

SCANNING ELECTRON MICROSCOPY OF LARVAL INSTARS AND IMAGO OF *RHINOESTRUS USBEKISTANICUS* GAN, 1947 (OESTRIDAE)

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Summary :

Rhinoestrus usbekistanicus is a larval parasite of the nasal cavities, turbinates and sinus of horse, donkey and Burchell's zebra. This study is the first description using scanning electron microscopy. The first larval instar shows minor differences with *Rhinoestrus purpureus*. The second larval instar has never been described before. It is characterized by prominent antennary lobes and reduced cephalic hooks. Pores of stigmatic plates are less numerous than with *Rhinoestrus purpureus*. The peritremes of the third larval instar are broader rather than higher in contrast to *Rhinoestrus purpureus*. New fine structures of this larval instar, especially sensorial, are described. This study shows distinctive features between imago of *Rhinoestrus usbekistanicus* and *Rhinoestrus purpureus*.

KEY WORDS : *Rhinoestrus usbekistanicus*, larvae, imago, ultrastructure, scanning electron microscopy, Oestridae.

Résumé : STADES LARVAIRES ET ADULTES DE *RHINOESTRUS USBEKISTANICUS* GAN, 1947 (OESTRIDAE) EN MICROSCOPIE ÉLECTRONIQUE À BALAYAGE

Rhinoestrus usbekistanicus est un parasite larvaire des cavités nasales, de l'ethmoïde et des sinus du cheval, de l'âne et du zèbre de Burchell. Cette étude en microscopie électronique à balayage est la première le concernant. Le premier stade larvaire montre des différences minimales avec *Rhinoestrus purpureus*. Le second stade larvaire n'a jamais été décrit auparavant. Il est caractérisé par des lobes antennaires proéminents et des crochets céphaliques réduits. Les pores des plaques stigmatiques sont moins nombreux que chez *Rhinoestrus purpureus*.

Les péritremes du troisième stade larvaire sont plus larges que hautes contrairement à celles de *Rhinoestrus purpureus*. De nouvelles structures fines de ce stade larvaire, notamment sensorielles, sont décrites. Cette étude montre les caractères distinctifs entre les adultes de *Rhinoestrus usbekistanicus* et de *Rhinoestrus purpureus*.

MOTS CLÉS : *Rhinoestrus usbekistanicus*, larves, imago, ultrastructure, microscopie électronique à balayage, Oestridae.

INTRODUCTION

Zumpt (1965) achieved a complete revision of the Oestridae and recapitulated previous descriptions. In *Rhinoestrus* genus, the type-species, *Rhinoestrus purpureus* (Brauer, 1858) from horse in Austria was very precisely described for the larval instars as well as for the imago. On the other hand, *Rhinoestrus usbekistanicus* Gan, 1947 from horse in Usbekistan was briefly described and the second larval instar was unknown.

Scanning electron microscopy allows for many groups, especially parasitic ones, an accurate morphological knowledge and permits the study of new characters of taxonomic importance (Hirschmann, 1983 ; Guitton, 1994). Such studies had been previously done for some Oestridae (Guitton & Dorchies, 1993; Colwell & Scholl, 1995).

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The aim of this paper is to improve the morphological description of imago and larval instars of *R. usbekistanicus* using scanning electron microscopy and to stress differences with *R. purpureus*.

MATERIALS AND METHODS

Larval instars were collected in Dakar's abattoir (Senegal) from nasal cavities and frontal sinuses of donkey (*Equus asinus*) naturally infected according to standardized protocol (Yilma & Dorchies, 1991). Imagos were obtained by pupation from third larval instar in dry earth. Samples were fixed in cold 70° ethanol, washed several times by projection and ultrasonized for between 30 sec and 3 min. They were dehydrated by passage in progressive ethanol concentrations up to absolute ethanol. Residual humidity was extracted to the critical point. For observation, samples were stuck on studs with self adhesive tape. They were covered with gold with M scope 500. The scanning electron microscope used was HITACHI S 520 under 20 KV. One to four samples of each larval instar were observed. For the imago, only males were studied.

RESULTS

FIRST INSTAR LARVAE (L1) (Figs 1, 4, 7)

Pseudocephalon

Antennary lobes are very prominent and separated by a deep groove. The buccal funnel being strongly structured. Anterior hooks are large, directed ventrally and obliquely towards the exterior as with *R. purpureus*. A spine-crown almost completely surrounds the anterior end with relatively few rows of spines (Fig. 1).

Segments 3 to 11

Ventral view

Ventral spines are arranged regularly along the body in four complete rows on the anterior part of each metamere (Fig. 4). Sometimes, some additional spines are present in an incomplete row. These spines are single and more massive and sharply pointed than the crown-spines of the pseudocephalon.

