ijpbs 2020; doi 10.22376/ijpbs.2020.11.4.b96-106

International Journal of Pharma and Bio Sciences

Original Research Article

Morphometric for better Epidemic Identification

Morphometric geometry study on wings of grasshoppers: reflect more evolutionary flexibility in hind wings.

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Abstract: Evolutionary changes in wings of neopteran insects has been examined in different groups to large extent but less in grasshoppers . Wings of insects have been considered as favourite structures to analyse diversity among the species. Morphometric Geometry studies, applied to analyse the wing structure in twenty species of grasshoppers indicated both forewings and hind wings have followed a similar pattern of vein organisation, specific to both of these wing pairs. The fore wings had all the vein complements emerging from their respective sclerite in the cubital lobe.The anal lobe of fore wings had reduced size and had two narrowly separated parallel running anal veins with restricted flexibility . This structural organization of the fore wing helped in straight positioning of these wings over folded hind wings extended over the abdomen and these serve as important structures for camouflage. Our Observations revealed that the hind wings irrespective of the species had all the vein complements in the cubital lobe plus vein complements ranging between 3-10 in the anojugal lobe. also indicated that radial veins of hind wings had more number of branches than other vein complements that has contributed towards the increase of wing nervures and cells in between. Wide angled placement of veins in both the lobes increased the laminar space of hind wings as well its curvature in all the twenty species. The cross vein links are more prominent in hind wings as squares but rectangular in fore wings. The critical statistical analysis revealed three way clustering of grasshoppers relatedness, majorly influenced by the number of anal veins and degree of divergence of different veins in both the pairs of wings. The wing venation pattern of grasshoppers bear evolutionary significance and represents a typical orthoptera wing type.

Keywords: cubital lobe, anal veins, nervures,Degree of divergence, morpho space.

Article History	**Date of Receiving**	29 July 2020	**Date of Revision**	01 September 2020
	Date of Acceptance	09 September 2020	**Date of Publishing**	02 October 2020

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Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Int J Pharma Bio Sci., Volume11., No 4 (October) 2020, pp b96-106

CItation MeghaUrs T.S1, Alwyn D'souza1, shakuntala.V1 and Channaveerappa, H*.1,2 , Morphometric geometry study on wings of grasshoppers : Reflect more evolutionary flexibility in hind wings..(2020).Int J Pharm Sci.11(4), b96-106 http://dx.doi.org/10.22376/ijpbs.2020.11.4.b96-106

1. INTRODUCTION

Morphometric geometry technique is emerging as a useful technique to understand structures in all forms of science including engineering and Medical sciences. Morphometric Geometry has become one of the major tools to assess diversity in between or among the insect species[1]. Wings of insects have been considered as favourite structures to analyse diversity within or in between the species[2]. Wing morphometry is used as a tool to asses Tsetse fly population genetics,species identification as well to help in elimination of these insects to protect human from trypanosomiasis[3], this technique is also usedto identify epidemic vector mosquito species particularly in densely populated area[4] and has helped to take measures to stop spreading of disease by controlling these mosquitoes.Grasshoppers are winged insects with an exception of a few wingless species, displaying a typical orthoptera wing pattern of protective and flight wings. For insects, wings are important flight organs that have contributed to their distribution success. The size, shape and structure of the insect varies from group to group, such variations reflect the ability of the insect's for their flight [5]and flight behaviour [6,7]. Wings show a great range in size and venation, stiffness and flexibility, pigmentation [8]and are subjected to strong selective pressure under specific ecological conditions[9].The physical organisation of the insect wing represents slender two dimensional membrane structures having crisscrossed network of tubular veins. The venation network allows fluid and nutrient transport across the structure and also provides mechanical support to wings for stiffening [10-12]. The pattern of vein arrangement in wings, demarcate the wing into a few domains in many insects. Wing domains and wing structure are widely studied using geometric morphometry principles in the taxonomic consideration of insects, to establish the relationship between the closely related taxa and to find variations in different ecological as well evolutionary contexts [13,14] In some insects phylogenetic analysis and morphometrics have been deployed to understand the variation of wings and the selection pressure on the wings [15-17] but such studies are limited to a few species or orders.In Comstock and Needham system [18,] the wings are divided into three major domains the Cubital, Anal &Jugal lobes. The cubital lobe has Costal(Co), Subcostal(SCo), Radial(R), Median(M) and Cubital(Cu) veins. The median, radial and cubital veins may have branches named as anterior or posterior or 1 or 2 and so on. Whereas in anal and Jugular lobe the veins vary in number may or may not be branched. The Jugular lobe is not represented in many groups of insects but cubital and anal lobes form the definitive part of all insect wings. In orthoptera insects the term anojugular is adapted by Kukkalova-Peck and Lawrence [19]because the jugular lobe is formed of single or two veins and the lobe is not distinct as in other insect groups. The venation architecture of forewings and the hind wings differ to a greater extent though both the wings have the same lobes. In grasshoppers, the differentiation of anal and jugular lobes is not prominent in their hind wings compared to other insects. Variations in the venation of insect wings have been studied using morphometric geometry analysis [20, 21] in a few insect species but in grasshopper such an effort is comparatively less. In this study, distribution and numerical difference of veins in grasshoppers' wings have been analysed to understand whether there exists a similarity between the individuals of different species or not. We have examined which part of the forewing and hind wing has shown a wide range of variations to understand the changes that have taken place among the species of grasshoppers involved in this study.

2. MATERIALS AND METHODS

Twenty species of grasshoppers collected from their natural habitat are used in this study (for species names see fig.(4-10). Forewings and hind wings of both the sexes separated, neatly spread on a glass surface and photographs were taken along with a measuring scale placed on side. Morphometry was done by following the procedure of Bellaza and Demayo[22]with suitable modifications. The photo records were converted into JPEG image file, along with the scale and imported to Adobe photoshop[23].The images were layered to place the markings like dot, name of the vein or line in between the veins using a measuring tool on the toolbar. Drawing lines from a marked deviation point of each vein, the degree of deviation as well as length of the wing vein measures recorded automatically. Even the length of wing or lobe of wings also recorded following this method. The measurements of the structure were done by placing markers from one end (either length or width) of the structure to the other end, the straight line was drawn from each point on to scale in the image, that indicated the actual length in between the two points. In cubital lobe markings include Costal (Co), Subcostal (Sco), Radial (R), Median (M) and Cubital Veins (Cv). Anal +jugular lobes may are may not be branched, If branched named those as A1, A2…An. As shown in the Figures (1 -3) the last number in the A1 to A-n series represents the jugular vein.

