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Ovipositors of grasshoppers exhibit in between species variations

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Abstract
Ovipositors in grasshoppers have been examined by several workers in functional and taxonomic context. These structures are essential for formation of egg pot and to bury the egg pod in the soil by digging a small tube like chamber. One of the means to understand inter specific variable characters is by comparison of structures. Comparison of the ovipositor of eleven species of female grasshoppers has been carried out in this study to understand variation in structural components of ovipositors. The ovipositors of eleven species had striking differences in the ovipositor valves in margins such as toothed, tuberculate or smooth condition, shape and size, shape of the apical tip. The apodemes also had variations in the length, width, shape and stainable tissue component. Grasshoppers involved in this study belonged to sub families Acridinae, Oedipodinae, Cyrtacanthacridinae, Catantopinae, Gomphocirinae and Hemiacridinae of the family Acrididae and Family pyrgomorphinae. These structure seems to have taxonomic significance as these showed considerable variations in between species and could be used in classification of grasshoppers.

Keywords: Valves, shape and size, margins, apodemes, stainable margin, egg guide, taxonomic significance

Introduction
In grasshoppers the ovipositor composed of two pairs of shovel shaped valves used to dig an egg chamber to place the eggs in the ground and help in manipulating and capping the eggs in a frothy moist substance to protect eggs from damage. During oviposition the valves cyclical movements of opening, closing. Protraction and retraction movements operated by contraction of ten pairs of muscles [1]. The female genital structure is different from that of male phallic structures in grasshoppers, because the female phallic structures are designed to perform two functions: 1, To receive the sperms transferred from male during copulation.2.To release eggs outside to be placed in the soil for which ovipositor has evolved. Studies have been carried out by different worker on taxonomic utility of female genitalic structures particularly on spermatheca indifferent sub families of Acrididae and have concluded that spermatheca has specificity to classify grasshoppers to different sub families [2-5] Agarwal [6-8] carried out a comparative study of ovipositors in many species of grasshoppers and importance of ovipositors for taxonomic consideration is discussed by Usmani and Shafee [9] and Usmani [10]. The present study on the structural variations in the ovipositors of eleven species of grasshoppers belong to six sub families of Acrididae and family pyrgomorphinae has carried out to investigate whether there are any taxonomic signal borne in the structural components of ovipositors.

Ovipositor an organ meant for deposition of eggs, shows a definite structural organization, composed of a pair of dorsal valve, a pair of ventral valve, a mesial valve located in between dorsal and ventral valves. The valves other than mesial valve, are strongly sclerotized look like prongs, have sharp curved process facing opposite directions. The basal part of the ovipositor impart support to form the base of the genital chamber. Thin sclerotized lateral sclerites are associated with the basal sclerites at their edge are clearly visible. The ventral valves are supported by thick basal sclerite and lateral sclerite are well marked at the ventral edge are the real digging structures.

The two valves are separated in half of their length. The base of the dorsal valveis irregularly flattened and its condyle fused with the base of the apodeme, The ventral condyle of apodeme articulates with the ventral valve sclerites. The apodeme projects in to body cavity, three edged, narrow spathulate in shape supported by a chitinous rod.

These are most helpful structures for the movement of valves and provide space for attachment of muscles [11] (Agarwala 1951). A detailed pictorial profile of this structure is provided. The basic morpho plan of the ovipositor of short horned grasshoppers has a pair of dorsal valve and a pair of ventral valve, articulating condyles, apodemes providing space for muscular attachment as well to support digging action of ovipositors. Within this basic plan the variation in size and shape of both the valves, the terminal parts of the valves have been examined by the earlier workers in different contexts [1-11]. In this study we have examined morphological and morphometric variations of ovipositors in between the eleven species of grasshoppers in view of possible taxonomic utility of these structures.

Materials and Methods

Adult females of grasshopper collected from the wild at University of Agriculture Sciences campus Dharwad. The insects were killed, dried, and preserved. The preserved specimen of different species of grasshoppers transferred to 70% alcohol for full relax by wetting. The relaxed specimen held by the abdomen between the fingers and the complex exposed by pulling back genital plate. The female ovipositor pulled out along with the muscles encircling the apodemes by inserting needle or forceps on either side of the structures without altering natural position of the membranes. The dissected portion placed in 10% solution of Potassium hydroxide for 15 to 20 minutes. All the extraneous materials removed using needles or forceps to allow complete study of the membranes and sclerotized structures.

The removed ovipositor of female were observed under stereo zoom and neat images recorded in an inbuilt camera. To discriminate the internal tissue structure of phallic complex and other tissues used crystalline violet stain for staining the structures before photo imaging. Clear images are used for description and discussion.

In total eleven species of short horned grasshoppers belonging to sub families Acridinae: *Acrida gigantea*, *Acrida exaltata*, Oedipodinae: *Locusta migratoria*, *Oedaleus abruptus*, *Cyrtacanthacridinae*: *Cyrtacanthacris tatarica tatarica*, Catantopinae: *Cantantops pinguis innotabilis*, Gomphocerinae: *Aulacobothrus luticeps luticeps* Hemiacridinae: *Hieroglyphis banian*, and Pyrgomorphinae*: Atractomorpha crenulata crenulata*, *Pyrgomorpha bispinosa bispinosa*, *Chrotogonus oxypterus*, are used in this study.

Morphometry of ovipositors done as follows, the ovipositors are placed parallel to the m m marked scale, photographed and image converted to JPG image on computer, later these images along with the recorded scale used for measuring the length through Adobe Photoshop measurement programme on computer. All measurements were done involving a maximum of three different images, all images showed same measured values, a few showed variation at third decimal place which was of negligible size, hence those values are not considered in this study.

