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**Research Article****Diagnostic characters of the immature stages of the desert locust, *Locusta migratoria* L. (Orthoptera: Acrididae: Oedipodinae)****Fakhra Soomro, Shahar Bano, Sajjad Ali Larik, Paras Soomro**

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ABSTRACT

This study examined the morphological variation of the nymphs of the desert locust, *Locusta migratoria*. Nymphs of various stages of *Locusta migratoria* were collected from agricultural and nonagricultural fields of Sindh, Pakistan. Insect culture was maintained under laboratory conditions and reared to maturity at temperature $28 \pm 2^\circ\text{C}$ to $39 \pm 2^\circ\text{C}$, relative humidity 28-61% and photo period of 12:12 (L: D). Cautiously sorting of instars showed that *L. migratoria* comprises on 5th nymphal stages in both the sexes with the exception of morphometric and morphological differences in each stage. This manuscript recruits comparative diagnostic features, identification key along with illustration. During the laboratory rearing, we examined that solitary phase of *L. migratoria* usually has two generations per annum. However, a field survey revealed that higher temperature ($> 42^\circ\text{C}$) in the desert three generations were usually captured. Furthermore, the sex ratio favored female instars only. Locust instars remain in groups after hatching and rush to fresh green foliage for feeding. Each stage of instars differentiated on the basis of wing pad development, along with significant increases in the size of the first abdominal segment. 5th instar has thicker venation, usually cross the 4th abdominal segment and displayed distinct color variation.

Keywords: Gregarious phase; hatching; immature stages; identification key; Illustration; solitary phase.

INTRODUCTION

The Desert locust *Locusta migratoria* L. has formed plagues since prehistory and it can cause heavy loss to agriculture. They attack not only the cultivated but also the rangeland fields and important crops and grazing land (Panhwar et al., 2024; Usmani, 2014; Steedman, 1988; Kirby, 1914; Bennett, 1993). Moreover, due to its capacity for rapid reproduction, long-distance migration, and agricultural destruction, the desert locust is regarded as the most hazardous migratory pest among locust species (FAO, 2020a; Joshi et al., 2020; Cressman, 2016; Steedman, 1990). Desert locust swarms can travel up to 150 kilometers when flying in the same direction of the wind (Zhang et al., 2019) comprising a population of billions of insects (FAO, 2020b). Although they are regarded as destructive pests and seriously harm crops, they can be utilized as a substitute for nutrient-dense food and feed because they are edible (Panhwar et al., 2023; Mariod et al., 2017).

In 2006, an outbreak of *L. migratoria* in Ladakh of India destroyed the pastures, crops and affecting people adversely (Contreras, 2011). This locust is chiefly graminivorous, occupying grass belt near wetlands. Among all locusts and grasshoppers, *L. migratoria* has the broadest geographical distribution, ranging from subtropical and tropical parts of the Eastern hemisphere and through the old-world including Europe, Africa, India, Pakistan, China, Japan, Australia, New Guinea and New Zealand (Vickery, 1983).

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Solitary nymphs and adults of *L. migratoria* damage vegetables, rice, maize, wheat, cotton and different food crops, as well as plantation of volatile oil, hayfield and pastures (Gómez et al., 2012). In this respect, millet is a primary food source for millions of people in hot, dry areas. The cereal plants of locust are particularly preferred by this locust species. In the solitary phase, these locusts live in isolation and implying that they are independent of their neighbor (Samejo et al., 2021; Panhwar & Mustafa, 2022). The solitarious form of *L. migratoria* shared morphometric similarities with their gregarious ones (Hewitt, 1982). Just a decade before, in 2006 outbreak of *L. migratoria* in Ladakh, India, affected the pastures, crops and peoples adversely (Hoell et al., 1998). Overall *L. migratoria* produces two generations within a year and its life cycle depends naturally on seasonal changes and embryonic diapauses (Latchininsky, 2008). The devastation that the locust plagues caused is highly alarming. It caught the attention and interest of workers. Typically, this species develops through five instars before becoming adults. However, little attention is given to the morphological differences of the nymph stages of the *L. migratoria* and comparative data are yet available. Therefore, this study aimed to better understand the phenology of this locust species via field and laboratory investigations. Immatures were collected from the field and raised in the laboratory. This study provided the first record of the morphological variations of their diagnostic characters. Also, morphological differences at the nymph stages may be beneficial to differentiate between pest and non pest species. The study of immature insects is a crucial step in integrated pest Management strategies.

