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Phylogenetic relationship of acrididae and pyrgomorphidae: Paraphyletic relationship between species of sub families of acrididae differ with each criteria

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Abstract

Super family Acridoidea includes eleven families, of these family Acrididae is the largest and the super family Pyrgomorphidea has been considered to be a derivative of Acrididae. In Indian sub-continent only representatives of the family Acrididae and Pyrgomorphidae are found. The phylogenetic relationship between the insects of these two families has been analysed in this work based on RAPD and proteins polymorphism. Species of grasshoppers involved in this work showed greater diversity for both these criteria. The phylogenetic trees constructed based on the diversity of these profiles reflected variations in cladogenic branch patterns for proteins, DNA, as well in the tree generated by combining these two factors. The protein data-based tree resulted in more shuffled combinations of Acrididae and Pyrgomorphidae species also in between the taxa of the subfamilies of Acrididae. The DNA profile tree resulted in almost separate congregation of the species of Acrididae and Pyrgomorphid species but with slight mixing of both the families. The combined protein and DNA phylogenetic tree profile resulted in separate clustered appearance of Pyrgomorphidae and Acrididae but There was no monophyletic representation among the members of the sub families Acridinae, Oedipodinae and Gomphocerinae of Acrididae hence the members of sub families reflected paraphyly that differed with each criterion. The Pyrgomorphidae species are found to be separated from the common Acridid ancestors of 4th and 5th order ancestral lineages, these two families appeared to be sister groups.

Keywords: RAPD, protein bands polymorphism, divergence, ramification, terminal taxa, sister group, paraphyletic

Introduction

In the super family Acridoidea that includes eleven families, only members of the family Acrididae and the superfamily Pyrgomorphoidea having only family Pyrgomorphidae, represented by a few species distributed in this geographical region of Indian sub-continent.Whereas in other parts of the world,different families of grass hoppers are found along with these two. About 350 species of grasshoppers are found in India; regard to unique state of their existence; analysis of phylogenetic relationships between the species can throw a light on the broad evolutionary process in the Indian grasshoppers. Though there are many works on the phylogeny of grasshoppers, contradictions have prevailed regard to relationship between the members of the sub families of Acrididae [1-5] and such phylogenetic analysis on grasshoppers of Indian subcontinent is limited.

Knowledge of phylogeny of Pyrgomorphidae is little, compared to the other Orthopteran insects. In this work species of Pyrgomorphidae and Acrididae were put together for analysis of phylogenetic relationship between the members of these two families. Our earlier work on six species of Pyrgomorphidae [6, 7] revealed only inter relationship among the species of the same family but the present work deals with the derivation of Pyrgomorphids and their phylogenetic relation with other species of Acrididae, as well in between species relationships of the sub familiesof Acrididae.

Material and Methods.

Twenty five species of grasshoppers of two families: 1. Acrididae-, Sub family –Acridinae – *Acrida exaltata* (Walker,1859), *Acrida gigantea* (Herbst, 1794), *Phlaeoba panteli* (Bolivar,1902), Sub family –Oedipodinae- *Dittopternis venusta* (Walker, 1870), *Heteropternis respondens*(Walker,1859), *Morphacris citrina* (Kirby, 1910), *Oedaleous abruptus* (Thunberg, 1815), *Oedaleus senegalensis* (Krauss,1877), *Trilopidia annulata* (Thunberg,1815), *Acrotylus humbertianus* (Saussure, 1884),*Aiolopus thalasinus tamulus* (Fabricius,1870), Sub family –Calliptaminae- *Aucorypha glaucopsis* (Walker,1870), Sub family – Catantopinae- *Catantops pinguis innotabilis* (Walker,1870), Sub family –Eyprepocnomidinae – *Eyprepocnomis alacris alacris* (Survillae,1838),Sub family –Hemiacridinae- *Spathosternum presiniferum presiniferum* (Walker,1871), Sub family –Gomphocerinae –*Leva cruciate* (Bolivar, 1914),*Gelastorhinus semipictus* (Walker,1870) and 2.Family-

Pyrgomorphidae- *Atractomorpha crenulata crenulata* (Fabricius,1793), *Chrotogonus oxypterus* (Blanchard,1836), *Chrotogonus trachypterus* (Blanchard,1836), *Neorthocris acuticeps acuticeps* (Bolivar,1902), *Poikilocera picta* (Fabricius,1775), *Pyrgomorpha bispinosa bispinosa* (Walker,1870).are used for this study, these species were given accession numbers A1-A12, B13-B19 for nineteen species of Acrididae and P20-P25 for six species of Pyrgomorphidae.

SDS PAGE analysis of proteins was done following the established protocols for proteins electrophoresis and for RAPD-PCR (Random Amplified polymorphic DNA-Polymerase Chain Reaction) is followed in the same line of our earlier work [8].

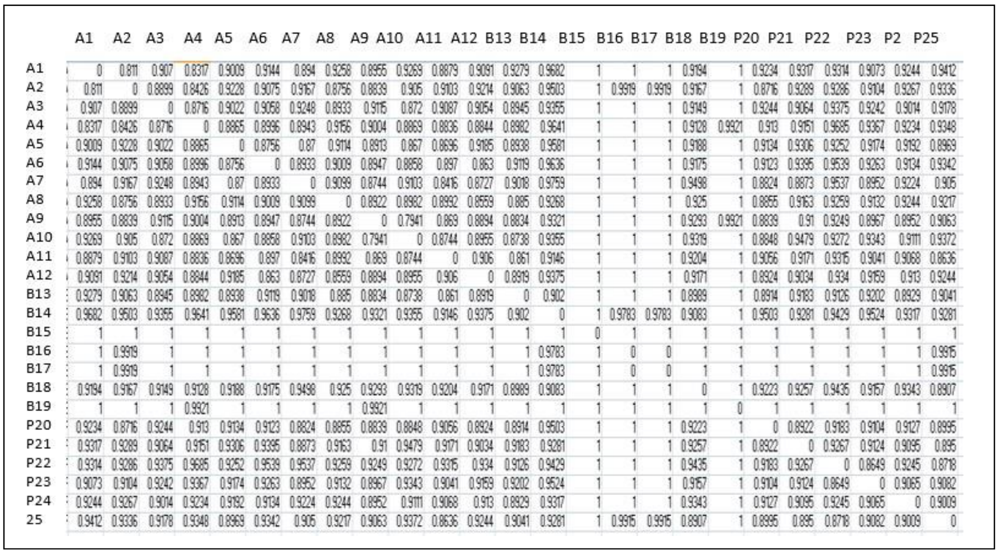
The computation of distance matrix and tree construction

