Sexual Dimorphism revealed through Craniometrical Analysis in *Macaca mulatta* from Northern Pakistan

Muhammad Ammar[1], Muhammad Tahir Waseem[2],*Abdul Majid Khan[1], Ayesha Iqbal[1], Sania Zubaid[1] Arshia Naseer[1] and Sehri Shahid[1]

[1]Ecology and Evolutionary Biology Lab, Institute of Zoology, University of the Punjab Lahore (54590)

[2]Zoological Science Division, Pakistan Museum of Natural History, Islamabad, Pakistan (44000)

ABSTRACT:

The Rhesus monkey (*Macaca mulatta*) is a significant non-human primate species found in Pakistan's northern areas. *Macaca mulatta* has evolved to survive in a wide range of environmental conditions, depending upon the variety of food items, and is considered to be the most scattered primate on the earth. Craniometry, the fundamental morphometric approach, has been used to identify differences in the skulls of males and females of this species. Eight preserved skulls—four of each gender—were used in the current study and were acquired from the Zoological Museum at the University of the Punjab, Lahore, Pakistan. A total of 48 skull variables (39 cranial and 9 mandibular) were analysed to find out the differences in male and female skulls. Most of the skull variables did not show any statistically significant difference except for a few variables. Results indicated that the males of *Macaca mulatta* have larger neurocranium, a slightly broader zygomatic, longer nasal bone, higher as well as longer facial bone, and wider ramus of the mandible as compared to the female's skull. These craniometric variations may be a result of several factors including sexual dimorphism, hormonal influence, developmental processes, genetic, and environmental factors. This study provides the baseline data of craniometry and insights into sexual dimorphism in *Macaca mulatta* from Pakistan and may have implications for understanding the evolution and ecology of this species.

Key Words: Craniometric analysis, *Macaca mulata,* Sexual dimorphis, Skull morphology.

INTRODUCTION

The Rhesus monkey (*Macaca mulatta*) is a significant non-human primate species belonging to old-word monkeys (*Cercopithecidae*) (Napier & Napier, 1970; Roberts, 1997). Macaques are an incredibly diversified genus of Old World monkeys comprising 22 species and are considered the most scattered non-human primate on the planet. Macaques have adapted to a wide range of environmental conditions and feed on a variety of food items including fruits, seeds, flowers, leaves, invertebrates, and human food products (Fooden, 2000). There are numerous inter- and intraspecific differences in physiology, morphology, behavior, ecology, reproductive biology, and genetics, showing a long and complicated evolutionary history as well as ecologically, considered the most scattered primate on the planet (Roos & Zinner, 2015). Seven Species of macaques are reported in South Asia (IUCN), of which the *Macaca mulatta* are distributed in the form of patches all around the latitudinal areas and reside in a variety of habitats, including as dry plains, mountains, rain forests, and deserts. They have adjusted to colonization by humans that's why we can find them in remote areas of big cities as well as villages, towns, temples, isolated sections of cities, and near to railway stations (Ciani, 1986; Makwana, 1978; Roonwal & Mohnot, 2013). *Macac mulatta* are inhibited to locations with hot, humid, and rainy summers and frigid, dry winters, these climate conditions resemble that of Pakistan where usually the rhesus monkeys can be seen in areas such as Murree Hills, Margala Hills, and the northern Pakistan (S. Goldstein & A. Richard, 1989).

Many studies can be found on the distribution, habitat, feeding, taxonomy, evolution, and systematics of *M.mulatta* in South Asia as well as in Pakistan (S. J. Goldstein & A. F. Richard, 1989; Minhas et al., 2013; Minhas et al., 2018; Roos et al., 2022; Roos & Zinner, 2015; Wenyuan, Yongzu, Manry, & Southwick, 1993). Even though few studies have been conducted on the cranial morphology of different species of primates (Botero-González, Ortiz, & Herrera-Rubio, 2021; de Freitas Burity, Mandarim-de-Lacerda, & Pissinatti, 1997; Nahallage, 2015).

