FINE STRUCTURE OF THE RESPIRATORY SYSTEM
IN MITES FROM THE FAMILY PARASITIDAE
(ACARI, MESOSTIGMATA) *

BY

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ABSTRACT

The paper reports results of ultrastructural studies (TEM and SEM) on the respiratory system in 9 species of mites belonging to the family Parasitidae. The respiratory system of mites includes pairs of stigmata, peritremes, atria, as well as arising from atria tracheae and tracheoles. The role of peritremes and minute spines covering their walls is discussed. TEM studies have also revealed a specific morphology of tracheal wall in mites, differing from that hitherto described in insects.

RÉSUMÉ

L'ultrastructure des organes de la respiration, étudiée à l'aide de TEM et SEM chez 9 espèces d'Acariens de famille Parasitidae est décrite. Les organes de la respiration se composent d'une paire de stigmates, d'une paire de péritrèmes et d'une paire d'atriums, ainsi que de trachées et trachéoles. L'auteur discute le rôle des péritrèmes et des épines cuticulaires sur leurs parois.

L'auteur décrit la structure des parois des trachées, qui est très spécifique et différente de celle décrite jusqu'à présent chez les Insectes.

INTRODUCTION

Respiration by means of tracheae is characteristic for most mites, except the youngest larval stages and the numerous representatives of the suborder Acaridida. In mites belonging to the suborder Mesostigmata the tracheal system begins with two openings (stigmata) localized symmetrically at both sides of the body above coxae, between the 3rd and 4th pair of legs. Forwards from the each stigma runs a though-shaped protrusion of the cuticle, which is called peritreme. The mites from the family Parasitidae possess three instars : a larva, a protonymph, and a deutonymph. The larva lacks the tracheal system and both, stigmata and peritremes. The later stages possess tracheae. The protonymph possesses stigmata, but their peritremes are very short ; in deutonymphs and the adult mites the whole respiratory system is fully developed.

Although the anatomy of the respiratory system in mesostigmatic acari is known (NEUMANN, 1941 ; HUGHES, 1959 ; JAKEMAN, 1961 ; WOODRING and GALBRAITH, 1976), the ultrastructural studies have not been yet performed. Some preliminary notes concerning this subject are presented in this study.

MATERIAL AND METHODS

The material for light microscopical and scanning (SEM) electron microscopical studies included males and females of mites belonging to the family Parasitidae (Mesostigmata) and to the following species : *Holoparasitus tirolensis* Sellnick, 1968, *Leptogamasus gimescus* Athias, 1970, *Paragamasus manicatellus* Athias, 1967, *P. monticola* Willm., 1953, *Parasitus lunulatus* (Müll., 1859), and *Pergamasus quisquiliarum* (Can., 1882), collected from the beech forest litter near Graz (Austria) as well as *L. succineus* Witaliński, 1973, *Parasitus niveus* (Wankel, 1961), and *Pergamasus viator* Halaškova, 1959, collected from the beech forest litter near Krakow, Southern Poland. For light microscopic purposes preparations of whole or sectioned mites in Berlese's solution were used. For SEM studies mites were killed with gaseous carbon dioxide, dried in the air, coated with carbon and gold and examined in Cambridge Stereoscan II A and JSM-35 scanning electron microscopes.

For transmission (TEM) electron microscopical studies only the females of *Pergamasus viator* were used, fixed at room temperature in 3.7 % glutaraldehyde buffered with 0.05 M cacodylate buffer pH 7.4 and postfixed in 1.4 % osmium tetroxide. All the mentioned solutions had their osmolarity adjusted to approx. 500 m0sm. The material was then conventionally embedded in Epon, and the thin sections were examined under Tesla BS 500 electron microscope.