Two terms are added in the morphometric description of Ovipositors 1. Total Length =Measured length from tip of the ventral valve to end of the apodeme. 2. In between free end gap= distance in between the free end of apodemes.

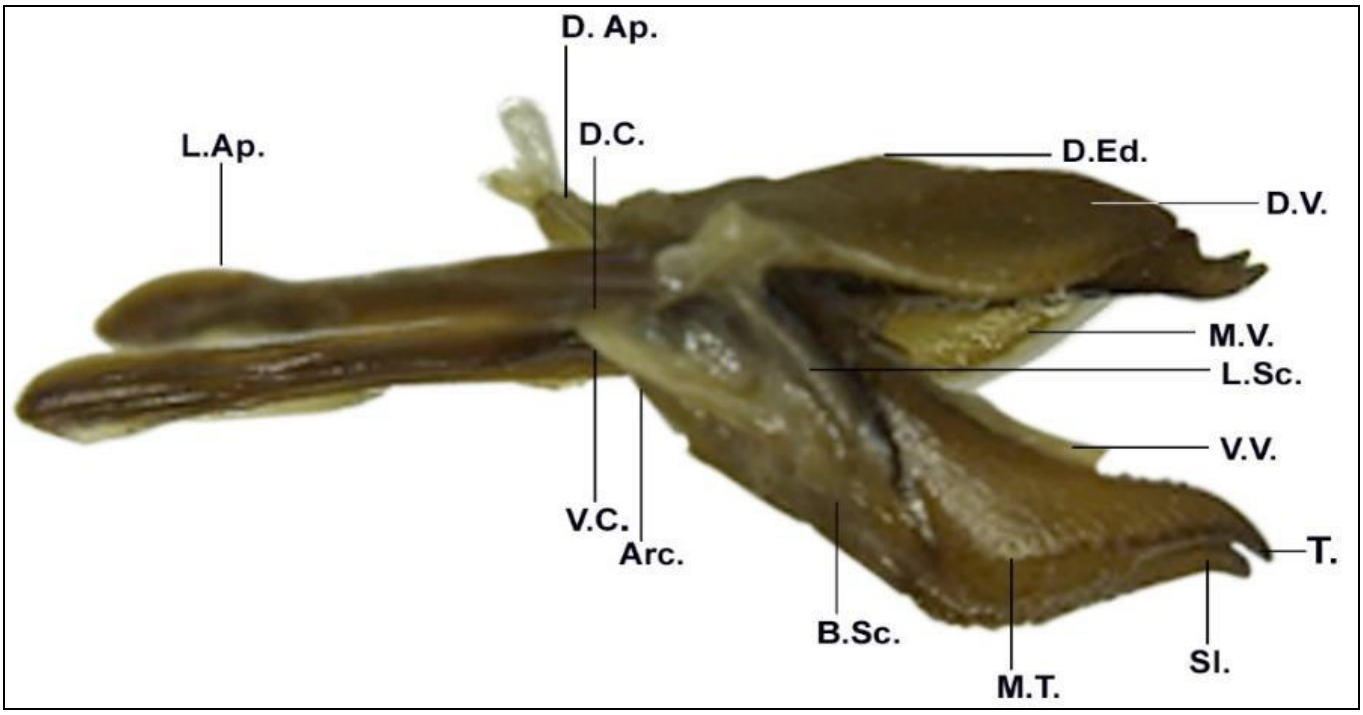
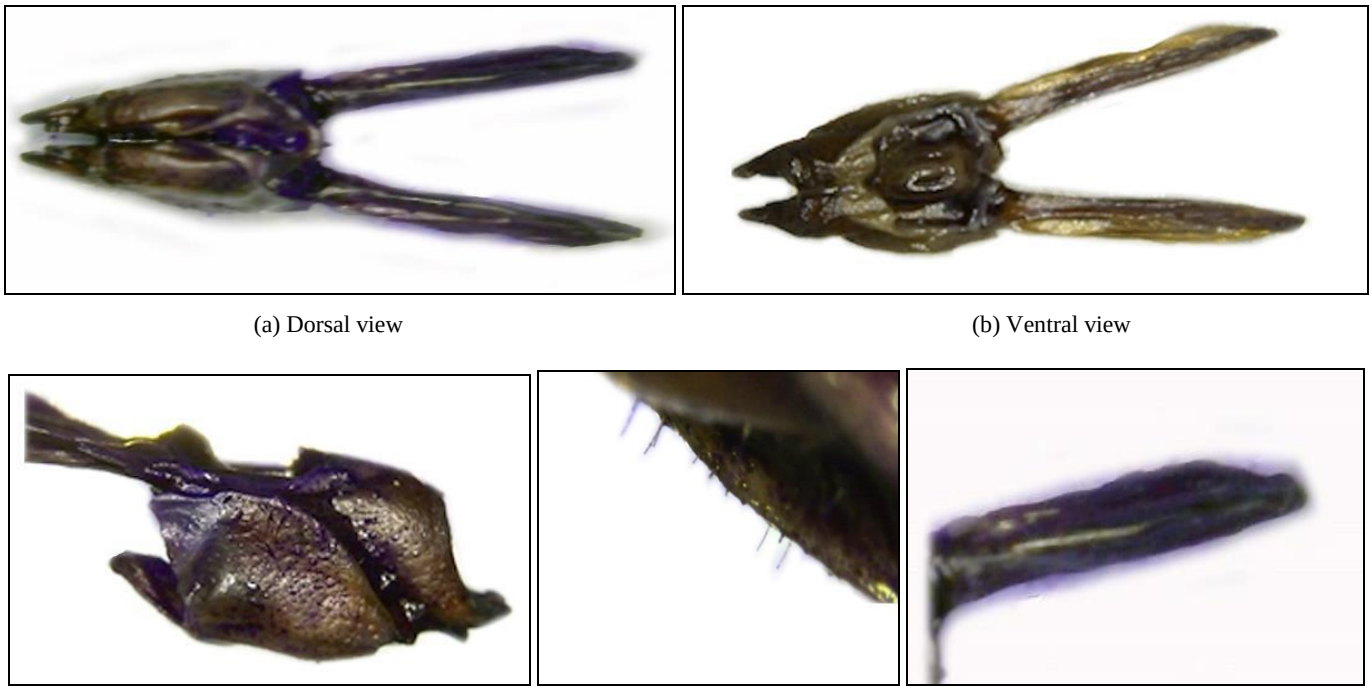


