

Larvae and adult flies of *Rhinoestrus purpureus* and *R. usbekistanicus*: morphology and pupation (Diptera: Oestridae)

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Keywords

Acropod,
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Ethmoid,
Myiasis,
Ovipositor,
Rhinoestrus purpureus,
Rhinoestrus usbekistanicus,
Verruciform.

Summary

Rhinoestrus species larvae are considered obligatory parasites of the nasal cavities of equine. This type of myiasis is characterised by sneezing, coughing, olfactory nerve damage and encephalomyelitis. Also, it has a zoonotic importance as the larvae might cause ophthalmomyiasis and conjunctivitis in human. While few studies describing *R. purpureus* adult fly antennal sensillae are available, the *R. usbekistanicus* antennal sensillae have never been described. Also, scanty data are available on the adult flies of *Rhinoestrus* species morphology. For this reason, the current study aimed at identifying and comparing different *Rhinoestrus* species (larvae, adult flies and time of pupation). Using light and scanning electron microscopy, we have evidenced differences between *R. purpureus*, *R. usbekistanicus* larvae in spination pattern, shape of spines, peritremes shape and ultrastructures. The study also showed that for both species the pupa required 15-21 days at room temperature to develop into adult flies, identified the adult male flies and female external genitalia have been identified. As well, the gross features of *R. purpureus* and *R. usbekistanicus* adult flies which included the disposition of the parafrontalia and parafascialia tubercles, mesonotal weals, wings and abdominal pellation have been characterised and the sensillae compared.

Introduction

Rhinoestrus species larvae considered obligatory parasites of the nasal cavities of equine. They included *R. purpureus*, *R. usbekistanicus* and *R. latifrons* which induced nasal myiasis in horses, donkeys and zebras. *Rhinoestrus purpureus* had been reported in Egypt by Zayed (Zayed 1992) and *R. usbekistanicus* in Senegal (Deconinck *et al.* 1996), in Niger (Tibayrenc *et al.* 1999) and in Sicily (Di Marco *et al.* 2001). Both species were recorded in Southern Italy by Otranto and colleagues (Otranto *et al.* 2004) and in Egypt by Hilali and colleagues (Hilali *et al.* 2015).

The life span of adult flies was short (one week), so the females emerged from the pupa containing fully developed eggs. After fertilization, the adults released 8-40 larvae (L1) on equine nostril and then L1 moved to nasal cavity and pharynx where they developed into L2 and L3. L3 emerged outside through the nostril to pupate within a few hours and transformed into adult after 15-32 days (Colwell *et al.*

2006). In relation to the manifested clinical signs, it is noticed that L1 did not induce any signs in infected hosts, L2 and L3 induced sinusitis and adult induced sneezing during their larviposition (Angulo-Valadez *et al.* 2010).

The larvae induced inflammatory changes in the nasal cavities, sinuses and pharynx which manifested in the form of sneezing, coughing, dyspnea, olfactory nerves damage and encephalomyelitis resulted from larval penetration to ethmoid bone and soft cerebral membrane (Zumpt 1965). Also, ophthalmomyiasis and conjunctivitis were noticed in human (Peyresblanques 1964).

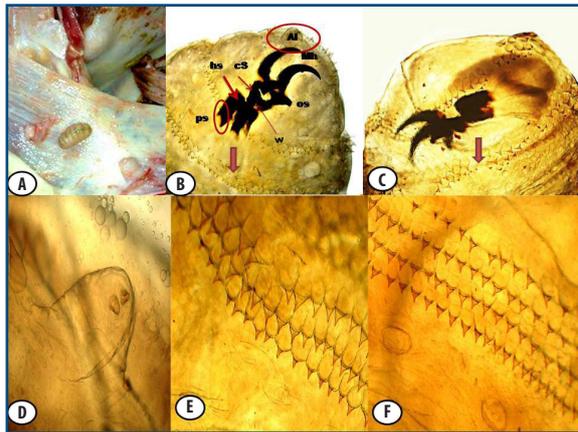
The larvae (L3) of *Rhinoestrus purpureus* and *R. usbekistanicus* have been previously described (Guitton *et al.* 1996, Hilali *et al.* 2015, Otranto *et al.* 2004) but fewer studies (Guitton *et al.* 1996, Liu *et al.* 2015) are available on adult fly antennal sensillae and male and female external genitalia especially for *R. usbekistanicus*. So, this study aimed to characterize

Table 1. Differences in spination pattern and posterior spiracles between *Rhinoestrus purpureus* and *R. usbekistanicus*.

Item	<i>R. purpureus</i>	<i>R. usbekistanicus</i>
Spine crown	Complete (Figure 2B and 5B)	Interrupted (Figure 2C and 5A)
Two rows of spines on dorsal surface of 3 rd segment	Complete (Figure 1B and 5B)	Interrupted (Figure 2C and 5A)
Spines on ventral surface of 4 th segment	4 rows (Figure 2F)	3 rows (Figure 2E)
1 st row of spines on ventral surface of segment 5-10	Complete (Figure 2F)	Interrupted (Figure 2E)
Posterior spiracles	Longer than wide (Figure 3C)	As long as wide (Figure 3D)
Spine shape	Triangular-shaped with a wide basis and recurved towards the body (Figure 3B and 7B)	Triangular-shaped with a wide basis and pointed extremity (Figure 3A and 7C)

The anterior spiracle appeared as a thin tube ended with characteristic funnel (Figure 4A).

