Entomologica, XXIV, Bari, 30-XII-1989

ISIDORO N.* - LUCCHI A.*

Eggshell fine morphology of *Allocontarinia sorghicola* (Coq.) (Diptera: Cecidomyiidae)**

ABSTRACT

The fine morphology of the Sorghum midge *Allocontarinia sorghicola* (Coq.) eggshell has been investigated, in ovary and laid eggs, with scanning and transmission electron microscopes. The eggshell consists of three layers: exochorion, endochorion and vitelline membrane. The micropylar apparatus, situated at the egg anterior pole, has been studied in detail. It is formed by an exochorion invagination delimiting a sort of atrium with one external and several internal apertures. At the egg posterior pole stands out a long appendage becoming wrinkled few hours after oviposition and probably having mechanical function facilitating the egg entering and passing through the female reproductive ducts.

Key words (in addition to those in the title): chorion, micropyle, vitelline membrane, ultrastructure.

CONTENTS

1. INTRODUCTION

The insects' eggshell, composed of chorion and vitelline membrane, is produced by the follicular cells (MARGARITIS *et al.*, 1980). Several important fun-

* Agricultural Entomology Institute, Perugia University, Borgo XX Giugno, 06100 Perugia, Italy.

** Research financially supported by Italian Ministry of Universities and Research in Science and Technology (MURST /60%).

ctions are attributed to the eggshell; its main purpose is to protect the embryo from environmental hazards such as temperature fluctuations, humidity, dryness and bacterial attack. It is also necessary that the eggshell allows the sperm to enter and to insure an adequate air exchange to the developing embryo. Although similarities do exist in eggshell structures among various insect species, nevertheless there are some important variations in relation to the broad diversity in egg-laying substrate. The egg fine structure of numerous species belonging to many Dipteran families is well reported in the literature (HINTON, 1981; MARGARITIS, 1985), but only one paper deals with a Cecidomyiidae egg (MAZZINI, 1977). Our aim was to investigate, through scanning and transmission electron microscopy, the eggshell fine morphology of *Allocontarinia sorghicola* (Coq.), the main insect pest of *Sorghum vulgare*L. in the regions of the world where the crop is grown (ISIDORO, 1987).

2. MATERIALS AND METHODS

Ovary eggs extracted by dissection and laid eggs of *Allocontarinia sorghicola* (Coq.), collected from *Sorghum vulgare* L. in the field near Papiano (PG), were observed with scanning and transmission electron microscopes. For S.E.M. observations the ovary eggs were immersed in Karnowsky's fixative (1965) with 2% Acrolein for 2 h; after washing in cacodylate buffer, the eggs were postfixed in 1% Osmium tetroxide for 1 h, rinsed in the same buffer, dehydrated in ethanol, treated with the critical point drying method with a Balzers apparatus (CPD 020), gold coated in a Balzer SCD 040 vacuum evaporator and viewed in a Philips 501 B. Instead the laid eggs, without any fixation, were gold evaporated and observed as previously described. For T.E.M. observations, ovary eggs and laid eggs were immersed in Karnowsky's fixative (1965) with 2% Acrolein for 3 h; after rinsing in the same buffer, the eggs were postfixed in 1% Osmium tetroxide for 1 h, dehydrated in ethanol and embedded through propylene oxide in Epon-Araldite. Thin sections, cut with an LKB «Nova» ultramicrotome, sequentially stained with Uranyl acetate and Lead citrate, were examined in a Philips EM 400 T.

Explanation of symbols used in the Figs:

AP, appendage of the egg
AT, micropylar atrium
EC, egg cytoplasm
EN, endochorion
EX, exochorion
FC, follicular cell
iM, inner micropylar aperture(s)
M, micropylar apparatus
MA, micropylar area
MV, microvilli
VM, vitelline membrane

3. RESULTS AND DISCUSSION

3.1 Egg outward aspect

The egg of *Allocontarinia sorghicola* has an elongated ellipsoidal shape (0.35 × 0.9 mm) and bears, on the posterior pole, a narrow appendage (0.18 × 0.01 mm) which becomes wrinkled few hours after oviposition (Fig. 1a). With the S.E.M. the external surface of the laid egg appears uniformly smooth (Fig. 1b). No respiratory structures were found. The anterior pole of ovary egg shows a circular micropylar area (about 14 μm in diameter) with a central subcircular aperture (about 1.7-2.5 μm in diameter) (Fig. 2). In the laid egg, instead, this aperture is not easily visible because it is occluded after oviposition (Fig. 1c).

