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Morphometric Study of the Genus *Garra* Found in Arunachal Pradesh, Northeast India

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ABSTRACT

Background: The fishes of genus *Garra* are one of the most abundant and taxonomically confusing groups which requires further studies to evaluate their taxonomical ambiguity. Therefore, the present investigation was conducted on the genus to understand their detailed morphometric and meristic features.

Methods: A total of 122 fish specimens belonging to 10 species were collected from the hill stream rivers of Arunachal Pradesh. Eleven morphometric and six meristic parameters were applied for each fish specimen. Identification key was developed after the detailed examination of morphological features. Fin formula was also generated for each species by counting the meristic characters. Phylogenetic tree was developed using morphological characters to understand the evolutionary relationship among the species.

Result: Present study observed the maximum length (54.8-135.0 mm) in *G. annandalei* and minimum length (64.1-70.2 mm) in *G. rufa*. And this study also confirmed the presence or absence of lobes and tubercles, rostral cap, proboscis, gular disc and fins' shape as key identifying features. Constant in morphometric measurements and meristic counts reveals the uniqueness of the species which was noticed from the generation of fin formula. Further, species were assigned with latest IUCN Red data, *G. rupecula* was identified as NT, 3 species were under NE and 6 species in LC category. Analysis of phylogenetic tree through neighborjoining (NJ) clustering method with bootstraps 100 depicts that *G. lissorhynchus, G. rufa* and *G. rupecula* were belonged to the common ancestral group and *G. annandalei* and *G. quadratirostris* were distantly related to *G. arunachalensis* and *G. lamta*. A strong positive and linear LLRs were found in between TL and other morphometric characters with r value >0.765. SL showed significantly ($p<0.01$) higher correlation with TL in all the species.

Key words: Fin formula, Genus *Garra*, LLRs, Morphometry, Phylogenetic tree.

INTRODUCTION

Arunachal Pradesh is a part of Indian Himalayan biodiversity hotspot and home to diverse fish species with high endemism level (Vishwanath, 2021). The state consists of large number of hill streams, rivers and rivulets with variations in altitude and climatic conditions which provide diverse habitats for the high-altitude fishes (Gogoi *et al*., 2024). The hill stream faunal diversity serves as an integral part of the riverine ecosystem and environmental stability (Mudoi *et al*., 2022). Considering the availability of fish species, currently 38 species of genus *Garra* are reported from different hilly terrains of northeast India, among the states Arunachal Pradesh is recognized as home to 17 identified species of genus *Garra* (Zirkunga *et al.,* 2023). This genus contributes significantly in maintaining the ecological integrity of a hill stream ecosystem through their roles in sustaining the nutrient cycle, habitat creation and controlling algal growth. The conservation of cyprinid fishes *Garra* (Hamilton 1822) is prime important for preserving the aquatic biodiversity and ecological functions of torrential habitats. They are small to medium sized fishes having a cylindrical, elongated body with modified sucker-like lower lips. The fishes are bottom dwellers and inhabitant to fast flowing waters (Chen *et al.,* 2009). The distribution of tubercles on the snout, the oromandibular features and the proboscis aspect of the *Garra* species vary morphologically and these differences have been utilized to distinguish between the species within the group (Nebeshwar and Vishwanath, 2017).

Fish morphometric and meristic study is a crucial tool for the precise identification of fish species (Cavalcanti *et al.,* 1999). Compared to other vertebrates, fish show greater diversification in terms of morphological features both within and between the populations. Morphometric measurements, statistical analysis and their relationships are pivotal for the taxonomic evaluation of species (Tandon *et al.,* 1993). Fish meristic counts are also used in the development of fin formula for a particular fish species. This variation of fish population in morphometric and meristic traits are also useful in phylogenetic studies. However, the available

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literature on taxonomical description of *Garra* is not enough to support the well-developed hypothesis of fish identification, it requires further studies using morphological datasets. Hence, the present study aims to generate comprehensive data on morphometric and meristic characters of *Garra* species found in Dibang and Noa dihing river of Arunachal Pradesh, NE India.

