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## Mandibles of twenty species of short horned grasshoppers (Orthoptera: Caelifera) have variations among different species

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#### Abstract

Mandibles are prominent masticatory structures in the mouth parts of grasshoppers provided an opportunity to analyze the structural variations among different species of grasshoppers. In this study structural variations in mandibles of twenty species of short horned grasshoppers are examined for two reasons - whether the grasshopper mandibles bear any taxonomic significance and to test if one could use mandible structures to establish inter species relationships among the grasshoppers. The morpho plan of mandibles having incisor, molar and basal regions remained consistent. The incisor region had variations in its teeth or denticles, the molar region had an architecture prominently represented in the form of ridges and grooves running along the length. The number of ridges and projections varied from species to species. The morphometric values of these regions showed variations between the species. The dendrogram constructed based on morphometric values followed three ways clustering. The molar structures in two species of Pyrgomorphidae exhibited different structures from that of Acrididae members. Adaptation of mandibles to the food habit and possible relationships among species of grasshoppers are discussed.

Keywords: Morphoplan, interspecific variation, ridges and grooves, adaptation, food habit, dendrogram

#### Introduction

Acridids have biting and chewing mouthparts. Their structures have been used to understand several aspects of evolution and phylogenetic relations by different scientists. The mandibles of grasshoppers are of dicondyle type that has an impact on shape of the head and experiencing evolutionary stasis Blanke (2019)<sup>[1]</sup>. Diversity in shape of mouthparts in arthropods initiated around 419 million years ago Misof *et al.* (2014)<sup>[18]</sup> and in extant forms this change in shape is often related to food type Chapman and Boer (1995)<sup>[4]</sup>, Evans and Forsyth (1985)<sup>[6]</sup>. In grasshoppers the relationship between mouth parts and food type is well established and first identified by Snodgrass (1935)<sup>[22]</sup>. Ecological innovations during diversification of insects that encountered new food sources and their adaptation to these food resources by modifying the mouthparts has been considered to be an important biological trait by evolutionary biologists Brues (1939)<sup>[3]</sup> as well as taxonomists Mulkern (1967)<sup>[17]</sup>.

The present architecture of functional mandibles of insects has derived from the biramus appendages of crustacean origin Blanke (2019)<sup>[1]</sup>. The mandibles of grasshoppers vary in different groups corresponding to their feeding habit (Gangwere, 1965; Chapman, 1990; Gangwere, 1991; Hsiao *et al.*, 2017)<sup>[7, 4, 8, 10]</sup>. A typical mandible morphological plan of grasshoppers has an incisor area, a molar area in its head region and a basal region that hinges in to head capsule by condyles. The mandibles of both sides extend transversely to meet below or in

Front of the mouth, operated by abductor muscles. The mandibles are slightly uneven in size but the architecture specific to a species is consistent.

In general grasshoppers are phytophagous and have specific plants as their food sources Mulkern (1967)<sup>[17]</sup> and they are classified into two categories based on the preference of food plant 'specialist' and 'generalist'. Specialist adapted to feed on one particular food type and generalists which feed on both grass and other herbaceous plants, this type of feeding habit is mainly assisted by mandibular architecture and physiological changes. Mandibles are studied as evidence of adaptation in grasshoppers by different workers around the world.

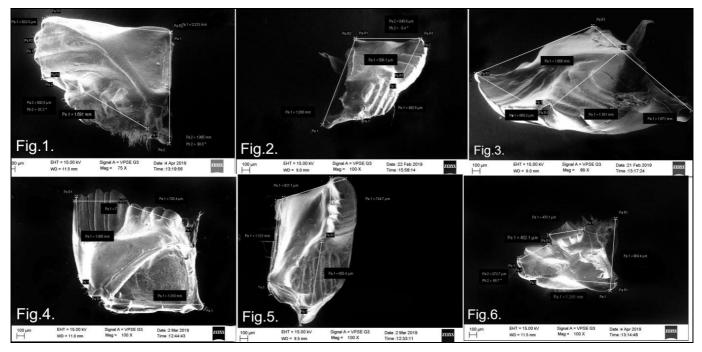
Hsiao *et al.* (2017)<sup>[10]</sup> studied mandibles of twenty species of grasshoppers belonging to six different families, as well in thirteen species of grasshopper of three families studied by Smith and Capinera (2005)<sup>[21]</sup> from Florida, structural adaptation of mandibles to specific food by different Mangolian grasshoppers of Pyrgomorphidae, and three subfamilies of Acrididae by Le Kang *et al.* (1999)<sup>[12]</sup> provide an evidence on the importance of mandibles for ecological adaptation related studies.

