

Morphometric Analysis of Tropicopolitan Bug *Triatoma rubrofasciata* (De Geer) In Two Different Parts of India

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Abstract: Quantitative analysis of morphometric characters of different body parts were used to understand the variation between three *Triatoma rubrofasciata* individuals collected from three different places in India. These individuals were collected from different regions in India by simple hand picking method. These morphometric comparisons provided valuable data for showing a clear variation between the measurements of different body segment for all three specimens. Measurement of total body length for the individuals collected from three different parts of India i.e. Gorakhpur (Subash Nagar), Trivandrum (Thoothoor) and Chennai (Nungampakam) were 20.70mm, 21.35mm and 21.06mm respectively. The length of rostral segments in relation to head capsule is continuously subjected for taxonomic discussion. The total length of the rostrum recorded was 2.50 mm, 2.72mm and 2.78mm respectively for Gorakhpur, Trivandrum (Thoothoor) and Chennai (Nungampakam) specimens. Gorakhpur specimen showed variation in most of the morphometric data recorded from other two specimens which were collected from two different localities of south India, though all three were adult healthy females. Shorter body segments of Gorakhpur specimen indicated that these morphometric changes could be due to unfavourable climatic condition and feeding habit of the individuals in north India (Gorakhpur).

Keywords: *Triatoma rubrofasciata*, Assess population, Morphometric parameters.

1. INTRODUCTION

The haematophagous bugs (Hemiptera: Reduviidae) are commonly called Kissing bugs or conenose bugs belong to the subfamily Triatominae and comprise 118 species and 14 genera (Ambrose 2007). The subfamily Triatominae contains numerous species which requires blood meal for their complete development. Most species feed on small terrestrial or arboreal mammals, especially didelphids, edentates, and rodents; others are associated with bats, birds for their meals (Lent and Wygodzinsky, 1979). Apart from the bite people receive and the blood loss they experience, the triatomines transmit *Trypanosoma cruzi*, the causative agents of Chaga's disease or South American Trypanosomiasis (Ambrose 2007).

Triatoma rubrofasciata (De Geer, 1773), cosmopolitan species distributed geographically between the tropics of Cancer and Capricorn, is most commonly found in Asia, Oceania, Africa and Central America. *Triatoma rubrofasciata* occurs both in the New World (mainly North East Brazil) and is also recorded from tropical and subtropical port areas in many parts of Old World (Gorla 1997). The origin of *T. rubrofasciata* has long been a mystery. The first species of the genus *Triatoma* DE GEER (1773) was from a specimen collected in the then Dutch East Indies (now Indonesia). It has since been recorded from domestic habitats in the port areas throughout the tropics and sub-tropics, and from more inland regions (Dias and Neves, 1943; Lucena and Marques, 1955; Abad Franch et al. 2001). In some parts of north-east India (Assam) *T. rubrofasciata* is commonly infected with the rat trypanosome, *Trypanosoma conorhini* (Donovan) and which, is transmitted by host contamination with infected bugs faeces (Hoare, 1972; Patterson et al. 2001).

Óómez Nùñez sensor have been adopted for studies on domestic infestation by *T. rubrofasciata* (Sherlock and Serafim 1974; Brasil and Silva 1983). India was witnessed for having two species of conenose bug in Assam (Awati, 1921-1922; Hoare, 1969; Knowles et al. 1923), Kolkatta, Mumbai (Wu, 1935), Pondichery (Neiva and Lent, 1941), Mysore, Ranchi, Delhi, Chennai (Haridass and Ananthakrishnan, 1980), Bangalore (Parameswaran and Sankara, 1977), Goa (Lent and Wygodzinsky, 1979), Tirunelveli and Kanyakumari (Ambrose and Rajan, 1992; Rajan, 2011).

T. rubrofasciata is known to be a morphologically variable species, ranging from orange marked specimens from south India (Gillett, 1934) to almost melanic forms in Hawaii (Lent and Wygodzinsky, 1979). However, several populations show superficial similarities to some North American species such as *T. sanguisuga* (Le Conte) and *T. rubida* (Uhler), which is also strongly associated with rodents (*Neotoma* spp.) (Usinger, 1944). *T. rubrofasciata* is now difficult to collect from many of the old world localities from where it has previously been recorded (Patterson et al. 2001). The Triatominae is divided into five tribes and seventeen genera; most species are known only from the new world, six species (*Linshcosteus*) are found only in India, and seven species (*Triatoma*) are known only from South East Asia ((Lent and Wygodzinsky 1979; Patterson et al. 2001).