Posterior spiracles (peritremes) were deeply located inside, depressed cuticle of the larvae caudal end. Such depression was enclosed within lower and upper lips. In lower lip, the anus was surrounded by several rows of large dumpy spines. There were two pairs of big papillae on dorsal lip, two pairs of small papillae on ventral lip, one pair of anal papillae and multiple rows of small spines surrounding the opened peritremes (Figure 6 A-D). The papillae were conical-shaped.

**Figure 2.** Photos and microphotos of *Rhinoestrus* species.

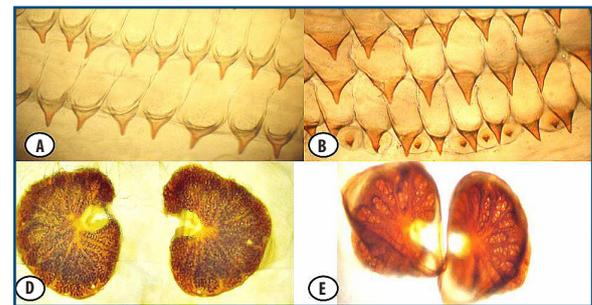
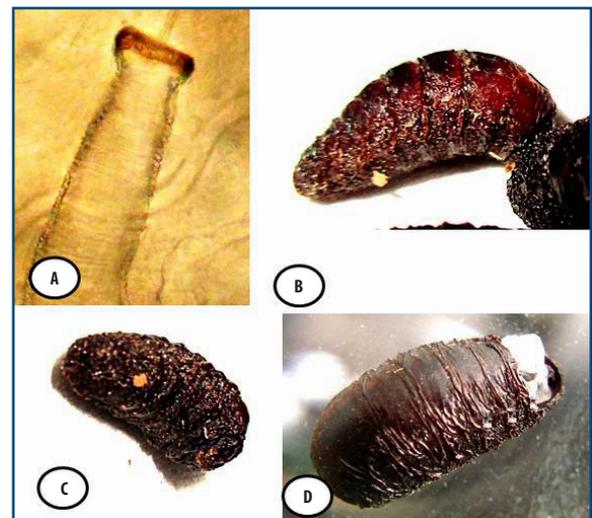
A. *Rhinoestrus* species in nasal cavity of donkey. Photomicrographs of L3 (B-F). **B.** The pseudocephalon of *R. purpureus* with antennal lobes (Al) and cephalopharyngeal skeleton composed of mouth hooks (Mh), oral sclerite (os), cross-bridge (cs), window (w), hypopharyngeal sclerites (hs) and pharyngeal sclerites (ps), showing complete spine crown and spination pattern of 3rd segment (arrow). **C.** *R. usbekistanicus* with interrupted spine crown and spination pattern of 3rd segment (arrow). **D.** Higher magnification of antennal lobe carrying verruciform sensillae. **E.** *R. usbekistanicus* with 3 rows spines on ventral surface of 4th segment. **F.** *R. purpureus* with 4 rows spines on ventral surface of 4th segment (digital camera).

Each peritreme was sickle-shaped with numerous respiratory units (Figure 3 C, D). The unit appeared kidney-shaped with linear respiratory slits (Figure 7 D, E). The peritremes internal channels were long and included a central button (Figure 7D). Highly elevated structures were visible on the posterior margin of the peritremes (Figure 7F). The pores were numerous and very small. The anal outline carried multiple rows of small spines (Figure 6A).

A few hours later after collection of larvae from freshly slaughtered donkeys, the third stage larvae were transformed into pupa. It reached 12 mm and was black brown colored. In *R. usbekistanicus*, pupa was slightly concave ventrally and strongly convex dorsally (Figure 4B) while in *R. purpureus* it was ventrally flattened (Figure 4C).

Adult flies morphological identification

After 15-21 days at 20-30 °C and 46-55% RH, the adult

**Figure 3.** A. *R. usbekistanicus* spination pattern with pointed extremity. B. *R. purpureus* spination pattern with recurved extremity. C. *R. purpureus* posterior spiracles. D. *R. usbekistanicus* posterior spiracles (digital camera).**Figure 4.** A. Anterior spiracle. B. *R. usbekistanicus* pupa with strongly convex dorsal surface. C. *R. purpureus* ventrally flattened pupa. D. *R. usbekistanicus* pupa after adult fly emergence (digital camera).

flies emerged through the puparium (Figure 4D) and reached 8-11 mm in length.