MATERIALS AND METHODS

Sampling, Killing and Preservation

Stock culture for rearing was maintained from newly hatched egg pod immature (*Locusta migratoria*) were collected from various non-agricultural and agricultural fields such as different crops, vegetables, herbs, water channels and wetlands at Nara District of Sindh, Pakistan. Immature stages were kept in laboratory conditions and raised to maturity at temperature $28 \pm 2^\circ\text{C}$ to $39 \pm 2^\circ\text{C}$, relative humidity of 28-61% (RH), and photoperiod of 12:12 (L: D). They were regularly fed with fresh leaves and soft stems of the host plants (preferred by insects). Before feeding, these leaves and soft stems were sterilized in a 5% sodium hypochlorite solution. Methods of insect collection and maintenance followed the standardized procedures of other studies (Kirby, 1914; Parihar, 1987).

Taxonomic Identification, Illustration, and Rearing of Insect

With the use of species descriptions and identification keys found in books and online resources, every specimen that was gathered was identified. In Immature stages of *L. migratoria* were sorted out under the stereoscopic binocular dissecting microscope based on various diagnostic characters, such as antennal segments, head, pronotum, wing pads, femur, and coloration. Body parts of early stages (1st to 3rd instar) were measured with a 2X ocular graph fixed in the microscope, and the advanced insect stages were determined with a Vernier caliper. Crucial features such as head, pronotum, and wing pads were sketched through the software Adobe Illustrator CC 2015. Lateral view of instars was captured with a digital camera (Nikon Coolpix P520). Occasionally, some nymphs were also reared to yield the desired instar. The data collected from the experimental groups was examined by a one-way analysis of variance (ANOVA) using repeated measurements using SPSS 16.0 software. The difference in mean value was calculated using the least significant test (LSD).

RESULTS AND DISCUSSION

Key for the immature stages of the *Locusta migratoria*:

1. Wing pads not developed, antenna with 13-14 segments..... 1st Instar
2. Wing pads with rounded margin directed downwards, antenna with 15-16 segments
.....2nd Instar
3. Wing pads with triangular margin directed downwards, antenna with 17-20 segments
.....3rd Instar
3. Wing pads turned backwards up to first abdominal segments
.....4th instar
4. Wing pads turned back ward up to fifth abdominal segments
..... 5th Instar

1st Instar

Antenna filiform having 13–14 segments, eyes dark brown with a golden-brown rim and a diagonal bright brown stripe crossing the top half. The pronotum's integument is smooth and not tactiform, and it is slightly longer than the head. Tegmina and wing pads did not emerge, and the pronotum did not cover the mesosternal and metanotum. The upper lobe is brown and shaped like a moon, whereas the hind femur is light and significantly narrow toward the knee joint (Figure 1, 2, and 3)

2nd Instar

Antenna 15–16 segments, apical segments darker. The eyes' oblique golden-brown band reached just a little bit up to the pronotum. The integument is marginally granulose towards metazoans, the pronotum is less strong, and it is slightly tactiform towards the middle carina. The pronotum partially covered the mesonotum, thick, rounded edges were seen on the tegmina and wing pads. The ventral side of the hind femur is typically dark brown without ventral bands, while the basal joint is large and the knee joint is significantly narrower (Figure 1, 2, and 3).

3rd Instar

Antenna with 17-20 segments in both sexes. Blue-shining mandibles with a flat vertex fastigium and a rounder, wider middle. Moderately tactiform and robust, with a significantly elevated pronotal arch in females. The pronotum nearly covers the mesonotum, the wing pad curves slightly backward, and the tegminal pad is oriented downward. The hind femur's dorsal side lacked bands and had an uneven, slightly dark chevron (Figure 1, 2, and 3).

4th Instar

Antenna with 21-23 segments. The vertex bulb-like fastigium has a flat apex that is somewhat invaginated, lateral borders, and equally spaced black bristles and grey dots. The integument is more granulose in the brown form than the green one, and the pronotum is more robust and tactiform, rising towards the median carina. The structure is leaf-like, with wing pads covering the tegminal pads and a curved upward border that overlaps with the first tergite. The tibia has nine outer black tip spines and eleven interior spines, while the hind femur grows large and powerful at the basal joint (Figure 1, 2, and 3).

5th Instar

Both sexes had a filiform antenna with 24-26 segments; the basal segments were longer, and the apical segments were smaller. The vertex's fastigium is bulb-shaped, the apex is flat, the lateral borders slope downward in a round manner, and the fastigial foveolae are small and conical. Wing pads cover the tegminal pads, which extend up to four and a half segments in female instars and cross the fifth segment in male instars. The pronotum is tactiform, significantly elevated from the head, and has integument that is densely granulose in brown and mildly granulose in green. A chevron with broad segmentations and a deeper tint, the hind femur becomes broader and more robust towards the basal joint and significantly narrower towards the knee joint (Figure 1, 2, and 3).