Were carried out by using software GEL QUEST version 1.0. The gel images uploaded to the input format, FSA files form automatic sequence and gel images. The output format 01 matrix for the analysis of PAUP future version carried out the complete cluster analysis and visualization of the dendrogram. The distance matrix was generated by the software applying Jaccard's method and Neighbour joining phylogenetic trees generated based on these results.. Each node of the dendrogram is represented using Alphabets(A,B,C--) The subsequent lineage ramified from its ancestral node in to more than one branch is designated with similar alphabet with different characters (A1, A2, B1B2.).

Results

The protein profile by SDS PAGE revelation of twenty-five species generated a total of 286 bands including fifty repeats. Of the 286 bands, 56 bands with a range of molecular weights between 116.5 KDa to 11.0KDa recorded in six members of Pyrgomorphidae and remaining 230 bands manifested in nineteen members of Acrididae. (Fig1). A common band of proteins for all twenty-five species was not found. The RAPD -PCR genomic DNA profile generated by ten DNA primers listed in fig (2) had 1086 bands in total for the twenty five species (fig.2). Family wise analysis recorded a minimum of 29 bands generated by OPAA-09 and a maximum of 156 bands were generated by OPC-06 for 19 species of Acrididae, followed by 114 bands (OPC-07), remaining primers generated variable number of bands between 64 to 99 with a total of 866 bands. For the six species of Pyrgomorphidae the same ten primers generated 212 bands in total, in these the primer 970-11 generated least number of 07 bands and OPC-06 generated 41 bands. For all the species a common RAPD band was not generated from the ten primers used. The distance and similarity matrix for protein profiles of all the twenty five species ranged between 0.75 to 1.0,the distance and similarity matrix generated for the RAPD profile of twenty five species of grasshoppers ranged between 0.77901 to 0.93855.The combined computation of proteins and DNA ranged between 0.811 to 1.0.(Table.1). Separate distance matrices for each profile was auto selected for construction of phylogenetic trees for proteins, RAPD genomic DNA and combination of both. The resulted tree profile is presented in the next paragraphs. A hypothetical time line is given in tree profile to measure approximate time of each lineage starting from basal node to terminal node, based on the estimated time scale provided by other workers. All the dendrograms are generated without using a out group because a suitable out group was not found for relative Primer RAPD profiles or proteins.

Table 1: Similarity and distance matrix for combined protein and DNA profiles of twenty-five species of grasshoppers



