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Morphology and Molecular Phylogeny of Four Acridid Species (Acrididae: Orthoptera) from Sindh

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Abstract: This study has been conducted to explore the four species of Acrididae on the basis of their morphological and molecular studies in Sindh. Surveys were conducted in 10 different districts of upper Sindh province to determine how common acrididae present in different hot plant ecosystems i.e., Dadu, Ghotki, Jacobabad, Kashmore, Khairpur Miras, Larkana, Naushahro Feroz, Qambar & Shahdadkot, Shikarpur and Sukkur, it was conducted a weekly field study of several different growing crops in the Kharif season (April 2021-October 2021 and April 2022 - October 2022) specimens were noticed in drawn times. For morphological study we studies the morphology, general coloration, morphometric variation, global distribution and host plants of four species. For molecular study we got DNA sequences of four species of Acrididae: *Acrida willemsei* belong to subfamily Acridine, *Oxya hyla* belong to subfamily Oxyinae, whereas *Aiolopus simulatrix* and *Aiolopus thalassinus* belong to subfamily Oedipodinae.

Keywords: Acrididae, morphology, phylogeny, distribution, Sindh.

信德省四种蝗虫(蝗科:直翅目）的形态学和分子系统发育

摘要：本研究旨在根据信德省的形态学和分子学研究探索四种蝗科植物。在上信德省的10个不同地区进行了调查，以确定不同热植物生态系统中常见的蝗科。例如，骰子、哥特基、雅各布巴德、卡什莫尔、凯尔布尔米拉斯、拉尔卡纳、瑙沙赫罗费罗兹、坎巴尔和沙赫达科特、希卡普尔和苏库尔，每周对哈里夫季节（2021年4月至2021年10月和2022年4月-2022年10月）标本在规定时间内被发现。对于形态学研究，我们研究了四个物种的形态、一般着色、形态变化、全球分布和寄主植物。对于分子研究，我们获得了四种蝗虫的脱氧核糖核酸 序列：蝗虫属于吖啶亚科，雨蛙属于亚科犀牛亚科，而拟拟鹦鹉和拟南芥属于足亚科.

关键词：蝗科，形态，系统发育，分布，信德.

1. Introduction

Acridids are the most prevalent orthopteran species found in the Pakistan subcontinent, and they are short-horned grasshoppers. These insects are part of the Acridoidea super family. Acrididae grasshoppers provide an outstanding opportunity to apply modern phylogenetic analysis because of their diversity and abundance. These insects dominate because they include pests that do massive damage to different crops and many insects that we don't acknowledge much about. These include biogeography, paleontological divergence, and where they exist. Many locations around the world still don't know how grasshoppers are structured because they are so spread out [1]. However, there has not been more work on the Phylogenetics grasshoppers from the area of Sindh province. This is true whether you look at physical differences, differences at the molecular level, or differences in genes. In consideration of the Sindh grasshopper phylogeny research' lacuna survive and compare the phylogenetic relationships between the different species of Acridids in the Acrididae families. Using biochemical methods to show how the genes of different types of grasshoppers are different and to examine the phylogenetic connection based on how their genes differ and are similar. By classifying protein bands according to their strength and mass, the SDS-PAGE has been used as an efficient technique for understanding genetic variations. This method is often used in modern biochemistry and molecular biology to compare proteins in insect hemolymph and muscle. Proteins are the most different regarding size, shape, and function, and there are also differences in genes because these molecules are put together with the help of DNA. The SDS-PAGE technique has been approved to be used with confidence to determine the molecular weight of proteins [2]. DNA bar-coding measures an organism's level of DNA sequence similarity to the set of reference species to identify it. Typically, the mitochondrial COI gene segment amplified is used for this purpose amplified by the "universal primers" of [3]. DNA bar-coding is normally examined as a reliable, cost-effective and easy molecular identification tool with a wide applicability across the metazoan taxa [4, 5]. DNA bar-coding is one of the most secure and efficient ways to identify organisms, especially compared to identifying new species by knowing their structure characteristics. Identification based on how a species looks isn't real or reliable because environmental and ecological factors can affect the same species in the same way; consequently, poly morphism in Acrididae species is common. Because of polymorphism and phenotypical patterns, the same species may appear to vary, which makes it difficult to identify species. Because this method is so complicated and hard to predict, our identification is not real.

DNA bar-coding must be used to ensure the integrity of the work. DNA bar-coding is a standard method that uses short pieces of the mitochondrial DNA gene, Cytochrome C oxidase submit I (COI), and polymerase chain reaction PCR and gel electrophoresis methods are used to amplify specific COI gene, require sequence data is used to identify unknown species by Blast of NCBI website there are few steps we simply follow we are focused in DNA sequencing. Several trade kits are available for isolating DNA from various biological materials. Varied DNA kits have different polymerase chain reaction (PCR) detection sensitivity levels. Motor proteins like myosin, kinesin, and dynein have been used to form phylogenetic trees. These trees show how Arthropods are related to each other by other proteins muscle like acting [6]. The abundance of species suggests that research into the muscles and genes of invertebrates can help clarify phylogenetic relationships and evolution [7, 8]. The polymerase chain reaction technique has changed Molecular Biology since it was first used. It is now a powerful tool that can be used in many ways. RAPD markers are usually used in insect research, which includes molecular fingerprinting [9, 10] analysis of phylogeny [11, 12]. In developmental biology and systematic studies on phylogenies, suitable phylogenies provide information on the patterns and processes of species growth.

