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* Corresponding author.

lr_zoo@yahoo.co.in

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Studies on the Genus *Garra* (Teleostei: Cyprinidae) from the Kaladan Drainage of Mizoram, India, and Synonymization of *G. tyao* with *G. rakhinica*

Mual Chin Zirkunga¹, Mahender Singh², Lalramliana^{3*}

¹ Department of Life Sciences, Pachhunga University College, Aizawl, Mizoram, India

² Molecular Biology and Biotechnology Division, ICAR-National Bureau of Fish Genetic Resources, Uttar Pradesh, Lucknow, Uttar Pradesh, India

³ Department of Zoology, Pachhunga University College, Aizawl, Mizoram, India

Abstract

Objectives: An integrative taxonomical approach to study the genus *Garra* from the Kaladan drainage was undertaken to evaluate the morphological characteristics as well as the phylogenetic relationship of the species. **Methods:** Fish samples were collected using nets and employing local fishermen. Tissues were collected preferably from the right side of the body. Whole specimens were preserved in 10% formalin or 90-95% ethanol. Genomic DNA was extracted and selected genes markers were quantified. Partial mitochondrial cytochrome c oxidase subunit I (COI) was used as the selected marker. **Findings:** A total of 152 specimens were collected which accounts for 8 species of the genus *Garra*. Based on the study, it was also concluded that *Garra tyao* is a junior synonym of *Garra rakhinica*. **Novelty:** This research is primarily to investigate the genus *Garra* from the Kaladan drainage and to provide a systematic account as well as to establish the phylogenetic lineage of the species from its congeners.

Keywords: Fishes of Mizoram; Integrative Taxonomy; Mitochondrial COI; Phylogeny; *Garra*

1 Introduction

Species of the genus *Garra* (Teleostei: Labeoninae: Cyprinidae) are mostly benthic fishes found in rapid waters such as riffles, torrents and waterfalls⁽¹⁾. Fishes of this genus are characterized by a streamlined sub-cylindrical body, a paired horizontal fin and a peculiar ventrally located suctorial disc^(2,3). The suctorial disc is a modified lower lip that is used for adhering to rocks and pebbles in rapid waters⁽⁴⁾. Menon reviewed the genus and recognize 37 species that are divided into 4 groups consisting of 9 complexes⁽⁵⁾. Currently, there are around 180 valid species of the genus *Garra* that are widely distributed from Borneo, China and Southern Asia through the Middle East, Arabian Peninsula and East to West Africa⁽⁶⁻⁸⁾. There are 51 species that are widely

distributed in the Indian waters and the northeastern regions of India alone harbors around 38 species of *Garra*^(9,10). Among the species of *Garra*, the snout morphology as well as distribution and pattern of the tubercles plays a significant taxonomical role in differentiating between species⁽¹¹⁾. Based on the snout morphology, species of the genus *Garra* can be classified as: (i) smooth snout species (ii) transverse lobe bearing species (iii) proboscis bearing species (iv) rostral flap bearing species and (v) rostral lobe bearing species⁽¹²⁾.

Ichthyological surveys conducted on the Kaladan river and its tributaries resulted in the collection of several specimens of *Garra*. Morphological and molecular analysis was carried out for the collected samples. The present study discusses the phylogenetic relationships among the genus *Garra* from the Kaladan drainage. It also provides species accounts and keys for the identification of species of *Garra* from the Kaladan drainage and its tributaries, the Tiau river and Cheu River.

2 Methodology

2.1 Study and Sampling area

The Kaladan drainage (Koladyne) originates from the western regions of the Chin Hills in Myanmar and flows through the border of eastern Mizoram where it forms an international border between India and Myanmar. The Tiau river which also forms the international border between Myanmar and India joins the Kaladan at $22^{\circ}47'10''\text{N}$ $93^{\circ}05'44''\text{E}$ where it flows through Mizoram and eventually drains into the Bay of Bengal at Sittwe, Myanmar. Other tributaries of the Kaladan include the Mat River, Khawchhak Tuipui and Tuichang River. Another tributary of the Kaladan, the Cheu River, is a small stream originating from the foothills of Phawngpui Mountain. Samples were collected from Kaladan and its tributaries using fishing nets, drag nets and also by employing local fishermen. Upon capturing the fish, tissue samples were collected from either fin clips or muscle tissues. Whole specimens were preserved in 90-95% ethanol or 10% formalin. Figure 1 represents the map of Mizoram showing only the Kaladan drainage with its tributaries.