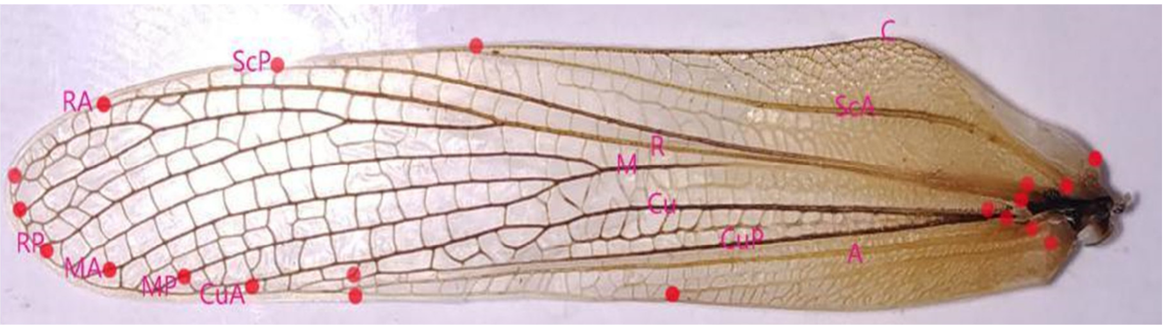

Fig 1: Landmark digitization of grasshopper forewing

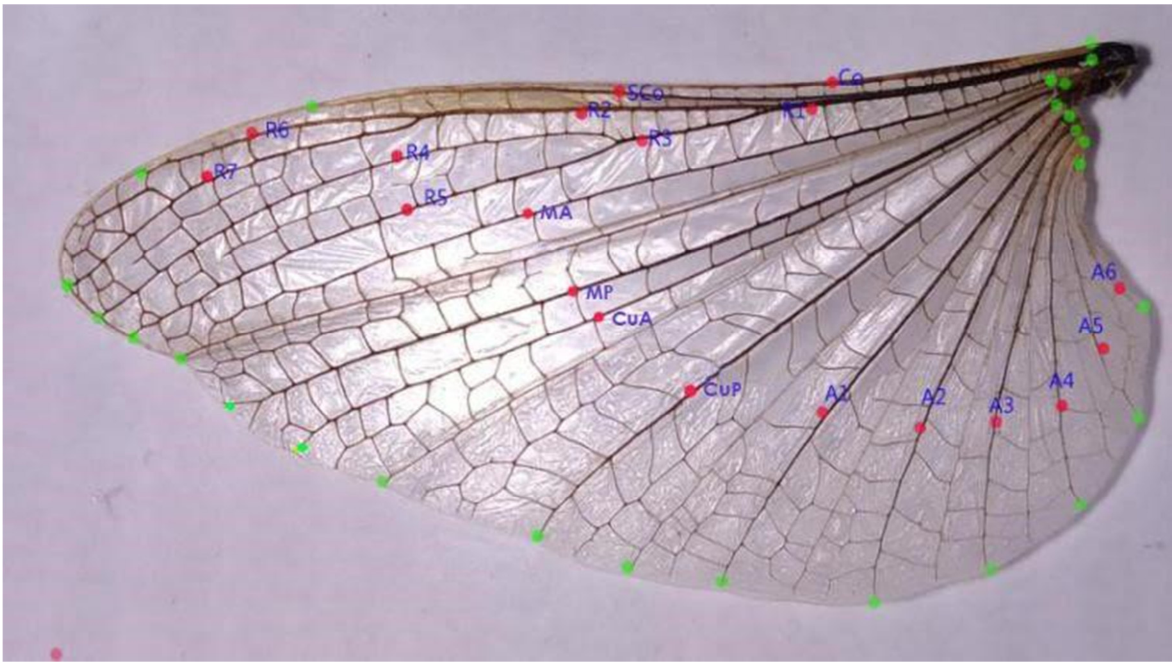

Fig 2: Landmark digitization of grasshopper hind wing

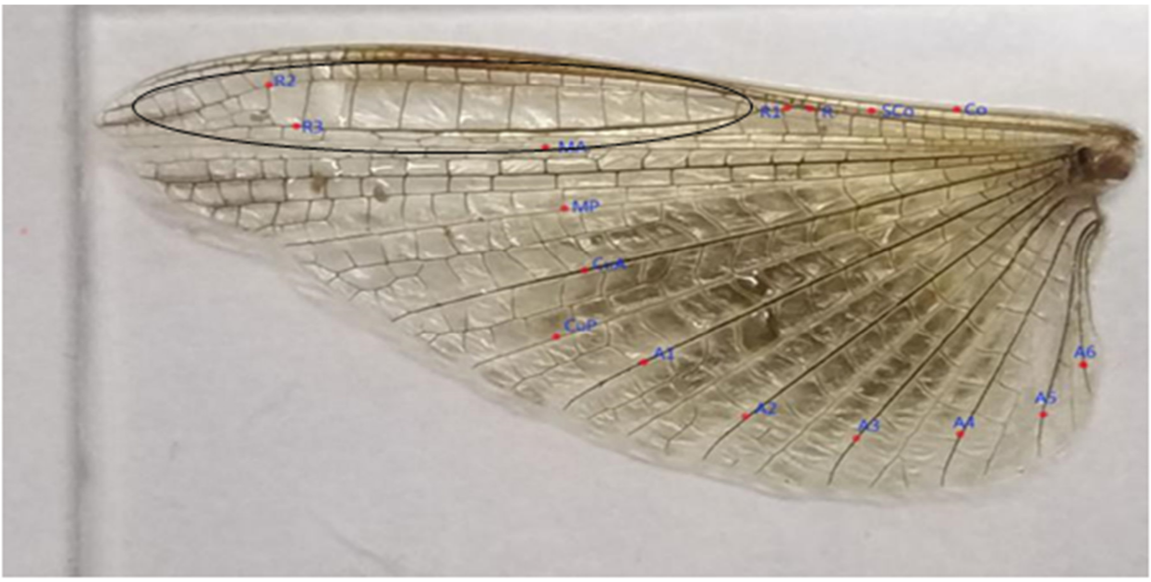

Fig 3: Hind wing of *A. exaltata* displaying semi-transparent area

3. STATISTICAL ANALYSIS

The technique used here is a programme on Adobe Photoshop, meant for digital image measuring. All the values obtained are tabulated for statistical applications. The tabulated contents analysed using IBM SPSS v22 software to construct hierarchical clusters as well to draw graphic representations.

4. RESULT

The study on wing geometric morphometry of twenty species of grasshoppers, in regard to the size and width of both fore wings and hind wings showed variations between all the species. The smallest forewing of 10.6mm and hind wing of 9.36mm was present in *S.p.prasiniferum*. The largest forewing of 52.12mm and hind wing of 51.21mm was present in *L.migratoria*. The remaining 18 species had hind wing length ranging between 14.01mm in *T.annulata* to 21.68mm in *C.p.innotabilis*. Forewing length ranged from 15.63mm in *T.annulata* to 22.6mm in *C.p.in notabilis* .In larger species the length of forewing ranged between 36.32mm to 50.68mm including two congeneric grasshoppers *A.exaltata* and *A.gigantea* the range was from 41.13mm to 43.29mm. (Figure.4and Figure.5)

