

Research Article



Available on <https://www.joarps.org>
Journal of Applied Research in Plant Sciences
(JOARPS)
ISSN: 2708-3004 (Online), 2708-2997 (Print)



Morphometric analyses & DNA barcoding of Acrididae (Orthoptera: Caelifera) using cytochrome oxidase subunit I gene

Abdul Aziz Babar, Riffat Sultana, Santosh Kumar, Muhammad Irfan Bozdar

Department of Zoology, University of Sindh, Jamshoro,
Department of Zoology, University of Sindh, Jamshoro,
Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Punjab,
Department of Zoology, University of Sindh, Jamshoro
Corresponding author email: riffat.sultana@usindh.edu.pk

Article Received 11-10-2022, Article Revised 28-11-2022, Article Accepted 13-12-2022

Abstract

DNA barcode data of Acrididae is limited in global data bases from Sindh, Pakistan. Hence, the present study was aimed to collect some selected Acridid species from the Sindh and generate DNA barcode data to improve the global database sequencing. Specimens were collected from cultivated, sandy, rocky, vegetation, grassy, desert, semi desert, roadside and open area of the upper Sindh districts i.e., Dadu, Ghotki, Jacobabad, Kashmore, Khairpur Miras, Larkana, Naushahro Feroz, Qambar & Shahdaktot, Shikarpur, Sukkur. Seven species of Acrididae were under discussion i.e., (24%) followed by *Acrida willemsei* (19%), *Truxalis eximia eximia* (15%), *Aiolopus thalassinus thalassinus* (13%), *Acrotylus longipes longipes* (10%), *Aiolopussimulatrix* (10%) and *Sphingonotus savignyi* (09%) belong to 3 sub-families i.e., Acridinae, Oedipodinae and Oxyinae. Moreover, maximum number of specimens belonged to Oxyinae. The present study suggests the collection of multiple specimens from different geographical locations and the generation of more DNA barcode data would facilitate the actual diversity amongst this taxon.

Keywords: DNA bar-coding, biodiversity, Sequence, Acrididae, Sindh.

Introduction

Acridids are the short-horned grasshoppers, the most common orthopteran found in Pakistan sub-continent. These insects belong to super family Acridoidea. Acrididae grasshoppers provide an excellent opportunity to apply modern phylogenetic analysis due to their diversity and abundance. These insects are important because they include pests that do a lot of damage to crops and many insects that we don't recognize much about. Even though the phylogeny of this group is studied from different angles and taxonomic levels, there are still many questions that haven't been answered (Song & Buchel, 2010). These include paleontological divergence, biogeography, and where they live. The systematic of grasshoppers still have not been figured out in many parts of the world, given how they are spread out (Song & Buchel, 2010). To the best of my knowledge, there hasn't been a single reliable work on the Phylogenetics from the area of upper Sindh grasshoppers. This is true whether you look at morphological features, molecular differences, or genetic differences. Considering the lacuna survive in the phylogenetic studies on upper Sindh grasshoppers and analyze phylogenetic relationships among different species of

Acridids classify under the families Acrididae. This has been carried out in two planes. The first one is using biochemical techniques to point out the genetic differences between different species of grasshoppers. The second is to study the phylogenetic relationship based on how similar and different their genes are. The SDS-PAGE has been used as a powerful tool that helps understand genetic differences by separating protein bands based on how strong they are and how much they weigh. This method is used a lot in modern biochemistry and molecular biology to look at differences in proteins in haemolymph and muscle tissues of insects. Proteins are the most variable when it comes to size, structure and function as well consider the genetic difference as these molecules are assembled by direct involvement of DNA. (Hooper & Thuma, 2005). SDS PAGE technique has been endorsed to be used with confidence in resolve of molecular weight of proteins (Weber & Osborn, 1969) for different purpose because the changeable structure and function of these molecules. Almost each single cell has specific proteins responsible for intracellular convey and contractile exercise. These molecules have stayed the same for a long time and have changed slowly in insects. (Hooper & Thuma, 2005). Phylogenetic trees have been made using motor

proteins like myosin, kinesin, and dynein to figure out how Arthropods are related (Ronitz *et al.*, 2009) another muscle proteins like actin (Mounier & Sparrow, 1993). MRF families have been generally used in phylogenetic studies of dissimilar insect groups (Oota & Saitou, 1999). The great diversity proposes that studying of invertebrate muscle proteins and genes can be tried to resolve phylogenetic relationship and evolution (Hooper & Thuma, 2005; Ohta, 1991). As such it could be very useful to routinely identify difficult taxa of economic and medical importance. However, despite these highly positive claims, DNA barcoding also seems to suffer from a number of potential limitations when used for the identification of insects. The main aim of studying hereditary diversity in this work is to acknowledge the scope of differentiation between the species of grasshoppers and try it to sort out the phylogeny. DNA molecule is the base of hereditary differences between the species or specific. By comparing the electrophoretic profiles, it is easy to see how the species in question are different. Random Amplified Polymerase DNA was first reported by (Welsch & McClland, 1990; Williams *et al.*, 1993). DNA was first used, the polymerase chain reaction technique has changed Molecular Biology and become a powerful tool with many applications. RAPD markers are generally used in insects affiliated research that comprise molecular finger printing (Fakrudin & Patil, 2005; Senthil Kumar & Gurusubramanian, 2011), phylogenetic analysis (Zhou *et al.*, 2000; Fakrudin *et al.*, 2012). In developmental biology and systematic studies phylogenies are central to research. Appropriate phylogenies provide information on patterns and procedure of growth of species.

Material Methods:

Study Sites: Surveys were recorded to assess the relative incidence of the acrididae in different hot plants ecosystem in 10 locations from upper Sindh districts i.e., Kashmore, Jacobabad, Shikarpur, Ghotki, Qambar & Shahdadkot, Sukkur, Khairpur, Larkana, Naushahro Feroz and Dadu, weekly field survey of largely different growing crops and it was conducted in Kharif season (April 2021-October 2021 and April 2022- October 2022) specimens were recorded in drawn times.