3.2 Eggshell ultrastructure

Chorion (EX, EN) and *Vitelline membrane* (VM). The egg cross sections show, everywhere, three distinct layers: the exochorion, the endochorion and the vitelline membrane. The exochorion (EX), consisting of a finely fibrous material, exibits two distinct parts: a more electrondense outer one (about 0.038 μm thick) and a less electrondense inner part (about 0.12 μm thick) in which a granular component is visible at high magnification (Figs 3, 7). The endochorion (EN) consists, in the ovary egg, of electrondense parallel laminae separated from one another by irregular electron translucent sublayers (Figs 3: a, b; 6b, 7). The parallel laminae disappear in the laid egg and only an empty space remains. The innermost layer is the vitelline membrane (about 0.25 μm in the egg main body). In the ovary egg it is crossed by longitudinal and transversal channels (Figs 3: a, b; 7); in the laid egg, instead, the channels disappear and the vitelline membrane is more compact (Fig. 3c).

The eggshell layers of several Diptera families seem to share common characteristics, but also diverse features closely connected with the broad diversity of the oviposition sites. The eggshell structure of the *Allocontarinia sorghicola* egg, like in some Muscidae and Culicidae, is very simple compared with the highly specialized eggshell structure such as in Drosophilidae, Trypetidae and Calliphoridae, where we can find, over the vitelline membrane, a wax layer, an innermost chorionic layer, an endochorion and an exochorion.

Micropylar apparatus (MA). A longitudinal section through the micropylar apparatus, situated at the egg anterior pole (Fig. 4), shows a sort of atrium, delimited by exochorion invagination, having an outer opening narrower than

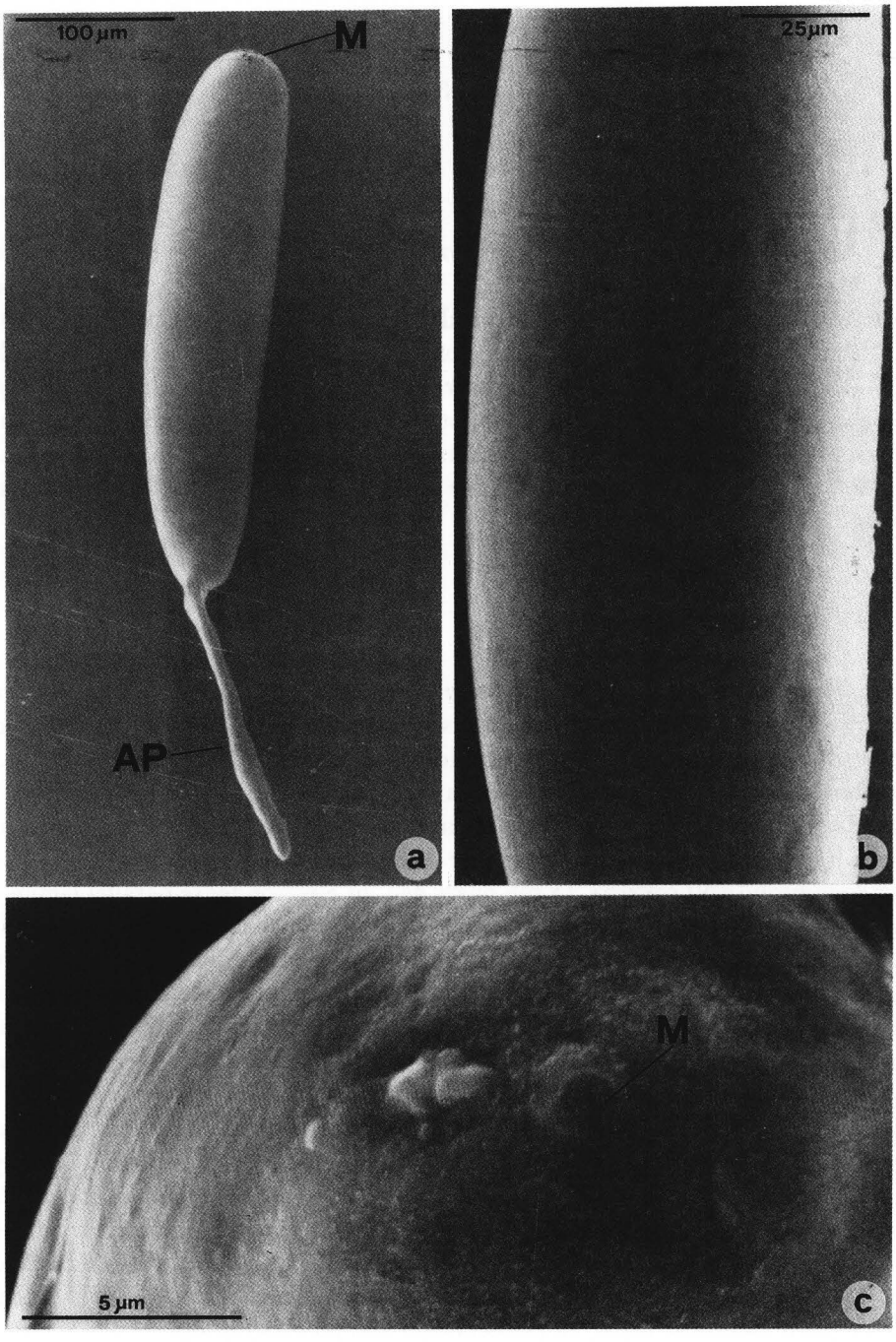


Fig. 1 - S.E.M. micrographs of the *Allocontarinia sorghicola* laid egg few minutes after oviposition: a) general aspect (anterior pole, upside); b) detail of the main body surface; c) detail of anterior pole showing the micropyle outer appearance.