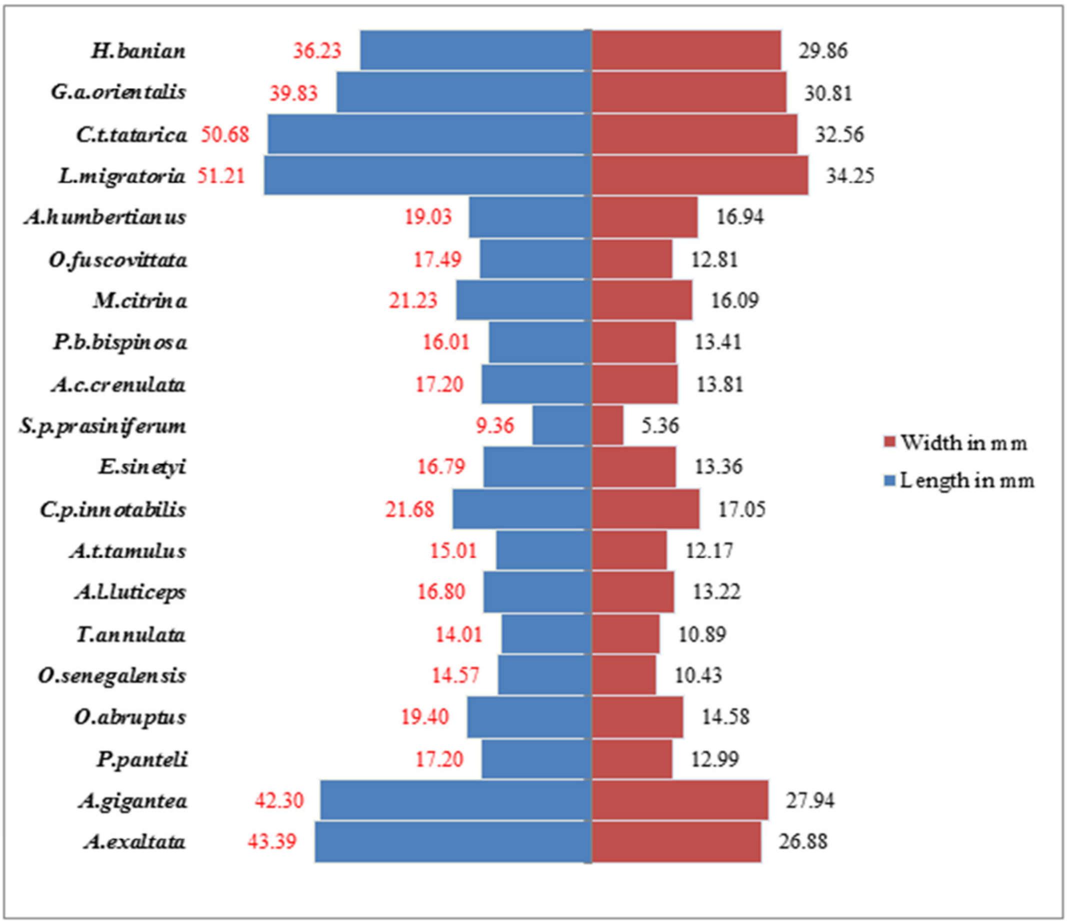

Fig 4: Length and width of hind wing of 20 species of grasshoppers

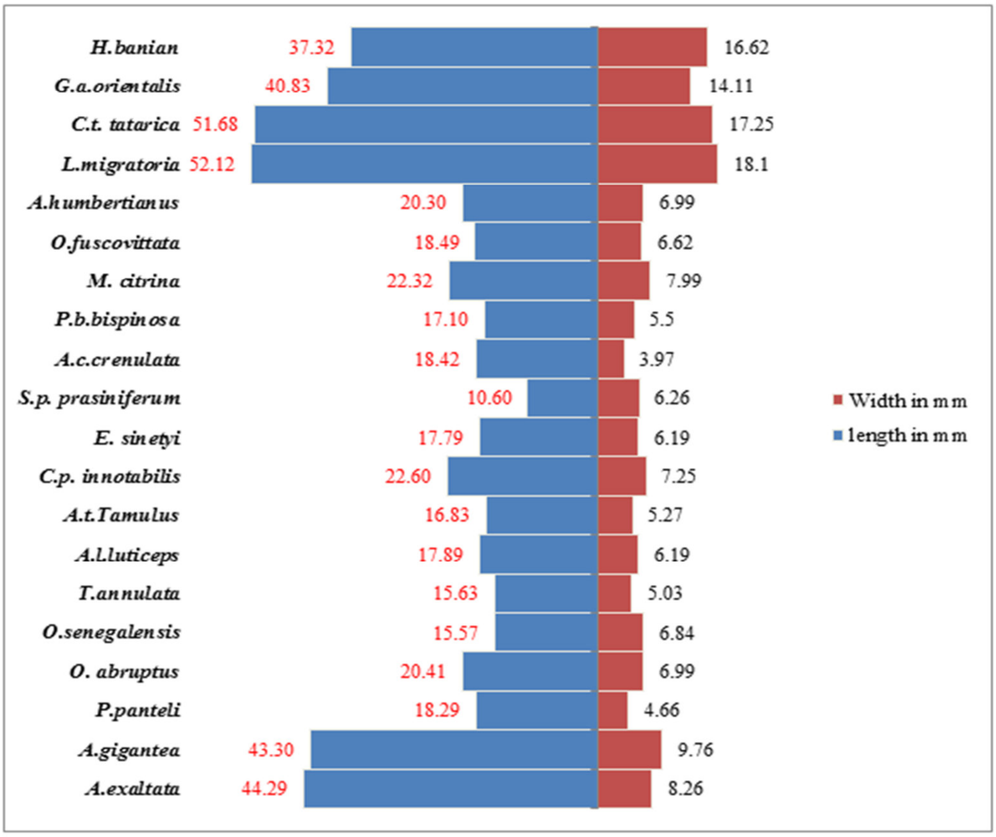

Fig 5: Length and width of fore wing of 20 species of grasshoppers

The degree of vein divergence of hind wings was 0.31^{0}in *A.c.crenulata* to 1.38 in *C.t.tatarica*, in between Co/SCo.0.24^0 in *A.humbertianus* to1.42^0 *L.migratoria,* in between Sc/R0.73^{0}in *A.humbertianus*to3.63 in *L.migratoria*, in betweenA.0.19^0 in *A.c.crenulata*to 3.07^0 *L.. migratoria*in between MA/MP.0.34^{0}in*A.humbertianus*to2.39^0(*L.migratoria*) in between MA/CuA. 0.23^{0}in*C.p.innotabilis* to 1.92^{0}of *A.gigantea*, in between CuA/CuP0.23^{0}in *C.p.in nobilis* to 1.92^{0}in *A.gigantea*.(Figure.6)