In *R. usbekistanicus*, eyes were less separated than *R. purpureus*. The parafrontalia had uniform, well separated tubercles with central spine. The parafascialia was with irregularly arranged and elevated tubercles (Figure 8 C, F and 10 A). In *R. purpureus*, the parafrontalia bore big and rounded tubercles while parafascialia was with small, flat and dark brown tubercles which developed like papulae (Figure 8 A, E and 10C). Mesonotum carried black brown weals in *R. purpureus* (Figure 8A). In

R. usbekistanicus, the thorax carried long and fine hairs on the ventral surface (Figure 8D and 10B) and without weals dorsally (Figure 8C). In both species, wings were hyaline with three black spots in the basal part near alula. The third longitudinal wing vein r4+5 did not recurrent made a characteristic closed wing cell r4+5. Also, the discal cross-vein (r-m) was situated beyond level the subcostal apex (Figure 9C). The legs were yellowish brown with more darkened femur. They were composed of coxa, trochanter, femur, tibia, tarsus, and acropod (Figure 9D). By scanning electron microscope (SEM),

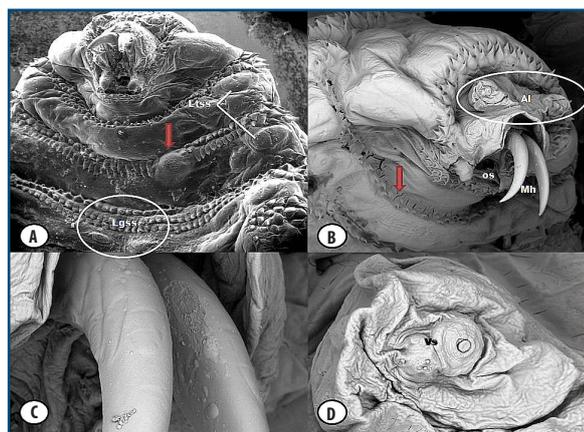


Figure 5. Scanning electron microscope. **A.** *R. usbekistanicus* larval anterior end showing bilateral sensorial structure (Ltss) associated with posteriorly located group of spines, showing large sensorial structures in between segments (Lgss) and interrupted spination pattern of 3rd segment (arrow, 61X). **B.** *R. purpureus* larval anterior end showing antennal lobes (Al), mouth hooks (Mh), oral sclerite (os) and complete spination pattern of 3rd segment (arrow, 100X). **C.** Distally grooved mouth hooks (350X). **D.** Higher magnification of antennal lobe with verruciform sensillae (800X).

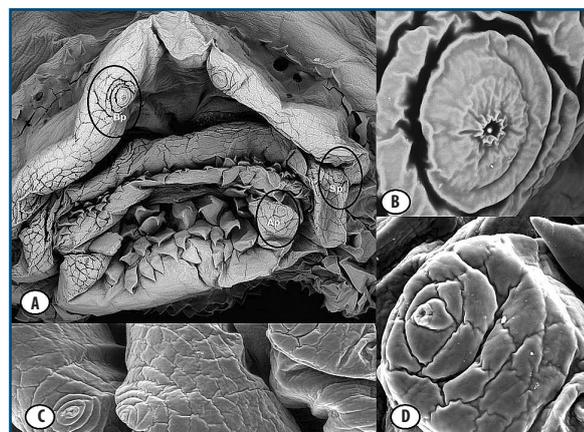


Figure 6. Scanning electron microscope. **A.** The last abdominal segment showing two pairs of big papillae (Bp) on dorsal lip, two pairs of small papillae (Sb) on ventral lip, one pair of anal papillae (Ab, 120X). **B.** Higher magnification of big papillae (2,000X). **C.** Higher magnification of small papillae (250X). **D.** Higher magnification of anal papillae (500X).

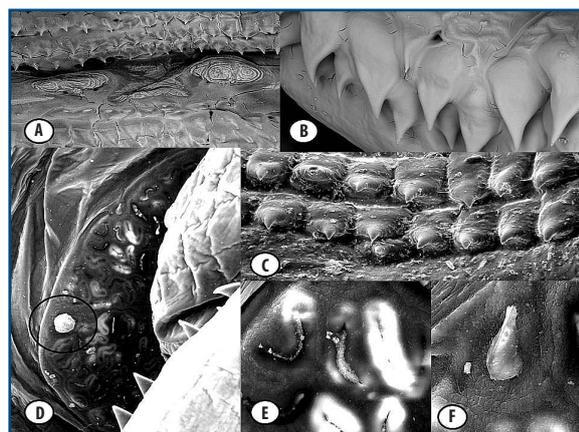


Figure 7. Scanning electron microscope. **A.** Large sensorial structures in between segments (100X). **B.** *R. purpureus* spination pattern with recurved extremity (800X). **C.** *R. usbekistanicus* spination pattern with pointed extremity (400X). **D.** Opened peritreme with central button (circle, 500X). **E.** Kidney-shaped respiratory unit with linear respiratory slits (4,000X). **F.** Highly elevated structures were visible on the posterior margin of the peritremes (2,000X).

