Taxonomic studies of subfamily Deltocephalinae and Typhlocybinae (Hemiptera: Cicadellidae) from District Faisalabad Punjab Pakistan with a key based on their Morphological Characters

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Abstract

This study was performed to identify the species of two subfamilies Deltocephalinae and Typhlocybinae at Taxonomic lab Department of Entomology University of Agriculture Faisalabad Pakistan.The Leafhopper specimens were collected with the help of sweep netting from different locations on vegetable and grasses etc. The specimens after collection were killed by placing them in poison bottle. Poison bottle was made by Potassium cyanide which is used for killing specimens. For preservation of specimen's entomological pins were used and had placed them in wooden box. To protect specimens from other insects like ants were used naphthalene bolls. Stereo light microscope digital camera fitted B41 Olympus was used with magnification power Omax 3.5X- 90X USB3 18 MP and variable magnification to identify key characters of leafhoppers from District Faisalabad. Two genera were identified from subfamily Deltocephalinae which are Exitianus indicus and Exitianus ball which are mostly feed on different grasses. These genera were first time studied in Punjab Pakistan. Two species were identified from subfamily Typhlocybinae which are Amritodusatkinsoni commonly known as cotton jassid and Empoascafabae which is called potato leafhopper. These are serious pest of Agricultural crops and cause huge losses all over Pakistan. So, identification is very important to control these species.

Keywords - *Taxonomy, Morphology, Identification, Faisalabad, Punjab*

I. Introduction

Cicadellidae are the most important family of leafhoppers in the world. It consists of 22,600 described species and 36 described subspecies (Dietrich, 2013). Most of these subfamilies are not properly categorized. Deltocephalinae which is the subfamily of Cicadellidae that feed on different grasses and Agricultural crops (Dellape and Paradelle, 2013) Deltocephalinae subfamily contain 75% vectors that transfer plant diseases (Weintraub and Beanland, 2006) The size of leafhoppers in between 2-10mm Leafhoppers having rows of spines on hind tibia. They are distributed worldwide from tropical rainforest to arctic tundra and from sea level to 4000-meter elevation and may be found feeding on nearly all major groups of vascular plants. They have unique sucking mouthparts for extraction of liquids from plants. They suck the sap of different crops and found in wild and Agricultural habitats (Novotny, 1994; Hamilton and Whitecomb, 2010). Cicadellidae largest subfamily Deltocephalinae and it is the biggest and most assorted subfamily of Cicadellidae with 6200 described species set in more than 850 genera, in 36 tribes (Zahniser and Dietrich, 2010). Individuals from the subfamily are likewise imperative vectors of plant sicknesses (Weintraub and Beanland, 2006) and represent 117 of the 151 cicadellid vector species recorded. A very little work done on Deltocephalinae in Pakistan and very little publications particularly that feed on grasses. This subfamily comprises of very soft bodied leafhoppers when contrasted with subfamily Deltocephalinae and attack on financially significant woody and non-woody plants including natural products, Potato, Tomato, Brinjal etc. The body length ranges 2.5mm to 5.6mm. Cross veins are not present in front wing venation before apical cells. They are in lacking cross veins before apical cells in forewing venation. Ahmad colleagues working to find out the new species of this subfamily since the year 1967 and found various species and new genera. After basic work done on genus Dworakewska which supports this subfamily. (Phuthi, 1940) consider first scientist to work on genus Empoasca from region of Subcontinent. And know at present times almost 126

species and 51 genera of this genus identified from different regions of Pakistan. Today lot of work done on the species of this subfamily because economically it is very important to identify their species. The specimens belong to this subfamily cause serious losses to important cash and food crops. Typhlocybinae recognized to study their forewing coming up short on an addendum, hindwing with every single longitudinal vein finishing at the sub marginal vein, and this vein coming to yet not surpassing the MP+R (Zhang, 1990). Now the identified genera 65 have been perceived appropriated around the world. The jassid that attack on cotton crop is a serious pest in the southern region of China (Zhang, 1990, Kuoh; 1966). In this present study two genera were identified from subfamily Deltocephalinae *Exitianus indicus* and *Exitianus ball*. These genera feed on grasses like lawns. Dark coloured single correct line is present on Exitianus indicus head and dark coloured spots are present on pronotum and couple of wedge-shaped faint spots are present on their scutellum. Four kind of apical cells are present on their front wing and preapical cells are three, size of appendix is average. Exitianus ball consider as medium sized body insects among 4 and 5mm and black brown patterns on their body. Two specimens were identified from subfamily Typhlocybinae. *Amrascabigutulla* which is the serious pest of cotton crop in Pakistan. Adult size is about 3.5mm long and body colour is pale green and wedged shape. Forewings and vertex contain two black spots. Adults are very active and fly when distributed. This species is cosmopolitan and found India, Pakistan, Iran, Turkey, Egypt, South Africa, Greece, Italy and Spain. And second most important pest from this subfamily is *Empoascafabae* which is the serious pest of vegetable crops. Mostly feed on potato, cucumber etc. Adult potato leafhopper Colour is pale green or yellowish and rows of six white spots behind the head and identified with use of lenses. White H shaped mark are present between their head and wing based. Body size is approximately 3mm long and on front wing near its tip a cross vein is present. Potato leafhopper found throughout the world Pakistan, India, China, Colombia, California, New York, North Carolina, Columbia etc.