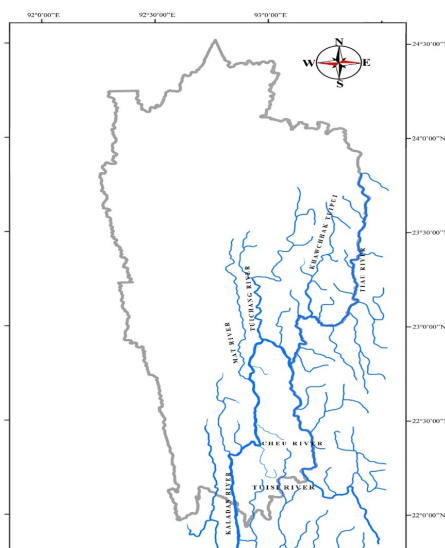


Fig 1. Map of Mizoram showing the Kaladan drainage with its tributaries

2.2 Morphological analysis

Specimens were measured using digital calipers rounded to the nearest 0.2 millimetres. A stereo zoom microscope (OLYMPUS SZX10) was used for counting the number of scales and fin rays. Counts and measurements follow Kullander & Fang⁽¹³⁾,

where the last two rays articulating on the same pterygiophore are counted as $1\frac{1}{2}$. Snout morphology and terminology of oromandibular structures follow Nebeshwar & Vishwanath⁽¹⁴⁾. Identification and morphological comparisons were done by comparing specimens from the Pachhunga University Museum of Fishes (PUCMF). Specimens are deposited in PUCMF, Pachhunga University College, Mizoram. Measurement data is given in percentage of standard length, ventral-anal fin origin distance and head length.

2.3 Molecular analysis

Total DNA extraction from muscle tissue of specimens of was performed using the Phenol Chloroform Isoamyl alcohol method following Makkapan *et al*⁽¹⁵⁾. Amplification of the cytochrome c oxidase subunit I (COI) gene was performed using ProFlex PCR System. The following PCR conditions were used: initial denaturation of 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 53°C for 1 minute, elongation at 72°C for 1 minute and final elongation at 72°C for 10 minutes. The universal primers FishF1: 5'-TCAACCAACCACAAAGACATTGGCAC-3' and FishR1: 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3' were used for amplifying the selected gene⁽¹⁶⁾. Samples were sent to Agrigenome Labs, Kochi, Kakkanad, Kerala for sequencing. The sequences obtained were edited Finch TV software version 1.4.0 (Geospiza Inc., Seattle, WA) and aligned using ClustalW algorithm in MEGA 11⁽¹⁷⁾. In the present study, COI gene sequences of eight species of *Garra* were generated (NCBI Accession Number: OQ269351 – OQ269362). The genetic divergence between the sequences was calculated using the Kimura 2-parameter distance model (K2P) in MEGA11. Maximum Likelihood (ML) was analysed using MEGA11, with the nucleotide substitution model HKY+G+I. The maximum likelihood supports were determined by bootstrapping 1000 replicates. DNA sequences of the partial mitochondrial COI gene of the congeneric species of *Garra* from the same drainage as well as from neighbour drainages were obtained from GenBank, and added to the analysis. *Psilorhynchus khopai* was used as the outgroup for rooting the tree (GenBank accession number: KF598827.1). (See Table 1).

Table 1. Species ID and GenBank accession numbers for taxa included in this study for COI

