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Ootaxonomic investigation of three species of *Mycomya* (Diptera, Mycetophilidae): a scanning electron microscope study

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ABSTRACT

The egg-shell structure of three fungus-gnat species of the genus *Mycomya* (*M. occultans, M. marginata, M. fimbriata*) was investigated by scanning electron microscope (SEM). Each species, pear-shaped with a single micropyle located at a pole, shows some distinctive characters in egg morphology, namely different egg shape and size, together with chorionic sculpturing of the micropylar area, egg surface and a specialized ventral area. The latter, interpreted as a plastron, is detected for the first time in the eggs of Mycomyinae. Ootaxonomic data of these species are compared with those of other Diptera and discussed in relation to adult behaviour and the microclimatic conditions of the laying biotope.

KEY WORDS: Ootaxomomy - Egg - Chorion - Diptera Mycetophilidae.

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INTRODUCTION

The ecology of the holoarctic species *Mycomya* is still not clear in all its details. However from what is presently known, it appears that adults live in dark and shady forests characterized by rotten wood and rich fungal flora (Vaisanen, 1984). This mostly humid environment makes the search for an egg laying site rather problematic and suggests that these species might have evolved egg shell structures suitable for adherence to a moist substrate.

In insects, egg shells are known to be complex three-dimensional structures allowing gas exchange, adherence to the substratum and protection against desiccation. Depending on the environment conquered by the species, nature has devised different structures as a solution to these functional constraints. Investigations to clarify these structural details by scanning electron microscopy have shown that differences between related species of insects may be used as reliable taxonomic markers (Hinton, 1968, 1981; Hinton & Service, 1969; Horsfall *et al.*, 1970; Matsuo *et al*, 1974a, b; Ward & Ready, 1975; Zimmerman *et al.*, 1977; Hillen & Southern, 1979; Stark & Szczytko, 1982; Cogley & Anderson, 1983; Gasc *et al.*, 1984; Yule & Jardel, 1985; Mazzini, 1987; Mazzini *et al.*, 1987; Gaino *et al.*, 1989).

From this standpoint, chorionic structures of Diptera Mycetophilidae eggs have been examined by several authors (Plachter, 1981; Mazzini & Santini, 1983; Santini & Mazzini, 1983, 1989) who have provided a wealth of observations that have helped the identification of Mycetophilidae species.

In this study, the chorionic surface of three species of *Mycomya* - *M. occultans* (Winn.), *M. marginata* (Meig.) and *M. fimbriata* (Meig.) - was examined by scanning electron microscopy, with a view to clarifying how the egg shell in these species is structurally adapted to be laid in such an unusual environment and to obtain further data for their taxonomy and phylogeny.

MATERIALS AND METHODS

Mature eggs were taken from adult females collected at San Rossore, Migliarino Pisano and Montefoscoli (Pisa) during the autumns of 1987 and 1988. The eggs were immersed in Karnovsky's fixative (1965) in 0.1 M, pH 7.2 sodium cacodylate buffer for 2 h at 4° C, then washed in the same buffer, post-fixed in 1% Osmium tetroxide for 1 h at 4° C and dehydrated in a graded ethanol series. The eggs were dried by the critical point method using liquid CO_2 in a Balzers CPD 020 apparatus, attached to specimen holders with double sticky tape and coated with a layer (80 nm) of gold in an MED 010 Sputter Coater (Balzers Union). Egg surface observations were carried out with a Jeol JSM 5200 scanning electron microscope operating at 15 kV. Micrographs were taken on Polaroid PN 52 Film.

