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Morphological profile of the forelimb long bones and ribs in the Ganges River dolphin, *Platanista gangetica* (Lebeck, 1801) (Mammalia: Cetacea: Platanistidae)

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We conducted a study on the morphology of the forelimb long bones and ribs in the Ganges River dolphin, *Platanista gangetica* (Lebeck, 1801) using traditional morphometric methods. A total of 17 morphometric measurements of six specimens of *P. gangetica* were taken. The humerus was the longest bone in the forelimb morphology, with its length ranging from 41.75 to 52.78 mm (mean: 45.57, SD: ± 5.07) in juveniles/subadults and 60.56 to 90.92 mm (mean: 76.75, SD: ± 22.90) in adults. The length of the scapula was 85.84 to 135.46 mm (mean: 102.18, SD: ± 23.00) in juveniles/subadults and 171.79 to 251.95 mm (mean: 211.87, SD: ± 56.68) in adults. Of 10 ribs, the sixth rib was the longest (mean: 133.55, SD: ± 24.12) in juveniles/subadults and the seventh (mean: 240.62, SD: ± 54.24) in adults. All measurements (except the two variables of the scapula) were significantly correlated ($P < 0.05$) with specific bone length. The allometric coefficients of all correlated variables of the humerus, radius, ulna, scapula, and the depths of ribs exhibited isometry against the length of the particular bone, respectively. We discussed the morphological patterns of the forelimb long bones and ribs in *P. gangetica* along with the structural and functional aspects of the aquatic lifestyles.

Key words: Allometry, forelimb, morphological pattern, *Platanista gangetica*, ribs

Introduction

The appendicular skeleton of cetaceans can be divided into the forelimbs, pectoral girdle, and pelvic girdle (Kipps et al., 2002; Cooper, 2009). Some aquatic mammals, including pinnipeds show appendicular skeletal similarities with terrestrial mammals (Endo et al., 1999; Shil et al., 2022). However, the structure of cetaceans, called flippers that lack separate fingers, is different from that of terrestrial mammals (Carwardine, 1996; Cooper et al., 2007). Instead, flippers consist of elongated and flexible bones covered by layers of tissue that forms a paddle shape (Muller, 2021). The forelimb long bones consist of three components, the humerus, radius, and ulna, which collectively contribute to its structure and function (Casteleyn and Bakker, 2019; Das et al., 2023). The scapula, often referred to as the shoulder blade, serves as an important bridge between the forelimb and the axial skeleton, facilitating the connection of these limbs to the body (Cooper, 2009). This scapula bone provides vital support and attachment, and enables dolphins to execute a wide range of movements with their flippers (Muller, 2021).

Ribs are essential skeletal structures that form the ribcage, which plays an important role in protecting the internal organs (Noble and Sooraj, 2023). In aquatic mammals, rib structures have undergone remarkable adaptations to suit an aquatic lifestyle and often exhibit distinctive features, notably a flatter and wider shape compared to those of their terrestrial counterparts (Villanueva et al., 2015; Ando and

Fujiwara, 2016). The strongly curved ribs connect with the vertebral column in the back and the sternum in the front. This union collectively shapes the rib cage (Marchesi et al., 2016). In addition to enclosing the thoracic region, cetacean ribs provide structural support and contribute to the streamlining of the body, aiding in the reduction of drag (Kallal et al., 2012).