MATERIALS AND METHODS

Current investigation was carried out in 2 hill stream rivers of Arunachal Pradesh *viz*., Dibang (28°05′21″N 95°42′13″E) and Noa dihing (27°31′07″N 95°58′59″E) river (Fig 1). A total number of 122 fish specimens belonging to 10 species were collected using gill and cast net, electrofishing devices and different traditional methods. Seasonally caught (2020 to 2024) fish specimens were fixed in 10% formalin and preserved in 70% alcohol for further analysis. Counts on meristic parameters (soft rays and spines of fins and scales on lateral line) and morphometric measurements such as total length (TL), standard length (SL), body depth (BD), head length (HL), dorsal fin length (DFL), pectoral fin length (PFL), ventral fin length (VFL), anal fin length (AFL) and caudal fin length (CFL) were recorded with the help of digital calliper to the nearest 0.1 mm (Fig 2) (Nebeshwar and Vishwanath, 2013). Statistical analysis such as LLRs, correlation coefficient and construction of phylogenetic tree based on morphological features was developed following SPSS 20.0 and PAST 4.0 software.

RESULTS AND DISCUSSION

In the present investigation, an attempt was made to evaluate the morphometric parameters of 10 different species of genus *Garra viz*., *G. annandalei, G. arunachalensis, G. gotyla, G. lamta, G. lissorhynchus, G. parastenorhynchus, G. rufa, G. rupecula, G. stenorhynchus, G. quadratirostris* found in Arunachal Pradesh. This genus is one of the most diverse and taxonomically confusing groups of the order Cypriniformes that has yet to be examined for proper evidence of morphological and meristic traits (Zirkunga *et al.,* 2023). In the current study, a total of 11 morphometric parameters were recorded that showed proportionate increase in length with increase in total length (Table 1), among the studied species, *G. annandalei* measured

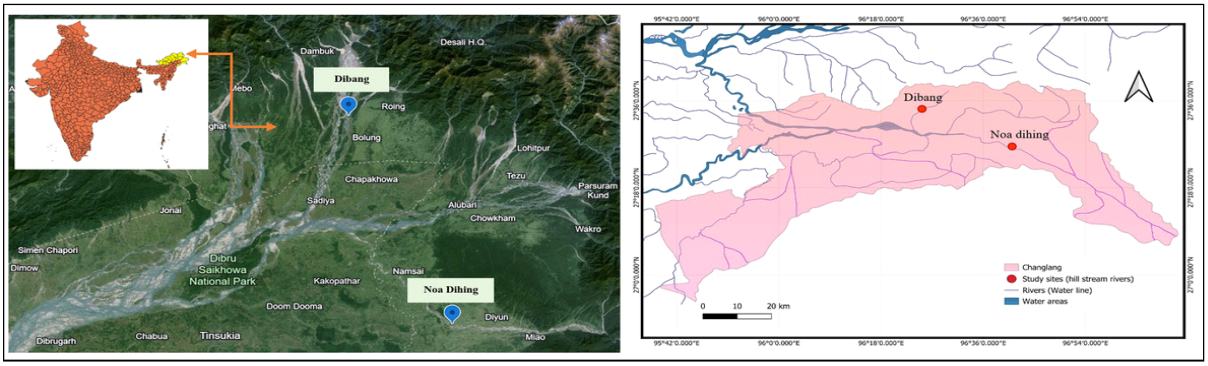
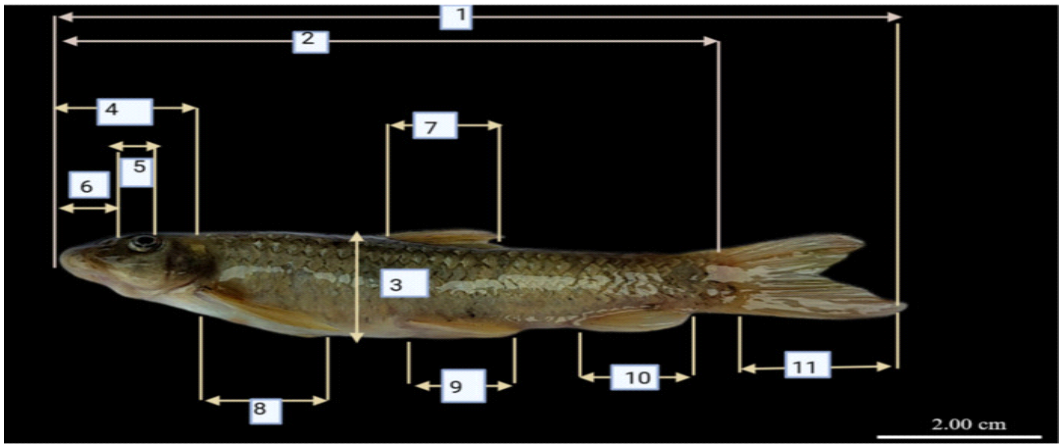


Fig 1: Location map of study sites in Arunachal Pradesh, NE India.