Collection of specimens: Adult specimens of acrididae were collected from different localities of the 10 mentioned districts of the upper Sindh province. The Specimens were collected from different habitats i.e. desert areas, semi desert, agricultural land, open ground, shrubs, grasses, herbs, rocky areas, vegetables, sandy areas, along road sides and others. At least 15 random and 15 non-random samples were collected from each area in farmers' field by moving diagonally in the field. The samples were collected from available fresh and Raton crop. Mostly the samples were collected in the morning time from 8 to 11 a.m. and in the evening time from 5

to 7 p.m. To study the diversity of acrididae, sampling was done with the help of insect hand net and hand-picking methods, covering a linear distance of 10 m, replicated four times taking different locations. Collected material was put into polythene bags or in plastic bottles.

Killing and preservation: After collection, the specimen were brought to Entomological and Bio control Research Laboratory (EBCRL-Laboratory) Department of Zoology, University of Sindh, Jamshoro then killed and preserved (Vickery & Kevan, 1983) and Standardized Entomological Method (Riffat & Wagan, 2012&2015). As usual, killing and pinning were done quickly after coming back from the field. This was done on the pronotum and slightly to the right of the median carina of the pronotum, behind the transverse sulcus. On the stretching board, the head was slightly pointed down. The long axis of the left wings was set at a right angle to the pins. There was little chance of breaking, and the back legs were bent under the body to take up the small space. The belly was pushed down by the wings. Specimens could be handled more easily if they were softened by spending 24 hours in the freezer. Soft specimens could be stretched, moved, and pinned more easily than hard specimens.

Results

The total seven species were collected from cultivated, sandy, rocky, vegetation, grassy, desert, semi desert, road side and open area of the upper Sindh and sort out in the Acrididae family and three sub-families Acridinae, Oedipodinae and oxyinae (Figure 01). More over maximum number of specimens collected belong to *Oxya hyla hyla* (24%) followed by *Acrida willemsei* (19%), *Truxalis eximia* (15%), *Aiolopus thalassinus thalassinus* (13%), *Acrotylus longipes longipes* (10%), *Aiolopus simulatrix* (10%) and *Sphingonotus savignyi* (09%) belong to Acrididae as shown in (Figure 02).

Acrida willemsei (Dirsh, 1954)

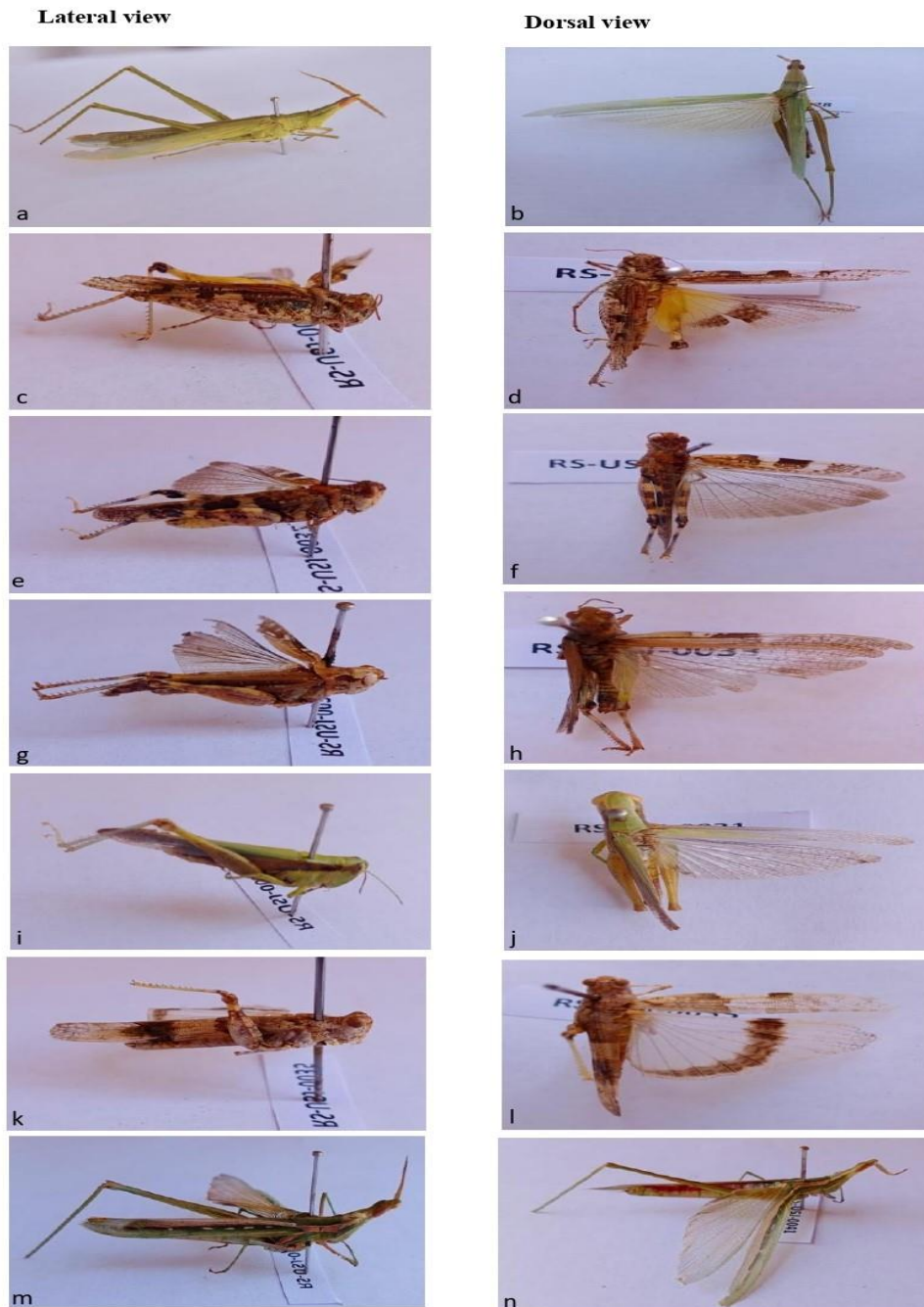
Morphological Description: This specimen body color is green and yellowish. Antennae are 17–18 segments, almost 13.5 mm and sword like structure and head of this conically and elongated in shape. Head length is shorter than the length of pronotum (Head 12 mm & Pronotum 10.5 mm). This specimen's tegmina are larger 47 mm than the wings in the length 42 mm. It has almost 29 spines on its leg and this specimen total length of body approximately 56 mm in size. Acridid grasshoppers eat many different kinds of plants, but most species only eat a few as their main source of food. Locusts' species are change color and act differently when there are a lot of them. They are a significant category of Orthopterous pests from an economic point of view that attack both cultivated and uncultivated crops and morphometric of various body parameters shown in table 1.

