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Cover: Emperor Tamarin *Saguinus imperator*: a look into a better world through the mustache lens – mixed media illustration. © Maya Santhanakrishnan.

INTRODUCTION

Nepal's fish taxonomy is in its early stages of investigation. According to Kottelat & Whitten (1996), Nepal is one of the Asian countries where some fish data are accessible, but their quality and geographical gaps necessitate substantial field investigation. The cyprinid genus *Garra* is a distinct group of bottom-dwelling fish found in fast-moving waters. They cling to the rocks using a small sucking disc on their lower lip (Zhou et al. 2015). In swift-moving water, the modified lower lip known as the suctorial disc is used to cling to rocks and pebbles (Rath et al. 2019). Currently, the genus *Garra* contains approximately 180 recognized species that range from Borneo, China, and southern Asia via the Middle East, Arabian Peninsula, and eastern to western Africa (Fricke et al. 2023).

During the ichthyofaunal survey, conducted from 02 September 2022 to 21 March 2023 in Lohandra River, four individuals of *Garra* species were collected which were not identifiable to the species level at the field. After close observation of morphometric and meristic characters and analyzing the mitochondrial cytochrome c oxidase subunit I (COI) gene sequences, those four specimens were confirmed to belong to *Garra kempfi*. The occurrence of *G. kempfi* from the Lohandra River in eastern Nepal is a new record for Nepal.

MATERIALS AND METHODS

Study area

The Lohandra River is one of the tributaries of the Koshi River system, flowing from the Letang Municipality in Morang District of southeastern Nepal, located between the Mahabharat hills and the Churia hills. The Lohandra River (Figure 1) is one of the most important water sources for irrigation and agriculture in Morang District. The study area is bordered by Warangi to the north, Biratnagar to the west, India to the south, and Rangeli to the east. The Lohandra River basin has a subtropical climate with an average yearly temperature of 30.9°C (Khanal 2015; Limbu et al. 2023). The riverbed predominantly consists of sand, gravel, cobble, and pebble whereas bamboo and bushes are the dominant vegetation.

Sampling, preservation, and measurements

Fish were collected from Lohandra River by using cast net, and local fishing gears (Dhadiya, Ghorlang, and Mosquito net) from 02 September 2022 to 21 March

2023. Collected specimens were preserved in 10% formalin for morphological examination by making their head upright to protect their caudal fin. For molecular study, the caudal fins of two individuals were preserved in 95% ethanol in the field and then transferred to 75% ethanol. All specimens were assigned a collection number to facilitate sample tracking. Voucher specimens were deposited at the Museum of the Central Department of Zoology, Tribhuvan University, Kirtipur Kathmandu, Nepal. Morphological measurements and meristic records were done according to Ng & Edds (2005). A digital Vernier caliper was used for point-to-point measurement, and data was recorded from the specimen's left side to the nearest tenth of a millimeter. Furthermore, water temperature, depth, velocity, and pH were also measured for the sampling locality.

DNA extraction, PCR, and sequencing

Total genomic DNA was extracted from the caudal fin using the Tiangen genomic DNA purification kit (Tiangen Biotech, Beijing, China). A partial fragment (~665 bp) of cytochrome c oxidase subunit I (COI) was amplified with the forward primer 5'-CGCTGATTCTTCTCTACCAAYCAYAAAGA-3' and the reverse primer 5'-ACTTCTGGGTGGCCGAAGAAYCARAA-3'. The PCR was performed in a 20 µl reaction volume containing 10 µl Taq Master Mix (Vazyme), 6 µl deionized nuclease-free water, 2 µl DNA, and 1 µl of each primer. The PCR protocol included 35 cycles with the following steps: initialization at 95°C for 2 mins, denaturation at 95°C for 30 s, annealing at 55°C for 45 s, elongation at 72°C for 45 s, and final elongation at 72°C for 5 mins. The amplified products were checked on a 1% agarose gel before sequencing. Successful amplicons were sequenced in both directions using the same primers and a BigDye Terminator Cycle Kit v.3.1 (Invitrogen) on an ABI 3730XL sequencer (Applied Biosystems).