The layers of cuticle were distinguished exclusively on the ground of their morphology as observed in TEM studies.
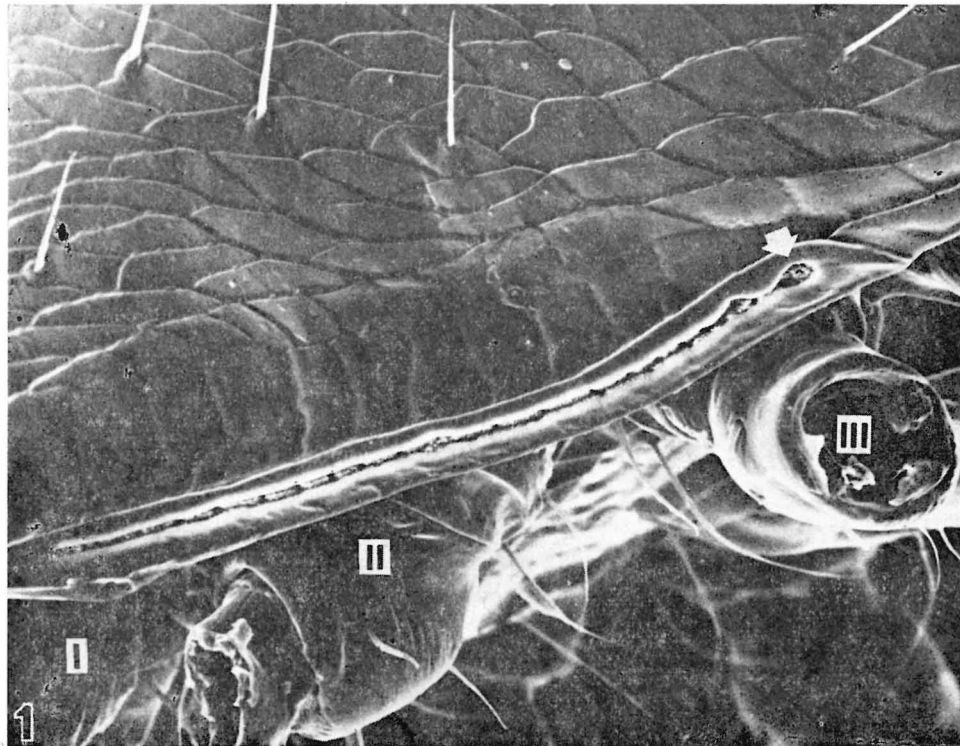

FIG. 1. — SEM micrograph of the border of dorsal shield with the stigma (marked by arrow) and peritreme running forwards. I-III) bases of legs, I-III pair. *H. tirolensis*. × 950.