The length of the rostral segments in relation to the head capsule of triatomines continues to be a subject of taxonomic discussion (Davis, 1969). Moreover, morphometry is the measurement and analysis of form, and the main premise of morphometrics is that a statistical analysis of genetic variability expressed by morphological character, a measure of population differentiation, and ultimately of speciation (Sorensen and Footitt, 1992; Dujardin et al, 1999, 2000). Hence an effort was made to collect *Triatoma rubrofasciata* by simple hand picking methods in Gorakhpur, Uttar Pradesh, north India localities and the morphological parameters especially rostrum were compared with the specimens collected from Trivandrum, Chennai, South India for taxonomic comparison to study the ecotypic variation.

2. METHODS

Collection of *T. rubrofasciata* was carried out with much precaution and the survey was done each time after the sunset in the localities of the Rapti River basin. At the site of collection climatic data was recorded as to match the arousal of the bug and climatic condition which prefers it. With the help of two more entomologists a single female individual was collected by simple hand picking method, when the wind was cool and flowing continuously at Rapti River basin (Fig. 2 & 1). The temperature and humidity recorded at the time of collection were 24°C and 84% respectively. The individual was collected mainly from mud wall cracks of the house which was very near to cow shed and chicken coops. Female *T. rubrofasciata* from Trivandrum and Chennai were borrowed from St. Xavier's College, Palayankottai, Trivandrum and Loyola College, Chennai. The taxonomic details were drawn with the help of camera lucida and morphometry was carried out under microscope with ocular and stage micrometer.

The following key taxonomic characters of *T. rubrofasciata* were used for identification and further studies 1. 1st antennal segment surpassing tip of head, 2. Short hairs on mouthparts becoming progressively longer towards tip, 3. Scutellum broad, triangular to tip, 4. Orange-red margin on sides of pronotum, 5. Orange-red margin on outer edge of abdomen, extending horizontally between segments 6. Abdomen flattened longitudinally underneath. Further the taxonomic characters of three populations were also compared.

3. RESULTS AND DISCUSSIONS

3.1. Morphometric Illustrations

All the specimens examined were dark brown to black in colour, and have a lateral abdominal margin that was tan. The total body length measure for Gorakhpur, Trivandrum and Chennai female *T. rubrofasciata* were 20.70mm, 21.35mm and 21.06mm respectively (Table 2). Relatively similar measurements of body length were also recorded 19.75mm from the *T. rubrofasciata* specimen collected from Arabia, Ceylon, China, Malaya, Maldives, Okinawa, Siam, West Africa, Taiwan, Honolulu, Thailand, Philippine islands, South India and Brazil (Gorla et al. 1997). But the average body length for Indian species was calculated to be 21.03mm which is slightly bigger than what was reported by Gorla et al. 1997. Among three Indian species also we observed that species collected from Trivandrum and Chennai were larger in size than Gorakhpur species. This size difference may be due to warm and humid climate of south India which provides better development conditions for the *T. rubrofasciata*.

3.2. Head

The head of *T. rubrofasciata* is typically cone shaped with piercing and sucking type of mouth parts (Fig 1). The total measurement of the head length for the specimens collected from Gorakhpur was least when compared with other two specimens from South India (Table 1a).

3.3. Cephalic Appendages

Antennae: Length of antenniferous tubercle and Ante ocular distance was recorded for only Trivandrum specimen, which measured about 0.63 and 1.31mm respectively (Table 2). These measurements couldn't get a chance to compare with Gorakhpur specimen as antennae were damaged during transmits.