Fig 1: Ovipositor in lateral view (General).

Abbreviations:-Arc. - Arcus; B.sc. -Basal sclerite; D.Ap.-Dorsal apodeme; D.C.- Dorsal condyle; D.Ed.- Dorsal edge; D.V.- Dorsal valve; L. Ap.- Lateral apodeme; L. Sc.-Lateral sclerite; M.T.- Mesial tooth; M.V.- Mesial valve; Sl.-Slope; T.-Tip; V.C.- Ventral condyle; V.V.- Ventral valve.

Results

***Locusta migratoria*:** Valves were robust and elongated, the dorsal valves (Fig 2a) was 6.00 mm long and 1.75 mm wide, the ventral valve was 6.2mm long and 2.05 mm wide (Fig 2b) both the valves had strong walls, the upper surface of dorsal valve had tubercules and bristle the outer edge of margin serrated (Fig 2c, d). The mesial valve was not seen the apical tips of dorsal valves curved outward and ventral valves tips were sharp slightly curved. The apodemes articulated with ventral valve through the sclerites and condyles, The ventral valve with slope slightly concave with angular external lateral project. The dorsal valve broad, wide, apical tip short broad, blunt external edge finely serrated, dorsal and ventral condyle blunt. The apodemes were longer than both the valves measured 8.5 mm long and were 1.00 mm wide. The apodeme had large stainable tissue and both the apodemes separated at the free end (Fig 2e) with a gap of 4.1 mm. The apodeme tips were blunt.Table.1.



(a) Dorsal view (b) Ventral view

(c) Lateral view (d) Bristles in lateral margin (e) Apodeme – stained

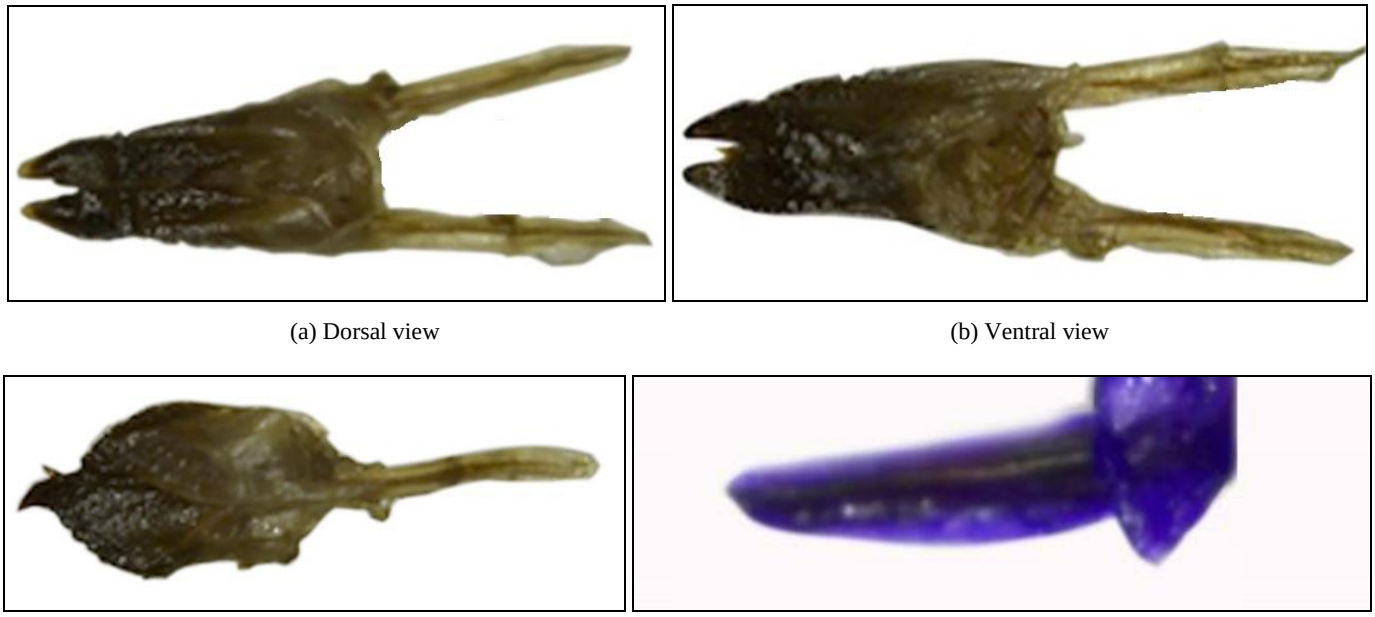
Fig 2: *Locusta migratoria*

***Oedaleus abruptus*:** Dorsal valve broad at the base measured 2.0 mm long,1.0 mm wide (Fig) and ventral valve slightly longer than dorsal valve with 2.25mm long and 1.07mm wide (Fig 3a). The tip of dorsal valve was blunt. The ventral valve concave with incurved pointed tips. The sclerites tuberculate and the condyles prominent (Fig b). The apodemes were long 3.02 mm and 0.6 mm wide. The apodemes gap free end had 1.08mm and had large s tainable tissue component. (Fig 3c) (Table.1)