Morphometry

During the present study detailed diagnostic characteristics of various instars were provided in earlier stages from 1st to 3rd instar were mostly indistinguishable while the last stage was somewhat morphological identical with adults. Progressive and morphometric variation in different body parts i.e. Head, pronotum, wing pads and femur were observed in all developmental stages. Furthermore, With the exception of the 1st instar, when the number of antennal segments varied for both species, the morphometric means of the different instars of *Locusta migratoria* reveal notable differences in the measurement of several body parameters Table (1). However, there are notable changes in the length of the femur and overall body length between male and female *Locusta migratoria* 2nd instars. The pronotum and overall body length of the 3rd instar male were much shorter than those of the female. From the perspective of the 4th instar, females have more antennal segments than males, and their bodies and femoral are longer. In addition to the other metrics, the 5th instar also exhibited a notable variation in wing pad and tegmina length. In general, female instars had a greater total body length than male instars Table (1).

Table (2) demonstrates that the 1st instar's hatching was seen starting in mid-May and was gathered in a moderately dominant percentage. On the other hand, the second generation was observed in August, and the majority of the initial instars were found on the outskirts of *Sorghum bicolor*. In May and June, however, a mixed population of all nymphal stages continued to be moderately dominant. Moreover, a more dominant percentage of various nymphal stages was recorded from August to September. However, advance stages come in collection from October to December in less number or absent. However, variations in the population size of different species during different months of the year may also be attributed to climatic factors that determine population size. During this study, we have observed only 5th nymphal instars in both sexes with no extra molt tend to have longer developmental times.

