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Morphobiological characterization of *Oestrus ovis*

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Abstract
The present study was undertaken to identify and characterize morphological features of *Oestrus ovis* in Telangana. *Oestrus ovis* is the most predominant nasal bot fly causing nasal myiasis in India. The *Oestrus ovis* third instar. larvae were reared in the laboratory in a cage and the adult fly emerged within 28 days. The larvae collected from Jiyaguda slaughter house, Hyderabad were separated into L_2 and L_3 larvae and washed with normal saline and PBS P^H 7.4 and were preserved in 70 % ethanol for identification. The larvae were speciated by light microscopic examination based on morphological features such as ventral spines, anterior hooks and "D" shaped posterior spiracles. Notably, L_2 larvae were smaller, averaging 1.38±0.17 cm in length, while L_3 larvae measured 2.28±0.15 cm. Various measurements, such as spiracle diameters and hook dimensions, exhibited discernible changes between the larval stages, providing insights into their developmental characteristics.

Keywords: Anterior hooks, Nasal myiases, *Oestrus ovis*, posterior spiracles, ventral spines

1. Introduction
Oestrus ovis larvae, as obligatory parasites, induce nasal myiases in sheep and goats and thus commonly referred to as nasal bot fly Soulsby (2012) [4]. This parasitic dipteran species has been widely observed to infest a variety of vertebrate animals, including livestock, and on rare occasions, humans. The significant impact of this parasite on livestock has been thoroughly documented worldwide, with *Oestrus ovis* larvae functioning as obligate parasites that instigate histopathological alterations in nasal tissues. These alterations give rise to allergic reactions, bacterial infections, and consequent economic losses to the livestock industry (Dorchies *et al.* 1998) [2].
The morphological characterization of *Oestrus ovis* larvae and adult flies holds paramount importance in understanding the lifecycle, development, and pathology induced by this notorious nasal bot fly. Detailed examination of the morphological features, such as the structure of ventral spines, anterior hooks and posterior spiracles, as well as the size and dimensions of larvae at different stages, provides crucial insights into their developmental progression. These morphological attributes not only aid in species identification but also contribute significantly to understanding the pathogenic mechanisms and impact on host tissues Zumpt (1965) [6]. By scrutinizing the distinct morphological characteristics of *Oestrus ovis*, researchers can better comprehend how these parasites infest and cause damage to nasal tissues in hosts, leading to allergic reactions, bacterial infections, and subsequent economic losses in livestock (Tabouret *et al.* 2003) [5]. Furthermore, understanding the morphology of adult flies enables the identification and tracking of these parasites, aiding in devising effective control and management strategies to mitigate their impact on animal health and agricultural productivity. Ultimately, the morphological analysis serves as a cornerstone in unraveling the intricate dynamics of this parasite-host interaction and guides efforts toward mitigating its detrimental effects.

2. Materials and Methods
The present study focused on the collection, quantification and morphometric analysis of *Oestrus ovis* larvae. The categorization of larvae into different developmental stages was carried out following established criteria from previous research. Thin sections of the posterior spiracles from second and third stage larvae were carefully excised using a sterile blade. These sections were subsequently placed into a clean, sterile test tube containing a 10 % KOH

solution and subjected to boiling for a duration of 3 to 5 minutes over a spirit lamp. After boiling, thin sections of posterior spiracles were cooled down and dehydrated in ascending grades of alcohol *viz.* 70 %, 90 %, and absolute alcohol for 3 hours, 24 hours, 30 min and 10 min, respectively. The dehydrated spiracles were cleared for 5 to 10 minutes in carboxylol solution and were mounted on a clean glass slide using DPX mountant with the help of a cover slip. After mounting, posterior spiracles, anterior hooks and ventral spines were identified under microscope.