2. Methodology

2.1. Study Sites

Surveys were conducted in 10 different districts of upper Sindh province to determine how common acrididae present in different hot plant ecosystems i.e., Dadu, Ghotki, Jacobabad, Kashmore, Khairpur Miras, Larkana, Naushahro Feroz, Qambar & Shahdadkot, Shikarpur and Sukkur, it was conducted a weekly field study of several different growing crops in the Kharif season (April 2021-October 2021 and April 2022-October 2022) specimens were noticed in drawn times.

2.2. Collection of Specimens

The ten districts of the upper Sindh province's diverse locations were used to collect adult acrididae specimens and the Specimens were collected from different habitats i. e. agricultural land, desert areas, semi desert, shrubs, grasses, rocky areas, vegetables, , sandy areas, herbs, open ground and, along road sides. Moving diagonally around the field, at least 15 random and 15 non-random samples were taken from each part of the farmer's field, and samples from the readily available fresh and Raton crop were also taken. Most of the samples were taken around 8 and 11 in the morning and in the evening 5 to 7 p. m. To study the variety of acrididae, samples were taken with the help of an insect hand net and hand-picking methods, covering a

distance of 10 m in a straight line, repeated four times, also every time in a different location. The specimens that were collected were put in polythene bags or plastic bottles.

2.3. Killing and Preservation

After collecting the specimens in jars and killing them with chloroform as usual, they were quickly killed and pinned on the pronotum and just to the right of the median carina of the pronotum behind the transverse sulcus. On the stretching board head was directed slightly downwards. The left wings were positioned so that the body's long axis formed a right angle with the pins. The rear legs were bent under the body with little chance of breaking and to fill the space. With wings, the abdomen was dropped. If specimens are kept in a decicater for 24 hours, their bodies become soft, allowing for easier stretching, movement, and pinning than with hard specimens.

2.4. Identification of Samples

The collected and persevered pinned acrididae specimens brought from the EBCRL museum for identification process was carried out by the using of stereoscopic dissecting binocular microscope with the help of experts, keys and descriptions available in the literature and on the website "http:://www.orthoptera.org." After collection, samples were identify with standardized methods located in the Entomological & Bio-control Research Laboratory (EBCRL-Laboratory) Department of Zoology, University of Sindh, Jamshoro and we have sent our specimen to know the proper DNA bar-coding to iBOL (International Barcode of Life). In the DNA bar-coding research as usual there are four steps of the coding such as.

Step 1. Isolate DNA from the sample, 2. Amplify the target DNA barcode region using PCR, Step 3. The sequence of the PCR products and steps 4. Compare the resulting sequences are against reference databases to find the matching species.

A procedure will be used to extract intact genomic DNA from the muscle of the hind femur and the primer pair designed for Orthoptera will be consulted while amplifications will be performed using a TaKaRa PCR Thermal Cycler for the PCR product will be subjected for to sequencing after distillation.

3. Results

3.1. *Acrida Willemsei*

3.1.1. Morphology

Antennae are 17–18 segments long, about 13.5 mm wide and have a sward-like structure and are elongated in shape. Pronotum length is longer than head length (Pronotum 10.5 mm and Head12 mm). tegmina are larger in this specimen (47 mm) than in the wings (42 mm) are long. It has almost 29 spins on its leg, and the whole length of this specimen's body is about 56 mm. Although acridid grasshoppers consume various plants, most species only use few them as their primary source of nutrition. When there are masses of a certain kind of locust, they change colour and act differently. From an economic perspective, they are an important group of orthopterous pests that attack both cultivated and wild crops (Table 1, Figure 1).

3.1.2. General Coloration

Due to colour morphism, they can be green or brown, but their bodies are mostly green. The eyes are a golden brown color and the antennae are brown. A small line of green, white, and light yellow can be seen on the head and pronotum Tegmina pointed apically, with or without a row of white spots in the centre. The wings are clear and slightly bigger than the tegmina.