Dorsal view

The rows of spines are interrupted medially from the fourth segment. On the sixth segment only a few spines were present laterally. The other segments are not studied.

Segment 12

Ventrally there are 6 to 7 rows of spines. Dorsally many small spines are present on a few rows medially. Stigmatic plates are large having few pores which are very big. Anal outline is surrounded with numerous, dumpy, long and sharply pointed spines (Fig. 7). Stigmatic margins show ten sensorial ciliated papillae which are regularly distributed.

SECOND LARVAL INSTAR (L2) (Figs 2, 5, 8)

Pseudocephalon

Antennary lobes are more prominent than with the first larval instar giving to pseudocephalon a triangular shape dorsally. The buccal funnel remains structured and the spine-crown is reduced in the number of rows and in the diameter of spines. Cephalic hooks are reduced in length and are directed less obliquely than for the first instar (Fig. 2). The second segment carries medio-ventral hair-like structures which are probably sensorial.

Segments 3 to 11

Ventral view

Three to four spines rows are regularly found along all the body on anterior end of each metamere. Spines are strong and cone-shaped (Fig. 5). The third and

fourth segments show identical sensorial structures to the second segment. On the other segments they are replaced by an ovalar structure without an appendage.

Dorsal view

Segments 3 and 4 carry uninterrupted spines rows. Segment 5 shows a medio-dorsal interruption. The following segments (6 and 7) show a few lateral spines. Last segments (8-11) are bare.

Segment 12

Stigmatic plates are very small and triangular-shaped with rounded angles. Pores are big and shaped like coffee-beans but are less numerous than with *Rhinoestrus purpureus* (about twenty) (Fig. 8). There is no spines on the dorsal margin of the stigmatic plates. Numerous and strong spines lie on both sides of the anal outline (Fig. 8). The stigmatic margins showed ten ciliated sensorial papillae.

THIRD LARVAL INSTAR (L3) (Figs 3, 6, 9, 10-12)

Pseudocephalon

Antennary lobes are less prominent than for the precedent instars. Cephalic hooks are long, massive, and sharply-pointed, directed ventrally and lightly obliquely. The spine-crown is uninterrupted and shows many rows of triangular-shaped spines (Fig. 3).

Segments 2 to 11

Ventral view

There are massive triangular-shaped spines with a wide basis and pointed extremity on three or four rows for each metamere (Fig. 6). The sensorial structures are the same as those of the second instar.

