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Morphological Description of the Three Larval Instars of *Wohlfahrtia nuba* (Diptera: Sarcophagidae)

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ABSTRACT

Background: Accurate identification of the larval stages of *Wohlfahrtia nuba*, a flesh fly species within the Sarcophagidae family, is crucial in forensic and medical entomology. This species may contribute to estimating the postmortem interval (PMI) and *is* associated with causing *myiasis* in livestock and humans. The insufficient data regarding the morphology of *the immature stages of W. nuba* hinders the taxonomic identification of this species' larvae in carcasses without raising adults. Therefore, this study provides a comprehensive examination of the morphological characteristics of three instars of *W. nuba* larvae using light microscopy.
Methods: It includes an examination of the cephalopharyngeal skeleton, pseudocephalon, antennal complex, oral ridges, anterior spiracles and their papillae number and arrangement, thorax and abdomen spinulation, number and distribution of papillae around the entrance of the spiracular cavity and posterior spiracles.
Result: The present study's findings demonstrate the distinct morphological characteristics that differentiate the three larval instars, facilitating accurate identification and developmental staging. The three larval stages have two curved mouth hooks. A large median hook is absent. The labrum is a reduced structure exhibiting four highly sclerotized teeth between the mouth hooks. The dorsal arch is short, curved and does not extend to the parastomal sclerite. The anterior spiracles, located laterally on the first thoracic segment, exhibited variability in the number of papillae across the three instar larvae. The first larval instar exhibited a total of eight papillae, which increased to 10 in the second instar and reached 11 in the third instar. These papillae are arranged in a row resembling an arch. The spines encircling the cephalic collar are thin and set into 6-7 rows, separating the first segments of the thorax. The posterior spiracle contains two incomplete peritremes hidden in a deep cavity. It is characterized by a single aperture divided at its upper section and shielded by thin hair-like structures during the first larval instar. Each incomplete peritreme contained two slits for the second larval instar and three slits for the third larval instar. The peritreme lacks a ventral arch and the button is not easily noticeable. A ring of 12 cuticular papillae encircles the anal cavity. These data add new morphological features to differentiate between *W. nuba* larvae in clinical and forensic contexts. This will help with taxonomic and forensic species identification studies and benefit farmers and veterinarians.

Key words: Cephalopharyngeal skeleton, Forensic entomology, Identification, Larvae, Morphology, *Wohlfahrtia nuba*.

INTRODUCTION

Forensic entomology plays a crucial role in estimating the postmortem interval (PMI) by analyzing the temporal succession of adult flesh flies and their larvae. *Wohlfahrtia nuba*, a species in the Sarcophagidae family, is attracted to decomposing corpses and plays a crucial role in the early stages of infestation during decomposition. This characteristic renders it an important biological indicator for estimating the PMI (Spradbery, 1994; Introna *et al*., 1998; Sotiraki *et al*., 2010; Al-Mesbah *et al*., 2011; Szpila *et al*., 2014; Sharawi *et al*., 2024). Additionally, *W. nuba* is an important flesh fly relevant to medical and veterinary management due to its ability to cause traumatic myiasis in humans and domestic animals as a facultative parasite, leading to economic losses and human suffering worldwide (Hall and Wall, 1995; Hall and Farkas, 2000; Courtney *et al*., 2009).

Numerous studies have thoroughly investigated the morphology of the immature stages of the Sarcophagidae and Calliphoridae families (Zumpt, 1965; Erzinçlioğlu, 1985; Sandeman *et al*., 1987; Ruiz-Martinez *et al*., 1989, 1990; Bonatto and Carvalho, 1996; Szpila and Pape, 2008; Florez and Wolff, 2009; Mendonça *et al*., 2010; Szpila, 2010; Singh *et al*., 2012; Midgley and Villet, 2021; Hanan, 2018 Alotaibi *et al*., 2025), focusing on the morpho-logical characteristics essential for species identification and developmental estimation. Studies examining the early developmental stages have been conducted across various

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species, including *Sarcophaga africa, Sarcophaga cultellata, Chrysomya bezziana*, *Cochliomyia hominivorax* and *Wohlfahrtia magnifica* (Lehrer and Fromunda, 1986; Szpila *et al.*, 2008, 2014; Szpila and Villet, 2011; Klong-Klaew *et al.*, 2012; Grzywacz *et al*., 2012; Sanit *et al*., 2012; Semelbauer and Kozanek, 2012; Buenaventura, 2013; Szpila *et al*., 2013 and 2014; Velásquez *et al.*, 2010, 2013; Ubero Pascal *et al*., 2015; Vairo *et al*., 2015; da-Silva-Xavier and de Carvalho Queiroz, 2016; Szpila and Wallman, 2016; Faraj and Mawlood, 2018). Similarly, Hilton (1973) studied Walker, (1937) investigated Wohlfahrtia vigil.

Identifying flesh fly larvae poses significant challenges due to their simplified external morphology and the notable similarity among species. Despite the significance of *W. nuba* in estimating the PMI and its medical and veterinary relevance, there is insufficient information regarding the morphology of its immature stages for accurate identification. This knowledge gap underscores the need to investigate the morphological characteristics of the three larval instars. Consequently, this study aims to examine the morphological features of the three developmental stages of *W. nuba* using light microscopy, the primary technique utilized by forensic entomologists (Szpila and Villet, 2011; Ubero-Pascal *et al*., 2015). Although light microscopy has limitations relative to advanced imaging techniques, it is still a sufficient and widely recognized method for identifying the morphological characteristics essential for species differentiation in forensic entomology. Light microscopy offers a practical and efficient method for data collection in forensic contexts, particularly when time and resources are limited. Various morphological characteristics are examined, including the cephalopharyngeal skeleton, pseudocephalon, antennal complex, oral ridges, anterior spiracles and their papillae number and arrangement, spinulation of thorax and abdomen, number and distribution of papillae around the entrance of the spiracular cavity and posterior spiracles. This study is characterized by its specific examination of the immature stages of *W. nuba*, in contrast to earlier research that predominantly focused on different flesh fly species or provided minimal morphological information on related species. Thereby providing new data to the field of forensic entomology.