Rostrum: The rostrum of *T.rubrofasciata* was divided into three segments like other reduviids, and the length of rostral segments in relation to head capsule is continuously subjected for taxonomic discussion (Davis, 1969; Murugan et al. 1996). The rostrum of collected specimens was not curved like in other reduviids; but it is straight and ends well before the forelegs (Krinsky, 2009). The first rostral segment of all the three specimens were covered less hairs when compared to other two segments. Similar to the above observations the rostral segments for Trivandrum (Thoothoor) and Chennai (Nungampakam) specimens was larger than Gorakhpur specimen (Table 1a). There was a slight exception in the third rostral segment, where Chennai specimen was slightly less when compare to Gorakhpur specimen. More variation was recorded in the second rostral segments, where largest measurement was noted for Chennai specimen (Table 1a, Fig 2). The length of the 3rd segment was measured to be 0.50mm, 0.56mm and 0.49mm respectively for three different places mentioned above. This data gave a clear idea that the third rostral segment was very much similar in length for all three specimens collected from different sites but other two segments, specially second rostral segment had large variation in lengths. The total length of the rostrum recorded was 2.50 mm, 2.72mm and 2.78mm respectively for Gorakhpur, Trivandrum (Thoothoor) and Chennai (Nungampakam) specimens. The total length shows clear difference between north and south Indian specimens, which might be due to change of climatic condition, altitude, rainfall and feeding habit, but there was no change in shape and density of hairs present on it. The ratio between 2nd and 1st, 3rd and 1st, and 2nd and 3rd rostral segments of *T.rubrofasciata* collected from Gorakhpur were calculated as 1.50, 0.63 and 2.40 respectively whereas, for Trivandrum (Thoothoor) specimen the values were 1.541, 0.658 and 2.339 respectively and for Chennai (Nungampakam) specimens the values were 1.632, 0.563 and 2.89 respectively (Table2). The ratio observed between 2nd and 3rd rostral segment varied much for Gorakhpur and Chennai specimens whereas other values observed in this segment were much similar, though its importance in morphometric analysis can't be considered less (Fig3). This variation may be due to ecological and geographical changes and due to variant type of host associated to these bugs in different area. Similarly more matching values were recorded by Gorla et al. 1997 in different specimen collected from various localities. The last segment of the rostrum was thickly covered with hair on the tip (Fig.1). According to (Cobben 1978) apical segment that serves as exploratory device in the section of substrate varies in its length architecture depending on the nutritional ecology and feeding strategy of these sucking insects.

Eyes: Maximum numbers of ommatidia in eye were recorded for Chennai specimen and the least for Gorakhpur specimen (Table 1a). The Maximum diameter (E1) of each eye and eye width (ED1) for the Gorakhpur specimen were noted to be 0.85mm and 1.45mm respectively, whereas reading of (E1) and (ED1) for Trivandrum (Thoothoor) were 0.86mm and 1.68mm respectively and for Chennai (Nungampakam) were 0.89mm and 1.52mm respectively Table 1). Similar morphological variations were also recorded by Patterson et al. (2001) with slight variation in the measurement.

The diameter of ocelli was measured to be 0.14mm for Gorakhpur individual, where as ocelli width (OD1) and synthlipsis (SY1) value recorded were 0.90mm and 0.75mm respectively (Fig. 2) (Table 1a). These above mentioned morphometric measures were recorded least for Gorakhpur specimen, which provides information that these bugs do not attain larger size then south Indian species. This might be due to favourable climatic condition of south India.

The value for Pronotum width was least in case of Trivandrum specimen but was very close to the recorded vale for Gorakhpur specimen (Table 2). Eye width: head length ratio calculated for Gorakhpur specimen was least as compared to other two specimens (Table 2). Whereas the ratio calculated for synthlipsis: eye width and Post-ocular length: pronotum widths for all three individuals showed only slight variation in the values (Fig. 2 & 3). Similar metric variables as above mentioned were also recorded by Gorla et al. (1997).

3.4. Thorax

Though our above measurements reveal that Gorakhpur specimen was lesser in body measurement than other two individuals but in case of thorax measure it was vice-versa (Fig. 2, Table 1a).

Scutellum: In case of measurement for Scutellum length (S1) and width (S2) also Gorakhpur specimen was significantly larger than other two specimens (Table 1a). The least measurement recorded for pronotum was of Trivandrum (Thoothoor) specimen and the readings for Chennai (Nungampakam) and Gorakhpur were very similar (Table 2). Similar observation was also made by Gorla et al. (1997) again with slight varied values.

3.5. Wing

The shape and texture of the wings of all the three specimens collected from three different localities of India were similar, but for the total wing length maximum value was recorded for Trivandrum specimen and minimum value was again recorded for Gorakhpur specimen (Table 1a). Recorded data for wings measurement shows that as the body length decreases the wings also reduce in size accordingly.

3.6. Leg

Taking very precise measurement for each parts of Fore leg, Mid leg and hind leg for all three specimens collected from different region of India, we found in each measurement Gorakhpur specimen was having lesser measurement than other two individuals (Table 1b). Though some parts of limbs were not measured because these parts were damaged during transmit. But still data again pointed that Gorakhpur specimen was much smaller in size.