Sl. no.	Species	Accession No.	Country/Source	References
1	<i>Garra lamta</i>	MK572210	Bangladesh	Rahman <i>et al</i> ⁽¹⁸⁾
2	<i>Garra nasuta</i>	JX074219	Aquarium	Yang <i>et al</i> ⁽¹⁹⁾
3	<i>Garra gotyla</i>	MN342593	Unknown	GenBank
4	<i>Garra litanensis</i>	MK714214	India	GenBank
5	<i>Garra gravelyi</i>	KX951811	India	Genbank
6	<i>Garra quadratirostris</i>	MN832847	India	GenBank
7	<i>Garra elongata</i>	MG182193	India	GenBank
8	<i>Garra kempi</i>	OK036441	India	GenBank
9	<i>Garra naganensis</i>	KX951813	India	GenBank
10	<i>Garra annandalei</i>	MK610283	India	GenBank
11	<i>Garra arupi</i>	KF511538	India	GenBank
12	<i>Garra rakhinica</i>	KU667322	India	GenBank
13	<i>Garra paralissorhynchus</i>	KJ909429	India	GenBank
14	<i>Garra lissorhynchus</i>	MN832846	India	GenBank
15	<i>Garra flavatra</i>	JF915609	Unknown	GenBank
16	<i>Psilorhynchus khopai</i>	KF598827	India	Genbank
17	<i>Garra cf. khawbungii</i>	OQ269362	India	This study
18	<i>Garra nigricollis</i>	OQ269361	India	This study
19	<i>Garra cf. manipurensis</i>	OQ269354 -57	India	This study
20	<i>Garra koladynensis</i>	OQ269352-53	India	This study
21	<i>Garra flavatra</i>	KF598823	India	This study
22	<i>Garra rakhinica</i>	OQ269351	India	This study
23	<i>Garra</i> sp. 1	OQ269358	India	This study
24	<i>Garra</i> sp. 2	OQ269359-60	India	This study

3 Results and Discussion

3.1 Morphological analysis

Based on the morphological comparison with relevant literature, a total of 8 species of *Garra* were collected from the Kaladan drainage. Figure 2 shows different species of the genus *Garra* collected from the Kaladan drainage. The species account for each specimen is given below:

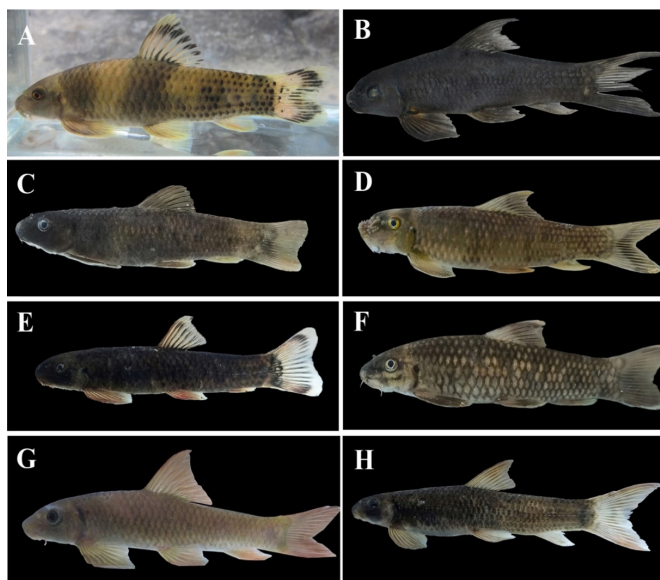


Fig 2. *Garra* species from the Kaladan drainage (A) *Garra flavatra* (B) *Garra khawbungii* (C) *Garra manipurensis* (D) *Garra koladynensis* (E) *Garra* sp. 2 (F) *Garra rakhinica* (G) *Garra nigricollis* (H) *Garra* sp. 1

Species account

Garra flavatra Kullander & Fang, 2004

Remarks: The predorsal region is scaled; there are 27–29 lateral line scales and 16 circumpeduncular scale rows, as well as light yellowish vertical bars along the body, a blackish spot above the gill opening, and two vertical rows of black spots that are asymmetrically spaced across the middle of the caudal fin. A rostral flap is present with conical tubercles. The snout lacks a proboscis.

Location: Tuisi River, Mizoram

Garra cf. khawbungii Arunachalam, Nandagopal & Mayden, 2014

Remarks: The snout is rounded with a weakly developed transverse groove and irregularly spaced tubercles, a forked caudal fin, scales on the breast and abdomen, and a black band on one or two rays of the lower lobe of the caudal fin; 8 branched dorsal fin rays, 5 branched anal fin rays, 36–37 lateral-line scales, and 16 circumpeduncular scales.