In primates striking inter- and intraspecific differences along a wide array of physical, ecological, and behavioral adaptations can be observed. The Cercopithecines have cheek pouches, shorter gut to digest fruits, broader incisors, and molar teeth with high crowns and relatively low cusps (Caton, 1998). While considering the cranial anatomy, cercopithecines have a narrow interorbital space, and both the maxillary and lacrimal bones combine to form the lacrimal canal (Fleagle, 2013). Morphologically, male rhesus macaques are generally larger than females. Adult males can weigh up to 20 kilograms, while females usually weigh between 4 to 12 kilograms (Kapsalis, 2004). The males also have larger heads and canines, which are used in inter-male aggression and displays of dominance (Kapsalis, 2004; Nath et al., 2006). In contrast, females have a smaller body size, shorter and lighter tails, and a narrower face (Smithsonian's National Zoo, n.d.). The female rhesus macaque also has a unique characteristic known as "perineal swelling," which occurs during the fertile period of the menstrual cycle and is a signal to males of reproductive readiness (Kapsalis, 2004).

The study aimed to provide a comprehensive analysis of the cranial morphology of this species and to find out variations in the craniometric variables of males and females which is important for understanding their evolutionary history and their adaptation to different habitats.

Craniometry is referred to as the scientific measurement of the skull to analyze its features concerning race, sex, and body type, interspecific variability, intraspecific (Swiderski et al., 2018). It is a useful standard morphometric technique for evaluating multiple co-related variables that are probably primarily due to genetics, evolutionary biology, and ethology that exist in crania over time.

MATERIALS AND METHODS:

This study involves a total of 8 adult skulls (4 males and 4 females of each species). These skulls were already collected from northern areas of Pakistan and are preserved in the Zoological Museum, University of the Punjab, Lahore, Pakistan. To determine the gender of each skull, we followed the methodology used by Cave and Steel (1964) and keenly observed the morphological characteristics of all skulls. The third molar eruption of the skulls assisted to distinguish them into adult groups. By the use of digital Vernier-Caliper having an accuracy level of ±0.01 mm, a total of 48 linear measurements (39 cranial and 9 mandibular) of skulls were taken to explain their morphology by following the methodology of Marroig (2007), Marroig and Cheverud (2001) and Nahallage (2015). By the use of tps dig2 software different landmarks were applied on anterior, superior, lateral, and ventral views of the cranium and mandible Figure 1 (A-G), and the distance between these landmarks was determined. The description of these landmarks along with their designation and position is elaborated in the tables (1, 2, and 3).

Table 1: Landmarks applied on the skulls of *Macaca mulatta*. 'A' indicates the anterior and the 'P' indicates the posterior designation. The position(s) where these landmarks were recorded are also indicated.

Sr. No.	Landmarks	Description	Designation	Positions(s)
1	IS	Intradentale superior	A	Midline
2	PM	Premaxillary suture at the alveolus	A	Right, Left
3	NSL	Nasale	A	Midline
4	NA	Nasion	A	Midline
5	FM	Fronto-malare	A	Right, Left
6	ZS	Zygomaxillare superior	A	Right, Left
7	ZI	Zygomaxillare inferior	A	Right, Left
8	MT	Maxillary tuberosity	A	Right, Left
9	PNS	Posterior nasal spine	A	Midline
10	APET	Anterior petrous temporal	A	Midline
11	EAM	Anterior external auditorymeatus	A	Right, Left
12	PEAM	Posterior external auditorymeatus	A	Right, Left
13	ZYGO	Inferior zygo-temporal suture	A	Right, Left
14	TSP	Temporo-spheno-parietal junction	A	Right, Left
15	OPI	Opisthion	AP	Midline
16	TS	Temporo-sphenoidal junction at the petrous	AP	Right, Left
17	JP	Jugular process	AP	Right, Left
18	BR	Bregma	AP	Midline
19	PT	Pterion	AP	Right, Left
20	BA	Basion	AP	Midline
21	LD	Lambda	P	Midline
22	AS	Asterion	P	Right, Left

Table 2: A total of 39 standard linear cranial measurements and membership in the sixfunctional/ developmental group and two major cranial regions as shown in Figure 1 (A-D).