RESULTS

The respiratory organ of the studied mites consists of two stigmata, two peritremes and the related systems of tracheae and tracheoles. The stigmata and peritremes (Figs. 1, 7, 8) are localized in the peritremal shields. In females these shields can be either free or fused with dorsal or ventral plate, in males all the mentioned elements are connected. The respiratory system does not show a sexual dimorphism.
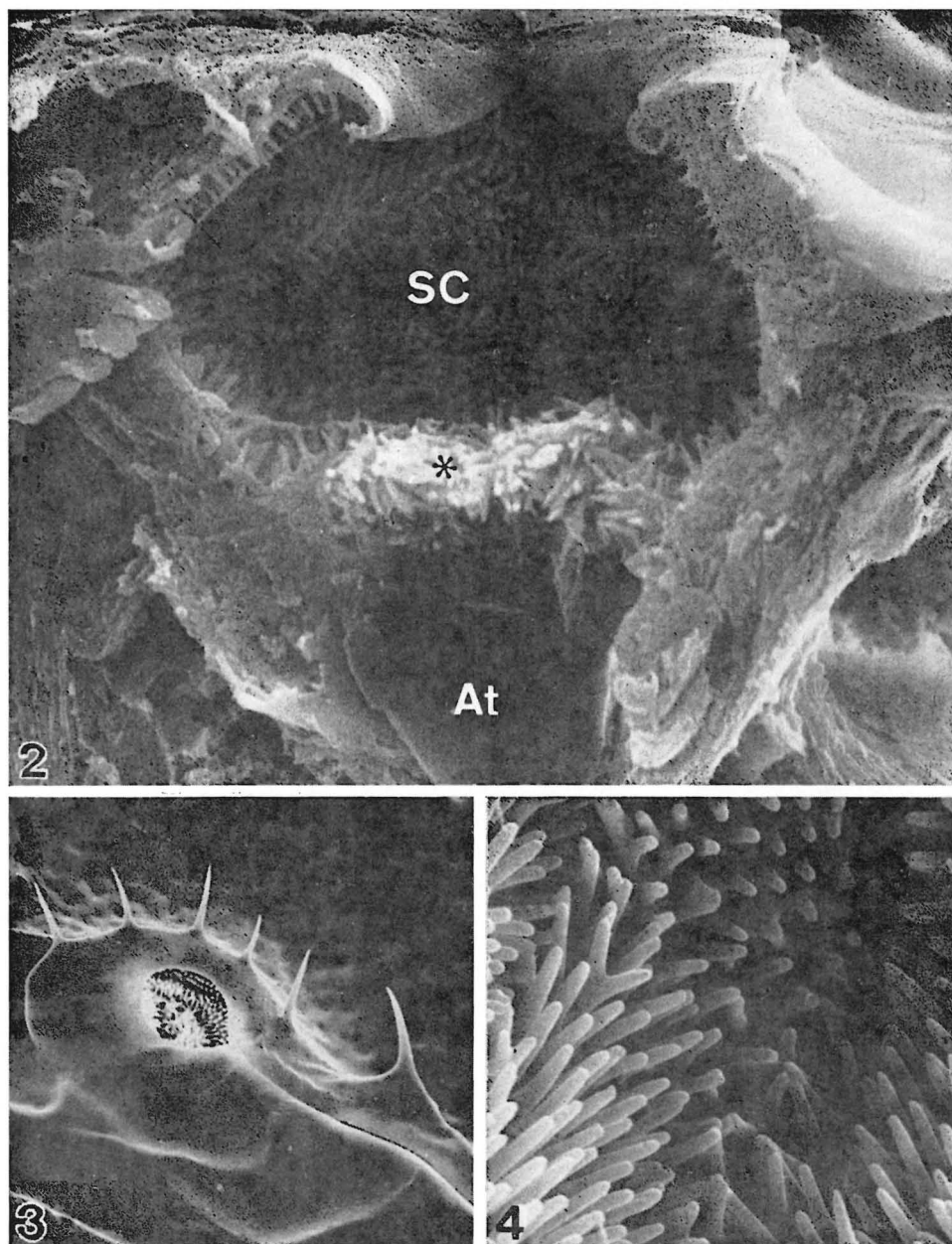
The stigmata.

