the COI sequences generated in this study were submitted to NCBI GenBank and BOLD. The accession numbers are given in the material examined section as well as in the phylogenetic tree and are marked by orange stars. We also retrieved COI sequences from NCBI, and BOLD. Representative sequences for 22 clearly defined channid taxa from India as referred in Conte-Grand et al. (2017) were retrieved from databases. Further, the sequences of a few recently described species whose accessions are referred by their original authors were retrieved from NCBI. The dataset of 157 COI sequences included an outgroup *Parachanna obscura* (MK074551). Genetic divergence analyses and the neighbor-joining phylogenetic tree visualization were performed in MEGA7.0 (Kumar et al. 2016). Bayesian inferences were drawn in Mr. Bayes (Ronquist & Huelsenbeck 2003) and the tree topology was developed in iTOL (Letunic & Bork 2007). We used the Kimura 2-parameter model, mostly applied in DNA barcoding studies (www.bold.org), to calculate the mean genetic distance between the groups. The study is limited by the lack of COI sequence of a few of the recent species, like, *C. brahmacharyi*, *C. pomanensis*. Further, we limit to discuss only the conspecific status of the COI sequences generated in the study, *C. harcourtbutleri* in particular.

Materials examined

1. *C. gachua* (Hamilton, 1822): FBRC/ZSI/F1979, 1, Himayat Sagar, Telangana, 17.35N & 78.42E, GenBank accession: KM272635; FBRC/ZSI/F/3317, 1, Small stream at Basavagu village, Andhra Pradesh, 17.70N 81.02E, GenBank accession: MT118102; FBRC/ZSI/F/3450, 6, Stream at Mothugudem, Andhra Pradesh, 17.80N & 81.64E, GenBank accessions: MW002473, MW002474, MW002475; FBRC/ZSI/F/2662, 1, Manjeera Dam, Telangana, 17.692N & 78.171E, GenBank accession: MH795975; FBRC/ZSI/F/3628, 1, Gubbagurthi near Wyara Lake, Telangana, 17.27N & 80.37E, GenBank accession: MW002494.

2. *C. harcourtbutleri* (Annandale, 1918): FBRC/ZSI/F/3393, 3, Papikonda National Park at G. M. Valasa Road, Andhra Pradesh, 17.59N & 81.68E, GenBank accession: MW002468; FBRC/ZSI/F/3615, 4, 62.0-83.0 mm SL, Stream at Mothugudem-Donkarayi Road, E. Godavari, Andhra Pradesh, 17.84N & 81.67E, GenBank accession: MW002479; FBRC/ZSI/F/3630, 2, 72.0–78.0 mm SL, River Pamuleru at Egavalasa village, Andhra Pradesh, 17.7N & 81.78E, GenBank accession: MW002470 (Image 1).

3. *C. kelaartii* Gunther,1868: FBRC/ZSI/F/3124, 13, Nilavoor Lake, Tamil Nadu, 12.56N & 78.64E, GenBank accessions: MT720842, MT720843, MT720844, MT720845, MT720846, MT720847; FBRC/ZSI/UN9604/DNA582, 1, Small pond at Valvanthinadu village, Namakkal District, Tamil Nadu, 11.280N & 78.364E, GenBank accession: MN685707.

4. *C. punctata* (Bloch, 1973): FBRC/ZSI/F/2405, 1, Kaddem Dam, Telangana, 9.026N & 76.385E, GenBank accession: MF601323; FBRC/ZSI/F/2717, 1, Maharashtra, 20.450N & 74.403E, GenBank accession: MH795988; FBRC/ZSI/F/3627, 1, Gubbagurthi near Wyara Lake, Telangana, 17.27N & 80.37E, GenBank accession: MW002493; FBRC/ZSI/F/3421, 1, Small stream near Basavagu Village, Andhra Pradesh, 17.70N & 81.02E, GenBank accession: MT654658.

5. *C. striata* (Bloch,1793): FBRC/ZSI/DNA357, 1, Namsai, Assam, 27.57N & 95.39E, GenBank accession: MK681748.