3.7. Abdomen

The abdomen length and width of the specimen collected from Gorakhpur were 14.1 and 6.5 respectively; this measurement was also least when compared to other two south Indian specimens (Table 1a, 2 and Fig. 2).

Gorakhpur specimen showed variation in majority of the morphometric data recorded from other two specimens which were collected from two different localities of south India, though all three were adult healthy females (Fig. 3). Shorter body segments of Gorakhpur specimen indicated that these morphometric changes could be due to unfavourable climatic condition and feeding habit of the individuals in Gorakhpur, as it is situated at extreme north-east part of India which has extreme winters and summer season as compared to Trivandrum (Thoothoor) and Chennai (Nungampakam) which is situated in south India where the temperature never drops below 19- 20°C and never rise above 35°C. Thus getting appropriate condition for *T.rubrofasciata* to breed and develop for establishing its colony is necessary.

T.rubrofasciata is known to be a highly variable species, ranging from bright orange- red- marked specimens in south India (Gillett, 1934) to almost melanic forms in Hawaii (Lent and Wygodzinsky, 1979). The observed morphometric differences between the individuals collected from north India and south India can be the result of evolutionary processes persuaded by isolation derived from latitudinal restriction in the distribution of *T.rubrofasciata*. Morphometry is the measurement and analysis of form, and the main premise of morphometrics is that a statistical analysis of genetic variability expressed by morphological character, a measure of population differentiation, and ultimately of speciation (Sorensen and Footitt, 1992; Dujardin et al., 1999, 2000). Morphometric analysis is being increasingly applied to the phylogenetics of Triatominae (Dujardin et al., 1997, 1998, 1999, 2000; Gorla et al., 1997).

In the area like Subash Nagar (Gorakhpur), people are totally unaware of its presence and disease caused by it elsewhere in the world. The symptoms include confusion, reduced coordination, and disruption of the sleep cycle, with bouts of fatigue punctuated with manic periods, leading to daytime slumber and night time insomnia reported by (Lent and Wygodzinsky 1979). Even though the population of Gorakhpur, *T. rubrofasciata* was existed in the house of local people but still their awareness about this bug largely nil. The feeding of Triatoma on human inhabitants has been a serious annoyance; severe allergic reactions have occurred.

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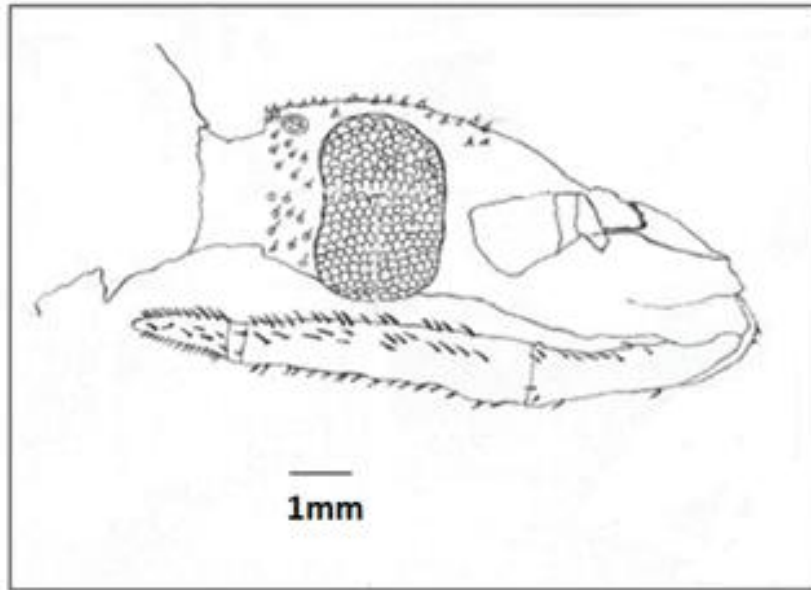


Fig1. Lateral view of the head of *Triatoma rubrofasciata* collected from Gorakhpur.