The Ganges River dolphin, *Platanista gangetica* (Lebeck, 1801), inhabits the freshwater systems of the Ganges-Brahmaputra-Meghna and Karnaphuli-Sangu rivers, spanning across India, Bangladesh, Nepal, and Bhutan (Mohan et al., 1997; Smith et al., 2001; Dewhurst-Richman et al., 2020). Within these river networks, *P. gangetica* navigates various aquatic habitats, from deep channels to shallow sandy areas, and occasionally ventures into tidal estuaries near the coast (Smith et al., 2006, Khan, 2019). The IUCN Red List (2022) officially designates this species as "Endangered" due to recent substantial reduction in geographic range and population size (Choudhury et al., 2006; Braulik and Smith, 2017; Bordoloi and Sahara, 2021; Kelkar et al., 2022). Several recent studies have been conducted on the morphology of the forelimb and ribs of aquatic mammals (Amson, et al., 2014; Castillo, et al. 2014; Ando and Fujiwara, 2016; Botton-Divet et al., 2016, 2017; Cooper et al., 2017; Hocking et al., 2018; Zadravec et al., 2020), most of which focused on adaptation in aquatic life and evolution of the bones. Two historical studies were published documenting variation in the forelimb (mostly numbers of carpals and phalanges) of *P. gangetica* through the use of radiograms. However, these did not include measurements (Turner, 1910; Pilleri and Gihr, 1976). Morphological studies have an important role in the field of biology, facilitating a comprehensive understanding of physical features, including size, shape, and structural characteristics (Meiri and Liang, 2021). Morphometric study of the forelimb and ribs of aquatic mammals can provide useful insights into their adaptations to aquatic life (Reidenberg, 2007). Thus, the present study focuses on morphological patterns of the forelimb long bones and ribs in *P. gangetica*, with particular reference to its distribution in the Halda River of Bangladesh.

Material and Methods

Specimen collection and measurements

The skeletons of *P gangetica* preserved in Halda River Research Laboratory, University of Chittagong (Fig. 1) as well as those buried on the different bank sides of the Halda River were examined at the Department of Zoology, University of Chittagong, Bangladesh.

A total of 17 morphometric measurements were taken using six specimens of different ages (Table 1; Fig. 2). Based on relative measurements and surface smoothness, four of the six specimens were considered juvenile or subadult (juv/subadult) and two as adult (Elbroch, 2006). Sex was unknown, as specimens had been buried without biological examination. Bone measurements were obtained with digital slide calipers to the nearest mm following established methods (Perrin, 1975; Peleg et al., 2020; Muller, 2021).

Table 1: List of measurements and abbreviations used for the forelimb long bones and ribs in *Platanista gangetica.*

Bones	Abbreviation	Measurement
Humerus	DLH	Deltoid length of humerus
	WH	Width of humerus
	DEH	Diameter of epicondyles of humerus
	HL	Length of humerus
Radius	RL	Length of radius
	RW	Width of radius
Ulna	UL	Total length of ulna
	FUL	Functional length of Ulna
	OL	Olecranon length
	WU	Width of ulna
Scapula	LOSc	Length of scapula with cartilage
	HOSc	Height of scapula with cartilage
	AMaxH	Acromion max height
	AMinH	Acromion min height
	SFW	Supraspinous fossa width
Ribs	Length	Length of ribs
	Depth	Depth of ribs

Figure 1: Forelimb long bones and ribs of *Platanista gangetica*: (A) humerus, (B) radius, (C) ulna, (D) scapula, and (E) ribs.

