



## RESEARCH ARTICLE

### DETERMINATION OF THE DIFFERENT NYMPHAL INSTARS OF *TRILOPHIDIA ANNULATA* (OEDIPODINAE: ACRIDIDAE: ORTHOPTERA)

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#### ABSTRACT

*Trilophidia annulata* is one of the most important agricultural pests in the low lying grasslands. It feeds on a wide range of crops but is primarily graminivorous. It's all developmental stages cause damage to varieties of crops but their immature seem most destructive. During the present study immatures of *T. annulata* was discussed. It passes through 5<sup>th</sup> nymphal instars in male and 6<sup>th</sup> in female. All the morphological differences were shown through illustration and photographs. It was noted that there is significant difference in the head, pronotum and wing pads of different nymphal stages. Color pigmentation is also unique and prominent feature in these developmental stages. Still now this, parameter was untouched. Present study is being carried out for the first time.

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## INTRODUCTION

*Trilophidia annulata* (Thunberg, 1815) is chiefly geophelous grasshopper widely distributed in Oriental region and Palaerctic region (Hollis, 1965). *Trilophidia* species are polyphagous considered as sporadic pest potentially very destructive. But infrequency in the crop loss was noticed therefore, remained overlooked by earlier workers. *T. annulata* is widely distributed on various cereal crops i-e Sorghum, Pearl-millet, Wheat and Maize, vegetables; Brinjal, Okara and Cabbage, grasses; Lawn grass, Berseem and Kasni and also found relaxing in open grounds (Moonis and Aziz, 1977). Considerable taxonomic work on the adults of this species have been done by earlier worker by (Hollis, 1965; Wagan, 1990; Mahmood *et al.*, 2008; Tan, 2010, 2012; Bughio *et al.*, 2014; Kumar, 2014; Parbakar *et al.*, 2015). But study on the nymphal stages of *T. annulata* was remained untouched except some primary work on the biology, food plants and seasonal distribution was done by (Moonis and Aziz, 1977; Soomro *et al.*, 2015), but they don't provide any morphological description and identification of the nymphal stages of species. Taxonomy of immature stages never paid attention with that of adults (Hayes, 1941). For getting clear picture of pest status it is essential to have complete set of nymphal stages either from field or reared in laboratory. Each stage of immature has potential to threaten the crops and can

reach to density by successful molting. Identification and characteristic of nymphal stages as general rule are as impotent as the taxonomy of adults (Emden, 1957). Nymphal stages of grasshoppers are usually variable in number (Sahadler and Witsack, 1999). This study is very important because in field there are numbers of immatures are available at the time. Present data will help to sort out the nymphs of this injurious insect before raising their densities to cause damage in any valued crop.

## MATERIALS AND METHODS

### Habitat and Sampling

Collection was made from 2014-2015 from cereal crops, vegetable, grasses and sunny areas (open grounds) with no intensive cultivations. Nymphal stages mainly rely on cryptic coloration and motionless postures to avoid detection. When vegetations and pebbles were disturbed they jumped quickly and tried to escape, their position was noticed quickly netted (with hand net 20 cm in diameter) or picked by hand.

### Laboratory techniques

### Preservation methodology

For the killing and preservation of samples method given by (Vickery and Kevan, 1983) and (Riffat *et al.*, 2012) was applied.

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## Identification and morphometry

Identification was carried out under stereoscopic dissecting binocular microscope and immatures were sort out into different stages. Body parts of instars were measured with vernier caliper except 1<sup>st</sup> and 2<sup>nd</sup> instars and antennae were measured by 2x ocular graph fixed in microscope. Occasionally early stages were reared to obtain the desired instar.

## Photographs and Drawing lines

Line drawing of important taxonomic characters of nymphs (i-e head, pronotum, wing pad, posterior abdominal segments dorsal and lateral view of both sexes) were drawn under binocular dissecting microscope with 2x ocular graph. Photographs of lateral view of instars were taken with (Nikon coolpix P520 camera) to highlight changes in coloration and morphology of important structure after each molting.

## Explanations of terms used in drawing line

ff = fastigial foveolae, fv = fastigium of vertex, c = circus, dv = dorsal valve, vv = ventral valve, sap = supra-anal plate, p = paraproct, sb = sub-genital plate, dv = dorsal valve, sVIII = sternite 8.