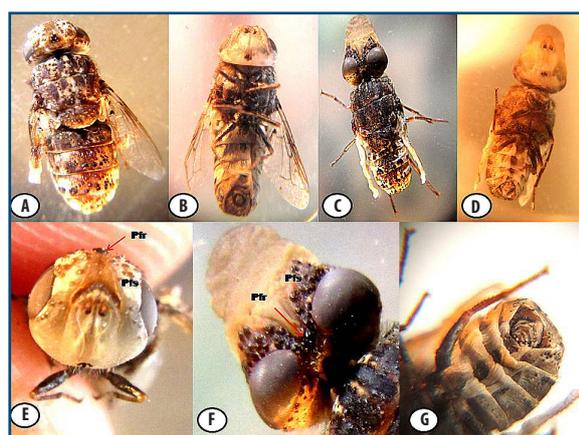


Figure 8. Photographs of adult flies. **A.** Dorsal view of *R. purpureus*. **B.** Ventral view of *R. purpureus* female. **C.** Dorsal view of *R. usbekistanicus*. **D.** Ventral view of *R. usbekistanicus* male. **E.** Head of *R. purpureus* carried big and rounded parafrontalia (Pfr) tubercles and small, flat and dark brown parafascialia (Pfs) tubercles. **F.** Head of *R. usbekistanicus*, carried uniform well separated parafrontalia (Pfr) tubercles and irregularly arranged and elevated parafascialia (Pfs) tubercles. **G.** Male terminalia.

the acropod consisted of a single median structure called unguitractor, and one pair of laterally located and symmetrical basipulvilli. The basipulvilli carried a pair of densely packed pulvilli with setae. The unguitractor ended with empodium and carried a pair of lunulate claws with tapered ends and thick base. The base of the claws carried several sensillae and setae (Figure 9E). In *R. purpureus*, the abdomen was yellowish brown with small black tubercles and white pellonisy (Figure 8 A, B). In *R. usbekisticus*, the abdomen had fewer numbers of tubercles with cloudy whitish yellow pellonisy which changed according to light incidence (Figure 8 C, D). These tubercles were flat and triangular shaped with black backwards directed hairs (Figure 10A).

There was one pair of antennae located in between the eyes (Figure 8 B, D, and 10 B, C). The antenna consisted of scape, pedicle, funiculus, and arista (Figure 10D and 11A). The base of arista carried sparse microtrichiae. Scape was the shortest segment. It carried numerous microtrichiae. Scape and pedicle had long hairs inserted in sockets called mechanoreceptors (Figure 11A). They appeared grooved in *R. usbekisticus* (Figure 11B) and appeared smooth in *R. purpureus* (Figure 11C). Funiculus carried different types of sensillae and sensory pits. The sensillae included:

1. Basiconic sensillae: included Ba I and Ba II. In *R. purpureus*, basiconic sensillae were digitiform with broad tips. Ba I ($7.44 \times 1.82 \mu\text{m}$) was longer than Ba II ($6.73 \times 1.65 \mu\text{m}$, Figure 11D). In *R. usbekisticus*, Ba I measured $7.38 \times 1.21 \mu\text{m}$

(Figure 12A) and appeared with broad tips, but Ba II measured $2.37 \times 0.63 \mu\text{m}$ and appeared with branched tips (Figure 12B), shorter and wider than Ba I.

2. Coeloconic sensillae: the shortest sensillae located mainly inside the sensory pits and characterized by longitudinal ridges (Figure 12C, $3.74 \times 1.30 \mu\text{m}$).
3. Clavate sensillae: club like with apical dilatation in *R. usbekisticus* (Figure 12D, $5.03 \times 1.26 \mu\text{m}$) and with rounded, broad tip in *R. purpureus* ($8.93 \times 1.86 \mu\text{m}$, Figure 11D).

Trichoid sensillae were absent in both *Rhinoestrus* species.

At the female adult fly end, the ovipositor consisted of tergites, sternites (6-8), epiproct, hypoproct and cerci. Tergite and sternite 8 appeared subdivided into two halves. Sternite 8 was like a horse-shoe-shaped and medially divided except at its rounded apex. Sternite 7 was narrowed at the mid ventral and appeared V-shaped with posterior opening. The epiproct was in the form of semicircular sclerite, while the hypoproct was surrounded by cerci (Figure 8B and 9A).

Male terminalia was composed of epandria, surstyli and cerci. Cerci were slender and diverge distally with broad tips. The surstylus was enlarged proximally and gradually tapered posteriorly. Its proximal half was covered with strong bristles. The epandria were provided with microtrichia and long bristles. The anal