Economic Importance: From the upper Sindh region of Pakistan, they do the most damage in the summer

and spring, and their numbers also rise after it rains. This costs money not only to Sindh and Pakistan, but

also to other countries. It hurts fodder crops, plants, jowar, and usually Bermuda grass.

Figure 1. (a-b) *Acridawillemsei* (c-d) *Acrotyluslongipeslongipes* (e-f) *Aiolopussimulatrix* (g-h) *Aiolopusthalassinusthalassinus* (i-j) *Oxyahylahyla* (k-l) *Sphingonotussavignyi* (m-n) *Truxaliseximiaeximia*



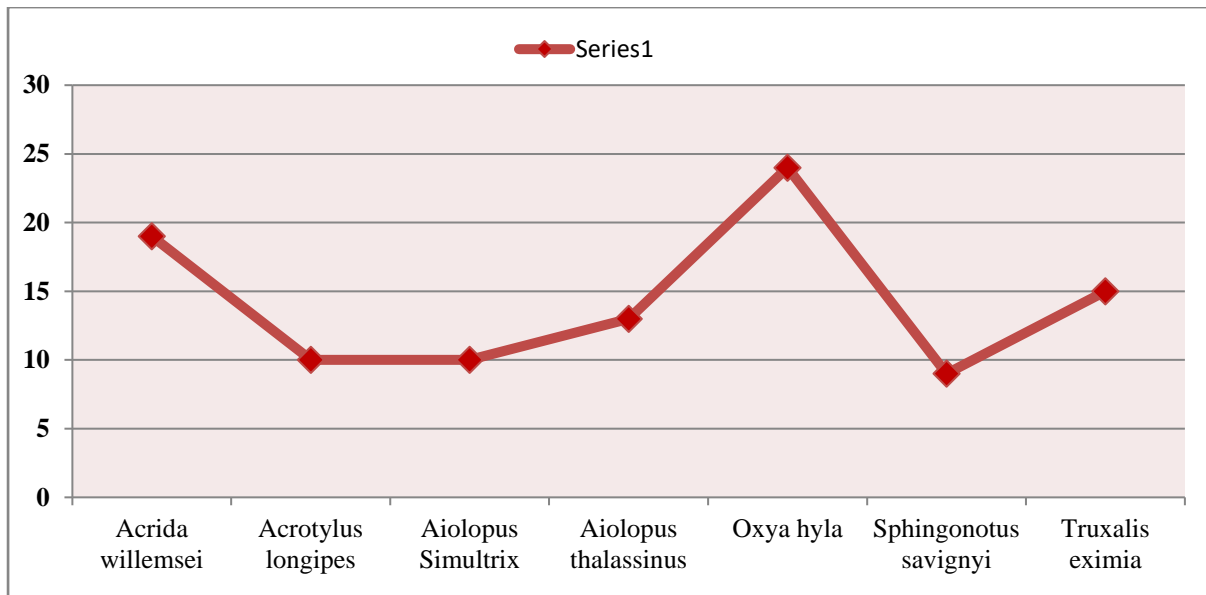


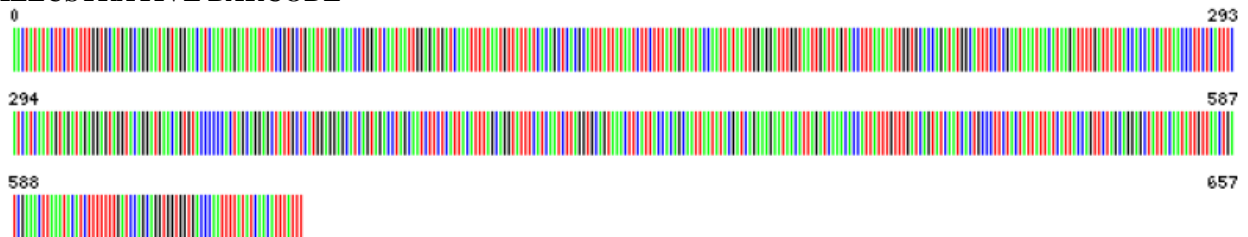
Figure 2. A number of specimens were collected from various localities of upper Sindh.

Table 1. Morphometric analysis of various body parameters of *Acrida willemsei*

Body Parameter	Male
	Mean \pm SD (n5)
Length of head	12.07 \pm 0.12
Length of antenna	12.05 \pm 0.1
Length of pronotum	10.52 \pm 0.08
Length of Femur	35.91 \pm 0.38
Length of tibia	32.684 \pm 0.26
Length of wings	42.424 \pm 0.38
Length of tegmina	47.52 \pm 0.50
Distance between compound eyes	1.226 \pm 0.02
Total body length	56.794 \pm 0.47

Genomic observation

ILLUSTRATIVE BARCODE



Nucleotide sequence:

AACTATATACTTCTTATTTGGTGCATGAGCAGGAATAGTAGGAACATCAATAAGAATAATTATCCG
 TGCTGAATTAGGACAACCTGGATCAATAATTGGAGATGATCAAATTTATAATGTTATTATCACAGC
 TCACGCATTTATTATAATCTTCTTTATAGTAATACCAATTATAATTGGAGGATTTGGTAATTGATTA
 GTACCTTTAATAATTGGTGCACCAGATATGGCATTTCCTCGAATAAATAACATAAGATTTTGATTA
 TTACCACCATCATTAACCTTCTCATTTTCATCATCAATAGTAGATAGAGGAGTAGGTACAGGATGA
 ACAGTGTACCCCCACTAGCAGGAGCTATTGCTCATGGAGGAGCATCAGTAGACCTAGCAATCTTC
 TCATTACATTTAGCAGGTATTTTCATCAATCTTAGGTGCAGTAACTTCATTACCACAGCAATTAATA
 TACGATCAGAAAGAATAACATTAGATCAAACACCATTATTTGTTTGATCAGTATCAATCACTGCCC
 TTCTACTATTATTATCATTACCAGTTCTAGCAGGAGCTATTACAATATTGTAACTGATCGAAACTT
 AAATACATCTTTTTTTGATCCAGCAGGTGGTGGTGACCCAATTTTATATCAACATTTATTT