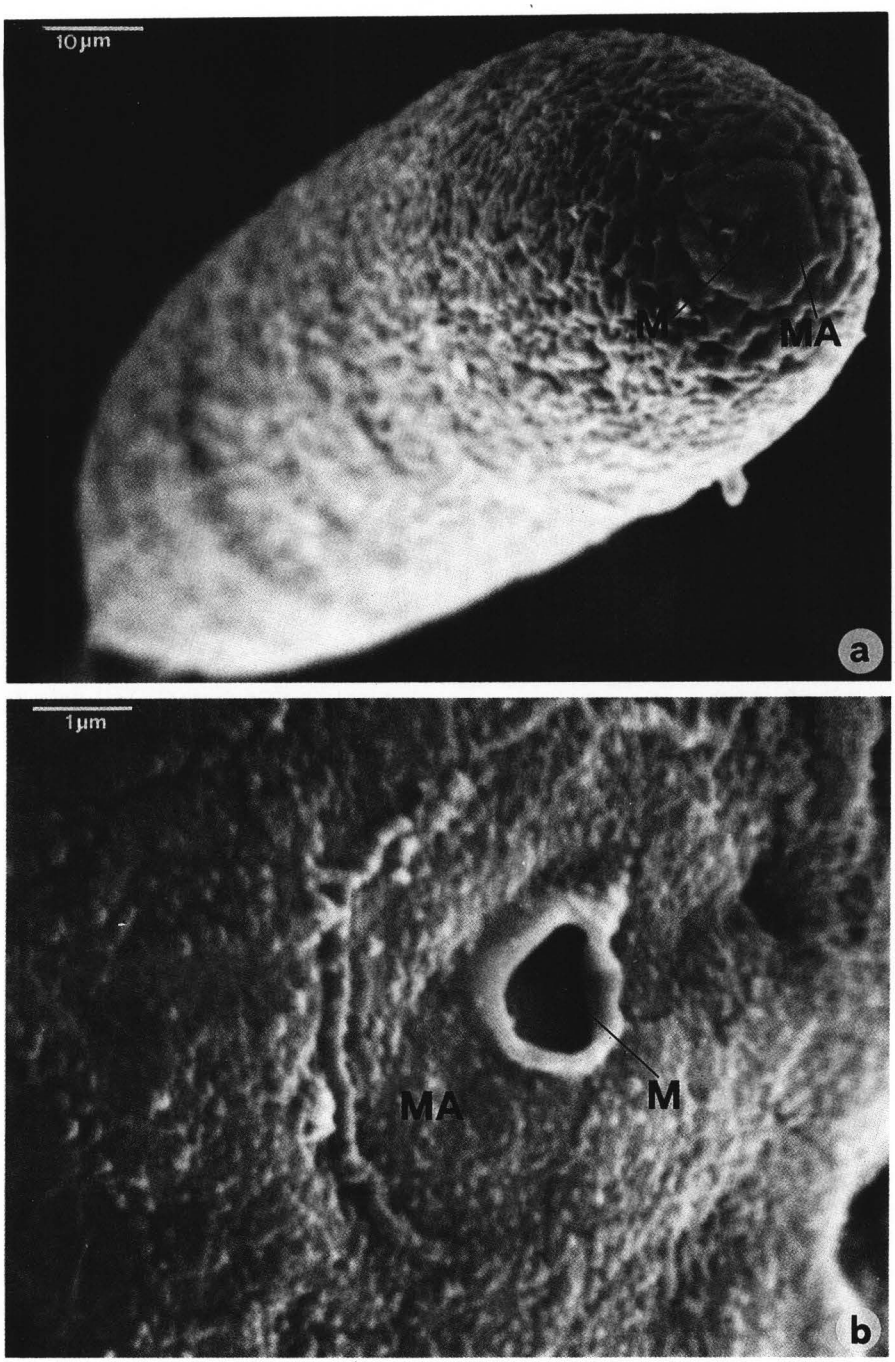


Fig. 2 - S.E.M. micrographs of an ovary egg: a) anterior view showing micropylar area and micropyle (surface shrinkage is an artefact); b) the same (from another specimen) displaying micropylar outer opening.