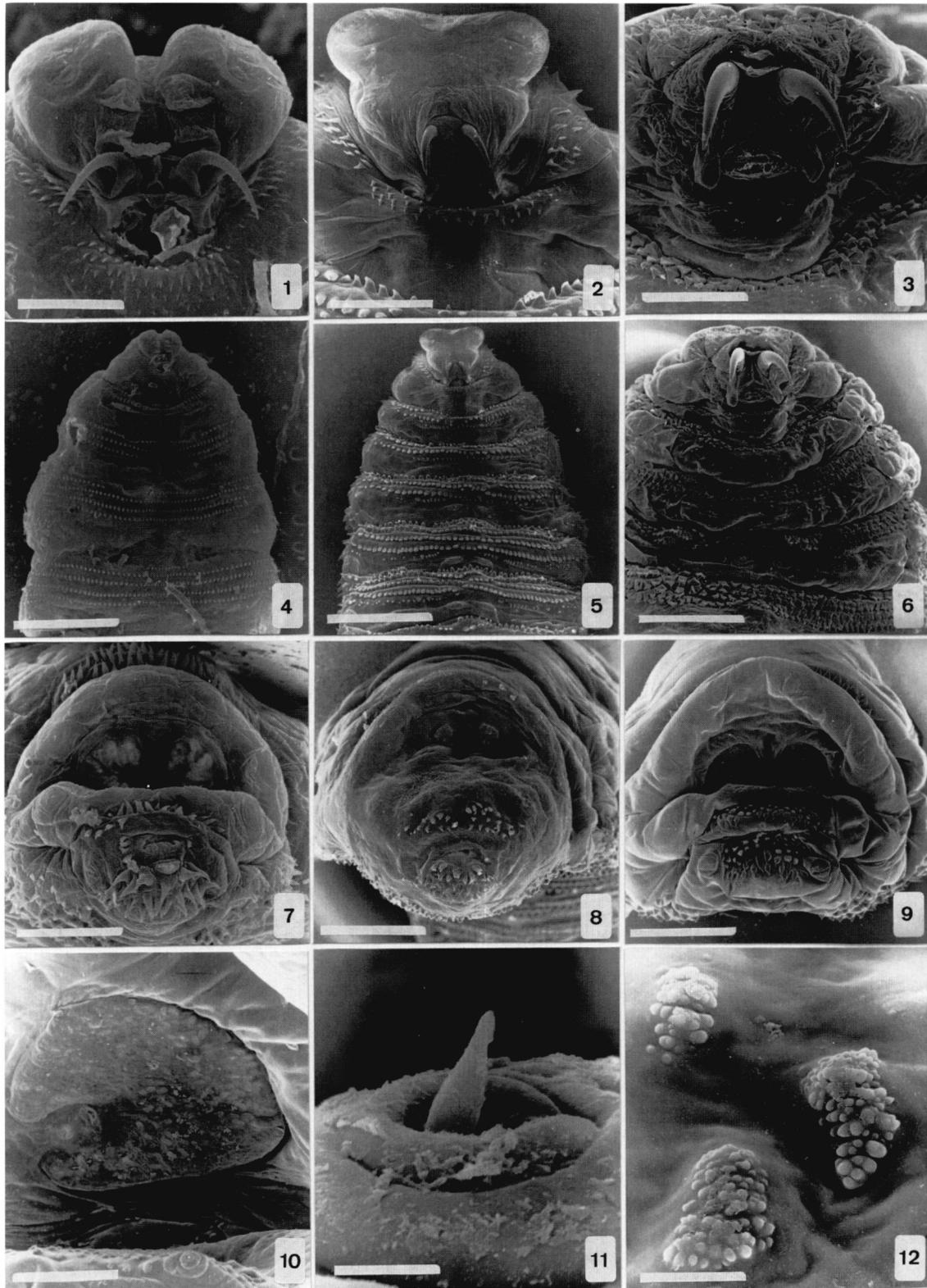
Dorsal view

Spines rows are interrupted medially on the segments 3, 4, 5. The fifth segment shows a few spines laterally. Segments 7 to 11 are bare.