The stigma is composed of three elements : 1) an external orifice, mostly circular in shape, 2) stigmal chamber, lying beneath the external orifice, and 3) internal orifice which connects the stigmal chamber with a tracheal ramification (atrium) (Fig. 2). The external orifice is localized on a small elevation of cuticle, the latter is mostly smooth, but sometimes forms characteristic thorns (*P. manicatellus*, Fig. 3). The diameter of the external orifice depends on the size of the mite, and ranges from 29.5 μm in the largest studied species (*P. quisquiliarum*) to 5.0 μm in the smallest one (*L. succineus*). The stigmal chamber, lying beneath the orifice has a diameter approximately twice as big as the orifice in a given species. The walls of the chamber are covered with densely packed fingerform cuticular processes (minute spines) (Fig. 4), 0.2 to 0,3 μm in diameter and 2.2 to 3.0 μm in height (*P. viator*). The minute spines have rounded tops and lack pores or infoldings. Except the minute spines, in all the investigated species occur more or less branched, complex cuticular processes (Figs. 5 and 6). As compared with the external orifice, the internal one has a diameter slightly smaller, additionally diminished by centrally directed minute spines and complex processes localized on its boundary.
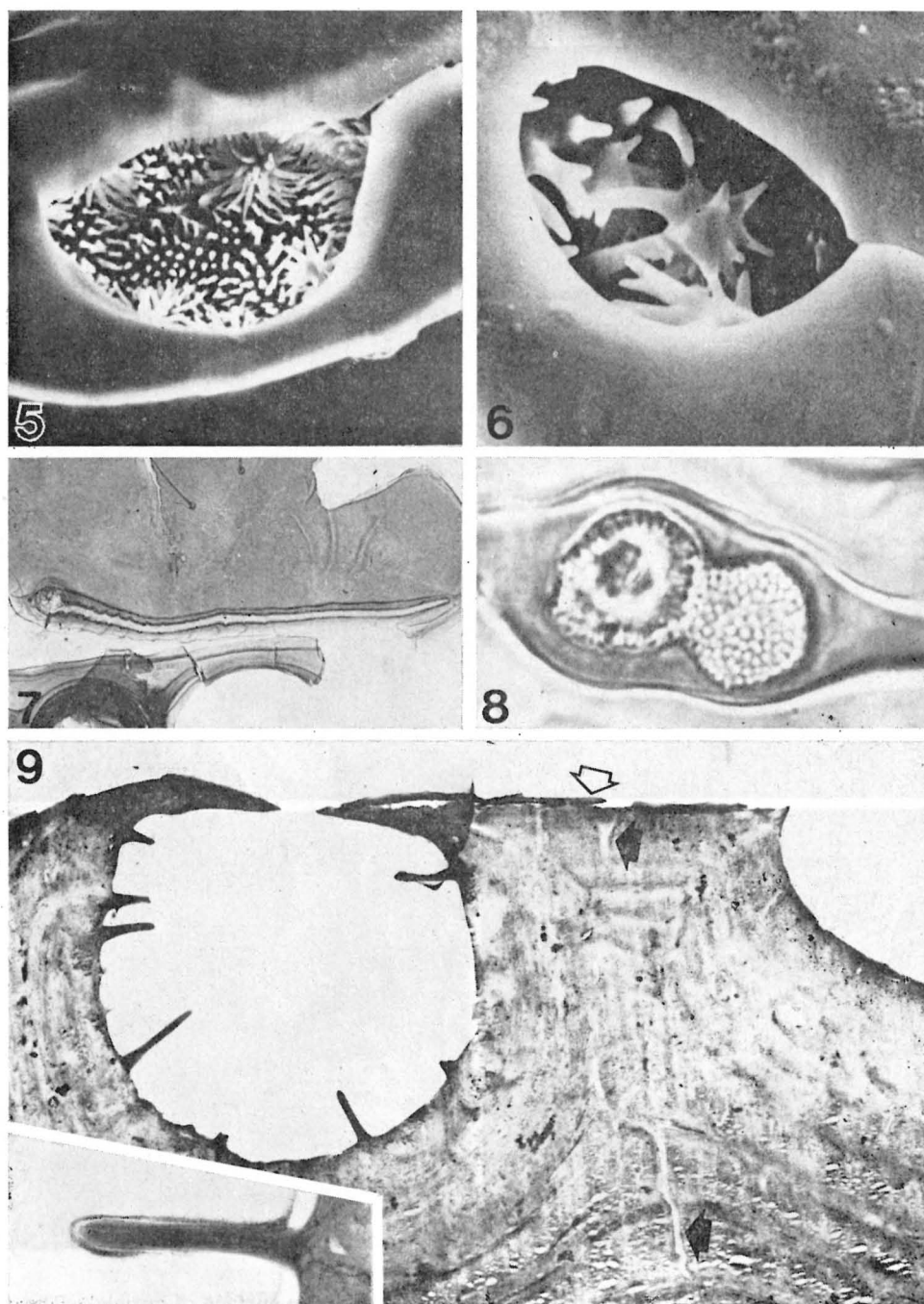
The peritremes.