RESULTS

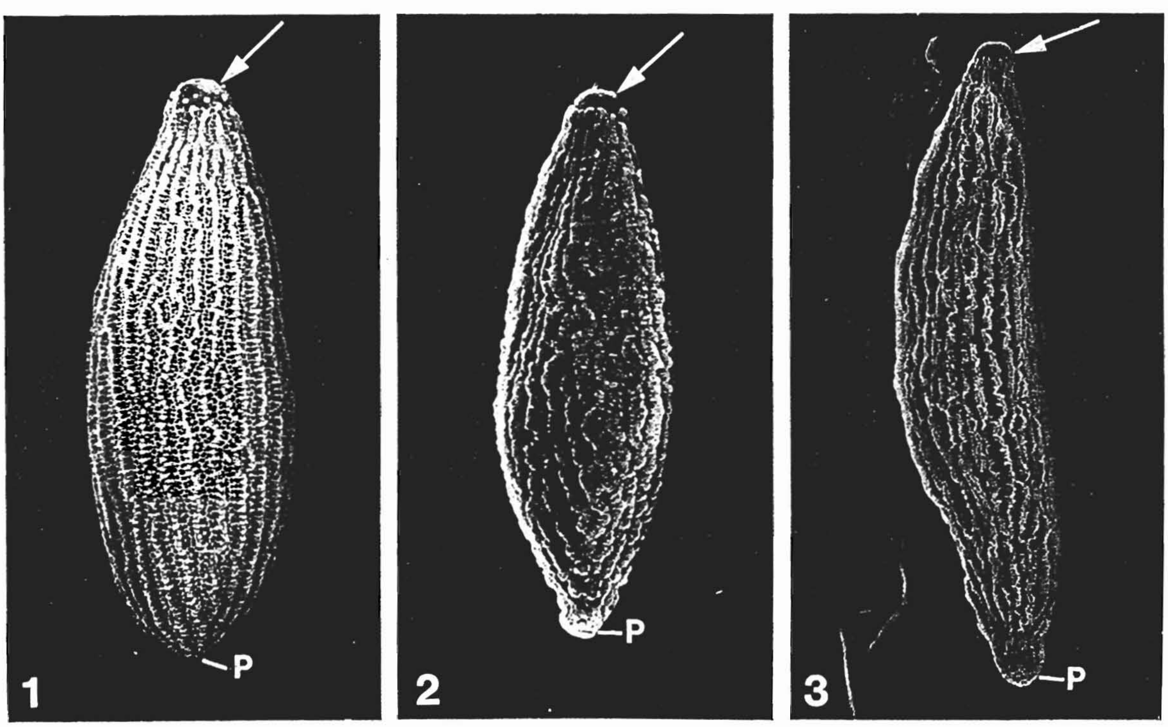
The eggs of the three species of *Mycomya* (*M. occultans, M. fimbriata* and *M. marginata*) are oblong in shape and show unequally rounded poles, but are different in size (Figs. 1, 2, 3). The eggs of *M. occultans* and *M. fimbriata* (Fig. 2) measure 0.39 mm and 0.36 mm in length, respectively (Figs. 1, 2), while those of *M. marginata* are about twice as long (0.86 mm) (Fig. 3).

All the species show longitudinal, occasionally branching ridges covering the egg surface and one of the poles, with the other pole occupied by the micropylar area (Figs. 1, 2, 3). In this region there is a centrally-located micropyle (Figs. 4, 5, 6). Around the micropylar area, the longitudinal ridges terminate in isolated plugs arranged in two concentric circles in *M. occultans* (Fig. 4), but suddenly terminate without plug formation in *M. fimbriata* and *M. marginata* (Figs. 5, 6). However only in *M. fimbriata* is it possible to observe a clear boundary between the micropylar region and the surrounding egg surface (Fig. 5). In addition, the basal layer of the chorionic structure of the micropylar area is raised in centrally perforated papillae in *M. occultans* (Fig. 4, inset), while in *M. fimbriata* and *M. marginata* it forms sausage-shaped appendages (Figs. 5, 6 insets).