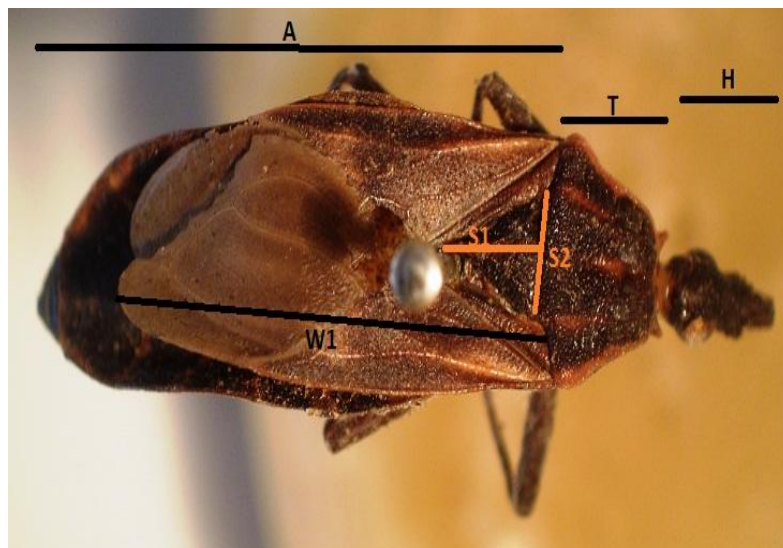
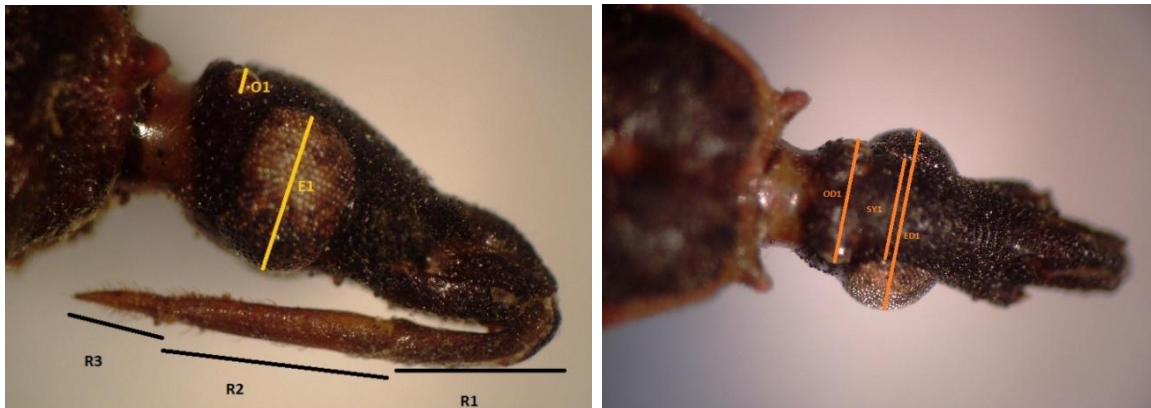


Fig2. Lateral and Dorsal views of the head including morphometric measurements taken. Length of Ocelli (O1), Rostrum 1st segment (R1), Rostrum 2nd segment (R2), Rostrum 3rd segment (R3), Distance between two Ocelli (OD1), Distance between two Eye (ED1), Abdomen (A), Thorax (T), Head (H), Scutulum length (S1), Scutulum width (S2), Wing length (W1), Synthlipsis (S1).

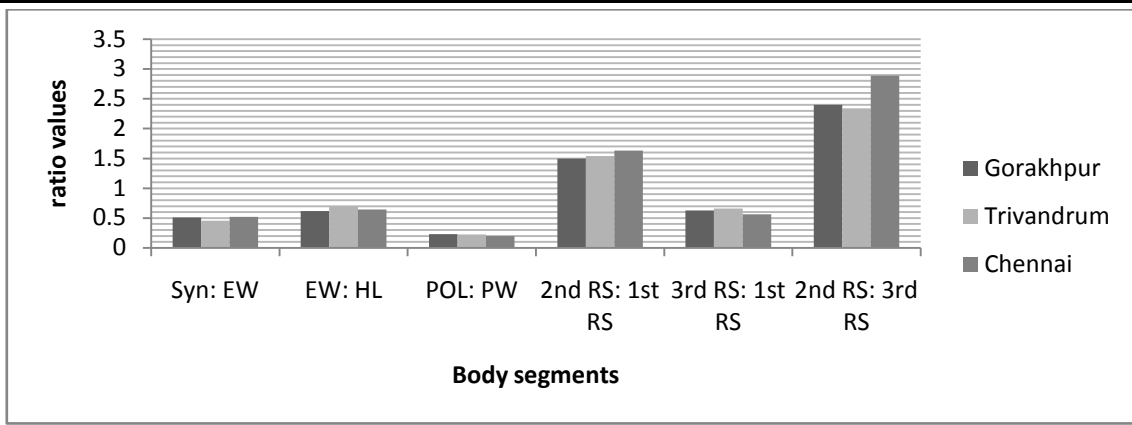


Fig3. Comparison of some selected morphometric measurement of *T. rubrofaciata* from three different locations in India (Synthlipsis- Syn, Eye width- EW, Head length- HL, Post ocular length- POL, Pronotum Width- PW, Rostal segment- RS).