Data analysis

The resulting sequences from the primer pairs were assembled using Geneious Prime 9.0.2 (<https://www.geneious.com>) software and aligned using MEGA 11 (Tamura et al. 2021). The noisy sequences of both ends were trimmed before subsequent sequence analysis. The aligned sequences were submitted to the GenBank database (GenBank accession numbers: OR889731, OR898805). The sequences of *G. kempfi* from Nepal were used to search homologous sequences of congeners from the GenBank database using the MegaBlast tool. The homologous sequences from other *Garra* species were

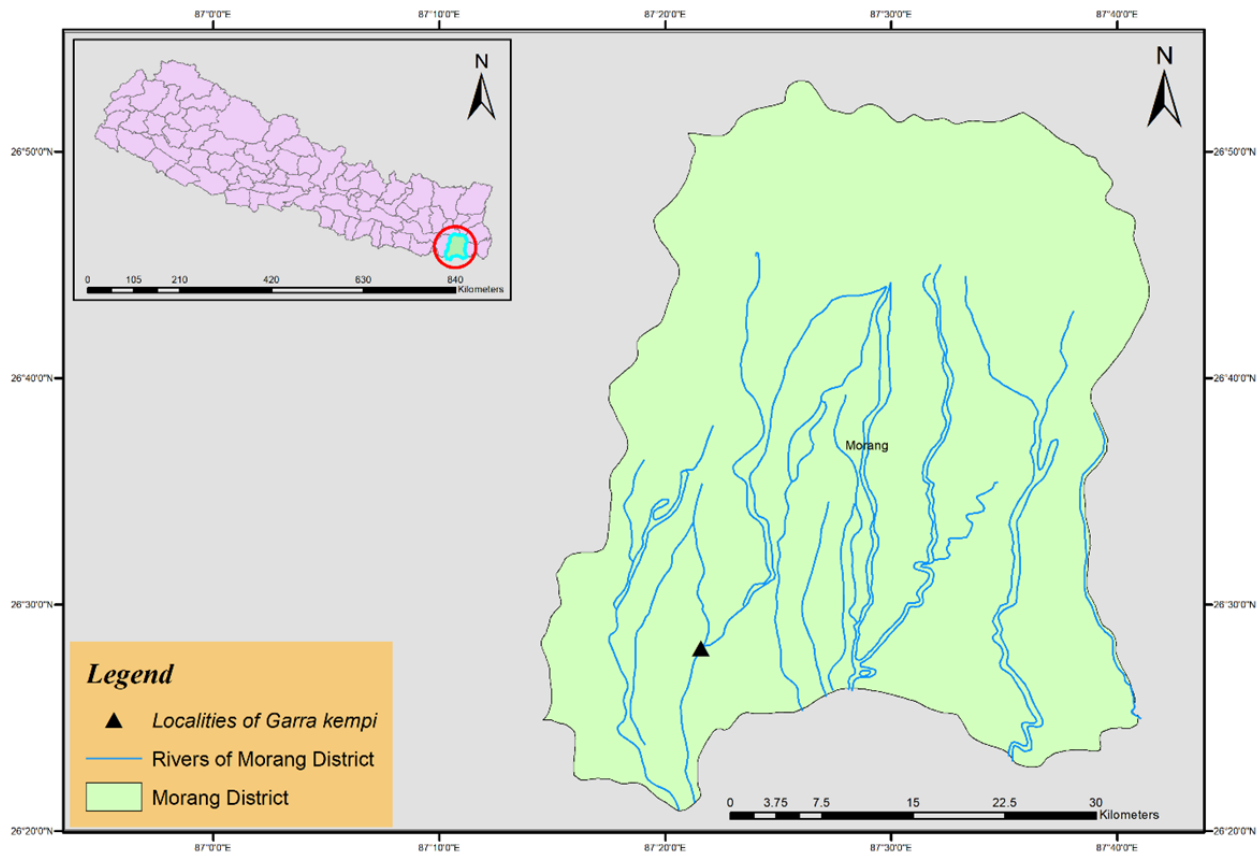


Figure 1. Map showing the locality of *Garra kempfi* sampling in eastern lowland of Nepal.

downloaded and aligned using the ClustalW algorithm in MEGA 11 (Tamura et al. 2021). The COI sequence of *Anguilla bengalensis* was used as an outgroup for the phylogenetic tree construction. The final alignment of 665 bp was used for the phylogenetic analysis.

The maximum likelihood (ML) phylogenetic tree was constructed using the RAxML-HPC Blackbox 8.2.12 tool (Stamatakis 2014) in CIPRES Science Gateway V 3.3 (Miller et al. 2010) online platform (<https://www.phylo.org/>) with GTR+G evolutionary model and 1,000 bootstrap replicates. The resulting tree was visualized in FigTree v. 1.4.4 (Rambaut 2018).

Pairwise evolutionary distances among the *Garra* species in 665 bp long COI gene sequences were calculated using MEGA 11 (Tamura et al. 2021) using the Kimura-2 parameter (K2P) (Kimura 1980).

