MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *GARRA* (TELEOSTEI: CYPRINIDAE: LABEONINAE) FROM MIZORAM, NORTH-EAST INDIA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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BY

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Submitted

In partial fulfillment of the requirement of the Degree of Doctor of Philosophy in Life Sciences in Mizoram University, Aizawl

CERTIFICATE

I certify that the thesis entitled **"Morphological and Molecular Characterization of *Garra* (Teleostei: Cyprinidae: Labeoninae) from Mizoram, North-East India"** submitted to the Mizoram University for the award of the degree of Doctor of Philosophy in Life Sciences by **M. C. Zirkunga** is a record of the research work carried out during the period 2020 - 2024 under my supervision and guidance and that this work has not formed the basis for the award of any degree, diploma, associateship, fellowship or other titles in this University or any other University or institution of higher learning.

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DECLARATION

Mizoram University

November, 2024

I **M. C. Zirkunga**, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/ Institute.

This is being submitted to the Mizoram University for the degree of **Doctor of Philosophy** in Life Sciences.

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Abbreviations

Mt.	Mountain
asl	Above sea level
UAE	United Arab Emirates
PUCMF	Pachhunga University College Museum of Fishes
NCBI	National Centre for Biotechnology Information
RL	Rostral lobe
RF	Rostral flap
PRB	Proboscis
SG	Sublachrymal groove
RCG	Rostral cap groove
TRG	Transverse groove
TL	Transverse lobe
DRS	Depressed rostral surface.
PPF	Pleated papilliferous fold
SL	Standard length
HL	Head length
TES	N-tris (hydroxymethyl) Methyl-2-aminoethane Sulphonic Acid
PCI	Phenol Chloroform Isoamyl alcohol
CI	Chloroform Isoamyl alcohol
TE	Tris EDTA (Ethylenediaminetetraacetic acid)
coi	*cytochrome c oxidase subunit* 1
cytb	*cytochrome b*
HKY	Hashegawa-Kishono-Yano model
G+I	Gamma + Invariant sites
ZSI	Zoological Survey of India
MUMF	Manipur University Museum of Fishes
BI	Bayesian Inference

General Introduction

Mizoram located in the Northeastern part of India is bounded on the north by Assam, Manipur and Tripura while the western and eastern part is bounded by Bangladesh and Myanmar (Burma) respectively (Joshi, 2005). The topography of Mizoram is characterized predominantly by a series of parallel hill ranges extending from north to south (Singh, 1996; Pachuau, 2009). The elevation gradually increases from west to east ranging from approximately 21 m asl at Tlabung to 2,157 m asl at Mt Phawngpui peak (Pachuau, 2009). These hilly terrains gave rise to numerous rivers, streams and brooks that drain into three distinct river drainages namely, the Karnaphuli River drainage system, the Kaladan (Koladyne) River drainage system and the Barak-Meghna River drainage system (Kar & Sen, 2007).

The Karnaphuli River (locally called Khawthlangtuipui) forms the Southwestern drainage system. The river Karnaphuli, originates near Phuldungsei village in Mamit District, Mizoram, after which it flows southward and joins the Mar River (Lalnunmawia, 2022). It then continues to flow southward where it joins with the northwardly flowing Tuichawng River and eventually enters Bangladesh in the Tlabung sub-division. The Karnaphuli River ultimately drains into the Bay of Bengal at Chittagong Port (Pachuau, 2009). Some important tributaries of the Karnaphuli River also include De River, Keisalam River, Aivapui River, Seling River, Ruangte River, Kau River and Phairuang River. Figure 1 shows the Karnaphuli River drainage with its tributaries.

The Kaladan River and its tributaries drain the northeastern and southeastern part of Mizoram (Pachuau, 2009). The largest river of Mizoram, the Kaladan River or Chhimtuipui River originates from the western part of Myanmar and enters Mizoram near Chapi village and flows in the northward direction forming a boundary between Mizoram and Myanmar (Lalnunmawia, 2022). The Tiau River which also forms a boundary between Mizoram and Myanmar flows southwards and joins Chhimtuipui River after which the river flows briefly in the westward direction and eventually flows in the southward direction. The Kaladan River then enters Myanmar near Lomasu Village in Mizoram and eventually drains at Sittwe, Myanmar (Lalnunmawia, 2022). Some of the major tributaries of the Kaladan River drainage are Tiau River, Mat River, Tuichang River, Khawchhak Tuipui, Kawlawh River and Ngengpui River. Smaller tributaries of the Kaladan River are Cheu River, Tuitlawk River, Vansang River, Tuisi (Tisi) River, Lungbun River, Tuikum River and Palak River. Figure 2 shows the Kaladan River drainage with its tributaries.

The Barak River and its tributaries drain the central and northern parts of the state (Kosygin, 2012). The Barak River, the headstream of the Meghna River originates near Mt. Japfu peak in Manipur, where it is met by the Tuivai River at 24.235374N, 93.021962E after which it flows through the southern part of Assam and eventually drains in the Bay of Bengal after merging with the Brahmaputra River (Chaturvedi, 1985). The Barak River system in Mizoram is confluent mostly by northwardly flowing rivers. Major rivers of the Barak-Meghna River system in Mizoram are the Tlawng River (Dhaleshwari), Tuivai River, Tuirial River (Sonai), Tuirini River, Tut River, Teirei River and Serlui River. Smaller tributaries of the Barak River are Tuiphal River, Tuivawl River, Phalte River, Dialdawk River, Hmawngva River, Chite River, Langkaih River etc. The river Tuirial and Tuirini merge at 23.851228N, 92.829018E and flows north eventually joining the Barak River in Assam. The river Tlawng and Tut joins at 24.035815N, 92.537748E after which it is eventually confluent by the Teirei River. The larger Tlawng River then joins the Barak River at 24.827549N, 92.637520E in Assam. The Langkaih River forms the northern border between Mizoram and Tripura and eventually joins the larger Barak River. Figure 3 shows the Barak River drainage with its tributaries within Mizoram.

A well-accepted concept is that freshwater fishes are distributed in a particular river basin and are separated (with few to several morphological distinctions) from their congeners in different basins (Vishwanath & Linthoingambi, 2008). Given this context, the existence of three drainage systems that are geographically not connected may plays a crucial role in the high fish diversity and variability in Mizoram. Further, Mizoram is one of the most important regions of the Indo-Burma biodiversity hotspot (Lalremruata, 2023) and since freshwater fishes represent the most threatened taxonomic group globally (Carrizo et al., 2013), taxonomical analysis and documentation of *Garra* from Mizoram is a critical necessity.

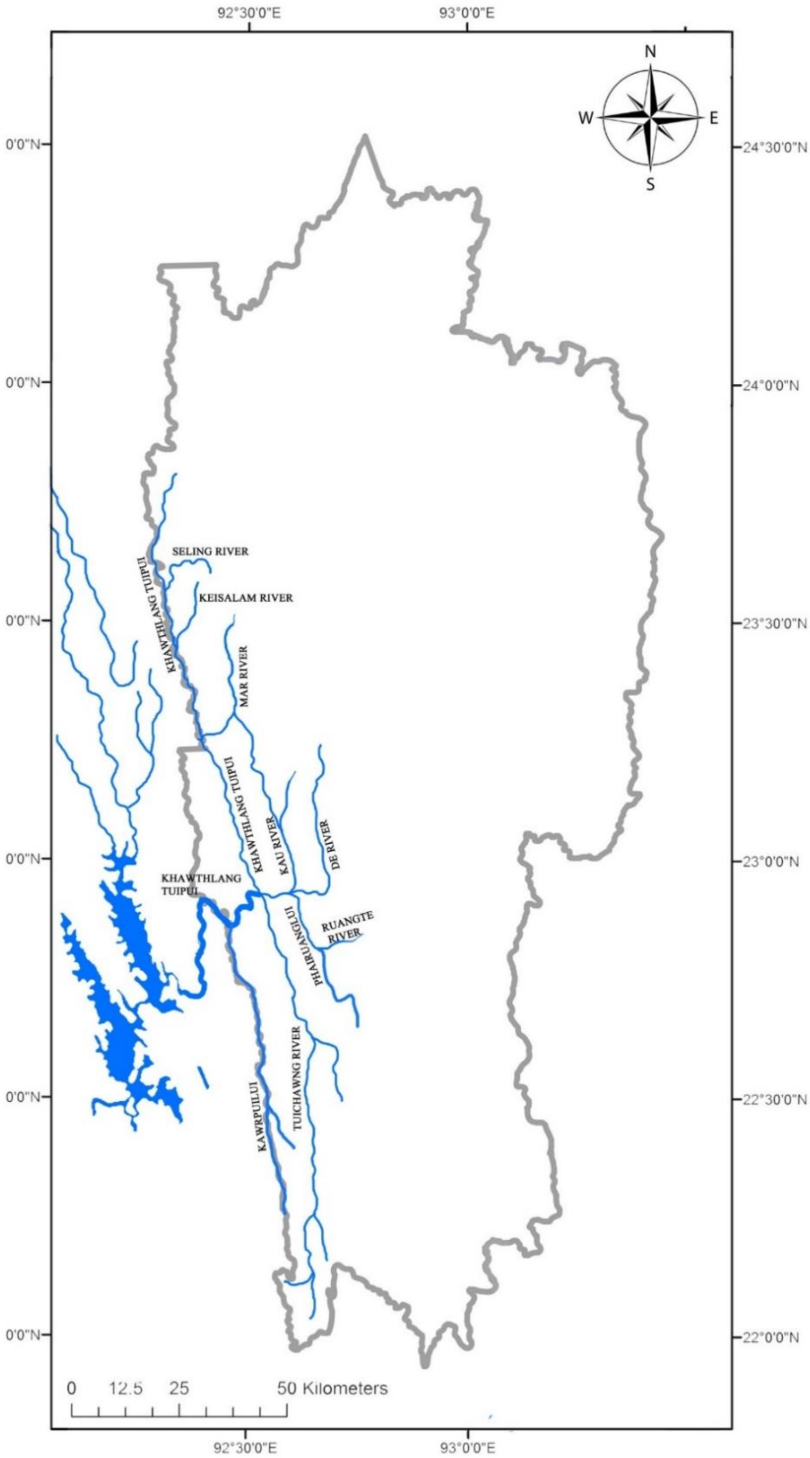


Fig. 1: Drainage map of the Karnaphuli River and its tributaries

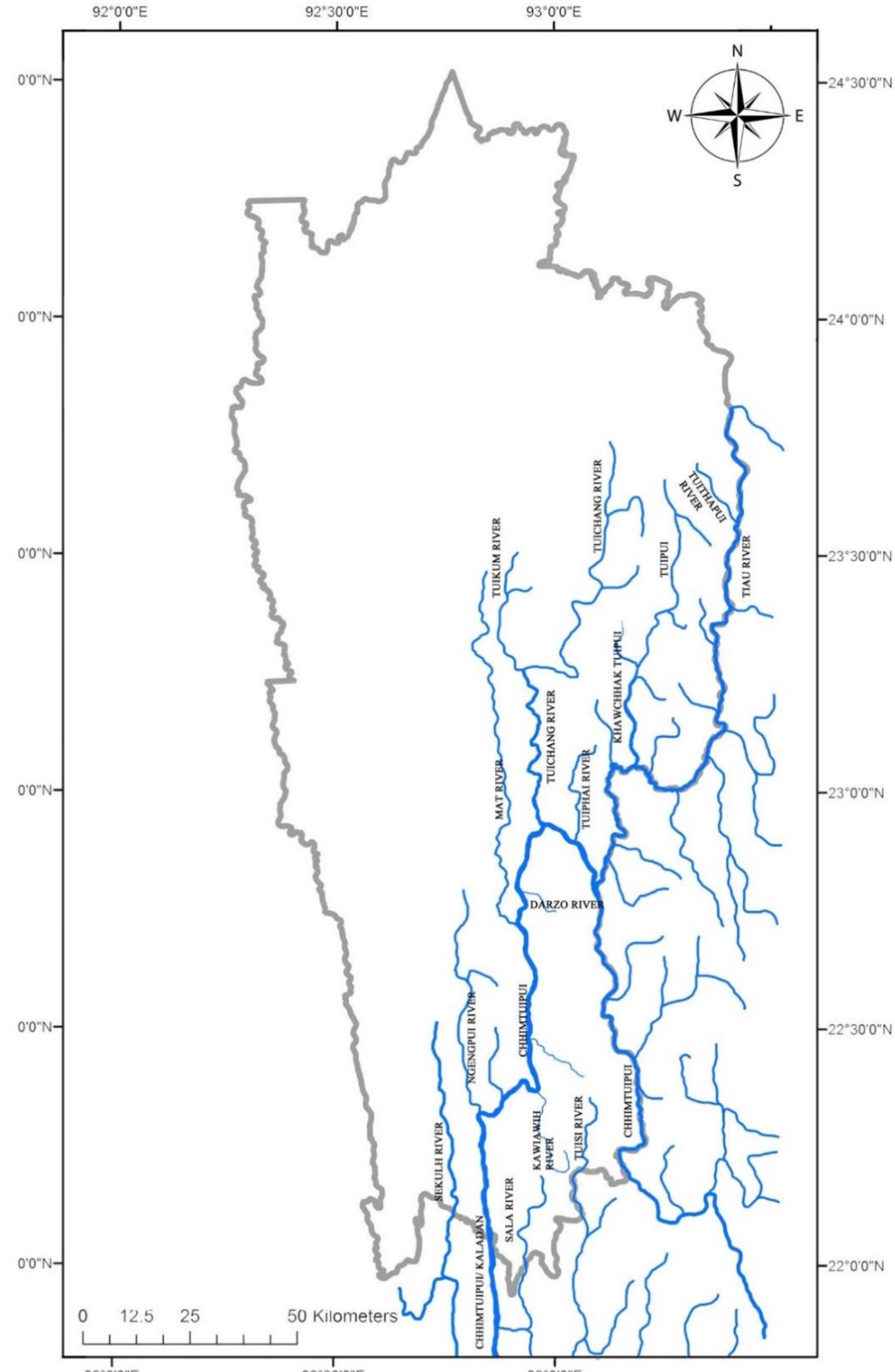


Fig. 2: Drainage map of the Kaladan River and its tributaries

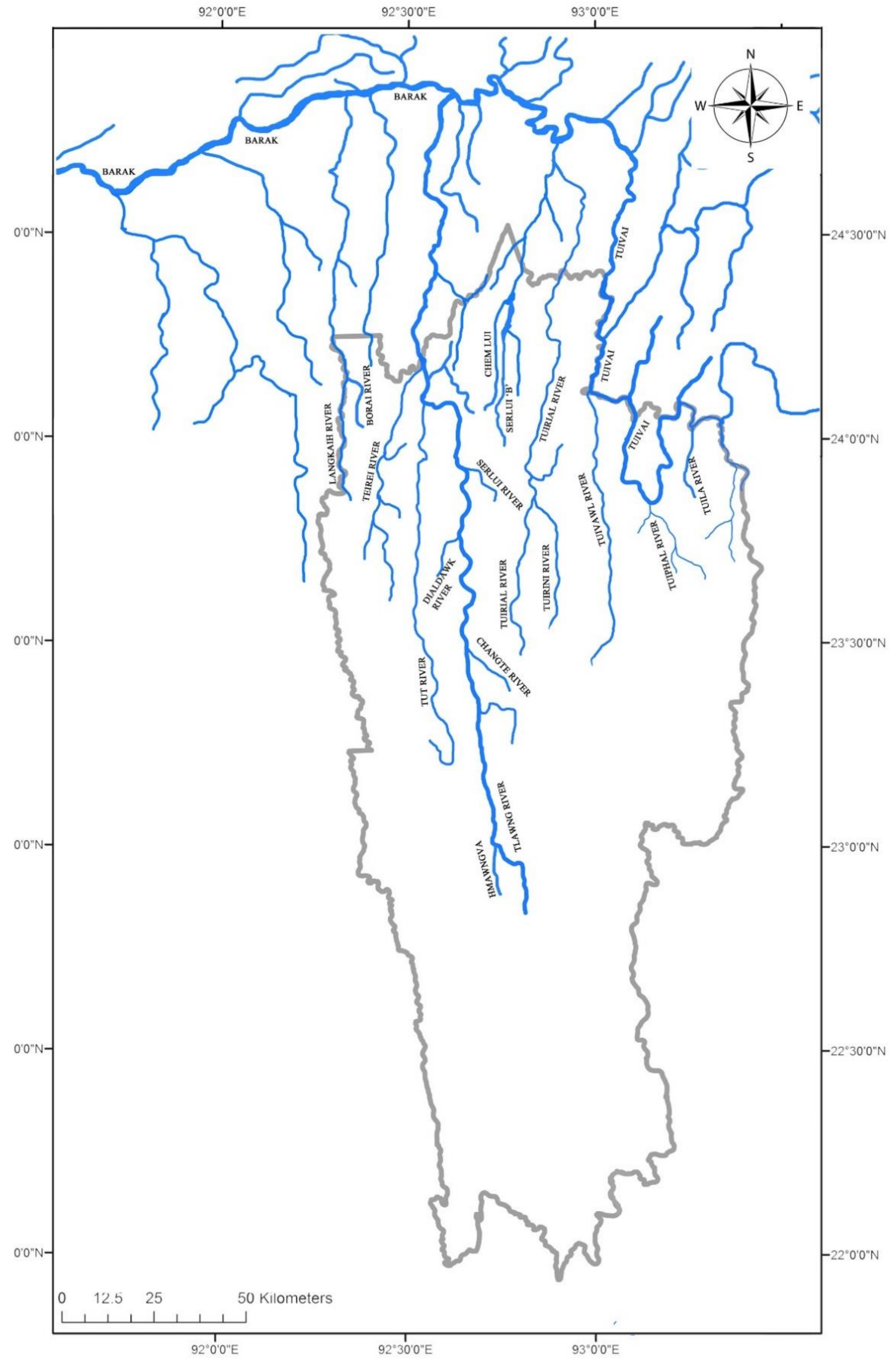


Fig. 3: Drainage map of the Barak River and its tributaries.

Systematic Position of the genus *Garra*

Phylum Chordata

Class Actinopterygii

Order Labeoninae

Family Cyprinidae

Subtribe Garraina

Genus *Garra* Hamilton, 1822

Cyprinid fish of the genus *Garra* are mostly benthic fish that are characterized by having a slender subcylindrical body, a pair of horizontally extended pectoral and pelvic fins and a modified lower lip that resembles a disc-like apparatus often called mental adhesive disc or gular disc **(**Rahman, 2016; Tamang et al., 2019; Kosygin, 2021). Members of this genus usually inhabits mountain torrents, rapid streams and riffles (Ezung et al., 2020; Zirkunga, 2023), brackish water (Getahun, 1999), lakes (Stiassny & Getahun, 2007) and caves (Bannister, 1987). The cave-dwelling species, *G. typhlops* Bruun & Kaiser (1948), *G. lorestanensis* Mousavi-Sabet & Eagderi (2016a) and *G. tashanensis* Mousavi-Sabet et al. (2016b) are morphologically distinct from their congeners by lacking pigments and eyes (Mousavi-Sabet et al., 2016b).

The presence of an oral sucking disc is a characteristic feature of the genus *Garra* along with other disc-bearing cyprinid such as *Ageneiogarra* (Garman, 1912), *Discogobio* (Lin, 1931), *Placocheilus* (Wu, 1977), *Discocheilus* (Zhang, 1997), *Vinagarra* (Nguyen & Bui, 2009), *Sinigarra* (Zhang & Zhou, 2012), *Ceratogarra* (Kottelat, 2020), *Guigarra* (Wang et al. 2020), *Pseudoplacoheilus* (Li et al., 2024) and *Supradiscus* (Li et al., 2024). The genus is widely distributed in North and Central Africa, parts of the Middle East and Southeast Asia regions including South China (Zhang & Chen, 2002; Kottelat, 2013).

Brief taxonomic History of *Garra*

The genus was first erected by Hamilton (1822). *Cyprinus lamta* (now *Garra lamta*) was the first species of *Garra* described from Bihar and Rapti River, Gorakhpur District, India. The Indian species of *Garra* were revised and illustrated by Hora (1921) along with a brief comment on foreign species. Hora (1921) synonymized the genus *Discognathus* with *Garra* based on the developmental analysis of the mental adhesive disc and further described nine new species of *Garra*, of which only 3 species namely *G. annandalei, G. kempi* and *G. naganensis* were recognized as valid species by Menon (1964).

The first global revision of the genus was conducted by Menon (1964). Menon (1964) in his book 'Monograph of the cyprinid fishes of the genus *Garra* Hamilton', discussed the morphological characteristics of 37 species of *Garra* from different parts of the Asian and African continents. Based on the comparison of the morphological characteristics of 37 species of *Garra* and their distribution, Menon (1964) provides the following important assumptions: -

1. Species of West Asia and Africa are the most primitive and generalized.
2. Highly specialized species were found mostly in Southeast Asia.
3. Southwest China has the highest number of species and Southwest China may be the ancestral home of the genus.

Current Taxonomic information

Since the erection of the genus by Hamilton (1822), several additional species have been described. According to the Eschmeyer Catalogue of Fishes (Fricke et al., 2024), currently, there are about 200 valid species of *Garra*. Several taxonomic revisions have been conducted on the genus. The Chinese *Garra* species were reviewed by Zhang et al. (2000) while Nebeshwar & Vishwanath (2017) reviewed and classified the South and Southeast Asian species based on the snout morphology and oromandibular structures. The southwest Asian *Garra* were studied and reviewed by Krupp (1983), Hamidan et al. (2014), Sayyadzadeh et al. (2015), Esmaeili et al. (2016) and Freyhof (2016). The African *Garra* species were also revised and classified based on the type of mental adhesive disc by Stiassny & Getahun (2007) and Moritz et al. (2019).

However, global revision of the genus has not been conducted after Menon (1964), and although several morphological and molecular-based revisions have been done, much of the research on the genus were geographically restricted (Kottelat, 2020). This may be due to the wide geographic distribution, unavailability of many of the type species (for comparison) due to its remote location, damaged museum specimens as well as limited genetic information of several species.

Taxonomy of *Garra* from Mizoram

Taxonomical analysis of ichthyofauna from Mizoram is still in the infantile stage as this may be attributed to the limited ichthyofaunal exploration and extensive survey of the riverine system due to the difficult topography, unavailability of road access and rough terrains. However, few researches were carried out on the genus within Mizoram. Solo & Lalnuntluanga (2018) reported seven species of *Garra* from the Kaladan River drainage. Lalronunga (2016) in an unpublished thesis reported four species of *Garra* from the Barak River system within Mizoram. Lalramliana et al., (2017) reported five species of *Garra* from the Karnaphuli River drainage in the Dampa Tiger Reserve.

Due the limited genetic data in the NCBI GenBank, insufficient morphological data and few distributional studies of the genus, the major purpose of the research aims at the following objectives:

1. To study the diversity and distribution of *Garra* in Mizoram.
2. To perform morphological and molecular characterization of *Garra* from different rivers of Mizoram.
3. To study the phylogenetic relationship and establish DNA barcode data of *Garra* from Mizoram.

CHAPTER – 1

Diversity and Distribution of *Garra* in Mizoram

Introduction

Global diversity and distribution of *Garra*

Garra are widely distributed in the tropical and subtropical regions of Africa, South and Southeast Asia, the Middle East and the Arabian Peninsula (Zhang & Chen, 2002; Tangjitjaroen et al., 2023). Li et al. (2024) infers that species belonging to the genus *Garra* are distributed in the Mekong River basin, west of the Mekong River basin in Southeast Asia and extending to Africa. Based on molecular phylogenetic results, Li et al. (2024) further indicated that species from the east of Mekong described as *Garra* were neither closely related nor share a recent common ancestor with *Garra*.

Menon (1964) performed a global revision of the genus *Garra* where he recognized 37 species that are distributed across South China, Southeast Asia including India, Southwest Asia and West, East and Central Africa. However, after Menon (1964), global revision on *Garra* has not been carried out and recent revisions are geographically restricted. Some of the most recent revisions of the genus are: -

1. Moritz et al. (2019) reviewed and reported 25 species of *Garra* from Africa and the Nile basin (Ethiopia, Cameroon, Eritrea, Congo, Kenya, Nigeria, Sudan, East and West Africa).
2. From the Middle East regions, Esmaeili et al. (2016) reviewed the genus *Garra* and reported 11 species from Iran. Zamani-Faradonbe et al. (2021) also reported 11 species of *Garra* from the Tigris River and coastal rivers of Iran. Kirchner et al. (2020) reported 5 species of *Garra* from the Hajar Mountains (UAE and Oman).
3. The most recent review of the genus from South and Southeast Asia was carried out by Nebeshwar & Vishwanath (2017) where they reported 79 species of *Garra*. However, many new species have been described since.

Diversity and distribution of *Garra* in Northeast India

Northeast India is constituted by eight states namely Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura. The rivers in the northeastern part of India are drained by the Ganga-Brahmaputra-Barak River drainage, the Kaladan River drainage, the Chindwin River drainage (Dikshit & Dikshit, 2014) and the Karnaphuli River drainage respectively (see General Introduction). Further, each drainage is uniquely characterised by having a high diversity of endemic fishes (Goswami et al., 2012) including the genus *Garra*. Currently, there are about 50 species of *Garra* that are distributed in the different drainages of northeast India (Nebeshwar & Vishwanath, 2017; Shangningam et al., 2019; Ezung et al., 2020; Fricke et al., 2024).