MATERIALS AND METHODS

Insects

Wohlfahrtia nuba adult specimens were sourced from a colony maintained at the Biology Laboratory of Hafr Al Batin University in Saudi Arabia. These adult flies were placed in rearing cages measuring 30×30×30 cm, where they were provided with granulated sugar, water and powdered milk for nourishment and allowed to mate and lay larvae. A plastic cup with approximately 60 g of fresh beef was placed inside the cage to serve as a substrate for larval deposition, with a new cup of fresh meat replacing it daily. Observations were made every 12 hours to track larval deposition over two weeks. Larvae were raised on fresh beef in 500-ml jars, which were covered with a cloth with an elastic ring and contained a 2 cm layer of damp sand at the bottom. The study was conducted in October 2023 at the biology laboratory of Hafr Al Batin University, maintaining conditions at 30°C±2, with 50-70% humidity and a light-dark cycle of 12:12 hours.

Specimen preparation

Ten larvae from each stage of development and ten prepupae were randomly selected and weighed using a highly accurate electronic balance (GR-200 balance; A and D Company, Limited, Tokyo, Japan). The selection of the first instars occurred one hour post-larviposition. To maintain the elongated shape of the pseudocephalon and avoid any deformations during storage in 80% ethanol, 20 larvae from each developmental stage and 20 prepupae were briefly immersed in hot water (80 to 95°C) for thirty seconds, following the procedure described by Adams and Hall (2003). The dimensions, including length and width, of three instar larvae and prepupae, were measured using an ocular micrometer attached to a light microscope (Olympus U-CMAD3 BX50, Japan). The length of the cephalopharyngeal skeleton was also measured. Subsequently, the larvae were mounted on slides and examined under a light microscope. The posterior end of the larvae was cut off using a razor blade to prepare a specimen suitable for microscopic examination of the posterior spiracles. Both sections of each larva were cleared in a solution of absolute ethanol saturated with phenol crystals (Smith and McFadden, 1981). Photomicrography was conducted using a Leica Vario-Summilux H-2.4/16-80 ASPH digital camera mounted on a Kyowa, Tokyo 070039, Japan. The morphological terminology for cephalopharyngeal skeleton, pseudocephalon and general morphology follows Courtney (2000), Szpila *et al*. (2008), Vairo *et al.* (2015) and Buenaventura (2013).

Statistical analysis

The data were statistically analyzed using SPSS 26.0 and presented as mean±standard error (S.E.). Before analyzing variance (ANOVA) to assess the differences in means, the data for normality and homoscedasticity were evaluated. The subsequent LSD test was conducted to determine which specific instar larvae exhibited significant differences in their development.

RESULTS AND DISCUSSION

General morphology

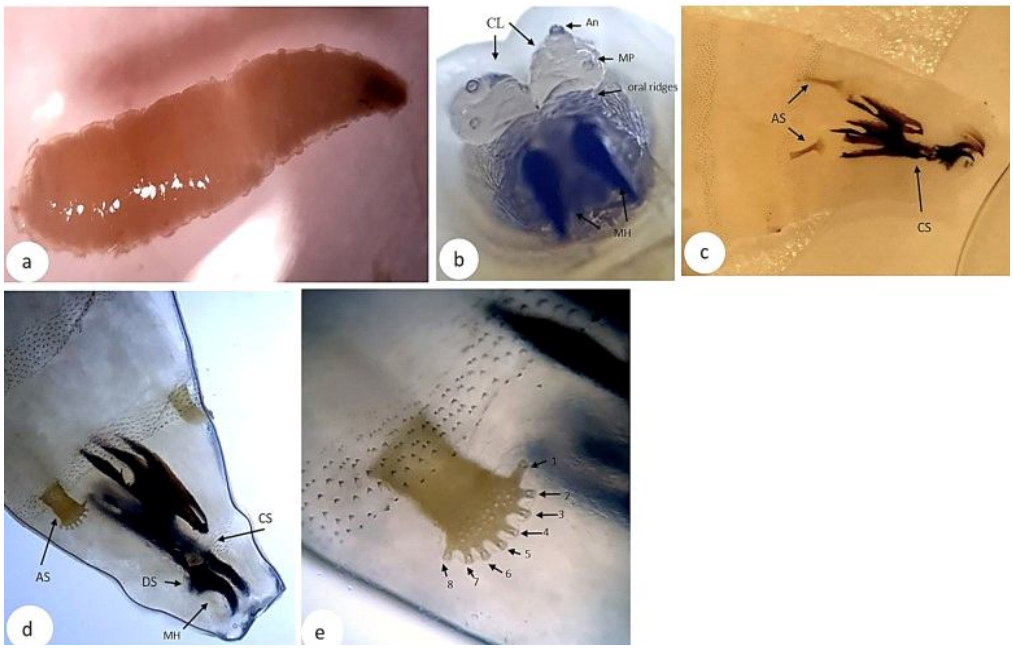
The larval body of *Wohlfahrtia nuba* exhibits a vermiform morphology, featuring a tapered front and a wide posterior (Fig 1a). The anterior region of the larval body houses pseudocephalon, comprising two cephalic lobes. Following the anterior region are three thorax segments, seven abdomen segments and a caudal segment containing the posterior spiracles. Larval stages possess a three-dimensional cephalopharyngeal skeleton, necessitating

the capture of two microscopic images: one before the coverslip and another after compression (Fig 1b, c, d). The anterior segment of the thorax features two broad anterior spiracles. The first and second larval instars bear morphological similarities to the third larval instar, irrespective of anterior and posterior spiracles, as well as variations in size and the degree of sclerotization.

First larval instar

The length of the first instar larvae, including the cephalopharyngeal skeleton, was measured to be 4.95±0.054 mm, with a width of 1.21 ± 0.038 mm (n = 20). The length of the cephalopharyngeal skeleton was 1.63±0.013 mm (n = 20). The weight of the first instar larvae was the lowest, measuring 12.18±0.248 mg (n = 10) (Table 1). The pseudocephalon displays a bilateral structure consisting of two cephalic lobes. Each cephalic lobe has a ventral maxillary palpus, a dorsal antennal complex and oral ridges in the middle on its anterior surface. A short antennal dome and a basal ring constitute the antennal complex. The oral ridges extend laterally to the ventral side of the pseudocephalon adjacent to the mouth aperture, forming a facial mask surrounding the mouth opening (Fig 1b).