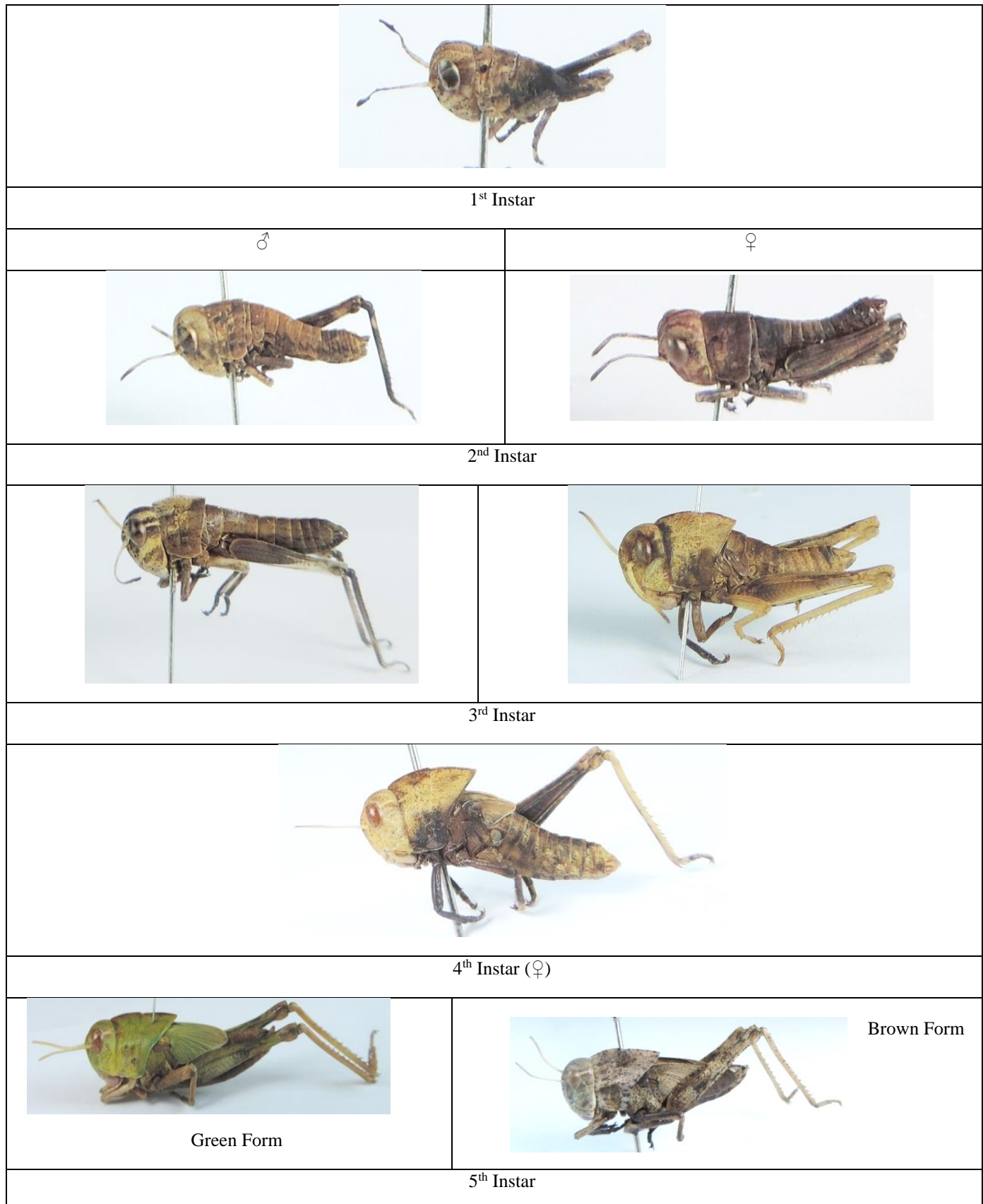


Figure 1. Male and female lateral view of different nymphal instars of *Locusta migratoria*.