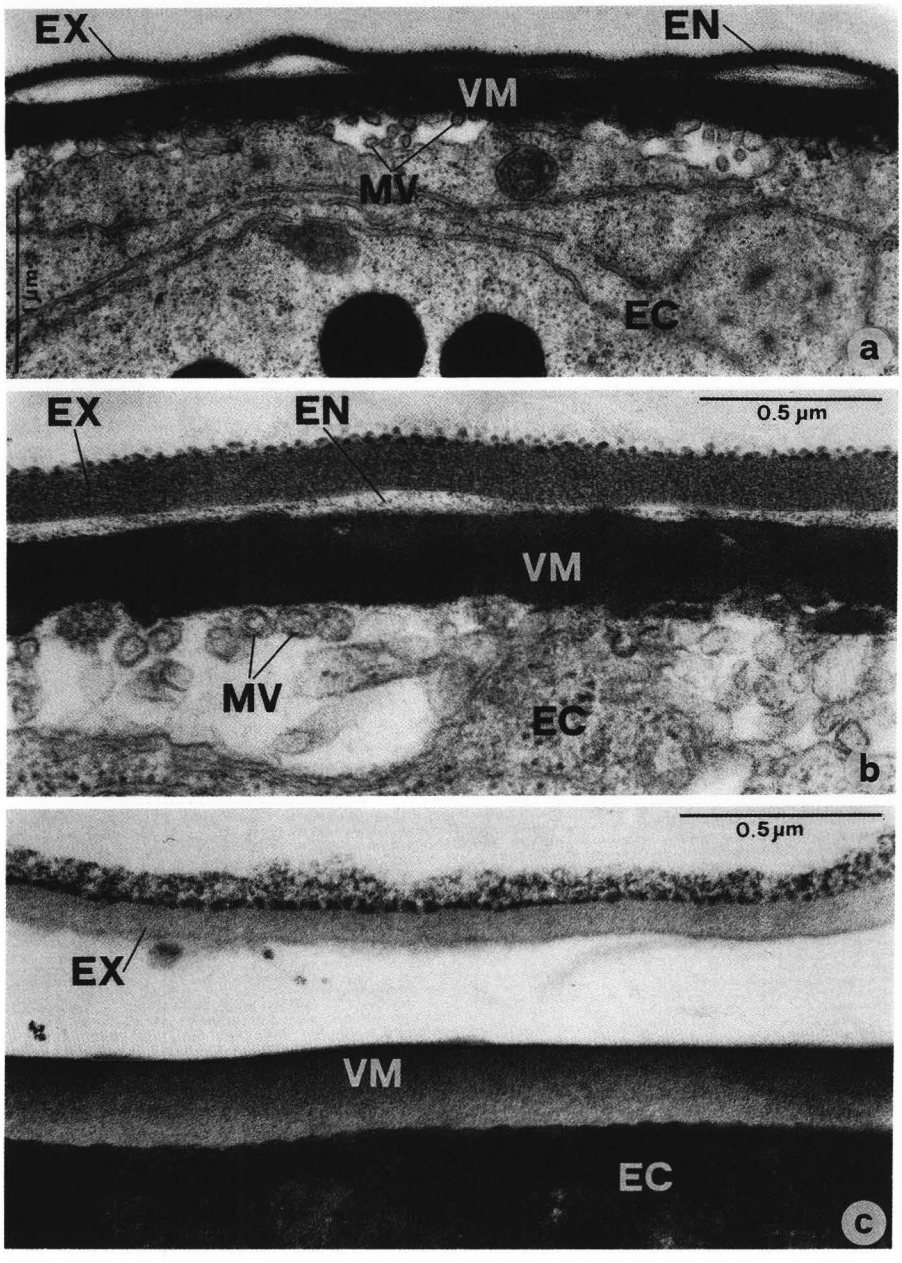


Fig. 3 - Details of ovary (a, b) and laid (c) egg cross sections through the egg main body.

the bottom which bears, all around, several irregular inner apertures crossing both the exochorion and the endochorion (Fig. 5a, b, c). At the level of the micropylar area the exochorion thickness remains constant, while the endochorion becomes thicker due to spreading of the electron translucent areas. As a rule, the vitelline membrane thickness also increases in this region (about 0.62-0.89 μm, Fig. 5a, b, c). In the laid egg, the exochorion surfaces delimiting the