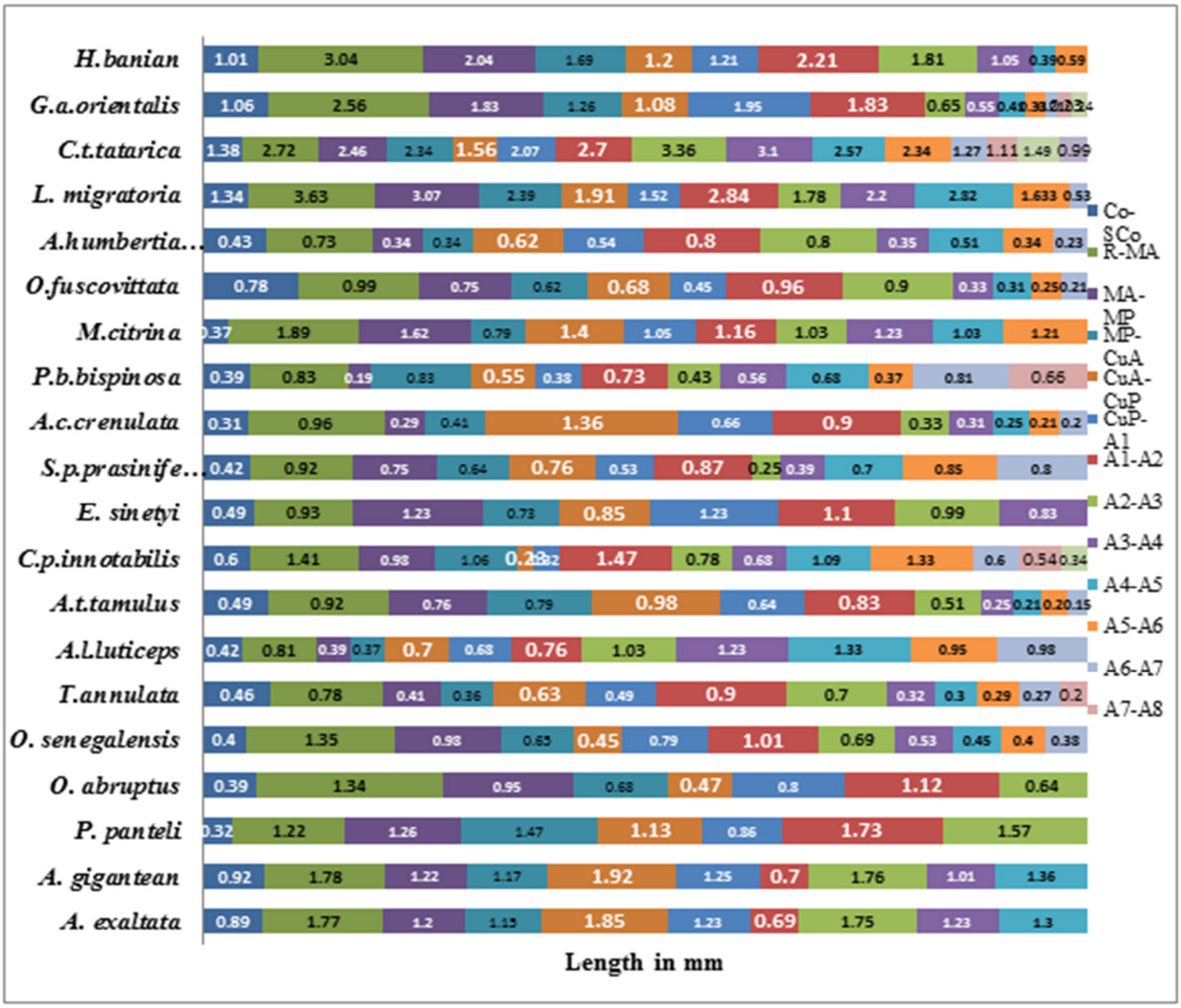

Fig 6: Divergence angle between each vein of hind wing of 20 species of grasshoppers

In forewings the degree of divergence (Figure.7) is recorded least divergence between Co/Sc 0.58° was in *P.panteli*, highest 4.45° recorded in *H.banian*. Least divergence between Sc/ScP is 0.0 in *A.c.crenulata* and highest 2.86°were in *H.banian*. The divergence between ScP/R was 0.1° in *A.humbertianus* to highest 0.75° in *L.migratoria*. Least divergence between R/M was 0.18°in *S.p.prasiniferum* to 0.86° in *L.migratoria*.. The lowest to highest between M/CuA was 0.53° in *O.senegalensis* and 2.86mm in *L.migratoria*. Least divergence between CuA/CuP was 0.0 in *A.c.crenulata*to highest 1.54° in *G.a.orientalis*. Thus the degree of divergence also varies from species.Main Radial veins that emerged from the base were typically two in number but the length of these two differed from species to species ranging between 2 to 8 branches in hind wings and all the branches are numbered likewise from1-n including the main radials, the number of radial branches was more in *L.migratoria* ,*C.t.tatarica,*and were least in *A.exaltata*, *A.gigantea*, *P.panteli*and *O.abruptus*. In the fore wings also radial veins RA and RP showed terminal branching ranging between 2-3 .

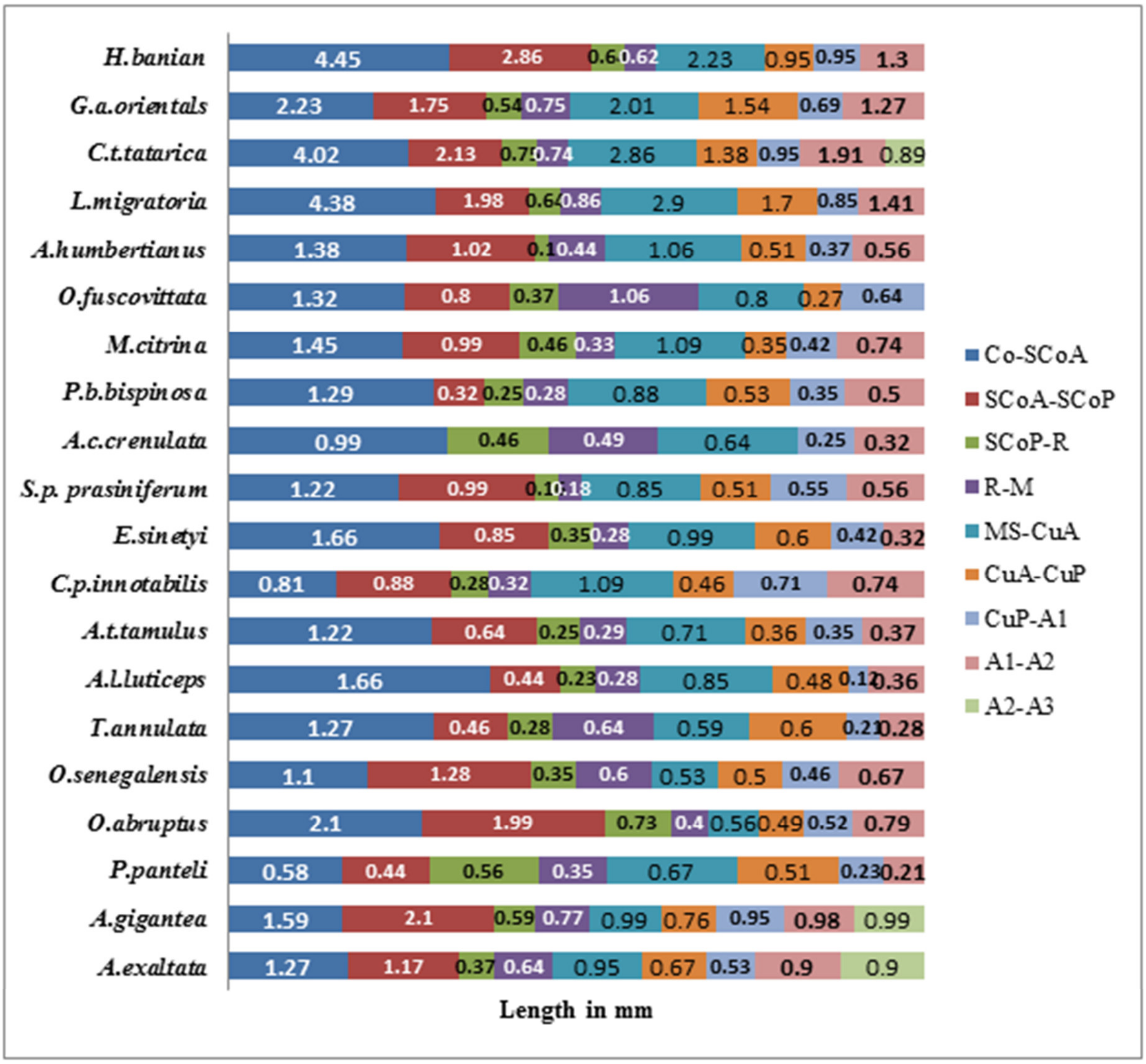

Fig 7: Divergence angle between each vein of fore wing of 20 species of grasshoppers