In the Ganga-Brahmaputra-Barak River drainage, the following *Garra* species are reported: *G. lamta* (Hamilton 1822), *G. gotyla* (Gray 1830), *G. nasuta* (McClelland 1838), *G. rupecula* (McClelland 1839), *G. lissorhynchus* (McClelland 1842), *G. annandalei* Hora 1921, *G. kempi* Hora 1921, *G. naganensis* Hora 1921, *G. chaudhurii* Hora 1921, *G. prashadi* Hora 1921, *G. arupi* Nebeshwar, Vishwanath & Das 2009, *G. kalpangi* Nebeshwar, Bagra & Das 2012, *G. arunachalensis* Nebeshwar & Vishwanath 2013, *G. birostris* Nebeshwar & Vishwanath 2013, *G. magnidiscus* Tamang 2013, *G. quadratirostris* Nebeshwar & Vishwanath 2013, *G. binduensis* Das et al., 2016, *G. tamangi* Gurumayum & Kosygin 2016, *G. biloborostris* Roni & Vishwanath 2017, *G. clavirostris* Roni, Sarbojit & Vishwanath 2017, *G. substrictorostris* Roni & Vishwanath 2018, *G. magnacavus* Shangningam, Kosygin & Sinha 2019, *G. ranganensis* Tamang et al., 2019, *G. paratrilobta* Roni *et al*. 2019, *G. chathensis* Ezung, Shangningam & Pankaj 2020, *G. langlungensis* Ezung, Shangningam & Pankaj 2021, *G. manipurensis* Vishwanath & Sarojnalini 1988, and *G. lungongza* Catherine & Lithoingambi 2023.

From the Chindwin River drainage, 17 species of *Garra* are reported, namely *G. abhoyai* Hora 1921, *G. litanensis* Vishwanath 1993, *G. compressus* Kosygin & Vishwanath 1998, *G. elongata* Vishwanath & Kosygin 2000, *G. nambulica* Vishwanath & Joyshree 2005, *G. paralissorhynchus* Vishwanath & Devi 2005, *G. namyaensis* Shangningam & Vishwanath 2012, *G. chakpiensis* Nebeshwar & Vishwanath 2015, *G. ukhrulensis* Nebeshwar & Vishwanath 2015, *G. cornigera*

Shangningam & Vishwanath 2015*, G. trilobata* Shangningam & Vishwanath 2015*, G. chindwinensis* Premananda et al. 2017, *G. moyonkhulleni* Arnkumar & Moyon 2018, *G. ngatangkha* Arunkumar & Moyon 2019*, G. chivaensis* Moyon & Arunkumar 2020, *G. chingaiensis* Abonmai et al., 2023 and *G. hexagonarostris* Nebeshwar et al., 2024.

In the Kaladan River drainage within Mizoram, 7 species of *Garra* were reported by Solo & Lalnuntluanga (2015) namely, *G. flavatra* Kullander & Fang 2004*, G. khawbungi* Arunachalam, Nandagopal & Mayden 2014*, Garra* cf. *koladynensis* Nebeshwar & Vishwanath 2017*, G. manipurensis* Vishwanath & Sarojnalini 1988*, Garra* cf. *matensis* Nebeshwar & Vishwanath 2017*, G. nigricollis* Kullander & Fang 2004*, G. rakhinica* Kullander & Fang 2004.

From the Karnaphuli River drainage within Mizoram, Lalramliana et al. (2017) reported five species of *Garra* namely *Garra* aff. *lissorhynchus*, *G. dampaensis* Lalronunga et al., 2013, *G. nigricollis*, *G. manipurensis* and an unidentified species of *Garra* bearing a proboscis.

The present chapter aims to provide the diversity and distribution of *Garra* in Mizoram and resolve any uncertainties related to the distribution of *Garra* within Mizoram.

Materials and methods

Sampling site

The study area comprises three river drainage systems of Mizoram along with their tributaries. Sampling was conducted on all the major tributaries of the Kaladan River (Tuipui River, Tuichang River, Mat River and Tiau River), Barak River (Tut River, Tlawng River, Tuirial River, Tuirini River and Tuivai River) and Karnaphuli River (Mar River, De River and Tuichawng River) along with several smaller tributaries. Table 1, 2 and 3 shows the different sampling sites and sampling periods for the collection of *Garra*.

Fish Sampling

Fish sampling was conducted from October, 2020 – March, 2024. Sampling was done as per the regulations provided by the Fisheries Department, Government of Mizoram. Since Mizoram is considered vulnerable to natural hazards such as cyclones, landslides and flash floods (Chenkual, 2016) sampling during the heavy monsoon period (June-September) was usually not undertaken.

Fish specimens were collected using cast nets, drag nets, gill nets and, also, by employing local fishermen. Live fishes were collected and stored in aerated containers and the water was regularly cycled. Specimens were identified and counted in the field after which they were released back into the sampling sites.

For morphological studies, some specimens were euthanised using clove oil following Fernandes et al. (2016). Euthanised specimens were then fixed in 5-10% formalin and stored in 90-95% ethanol. Specimens were deposited in Pachhunga University College Museum of Fishes (PUCMF).

Table 1: Sampling sites, period, location and geocoordinates of the Kaladan River drainage within Mizoram.

Sl. No	River	Location	Sampling period	District	Geocoordinates
1	Tuichang River	Near Keitum village	January 2021	Serchhip District	From 23.225 N 92.9327 E to upstream direction
2	Tiau River	Champhai	March 2021	Champhai District	From 23.4619 N 92.3941 E to upstream direction
3	Tiau-Tuipui River	Near Sangau Village	March, 2021	Lawngtlai District	From 22.7861 N 93.0961 E to upstream direction
4	Mat River	Near Thualthu Village	April, 2022	Lunglei District	From 22.7677 N 92.8572 E to upstream direction
5	Vansang River	Near Thualthu Village,	April, 2022	Lunglei District	From 22.7502 N 92.7955 E to upstream direction
6	Tuisi River	Near Tisi Village	January 2023	Siaha District	From 22.3519 N 93.0775 E to upstream direction

Table 2: Sampling sites, period, location and geocoordinates of the Barak River drainage within Mizoram.

Sl. No	River	Location	Sampling period	District	Geocoordinates
1	Tuirial River	Near Tuirial village	January 2021	Aizawl District	From 23.8405 N 92.8188 E to upstream direction
2	Tuirini River	Champhai	January 2021	Saitual District	From 23.7066 N 92.8880 E to upstream direction
3	Tuivai River	Near Khawkawn Village	December, 2021	Saitual District	From 24.0369 N 93.2519 E to upstream direction
4	Tuila River	Near Khawkawn Village	December, 2021	Saitual District	From 24.0225 N 93.2522 E to upstream direction
5	Tlawng River	Near Aizawl	April, 2022	Lunglei District	From 23.7072 N 92.6661 E to upstream direction
6	Tut River	Near Dapchhuah Village	March 2022	Mamit District	From 23.7650 N 92.5219 E to upstream direction
7	Teirei River	Near Keitum village	March 2022	Mamit District	From 23.9063 N 92.4194 E to upstream direction

Table 3: Sampling sites, period, location and geocoordinates of the Karnaphuli River drainage within Mizoram.

Sl. No	River	Location	Sampling period	District	Geocoordinates
1	Ruangte River	Near Hauruang village	March 2021	Lunglei District	From 22.8275 N 92.6397 E to upstream direction
2	De River	Near Hauruang town	March 2021	Lunglei District	From 22.8400N 92.7072 E to upstream direction
3	Phairuang River	Near Phairuangkai	March, 2021	Lunglei District	From 22.8183 N 92.6480 E to upstream direction
4	Tuichawng River	Near Chawngte	April, 2021	Lunglei District	From 22.6205 N 92.6497 E to upstream direction
5	Keisalam River	Near Phuldungsei Village	April, 2023	Mamit District	From 23.4544 N 92.3374 E to upstream direction
6	Seling River	Near Phuldungsei Village	April 2023	Mamit District	From 23.5161 N 92.3722 E to upstream direction
7	Mar River	Near Marpara village	January 2024	Mamit District	From 23.2686 N 92.4269 E to upstream direction

Data analysis for population dynamics of *Garra* from Mizoram

Shannon-Weiner diversity index

Species diversity was calculated using Shannon-Weiner index (H'). The Shannon-Weiner diversity (H') is represented by the equation: -

$$H' = -\Sigma P_i \ln P_i$$

Where, H'= Shannon-Weiner index;

$P_i = n_i/N$

n_i is Number of individuals of each species in the sample.

N is Total number of individuals of all species in the sample

Jaccard Similarity index

Qualitative similarity between the drainage is calculated using Jaccard index (Krebs, 1989). The calculated results of the Jaccard index was used for comparing species similarities between two sites. This Jaccard Similarity index is represented by the following equation: -

$$C_j = \frac{j}{(a+b-j)}$$

Where, *j* is the number of species found at both sites,

a is the number of species recorded at site A, and

b is the number of species recorded at site B.

All calculations were performed in Past software version 4.03 (Hammer & Harper, 2001)

Results

Based on the sampling conducted during 2020-2024 on various rivers of the Kaladan River drainage, Barak River drainage and Karnaphuli River drainage, fourteen (14) species of *Garra* were recorded.

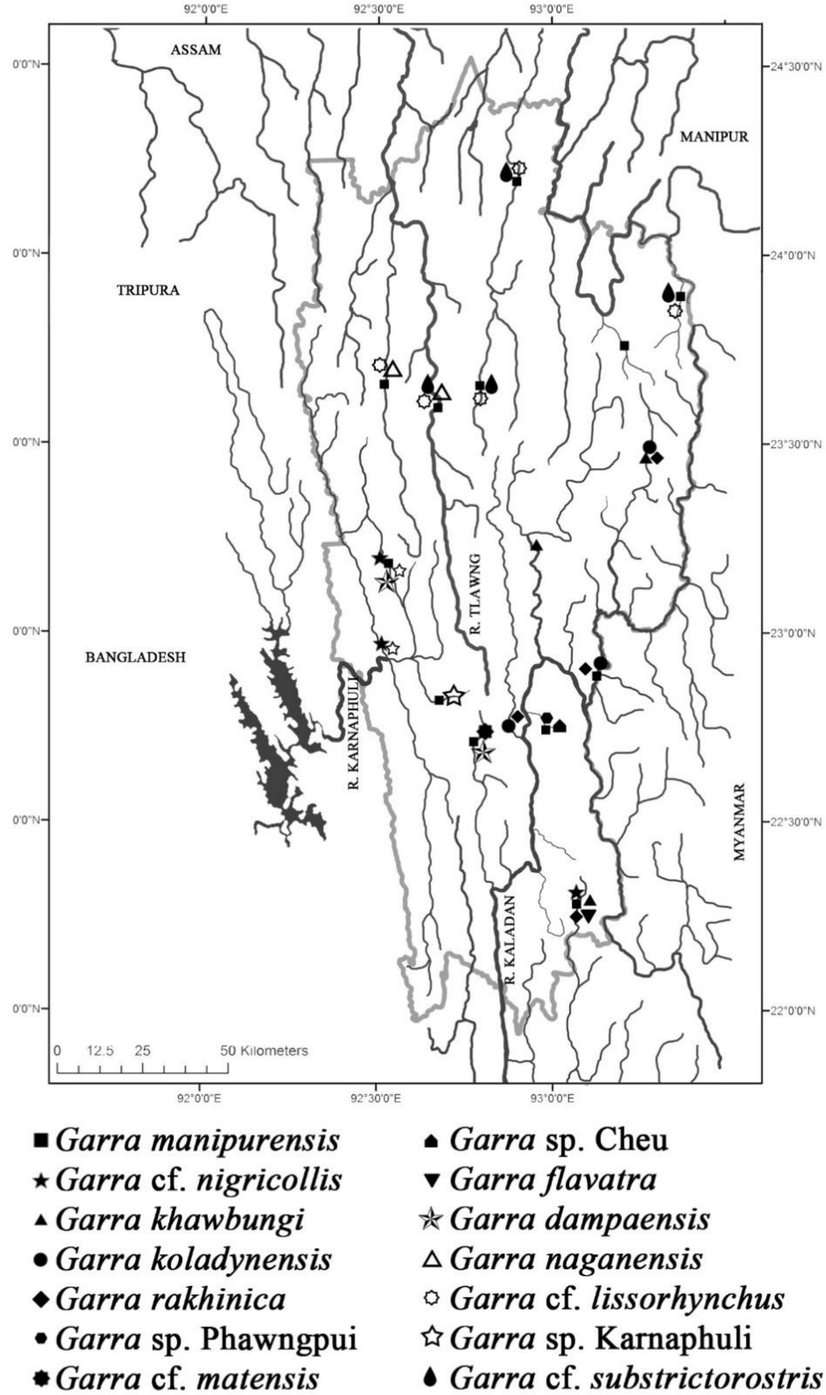
- From the Kaladan River drainage and its tributaries, nine (9) species of *Garra* were recorded namely, *G. manipurensis, G. nigricollis, Garra* cf. *matensis, G. rakhinica, G. flavatra, G. koladynensis, G. khawbungi, Garra* sp. Cheu and *Garra* sp. Phawngpui. Further, *G. flavatra* and *Garra nigricollis* were collected from the Tisi/Tuisi River only.
- From the Barak River drainage and its tributaries four (4) species of *Garra* were recorded namely *G. manipurensis, G. naganensis, Garra* cf. *substrictorostris* and *Garra* cf. *lissorhynchus*.
- From the Karnaphuli River drainage, four (4) species of *Garra* were recorded namely *G. nigricollis, G. dampaensis, G. manipurensis* and *Garra* sp. Karnaphuli.

Based on the sampling results, it was also observed that some species of *Garra* are confined to a particular river drainage. For instance, *G. matensis, G. koladynensis, G. khawbungi, Garra* sp. Cheu and *Garra* sp. Phawngpui were only recorded from the Kaladan River drainage. *G. naganensis, Garra* cf. *lissorhynchus* and *Garra* cf. *substrictorostris* were found only in the Barak River drainage while *Garra* sp. Karnaphuli and *G. dampaensis* were found only in the Karnaphuli River drainage. Interestingly, *G. manipurensis* is collected from all three river drainages. Table 4 shows the drainage-wise distribution of *Garra* in Mizoram. Figure 4 shows the distribution of different species of *Garra* in Mizoram.

Table 4: Drainage-wise distribution of *Garra* species from Mizoram, Northeast India.

Sl. No	Species	Kaladan drainage	Barak drainage	Karnaphuli drainage
1	*G. manipurensis*	+	+	+
2	*G. nigricollis*	+	-	+
3	*Garra* cf. *matensis*	+	-	-
4	*G. rakhinica*	+	-	-
5	*G. flavatra*	+	-	-
6	*G. koladynensis*	+	-	-
7	*Garra* sp. Cheu	+	-	-
8	*Garra* sp. Phawngpui	+	-	-
9	*G. khawbungi*	+	-	-
10	*Garra* cf. *lissorhynchus*	-	+	-
11	*Garra* cf. *substrictorostris*	-	+	-
12	*G. naganensis*	-	+	-
13	*G. dampaensis*	-	-	+
14	*Garra* sp. Karnaphuli	-	-	+

Fig. 4: Map of Mizoram showing the distribution of 14 species of *Garra*.



Population dynamics of *Garra* from Mizoram

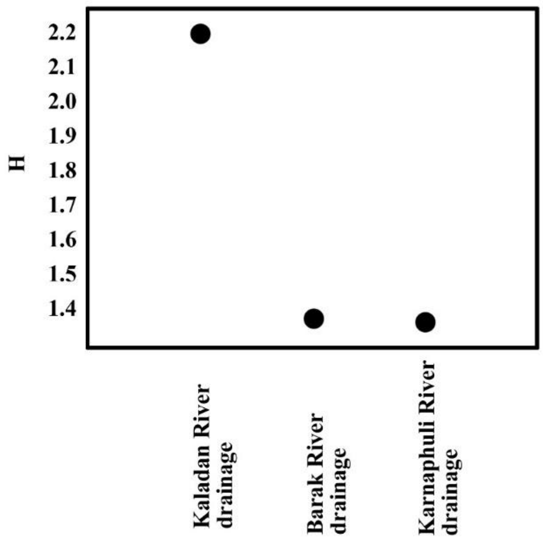
Shannon Diversity Index

The analysis consists of 318 *Garra* specimens from the Kaladan River, 184 specimens from the Barak River and 156 specimens from the Karnaphuli River. All the specimens collected account for fourteen (14) species of *Garra*. The calculated result of the Shannon diversity index is shown in Table 5. Figure 5 shows the graphical representation of the Shannon diversity index for *Garra* species from the Kaladan, Barak and Karnaphuli River drainages respectively. Based on the diversity analysis, the diversity of the *Garra* species is comparatively highest in the Kaladan River drainage (H'=2.19). The Barak and the Karnaphuli River drainages have a comparatively lower diversity index value of H'=1.38 and H'=1.38 respectively.

Table 5: Shannon diversity index of *Garra* species between three drainages of Mizoram. Diversity indices were calculated using Past software version 4.03 (Hammer & Harper, 2001).

	Kaladan drainage	Barak drainage	Karnaphuli drainage
No. of species	9	4	4
Shannon H'	2.19	1.38	1.38

Fig. 5: Graph showing the Shannon Diversity index based on the number of taxa of *Garra* from the Kaladan, Barak and Karnaphuli River drainages respectively. Graph was plotted in Past software version 4.03 (Hammer & Harper, 2001).



Jaccard's Similarity Index

The Jaccard's similarity index was also calculated for the three river drainages. The analysis consists of 14 species (9 species of *Garra* from the Kaladan River drainage, 4 species from the Barak River drainage and 4 species from the Karnaphuli River drainage). The similarity between the Barak River drainage and the Karnaphuli River drainage is the highest ($C_j = 0.18$) while the lowest similarity index ($C_j = 0.08$) is observed between the Kaladan River drainage and the Barak River drainage. Table 6 shows the Jaccard's similarity indices between the Kaladan, Barak and Karnaphuli River drainages.

Table 6: Jaccard's similarity index analysis of *Garra* from the Kaladan, Barak and Karnaphuli River drainages. Jaccard's similarity index was calculated using Past software version 4.03 (Hammer & Harper, 2001).

	Kaladan River drainage	Barak River drainage	Karnaphuli River drainage
Kaladan River drainage		0.08	0.18
Barak River drainage	0.08		0.14
Karnaphuli River drainage	0.18	0.14	

Discussion

Mizoram along with Meghalaya, Nagaland, Manipur and Assam (south of the Brahmaputra River) belong to the Indo-Myanmar (Indo-Burma) region (Myers et al., 2000). These regions are a hotspot for freshwater fishes with more than 440 species of fishes that account for about 62.81% of the total freshwater fishes of India (Goswami et al., 2012). The high diversity of freshwater fishes in the region can be attributed to the geomorphology that consists of a variety of torrential hill streams and rivers. Further, the tectonic development of the Indo-Burma ranges, created by the collision of the Indian, Chinese, and Burmese plates, significantly contributes to the variety of fish species (Goswami et al., 2012).

Based on the current research findings, among the three river drainages of Mizoram, the Kaladan River drainage has the highest diversity of *Garra.* Nine (9) species of *Garra* were collected from the Kaladan River drainage namely *G. flavatra, G. rakhinica, G. manipurensis, G. koladynensis, G. khawbungi*, *Garra* cf. *matensis, G. nigricollis*, *Garra* sp. Phawngpui and *Garra* sp. Cheu. Of these nine species, *G. flavatra* and *G. nigricollis* were only collected from Tuisi/Tisi River near Tisi Village, while the two unidentified species, *Garra* sp. Cheu and *Garra* sp. Phawngpui were only recorded from Cheu stream, near Sangau Village in Lunglei District, Mizoram.

The Kaladan River drainage is isolated (Lalramliana et al., 2018), bordered to the west by the Chittagong hill tract, which separates it from the Barak-Meghna drainage, and to the east by the Rakhine Yoma hill range, which isolates it from the Chindwin-Irrawaddy (Vishwanath, 2021). The Kaladan River drainage, being the largest river in Mizoram (Lawlor & Lalthanzara, 2021) and the most important river in southern Mizoram (Dikshit & Dikshit, 2014), is often overlooked, partly because of its remote location as well as difficulty in accessibility. The Kaladan River drainage however shows high fish diversity with distinct separation from other species, indicating that it represents an ecologically unique region (Barman et al., 2018). Further, the ichthyofauna of the Kaladan River drainage is poorly explored and there may be many endemic species (Anganthoibi & Vishwanath, 2010). This suggested that the Kaladan River drainage is equally important as the other major drainages of Northeast India.

Jaccard similarity index analysis shows that the Kaladan River drainage has lower similarity with the Barak River drainage and the Karnaphuli River drainage. Based on the current sampling result, *G. rakhinica, G. manipurensis, G. nigricollis*, and *G. flavatra* which were described and reported from the Rakhine Yoma by Kullander & Fang (2004), were also found in the Kaladan River drainage suggesting that the Kaladan River drainage, instead resembled the Rakhine River elements. These findings also support the drainage-based division of the freshwater fish fauna by Vishwanath (2021) which suggested that the region around the Kaladan River drainage and drainages along the western face of the Rakhine-Yoma represent a distinct geographic unit or ecoregion.

Further, the current findings also indicated that the Kaladan River drainage has higher *Garra* diversity compared to the Barak and Karnaphuli River drainage. The higher diversity in the Kaladan River drainage may be significantly influenced by the geographical location of the Kaladan River drainage. The Kaladan River basin and the few rivers of the Rakhine River drain along the coast of the Bay of Bengal at Sittwe. Consequently, it is hypothesized that the complex river system of the Kaladan River and the Rakhine River at the flood plains of Sittwe could possibly be led to overlapping in river courses, thereby mediating the exchange of fishes. Further, a preliminary analysis result based on molecular phylogenetics by Wang et al. (2024) suggested that the Irrawaddy River basin may be the origin of the speciation of *Garra* and that *Garra* species have undergone dispersal as well as reverse dispersal in these regions. Consequently, the higher species diversity and lower similarity of *Garra* in the Kaladan River drainage with nearby drainages within Mizoram could be due to the geographical proximity of the Kaladan River basin with the Rakhine River basin and alternately the adjacency of the Irrawaddy River basin and the Rakhine Rivers.

Based on the results obtained from the current analysis, it is observed that the Barak River drainage and the Karnaphuli River drainage show lower species diversity compared to the Kaladan River drainage. A total of 4 species were reported from the Barak River drainage (Roni et al., 2019). The Barak River drainage, which joins the greater Ganga-Brahmaputra River basin near the mouth is expected to have a higher fish diversity, yet, only four species of *Garra* namely *G. naganensis, G. manipurensis, Garra* cf. *substrictorostris* and *Garra* cf. *lissorhynchus* were collected during the entire study period. Recent studies have shown that five species of *Garra* were reported from the lesser studied Karnaphuli drainage (Lalramliana et al., 2017), however, only four species *of Garra* namely *G. dampaensis, G. manipurensis, G. nigricollis* and *Garra* sp. Karnaphuli were collected during the entire study period. The lower species diversity in the Barak River drainage and the Karnaphuli River drainage could be attributed to the fact that many parts of the rivers are close to human settlements and, as proposed by Damor (2024), loss of biodiversity and progressive destruction of habitat is hugely affected by anthropogenic activities such as deforestation, expansion of agricultural lands, pesticides, water pollution, illegal fishing and overfishing which consequently, may be responsible for lower *Garra* diversity in these river drainages. Further, the Barak River drainage and the Karnaphuli River drainage show a comparatively higher species similarity compared to the Kaladan River drainage. It is hypothesized that the complex river system of the Barak River drainage and the Karnaphuli River drainage at the flood plains of western Bangladesh could possibly be led to overlapping in river courses which may mediate the exchange of fishes.

The current findings show how little is understood about the divergence and dispersal of *Garra*. Also, the research findings could not conclude on how dispersal and speciation occur in the Kaladan, Barak and Karnaphuli River drainages. As a result, there is an urgent need to investigate the phylogeny, biogeography and diversification of the cyprinid *Garra* in these regions.

In conclusion, fourteen (14) species of *Garra* are currently recorded from Mizoram, that accounts for about 25% of the total species recorded from the northeastern regions of India. This indicated that Mizoram serves as a favourable hotspot for *Garra*. This may be attributed to the presence of clean freshwater, swift streams and riffles along with many pebbles and rocky substratum that provide suitable habitat for the rheophilic *Garra* to flourish.

CHAPTER 2

Morphological and molecular characterization of *Garra* from Mizoram

Introduction

The genus *Garra* predominantly consists of benthic fish that typically reside in fast-flowing rivers, streams, and riffles, where they secure themselves to the substrate with their adhesive mouthparts (Rath et al., 2019**;** Nagar et al., 2012; Shangningam et al., 2021). Some members of the genus are also found in pools, lakes, brackish water and caves (Arunachalam et al., 2013; Mousavi-Sabet & Eagderi, 2016). The cyprinid *Garra* is morphologically characterized by having an elongated subcylindrical body, a crenulated rostral fold, a modified lower lip that generally forms either a circular or ovoid callous pad and a suctorial disc with an anteromedian fold (Talwar & Jhingran, 1991; Stiassny & Getahun, 2007; Lothongkham et al., 2014). The mental adhesive apparatus is also called the mental adhesive disc, gular disc, labial suctorial disc or oral adhesive disc (Kottelat, 2020).