The cephalopharyngeal skeleton has a pair of large, symmetrical and heavily sclerotized mouth hooks. Each mouth hook is connected to a basal dental sclerite with an anteriorly projecting chitinous process. These mouth hooks are highly curved with a pointed end directed ventrally, resembling a talon. The absence of the large median hook is observed (Fig 1b, c, d). A pair of anterior spiracles are on either side of the spiny band of the prothorax (Fig 1c). The anterior spiracles have eight papillae arranged in a row,



Abbreviations: An: Antennal complex; AS: Anterior spiracle; CL: Cephalic lobes; CS: Cephalopharyngeal skeleton; DS: Dentate sclerite; MH: Mouth hooks; MP: Maxillary palpus.

Fig 1: Morphology of the first instar *Wohlfahrtia nuba* larvae: a: Lateral view of the entire first larval instar body; b: Pseudocephalon and mouth hooks. c, d: Lateral view of the anterior end of the larval body; e: Anterior spiracle.

Table 1: Average measurements of length, width and weight across the three larval instars and prepupal stage, including cephalopharyngeal skeleton length in *Wohlfahrtia nuba.*

Development stage	Whole larval body			Cephalopharyngeal skeleton
	Length (mm)	Width (mm)	Weight (mg)	Length (mm)
	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±SE
1st larval instar	4.95±0.054[a]	1.21±0.038[a]	12.18±0.248[a]	1.63±0.013[a]
2nd larval instar	17.83±0.049[b]	3.19±0.028[b]	84.87±0.396[b]	1.82±0.037[b]
3rd larval instar	24.32±0.281[c]	5.45±0.117[c]	182.15±0.642[c]	2.51±0.041[c]
Prepupal stage	19.48±0.417[d]	6.29±0.053[d]	123.74±0.443[d]	1.94±0.019[d]

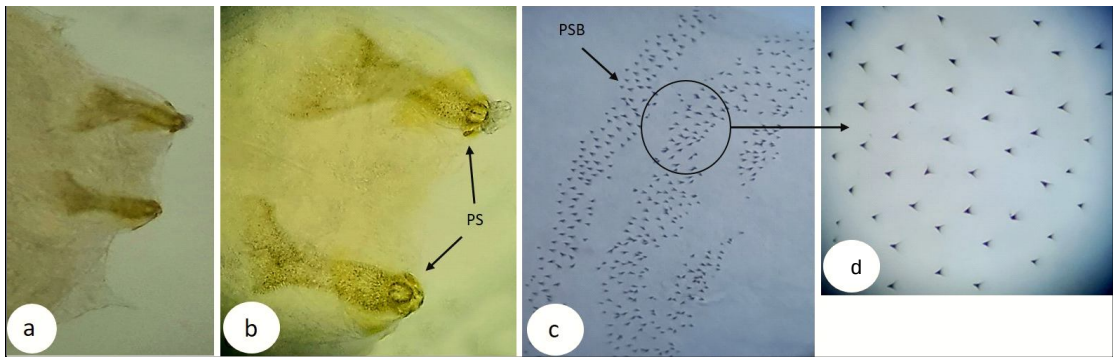
Means with the same letter in each column are not statistically different at a significance level of P<0.05 based on the LSD test performed after analysis of variance (ANOVA).

forming an arch-like shape (Fig 1d). The anterior bands of posteriorly directed spines, form a complete circle on the dorsal side and are interrupted laterally along the margins of all abdominal segments (Fig 2c,d). Both the anterior and posterior bands feature elongated, pointed spines. The anal pads are prominently extended (Fig 1a, 2a). The posterior spiracular plates are more distantly spaced, with the peritreme lacking development on the ventral side. They feature a single aperture split in their upper portion and are shielded by thin, hair-like structures (Fig 2b).

Second larval instar

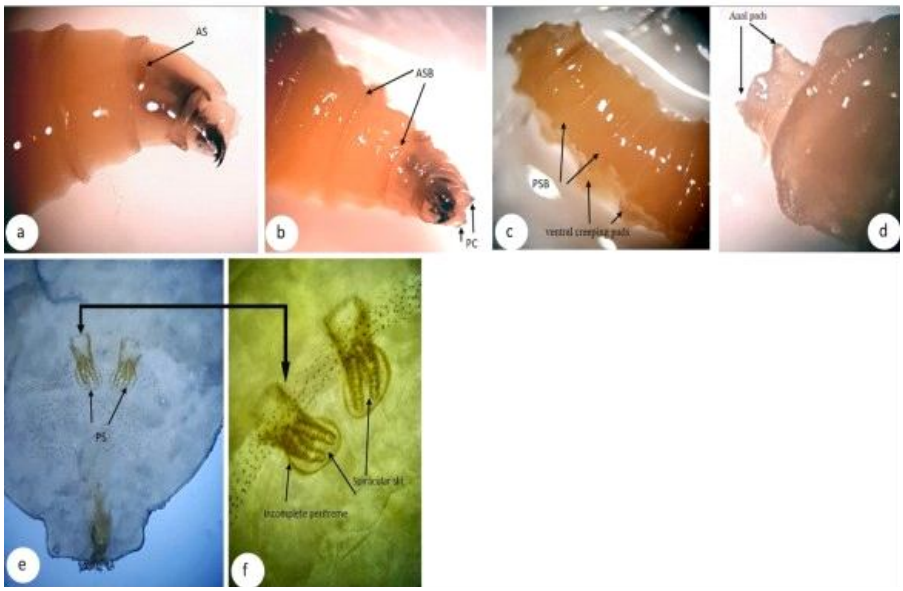
The second larval instar had a length of 17.83±0.049 mm, including the cephalopharyngeal skeleton and a width of 3.19±0.028 mm (n = 20). The second instar larvae showed a significant weight gain, increasing to more than six times the weight of the first instar larvae, reaching 84.87±0.396 mg (n = 10), which was highly significant (P<0.05). The length of the cephalopharyngeal skeleton increased by 1.82± 0.037 mm (n = 20) (Table 1).