Aminoacid Sequence:

TMYFLFGAWAGMVGTSMSMIIRAELGQPGSMIGDDQIYNVIITAHAFIMIFFMVPIMPIMIGGFGNWLVLPL
 MIGAPDMAFPRMNNMSFWLLPPSLTLLISSMVDSGVGTGWTVPPLAGAIHGGASVDLAIIFSLHLA

GISSILGAVNFITTAINMRSESM TLDQTPLFVWSVSITALLLLSLPVLGAI TMLLTDRNLNTSFFDPAG
 GGDPILYQH L F

Acrotylus longipes longipes (Charpentier, 1845)

Morphological Description: Small to medium body that is hairy and rough. Filiform antennae with 23-24 antennal segments. Head thicker, vertex concave with lateral carinulae and angular. Pronotum less or more smooth on disc, phthisic, short, narrow in prozona, distinct and lateral carinae irregular tuberculate. Wings and tegmina fully developed. Wings hyaline, without dark bands and yellow or orange at the base. Tegmina apex are noticed without dark speckles. Short ovipositor having curved valves straight and conical. Light yellowish brown body and dirty brown

in color. Brown antennae and base of antennae with spots. Pronotum with brownish speckles. Head brown with lower part whitish. Wings transparent with some dark veins. Hind tibia brownish or sometime found bluish and morphometric of various body parameters shown in table 2.

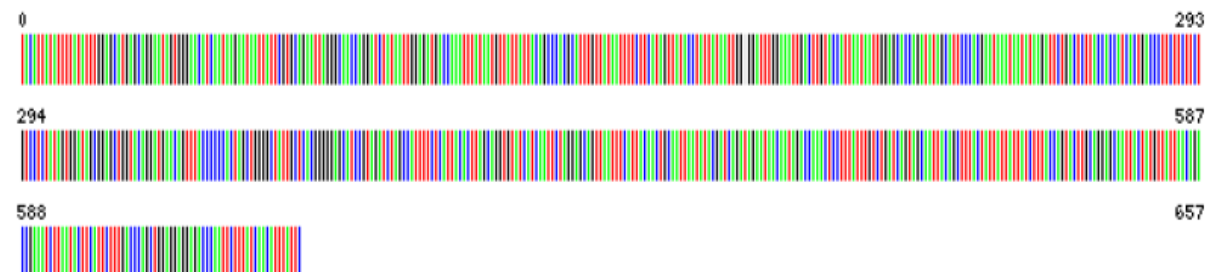
Economic Importance: This species of great economic importance because they found an importance group of pest and pose a constant threat to cereal crop, orchards, vegetables, grassland species.

Table 2. Morphometric analysis of various body parameters of *Acrotylus longipes longipes*

Body Parameter	Male
	Mean ± SD (n5)
Length of head	2.69 ±0.02
Length of antenna	3.906 ±0.20
Length of pronotum	3.082 ±0.08
Length of Femur	12.45 ±0.44
Length of tibia	10.154 ±0.08
Length of wings	19.5 ±0.5
Length of tegmina	17.18 ±0.22
Distance between compound eyes	0.882 ±0.03
Total body length	22 ±0.4

Genomic observation

Illustrative Barcode



Nucleotide Sequence

TACATTATATTTTATATTTGGAGCATGAGCAGGAATAGTGGGAACATCAATAAGAATAATTATTCG
 TGCAGAATTAGGGCAACCAGGATCTATAATTGGAGATGACCAAATTTATAATGTTATTATTACAGC
 CCACGCATTTGTTATAATTTTCTTCATAGTTATACCTATTATAATTGGNGGATTTGGAAATTGACTT
 GTACCATTAATAATTGGAGCACCAGATATAGCATTCCACGAATAAATAATATAAGATTCTGACTC
 TTACCACCATCACTGACCCTTCTTCTTCTGTCTCTATAGTGGATAGCGGAGCTGGTACAGGATGA
 ACAGTTTACCCCCACTAGCTGGGGCTATTGCTCACGGGGGAGCATCCGTAGATCTAGCCATTTTC
 TCATTACACTTAGCAGGTGTATCATCAATTCTAGGAGCAGTTAATTTTATTACA ACTGCAATTAAT
 ATACGATCAGATAGAATAACAATAGACCAA ACTCCTTTATTTGTCTGATCAGTAGCAATTACAGCT
 TTACTATTATTATTATCTTTACCAGTACTTGCAGGAGCAATTACTATGTTATTAACAGACCGAAATC
 TTAATACTTCATTCTTTGACCCAGC

Amino Acid Sequence

TLYFMFGAWAGMVGTSMSMIIRAELGQPGSMIGDDQIYNVIITAHAFVMIFFMVMPI MIXGFGNWLVP
 LMIGAPDMAFPRMNNMSFWLLP PSLTLLLLSSMVDSGAGTGWTVY PPLAGAI AHGGASVDLAI FSLHL
 AGVSSILGAVNFITTA INMRSDSMTMDQTPLFVWSVAITALLLLSLPVLGAI TMLLTDRNLNTSFFDP
 AGGGDPILYQH L F

***Aiolopus simulatrix* (Walker, 1870)**

Morphological Description: Antenna with 20-24 antennal segments. Usually medium size in body. Slightly saddle shaped pronotum, constricted in prozona. Wings hyaline, slightly opaque at apex. Body color paler, greenish brown, brownish or dusty brown. Brown color antenna. Head reddish brown. Fastigium of vertex green, brownish or pink reddish. Transparent tegmina, brownish with irregular blackish

speckels, wings hyaline and colorless, morphometric of various body parameters shown in table 3.

Economic Importance

This species noticed the serious pest of grain, and also sometime other crops, many parts of its range by the most important species as a pest of grain crops in the different localities of Sindh, Pakistan, cultivated, sandy, rocky, desert and others.