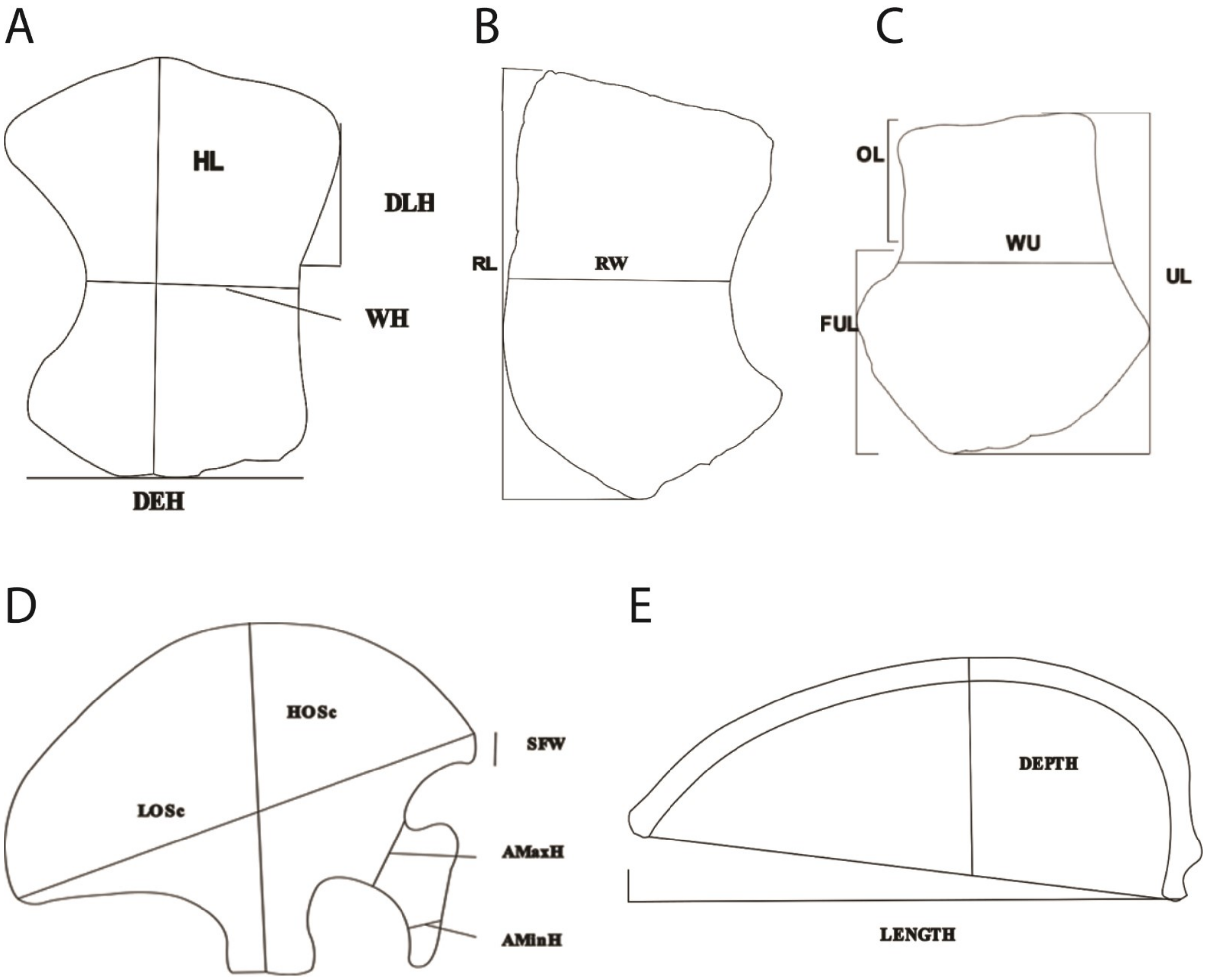

Figure 2: Morphometric measurements used in *Platanista gangetica*: (A) humerus, (B) radius, (C) ulna, (D) scapula, and (E) rib. Explanation of measurements is presented in Table 1.

Analyses of morphometric measurements

Basic statistical parameters [mean (M), standard deviation (SD), minimum and maximum values] were calculated using measurements of the forelimb long bones, scapula, and ribs of juv/subadult and adult samples separately. The Mann-Whitney *U* test was used to compare right and left sides of the humerus, radius, ulna, scapula, and ribs (Das et al., 2023). As no significant difference was observed between right and left side values of the humerus, scapula, and ribs (P > 0.05), right sided values were considered for further analyses (Das et al. 2023). We calculated the correlation between M and SD for all measurements separately to assess patterns of variability in both age groups (see Polly, 1998). We also used the Mann-Whitney *U* test to analyze the differences between juv/subadult and adult samples.

Pearson correlation coefficients were calculated to examine the relationship of the variables of the humerus, radius, ulna, and scapular bones against the length of the humerus (HL), ulna (UL), radius (RL), and scapula with cartilage (LOSc), respectively (Biswas and Motokawa, 2019; Das et al., 2023). Allometric analysis was performed for variables of the humerus, radius, ulna, scapular bones with corresponding bone length (Das et al., 2023), i. e. HL, UL, RL, and LOSc served as an independent variable (Biswas and Motokawa, 2019; Das et al., 2023). The correlation coefficients and allometry of all rib depths were also examined against the respective rib length. For these analyses (Pearson correlation coefficients and allometry), combined data of juv/subadult and adult samples were considered. We used a similar method (ordinary least square regression) to examine allometric coefficients following Biswas and Motokawa (2019). The statistical program 'Past' (ver. 4.09 b) (Hammer et al., 2001) was used to analyze morphological data.