The pseudocephalon in the second larval stage closely resembles that in the first stage. The only things that make them different are their level of sclerotization and size. The pair of mouth hooks exhibit increased sclerotization and pronounced tapering, resembling talons (Fig 3a, b and 4a). The mouth hooks are attached to the more extensive dental sclerite, which is hooked in shape (Fig 4a-c). The parastomal sclerite exhibits increased distinctness and elongation (Fig 4b). Additionally, the labial sclerite exhibits increased prominence and lateral elongation (Fig 5b). No



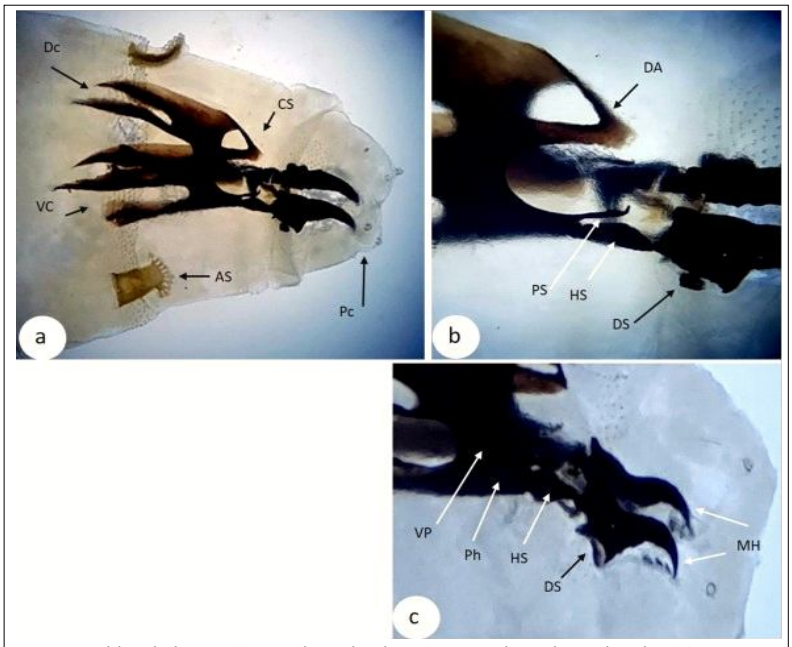
Abbreviations: PS: Posterior spiracles; PSB: Posterior spinose bands.

Fig 2: Morphology of the first instar *Wohlfahrtia nuba* larvae: a, b: Posterior end of the larval body with Posterior spiracles; c, d: Posterior spinose bands spines.



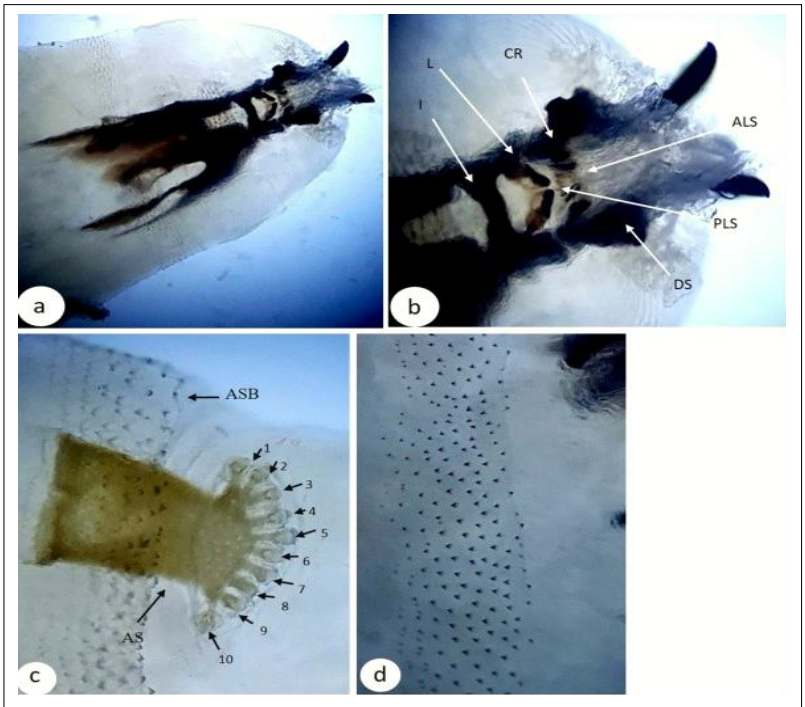
Abbreviations: AS: Anterior spiracle; ASB: Anterior spinose bands; PC: Pseudocephalon; PS: Posterior spiracles; PSB: Posterior spinose bands.

Fig 3: Morphology of the second instar *Wohlfahrtia nuba* larvae: a: Lateral view of the anterior end; b: Ventral view of the anterior end; c: Lateral view of the posterior end; d: Caudal segments; e, f: Posterior spiracles.



Abbreviations: AS: Anterior spiracle; ASB: Anterior spinose bands; CS: Cephalopharyngeal skeleton; DA: Dorsal arch; DC: Dorsal cornua; DS: Dentate sclerite; HS: Hypostomal sclerite; MH: Mouth hooks; PC: Pseudocephalon; Ph: Pharyngeal sclerite; PS: Parastomal sclerite; VC: Ventral cornua; VP: Vertical plate.

Fig 4: Morphology of the second instar *Wohlfahrtia nuba* larvae: a: Lateral view of anterior end of the body; b, c: Cephalopharyngeal skeleton.



Abbreviations: ALS: Anterior labial sclerite; AS: Anterior spiracle; ASB: Anterior spinose band; CR: Chitinous ventral ring; DS: Dental sclerites; L: Labial sclerite; I: Intermediate sclerite.

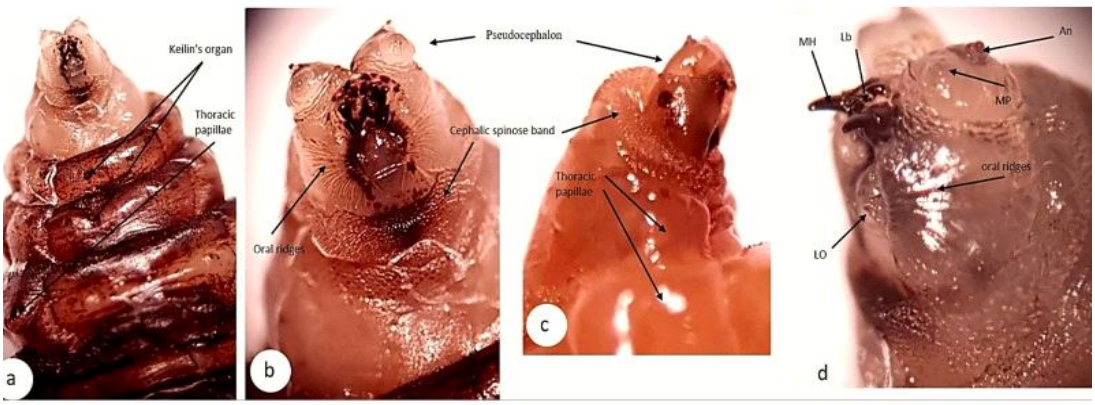
Fig 5: Morphology of the second instar *Wohlfahrtia nuba* larvae: a, b: Ventral view of the cephalopharyngeal skeleton; c: Anterior spiracle; d: Anterior spinose bands.