	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	B13	B14	B15	B16	B17	B18	B19	P20	P21	P22	P23	P2	P25
A1	0	0.811	0.907	0.8317	0.9009	0.9144	0.894	0.9258	0.8955	0.9269	0.8879	0.9091	0.9279	0.9682	1	1	1	0.9194	1	0.9234	0.9317	0.9314	0.9073	0.9244	0.9412
A2	0.811	0	0.8899	0.8426	0.9228	0.9075	0.9167	0.8756	0.8839	0.905	0.9103	0.9214	0.9063	0.9503	1	0.9919	0.9919	0.9167	1	0.8716	0.9289	0.9286	0.9104	0.9267	0.9336
A3	0.907	0.8899	0	0.8716	0.9022	0.9058	0.9248	0.8933	0.9115	0.872	0.9087	0.9054	0.8945	0.9355	1	1	1	0.9149	1	0.9244	0.9064	0.9375	0.9242	0.9014	0.9178
A4	0.8317	0.8426	0.8716	0	0.8865	0.8996	0.8943	0.9156	0.9004	0.8869	0.8836	0.8844	0.8982	0.9641	1	1	1	0.9128	0.9921	0.913	0.9151	0.9685	0.9367	0.9234	0.9348
A5	0.9009	0.9228	0.9022	0.8865	0	0.8756	0.87	0.9114	0.8913	0.867	0.8696	0.9185	0.8938	0.9581	1	1	1	0.9188	1	0.9134	0.9306	0.9252	0.9174	0.9192	0.8969
A6	0.9144	0.9075	0.9058	0.8996	0.8756	0	0.8933	0.9009	0.8947	0.8858	0.897	0.863	0.9119	0.9636	1	1	1	0.9175	1	0.9123	0.9395	0.9539	0.9263	0.9134	0.9342
A7	0.894	0.9167	0.9248	0.8943	0.87	0.8933	0	0.9099	0.8744	0.9103	0.8416	0.8727	0.9018	0.9759	1	1	1	0.9498	1	0.8824	0.8873	0.9537	0.8952	0.9224	0.905
A8	0.9258	0.8756	0.8933	0.9156	0.9114	0.9009	0.9099	0	0.8922	0.8982	0.8992	0.8559	0.885	0.9268	1	1	1	0.925	1	0.8855	0.9163	0.9259	0.9132	0.9244	0.9217
A9	0.8955	0.8839	0.9115	0.9004	0.8913	0.8947	0.8744	0.8922	0	0.7941	0.869	0.8894	0.8834	0.9321	1	1	1	0.9293	0.9921	0.8839	0.91	0.9249	0.8967	0.8952	0.9063
A10	0.9269	0.905	0.872	0.8869	0.867	0.8858	0.9103	0.8982	0.7941	0	0.8744	0.8955	0.8738	0.9355	1	1	1	0.9319	1	0.8848	0.9479	0.9272	0.9343	0.9111	0.9372
A11	0.8879	0.9103	0.9087	0.8836	0.8696	0.897	0.8416	0.8992	0.869	0.8744	0	0.906	0.861	0.9146	1	1	1	0.9204	1	0.9056	0.9171	0.9315	0.9041	0.9068	0.8636
A12	0.9091	0.9214	0.9054	0.8844	0.9185	0.863	0.8727	0.8559	0.8894	0.8955	0.906	0	0.8919	0.9375	1	1	1	0.9171	1	0.8924	0.9034	0.934	0.9159	0.913	0.9244
B13	0.9279	0.9063	0.8945	0.8982	0.8938	0.9119	0.9018	0.885	0.8834	0.8738	0.861	0.8919	0	0.902	1	1	1	0.8989	1	0.8914	0.9183	0.9126	0.9202	0.8929	0.9041
B14	0.9682	0.9503	0.9355	0.9641	0.9581	0.9636	0.9759	0.9268	0.9321	0.9355	0.9146	0.9375	0.902	0	1	0.9783	0.9783	0.9083	1	0.9503	0.9281	0.9429	0.9524	0.9317	0.9281
B15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
B16	1	0.9919	1	1	1	1	1	1	1	1	1	1	1	0.9783	1	0	0	1	1	1	1	1	1	1	0.9915
B17	1	0.9919	1	1	1	1	1	1	1	1	1	1	1	0.9783	1	0	0	1	1	1	1	1	1	1	0.9915
B18	0.9194	0.9167	0.9149	0.9128	0.9188	0.9175	0.9498	0.925	0.9293	0.9319	0.9204	0.9171	0.8989	0.9083	1	1	1	0	1	0.9223	0.9257	0.9435	0.9157	0.9343	0.8907
B19	1	1	1	0.9921	1	1	1	1	0.9921	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
P20	0.9234	0.8716	0.9244	0.913	0.9134	0.9123	0.8824	0.8855	0.8839	0.8848	0.9056	0.8924	0.8914	0.9503	1	1	1	0.9223	1	0	0.8922	0.9183	0.9104	0.9127	0.8995
P21	0.9317	0.9289	0.9064	0.9151	0.9306	0.9395	0.8873	0.9163	0.91	0.9479	0.9171	0.9034	0.9183	0.9281	1	1	1	0.9257	1	0.8922	0	0.9267	0.9124	0.9095	0.895
P22	0.9314	0.9286	0.9375	0.9685	0.9252	0.9539	0.9537	0.9259	0.9249	0.9272	0.9315	0.934	0.9126	0.9429	1	1	1	0.9435	1	0.9183	0.9267	0	0.8649	0.9245	0.8718
P23	0.9073	0.9104	0.9242	0.9367	0.9174	0.9263	0.8952	0.9132	0.8967	0.9343	0.9041	0.9159	0.9202	0.9524	1	1	1	0.9157	1	0.9104	0.9124	0.8649	0	0.9065	0.9082
P24	0.9244	0.9267	0.9014	0.9234	0.9192	0.9134	0.9224	0.9244	0.8952	0.9111	0.9068	0.913	0.8929	0.9317	1	1	1	0.9343	1	0.9127	0.9095	0.9245	0.9065	0	0.9009
25	0.9412	0.9336	0.9178	0.9348	0.8969	0.9342	0.905	0.9217	0.9063	0.9372	0.8636	0.9244	0.9041	0.9281	1	0.9915	0.9915	0.8907	1	0.8995	0.895	0.8718	0.9082	0.9009	0

Table-1.A1-*A.exaltata, A2-A.gigantea,A3- A.humbertianus,A4- A.t.tamulus,A5- D.venusta, A6- G.semipictus A7-H.respondens A8-M.citrina, A9- O.abruptus,A10- O.senegalensis,A11- P.panteli,A12-T.annulata,A13- A.glaucopsis,B14- C.p. innotabilis,B15- E.sinetyi,B16- C.tatarica,B17- E.a. alacris,B18- S.prasiniferum B19-L.cruciata,P20- A.c. crenulata,P21-- C.oxypterus,P-22- C.trachypterus P23N.a. acuticeps, P24-P P.picta, P25- P.b.bispinos*