3. Results and Discussion

3.1. Rearing of Adult *Oestrus Ovis.*

In a laboratory environment, the adult flies emerged from third instar larvae. Within 28 days successfully. The adult flies were kept dry and preserved in the laboratory as expalained in Cepeda-Palacios *et al.* (1999) [1]

3.2. Morphological Studies

3.2.1. Morphology of young (L_2) and mature (L_3).

The young larvae were white or slightly yellow, in color with, dark transverse bands present on the dorsal aspects of the segments. The fully grown larva was approximately 3 cm in length and it tapered towards the anterior end with a flat surface posteriorly. The larvae of this species, specifically the L2 and L3 stages, display distinct differences in size. L2 larvae were relatively smaller, with an average length of 1.38±0.17 cm and a width of 0.65±0.05 cm, while L3 larvae were noticeably larger, measuring 2.28±0.15 cm in length and 0.68±0.04 cm in width. These variations reflect the growth and development of the larvae as they progress through their stages as described by Zumpt (1965) [6].

Table 1: Morphometry of length and width L_2 and L_3

S. No	Morphometry	Length (cm)	Width (cm)
1.	2nd stage larva	1.38±0.17	0.65±0.05
2.	3rd stage larva	2.28±0.15	0.68±0.04

3.2.2. Posterior spiracles of larvae of *Oestrus ovis*

The black 'D'-shaped stigmal plates are conspicuously located on the posterior surface. Posterior spiracles of six *Oestrus ovis* larvae longitudinal diameter exhibited a progression from 0.24±0.013 mm in L2 to 0.28±0.007 mm in L3, while the lateral diameter similarly advanced from 0.20±0.009 mm in L2 to 0.24±0.004 mm in L3.

Table 2: Morphometry of posterior spiracles of larvae

S. No	Morphometry	Logitudnal diameter (mm)	Lateral diameter (mm)
1.	2nd stage larva	0.24±0.013	0.20±0.009
2.	3rd stage larva	0.28±0.007	0.24±0.004

3.3. Anterior hooks of larvae of *Oestrus ovis*

The mature larva possessed of large black oral hooks, connected to an internal cephalopharyngeal skeleton. The length of the anterior hooks showed a growth from 0.21±0.007 mm in L2 to 0.23±0.007 mm in L3. while their width remained consistent at 0.05±0.002 mm for two instars.

Table 3: Morphometry of anterior hooks of larvae

S. No	Morphometry	Length(mm)	Width (mm)
1.	2nd stage larva	0.21±0.007	0.05±0.002
2.	3rd stage larva	0.23±0.007	0.05±0.001

3.4. Ventral spines of larvae from *Oestrus ovis*

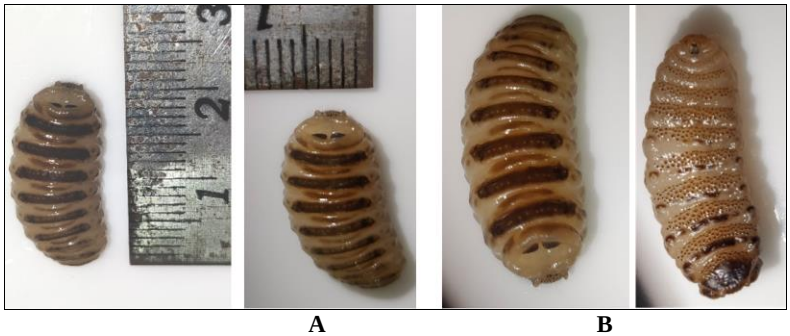
Furthermore, the ventral surface of the larva with rows of small spines. The length of the ventral spines increased 0.03±0.003 mm in L2 to 0.04±0.003 mm in L3, while the base increased 0.02±0.001 mm in L2 to 0.03±0.003 mm in L3 as seen in Sen and Fletcher (1962) [3].