Results

Forelimb long bones and scapular morphology

The humerus was the longest bone in the forelimb morphology, with the length ranging from 41.75 mm to 52.78 mm (mean: 45.57, SD: ± 5.07) in juv/subadults and 60.56 mm to 90.92 mm (mean: 76.75, SD: ± 22.90) in adults (Table 2). The radius length ranged from 22.43 mm to 40.77 mm (mean: 31.47, SD: ± 9.17) in juv/subadults and 43.74 mm in adults (Tabel 2). The length of the ulna varied from 23.05 mm to 36.25 mm (mean: 29.56, SD: ± 6.60) in juv/subadults and 41.21 mm in adults (Table 2). The mean values of forelimb variables were significantly different between juv/subadult and adult samples (Mann-Whitney U = 15, P < 0.01). However, the mean values of the humerus showed positive correlation with SD in both juv/subadult (r = 0.608, P = 0.392) and adult (r = 0.976, P = 0.024) samples (Fig. 3A).

Scapular length with cartilage was 85.84 mm to 135.46 mm (mean: 102.18, SD: ± 23.00) in juv/subadults and 171.79 mm to 251.95 mm (mean: 211.87, SD: ± 56.68) in adults (Table 2). The means of scapular variables did not differ significantly between juv/subadult and adult samples (Mann-Whitney U = 15, P < 0.01). The mean values of the scapular variables showed positive correlation with SD in both juv/subadult (r = 0.953, P = 0.012) and adult (r = 0.989, P = 0.001) samples (Fig. 3B).

Rib morphology

The length of R1 exhibited the lowest range, from 49.86 mm to 66.68 mm (mean: 57.96, SD: ± 7.53) in juv/subadults and from 98.57 mm to 134.71 mm (mean: 116.64, SD: ± 25.55) in adults (Table 3). The highest mean value was found for R6 [113.32 mm to 168.40 mm (mean: 133.55, SD: ± 24.12)] in juv/subadults, whereas for R7 [202.26 mm to 278.97 mm (mean: 240.62, SD: ± 54.24)] in adults (Table 3). The mean values of rib lengths were significantly different between juv/subadult and adult samples (Mann-Whitney U = 7, P < 0.01). However, the mean values of rib lengths showed positive correlation with SD in both juv/subadult (r = 0.913, P < 0.001) and adult (r = 0.916, P < 0.001) samples (Fig. 4A).

The highest mean was observed for the depth of R5 in both juv/subadults [38.71 mm to 56.04 mm (mean: 44.12, SD: ± 8.15)] and adults [67.37 mm to 86.07 mm (mean: 86.72, SD: ± 13.22)] (Table 3). The lowest mean was detected for the depth of R1 in juv/subadults [20.76 mm to 30.37 mm (mean: 24.85, SD: ± 4.20)], whereas for R10 in adults [44.38 mm to 45.17 mm (mean: 44.78, SD: ± 0.56)] (Table 3). The mean values of rib depth also differed between juv/subadult and adult samples (Mann-Whitney U = 00, P < 0.01). A positive correlation was found between mean values of rib depth and SD in both juv/subadult (r = 0.671, P < 0.05) and adult (r = 0.505, P = 0.136) samples (Fig. 4B).

Correlation and allometry in forelimb long bones and scapula

All variables measured for the humerus (DLH, WH, DEH) showed significant positive correlation with humerus length (Table 4). The coefficient of determination ranged from 0.91 to 0.95 (mean: 0.93, SD: ± 0.02). The allometric coefficients ranged from 1.09 (DLH) to 1.27 (WH) and showed isometry with HL (Table 4). Radius width showed strong correlation (r = 0.998, P < 0.05) with radius length and showed isometry. All measured variables for the ulna (WU, OL, FUL) exhibited significant correlation with UL (Table 4). The coefficient of determination ranged from 0.93 to 0.95 (mean: 0.94, SD ± 0.01). The allometric coefficients ranged from 0.67 (WH) to 1.50 (OL) and exhibited isometry with UL (Table 4).