The anal veins of hind wings also had shown greater variation in number ranging between 4 to 10. Anal veins radiates from the base to the tip. Least number of anal veins was there *in P.panteli,O.abruptus*, and *E.sinetyi*. Highest number of 10 anal veins was recorded in *C.t.taratarica*. In all other species the range was between 5 to 9 veins. Number of five anal veins was more common.In case of forewings, least number of anal veins was present in *O.fuscovitatta*i.e two anal veins.(Figure.8)

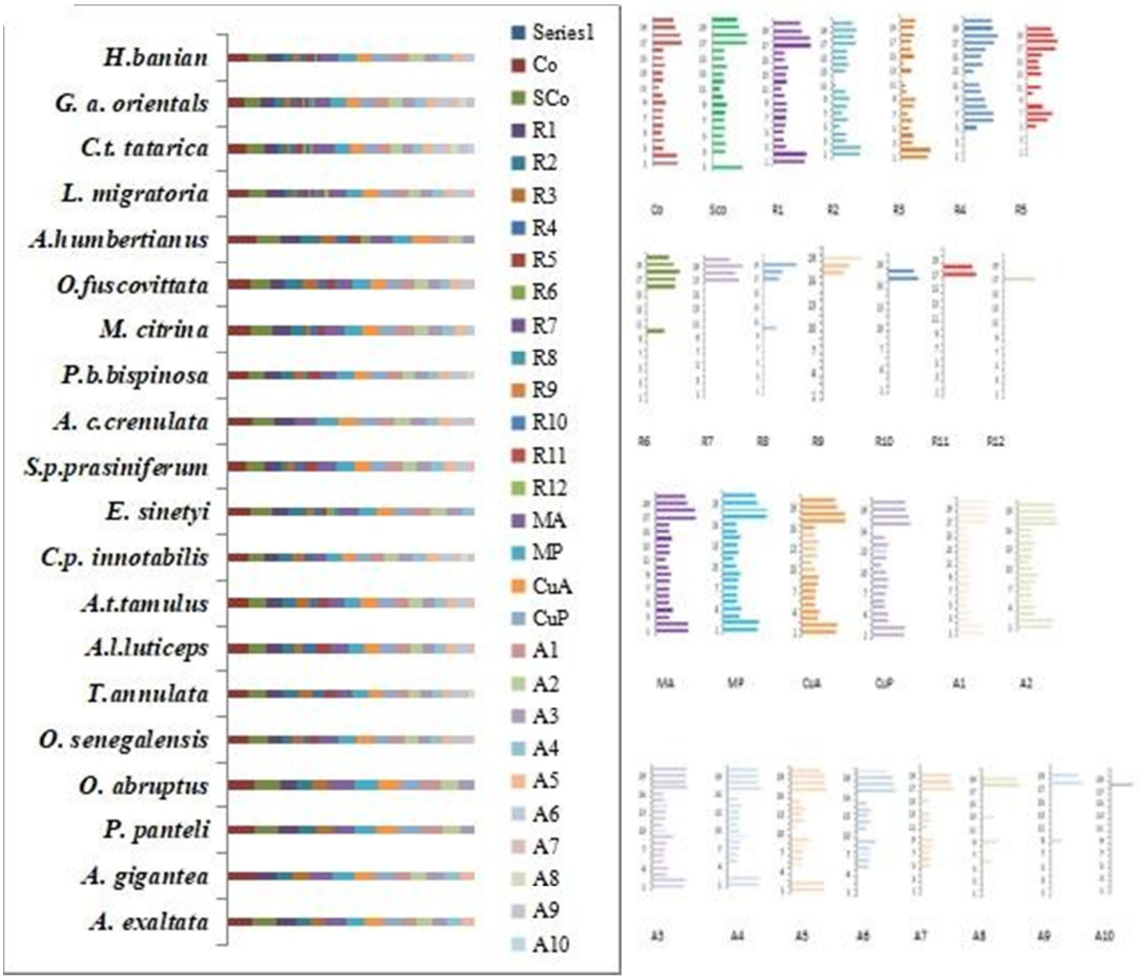

Fig 8: Length of each vein of hind wing of 20 species of grasshoppers (in mm)

The curvature of forewings and hindwings differed. In hind wing, the highest curvature of 121mm was in *L.migratoria* and least curvature of 29.15mm was *in E. sinetti*. In forewing, highest curvature of 24.5mm was in *L.migratoria* and least curvature of 4.25mm was in *P.b.bispinosa*. In *A.gigantea* and *A.exaltata* 76.8mm and 75.9mm is the curvature of hind wing and13.99mm, 12.56mm is the curvature of fore wing respectively.(Figure.9) Addition to these fore wings had pigmentations of different shades of brown colour as well white patches in 13 species. Such variegated patchy appearance of fore wings and plain green colour in *A.c.crenulata,O.fuscovittata* to plain brown colour in grasshoppers such as *C .p.innotabilis* and *T.annulata*with respective background of these insects. The hind wings were hyaline had pigmentation either in tignes or in the form of bands. In two species *A.exaltata* and *A.gigantea* a pale semi-transparent landmark was prominent in the hind wings with large squarish cells of cross veinules(Figure.3).The statistical analysis to establish the relatedness of species based on their variation in number of veins, wing structure reflected the formation of different sub clusters. The closest cluster was between *A.exaltata* and A.gigantea, the two congeneric species. The cluster depicted three way divergences of wings in twenty species of grasshopper (Figure. 10) of which thirteen formed a congregation of many sub clusters. The graph represents details of species clustered together at terminals that have ramified from different branches which in turn joined to a common base.