II. Materials and Methods

A. Study site:

The research work for purpose of species identification was conducted In Taxonomic lab, Department of Entomology, University of Agriculture, Faisalabad during year 2018-2019.

B. Collection of Specimens:The Leafhopper specimens were collected with the help of sweep netting from different locations on vegetable and grasses etc. The specimens after collection were killed by placing them in poison bottle. Poison bottle was made by Potassium cyanide which is used for killing specimens. For preservation of specimen's entomological pins were used and had placed them in wooden box. To protect specimens from other insects like ants were used naphthalene bolls. Stereo light microscope digital camera fitted B41 Olympus was used with magnification power Omax 3.5X- 90X USB3 18 MP and variable magnification to identify key characters of leafhoppers from District Muzaffargarh. A comprehensive survey and field visits were conducted throughout the District during the whole year 2019. During field surveys mostly, agriculture fields grasses were visited. The specimens were identified with help of Taxonomic keys of different researchers.

C. Agriculture Field Area:Leafhopper mostly found on agricultural crops like potato, cucumber, sugarcane, Legumaceous plants like Alfalfa and spinach etc. Faisalabad District is agriculture-based area and different crops grown so collection was very easy. Collection of jassid and potato leafhopper was done on cotton, potato and tomato crops. The specimens of Deltocephalinae were collected from different grasses.

D. Preservation:After collection of specimens the most important step is to preserve. For preservation first insects were pinned very carefully. Entomological pins 20 no were used. Wooden boxes mostly used for preservation of these insects. Large specimens mostly pinned but small specimens were glued to right side of thorax. Wing and legs spreading are most important for successful preservation. To protect insects from different harmful insect's naphthalene tablets were used.

E. Killing Jar:Killing jar is compulsory to kill the collected insects and Entomologists mostly used this tool to protect insects from further injuries. After collection specimens were killed by potassium cyanide killing jar. Generally killing agents are chloroform, ether and ethyl acetate are used. But researchers think that cyanide is more toxic and mostly used for hard bodied insects.

F. Description:Morphological characters were used to describe the adult specimens like (Head, thorax, abdomens etc.) Taxonomic keys were used from Khatri (2010), Dietrich (2005), Distant (1908) and British Museum of Natural History.

III. Results and Discussion

A. Taxonomic study:

The outputs of this present study were to find out different species of two subfamilies Deltocephalinae and Typhlocybinae fauna from District Faisalabad Punjab Province. Two specimens were identified from subfamily Deltocephalinae which are *Exitianus indicus* and *Exitianus ball*. Two specimens were identified from subfamily Typhlocybinae Included *Amrascabigutulla* and *Empoascafabae* which are

serious pests of Agricultural crops. These specimens were recognised based on their morphological characters head, thorax and abdomen.

B. Subfamily Typhlocybinae:

This subfamily comprises of very soft bodied leafhoppers when contrasted with subfamily Deltocephalinae and attack on financially significant woody and non-woody plants including natural products, Potato, Tomato, Brinjal etc. The body length ranges 2.5mm to 5.6mm. Cross veins are not present in front wing venation before apical cells. They are in lacking cross veins before apical cells in forewing venation. Ahmad colleagues working to find out the new species of this subfamily since the year 1967 and found various species and new genera. After basic work done on genus Dworakewska which supports this subfamily. (Phuthi, 1940) consider first scientist to work on genus *Empoasca* from region of Subcontinent And know at present times almost 126 species and 51 genera of this genus identified from different regions of Pakistan. Today lot of work done on the species of this subfamily because economically it is very important to identify their species. The specimens belong to this subfamily cause serious losses to important cash and food crops.

C. Genus: 3 Amrascabiguttula (Ishida, 1912; Chlorita)

Diagnostic characters: Adult size is about 3.5mm long and body colour is pale green and wedged shape. Forewings and vertex contain two black spots. Adults are very active and fly when distributed.

Head: Head scutellum and pronotum are pale ochraceous and two tiny dark coloured spots are present Plate like of head contain white greyish spots are present near side of anterior margin

Thorax: Front wing are mostly pale ochraceous and dark coloured spots are present on the surrounding side wing. Forewings and vertex have two black spots.