6. *C. marulius* (Hamilton, 1822): FBRC/ZSI/F2337, 1, Singur Dam, Telangana, 17.802N & 77.892E, GenBank accession: KY694512; FBRC/ZSI/F/2715, 1, Maharashtra, 20.450N & 74.403E, GenBank accession: MH795986;

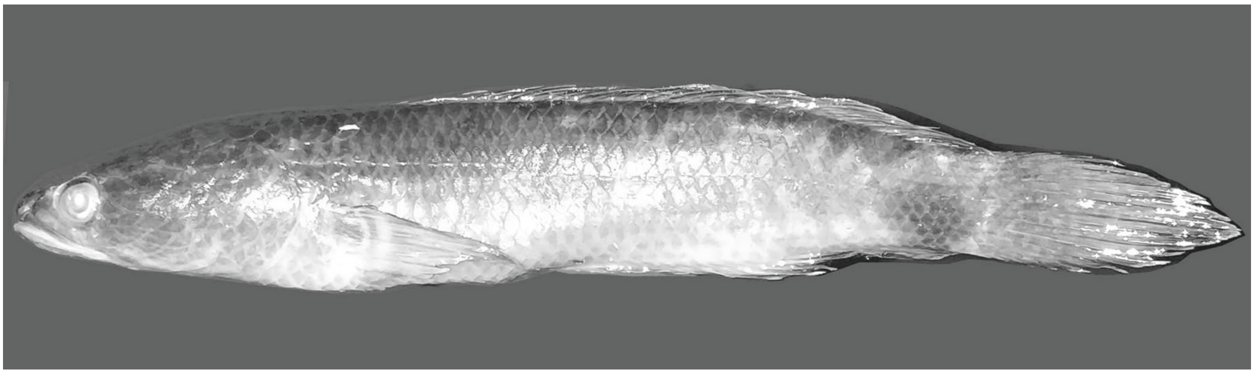

Image 1. ***Channa harcourtbutleri*** **collected from River Pamuleru, Andhra Pradesh (preserved specimen, FBRC/ZSI/F/3630). © Boni Amin Laskar.**

Table 1. Estimated genetic divergence (% K2P) between the studied groups in the genus *Channa*. The analysis reveals a low K2P genetic divergence among the specimens of *C. royi* Andaman Islands, *C. harcourtbutleri* Myanmar and *C. harcourtbutleri* India, suggesting their conspecific status.

Grouped taxa	Within-group (K2P %)	Between-group (K2P %)																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1. *C. harcourtbutleri* Myanmar	0.013																								
2. *C. harcourtbutleri* India	0.002	2.3																							
3. *C. royi* Andaman Islands	0.003	2.3	1.4																						
4. *C. gachua* Godavari	0.002	17.3	17.4	16.7																					
5. *C. gachua* Topotypic	0.006	19.3	19.5	19.3	3.2																				
6. *C. andrao*	0.000	16.0	14.7	15.7	18.6	19.5																			
7. *C. aurantimaculata*	0.001	12.7	13.4	13.1	15.4	16.9	15.7																		
8. *C. aurantipectoralis*	0.001	16.1	16.0	16.9	17.2	16.9	18.3	14.9																	
9. *C. barca*	0.000	16.9	16.3	17.5	15.2	16.5	15.4	8.8	15.4																
10. *C. bipuli*	n/c	17.7	17.8	18.8	15.3	18.2	16.1	12.3	18.2	12.2															
11. *C. bleheri*	0.001	17.4	16.8	16.8	15.4	16.9	16.7	14.8	17.0	14.0	12.4														
12. *C. brunnea*	0.000	16.1	16.1	16.4	16.4	17.2	17.2	14.5	17.5	14.9	12.3	9.7													
13. *C. diplogramma*	0.004	22.8	23.3	24.1	22.7	22.0	22.3	22.0	23.0	23.1	25.8	23.9	25.0												
14. *C. kelaartii*	0.007	16.3	15.5	15.8	10.5	9.9	19.6	16.3	16.4	17.7	16.5	16.2	16.5	22.1											
15. *C. lipor*	n/c	15.1	15.6	15.6	16.0	14.4	17.2	14.5	11.9	15.7	21.4	16.7	17.7	22.6	15.5										
16. *C. marulius*	0.004	24.6	24.3	24.7	21.0	24.7	19.5	21.1	21.1	20.3	18.6	18.4	21.7	19.1	21.2	23.3									
17. *C. melanostigma*	0.008	14.5	15.5	15.4	16.3	17.8	14.6	6.3	18.8	10.3	11.7	14.7	13.4	23.6	15.5	15.7	21.4								
18. *C. pardalis*	0.002	16.2	16.5	17.2	14.8	16.2	14.7	11.2	16.4	10.9	5.8	12.0	12.2	23.1	13.8	17.2	18.2	12.9							
19. *C. pseudomarulius*	0.000	23.3	22.2	23.1	22.8	25.0	18.7	19.8	20.5	19.8	19.2	19.2	23.1	16.9	20.7	24.2	4.3	21.0	18.0						
20. *C. punctata*	0.015	21.4	20.6	19.9	19.3	19.9	19.1	19.0	20.1	20.0	17.6	18.2	20.3	19.6	17.9	21.5	16.1	16.8	19.5	15.4					
21. *C. quinquefasciata*	0.008	14.9	14.3	15.3	16.8	17.2	15.9	12.6	16.0	13.3	15.0	15.7	14.4	22.2	15.7	15.4	19.2	12.3	13.7	19.1	19.3				
22. *C. rara*	0.002	16.1	16.6	15.7	9.4	10.8	19.2	16.3	15.6	18.0	18.8	17.3	18.3	22.1	8.8	14.5	25.4	17.1	15.2	25.0	19.7	18.4			
23. *C. stewartii*	0.005	17.1	16.6	17.7	17.9	19.0	16.6	12.6	15.8	13.8	15.8	16.4	12.8	21.6	15.7	16.3	20.7	12.3	12.2	19.3	19.1	8.3	19.1		
24. *C. stiktos*	0.013	23.6	22.3	22.2	21.3	21.2	22.8	21.3	20.2	19.6	18.7	17.7	19.9	24.2	20.1	20.7	21.4	20.6	18.6	22.5	23.0	21.3	22.1	18.4	
25. *C. striata*	0.001	21.1	19.8	18.4	22.0	23.2	19.3	21.0	24.0	21.2	17.0	22.2	23.8	20.3	20.6	23.0	18.1	18.7	19.4	17.5	16.7	21.7	22.1	22.0	23.7