3.1.3. Morphometric

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

Body Parameter	**Mean ± S. D (mm)**	
	Male (n = 05)	**Female (n = 05)**
Length of head	6.14 ± 6.15	12.07 **±** 0.12
Length of antenna	9. 95 ± 0.39	12.05 **±** 0.1
Length of pronotum	5. 08 ± 0.33	10.52 **±** 0.08
Length of Femur	22. 5 ± 0.92	35.91 **±** 0.38
Length of tibia	21.65 ± 0. 52	32.684 **±** 0.26
Antennae Segments	18.42 ± 0.89	20.73 ± 1.32
Length of wings	26.4 ± 1.55	42.424 **±** 0.38
Length of tegmina	28.05 ± 1.71	47.52 **±** 0.50
Distance b/w compound eyes	0.7 ± 0.22	1.226 **±** 0.02
Total body length	33.05 ± 1.62	56.794 **±** 0.47

3.1.4. Global Distribution

This type of grasshopper lives in Asia and is in the family Acrididae and sub family of Acridine the distribution recorded of this species includes India, Malaysia, Southern China, Taiwan and Indi-China [13] and the first person to describe this species was[14]. Since the beginning of time, grasshoppers and locusts have plagued green crops. Sindh has a flourishing agricultural sector owing to its extensive irrigation infrastructure and extensive river system. An extensive survey was conducted in Sindh, Pakistan, to learn more about the types and geographic distribution of grasshoppers and locusts belonging to the Acrididae family. In this study, data on *Acrida willemsei* were gathered from Pakistan's upper Sindh province. The samples were from various environments and host plants.

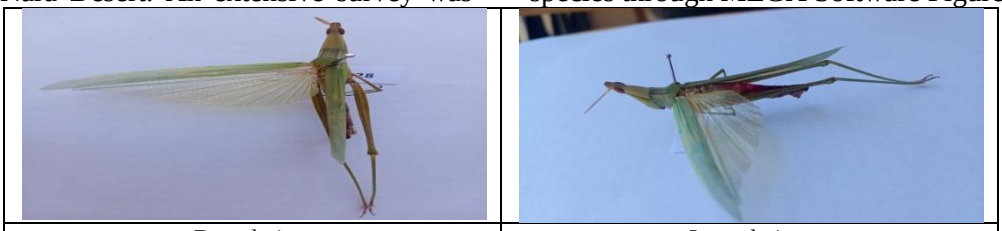
3.1.5. Host Plants

This species was discovered in sand, grass, and by the sides of roadways. The color of the body is identical to the plants. Found in fodder crops and corn (*Zea mays*), Jower (*Sorghum vulgare*) Commonly cause affection to Desert grass (*Panicum turgidum*),

Ziziphus (*Ziziphus nummularia*), milk weed (*Calotropis procera*), Bermuda grass (*Cynodondectylon*), Euphoriba (*Euphorbia neriifolia*) and Indian Bdellium (*Commiphorawightii*).

3.1.6. Remarks

This species was first discovered and recorded from locations in upper Sindh, Pakistan, as well as from various host plants. This species observed similarities almost the same as the *Acrida exaltata*. This species is prevalent on grass, cultivated ground, at the side of roads, and along vegetation-lined channels. It is common and found at various locations in the upper Sindh and the Nara Desert. An extensive survey was conducted in Sindh, Pakistan, to learn more about the types and geographic distribution of grasshoppers and locusts belonging to the Acrididae family. When seen in the field, male specimens exhibited moderate to good activity. In females moved fairly slowly, in contrast, and this species was initially reported by[14] and this species includes India, Taiwan and Indi-China, Malaysia, Southern China [13]. The specimens were collected from different habitats and host plants. There are described sequence producing alignment, Table 2 and tree reconstruction nearest neighbor detail through the iTOL website (Figure 2). Another tree was reconstructed from BIN and nearest neighbor of this species through MEGA Software Figure 3.



Dorsal view | Lateral view

Fig. 1 Dorsal and lateral views of *Acrida willemsei*

Table 2 Sequence producing significant alignment: *Acrida willemsei*

S. No.	Species	Marker	Maximum score	Query cover	Percent identification	Accession Number
01.	*Acridawillemsei*	COI	1181	99%	99.09%	KJ889496.1
02.	*Acridawillemsei*	COI	1175	99%	98.93%	KJ889697.1
03.	*Acrida cinerea*	COI	1147	99%	98.17%	HM180414.1
04.	*Acrida cinerea*	COI	1142	99%	98.02%	KX673195.1
05.	*Acrida bicolor*	COI	865	84%	94.62%	KC261403.1
06.	*Acridaoxycephala*	COI	837	84%	93.73%	KC261404.1
07.	*Amblytropidiamysteca*	COI	793	99%	88.43%	HQ983716.1
08.	*Chorthippuspullus*	COI	776	99%	87.98%	HM422219.1
09.	*Stenobothruslineatus*	COI	774	98%	88.15%	GU706145.1
10.	*Ceracriskiangsu*	COI	773	100%	87.88%	KJ667307.1