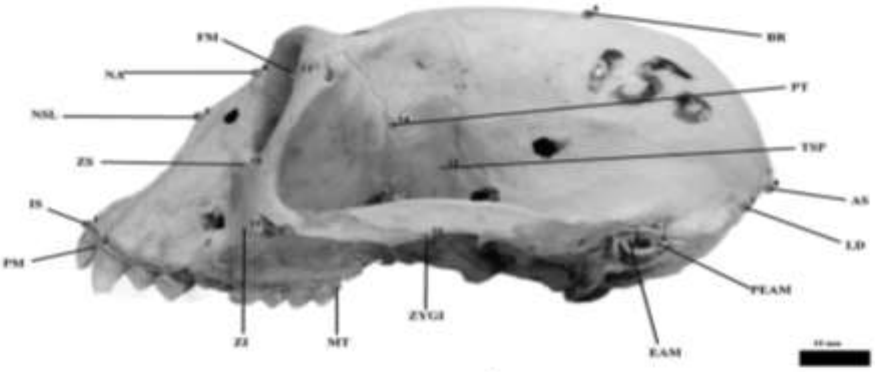
RESULTS:

A total of forty-eight standard cranial and mandibular measurements were taken to find out the differences and similarities in the skull morphology of both species (Tables 1, 2, and 3). The mean and standard deviation of all the specimens were expressed after statistical analysis. The statistical analysis was performed by using SPSS20 software. An Independent sample t-test was applied to find out the intraspecific variations in the skulls of both species. The detailed results are explained in Table 4.

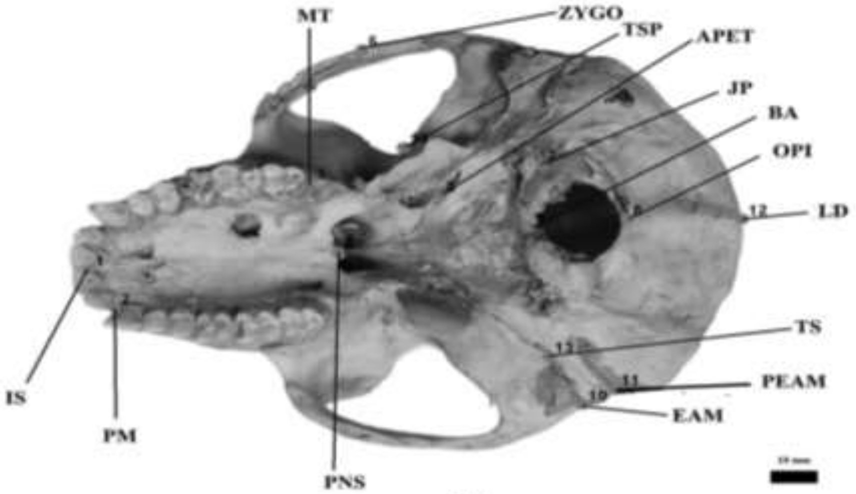
Sr. No.	**Parameters**	**Functional / Developmental group**	**Region**
1	IS-PM	Oral	Face
2	IS-NSL	Nasal	Face
3	IS-PNS	Oral, Nasal	Face
4	PM-ZS	Oral	Face
5	PM-ZI	Oral	Face
6	PM-MT	Oral	Face
7	NSL-NA	Nasal	Face
8	NSL-ZS	Nasal	Face
9	NSL-ZI	Oral, Nasal	Face
10	NA-BR	cranial vault	Neurocranium
11	NA-FM	Orbit	Neurocranium
12	NA-PNS	Nasal	Face
13	BR-PT	cranial vault	Neurocranium
14	BR-APET	cranial vault	Neurocranium
15	PT-FM	Orbit	Neurocranium
16	PT-APET	cranial vault	Neurocranium
17	PT-BA	cranial vault	Neurocranium
18	PT-EAM	cranial vault	Neurocranium
19	PT-ZYGO	Zygomatic	Face
20	PT-TSP	cranial vault, zygomatic	neurocranium, Face
21	FM-ZS	Orbit	Neurocranium
22	FM-MT	Zygomatic	Face
23	ZS-ZI	Oral	Face
24	ZI-MT	Oral	Face
25	ZI-ZYGO	Zygomatic	Face
26	ZI-TSP	Zygomatic	Face
27	MT-PNS	Oral	Face
28	PNS-APET	cranial base	Neurocranium
29	APET-BA	cranial base	Neurocranium
30	APET-TS	cranial base	Neurocranium
31	BA-EAM	cranial base	Neurocranium
32	EAM-ZYGO	Zygomatic	Face
33	ZYGO-TSP	Zygomatic	Face
34	LD-AS	cranial vault	Neurocranium
35	BR-LD	cranial vault	Neurocranium
36	OPI-LD	cranial vault	Neurocranium
37	PT-AS	cranial vault	Neurocranium
38	JP-AS	cranial base	Neurocranium
39	BA-OPI	cranial base	Neurocranium