Two variables measured for the scapula (HOSc and SFW) showed significant correlation with scapular length (Table 4). The coefficient of determination ranged from 0.44 to 0.98 (mean: 0.74, SD: ± 0.27). The correlated variables (HOSc and SFW) showed isometry with LOSc (Table 4).

Correlation and allometry in ribs

The depths of all ribs showed significant positive correlation against respective rib length (Table 5). The coefficient of determination ranged from 0.711 (depth of R3) to 0.995 (depth of R6) (mean: 0.939, SD: ± 0.99). Allometric coefficient varied from 0.77 to 1.08 (mean: 0.96, SD: ± 0.99). The depths of all rib bones (except depth of R2) showed isometry against respective rib length (Table 5).

Table 2: Descriptive statistics of morphometric measurements (in mm) of the forelimb and scapular bones in *Platanista gangetica* (SD: standard deviation, Min: minimum, Max: maximum).

Bones	Variables	Juvenile/Subadult (N= 4, N= 3 for radius and ulna)				Adult (N= 2, N= 1 for radius and ulna)			
		Mean	SD	Min	Max	Mean	SD	Min	Max
Humerus	DLH	20.68	3.43	17.32	24.57	37.68	6.32	33.21	42.15
	WH	19.37	4.34	15.30	24.03	37.95	8.13	32.20	43.70
	DEH	22.76	4.92	18.54	28.91	41.34	11.65	33.10	49.58
	HL	45.57	5.07	41.75	52.78	76.75	22.90	60.56	92.94
Ulna	UL	29.56	6.60	23.05	36.25	41.21	-	-	-
	FUL	19.59	6.42	12.38	24.71	29.90	-	-	-
	OL	13.90	4.91	10.19	19.47	23.32	-	-	-
	WU	22.44	2.68	19.45	24.62	29.88	-	-	-
Radius	RL	31.47	9.17	22.43	40.77	43.74	-	-	-
	RW	15.79	5.05	10.70	20.79	23.82	-	-	-
Scapula	LOSc	102.18	23.00	85.84	135.46	211.87	56.68	171.79	251.95
	HOSc	71.42	9.45	64.91	85.43	133.42	28.96	112.94	153.90
	AMaxH	19.67	4.41	16.96	26.25	41.84	12.73	32.84	50.84
	AMinH	11.53	1.86	10.41	14.31	22.82	3.79	20.14	25.50
	SFW	13.31	2.03	11.00	15.21	24.20	6.68	19.48	28.92

Table 3: Descriptive statistics of morphometric measurements (in mm) of the ribs in *Platanista gangetica* (SD: standard deviation, Min: minimum, Max: maximum).

Bones	Variables	Juvenile/Subadult (N= 4)				Adult (N= 2)			
		Mean	SD	Min	Max	Mean	SD	Min	Max
Length of Ribs	R1	57.96	7.53	49.86	66.68	116.64	25.55	98.57	134.71
	R2	98.62	15.03	86.71	120.47	187.33	41.20	158.19	216.46
	R3	120.64	17.34	109.44	146.50	215.54	52.58	178.36	252.72
	R4	127.52	19.98	111.76	156.46	226.98	61.13	183.75	270.20
	R5	131.37	22.84	113.32	164.34	236.51	60.78	193.53	279.49
	R6	133.55	24.12	113.96	168.40	237.07	57.47	196.43	277.70
	R7	127.25	22.97	107.89	160.20	240.62	54.24	202.26	278.97
	R8	124.34	25.02	100.42	159.21	236.46	53.00	198.98	273.94
	R9	121.17	24.30	99.89	155.47	223.95	45.51	191.77	256.13
	R10	108.82	19.81	91.47	136.05	211.95	52.01	175.17	248.73
Depth of Ribs	R1	24.85	4.20	20.76	30.37	48.62	9.43	41.95	55.29
	R2	34.54	5.59	30.77	42.86	60.21	10.54	52.75	67.66
	R3	40.40	6.14	36.42	49.56	58.90	29.27	38.20	79.59
	R4	42.75	7.86	35.47	53.92	75.61	21.57	60.36	90.86
	R5	44.12	8.15	38.71	56.04	76.72	13.22	67.37	86.07
	R6	43.47	7.22	38.72	54.00	75.89	18.33	62.93	88.85
	R7	39.27	9.79	31.83	53.15	75.44	18.21	62.57	88.31
	R8	33.89	7.58	27.39	43.79	68.45	8.85	62.19	74.71
	R9	31.22	6.71	26.05	40.76	61.27	9.87	54.29	68.25
	R10	26.34	5.89	18.90	33.30	44.78	0.56	44.38	45.17