Table 3. Morphometric analysis of various body parameters of *Aiolopus simulatrix*

Body Parameter	Male
	Mean ± SD (n5)
Length of head	3.148 ±0.008
Length of antenna	6.17 ±0.14
Length of pronotum	4.008 ±0.14
Length of Femur	12.61 ±0.38
Length of tibia	10.116 ±0.80
Length of tarsi	2.906 ±0.49
Leg spins	11.6 ±0.54
Length of wings	20.84 ±0.21
Length of tegmina	21.66 ±0.15
Distance between compound eyes	1.012 ±0.04
Total body length	22.78 ±0.69

Genomic Observation

Illustrative Barcode



Nucleotide Sequence

CGAGCAGAATTAGGACAACCAGGATCAATAATTGGAGATGATCAAATCTATAATGTAATTATTAC
 AGCACATGCATTCGTTATAATTTTCTTTATAGTTATAACCAATTATAATTGGTGGATTTGGAAATTGA
 CTTGTACCATTAATAATTGGAGCACCAGATATAGCATTTCACGAATAAATAACATAAGATTTTGA
 CTTTTACCACCATCATTAAATTTACTCATTTCGTCATCTATGGTTGATAACGGAGCTGGTACGGGTT
 GAACAGTTTACCCCCACTCGCAGGAGCAATTGCACACGGGGGAGCATCAGTTGATCTAGCAATTT
 TCTCACTACATTTAGCAGGTATTTTCATCAATTCTAGGAGCAGTTAATTTTCATTACAACAGCAATTA
 TATACGATCAGAAAGAATAACTATAGATCAAACACCCTTATTTGTTTGATCAGTAGCAATTACAGC
 ATTATTACTTTTATTATCATTACCAGTTTTAGCCGGAGCTATTACAATATTATT

Amino Acid Sequence

RAELGQPGSMIGDDQIYNVIITAHAFVMIFFMVMPIGIGFGNWL VPLMIGAPDMAFPRMNNMSFWLL
 PPSILLISSMVDNGAGTGWTVYPPLAGAIAHGGASVDLAI FSLHLAGISSILGAVNFITTA INMRSESM
 TMDQTPLFVWSVAITALLLLSLPVLGAI TMLX

***Aiolopus thalassinus thalassinus* (Fabricius, 1781)**

Morphological Description: Antenna filiform with 21-23 antennal segments. Body usually observed medium in size. Pronotum longer than head subconical. Pronotum slightly saddle shaped. Angled, long, and concave at the tip, with well-developed lateral carinulae. The middle part of the tegmen, where the intercalary vein is fully formed. Messosternal interspace almost square. Both wings and tegmina grew. The tips of the wings are a bit dusty. The back femur is long and thin. The dorsal carina is smooth, and the dorsal genicular lobes are

round. The colour of the body is lighter, browner, greener, or dusty brown. Head reddish brown. The tip of the fastigium is green, brownish, or pinkish red. Tegmina is clear, brown, and has irregular black spots. Colorless and hyaline wings. Darker brownish on the back femur. Straw-colored hind tibia and morphometric of various body parameters shown in table 4.

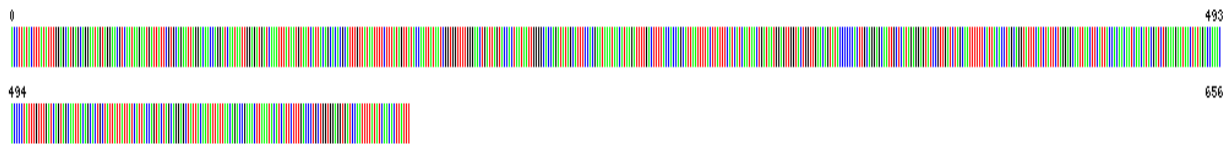
Economic Importance: This pest is generally a minor pest, through it sometimes does considerable damage and especially this was attack in the seeding stage.

Table 4. Morphometric analysis of various body parameters of *Aiolopus thalassinus thalassinus*

Body Parameter	Female
	Mean ± SD (n5)
Length of head	2.85 ±0.015
Length of antenna	5.25 ±0.60
Length of pronotum	3.95 ±0.34
Length of Femur	12.8 ±0.38
Length of tibia	10.16 ±0.80
Length of wings	19.84 ±0.21
Length of tegmina	21.66 ±2.15
Distance between compound eyes	1.12 ±0.04
Total body length	22.78 ±0.69

Genomic observation

Illustrative Barcode



Nucleotide Sequence

ACCTTATACTTTATATTTGGAGCATGAGCAGGAATAGTAGGAACGTCAATAAGAATGATTATTCGT
 GCAGAATTAGGACAACCAGGATCAATAATTGGAGATGATCAAATTTATAATGTAATCATTACAGC
 ACACGCATTTGTTATAATTTTCTTTATAGTTATAACCAATTATAATCGGTGGTTTTGGGAACGATTA
 GTACCACTAATAATTGGGGCACCAGATATAGCATTTCCACGAATAAATAACATAAGATTTTGACTT
 TTACCACCAGCATTAAATTTTACTTATTTTCATCATCAATAACTGATAATGGAGTTGGTACTGGTTGAA
 CAGTATACCCCCCACTTGCAGGAGCAATTGCTCATAGAGGAGTATCCGTTGATCTAGCAATTTTTT
 CATTACACCTAGCAGGTATTTTCATCTATTCTAGGAGCAATTAACTTCATTACCACAACAATCAACA
 TACGATCTGAAAGAATAACTATAGACCAACACCCCTATTTGTTTGATCAGTAGCAATTACAGCAC
 TGCTATTATTATTACATTACCAGTACTAGCAGGAGCTATTACAATATTATTAACAGACCGAAACT
 TAAATACATCATTCTTTGACCCTGCTGGTGGAGGTGATCCAATTTTATATCAACACTTATTT

Amino Acid Sequence

TLYFMFGAWAGMVGTSMSMIIRAELGQPGSMIGDDQIYNVIITAHAFVMIFFMVMPIGIGFGNWLVP
 LMIGAPDMAFPRMNNMSFWLLPPLALLISSMTDNGVGTGWTVYPPLAGAIHSGVSVDLAIFSLHLA
 GISSILGAINFITTTINMRSESMTMDQTPLFVWSVAITALLLLSLPVLGAIITMLLTDRLNNTSFFDPAG
 GGDPILYQHFLF

***Oxya hyla hyla* (Serville, 1831)**

Morphological Description

Antennae filiform and 24 – 26 segments. Medium size in body, green to paler green in color. Fastigium of vertex lack mid-longitudinal carina. Pronotum longer than head shorter. Flattened pronotum and very narrowing forward rounded posteriorly. On the back side of the pronotum, there are three transverse sulci. Prosternum conical in shape. Mesosternum open type. Wing and tegmina well developed. Femur shape looks cylindrical. Tibia expanded in apical half. This species body color green to paler green, eyes and antennae brownish in color. Wings are hyaline. Light green

femur, tibia white in color and morphometric of various body parameters shown in table 5.