morphological differences are observed in the dorsal arch between the first and second larval instars (Fig 1c, d, 4b). The lateral view of the cephalopharyngeal skeleton shows the parastomal sclerite's connection to the intermediate sclerite. (Fig 4b, c). The width of the vertical plate is equal to one-third of the ventral cornua. The widths of the dorsal and ventral cornuae are identical and conical. However, the dorsal cornua is longer than the ventral cornua and has a posterior bifurcation (Fig 4a). The hypostomal sclerite is small (Fig 4b, c). The dorsal arch is short and curved. It does not extend to the parastomal sclerite (Fig 4c). The ventral view of the cephalopharyngeal skeleton reveals H-shaped intermediate sclerites, short parastomal sclerites and the labial sclerites. The vertical plate is heavily sclerotized (Fig 5a, b).

No morphological differences exist between the thoracic and abdominal segments of the first and second larval instars, except for size variations. A pair of more prominent anterior spiracles exhibits a rise in papillae, with approximately 10 organised in a row, forming an arch-like structure (Fig 4a, 5c). The anterior bands of spines that point backwards form a continuous ring around the edges of all thoracic segments (Fig 5c, d). Each abdominal segment possesses a pair of ventral creeping pads covered with spines (Fig 3c). Figures 3c and e illustrate the eighth abdominal segment, the caudal segment. The caudal segment of the second larval instar looks a lot like the first, but the posterior spiracles are more developed and sclerotized. Each posterior spiracle contains two internal slits within a peritreme. The peritreme lacks a ventral arch that is sclerotized. The button exhibits invisibility (Fig 3e, f).

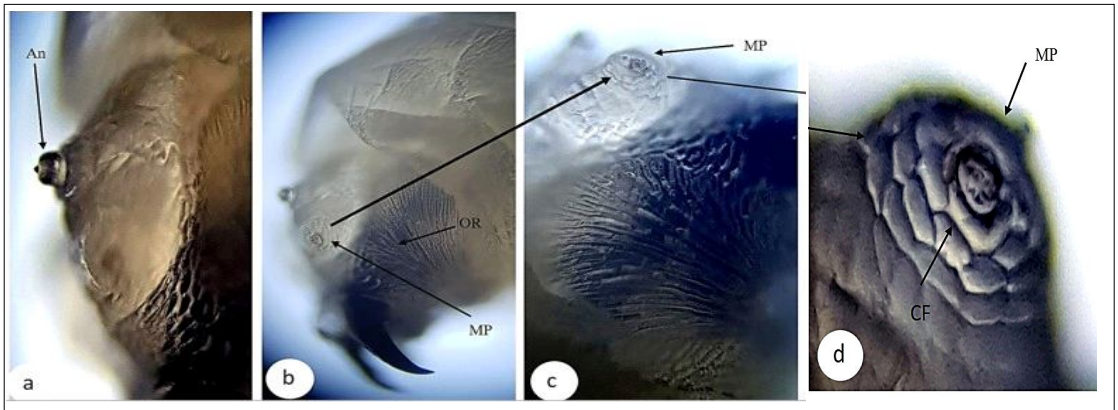
Third larval instar

Sclerotization and size are the only morphological distinctions between the third and second instars. The length of the third larval instar, including the cephalopharyngeal skeleton, significantly increased ($P<0.05$) to 24.32±0.281 mm, with a width of 5.45 0.117 mm (n = 20). As shown in Table 1, there was a significant rise in weight, approximately 15 times greater than that of the first instar larvae, reaching



Abbreviations: An: Antennal complex; MP: Maxillary palpus; Lb: Labrum; MH: Mouth hook; LO: Labial organs.

Fig 6: Morphology of the third instar *Wohlfahrtia nuba* larvae: a: Ventral view of the thoracic segments with Keilin's organ; b: Ventral view of the Pseudocephalon; c: Lateral view of the Anterior end of the body with thoracic papillae; d: Lateral view of the Pseudocephalon.



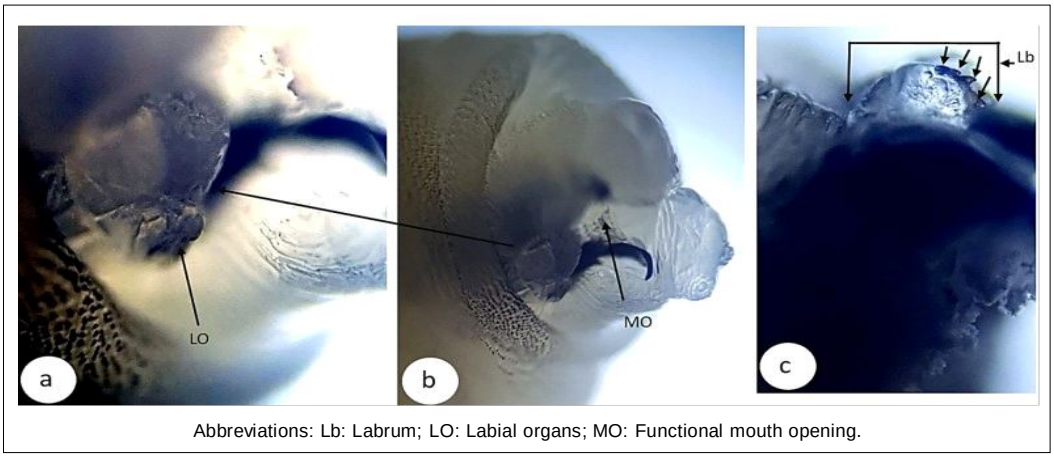
Abbreviations: An: Antennal complex; MP: Maxillary palpus; OR: Oral ridges; CF: Cuticular folds.

Fig 7: Morphology of the third instar *Wohlfahrtia nuba* larvae: a, b: Antennal complex; c, d: Maxillary palpus.

182.15±0.64 mg (n = 10), with a high degree of statistical significance (P<0.05). The length of the cephalopharyngeal skeleton was 2.51±0.041 mm (n = 20).