Table 4: Patterns of correlation and allometry of the forelimb long bones and scapula in *Platanista gangetica* (r: Correlation coefficient; r2: coefficient of determination; α: Coefficient of allometry; I: isometry, and P_{ISO}: deviation from isometry).

Bones	Variables	r	r^2	P	α	t	P_{ISO}
Humerus	DLH	0.96	0.92	< 0.05	1.09 I	6.89	> 0.05
	WH	0.95	0.91	< 0.05	1.27 I	6.35	> 0.05
	DEH	0.97	0.95	< 0.05	1.19 I	8.63	> 0.05
Ulna	WU	0.97	0.93	< 0.05	0.67 I	5.24	> 0.05
	OL	0.97	0.95	< 0.05	1.50 I	6.10	> 0.05
	FUL	0.97	0.94	< 0.05	1.44 I	5.57	> 0.05
Radius	RW	0.998	0.996	< 0.05	1.16 I	21.59	> 0.05
Scapula	HOSc	0.99	0.98	< 0.05	0.81 I	16.2	> 0.05
	AmaxH	0.67	0.44	> 0.05	-	-	-
	AminH	0.77	0.59	> 0.05	-	-	-
	SFW	0.97	0.95	< 0.05	0.796 I	8.42	> 0.05

Table 5: Patterns of correlation and allometry of the ribs in *Platanista gangetica* (r: Correlation coefficient; r2: coefficient of determination; α: Coefficient of allometry; I: isometry, N: negative allometry, and P_{ISO}: deviation from isometry).

Variables	r	r_2	p	α	t	P_{ISO}
Depth of R1	0.995	0.991	< 0.01	0.98 I	20.63	> 0.05
Depth of R2	0.992	0.984	< 0.01	0.88 N	15.84	< 0.05
Depth of R3	0.843	0.711	< 0.05	0.77 I	3.14	> 0.05
Depth of R4	0.996	0.991	< 0.01	1.02 I	21.42	> 0.05
Depth of R5	0.993	0.986	< 0.01	0.94 I	17.08	> 0.05
Depth of R6	0.998	0.995	< 0.01	0.96 I	29.33	> 0.05
Depth of R7	0.989	0.979	< 0.01	1.08 I	13.50	> 0.05
Depth of R8	0.984	0.969	< 0.01	1.07 I	11.14	> 0.05
Depth of R9	0.991	0.983	< 0.01	1.07 I	15.23	> 0.05
Depth of R10	0.893	0.797	< 0.05	0.78 I	3.96	> 0.05