Abdomen: Male subgenal plate exceeded through the anus tube and the diameter of tube reached through 1/3 part of male plate. The size of style increased from base and shape of apophysis become saw like. Very simple kind of tube present in Aedeagus and at the side of apical margin is bowl-shaped.

Measurements: Male total length 2.8, forewing length 2.24, crown length at middle 0.24, crown width across eyes 0.68, interocular width at anterior 0.35, eyes length in cross 0.34, pronotum width 0.67, pronotum length 0.31, mesonotum length 0.15, scutellum length 0.23.

Distribution: This species is cosmopolitan and found India, Pakistan, Iran, Turkey, Egypt, South Africa, Greece, Italy and Spain.

Material Examined: Cotton field, Toba Tek Singh 23, 12- ix- 2019, Shah Kot 17, 15- x- 2019,

Remarks: Cosmopolitan species and mostly feed on cotton crop. It causes reasonable losses to cotton crop.

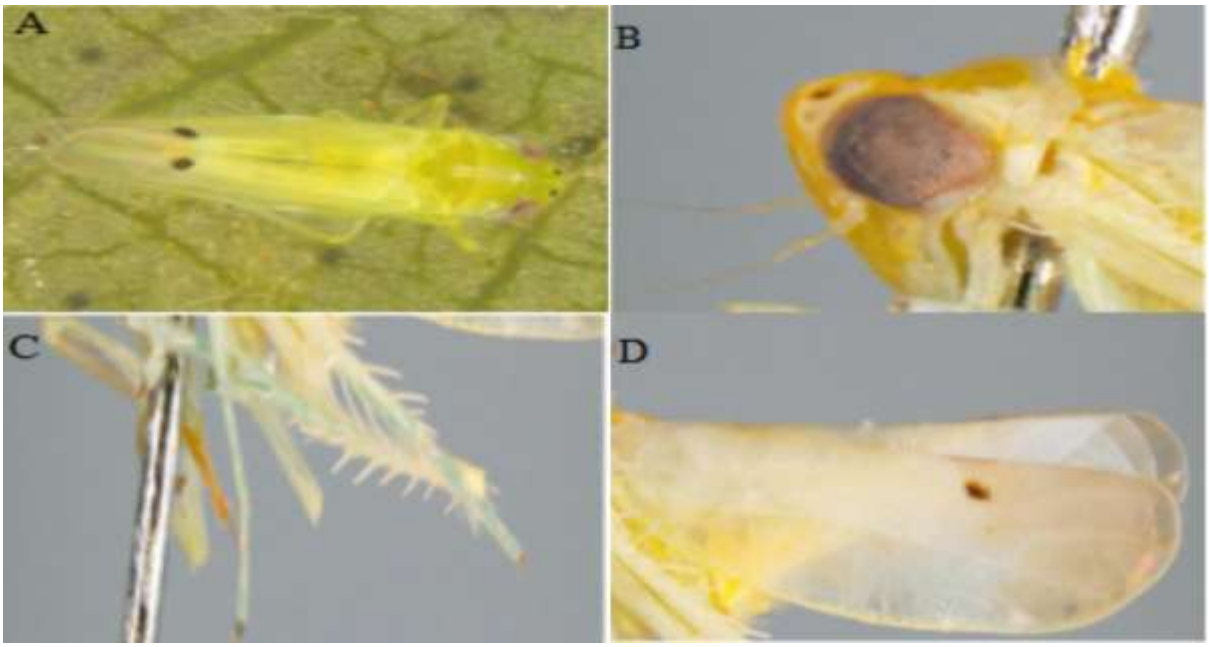

Figure 1: (A)Complete view of Amrascabiguttula (Ishida, 1912; Chlorita) (B) Head view Amrascabiguttula (Ishida, 1912; Chlorita)(C) Legs view Amrascabiguttula (Ishida, 1912; Chlorita) (D) Wings venation Amrascabiguttula (Ishida, 1912; Chlorita)

D. Genus: 4 Empoascafabae (Harris 1841)

Diagnostic characters:

Adult potato leafhopper Colour is pale green or yellowish and rows of six white spots behind the head and identified with use of lenses. White H shaped mark are present between their head and wing based. Body size is approximately 3mm long and on front wing near its tip a cross vein is present.

Head: Identification from dorsal view crown is clearly longer then next to eye. Crown particularly shorter then width between eyes. Head is almost subequal to that of pronotum and wider then maximum width of pronotum. Coronal suture broadened well beyond crown mid length but not onto face. Lateral frontal suture increased to or near ocelli. When eyes seen from dorsal side narrower then distance. Maximum length of face 1- 1.5 X and ante clypeus of male flat or slightly convex. Rostrum not extended to hind coxa.