## RESULTS AND DISCUSSION

During the field survey large number of nymphs of *T. annulata* were captured they brought to laboratory and sorted out into five nymphal instars in male and six nymphal instars in female all the immature stages having significant difference with each other however key differences occurring in head and wing pads are discuss below:

### Comparative morphology of head and pronotum of various instars (Plate 1-3)

During the present study for the separation and identification of nymphal stages we have used combine characters i.e head, thorax, pronotum, wing pads, hind femur with tibia and posterior abdominal segments to highlight changes occur at each developmental stage in male and female instar. (Roonwal, 1952) reported that number of antennal segments varies in the instars of same stage during post embryonic development in many acridids species. Body shape and color change with age. Head globular, First instar hopper light brown in color at advance stages it become dark with grey spot scattered throughout with white and black hair. Fastigium of vertex bulb shape concave, tubercles appeared from base of vertex moving towards eyes forming somewhat D-shape structure become distinct in third instar and fastigial foveolae become visible. Three transverse tubercles appeared before eyes in fourth instar. Fifth instar with light median carinae appeared toward apex, vertex slightly constricted towards anterior part. Median transverse tubercles before vertex forming U-shape structure, fastigial foveolae concave distinct somewhat oval shape, In the sixth instar (female only) U-shape structure become clear, apex of vertex wide, median carinae visible and elongated (head in D-form instar similar to that of fifth instar). Each instar under goes marked differentiation in the thorax after each molt. (Chesler, 1938) use combined characters for identification of instars include length of body, hind femur, antennae, and wing rudiments. In first instar pronotum

tuberculate hairy with three weak transverse sulci become distinct at advance stages, median carina sharp, raised, forming two tooth like projection towards prozona, from second instar to fourth instar it become more strong, raised, posterior margin tactiform pointed. Lateral tubercles become distinct with lateral carinae appear from fourth instar become thicker in fifth instar. Wing pads and elytron become thicker directed downwards in second instar become pointed, curved backwards in third instar, fourth instar wing pads leaf like pointed cover tegmina and dorsum up to margin of first abdominal segment or up to middle of second abdominal segment. (Albercht, 1955) also suggested that position of tegmina and wing pad is an authentic character to describe stage of instars. Fifth instar wing pads slightly variable extended from fourth up to six abdominal segment, veins with blackish spots. (Moonis and Aziz, 1977) reported the five male and six female instars of *T. annulata* from India but not provide any morphological description of this species. (Collins, 2001) reported that acridids grasshopper *Chorthippus brunneus* simultaneously have four or five instars in female while male have constantly four instars cycle. At present single specimen sixth instar female was collected, wing pads up to four and half abdominal segments, much wider, veins much thicker with narrow spaces. Outer margin of wing pad from anal to apical area with dark brown coloration. (Edis et al., 2004) recorded the variation in *Cornops aquaticum* in the number of instar in both sexes occurs due to the change in photoperiod, temperature, climatic condition of the region. Deformed male and female instar having slightly smaller pronotum as compared to fifth instar, wing pads narrow, extended up to middle of supra-anal plate, abdominal segments narrow. Earlier (Popov, 1989) remain unsuccessful to describe exact number of instars in *T. repleta*.

### Morphology of posterior abdominal segments and external genitalia

#### Dorsal view (Plate-4)

Sex and stage of instars are also identified on the basis of morphology of genitalia. First instar sex remain undistinguished from ventral view while supra anal plate narrow and pointed. In second instar, this started to become thick and wide apex become blunt. The third and fourth instar supra-anal plate still falls short of the paraproct tip. Supra-anal plate of fourth and fifth instar towards median base indented forming V shape structure. Cerci smaller than paraproct become thicker in advanced stages and pointed in male instars than female instars. Fifth female instar having upper valve of ovipositor thin, jointed and considerably extended beyond supra-anal plate whereas, sixth instar upper valves of ovipositor are thicker, pointed curved upwards lower valve well differentiated and distinct from dorsal view. Abdominal segments (VII-X) become wider in each advance stage while the XI segment of female instar medially pointed forward than male instars

#### Ventral view (Plate-5)

Second instar sub genital plate indented short. In third instar its somewhat rounded toward apex covered by paraproct. In the fourth instar this becomes conical and straight. Fifth instar having considerable elongated sub genital plate and tilted upwards and slightly extended from paraproct. For female, second instar dorsal ovipositor valve are triangular touch the base of ventral valve on ninth abdominal segment.