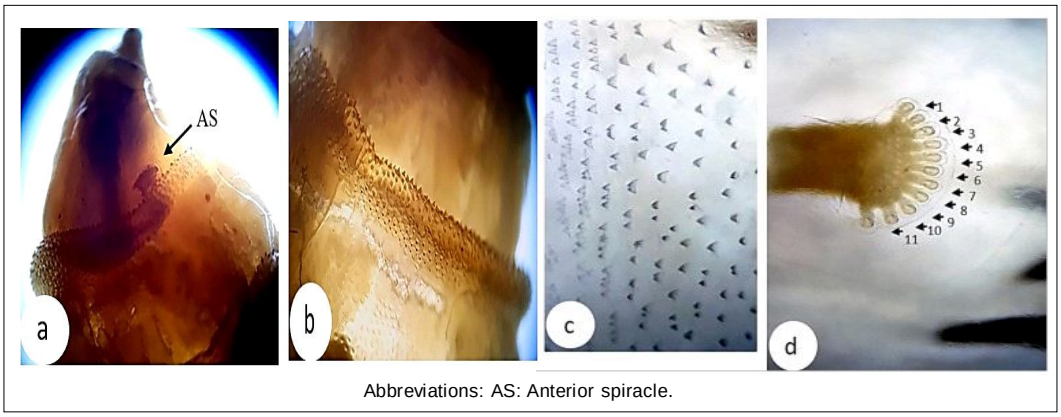
The pseudocephalon and cephalopharyngeal skeleton exhibit morphological similarities to those observed in the second larval stage, as depicted in Fig 6a-d, except for size and additional differentiation. The two mouth-hooks exhibit greater robustness, apical tapering, heavy sclerotization, increased thickness and more remarkable similarity. The dental sclerite is larger and hook-shaped than the one in the second larval instar. The parastomal sclerite exhibits greater prominence compared to the second larval instar. In the antennal complex, it was observed that the diameter and height of the basal ring are more significant than that of the antennal dome (Fig 7a, b). A center cluster of sensilla, comprising two basiconic and three coeloconic sensilla, forms the maxillary palpus. These sensilla are surrounded by several cuticular folds (Fig 7c, d). The oral ridges extend laterally to the ventral side of the pseudocephalon adjacent to the mouth aperture, forming a facial mask that surrounds the mouth opening (Fig 6b and 7b). Additionally, the mouth opening has a labial lobe in the middle, with labial organs on both sides (Fig 8a, b). The absence of the large median hook is observed. The dental sclerite is of considerable size and fused to the mouth hook's base. Labrum is a reduced structure exhibiting four highly sclerotized teeth between the mouth hooks (Fig 8c). The cephalic spinose band, which consists of spines directed posteriorly, differentiates the cephalic region from the first segment of the thorax. The spines on the thoracic and abdominal segments exhibit a similar arrangement and pattern as observed in the preceding larval instars. The spines of the thoracic spinose band exhibit both single and double tips in their arrangement. The spines exhibit a tapering shape at the apex and a broad shape at the base. The spines are dorsally arranged in 7-9 rows and ventrally arranged in 9-10 rows (Fig 9a-c).

Similar to the first and second larval stages, each thoracic segment contains laterally a pair of anterior spiracles and Keilin's organ ventrally (Fig 6a). These segments are bordered anteriorly by spiny bands with uniform, posterior-facing spines (Fig 6a). Additionally, each



Abbreviations: Lb: Labrum; LO: Labial organs; MO: Functional mouth opening.

Fig 8: Morphology of the third instar *Wohlfahrtia nuba* larvae: a: Anterolateral view of the labial lobe with labial organs; b: Functional mouth opening; c: Lateral view of the labrum.



Abbreviations: AS: Anterior spiracle.

Fig 9: Morphology of the third instar *Wohlfahrtia nuba* larvae: a: First thoracic segment with an anterior spiracle and a second thoracic segment; b: First thoracic spinose band; c: Thoracic band spines; d: Anterior spiracle.

spiny band is accompanied by thoracic papillae on both sides (Fig 10a-d). An anterior spiracle pair of eleven papillae arranged in a row resembling an arch (Fig 9d). A pair of spiny creeping pads is present on the ventral side of each abdominal segment (Fig 10c).

Fig 11a, b and c show the caudal segment with a pair of large, strongly protruding pads called anal pads, along with posterior spiracles that are fully developed. Twelve cuticular papillae encircle the spiracular cavity. The cuticular papillae are categorized into three pairs on the dorsal and dorsolateral regions and three on the lateral and ventral regions (Fig 11e). The interband areas of larval body segments lack spines but exhibit small granular structures called warts. The spines within the spiny bands exhibit apical tapering and share a consistent outline. The posterior spiracle consists of three dorsoventrally oriented slits enclosed by an incomplete peritreme. The slits are slightly curved. The button is absent (Fig 11d, e and 12a-c).

Prepupae

The morphology of prepupae closely resembles that of third-instar larvae; however, minor morphological differences differentiate these stages. The posterior spiracle exhibits internalization while maintaining three spiracular slits, indicating an early adaptation for the prepupal stage. The

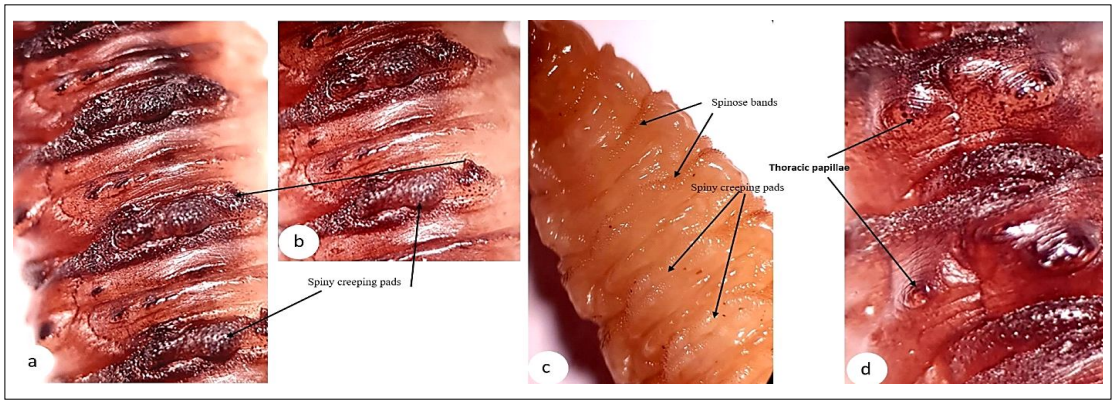


Fig 10: Morphology of the third instar *Wohlfahrtia nuba* larvae: a, b, c: Ventral view of the abdomen with posterior spinose bands and spiny creeping pads; d: Lateral view of the thoracic segments with thoracic papillae.