RESULTS

Materials examined: LR1001, LR1002; 68–71.5 mm, Lohandra River, Nepal, 6 km from Biratnagar Sub-metropolitan City, 26.54611°N & 86.9383°E, 70 m;

Limbu & Rajbanshi, 02 September 2023.

Diagnosis: The photographs and morphometric data are given in image 1 and table 1. The snout of *G. kempfi* is rounded, the proboscis is absent, lateral surface of the snout in front of the nostrils is slightly raised from the general surface. Scales are absent on the chest and sparsely present along the midline of the belly. The pelvic fin is not reaching the anal origin, and 40–42 lateral line scales are present. Eight rows of scales are present between the bases of the dorsal and ventral fins. It has 7–8 branched dorsal fin rays. The pectoral fin has 12 branched fin rays whereas the pelvic fin has eight branched rays. The anal fin contains five branched fin rays. The caudal fin contains 10+9 principle fin rays. There are 40–42 lateral line scales whereas 11–12 predorsal and 15–16 circumpenducular scales.

Coloration: Dorsal and lateral surface body of this species is black, while the ventral part is dull white.

Dorsal and caudal fins are grayish. The dorsal part of paired fins is grayish whereas ventrally dull.

Distribution: Recorded from the Lohandra River in the eastern lowland.

Remarks: All specimens were collected from the

Table 1. Morphometric data of *Garra kempfi*. N = number of specimens.

Characters	<i>Garra kempfi</i> (n = 2)
Standard length (mm)	68–71.5
Percent of standard length (% SL)	
Head length	26.4–27.6
Body depth at dorsal-fin origin	20–23.3
Predorsal length	46.5–48.1
Preanus length	65.9–67.8
Prepectoral length	19.9–20.5
Prepelvic length	49.7–50.9
Dorsal fin base length	15.9–18.1
Dorsal fin length	21.7–23.8
Pectoral fin length	18.5–20.1
Pelvic fin length	17.9–19.2
Anal fin base length	5.8–6.7
Anal fin length	17.5–18.7
Caudal peduncle length	15.7–17.1
Percent of head length (% HL)	
Head depth	67.3–68.6
Snout length	52.4–53.8
Eye diameter	20.8–21.5
Interorbital space	42.1–43.7

fast-flowing water of the Lohandra River with rocky substratum. Water temperature of 24 °C, water velocity of 0.9 m/sec, water depth of 0.5 m, and pH 8.5 was recorded during the ichthyological survey. Altitude ranges 80–90 m. This species is also reported from Tibet (China) and India (Arunachal Pradesh, Manipur, Meghalaya, Mizoram, and Nagaland).

Economic importance: This fish is a prominent local food fish.

Phylogenetic analysis: The phylogenetic relationship of the *G. kempfi* from Nepal was assessed with other 19 known species under the genus using cytochrome c oxidase subunit I (COI) sequences. The ML tree with a strong bootstrap support revealed that the recently discovered Nepalese *Garra* species forms a monophyletic clade with *Garra kempfi* (Accession Number OL440722.1) from India (Figure 2). The Nepalese *G. kempfi* had a genetic distance of just 1.8% with the *G. kempfi* from India, 4.1% with *G. fluviatilis*, 7.4% with *G. spilota*, 9.0% with *G. yajiangensis*, 9.3% with *G. gotyla* and 9.6% with *G. qiaojiens* (Table 2). The GC content of the COI sequences of *G. kempfi* in Lohandra River is 26.9% and 15.7%, respectively, which is nearly identical