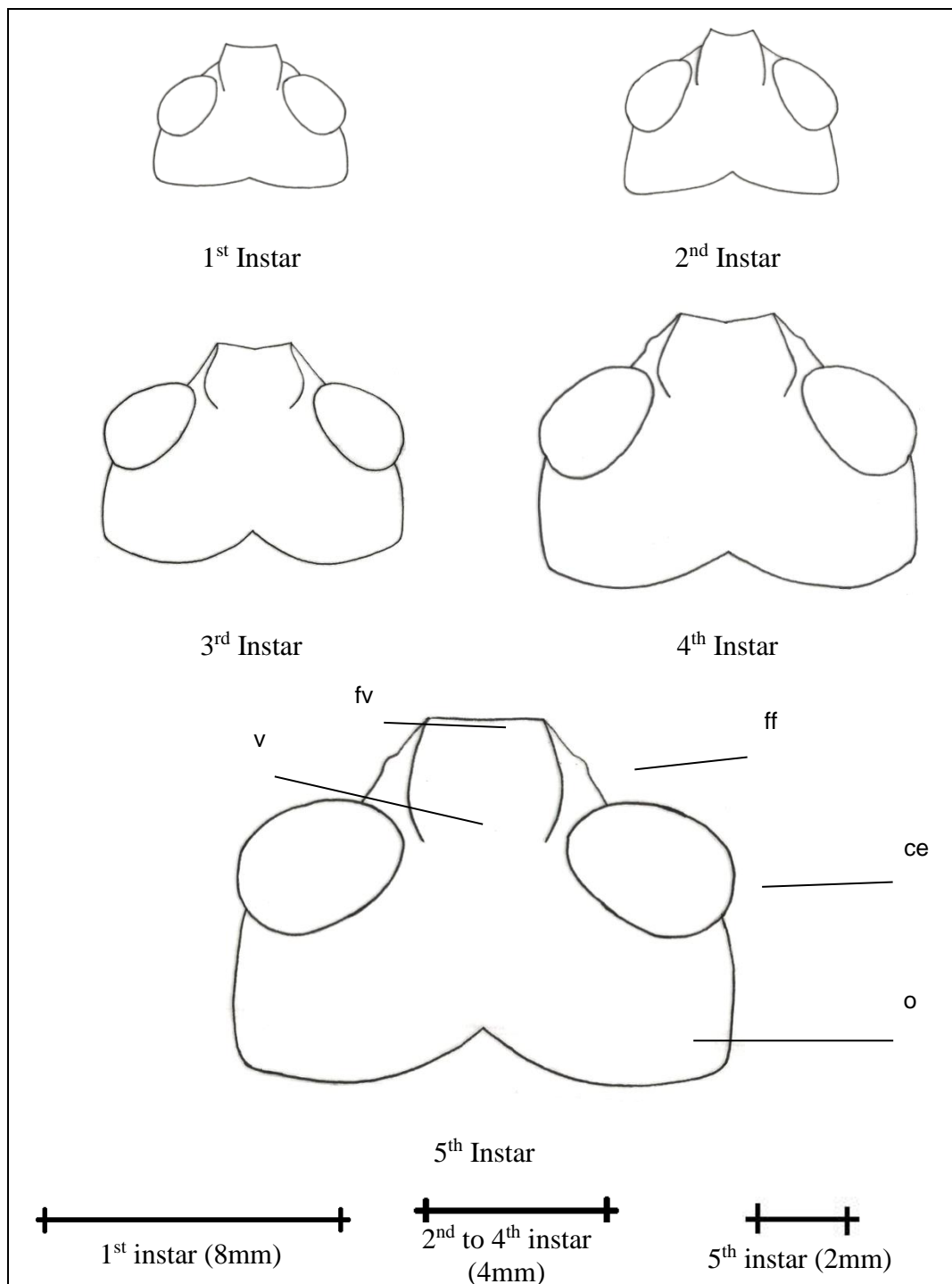


Figure 2. Dorsal view of head of different Instars of *Locusta migratoria* ff = fastigial foveolae, tg= tegminal pad, o= occiput, ce= compound eye, v= vertex

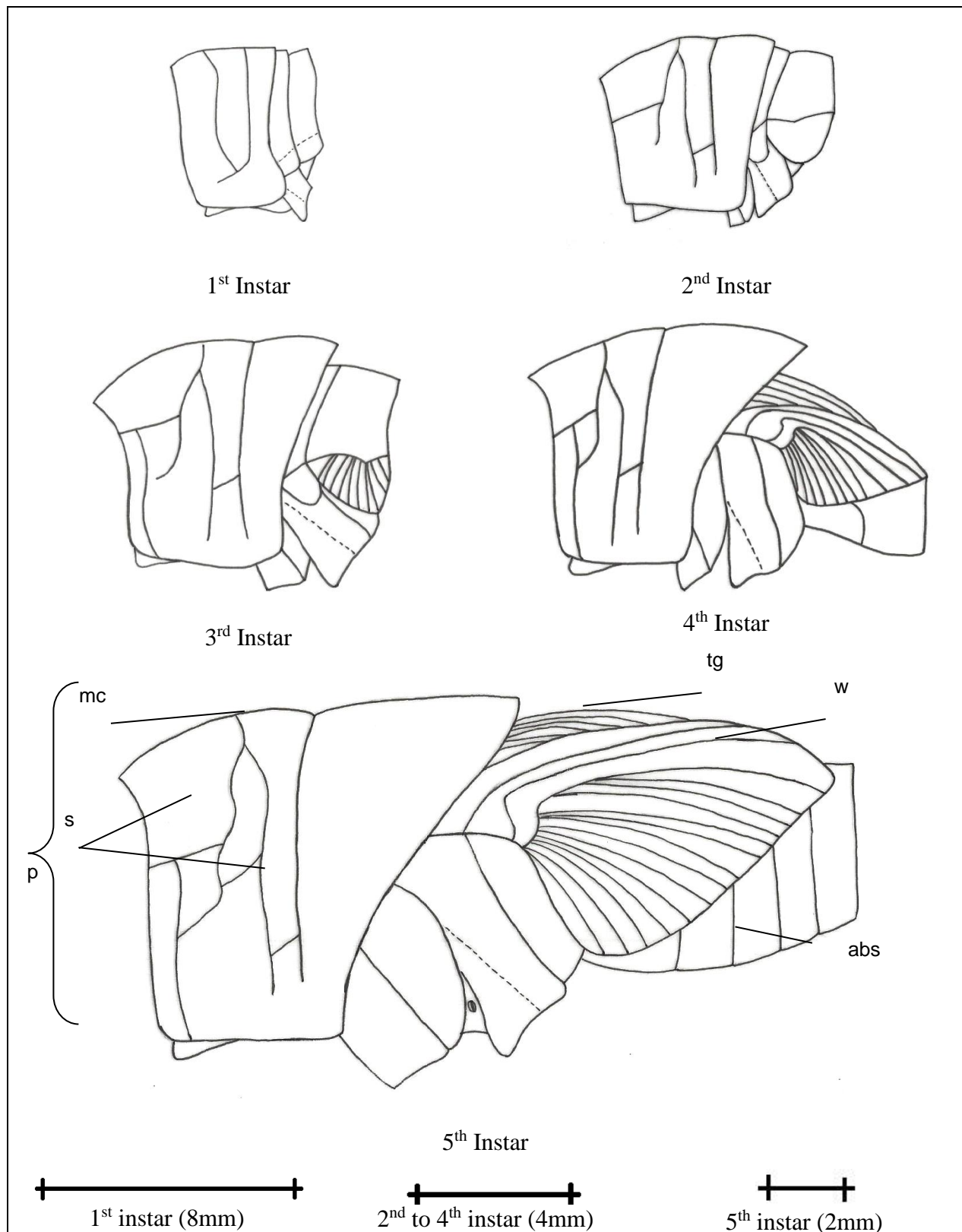


Figure 3. Lateral view of wing pads of different nymphal instars of *Locusta migratoria* abs = abdominal segment, ff = fastigial foveolae, fv = fastigium of vertex, mc= median carina, p = pronotum, tg= tegminal pad, w = wing pad