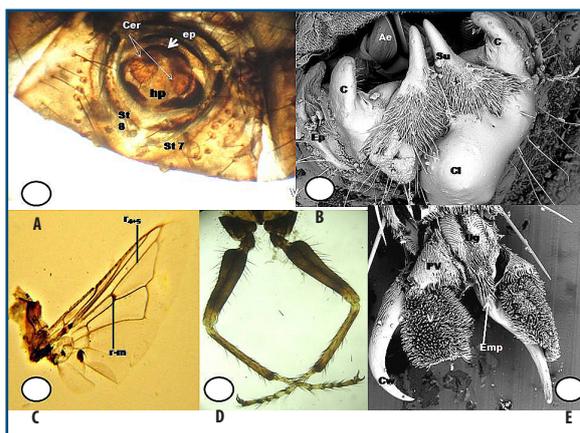


Figure 9. A. Micro photo of female ovipositor consisting of sternites (st7-st8), epiproct (ep), hypoproct (hp) and cerci (Cer). B. Scanning electron microscope, male terminalia composed of epandria (Ep), surstyli (Su), cerci (C) and ended with clasper (Cl), Ae (aedeagus, 300X). C. Micro photo of wing showing non recurrent wing vein r4+5 and discal cross-vein (r-m) beyond level the subcostal apex. D. Legs. E. Scanning electron microscope, the acropod consisting unguitractor (Ug), basipulvilli (Pv) carried densely packed pulvilli (V) with setae. The unguitractor ended with empodium (Emp) and carried a pair of claws (Cw) with tapered end (800X).

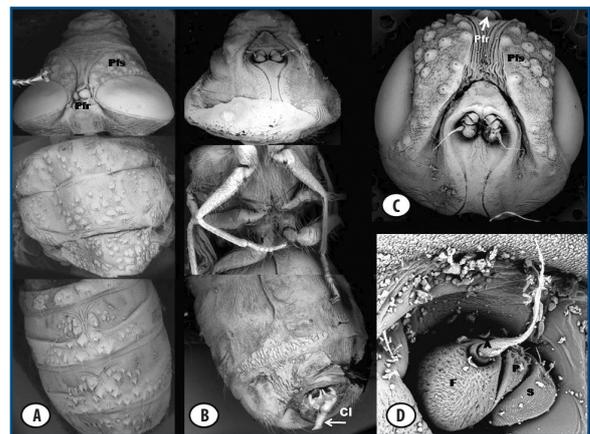


Figure 10. Scanning electron microscope. A. Dorsal view of *R. usbekisticus* showing parafrontalia (Pfr) tubercles, parafascialia (Pfs) tubercles, thoracic and abdominal tubercles was flat and triangular shaped with black backwards directed hairs (70X). B. Ventral view of *R. usbekisticus* showing thorax carried long and fine hairs and showing the male terminalia ended with clasper (Cl) (70X). C. Head of *R. purpureus* carried big and rounded parafrontalia (Pfr) tubercles and small, flat and dark brown parafascialia (Pfs) tubercles and the antennae located inside groove in between eyes (90X). D. The antenna consisted of scape (S), pedicle (P), funiculus (F) and arista (A, 500X).

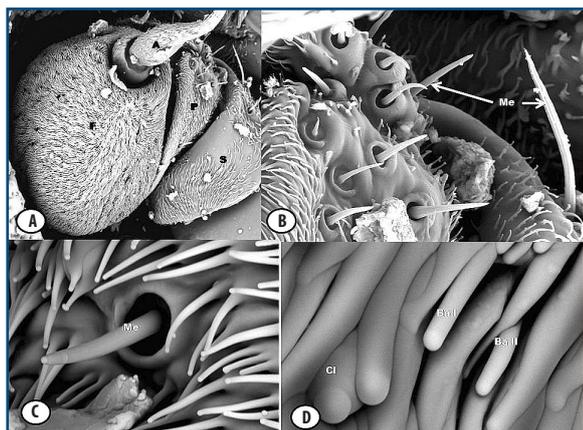


Figure 11. Scanning electron microscope. **A.** Microtrichiae covered the funiculi and the base of arista carried. Also the funiculi carried numerous sensory pits (1,000X). **B.** *R. usbekistanicus*, scape and pedicle had grooved mechanoreceptors (4,000X). **C.** *R. purpureus*, the mechanoreceptors appeared smooth and inserted in sockets (12,000X). **D.** *R. purpureus* with digitiform Basiconic sensillae (Ba I and Ba II) with broad tips and rounded broad tip clavate sensillae (Cl, 24,000X).

plate was characterized by large ventral expansion (copulatory cercal claspers, Figure 8 D, G, 9b and 10B).

Discussion

The current study described the morphological differences between *R. purpureus* and *R. usbekistanicus* third stage larvae (L3) spination pattern, peritreme fine structures, pupa and adult flies. Although there was a single study described *R. purpureus* adult fly antennal sensillae (Liu *et al.* 2015), no studies have described *R. usbekistanicus*.

There were four morphotypes of *R. purpureus* and *R. usbekistanicus* larvae recorded in Italy (Otranto *et al.* 2004), but the study and Hilali and colleagues (Hilali *et al.* 2015) detected only two morphotypes in Egypt. This might be due to climatic changes and seasonal variations (warmer temperature required by L1 to develop into L2 and by L3 to immerse outside host and pupate (Angulo-Valadez *et al.* 2010).