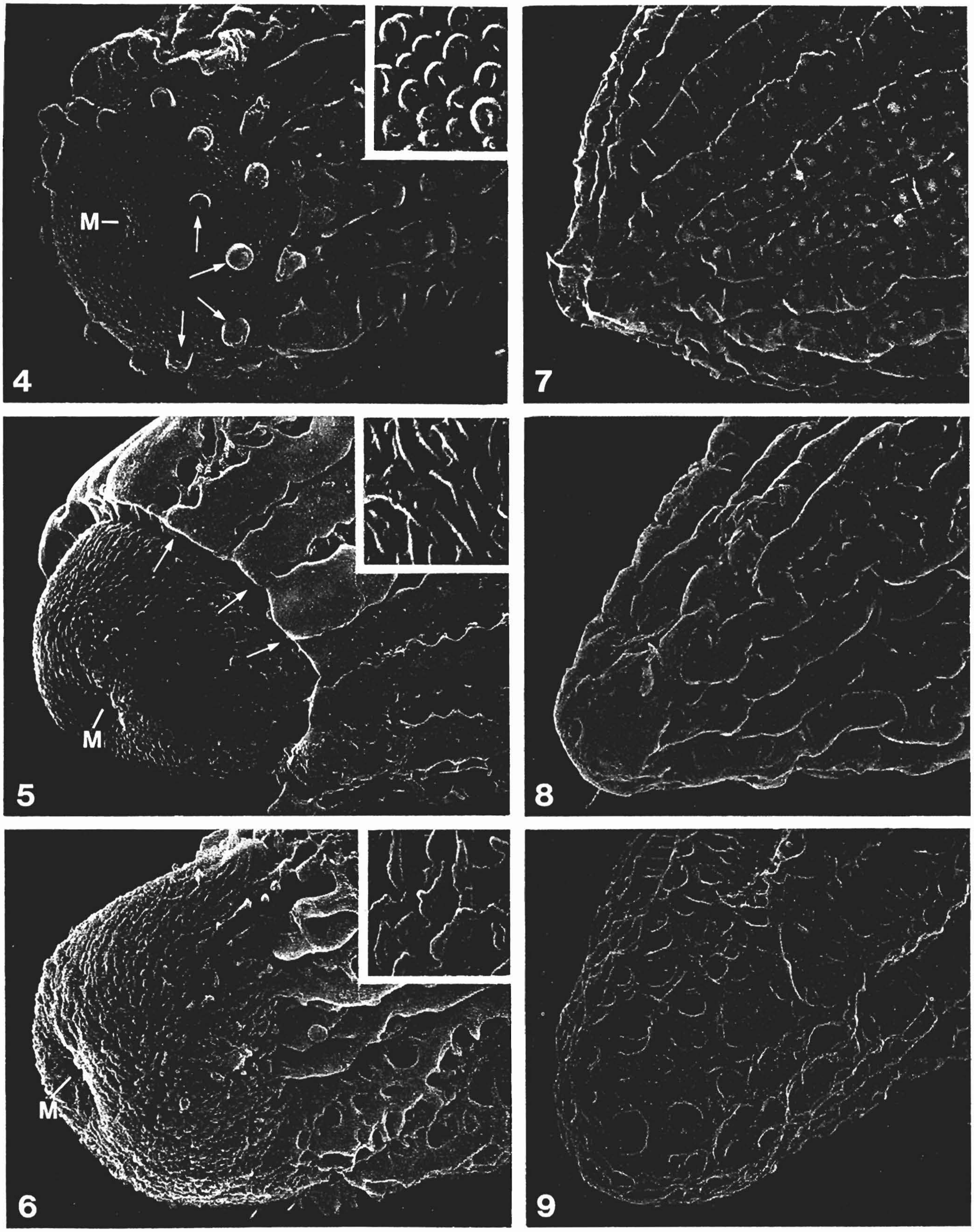
The posterior pole, rounded differently for each species (Figs. 7, 8, 9), is covered with longitudinal ridges. In *M. occultans* these are separate as far as the apex of the pole (Fig. 7), while in *M. fimbriata* and *M. marginata* they fuse together near the polar region. In this case, the posterior pole consists of a sieve-like chorionic structure (Figs. 8, 9).