Thorax: Forewings venation contain 2 apical cells and 3 both quadrates basally and RP and MA (anterior side) veins are free and connected with cross vein or apical cells 2 petiolate. Forewings veins SCR second longitudinal vein and M fourth longitudinal vein free preapically. The shape of apical cells 4 tapered near base and MCU fourth and fifth longitudinal vein ended near posterior end of apical margin. The shape of forewing vein MCU is distinctly curved. Minute spots with veins absent or different. The first apical cells of hind wings are 2x longer then wide. The base of hind wing first apical cells towards or near the distal part of apex.

Legs: Rows of front femur AV with 1 basal seta clearly extended and AM1 front femur clearly extended. Middle femur consists of dorsoapical macrochaetae or with dorsoapical macrochaeta. Rows of hind tibia AV consist of preapical macrochaetae.

Abdomen: Internal ridges or ingrowths from exoskeleton extended to but little or no beyond to the ventral portion of segment of abdomen (Sternite III). Second thickened dorsal plate (tergal structure) if developed at all with only a ventrally directed low wall less developed or absent medially. Third internal ridges (tergal) of thickened dorsal plate (apodemes) are absent.

Distribution: Potato leafhopper found throughout the world Pakistan, India, China, Colombia, California, New York, North Carolina, Columbia etc.

Material Examined: Agriculture University, vegetables field, 14, 12 – xi- 2019, Horticulture, field, 13, 11-xii- 2019.

Remarks: Potato leafhopper mostly feed on vegetables like potato, tomato, Brinjal. It transmits viral diseases and destroyed crops.

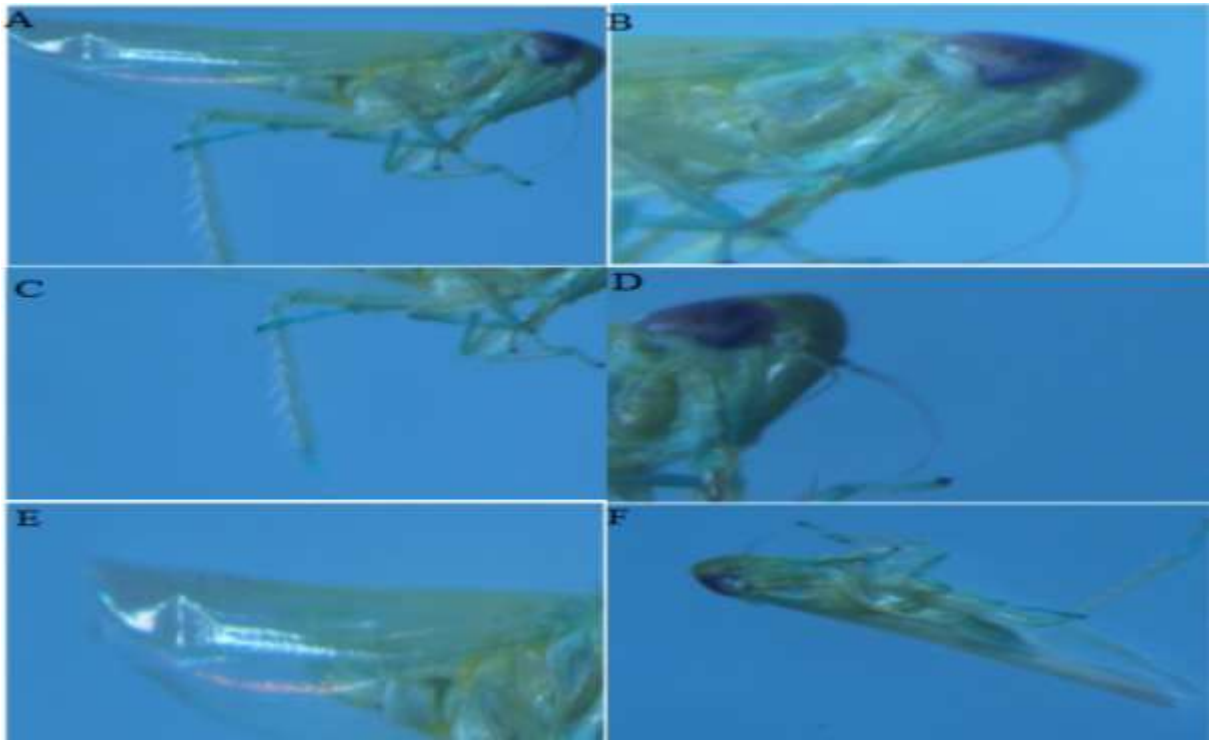

Figure 2:(A)Complete body view Genus: Empoascafabae (Harris 1841) (B) Head view Genus Empoascafabae (Harris 1841) (C) Legs view Genus: Empoascafabae (Harris 1841) (D) Antennal and eyes view Genus: Empoascafabae (Harris 1841)(E)Wings venation Genus: Empoascafabae (Harris 1841)(F) Lateral view F FF. Genus: Empoascafabae (Harris 1841)