(a) Dorsal view (b) Ventral view (c) Apodeme- stained

Fig 3*:* *Oedaleus abruptus*

***Pyrgomorpha bispinosa bispinosa*:-**The dorsal valve wide at base and narrows towards tip, so also the ventral valve (Fig 4 a, d). The dorsal valves were longer 3.02mm than the width 2.2mm. The ventral valves slightly longer (Fig 4 b) than dorsal valves measured 3.80mm long and 2.05mm wide. The apodemes was shorter than both the valves and had a 2.75mm free end gap between the apodemes (Table.1). The outer margin of the valves was serrate. The tip of the dorsal valves out curved to form a sharp spine. The ventral valve out curved had slightly slant inner edge, the tip out curved sharp pointed. The lateral sclerites and condylar process were prominently seen. The apodemes had large stainable tissue component as well thicker unstained sclerite rod (Fig. 4d).

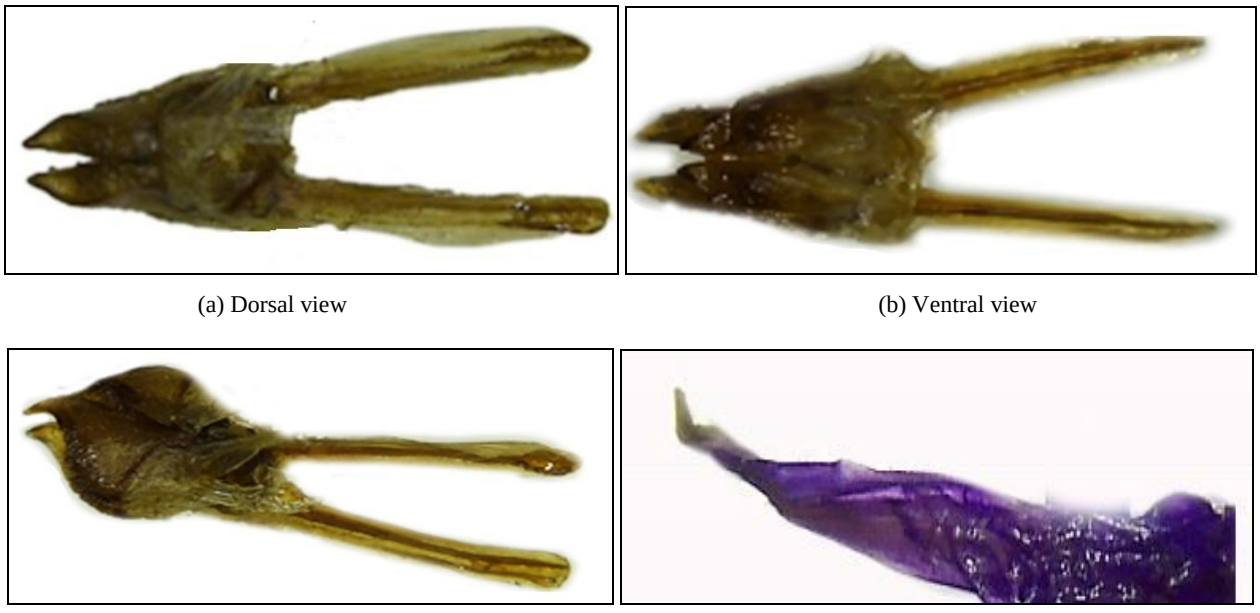


(a) Dorsal view (b) Ventral view

(c) Lateral view (e) Apodeme – stained

Fig 4: *Pyrgomorpha bispinosa bispinos*a

***Atractomorpha crenulata crenulata*:** The valves appear narrowing triangular in shape (Fig 5 a). The dorsal valves supported by thick sclerites with concave inner margin to form a shape curved tip, this valve measured 2.25mm long, 1.09mm wide. The ventral valve larger (Fig 5 b) 3.0 mm and was 2.00mm wide with inner concavity, the tip out curved blunt and thick at the base of the spine. The condylar and articulating sclerites prominently visible. The apodeme was 3.01mm long, 0.8mm wide, with free end gap of 2. 05mm (Table1). The stainable tissue of apodeme is greater than other species the unstained rod appeared streak, like at the tip, but slightly wider at the base (Fig 5c, d).