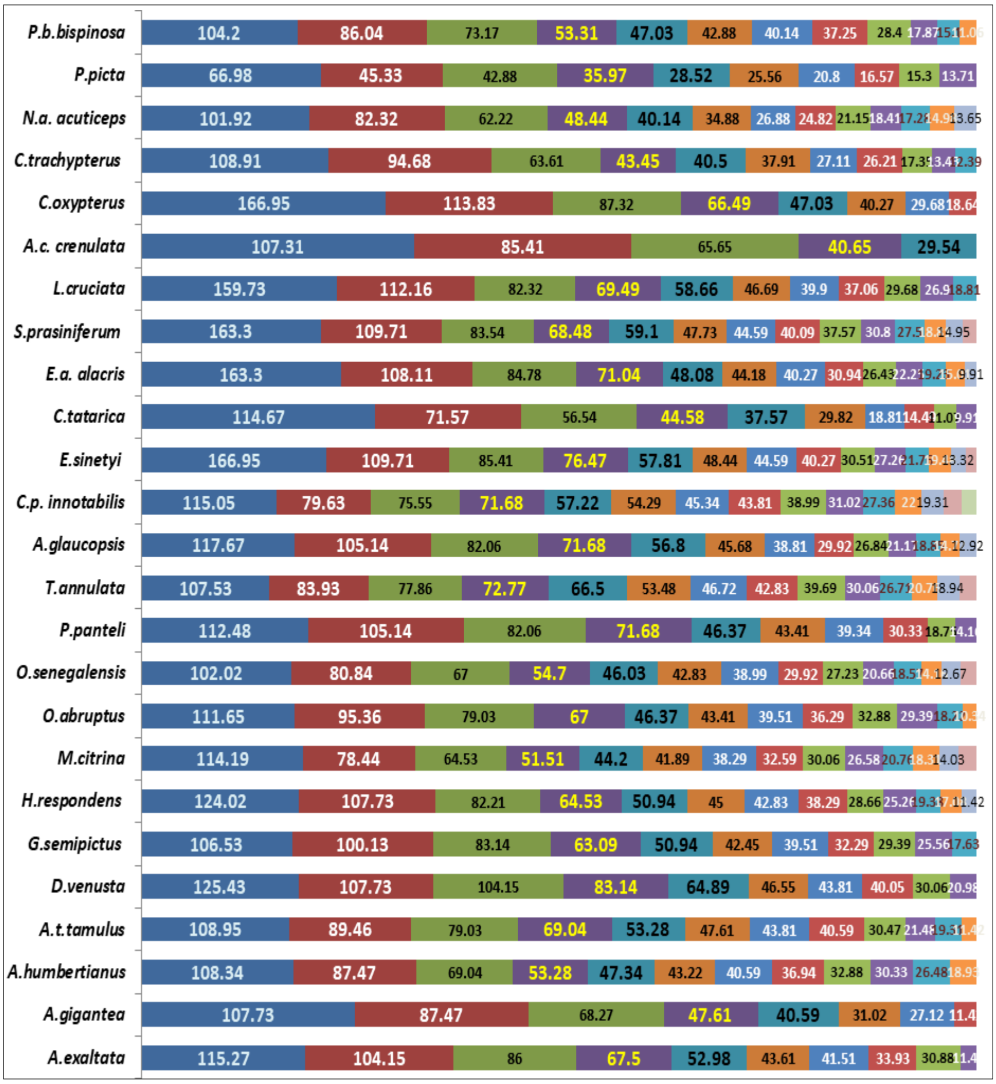


Fig 1: Molecular weight values for bands Electrophoresed in proteins- color indicate band, inserted numbers are molecular weight of protein.

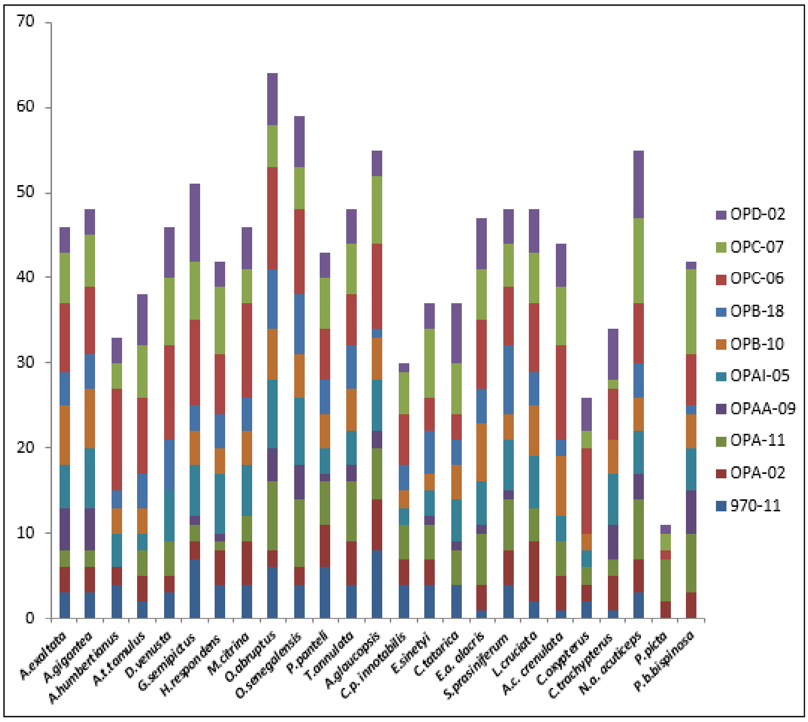


Fig 2: DNA polymorphism of 25 species of grasshoppers. Each of the colored bands represent different RAPD primers. Missing color indicates absence of bands. Length of color represent number of bands.

DNA data based phylogenetic tree.

The phylogenetic tree for twenty-five species of grasshoppers belonging to families Acrididae and Pyrgomorphidae showed the origin from a common root. Two species *A.exaltata* and *A.gigantea*, of the subfamily Acridinae ramified early from the root as long branches of different branch values. The third long branch that ramified from the innermost node ‘A’ shared by *A.humbertianus* and node ‘B’ second inner ancestral lineage extended to produce two subsequent ancestral lineages, ‘C$_1$’ and ‘C$_2$’. The node ‘C1’ ramified to derive two taxa, A.*t.tamulus* of Oedopodinae and *G.semipictus* of Gomphocerinae. The fourth order ancestral lineages ‘D$_1$’ and ‘D$_2$’ are derived from ‘C$_2$’ node both these lineages have extended to generate two ancestral lineages each ‘E$_1$’, ‘E$_2$’, ‘E$_3$’ and ‘E$_4$’. The node ‘E$_1$’ ramified to generate sixth ancestral lineages ‘F$_1$’ that derives two species *D.venusta* and *C.p.innotabilis* and from node ‘F$_2$’ other two species *C.tatarica* and *E.a.alacris* are resulted as its extant species. From the node ‘E$_2$’ two ancestor ‘F3’ and ‘F4’ are formed each of these too further generated ‘G$_1$’,’G$_2$ ’and’G$_3$’ lineages. Also, from the ‘F$_4$’ node species *C.oxypterus* a Pyrgomorphid is derived, the node ‘G$_1$’ branched to generate species *H.respondens* and a node ‘H$_1$’ as outer most node, ramified in to two terminal taxa *O.abruptus* and *O.senegalensis* of Oedipodinae and tribe locustini, thus these two species appeared as younger lineage with other two sets of species. From the ancestor ‘G$_2$’,‘H$_2$’ and ‘H$_3$’ extant ancestral lineages, of 8th order in the tree are derived, both these resulted in short branched terminal taxa, *M.citrina* with *E.sinetyi* and *P.panteli* with *S.p.prasiniferum* of different sub families. The node ‘G$_3$’ ramified to generate *T.annulata* and *A.glaucopsis*. The consequent derivatives of the node ‘D$_2$’ are unique being represented by five species of Pyrgomorphidae and one species of Acrididae, further ramified to yield to ancestral generations ‘E$_3$’ and E$_4$; and the node ‘E$_3$’ bifurcated to derive *L.cruciata* and *A.c.crenulata* where as ‘E$_4$’ advanced to derive species *C.trachypterus* and node ‘F$_4$’ finally resulted in a pair of outer most taxa *P.picta* and *P.b.bispinosa*; as younger derivatives in the lineage.