FBRC/ZSI/DNA267, 1, Tungabhadra River, Andhra Pradesh, 16.169N & 77.934E, GenBank accession: MK336898.

7. *C. bleheri* Vierke, 1991: FBRC ZSI DNA354, 1, Tinsukia, Assam, 27.57N & 95.39E, GenBank accession: MK632315.
8. *C. aurantimaculata* Musikasinthorn, 2000: FBRC/ZSI/DNA359, 1, Tinsukia, Assam, 27.57N & 95.39E, GenBank accession: MK632318.
9. *C. stewartii* (Playfair, 1867): FBRC/ZSI/DNA356, 1, Tinsukia, Assam, 27.57N & 95.39E, GenBank accession: MK632316.

RESULTS

The phylogenetic tree shows distinctive cladding of the Asian channid taxa with reference to the African *Parachanna* used herein to root as out-group. The generated sequences of the *C. gachua* species-group from the Yelagiri Hills in Tamil Nadu form a distinct clade that comprises of a few database sequences that were referred in a recent study as *C. kelaartii*. Thus, this study contributes further specimens of *C. kelaartii* from the southern Eastern Ghats. The sequences of the *C. gachua* like specimens collected from near to the type locality of *Ophicephalus marginatus* and various other localities in the Godavari River basin are nested as a subclade to the topotypic *C. gachua*. Our generated sequences of other congeners like *C. marulius*, *C. punctata*, *C. stewartii*, *C. bleheri*, *C. aurantimaculata*, and *C. striata* show distinct cladding and each comprises of conspecific database sequences wherever available. The sequence of *C. pomanensis* (referred in Praveenraj et al. 2019) was found to be cohesively claded with a sequence of *C. melanostigma*. Hence, in the absence of further specimens for both the taxa, the identity of the two sequences is not confirmed and remained beyond the limit of this study. Our sequences of *C. stewartii* are nested in the 65th clade of *C. stewartii* of Conte-Grand et al. (2017) (BIN:AAF3764) while status of the taxonomic assignment of the 64th clade of *C. stewartii* of Conte-Grand et al. (2017) (BIN:AAF3772) is also beyond the limit of this study. However, the generated sequences of the specimens in *C. gachua* species-group from near the Papikonda National Park, East Godavari District, in the Eastern Ghats, are cohesively claded with the two database sequences from southern India of Conte-Grand et al. (2017) (MF462283 BOLD:ADL6569, MF462269) and formed a sub-clade to the species *C. harcourtbutleri* from Myanmar (BIN:AAC3926) suggesting their close genetic similarity with the latter, hence referred hereafter as *C.*