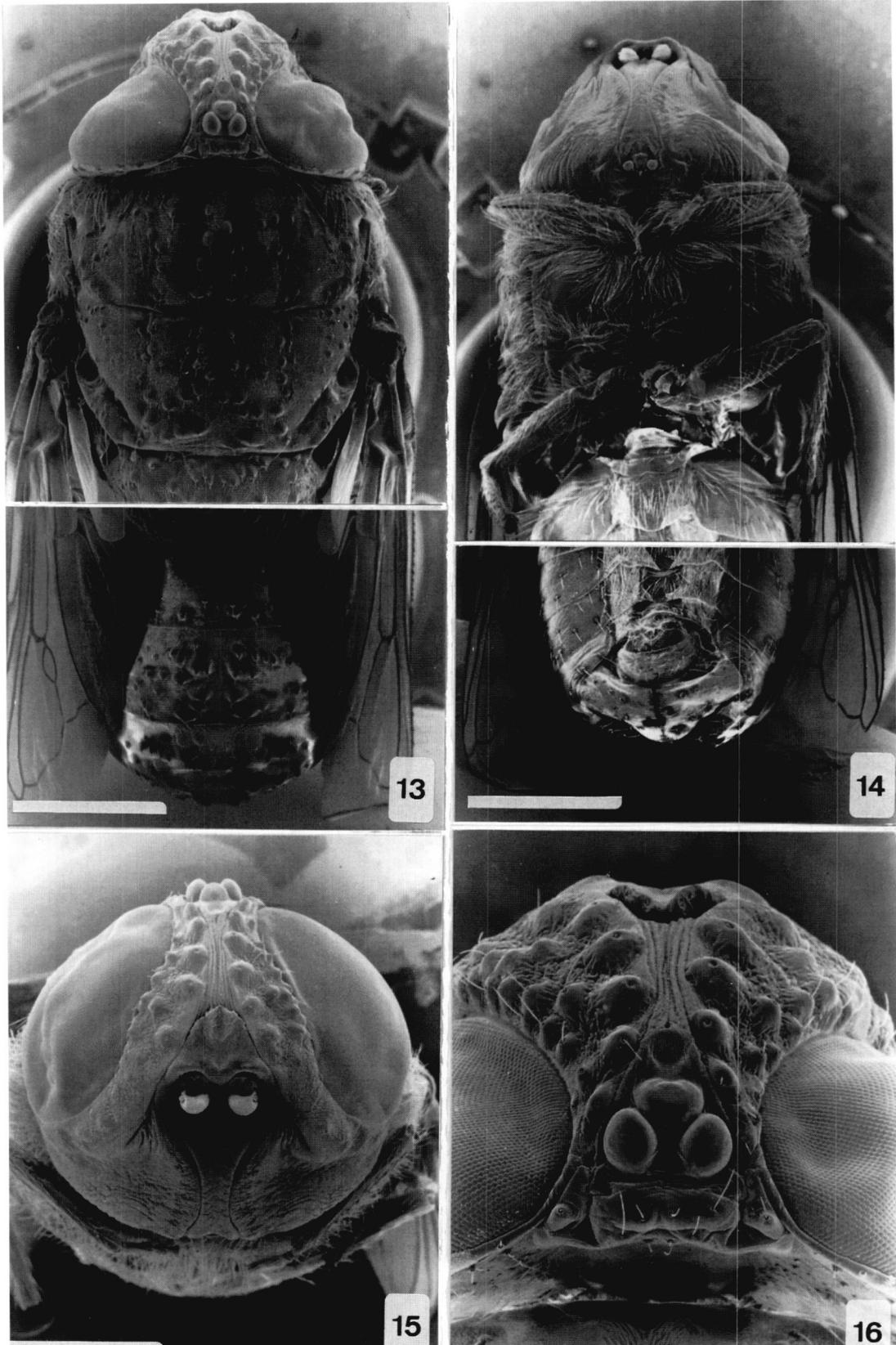
Segment 12

A few spines are present medially on the dorsal face. The peritremes are broader rather than higher in contrast to *R. purpureus*. The internal channel is long. The central button is included in peritremes (Fig. 10). The stigmatic margins bear ten sensorial ciliated papillae (Fig. 11). Sometimes, near these, are found orifices which are probably excretory papilla. Heaped structures are visible on the ventral margin of the peritremes. Their function is unknown (Fig. 12).

Stigmatic plates are of average size but more developed than for the second instar. The pores are very small and very numerous. Anal outline is surrounded by numerous and dumpy spines (Fig. 9).



Figs 1-12. – *Rhinoestrus usbekistanicus*. Fig. 1. – Ventral view of the pseudocephalon of first larval instar. Bar = 50 μ m. Fig. 2. – Ventral view of the Pseudocephalon of second larval instar. Bar = 250 μ m. Fig. 3. – Ventral view of the pseudocephalon of third larval instar. Bar = 600 μ m. Fig. 4. – Ventral view of anterior part of first larval instar. Bar = 300 μ m. Fig. 5. – Ventral view of anterior part of second larval instar. Bar = 860 μ m. Fig. 6. – Ventral view of anterior part of third larval instar. Bar = 1,360 μ m. Fig. 7. – Posterior view of posterior end of first larval instar. Bar = 176 μ m. Fig. 8. – Posterior view of posterior end of second larval instar. Bar = 600 μ m. Fig. 9. – Posterior view of posterior end of third larval instar. Bar = 1,200 μ m. Fig. 10. – Posterior view of sigmatic plate of third larval instar. Bar = 250 μ m. Fig. 11. – Peri-stigmatic ciliated papillae of third larval instar. Bar = 8.6 μ m. Fig. 12. – Heaped structures on the ventral margin of peritremes of third larval instar. Bar = 27 μ m.



Figs 13-16. – *Rbinoestrus usbekistanicus*. Fig. 13. – Dorsal view of the imago. Bar = 1,500 µm. Fig 14. – Ventral view of the imago. Bar = 1,500 µm. Fig. 15. – Frontal view of the head of the imago. Bar = 1,200 µm. Fig. 16. – Dorsal view of the head of the imago. Bar = 500 µm.

IMAGO (Figs 13-16)

Eyes are less separated from one another than for *R. purpureus*. The male-front, in its narrowest point, measures about one-third of eye-length (Figs 13, 15). The parafrontalia bears well-separated and almost-symmetrical tubercles (Fig. 16). The mesonotum is without black or black-brown weals as with *R. purpureus* (Fig. 13).

All the dorsal face shows tubercles with central spines. One can see wide patches of very small hairs between these tubercles (Fig. 13).

Ventrally, the thorax is entirely covered with long and fine hairs. Few tubercles are visible on lateral side of the abdomen. A partial observation of wings does not permit to find significant differences with *R. Purpureus* (Fig. 14).

DISCUSSION

The scanning electron microscopy study of larval instars and imago of *R. usbekistanicus* gives us a finer morphologic knowledge of this species, the second larval instar of which had not been described before.

This study also allows one to differentiate clearly *R. usbekistanicus* from the type-species of the genus *R. purpureus*. The first larval instars of both species are very similar. The second larval instar of *R. usbekistanicus* differs from the one of *R. purpureus* by the morphology of its antennar lobes and the number of pores of the stigmatic plates. The third larval instar differs by the shape of stigmatic plates; the imago by numerous features especially the distance between eyes, the disposition of the tubercles of parafrontalia and the absence of black-brown weals on the mesonotum.

Finally, this study allows the discovery of new features, like that of the structure of pre-stigmatic sensorial papilla previously described for *Gasterophilus intestinalis* and *Oestrus ovis* (Colwell & Scholl, 1995), which might be very informative for phylogenetic analysis on the genus *Rhinoestrus* and, more generally, in the Oestridae family.

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Reçu le 2 novembre 1995
 Accepté le 10 janvier 1996