Isley (1994) classified grasshoppers into three groups based on mandibles general structure and their diet as Graminivorous (grass feeding type) where grinding molar and incisors typically fused into a scythe-like cutting edge. Forbivorous (broad leaf feeding type) where the molar region consist of depression surrounded by raised teeth and sharp interlocking incisor teeth. Herbivorous (mixed plant feeding type) have characteristics of both the types. In this study, we have examined the structural variations in mandibles of twenty species of short horned grasshoppers for two reasons; 1. To know the taxonomic significance, and 2. to establish inter species relationships among grasshoppers.

#### **Material and Methods**

For the current study Specimens (Table 1) were collected in and around the 'Manasa Gangotri Campus University of Mysore, Mysuru. Grasshoppers of both the (Three individuals from each sex) were collected, and identification of the species was done by the taxonomist Prof. C.A. Viraktamath, Entomologist and Emeritus professor at GKVK Campus University of Agricultural Sciences, Bengaluru, preserved in 70% alcohol for further use. The mandibles dissected out under the stereomicroscope, using scissor and dissection needle, into physiological saline (7% NaCl) and transferred to 2% KOH for 20 min to degrade the muscles attached to the mandibles. Three males and three females of each species were used for the study of mandibles as well to check the structural variations in mandible.

Sl. No.	Family	Sub Family	Species	Described By
	Acrididae		Acrida gigantea	Herbst, 1794
		Acridinae	Acrida exaltata	Walker, 1859
			Phlaeoba panteli	Bolivar, 1902
			Trilophidia annulata	Thunberg, 1815
		Γ	Oedaleus senegalensis	Krauss, 1877
			Acrotylus humbertianus	Saussure, 1884
		Oedipodinae	Aiolopus thalassinus tamulus	Fabricius, 1870
			Morphacris citrina	Kirby, 1910
1			Oedaleusabruptus	Thunberg, 1815
			Gastrimargus africanus orientalis	Sojosedt, 1928
		Coptacrinae	Epistaurus sinetyi	Bolivar, 1902
		Hemiacridinae	Aulocobathrus luticeps luticeps	Walker, 1871
		Catantopinae	Catantops pinguis innotabilis	Walker, 1870
		Spathosterninae	Spathosternum prasiniferum prasiniferum	Walker, 1871
		Calliptaminae	Acorypha glaucopsis	Walker, 1871
		Cyrtacathocridinae	Cyrtacanthacris tatarica tatarica	Linnaeus, 1758
		Oxynae	Oxya fuscovittata	Marrschall, 1836
	Pyrgomorphidae		Pyrgomorpha bispinosa bispinosa	Walker, 1870
2		Pyrgomorphinae	Atractomorpha crenulata crenulata	Fabricius, 1793
			Neorthocris acuticeps acuticeps	Bolivar, 1902



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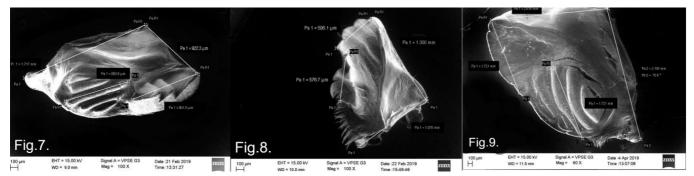


Fig 1: Right mandible of Acorypha glaucopsis, (2): Left mandible of Acrida exaltata, (3): Right mandible of Acrida gigantea, (4): Left mandible of Acrotylus humbertianus,(5):Left mandible Aiolopus thalssinus tamulus,(6):Right mandible of Atractomorpha crenulata crenulata,(7):Left mandible of Aulocobothrus luticeps luticeps,(8):Right mandible Catantops pinguis innotabilis, (9): Left mandible of Cyrtacanthacris tatarica tatarica