Except for a limited area of the ventral surface occupied by a round plate, the egg surface is characterized by the longitudinal ridges. For each species, these structures show different morphology. In *M. occultans*, each ridge consists of a continuous bulge, supported by a single row of broad pyramidal columns (Figs. 10, 11). Between them the chorionic structures form an interstitial network below which a granular basal layer appears (Fig. 10). *M. fimbriata* shows broad wavy longitudinal ridges breached by circular openings (Figs. 12, 13). The same arrangement is observed in the chorionic network of the interridge areas (Fig. 12). In contrast with the species mentioned above, the ridges of *M. marginata* consist of double longitudinal bulges (Figs. 14, 15). Each bulge consists of cylindrical columns of 7 μm in length and 1.8 μm in diameter united apically to form a continuous strip (Fig. 15). The basal layer between the ridges exhibits a granular morphology and in its central portion a compact cord with a characteristic zig-zag course (Fig. 14).

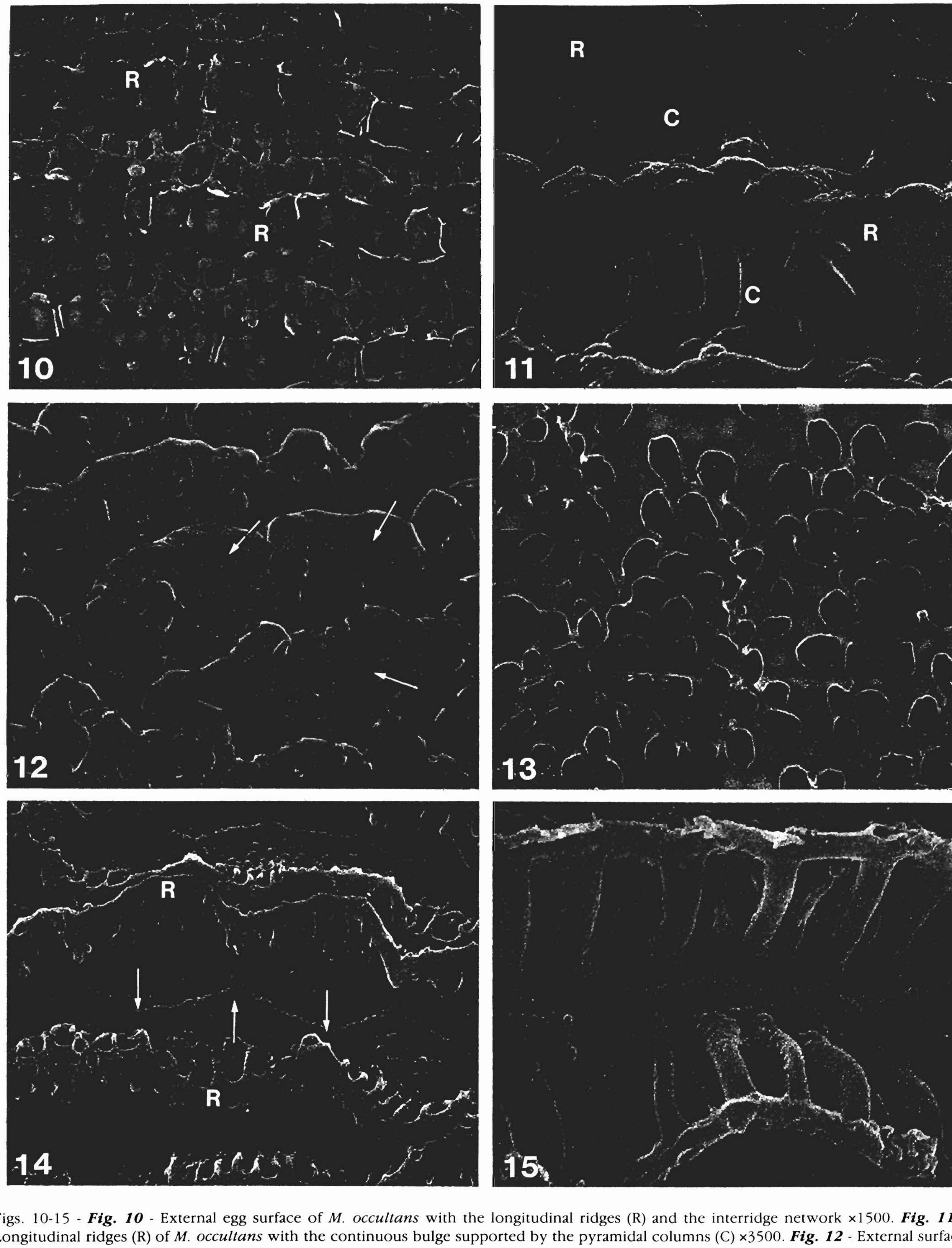
The ventral plate (about 30 μm in diameter) is always circular in shape, but shows some morphological differences in the three species (Figs. 16, 18, 20). The plates of *M. occultans* (Fig. 16) and *M. marginata* (Fig. 20) are more similar to each other than to that of *M. fimbriata* (Fig. 18), being separated from the surrounding egg surface by a clear boundary and subdivided by a thin cord-like structure into polygonal areas (Figs. 16, 20). The plate of *M. fimbriata* has no polygonal pattern and its peripheral outline is not easily recognizable (Fig. 18). At higher magnification the fine morphology of the three plates is different. It is constituted by a network of chorionic fibres delimiting irregular cavities in all three species but these fibres are thin and convoluted in *M. oc-*