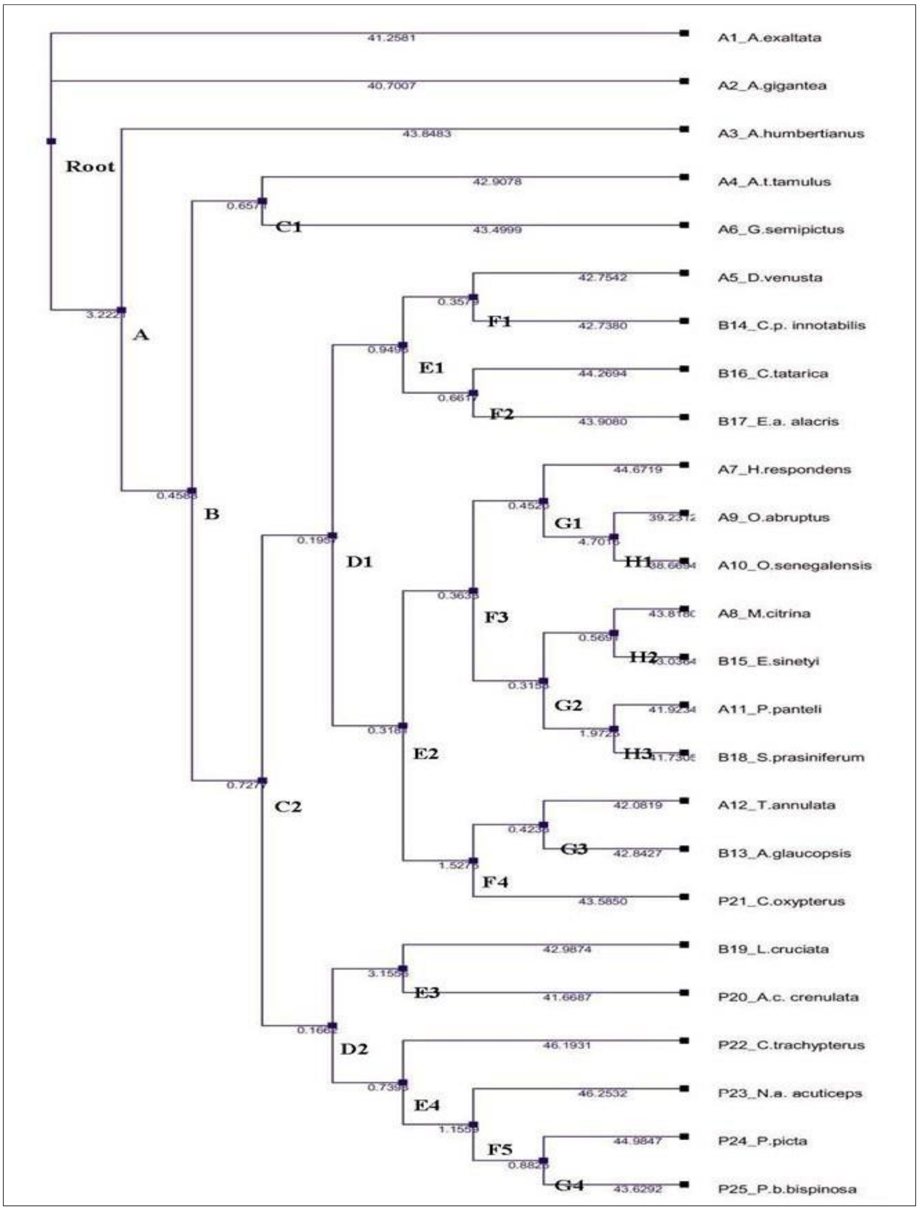


Fig 3: Phylogenetic tree based on RAPD profile.

Phylogenetic tree based on Protein data.

This tree is characterized by shuffling of species compared to DNA tree and imparted more paraphyletic appearance of many species. The root extends to generate node ‘A’, also simultaneously ramified two taxa *A.exaltata* and *O.senegalensis* derived from the root. The second ancestral lineage ‘B_1’ and ‘B_2’ were generated by the ancestor ‘A’. The cluster generated by these two nodes is interesting, the ‘B_1’ derivatives are all members of Acrididae but cluster ‘B_2’ derivatives include both members of Acrididae and Pyrgomorphidae. The lineages ‘C_1’, ‘C_2’ are derived from ‘B_1’ and the other node ‘C_3’ is derived from ‘B_2’. The node ‘C_1’ extends to generate two nodes ‘D_1’and ‘D_2’ both these ramify to derive two taxa each *A.gigantea* plus, *H.respondens* and *D.venusta*,*G.semipictus* of three different subfamilies. Two species *A.humbertianus* and *A.t.tamulus* are derived from the ancestor ‘C_2’. The ancestor ‘B_2’ has extended to derive a species on one ramification, simultaneously resulting in node ‘C_3’, that further ramifies into ‘D_3’ and ‘D_4’ nodes, that extend and ramify to generate the 5th ancestral lineages ‘E_1’, ‘E_2’ and ‘E_3’, ‘E_4’; the node ‘E_3’ and ‘E_4’ branches to result in terminal taxa. *O.abruptus* and *C. trachypterus* along with other set of taxa *C.p.innotabilis* and *P. picta*. Series of ramification have taken through ‘D_3’ node; the node ‘E_1’ a derivative of ‘D_1’ generates two ancestor ‘F_1’ and ‘F_2’ nodes. From the node ‘F_1’ two lineages are derived one resulting in a taxa *L.cruciata* and other one deriving ancestral node ‘G’ that terminates in nodes ‘H_1’ and ‘H_2’; the node ‘H_2’ extend to generate two sub terminal taxa *A.c.crenulata*, *C.oxypterus*: the H1 node extend to generate another node ‘I’ that branches to generate *P.b.bispinosa.* Node ‘I’ extend in to node ‘J’ that ramifies to bear two terminal most taxa *M.citrina* and *N.a.acuticeps*, belong to Acrididae and Pyrgomorphidae respectively, also species *S.p.prasiniferum* is derived from the node ‘I’ and in the other clade of this cluster, the node ‘F_2’ branches to generate two species *E.sinetyi* and *E.a.alacris.* The node ‘D_3’ derives ancestral lineage ‘E_2’ that extend to generate the lineage ‘F3’ that end in two taxa *P.panteli* and *A. glaucopsis;* also the species *T.annulata* is derived from node ‘E_2’. Even in this tree, members of Acrididae and Pyrgomorphidae failed to form separate clusters.