E. Subfamily Deltocephalinae, Feiber, 1869

This subfamily considers as most diverse and second largest subfamily with almost 800 genera and 6500 species worldwide the 1/3 specimens in this subfamily belongs to mostly grasslands and forest ecosystems. The specimens belong to this subfamily considered as the most destructive pests of most important crops and affect the crops by feeding directly as well transfer very viral diseases. A very little work done on Deltocephalinae in Pakistan and very little publications particularly that feed on grasses. Complete morphological description of subfamily Deltocephalinae was provided by (Zahniser and Dietrich, 2010).

Diagnostic Characters: Size of these leafhoppers are small to large and wedged shaped. Head consist of simple eyes on the anterior side and very near to the eyes. Lateral frontal suture prolonged to ocelli. Forewings brachypterous to macropterous. Male pygofer with basolateral oblique and subgenal plates articulated with each other.

G. Genus: 5 Exitanus indicus (Distant, 1908)

Diagnostic characters:

Dark coloured single correct line is present on their head and dark coloured spots are present on pronotum and couple of wedge-shaped faint spots are present on their scutellum. Four kind of apical cells are present on their front wing and preapical cells are three, size of appendix is average. Pygofer consist of two spines at their apex and six to eight macrosetae present on the subgenal plate and shape of anal tube is chitinous. Medially pair of processes present on aedeagal shaft. Laterally aedeagus pointed and ingrowths present on basal side are square shaped. At the dorsal side of aedeagus gonopore present and apophysis which is Y shaped are strongly attached with style. The shape of apophysis is sharped.

Measurements: Total body size of male, 4.68, Length of front wing, 3.8, Length of crown at middle, 0.35, Size of crown transversely at eyes, 1.43, at the front side, 0.88, crosswise eye length, 0.57, girth of frontal plate, 1.33, Length of pronotum, 0.7, Length of mesonotum, 0.26 and the length of scutellum is 0.39.

Distribution: This is cosmopolitan and found on grasses. Pakistan, China, India and Australia etc.

Material Examined: Jaranwalla 15, 16-xx-xi-2019, Shah Kot 14, 11xi-xv-2019, UAF Horticulture Field - iv-v-2019.