The snout morphology and oromandibular structures are crucial for the morphological identification and classification of *Garra* from South Asia and Southeast Asia (Nebeshwar and Vishwanath, 2013, 2017). The terminology used for describing the oromandibular structures of *Garra* and other disc-bearing cyprinids often differ. Zhang et al. (2002) described the oromandibular structures as follows: The fleshy flap of skin anterior to the callous pad that is formed by the anterior border of the mental adhesive disc is called the '*anteromedian fold*' while Kottelat (2020) termed this as the '*torus*'. The '*anterolateral lobe*' is the lobe-like structure, discretely marginated at both the lateral ends of the anteromedian fold that bridges the lateroposterior flap and the rostral fold. Kottelat (2020) renamed the anterolateral lobe as the '*labellum*'. The '*central callous pad*' is the central part of the mental adhesive disc, constituted by a fleshy pad of skin that represents most of the mental adhesive disc and is termed the '*pulvinus*' by Kottelat (2020). The '*latero-posterior flap*' is the membranous flap at the periphery of the lateral and posterior part of the central callous pad, designated as *'labrum*' by Kottelat (2020). Further, Kullander & Fang (2004) named the small papilliferous fold extending from each corner of the mouth the ‘pleated papilliferous fold’, which Kottelat (2020) termed the ‘*subtoral fold*’. However, these terminologies are used interchangeably. Figure 6 shows a schematic diagram of the mental adhesive disc (gular disc) of *Garra* with terminologies of mouthparts.

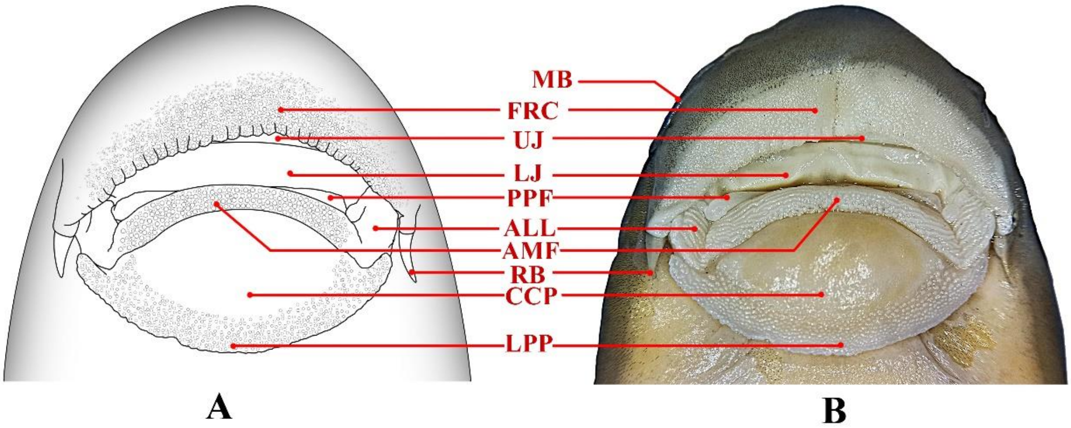


Figure 6. Oromandibular structure and terminologies of *Garra* sp. as suggested by Zhang et al. (2002) and Kottelat (2020). A) line drawing of mouth part of *Garra* sp Phawngpui and B) Photograph of mouth of *Garra* sp. Phawngpui showing MB, maxillary barbel; FRC, fimbriated rostral cap; UJ, upper jaw; LJ, lower jaw; PPF, pleated papilliferous fold; ALL, anterolateral lobe; AMF, anteromedian fold; RB, rostral barbel; CCP, central callous pad; LPP, lateroposterior flap.

Snout types of Southeast Asian *Garra*

Following Nebeshwar & Vishwanath (2017), *Garra* from the South and Southeast Asian regions are collectively classified into 5 different groups: (1) smooth snout (2) snout with rostral lobe (3) snout with rostral flap (4) snout with proboscis and (5) snout with transverse lobe. The division of species group was revised by Vishwanath (2021), whereby the grouping was simplified into 3 groups: (1) snout with smooth dorsal surface; (2) snout with transverse lobe (3) snout with proboscis.

However, the present study follows and discusses the classification based on Nebeshwar & Vishwanath (2017) instead of Vishwanath (2021) as the molecular phylogenetic analysis result based on *coi* and *cytb* conforms more towards the former (See Chapter 3).

According to Nebeshwar & Vishwanath (2017), *Garra* species with a '*smooth snout group*' (Fig. 7a) are characterized by having a smooth snout usually with a more or less rounded dorsal surface. Other characteristics such as rostral flap, rostral lobe, proboscis and transverse lobe are absent in this group. '*Snout with rostral lobe group*' (Fig. 7b) on the other hand has a fleshy, narrowly pointed triangular lateral projection that originates just above the origin of the rostral barbel directed posteriorly. The distal end of the rostral lobe is enclosed in the sublachrymal groove. The rostral lobe usually has small conical tubercles on its surface (Zhang & Kottelat, 2006). '*Species with rostral flap group*' (Fig. 7c) are characterized by having a pair of rostral flaps (a fleshy triangular lateral projection that may be broadly rounded or truncated and not elevated from the surface of the snout). The paired rostral flap is situated on the lateral side of the snout, slightly anterior to and above the origin of the rostral barbel. The '*proboscis bearing species group*' (Fig. 7d) is characterized by the presence of a fleshy protuberance that protrudes anterodorsally above the dorsal surface of the snout but does not extend beyond the snout tip. The proboscis may be elevated above the snout or embedded on the depressed rostral surface of the snout. Further, the proboscis may be trilobed, bilobed, unilobed or quadrate. Species of *Garra* with proboscis are usually characterized by having a transverse lobe. The proboscis and transverse lobe are generally tuberculated with small to large-sized tubercles. '*Species with transverse lobe group*' (Fig. 7e) on the other hand are characterized by having a transversely elongated fleshy fold that is posteriorly demarcated by a shallow or deep transverse groove over the anterodorsal surface of the snout. Further, the transverse lobe may be prominent, slightly elevated or indicated by an irregular patch of tubercles transversely arranged across the anterodorsal region of the snout. Table 7 shows the snout characteristics of different groups of *Garra* from Southeast Asia.

Table 7: Snout characteristics of different groups of *Garra* from Southeast Asia as suggested by Nebeshwar & Vishwanath (2017).

	Proboscis	Transverse lobe	Rostral flap	Rostral lobe
Proboscis bearing species	Present	Usually present	Absent	Absent
Rostral flap-bearing species	Absent	Occasionally present	Present	Absent
Rostral lobe-bearing species	Absent	Absent	Absent	Present
Transverse lobe-bearing species	Absent	Present	Absent	Absent
Smooth snout species	Absent	Absent	Absent	Absent

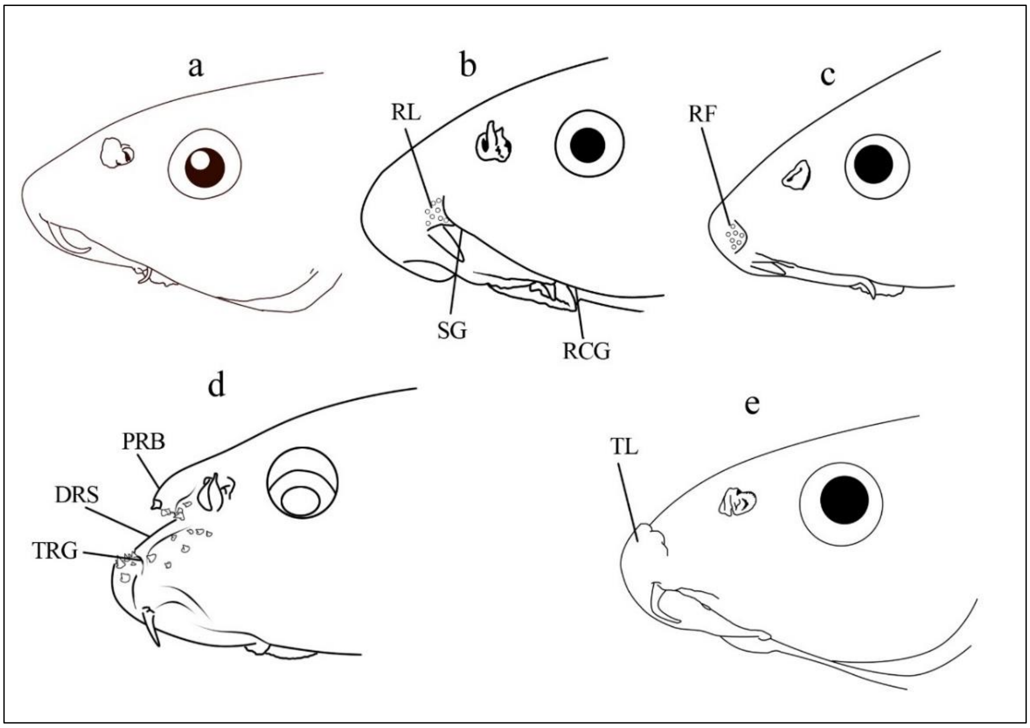


Figure 7. Different snout structures of *Garra* from South and Southeast Asia as proposed by Nebeshwar & Vishwanath (2017): a) species with smooth snout, b) species with rostral lobe, c) species with rostral flap, d) species with proboscis and transverse lobe and d) species with transverse lobe. RL, rostral lobe; RF, rostral flap; PRB, proboscis; SG, sublachrymal groove; RCG, rostral cap groove; TRG, transverse groove; TL, transverse lobe; DRS, depressed rostral surface.

Garra from Africa and the Middle East

Stiassny & Getahun (2007) classify *Garra* from Africa based on the disc type. Moritz et al. (2019) revised the African *Garra* and classified the 25 species of *Garra* from Africa based on the disc morphology. The disc type according to Stiassny & Getahun (2007) is as follows: Type A: Mental adhesive disc is weakly developed with a diminutive lateroposterior flap, mental adhesive disc often only consists of a central callous pad. Type C: Mental adhesive disc is well-developed with a distinct papillated lateroposterior flap. Type B: Mental adhesive conspicuous but usually has a partial and narrow lateroposterior flap that usually lacks papillae; intermediate between Type A and Type C.

On the other hand, the snout and oromandibular descriptions of most species of *Garra* from the Middle East are poorly provided, however based on relevant literature, some species viz. *G. shamal*, *G. sharq*, *G. amirhosseini*, *G. mondica, G. turcica, G. orontesi, G. meymehensis, G. tiam, G. hormuzensis* and *G. lorestanensis* have been mentioned to possess a proboscis on the snout (Sayyadzadeh et al., 2015; Esmaeili et al., 2016; Baycelebi et al., 2018; Kirchner et al., 2020; Zamani-Faradonbe et al., 2021a; Zamani-Faradonbe et al., 2021b; Baycelebi et al., 2021), while in other species, detailed morphology of the snout and oromandibular structures are poorly provided.

Other disc-bearing cyprinids

Other disc-bearing cyprinids in Southeast Asia are *Ageneiogarra* Garman, 1912, *Discogobio* (Lin, 1931), *Placocheilus* (Wu, 1977), *Discocheilus* (Zhang, 1997), *Vinagarra* (Nguyen & Bui, 2009), *Sinigarra* (Zhang & Zhou, 2012), *Ceratogarra* (Kottelat, 2020), *Guigarra* (Wang et al. 2022), *Pseudoplacoheilus* (Li et al., 2024) and *Supradiscus* (Li et al., 2024).

Members of the genus *Garra* from Southeast Asia are characterised by having a well-developed mental adhesive disc (Thoni, Gurung, and Mayden 2016) and are distinguished from other disc-bearing cyprinid by having the rostral cap crenulated (vs. non-crenulated in *Discocheilus*), anterolateral lobe and anteromedian fold joined (vs. separated in *Ageneiogarra*, *Discogobio*, *Vinagarra, Placocheilus* and

Pseudoplacocheilus), boundaries between central callous pad and lateroposterior flap conspicuous (vs. inconspicuous in *Guigarra*), presence of a well-developed anteromedian fold (vs. absent in *Sinigarra*), presence of a pair of rostral and maxillary barbel (vs. only a pair of maxillary barbel in *Ceratogarra*), and posterior end of adhesive disc (lateroposterior flap) continuous without a notch (vs. discontinuous with notch in *Supradiscus*) (Kottelat, 2020; Wang et al., 2022; Li et al., 2024).

Materials and Methods

Morphological analysis

Methods of morphological measurements, terminologies of oromandibular structures and classification based on the morphology of the snout follow Nebeshwar & Vishwanath (2013, 2017) where lateral lines are counted from the first pored scales above the pectoral girdle to the last pored scale on the caudal fin base. Transverse scales above lateral line scales are counted from the scale adjacent to the dorsal fin origin and downwards transversely to the scale above the lateral line scale. Transverse scales between the pelvic and anal fin origin are counted from the first scale anterior to the pelvic and anal fin origin respectively and upwards transversely to the scale below the lateral line scale. Scale adjacent to the dorsal fin-, pelvic fin- and anal fin origin are counted as ½. Predorsal scales are counted only if the predorsal scales are arranged regularly. Circumpeduncular scales are counted on the shallowest regions of the caudal peduncle. Dorsal fin-base scales and anal-fin base scales are counted from the first small scale originating from the base of the dorsal fin origin to the last small scale originating from the base of the last dorsal fin ray. Measurement data is given in percentage of standard length (SL), head length (HL), and percentage of distance between the pelvic fin origin and anal fin origin.

Fin ray count follows Kottelat (2001), where the last dorsal- and anal-fin rays articulating with the same pterygiophore as the preceding ray is counted as ½. For the caudal fin rays, the branched ray along with the principal caudal fin ray is counted. Morphometric data used in the identification and comparison of species is provided in Appendix 1.

Clearing and staining of specimens was done by modification of Taylor & Van Dyke (1985) where specimens are treated with 10% KOH and alizarin red S. Tissues were cleared and visualized under a microscope for osteology. The Weberian apparatus is considered equivalent to four vertebrae, hence counted as four (4). Counting of the abdominal vertebrae was done from the Weberian apparatus to the vertebrae with hemal spine situated anterior to the first anal fin pterygiophore. Counting of the caudal vertebrae was done from the vertebrae with hemal spine situated immediately behind the first anal fin pterygiophore to the last vertebrae. Counting of the predorsal vertebrae was done from the Weberian apparatus to the vertebrae with its neural spine immediately anterior to the first dorsal fin pterygiophore. Morphometric and meristic are provided in Figure 8.

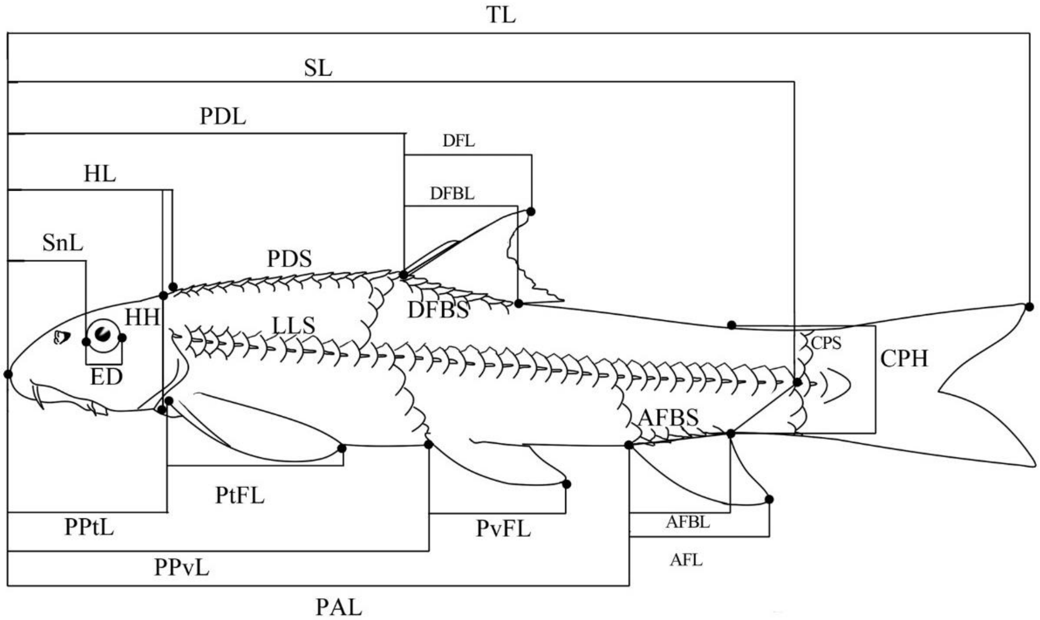


Figure 8. Illustration of *Garra* with smooth snout showing the morphometrics and meristic (TL, total length; SL, standard length; PDL, predorsal length; HL, head length; SnL, snout length; HH, head height; ED, eye diameter; PPtL, pre-pectoral length; PPvL, pre-pelvic length; PAL, preanal length; PtFL, pectoral fin length; DFL, dorsal fin length; DFBL, dorsal fin base length; PvFL, pelvic fin length; AFL, anal fin length; AFBL, anal fin base length; CPL, caudal peduncle length; CPH, caudal peduncle height; PDS, predorsal scales; LLS, lateral line scales; LTrS, lateral transverse scales; DFBS, dorsal fin base scales; AFBS, anal fin base scales; CPS, caudal peduncle scales)

Molecular analysis

DNA extraction

Tissues for DNA extraction were taken from ethanol-preserved specimens. Tissues were taken from the left side of the body. DNA extraction protocol follows Asahida et al. (1996) and Sambrook et al. (2001). Tissues were stored in molecular grade ethanol (*Omnis* Ethanol absolute, Jensen & Jensen GmbH & Co. Germany). Steps involved in the extraction of genomic DNA are given below:

20-40 gm of muscle tissues were taken and homogenized in 500 µL of high urea TES buffer (3M Urea)

↓

Adding 10-15 µL of Proteinase K (20mg/ml) and incubating it for 1 hour at 50°C.

↓

Adding 500 µL of Phenol Chloroform Isoamyl alcohol (PCI) and gently shaking the samples for 10-15 minutes followed by centrifugation at 14000 rpm for 10 minutes.

↓

Supernatant collected in a fresh centrifuge tube

↓

Adding 500 µL Chloroform Isoamyl alcohol (CI) and gently shaking the samples for 10-15 minutes followed by centrifugation at 14000 rpm for 10 minutes.

↓

Supernatant collected in a fresh centrifuge tube

↓

Adding chilled absolute ethanol (double the amount of supernatant) and storing overnight for ideal precipitation of DNA

↓

Centrifugation at 14000 rpm for 10 minutes.

↓

Decanting ethanol and washing with 70% ethanol followed by centrifugation at 14000 rpm for 5 minutes.

↓

Drying at room temperature

↓

Resuspension using 20 µL nuclease free water or 20 µL TE buffer.

Gene amplification and sequencing

Two mitochondrial markers, *cytochrome c oxidase subunit* I (*coi*) and *cytochrome b* (*cytb*) were selected for genetic analysis. For amplification of the *coi* gene Primer Fish F1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and Fish R1 (5'- TAGACTTCTGGGTGGCCAAAGAATCA-3') were used for amplifying the selected gene marker (Ward et al., 2005). The following are the PCR conditions used for amplification of the *coi* marker: Initial denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 53°C for 45 seconds and elongation at 72°C for 1 minute followed by final elongation at 72°C for 10 minutes. The *cytb* gene marker is amplified using the primer LACyp (5'-ATGGCA AGCCTACGAAAAAC-3') and HACyp (5'-TCGGATTACAAGACCGAT GCTT-3') following Tang et al., (2018). The following are the PCR conditions used for amplification of *cytb* marker: Initial denaturation at 94°C for 3 minutes followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds and elongation at 72°C for 1 minute and final elongation at 72°C for 10 minutes. Amplified DNA sequences are sent to Eurofins Genomics India Pvt. Ltd. Bangalore, India for Sanger dideoxy sequencing. Sequences are obtained in a FASTA format file and chromatograms are edited using FinchTV 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com). DNA sequences of both *coi* and *cytb* gene were blasted in NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) to find the nearest similar species.

Analysis of pairwise genetic distances

MEGA X version 10.2 (Kumar et al., 2018) was used to determine the pairwise genetic distances between the species. For the estimation of genetic distance, the variance estimation method was set to bootstrap method with 1000 bootstrap replicates. *p*-distance method was selected for the estimation of the pairwise genetic distances and rates of substitution among sites were considered to be gamma-distributed with Invariant sites (G+I). Further, only published data available on NCBI GenBank were used for the calculation of genetic distances. Sequences used in the analysis are provided in Table 19.

Results

Species Account

1. *Garra koladynensis* Nebeshwar & Vishwanath, 2017

Fig. 9: *Garra koladynensis,* 79.3 mm SL, 22.790710N, 93.097869E, Tiau River, Kaladan River drainage, near Sangau Village, Lawngtlai District, Mizoram, India.

Diagnosis: Morpho-meristic data are provided in Table 8 and Table 12 respectively. *G. koladynensis* is characterized by the following combination of characters: Proboscis trilobed; median lobe prominent with 3 large conical tubercles; lateral lobes with 3–4 tubercles. Anteromedian fold of mental adhesive disc slightly arched, reaching till half the length of the lower jaw. 33–34 lateral line scales, 8½ branched dorsal fin rays. 5½ branched anal fin rays. Transverse scales rows; above lateral line 4½; between lateral line and anal fin origin 4½. 3–4 scales between anus and anal fin origin. 5–6 dorsal fin base scales. 4–5 anal fin base scales.

Distribution: Kaldan drainage and its tributaries

2. ***Garra rakhinica* Kullander & Fang, 2004**

Fig. 10: *Garra rakhinica*, 68.2 mm SL, 22.791132N, 93.097944E, Tiau River, Kaladan River drainage, near Sangau Village, Lawngtlai District, Mizoram, India.

Diagnosis: Morpho-meristic data are provided in Table 8 and Table 11 respectively. *Garra rakhinica* is characterized by the following combination of characters: Snout smooth and rounded with rostral flap; rostral flap with 3-5 conical tubercles. Presence of a black horizontal stripe across the cheek arising from the anterolateral surface of the snout behind the sublachrymal groove and extending slightly beyond the posterior margin of the eye. Thick indistinct band on the upper half of the dorsal fin. Caudal fin deeply emarginate with thick dark indistinct W-shaped band on the posterior half. 6½ branched dorsal fin rays. 7½ branched pelvic fin rays 4½ branched anal fin rays. 30–31 lateral line scales. Transverse scale rows above lateral line 4½; between lateral line and anal fin origin 4½. Circumpeduncular scale rows 16. Predorsal scales 10–12. Scales between anus and anal fin origin 2–3. Dorsal-fin base scales 5–7. Anal-fin base scales 3–4.

Distribution: Kaldan drainage and its tributaries

3. ***Garra flavatra*, Kullander & Fang 2004**

Fig. 11: *Garra flavatra*, 50.9 mm SL, 22.350544N, 93.078315E, Tuisi/ Tisi River, Kaladan River drainage, near Tisi Village, Siaha District, Mizoram, India.

Diagnosis: Morpho-meristic data are provided in Table 8 and Table 11 respectively. *Garra flavatra* is characterized by the following combination of characters: Snout smooth and rounded. Rostral flap present; flap with 3-5 conical tubercles. Thick yellowish blotch on dorsal and lateral surface of body; yellowish bar/blotch located anterior to dorsal fin, immediately posterior to dorsal fin and on caudal peduncle region. Dorsal fin and anal fin with distinct submarginal band. Caudal fin with vertical black bands on lobe tips and vertical rows of irregular black spots across the middle of the caudal fin; caudal fin with W-shaped band. 6½ branched dorsal fin rays. 4½ branched anal fin rays. 7½ branched pelvic fin rays. 31 lateral line scales. Transverse scale rows above lateral line 3½. Transverse scale rows between lateral line and anal fin origin 4½. Gill rakers 13–14. Predorsal scales 9–11.

Distribution: Tuisi/ Tisi River, Kaladan River drainage

4. ***Garra dampaensis* Lalronunga et al., 2013**

Fig. 12: *Garra dampaensis*, 47.5 mm SL, 23.459881N, 92.337714E, Keisalam River, Karnaphuli River drainage, near Phuldungsei Village, Mamit District, Mizoram, India.

Diagnosis: Morphometric and meristic data are provided in Table 8 and Table 12 respectively. *Garra dampaensis* is characterized by the following combination of characters: Snout smooth with rostral flap; rostral flap with 5–10 conical tubercles. PPF reduced. Anteromedian fold of mental adhesive disc highly arched, reaching beyond the mid-length of lower jaw. 6½ branched dorsal fin rays. 31 lateral line scales. Transverse scales above lateral line 3½; between lateral line and anal fin origin 4–4½. Predorsal scales 10–12. Chest and belly scaled; scales deeply embedded. Gill raker 13. Scales with blackish spot at the center forming 6 narrow indistinct black stripes on caudal peduncle. Dorsal fin with submarginal black band. W-shaped band present on caudal fin.