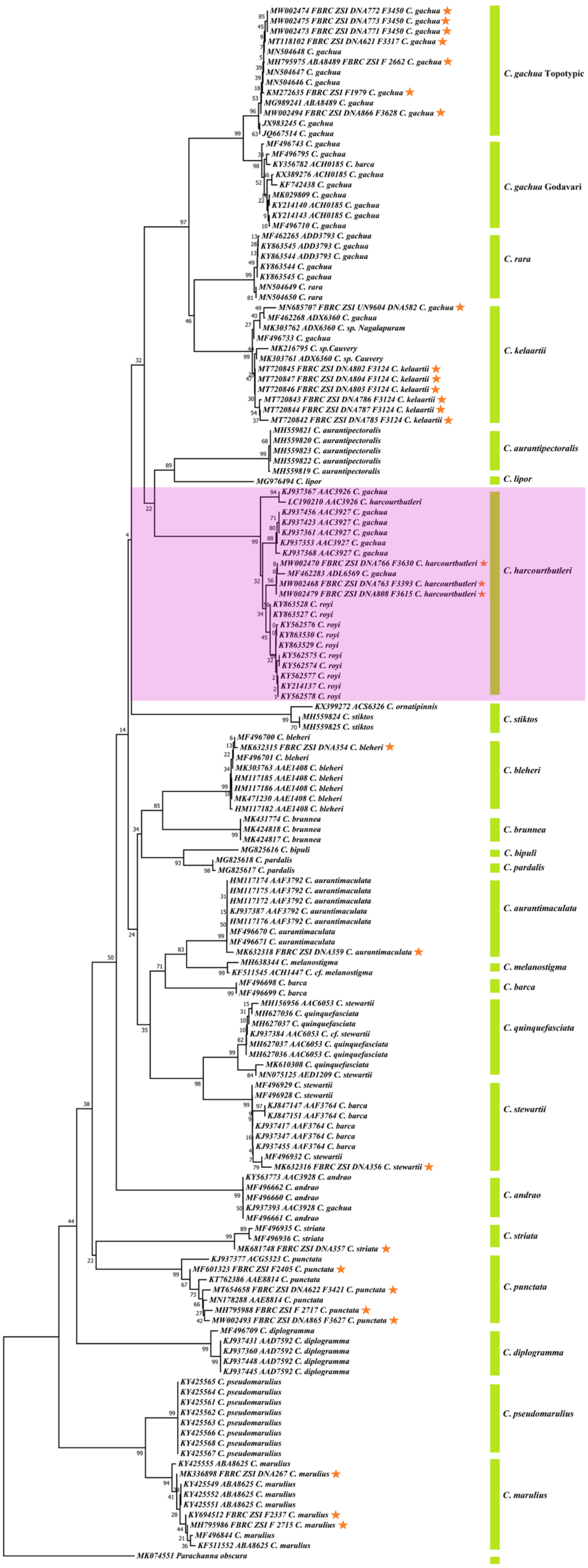

Figure 1. Neighbour-Joining phylogeny based on K2P divergence on partial mtCOI gene of the Channid taxa In India. The cohesive cladding (pink shape in figure) of the sequences of *C. harcourtbutleri* Myanmar, *C. royi* Andaman Islands, and *C. harcourtbutleri* India suggest their conspecific status due to low genetic divergence.

harcourtbutleri India.

The present dataset is comprised of representative sequences from the clearly defined taxa. However, in order to estimate the range of intra-species genetic divergence, the sequences are grouped based on the clustering inferred from neighbor-joining phylogeny (Figure 1). Sequences in the cluster with topotypic *C. gachua* (referred in Britz et al. 2019) are named as *C. gachua* Topotypic. The sequences of *C. gachua* like specimens from near to the type locality of *O. marginatus* are named as *C. gachua* Godavari. Similarly, the sequences of *C. royi* from Andaman Islands (Praveenraj et al. 2019) are tentatively named as *C. royi*, sequences in the cluster with *C. harcourtbutleri* BIN:AAC3926 from Myanmar (Conte-Grand et al. 2017) are named as *C. harcourtbutleri* Myanmar, and the sequences of *C. gachua* species-group from near the Papikonda National Park, East Godavari District, along with other database sequences in the same cluster are named as *C. harcourtbutleri* India. The overall genetic divergence between groups in the dataset is lying in the range from 1.4–25.8 % (Table 1). The divergence matrix revealed that the sequences of *C. gachua* Godavari are genetically diverged by 3.2% K2P distance from *C. gachua* Topotypic, and maintained 9.4–22.8 % K2P distance from all the congeners. *C. pseudomarulius* is diverged by 4.3% K2P from *C. marulius*, *C. pardalis* is diverged by 5.8% from *C. bipuli*, *C. melanostigma* by 6.3% from *C. aurantimaculata*, and so on. Similarly, the divergence matrix revealed that the sequences of *C. harcourtbutleri* India are genetically diverged by 1.4% K2P distance from *C. harcourtbutleri* Myanmar (BIN: AAC3926), 2.3% K2P distance from *C. royi* Andaman Islands, and maintained 13.4–24.3 % K2P distance from all the congeners.