Table 1. Morphometry of different nymphal instars of *Locusta migratoria* (♀) and (♂)
 Mean in the same row followed by the same letters is not significantly different from one another at 5% level of probability

Parameters (mm)	1 st Instar (n=15) (Mean±S.E)		2 nd Instar (n=15) (Mean±S.E)		3 rd Instar (n=15) (Mean±S.E)		4 th Instar (n=15) (Mean±S.E)		5 th Instar (n=15) (Mean±S.E)	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
AS	13.46±0.13 ^d	15.53±0.13 ^d	15.53±0.13 ^d	15.53±0.13 ^d	0.79±0.01 ^b	18.86±0.27 ^c	-----	21.93±0.20 ^b	24.80±0.20 ^a	25.06±0.20 ^a
LA	2.30±0.02 ^d	2.82±0.06 ^d	2.82±0.06 ^d	2.82±0.06 ^d	2.46±0.04 ^c	4.10±0.10 ^c	-----	5.43±0.23 ^b	7.38±0.06 ^a	8.08±0.05 ^a
DBE	0.51±0.01 ^d	0.64±0.01 ^d	0.64±0.01 ^d	0.64±0.01 ^d	0.63±0.01 ^c	0.97±0.03 ^c	-----	1.38±0.04 ^b	1.52±0.02 ^a	1.82±0.02 ^a
LH	1.11±0.02 ^c	1.40±0.02 ^d	1.40±0.02 ^d	1.40±0.02 ^d	1.22±0.02 ^c	2.14±0.07 ^c	-----	2.77±0.05 ^b	3.27±0.04 ^a	3.72±0.04 ^a
LP	1.24±0.03 ^c	1.73±0.06 ^d	1.73±0.06 ^d	1.73±0.06 ^d	1.32±0.02 ^c	3.29±0.16 ^c	-----	6.19±0.16 ^b	6.85±0.13 ^a	9.29±0.25 ^a
LTG	-----	0.66±0.01 ^c	0.77±0.02 ^d	0.77±0.02 ^d	0.66±0.01 ^c	0.93±0.02 ^c	-----	4.04±0.10 ^b	7.45±0.13 ^a	9.47±0.08 ^a
LW	-----	0.79±0.02 ^c	0.86±0.02 ^d	0.86±0.02 ^d	0.79±0.02 ^c	1.06±0.03 ^c	-----	3.62±0.12 ^b	6.74±0.09 ^a	8.87±0.06 ^a
LF	3.32±0.06 ^c	3.49±0.05 ^c	4.11±0.09 ^d	4.11±0.09 ^d	.49±0.05 ^c	6.55±0.23 ^c	-----	12.16±0.10 ^b	12.20±0.27 ^a	16.56±0.26 ^a
LT	2.91±0.03 ^c	2.97±0.03 ^c	3.58±0.08 ^d	3.58±0.08 ^d	2.97±0.03 ^c	5.88±0.22 ^c	-----	11.36±0.07 ^b	10.97±0.18 ^a	15.10±0.32 ^a
WF	0.72±0.02 ^c	0.79±0.01 ^c	0.95±0.02 ^d	0.95±0.02 ^d	0.79±0.01 ^c	1.53±0.06 ^c	-----	2.28±0.03 ^b	3.39±0.09 ^a	4.21±0.04 ^a
WTG	-----	0.61±0.02 ^c	0.71±0.01 ^c	0.71±0.01 ^c	0.61±0.02 ^c	0.80±0.02 ^c	-----	1.22±0.03 ^b	1.69±0.02 ^a	2.03±0.05 ^a
WW	-----	0.69±0.01 ^c	0.80±0.02 ^d	0.80±0.02 ^d	0.69±0.01 ^c	0.98±0.02 ^c	-----	2.00±0.04 ^b	3.71±0.04 ^a	4.53±0.07 ^a
TB	5.59±0.05 ^d	6.19±0.09 ^c	7.81±0.11 ^d	7.81±0.11 ^d	6.19±0.09 ^c	11.90±0.38 ^c	-----	17.96±0.19 ^b	20.23±0.26 ^a	23.72±0.32 ^a

Table 2. Distribution of various instars of *Locusta migratoria* in different months during 2022-2023.