In the present study, both *Rhinoestrus* morphotypes had a lateral sensorial structure on 2-10 segments as described by Hilali and colleagues (Hilali *et al.* 2015). The morphological identification of *R. purpureus* and *R. usbekistanicus* larvae based on the spination shape, pattern, and shape of peritremes resembled the descriptions of (Dong *et al.* 2017, Guitton *et al.* 1996, Guitton *et al.* 1997, Hilali *et al.* 2015, Otranto *et al.* 2005). L3 spines and powerful hooks made the larvae able to crawl outside the donkeys to pupate and transformed into adult flies. Also, the grooves in oral hooks might act as gustatory sensors or as rasping structures to tear the tissue where larvae feed, such as in *Sitodiplosis mosellana* (Diptera:

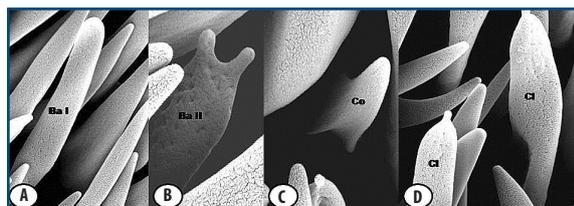


Figure 12. **A.** *R. usbekistanicus*, Ba I appeared with broad tips (15,000X). **B.** *R. usbekistanicus*, Ba II appeared with branched tips (60,000X). **C.** Coeloconic sensillae (Co) characterized by longitudinal ridges (30,000X). **D.** Club like clavate sensillae like with apical dilatation in *R. usbekistanicus* (Cl, 30,000X).

Cecidomyiidae) (Wang *et al.* 2016). The description of the antennal lobes and the cephalopharyngeal skeleton was similar to that of Dong and colleagues (Dong *et al.* 2017). The present description of respiratory units and slit ultrastructures agreed with that of Zayed and colleagues (Zayed *et al.* 2008). The presence of highly elevated structures on the peritreme ventral margin was also recorded by Guitton and colleagues (Guitton *et al.* 1996) who could not detect their function. The anterior spiracle of *Rhinoestrus* species composed of thin tube ended with characteristic funnel and resembled the description of Grunin (Grunin 1966).

In relation to the number of caudal papillae, the current study recorded two pairs of big papillae, two pairs of small papillae, one pair of anal papillae like those stated by Zayed and colleagues (Zayed *et al.* 2008) for *R. purpureus* and Guitton and colleagues (Guitton *et al.* 1997) for *R. usbekistanicus* larvae. In contrast, Dong and colleagues (Dong *et al.* 2017) recorded only the big and small papillae for *R. purpureus* in China.

The characteristic features including the distance between the eyes, the disposition of the parafrontalia and parafascialia tubercles, mesonotal weals, wings, abdominal pellation and pupa were similar to that described by Guitton and colleagues, Pape, and Zumpt (Guitton *et al.* 1996, Pape 2001, Zumpt 1958, Zumpt 1965).

The current study showed that the acropod consisted of unguitector and one pair of basipulvilli which carried densely packed pulvilli with setae. The unguitector carried an empodium and a pair of claws with tapered end. This description was similar to the described features of *Chrysomya chani* tarsomere recorded by (Sukontason *et al.* 2006). Adult fly could use the claw tips to grasp rough particles (Bräuer *et al.* 2016) and as a mechanical carrier for microorganisms (Greenberg 1971). Also, the glandular setae carried on pulvilli were coated with sticky secretions that enable the fly to walk on different surfaces (vertical or inverted smooth) (Resh and Carde 2009).

In relation to adult fly antennal sensillae, there were numerous sensory pits in both species like that noticed in *R. purpureus* in North China (Liu *et al.* 2015) and other dipterans (Hunter and Adserballe 1996, Poddighe *et al.* 2010, Zhang *et al.* 2012). The appearance of branched tipped basiconic sensillae (II) in *R. usbekistanicus* resembled chaetic sensilla type 2 in *Sitophilus zeamais* (Coleoptera: Curculionidae) in Italy (Romani *et al.* 2019). In *R. purpureus*, basiconic sensillae (I and II) were digitiform with broad tips like that of *Gasterophilus intestinalis* (Abdel Rahman *et al.* 2018, Zhang *et al.* 2016) and in contrast to Liu and colleagues (Liu *et al.* 2015) who observed grooved mechanoreceptors on the scape and pedicle, Ba I with sharp tipped and Ba II with short branched apical part for *R. purpureus*. The variation in shape between mechanoreceptors, Ba I and Ba II in the currently studied *R. purpureus* in Egypt and previously studied in China, might be due to the presence of different morphotypes of *R. purpureus* in different countries. The absence of trichoid sensillae which were responsible for pheromone identification (Clyne *et al.* 1999) explained the presence of clasper in male terminalia and the traumatic insemination where male flies chase any same sized female flies and wait at mating sites. Also, the increased number of sensory pits and the branched tipped sensillae facilitated the capacity of odor detection (Liu *et al.* 2015, Poddighe *et al.* 2010, Zhang *et al.* 2012). In addition, the grooves in coeloconic sensillae might act as thermo and chemo-sensors which direct the females toward the healthy hosts to deposit their larvae on their nostrils (Colwell *et al.* 2006, Liu *et al.* 2015).