(a) 1<sup>st</sup>



(b) 2<sup>nd</sup> ♂



(b) ♀



(c) 3<sup>rd</sup> ♂



(c) ♀



(d) 4<sup>th</sup> ♂



(d) ♀



(e) 5<sup>th</sup> ♂



(e) ♀



(f) 6<sup>th</sup> ♀



(g) de-form ♂



(g) ♀

Plate 1. (a-g) Various nymphal instars of *Trilophidia annulata*

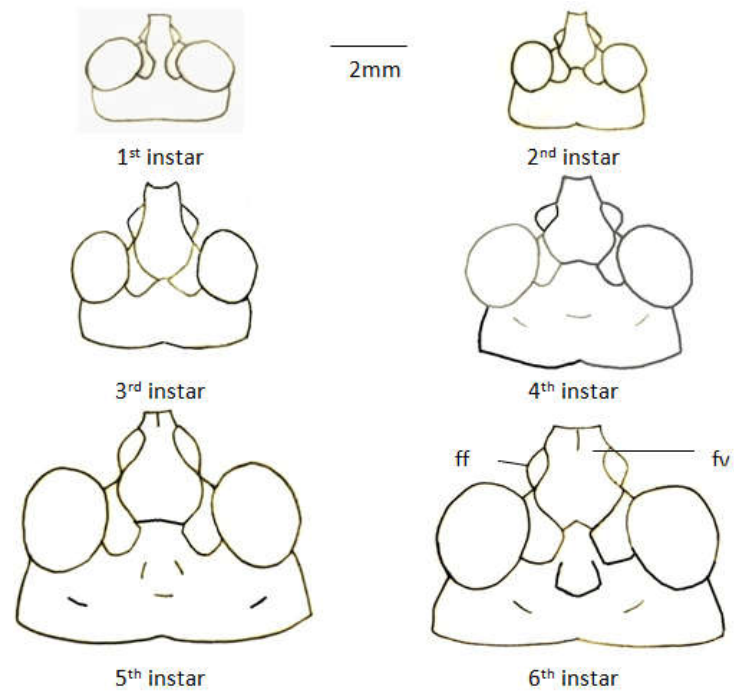


Plate 2. Differentiation of head in various nymphal instars of *T. annulata*

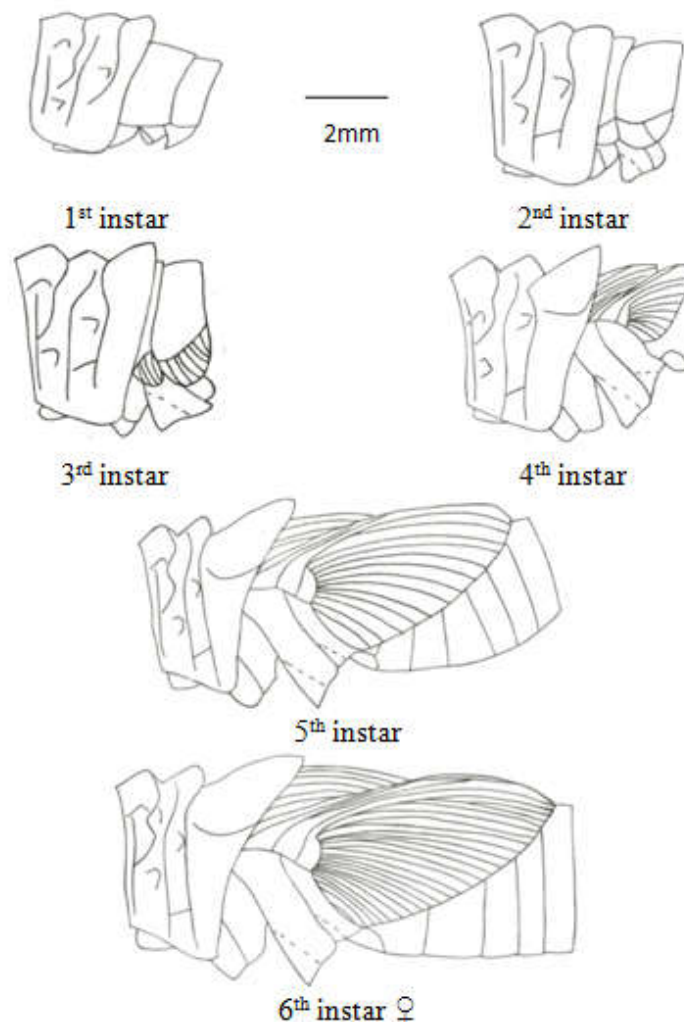