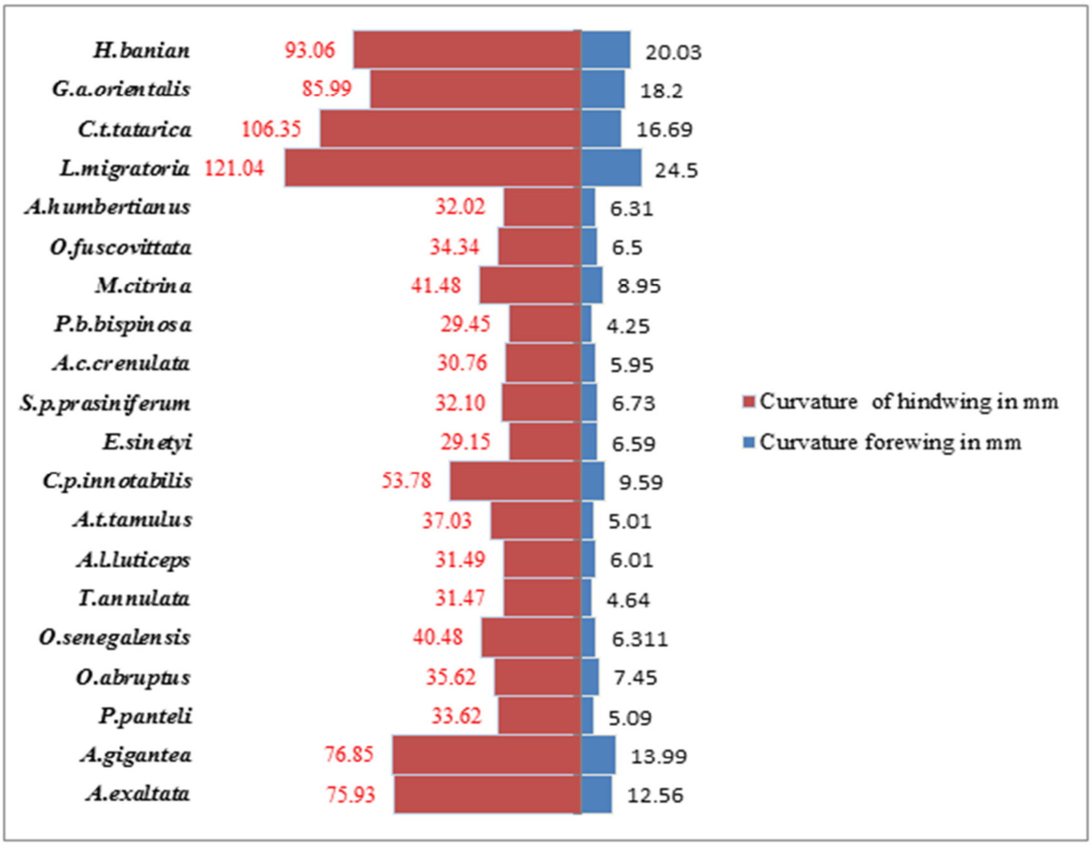

Fig 9: Curvature of hind wing and fore wing of 20 species of grasshoppers

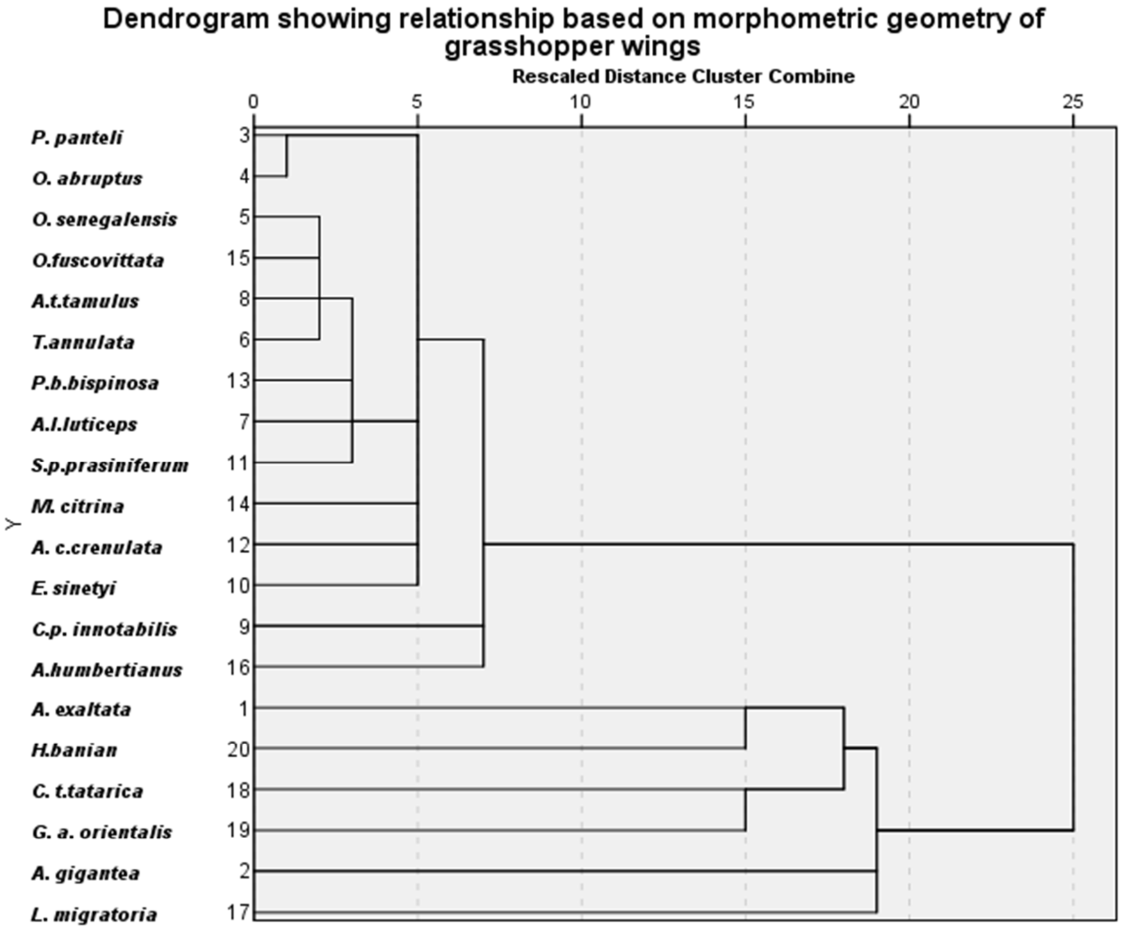

Fig 10: Dendrogram showing divergence and clustering of twenty species

5. DISCUSSION

In twenty species of grasshoppers examined, similar trends of evolution of wing lobes can be traced. Existence of dissimilarities in the anal lobe of hind wings and fore wings of all the species could be easily visualized. The fore wings had the vein complements originating from respective basal sclerites and extended further to the end of the wing margin. Fore wings are leathery mainly meant for protecting the hind wings and assist in flight control [24,25]Whereas the hind wings help in aerodynamic lift and thrust during stroke of wings [26].The fore wing veins of cubital lobe have very narrow gap between each compared to hind wing, thus the cross vein links appear congregated. The narrow and compactness of wing cells might have evolved to impart stiffness to wings. The veins of the fore wing are large at the base and taper towards the end such an arrangement provides resistance[27]. Contrast to the cubital lobe, the anal lobe of fore wing has only two veins running up to the up to the anal lobe edge which is very small. In a few cases one of the anal vein was shorter ended half way in the anal lobe. Such a reduction in the size of the anal lobe has a significance in maintaining required size to fold over the fan like hind wing but for this evolutionary curtail and fixed size of fore wing anal fold, the wings have either could have to be crossed over each other or could have had to be horizontally spread or folded vertically as in butter flies. Same type of evolution of wings could be found in other insects that shared common ancestry with Acrididae [28]. The present day wing type of Acrididae evolved as a monophyletic lineage pleiconeoptera derived from Neopteranancestor. The pleiconeopterans have wings that could be folded fan like with large number of anal veins. In Acridid grasshoppers the hind wings are polyneurous and main veins are slightly heavier than the cross veins. The main veins are spaced apart from each other with distinct gaps and run parallel. Interlink between these veins through cross veins resulted in square or rectangular cells generally placed perpendicular to the main axis.In locusts the arrangement of longitudinal veins and cross links cell formation has been studied, such an arrangement of veins has inferred to provide resistance to torsion[29]and the wing membrane acts as stressed skin with its cells enabling structural stiffness with flexibility during wing movement .Among the extant orthopteran insect lineage the short horned grasshoppers have originated about 65 million years ago and the members of pyrgomorphidae are the latest to be formed from Acridomorpha[30].The type of venation in wings has the same basic plan in cubital lobe in both Acrididae and Pyrgomorphidae but the number of anal veins are found to be more in members of pyrgomorphidae examined indicating evolutionary trend to increase in the number of anal veins. Among the twenty species of grasshoppers examined, number of anal veins ranged between 3 to 10, thus anal vein with three can be assumed to be the basic plan and increase in their number to be a derived type. whereas the anal lobe of fore wing 18 species had a consistent number with two veins running parallel only in species *A.exaltata* and *A.gigantea* three veins recorded in the anal lobe of fore wing. Addition to flight support the fore wings of grasshoppers have evolved to perform two protective functions, one - the cover folding over the body and the other imparting camouflage with the wing colour to match the background. The markings on the fore wings vary from species to species also between the individuals of the same species indicate adaptive flexibility in the fore wings of grasshoppers. The hind wings are generally hyaline having tinges of one colour except in band winged grasshoppers with bright colour bands. Increase in number radial vein branches in the hind wings has not only helped increase in space of hind wing but also increased the number of wing cells in that region and contributed to the enhancement of curvature of the cubital lobe. This trend of curvature increase recorded in the present study indicates revolutionary processes operating on wings of these grasshoppers since the origin of these insects. The anal lobe seemed to be more prone to increase in size by increasing the number of diverging parallel running veins from base to margin of the wing. This has also contributed to increase in space of the hind wing but anal fold region of fore wings did not bear any trend towards increase of size as it was seen in hind wings. The anal lobe was consistent with two anal veins though two species had three anal veins the last vein was too short extended less than half length of the lobe and had no effect on change on space of anal fold region of the fore wing.The wing size vary with the sex in grasshopper as sex size dimorphism is prominent in these insects and wing size found to be correlated with their body size[31]but shape ,number of veins of cubital and anal lobes remain the same in both the sexes. Hind wings assist in speed flights through their full expanse as examined in lepidopterans, such an increased speed help to escape from enemies.