[1. Total length (TL), 2. Standard length (SL), 3. Body depth (BD), 4. Head length (HL), 5. Eye diameter (ED), 6. Pre orbital length (Pre OL), 7. Dorsal fin length (DFL), 8. Pectoral fin length (PFL), 9. Ventral fin length (VFL), 10. Anal fin length (AFL), 11. Caudal fin length (CFL)].

Fig 2: *Garra* sp. showing morphometric measurements .

Table 1: Morphometric indices of the genus *Garra* reported in Arunachal Pradesh, NE India.

Fish species	TL (min-max) mm	SL mm (min-max) mm	TW (min-max) g	BD (min-max) mm	HL (min-max) mm	Pre. OL (min-max) mm	ED (min-max) mm	DFL (min-max) mm	PFL (min-max) mm	VFL (min-max) mm	AFL (min-max) mm	CFL (min-max) mm
Garra annandalei	54.80	39.57-	9.25-	10.00-	10.50-	4.70-	2.00-	14.00-	16.00-	13.00-	14.12-	15.23
	-135.00	110.00	23.95	25.00	20.00	11.00	4.10	21.00	20.00	17.00	21.00	25.00
Garra arunachalensis	110.00-	88.00-	23.38-	18.00-	19.00-	9.00-	3.00-	19.00-	17.00-	9.00-	15.00-	19.00-
	131.00	105.00	37.10	25.00	25.00	13.00	4.00	25.00	23.00	9.00	19.00	25.00
Garra gotyla	49.56 -	34.56-	8.12-	9.00-	10.00-	4.00-	1.80-	13.00-	15.00-	13.84-	14.00-	15.00-
	110.00	95.00	16.62	21.00	17.00	8.00	4.00	18.00	17.00	15.00	16.00	18.10
Garra lamta	71.66 -	56.66-	25.06-	17.20-	16.00-	8.00-	3.00-	18.00-	19.00-	16.00-	15.00-	15.00-
	110.56	90.56	30.00	20.00	19.00	9.50	3.50	20.00	20.00	16.50	16.00	20.00
Garra lissorhynchus	80.10-	69.60-	14.50-	11.00-	12.00-	6.00-	3.00-	13.00-	12.00-	10.00-	10.00-	10.50-
	100.56	89.56	22.20	15.00	13.00	6.30	3.40	14.00	13.00	11.00	11.00	11.00
Garra parastenorhynchus	70.95 -	55.70-	15.24-	15.00-	15.30-	7.00-	3.00-	16.00-	15.00-	13.00-	12.00-	15.25-
	131.00	110.00	35.00	25.00	25.00	13.00	4.00	28.00	23.00	22.00	20.00	30.00
Garra rufa	64.10 -	54.10-	7.91-	10.50-	12.00-	6.00-	3.00-	14.00-	14.00-	11.50-	10.00-	10.00-
	70.24	59.74	20.00	11.00	13.00	6.50	3.20	15.00	14.50	12.00	11.00	10.50
Garra rupecula	75.39-	66.14-	20.42-	8.42-	10.10-	5.00-	2.00-	11.05-	10.75-	10.70-	10.80-	9.25-
	82.18	72.11	23.84	12.07	11.14	5.20	2.10	11.21	11.05	11.00	11.12	10.00
Garra stenorhynchus	65.57-	49.57-	9.17-	10.00-	10.00-	4.00-	1.50-	14.00-	12.00-	11.00-	11.00-	16.00-
	133.00	110.00	18.40	22.00	21.00	13.00	4.00	28.00	22.00	22.00	20.00	25.00
Garra quadratirostris	76.97-	66.97-	9.23-	16.00-	13.00-	6.00-	2.50-	15.60-	15.00-	14.00-	11.50-	10.00-
	125.00	102.00	11.54	25.00	24.00	12.00	4.00	25.00	23.00	22.00	19.00	30.00