Location: Tuisi River, Mizoram

Garra manipurensis Vishwanath & Sarojnalini, 1988

Remarks: The snout is smooth and rounded; rostral lobe is present; the rostral lobe has 3–6 little conical tubercles. 31–32 lateral line scales; $7\frac{1}{2}$ branched dorsal fin rays and $4\frac{1}{2}$ branched anal fin rays; the mental adhesive disc is posteriorly positioned; Dorsal, pectoral, pelvic, anal and caudal fins grayish, posterior halves of each distal $\frac{2}{3}$ length of interradial membrane of dorsal fin black; faint mid-lateral stripe present, more distinct towards caudal peduncle and extending to median rays of caudal fins.

Location: Tiau River and Cheu River near Sangau Village

Garra koladynensis Nebeshwar & Vishwanath, 2017

Remarks: Snout with proboscis and transverse lobe; proboscis tri-lobed, transverse lobe with 11–23 tubercles; 30–31 lateral line scales; $8\frac{1}{2}$ branched dorsal fin rays; $5\frac{1}{2}$ branched anal fin rays; circumpeduncular scale rows 16; chest and belly scaled; caudal fin forked; six narrow dark black stripes on caudal peduncle; Black spot at upper angle of gill opening.

Location: Tiau River, near Sangau Village

Garra rakhinica Kullander & Fang, 2004

Remarks: Snout smooth with rostral flap; chest and abdomen scaled; 28 lateral line scales; 16 circumpeduncular scale; caudal fin emarginate; black horizontal stripe across the snout, originating from base of anterior barbel and extending to the preopercle; Black spot at upper angle of gill opening; Black spots along the base of the dorsal fin.

Location: Tiau River, near Sangau Village

***Garra nigricollis* Kullander & Fang, 2004**

Remarks: 33 lateral line scales; circumpeduncular scale rows 16; Snout smooth and rounded with rostral lobes; chest and abdomen scaled; presence of black bar extending from pectoral fin base dorsally across the nape to trans-lateral pectoral fin base across the posterior margin of the head; Dark grey rounded blotch on lateral side of caudal peduncle.

***Garra* sp. 1**

Remarks: Snout smooth without transverse lobe or proboscis; chest and abdomen scaled; 37-38 lateral line scales; 16 circumpeduncular scale rows; dorsal, pectoral and anal fin hyaline; caudal fin forked; head wider than deep; vent situated midway between the anal and pelvic fin origin; $4\frac{1}{2}$ / $1/4\frac{1}{2}$ transverse scale rows.

Location: Cheu River, near Sangau Village

***Garra* sp. 2**

Remarks: Snout smooth; rostral flaps with 4–5 small tubercles; Caudal fin emarginate; W-shaped band present on caudal fin; chest and belly scaled; Predorsal scales 10-12; 16 circumpeduncular scales; 27-28 lateral line scales; Vent to anal fin distance is 18-24 % of pelvic to anal distance.

Location: Tiau River, near Sangau Village.

3.2 Molecular Analysis

The total length of the COI sequence developed is approximately 660 bp. Figure 3 shows ML tree inferred from the COI sequence comprising 22 species of *Garra* with one outgroup species. The ML tree analyses showed five divergent clades supporting morphological distinction. Species possessing snout with proboscis are grouped in Clade I viz. *G. koladynensis*, *G. lamta*, *G. nasuta*, *G. gotyla*, *G. litanensis*, *G. gravelyi*, *G. quadratirostris*, and *G. elongata*. Species with transverse lobe are grouped in Clade II viz. *G. arupi* and *G. kempi*. Smooth snout species are grouped in Clade III viz. *G. annandalei*, *G. naganensis*, *Garra* sp. 1, *Garra* cf. *khawbung* and *Garra nigricollis*. Species bearing rostral flap are grouped together in Clade IV viz. *G. rakhinica*, *G. paralissorhynchus*, *G. lissorhynchus*, *G. flavatra* and *Garra* sp. 2. Rostral lobe-bearing species are grouped in Clade V viz. *G. manipurensis*. The data obtained, based on p-distance matrix, showed the nucleotide inter-specific distance ranges from 3.8-20.5%. The unidentified species *Garra* sp. 1 has a smooth snout without a rostral lobe/flap and shares similar morphological characteristics with *G. annandalei* and *G. naganensis*. However, analysis of p-distance revealed that *Garra* sp. 1 has an interspecific distance of 9.6% from *G. annandalei* and 7.2% from *G. naganensis*. Also, the unidentified species *Garra* sp. 2 bears a rostral flap and falls within Clade III. However, the interspecific distance of the species with other species from the same clade ranges from 9.5-12% suggesting that *Garra* sp. 1 and *Garra* sp. 2 may be undescribed species.