Distribution: Karnaphuli River drainage

5. *Garra khawbungi* Arunachalam et al., 2014

Fig. 13: *Garra khawbungi,* 75.1 mm SL, 22.352904N, 93.076279 E, Tisi/ Tuisi River, Kaladan River drainage, near Tisi Village, Siaha District, Mizoram, India.

Diagnosis: Morpho-meristic data are provided in Table 8 and Table 11 respectively. *Garra khawbungi* is characterized by the following combination of characters: Snout with transverse lobe; proboscis absent. Transverse lobe of snout slightly elevated from snout tip with small to large sized tubercles. Medium to large-sized tubercles (16–22) present on the dorsal side of the snout. Black spot present at the upper angle of gill opening. Mental adhesive disc elliptical, width greater than length (disc width 1.5–1.7 times disc length). Anteromedian fold of mental adhesive slightly arched and not reaching the mid-length of the upper jaw. PPF present. 8½ branched dorsal fin rays. Anus closer to anal fin origin (38.3–41.4% distance between anal fin origin and pelvic fin origin. 36–37 lateral line scales. Gill raker count 22.

Distribution: Kaldan drainage and its tributaries

6. ***Garra naganensis* Hora, 1921**

Fig. 14: *Garra naganensis,* 73.5 mm SL, 24.015197N, 93.245971E, Tuila River, Barak River drainage, near Khawkawn Village, Saitual District, Mizoram, India.

Diagnosis: Morpho-meristic data are provided in Table 9 and Table 12 respectively. *Garra naganensis* is characterized by the following combination of characters: Snout smooth and rounded; without rostral flap or rostral lobe. Dorsal, pectoral, pelvic and anal fin hyaline. Anteromedian fold slightly arched barely extending beyond the base of the lower jaw. Central callous elliptical, relatively large; width greater than length (width 1.7–2.1 times length of callous pad). Groove between the central callous pad and the anteromedian fold narrow. PPF present, well-developed. 8½ branched dorsal fin rays. 7½ branched pelvic fin rays. 5½ branched anal fin rays. Caudal fin forked. Lateral line scales complete with 36–37 scales. Transverse scale rows between lateral line and dorsal fin origin 4½; between lateral line and anal fin origin 3½–4½. Predorsal scales 12. Chest and belly scales; chest scales deeply embedded. Gill raker count 19.

Distribution: Barak River drainage and its tributaries

7. *Garra manipurensis* Vishwanath & Sarojnalini, 1988

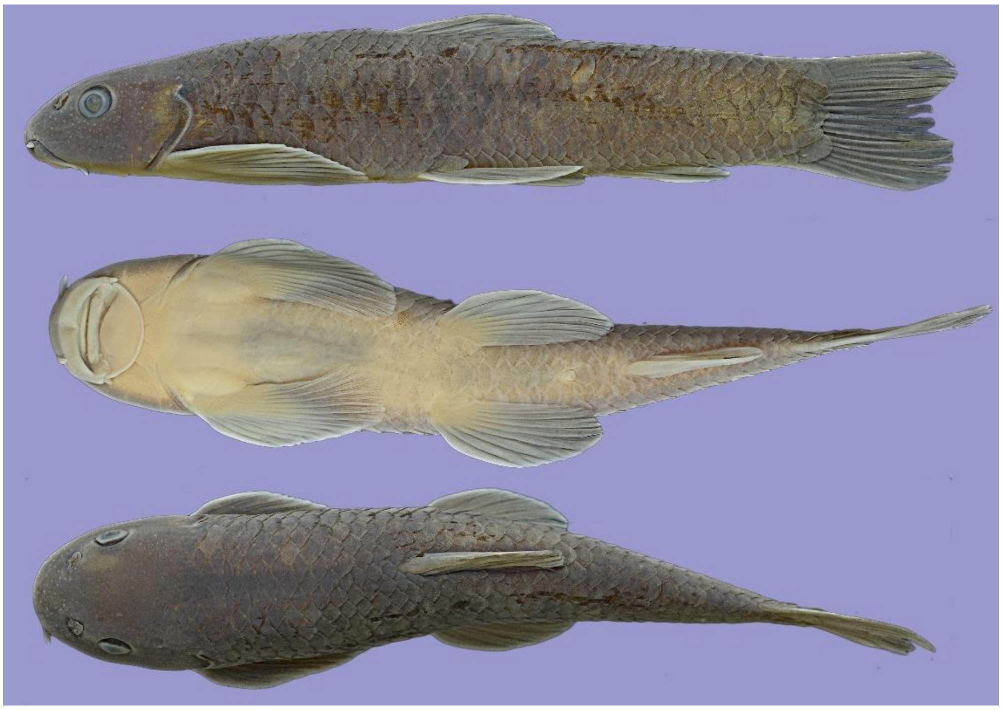


Fig. 15: *Garra manipurensis,* 51.6 mm SL, 22.809891N, 93.099266E, Tiau River, Kaladan River drainage, near Sangau Village, Lawngtlai District, Mizoram, India.

Diagnosis: Morpho-meristic data are provided in Table 9 and Table 12 respectively. *G. manipurensis* is characterized by the following combination of characters: Snout smooth and rounded. Rostral lobe present; rostral lobe with 3–6 small conical tubercles. Dark mid-lateral stripe present is smaller specimens, extending till the median lobe of the caudal fin. Anteromedian fold slightly arched; lateral extension of anteromedian fold reaching beyond the lateral margin of the central callous pad. PPF present. 7½ branched dorsal fin rays. Anus closer to anal fin origin than to pelvic fin origin (25–27% distance between anal fin- and pelvic fin origin). 34–35 lateral line scales. 3½–4½ transverse scales rows above lateral line, 4–4½ between lateral line and anal-fin origin. Predorsal scales 11–14. Chest and belly scaled; scales on chest deeply embedded. Scales between anus and anal fin origin 3–4. Dorsal-fin base scales 4–5. Anal-fin base scales 3–4. Total vertebrae 33–34.

Distribution: Kaladan River, Barak River and Karnaphuli River drainage.

8. ***Garra* cf. *substrictorostris* Roni & Vishwanath, 2018**

Fig. 16: *Garra* cf. *substrictorostris,* 89.2 mm SL, 23.793755N, 92.802974E, Tuirial River, Barak River drainage, near Tuirial Village, Aizawl District, Mizoram, India.

Diagnosis: Morpho-meristic data are provided in Table 9 and Table 12 respectively**:** *Garra* cf. *substrictorostris* is characterized by the following combination of characters: Proboscis present; proboscis unilobed with 5–6 tubercles. Mental adhesive disc relatively wider than long. 8½ branched dorsal fin rays. 5½ branched anal fin rays. Anus closer to anal-fin origin than to pelvic-fin origin (25–27% distance between pelvic fin origin and anal fin origin). 33–34 lateral line scales. Transverse scale rows; above lateral line 4½; between lateral line and anal fin origin 4½. Scales on chest and belly conspicuous. Predorsal scales 10. 3–4 scales between anus and anal fin origin. 5–6 dorsal fin base scales.

Distribution: Barak River drainage and its tributaries

9. ***Garra* cf. *matensis*, Nebeshwar & Vishwanath 2017**

Fig. 17: *Garra matensis*, 47.5 mm SL, 22.763575N, 92.826023E, Vansang River, Tributary of Ngengpui River, Kaladan River drainage, near Thualthu Village, Lunglei District, Mizoram, India.

Diagnosis: Morpho-meristic data are provided in Table 9 and Table 11 respectively. *Garra* cf. *matensis* is characterized by the following combination of characters: Snout rounded. Rostral flap present with 3–5 conical tubercles. Disc elliptical, disc width greater than length (disc width 48–51% HL, Disc length 34–35% HL). Papillated anterolateral lobe. Lateroposterior flap moderately rounded with relatively large papillae. 6½ branched dorsal fin rays. 7½ branched pelvic fin rays. 4½ branched anal fin rays. Anus closer to anal-fin origin than to pelvic-fin origin (22–23% distance between anal fin- to pelvic fin origin). 30–31 lateral line scales. Transverse scales rows above lateral line 3½; between lateral line and anal fin origin 3½–4½. Caudal fin deeply emarginated; distinct W-shaped band on caudal fin.

Distribution: Vansang River & Ngengpui River, Kaldan drainage.

10. *Garra nigricollis* Kullander & Fang, 2004

Fig. 18: *Garra nigricollis* 76.7 mm SL, 22.185262N, 93.041664E, Tusi River, Kaladan River drainage, near Khopai Village, Siaha District, Mizoram.

Diagnosis: Morpho-meristic data are provided in Table 10 and Table 11 respectively. *G. nigricollis* is characterized by the following combination of characters: Snout with transverse lobe; transverse lobe indistinct, demarcated from the snout by a shallow and indiscrete transverse groove. Dark brown or blackish bar extending from the pectoral fin base, across the nape to the trans-pectoral fin base. Anus closer to anal-fin origin than to pelvic-fin origin (35–37% distance between anal fin- to pelvic fin origin). Mental adhesive disc relatively small; elliptical, width greater than length (disc width 39–40 %HL, Disc length 23–28 %HL). Anteromedian fold of mental adhesive disc slightly arched, not reaching the mid-length of the lower jaw.

Distribution: Tuisi River, Kaladan River drainage and Keisalam River, Karnaphuli River drainage.

11. ***Garra* cf. *lissorhynchus* (McClelland, 1842)**

Fig. 19: *Garra* cf. *lissorhynchus*, 60.3 mm SL, 24.015197N, 93.245971E, Tuila River, Barak River drainage, near Khawkawn Village, Saitual District, Mizoram, India.

Diagnosis: Morpho-meristic data are provided in Table 10 and Table 11 respectively. *Garra* cf. *lissorhynchus* is characterized by the following combination of characters: Snout smooth with rostral flap. Posterior end of flap continuous with the sublachrymal groove. Anteromedian fold highly arched. Submarginal band on the dorsal fin. Posterior periphery of scales with small minute black spots, few scales with large black spots. 6½ branched dorsal fin rays. 7½ branched pelvic fin rays. 4½ branched anal fin rays. 33 lateral line scales. Transverse scale rows above lateral line 4½. Transverse scale rows between lateral line and anal fin origin 4½. 14 predorsal scales. Vent closer to anal fin origin than pelvic fin origin (34–36% distance between anal fin- and pelvic fin origin); scales between anus and anal fin origin 3–4. Gill rakers count 15.

Distribution: Barak River drainage and its tributaries.

12. ***Garra* sp. Cheu**

Fig. 20: *Garra* sp. Cheu, 42.7 mm SL, 22.677892N, 93.028165E, Cheu stream, Kaladan River drainage, near Sangau Village, Lawngtlai District, Mizoram, India.

Diagnosis: Morpho-meristic data are provided in Table 10 and Table 11 respectively. *Garra sp.* Cheu is characterized by the following combination of characters: Snout rounded and blunt. Rostral flap present with 3–5 conical tubercles. Distinct submarginal band on upper half of the dorsal fin. Disc elliptical, disc width greater than length (disc width 53–64% HL, disc length 30–42% HL). Groove between the central callous pad and the anteromedian fold narrow. 6½ branched dorsal fin rays. 7½ branched pectoral fin rays. 4½ branched anal fin rays. Anus closer to anal-fin origin than to pelvic-fin origin (28–32% distance between anal fin origin to pelvic fin origin). Distinct W-shaped band on caudal fin. 30–31 lateral line scales. Transverse scale rows; above lateral line 4½; between lateral line and pelvic fin origin 3½; between lateral line and anal fin origin 4½. Predorsal scales 10–12; arranged regularly. Scales on chest deeply embedded. Scales between anus and anal fin origin 2–3.

Distribution: Cheu stream near Sangau Village, Kaldan drainage.

13. *Garra* sp. Phawngpui

Fig. 21: *Garra* sp. Phawngpui, 99.5 mm SL, 22.677892N, 93.028165E, Cheu stream, Kaladan River drainage, near Sangau Village, Lawngtlai District, Mizoram, India.

Diagnosis: Morpho-meristic data are provided in Table 10 and Table 12 respectively. *Garra* sp. Phawngpui is characterized by the following combination of characters: Snout smooth and rounded without proboscis and transverse lobe; rostral flap and rostral lobe absent. Anteromedian fold of adhesive disc moderately arched narrowly reaching base of lower jaw. Central callous pad of mental adhesive disc wider than long (width of central callous pad 2–2.2 times the length of central callous pad); 40–41 lateral line scales; 8½ branched dorsal fin rays; 12–13 predorsal scales; 6–7 scales between anus and anal fin origin; Total vertebrae 41. Transverse scale rows; above lateral line 4½, between lateral line and anal-fin origin 4½. Chest and belly scaled; scales on chest deeply embedded.

Distribution: Cheu stream**,** Kaldan River drainage.

14. ***Garra* sp. Karnaphuli**

Fig. 22: *Garra* sp. Karnaphuli*,* 82.6 mm SL, 23.266721N, 92.395053E, Mar River, Karnaphuli River drainage, Mamit District, Mizoram, India.

Diagnosis: Morpho-meristic data are provided in Table 10 and Table 12 respectively. *Garra* sp. Karnaphuli is characterized by the following combination of characters: Proboscis present; proboscis unilobed. Transverse lobe with 16–22 tubercles. Mental adhesive disc relatively wider than long (disc width 46–55% HL, disc length 34.6–38.6% HL). 8½ branched dorsal fin rays. 7½ branched pelvic fin rays. 5½ branched anal fin rays. Anus closer to anal fin origin than to pelvic-fin origin (26–27% distance between pelvic fin origin and anal fin origin). 33–34 lateral line scales. Transverse scale rows; above lateral line 4½; between lateral line and anal fin origin 4½. Predorsal scales 10; regularly arranged. 3–4 scales between anus and anal fin origin. 5–6 dorsal fin base scales. 4–5 anal fin base scales.

Distribution: Karnaphuli River drainage and its tributaries.

Table 8: Morphometric data of *G. koladynensis, G. rakhinica, G. flavatra, G. dampaensis* and *G. khawbungi*.

	Garra koladynensis	*Garra rakhinica*	*Garra flavatra*	*Garra dampaensis*	*Garra khawbungi*
Standard length	68.6-130.6	44.7-97.7	45.2-51.2	54.5-71.5	71.2-132.6
% standard length					
Body depth	21.5 - 27.7	25.1-28.3	20.4-21.5	21.0-23.6	22.6-25.3
Head length	23.8 - 28.4	25.2-27.3	24.5-26.5	25.5-28.2	25.8-29.9
Head height at eye	15.9 - 17.8	13.7-16.5	13.5-14.8	13.3-14.5	14.5-16.5
Head height at occiput	18.2 - 20.9	18.1-21.6	15.4-16.8	15.3-16.2	16.3-19.0
Head width at opercle	20.6 - 22.7	18.5-20.4	19.0-20.3	20.1-22.4	17.6-20.3
Head width at nare	16.8 - 17.6	15.9-17.0	15.4-16.5	15.7-16.9	13.7-16.6
Body width at dorsal	20.5 - 24.3	15.6-17.8	15.9-17.1	16.1-18.4	16.6-18.4
Body width at anal	12.2 - 14.4	8.5-9.6	9.6-10.5	8.1-9.7	9.8-12.6
Caudal peduncle length	12.9 - 15.1	17.6-19.3	15.4-16.8	16.1-16.9	14.5-17.3
Caudal peduncle height	13.1– 15.9	16.6-18.4	15.0-16.5	14.8-16.4	13.3-15.7
Dorsal fin length	25.4 - 28.4	28.5-32.1	24.2-26.4	21.2-23.4	28.7-33.8
Dorsal fin base length	18.2 - 20.3	16.7-18.3	14.7-15.9	12.2-13.9	18.0-18.9
Pectoral fin length	22.1– 27.4	25.1-27.5	23.6-24.6	22.9-25.0	26.0-29.7
Pelvic fin length	21.3 - 24.8	22.9-25.2	20.4-21.2	19.5-21.5	22.4-24.8
Anal fin length	18.7 - 24.1	21.5-24.2	18.9-20.9	17.5-20.4	21.9-24.7
Anal fin base length	7.1– 9.8	7.1-8.4	6.7-7.6	5.6-9.2	7.4-9.0
Upper caudal fin lobe length	29.9 - 32.3	31.8-33.4	25.4-26.7	24.1-27.6	32.2-36.2
Lower caudal fin lobe length	31.3 - 33.2	28.7-30.5	25.7-28.9	24.0-25.4	31.0-33.7
Median caudal fin length	16.4 – 17.8	19.8-22.4	20.6-21.8	17.7-19.0	18.0-19.5
Pre-anal length	78.4 - 82.1	78.1-80.2	80.1-83.9	75.1-80.8	77.2-80.6
Pre-anus length	72.6 - 75.7	68.8-70.7	67.5-69.0	70.3-74.5	65.5-70.2
Pre-ventral length	53.2 - 56.1	53.7-56.2	54.6-56.5	50.2-54.5	52.7-55.0
Pre-dorsal length	48.0 - 52.7	52.3-55.8	50.6-53.7	53.4-55.0	49.0-51.9
Pre-pectoral length	20.2 - 23.4	23.5-25.1	22.9-23.7	21.9-24.3	24.2-26.8
Pelvic to anal length	23.1– 26.2	25.8-27.2	27.0-29.3	25.0-28.2	26.5-27.6
% vent to anal (pelvic-anal)					
Vent to anal fin length	21.8 - 30.3	28.4-32.5	45.7-47.3	20.1-22.4	38.3-41.4
% Head length					
Snout length	56.8 - 61.4	48.8-53.2	49.7-53.8	50.8-54.7	51.3-55.5
Eye diameter	16.6 - 20.2	20.2-24.6	17.3-18.6	17.7-20.6	17.9-22.5
Inter-orbital space	44.4 - 47.5	48.7-50.8	46.7-50.3	46.4-49.9	41.6-46.0
Disc length	37.5 - 42.8	32.5-34.7	34.1-34.6	29.8-34.3	23.6-28.2
Disc width	53.5 - 57.2	53.7-55.4	50.9-51.6	47.5-55.5	37.9-40.2
Callous pad length	26.2 - 29.3	25.2-26.8	23.8-26.9	19.8-25.1	11.6-15.1
Callous pad width	34.8 - 37.5	40.3-44.7	37.7-41.1	33.6-41.0	24.2-28.8

Table 9: Morphometric data of *G. naganensis, G. manipurensis. Garra* cf. *substrictorostris, Garra* cf. *matensis.*

	Garra naganensis	*Garra manipurensis*	*Garra* cf. *substrictorostris*	*Garra* cf. *matensis*
Standard length	66.1– 82.0	44.8 - 62.2	57.51– 99.94	47.54 - 48.06
% to standard length				
Body depth	21.6-23.4	18.5-20.9	21.1-25.4	22.3-23.7
Head length	22.9-24.1	24.2-27.5	24.9-26.6	25.6-27.5
Head height at eye	12.3-12.9	12.4-14.4	15.1-16.1	13.8-14.3
Head height at occiput	14.3-15.4	14.5-16.8	16.6-18.4	14.9-16.4
Head width at opercle	17.1-17.4	19.3-22.3	18.1-18.9	20.2-20.6
Head width at nare	12.2-12.2	14.4-15.8	15.3-16.6	15.5-16.4
Body width at dorsal	13.7-12.4	16.1-18.2	15.4-17.3	16.9-18.5
Body width at anal	7.3-7.3	8.8-10.9	8.3-10.4	9.1-10.6
Caudal peduncle length	15.9-14.6	15.8-17.5	13.2-15.5	14.5-15.7
Caudal peduncle height	13.2-13.4	12.2-14.6	13.2-14.5	13.9-15.0
Dorsal fin length	24.1-26.4	21.8-23.7	25.7-26.9	22.7-23.8
Dorsal fin base length	15.4-16.4	12.0-14.4	17.2-19.2	13.0-14.7
Pectoral fin length	21.5-24.4	24.7-26.4	22.4-24.3	24.7-24.8
Pelvic fin length	19.7-20.7	20.3-23.0	20.6-23.0	20.1-20.2
Anal fin length	18.4-20.0	17.7-19.8	19.3-21.3	18.8-19.2
Anal fin base length	7.8-8.7	6.5-8.5	7.5-9.6	7.5-8.9
Upper caudal fin lobe length	28.7-29.2	27.1-28.1	28.1-30.7	24.0-24.3
Lower caudal fin lobe length	27.0-29.1	27.0-29.7	29.6-31.2	24.0-26.0
Median caudal fin length	17.3-18.3	20.6-22.5	15.3-17.2	16.5-17.2
Pre-anal length	79.9-78.6	78.3-80.4	78.4-81.3	77.0-80.5
Pre-anus length	66.3-67.3	70.1-72.7	71.4-73.8	72.9-73.3
Pre-ventral length	50.2-52.8	51.2-53.9	54.4-55.9	54.6-57.7
Pre-dorsal length	47.7-50.3	50.7-52.7	48.7-50.2	52.2-53.8
Pre-pectoral length	21.5-21.0	20.9-22.9	23.6-24.7	22.6-24.1
Pelvic to anal length	30.0-27.9	25.3-28.7	25.9-28.6	25.0-26.4
% vent to anal (pelvic-anal)				
Vent to anal fin length	40.8-41.6	25.3-27.2	24.9-26.4	22.8-23.0
% to Head length				
Snout length	50.7-51.0	49.8-52.5	52.5-61.5	45.0-50.2
Eye diameter	19.4-20.9	20.2-24.4	17.0-20.2	19.2-20.8
Inter-orbital space	48.5-45.4	49.2-52.6	44.1-46.3	49.6-50.4
Disc length	22.4-24.1	26.6-30.5	34.6-38.6	34.4-35.4
Disc width	39.9-42.4	43.9-48.2	45.8-54.5	47.8-50.5
Callous pad length	18.1-15.5	16.0-19.1	22.8-25.5	23.8-25.0
Callous pad width	30.4-31.1	30.3-33.5	33.0-35.7	34.8-36.5

Table 10: Morphometric data of *G. nigricollis*, *Garra* cf. *lissorhynchus*, *Garra* sp. Cheu, *Garra* sp. Phawngpui and *Garra* sp. Karnaphuli.

	Garra. nigricollis	*Garra* cf. *lissorhynchus*	*Garra* sp. Cheu	*Garra* sp. Phawngpui	*Garra* sp. Karnaphuli
Standard length	76.7–117.5	50.1-90.6	41.6-50.3	69.5-112.9	57.3-110.3
% to standard length					
Body depth	24.9-25.9	20.7-23.4	17.2-20.9	17.3-26.6	19.4-22.9
Head length	27.6-30.4	24.4-24.8	24.4-27.1	22.6-25.8	23.2-31.6
Head height at eye	14.5-15.4	12.7-13.1	11.3-13.0	11.0-12.5	13.6-15.6
Head height at occiput	17.8-18.0	14.8-15.3	12.8-14.6	12.2-14.1	15.1-17.6
Head width at opercle	19.4-21.0	17.7-18.1	18.1-20.5	16.9-18.8	16.7-20.8
Head width at nare	14.8-15.5	13.5-14.0	14.0-17.1	12.6-15.4	14.2-19.2
Body width at dorsal	14.3-19.3	12.1-14.1	13.6-15.8	12.2-14.1	15.2-17.7
Body width at anal	10.0-11.6	8.4-9.0	7.7-10.4	6.9-8.8	8.8-10.0
Caudal peduncle length	14.4-15.5	13.9-14.4	12.4-18.1	13.4-16.1	15.1-17.8
Caudal peduncle height	13.2-14.1	13.8-14.2	11.8-13.0	11.7-12.9	13.7-14.0
Dorsal fin length	26.0-27.1	21.1-21.5	19.5-21.8	20.6-24.2	22.8-27.6
Dorsal fin base length	15.9-16.8	13.6-15.0	10.5-12.8	14.3-15.7	14.6-18.0
Pectoral fin length	24.8-29.1	22.8-23.3	21.7-23.0	20.3-23.6	21.8-23.6
Pelvic fin length	22.7-25.7	19.2-20.1	18.4-20.2	18.7-20.3	18.4-21.3
Anal fin length	20.9-22.7	17.9-19.4	16.6-17.8	17.1-18.5	19.1-20.0
Anal fin base length	7.9-9.2	7.2-7.4	6.2-8.1	6.8-8.7	7.8-8.4
Upper caudal fin lobe length	31.0-34.0	23.8-26.9	21.4-24.4	29.1-31.7	27.6-31.4
Lower caudal fin lobe length	30.7-34.8	24.0-27.2	20.6-23.5	28.3-30.2	27.1-32.7
Median caudal fin length	17.9-22.0	17.3-18.8	16.5-18.8	17.6-20.4	15.5-16.7
Pre-anal length	79.2-80.5	77.7-79.3	10.6-79.2	78.6-80.9	78.1-79.9
Pre-anus length	69.5-71.0	70.0-70.1	69.5-72.3	66.0-69.2	69.3-72.7
Pre-ventral length	53.6-55.4	54.4-55.2	53.4-56.7	49.9-55.2	51.6-53.5
Pre-dorsal length	50.5-54.8	52.0-53.8	51.8-54.1	47.4-49.7	45.7-49.7
Pre-pectoral length	25.2-27.5	22.2-22.3	22.3-24.4	20.0-23.3	23.2-25.2
Pelvic to anal length	27.1-27.9	23.9-25.0	23.2-26.1	27.9-30.3	27.7-29.2
% vent to anal (pelvic-anal)					
Vent to anal fin length	35.5-37.3	33.9-36	27.9-31.5	40.2-43.8	25.8-27.0
% to Head length					
Snout length	51.0-56.2	45.7-51.1	46.5-53.7	50.7-53.8	53.7-59.6
Eye diameter	19.8-21.9	19.5-20.5	13.9-21.5	14.2-17.2	15.8-25.3
Inter-orbital space	42.5-48.1	47.2-47.8	46.6-52.2	44.1-51.6	40.2-45.3
Disc length	23.0-28.1	32.8-34.5	29.8-42.6	22.9-25.7	30.8-37.8
Disc width	39.2-40.9	48.2-50.5	53.2-63.9	48.7-52.5	44.7-58.8
Callous pad length	13.7-17.6	20.0-24.5	18.3-26.2	16.1-18.2	19.6-24.4
Callous pad width	23.6-27.8	33.4-36.0	33.0-42.6	35.5-39.8	31.3-38.1

Table 11: Meristic data of *G. nigricollis, G. khawbungi, Garra* cf. *matensis, G. flavatra, G. rakhinica, Garra* sp. Cheu and *Garra* cf. *lissorhynchus*.