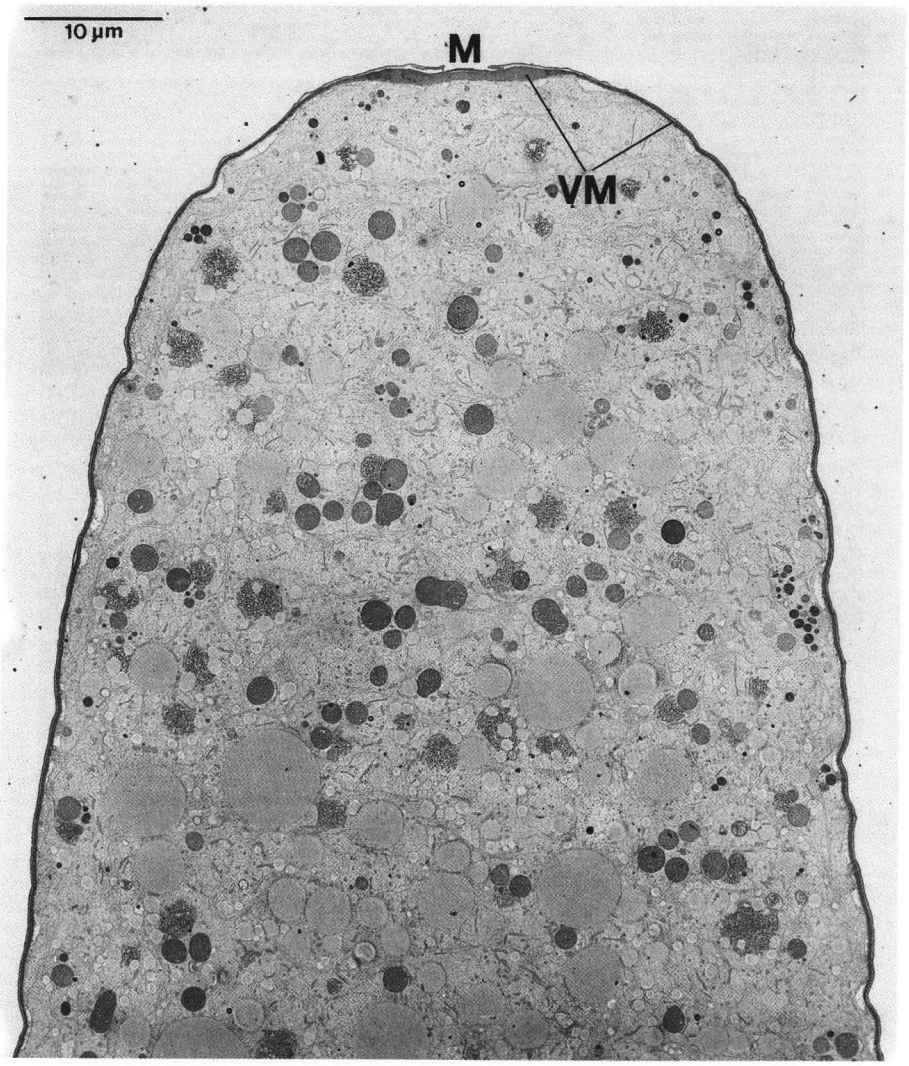
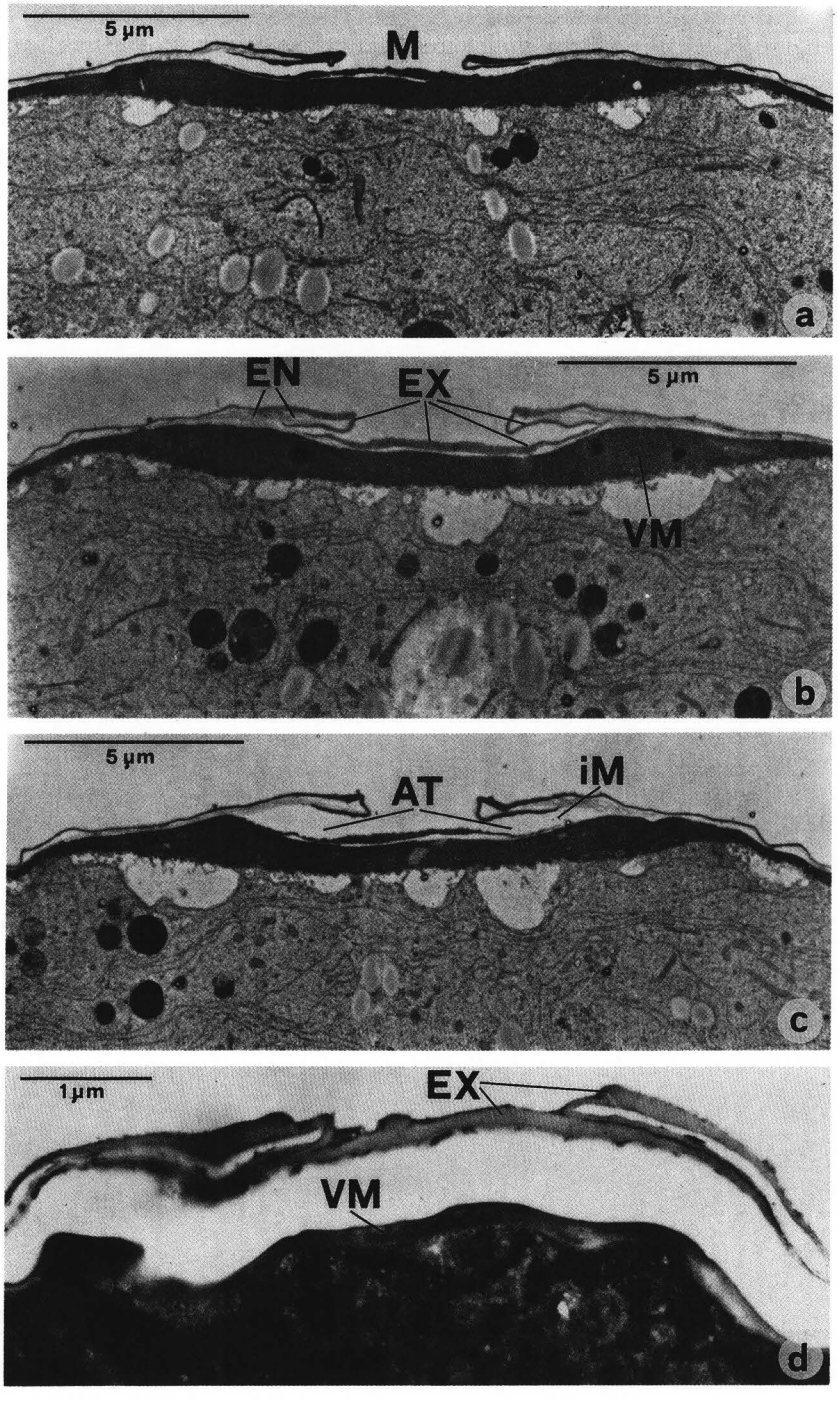


