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.

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Summary :

Second stage larvae of three Oestridae species, *Oestrus ovis* (Linnaeus, 1758), *Cephalopina titillator* (Clark, 1816) and *Rhinoestrus usbekistanicus* (Gan, 1947) were described in detail using scanning electron microscopy. Their morphological features were compared.

O. ovis and *R. usbekistanicus* show several common characteristics which make them quite different from *C. titillator*. These observations seem to validate the cladogram of adult Oestridae genus established by Papavero (1977).

KEY WORDS : *Rhinoestrus usbekistanicus, Oestrus ovis, Cephalopina titillator,* ultrastructure, second stage larvae, scanning electron microscopy, Oestridae.

Résumé : MORPHOLOGIE COMPARÉE DES SECONDS STADES LARVAIRES D'*OESTRUS OVIS* (LINNAEUS, 1758), *CEPHALOPINA TITILLATOR* (CLARK, 1816) ET *RHINOESTRUS USBEKISTANICUS* (GAN, 1947) (OESTRIDAE) EN MICROSCOPIE ÉLECTRONIQUE À BALAYAGE

Les seconds stades larvaires de trois espèces d'Oestridae, Oestrus ovis *(Linnaeus, 1758),* Cephalopina titillator *(Clark, 1816) and* Rhinoestrus usbekistanicus *(Gan, 1947) sont décrites dans le détail grâce à la microscopie électronique de balayage. Leurs caractéristiques morphologiques sont comparées.*

O. ovis et R. usbekistanicus *ont de nombreux caractères communs qui les distinguent nettement de* C. titillator. *Ces observations semblent valider le cladogramme établi par Papavero (1977) sur les genres d'Oestridae adultes.*

MOTS CLÉS : Rhinoestrus usbekistanicus, Oestrus ovis, Cephalopina titillator, *ultrastructure, second stade larvaire, microscopie électronique à balayage, Oestridae.*

INTRODUCTION

The external morphology of Oestridae parasites of the upper respiratory system of mammals has a great taxonomic importance. Imagos and third stage larvae had been subject to precise descriptions with optical microscopy. They allowed to make the current classification (Zumpt, 1965) and Papavero (1977) proposed a cladogram of Oestridae genus (Fig. 1) based on morphological features of imago, especially wings structures. Tiny first stages larvae (L1 and L2) are too much small to be completely described with optical techniques. Scanning electron microscopy showed to be a very useful mean of studying their morphology (Guitton & Dorchies, 1993; Colwell & Scholl, 1995; Guitton, Dorchies & Morand, 1996). Using this technique, we observed second stage larvae of three different Oestridae species of genus studied by Papavero: *Oestrus ovis* (Linnaeus, 1758) the sheep nasal bot, *Cephalopina titillator* (Clark, 1816) the camel nasal bot and *Rhinoestrus usbekistanicus* (Gan, 1947) an equine nasal bot. The aim of this comparative study was to gain a better understanding of the differences and the similarities between these three species. The principal motivation was to check whether these observations, based on larvae, genetically similar but morphologically very different with imago, were in concordance with Papavero's cladogram (1977) or not.

MATERIALS AND METHODS

Second stage larvae were collected in different abattoirs: Pamiers, France *(Oestrus ovis)*, Dire Dawa, Ethiopia *(Cephalopina titillator)* and Dakar, Senegal *(Rhinoestrus usbekistanicus)*. They were collected by head-dissections according to standardized protocol (Yilma & Dorchies, 1991). Samples were fixed in cold 70° ethanol, washed several times by projection and ultrasonized for 30 sec. to 3 min. They were deshydrated 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