Economic Importance

Of considerable economic importance in upper Sindh, causing the most damage in September and early October when the rice fields seem to be huge of this one. It is principally a pest of rice. Mostly damage to rice is caused when the young adults appear in late summer in large numbers. Later they scatter and lose their importance as pests. Barley, cotton, grass, crow foot grass and desert grass but also samples were collected in Pearl millet and jowar were noticed pest that causes become huge loses.

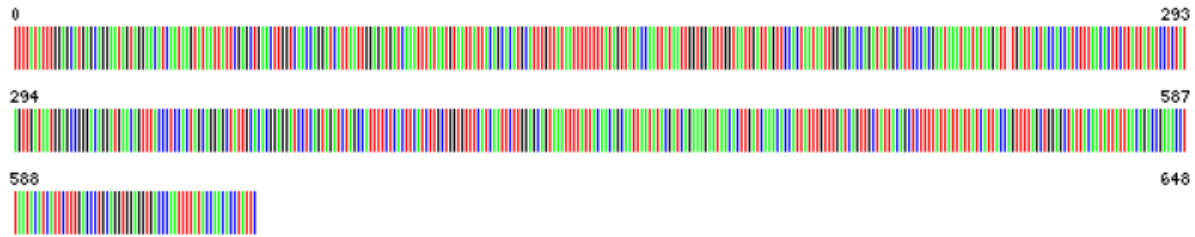
Table 5. Morphometric analysis of various body parameters of *Oxya hyla hyla*

Body Parameter	Male
	Mean ± SD (n5)
Length of head	4.356 ±0.04
Length of antenna	6.4 ±0.20
Length of pronotum	6.062 ±0.04
Length of Femur	17.504 ±0.50
Length of tibia	14.7 ±0.25

Length of wings	24.36 ±0.27
Length of tegmina	27.36 ±0.43
Distance between compound eyes	0.852 ±0.05
Total body length	33.134 ±0.39

Genomic observation

Illustrative Barcode



Nucleotide Sequence:

TTTTATATTTGGAGCATGAGCAGGAATAGTAGGAACATCAATAAGTATAATTATTCGAGCTGAACT
 TGGTCAACCAGGATCATTAAATTGGAGATGATCAAATTTATAATGTAATTATTACAGCACATGCATT
 TGTATAAATTTTTTTATAGTTATACCAATTATAATTGGTGGATTTGGTAATTGATTAGTTCCACTA
 ATAATTGGAGCACCAGATATAGCATTCCCACGAATAAATAATATAAGATTNTGATTACTACCACCA
 TCTTTAACACTTCTTATTATATCCTCTATAGTTGATAATGGAGCCGGGACAGGATGAACAGTTTACC
 CTCCACTAGCAGGAGCTATTGCACACGGAGGATCCTCAGTAGATCTAGCCATTTTCTCACTTCATC
 TTGCTGGTGTTCATCAATTCCTGGAGCAGTAAATTTTATTACAACAGCAATTAATATACGATCAG
 AAAGAATAACACTTGATCAAACACCATTATTTGTTTATGATCAGTTGCTATTACAGCTCTTTTATTATT
 ATTATCATTACCAGTTTTAGCTGGAGCTATTACAATATTATTAACAGACCCGAAACCTTAATACATC
 ATTCTTTGACCCTGCAGGTGGAGGTGACCAATTTTATACCAACACCTATTC

Amino Acid Sequence

FMFGAWAGMVGTSMSMIIRAE LGQPGSLIGDDQIYNVIITAHAFVMIFFMVMPI MIGGFNWL VPLMIG
 APDMAFPRMNNMSXWLLP PSLTLLIMSSMVDNGAGTGWTVYPPLAGAI AHGGSSVDLAI FSLHLAGV
 SSILGAVNFITAINMRSE SMTLDQTPLFVWSVAITALLLLSLPVLAGAITMLLTDRNLNTSFFDPAGG
 GDPILYQHFLF

***Sphingonotus savignyi* (Saussure, 1884)**

Morphological Description: Filiform antennae having 26–28 antennal segments. Small to medium sized body. Lateral and median carinulae on the vertex. Paler brown body with whitish bands. Three transverse sulci, lateral carinae, and median carinae are linear on the dorsal side of the pronotum. Pronotum posterior margin obtuse angular. Tegmina, which are long and narrow, have two brown stripes at the base. Wings have a transverse band. Femur is cylindrical, and the arolium is small. At base wings are colorless. Femur yellow in color on inner side. Tibia pale and bluish. Reddish-gray body with dark and white spots. White head, brown antennae with light rings. Tegmina colorless in apical third.

Colorless, clear wings with a dark, narrow, curved fascia and dark spots at the apex. The inside of the femur is pale buff, and the fascia is black. The back tibia is yellow and the inside of the bases are black and morphometric of various body parameters shown in table 6.

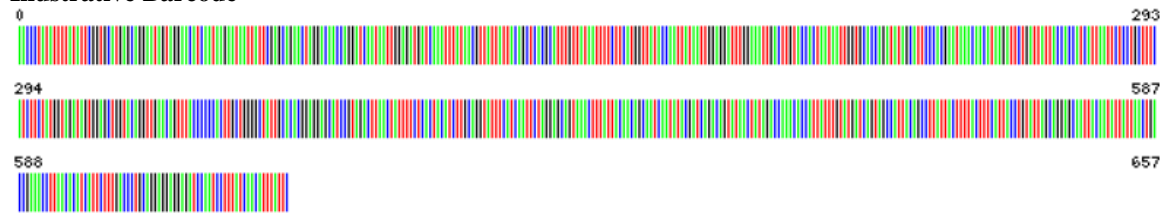
Economic Importance: This species feed the wide range of food plants this is found in rocky, sandy and vegetation. Nymphal stages are more epidemic than no functional wings to fly and all time are eating vigorously. Mostly they feed on blades of short grasses and seeding of crops consumes large biomass. This is direct effect on the economics.