3.3 Synonymization of *G rakhinica* with *G tyao*

G. rakhinica is described by Kullander & Fang from specimens of Taunggok, Thade River drainage, Rakhine State, Myanmar (the Rakhine Yoma) and had recently been reported from the Kaladan drainage of Mizoram by Nebeshwar & Vishwanath^(13,14). Arunachalam *et al* described two new species of *Garra* from the Kaladan drainage: *G. khawbung* from the Tuipui River and *G. tyao* from the Tiau River⁽²⁰⁾. There is no doubt about the identity of *G. khawbung* when compared to the previously described species of *Garra*, however, the identity of *G. tyao* is doubtful as it closely resembles *G. rakhinica*.

The distinguishing features between *G tyao* and *G. rakhinica*, as mentioned by Arunachalam *et al*⁽²⁰⁾, are that *G. tyao* possessed more lateral-line scales (31 vs. 28-29 in *G. rakhinica*), fewer branched caudal-fin rays (9+8 vs. 10+9 in *G. rakhinica*) and W-shaped band on posterior margin of caudal fin (vs. absent in *G. rakhinica*). Upon thorough examination of specimens collected from Tiau River and Kaladan River, it was observed that they belong to the same species and the reported morphological difference between the two species is due to the different methods employed by the authors. Arunachalam *et al*⁽²⁰⁾ follow Hubbs & Lagler⁽²¹⁾ for morphometric analysis where the lateral-line scales count includes scales on the caudal-fin base and the caudal-fin rays counting includes only the branched rays. In contrast, Kullander & Fang⁽¹³⁾ follow the method where the lateral-line scales were counted from the anterior-most scale in contact with the shoulder girdle to the end of the hypural plate (excluding those on the caudal fin), and the caudal-fin rays counting includes all the principal fin rays. Comparing the two species following Hubbs & Lagler⁽²¹⁾, the lateral-line scales count of *G. tyao* (31) does not differ from *G. rakhinica* (30–31), and also, the caudal fin rays of *G. tyao* (9+8) also conform to *G. rakhinica* (9+8). Subsequently, the reported presence of a W-shaped band on the caudal fin in *G. tyao* may be attributed to the difference in terminologies used for the description

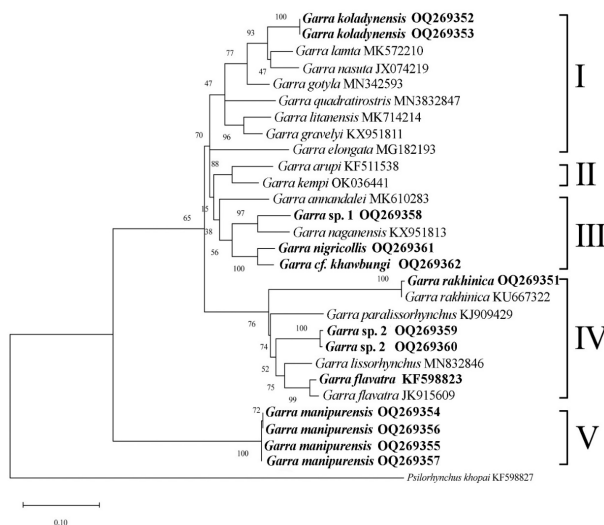


Fig 3. Maximum Likelihood tree inferred from COI for *Garra* from the Kaladan drainage

of the species. Upon looking at the picture of *G. tyao* {see Figure 4 (A) of Arunachalam *et al*⁽²⁰⁾}, the W-shaped band is less conspicuous compared to those observed in 'lissorhynchus complex', rather, more appropriate to be stated as 'a greyish caudal fin with a white lobe tip, a blackish blotch close to the tip and a grey curved bar across the middle of the caudal fin' as described by Kullander & Fang⁽¹³⁾.

The main distinguishing character of *Garra rakhinica* from all other congeners is that it possessed a blackish horizontal stripe across the snout extending to the preopercles⁽¹³⁾ which we also observed in all our studied specimens. The same character, though not mentioned at all in the original description, can also be seen in *G. tyao* {see Figure 4 (C) of Arunachalam *et al*⁽²⁰⁾}.