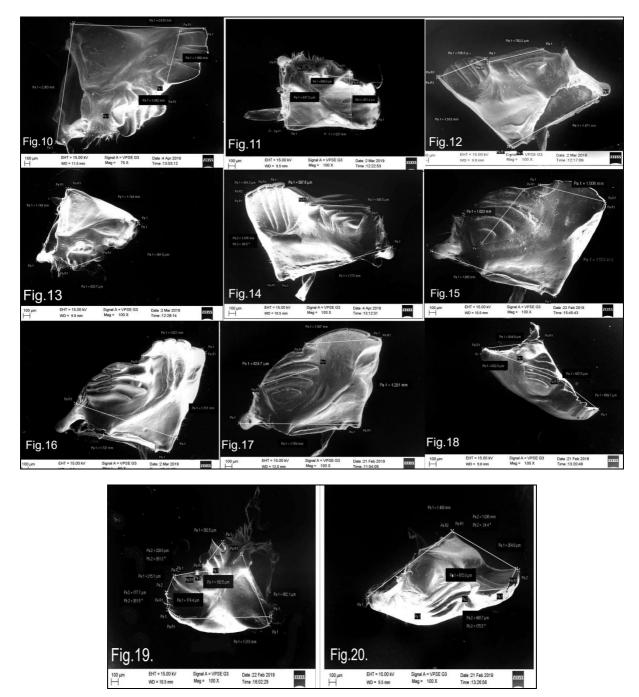


Fig 2: Right mandible of Gastrimargus africanus orientalis,(11): Left mandible of Epistaurus sinetyi, (12):Right mandible Morphacris citrina, (13): Left mandible of Neorthocris acuticeps acuticeps, (14): Right mandible of Oedaleus abruptus, (15): Left mandible of Oedaleus senegalensis, (16): Left mandible of Oxya fuscovittata, (17): Right mandible of Trilophidia annulata, (18): Left mandible of Phlaeoba panteli, (19): Right mandible of Pyrgomorpha bispinosa bispinosa, (20): Left mandible of Spathosternum prasiniferum prasiniferum

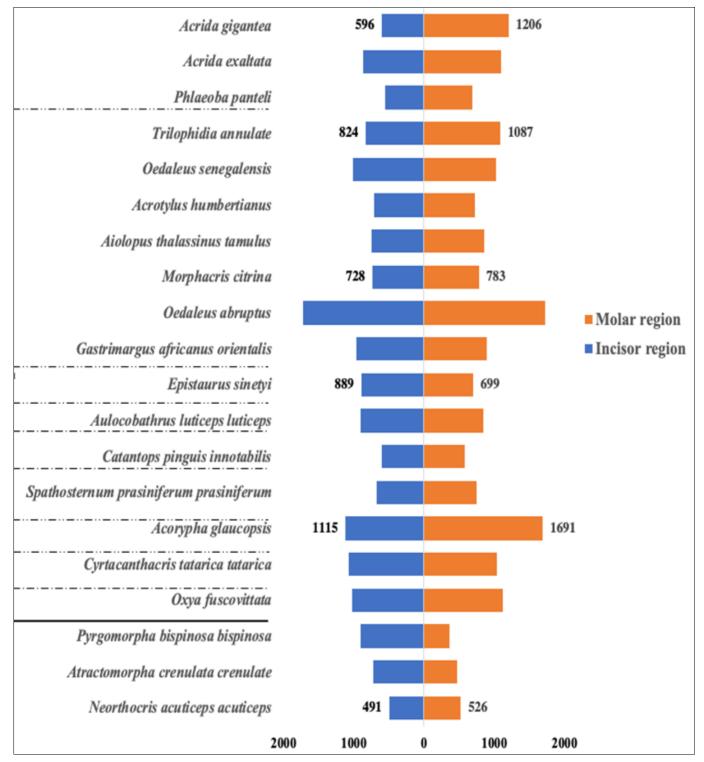


Fig 3: Morphometric values in length of incisor and molar regions of mandibles in 20 species of grasshoppers, data labels depict the range of length of incisor and molar regions in μm, black line in the Y axis categorize two families of grasshopper and dotted lines represent the sub families.