Fig. 4 - Anterior portion of a median longitudinal section through the micropyle of an ovary egg.


5 µm
M
a
EN
EX
5 µm
VM
b
5 µm
AT
iM
c
1µm
EX
VM
d

micropylar atrium adhere to one another and shut both the outer and inner micropylar apertures (Fig. 5d).

The micropyle structure above described is not common in other insect orders and it suggests two main functions: the first is that the micropylar atrium serves, in the ovary egg, as a structure to receive from spermatheca the immotile sperms (BACCETTI & DALLAI, 1976), thus allowing passive finding and entering the inner micropylar apertures; at the same time, the absence of an egg specialized respiratory system suggests that the micropyle also plays a role in respiration, providing a route for oxygen from the outside into the endochorion. Given the absence of discrete aeropyles, the oxygen stored in the endochorion, enclosed between the electrondense parallel laminae, is probably enough for normal embryo respiration during development.

Egg appendage (AP). A longitudinal section through the appendage of an ovary egg shows an increment of the eggshell thickness devoted exclusively to two layers (Fig. 6a): starting from the base of the appendage, the exochorion progressively increases to about 1.05 μm and then remains constant up to the apical end. Also the thickness of the vitelline membrane increases but it varies along the appendage. A longitudinal section through a wrinkled laid egg appendage displays only the exochorion, whereas both the endochorion and the vitelline membrane disappear (Fig. 6c).

The literature reports many instances of eggs with one or more appendages situated either at the anterior pole or at the posterior pole (HINTON, 1981). In the first case it very often serves as a respiratory appendage, while when it is situated at the posterior pole, it usually functions to anchor the egg to the substrate. In the *Allocontarinia sorghicola* egg the appendage is at the posterior pole, but it has no anchorage function since it becomes wrinkled few hours after oviposition. On the other hand, the presence of a remarkably thick exochorion in the ovary egg appendage suggests that it probably has a mechanical function facilitating the egg entering and passing through the female reproductive ducts.

Fig. 5 - Ovary egg (a, b, c) median longitudinal sections' details showing micropylar apparatus from different specimens; d) same kind of section from a laid egg displaying micropyle naturally closed.