Thus, the similarity between the two species was beyond doubt when morphological and meristic comparison was performed with the published data of *Garra tyao* and a combined data of 53 specimens of *G. rakhinica* from the Tiau river and the Kaladan river along with the original description of *G. rakhinica*. Furthermore, the difference between the two specimens is attributed to the difference in the methodology as stated above. It is therefore concluded that *G. tyao* is a junior synonym of *G. rakhinica*. Figure 4 (A-C) shows a comparison between *G. rakhinica* and *G. tyao*.

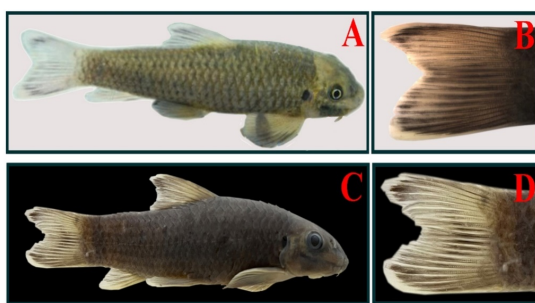


Fig 4. A- Lateral view of *Garra tyao* (from Fig 4(A) of Arunachalam *et al*⁽²⁰⁾). (B) Caudal fin of *Garra tyao* (from Fig 4(C) of Arunachalam *et al*⁽²⁰⁾). (C) Lateral view of *Garra rakhinica* from Kaladan. (D) Caudal fin of *Garra rakhinica* from Kaladan

Keys for identification of *Garra* from the Kaladan drainage

1. Snout without proboscis.....2
- Snout with proboscis, proboscis trilobed*Garra koladynensis*
2. Snout smooth without transverse lobe or transverse groove.....3

- Snout with transverse groove, 14-15 branched pectoral fins; $4\frac{1}{2}$ -5 anal scale rows; 10 predorsal scales *Garra cf. khawbung*
3. Snout smooth without rostral lobe or rostral flap.....4
- Snout smooth with rostral lobe or rostral flap5
4. Lateral line scales 33, Dark brown or black bar extending from pectoral fin base dorsally across nape to translateral pectoral fin-base*Garra nigricollis*
- Lateral line scales 37-38, Presence of black spot at the upper angle of the gill opening.....*Garra* sp. 1
5. Snout smooth with rostral lobe, caudal fin emarginated with grayish strip over lateral line extending to median rays of caudal fin.....*Garra manipurensis*
- Snout smooth with rostral flap, presence of W-shaped band or vertical spots/ bar on the caudal fin6
6. Lateral line scales 30 or more *Garra* sp. 2
- Lateral line scales less than 30 7
7. Caudal fin with two vertical rows of black spots, inframarginal band along the posterior end of caudal fin*Garra flavatra*
- Black horizontal stripe from base of anterior barbel to preopercle*Garra rakhinica*

4 Conclusion

There are numerous small streams and rivers in Mizoram and many of these waterbodies are difficult to access due to the region's hilly topography and the challenging terrain. Efforts were made to perform a thorough taxonomic investigation of the genus *Garra* from the Kaladan drainage. Furthermore, ichthyofaunal researches from Mizoram are still very few. Although Beihrosa *et al.*⁽²²⁾ conducted a rather similar investigation on *Garra*, more recent taxonomic research along the Kaladan drainage has shown the presence of a few other species, which are represented here as *Garra* sp. 1 and *Garra* sp. 2.

Based on the taxonomic investigation of the genus *Garra* from the Kaladan drainage, in addition to the previously recorded 6 species, the total number of *Garra* species from the Kaladan drainage is now 8, which accounts for about 20% of the total species found in the northeastern regions of India. Also, based on the morphological analysis and comparison of data, it was concluded that *Garra tyao* is a junior synonym of *Garra rakhinica*.

In this study, morphological and molecular phylogenetic analysis were used to identify the species. Morphological analysis is carried out using the identification keys provided by various authors as well as other relevant literature. When it comes to identifying morphologically similar groups, there is, however, a lack of information regarding the identification and categorization of species, which frequently leads to misidentification. We therefore recommend the use of a common method of morphometric measurements as well as integrative taxonomical approach for all species of *Garra*.

5 Declaration

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