Months	1 st Instar	2 nd Instar	3 rd Instar	4 th Instar	5 th Instar
Trip-I					
January	Absent	Absent	Absent	Absent	Absent
February	Absent	Absent	Absent	Absent	Absent
March	Absent	Absent	Absent	Absent	Absent
Trip-II					
April	Absent	Absent	Absent	Absent	Absent
May	Present	Present	Present	Present	Absent
June	Present	Present	Dominant	Dominant	Dominant
Trip-III					
July	Present	Present	Present	Present	Present
August	Dominant	Dominant	Dominant	Dominant	Dominant
September	Dominant	Dominant	Dominant	Dominant	Dominant
Trip-IV					
October	Absent	Absent	Absent	Absent	Absent
November	Absent	Absent	Absent	Absent	Absent
December	Absent	Absent	Absent	Absent	Absent

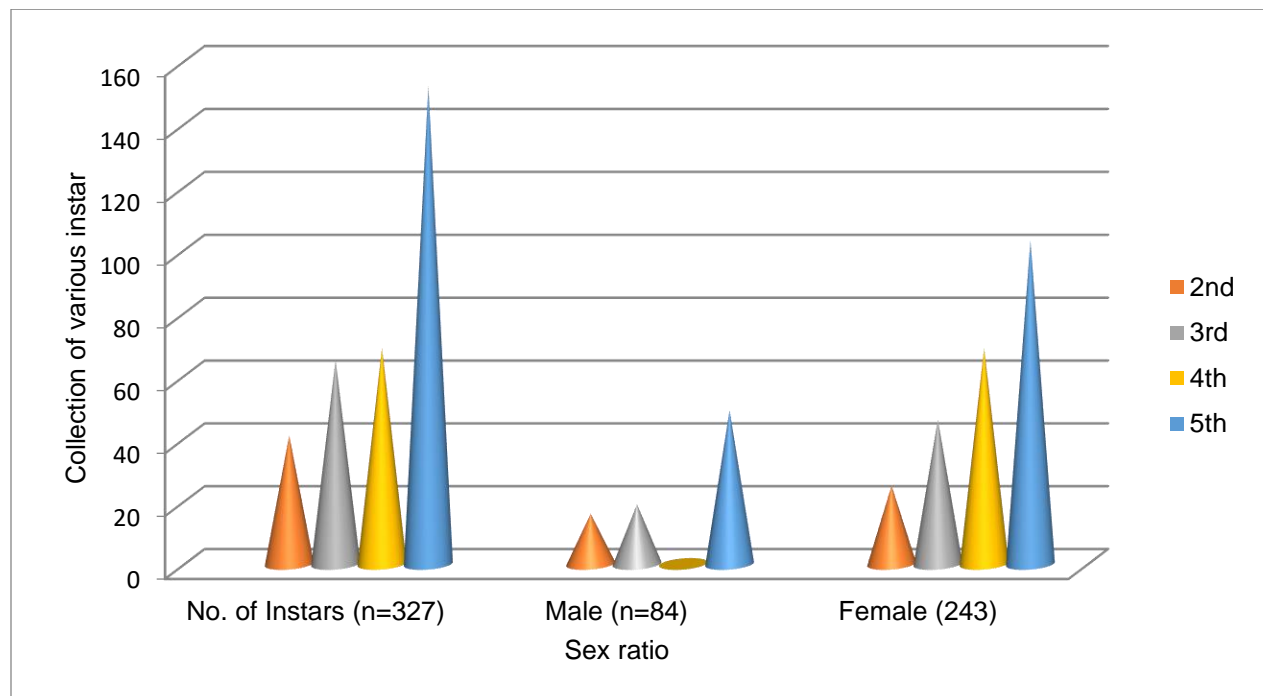
It has been observed from Table (3) that distribution of various instars of the desert locust, *Locusta migratoria*, across different districts in Sindh, Pakistan, during the year 2022-2023. It shows the total number of immature locusts collected, categorized by their developmental stages (1st to 5th instar) in each district. The table indicates that the highest incidence of locusts was found in (Nara) Khairpur, with 35.98% of the total collected, and followed by 12.85% in Dadu, 11.56 % Tharparkar, and 9.76% in Umerkot respectively. Other districts had significantly lower percentages such as 2.31% in Jamshoro, 4.59% in Karachi, 5.14 % Thatta and 5.65% in Sanghar respectively.