**Image 1 . *Garra kempfi*: A—lateral view | B—dorsal view | C—ventral view. © Jash H. Limbu.**

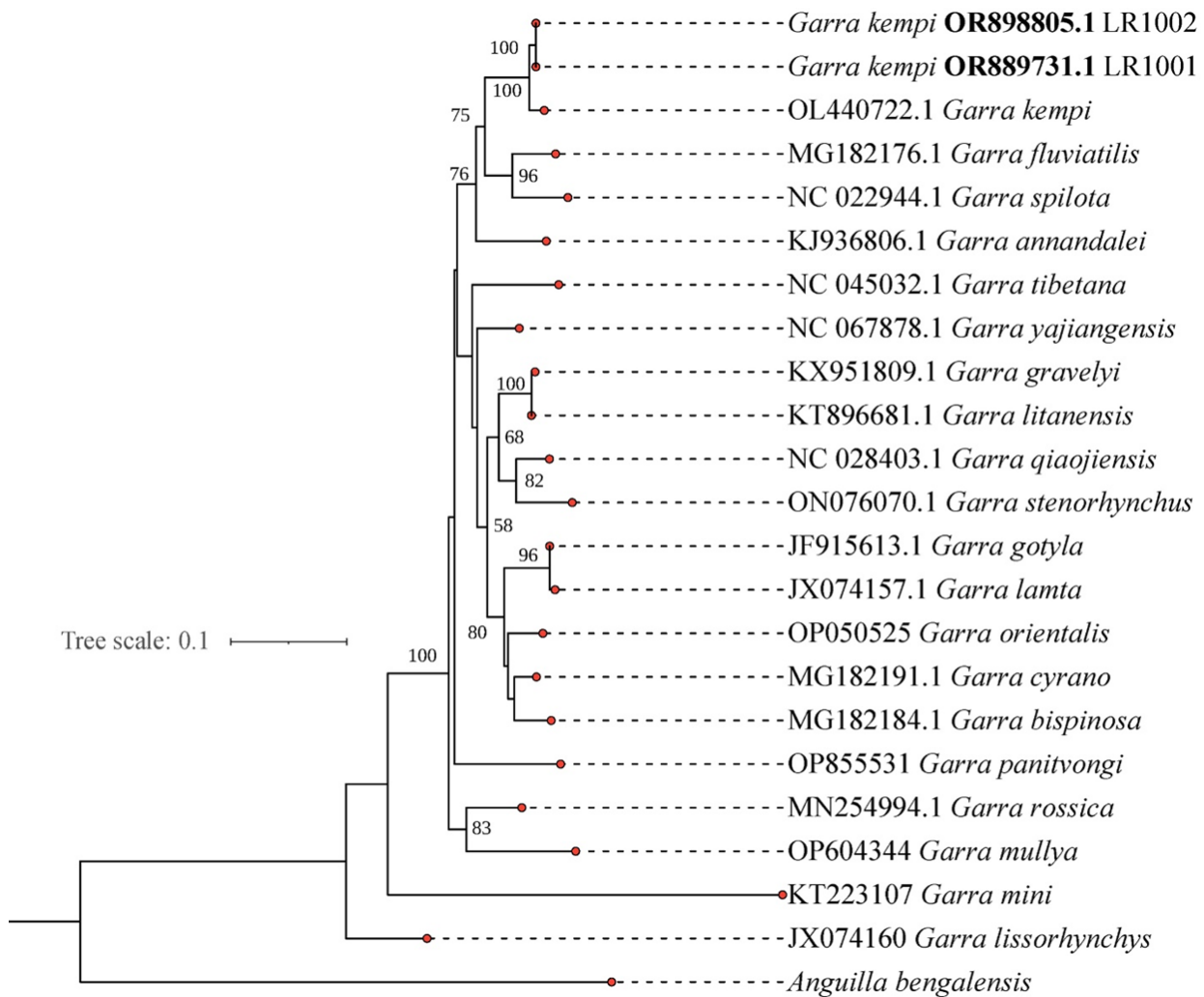


Figure 2. Maximum-likelihood (ML) tree of newly recorded *Garra kempfi* from Lohandra River in the eastern lowland of Nepal (GenBank accession numbers: OR889731.1, OR898805.1; sample tracking numbers: LR1001, LR1002) and other species of the *Garra*, based on sequences (665 bp) covering the partial barcoding region of cytochrome c oxidase subunit I (COI) gene.

to previously deposited sequencing data from China and India (26.5% & 15.7%).

DISCUSSION

This study reports the first reliable record of *Garra kempfi* in Nepal. Previously, *G. kempfi* was only known from swift-moving mountain streams in northern India and Tibet (China) (Menon 1999; Nebeshwar et al. 2009). Since this species has not been documented at an elevation below 100 m, this finding is quite intriguing. Specimens were collected from fast-flowing water (0.9 m/sec) with a rocky substrate, a water temperature of 24°C, a depth of 0.5 m, and a pH of 8.5. The cyprinid genus *Garra*, typically found in swiftly moving waters,

is known for bottom-dwelling fish that adhere to rocks using a tiny sucking disc on their lower lip (Zhou et al. 2015). Mitochondrial cytochrome c oxidase subunit I sequences of *G. kempfi*, along with those of 19 other species in the genus, were analyzed to validate the new sequences and annotations. Molecular phylogenetic analysis revealed a clearly defined monophyletic clade that included *G. kempfi* from Nepal and northeastern India. The rivers in eastern Nepal and northeastern India share similar climatic conditions and eventually merge to form large rivers that drain into the Indian Ocean, likely facilitating the dispersal of *G. kempfi* into Nepalese rivers. Besides the Lohandra River, this species might inhabit other fast-flowing water bodies of Himalayan origin. Therefore, a detailed ichthyofaunal survey is essential in Nepal to properly document its fish diversity.