Table 3: The standard Mandibular Measurements, also defined in Figure 1 (E-G).

Sr. No.	**Parameters**	**Landmarks**	**Measurements**	**Description**
1.	SH	1-8	Symphysis Height	Between infradentale and gnathion
2.	BCB	1b-2b	Bi Condylar Breadth	Between two condyla lateralia
3.	BGB	3b-4b	Bi Gonial Breadth	Between two gonia
4.	BMEB	2a-3a	Bi Mental Breadth	Between two mental eminence
5.	MRB	5-6	Minimum Ramus Breadth	Minimum distance between the anterior and posterior borders of ramus
6.	MBL	1-9	Mandibular Body length	Straight distance from gnathion to gonion
7.	MBH	3-4	Mandibular Body height	Height of the mandible between the Ml and M3
8.	MBB	5b-6b	Mandibular Body Breadth	Maximum thickness of the body at the level of second molar
9.	HOR	2-7	Height of Ramus	Between gonion and highest point of mandibular condyle


FM
NA
NSL
ZS
IS
PM
BR
PT
TSP
AS
LD
PEAM
EAM
ZYGI
MT
ZI
10 mm

A


MT
ZYGO
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APET
JP
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OPI
12
LD
TS
11
PEAM
10
EAM
IS
PM
PNS
10 mm

B


NA
NSL
ZS
FM
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113
monkey
10 mm