DISCUSSION

Conte-Grand et al. (2017) recovered a total number of 90 BINs in their dataset having a total number of 38 valid species at time, and inferred higher species diversity in snakeheads. However, they neither included any COI sequence data of *C. limbata* in the phylogenetic analysis nor assigned any BIN for the species. As of now, a search for BINs with the name '*Channa*' in BOLD yielded a record of 93 BINs. Conte-Grand et al. (2017) had an extensive dataset covering various geographical areas and populations. In fact, the representative sequences for almost all the species described or validated after 2017 were present either as an unnamed clade (potential new BIN in Conte-Grand et al. 2017) or has been assigned with BOLD BIN.

The taxonomic history of the snakehead fishes finds two descriptions, *O. marginatus* and *O. coramota*, with their type locality in Vizagapattam (=Visakhapatnam, Andhra Pradesh), part of the Eastern Ghats region. However, both these species have been synonymized with *C. gachua* (Roberts 1993; Ng et al. 1999; Courtenay & Williams 2004; Kottelat 2013). In fact, Britz et al. (2019) examined specimens of topotypic *O. marginatus*, and found a very similar colour pattern as well as a very little genetic difference with topotypic *C. gachua* (2.4% uncorrected p-distance). Britz et al. (2019) pointed out several flaws in the description of *C. shingon* by Endruweit (2017) and provided various valid reasons to consider *C. shingon* as a junior synonym of *C. harcourtbutleri*. Britz et al. (2019) was also not convinced enough by the morphological descriptions to consider *C. royi* as a distinct species from *C. harcourtbutleri*, and a very low genetic distance of 2.4–2.8 % uncorrected p-distance was stated to be in the range of intra-species variation, and therefore considered Andaman *C. royi* as a junior synonym of *C. harcourtbutleri*. However, the distribution limit of *C. harcourtbutleri* and *C. limbata* is not yet clear. Conte-grand et al. (2017) mentioned an unexpected placement of two specimens from southern peninsular India in the middle of the eastern lineage of the *C. gachua* species-complex. The same statement was repeated in Ruber et al. (2019). Conte-grand et al. (2017) showed that the two specimens from southern peninsular India (one from Chunchi falls, Cauvery River, 12.351N & 77.443E; and the other from Kali River, 15.381N & 74.403E) were included in a Putative BIN:ACM5826 new that claded away from the BIN of *C. harcourtbutleri* (AAC3926).

Following the previous studies, it may be figured out that the nominal species in the genus *Channa* with fewer or no morphological differences can have intra-species genetic divergence as high as 2.2–2.4 %, and the nominal taxa falling within such range of genetic divergence could be considered as a single species. Therefore, a clear understanding of the range of intra-species genetic divergence would be helpful in taxonomic assignment of the channid taxa. Based on the analysis of COI barcode sequences, we confirm that the specimens of *C. gachua* species-group from East Godavari District, Eastern Ghats, India, along with the sequences from southern India, are actually a single species which may be named as *C. harcourtbutleri* because of low genetic divergence with the conspecific sequences from Myanmar. Thus, this study claims the presence of *C. harcourtbutleri* in the wild in Eastern Ghats region, in mainland India. Nonetheless, *C. harcourtbutleri* has already been recorded to be distributed

Image 2. *Channa harcourtbutleri*, image taken in the field immediately after collection. © Boni Amin Laskar.

in Andaman Islands through the synonymization of *C. royi* with *C. harcourtbutleri*. Hence, this study reports for the first time the occurrence of *C. harcourtbutleri* in mainland India (Image 2). Unexpectedly, no specimens from northeastern India are available to place in the clade of *C. harcourtbutleri*. In this background, the distribution of *C. harcourtbutleri* is appearing disjunct.

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Communications

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