The peritreme is a groove in cuticle, possessing a length depending on the developmental stage of the mite. The larvae which are devoid of stigmata, do not possess peritremes. The protonymphs have already stigmata and very short peritremes, with their length only fractionally exceeding the diameter of stigma. The deutonymphs and adults possess fully developed peritremes running from stigmata forwards, above the bases of legs and ending blindly near the bases of the 1st or 2nd pair of legs (Figs. 1 and 2). The only exception is a small group of species belonging to the genus *Leptogamasus*, whose adults possess very short, " protonymphal " peritremes (Fig. 8) (ATHIAS-HENRIOT, 1967 ; WITALINSKI, 1973). Unfortunately, due to their scarcity there are no data concerning the morphology of peritremes in earlier developmental stages of that genus.

The peritreme as seen in cross section is roughly circular, and the diameter ranges from 15 μm (*P. quisquiliarum*) to 4.5 μm (*L. gimescus*). The boundaries of cuticular folds covering the peritreme are sporadically accreted, but only over short distances. Usually a fissure occurs between them, max. 3.0 μm in width in most species excluding the representatives of genera *Holoparasitus* and *Parasitus*, where the fissure is wider (Figs. 1 and 3). In all the investigated species the peritremal walls, similarly to the walls of the stigmal chamber are coated with minute spines.

The TEM observations showed, that the cuticle which forms peritreme does not differ with respect to its thickness and structure from the cuticle of the sclerotized shields. It is composed,

FIGS. 2-4 : 2) A section through stigma, running perpendicularly to the surface of cuticle. The picture shows fragment of the external orifice, stigmal chamber (SC) lying beneath it, with walls covered by minute spines, which can be seen also on the boundary of the internal orifice (asterisk). At) part of the smooth wall of atrium. *P. viator* ; SEM. × 4 100 ; 3) Stigma and fragment of peritreme in *P. manicatellus*. Minute spines inside the stigma are clearly visible. SEM. × 1 600 ; 4) Fragment of the wall of stigmal chamber coated with minute spines. *P. viator* ; SEM. ×11 100.

FIGS. 5-9 : 5 and 6) SEM micrographs of stigmata with both, singular and complex (branching) cuticular processes. 5) *P. viator*. × 3 300 ; 6) *L. gimescus*. × 9 900 ; 7 and 8) Light micrographs of stigmata and peritremes of *P. viator* (7) and *L. succineus* (8). × 260 and × 1 750, respectively ; 9) TEM micrograph showing a transversal section through peritreme in *P. viator*. Minute spines can be seen inside the peritremal channel. The cuticle, which forms peritreme contains pore canals reaching its surface (dark arrows). A fragment of epicuticular layer partially preserved during preparation of the material for TEM is marked by light arrow. × 6 200.
    Inset : a higher magnification of a single minute spine covered by the fine epicuticular layer. × 16 600.