C

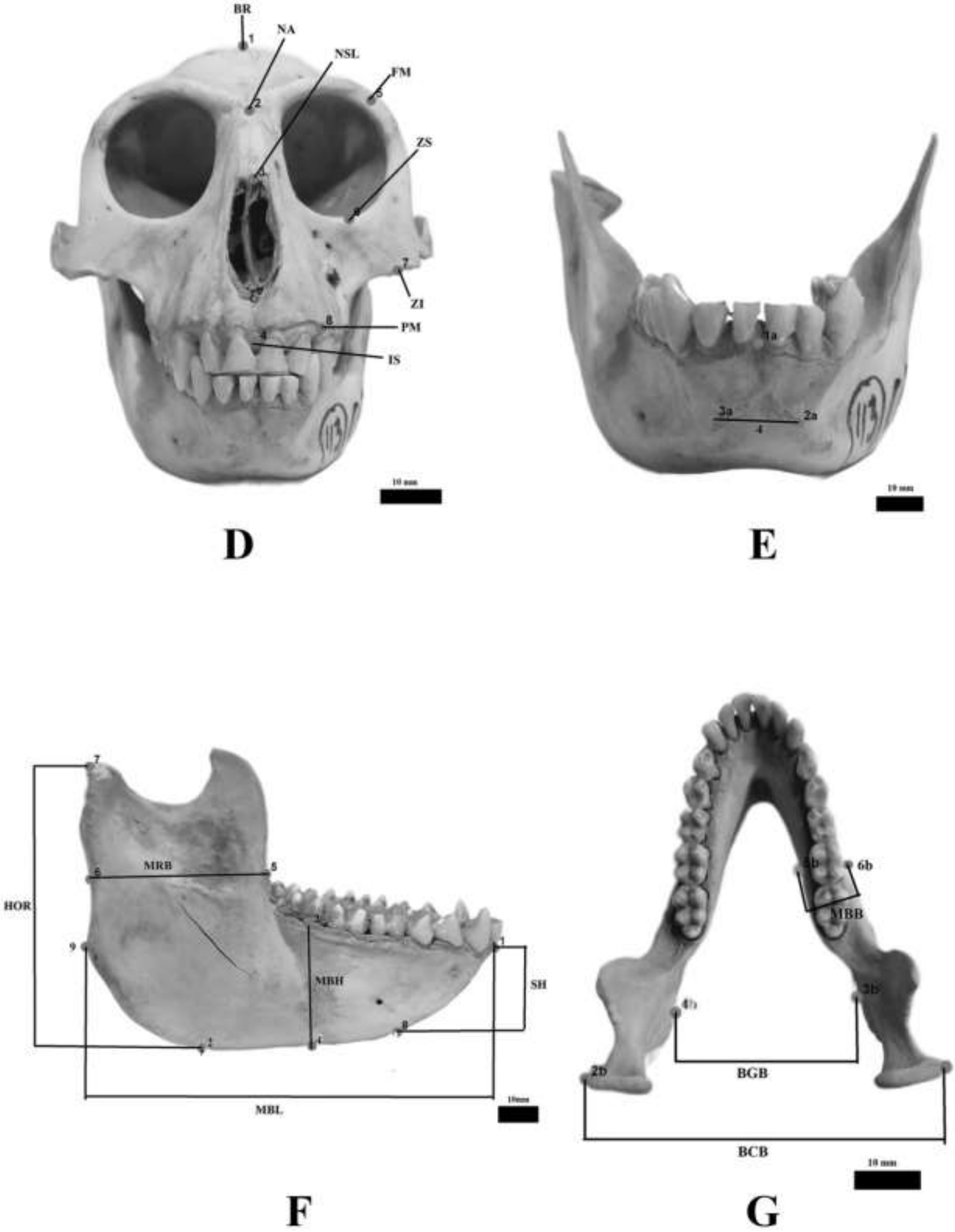

Fig 1: A, B, C, and D represents landamrks on lateral, ventral, superior and frontal view of upper jaw respectivley, while the E, F, and G are showing landmarks on anterior, lateral and ventral view respectively of lower jaw of *Macaca mulatta* skull.

Table 4. The Mean, standard deviation, and the independent sample t-test of craniometrical characters of *Macaca mulatta*.