		Garra nigricollis	*Garra khawbungi*	*Garra* cf. *matensis*	*Garra flavatra*	*Garra rakhinica*	*Garra* sp. Cheu	*Garra* cf. *lissorhynchus*
Branched dorsal fin rays		8½	8½	ii, 6½	6½	7½	6½	6½
Branched pectoral fin rays		13½-14½	14½	13½	13½	13½	13½	12½
Branched pelvic fin rays		7½	7½	7½	7½	7½	6½	6½
Branched anal fin rays		5½	5½	4½	4½	4½	4½	4½
Caudal fin rays		19	19	19	19	19	19	19
Total lateral line scales		34-35	36-37	30-31	31	30-31	32-33	32-33
Lateral transverse scales	Dorsal fin origin to lateral line	4½	4½	3½	3½	3½	3½	4½
	Pelvic fin origin to lateral line	3-3½	3	3–3½	3	3-3½	3½	3-3½
	Anal fin origin to lateral line	3½	4½	3½–4½	4-4½	4½	4½	4½
Pre–dorsal scales		11-12	11	10-11	9-11	10-11	11–13	14
Circumpeduncular scales		16	16	16	16	16	16	16
Scales between anus and anal–fin origin		4-5	5-6	2-3	6	3	3-4	3-4
Total vertebrae		34	34	29-30	30	30	32	31
	Abdominal vertebrae	23-24	24	19-20	20	19	21	21
	Caudal vertebrae	10-11	10	10-11	10	11	11	10
	Predorsal vertebrae	10-11	10	10	10	9	11	11
Gill rakers		14-15	22	14	13-14	15	13	15
Pharyngeal teeth		5,5,2	5,3,2	5,3,2	5,4,2	5,5,2	5,4,2	5,5,2
Dorsal fin base scales		5-6	6-8	5-7	4	6-7	5-6	5-6
Anal fin base scales		4-5	4-5	2-4	3	3-4	3-4	3-4

Table 12: Meristic data of *Garra dampaensis, Garra* sp. Phawngpui, *Garra naganensis, Garra* sp. Karnaphuli, *Garra* cf. *substrictorostris, Garra manipurensis* and *Garra koladynensis*.

		Garra dampaensis	*Garra* sp. Phawngpui	*Garra naganensis*	*Garra* sp. Karnaphuli	*Garra* cf. *substrictorostris*	*Garra manipurensis*	*Garra koladynensis*
Branched dorsal fin rays		6½	8½	8½	8½	8½	7½	8½
Branched pectoral fin rays		12½-13½	13½	12½	13	13½	12½-13½	12½
Branched pelvic fin rays		6½	8½	7½	7½	7½	7½	7½
Branched anal fin rays		4½	5½	5½	5½	5½	4½	5½
Caudal fins		19	19	19	19	19	19	19
Total lateral line scales		30-31	40-41	37	34	32-33	33-34	32-33
Lateral transverse scales	Dorsal fin origin to lateral line	3½	4½	4½	4½	4½	4½	4½
	Pelvic fin origin to lateral line	3½	2½–3½	3½	3½	3	3-3½	2½
	Anal fin origin to lateral line	4	3½–4½	3½-4½	4½	4½	4½	4½
Pre–dorsal scales		10-12	12–13	12	10	10	11-12	9-11
Circumpeduncular scales		16	16	16	16	16	16	16
Scales between anus and anal–fin origin		2-3	6–7	6-7	4	3-4	3-4	3-4
Total vertebrae		30	40–41	37	32	32	32-33	32
	Abdominal vertebrae	19-20	27	25	21	20-21	20-21	21
	Caudal vertebrae	10-11	13–14	12	11	11-12	12	11
	Predorsal vertebrae	10	12–13	11	9	9	9-10	9
Gill rakers		13	13	19	22	21	11	21
Pharyngeal teeth		5,4,2	5,3,2	5,5,2	5,4,2	5,4,2	4,3,2	5,3,2
Dorsal fin base scales		5-6	6–7	5-6	5-6	6-7	4-5	5-7
Anal fin base scales		4-5	4	3-4	4-5	4-5	3-4	3-5

Grouping of *Garra* from Mizoram based on the snout type

Following Nebeshwar and Vishwanath (2017), *Garra* from Mizoram can be classified into five different groups based on the morphology of the snout and oromandibular structures. Detailed classification of *Garra* from Mizoram based on the morphology of the snout is provided in Table 13.

Table 13: Classification of *Garra* from Mizoram based on the snout morphology

Snout type	Species	Distribution
Snout with proboscis	*Garra koladynensis*	Kaladan River drainage
	Garra cf. *substrictorostris*	Barak River drainage
	Garra sp. Karnaphuli	Karnaphuli River drainage
Snout with rostral lobe	*Garra manipurensis*	Barak, Karnaphuli and Kaladan River drainage
Snout with rostral flap	*Garra* cf. *matensis*	Kaladan River drainage
	Garra flavatra	Kaladan River drainage
	Garra rakhinica	Kaladan River drainage
	Garra sp. Cheu	Kaladan River drainage
	Garra dampaensis	Karnaphuli River drainage
	Garra cf. *lissorhynchus*	Barak River drainage
Snout with transverse lobe	*Garra khawbungi*	Kaladan River drainage
	Garra nigricollis	Kaladan & Karnaphuli River drainage
Snout smooth	*Garra naganensis*	Barak River drainage
	Garra sp. Phawngpui	Kaladan River drainage

Morphomeristic differences of smooth snout group

Two species belonging to the smooth snout group are collected namely, *G. naganensis* and *Garra* sp. Phawngpui. Morphological differences between the two species are provided in Table 14. Figure 23 shows the oromandibular structures of *G. naganensis* and *Garra* sp. Phawngpui.

Table 14: Morphomeristic differences among smooth snout species of *Garra* from Mizoram.

	Garra naganensis	*Garra* sp. Phawngpui
Disc width (% HL)	39.9 - 42.4	48.7-52.5
Head width at nare (%HL)	12.2 - 12.2	12.6 - 15.4
No. of lateral line scales	37-39	40-41
Abdominal Vertebrae	25	27
Gill raker count	19	13
Distribution	Barak River drainage	Kaladan River drainage

Morphomeristic differences of species with transverse lobe on snout

Two species of smooth snout species are collected and recorded namely *G. khawbungi* and *G. nigricollis*. Morphological differences between the two species are provided in Table 15. Figure 24 shows the oromandibular structures of *G. khawbungi* and *G. nigricollis*.

Table 15: Morphomeristic differences among *Garra* with snout with transverse lobe from Mizoram.

	Garra nigricollis	*Garra khawbungi*
Anus-anal fin origin distance (% distance between pelvic fin- to anal fin origin)	35.5–37.3	38.3–41.4
Number of lateral line scales	34–35	36–37
Transverse lobe on snout	Weakly developed	Prominent
Trans-pectoral band	Present	Absent
Distribution	Karnaphuli & Kaladan River drainage	Kaladan River drainage

Morphology of the rostral lobe-bearing species

G. manipurensis is the only species collected from Mizoram that have a rostral lobe on the snout. For morphometric and meristic measurements, see Tables 9 & 12.

Morphomeristic differences of species with rostral flap on snout

Six species of *Garra* having rostral flap are collected from Mizoram namely, *Garra* cf. *matensis, G. flavatra, G. rakhinica, Garra* sp. Cheu, *Garra* cf. *lissorhynchus* and *G. dampaensis*. Morphological differences among rostral flap-bearing species are provided in Table 16. Figure 26 shows the oromandibular structures of the rostral flap-bearing species.

Table 16: Morphomeristic differences among 'snout with rostral flap species group' of *Garra* from Mizoram.

	Garra matensis	*Garra flavatra*	*Garra rakhinica*	*Garra* sp. Cheu	*Garra* cf. *lissorhynchus*	*G. dampaensis*
Lateral line scales	30–31	31	30–31	32–33	32–33	30–31
Disc width (%HL)	47.8–50.5	50.9–51.6	53.7–55.4	53.2–63.9	32.8–34.5	47.5–55.5
Anus to anal fin origin distance (% distance between pelvic fin- to anal fin origin)	22.8–23.0	45.7–47.3	28.4–32.5	27.9–31.5	33.9–36	20.1–22.4
Total vertebrae	29–30	30	30	32	31	30
Branched pelvic fins	**7½**	7½	7½	6½	6½	6½
Branched dorsal fin rays	6½	6½	7½	6½	6½	6½
Distribution	Kaladan River drainage	Kaladan River drainage	Kaladan River drainage	Kaladan River drainage	Barak River drainage	Karnaphuli River drainage

Morphomeristic differences of species with proboscis on the snout

Three species of *Garra* having a proboscis on the snout are collected namely, *G. koladynensis, Garra* sp. Karnaphuli and *Garra* cf. *substrictorostris*. Morphological differences of the three proboscis-bearing species are provided in Table 17. Figure 27 shows the snout and oromandibular structures of the proboscis-bearing species.

Table 17: Morphomeristic differences among *Garra* with 'snout with proboscis species group' from Mizoram

	Garra koladynensis	*Garra* sp. Karnaphuli	*Garra* cf. *substrictorostris*
Proboscis	Trilobed	Unilobed	Unilobed
Body width at dorsal	20.5–24.3	15.2–17.7	15.4–17.3
Lateral line scales	32–33	34	32–33
Sublachrymal groove	Shallow, continuous or discontinuous with rostral cap groove	Shallow, discontinuous with rostral cap groove	Deep, discontinuous with rostral cap groove
Upper lip	Reduced or absent	Reduced as a thin membranous flap	Prominent, covering the upper jaw
Distribution	Kaladan River drainage	Karnaphuli River drainage	Barak River drainage

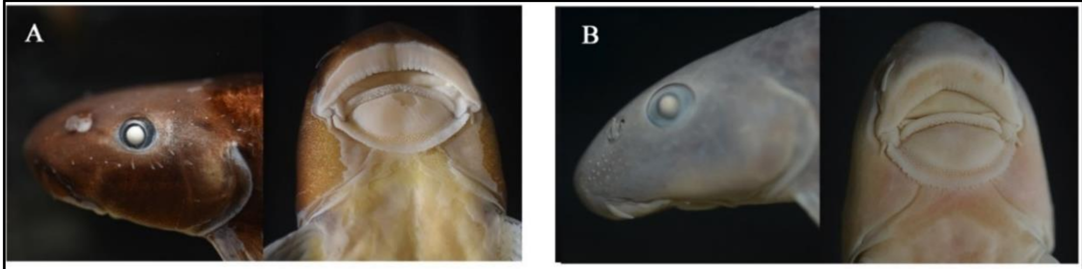


Fig. 23: Snout and oromandibular structure of *Garra* with smooth snout (**A**) *Garra* sp. Phawngpui (**B)** *G. naganensis.*

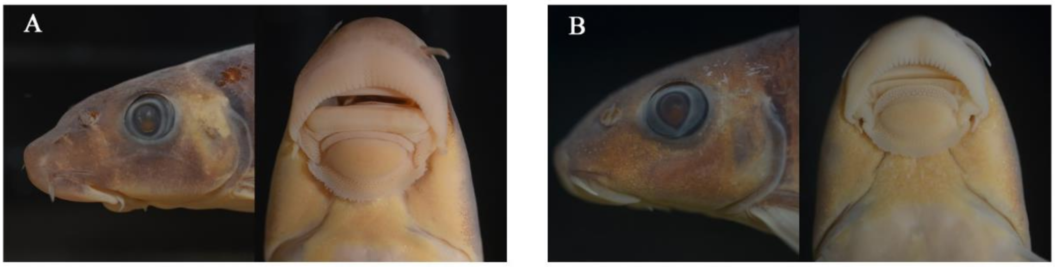


Fig. 24: Snout and oromandibular structure of *Garra* with transverse lobe from Mizoram (**A**) *G. nigricollis* from Karnaphuli River drainage (**B)** *G. nigricollis* and **(C)** *G. khawbungi* from Kaladan River drainage

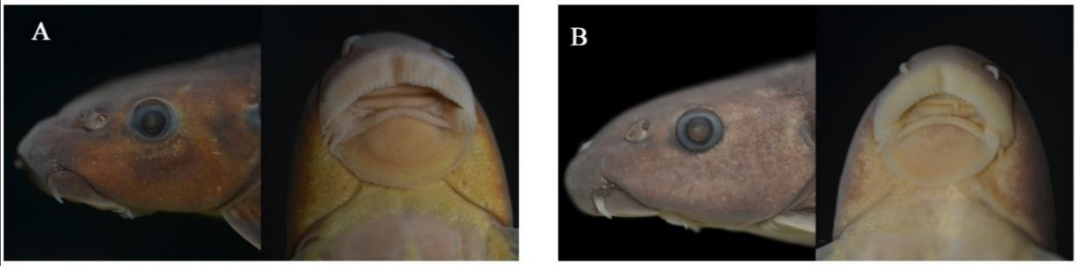


Fig. 25: Snout and oromandibular structure of *G. manipurensis* from (**A**) Barak River drainage (**B)** Kaladan River drainage (**C)** Karnaphuli River drainage

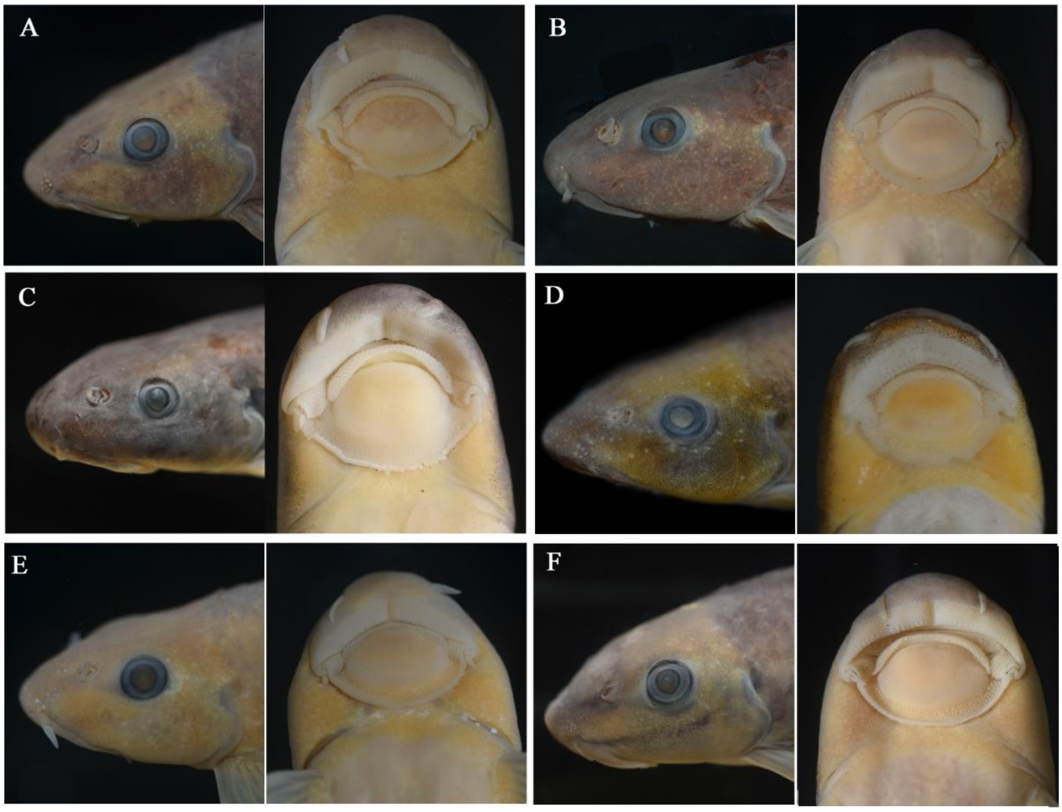


Fig. 26: Snout and oromandibular structure of *Garra* with rostral flap from Mizoram (**A**) *G. dampaensis* (**B**) *Garra* cf. *lissorhynchus*, (**C**) *Garra* sp. Cheu, (**D**) *Garra* cf. *matensis*, (**E**) *G. flavatra*, (**F**) *G. rakhinica*

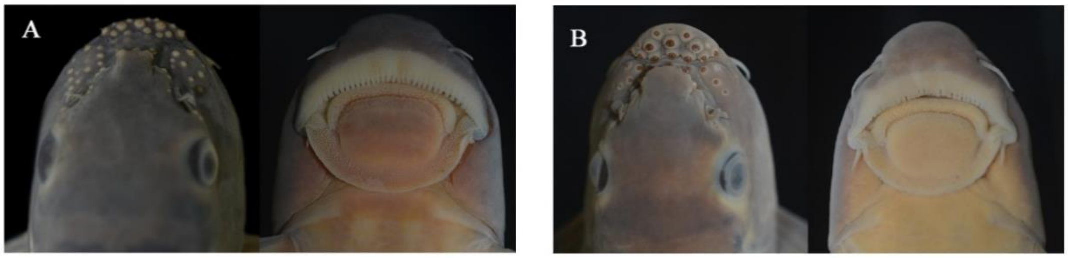


Fig. 27: Snout and oromandibular structure of *Garra* with proboscis from Mizoram (**A**) *G. koladynensis*, (**B**) *Garra* cf. *substrictorostris*, (**C**) *Garra* sp. Karnaphuli

General Morphological characteristics of *Garra* from Mizoram

Based on the morphological analysis, all species of *Garra* collected and identified from Mizoram share several morphological similarities. *Garra* from Mizoram are all collectively characterized by the following combination of characters:

1. Two pairs of barbels present (rostral and maxillary barbel)
2. Rostral cap is well-developed, papillated and fimbriated. Rostral cap may either partially or fully cover the upper jaw.
3. Mental adhesive disc well-developed, anteromedian fold prominent. Anteromedian fold separated from the central callous pad by a shallow to deep narrow transverse groove. Lateroposterior flap and central callous pad well demarcated. Anterolateral lobe present in all species. Pleated papilliferous fold may be present, reduced or absent.
4. Lateral line complete, lateral line scale count ranges between 30–41.
5. Predorsal region scaled and regular. Predorsal scale count ranges from 9–14.
6. Number of scales between anus and anal fin origin (range 2–7)
7. Anus/Vent closer to anal fin origin than to pelvic fin origin. Shortest distance between anus and anal fin origin recorded in *G. dampaensis* (20–22% distance between anal fin origin and ventral fin origin). Largest distance between anus and anal fin origin in *G. flavatra* (45–47% distance between anal fin origin and ventral fin origin).
8. Three rows of pharyngeal teeth.
9. Circumpeduncular scale count 16.

Key to species of *Garra* from Mizoram

1. Snout with proboscis ……………………………………………………..…...……2
   Snout without proboscis ……………..……………………………………………4
2. Proboscis trilobed……………………………..……..……..…..*Garra koladynensis*
   Proboscis unilobed ………………………………………………………………….. *3*
3. Caudal peduncle length 15-18 %HL; Upper lip present and covering the upper jaw; 32-33 lateral line scales ……………..………..… *Garra* cf. *substrictorostris*
   Caudal peduncle length 13-15 %HL; Upper lip present, reduced as thin membranous flap; 33-34 lateral line scales ……………… *Garra* sp. Karnaphuli
4. Snout smooth without transverse lobe ………..……………………..………… 5
   Snout with or without transverse lobe; rostral flap may be present…………… 7
5. Snout smooth with rostral lobe; dorsal fin rays 7½ ……...….. *Garra manipurensis*
   Snout smooth without rostral lobe; dorsal fin rays 8½ ....……………………..6
6. Total lateral line scales 40-41; abdominal vertebrae 27 …. *Garra* sp. Phawngpui
   Total lateral line scales 36-37; abdominal vertebrae 25 ….. *Garra naganensis*
7. Snout with transverse lobe; rostral flap absent ……………..……………….. 8
   Snout with or without transverse lobe; rostral flap present ….………………..… 9
8. Transverse lobe prominent with tubercles, black spot at upper gill opening; transverse scales rows 4½/1/4½ ……………………………..… *Garra khawbungi*
   Transverse lobe inconspicuous; transverse scale rows 4½/1/3½, black band behind opercle extending above head to trans-pectoral fin origin.… *Garra nigricollis*
9. Dorsal fin with 7½ branched fin rays ..………………………….….. *Garra rakhinica*
   Dorsal fin with 6½ branched fin rays; distinct W-shaped band on caudal fin .. 10
10. Total lateral line scale counts 32 or more……………………...…………… 11
    Total lateral line scale counts less than 32 ……..……………………………. 12
11. Gill rakers 13; Disc width 53.2-63.9 %HL ..…….……………… *Garra* sp. Cheu
    Gill rakers 15; Disc width 32.8-34.5%HL ..…...……..… *Garra* cf. *lissorhynchus*
12. Anus closer to anal fin origin (distance between anus and anal fin origin 20-23 %distance between anal fin- and pelvic fin origin) …………………………… 13

Anus at nearly midpoint (distance of anus 45-47 %distance between anal fin- and pelvic fin origin); Vertical rows of irregular black spots on caudal fin …………………………………...…………………………..……... *Garra flavatra*

13. Snout length 45-50 %HL; branched pelvic fin rays 7½, anus to anal fin origin distance 22-23 %distance between anal fin- and pelvic fin origin …………………………………………………..………..…….. *Garra* cf. *matensis*

    Snout length 51-55 %HL; branched pelvic fin rays 6½, anus to anal fin origin distance 20-22 %distance between anal fin- and pelvic fin origin ….…………………………………………….…….……...….. *Garra dampaensis*

Genetic distances

Pairwise genetic distances were calculated for all available *coi* and *cytb* sequences in NCBI GenBank. Table 19 shows the DNA sequences with references used for the estimation of pairwise genetic distances. Table 18 shows the pairwise genetic distance between *Garra* species.

Based on the analysis of *coi* and *cytb* gene, the following observations were made: -

1. Interspecific genetic distances of all species of *Garra* included in the analysis (47 species) range from 3.7–36.6% in *coi* gene and 4.8–32.2% in *cytb* gene.
2. Closest genetic similarity is observed between G. *nasuta* (NCBI Accession number: JX074219) and *Garra* cf. *substrictorostris* from the Barak River drainage (pairwise genetic distance = 3.7% in *coi* gene and 4.8% in *cytb* gene).
3. The interspecific distance between the 14 species of *Garra* collected from Mizoram ranges from 4.1%–25.8% in *coi* gene and 7.9–30.2% in *cytb* gene.
4. The closest interspecific distance (between *Garra* from Mizoram) is observed between the transverse lobe-bearing species, *Garra khawbungi* and *Garra nigricollis* (*p*-distance = 4.1% in *coi* gene)
5. The largest interspecific genetic distance (between *Garra* from Mizoram) is observed between the rostral flap-bearing G. *rakhinica* and the rostral lobe-bearing species *G. manipurensis* (*p*-distance = 25.8% in *coi* gene).

Table 18: Pairwise distance analysis of *Garra* from Mizoram. Evolutionary Divergence was estimated using pairwise distance analysis in MEGA 11 (Tamura et al., 2021). This analysis involved 14 nucleotide sequences (*coi*) and the number of nucleotide base differences between sequences are shown.