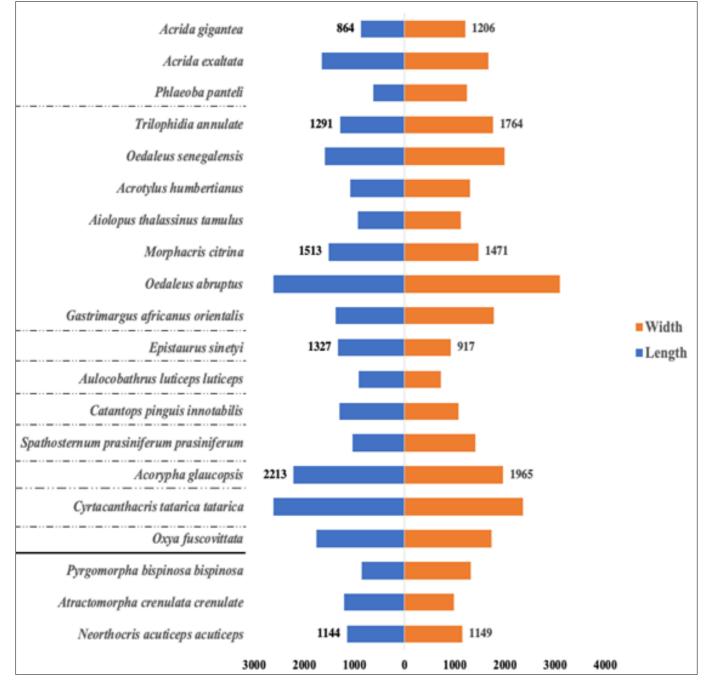


Fig 4: Length and width of mandibles in 20 species of Grasshopper, data labels depict the range of length and width of mandibles in μm, black line in the Y axis categorize two families of grasshopper and dotted lines represent the sub families.

Acrida gigantea			3	5	4	3				
Acrida exaltata		i	4	4	4	4				
Phlaeoba panteli			5	5	3	5				
Trilophidia annulate			4	4	3	4	τ.			
Oedaleus senegalensis			4	4	3	4				
			•			•				
Acrotylus humbertianus			3	4	5		4			
Aiolopus thalassinus tamulus			5	5	5		4			
Morphacris citrina			3	4	5		4			
Oedaleus abruptus			5	5	5		5			
Gastrimargus africanus orientalis			4	5	3	4				<ul> <li>Left mandible Incisor teeth</li> <li>Left mandible Molar ridges</li> </ul>
Epistaurus sinetyi		-4		7	5		5			Right mandibleIncisor teeth
Aulocobathrus luticeps luticeps			3	5	2	4				Right mandible Molar ridges
Catantops pinguis innotabilis		3		7	4		9			
Spathosternum prasiniferum prasiniferum			3	4	14					
Acorypha glaucopsis		3		7	4		7			
Cyrtacanthacris tatarica tatarica		3	10		4		10			
Oxya fuscovittata			4	4	3		9			
Pyrgomorpha bispinosa bispinosa		3	9		5		9			
Atractomorpha crenulata crenulate		4	8	}	5		8			
Neorthocris acuticeps acuticeps		4		7	4		7			
	15	10	5		0	5	10	15	20	

Fig 5: Number of denticles or teeth, in incisor and ridges in molar region of left and Right Mandible in 20 species of Grasshoppers, black line in the Y axis categorize two families of grasshopper and dotted lines represent the sub families.

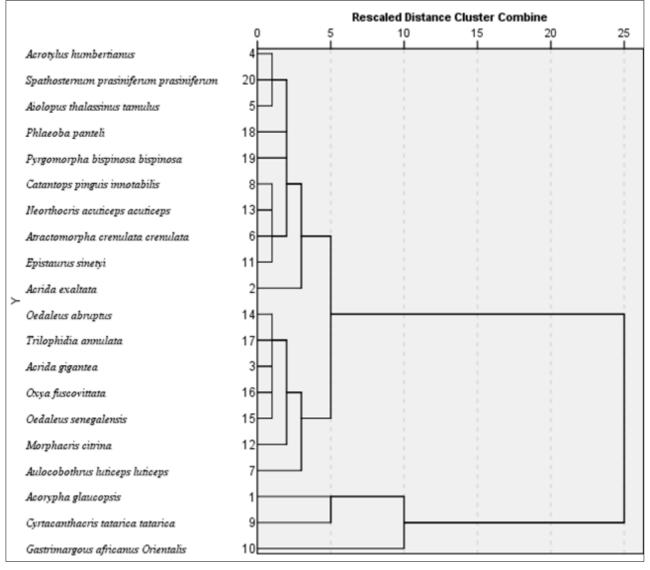


Fig 6: Dendrogram based on Morphometric Values of Mandibles, clusters showing squared Euclidian distance among 20 species of grasshopper.

Table 2: Pearson correlation between morphometric values of mandibles in 20 species of grasshopper

	Incisor Region	Molar Region	Length	Width
Incisor Region	1	.708**	.785**	.875**
Molar Region	$.708^{**}$	1	.692**	.762**
Length	.785**	.692**	1	.806**
Width	.875**	.762**	.806**	1

\*\*. Correlation is significant at the 0.01 level (1-tailed).