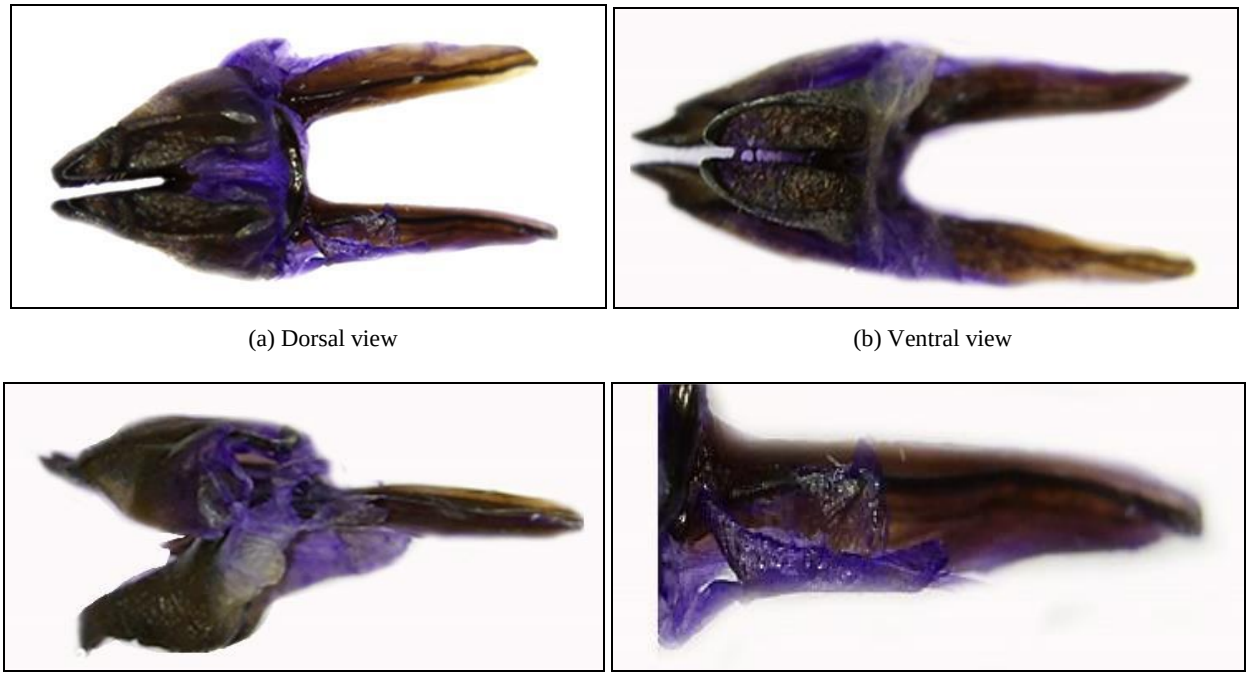


(a) Dorsal view (b) Ventral view

(c) Lateral view (d) Apodeme - stained

Fig 5: *Atractomorpha crenulata crenulata*

***Chrotogonus oxypterus*:** The dorsal valve broad, robust supported by strongly built sclerite with tubercles (Fig 6a). This valve had slightly inner margin concavity forms an out curved pointed tip, the valves measures 2.0mm long, 2.08mm wide. The ventral valve longer than dorsal valves (Fig 6 b), had stainable inner margins and concave outer margins at towards the end to form a prominent out curved blunt tip. The mesial valve was short triangular. The ventral valve measured 3.0mm, 2.9mm wide (Fig 6c). The apodemes were strongly built with large un stainable sclerotized arm (6d). The apodeme was 3.08mm long and 0.9mm wide; with in between arm separated gap of 2.0mm (Table1). The articulating condylar and sclerite process were prominent and large.

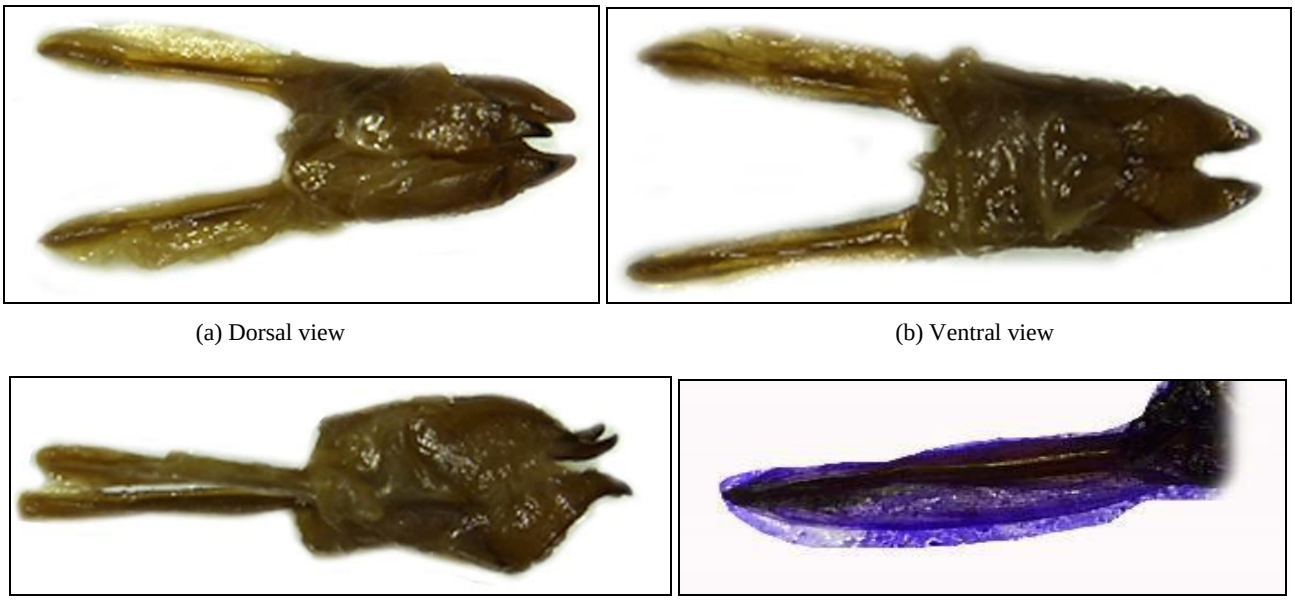


(a) Dorsal view (b) Ventral view

(c) Lateral view (d) Apodeme– stained

Fig 6: *Chrotogonus oxypterus*

***Aulacobothrus luticeps luticeps*:** Both dorsal and ventral valves (Figs 7 a, b) are narrow triangular in shape. The dorsal valves supported by an elongated lateral sclerite, the valve was 3.08mm long and 2.01mm wide, the ventral valve was broader with 4.00 mm length and 2.70mm width. The apodemes were 4.03mm long and 1.00mm wide, the apodemes of separated at the free end with 2.08mm gap (Table1). The articulating and sclerites bearing thick dark colour. The stainable area of apodeme was greater than unstainable area (Fig 7d). The dorsal valves and ventral valve had inner margin concavity, had terminal out curved sharp tips. The mesial valve appeared as very short structure (Fig 7c).