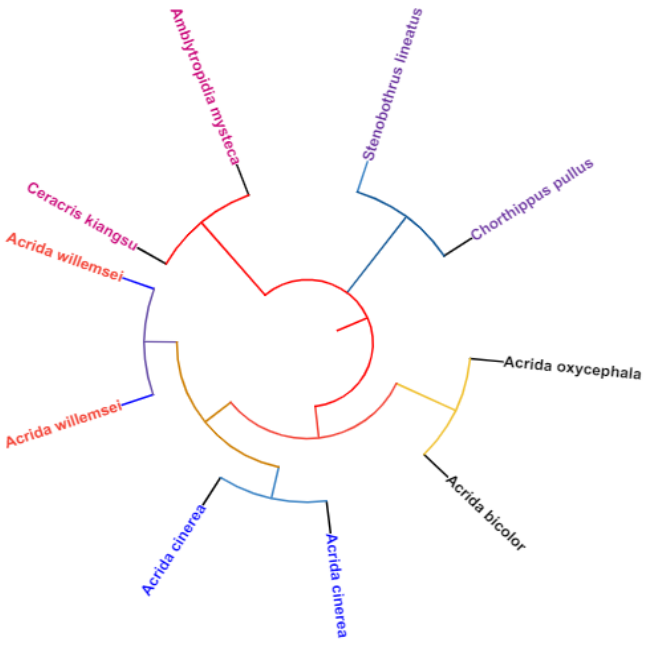


Fig. 2 Tree reconstruction of bin and nearest neighbor detail of *Acrida willemsei*

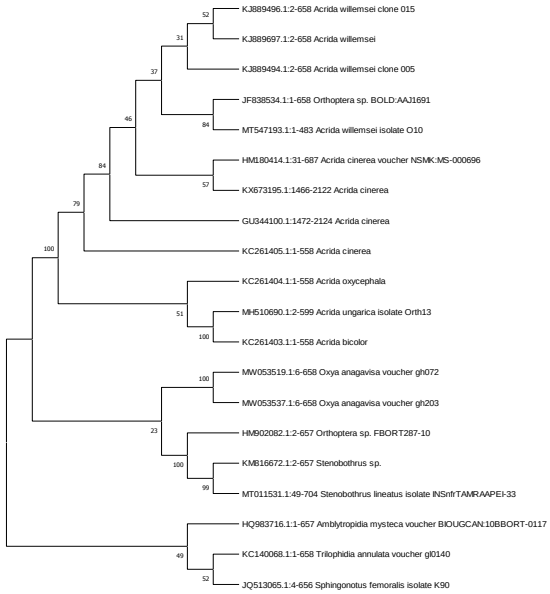


Fig. 3 Tree construction of BIN and nearest neighbor detail of *Acrida willemsei*

3.2. *Oxya Hyla*

3.2.1. Morphology

Filiform antennas with 24–26 segments. Body size

was on the average. The vertex's fastigium lacks a mid-longitudinal carina. The head is shorter than the pronotum. The top of the body is flat, and the back is rounded and gets very narrow in front. On the back of the pronotum, there are three transverse sulci. Prosternum is shaped like a cone. Mesosternum open type. Well-developed wings and tegmina. Femur appears cylindrical in shape. The Tibia grew in the apical half and morphometric of various body parameters shown in Table 3, Figure 4.

3.2.2. General Coloration

Body green to paler green. This species' bodies are green to pale green, and its eyes and antennae are brownish. Wings are hyaline, pale green femur, tibia white.

3.2.3. Morphometric

Table 3 Morphometric analysis of various body parameters of *Oxya hyla*

Body Parameter	**Mean ± S. D (mm)**	
	Male (n = 05)	**Female (n = 05)**
Length of head	4.35 ± 0.04	3.75 ± 0.37
Length of antenna	6.4 ± 0.20	7.8 ± 0.88
Length of pronotum	6.06 ± 0.04	6.05 ± 0.33
Length of Femur	17.5 ± 0.50	16.95 ± 1.05
Length of tibia	14.7 ± 0.25	16.05 ± 0.78
Antennae Segments	25.33 ± 1.16	26.45 ± 1.18
Length of wings	24.36 ± 0.27	27.95 ± 1.32
Length of tegmina	27.36 ± 0.43	30.95 ± 1.15
Distance b/w compound eyes	0.85 ± 0.05	0.88 ± 0.18
Total body length	33.13 ± 0.39	30.15 ± 3.22

3.2.4. Global Distribution

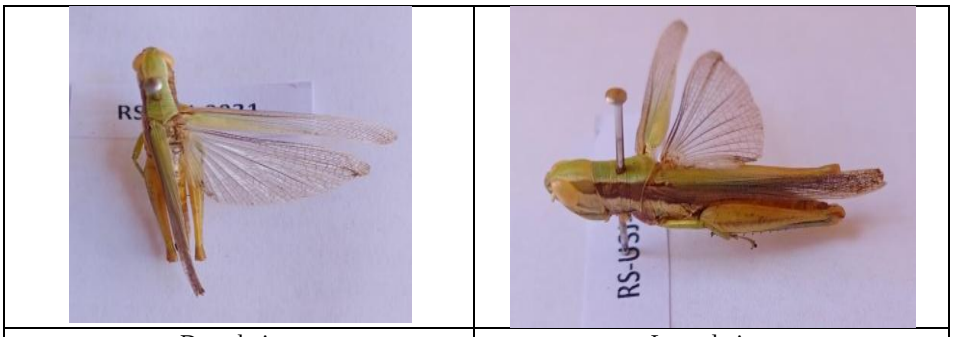
This subspecies are noticed in Afghanistan, Africa, Kenya, Iran, Bangladesh and Pakistan. Its presence in upper Sindh, Pakistan.