as found in other mesostigmatic acari (WHARTON et al., 1968) of three layers : the endo-, exo-, and epicuticle, although the latter is quite often removed during the preparation of material for EM purposes. The first two layers are penetrated by scarcely ramified and well demarcated pore canals. The minute spines of stigmal chambers and peritremes are formed by exocuticle covered by epicuticle. At their base are frequently visible the pore canals with their delicate ramifications seemingly entering at least some minute spines (Fig. 9).
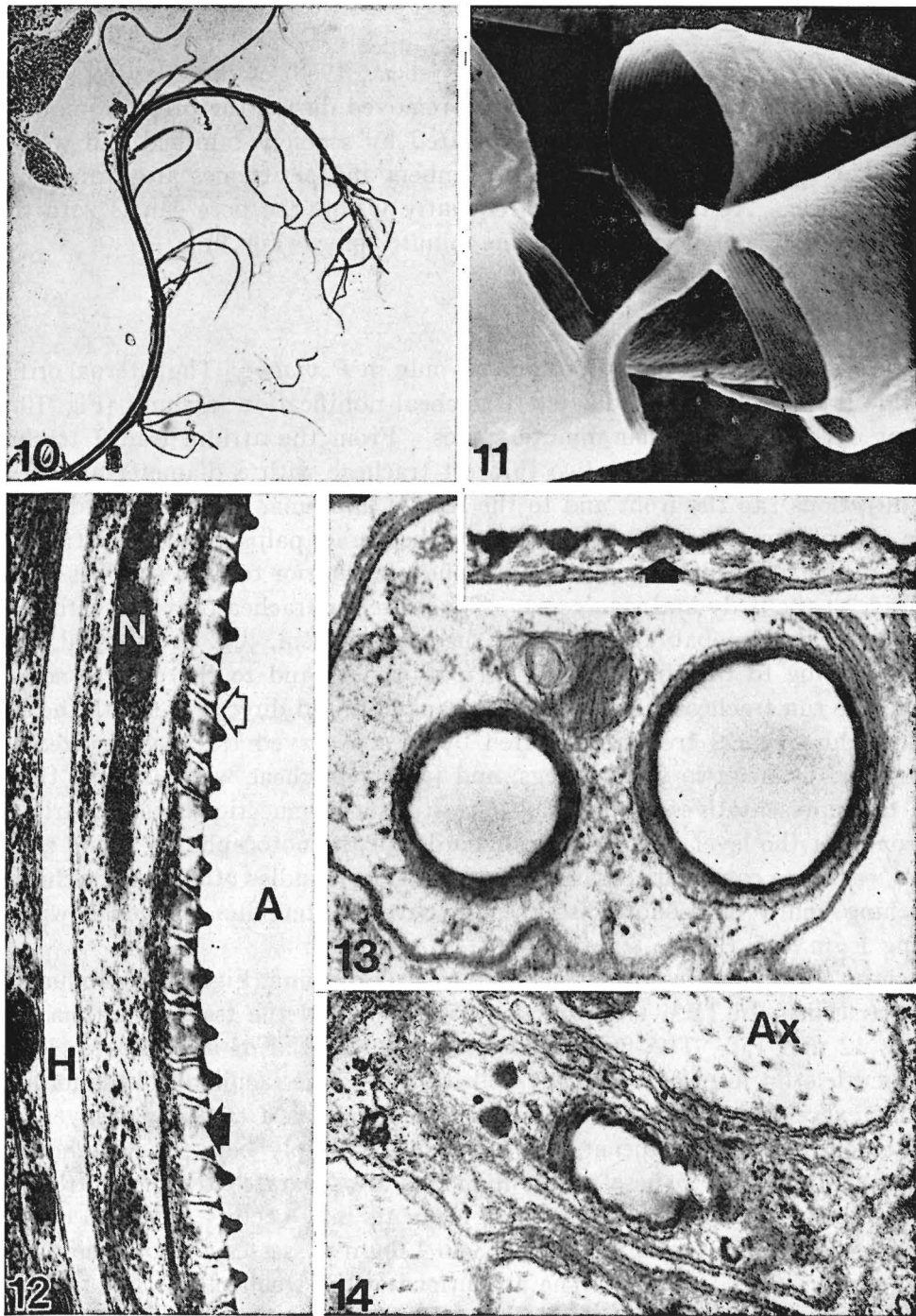
The tracheal system.

The tracheal system was studied profoundly only in *P. viator*. The internal orifice of stigma leads to a wide (approx. 45 µm in diameter) tracheal ramification (atrium) (Fig. 10). Its walls are smooth or covered by singular minute spines. From the atrium lead 7 tracheae, part of which immediately bifurcates. The two thickest tracheae with a diameter approx. 18 µm run in opposite directions : to the front and to the rear of idiosoma, along the borders of the dorsal shield. The anterior trachea conducts the air to chelicerae, palps and the first and second pair of legs. Part of the tracheoles originating from the left anterior trachea supplies organs localized at the right side of the body and vice versa. The posterior trachea provides with the air mainly the genital system and probably part of the digestive system. At the ventral side of atrium begin trachea leading to the third and fourth pair of legs and to the central nervous system. At the dorsal side run tracheae supplying the organs localized directly beneath the dorsal shield. The left and right tracheal tree is connected by means curved trachea localized near sternal shield at the level of last two pairs of legs, and joining tracheae which supply the fourth pair of legs. In the representatives of the genus Parasitus such connection occurs clearly at the dorsal side of idiosoma, at the level of the posterior border of the notocephalon. The tracheae divide several times, each one can send alternately to both sides bundles of several tracheoles (Fig. 10), before branching finally on a short distance into seven to ten thin tracheoles with a diameter not exceeding 1 µm.