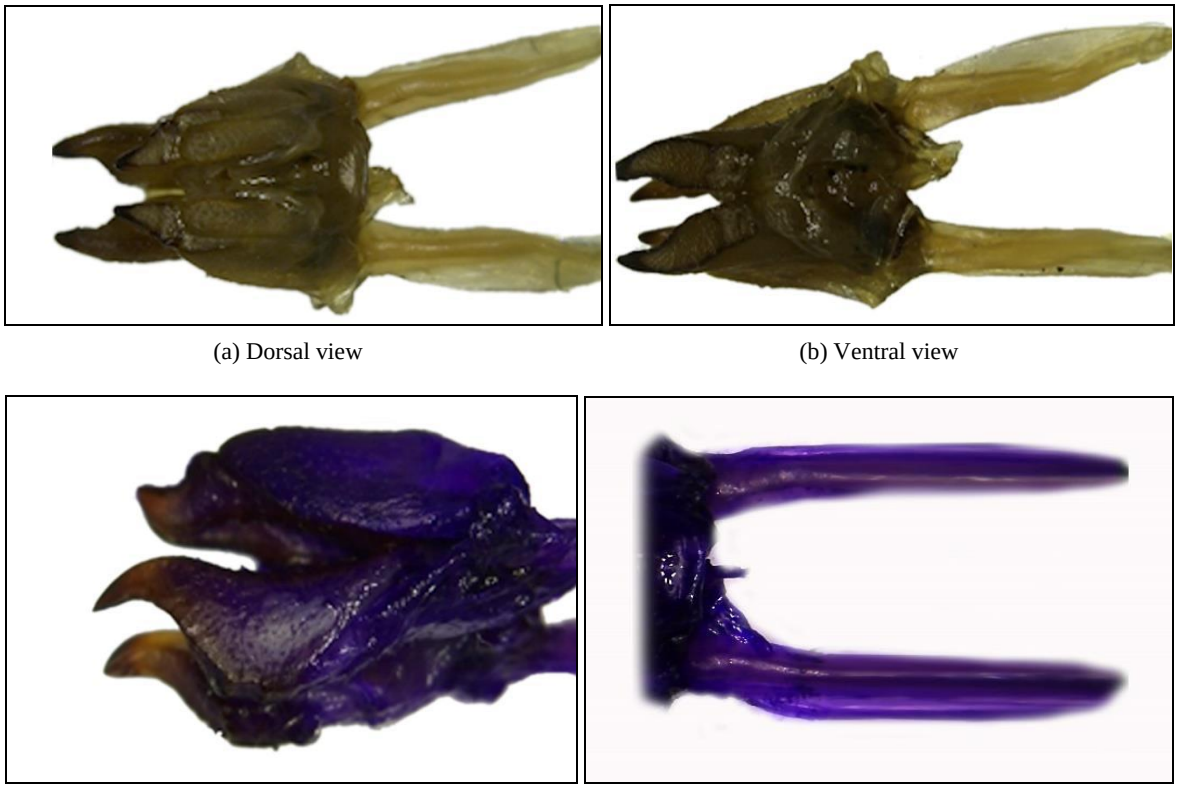


(a) Dorsal view (b) Ventral view

(c) Lateral view (d) Apodeme - stained

Fig 7: *Aulacobothrus luticeps luticeps*

***Cyrtacanthacris tatarica tatarica*:-**Both dorsal and ventral valves robust (Figs 8 a, b), almost bear pincer like appearance in dorsal side photographic profiles. The dorsal valves are supported by strong, thick sclerite wall. The dorsal valves were 5.9mm long and 4.02mm wide. The ventral valve was 6.09mm wide and 4.00 mm wide. The apodemes were 7.0 mm long and 1.25mm wide with their free end gap of 5.01mm (Table1). The inner margin of ventral valves was slant with terminal concavity ending is out curved robust pointed hook like spine. The margins of valves are serrated and the valve body had many pegged structures. The apodeme had large stained area (fig 8d) and a long chitinous rod like of equal size unstained structure. The inner margin of each valves had two-three conical projections (Fig 8c).