Female external genitalia (ovipositor) consisted of tergites, sternites (6-8), epiproct, hypoproct and cerci as stated by Colwell and colleagues, Kurahashi

and Samerjai, and Sukontason and colleagues (Colwell *et al.* 2006, Kurahashi and Samerjai 2018, Sukontason *et al.* 2014). The tergite and sternite 8 appeared subdivided into two halves and this was like *Hypoderma* species. Like *Gasterophilus intestinalis*, the sternite 8 of *Rhinoestrus* species was horse-shoe-shaped, sternite 7 was V-shaped and narrowed at the mid ventral with posterior opening and finally the epiproct appeared as semicircular sclerite (Colwell *et al.* 2006).

The studied structures of male terminalia showed resemblance among *Stomorhina discolors* (Moophayak *et al.* 2017) and other Oestrid flies (Colwell *et al.* 2006). Kurahashi and Samerjai (Kurahashi and Samerjai 2018) stated that the surstylus appeared less chitinized than the cercus and described it as an outgrowth of the epandrium pleural margin. Resh and Carde (Resh and Carde 2009) demonstrated that the clasper's function is to grasp a portion of the female body, pierce the side of female abdomen and deposit the sperm (traumatic insemination).

Future study

The study of life cycle and ultrastructure of sensillae will help to perform further studies on the effect of different materials on chemical receptors carried on sensillae and so to draw effective control strategies.

Statement of animal rights

This study was approved ethically by ZU-IACUC Committee, Zagazig University, Egypt with number ZU-IACUC/2/F/75/2018.