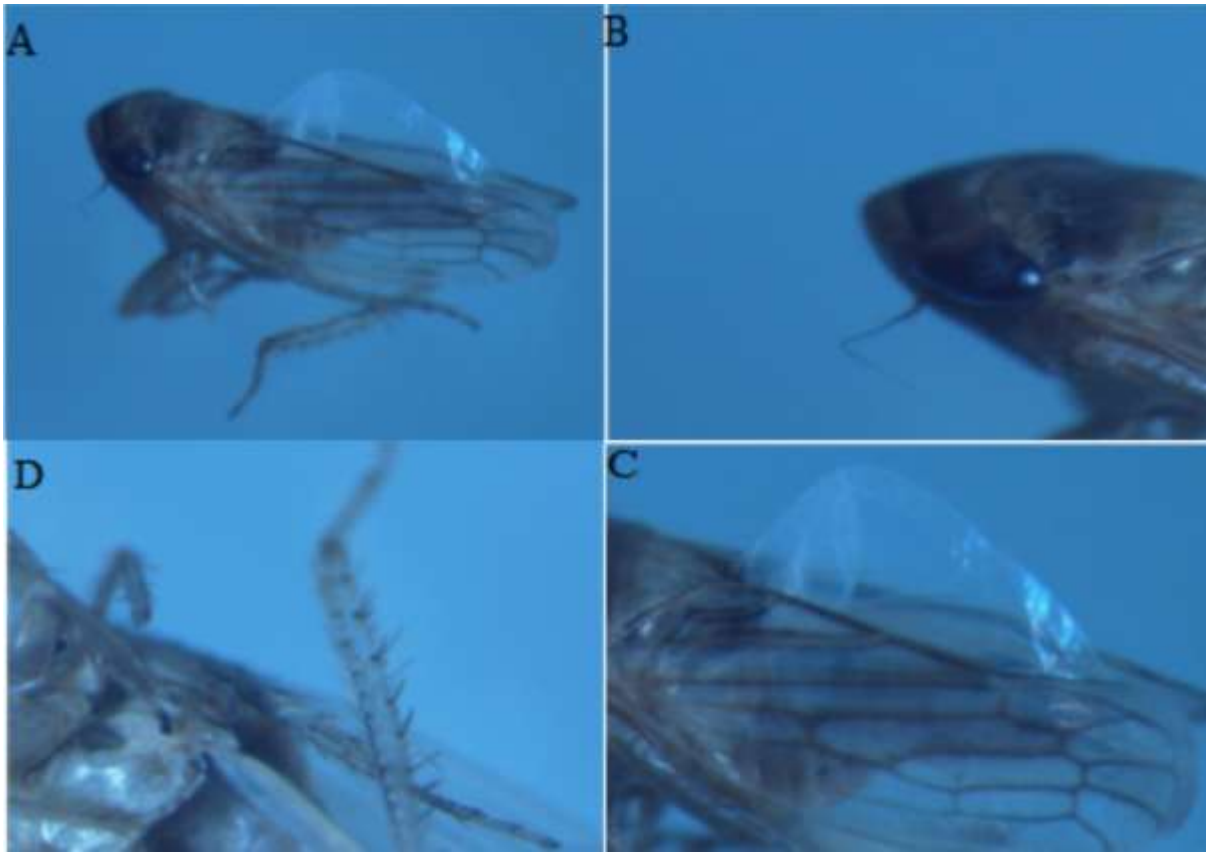

Figure 3: (A) Complete body body view of Exitianus indicus (Distant, 1908) (B)Head dorsal view of Exitianus indicus (Distant, 1908) (C)Wing venation of Exitianus indicus (Distant, 1908) (D) Legs view of Exitianus indicus (Distant, 1908)

H. Genus: 6 Exitianus Ball, 1929

Diagnostic Characters: The specimens belong to this genus consider as medium sized body insects among 4 and 5mm and black brown patterns on their body.

Head: Simple eyes are present dorsally and head is wider then pronotum. Mesonotum and pronotum contain various kind of patterns. Face broader then long and ledges on antennae are absent. The size of ocelli is large and separated from eyes the distance is slightly greater than their own width. The clypeus is tapering towards apex.

Thorax: Forewing appendix have been very huge and wing apex prolonged. Brown coloration are present or absent on their anal veins. The size of pronotum is more than vertex and lateral margins are short. The inner apical cells of forewings are open.

Abdomen: Two to six black spines are present with apical margin of male pygofer. At the middle male subgenal plates have touched each other and shape of stem is Y shaped. The shape of aedeagus from base is broad and from side of apex is slim. Articular structure present between base and shaft, structure of shaft is very simple. At the dorsal side gonopore is present. The ovipositor of female is extended towards considerable distance.

Distribution: This specie is cosmopolitan and found in all geographical distribution.

Material Examined: UAF grass field 16, 14 -xiv- 2019, Shah Kot 15, 16 -xv- 2019, Toba Tek Singh 12, 11 -xv- 2019

Remarks: Mostly found on grasses, Lawns and it is cosmopolitan species.