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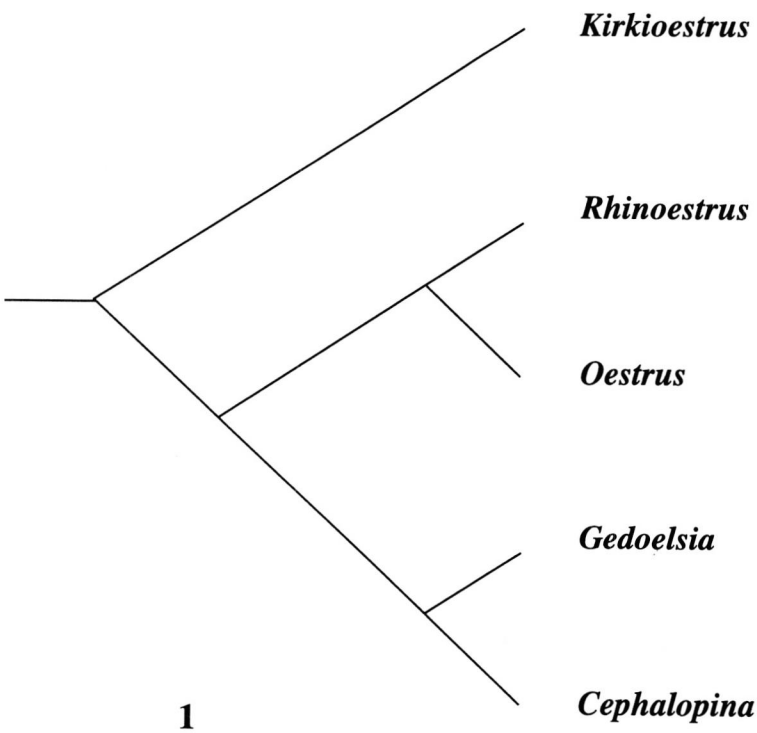
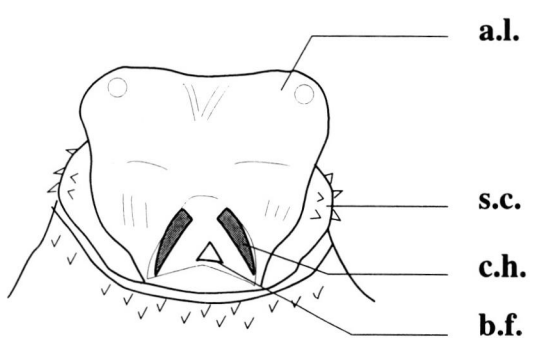


Fig. 1. — Papavero's cladogram of adult Oestridae (1977)



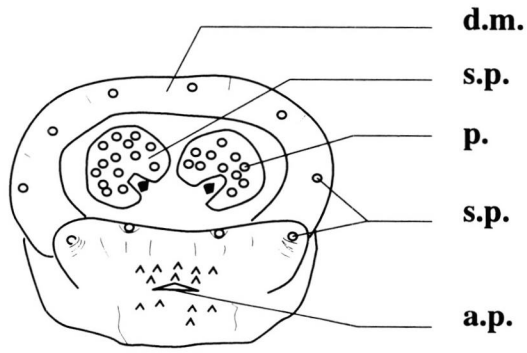


Fig. 2. — Pseudocephalon in ventral view. a.l. antennary lobes. s.c. spine crown. c.h. cephalic hooks. b.f. buccal funnel.

Fig. 3. — Metamere 12 in posterior view. d.m. dorsal margin. s.pl. stigmatic plates. p. pores. s.pa. sensorial papillae. a.p. anal primordium.