Table 6. Morphometric analysis of various body parameters of *Sphingonotus savignyi*

Body Parameter	Male
	Mean ± SD (n5)
Length of head	2.56 ±0.11
Length of antenna	5.318 ±0.68
Length of pronotum	4.424 ±0.08
Length of Femur	11.23 ±0.17
Length of tibia	9.54 ±0.38
Length of wings	24.62 ±0.39
Length of tegmina	25.72 ±0.46
Distance between compound eyes	1.034 ±0.01
Total body length	24.564 ±0.38

Genomic observation

Illustrative Barcode



Nucleotide Sequence:

AACCCTATATTTTATATTCGGTGCATGAGCAGGAATAGTAGGAACATCAATAAGAATAATTATTCG
 AGCAGAACTAGGACAACCAGGATCAATAATTGGAGATGATCAAATTTATAACGTTATTATTACAG
 CTCACGCATTTGTTATAATTTTCTTCATGGTTATACCAATTATAATTGGAGGATTTGGAAATTGACT
 TGTACCACTAATAATTGGTGCACCAGATATAGCATTCCCACGAATAAACACATAAGATTCTGATT
 ATTACCACCATTAATTCTCCTGCTTTCATCTTCTATGGTAGATAGTGGAGCTGGTACAGGTTGA
 ACAGTTTACCCCTTGTCTGGGGCTATTGCACACGGAGGAGCATCCGTAGACTTAACTATTTTC
 TCACTACATCTTGCAGGTATTTTCATCAATTCTAGGAGCAGTAACTTTATTACAACAGCAATCAAT
 ATACGATCAGACAGTATAACTATAGACCAAACACCATTATTTGTATGATCAGTAGCCATTACAGCC
 TTATTACTTTTACTTTTCATTACCTGTATTAGCAGGAGCAATTACAATATTATTAAGTACCGAAACC
 TTAACACATCATTCTTTGACCTGCAGGAGGAGGAGATCCAATCCTTTATCAACATTTATTC

Amino Acid Sequence

TLYFMFGAWAGMVGTSMSMIIRAELGQPGSMIGDDQIYNVIITAHAFVMIFFMVMPIIMIGGFGNWLVP
 LMIGAPDMAFPRMNNMSFWLLPSSLILLSSMVDGAGTGWTVYPPLAGAIHGGASVDLTIFSLHLA
 GISSILGAVNFITAINMRSDSMTMDQTPLFVWSVAITALLLLSLPVLGAIITMLLTDRNLNTSFFDPA
 GGGDPILYQHLF

***Truxalis eximia eximia* (Eichwald, 1830)**

Morphological Description: Ensiform antennae with 18 segments. The body is big, long, and almost stick-like. It is green, and a pale line runs from behind the eyes on each side of the head, along the pronotum and tegmina. The head is longer than the pronotum, and the vertex is narrower from the front. A posterior sulcus crosses the dorsal side of the pronotum. Medium size of arolium. Hind femur elongate and narrow. Female's wings are purplish blue from dorsal side. A pale-colored hind tibia has 25–28 black-tipped spines on either side. The entire body is green with a pale line on either side of the head starting from

behind the eyes. Reddish brown eyes, antennae, and tegmina. Hyaline wings in females are purple blue on the dorsal side. Dusty brownish color and an angular apex on the hind femur. Brownish or black-tipped spines on either side of the tibia and morphometric of various body parameters shown in table 7.

Economic Importance: This species harmful for the agricultural crops, forests, vegetables, orchards and also wide varieties of fruits this attack mostly in summer seasons and also spring seasons and this way happen the economic loss of the agricultural field and attack become on the crops, vegetables, grasses, jowar and others

Table 7. Morphometric analysis of various body parameters of *Truxalis eximia eximia*

Body Parameter	Female
	Mean ± SD (n5)
Length of head	11.75 ±0.30
Length of antenna	19.05 ±0.65
Length of pronotum	10.44 ±2.05
Length of Femur	41.23 ±2.25
Length of tibia	38.5 ±3.05
Length of wings	51.62 ±3.05
Length of tegmina	53.5 ±3.06
Distance between compound eyes	1.26 ±0.18
Total body length	63.2 ±2.75

Genomic Observation

Illustrative Barcode



Nucleotide Sequence

AACTATATACTTCTTATTTGGTGCATGAGCAGGAATAGTAGGAACATCAATAAGAATAATTATTCCG
TGCTGAATTAGGACAACCTGGATCAATAATTGGAGATGATCAAATTTATAATGTTATTATCACAGC
TCACGCATTTATTATAATCTTCTTTATAGTAATACCAATTATAATTGGAGGATTTGGTAATTGATTA
GTACCTTTAATAATTGGTGCACCAGATATGGCATTTCCTCGAATAAATAACATAAGATTTTGATTA
TTACCACCATCATTAAACCCTTCTCATTTTCATCAATAGTAGATAGAGGAGTAGGTACAGGATGA
ACAGTGTACCCCCACTAGCAGGAGCTATTGCTCATGGAGGAGCATCAGTAGACCTAGCAATCTTC
TCATTACATTTAGCAGGTATTTTCATCAATCTTAGGTGCAGTAAACTTCATTACCACAGCAATTAATA
TAGCATCAGAAAGAATAACATTAGATCAAACACCATTATTTGTTGATCAGTATCAATCACTGCC
TTCTACTATTATTATCATTACCAGTTCTAGCAGGAGCTATTACAATATTGTTAACTGATCGAAACTT
AAATACATCTTTTTTTGATCCAGCAGGTGGTGGTGACCCAATTTTATATCAACATTTATTT

Amino Acid Sequence

TMYFLFGAWAGMVGTSMSMIIRAELGQPGSMIGDDQIYNVIITAHAFIMIFFMVPIMIGGFNWLVLPL
MIGAPDMAFPRMNNMSFWLLPSSLTLLISSMVDSGVGTGWTVYPPLAGAIHGGASVDLAIIFSLHLA
GISSILGAVNFITAINMRSEMTLDQTPLFVWSVSITALLLLSLPVLGAIITMLLTDRNLNTSFFDPAG
GGDPILYQHLF