#### Scanning Electron Microscope Imaging of Mandibles

The procedure by Kim and Fung (2005) <sup>[21]</sup> as adopted by Kavya et.al. (2018) <sup>[13]</sup> is followed for SEM imaging. Scanning electron microscope studies were done using Carl Zeiss Electron Microscope at IOE University of Mysore, Mysuru. India The mandibles to be imaged taken from the grasshoppers were preserved in alcohol, the samples of mandibles separated and critically dried and mounted onto aluminum holder stubs using a double sticky carbon tape placed on the grid, the position of each sample noted. The values of the nitrogen gas tank (less than 100 psi) kept open that are regulated by commands on the computer, vent Button of the microscope operated to start. This program is entirely automated.

#### **Statistical Analysis**

Data obtained through measurement of mandibles of both

sides are analyzed using IBM SPSS statistics Version 22.0. Armonk, NY: IBM Corp. Hierarchical cluster analysis was done by applying agglomeration schedule, dendrogram was plotted by calculating squared Euclidian distance between mandible dimensions of 20 species of grasshopper, paired Pearson correlation was done on the compiled data of morphometric values of mandibles and graphs of measurement of length and width were constructed using Microsoft Excel 2010, following abbreviations are used to represent morphometric measurements of mandible Length-ML and Width-MW, in the text.

#### Results

 Acorypha glaucopsis is herbivorous and the mandibles were large in size (ML=2213 μm, MW=1965 μm, Fig. 22) Right mandible Fig. 1; had four prominent incisor teeth pointed at the apex had two ridges and three grooves. In the molar region of both left and right mandible irregularly arranged six projections were present that had a similar look of mammal's canine. Left mandible had three prominent incisor teeth, two ridges and three grooves (Fig. 23); the molar part had six molar projections, the same as in the right mandible.

- 2. Acrida exaltata is graminivorous and the mandibles were relatively small in size (ML=864.20μm, MW=1206 μm, Fig. 22). Right side incisor region had three major teeth and two minor teeth with five prominent grooves. Molar area had five molar ridges placed adjacent and running from one end to the other end of the molar area. Left side mandible's Fig. 2; incisor area had five indistinct broad incisor teeth with four grooves Fig. 23; and its molar area had five distinct ridges and same as in the right mandible.
- 3. Acrida gigantea belongs to the graminivorous group and the mandibles were comparatively medium in size (ML=1656  $\mu$ m, MW=1671  $\mu$ m, Fig. 22). Right mandible Fig. 3; had five plates like indistinct incisor teeth with one groove and two ridges along with four short and hard prominent molar ridges. Whereas in the left mandible had three incisor teeth. Except for three incisor teeth the architecture is the same as right mandible Fig. 23.
- 4. Acrotylus humbertianus Grouped under graminivorous type, the mandibles were comparatively small (ML=1085  $\mu$ m, MW=1310  $\mu$ m, Fig. 22). Left mandible Fig. 4; had prominent five incisor teeth with four ridges and grooves which were extremely hard and the molar area had four distinct ridges extended from one end to other of the molar area. Right mandible had four distinct incisor teeth with two prominent ridges and in the molar area it had four distinct molar teeth like projections which were broad, angular and its depression begins in the incisor area Fig. 23; and is extended up to the molar area.
- 5. Aiolopus thalassinus tumulus comes under group graminivores, the mandibles were of relatively small size  $(ML=931.10 \ \mu m, MW=1121 \ \mu m, Fig. 22)$ . Left mandible Fig. 5; incisor had three major teeth with two minor teeth and four distinct grooves. Molar area had five molar ridges which were distinct that runs from one end of the molar region to the other. Right mandible had four indistinct incisor teeth and in the molar area with five molar ridges similar to that of the left mandible Fig. 23.
- 6. Spathosternum prasiniferum prasiniferum comes under the group graminivorous species. The mandibles were comparatively small in size (ML=1036  $\mu$ m, MW=1409  $\mu$ m, Fig. 22). Left mandible had four distinct incisor teeth and two prominent ridges with three grooves. Molar area had five long and slender tth like structures. Right mandible Fig. 6; had indistinct incisor teeth without any prominent ridges and grooves. Molar area had five uniformly arranged ridges Fig. 23.
- 7. Aulocobathrus luticeps laticeps is a graminivorous grasshopper and the mandibles were comparatively of medium size (ML=922.30 μm, MW=1717 μm, Fig. 22). Left mandible Fig. 7; incisor area had three distinct incisor teeth with two prominent grooves. Molar area had five prominent molar teeth like projections, long and slender, running from one end to other. Right mandible incisor area had indistinct incisor teeth without any prominent ridges and grooves. The molar area had four distinct molar ridge with one adjacently placed indistinct molar ridge Fig. 23.
- 8. Catantops pinguis innotabilis comes under the group

forbivores. The mandibles were comparatively small in (ML1300  $\mu$ m, MW1708  $\mu$ m, Fig. 22). Right mandible Fig. 8; had four distinct incisor teeth, two prominent ridges with three grooves, that almost had leaf like appearance, Molar area consisted of one longitudinal groove and eight molar transverse ridges around the longitudinal groove. Left mandible had three distinct incisor teeth with two ridges Fig. 23; and two grooves, the molar region was the same as the right mandible.