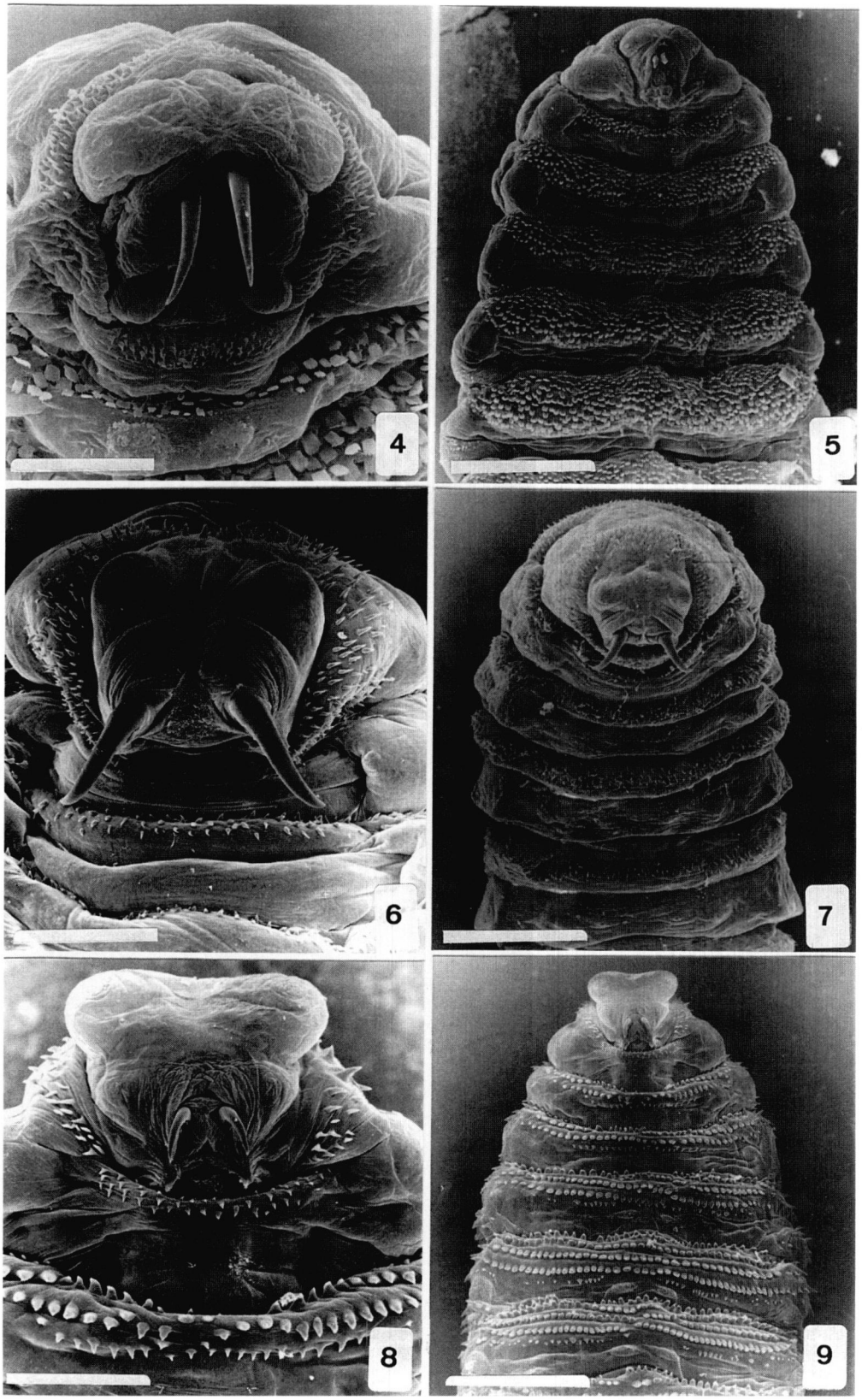
HITACHI S 520 under 20 kV. One to three samples of each larval instar were observed.

RESULTS

Morphological characters investigated in this study are shown on Figure 2 (pseudocephalon) and Figure 3 (segment 12).

PSEUDOCEPHALON (Figs. 4, 6, 8)

Antennary lobes of *C. titillator* are small (Fig. 6) but quite developed on *O. ovis* (Fig. 4) and specially on *R. usbekistanicus* (Fig. 8). On this species they are particularly prominent: this explains the triangular shape of the pseudocephalon which can be easily observed on ventral view. The buccal funnel is already well structured on *O. ovis* (Fig. 4) and *R. usbekistanicus* (Fig. 8) but is not really lined on *C. titillator* (Fig. 6). Cephalic hooks are large on *O. ovis* and *C. titillator* (Fig. 6) (length = 2/3 of the pseudocephalon height); they are short on *R. usbekistanicus* (Fig. 8) (length = 1/3 of the pseudocephalon height). These hooks are ventrally curved on the three species but are obliquely out oriented on *C. titillator* and vertically oriented on the two other species.



Figs. 4-21. — Second larval instar. Figs. 4-5. *Oestrus ovis*. Fig 4. Ventral view of the pseudocephalon of second larval instar; Bar = 176 µm Fig. 5. Ventral view of anterior part of second larval instar; Bar = 860 µm. Figs. 6-7. *Cephalopina titillator*. Fig. 6.- Ventral view of the pseudocephalon of second larval instar; Bar = 380 µm. Fig. 7. Ventral view of anterior part of second larval instar; Bar = 1000 µm. Figs. 8-9. *Rhinoestrus usbekistanicus*. Fig. 8. Ventral view of pseudocephalon of second larval instar; Bar = 300 µm. Fig. 9. Ventral view of anterior part of second larval instar; Bar = 860 µm.