Note: TL: Total length, SL: Standard length, BD: Body depth, HL: Head length, ED: Eye diameter, Pre OL: Pre-orbital length, DFL: Dorsal fin length, PFL: Pectoral fin length, VFL: Ventral fin length, AFL: Anal fin length, CFL: Caudal fin length, Minimum (min), Maximum (max), Millimetre (mm), Gram (g).

with the maximum length (54.80 - 135.00 mm) and *G. rufa* with minimum length (64.10 - 70.24 mm). Similarly, Ujjania *et al.* (2012) reported positive increase in morphometric parameters with increase in total length of cold-water fish from Bari Talab, Udaipur, India. Same report was also observed on the morphometric parameters of genus *Garra* in the Himalayan foothills of Himachal Pradesh (Kanwal and Pathani, 2011; Brraich and Akhter, 2015). Additionally, Basudha *et al*. (2018) and Zhang *et al*. (2023) revealed the importance of morphological variation in the identification of freshwater Cyprinidae.

Identification key was developed by examining the external morphological characteristics such as shape of body and head, eye, structure of mouth, rostral cap and lips, presence of lobes and tubercles in the snout, shape of fins and colour pattern (Fig 3 and Table 2). In *Garra*, the morphology of snout and distribution pattern of tubercles in the snout bore significant role in taxonomical differentiation among the species (Sophiya *et al.,* 2020). Considering the morphology of snout, they can be classified as: (i) species with smooth snout (ii) species with transverse lobe (iii) species with proboscis (iv) species with rostral flap and lobe (Ezung *et al.,* 2021). Different species of *Garra* which was described recently from Northeast India includes: *G. gotyla gotyla, G. lamta, G. lissorhynchus, G. arupi, G. nasuta, G. rupecula, G. elongate, G. kempi, G. kimini* which differs by the presence of short tubercles on proboscis, transverse groove, variation in fin rays and lateral line scales (Vishwanath, 2021). The current study also confirms the presence of smooth snout with absence of proboscis in *G. annandalei,* transverse lobe with probosis, rostral flap and lobe in *G. arunachalensis, G. gotyla, G. lamta, G. parastenorhynchus, G. rufa, G. rupecula, G. stenorhynchus* and *G. quadratirostris*. Similarly, Cıcek *et al*. (2016) also reported the importance of morphological traits in identification of *Garra* species from Tigris River system of Southeast Turkey.

Fins plays an important role in identification of most of the freshwater fish species (Kullander and Fang, 2004). Generated fin formula of the species was based on the meristic characters such as numbers of spines and rays in the fins and scales present in lateral line (Table 2). Arunachalam *et al*. (2013) reported that single branched pectoral fin ray was the main distinguishing character of *Garra* present in the Indian subcontinent. In this study, all the species of *Garra* had atleast one branched ray in the pectoral fin, whereas, in case of *G. annandalei* both pectoral and caudal fin was deeply serrated. *G. lissorhynchus* showed the presence of W-shaped band on the caudal-fin and absence of scales near the dorsal fin. This finding was similar with the characters described by Hora (1921) and Bhakta *et al.* (2022). Additionally, Rath *et al*. (2019) also reported on the importance of fin ray counts, fin shapes, relative sizes and its positioning as distinguishing characters in differentiating *Garra* species. Further, IUCN status (ver. 2024-1) was also assigned for the studied species, *G. rupecula* was identified as near threatened (NT) species, *G. arunachalensis, G. parastenorhynchus, G. quadratirostris* were under not evaluated category (NE) and *G. annandalei, G. gotyla, G. lamta, G. lissorhynchus, G. rufa, G. stenorhynchus,* were under least concerned (LC) category (Table 2).

The regression analysis for LLRs was established to assess the symmetrical growth pattern in relation to TL with SL, BD and HL (Table 3). A significant positive relationship ($p<0.01$ and 0.05) was observed with 'r' values > 0.765. The values of coefficient of correlation have been found to be highly significant ($p<0.01$) for total length (TL) and standard length (SL) in all the species of genus *Garra.* Similarly, the value was significantly ($p<0.05$) higher in all other parameters except for head length (HL) and body depth (BD) in *G. annandalei, G. lissorhynchus, G. parastenorhynchus* and *G. rufa* (Table 3). The analysis from regression equation clearly revealed that the lengths of