Table 2. Pairwise genetic distances among the species of the genus *Garra* based on COI sequences (665 bp) and Kimura 2 parameter computed in MEGA 11 (genetic distance below diagonal, standard error above diagonal).

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<i>G. kempī</i> Nepal		0.006	0.009	0.011	0.013	0.014	0.013	0.013	0.013	0.014	0.014	0.014	0.014	0.014	0.013	0.014	0.015	0.016	0.017	0.019
<i>G. kempī</i>	0.018		0.010		0.013	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.014	0.016	0.016	0.015	0.016	0.016	0.018	0.020
<i>G. fluviatilis</i>	0.041	0.052		0.009	0.010	0.010	0.010	0.010	0.010	0.010	0.011	0.010	0.011	0.012	0.012	0.011	0.011	0.012	0.012	0.016
<i>G. spilota</i>	0.074	0.080	0.044		0.014	0.014	0.015	0.014	0.014	0.014	0.013	0.014	0.014	0.015	0.014	0.017	0.013	0.015	0.015	0.019
<i>G. yajiangensis</i>	0.090	0.089	0.048	0.109		0.011	0.011	0.011	0.011	0.011	0.013	0.012	0.013	0.012	0.012	0.012	0.013	0.015	0.016	0.019
<i>G. qiaojians</i>	0.096	0.104	0.059	0.116	0.071		0.011	0.010	0.011	0.011	0.014	0.011	0.012	0.011	0.013	0.011	0.015	0.017	0.017	0.020
<i>G. gravellyi</i>	0.090	0.104	0.052	0.114	0.065	0.062		0.002	0.011	0.011	0.015	0.011	0.014	0.011	0.011	0.012	0.015	0.014	0.016	0.020
<i>G. litanensis</i>	0.090	0.104	0.048	0.110	0.065	0.059	0.003		0.010	0.011	0.014	0.010	0.014	0.011	0.011	0.012	0.014	0.014	0.015	0.020
<i>G. gotyla</i>	0.093	0.103	0.052	0.103	0.073	0.074	0.070	0.068		0.003	0.014	0.010	0.014	0.011	0.011	0.014	0.013	0.015	0.017	0.019
<i>G. lamta</i>	0.095	0.105	0.054	0.104	0.075	0.077	0.072	0.070	0.005		0.014	0.010	0.014	0.011	0.011	0.014	0.014	0.016	0.017	0.019
<i>G. rossica</i>	0.096	0.098	0.059	0.096	0.086	0.106	0.107	0.103	0.105	0.102		0.013	0.015	0.013	0.013	0.016	0.015	0.014	0.016	0.020
<i>G. cyrano</i>	0.099	0.112	0.057	0.113	0.076	0.074	0.076	0.072	0.055	0.060	0.097		0.013	0.009	0.009	0.013	0.013	0.015	0.015	0.019
<i>G. tibetana</i>	0.099	0.098	0.065	0.111	0.093	0.085	0.096	0.096	0.102	0.102	0.114	0.092		0.014	0.014	0.014	0.016	0.015	0.016	0.019
<i>G. orientalis</i>	0.102	0.112	0.070	0.120	0.074	0.071	0.069	0.069	0.064	0.069	0.100	0.043	0.094		0.009	0.014	0.014	0.016	0.017	0.021
<i>G. bispinosa</i>	0.103	0.120	0.066	0.107	0.083	0.083	0.072	0.074	0.064	0.065	0.093	0.045	0.097	0.050		0.014	0.014	0.015	0.016	0.019
<i>G. stenorhynchus</i>	0.105	0.112	0.063	0.138	0.079	0.064	0.072	0.072	0.098	0.102	0.114	0.092	0.106	0.093	0.097		0.016	0.016	0.016	0.020
<i>G. panitvongi</i>	0.110	0.115	0.074	0.110	0.099	0.112	0.113	0.113	0.101	0.106	0.114	0.095	0.122	0.104	0.103	0.126		0.017	0.018	0.020
<i>G. mullya</i>	0.123	0.121	0.075	0.124	0.111	0.127	0.106	0.102	0.126	0.132	0.101	0.125	0.119	0.126	0.125	0.125	0.137		0.017	0.022
<i>G. lissorhynchus</i>	0.148	0.159	0.085	0.139	0.138	0.135	0.126	0.123	0.142	0.143	0.128	0.135	0.143	0.147	0.146	0.141	0.155	0.146		0.020
<i>G. mini</i>	0.179	0.179	0.127	0.178	0.181	0.192	0.199	0.196	0.193	0.193	0.178	0.183	0.173	0.204	0.187	0.193	0.192	0.206	0.188	

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