- 9. Cyrtacanthacris tatarica tatarica belongs to forbivore, The mandibles were comparatively large (ML2610 μm, MW2363 μm, Fig. 22). Left mandible Fig. 9; had three distinct incisor teeth with three prominent grooves. Molar area had ten molar teeth like structures with one longitudinal groove. Right mandible had four distinct incisor teeth and two prominent ridges. Fig. 23; Molar area was very similar to the left mandible.
- 10. Gastrimargus africanus orientals grouped under the graminivorous grasshoppers. The mandibles were comparatively large in size (ML2617  $\mu$ m, MW3100  $\mu$ m, Fig. 22). Right mandible Fig. 10; had three indistinct incisor teeth that had almost plate like, hard structure without any ridges and grooves. Molar area had five ridges in which 4th and 5th were running from one end to another end. Left mandible had three distinct incisor teeth with three ridges and grooves. Molar area had distinct Fig. 23; and prominent five molar ridges similar to the right mandible.
- 11. *Epistaurus sinetyi* comes under the group herbivorous grasshoppers. The mandibles were relatively small in size (ML1327  $\mu$ m, MW917.2  $\mu$ m, Fig. 22). Right mandible had four distinct incisor teeth sharp at the apex region and had three grooves. Molar area had six molar teeth, it almost appeared like 'v' 'I' and dot-like structures. Left mandible Fig. 11; had three major and two minor incisor teeth with four prominent ridges. Fig. 23; along with six molar teeth at the molar area.
- Morphacris citrine is a graminivore, the mandibles were medium in size (ML1513 μm, MW1471 μm, Fig. 22) Left mandible had five distinct incisors teeth with two minor and three major ridges. Molar area had four short and strong teeth like structures placed adjacently; it covered almost the entire molar area. Right mandible Fig. 12; had four indistinct incisor teeth without any prominent ridges and grooves. Molar areas had four distinct molar teeth and were similar to left mandible Fig. 23.
- 13. *Oedaleus abruptus* placed under graminivores. The mandibles were medium in size (ML1376 μm, MW1773 μm, Fig. 22) Left mandible Fig. 13; had five distinct incisor teeth with four grooves and four ridges and was blunt at the apex. Molar area was regular with five molar teeth bearing large sized ridges. Right mandible had four indistinct incisor teeth without any ridges and grooves and Fig. 23; the molar area was very similar to that of the left mandible.
- 14. *Oedaleus senegalensis* comes under the group graminivorous. The mandibles were comparatively medium in size (ML1593 μm, MW1985 μm, Fig. 22). Right mandible Fig. 14; had three indistinct incisor teeth without any prominent ridges and grooves. Molar area had four distinct molar teeth without any longitudinal groove. Left mandible had three distinct incisor teeth with two conspicuous ridges and Fig. 23; in the molar

area five distinct molar ridges were present, similar to that of the right mandible.