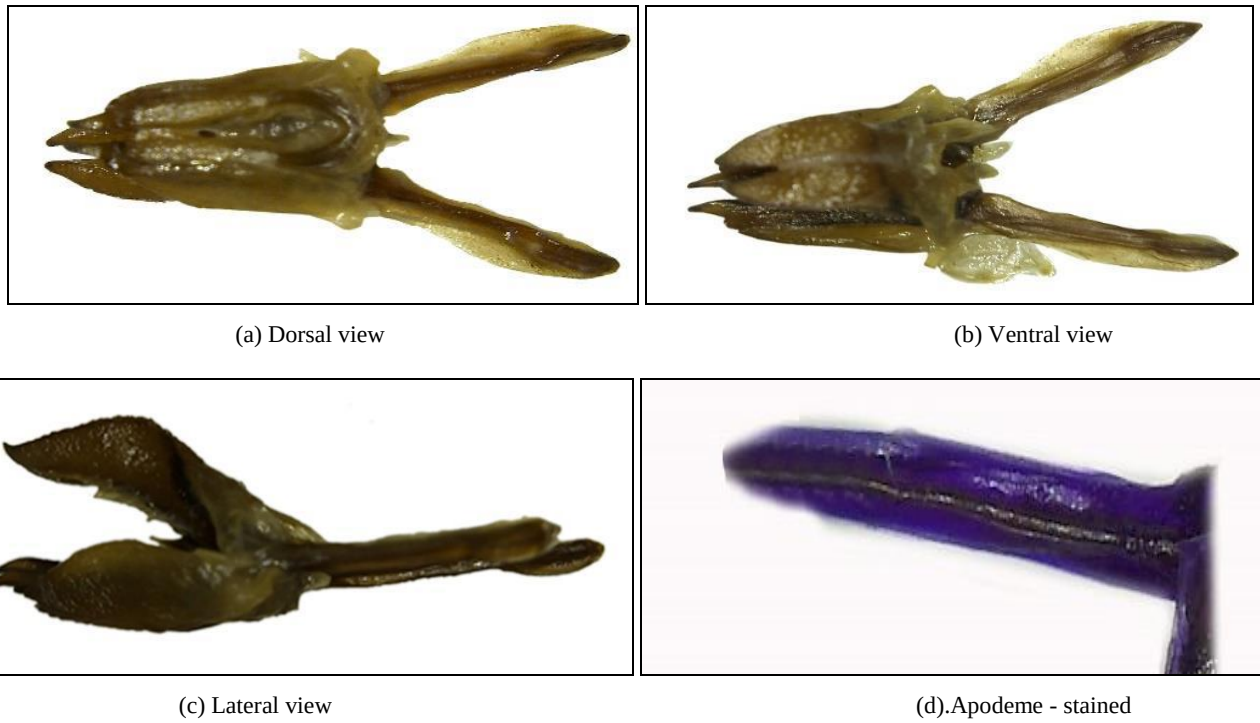


(a) Dorsal view (b) Ventral view

(c) Lateral view (d) Apodeme - stained

Fig 8: *Cyrtacanthacris tartarica tatarica*

***Hieroglyphis banian*:** The valves medium large in size, the dorsal valve supported by sclerite wall almost its length, the dorsal side of the valve tuberculata (Fig 9 a, b). The dorsal valve was 2.08mm and 2.01mm wide, whereas ventral valve was 3.08mm long and 2.03mm wide. The apodeme was 2.75mm long and 0.8mm wide with a free end gap of 2.75mm. The inner margin of the dorsal valves slightly concave and tip had outward curved sharp pointed hook like structure. The ventral valve was slant behind, directed upward at anterior end to result in sharp strong teeth. The mesial valve was small streak like in its visibility (Fig 9c). The apodeme (fig 9d) was 2.75mm long and 0.8 mm wide, both the apodemes separated by a gap of 2.75mm at the free end (Table1). The apodemes had large stainable area and chitinous non stained rod structure extended uniformly along the length of the apodeme. The articulating area sclerites and condyles were more prominent and thick.



(a) Dorsal view (b) Ventral view

(c) Lateral view (d).Apodeme - stained

Fig 9: *Hieroglyphics banian*

***Cantantops pinguis innotablis*:** The dorsal and ventrali mages of the ovipositor look like a narrow triangular structure (Figs 10 a, b), the valves were longer than the width. The dorsal valve measures 5.00m long and 2.01mm wide, the ventral valve had a length of 5.01mm and width of 2.25mm. The apodemes were shorter than valves measured about 4.10mm long and 1.0mm wide. The separation gap at the free end of apodemes was 4.01mm (Table1). The dorsal valves were supported by strong chitinous structure had no tubercules on the surface. The dorsal valve tip was slightly concave terminated in a thick slightly curved blunt tip. The ventral valves had slight concavity at lower end and upper position was slightly slant. The ventral tip was blunt strong little curved outward (Fig 10 c). The apodeme had wide stainable tissue covering and the supportive rod of chitin (Fig 10 d). The condyles and sclerites articulation was prominent and projected.

(a) Dorsal view (b) Ventral view

(c) Lateral view (d) Apodeme - stained

Fig 10: *Catantops pinguis innotabilis*

***Acrida exaltata*:-**The ovipositor valves were of medium sized, the dorsal valves were of medium size, were supported by strongly built lateral sclerites. Composed to its size measured 3.90mm long and 4.08 wide. The dorsal view of the photo image looked pincer like (Fig 11 a, b). The outer margin slightly concave and outwardly curved with sharp but short tip. The inner margin of the valves was slant. The ventral valves (Fig 11 b) measure 5.0mm long and 2.02mm wide. This valve too supported by strong lateral sclerites. The inner margin was slant and slightly concave and outer margin in curved outward, thick and blunt (Fig 11a, b). The dorsal condyles and the articulating sclerites distinctly seen. The apodeme were 6.75mm long, 0.8 mm wide, the free end separation was 3.00mm (Table1). The stained area of the apodemes was large (Fig 11c).

(a) Dorsal view (b) Ventral view (e) Apodeme – stained

Fig 11: *Acrida exaltata*

***Acrida gigantea*:-**The dorsal valve is broad measure 2.01mm wide and 2.25mm long (Fig 12a)ventral valves was 3.9mm long and 2.01mm wide (Fig 12b) both the valves had supported by strongly built sclerites. The outer margin of the ventral valves was concave curved downward to produce a sharp spine. The dorsal valve had slightly slant outer margin little concave inner margin. The valve ended with broad spine pointed at the tip. The mesial valve was thin triangular slender structures (Fig 12 b). The apodemes was 4.0mm long and 0.8 mm wide both the apodemes separated at their free end with a gap of 2.0 mm. The total length of ovipositor including apodemes was 9.0mm (Table1). The apodeme hooked like spatula had large stained area (Fig 12c).