Table1a. Comparative morphometric measurements of three female individuals of *T. rubrofaciata* collected from three different regions of India

Body Regions (In mm)	Gorakhpur (Subash Nagar)	Trivandrum (Thoothoor)	Chennai (Nungampakam)
Head (H)	2.30	2.45	2.36
Thorax (T)	4.30	3.70	3.55
Abdomen (A)	14.10	15.20	15.15
Total body	20.70	21.35	21.06
Rostrum (segment-wise)	R1	0.80	0.85
	R2	1.20	1.31
	R3	0.50	0.56
Total length	2.50	2.72	2.78
Eye: Maximum diameter	0.85 (E1)	0.86	0.89
Eye: Number of Ommetidia	230	250	284
Eye width	1.45 (ED1)	1.68	1.52
Ocelli: Maximum diameter	0.14 (O1)	0.16	0.17
Ocelli: Number of Ommetidia	2	2	2
Ocelli width	0.90 (OD1)	1.01	0.81
Wing length	14.1 (W1)	15.29	15.20
Scutullum			
Length (S1)	2.50	1.90	1.73
Width (S2)	2.48	1.87	1.62

Table1b. Comparative morphometric measurements of three female individuals of *T. rubrofaciata* collected from three different regions of India.

Body Regions (In mm)	Gorakhpur (Subash Nagar)	Trivandrum (Thoothoor)	Chennai (Nungampakam)
Coxa (C) Fore leg	0.45	0.46	0.48
	0.47	0.48	0.50
	-	0.51	0.53
Femur (F) Fore leg	3.25	3.40	4.12
	3.90	4.22	5.11
	-	7.12	7.83
Tibia (Ti) Fore leg	3.10	3.84	4.54
	4.80	5.35	6.23
	-	8.23	8.85
Tarsus (Ta) Fore leg	0.80	-	0.84
	0.79	-	-
	-	0.81	-
Pretarsus (Pt)Foreleg	0.17 (1claw)	-	0.18(1claw)
	0.20 (2 claws)	-	-
	-	-	-
Total length Fore leg	7.77	-	10.16
	10.16	-	-
	-	-	-

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Table2. Taxonomic metric variables of three specimens of *Triatoma rubrofasciata* collected from three localities in India.

Variables	Gorakhpur	Trivandrum	Chennai
Body length (mm)	20.70	21.35	21.06
Width of abdomen (mm)	6.50	7.82	8.12
Width of pronotum (mm)	2.07	1.98	2.09
Eye width (mm) (Distance between eyes)	1.45	1.68	1.52
Post – ocular length (mm)	0.48	0.45	0.42
Synthlipsis (mm)	0.75 (SY1)	0.77	0.79
Synthlipsis: eye width	0.51	0.458	0.519
Eye width: head length	0.62	0.685	0.644
Post-ocular length: pronotum width	0.231	0.227	0.200
2nd rostral segment: 1st rostral segment	1.50	1.541	1.632
3rd rostral segment: 1st rostral segment	0.63	0.658	0.563
2nd rostral segment: 3rd rostral segment	2.40	2.339	2.890
Length of antenniferous tubercle(mm)	-	0.63	-
Anteocular distance (mm)	-	1.31	-

4. CONCLUSION

Triatoma rubrofasciata has been reported from different parts of India in time to time; quite frequently from south India in recent times. In our present effort we were able to report this bug from Gorakhpur (north India) for the first time. Since collection and determination of these bugs are very difficult because of their nocturnal behaviour and blood feeding habits too. This study, *T. rubrofasciata* collected from Gorakhpur was compared with individuals from different parts of India by morphometric observations in order to gain knowledge about their shape and size variations. This study would help to clarify the identification difficulties of this species to other species of *Triatoma* which were reported in Karachi, Pakistan and elsewhere in India (Muthupet, Tamil Nadu) as quoted as *T. sanguisuga*. This serious effort brought out the small and important regional variations of this species so that geographic variation in the northern Indian and southern ones can be realised. This study revealed that north Indian species was significantly smaller in size than the individuals collected from two different places in south India; this may be due to extreme weather in northern part of India and their host availability which directly affect the biology of these hematophagous bugs.

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