Plate 3. Differentiation of pronotum and wing pad in various nymphal instars

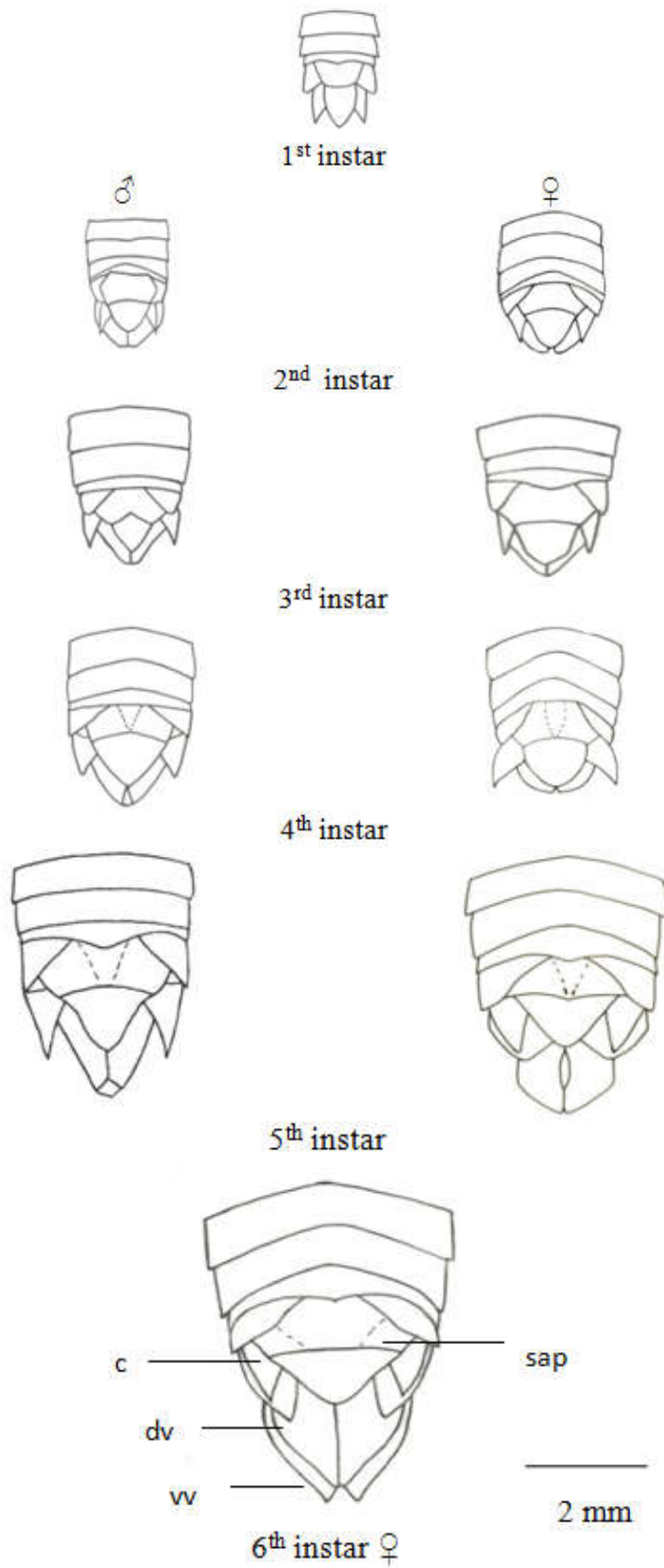


Plate 4. Dorsal view of the tip of abdomen of various instars of *T. annulata*

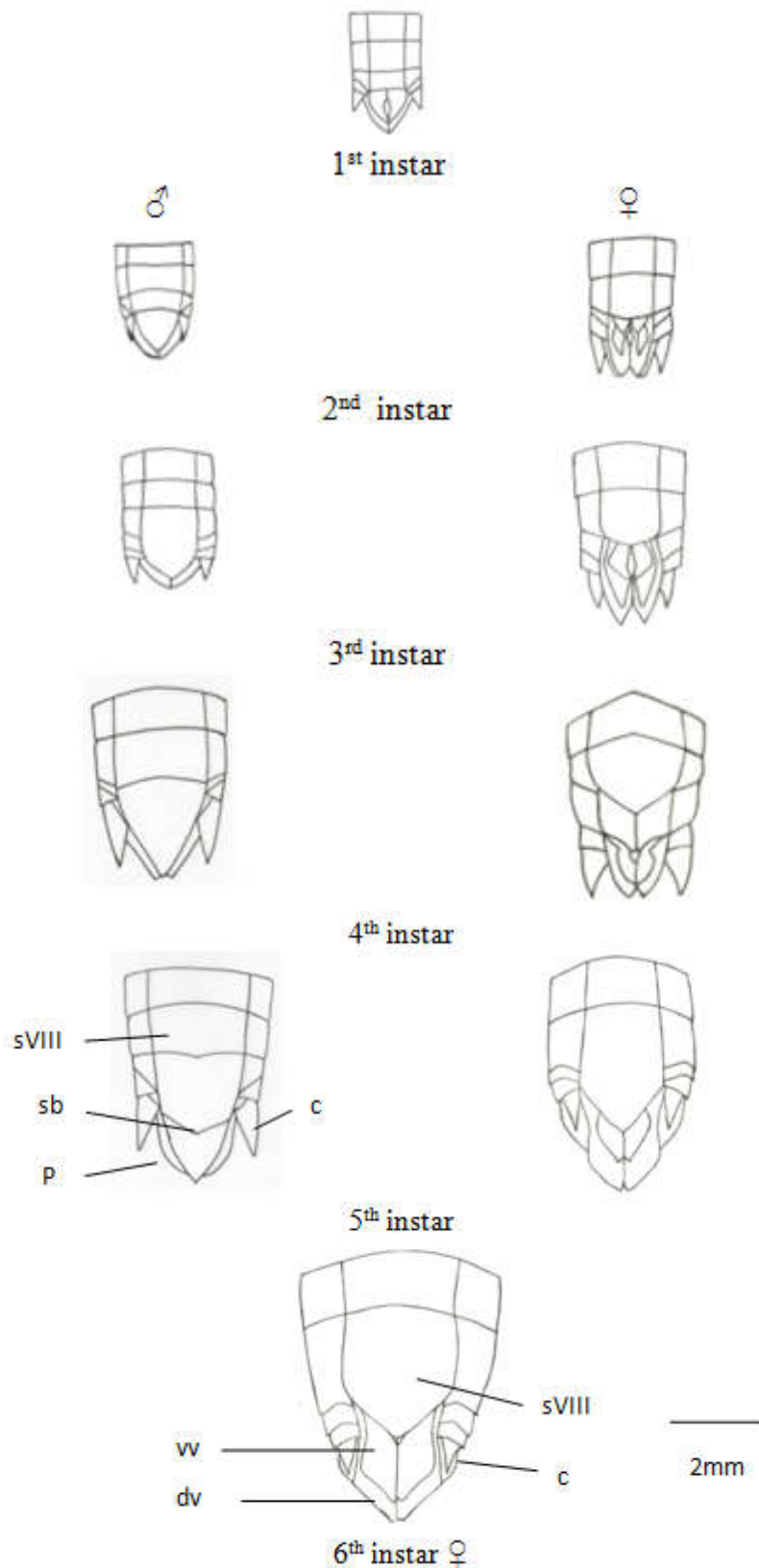


Plate 5. Ventral view of the tip of abdomen various instars of *T. annulata*

In the third instar, ventral valve forming hook like structure and reach up to midline of dorsal valve, fourth instar valve apex directed outwards and remain inside of paraproct. in the fifth instar valves slightly cross paraproct, while ventral valve

still remained thin and shorter than dorsal valve, in the sixth instar ninth sternite much longer and wide with conical apex, ventral valve become thick and strong about to touch the apex of dorsal valve.

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