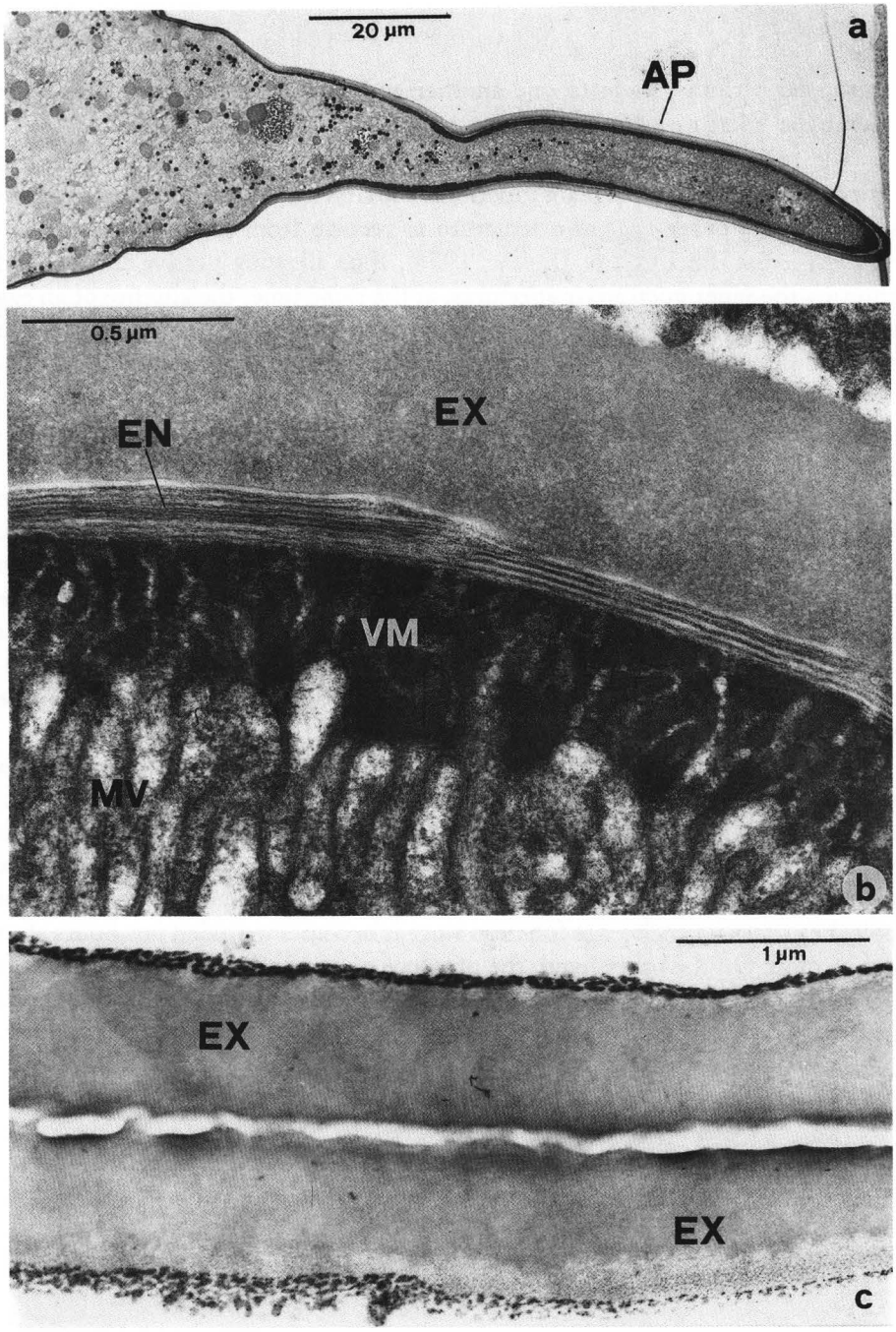


Fig. 6 - a) median longitudinal section of ovary egg posterior end; b) detail of intermediate cross section through the egg appendage; c) median longitudinal section detail of the same appendage from laid egg.