Parameters		**Sex**	**Mean (Cm)**	**Std. Deviation**	**Two sample t-test and P value**	
					T	**P**
1	**IS-PM**	Male	1.16	0	0.769	0.035*
		Female	1.063	0.21779	0.769	
2	**IS-NSL**	Male	2.636	0.43684	-1.475	0.424
		Female	2.19	0.29	-1.475	
3	**IS-PNS**	Male	4.096	0.70465	0.282	0.238
		Female	4.22	0.28	0.282	
4	**PM-ZS**	Male	2.873	0.54857	-0.931	0.029*
		Female	2.575	0.085	-0.931	
5	**PM-ZI**	Male	3.18	0.83108	-0.167	0.036*
		Female	3.1	0.02	-0.167	
6	**PM-MT**	Male	4.036	0.51433	0.129	0.208
		Female	4.08	0.27	0.129	
7	**NSL-NA**	Male	1.556	0.45391	1.693	0.074
		Female	2.005	0.065	1.693	
8	**NSL-ZS**	Male	2.475	0.365	0.259	0.381
		Female	2.38	0.52115	0.259	
9	**NSL-ZI**	Male	4.285	0.565	0.668	0.552
		Female	3.883	0.87558	0.668	
10	**NA-BR**	Male	5.385	0.035	-0.159	0.099
		Female	5.466	0.8867	-0.159	
11	**NA-FM**	Male	3.385	0.115	0.735	0.066
		Female	3.073	0.72528	0.735	
12	**NA-PNS**	Male	3.815	0.085	0.497	0.032*
		Female	3.656	0.54501	0.497	
13	**BR-PT**	Male	4.065	0.265	0.617	0.415
		Female	3.876	0.45764	0.617	
14	**BR-APET**	Male	5.225	0.155	0.79	0.178
		Female	5.01	0.44542	0.79	
15	**PT-FM**	Male	1.67	0.34	-0.032	0.585
		Female	1.68	0.41581	-0.032	
16	**PT-APET**	Male	3.185	0.145	0.164	0.123
		Female	3.13	0.56312	0.164	
17	**PT-BA**	Male	4.875	0.055	1.215	0.087
		Female	4.33	0.77485	1.215	
18	**PT-EAM**	Male	3.513	0.54418	-0.481	0.032*
		Female	3.36	0.09	-0.481	
19	**PT-ZYGO**	Male	3.17	0.24	0.794	0.272
		Female	2.96	0.39	0.794	
20	**PT-TSP**	Male	1.55	0.06	0.779	0.085
		Female	1.336	0.47057	0.779	
21	**FM-ZS**	Male	2.375	0.075	0.652	0.052
		Female	2.26	0.29597	0.652	
22	**FM-MT**	Male	4.47	0.45	0.614	0.288
		Female	4.15	0.78307	0.614	
23	**ZS-ZI**	Male	2.1	0.01	0.676	0.02*
		Female	1.876	0.57239	0.676	
24	**ZI-MT**	Male	2.243	0.22234	-1.317	0.04*
		Female	2.07	0.05	-1.317	
25	**ZI-ZYGO**	Male	2.775	0.315	0.385	0.2
		Female	2.627	0.58859	0.385	

26	**ZI-TSP**	Male	2.97	0.63	-0.486	0.443
		Female	3.283	0.92219	-0.486	
27	**MT-PNS**	Male	1.19	0.04	-1.52	0.069
		Female	1.31	0.13077	-1.52	
28	**PNS-APET**	Male	1.795	0.105	-0.938	0.247
		Female	1.95	0.26627	-0.938	
29	**APET-BA**	Male	1.735	0.225	0.337	0.756
		Female	1.68	0.17088	0.337	
30	**APET-TS**	Male	1.275	0.025	-1.514	0.178
		Female	1.323	0.04933	-1.514	
31	**BA-EAM**	Male	3.335	0.185	0.158	0.182
		Female	3.263	0.76592	0.158	
32	**EAM-ZYGO**	Male	2.205	0.035	-2.104	0.072
		Female	2.723	0.42525	-2.104	
33	**ZYGO-TSP**	Male	1.925	0.225	-0.902	0.719
		Female	2.096	0.2409	-0.902	
34	**LD-AS**	Male	2.565	0.015	1.263	0.111
		Female	2.396	0.23029	1.263	
35	**BR-LD**	Male	4.765	0.075	0.638	0.043*
		Female	4.583	0.48758	0.638	
36	**OPI-LD**	Male	2.795	0.105	1.394	0.055
		Female	2.473	0.38553	1.394	
37	**PT-AS**	Male	5.22	0.19	0.724	0.224
		Female	4.906	0.72501	0.724	
38	**JP-AS**	Male	3.035	0.335	0.998	0.832
		Female	2.756	0.34819	0.998	
39	**BA-OPI**	Male	2.18	0.54	0.515	0.565
		Female	1.926	0.66003	0.515	
40	**SH**	Male	1.525	0.105	-4.298	0.801
		Female	1.906	0.1124	-4.298	
41	**BCB**	Male	6.635	0.395	-0.402	0.128
		Female	6.886	1.01115	-0.402	
42	**BGB**	Male	4.865	0.915	-0.657	0.773
		Female	5.376	0.99158	-0.657	
43	**BMEB**	Male	1.39	0.08	0.175	0.169
		Female	1.338	0.507	0.175	
44	**MRB**	Male	2.696	0.04	-0.621	0.036*
		Female	2.53	0.46307	-0.621	
45	**MBL**	Male	6.725	0.315	-0.253	0.099
		Female	6.887	1.06733	-0.253	
46	**MBH**	Male	1.69	0.12	-1.689	0.161
		Female	1.966	0.25716	-1.689	
47	**MBB**	Male	0.785	0.065	-0.032	0.834
		Female	0.786	0.06351	-0.032	
48	**HOR**	Male	3.875	0.115	-0.835	0.088
		Female	4.186	0.63611	-0.835	