3.2.5. Host Plants

A fair number of this species' specimens were found in thick vegetation along the roads and agricultural fields although a few specimens were found in grass (*Cynodondactylon*) and Jowar (*Sorghum vulgare*).

3.2.6. Remarks

This species was taken from the rice fields [15], [16]. This species was found in Jharkhand, India, in the upper half of the cereal fields, usually paddy. Further, [17] collected this fodder crops, herbs and shrubs of Umerkot, Sanghar, Tharparkar, and Badin districts of Thar Desert in 2013. This species was also been recorded by [18] in all types of vegetation, grasses and rice field. During this survey, samples from a fair number of districts in upper Sindh were collected and shown. Mostly specimens were collected from agricultural fields, fodder, corn and grass crops during July to November and less in coldest season of the year. There are described sequence producing alignment, Table 4 and tree reconstruction nearest neighbor detail (Figure 5). Another tree was reconstructed of BIN and nearest neighbor of this species through MEGA Software Figure 6.



Dorsal view | Lateral view

Fig. 4 Dorsal and lateral views of *Oxya hyla*

Table 4 Sequence producing significant alignment: *Oxyahylahyla*

S. No.	**Species**	**Marker**	**Maximum score**	**Query cover**	**Percent identification**	**Accession Number**
01.	*Oxyahyla*	COI	1190	100%	99.69%	JF838507.1
02.	*Oxyahyla*	COI	1188	100%	99.54%	KY844961.1
03.	*Oxyafuscovittata*	COI	1098	100%	96.92%	JN306017.1
04.	*Oxyahyla*	COI	1092	91%	99.50%	KY834529.1
05.	*Oxyafuscovittata*	COI	1059	100%	95.99%	JF838510.1
06.	*Oxyasinuosa*	COI	856	99%	90.45%	MN609344.1
07.	*Oxya chinensis*	COI	850	99%	90.29%	KC140003.1
08.	*Oxyavelox*	COI	845	99%	90.14%	HM180750.1
09.	*Oxya chinensis*	COI	839	99%	89.98%	KF966601.1
10.	*Oxya chinensis*	COI	839	99%	89.98%	MW085472.1

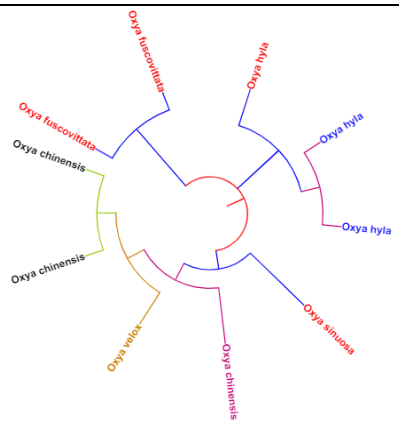


Fig. 5 Tree reconstruction of bin & nearest neighbor detail of *Oxyahylahyla*

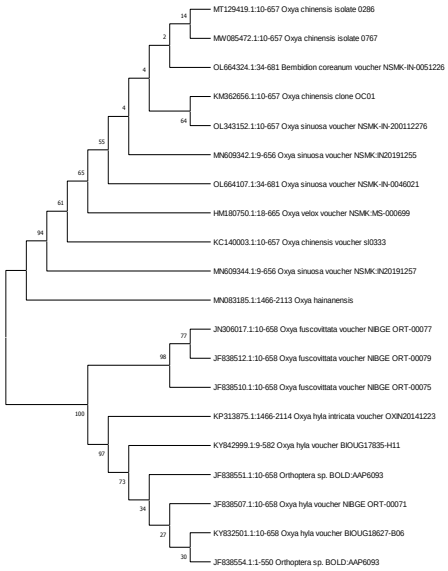


Fig. 6 Tree construction of BIN and nearest neighbor detail of *Oxya hyla*

3.3. *Aiolopus Simulatrix*

3.3.1. Morphology

Medium stature, more highly rugulose integument, and antennae with 22–24 segments in males, as long as head and pronotum together; pentagonal fastigium of the vertex, slightly concave, little wider than it is long, narrowly rounded apex; oval foveolae eyes, rather thin pronotum, protozoal median carina strength. Hyaline hind wings shorter with hind tibia, nine outer and ten inner spines on the hind femur and morphometric of various body parameters are shown in Table 5, Figure 7.

3.3.2. General Coloration

Brown with orchraceous, consistently brown, or occasionally greenish. Two black patches on the top side of the hind femur, dark on the back knee, hind tibia blackhind femur with two dark spots at upper surface its. Identical to males but larger and more robust in the female.