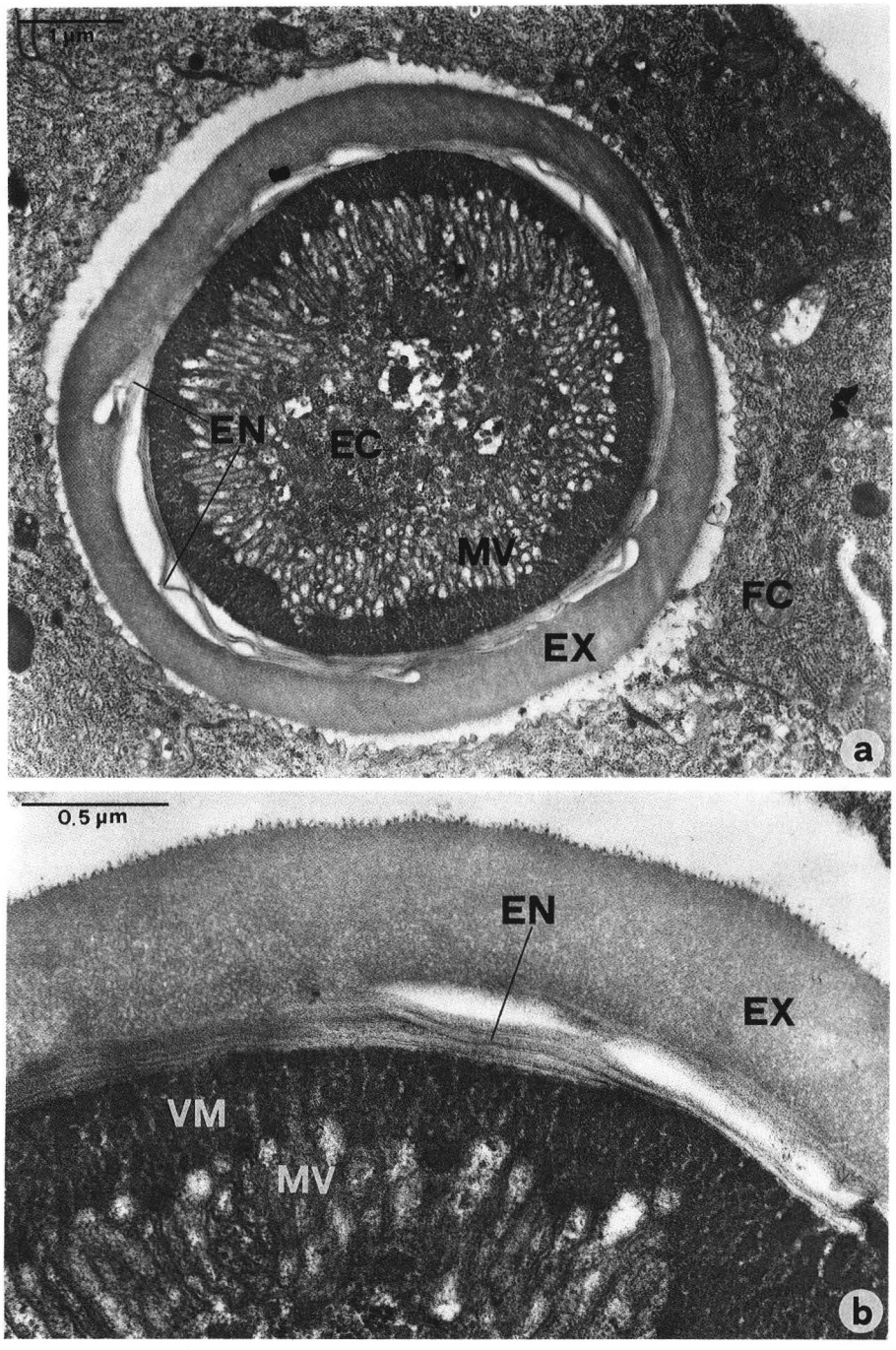


Fig. 7 - Ovary egg: a) appendage cross section; b) detail of the same.

4. ACKNOWLEDGEMENTS

We are very grateful to: Professor Mario Solinas' critical reading of the manuscript, Mr Enzo Mariucci's decorative arranging of the figures; Mr. Aldo Mommi's film processing and photographic printing; Mr. Luigi Bonuomo's careful typing and editing of the manuscript; Mary F. Traynor, English lecturer, Agricultural Faculty of Perugia University, for reviewing the English form of the manuscript.

5. RIASSUNTO

MORFOLOGIA FINE DEGLI INVOLUCRI (CORION E MEMBRANA VITELLINA) DELL'UOVO DI *ALLOCONTARINIA SORGHICOLA* (COQ.) (DIPTERA: CECIDOMYIIDAE)

Sono riportati i risultati di una indagine condotta al microscopio elettronico a scansione ed a trasmissione relativi alla struttura del corion e della membrana vitellina di uova ovariche e deposte di *Allocontarinia sorghicola*. La regione micropilare, situata al polo anteriore dell'uovo, è stata particolarmente studiata. Il micropilo è costituito da una invaginazione del solo esocorion delimitante una sorta di atrio con imboccatura (apertura micropilare esterna) più stretta del fondo nel quale tutt'attorno si aprono diversi fori laceri (aperture micropilari interne). Nelle uova deposte, le superfici dell'esocorion delimitanti l'atrio finiscono per aderire l'una all'altra, occludendo tutte le aperture micropilari. Il lungo peduncolo situato al polo posteriore ha molto probabilmente la funzione di facilitare il passaggio dell'uovo lungo i gonodotti femminili.

6. REFERENCES

BACCETTI B. & DALLAI R., 1976 - The spermatozoon of Arthropoda. XXVII. Uncommon axoneme patterns in different species of the Cecidomyd flies. *J. Ultrastruct. Res.*, 55: 50-69.

HINTON H. E., 1981 - Biology of insect eggs. Pergamon Press, Oxford.

ISIDORO N., 1987 - Allocontarinia (*Contarinia*) *sorghicola* (Coq.) (Diptera, Cecidomyiidae): morfologia, biologia, danni, controllo. *Entomologica*, XXII: 35-73.

KARNOWSKY M.J., 1965 - A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.*, 27: 137 A-8.

MARGARITIS L.H., 1985 - Structure and Physiology of the Eggshell. Comprehensive Insect Physiology Biochemistry and Pharmacology, vol. 1: 153-230.

MARGARITIS L.H., KAFATOS F.C. & PETRI W.H., 1980 - The egg of *D. melanogaster*. I Fine structure of the layers and regions of the wild type eggshell. *J. Cell Sci.*, 43: 1-35.

MAZZINI M., 1977 - Fine structure of the Insect micropyle. Ultrastructure of the egg in a primitive Cecidomyid Dipteran, *Contarinia* sp. Monitore Zool. Ital., 11: 47-56.