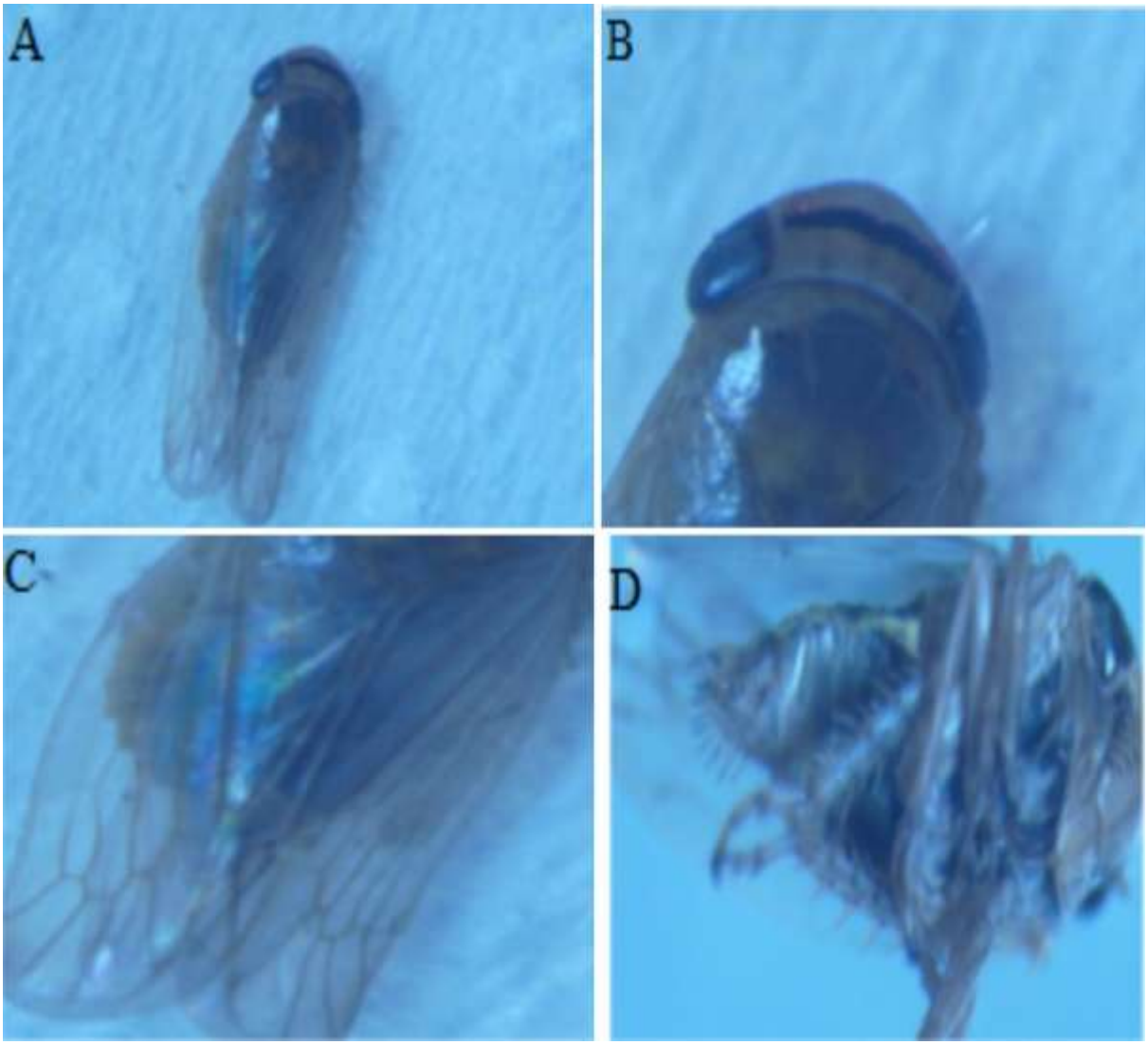

Figure4: (A) Complete body view Genus: Exitianus Ball, 1929 (B) Head view of Genus: Exitianus Ball, 1929 (C) Wing venation of Exitianus Ball, 1929(D) Lateral view of Exitianus Ball, 1929

I. Keys to genera and Tribes of subfamily Typhlocybinae from District Faisalabad Punjab Pakistan

1. Sub marginal veins are present on hind wing and extended towards wing apex beyond apex of vein R+M. Hind wing vein 1v and 2v are fused completely…….. ***Togaritettix Matsumura***

Sub marginal veins on hind wing absent or present on the side of wing and not extended outside the highest part of wing vein M+R……...***Erythroneurinii***

2. The lobe present on preapical styli are less or more advanced and shape of lobe is pointed ……***ZyginidiaHaupt***

3. Ventral pygofer process are not present and second extension of styli are long pointed, conical shaped……***HelionidiaZahvatkin***

- Pygofer process are present and styli are without subapical tooth……***Empoascinii***

4. Front wing consist of single apical vein which originate M cell and adeagal spines present but tiny……***EmpoascaWash***

- The colour of front wing is ochraceous and dark coloured spots are present, the basic figure of pygofer is elliptical and apical process is in serrated form……***Amrascabigutulla.***

J. Keys to the Deltocephalinae genera from Pakistan

1. Large wings……2

-Reduced wings, the shape of head is wide rounded…… ***Chiasmus***

2. -Forewing consist of various epithelial cells and head spatulate…….. ***Chiasmus***

Forewings consist of three subapical cells……7

3. Appendix of forewing extended beyond middle of outer apical cells……***Chiasmus***

-Appendix of forewing extended to second apical cells and there are no epithelial cells on in head……4

4. The top of apex on head is very short, length is unchanging and four time broader then wide between eyes and then long……***Balclutha***

-The size of vertex is more medially than to eyes……5

5. The pygofer of male consist of slender hook like processes lake of comb like serrations and the colour thorax and their head are golden yellow two rounded spots on……***Cicadullina***

The pygofer of male lack of these processes but consist of comb like serrations, head and thorax not as above……6

6. Subgenal plates of male are fused male pygofer lack of black stout spines……9

-Subgenal plates of male fused lake of black stout spines and head vertex is three time wider and eyes which are present between are long…….....***Goniagnathuss***