3.3.3. Morphometric

Table 5 Morphometric analysis of various body parameters of *Aiolopus simulatrix*

Body Parameter	**Mean ± S. D (mm)**	
	Male (n = 05)	**Female (n = 05)**
Length of head	3.148 ± 0.008	3.15 ± 0.55
Length of antenna	6.17 ± 0.14	6.25 ± 0.44
Length of pronotum	4.08 ± 0.14	4.37 ± 0.32
Length of Femur	12.61 ± 0.38	13.4 ± 0.62
Length of tibia	10.116 ± 0.80	12.2 ± 0.58
Antennae Segments	12.3 ± 0.85	12.7 ± 0.96
Length of wings	20.84 ± 0.21	22.05 ± 1.08
Length of tegmina	21.66 ± 0.15	23.8 ± 0.95
Distance b/w compound eyes	1.012 ± 0.04	0.9 ± 0.21
Total body length	22.78 ± 0.69	25.4 ± 0.51

3.3.4. Global Distribution

Afghanistan, Albania, Austria, Bangladesh, Ethiopia, France, Egypt, India, Iran, Oman, Pakistan, and Turkey are among the countries where this subspecies is widely dispersed. This species presence is also shown in upper Sindh province.

3.3.5. Host Plants

This species nature was noticed to be graminivorous. So, as expected, this species is collected from different cultivated fields. The most frequently it can be seen in the following fields and plantings: water melon (*Citrullus vulgaris*), lawn grass/Chhabargaah (*Cynodondactylon*), Jower (*Sorghum vulgare*), alfalfa-losan (*Medicago sativa*), corn (*Zea mays*), pearl millet (*Pennisetum glaucum*), barley (*Hordeum vulgare*) and Lady finger (*Abelmoschus esculentus*) crops.

3.3.6. Remarks

[17] reported this species from the Pakistani district of Jamshoro, although [19] it was reported from eastern and central Uttar Pardesh, India. [20] this species was procured in Iran. [21] recorded from tamil Nadu, India. [22] from Madhya Pradesh and Chhattisgarh, India. [23] noticed from Punjab (India). This similar to this species morphologically with *O. nigrofasciatus* but isolate by pronotum which is rounded sub-acute *O. senegalensis*. However, in *O. nigrofasciatus* its oval in shape. Unfortunately, only female specimens were gathered during the current collection from the grass, bajra, and Jowar fields and there are described sequence producing alignment, Table 6 and nearest neighbor detail of tree reconstruction (Figure 8). Another tree was reconstructed of BIN and nearest neighbor of this species through MEGA Software Figure 9.

Dorsal view

Lateral view

Fig. 7 Dorsal and lateral views of *Aiolopus simulatrix*

Table 6 Sequence producing significant alignment: *Aiolopus simulatrix*

S. No.	Species	Marker	Maximum score	Query cover	Percent identification	Accession Number
01.	*Aiolopus thalassinus tamulus*	COI	725	100%	91.89%	KC140014.1
02.	*Trimerotropics californica*	COI	719	100%	91.70%	KJ923360.1
03.	*Aiolopus thalassinus tamulus*	COI	713	100%	91.51%	KC140009.1
04.	*Spharagemon campestris*	COI	708	100%	91.31%	KM536074.1
05.	*Trimerotropismodesta*	COI	708	100%	91.31%	KJ923374.1
06.	*Trimerotropislatifasciata*	COI	708	100%	91.31%	KJ923367.1
07.	*Spharagemonbolli*	COI	706	100%	91.12%	KR143241.1
08.	*Conozatexana*	COI	695	100%	90.73%	JQ286499.1
09.	*Aiolopus thalassinus tamulus*	COI	671	99%	89.90%	KY845853.1
10.	*Trimerotropisochraceipennis*	COI	671	100%	89.77%	JQ286551.1

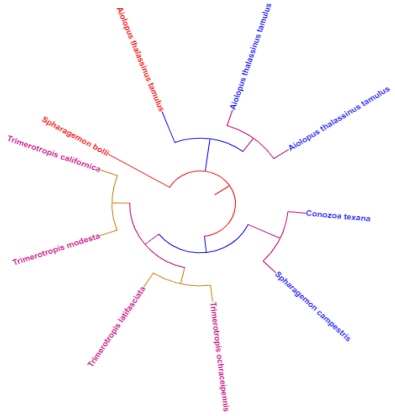


Fig. 8 Tree reconstruction of bin and nearest neighbor detail of *Aiolopus simulatrix*

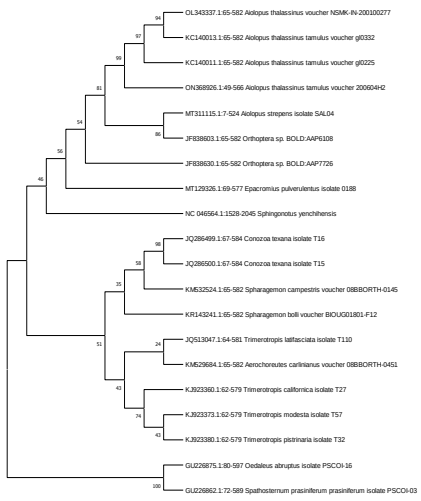


Fig. 9 Tree construction of BIN and nearest neighbor detail of *Aiolopus simulatrix*

3.4. *Aiolopus Thalassinus*

3.4.1. Morphology

Filiform antennas with 21–23 antennal segments. normally have a medium-sized body. Pronotum smaller than subconical head. Pronotum with a faint saddle form. angular vertex, elongate, developing lateral carinulae and concave. Trapezoidal fastigial foveolas. the saddle-shaped pronotum, limited to Prozona. Terminal medial region with fully formed intercalary vein. Square mesosternal interspace. developed tegmina and wings. Hyaline wings, minor opacity near the apex. The long hind femur, slender, not serrated on the dorsal carina, rounded dorsal genicular lobes, Table 7, Figure 10.