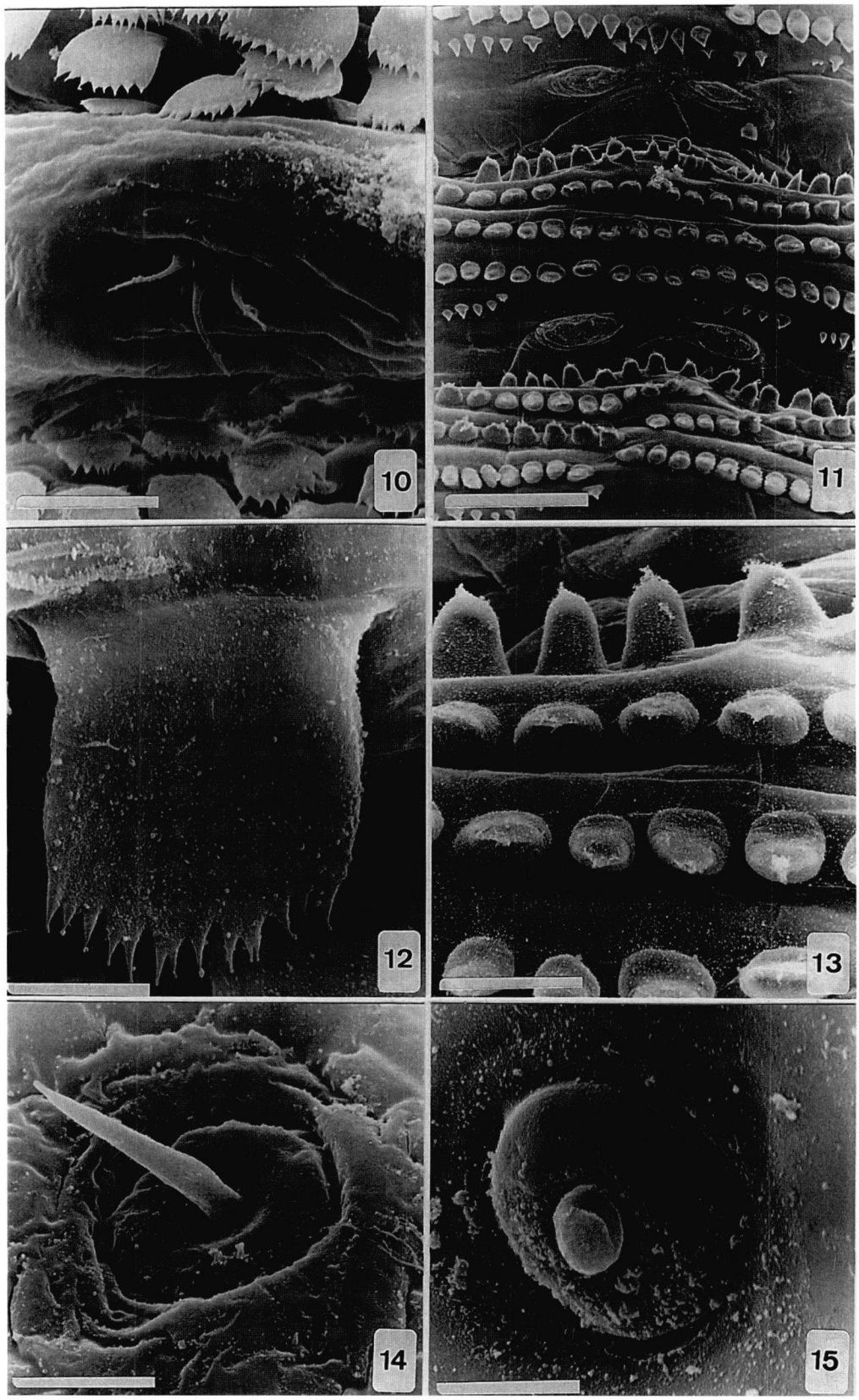