	Garra sp. Cheu	*Garra dampaensis*	*Garra flavatra*	*Garra* sp. Karnaphuli	*Garra khawbungi*	*Garra koladynensis*	*Garra* cf. *lissorhynchus*	*Garra manipurensis*	*Garra* cf. *matensis*	*Garra naganensis*	*Garra nigricollis*	*Garra* sp. Phawngpui	*Garra rakhinica*	*Garra* cf. *substrictorostris*
Garra sp. Cheu	0.0													
Garra dampaensis	5.2	0.0												
Garra flavatra	9.4	10.5	0.0											
Garra sp. Karnaphuli	17.1	17.9	16.0	0.0										
Garra khawbungi	15.6	16.0	15.3	11.9	0.0									
Garra koladynensis	16.2	18.0	13.7	5.1	12.4	0.0								
Garra cf. *lissorhynchus*	10.3	11.7	8.2	16.1	15.1	14.8	0.0							
Garra manipurensis	21.8	24.3	25.2	25.7	20.5	23.3	25.1	0.0						
Garra cf. *matensis*	4.3	5.7	8.8	16.1	13.6	15.5	8.9	21.0	0.0					
Garra naganensis	16.1	17.1	15.0	13.6	10.1	12.4	16.2	21.1	15.1	0.0				
Garra nigricollis	15.2	16.7	14.8	12.0	4.1	12.1	16.1	21.5	13.8	9.8	0.0			
Garra sp. Phawngpui	15.6	17.6	16.5	12.6	10.6	12.3	15.7	18.7	14.6	8.7	11.5	0.0		
Garra rakhinica	15.9	18.0	15.7	22.2	22.5	19.6	15.4	25.8	16.5	21.7	20.8	22.8	0.0	
Garra cf. *substrictorostris*	17.6	20.2	14.6	5.9	11.7	5.8	16.9	23.2	17.4	11.7	12.7	12.2	21.8	0.0

Discussion

During the entire study period between 2020-2024, fourteen (14) species of *Garra* were collected from Mizoram. The species of *Garra* from Mizoram comply with the morphological classification based on the snout types by Nebeshwar & Vishwanath (2017). Morphological, meristic, and genetic distance analysis indicated that species of *Garra* from Mizoram are characteristically and genetically unique from their congeners. Detailed discussions on *Garra* from Mizoram is given below:

Snout with rostral lobe

A species of *Garra* having a rostral lobe collected from all three drainages of Mizoram. The species closely resembles *G. manipurensis* and it is uniquely differentiated from all other *Garra* species from Mizoram by having a rostral lobe (vs. absent in all other species). *G. manipurensis* was described by Nebeshwar and Sarojnalini (1998) from the Barak River drainage. Nebeshwar and Vishwanath (2017) redescribed *G. manipurensis* from the Kaladan River drainage and synonymized *G. vittatula* Kullander & Fang (2004) with *G. manipurensis*. Morphometric and comparison of the snout morphology indicated that the rostral lobe-bearing species from Mizoram is *G. manipurensis.*

Snout with rostral flap

Rostral flap-bearing species from South and Southeast Asia are *G. flavatra, G. paralissorhynchus, G. nigricollis, G. propulvinus, G. poecilura, G. rakhinica*, *G. namyaensis* and *G. lissorhynchus* (Nebeshwar & Vishwanath, 2017). *G. dampaensis* from the Karnaphuli River drainage was placed among the smooth snout group by Nebeshwar & Vishwanath (2017). However, morphological observation of the holotype of *G. dampaensis* (PUCMF 12001) and paratype (PUCMF 12002, 10 exs) shows that the species possesses a rostral flap. *G. dampaensis* is therefore considered to be a member of the 'species with rostral flap group'.

Six species of *Garra* with rostral flap are collected from Mizoram. Based on morphological comparison with relevant literature and NCBI blast results, species such as *G. rakhinica, G. flavatra,* and *G. dampaensis* were identified at the species level. However, the taxonomy of three other species were uncertain and could not be concluded. The reasons are as follows:

From the Vansang River, Kaladan River drainage, a species of *Garra* that closely resembles *G. matensis* was collected. *G. matensis* was described from Mat River, near Thualthu Village in Mizoram by Nebeshwar & Vishwanath (2017). *G. matensis* is characterized by having a rostral flap with four to seven small conical tubercles; 30-31 lateral-line scales; 6½ branched dorsal-fin rays; central callous pad of mental adhesive disc small (width 34-38 % HL; length 22–24 % HL). Sampling was conducted at two different river systems, Mat River and Vansang River (a tributary of the Ngengpui River), Kaladan River drainage near Thualthu Village. *Garra* species that fit the description of *G. matensis* was not collected from Mat River however, the *Garra* species collected from Vansang River closely resemble *G. matensis.* Since the species is collected from a different river system and not the type locality, the species is designated here as *Garra* cf. *matensis*.

From the Barak River drainage, a species of *Garra* that resembles *G. lissorhynchus* was collected. McClelland (1842) described *G. lissorhynchus* as *Platycara lissorhynchus* from Kaysah Mountains (Khasi Hills), Assam and Menon (1964) indicated that the species are distributed in the Brahmaputra River system, Assam. However, *G. lissorhynchus* was also reported from the Tuivai River and Barak River drainage by Vishwanath (1993). Although the rostral flap-bearing species from the Barak River drainage closely resembles the morphological characteristics of *G. lissorhynchus*, yet the species could not be accurately identified. Also, *Garra* belonging to the Lissorhynchus complex (Menon, 1964) are poorly studied and understood. The species is therefore designated here as *Garra* cf. *lissorhynchus.*

From the Kaladan River drainage, a species of *Garra* that possesses a rostral flap on the snout was collected. It is characterized by having 32-33 lateral line scales, 3½ transverse scale rows between lateral lines and 4½ transverse scales between lateral line and ventral fin origin. Morphological and meristic characteristics analysis indicated that *Garra* sp. Cheu differs from other congeneric rostral flap-bearing species by having fewer branched pelvic fin rays (6½ vs. 7½ in *G. matensis*, *G. flavatra* and *G. rakhinica*), fewer predorsal scales (11–13 vs. 14 in *G. lissorhynchus*) and more vertebrae (32 vs. 30 in *G. dampaensis*). Based on the morpho-meristic comparisons, the species of *Garra* collected near Sangau Village does not fit the description of any *Garra* species and hence, is represented here as *Garra* sp. Cheu.

Pairwise genetic analysis based on the *coi* and *cytb* gene indicated that *Garra* cf. *matensis* is genetically distinct from other rostral flap bearing species by 4.2-16.5% in *coi* gene. *Garra* cf. *lissorhynchus* also differs from congeneric smooth snout species by 8.2-15.3% in the *coi* gene. The unidentified species, *Garra* sp. Cheu also differs from the other rostral flap-bearing species included in the study by a pairwise genetic difference of 4.2-15.9% in the *coi* gene.

Menon (1964) classified species of *Garra* having caudal fin with W-shaped band as belonging to the Lissorhynchus complex. Rostral flap-bearing species such as *G. paralissorhynchus, G. flavatra, G. dampaensis, Garra* cf. *matensis, Garra* cf. *lissorhynchus* and *Garra* sp. Cheu are characterized by having a distinct W-shaped band on the caudal fin except for *G. rakhinica,* where the caudal fin is characterized by a thick indistinct W-shaped band. It is therefore assumed that the presence of a rostral flap may be one important characteristic feature of the Lissorhynchus complex.

Snout smooth without rostral flap, rostral lobe, transverse lobe, or proboscis

From the Barak River drainage, a smooth snout species of *Garra* that closely resembles *Garra naganensis* was collected. *G. naganensis* was described by Hora (1964), and in the original description, the type locality was mentioned as Senapati stream near Karong village, Naga Hills, Assam District. Nebeshwar & Vishwanath (2017) also indicated that *G. naganensis* is distributed in the Barak-Meghna River drainage and belongs to the smooth snout species group. Morphological and meristic comparison of smooth snout species from the Barak River drainage in Mizoram conforms to the description of *G. naganensis*. Consequently, the species from the Barak River drainage is therefore identified as *G. naganensis*.

From the Kaladan River drainage, a smooth snout species of *Garra* was collected that is characterized by having 40–41 lateral line scales, 4½ transverse scale rows between lateral lines and dorsal fin origin, 8½ branched dorsal fin rays, 12-13 predorsal scales and 6–7 scales between anus and anal fin origin. Morphological and meristic characteristics of the smooth snout *Garra* from Kaladan River drainage do not comply with the morphological characteristics of congeneric smooth snout species. It differs from its smooth snout congeners by the following; from *G. annandalei* in having predorsal scales (12−13 vs. 8–9); from *G. lamta* and *G. chaudhurii* in having more lateral line scales (40–41 vs. 30–38); from *G. lungongza* and *G. compressa* in having more branched dorsal fin rays (8½ vs. 7−7½); from *G. chakpiensis* and *G. ukhrulensis* in having a greater disc width (48.7−52.5 % vs. 36–48 %HL); and from *G. chivaensis* in having more branched dorsal fin rays (8½ vs. 6); Based on the morphological comparisons, the species of *Garra* collected near Mt. Phawngpui does not fit the description of any *Garra* species and hence is represented here as an unidentified species, *Garra* sp. Phawngpui.

Analysis *coi* and *cytb* genes indicated that *G. naganensis* and *Garra* sp. Phawngpui are genetically distinct from their congeners. Analysis of 45 species of *Garra* based on the *coi* and *cytb* gene shows that the nearest genetically similar species of *G. naganensis* is *Garra* sp. Phawngpui (pairwise genetic difference 8.6% in *coi* gene) and the two species differ from their congeners by more than 10% pairwise genetic distance.

Snout with proboscis

Garra species having a proboscis that closely resembles *G. koladynensis* was collected from the Kaladan River drainage. Nebeshwar & Vishwanath (2017) described *G. koladynensis* from the Kaladan River drainage and placed it among the 'proboscis-bearing species group'. Morphological, meristic, snout morphology and oromandibular structures of the proboscis-bearing species from the Kaladan River drainage in Mizoram conform to the characteristics of *G. koladynensis,* hence identified here as *G. koladynensis*.

From the Barak River drainage, *Garra* species with proboscis on the snout was collected. Two species of *Garra* with proboscis, *G. substrictorostris* Roni & Vishwanath (2018) and *G. paratrilobata* (Roni et al., 2019) were described from the Barak River drainage. The species collected from the Barak River drainage is distinguished from *G. paratrilobata* in having a unilobed (vs. trilobed) proboscis on the snout. However, morphological and meristic analysis indicated that the species closely resembles *G. substrictorostris* except in the number of transverse lateral scales above the lateral line. The species is therefore designated here as *Garra* cf. *substrictorostris*.

Another species of *Garra* having a proboscis on the snout was collected from a Mar River near Marpara Village, Karnaphuli River drainage, Mamit District, Mizoram. The species is characterized by having a unilobed proboscis on the snout; 33-34 lateral line scales; Transverse scale rows above lateral line 4½; Scales on chest and belly embedded, conspicuous. Predorsal scales 10; 3–4 scales between anus and anal fin origin. 5–6 dorsal fin base scales and 4–5 anal fin base scales. Although the proboscis-bearing species from the Karnaphuli River drainage closely resemble *Garra* cf. *substrictorostris* from the Barak River drainage, genetic analysis indicated that it genetically differs from *Garra* cf. *substrictorostris* by a pairwise genetic difference of 5.9% in *coi* gene and from its congeners by 4.9-28.7% in the *coi* gene. The species is therefore designated here as *Garra* sp. Karnaphuli.

Snout with transverse lobe

The transverse lobe-bearing species of South and Southeast Asia according to Nebeshwar & Vishwanath (2017) are *G. arupi, G. jenkinsoniana, G. kempi, G. magnidiscus, G. prashadi, G. elongata, G. mcclellandi, G. mullya, G. periyarensis, G. nethravathensis* and *G. ceylonensis*. From the Kaladan River drainage, a species that resembled *G. khawbungi* was collected. *G. khawbungi* was described from the Kaladan River drainage by Arunachalam et al., (2014) and is characterized by having a transverse lobe on the snout. Morphological and meristic comparison of the transverse lobe–bearing species from Tuisi River, Kaladan River drainage conforms to the morphological descriptions of *G. khawbungi* and is identified herein as *G. khawbungi*.

Garra nigricollis

Garra species that closely resemble *G. nigricollis* was collected from both the Kaladan River drainage and the Karnaphuli River drainage. Earlier studies by Solo & Lalnuntluanga (2018) and Lalramliana et al. (2020) also shows that *G. nigricollis* is distributed in the Kaladan River drainage and the Karnaphuli River drainage respectively. *G. nigricollis* was described by Kullander & Fang (2004) from the Rakhine Yoma and in the original species description, it was indicated that it possesses a rostral lobe. However, Nebeshwar & Vishwanath (2017) suggested that *G. nigricollis* instead possess a rostral flap and thus was included among the 'species with a rostral flap group'. Morphometric and meristic analysis indicated that among many other similar results, the species have a black band originating from one side of the pectoral fin base, extending across the nape to the other side of the pectoral fin base, which is a distinguishing characteristic of *G. nigricollis*. The species is therefore identified as *G. nigricollis*.

However, detailed observation of the snout structure of *G. nigricollis* showed that the rostral lobe (Kullander & Fang, 2004), later indicated as rostral flap by Nebeshwar & Vishwanath (2017) is rather a lateral extension of the transverse lobe and not a true rostral flap. In *G. nigricollis* and *G. khawbungi*, both the lateral ends of the transverse lobe tapers and posteriorly directed on the anterolateral surface of the snout, appearing as a rostral flap. Further, all rostral flap-bearing species of Mizoram are characterized by having an emarginated to deeply emarginated caudal fin with a W-shaped band and reduced PPF. In contrast, *G. nigricollis* from Mizoram is characterized by having a forked caudal fin without a W-shaped band and well-developed PPF. Genetic analysis also indicates that *G. nigricollis* is genetically similar to congeneric transverse lobe-bearing species *G. khawbungi* (96.2% similarity in *coi* gene) rather than the rostral flap-bearing species (84.3-88.9% similarity in *coi* gene). Further, phylogenetic results (see Chapter 3 Figure 28) also shows that *G. nigricollis* forms the same clade as *G. khawbungi i*ndicating that *G. nigricollis* suggesting that it closely resembles *G. khawbungi*.

In conclusion, several studies on species revalidations, synonymization and descriptions of new species of *Garra* have been published in recent years. Some notable mentions are: a new genus *Ceratogarra* was established to accommodate *G. cambodgiensis* and *G. fasciacauda* (Kottelat, 2020); *G tyao* synonymized with *G. rakhinica* (Zirkunga et al., 2023); *G. minimus* synonymized with *G. quadratirostris*, *G. nigricauda* synonymized with *G. arunachalensis*, *G. alticaputus* and *G. kimini* synonymised with *G. birostris,* and *G. tyao* synonymized with *G. rakhinica* along with the description of a new species, *G. hexagonarostris* from Chindwin basin (Nebeshwar et al., 2024); a new genus *Supradiscus* and *Pseudoplacocheilus* erected by Li et al. (2024) to accommodate *G. micropulvinus* and *P. cryptonemus* respectively.

Revelations from these studies and earlier studies by experts along with the current research findings suggested that the genus is constantly subjected to taxonomical modifications, further suggesting the need for taxonomical revision at the global level. The present chapter provides the morphological and meristic characteristics of *Garra* from Mizoram along with the molecular data (*coi* and *cytb* gene) that will expectantly aid in future studies of *Garra*.

CHAPTER 3

Phylogenetic analysis of *Garra* from Mizoram

Introduction

Phylogeny studies the evolutionary history and descent of a group of taxa from a common ancestor and the time of their divergence (Patwardhan et al., 2014). The use of molecular data in phylogenetics reveals relationships between rates of genetic evolution and speciation, suggesting that rapid genetic changes often accompany speciation events (Webster et al., 2003). Combining morphological and molecular-based phylogenetic methods improves the accuracy of determining the phylogenetic relationships among organisms (Patwardhan et al., 2014).

The development of suitable taxonomic markers has allowed researchers in taxonomy and systematics to address taxonomic uncertainties and biodiversity challenges by establishing significant phylogenetic relationships (Esa et al., 2012). Mitochondrial genes are often utilized for phylogenetic studies because these sequences are easy to obtain and also because they contain various information essential for distinguishing between species and understanding the divergence of species within a population (Vrijenhoek, 1998; Galtier et al., 2009).

Recently, there has been growing interest in the application of molecular techniques for identifying fish species (Ward et al., 2009). Mitochondrial genes like *cytb* and *coi* genes are frequently utilized for DNA barcoding in fish (Esa et al., 2012). The mitochondrial *cytb* gene has been widely employed in systematic studies (Farias et al., 2001). However, the mitochondrial *coi* gene has also gained immense use in the barcoding of fish as well (Ward et al., 2005, 2009). The application of these mitochondrial genes has significantly contributed to taxonomic validation and the discovery of new species.

Phylogeny of Southeast Asian *Garra*

The phylogeny of the genus *Garra* remains ambiguous, partly due to the absence of a global phylogenetic study and a lack of adequate molecular data. While some researchers (Yang & Mayden 2010; Wang et al., 2022) consider *Garra* as a monophyletic group, others (Tang et al., 2009; Zheng et al., 2012) consider the genus as polyphyletic. Additionally, the evolutionary divergence of the genus in Southeast Asia, the Middle East, and Africa is not well understood. Several authors have conducted phylogenetic analyses of the genus *Garra* in Southeast Asia, which are summarized below:

Phylogenetic analysis of the African and Asian *Garra* by Tang et al. (2009) includes 7 species of *Garra* from Africa and Asia. Based on the phylogenetic results, Tang et al. (2009) infer that the genus *Garra* is monophyletic and further indicated that the genus *Garra* probably originated from Asia. Yang & Mayden (2010) also performs a comprehensive phylogenetic analysis of the Tribe Labeonini Bleeker, 1859 using four nuclear genes; *recombination activating gene*, *Rhodopsin*, *interphotoreceptor retinoid binding protein* gene and *early growth response protein* 2B gene. Based on the phylogenetic analysis that includes 12 species of *Garra* from Africa, South Asia, China and Southeast Asia, Yang & Mayden (2010) indicated that the genera *Garra* form a monophyletic group.

Zheng et al. (2010) also constructed a phylogenetic relationship for the subfamily Labeoninae Bleeker, 1859 using two nuclear genes and three mitochondrial genes (*coi, cytb* and *16s*). The analysis includes five *Garra* species from Africa, South and Southeast Asia. Zheng et al. (2010) concluded that the genus *Garra* was not monophyletic since two independent lineages were identified; one lineage included *G. orientalis*, *G. mirofrontis* and *G. tengchongensis* which formed a sister clade with *Akrokolioplax bicornis* and *Crossocheilus burmanicus*; while another lineage includes *G. imberba* and *G. micropulvinus* that form a sister clade with *Placocheilus cryptonemus*.

Yang et al. (2012) analyzed the phylogenetic relationship of the cyprinid tribe Labeonini using *coi, cytb, 16S ribosomal RNA, NADH dehydrogenase subunit 4* and

NADH dehydrogenase subunit 5 genes. The analysis includes 32 *Garra* species along with the type species of *G. lamta*. In the analyses, all the species designated as *Garra* (except for *G. imberba* and *G. micropulvinus)* were nested within the same clade, denoted as 'Subtribe Garraina Bleeker, 1863'. However, *G. imberba* and *G. micropulvinus* along with *Placocheilus, Bangana, Discogobio* etc. are nested in a separate clade. Yang et al. (2012) therefore revalidated the genus *Ageneiogarra* to accommodate *G. imberba* and *G. micropulvinus*. Further, Yang et al. (2012) resolved the taxonomic position of *Horalabiosa joshuai* and *Phreatichthys andruzzii* by synonymizing *H. joshuai* and *P. andruzzii* with *Garra*. Based on the phylogenetic result of Yang et al. (2012) all species included in the study form a monophyletic group except *G. fasciacauda* and *G. cambodgiensis.*

Zheng et al. (2012) analyze the phylogeny of the subfamily Labeoninae that includes 14 species that are designated as *Garra*. The phylogenetic analysis was conducted using *rag*1, *rh, egr2b* and *interphotoreceptor retinoid binding protein* (*irbp*) genes. The phylogenetic results of Zheng et al. (2012) indicated that all the species assigned as *Garra* (except *G. micropulvinus* and *G. imberba*) were nested within the same clade with *C. burmanicus* and *A. bicornis* suggesting that *Garra* is polyphyletic. It was also proposed from the phylogenetic results that the disc-bearing *Discogobio* and *Garra* have different ancestors. Further, Zheng et al. (2012) infer that the oromandibular characters of the subfamily Labeoninae, although morphologically similar in many ways, have evolved separately (parallelly) and addedly indicated that these morphological characters have little systematic and phylogenetic significance among the different genera.

Wang et al. (2022) perform a phylogenetic analysis of the Labeoninae fish while describing a new genus, *Guigarra* from Guangxi Zhuang Autonomous Region, China. The phylogenetic relationship was inferred from concatenated sequences of *coi, cytb* and *rag1* genes. Based on their phylogenetic results, Wang et al. (2022) suggested that the genus *Garra* was monophyletic. Although Wang et al. (2022) indicated that the genus *Garra* is monophyletic, the analysis included only two *Garra* species, *G. mirofrontis* and *G. orientalis,* which was not enough to confirm the monophyly of *Garra.* Further, Wang et al. (2022) could not give a conclusive remark on the taxonomic position of the genera *Sinigarra, Ageneiogarra, Vinagarra* and *Placocheilus* and indicated that these genera need further revisions.

The most recent phylogenetic analysis of the oromandibular disc-bearing genera of the subfamily Labeoninae was conducted by Li et al. (2024). In their study, Li et al. (2024) constructed the phylogeny of the disc-bearing genera by comparing 68 species belonging to 21 genera using three mitochondrial genes (*coi, cytb* and *ND4)* and two nuclear genes. The analysis includes 31 species that were designated as *Garra*. Based on the morphological analysis and phylogenetic results, Li et al. (2024) addressed the taxonomical ambiguities of some *Garra* encountered by Yang et al. (2012), Zheng et al. (2012) and Wang et al. (2022) which are explained below*:*
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- *Placocheilus dulongensis* was synonymized with *Garra* based on molecular and phylogenetic analysis.
- A new genus *Supradiscus* was erected to accommodate the phylogenetically distinct *Garra micropulvinus*.
- The genus *Ceratogarra* was revalidated to accommodate *Garra fasciacauda* and *G. cambodgiensis.*
- The genus *Vinagarra* was revalidated to accommodate taxonomically ambiguous *Garra findolabium*.
- The taxonomy of the genus *Ageneiogarra* was unclear, however Li et al. (2024) tentatively accommodated the species *Garra imberba*, *G. hainanensis* and *G. nujiangensis* within the genus *Ageneiogarra* and indicated that further study was required to elucidate the taxonomic status of these species.
- Li et al. (2024) also determined that based on the phylogenetic result, the genu *Garra* is monophyly, however, the monophyly of the genus could not be concluded and that future phylogenetic studies supplemented by more species of the subfamily Labeoninae is required.

Materials and Methods

Samples of *Garra* were collected from the three different river drainages of Mizoram. *Garra* specimens for molecular study were collected per the "The Mizoram Fisheries (Amendment), Act, 2022" (Act No. 2 of 2022) of the Department of Fisheries, Government of Mizoram. Ethical clearance (Appendix 2) was also obtained from the current institution, Pachhunga University College (PUC).

Members of the Tribe Garrini Bleeker 1863 were used as an ingroup following Li et al. (2024) and included the genus *Garra* and *Tariqilabeo*. Members of the Tribe Semilabeonini Li et al., 2024 were used as an outgroup and included the following genera: *Supradiscus, Placocheilus, and Pseudoplacocheilus*. Table 19 shows the mitochondrial sequences (*coi* and *cytb* gene) of species, source, sequence code, NCBI accession number and references. Mitochondrial DNA sequences of *Garra* from Africa and the Middle East were not included in the current phylogenetic analysis. The developed sequences were deposited to NCBI GenBank (see Table 20)

Phylogenetic Analysis

Alignment of the two mitochondrial gene sequences was done using the ClustalW algorithm in MEGA 11 (Tamura et al., 2021). Any inconsistencies, including gaps and missing data on the aligned sequences were checked manually and eliminated. The two mitochondrial sequences were concatenated using MEGA 11.

The best fitting nucleotide substitution model = HKY+G+I (Hasegawa-Kishino-Yano with gamma distribution and invariant sites) was estimated in jModelTest (Posada, 2008). A phylogenetic tree was constructed using the Bayesian Inference (BI) method in MrBayes version 3.2.7 (Ronquist et al., 2012). For BI analysis, the following settings were applied: nucleotide substitution model (nst) = 2, rates = invgamma, code = vertmt, number of generations=2000000 ($1x20^6$). All other commands were set to default. BI analysis was stopped when the SD reached 0.01 or lower.

Table 19: Taxa examined in the current study with specimen voucher, references and GenBank accession numbers for each gene. The species names of the downloaded DNA sequences were retained as uploaded to avoid confusion in the taxonomic status of the species.