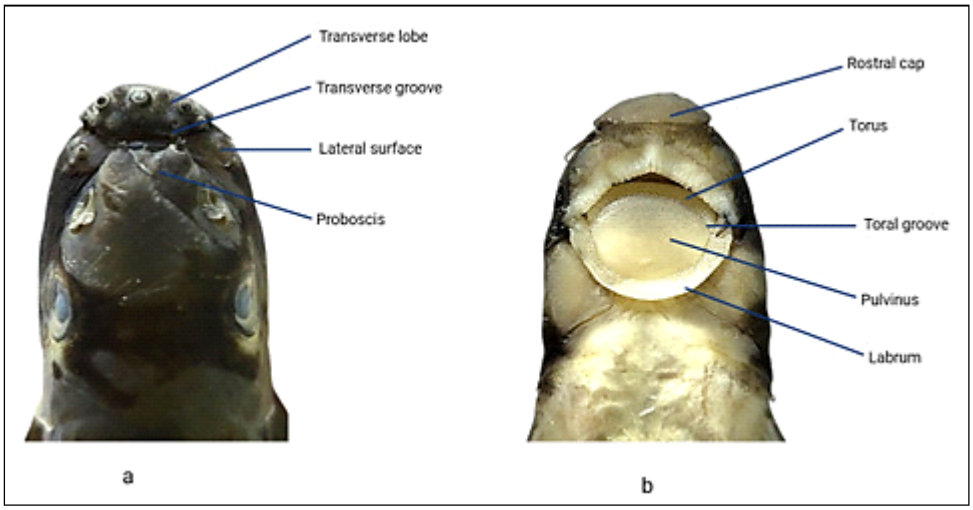


Fig 3: (a) Snout morphology and (b) Oromandibular structures of genus *Garra*.

Table 2: Reported identification keys and fin formulae of the studied specimens of genus *Garra.*

Fish species	IUCN status	Diagnosis character	Fin formula	Photograph
Garra annandalei (Hora, 1921)	LC	Dark grey and cylindrical body with slightly depressed head. Presence of smooth snout without proboscis. Mouth was small and semi-circular. Lower lip was modified into mantle disc, upper lip had minute fringes. Presences of two pairs of maxillary and rostral barbels. Pectoral fin was shorter than the head length. Both pectoral and caudal fin was deeply serrated.	$D_9 P_{14-15} V_{8-9} A_{6-9} C_{21}$ LLS $_{32-35}$	
Garra arunachalensis (Nebeshwar and Vishwanath, 2013)	NE	Body was greyish on dorsal side and pale ventrally. Body was compressed laterally near the caudal peduncle. Snout showed the presence of transverse lobe. Presence of quadrate proboscis with large unicuspidal tubercle. Rostral cap and lobe were distinct. Upper lip had poorly developed papillae. Large gular disc was present on lower lip while labellum was absent. Numerous scales were present in the ventral portion of the body.	$D_{8-9} P_{14-15} V_{8-9} A_{6-7} C_{19}$ LLS $_{34-35}$	
Garra gotyla (Gray, 1830)	LC	Cylindrical body, snout had transverse lobe with small tubercles. Proboscis was slightly bilobed with small tubercle in each lobe and slightly depressed rostral surface. Lower lip is modified into mantle elliptical disc.	$D_8 P_{12-13} V_{7-8} A_5 C_{21}$ LLS $_{33-35}$	
Garra lamta (Hamilton, 1822)	LC	Body dark brown and snout with a well-developed bilobed proboscis. Presence of transverse groove. Mouth was inferior with a large gular disc. Enlarged paired fins were present that were horizontally situated.	$D_{3/7-8} P_{1/12} V_{10} A_{2/5} C_{18}$ LLS $_{33-35}$	
Garra lissorhynchus (McClelland, 1842)	LC	Body was semi cylindrical. Snout was smooth without proboscis. Rostral lobe and cap were not prominent. Mouth inferior with small gular disc behind it. Minute fringes were present on the upper lips. Submarginal black band was	$D_{7-8} P_7 V_6 A_6 C_{10}$ LLS $_{30-33}$	

Table 2: Continue...