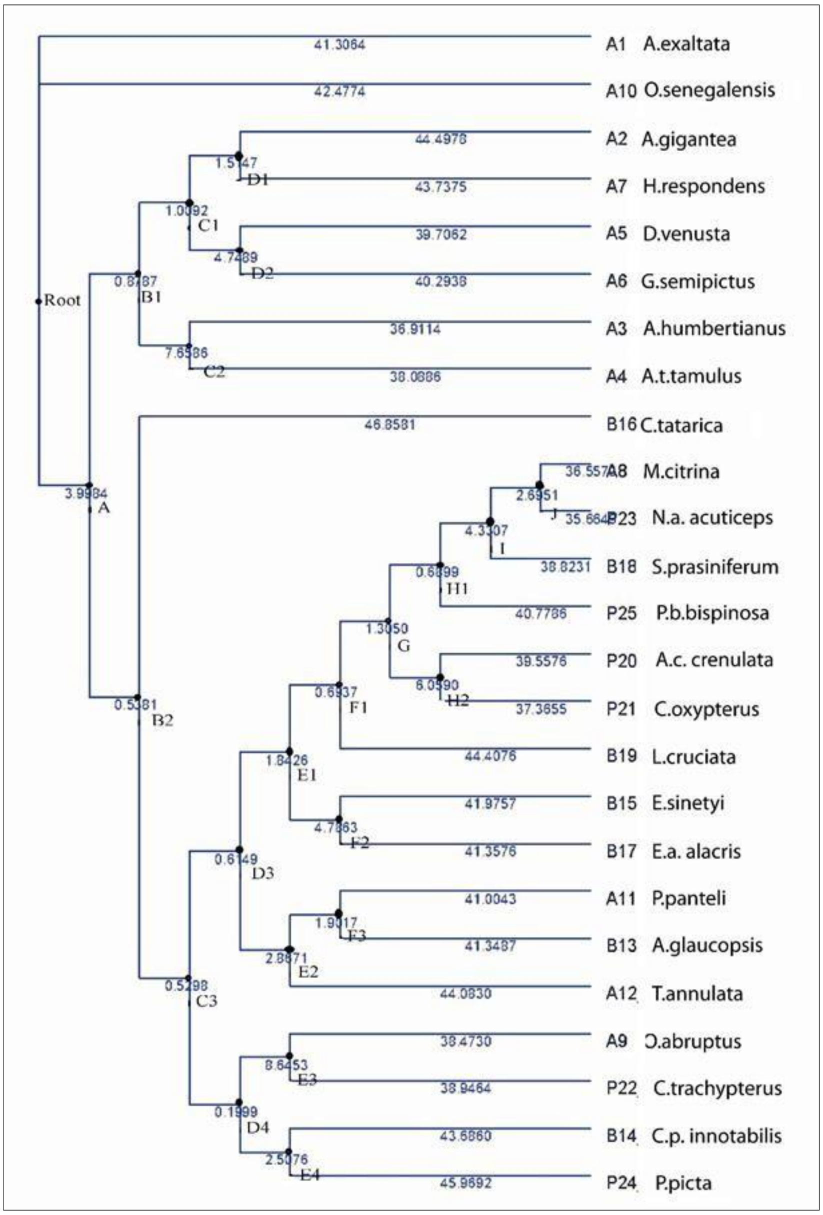


Fig 4: Phylogentic tree based on protein profile

Phylogenetic tree based on DNA and Protein data combined.

Unique feature of this tree lies in bringing together all the species of Pyrgomorphidae in to one cluster as well all members of Acrididae in to another cluster. This tree too had the same number of twenty two ancestral lineages (nodes) up to the terminal taxa appeared in ten sequential orders from node ‘A’ to node ‘J$_2$’. From the base of the root, initial diversification has taken by two species *A. exaltata* and *A. gigantea* of the subfamily Acridinae; also node ‘A’ derived from the root as the sub ultimate ancestral lineage has led to the formation of ancestral lineage ‘B’; the node ‘C’ and species *A. humbertianus* is formed from node ‘B’, whereas species *A.t.tamulus* has originated from the node ‘A’. The node‘D$_1$’ bifurcates in to two Oedipodine species *M. citrina* and *T. annulata* ; derivation of node ‘D$_2$’ is extensive, resulted in two nodes ‘E$_1$’ and ‘E$_2$’, the node ‘E$_1$’ extend to generate the node ‘F$_1$’ in one lineage and on the other terminates in a species *A.glaucopsis*. The node ‘F$_2$’ proceeds to generate the next generation of ancestral lineages ‘G$_1$’ and ‘G$_2$’; from the node ‘G$_2$’ two species *O. abruptus* and *O. senegalensis* are derived. The ancestor ‘G$_1$’ further routed to the formation two 8th order ancestral lineages ‘H$_1$’ and ‘H$_2$’ both of these ramified to generate two taxa each with short branches; *D.venusta* and *G.semipictus*, are derived from ‘H$_1$’, *H. respondens* and *P. panteli* are derived from ‘H$_2$’ node. The ‘D$_2$’ derived, ‘F$_3$’ node via E2 node, ramifies directly generating two taxa *A. C. crenulata* and *C.oxypterus*. The ‘F$_2$’ node extends to generate *P.picta* in one clade and the other led to form the node ‘G$_3$’ that advances to form ‘H$_3$’, ‘H$_4$’, these further extend in to ‘I$_1$’, ‘I$_2$’ and ‘I$_3$’ nodes. The ‘H$_4$’ node results in node in ‘I$_3$ and also in a species P.*b.bispinosa*; whereas ‘I$_3$ ramifies to end in *C.trachypterus* and *N. a. acuticeps*. The node ‘H$_3$’ and its subsequent ramifications gathered only members of Acrididae. Ramification of I$_1$ has derived *C. p. innotabilis* and *C.tatarica*, but the sub terminal branching of I$_2$ resulted in terminal nodes ‘J$_1$’and ‘J$_2$’ both bifurcated to end with two terminal taxa of shortest branches. Species *E.sinetyi* and *L.cruciata* derivatives of node ‘J$_1$’ and *C.tatarica* and *E. a. alacris* are derived as youngest forms in this lineage.