	Species	***Voucher***	***cytb***	***coi***	**Reference**
1	*Akrokolioplax bicornis*	KIZ05207	GU086552	N/A	Zheng et al. (2010)
2	*crossocheilus burmanicus*	CTOL3941	JX074224	JX074142	Yang et al. (2012)
3	*Garra arupi*	BOF31	N/A	KF511538	Dhar et al. (2015)
4	*Garra bicornuta*	CTOL3965	JX074238	JX074156	Yang et al. (2012)
5	*Garra ceylonensis*	CTOL3903	JX074252	JX074172	Yang et al. (2012)
6	*Garra dengba*	IHB:2016032482	MH243429	N/A	Deng et al. (2018)
7	*Garra dulongensis*	KIZ041106081	JQ864590	N/A	Wang et al. (2014)
8	*Garra flavatra*	N/A	AP011410	AP011410	Yang et al. (2012)
9	*Garra fuliginosa*	CTOL3897	JX074255	JX074176	Yang et al. (2012)
10	*Garra gotyla*	CTOL1919	JX074256	JX074177	Yang et al. (2012)
11	*Garra gravelyi*	N/A	N/A	LC189819	Kano et al. (2017)
12	*Garra hughi*	GH	MW314150	MW311120	Sudasinghe et al. (2021)
13	*Garra kalakadensis*	GAK_133	MW314203	N/A	Sudasinghe et al. (2021)
14	*Garra kempi*	CTOL3973	JX074243	JX074161	Yang et al. (2012)
15	*Garra lamta day*	CTOL3969	JX074239	JX074157	Yang et al. (2012)
16	*Garra lamta hamilton*	CTOL3970	JX074240	JX074158	Yang et al. (2012)
17	*Garra lissorhynchus*	CTOL3972	JX074242	JX074160	Yang et al. (2012)

18	*Garra mirofrontis*	KIZ20050325009	JQ864584	JQ864605	GenBank
19	*Garra mlapparaensis*	GMT1	MW314147	MW311116	Sudasinghe et al. (2021)
20	*Garra mullya*	CTOL3964	JX074237	JX074155	Yang et al. (2012)
21	*Garra nasuta*	CTOL3943	JX074295	JX074219	Yang et al. (2012)
22	*Garra orientalis*	N/A	JX290078	JX290078	Su et al. (2013)
23	*Garra periyarensis*	GPT2	MW314148	MW311118	Sudasinghe et al. (2021)
24	*Garra qiaojiensis*	CXY20060095	JQ864583	N/A	Wang et al. (2014)
25	*Garra salweenica*	UF:183869	N/A	MK902688	Page et al. (2019)
26	*Garra salweenica*	KIZ07194	JQ864593	N/A	Wang et al. (2014)
27	*Garra spilota*	N/A	AP011327	AP011327	Yang et al. (2012)
28	*Garra stenorhynchus*	GSTB	MW314153	MW311123	Sudasinghe et al. (2021)
29	*Garra surendranathanii*	GPT1	MW314157	MW311127	Sudasinghe et al. (2021)
30	*Garra tengchongensis*	KIZCXY20060137	GU086566	GU086604	Zheng et al. (2010)
31	*Placocheilus caudafasciatus*	KIZCXY20071036	JQ864588	N/A	Wang et al. (2014)
32	*Pseudoplacocheilus cryptonemus*	KIZ05169	GU086532	GU086570	Zheng et al. (2010)
33	*Supradiscus micropulvinus*	KIZWWY2008024	GU086567	GU086605	Zheng et al. (2010)
34	*Garra flavatra*	N/A	SGLRL-M14	KF598823	GenBank
35	*Garra* sp. Tuirivang	N/A	N/A	KF318331	GenBank
36	*Garra sp. Cheu*	ZK106A	OQ269359	OQ269359	This study
37	*Garra dampaensis*	ZK41B	PQ580751	PQ569611	This study

38	*Garra flavatra*	ZK220	PQ580747	N/A	This study
39	*Garra sp. Karnaphuli*	ZK221A	N/A	PQ569612	This study
40	*Garra khawbungi*	ZK217A	PQ580743	PQ569613	This study
41	*Garra koladynensis*	ZK101B	PQ580752	OQ269352	This study
42	*Garra cf. lissorhynchus*	ZK35A	PQ580742	PQ569614	This study
43	*Garra manipurensis*	ZK103B	N/A	OQ269354	This study
44	*Garra cf. matensis*	ZK140B	PQ580746	PQ569615	This study
45	*Garra naganensis*	ZK36A	PQ580753	PQ569616	This study
46	*Garra nigricollis*	ZK132A	N/A	OQ269361	This study
47	*Garra sp.* Phawngpui	ZK105A	PQ580744	OQ269358	This study
48	*Garra rakhinica*	ZK100A	PQ580745	OQ269351	This study
49	*Garra cf. substrictorostris*	ZK174A	PQ580748	N/A	This study
50	*Garra cf. substrictorostris*	ZK195	N/A	PQ569617	This study

Results

Approximately 660 and 1100 *coi* and *cytb* nucleotide sequences were obtained in the current study. The whole analysis included 50 species belonging to 5 genera of disc-bearing cyprinid of the Subfamily Labeoninae. The genus *Garra* and *Tariqilabeo* are members of the ingroup while the outgroup includes the genera *Supradicus*, *Pseudoplacocheilus* and *Placocheilus*. The final length of the concatenated alignment was 1777 bp. The concatenated sequence consisted of 786 conserved sites, 991 variant sites, and 680 parsimony information sites. The mean base composition of the sequence was: - A =28.9%, T(U) =29.5%, C =26.6% and G =14.9% respectively.

Phylogenetic analysis

The BI tree was generated in MrBayes version 3.2.7 (Ronquist et al., 2012) by running four simultaneous Markov chain Monte Carlo simulations for $1x20^6$ generations, sampling the tree every 1,000 generations. After $1x20^6$ generations, the analysis stopped at SD = 0.013. Also, the first 25% of samples were discarded as relative burn-in. The phylogenetic trees were visualized and edited using FigTree v. 1.4.2 (Rambaut, 2014). The BI tree *Garra* generated using the mitochondrial gene (*coi* and *cytb*) is presented in Figure 28. The phylogenetic tree of BI gives the following important information.

Eight clades were recovered from the mitochondrial gene tree which are elaborated as follows:

1. Clade I branched from the most recent ancestor of the genus and includes the species *G. arupi, G. dengba, G. dulongensis* and *G. tengchongensis.*
2. Clade II and Clade III are represented predominantly by *Garra* species from Peninsular India. Species such as *G. bicornuta, G. mlapparensis, G. periyarensis* and *G. hughi* are nested together in Clade II while *G. ceylonensis, G. mullya, G. surendranathanii* and *G. kalakadensis* are nested together in Clade III of the mitochondrial tree.

3. Members of the Lissorhynchus complex (Menon, 1964) that are characterised by the presence of a W-shaped band on the caudal fin are nested in Clade IV of the phylogenetic tree. Further, morphological analysis of some members of Clade IV shows that all the analysed species of Lissorhynchus complex are characterized by the presence of a rostral flap on the snout.
4. Clade V also branched with high probability value and includes all the proboscis bearing species included in the phylogenetic study. Members of the clade include *G. salweenica, G. fuliginosa, G. mirofrontis, G. orientalis, G. lamta, G. gotyla, G. qiaojiensis, G. gravelyi, G. nasuta, G. koladynensis, Garra* cf. *substrictorostris* and *Garra* sp. Karnaphuli.
5. Clade VI represent the morphologically distinct group of *Garra* that are characterised by neither having a proboscis nor a rostral flap. Species within this clade are also collectively characterised by having a pleated papilliferous fold between the anteromedian fold and the lower jaw. Members of the group includes *G. nigricollis, G. khawbungi, G. spilota* and *G. kempi*.
6. Clade VII is represented by *Garra* species lacking proboscis, rostral lobe or rostral flap. Members of the clade, *G. naganensis* and *Garra* sp. Phawngpui are nested together and are characterised by having a smooth snout without rostral lobe, rostral flap and/or a transverse lobe.
7. Clade VIII is represented by the only one species, *G. manipurensis*. Further, morphological analysis also shows that *G. manipurensis* is the only species included in the study that is characterized by the presence of a rostral lobe on the snout.
8. The ingroup species also include other members of the Tribe Garrini that is, the genus *Crossocheilus* (now *Tariqilabeo* Mirza & Saboohi, 1990). The clade is represented by two species of *Tariqilabeo, T. bicornis* (Wu 1977) and *T. burmanicus* (Hora 1936).
9. The outgroup species are represented by members of the Tribe Semilabeonini Li et al., 2024 namely *Pseudoplacocheilus cryptonemus*, *Supradiscus micropulvinus* and *Placocheilus caudafasciatus*.

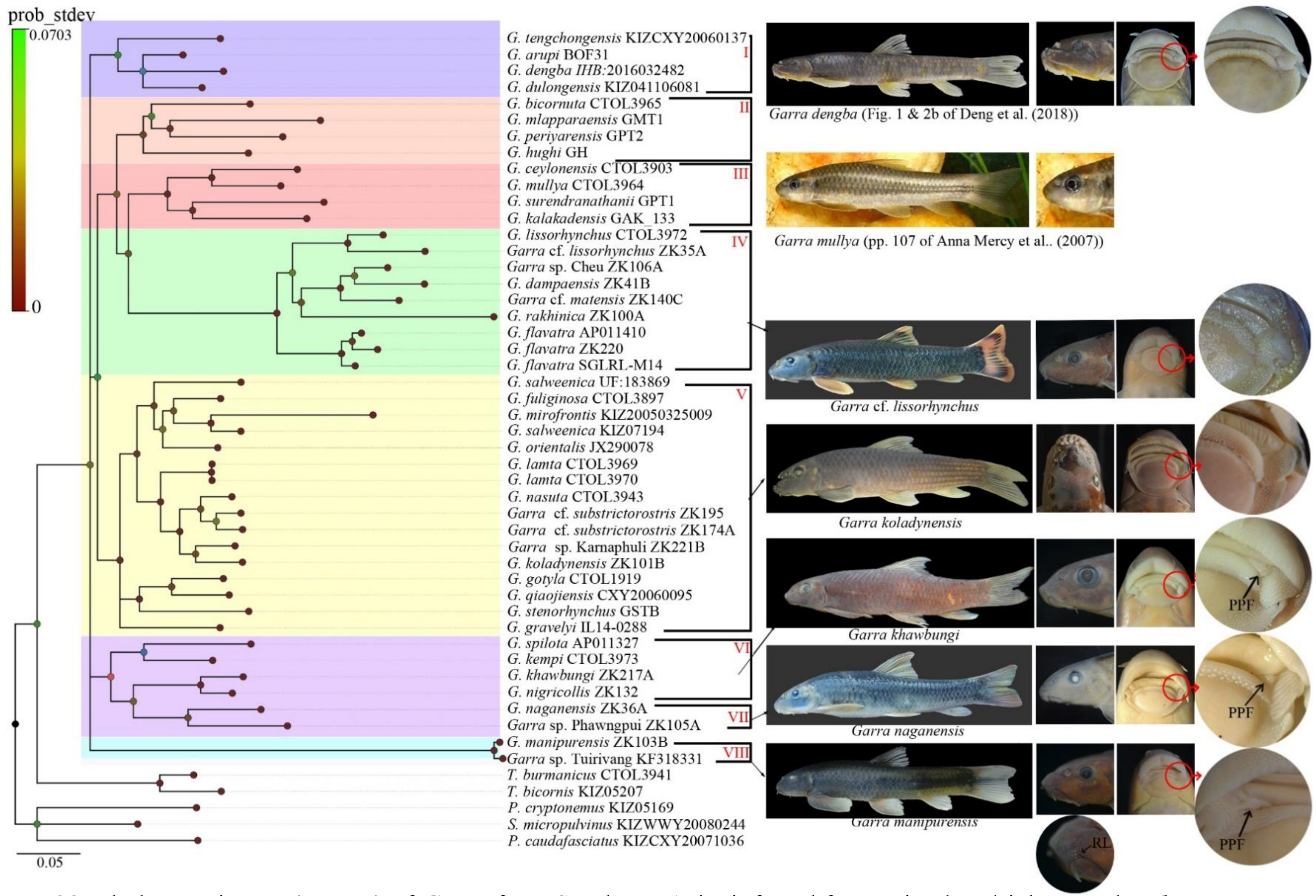

Figure 28: Phylogenetic tree (BI tree) of *Garra* from Southeast Asia, inferred from mitochondrial *coi* and *cytb* gene.

Table 20: Details of mitochondrial DNA sequence uploaded to NCBI GenBank.

Sl No	Species	Code	NCBI Accession number	
			coi	***cytb***
1	*Garra* sp. Cheu	ZK106A	OQ269359	PQ580750
2	*Garra dampaensis*	ZK41B	PQ569611	PQ580751
3	*Garra flavatra*	MF335	KF598823	-
4	*Garra* sp. Karnaphuli	ZK221A	PQ569612	-
5	*Garra khawbungi*	ZK217A	PQ569613	PQ580743
6	*Garra koladynensis*	ZK101B	OQ269353	PQ580752
7	*Garra* cf. *lissorhynchus*	ZK35A	PQ569614	PQ580742
8	*Garra manipurensis*	ZK103A	OQ269354	-
9	*Garra* cf. *matensis*	ZK140B	PQ569615	PQ580746
10	*Garra naganensis*	ZK36A	PQ569616	PQ580753
11	*Garra nigricollis*	ZK51XX	OQ269361	-
12	*Garra* sp. Phawngpui	ZK105A	OQ269358	PQ580744
13	*Garra rakhinica*	ZK100A	OQ269351	PQ580745
14	*Garra* cf. *substrictorostris*	ZK195	PQ569617	-
15	*Garra* cf. *substrictorostris*	ZK174A	-	PQ580748

Discussion

The genus *Garra* possibly originated during the Middle or Upper Miocene and have probably dispersed to Africa during the Pleistocene (Menon, 1964). A phylogenetic study by Wang et al. (2024) indicated that the common ancestor of the genus might have originated from the Irrawaddy River basin in Myanmar. Further, Li et al. (2024), based on molecular phylogenetic studies indicated that the genus *Garra* are distributed only in the Mekong River and basins to the west of the Mekong River including the North, West and Central Africa.

Molecular and morphological evidence suggested that the African, West Asia and the South and Southeast Asian species represent distinctly evolutionary groups (Menon, 1964; Krupp, 1982; Stiassny & Getahun, 2007; Englmaier et al., 2020, Tangjitjaroen et al., 2023). The morphological analysis also shows that most of the Middle East (West Asia) *Garra* are generally characterised by having fewer scales between the anus and anal-fin origin suggesting the vent is closely situated near the anal-fin origin (Hamidan et al., 2014; Sayyadzadeh et al., 2015; Esmaeili et al., 2016; Kirchner et al., 2020; Zamani-Faradonbe et al., 2021; Bayçelebi et al., 2021). The African and Middle East *Garra* are therefore considered the most primitive and generalised species by Menon (1964). On the other hand, *Garra* from Southeast Asia are considered a highly specialized species (Menon, 1964). The snout and oromandibular structures of *Garra* from Southeast Asia are relatively well-developed compared to their African and Middle Eastern counterparts.

Nebeshwar & Vishwanath (2017) noticed the highly specialised snout morphology and oromandibular structures of *Garra* from Southeast Asia and classified the genus *Garra* into 5 morphologically distinct groups. However, the classification is based only on morphological characteristics and no molecular analysis was conducted. The present study seeks to investigate if the highly modified snout and oromandibular features of *Garra* from Southeast Asia have evolutionary significance and to also assess if the morphological classification proposed by Nebeshwar & Vishwanath (2017) is supported by genetic evidences.

Species grouping of *Garra* from Southeast Asia based on phylogenetic analysis.

Seven clades for the genus *Garra* were recovered from the phylogenetic tree (BI tree) inferred from the mitochondrial *coi* and *cytb* gene. A detailed discussion of the phylogenetic tree is provided below:

1. **The proboscis-bearing species group**

   The mitochondrial phylogenetic tree indicated that the proboscis-bearing species are nested together in Clade V. Nebeshwar and Vishwanath (2017) collectively classify the species of *Garra* having a proboscis on the snout as belonging to the 'snout with proboscis species group'. Species included in the study namely, *G. salweenica, G. fuliginosa, G. mirofrontis, G. orientalis, G. lamta, G. gotyla, G. qiaojiensis, G. gravelyi, G. nasuta, G. koladynensis, Garra* cf. *substrictorostris* and *Garra* sp. Karnaphuli, are characterised by having a well-developed proboscis on the snout.

   The current findings comply with the species grouping by Nebeshwar & Vishwanath (2017). Therefore, based on the phylogenetic analysis, it is concluded that the proboscis-bearing species form a distinct species group.

2. **The smooth snout species group**

   Two smooth snout species (*G. naganensis* and *Garra* sp. Phawngpui) are also collected during the study. Both the *coi* and *cytb* gene sequences of the two smooth snout species are included in the phylogenetic analysis. Based on the phylogeny, the smooth snout species represent a phylogenetically distinct group complying with the species grouping of Nebeshwar & Vishwanath (2017).

3. **The transverse lobe-bearing species group**

   Two phylogenetically distinct clades of species having a transverse lobe on the snout are recovered from the phylogenetic analysis of the mitochondrial *coi* and *cytb*.

   Clade I is represented by four *Garra* species namely *G. arupi, G. dengba, G. dulongensis* and *G. tengchongensis*. Further observations of the snout and oromandibular structures from published data and relevant literature

(Nebeshwar et al., 2009; Chen et al., 2012; Deng et al., 2018; Sun et al., 2018) shows all species of Clade I except *G. tengchongensis* are characterized by the having a tuberculated transverse lobe on the tip of the snout. However, original description of *G. tengchongensis* (Zhang & Chen, 2002) indicated that the species possesses a smooth snout. The species group (Clade I) therefore appears to be ambiguous and needs further analysis. Also, the description of oromandibular structures and observations of figures of oromandibular structures from relevant literatures (Nebeshwar et al., 2009; Chen et al., 2012; Deng et al., 2018; Sun et al., 2018) indicated that the pleated papilliferous fold appears to be absent in these species. The species group is therefore tentatively represented here as 'species group with ambiguous snout structure'.

Other transverse lobe-bearing species namely *G. khawbungi* and *G. nigricollis* were also collected from the Kaladan River drainage in Mizoram, Northeast India. The two species are nested within the Clade VI of the phylogenetic tree. Morphological analysis of the snout structures shows that a weakly developed transverse lobe with a shallow depressed rostral surface is present in both *G. nigricollis* and *G. khawbungi*. Further, the lateral end of the extended transverse lobe tapers appearing as a rostral flap on both sides (This may be the reason why *G. nigricollis* was included as belonging to the 'species with rostral flap group' by Nebeshwar and Vishwanath (2017). Hora (1921) also stated that the snout of *G. kempi* has a bony area that is slightly elevated from the general surface.

Further, morphological analysis of the oromandibular structures of *G. nigricollis, G. khawbungi* and published data of *G. spilota* (Kullander & Fang, 2004) also shows that the species are characterized by having a distinct pleated papilliferous fold. However, the presence of a pleated papilliferous fold is not mentioned in the original description of *G. kempi*. The species within Clade VI other than the smooth snout species are therefore tentatively designated as 'species with weakly developed transverse lobe group'.

Based on the phylogenetic results of the mitochondrial *coi* and *cytb* gene, the transverse lobe-bearing species groups are therefore represented by two distinct lineages. However, in order to retain the species grouping by Nebeshwar & Vishwanath (2017), a revised species grouping is proposed here based on the phylogenetic results which are as follows:

i) 'Species group with ambiguous snout structure' and

ii) 'Species with weakly-developed transverse lobe group'

4. Rostral lobe-bearing species group

The rostral lobe-bearing species group (Clade VII) is solely represented by *G. manipurensis*. *G. manipurensis* is characteristically distinct from other species groups in having a rostral lobe on the snout. Based on relevant literature (Fig. 2 of Kullander & Fang, 2004), *G. propulvinus* also appears to possess a rostral lobe on the snout. Analysis of mitochondrial sequence of *G. manipurensis*, therefore, indicated that the rostral lobe-bearing species represent a phylogenetically distinct species group. However, genetic analysis of other rostral lobe-bearing species is highly suggested.

5. Rostral flap-bearing species group

'The Lissorhynchus group' (Clade IV) on the other hand is represented by morphologically distinct species of *Garra.* All morphologically analyzed species namely, *G. rakhinica, G. flavatra, G. dampaensis, Garra* cf. *matensis, Garra* sp. Cheu and *Garra* cf. *lissorhynchus* are characterized by having a rostral flap, a characteristic feature of 'snout with rostral flap species group' as suggested by Nebeshwar & Vishwanath (2017). Also, all analyzed species of the '*Lissorhynchus group'* are morphologically characterized by having a distinct W-shaped band on the caudal fin (Lissorhynchus complex of Menon (1964)). Although the 'rostral flap-bearing species group' appears to be genetically supported by the mitochondrial gene analysis, a thorough morphological analysis and comparison of other members of the Lissorhynchus complex (*G. abhoyai, G. nambulica, G. namyensis* and *G. lissorhynchus*) is suggested.

6. **Taxonomy of the Peninsular Indian *Garra* and the Lissorhynchus group**:

Clade II and Clade III are represented predominantly by *Garra* species from Peninsular India and Sri Lanka. It is clearly defined from the mitochondrial tree that the species from Peninsular India represent a phylogenetically distinct group. However, further morphological and molecular analysis is suggested for the species of *Garra* from Peninsular India.

Phylogeny of *Garra* based on snout morphology and oromandibular structures

Based on the current findings, it is observed that the BI tree inferred from mitochondrial *coi* and *cytb* is largely consistent with the morphological grouping of the Southeast Asian *Garra* by Nebeshwar & Vishwanath (2017). However, morphological evidences and phylogenetic results show that the smooth snout species and the species with weakly-developed transverse lobe species are nested together within a common clade (Clade VI of the mitochondrial tree) suggesting that the smooth snout species and the transverse lobe-bearing species although morphologically distinct, evolved from a common ancestral stock, forming a sister group. Further, based on the phylogenetic tree, it is proposed that the presence of a transverse lobe on the snout may not have evolutionary significance since other transverse lobe-bearing species *viz. G. arupi, G. dengba* and *G. dulongensis* form a separate clade. Also, morphological analysis of the oromandibular structures of the smooth snout species (*G. naganensis* and *Garra* sp. Phawngpui*),* weakly developed transverse lobe (*G. khawbungi* and *G. nigricollis*)*,* the rostral lobe-bearing species (*G. manipurensis*) and published data (Fig. 8 of Kullander & Fang (2004)) of *G. spilota* indicated that these species are characterized by having a well-developed pleated papilliferous fold. Based on the phylogenetic analysis, these species groups are therefore collectively grouped within the super-category 'Species with well-developed PPF'.

On the other hand, morphological analysis of the oromandibular structures of species such as *G. flavatra, G. rakhinica, G. dampaensis, Garra* cf. *lissorhynchus, Garra* cf. *matensis, Garra* sp. Cheu, *G. koladynensis, Garra* cf. *substrictorostris, Garra* sp. Karnaphuli as well as other members of Clade I, IV and V from relevant literature (Nebeshwar et al., 2009; Chen et al., 2012; Deng et al., 2018; Page et al., 2018) show that the members of these groups are characterized either by having a weakly developed or absent pleated papilliferous fold, and are therefore provisionally placed under the super category ‘Species with reduced/absent PPF’. The revised species grouping of the genus *Garra* from Southeast Asia is shown in Table 21.

Table 21: Tentatively proposed revised taxonomic grouping of Southeast Asian *Garra* based on phylogenetic results of mitochondrial *coi* and *cytb* gene. (* mark indicate the proposed group/supergroup for classification of the genus *Garra* from Southeast Asia)

Revised grouping based on phylogenetic analysis	**Taxonomic grouping based on the snout morphology by Nebeshwar & Vishwanath (2017)**	
*Species with reduced/ absent PPF	**1**	Snout with proboscis group
	2	*Species group with ambiguous snout structure
	3	Snout with rostral flap group
*Species with PPF	**4**	Smooth snout group
	5	Snout with weakly-developed transverse lobe group
	6	Snout with rostral lobe group

Position of the anus/vent position of *Garra* from Southeast Asia

Menon (1964) indicated that the vent position of the genus *Garra* is crucial for the study of the adaptation as well as evolutionary divergence. A part from the snout and oromandibular structures, the vent/anus positions with respect to the distance from the ventral fin origin and anal fin origin was analyzed. Two types of vent position are identified: i) ‘Vent anteriorly positioned’, where the anus/vent is anteriorly positioned and the distance of the anus/vent is closer to the pelvic fin origin than the anal fin origin. ii) 'Vent midway or posteriorly positioned', where the anus/vent is positioned medially or posteriorly and closer to the anal fin than the ventral fin origin. Based on the morphological analysis of available specimens as well as analysis of the vent distance from available literatures, it was observed that the species in Clade I are characterized by a vent that is anteriorly positioned (closer to the ventral fin origin than the anal fin origin) while in all other observed groups, the vent/anus is situated either at the midpoint or towards the anal fin origin (posteriorly positioned).

It is however important to highlight that molecular hypotheses derived from molecular phylogenetic analysis usually agree with traditional morphology-based systematics, although they may occasionally present contradictions (Meyer & Zardoya, 2003) and several morphomeristic characters of the Southeast Asian *Garra* are within the same range and sometimes overlap. Currently, only morphological characteristics of *Garra* species from Mizoram and published data of *Garra* from Southeast Asia are used for inferring the phylogeny. However, future morphological and molecular studies of all the congeneric species are critical necessity.

In conclusion, the phylogenetic tree based on the mitochondrial *coi* and *cytb* gene revealed that the morphological grouping of Southeast Asian *Garra* by Nebeshwar & Vishwanath (2017) is largely supported by molecular results, however, a revised grouping retaining the original species grouping by Nebeshwar & Vishwanath (2017) is proposed based on the phylogenetic results (see Table 21).

The sequenced mitochondrial DNA as well as the outcomes of the phylogenetic study will likely benefit future research on the genus *Garra* and may assist in the paleodrainage study of the Northeast India. Furthermore, the phylogenetic results indicate that the various drainages of Mizoram may play a significant role in understanding of the dispersal and divergence of the genus.