		present on the dorsal fin. Prominent W-shaped band was present on the caudal fin. Besides, the caudal fin was blunt in shape.		
Garra parastenorhynchus (Thoni, Gurung and Mayden, 2016)	NE	Body was laterally compressed, more tapered towards the caudal peduncle. Head was large and slightly depressed near the rostral lobe but dorsally it was rising over the snout that was continuous up to the origin of dorsal-fin and gently sloping towards the end of caudal peduncle. Eyes were dorso -laterally located with slightly convex inter-orbital area.	$D_8 P_{11-13} V_8 A_{6-7} C_{19-21}$ LLS $_{33-35}$	
Garra rufa (Heckel, 1843)	LC	Body shape was elongated with inflated head and small eyes. Mouth was ventrally located with lower lip modified into adhesive pad. Snout had minute tubercles with presence of proboscis and transverse groove. Presence of a pair of maxillary barbel.	$D_7 P_6 V_{5-6} A_6 C_{11-13}$ LLS $_{34-35}$	
Garra rupecula (McClelland, 1839)	NT	Body was fusiform. Presence of well-developed suctorial disc in the ventral side of mouth. Proboscis was absent. Presence of two pairs of barbels. A Black spot was present behind upper angle of gill opening.	$D_{5-7} P_6 V_{5-6} A_5 C_8$ LLS $_{32-35}$	
Garra stenorhynchus (Jerdon, 1849)	LC	Well-developed proboscis with transverse lobe on snout and adhesive pad on ventral side of snout. Presence of rostral cap. Paired pectoral and pelvic fin and anal fin; a black spot at upper angle of gill opening; a row of dark spots at base of branched dorsal fin rays.	$D_{10-15} P_{20-24} V_{9-12}$ $A_{10-11} C_{23-25}$ LLS $_{34-35}$	
Garra quadratirostris (Nebeshwar and Vishwanath, 2013)	NE	Body was elongated and slightly compressed laterally. Presence two pairs of barbels, maxillary barbel present at the corner of mouth, shorter than rostral barbel. Rostral cap well-developed and moderately fimbriate.	$D_{14-16} P_{14-15} V_{8-10} A_{8-10}$ C_{19-20} LLS $_{34-35}$	

Note: D: Dorsal fin ray; P: Pectoral fin ray; V: Ventral fin ray; A: Anal fin ray; C: Caudal fin ray; LLS: Lateral line scale.

Table 3: Length-length relationships (LLRs) between the total length and other body parameters of the studied species of genus *Garra*.

Species	Body parameters	Regression equation	r	P value
Garra annandalei (n=21)	SL	y = 0.7185x + 1.6127	0.934	0.008
	HL	y = 0.1335x+ 1.1914	0.791	0.061
	BD	y = 0.2569x + 0.1414	0.814	0.032
Garra arunachalensis (n=10)	SL	y = 0.7325x + 1.6548	0.982	0.025
	HL	y = 0.1315x+ 1.1812	0.885	0.056
	BD	y = 0.2614x + 0.1951	0.972	0.048
Garra gotyla (n=17)	SL	y = 0.8288x + 1.5721	0.924	0.004
	HL	y = 0.1155x + 1.1845	0.786	0.045
	BD	y = 0.2217x + 0.1335	0.812	0.035
Garra lamta (n=12)	SL	y = 0.6671x + 1.6456	0.845	0.008
	HL	y = 0.1238x + 1.1452	0.942	0.031
	BD	y = 0.2426x + 0.1975	0.821	0.028
Garra lissorhynchus (n=10)	SL	y = 0.8593x + 1.5257	0.912	0.007
	HL	y = 0.1348x + 1.1814	0.765	0.091
	BD	y = 0.2488x + 0.1751	0.886	0.079
Garra parastenorhynchus (n=12)	SL	y = 0.8785x + 1.6145	0.973	0.008
	HL	y = 0.1157x + 1.1647	0.875	0.085
	BD	y = 0.2575x + 0.1945	0.905	0.071
Garra rufa (n=10)	SL	y = 0.8571x + 1.5836	0.974	0.008
	HL	y = 0.1303x + 1.1848	0.769	0.090
	BD	y = 0.2773x + 0.1319	0.893	0.037
Garra rupecula (n=10)	SL	y = 0.8673x + 1.6124	0.983	0.008
	HL	y = 0.1256x + 1.1816	0.827	0.054
	BD	y = 0.2575x + 0.1945	0.829	0.036
Garra stenorhynchus (n=10)	SL	y = 0.8557x + 1.5795	0.943	0.007
	HL	y = 0.1386x + 1.1864	0.847	0.035
	BD	y = 0.2571x + 0.1345	0.793	0.031
Garra quadratirostris (n=10)	SL	y = 0.8548x + 1.6175	0.864	0.008
	HL	y = 0.1456x + 1.1879	0.951	0.050
	BD	y = 0.2547x + 0.1904	0.803	0.043

Note: n: Total of individual specimen; SL: Standard length; BD: Body depth: HL: Head length.