It has been noted that sex ratio was exclusively in the favorable for female hoppers with 31.49 % 5th instar and 21.10% 4th instar of total population. While, in the case of male nymphal instar advance stages like 5th instar was dominant with 14.98% respectively. While the lowest sex ratio such as 4.89% and 5.81% was recorded for 2nd and 3rd instar male respectively (Figure 4).

During the present study detailed diagnostic characteristics of various instars were provided from earlier stages. However, sex determination was mostly indistinguishable while the last stage was somewhat morphological identical with adults. Progressive and morphometric variation in different body parts i.e. head, pronotum, wing pads and femur were observed in all developmental stages. However, Chesler (1938) relied on the enlargement of the wing pads to identify the nymphal stages. In a similar way, Albrecht (1955) favored the placement of wing pads and tegmina solely for identifying the instar stage. However, in order to determine the stage of different instars in Oedipodinae species, Guibord (1969) and Crozier (1977) relied on antennal segments, external genitalia, and wing pads. The present study confirms the occurrence of five nymphal stages at laboratory and outdoor settings. Whereas, (Soomro et al., 2015, 2016, 2019) recorded six nymphal stages in various Oedipodinae species along with variation of instars in male and female sex in *Trilophidia annulata*. The current investigation supports the five nymphal stages were recorded in the subspecies *Locusta migratoria migratorioides* by Dirsh (1959). Additionally, he compared their nymphal phases to those of *Gastri margus nigericus*, which shares the same environment and mature morphology. Although *G. nigericus*'s 1st nymphal instar differs greatly from *L. migratorioides*'s, with a more arcuate pronotum, their external features gradually converge in later stages, giving them a similar appearance to adults. In certain ways, the current findings are also in agreement with (Madamba, 2014). For example, *L. migratoria* consistently produces five nymphal stages, which adapt the color of their environment as a protection technique, such as camouflage. The current study's findings indicate that both the male and female instars of both species, as well as the morphometric characteristics of the various developmental stages, differed significantly. Additionally, the mid-May collection showed the largest hatching of the 1st instar. Early stages remain in clusters; however, advance stages are found scattered and voracious on vegetation.

Table 3. District wise distribution of various instars of *Locusta migratoria* during the year 2022-23.

Districts	Developmental Stages					Total No. of Instars 389	(%)
	1 st	2 nd	3 rd	4 th	5 th		
Karachi	---	---	02	03	09	14	4.59
Hyderabad	---	---	---	---	---	---	---
Badin	---	---	---	---	---	---	---
Thatta	---	03	03	05	09	20	5.14
Jamshoro	---	---	03	---	06	09	2.31
Matiari	---	---	---	---	---	---	---
Dadu	02	08	08	11	21	50	12.85
Umerkot	---	07	09	07	15	38	9.76
Tharparkar	02	05	09	11	18	45	11.56
Sanghar	---	06	07	---	09	22	5.65
Shaheed Benazirabad	---	---	---	---	---	---	---
Khairpur	56	11	15	17	41	140	35.98
Sukkur	---	---	05	09	13	27	6.94
Shikarpur	---	---	---	---	---	---	---
Ghotki	---	---	---	---	---	---	---
Larkana	02	01	04	06	11	24	6.16
N. Feroz	---	---	---	---	---	---	---

Figure 4. Sex ratios in different instars of *Locusta migratoria* collected from field during the year 2022-2023.

Additionally, the August to September maximum percentage of different nymphal stages was recorded. However, fewer advance stages are collected until October. Similar finding was reported by (Soomro et al., 2015), that hatching began in May and the young remained in the field till October in the districts of Sukkur and Khairpur in Sindh, Pakistan. In contrast, (Azim & Reshi 2008) gathered the April nymphal stages. Moreover, final instars displayed significant color variation in accordance to vegetation. Precised identification key of nymphal instars is documented for the first time

with supportive images and drawing line comprehends the identification. The findings are crucial for the timely implementation of control measures.

CONCLUSION

In conclusion, this research provides valuable visions into the biology of the desert locust, emphasizing the significance of understanding its immature stages. By authenticating the morphological variations and life cycle patterns, the study contributes to the broader field of entomology and pest management. The findings offer practical knowledge that can aid in controlling this significant agricultural pest, ultimately helping to protect crops and ensure food security. Moreover, the study indicates a sex ratio that favors female instars, which has important implications for understanding the reproductive potential of the species.

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