3.4.2. General Coloration

Paler, brownish, greenish, or dusty brown skin tones. Having brown antennas. reddish-brown head. Vertex fastigium: green, brownish, or reddish-pink. Tegmina transparent, Brown with sporadic blackish flecks. Colorless and hyaline wings. Hind lighter brownish femur.straw-colored hind tibia.

3.4.3 Morphometric

Table 7 Morphometric analysis of various body parameters of *Aiolopus thalassinus*

Body Parameter	Mean ± S. D (mm)	
	Male (n = 05)	Female (n = 05)
Length of head	2.48 ± 0.08	2.55 ± 0.27
Length of antenna	4.94 ± 0.51	5.15 ± 0.72

Continuation of Table 7

Length of pronotum	3.94 ± 0.32	4.05 ± 0.64
Length of Femur	13.64 ± 0.40	14.05 ± 1.01
Length of tibia	11.9 ± 0.89	12.2 ± 0.84
Antennae Segments	21.9 ± 0.58	22.02 ± 0.95
Length of wings	20.34 ± 1.05	21.22 ± 3.25
Length of tegmina	21.7 ± 1.03	22 ± 3.80
Distance b/w compound eyes	0.93 ± 0.11	0.95 ± 0.28
Total body length	23.5 ± 0.79	24 ± 3.75

3.4.4. Global Distribution

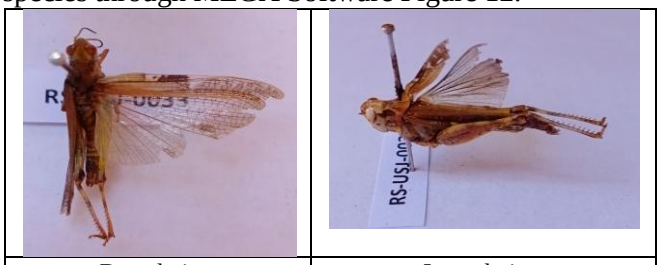
France, Japan, Australia, India, Pakistan, Sri Lanka, South Africa, Europe, Middle Europe, Switzerland, Locarno, and the Maggia Delta are among the countries where this species is present. Its presence in Nara Desert which is located in upper Sindh, Pakistan.

3.4.5. Host Plants

This species is graminivorous by nature. Although, this species was gathered from several agricultural fields frequently seen in Barley (*Hordeum vulgare*), Lady finger (*Abelmoschus esculentus*), Alfalfa-Losan (*Medicago sativa*), pearl millet (*Pennisetum glaucum*), water melon (*Citrullus vulgaris*), corn (*Zea mays*), Jower (*Sorghum vulgare*) and Lawn grass/Chhabar Gaah (*Cynodondactylon*) crops.

3.4.6. Remarks

Although this species' antennae are shorter than its head and pronotum, it can be distinguished by its thinner fastigalfoveolae. [24] and [25] reported that this subspecies is a significant crop pest. [26] recorded it during the monsoon in many Pakistani districts.[17] reported this from fodder crops of Nara Desert. The specimens in our possession had short, conical aroliums, which differ slightly from other known species. Presently, these species were taken from grasses that were cultivated in mixed and sporadic vegetation and there are described sequence producing alignment, Table 8 and tree reconstruction of nearest neighbor detail (Figure 11). Another tree was reconstructed of BIN and nearest neighbor of this species through MEGA Software Figure 12.



Dorsal view | Lateral view

Fig. 10 Dorsal and lateral views of *Aiolopus thalassinus*

Table 8 Sequence producing significant alignment: *Aiolopus thalassinu sthalassinus*

S. No.	Species	Marker	Maximum score	Query cover	Percent identification	Accession Number
01.	*A. thalassinus tumulus*	COI	1214	100%	110.00%	KY829992.1
02.	*A. thalassinus tumulus*	COI	1158	95%	100.00%	KY843882.1
03.	*Prumaarctica*	COI	802	99%	88.74%	KC139971.1
04.	*A. thalassinus tumulus*	COI	797	99%	88.57%	KC140013.1
05.	*A. thalassinus tumulus*	COI	791	99%	88.41%	KC140010.1
06.	*Sphingonotusningsianus*	COI	787	100%	88.28%	JQ513060.1
07.	*Prumaarctica*	COI	785	99%	88.28%	KC139970.1
08.	*Sphingonotustsinglingensis*	COI	782	100%	88.13%	JQ513059.1
09.	*Epacromiuspulverulentus*	COI	782	100%	88.16%	MT129326.1
10.	*Prumaarctica*	COI	774	99%	87.98%	KC139968.1