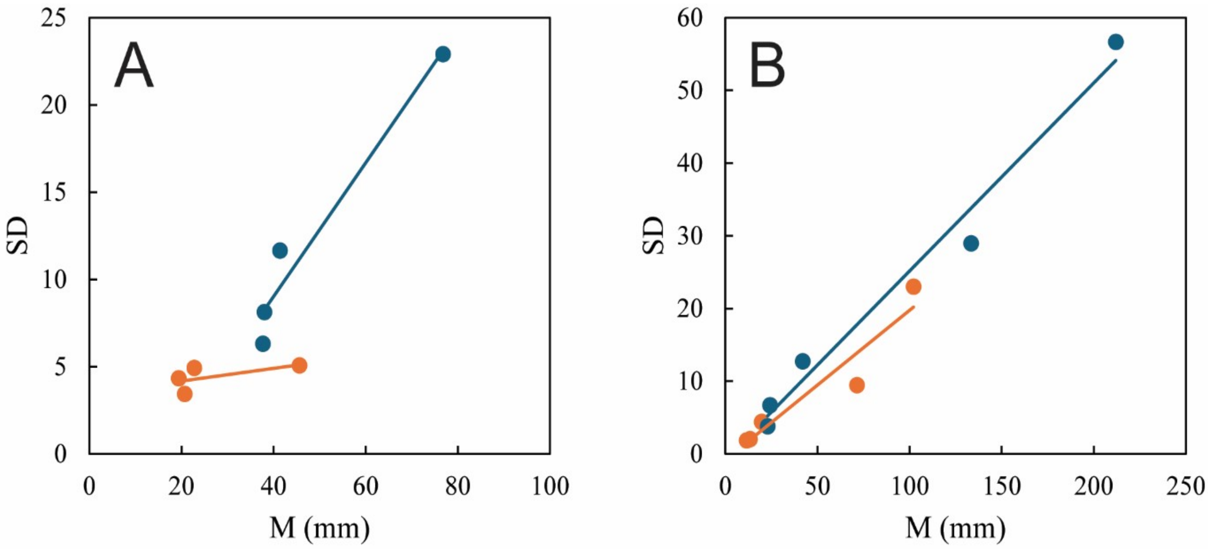

Figure 3: Plots of mean (M) versus standard deviation (SD) of humerus (A) and scapula (B) measurements in *Platanista gangetica*. Juvenile/subadult and adult specimens are represented by orange and blue colors, respectively.

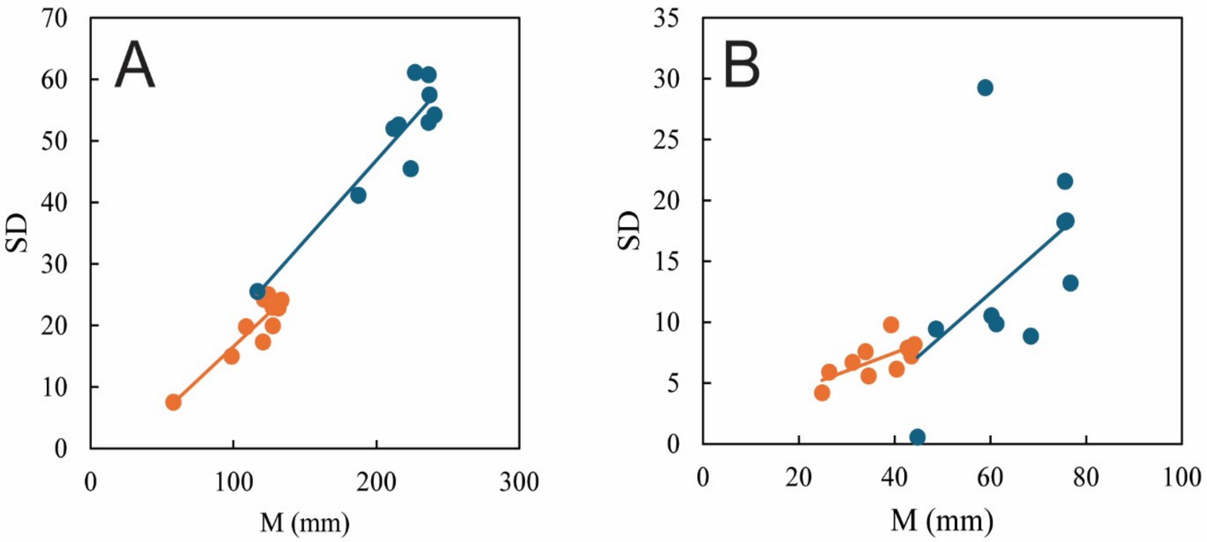

Figure 4: Plots of mean (M) versus standard deviation (SD) of rib length (A) and depth (B) in *Platanista gangetica*. Juvenile/subadult and adult specimens are represented by orange and blue colors, respectively.