References

- Abdel Rahman M.M.I., Hassanen E.A.A. & Abdel Mageed M.A. 2018. Light and scanning electron microscopy of *Gasterophilus intestinalis* (larvae and adult fly) infesting donkeys with emphasis on histopathology of the induced lesions. *EVMPJS*, **14**, 15-31.
- Angulo-Valadez C.E., Scholl P.J., Cepeda-Palacios R., Jacquet P. & Dorchie P. 2010. Nasal bots... a fascinating world! *Vet Parasitol*, **174**, 19-25.
- Bräuer P., Neinhuis C. & Voigt D. 2016. Attachment of honeybees and greenbottle flies to petal surfaces. *Arthropod-Plant Inter*, **11**, 171-192.
- Clyne P.J., Certel S.J., Bruyne M., Zaslavsky L., Johnson W.A. & Carlson J.R. 1999. The odor specificities of a subset of olfactory receptor neurons are governed by Acj6, a POU-domain transcription factor. *Neuron*, **22**, 339-347.
- Colwell D.D., Hall M.J.R. & Scholl P.J. 2006. The oestrid flies - Biology, host-parasite relationships, impact and management. CABI International, London, UK.
- Deconinck P., Pangui L.J., Githego A. & Dorchie P. 1996. Prévalence de *Rhinoestrus usbekistanicus* (Gan 1947) chez l'âne (*Equus asinus*) au Sénégal. *Rev Elev Méd Vét Pays Trop*, **49**, 38-40.
- Di Marco V., Riili S., Vullo S., Capeccchio M.T. & Dorchie P. 2001. One case of equine myiasis caused by *Rhinoestrus usbekistanicus*. Proc. 18th International Conference of the World Association for the Advancement of Veterinary Parasitology (WAAVP). Stresa, Italy, August 26-30, 120.
- Dong J., Bao H. & Mang L. 2017. Ultramorphological and molecular characteristics of the larval stages of the horse nasal-myiasis fly, *Rhinoestrus* sp. (Diptera: Oestridae) from Mongolian horse in China. *Microsc Res Tech*, 1-9.
- Greenberg B. 1971. Flies and disease. Ecology, classification and biotic associations. Princeton University Press, NJ.
- Grunin K.J. 1966. Oestridae. Die Fliegen der Palaearktischen Region. Schweizerbart'sche, Stuttgart.
- Guitton C., Dorchie P. & Morand S. 1996. Scanning electron microscopy of larval instars and imago of *Rhinoestrus usbekistanicus* Gan, 1947 (Oestridae). *Parasite*, **3**, 155-159.
- Guitton C., Dorchie P. & Morand S. 1997. Morphological comparison of second stage larvae of *Oestrus ovis* (Linnaeus, 1758), *Cephalopina titillator* (Clark, 1816) and *Rhinoestrus usbekistanicus* Gan, 1947 (Oestridae) using scanning electron microscopy. *Parasite*, **4**, 277-282.
- Hilali M.A., Mahdy O.A. & Attia M.M. 2015. Monthly variations of *Rhinoestrus* spp. (Diptera: Oestridae) larvae infesting donkeys in Egypt: morphological and molecular identification of third stage larvae. *J Adv Res*, **6**, 1015-1021.
- Hunter F.F. & Adserballe C.F. 1996. Cuticular structures on the antennae of *Hypoderma bovis* De Geer (Diptera: Oestridae) females. *Int J Insect Morphol Embryol*, **25**, 173-181.
- Kurahashi H. & Samerjai C. 2018. Revised keys to the flesh flies of Thailand, with the establishment of a new genus (Diptera: Sarcophagidae). *Med Entomol Zool*, **69**, 67-93.
- Liu X.H., Li X.Y., Li K. & Zhang D. 2015. Ultrastructure of antennal sensory organs of horse nasal-myiasis fly, *Rhinoestrus purpureus* (Diptera: Oestridae). *Parasitol Res*, **114**, 2527-2533.
- Moophayak K., Sanit S., Chaiwong T., Sukontason K., Kurahashi H., Sukontason K.L., Vogtsberger R.C. & Bunchu N. 2017. Morphological characteristics of terminalia of the wasp-mimicking fly, *Stomorhina discolor* (Fabricius). *Insects*, **8** (1), 11. doi:10.3390/insects8010011.
- Otranto D., Colwell D.D., Milillo P., Marco V.D., Paradies P., Napoli C. & Giannetto S. 2004. Report in Europe of nasal myiasis by *Rhinoestrus* spp. in horses and donkeys: seasonal patterns and taxonomical considerations. *Vet Parasitol*, **122**, 79-88.
- Otranto D., Milillo P., Traversa D. & Colwell D.D. 2005. Morphological variability and genetic identity in *Rhinoestrus* spp. causing horse nasal myiasis. *Med Vet Entomol*, **19**, 96-100.
- Pape T. 2001. Phylogeny of Oestridae (Insecta: Diptera). *Syst Entomol*, **26**, 133-171.
- Peyresblanques J. 1964. Myases oculaires. *Ann Oculistic*, **197**, 271-295.
- Poddighe S., Dekker T., Scala A. & Angioy A.M. 2010. Olfaction in the female sheep botfly. *Naturwissen*, **97**, 827-835.
- Resh V.H. & Carde R.T. 2009. Encyclopedia of insects. Academic Press, London.
- Romani R., Bedini S., Salerno G., Ascrizzi R., Flamini G., Echeverria M.C., Farina P. & Conti B. 2019. Andean flora as a source of new repellents against insect pests: behavioral, morphological and electrophysiological studies on *Sitophilus zeamais* (Coleoptera: Curculionidae). *Insects*, **10**, 171.
- Sukontason K.L., Bunchu N., Methanitkorn R., Chaiwong T., Kuntalue B. & Sukontason K. 2006. Ultrastructure of adhesive device in fly in families Calliphoridae, Muscidae and Sarcophagidae, and their implication as mechanical carriers of pathogens. *Parasitol Res*, **98**, 477-481.
- Sukontason K.L., Sanit S., Klong-Klaew T., Tomberlin J.K. & Sukontason K. 2014. *Sarcophaga (Liosarcophaga) dux* (Diptera: Sarcophagidae): a flesh fly species of medical importance. *Bio Res*, **47**, 14.
- Tibayrenc R., Garba D. & Dorchie P. 1999. Prévalence de *Rhinoestrus usbekistanicus* (Gan, 1947) chez l'âne (*Equus asinus*) dans la région de Niamey, Niger. *Rev Elev Méd Vét Pays Trop*, **52**, 113-115.
- Wang Y., Li D., Liu Y., Li X.J., Cheng W.N. & Zhu-Salzman K. 2016. Morphology, ultrastructure and possible functions of antennal sensilla of *Sitodiplosis mosellana* Gehin (Diptera: Cecidomyiidae). *J Insect Sci* (Online), **16**, 1-12.
- Zayed A.A. 1992. Studies on *Rhinoestrus purpureus* (Diptera: Oestridae) larvae infesting donkeys (*Equus asinus*) in Egypt. III. Pupal duration under controlled conditions. *Vet Parasitol*, **44**, 285-290.
- Zayed A.A., Abdel-Shafy S. & El-Khateeb R.M. 2008. Surface

- ultrastructure of posterior abdominal spiracles of third instars of nasal bots of *Cephalopina titillator*, *Oestrus ovis* and *Rhinoestrus purpureus* (Diptera: Oestridae) infesting camels, sheep and donkeys in Egypt. *Res J Parasitol*, **3**, 1-11.
- Zhang D., Wang Q.K., Hu D.F. & Li K. 2012. Sensilla on the antennal funiculus of the stomach bot fly, *Gasterophilus nigricornis* (Diptera: Oestridae). *Med Vet Entomol*, **26**, 314-322.
- Zhang D., Li X., Liu X., Wang Q. & Pape T. 2016. The antenna of horse stomach bot flies: morphology and phylogenetic implications (Oestridae, Gasterophilinae: Gasterophilus Leach). *Sci Report*, **6**, 34409.
- Zumpt F. 1958. On *Rhinoestrus steyni* n. sp. and *Gasterophilus zebrae* Rodhain & Bequaert (Diptera), parasites of Burchell's zebra (*Equus burchelli* Gray). *J Ent Soc S Africa*, **21**, 56-65.
- Zumpt F. 1965. Myiasis in man and animals in the old world. London, Butterworths.