Regarding intraspecific comparison, most of the craniometric features of males and females of *M.mulatta* showed similar values while some of the differences were also found in their skull morphology.

The values of IS-PM, PM-ZS, PM-ZI, PT-EAM, ZI-MT, MRB, ZS-ZI, NA-PNS, BR-LD, and MRB were found as statistically significant ($p<0.05$) and were found more in males (Fig 2).

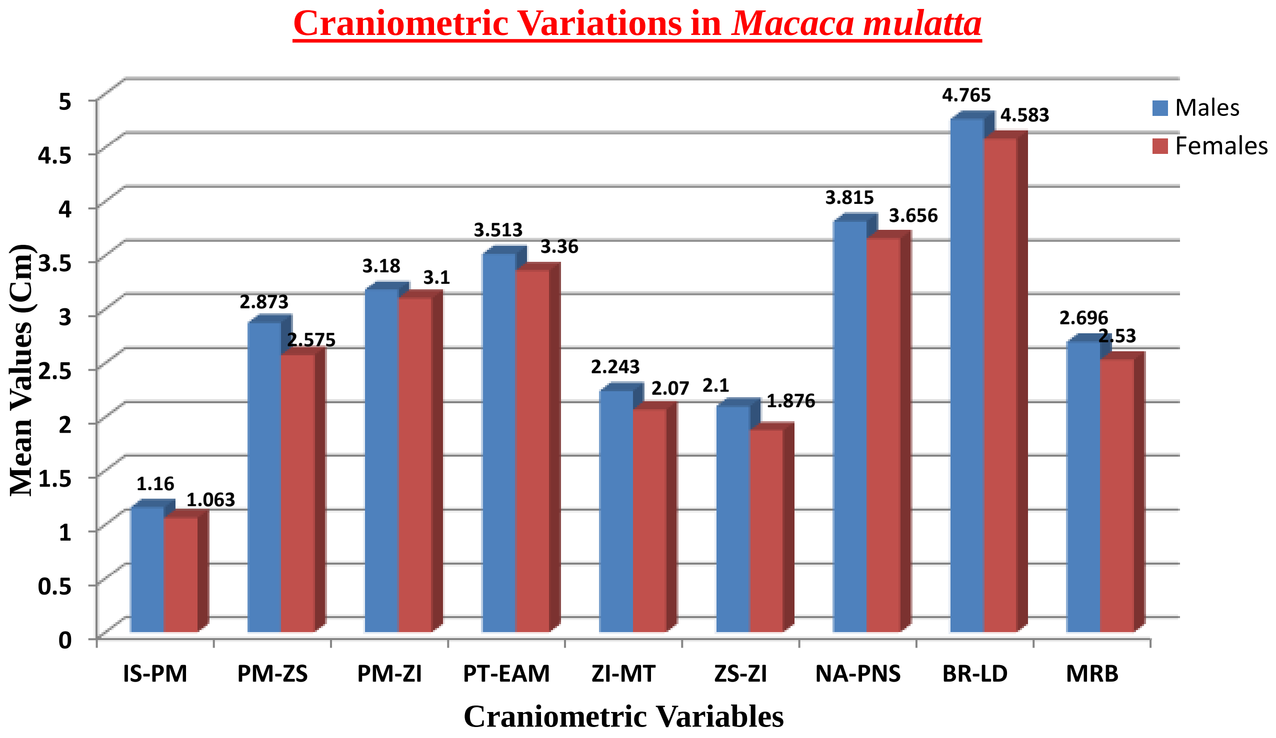

Fig 2: Chart showing a comparison of statistically significant craniometric variables of males and females of *Macaca mulatta*