- 15. Oxya fuscovittata is a member of graminivorous grasshoppers. The mandibles were medium size (ML=1751  $\mu$ m, MW=1737  $\mu$ m, Fig. 22) Right mandible had four distinct incisor teeth with two prominent ridges which almost appeared like a root of the plant. Molar area had five distinct strong, hard and large molar teeth. Left mandible Fig. 15; had three indistinct incisor teeth with five molar ridges and were similar to right mandible Fig. 23.
- 16. *Trilophidia annulata* comes under the group graminivores. The mandible was comparatively medium in size (ML=1291 μm, MW=1764 μm, Fig. 22). Right mandible has three indistinct incisor teeth that appeared almost plain structure without any ridges and grooves. Molar area had four distinct molar teeth like projections. Left mandible Fig. 16; had three distinct incisor teeth with two prominent ridges and Fig. 23; four distinct molar ridges.
- 17. *Phlaeoba panteli* comes under the group graminivores. The mandibles were comparatively small in size (ML=632  $\mu$ m, MW=1246  $\mu$ m, Fig. 22). Left mandible had five distinct incisor teeth, four ridges and three grooves are prominent in the incisor area. Molar area had five molar ridges regularly arranged. Right mandible Fig. 17; had four distinct incisor teeth and molar area similar to the left mandible Fig. 23.
- 18. Pyrgomorpha bispinosa bispinosa categorized under the group forbivores. The mandibles were comparatively smaller than other species (ML=862.10  $\mu$ m, MW=1313  $\mu$ m, Fig. 22). Left mandible Fig. 18; had four distinct teeth without any prominent ridges and grooves in the incisor area and which were sharp at the apex. Molar area had one longitudinal groove surrounded by nine transverse teeth like ridges; it almost appeared like a basket with the central depression. Right mandible incisor area had five incisor teeth larger than the left mandible incisor teeth with two ridges Fig. 23; and one groove. Molar area was the same as the left mandible.
- 19. Neorthocris acuticeps acuticeps comes under the group forbivorous. The mandible was comparatively small in size (ML=1144 μm, MW=1149 μm, Fig. 22). Left mandible had four indistinct incisor teeth without any prominent ridges and grooves which were blunt at the apex. Molar area had one longitudinal groove with seven indistinct molar projections. Right mandible Fig. 19; had four distinct incisor teeth with two prominent ridges Fig. 23; and the molar area was similar to left mandible.
- 20. Atractomorpha crenulata crenulata grouped under forbivorous grasshoppers. Mandibles were comparatively small in size (ML=1206 μm, MW=984.4 μm, Fig. 22). Left mandible Fig. 20; had five distinct incisor teeth sharp at the apex. Interestingly, the molar area had ten teeth like structures arranged around a longitudinal groove and appeared like a flower vase. Right mandible had four indistinct incisor teeth, very sharp and pointed at the apex region with two prominent ridges relatively larger than the left mandible. Molar area had eight molar teeth like structures similar to the left mandible Fig. 23.

Morphometric evaluation of mandibles carried out in all the twenty species, the details of the measurement records represented as graphical values Figs. 21-23; The largest size of mandible length 2616 µm recorded in G. a. orientalis followed by C. t. tatrica (2610µm) and the least length recorded 632µm Fig. 21; in P.panteli. The molar region measures ranged between 362.5µm to1721µm in species-P.b.bispinosa and G.a.orientalis respectively. The largest size of incisor region 1115 µm recorded in A. glaucopsis and least of incisor 491 µm recorded Fig. 21; in N.a. acuticeps. In G. a. orientalis both the regions were of equal size Fig. 21; least number of denticles or teeth in incisor region, four numbers recorded in seven species, five in five species, more than five in others. A maximum of 10 teeth were found in C. t. tatarica. Variations in the number of molar ridges also recorded, four ridges found in nine species, five in two species and more than five ridges in other species. Maximum of ten molar ridges recorded in C. t. tatarica. The Pearson correlations for morphometric values of mandibles found to be significant in between twenty species of grasshoppers Table 2.The dendrogram revealed three major clusters, one cluster had three species having larger mandibles, and the other two major clusters had seven and ten species, respectively. The major clusters had three sub clusters each Fig. 24.

#### Discussion

The relationship between mouthpart and diet of insects is well known (Smith and Capinera 2005) <sup>[21]</sup>, modifications of mouthparts, particularly mandibles, are more pronounced. Based on the modification of mandibles, the grasshoppers have been classified into graminivorous, forbivorous, herbivorous type Mulkern (1967) <sup>[17]</sup>, Smith and Capinera (2005) <sup>[21]</sup>. The type of biting cusps varies from species to species, grasshoppers that feed on soft broad leaf plants have small sharply pointed cusps, grass feeders have very long chistle-like edged cusps, distally but short flattened cusps proximally but in general mouthparts consistency is seen in subfamilies of grasshoppers, in particular mandible structure is highly consistent Smith and Capinera (2005) <sup>[21]</sup> that limits the change of host plant by the grasshopper.