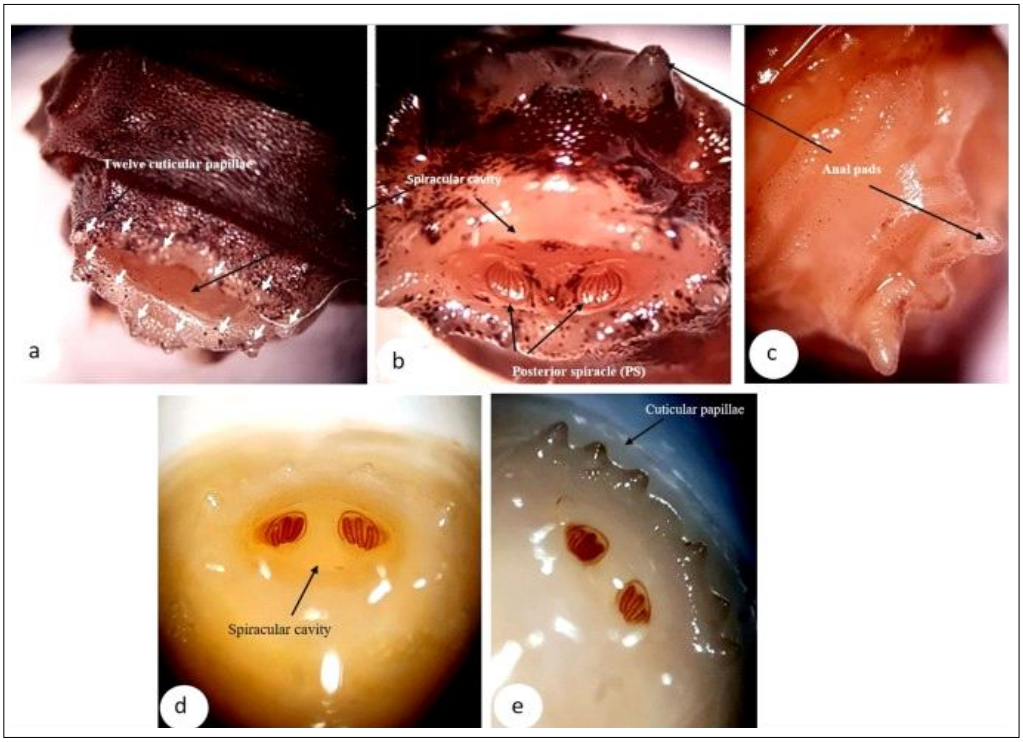


Fig 11: Morphology of the third instar *Wohlfahrtia nuba* larvae: a: Dorsal view of the caudal segment with twelve cuticular papillae; b: Spiracular cavity showing posterior spiracles; c: Ventral view of the caudal segment showing anal pads; d, e: spiracular cavity, posterior spiracles, cuticular papillae.

observable spiracular retraction is a key distinction during puparium formation, indicating preparation for pupa formation. As prepupae transition, the spiracles retract and become less prominent compared to the third-instar larvae, indicating a significant morphological change. Additionally, external hardening occurs during puparium formation. The puparium length, including the cephalopharyngeal skeleton, significantly decreased ($P<0.05$) to 19.48±0.417 mm (n = 20), while the width significantly increased to 6.286±0.053 mm (n = 20). There was a significant reduction in average weight ($P<0.05$) to 123.74±0.443 mg (n = 10). The length of the cephalopharyngeal skeleton decreased by 1.94±0.019 mm (n = 20).

Larval stages have two large mouth hooks that are strongly curved, resembling talons and aligned in parallel. It is worth noting that the medium hook is absent. The mouth hook data presented in this study are consistent with the findings reported by several previous researchers (Sukontason *et al*., 2010; Szpila, 2010; Szpila *et al*., 2015; Ubero Pascal *et al*., 2015). The cephalopharyngeal skeletons of *W. nuba*, distinguished by the presence of two mouth hooks and the absence of a median mouth hook, differ from those of *Wohlfahrtia vigil*, which possesses a single median mouth hook. *Wohlfahrtia pattoni* and *Wohlfahrtia magnifica* exhibit a median hook, as documented by Hilton (1973) and Szpila *et al*. (2014).

The vertical plate is highly sclerotized and measures one-third the length of the ventral cornua. In comparison to the ventral cornuam the dorsal cornua is longer and bifurcated posteriorly. Lopes and de Albuquerque (1982) reported the presence of hooked spines on the anterior margin of *Cochliomyia macellaria* and *Cochliomyia hominivorax*. Sukontason *et al*. (2005) presented a photograph of the cephalopharyngeal skeleton that closely resembles the one depicted. Hilton (1973) reported the larval cephaloskeleton of *W. pattoni*, which is very similar to what is described for *W. nuba*. However, the difference observed is shorter length of the ventral cornua in *W. nuba* across all instars. The first larval stage exhibits a diminutive hypostomal sclerite, whereas *Wohlfahrtia opaca* lacks a hypostomal sclerite entirely (James and Gassner, 1947). Ishijima (1967) highlighted the dorsal cornua as a distinctive characteristic of the Sarcophagidae family. In contrast, a study by Szpila and Pape (2007) found that the dorsal cornua is not a reliable characteristic for distinguishing the family Sarcophagidae.

The examinations suggest a correlation between the level of sclerotization of cephalopharyngeal skeleton sclerites and the age of the larvae. It appears that this correlation becomes more pronounced for the mouth hooks, which taper at the tip and lose some of their extreme concavity. Consequently, this observation indicates that each hook becomes thicker overall as the larvae age. The parastomal sclerites exhibit distinct elongation and differentiation. These results are in line with those of Vairo *et al*. (2015), who studied *Peckia (Sarcodexia) lambens* and Lopes and de Albuquerque (1982), who used cephalopharyngeal skeleton configurations to classify Sarcophagidae.