(a) Dorsal view (b) *Ventro-lateral- stained* (e) Apodeme - stained

Fig 12: *Acrida gigantea*

Table 1: Morphometric values of different components of ovipositor in eleven species of grasshoppers

Characters		Dorsal valve		Ventral valve		Apodeme		Distance between the free end of apodemes	Total length
Sl. No	Species	L	W	L	W	L	W		
1.	*Locusta migratoria*	6.0	1.75	6.2	2.05	8.5	1.00	4.1	15.05
2.	*Oedaleus abruptus*	2.0	1.0	2.25	1.07	3.02	0.6	1.08	6.0
3.	*Pyrgomorpha bispinosa bispinosa*	3.02	2.2	3.80	2.05	2.75	0.85	2.75	6.75
4.	*Atractomorpha crenulata crenulata*	2.25	1.09	3.0	2.00	3.01	0.8	2.05	6.03
5.	*Chrotogonus oxypterus*	2.0	2.8	3.0	2.9	3.08	0.9	2.0	6.85
6.	*Aulacobothrus luticeps luticeps*	3.08	2.01	4.00	2.7	4.03	1.0	2.08	8.0
7.	*Cyrtacanthacris tatarica ta tarica*	5.9	4.02	6.09	4.00	7.0	1.25	5.01	14.0
8.	*Heiroglyphis banian*	2.08	2.01	3.08	2.03	2.75	0.8	2.75	6.02
9.	*Cantantops pinguis innotablis*	5.00	2.01	5.01	2.25	4.10	1.0	4.01	10.25
10.	*Acrida exaltata*	3.9	4.08	5.0	2.02	6.75	0.8	3.0	11.35
11.	*Acrida gigantea*	2.25	2.01	3.9	2.02	4.0	0.8	2.0	9.0

Discussion

Morpho plan of ovipositor is same in all the species of Acridids examined till the date [11, 6, 7, 8, 2, 9, 12] including the present study. Within this common plan variations in the components are recorded particularly in dorsal valves that exhibited variations which are not of general type thus considered to have phylogenetic significance [6, 7]. The dorsal valves of eleven species examined in this work varied in upper wall surface that had elevation of definite shape in *L.migratoria, O. abruptus, P. b. bispinosa, C. oxypterus, A. l. luticeps, H. banian and A. exaltata C. t. tatarica C.p. innotabilis*; had tubercles in *L. migratoria, C. oxypterus*, and was non tuberculate in others whereas the side walls were highly sclerotized borne tubercles and bristles in *L. migratoria*, serrated in species *L. migratoria, A. c. crenulata, C. t. tatarica, H. banian ;* smooth in all others. A few had denticles on the inner margin in *L. migratoria, A. l. luticeps, C. t. tatarica, H. banian* The morphometric values of dorsal valves were not similar in any of the eleven species implying species specificity in their size. The tip points, inner wall curvature were different in each of the species. The ventral valves were longer than the width in all the species, supported by sclerotized basal and lateral walls. The tip of these valves generally found out to form either sharp or blunt hook like structure modified for digging the tubes in the soil. The dorsal valves had similar length in *O. abruptus* and *C. oxypterus* but differed in the width, like wise four species had similar width of dorsal valve but had different lengths. Two species *A. c. crenulata* and *C. oxypterus* had same length of 3.o mm but had variation in width related to apodemes none of the eleven species had same length but width wise four species had common width of 0.8 mm and three species had 1.0mm width in common. The free end gap distance of apodemes had variations in between the species but two species had similar gap distance of 2.omm. In spite of all these changes in other charters discussed, the total length was never been the same in eleven species examined. Where one or two of the characters considered here overlaps, the total length may be considered as a decisive to conclude the in between difference of species. The ovipositors of two congeneric grasshoppers *A. exaltata* and *A. gigantea* showed about 85% variations in their structural organization bur these two species had shown only about 25% variations at DNA and proteins level [13] as confirmed by molecular analysis. This high lights the possible utility of ovipositors as means of analyzing intra specific and intra generic variations.

The stainable tissue of apodeme is another important feature differed in each of the species. The length and width of this tissue is crucial for the attachment of muscles that has been described by other workers [11, 12, 1] to operate the valve movements during oviposition. The free end gap of apodemes may too is helpful in the angular movements of. The hard chitinous rod of apodemes articulate through its condyles to provide force for digging the soil during oviposition. The size of the articulating sclerites of valves too had variation in all the species it appeared large in *A. gigantea* (1.1mm) and *C. p. innotabilis* (1.14mm), in other species the length of this part was between 0.03mm in *A.l. luticeps* to 0.91 mm in *C. t. tatarica* hence this feature may have an additional taxonomic value. The mesial valve was seen clearly in a few species appeared as thin thread like extension but we could not identify significant variation among the species. Though Usmani and Kumar [14] have discussed the possibility of taxonomic utility of female phallic organs in grasshoppers, the importance of ovipositors in taxonomy is not considered.

Conclusion

Taxonomic assessment of a species is majorly done by reviewing key traits of organisms, sometimes molecular variations are used to confirm similarity and differences among closely related species of grasshoppers as in case of congeneric species. The ovipositors of grasshoppers have variations in between the species examined and morphometric values had striking differences among the species. The variations of the components of ovipositors have taken place without altering the common plan of this structures. All these

qualities make provision for taxonomic utility and phylogenetic analysis of grasshoppers. Whenever two closely related or cryptic species of grasshoppers could not be differentiated by established taxonomic key characters, in such cases ovipositor may become decisive which has to be confirmed by further evaluation.

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