SUMMARY

Diversity analysis

1. Kaladan River drainage: The Kaladan River drainage has the highest diversity of *Garra.* Nine (9) species of *Garra* namely *G. flavatra, G. rakhinica, G. manipurensis, G. koladynensis, G. khawbungi*, *Garra* cf. *matensis, G. nigricollis*, *Garra* sp. Phawngpui and *Garra* sp. Cheu are present.

   Jaccard similarity index analysis shows that the Kaladan River drainage has low similarity with the Barak River drainage and the Karnaphuli River drainage but instead is similar to the Rakhine Yoma River system elements.

2. Barak River drainage: Barak River drainage has lower species diversity compared to the Kaladan River drainage. Four (4) species namely *G. naganensis, G. manipurensis, Garra* cf. *substrictorostris* and *Garra* cf. *lissorhynchus* were collected.
3. Karnaphuli River drainage: Karnaphuli River drainage shows low species diversity. A total of 4 species of *Garra* namely *G. dampaensis, G. manipurensis, G. nigricollis* and *Garra* sp. Karnaphuli were collected.
4. The Barak River drainage and the Karnaphuli River drainage show a high species similarity. It is hypothesized that the complex river system of the Barak River drainage and the Karnaphuli River drainage at the flood plains of western Bangladesh could possibly lead to overlapping in river courses which may mediate the exchange of fishes.
5. Fourteen (14) species of *Garra* are currently recorded from Mizoram, that accounts for about 25% of the total species recorded from the northeastern regions of India.

Morphological analysis

6. Fourteen (14) species of *Garra* were collected from Mizoram that can be grouped into 5 distinct species groups based on Nebeshwar & Vishwanath (2017) as follows:
    - **i) Rostral flap-bearing group** (*G. manipurensis*)
    - **ii) Smooth snout species group** (*G. naganensis, Garra* sp. Phawngpui)
    - **iii) Transverse lobe species groups** (. *G. nigricollis, G. khawbungi*)
    - **iv) Proboscis bearing group** (*G. koladynensis, Garra* cf. *substrictorostris, Garra* sp. Karnaphuli)
    - **v) Rostral flap bearing group** (*G. flavatra, G. rakhinica, G. dampaensis, Garra* cf. *matensis, Garra* sp. Cheu, *Garra* cf. *lissorhynchus*)
7. *Garra nigricollis* was placed among the transverse lobe-bearing species group (rostral flap-bearing species in Nebeshwar & Vishwanath, 2017)
8. *Garra dampaensis* was placed among the rostral flap-bearing species group (smooth snout species group in Nebeshwar & Vishwanath, 2017).
9. *Garra tyao* from the Kaladan River drainage was synonymized with *G. rakhinica* Kullander & Fang, 2004.
10. Key to species and general morphological characteristics of *Garra* from Mizoram is also provided.
11. Interspecific genetic distances of all species of *Garra* included in the analysis (47 species) range from 3.7–36.6% in *coi* gene and 4.8–32.2% in *cytb* gene.
12. The interspecific distance between the 14 species of *Garra* collected from Mizoram ranges from 4.1%–25.8% in *coi* gene and 7.9–30.2% in *cytb* gene. The closest interspecific distance (between *Garra* from Mizoram) is observed between the transverse lobe-bearing species, *Garra khawbungi* and *Garra nigricollis* (4.1%–25.8% in *coi* gene and 7.9–30.2% in *cytb* gene). The largest interspecific genetic distance (between *Garra* from Mizoram) is observed

between the rostral flap-bearing G. *rakhinica* and the smooth snout *Garra manipurensis* (pairwise genetic distance = 25.8%).

13. Closest genetic similarity is observed between G. *nasuta* (NCBI Accession number: JX074219) and *Garra* cf. *substrictorostris* from the Barak River drainage (pairwise genetic distance = 3.7% in *coi* gene and 4.8% in *cytb* gene).

Phylogenetic analysis

14. Approximately 660 and 1100 *coi* and *cytb* nucleotide sequences were obtained in the current study.
15. A phylogenetic tree was constructed using mitochondrial genes (*coi* and *cytb*). The phylogenetic analysis includes 50 species of the subfamily Labeoninae.
16. A Bayesian Inference (BI) tree was constructed in MrBayes and the following were recovered: eight (8) clades for the genus *Garra*, one (1) clade consisting of the genus *Tariqilabeo* and outgroup of the Tribe Semilabeonini.
17. Based on the phylogenetic tree, the seven *Garra* clades are as follows: -

    Clade I: Species of ambiguous snout structures. Species having transverse lobe (*G. arupi, G. dengba, G. dulongensis*) and smooth snout species (*G. tengchongensis*). Vent position close to ventral fin origin in all four mentioned species.

    Clade II and Clade III: *Garra* species from Peninsular India.

    Clade IV: Members of the Lissorhynchus complex (Caudal fin with W-shaped band that may be distinct or indistinct). Also characterised by having a rostral flap on the anterior surface of the snout. Vent position close to anal fin origin.

    Clade V: Proboscis bearing species. Members of the clade include *G. salweenica, G. fuliginosa, G. mirofrontis, G. orientalis, G. lamta, G. gotyla, G. qiaojiensis, G. gravelyi, G. nasuta, G. koladynensis, Garra* cf.

substrictorostris and *Garra* sp. Karnaphuli. Vent position close to anal fin origin.

Clade VI: Neither having a proboscis nor a rostral flap. Presence of a pleated papilliferous fold between the anteromedian fold and the lower jaw. Members of the group include *G. nigricollis, G. khawbungi, G. spilota* and *G. kempi*. Vent situated midway between the anal fin origin and ventral fin origin.

Clade VII: Neither having a proboscis nor a rostral flap. Presence of a distinct pleated papilliferous fold between the anteromedian fold and the lower jaw. Members of the group include *G. naganensis* and *Garra* sp. Phawngpui.

Clade VIII: Rostral lobe bearing species.

Outgroups: Tribe Semilabeonini Li et al., 2024 (*Pseudoplacocheilus cryptonemus*, *Supradiscus micropulvinus* and *Placocheilus caudafasciatus*).

18. The species grouping based on the snout morphology by Nebeshwar & Vishwanath (2017) is largely supported by phylogenetic analysis of the mitochondrial *coi* and *cytb* genes. Although highly supported, based on the divergence, a new or revised grouping is proposed while retaining the original species grouping by Nebeshwar & Vishwanath (2017). The transverse lobe-bearing species are divided into two species groups based on the presence or absence of a pleated papilliferous fold and the position of the vent.
19. Phylogenetic analysis also reveals that the Peninsular Indian species are closely related to the Lissorhynchus complex while the proboscis-bearing species forms a monophyletic group. However, further morphological and genetic analysis is suggested to better understand their evolutionay divergence.

20. Phylogenetic analysis further reveals that the rostral lobe-bearing species (*G. manipurensis*) diverge distantly with a long branch length. Morphological analysis also reveals that *G. manipurensis* also possesses several characters that are distinct from the other species groups including presence of a rostral lobe.
21. The smooth snout species and the species with weakly-developed transverse lobe species appear to have a close common ancestor based on the phylogeny. Similar characters including vent positions, number of fin rays on the dorsal fin, presence of pleated papilliferous fold, number of fin rays on the anal fin etc. are observed between the species within this clade (Clade VI).
22. Morphological analysis also indicates that other species (other than Clade VI and Clade VII) are characterised by having reduced/absent pleated papilliferous fold.
23. Based on the current findings, two super groups are proposed: i) species with PPF and ii) species without/reduced PPF. A new species group is also proposed for species with doubtful snout structure that are also collectively characterised by having the vent closer to the ventral fin origin than the anal fin origin.

APPENDIX I

Comparative materials for morphological analysis:

Garra annandalei: ZSI FF 6042; 79.9 mm SL; East Kameng, Arunachal Pradesh, India. ZSI F 6082/2 (3exs); 52.2 – 59.5 mm SL; Kokha nallah, Kosi river, Barahak, Shitra, India.

Garra lissorhynchus: ZSI Calcutta, FF 8098/1; 73.05 mm SL. MUMF 4163-4166 (3exs); 68.2 – 85.9 mm SL; Khanphung, Barak River, Manipur, India.

Garra naganensis: ZSI F 9970/1; 87.5 mm SL; Senapati Stream, Naga hills, Assam, India. MUMF 4156-4159 (4exs); 78.2 – 103.4 mm SL; Tuivai River, India.

Garra gravelyi: MUMF 4173; 74.5 mm SL; Leimatak River, Manipur, India.

Garra abhoyai: Non-type; ZSI FF7983; 47.4 mm SL; Shingtha ahlu Chandel, Tunhring, Manipur, India.

Garra paralissorhynchus: PUCMF 21046: 49.3 mm SL; Near Gamphazol, Manipur River, Manipur, India.

Garra dampaensis: Holotype; PUCMF 12001; 45.6mm SL; Seling River, Karnaphuli River drainage, Mizoram, India. Paratypes; PUCMF 12002 (10 exs); 40.6–51.9 mm SL; Seling River, Karnaphuli River drainage, Mizoram, India. Cleared and stained; PUCMF 12004 (3 exs); 36.5–41.4 mm SL; Seling River, Karnaphuli River drainage, Mizoram, India.

Garra lamta: ZSI FF 4611 (2ex); 54.8-56.2 mm SL; Dhalsa River, Jalpaiguri, W. Bengal, India.

Additional morphometric data from: Hora (1921), Arunachalam et al. (2013), Arunachalam et al. (2014), Thoni et al. (2016), Abonmai et al. (2023), Catherine & Linthoingambi (2023), Marngar & Lokeshwor (2023), Thoidingjam et al. (2023), Ezung et al. (2020), Vishwanath & Joyshree (2005), Shangningam & Vishwanath (2012), Arunkumar & Moyon (2019), Nebeshwar et al. (2009), Tamang (2013), Vishwanath & Sarojnalini (1988), Kosygin & Vishwanath (1998), Nebeshwar & Vishwanath, (2015), Moyon & Arunkumar (2020), Roni & Vishwanath (2018), Nebeshwar & Vishwanath (2013), Shangningam & Vishwanath (2015), Roni et al. (2017), Premananda et al. (2017), Roni & Vishwanath (2017), Ezung et al. (2020), Tamang et al. (2019), Roni et al. (2019), Shangningam et al. (2019), Ezung et al. (2021), Nebeshwar et al. (2012), Arunkumar & Moyon (2018), Page et al. (2019), Devi (1992), Rayamajhi & Arunachalam (2017), Shangningam et al. (2019, Shangningam et al. (2021, Jadhav et al. (2023, Madhusoodana Kurup & Radhakrishnan (2010), Silas (1955), Devi (1992), Devi & Indra (1984), Arunachalam & Nandagopal (2014), Devi & Menon (1994), Arunachalam et al. (2013), Gopi (2001), Shaji et al. (1996), Kangrang et al. (2016), Tangjitjaroen et al. (2023), Thoni & Mayden (2015), Page et al. (2019), Lothongkham et al. (2014), Chen et al. (2009), Chen et al. (2009), Zhang (2006), Zhang & Chen (2002), Yu et al. (2016).

APPENDIX II

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No. PUC/IAEC/2019-21/023 23 May 2019

To

Dr Lalramliana
Assistant Professor
Department of Life Sciences/Zoology

Subjects: Approval of Application for Ethical Certificate.

I refer to your application to the IAEC of Pachhunga University College (dated 15/05/2019), and I am pleased to inform you that your proposed project, after scrutiny, is duly approved by the committee. The details are given below:

Project title	Molecular and morphological characterization of freshwater fishes of Mizoram, North East India
Animal handling involved	1. Fish
Approval number	PUC-IAEC-2019-A10
Approval date	23/05/2019

The approval is on the conditions that you shall:

- use and handle the animal models, i.e., fish, according to the ethical guidelines laid down by the Committee for the Purpose of Control of Supervision of Experiments on Animals (CPCSEA), and Animal Dissection Monitoring Committee of Pachhunga University College;
- submit annual progress report of the research;
- and facilitate inspection of the research environment from time to time.

Prof K. Lalchhandama
Chairman

PACHHUNGA UNIVERSITY COLLEGE AIZAWL 796001 MIZORAM

Prof H. Lalthanzara
Secretary

Institutional Animal Ethics Committee of Pachhunga University College

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BIO-DATA

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HSSLC	2008	Meghalaya Board of School Education	Science	First	61.8
B. Sc	2012	North Eastern Hill University	Zoology	First	68.5
M. Sc	2014	North Eastern Hill University	Zoology	First	68.5
NET JRF	2016	UGC-CSIR	Life Sciences	NA	NA
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PAPER PRESENTED IN SYMPOSIUM/CONFERENCE

INTERNATIONAL CONFERENCE/SEMINAR

1. Poster presentation at ‘International Conference on Biodiversity and Conservation (ICBC) organized by Dept of Zoology and Fishery Science, St. Anthonys College, Shillong, from 14-15 December 2022 on the topic ‘Integrative taxonomical analysis of *Garra* (Cyprinidae: Labeoninae) from Mizoram, Northeast India’
(**Awarded Best presenter**)
2. Oral presentation at ‘PUC Research conclave 2023 (An international Workshop on Research and Development organized by Research and Development Cell, Pachhunga University College) on the topic ‘Molecular and morphological characterization of the Genus *Garra* from Mizoram, Northeast India’.
3. Oral presentation at the ‘International Conference on Conservation of Biodiversity in Genomics Era’ held at Pachhunga University College, Aizawl during 22-23 March, 2024 on the topic ‘Assessment of ichthyofaunal population decline and recovery strategy of Tuirial River with emphasis on the genus *Garra’*
(Awarded 3rd place ‘Best presenter’)

NATIONAL CONFERENE/SEMINAR

1. Poster presentation at ‘Mizoram Science Congress held at Aijal Club, Aizawl during 24-25 November, 2022 on the topic ‘Studies on the genus *Garra* (Teleotei: Cyprinidae) from the Kaladan drainage of Mizoram, India, and synoymization of *G. tyao* with *G. rakhinica’*

TRAINING/WORKSHOP ATTENDED

1. Participated at the ‘Workshop on Traditional medicine under the aegis of the International Conference on Biodiversity and Conservation (ICBC)’ on 15th December 2022 at St. Anthony’s College, Shillong, organized by Bio Resources Development Center in collaboration with Department of Zoology and Fisheries Sciences, St. Anthony’s College, Shillong.

2. Participated at the Hands-on Workshop on Molecular Phylogenetics during 9th – 14th March 2023 sponsored by DBT- Advance State Level Biotech Hub & Mizoram University.
3. Attended Online Short Term Course on ‘Research and Publication Ethics’ organized by the UGC Human Resource Development Centre, Mizoram University during 01st – 07th October, 2021.

LIST OF PUBLICATIONS

1. **Zirkunga M** (2021) On the definition, status of research, diversity and prospect of exploration of the Genus *Garra* (Cypriniformes; Cyprinidae) from Mizoram, Northeast India. *Science Vision*, 21(1): 1–5.
2. Lalramliana, **Zirkunga MC** & Lalronunga S (2020) Ichthyofauna of Dampa Tiger Reserve Rivers, Mizoram, North-Eastern India. *Journal of Environmental Biology*, 41(4): 884–895. https://doi.org/10.22438/jeb/4(si)/ms_1903
3. **Zirkunga MC**, Singh M & Lalramliana (2023) Studies on the Genus *Garra* (Teleostei: Cyprinidae) from the Kaladan Drainage of Mizoram, India, and synonymization of *G. tyao* with *G. rakhinica*. *Indian Journal of Science and Technology*, 16: 62–69. https://doi.org/10.17485/ijst/v16sp1.msc9

PARTICULARS OF THE CANDIDATE

NAME OF THE CANDIDATE : **M. C. ZIRKUNGA**

DEGREE : **DOCTOR OF PHILOSOPHY**

DEPARTMENT : **LIFE SCIENCES**

TITLE OF THE THESIS : **MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *GARRA* (TELEOSTEI: CYPRINIDAE: LABEONINAE) FROM MIZORAM, NORTH-EAST INDIA**

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ABSTRACT

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *GARRA* (TELEOSTEI: CYPRINIDAE: LABEONINAE) FROM MIZORAM, NORTH-EAST INDIA

AN ABSTRACT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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DEPARTMENT OF LIFE SCIENCES

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SCHOOL OF LIFE SCIENCES

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ABSTRACT

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *GARRA* (TELEOSTEI: CYPRINIDAE: LABEONINAE) FROM MIZORAM, NORTH-EAST INDIA

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Submitted
In partial fulfillment of the requirement of the Degree of Doctor of Philosophy in Life Sciences in Mizoram University, Aizawl.

Cyprinid fish belonging to the genus *Garra* are mostly found in fast flowing streams and riffles. Members of the genus are characterized by having an elongated, sub-cylindrical body and a specialized lower lip that forms a disc-like apparatus known as the mental adhesive disc or gular disc. The genus *Garra* along with other genera such as *Ageneiogarra*, *Discogobio*, *Placocheilus*, *Discocheilus*, *Vinagarra*, *Sinigarra*, *Ceratogarra*, *Guigarra*, *Pseudoplacoheilus* and *Supradiscus* belonged to the subfamily Labeoninae and are morphologically characterized by having a oromandibular disc.

According to the Eschmeyer Catalog of Fishes, currently, there are about 200 valid species of *Garra* that are widely distributed in North and Central Africa, the Middle East and Southeast Asia including South China. Regional-wise taxonomical revisions on the genus are occasionally conducted on the species from Southeast Asia, Africa, Peninsular India and the Middle East (West Asia), however, global revision of the genus has not been conducted.

A widely accepted idea is that freshwater fish are found in specific river basins and are morphologically distinct from their congeners in other basins. However, there are instances where cross-drainage dispersal is also observed. Given this context, the existence of three separate and unconnected drainage systems in Mizoram may result in high ichthyofaunal diversity. The three drainage systems of Mizoram are constituted by i) The Karnaphuli River drainage that forms the Southwestern drainage system and drains into the Bay of Bengal at Chittagong Port, ii) The southwardly flowing Kaladan River drainage system that forms the southeastern drainage system of Mizoram draining at Sittwe in Myanmar and iii) The Barak River drainage system that drains the central and northern parts of the state which eventually drains in the Bay of Bengal after merging with the Brahmaputra River.

Mizoram occupies a significant area of the Indo-Burma biodiversity hotspot. Also, ichthyofaunal exploration within the region is still in the juvenile stage. Therefore, the current study is carried out to conduct the documentation, taxonomical analysis as well as phylogenetic analysis of *Garra* from Mizoram. The objectives incorporated in this study are broadly classified into three chapters:

Chapter 1: Diversity and Distribution of *Garra* in Mizoram

Fish sampling was conducted during October, 2020 – March, 2024. Fish specimens were collected randomly from selected tributaries of the three drainages. Based on the sampling data, species diversity for the genus *Garra* was calculated and it was observed that the Kaladan River drainage has the highest species diversity indicated by the presence of nine (9) species of *Garra* while the Karnaphuli and the Barak River drainage have a comparatively lower diversity with four (4) species each.

Species similarity was also calculated using Jaccard's similarity index. The calculated result of Jaccard's similarity index shows that the similarity between the Barak River drainage and the Karnaphuli River drainage is the highest ($C_j = 0.18$) while the lowest similarity index ($C_j = 0.08$) is observed between the Kaladan River drainage and the Barak River drainage.

Distribution analysis was also conducted and fourteen (14) species of *Garra* were found to be distributed in the three drainages of Mizoram. From the Kaladan River drainage, nine (9) species namely *G. flavatra, G. rakhinica, G. manipurensis, G. koladynensis, G. khawbungi*, *Garra* cf. *matensis, Garra* cf. *nigricollis*, *Garra* sp. Phawngpui and *Garra* sp. Cheu were collected. Of these, *G. koladynensis, G. khawbungi*, *Garra* cf. *matensis, Garra* sp. Phawngpui and *Garra* sp. Cheu were found only in the Kaladan River drainage.

From the Barak River drainage, four (4) species namely *G. naganensis, G. manipurensis, Garra* cf. *substrictorostris* and *Garra* cf. *lissorhynchus* were collected. Of these *G. naganensis* and *Garra* cf. *substrictorostris* were found only in the Barak River drainage. From the Karnaphuli River drainage, four (4) species namely *G. dampaensis, G. manipurensis, G. nigricollis* and *Garra* sp. Karnaphuli were collected. Of these, *G. dampaensis* and *Garra* sp. Karnaphuli were found only in the Karnaphuli River drainage.

Interestingly, *G. manipurensis* is found in all the three river drainages while *Garra* cf. *nigricollis* is found to be present in the Kaladan River drainage and Karnaphuli River drainage.

Chapter 2: Morphological and molecular characterization of *Garra* from Mizoram

Nebeshwar & Vishwanath (2017) placed *Garra* from the South and Southeast Asian regions into 5 different groups based on the snout morphology, which are as follows: (1) smooth snout species group (2) snout with rostral lobe species group (3) snout with rostral flap species group (4) snout with proboscis species group and (5) snout with transverse lobe species group. Based on the morphological analysis, all fourteen species of *Garra* from Mizoram can be collectively accommodated among the species group proposed by Nebeshwar and Vishwanath (2017).

G. manipurensis is uniquely differentiated from all other *Garra* species (except *G. propulvinus*) by having a rostral lobe (vs. absent in all other species) and is considered a member of the 'snout with rostral lobe species group'.

Six species of *Garra* belonging to the 'rostral flap-bearing species group' are also collected namely, *G. rakhinica, G. flavatra, Garra* cf. *matensis, Garra* cf. *lissorhynchus, Garra* sp. Cheu and *G. dampaensis.* All rostral flap-bearing species collected are also characterized by the presence of a W-shaped band on the caudal fin.

Two species belonging to the 'smooth snout species group' were also collected from Mizoram namely, *G. naganensis* and an unidentified smooth snout species designated here as *Garra* sp. Phawngpui. Further analysis of *coi* and *cytb* genes indicated that *G. naganensis* and *Garra* sp. Phawngpui are genetically distinct (*p*-distance = 8.7% in *coi* and 13% in *cytb* gene).

Members of the proboscis-bearing species group from Mizoram include *G. koladynensis*, *Garra* cf. *substrictorostris* and *Garra* sp. Karnaphuli. Among the proboscis-bearing species of Mizoram, *G. koladynensis* is morphologically characterized by having a trilobed proboscis while *Garra* cf. *substrictorostris* and *Garra* sp. Karnaphuli are characterized by having a unilobed proboscis.

Two species of *Garra* belonging to the 'transverse lobe species group' were collected namely, *G. khawbungi* (from the Kaladan River drainage) and *G. nigricollis* (from both the Kaladan River drainage and the Karnaphuli River drainage). The species are characterized by the presence of indistinct/ weakly developed transverse lobe on the snout.

Further, pairwise genetic distance analysis based on the mitochondrial *coi* and *cytb* gene shows that interspecific genetic distances of all species of *Garra* included in the analysis ranges from 3.7–36.6% in *coi* and 4.8–32.2% in *cytb* gene. Further, the closest genetic similarity is observed between G. *nasuta* (NCBI Accession number: JX074219) and *Garra* cf. *substrictorostris* from the Barak River drainage (*p*-distance = 3.7% in *coi* gene). The interspecific distance between the 14 species of *Garra* from Mizoram ranges from 4.1–25.8% in *coi* gene and 7.9–30.2% in *cytb* gene. The closest interspecific distance between *Garra* from Mizoram is observed between the transverse lobe-bearing species, *Garra khawbungi* and *Garra nigricollis* (p-distance = 4.1% in *coi* gene)

Chapter 3: Phylogenetic analysis

Using the sequenced data of mitochondrial *coi* and *cytb* and available mitochondrial gene sequences from NCBI GenBank, phylogenetic analysis was conducted to test the phylogeny of *Garra* from Southeast Asia. Based on the phylogenetic results, the species grouping based on the morphology of the snout and oromandibular structures by Nebeshwar and Vishwanath (2017) was revised.

The mitochondrial gene analysis includes 50 species belonging to 5 genera of the subfamily Labeoninae. The mitochondrial tree shows that instead of the 5 morphologically distinct species groups as suggested by Nebeshwar and Vishwanath (2017), 6 species group are tentatively proposed. A new species group '*species with ambiguous snout structures'* that are also characterized by having the anus closer to the ventral fin than the anal fin origin is proposed to accommodate the phylogenetically distinct species namely *G. arupi, G. dengba, G. dulongensis* and *G. tengchongensis.*

Further, based on the phylogenetic results and morphological analysis of the oromandibular structures, the six species groups are categorized as belonging to two super-groups that is i) Species with reduced/ absent PPF super group and ii) Species with well-developed PPF super group.

In summary, the present study analyses the diversity and distribution of species of the genus *Garra* from the three drainages of Mizoram. Further, morphological characters and diagnosis of fourteen species of *Garra* were provided. The study also analyses the phylogeny of *Garra* from Mizoram with its congeners from Southeast Asia and provides a revised species grouping of the Southeast Asia *Garra* species. The findings and results from the present study will likely be beneficial for future studies on the genus and possibly help in better understanding the taxonomy and phylogeny of the genus.