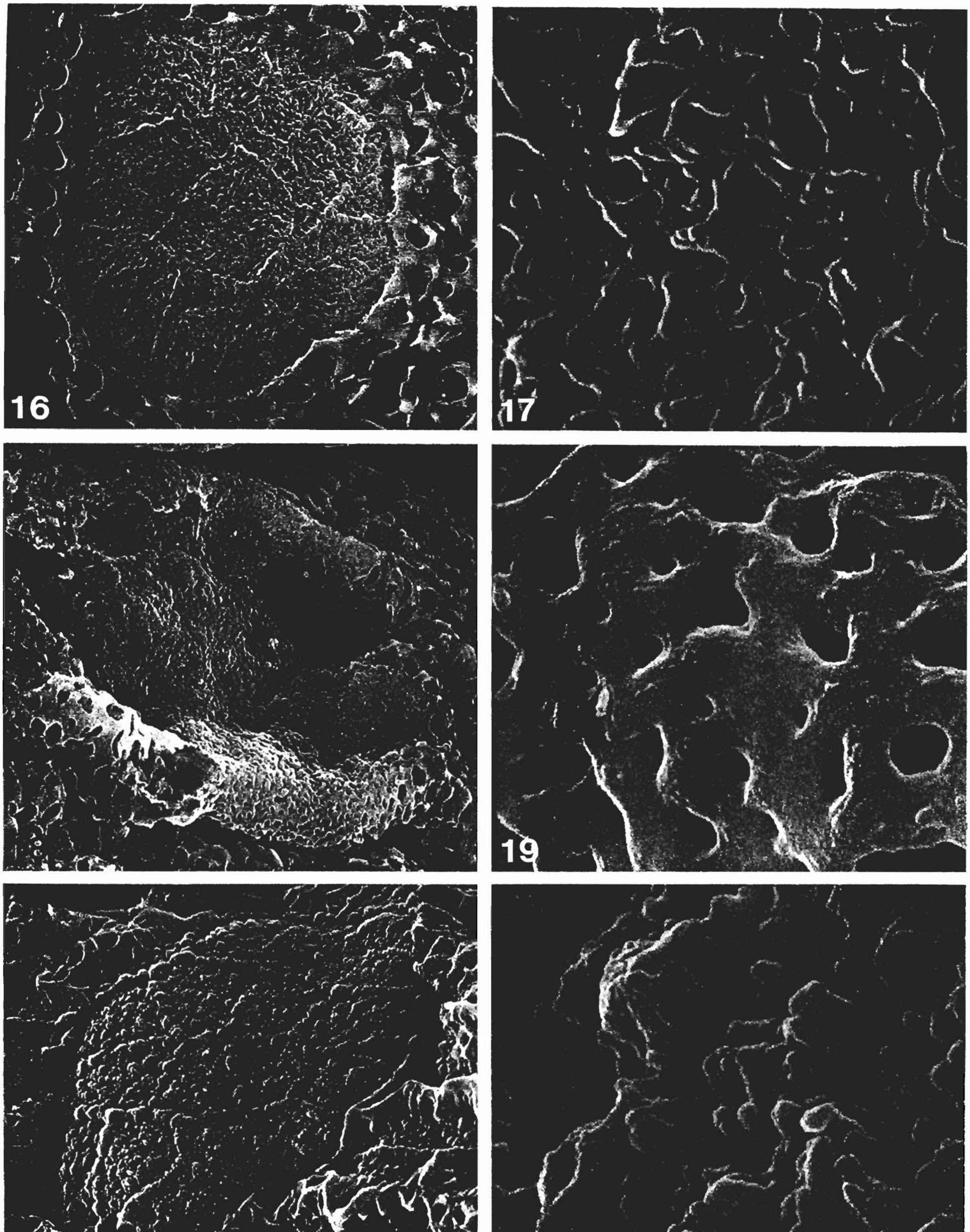
Figs 1-3 - Eggs of *Mycomya occultans* (1), *M. fimbriata* (2), *M. marginata* (3). Micropylar area (arrow), posterior pole (P). 1 and 2 ×200; 3 ×100.