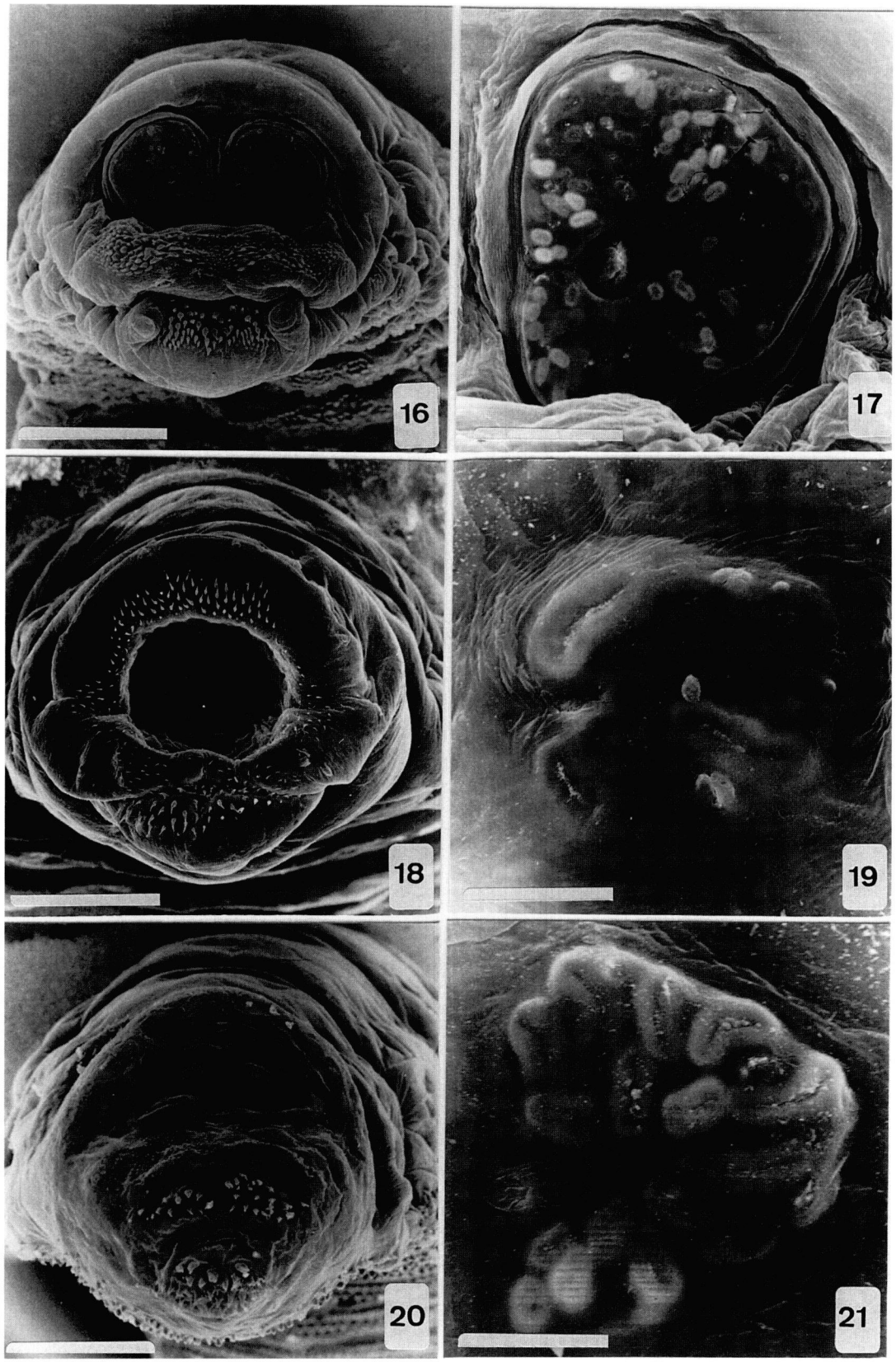