DISCUSSION:

This study presents the detailed craniometrical measurements of *Macaca mulatta*. This study's descriptions and linear measurements are a compilation of pertinent scientific data that allow for the identification of the species and allow it to be distinguished from other species, both living and extinct. Most of the craniometric variables showed similarities among the males and the females of *M.mulatta*. However, it was found that the males have larger neurocranium, a slightly broader zygomatic, longer nasal bone, and higher as well as longer facial bone as compared to the female's skull. It was also revealed that the ramus of the mandible of males is wider than females. These results are consistent with previous studies on *Macaca mulatta* (Li et al., 2012; Richtsmeier et al.,1993). There are some significant differences between the life histories of male and female rhesus monkeys that are connected to the social organization of wild rhesus communities. The basis of rhesus monkey society is the preservation of stable matrilines through sex-specific behavioral patterns (Altmann, 1962; Lindburg, 1971). Another gender difference in rhesus macaques is that although females are incorporated into the social structure of their native group, males join a new troop after reaching puberty (Colvin, 1986).

Craniometrical variations between males and females of *Macaca mulatta* can be attributed to several factors, including sexual dimorphism, developmental processes, hormonal influence, genetic factors, and environmental influences.

Sexual dimorphism is the most prominent factor contributing to craniometrical variations between male and female primates. In general, male rhesus macaques have larger and more robust skulls compared to females, which is likely due to the selective pressures and aggressive behavior associated with male-male competition for access to mates and resources, female primates, on the other hand, tend to have smaller and more gracile skulls, which may reflect the selective pressures associated with reproduction and parental care (Richtsmeier, 1989; Strier, 2015). The sexual dimorphism in cranial variables found in this study, such as neurocranial length, width and height, the width of the zygomatic bone, facial length and height, and the width of the ramus of the mandible may also reflect differences in the biomechanical stresses experienced by males and females during feeding, social behavior, and other activities (Roseman et al., 2010; Smith & Jungers, 1997). The developmental processes also play a role in craniometrical variations between male and female rhesus macaques. Studies have shown that male rhesus macaques tend to have faster growth rates and earlier maturation compared to females, which can lead to differences in cranial size and shape (Lee et al., 2018), while the females show slower but prolonged growth patterns. A similar prolonged growth pattern in female chimpanzees was observed (Leigh & Shea, 1995). Male growth ceased after the canines and third molars fully erupted, although female growth continued (Q. Wang, Strait, & Dechow, 2006). Variations in the growth pattern have been linked to feeding ecology, ontogenetic process social variations, and reproductive behavior (Leigh & Cheverud, 1991; O'Higgins, Moore, Johnson, McAndrew, & Flinn, 1990; Schillaci & Stallmann, 2005). Furthermore, environmental factors such as diet, habitat, and social interactions can also affect cranial morphology in rhesus macaques (Roseman et al., 2010). Hormones such as testosterone and estrogen play a critical role in the development of sexual dimorphism in primates, including rhesus macaques (Geschwind and Galaburda, 1985). Testosterone is known to promote bone growth and remodeling, resulting in larger cranial dimensions in males compared to females (Kanazawa, 2000). In contrast, estrogen is believed to have a suppressive effect on bone growth (Shi, Lin, & Su, 2015), which may contribute to the smaller cranial dimensions observed in females.

There may be genetic differences between males and females of both species that contribute to cranial variations. For instance, studies have identified how microcephaly genes are associated with cranial dimensions and brain development by having sex-specific effects in primates (Rimol et al., 2010; J.-k. Wang, Li, & Su, 2008).

As this is the first study on *Macaca mulatta* from Pakistan by using craniometric analysis, the interpretation of our results cannot be directly extended to the whole genus. Thus, more studies with a larger sample size and encompassing a wider geographic range, and especially combined with molecular and cytogenetic data will be needed to extend our knowledge of sexual dimorphism, diversity, and evolutionary patterns in both species.

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