The labrum is a reduced structure exhibiting four highly sclerotized teeth between the two mouth hooks. This supports the findings of previous research by Lopes and de Albuquerque (1982) and YuG and Dolin (1988), who did not classify the labrum as a third-mouth hook. The researchers classified it as a prominent labrum with a distinct hook shape, which they employed as a distinguishing characteristic for the subfamily Paramacronychiinae. Although our findings differ from previous reports, it is essential to note the contrasting conclusions reached by Schumann (1976), Valentyuk (1971), Lehrer and Fromunda (1986) and more recently by Szpila *et al*. (2014) regarding the labrum in *W. magnifica*. The labrum has been proposed to function as an additional mouth hook by Ruiz-Martinez *et al*. (1989) and 1990. Zumpt (1965) described the mouth hooks of the first larval instar of *Chrysomya bezziana* as toothed. Szpila *et al*. (2014) described the cephalo pharyngeal skeleton of *C. bezziana,* revealing that the labrum is a tiny structure hidden between two mouth hooks and the tips do not have highly sclerotized teeth.

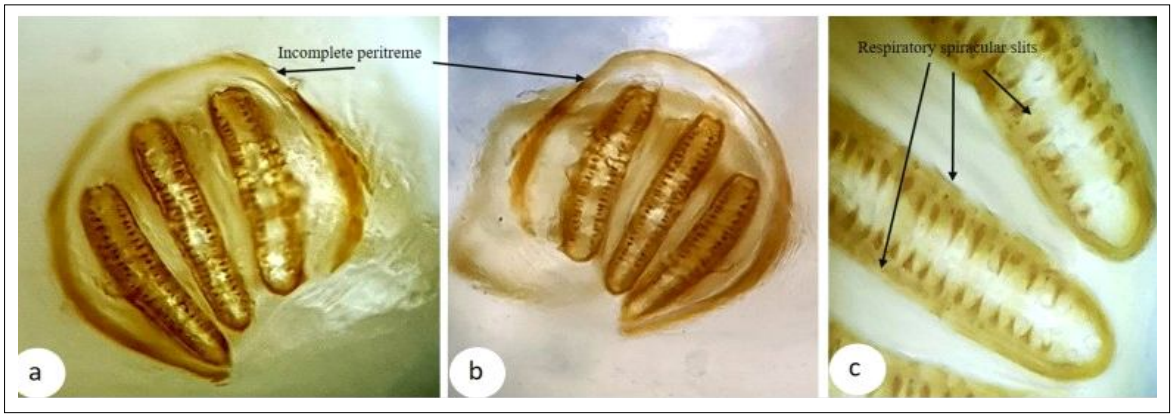


Fig 12: Morphology of the third instar *Wohlfahrtia nuba* larvae: a: Left posterior spiracle; b: Right posterior spiracle; c: Respiratory spiracular slits.

The pseudocephalon of *W. magnifica* larvae, as described by Szpila *et al*. (2014), is strikingly similar to the one mentioned in this article. The only difference is that the antennal dome is shorter than the basal circle's diameter and height. The findings of Vairo *et al*. (2015) align with our observations for *W. nuba*. Lopes and de Albuquerque (1982) employed the term "pseudocephalon" for broad taxonomic categorization, whereas Kirk-Spriggs (2003) regarded it as an interspecific anatomical feature.

The number of anterior spiracular papillae shows variability across the developmental stages observed in *W. nuba*. Each anterior spiracle typically consists of eight papillae in the first larval instar. This number increases to 10 papillae in the second larval instar. By the third larval instar, the spiracular papillae arrangement typically comprises 11 aligned rows resembling an arch. The findings presented in this study provide a different perspective than the observations made by Hilton (1973) regarding the number of papillae in the larval instars of *W. pattoni* and *W. vigil*. Hilton reported that these species possess 8–10 papillae in the second and third larval instars while lacking papillae in the first instar. Moreover, Szpila *et al*. (2015) reported that the first larval stage of *W. nuba* displays anterior spiracles with more than seven papillae, which aligns with the findings of Leite and Guevara (1993) regarding *C. hominivorax*. Mazza and Jörg (1939) reported that the anterior spiracle of *C. hominivorax* larvae exhibit a range of seven to ten papillae in the second instar and six to eleven papillae in the third instar.

The posterior spiracles' morphology, peritreme shape and internal spiracular slit are frequently employed as family-specific characteristics (Sukontason *et al*., 2010; Velásquez *et al*., 2010; Ubero Pascal *et al*., 2015). The findings presented in this study align with the results reported by Hilton (1973) regarding *W. pattoni*. According to Velásquez *et al*. (2010), the posterior spiracle slits in *Sarcophaga africa* are straight, while in other flesh fly larvae, they are curved. Based on the taxonomic classification provided by Ishijima, 1967 for *W. nuba* larvae, the absence of a sclerotized ventral arch is a distinguishing feature of the posterior spiracles peritreme. According to Richet *et al*. (2011), the larvae of *Sarcophaga caerulescens* exhibit a well-sclerotized ventral arch. The arrangement and number of papillae encircling the spiracular cavity are frequently employed in larval taxonomy (Szpila, 2010). Within *W. nuba*, 12 cuticular papillae surround the spiracular cavity. The arrangement of the cuticular papillae follows a pattern observed by Kano *et al*. (1951) and Sanjean (1957). There are six pairs: three on the dorsolateral and dorsal sides and three on the ventrolateral and ventral sides.

CONCLUSION

This study provides a detailed morphological description of the three instar larvae of the prevailing species of flesh flies, *W. nuba*, of high forensic value as an indicator for estimating PMI and medical and veterinary significance. Through an examination of the morphological characteristics, including the cephalopharyngeal skeleton, pseudocephalon, antennal complex, oral ridges, anterior spiracles and their papillae number and arrangement, thorax and abdomen spinulation, number and distribution of papillae around the entrance of the spiracular cavity and posterior spiracles, we have identified reliable features for distinguishing between instars. This study enhances the understanding of the larval development of *W. nuba* and offers essential tools for identification applicable in forensic and medical entomology. Additional research may examine the ecological factors that affect its development, distribution and behavior. The morphological data provided here lay the groundwork for subsequent research on this significant species.

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Data availability statement

All the datasets generated or analyzed during this study are included in this published article.

Ethical approval

This study was approved by the Ethics Committee at Zagazig University (approval number ZU-IACUC/1/F/425/2023).

Conflict of interest

The author declares no potential conflict of interest in the current study.

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