7. Predominately specimens are mostly green colour ……...***Nephotettix***

- Predominately colour of species is light brown……***Exiitanus***

8. Lateral sclerite are very large in size and visible behind eyes when see from dorsal side……11

The size of lateral sclerite is very small and behind eyes are not visible ……13

Lateral carina presents in forewing……***Grammacephallus***

- Lateral carina are not present in Carina……***Masiripiuss***

10. Species are green male pygofer lack of stout setae are not present in Center…….....***Hecallus***

The colour of species belong to this genus are mostly brown and stouts are present in centre……***Glossocratuss***

11. Ventral pair of processes present on Aedeagal shaft…….....***opisiuss***

Pair of processes are not present on Aedeagal shaft…….....***orosisuss***

12. Vertex consist of transverse black stripe dorsal marginal appendages on pygofer…….....***Paramesodes***

-vertex lack of transverse black stripe and no appendages on pygofer……***Maiestas***

Male connective with sterile hair like filament……***Scaphoideus***

Male connective without hair like filament……***Bampurius***

14. Subgenal plates of male with mesal process…… ***Neolimnus***

15. Subgenal plates of male are short……***Psamtettix***
Subgenal plates of male are longer……***Jilinga***

IV. Conclusion

The results of this study show that two genera were identified from subfamily Deltocephalinae included *Exitianus indicus* and *Exitianus ball* which are mostly found on grasses. Dark coloured single correct line is present *Exitianus indicus* head and dark coloured spots are present on pronotum and couple of wedge-shaped faint spots are present on their scutellum. Four kind of apical cells are present on their front wing and preapical cells are three, size of appendix is average. The specimens belong to this genus *Exitianus ball* consider as medium sized body insects among 4 and 5mm and black brown patterns on their body. Two species identified from subfamily Typhlocybinae included *Amrascabigutula* and *Empoascafabae* which are serious pest of Agricultural crops. *Amrascabigutula* adult size is about 3.5mm long and body colour is pale green and wedged shape. Forewings and vertex contain two black spots. Adults are very active and fly when distributed. Adult potato leafhopper (*Empoascafabae*) Colour is pale green or yellowish and rows of six white spots behind the head and identified with use of lenses. White H shaped mark are present between their head and wing based. Body size is approximately 3mm long and on front wing near its tip a cross vein is present. Specimens identification is very important for adapting control measures.

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V. References

[1] Dellape, G., and S.L., Paradelle. (2013). New records of Proconiini (Hemiptera: Cicadellidae) from Argentina. Revista de la Sociedad Entomológica Argentina. 72: (3-4).
[2] Dietrich, C.H., (2005). Keys to the families of Cicadomorpha and subfamilies and tribes of Cicadellidae (Hemiptera: Auchenorrhyncha). Florida Entomologist. 502-517.
[3] Dietrich. H.C. (2013). Overview of the phylogeny, taxonomy and diversity of the leafhopper (Hemiptera: Auchenorrhyncha: Cicadomorpha:Membracoidea: Cicadellidae) vectors of plant pathogens. International Proceeding Symposium. 47-69.
[4] Distant, W. L. (1908). Rhynchota- Homoptera. The Fauna of British India including Ceylon and Burma, 4: 1-501.
[5] Hamilton, K. A., and R.F, Whitcomb. (2010). Leafhoppers (Homoptera: Cicadellidae): a major family adapted to grassland habitats. Arthropods of Canadian grasslands. 1:169-197.
[6] Khatri, I., and MD, Webb. (2010). The Deltocephalinae leafhoppers of Pakistan (Hemiptera,Cicadellidae). Zootaxa. 2365: 1-47.DOI: 10.5281/zenodo.193658.
[7] Kuoh, C.L. (1966). Economic Insect Fauna of China. Fasc. 10. Homoptera Cicadellidae. Fauna Editorial Committee, Academia Sinica. Beijing, China. Science Press. 1–170.
[8] Novotny, V. (1994). Association of polyphagy in leafhoppers (Auchenorrhyncha, Hemiptera) with unpredictable environments. Oikos 70: 223–232.
[9] Pruthi, H.S. (1940). Descriptions of some new species of Empoascawalsh (Eupterygidae, Jassoidea) from North India. Indian Journal of Entomology. 2: 1-10.
[10] Weintraub, P.G., and L, Beanland. (2006). Insect vectors of phytoplasmas. Annual Review of Entomology. 51: 91-111.
[11] Zahniser, J.N., and C.H, Dietrich. (2010). Phylogeny of the leafhopper subfamily Deltocephalinae (Hemiptera: Cicadellidae) based on molecular and morphological data with a revised family-group classification. Systematic Entomology. 35(3):489-511.
[12] Zhang, Y.L. (1990). A taxonomic study of Chinese Ciadellidae(Homoptera).TianzeEldonejo. Yangling, Shaanxi, China. 218.