Discussion

DNA bar-coding measures an organism's level of DNA sequence similarity to a set of reference species in order to identify it. Typically, the mitochondrial COI gene segment amplified is used for this purpose amplified by the "universal primers" of (Folmer *et al.*, 1994). DNA bar-coding is normally examined as a reliable, cost-effective and easy molecular identification tool with a wide applicability across metazoan taxa (Hajibabaei *et al.*, 2006; Hebert *et al.*, 2004). Therefore, routinely identifying challenging taxa of economic and medical significance could be very helpful. This is especially true for many insect taxa that include a lot of well-known pests or disease-carrying insects, whose identification often requires very advanced taxonomic skills. In addition, DNA bar-coding could be pivotal for the identification of certain life stages (*e.g.* eggs, larvae, nymphs or pupae), which are often impractical to recognize otherwise. Although more actually, the truthful of insect DNA bar-coding may be questioned because insects involve >1,000,000 recount species and likely millions of still undescribed taxa (Footit, 2009). This exuberant species richness may, indeed, severely drive the ability of the DNA barcode reference databases to adequately constitute the enormous insect taxonomic diversity. Yet, other studies questioned the adequacy of DNA barcoding in Orthoptera (Trewick, 2007). The present study advocates the collection of multiple specimens from various geographical positions and the cohort of more DNA barcode data would facilitate the actual diversity of this Acrididae.

Conclusion

In this study we studied the morphological description, economic importance, and DNA sequence of seven Acrididae species from Sindh. Moreover, this study will be a source for species identification through DNA sequence. The present study suggests the collection of multiple specimens from different geographical locations and the generation of more DNA barcode data would facilitate the actual diversity amongst this taxon.

References

- Fakrudin, B., & Patil, B.V. (2005). Studies on genomic flux and molecular analysis of insecticide resistance in cotton bollworm, *Helicoverpa armigera* (Hbner) occurring in south Indian cotton ecosystems. — Final Report submitted to Department of Biotechnology, Government of India.
- Fakrudin, B., Prakash, S. H., Krishna reddy, K. B., Vijay kumar., Badari prasad, P. R., Patil, B. V., & Kuruvinashetti, M. S. (2004). Genetic variation of cotton bollworm, *Helicoverpa armigera* (Hbner) of south Indian cotton eco-system using RAPD markers. *Curr. Sci.* **87**, 1654–1657.
- Folmer O., Black M., Hoeh W., Lutz R., & Vrijenhoek R. (1994). DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol*, **3**, 294–299.

- Footitt, R. G., & Adler, P. H. (2009). Eds: *Insect biodiversity, science and society*. Chichester, UK: Wiley-Blackwell.
- Hajibabaei, M., Janzen, D. H., Burns, J. M., Hallwachs, W., & Hebert, P. D. N. (2006). DNA barcodes distinguish species of tropical Lepidoptera. *Proc Natl Acad Sci USA*, **103**, 968–971.
- Hebert, P. D. N., Penton, E. H., Burns, J. M., Janzen, D. H., & Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proc Natl Acad Sci USA*, **101**, 14812–14817.
- Hooper, S. L., & Thuma, J. J. B. (2005). Invertebrate muscle: muscle specific genes and proteins. *Physiological reviews*, **85**, 1001-1060.
- Mounier, N., & Sparrow, J. C. (1993). Muscle acting genes in insects. *Comp. Biochem. Physioland. Biochem. Mol. Biol.*, **105**, 231–238.
- Ohta, T. (1991). Multigene families and evolution of complexity. *Journal of Molecular Evolution*, **33**, 34-41.
- Oota, S., & Saitou, N. (1999). Phylogenetic relationship of muscle tissues deduced from super imposition of gene trees. *Molecular Biology Evolution*, **16**(6), 856-867.
- Riffat, S., & Wagan, M. (2012). Review of the genus *Hieroglyphus* Krauss 1877 (*Hemiacridinae: Acrididae: Orthoptera*) with a description of one new species from Pakistan. *Pakistan Journal of Zoology*, **44**(1), 43-51.
- Riffat, S., & Wagan, M. (2015). Grasshoppers and locusts of Pakistan. *Higher Education Commission, Pakistan*, 1-180.
- Ronitz, O. D., Becker, F. S., & Kollmar, M. (2009). Reconstructing the phylogeny of 21 completely sequenced arthropod species based on their motor protein. *BMC Genomics*, **10**, 173.
- Senthil Kumar, N., & Guru Subramanian. (2011). Random amplified polymorphic DNA (RAPD) markers and its applications. *Sci vis*. **11**(3), 116-124.
- Song, H., & Buchelib, S.R. (2010). Comparison of phylogenetic signal between male genitalia and non-genital characters in insect systematics. *Cladistics*, **26**, 23-35.
- Trewick, S. A. (2007). DNA Barcoding is not enough: mismatch of taxonomy and genealogy in New Zealand grasshoppers (Orthoptera: Acrididae). *Cladistics*, **23**, 1–5.
- Vickery, V. R., & Kevan, D.K.M. (1983). A monograph of the orthopteroid insects of Canada and adjacent regions. Vols. I and II. A monograph of the orthopteroid insects of Canada and adjacent regions. **I(II)**.1-13.
- Weber, K., & Osborn, M. (1969). The reliability of molecular weight determination by dodecyl sulfate poly acrylamide gel electrophoresis. *Journal Biological Chemistry*, **244**, 4406-4412.
- Welsh, J., & McClland, M. (1990). Finger printing genomics using PCR with arbitrary primers. *Nucleic Acid Res*, **18**, 7213-7218.
- Williams, J. G., Hannafey, M. K., Rabalski, J. A., & Tingey, S. V. (1993). Genetic analysis using random amplified polymorphic DNA markers. *Methods. Enzymol*, **218**, 704-740.
- Zhou, X., Faktor., Applebaum, S. W., & Coll, M.(2000). Population structure of the pestiferous moth *Helicoverpa assmigerain* the eastern Mediterranean using RAPD analysis. *Heredity*, **85**, 251-256.

Publisher's note: JOARPS remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. To

view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.