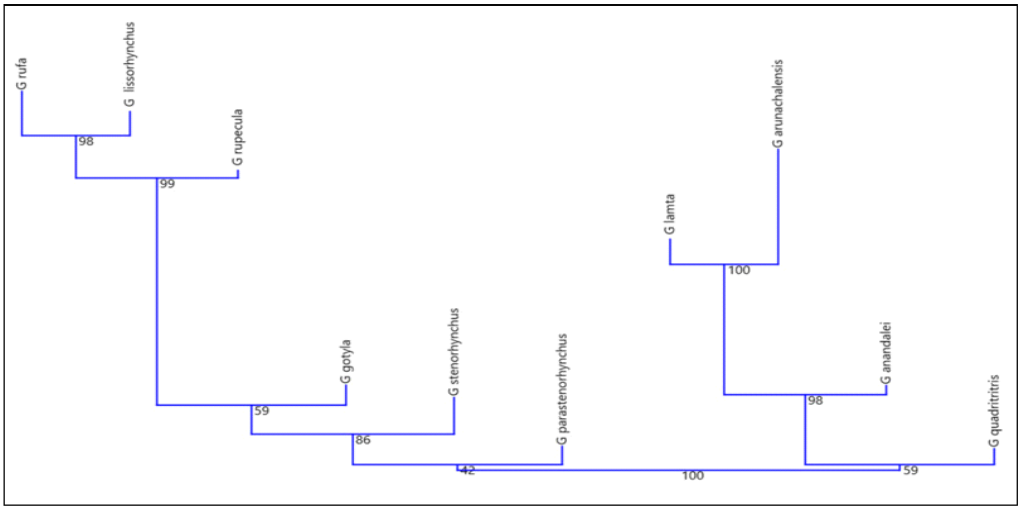


Fig 4: Phylogenetic tree based on morphometric parameters of the studied species of genus *Garra*.

the studied morphometric parameters were proportionate to the total length which was similar with the reports of Hossain (2010) and Alam *et al.* (2014). Further, Brraich and Akhter (2015) observed significant correlation between total length and external body parts in hill stream fish, *Crossocheilus latius latius*.

In the present investigation, a phylogenetic tree was constructed based on morphometric parameters through neighbor-joining (NJ) clustering method with bootstraps 100 which depicts that *G. lissorhynchus, G. rufa* and *G. rupecula* were in the common ancestral group. Species such as *G. gotyla, G. parastenorhynchus* and *G. stenorhynchus* were found related to each other. Further, the study also reports that *G. arunachalensis* and *G. lamta* were closely related species, whereas, *G. annandalei* and *G. quadratirostris* were distantly related to *G. arunachalensis* and *G. lamta* (Fig 4). Similar studies were also conducted on Selangat fish from Kelabat Bay and Tukak Strait, Indonesia where 12 morphometric characters confirmed the significance of morphometric variables in construction of phylogenetic tree (Aisyah and Syarif, 2019). Further, reports on evolution of bony fishes using multi-locus phylogenetic analysis found the remarkable importance of morphological factors (Broughton *et al.,* 2013). Betancur-R *et al*. (2013) also used the systematic concepts of construction of phylogenetic tree based on morphology to describe the relationships among fish lineages. Besides, Muser and Wagner (2015) from their study claimed that the stability and accuracy of phylogenetic trees constructed through molecular data set could be influenced by the morphological data.

CONCLUSION

The current study has provided additional data on the morphological characters of genus *Garra* found in the hill stream rivers of Arunachal Pradesh. The morphological characters such as presence and absence of lobes, tubercles, rostral cap, transverse groove, gular disc appears to be the key identifying features of the genus *Garra*. Linear length-length relationship was observed between the dependent character (TL) and other independent body characters. Constant in morphometric measurements and meristic counts of each fish reveals the uniqueness among the species of the genus which is also evident from the generation of fin formulae. Morpho-phylogenetic analysis confirmed the evolutionary relatedness among the species.

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Conflict of interest

The authors declare no conflicts of interest.

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