The present study on twenty species of grasshoppers' regard to mandibles variation confirmed above classified into three types, of these fourteen species are graminivorous -Acrotylus humbertianus, Acrida gigantea, Acrida exaltata, Aulcobathrus luticeps luticeps, Aiolopus thalassinus tumulus, Gastrimargus africanus orientalis, Morphacris citrina, Oedaleus abruptus, Oedaleus senegalensis, Oxyafusco vittata, Phlaeoba panteli, Spathosternum prasiniferum prasiniferum, Trilophidia annulata, they havestrongly modified sharp edge incissor region and molar folds extended, whereas Atractomorpha crenulata crenulata, Cyrtacanthacris tatarica tatarica, Neorthocris acuticeps acuticeps, Catantops pinguis innotabilis, Pyrgomorpha bispinosa bispinosa are forbivorous, adapted to feed on grass and smooth leaves. This type of classification based on mandibular modification have been documented by several works around the globe (Kang et al., 1999; Li et al., 1983; Li and Chen, 1985; Kang and Chen, 1994) <sup>[12, 15, 16]</sup>. There are reports on mandibles of other insects like horn beetles Hörnschemeyer et al. (2013)<sup>[9]</sup> and ants Brito et al. (2016). Mandibles are strong in beetles and used both for feeding and defense. In ants, the mandibles are used for biting in self-defense and feeding. In grasshoppers, the mandibles are used exclusively for feeding and there is no modification of mandibles for defense purpose in all the twenty species under study.

Mandibles in all the twenty species of grasshoppers had hard texture formed cuticles. The structural analysis revealed the

mandibles of forbivore grasshoppers had digitate incisor area that could be distinctly seen. The molar area had transverse ridges and grooves that enable the grasshoppers to feed on the leaves, flowers and grass. In graminivore mandibles, incisor teeth are indistinct with the smooth margins, the molar area had long ridges, in herbivores intercalary teeth were distinct compared to graminivores, the molar region had comparatively short longitudinal grooves. Irrespective of the diet type of grasshoppers, each species of grasshoppers had distinct structural variations in incisor and molar region that could be easily visualized. All the twenty species of grasshoppers had well defined morphological features to distinguish each of the species as a taxonomic unit (as evident in identification of these species). Even the two pairs of congeneric species had no overlapping values and there was no clutter in taxonomic status based on morphological traits. The mandibles in all the species exhibited variations in the molar, incisor region and in size specific to species that only could be considered as one of the defined traits of a species. Thus these variations in mandibles examined in context of taxonomic utility, found to be used only as an additional character in defining the species but in three species of Pyrgomorphidae, the molar profile was different from members of Acrididae, this unique trait seems to bear phylogenetic signal that has to be confirmed by further studies involving many more species of Pyrgomorphidae.

The structural variation in mandibles of grasshopper has been examined in context of evolution proposing niche variation hypothesis by Van Vale (1965)<sup>[23]</sup>, Patterson (1983)<sup>[19]</sup> which narrated biotic diversity at both genetic and phenotypic level relating to morphological variation in accordance with ecological habitat. According to this theory, grasshoppers occupying a broader ecological niche produce greater variation All grasshopper species involved in this study coexisted in an identical habitat and the mandibles of these grasshoppers were different from each other, meaning that mandibular structure is characteristic to species and phylogenetically conserved character. The statistical correlation obtained by paired correlation test has shown incisor and molar regions were positively correlated, indicating that increase in size at one region of the mandible (either incisor or molar) also had an increase in the other region. If there were to be dissimilarity by decreasing the size of one region and increase in the other region (either incisor or molar), as an evolutionary process, it would have been negatively correlated. Thus increase in one region of mandible might have an impact on increase in the other for functional coordination of these structures, during the course of evolution.

The statistical application to examine the relationship between species regarding the mandibular characters has shown three clusters,. Such clustering reveals similarity of species in relation to mandibular organization and indirectly reflects food habit. Thus the mandible morphology can also be used to understand ecological requirements and evolutionary history, but to use mandibles as a standard measure of taxonomic trait to distinguish between species has feeble chance. Though there were changes in the molar region of three species of Pyrgomorphidae *N.a. acuticeps*, *A. c. crenulata* and *P. b. bispinosa* well in one Acrididae member *E. sinetyi*, it could be considered up to family level identification particularly in Pyrgomorphidaeand could be used as added character in other species. In acridid grasshoppers we have examined, the mandibles are exclusively adapted for feeding and there was

no modification of mandibles for defense purposes.

#### Conclusion

Mandibles in grasshoppers have a well-defined morphometric plan varying to an extent in its architecture. This variation may be a result of adaptation to food types existing around the habitat of these insects. The shape of incisor structures, their numbers and the molar folds or projections reflect the type of phytophagy whether it is graminivorous or forbivorous. Such variation in mandible structures directly reflects the relation between the insect and its food type. In these twenty species analyzed, mandibles as taxonomic structures bear weak signals at species level, to confirm the taxonomic utility of mandibles more sampling and analysis has to be done.

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