Figs. 4-9 - ***Fig. 4*** - Micropylar area of *M. occultans* showing the central micropyle (M) and the two circles of plugs (arrows). ×1500. Inset shows the central perforated papillae of the basal layer. ×7500. ***Fig. 5*** - Micropylar area of *M. fimbriata* with the micropyle (M) and the clear boundary between it and the surrounding egg surface (arrows). ×1500. Inset shows the sausage-like appendages of the basal layer. ×7500. ***Fig. 6*** - Micropylar area of *M. marginata* with micropyle (M) and sausage-like appendages (Inset). ×1500 and inset ×7500. ***Figs. 7-8*** and ***9*** - Posterior egg pole of *M. occultans, M. fimbriata*, and *M. marginata*, respectively. Note the sieve-like structure of the poles of *M. fimbriata* (8) and *M. marginata* (9) ×1000.



Figs. 10-15 - ***Fig. 10*** - External egg surface of *M. occultans* with the longitudinal ridges (R) and the interridge network ×1500. ***Fig. 11*** - Longitudinal ridges (R) of *M. occultans* with the continuous bulge supported by the pyramidal columns (C) ×3500. ***Fig. 12*** - External surface of *M. fimbriata* with longitudinal bulges all perforated by circular openings (arrows) ×1500. ***Fig. 13*** - Detail of the interridge area of *M. fimbriata* ×3500. ***Fig. 14*** - External surface of *M. marginata* with longitudinal ridges (R) and the interridge network. Note the thin cord with a peculiar zig-zag course (arrows) ×1500. ***Fig. 15*** - Longitudinal ridges of *M. marginata* with double rows of cylindrical columns united apically to form a continuous strip ×3500.