Discussion

The forelimbs have evolved into streamlined flippers, optimized for swimming and diving skill in aquatic environments (Cooper et al., 2007; Sun et al., 2022). Our study found that the humerus was the longest bone in the forelimb of *P. gangetica* similar as in most aquatic mammals, with a length of 41.75 mm to 52.78 mm (mean: 45.57, SD: ± 5.07) in juv/subadults and 60.56 mm to 90.92 mm (mean: 76.75, SD: ± 22.90) in adults. The humerus length was 52.0–73.10 mm for the common dolphin (*Delphinus delphis*), 85.0–103.0 mm for the Risso's dolphin (*Grampus griseus*), 50.0–51.0 mm for the harbour porpoise (*Phocoena phocoena*), 51.5–53.0 mm for the vaquita (*Phocoena sinus*), and 46.5–53.0 mm for the pantropical spotted dolphin (*Stenella attenuata*) (Sanchez and Berta, 2010). Our study also found that the scapula length was 85.84 mm to 135.46 mm (mean: 102.18, SD: ± 23.00) in juv/subadults and 171.79 mm to 251.95 mm (mean: 211.87, SD: ± 56.68) in adults. Sanchez and Berta (2010) reported scapular lengths of 109.0–123.5 mm for *D. delphis*, 172.0–204.0 mm for *G. griseus*, 116.0–118.0 mm for *P. phocoena*, 122.0 mm for *P. sinus*, and 106.4–170.0 mm for *S. attenuata*. Based on these comparisons, it can be inferred that structural variation among the bones of different cetaceans is broadly related to their overall body size and shape.

We also found differences in the structure of the forelimb long bones and ribs between juv/subadult and adult specimens, indicating age variation. More studies covering samples of different ages are needed to explain the functional aspects of age variation. Furthermore, a positive correlation between mean values and standard deviations was detected in both juv/subadult and adult samples, as expected (Polly, 1998; Biswas and Motokawa, 2019).

Variables of forelimb long bones (humerus, radius, and ulna) exhibited a significant correlation with respective bone length and displayed an isometric relationship. Two variables of the scapula (HOSc and SFW) also showed significant correlation with scapular length and showed isometry. This may indicate somatic patterns of growth (a biological process that regulates progressive changes in body composition) for these bone characteristics of the forelimb and scapula (Biswas and Motokawa, 2019; Zaniqueli et al., 2020). These bony components play an important role in muscle attachment (Zhang and Ge, 2014; Biswas and Motokawa, 2019; Das et al., 2023). However, the minimum and maximum heights of the scapular acromion showed non-significant correlation with scapular length. These patterns may be related to functional stress on these bony components from early age (Doube et al., 2009; Das et al., 2023), as these areas are usually attached to the humeral head (Cooper et al., 2007). These components may be used for supporting loadings modes such as bending and torsion during flipper movement (Cooper et al., 2007; Doube et al., 2009). The interaction between the scapula and humerus is essential for achieving the range of motion required for swimming and locomotion in aquatic environments (Klima et al., 1980).

Among the 10 ribs, the middle bones (R5, R6, and R7) showed relatively greater length and depth than the others. The length and depth were gradually reduced in R1 and R10. Furthermore, we found significant positive correlations between all rib depths with the corresponding rib length. All rib depths (except the depth of R2) showed isometry against the respective rib length. These patterns may be related to the function of the skeletal framework in supporting and protecting the internal organs (Villanueva et al., 2015). The ribs exhibit a distinctive curvature, forming a connection with the vertebral column dorsally and the sternum ventrally. This curvature contributes to the structural integrity of the rib cage while simultaneously reducing the overall weight of the bone (Connolly et al., 1978). It enables the maintenance of a streamlined body profile in the water which reduces hydrodynamic drag and allows more efficient movement through the aquatic environment (William, 1983).

Conclusions

The present study provides preliminary data on the length, width, and shape of the forelimb long bones and ribs in *P. gangetica*, which will serve as a reference for further anatomical studies. Besides, the correlation and allometric patterns of the forelimb and ribs provide basic information about structural and functional aspects associated with an aquatic lifestyle.

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Author contributions

J.U.N.T., M.M.K., and J.K.B. conceived and designed the study, analyzed the data, and wrote the manuscript. J.U.N.T., A.K.B., and S.M.K. collected the specimens and carried out the morphological measurements. All authors reviewed the final article.

Conflicts of interest

All the authors declare that there are no conflicting issues related to this research article.

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