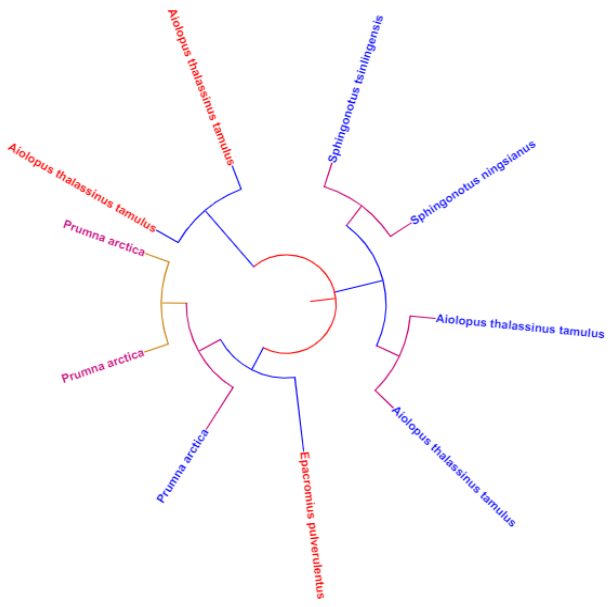


Fig. 11 Tree reconstruction of bin & nearest neighbor detail of *Aiolopu sthalassinu sthalassinus*

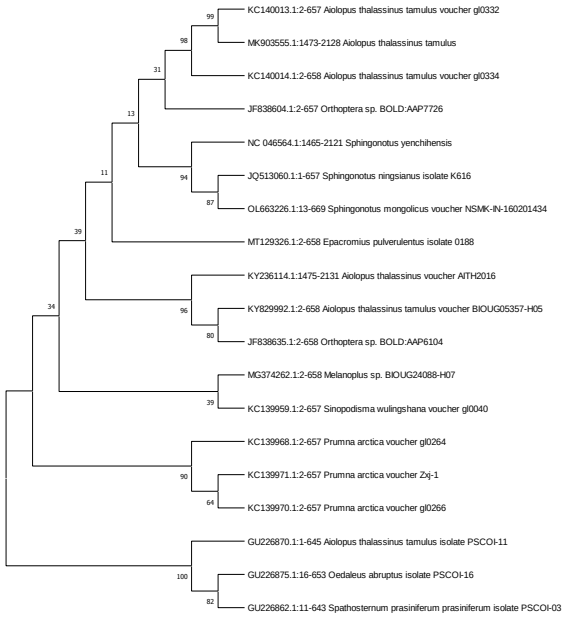


Fig. 12 Tree construction of BIN and nearest neighbor detail of *Aiolopus thalassinus*

4. Discussion

To identify an organism, DNA bar-coding analyses how closely its DNA sequence resembles that of a group of reference species Most of the time, "universal primers" are used to make a copy of the mitochondrial COI gene segment of [3]. DNA bar-coding is usually considered at as a reliable, cheap, and easy molecular identification tool that can be used for various metazoan taxa [4, 5]. Consequently, it could be highly beneficial to regularly discover difficult taxa with economic and medicinal value. This is particularly true of many insect species that contain several well-known pests or disease-carrying insects, whose identification frequently necessitates very sophisticated taxonomic knowledge. Additionally, DNA bar-coding may be essential for identifying particular life stages (e. g. eggs, larvae, nymphs or pupae), which are often difficult to see if you don't know them. The accuracy of insect DNA bar-coding, however, may be called into question because there are over a billion recorded species of insects, in addition to probably millions of more taxa that have yet to be uncovered [27, 28]. This large number of species may, in fact, make it difficult for DNA barcode reference databases to keep up with the huge taxonomic diversity of insects. Still, some studies have questioned whether or not DNA barcoding in Orthoptera is sufficient [29]. To ensure sure the work is correct, DNA bar-coding must be used. DNA bar-coding is a common method that uses short pieces of the mitochondrial DNA gene, Cytochrome C oxidase subunit I (COI) and polymerase chain reaction (PCR) and gel electrophoresis (GE) are used to make more copies of a specific COI gene. We simply follow a few steps, and we're mostly interested in DNA sequencing, by using Blast on the NCBI website, sequence data is used to identify unknown species.

5. Conclusion

In this study we studies the morphology and molecular phylogeny of *Acrida willemsei* belong to subfamily Acridine, *Oxya hyla hyla belong to* subfamily Oxyinae whereas *Aiolopus simulatrix and Aiolopus thalassinus thalassinus* belong to the subfamily Oedipodinae. For morphological study we studies the morphology, general coloration, morphometric variation, global distribution and host plants of four species. For molecular study, the accession numbers, markers, maximum score, query cover and percent identification of each species has been mentioned along with tree, iTOL tree and (COI) reconstruction of bin and nearest neighbor detail and got authentic identification through the iBOL Canada.

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