Fig. 10. *Oestrus ovis.* Little tufs of small diameter hairs; Bar = 30 µm. Fig. 11. *Rhinoestrus usbekistanicus.* Ovalar sensorial structures; Bar = 250 µm. Fig. 12. *Oestrus ovis.* Ventral currycomb-shaped spines; Bar = 10 µm. Fig. 13. *Rhinoestrus usbekistanicus.* Ventral sharp-shaped spines; Bar = 75 µm. Fig. 14. *Oestrus ovis.* Peri-stigmatic ciliated papillae; Bar = 8,6 µm. Fig. 15. *Cephalopina titillator.* Peri-stigmatic papillae with a central button; Bar = 6 µm.



Figs. 16-17. *Oestrus ovis*. Fig. 16. Posterior view of posterior end; Bar = 500 µm. Fig. 17. Posterior view of stigmatic plate; Bar = 150 µm. Figs. 18-19. *Cephalopina titillator*. Fig. 18. Posterior view of posterior end; Bar = 500 µm. Fig. 19. Posterior view of stigmatic plate; Bar = 60 µm. Figs. 20-21. *Rhinoestrus usbekistanicus*. Fig. 20. Posterior view of posterior end; Bar = 600 µm. Fig. 21. Posterior view of stigmatic plate; Bar = 50 µm.

The spine crown, surrounding the whole pseudocephalon is very similar for the three species, although the number of rows is lower for *R. usbekistanicus* (Fig. 8).

SEGMENTS 2 TO 11 (Figs. 5, 7, 9 to 13)

Ventral view: the ventral spines are disposed on several rows on the front edge of each segment on *O. ovis* (Fig. 5) and *R. usbekistanicus* (Fig. 9) but are sparse after the forth segment on *C. titillator* (Fig. 7). On *O. ovis* and *R. usbekistanicus*, the number of spine rows remains constant from the front to the bottom of the body (Fig. 5 and 9).
The ventral spines are sharp-shaped on *R. usbekistanicus* and *C. titillator* (Fig. 13) and have a special currycomb shape on *O. ovis* (Fig. 12). On the bottom part of each segment, on both sides of the vertical axis, two little tufts of small diameter hairs can be observed on the three species (Fig. 10). On the following segments, these tufts are replaced by two ovalar structures which may have a sensorial role (Fig. 11).

Dorsal view: the dorsal side has spine rows (sometimes sparse) only up to the forth segment, on the three species. The other segments are blade.

SEGMENTS 12 (Figs. 16 to 21).

Stigmatic plates are quite visible with a bottom view on *O. ovis* (Fig. 16) and *R. usbekistanicus* (Fig. 20), but are not so apparent on *C. titillator*, on which they are hidden in a deep depression.
On *O. ovis* (Fig. 16), stigmatic plates are pentagonal with rounded angles and are quite developed. On *C. titillator* (Fig. 18), they are kidney-shaped and smaller. On *R. usbekistanicus* (Fig. 20), they are also kidney-shaped, but are very small.
Pores are small and very numerous on *O. ovis* (Fig. 17), large and not numerous on *R. usbekistanicus* (Fig. 21), and very large and sparse on *C. titillator* (Fig. 19).
There are no spines on the dorsal margin surrounding the stigmatic plates on *O. ovis* (Fig. 16) and *R. usbekistanicus* (Fig. 20) but some exist on *C. titillator* (Fig. 18). Spines surrounding the anal outline exist on the three species; they are large on *O. ovis* (Fig. 16) and *R. usbekistanicus* (Fig. 20) but thin and sharp on *C. titillator* (Fig. 18).
Stigmatic margins show on the three species ten sensorial papillae regularly spaced. They are ciliated on *O. ovis* and *R. usbekistanicus* (Fig. 14) and have a central button on *C. titillator* (Fig. 15).

DISCUSSION

This study allows a complete and precise description of external morphological characteristics of the second stage larvae for three species belonging to *Oestrus*, *Rhinoestrus* and *Cephalopina* genera. Some of them were already known (Zumpt, 1965; Guitton & Dorchies, 1993; Guitton, Dorchies & Morand, 1996), others are new as the structure of the papillae surrounding stigmatic zones, of a great taxonomic interest. *O. ovis* and *R. usbekistanicus* have a lot of common characteristics which are the number of ventral spines rows, the structure of the papillae surrounding stigmatic zones, the structure of buccal funnel, the spines on the spiracle top margin, the prominent antennary lobs. On the basis on these characters *R. usbekistanicus* closely ressembles *O. ovis* and both differ from *C. titillator*. These observations seem to validate Papavero's cladogram which showed that *Oestrus* and *Rhinoestrus* were sister groups well separated of genus *Cephalopina* (Fig.1). Additional work on the two missing genera *Gedoelstia* and *Kirkioestrus* should allow the establishment of a cladogram, which could be compared with Papavero's one. This study also shows the interest, for many parasitic groups, of a combined approach on one hand based on adult morphology, on other hand based on larval morphology. The comparison of results of both approaches permit an excellent control of trees' reliability.

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