Figs. 16-21 - ***Fig. 16*** - Ventral plate of *M. occultans* showing the subdivision in polygonal areas ×2000. ***Fig. 18*** - Ventral plate of *M. fimbriata* lacking polygonal pattern ×2000. 20. Ventral plate of *M. marginata* showing a similar aspect to that of *M. occultans* ×2000. ***Figs. 17,19*** and ***21*** - Higher magnification of the network of the plates in *M. occultans* (17), *M. fimbriata* (19) and *M. marginata* (21) ×20 000.

cultans (Fig. 17) and thicker in *M. marginata* (Fig. 21). In *M. fimbriata* (Fig. 19) they are densely packed, and encircle larger cavities.

DISCUSSION

Scanning electron microscopy investigations of egg chorionic patterns have made it possible to characterize different subfamilies of Mycetophilidae (Plachter, 1981; Mazzini & Santini, 1983; Santini & Mazzini, 1983, 1989), confirming the validity of ootaxonomic analysis. In this regard, it is important to note that the three species of *Mycomya*, described in this paper, can be distinguished by their fine chorionic surface structure. Although they have some similar features, such as a pear-like shape, a single micropyle located at a pole, an external surface adorned with longitudinal ridges and a circular ventral plate, each of them exhibits peculiar characters useful for species-specific identification. These are: egg size, morphology of the micropylar area, longitudinal ridges, interridges and ventral plate.

Egg size is very different, *M. marginata* having eggs twice long as the other species. Two different types of chorionic sculpturing occur in the micropylar area. The first type, belonging only to *M. occultans*, shows two concentric circles of plugs and a papillar basal layer. The second type lacks plugs, and in the basal layer there are sausage-like appendages. A pattern similar to that of *M. occultans* was reported by Plachter (1981) in some Gnoristinae (*Boletina* sp., *Apolephthisa* sp.) and Leiinae (*Ectrephestoneura* sp.) (*sensu* Tuomikoski, 1966). Therefore this type of chorionic sculpturing of the micropylar area is presumably the most common in the subfamily.

The longitudinal ridges, characterizing the morphology of the external surface of the egg, are also present in some Gnoristinae (Plachter, 1981) and Leiinae (Plachter, 1981; our unpublished data). However, each species exhibits peculiar features of these longitudinal structures, so that it is possible to consider them a species-specific character.

Taxonomic significance can also be attributed to the ventral plate found in all three species examined and in *Leia bimaculata* (our unpublished data), in the same location, but with different features. In addition, the different morpholgy of this structure from the rest of the surface suggests a specialized function. It might be related to the respiration of the eggs. The developing embryo needs oxygen, which is normally absorbed through aeropyles, plastrons or respiratory appendages (Hinton, 1971; Cummings & O'Halloran, 1974; Mazzini, 1974, 1977). In Mycetophilidae, aeropylar openings have been reported by Santini & Mazzini (1989), whereas in certain Gnoristinae and Leiinae (*sensu* Tuomikoski, 1966) Plachter (1981) described a plastron restricted to the interior of the longitudinal ridges. We suggest that the peculiar structure of the ventral plate of *M. occultans, M. marginata* and *M. fimbriata* might be related to the plastron. Oxygen supply and gas exchanges in general are presumably limited to this area, while the remaining surface of the egg is probably adapted to protect against desiccation (limiting the loss of water), and for attachment to the substratum. In other insects the latter is usually accomplished by specialized chorionic structures (Gaino & Mazzini, 1987) or by adhesive material. This may be secreted by follicle cells during egg maturation or by the colleteric glands during egg passage through the oviduct. In the first case, it is regarded as a secondary covering, while in the second case as a tertiary extrachorionic layer. Some Mycetophilidae may have extrachorionic adhesive material, like the jelly layer of Sciophilinae (Plachter, 1981) and Keroplatinae (Santini, 1979, 1980; Santini & Mazzini 1983). However, SEM observations failed to reveal any adhesive material on the eggs of the three species of Mycomyinae. It is conceivable that the attachment functions in these eggs are mediated by the irregular chorionic sculpturing.

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