Table 4: Morphometry of ventral spines of larvae

S. No	Morphometry	Length (mm)	Base (mm)
1.	2nd stage larva	0.03±0.003	0.02±0.001
2.	3rd stage larva	0.04±0.003	0.03±0.003

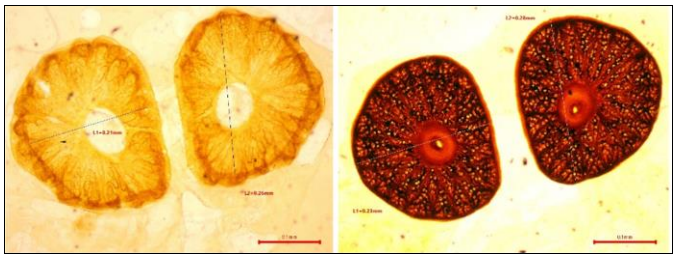
A **B**

Fig 1: Photograph of young (L_2) larva showing its length(A) and width(B).



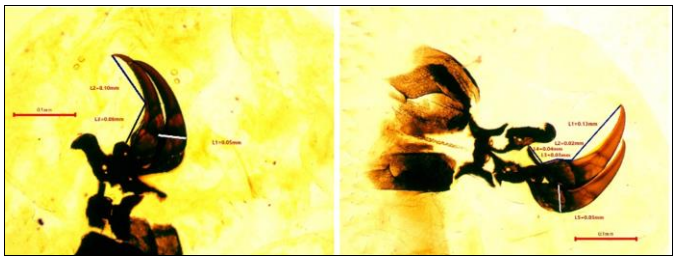
A **B**

Fig 2: Photograph showing of mature (L3) larva showing its length(A) and width(B).



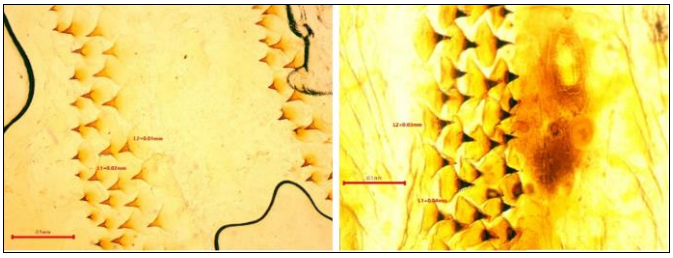
A B

Fig 3: Photomicrograph of L_2(A) and L3(B) larva showing posterior spiracle (4X).



A B

Fig 4: Photomicrograph of L_2(A) and L3(B) larva showing anterior hooks (4X).



A B

Fig 5: Photomicrograph of L_2 (A) and L3(B) larva showing ventral spines (4X).

3.5. Pupa and adult fly of *Oestrus ovis*

In this particular investigation, it was observed that the emergence of the adult fly from its pupal stage took precisely 28 days. The pupa itself is characterized by its measurements, boasting a length of 1.53±0.04 cm and a width of 0.53±0.02 cm. Serving as a critical transitional phase between larval and adult stages, it acts as a bridge in the fly's life cycle. Once the transformation is complete, the adult fly, emerged with its length - 1.1±0.03 cm, width- 0.53±0.02 cm, displays a body tinted in greyish-brown and adorned with numerous small black spots on its thorax. This thorax is delicately covered with fine, light brown hair. An intriguing feature of these adult flies is their rudimentary and non-functional oral mouthparts, rendering them entirely incapable of feeding.

A B

Fig 6: Photograph showing length(A) and width(B) of pupa.

A B

Fig 7: Photograph showing length(A) and width(B) of adult fly.

4. Acknowledgements
The research facilities provided by P. V. Narasimha Rao Telangana Veterinary University Rajendranagar, Hyderabad is being acknowledged.

5. Conclusion
The study of Oestrus ovis larvae and pupae provides valuable insights into their morphology and developmental stages. From the initial larvae to the mature third instar, distinct changes in size and anatomical features like posterior spiracles, anterior hooks, and ventral spines are observed. The pupal stage, crucial for metamorphosis into the adult fly, lasts 28 days and marks a critical phase in their life cycle. The emergence of the adult fly, characterized by its distinct appearance and non-functional mouthparts, underscores the species' adaptation strategies. This comprehensive morphological study enhances our understanding of Oestrus ovis development and life history.

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