[32]Large size of the wing could be attained by extension of veins till the tip of the wing and by deviating away by each other with a degree of divergence both at base of their origin and terminal points. Such a kind of tapering veins and expanded angle between veins at the terminus make it possible to increase in curvature of the wings to attain large size of hind wings but in fore wings of grasshoppers though there exist a degree of divergence of veins at the base of their origin terminal gaps do not widen resulting in a narrow curvature. In all the twenty species of grasshopper's same trend of venation supported by nerves towards increasing the curvature of hind wing could be seen. Fast flying grasshoppers such as *G.a.orientalis, H.banian. L.migratoria,C.t.tatarica ,C.p.innotabilis* have broad hind wings with greater curvature support our argument that lengthy veins in hind wing enabling greater curvature to increase the flight speed of grasshoppers. The wing size often has been considered to be proxy of the body size and the body size variations are influenced by Environmental gradients, temperature at developmental stages and solar radiations as observed in T.annulata[33]and by different geographical conditions in*N.a.acuticeps*[34]but in *D.melanogaster* the wing shape is affected by a heat shock protein Hs P 90[16].To avoid any such contradictions all the species of insects under analysis collected from the same geographical region.Characteristics recorded by morphometric geometry were statistically analysed to identify the similarity and differences of close or distant relationship among 20 species of grasshoppers. The cluster analysis showed three way clustering. The first cluster included *A.exaltata*, *A.gigantea* and *O.abruptus*. The second cluster included 13 species. The third cluster included four species. The clustering has reflected commonness of variations among the wing characters of 20 species of grasshoppers. The graph obtained also revealed more venation in *P.panteli* with least number of anal veins. The similarities in degree of divergence of number of anal veins were almost similar in 13 species but were different in other four species clustered together, that had five anal veins. The degree of divergence was also different with the insects grouped in all the three groups with a relative similarity. Hence the evolutionary divergence of wings in grasshopper could be predicted that has taken place in three different directions.In this study we have provided an additional taxonomic character in the form of wing morphometry of locusts that have drastic impact on economy and human health which we feel could be used as standard measure to inidentify or differentiate some of the pests like plague locust. Wing morphometric geometry though seems to be simple technique it has large scale utility potential in vector biology to identify and control the vectors[4]to safeguard the human health.This technique is also used in discriminating blow flies of forensic importance[35],that are potential source of pathogens having direct impact on human health ,the landmark based morphometric geometry[36] is also used in neuroscience to measure variations in brain folds.

6. CONCLUSION

As far the wings of grasshoppers a different evolutionary trend seems to be operative since their divergence from Neopteran ancestors .Our observation analysis has confirmed the trend is towards the increase in the morphological width of hind wing by increasing the sub braches of radial veins and increase in the number of anal lobe veins that has been followed through lineages of short horned grasshoppers. Simultaneously the trend is stringent or parsimonious in anal lobe of fore wings, never allowed an increase in veins in this lobe of fore wings. For this type of analysis, the morphometric geometry (technique) study on wings seems to be a good method to analyse evolutionary trends in orthopteroid insects.

7. ACKNOWLEDGEMENT

Authors are grateful to the chairperson department of studies in zoology, Manasagangotri, University of Mysore, Mysuru. For providing facilities to complete this work successfully.

8. AUTHORS CONTRIBUTION STATEMENT

All authors have made contributions towards this work. MeghaUrs contributed towards compiling the data for the work she has designed, Alwyn D'souza , assisted in complete statistical analysis and data organization, Dr.Shakuntala. And Dr.Channaveerappa has contributed in critical analysis of the data and discussion in line with the work designed.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