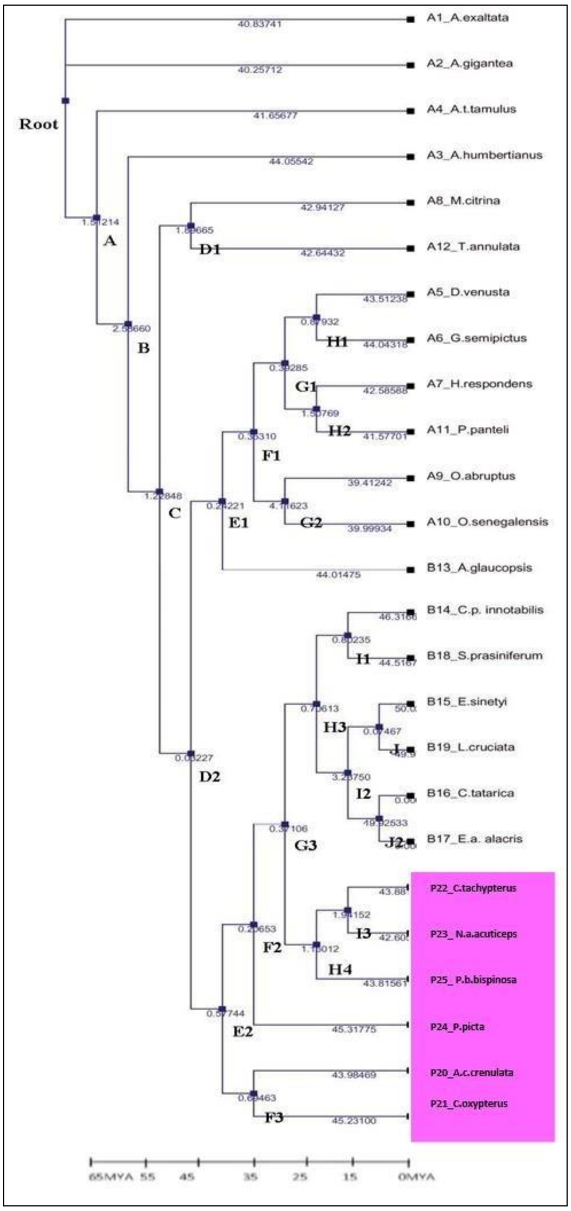


Fig 5: Phylogenetic tree based on Protein and RAPD profile

Discussion

In Phylogenetic analysis of twenty-five species of Acridids, twenty two ancestral nodes addition to root were conspicuous in each phylogenetic tree but the order of their arrangement and ramification from inner most node to outer most node changed with each criterion. The protein profile tree resulted in shuffled branch sharing between the members of the family Acrididae and Pyrgomorphidae. The members of sub family Acrididae too shared shuffled branch relationship among different taxa in the cladogram. The sub family Oedipodinae comprised of eight species did not show common clustered appearance, it was also true with the members of the sub families Acridinae and Gomphocerinae.

In DNA based tree profile, ramification of taxa and their relatedness was slightly different from that of protein-based tree. Here the basal divergence has taken place involving two species *A.exaltata* and *A.gigantea* of the same sub family Acridinae. Among the eight Oedipodines, *A.humbertianus* diverged earlier parallel to basal divergents with higher branch value, the remaining species *H.respondens, O.abruptus* and *O.senegalensis* shared a common root but the latter two species emerged as youngest with short branch length. Contrary to this same two species shared lineage with species of different sub family in the protein tree. The other four Oedipodines in the DNA tree showed variations in their relationship, *M.citrina* appeared with *E.sinetyi*, *T.annulata* with *A.glaucopsis*, *D.venusta* with *C.p.innotabilis* that belong to different sub families. Astonishingly five of the six Pyrgomorphid species appeared as a single cluster comprised of four sub clusters. Whereas the lone Pyrgomorphid species *C.oxypterous* shared its branch with the node that derived members of Acrididae. In our earlier work for tracing relationship among six Pyrgomorphids [6, 7] the branching behavior of this species differered to the greater extent and never appeared together with its congeneric form *C.trchypterous*, thus these two congeneric forms appeared to be derivatives of two ancestors. The noteworthy feature in DNA tree is the separation of Pyrgomorphid ancestral form has taken place at fourth order lineage in this phylogenetic derivation but advances of this in to the 5thorder ancestral lineage, have given rise to *A.c.crenulata* that shared ancestry with a member of Acrididae of sub family Gomphocerinae, *L.cruciata*. Subsequent progress of node E4 resulted as common ancestor of *N. a. acuticeps, P.picta* and *P.b.bispinosa* that are derived through sixth order ancestral lineage. From lineage G1-G4, eleven of the twenty five taxa of grasshoppers derived as terminal taxa.Thus all the taxa of Acrididae and Pyrgomorphidae had a common ancestral lineage up to the order of five nodes, depicted their relativity as sister groups.