10. REFERENCES

1. Laffont Rémi, FirmatC, Alibert P, David B, Montuire S, SaucèdeT. Biodiversity and evolution in the light of morphometrics: frompatterns to processes. C R Palevol.2011;10(2-3):133-42. doi:10.1016/j.crpv.2010.10.004.
2. Tatsuta H, Takahashi KH, Sakamaki Y. Geometric morphometrics in entomology: basics and applications. Entomological Science.2018;21(2):164-84. doi: 10.1111/ens.12293.
3. Wilke ABB, ChristeRdO, Multini LC, Vidal PO, Wilk-da-Silva R, de Carvalho GC, et al. Morphometric Wing Characters as a Tool for Mosquito Identification. PLoS ONE .2016 .11(8): e0161643. doi:10.1371/journal.pone.0161643
4. Patterson J.S. and C.J. Schofield . Preliminary study of wing morphometry in relation to tsetse population genetics. South African Journal of Science 101, March/April 2005, 132-134. https://www.researchgate.net/publication/279607228
5. SalcedoMaryK, HoffmannJordan, DonougheSeth, MahadevanLakshminarayanan. Size, shape and structure of insectwings.bioRxiv.2018. doi: 10.1101/478768, PMID 478768.
6. GrimaldiDavid, EngelMichaelS, EngelMichaelS. Evolution of the insects. Cambridge University Press; 2005.
7. JohanssonFrank, SöderquistM, BokmaF. Insect wing shape evolution: independent effects of migratory and mate guarding flight on dragonfly wings. BiolJLinnSoc.2009;97(2):362-72. doi: 10.1111/j.1095-8312.2009.01211.x.
8. ParchemRonaldJ, PerryMichaelW, PatelNipamH. Patterns on the insectwing. CurrOpin Genet Dev. *2007*;17(4):300-8. doi: 10.1016/j.gde.2007.05.006, PMID 17627807.
9. DeVriesPJ, PenzCarlaM, HillRyanI. Vertical distribution, flightbehaviour and evolution of wingmorphology in morphobutterflies.J AnimEcol.2010;79(5):1077-85. doi: 10.1111/j.1365-2656.2010.01710.x, PMID 20487088.
10. JongeriusSR, Lentink D. Structural analysis of a dragonflywing. Exp Mech. 2010;50(9):1323-34.doi: 10.1007/s11340-010-9411-x.
11. BrodskyAndreiKThe Evolution of Insect Flight.Oxford University Press; 1994.
12. WoottonRJ. Geometry and mechanics of insect hind wing fans: amodelling approach. Proc R SocLondB. 1995;262(1364):181-7. doi: 10.1098/rspb.1995.0194.
13. AdamsDC, RohlfFJ. Ecological character displacement in Plethodon: biomechanical differences found from a geometric morphometric study. ProcNatlAcadSci U S A. 2000;97(8):4106-11.doi: 10.1073/pnas.97.8.4106, PMID 10760280.
14. BenítezHugo Alejandro, VidalMarcela, BrionesRaul, JerezViviane. Sexual dimorphism and morphologicalvariation in populations of *Ceroglossuschilensis* (Eschscholtz, 1829)(Coleoptera, Carabidae).J Entomol Res Soc. 2010;12(2):87-95.
15. PélabonChristophe, FirmatCyril, BolstadGeir H, VojeKjetil L, HouleDavid, CassaraJason, Rouzic Arnaud Le, HansenThomas F. Evolution of morphological allometry. Ann N Y Acad Sci.

2014;1320:58-75. doi: 10.1111/nyas.12470, PMID 24913643.

16. DebatVincent, DebelleAllan, DworkinIan. Plasticity, canalization, and developmentalstability of the DrosophilaWing: jointeffects of mutations and developmental temperature. Evolution.2009;63(11):2864-76. doi:10.1111/j.1558-5646.2009.00774.x, PMID 19624729.
17. MacLeodNorman. Automated taxonidentification in systematics: theory, approaches and applications. CRC Press; 2007.
18. ComstockJH, NeedhamJG.The wings of insects. Am Nat.1898;32(373):43-8. doi: 10.1086/276766.
19. Kukalová-PeckJarmila, LawrenceJohnF. Relationships among coleopteransuborders and majorEndoneopteranlineages: evidence from hindwingcharacters. Eur J Entomol. 2004;101(1):95-144.doi: 10.14411/eje.2004.018.
20. LorenzCamila, SuesdekLincoln. Evaluation of chemicalpreparation on insectwingshape for geometricmorphometrics.Am J Trop Med Hyg.2013;89(5):928-31. doi: 10.4269/ajtmh.13-0359, PMID 24019438.
21. OutomuroDavid, AdamsDeanC, JohanssonF. The evolution of wingshape in ornamented-winged damselflies (Calopterygidae, Odonata).Evol Biol.2013;40(2):300-9. doi: 10.1007/s11692-012-9214-3.
22. BellezaBG. Cesar G D, Describing the Wing Shape of three local populations ofBrontispaLongissima Using Elliptic FourierAnalysis. AnnBiol Res. 2014;5(6):47-56.https://www.adobe.com/in/products/photoshop.html/photoshop elements
24. WoottonRJ, EvansKE,Herbert RC, Smith CW. The hind wing of the desert locust (Schistocerca gregaria Forskål). I. Functional morphology and mode of operation. JExpBiol.2000;203(19):2921-31. PMID10976029.
25. WalkerSimonM, ThomasAdrianLR, TaylorGraham K. Deformable wingkinematics in the desert locust: how and whydocamber, twist and topography vary through the stroke?J R Soc Interface.2009;6(38):735-47. doi: 10.1098/rsif.2008.0435, PMID 19091683.
26. WoottonRobinJ. Functional morphology of insectwings.Annu Rev Entomol.1992;37(1):113-40.doi: 10.1146/annurev.en.37.010192.000553.
27. DirksJan-Henning, TaylorDavid. Fracture toughness of locust cuticle. J Exp Biol. 2012;215(9):1502-8. doi: 10.1242/jeb.068221, PMID 22496286.
28. HamiltonKGAndrew. The insectwing, Part IV.Vocationaltrends and the phylogeny of the wingedorders. JKans Entomol Soc. 1972:295-308.
29. HerbertRC, Young PG, Smith CW, Wootton RJ, Evans KE. The hindwing of the desertlocust (SchistocercaGregariaForskal). III. A finite elementanalysis of a deployable structure. J ExpBiol.2000;203(19):2945-55. PMID 10976031.

30 Song.H, AmédégnatoChristiane, CiglianoMaria Marta, Desutter-GrandcolasLaure, HeadsSamW, HuangYuan, OtteD, WhitingMF. 300 million years of diversification: elucidating the patterns of orthopteran evolution based on comprehensive taxon and gene sampling. Cladistics.2015;31(6):621-51. doi: 10.1111/cla.12116.

31. DharbalMeghaS, DineshU, JayashreeH, SachidanandaM, ChannaveerappaH. Body size variation analysis: sexual dimorphism in grasshoppers fits in to Rensch’s rule. ProceedingsVIBHAVAT National conference.Eds .Vijaya Reddy and Jyothi R. Reddy janasangha first grade college.Bengaluru(2015);.68-71.
32. JantzenBenjamin, EisnerThomas. Hindwings are unnecessary for flight but essential for execution of normal evasive flight in Lepidoptera. Proc Natl AcadSci U S A. 2008;105(43):16636-40. doi: 10.1073/pnas.0807223105. PMID 18936482.
33. Bai Y, DongJia-Jia, GuanDe-Long, XieJuan-Ying, XuSheng-QuanGeographic variation in wing size and shape of the grasshopper Trilophidiaannulata (Orthoptera: Oedipodidae): morphological trait variations follow an ecogeographical rule. Sci Rep. 2016;6:32680. doi: 10.1038/srep32680. PMID 27597437

34 DharbalMeghaS, UdapiDinesh, JayashreeH, Sachidananda MurthyKL, ChannaveerappaH. Observation of body size in different geographical population of grasshopper *Neorthocris acuticeps acuticeps* (bolivar). I JPAZ.2015;3(4):354-7.

35 NarinSontigun, , Kabkaew L. Sukontason, Barbara K. Zajac, Richard Zehner, KomSukontason ,Anchalee Wannasanand Jens Amendt . Wing morphometrics as a tool in species identification of forensically important blow flies of Thailand. Sontigun et al. Parasites & Vectors .2017 10:229 .DOI 10.1186/s13071-017-2163-z

36. Chollet MB, Aldridge K, Pangborn N, Weinberg SM, DeLeonVB .Landmarking the Brain for Geometric Morphometric Analysis: An Error Study. PLoS ONE2014.9(1):e86005. doi:10.1371/journal.pone.0086005