DNA and protein profile combined tree yielded a different picture from individual protein or DNA trees. In this tree the members of three sub families Acrididae placed in the close proximity sharing nearest ramifications. The two members of the sub family Acridinae diverged as basal diversification from the root while the other *P.panteli* appeared as derivative of eighth order node that shared branch with *D.venusta*. Members of Oedipodinae appeared in single cluster derived from nodes 1st,2nd,4th,7th,8th order, along with two other taxa *G.semipictus* and *P.panteli* reflected paraphyletic representation. Our findings of paraphyletic derivation of sub families Oedipodinae,Acridinae and Gomphocerinae coincide with the analytical confirmations on subfamilies of Acrididae as non-monophyletic or paraphyletic in their cladogenic relationships [9, 10]. The two congeneric Oedipodines *O.abruptus* and *O.senegalensis* originated from a common node with short branches. The other member of Gomphocerinae and *E.sinetyi* co appeared from the node ‘ J’ as youngest taxa in the tree with shortest branches. So also *C. t.tatarica* of Cyrtacanthocridinae and *E.a.alacris* of Eypropocnidinae appeared as youngest taxa derived from ‘J 2’ node.Most important clustering in this tree is that all the six species of the family pyrgomorphidae appeared as a single group derived through four different nodes depicting the common ancestor of all pyrgomorphids is a fifth order lineage from which a few Acrididae and all pyrgomophidae, have originated as paraphyletic species. Thus in tracing the phylogenetic relations of twenty five species of grasshoppers using different markers revealed shuffled relationship between the members of Acrididae and Pyrgomorphidae in protein tree and did not bring together members of subfamily Oedipodinae as well sub families Acridinae and Gomphocerinae. The DNA based tree too resulted in shuffled appearance for the members of the sub family Oedipodinae,Acridinae and Gomphocerinae but provisioned the nearest clustering of species of pyrgomorphidae. Of these three trees combined DNA and Prtotein tree clustered together members of Oedipodinae and pyrgomorphidae in to respective groups but regard to cluster of Oedipodinae there was a mix with two members of the other subfamilies as sister group.The hypothetical time line drawn for the phylogenetic tree depicts the common ancestor existed about sixty five million years back the present Grasshoppers of Acrididae and pyrgomorphidae have originated after four or five Ancestral lineages of the Acridomopha, the common ancestor of all grasshoppers. The Pyrgomophids originated about thirty five million years ago passing through at least three evolutionary lineages to emerge as present day Pyrgomorphid species.

A few evidences are available on paraphyletic representation, as a common feature, of the sub families of Acrididae; based on the mitochondrial genomic analysis of cladogenic link of the members of sub families of Acrididae found to be not only paraphyletic also polyphyletic [12], where as in the phylogenetic tree based on Amylase enzymes as markers, the Pyrgomorphids appeared as para phyletic and sister groups of the Acrididan sub families [13], even the studies by combining molecular and morphological markers the members of pyrgomorphidae found to have paraphyly among the taxa [14] and phylogeny constructed based on acoustic features in orthopteroid insects could not produce a tree depicting monophyly either at subfamily level or at tribal level of these insects [15]. These studies have not recorded monophyletic origin of sub families of short horned grasshoppers but asserted that there is a degree of conflict between morphological traits and molecular variations in this group of insects. Our observations too has compliance with these findings, as monophyly for sub families of Acrididae could not be achieved with different criteria used and the paraphyletic assortment of members of sub families differed with each criteria.

Phylogenetic derivation of pyrgomorphidae

Phylogeny of Pyrgomorphidae is still a less understood topic in the evolutionary studies of Orthopteran insects. Based on the male phallic structure, the Pyrgomorphidae placed close to Pamphagidae or close to Lentulidae species of south African endemic grasshoppers; further Eades[16] discussed the Pyrgomorphoidae might have derived from the Pyrgacrimorphidae that are found in Mauritius and Reunion islands but Song and Marino perez [11] disagreed with this concept for two reasons that male phallic organ similarity may be due to convergence and the other, Mauritius and Reunion islands have formed only about 6.5mya i.e. after the origin of Pyrgomorphids. Elevation of the Pyrgomorphidae to be a family under Acridomorpha suggested by Eades [16] has been accepted by Song and Marino perez [17]. Phylogenetic placement of Pyrgomorphids has conflicts of views. Roberts [see 17] felt the Pyrgomorhidae should be placed with Pamphagidae because of their open ejaculatory sac associated phallic structures but Dirish [18] and Amedegnato [19] considered that Pyrgomorphidae are closey related to Lentulidae based on male phallic structure. Through the molecular studies, Eades [16] confirmed Pyrgomorphidae to be the sister group of Acridoidea. In this study, it was seen that the Pyrgomorphoidae are derived from a common ancestor of 4 - 5th order ancestral lineage, that had shared ancestry with Acrididae too; but *C.oxypterus* of Pyrgomorphidae showed paraphyly to share its relationship with two species of Acrididae in the DNA tree. In protein and DNA combined tree monophyly for all the 6 species of Pyrgomorphidae was recovered and arranged in to single cluster. In the absence of evidence from related families like Lentulidae or Pamphagidae, in Indian sub-continent, we are forced to comply with our opinion that Pyrgomorphidae are derived from Acrididae ancestral lineages that have undergone subsequent speciation events to result in the present day Pyrgomorphids.

Conclusion

The pattern of ramifications in the phylogenetic trees for the twenty five species of grasshoppers had shown differences in ramification, branch lengths, manifestation of terminal taxa between the protein and (RAPD) DNA based phylogenetic trees, Combination of DNA and protein profiles resulted in congregation of Acrididae and Pyrgomorphidae species in to separate clusters but failed to bring together the individuals of sub families of Acrididae, in to sub family wise cluster, thus majority of the taxa appeared paraphyletic. This raises the question did the individuals in each sub family which are categorized on the basis of taxonomic characters, are resulted due to convergent evolution of morphological characters ?,in spite of their molecular differences. This question has a wide open chance to review the evolution of grass hoppers in the direction of possible morphological traits convergence process among the species of different sub families. Regard to phylogeny of Pyrgomorphids, it is imminent to believe these species are derivatives of Acrididae.

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