The tracheae and tracheoles are lined with a cuticular intima (Fig. 11), a product of externally lying tracheolar cells. In TEM two principal components of the tracheal intima can be distinguished (Figs. 12 and 15). The first component is a material of a moderate electron density (probably exocuticle) in form of a helically coiled band. The taenidial fold is formed only with a part of that exocuticular band. The second component is a cuticulinar layer which covers the air surface of tracheae and penetrates more or less deeply between neighbouring coiles of exocuticular taenidial band. These two components are separated by an electron-lucent space, in which some fibrils connecting the two main elements may occur. The wall of the tracheoles (Figs. 13 and 14) with an internal diameter 0.3 to 1.0 µm possesses a two-layered intima, whose thickness range from 60 to 120 nm. The air surface of the tracheoles is lined with a circularly folded layer of electron dense substance, approx. 10 to 20 nm thick, being a continuation of the similarly electron-dense layer of the tracheal intima. Parallelly to insects (LOCKE, 1966) this layer is constituted presumably of cuticulin. It is separated from the surface of tracheolar cells by the layer of moderate electron density, 40 to 100 nm thick. In thick tracheoles in that layer the remainders of exocuticular taenidial band can be distinguished (Fig. 13, inset).
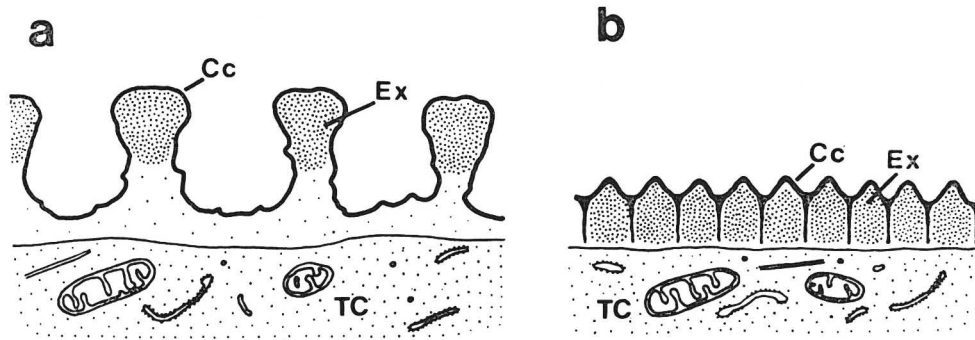
The diameter of the finest tracheoles, their scarce distribution, and their relatively thick intima point to a not highest oxygen requirements of the supplied organs. In tissues with a very high level of metabolism, e.g. in the flight muscles of insects, much thinner tracheoles, 0.1 to 0.2 µm in diameter, were observed (SMITH, 1961).

The tracheolar cells closely surrounding tracheae and tracheoles (Figs. 12, 13, 14) usually

Figs. 10-14 : 10) Light micrograph showing the isolated posterior trachea with the bundles of tracheoles. *P. viator.* × 315 ; 11) SEM micrograph of two ruptured tracheae, with their internal surface covered by taenidial folds exposed. *P. viator.* × 5 800 ; 12) A near longitudinal section through the trachea (approx. 5 μm in diameter) in *P. viator.* In the intima can be observed sections through taenidial folds with their exocuticular (dark arrow) and cuticulinar (light arrow) components separated from each other by an electron-lucent space. A) air space ; H) hemocoel ; N) fragment of the tracheolar cell nucleus. TEM. × 10 100 ; 13) Transversal section through two thick tracheoles surrounded by a common tracheolar cell. A two-layered structure of intima as well as sectioned transversely microtubules lying between tracheoles are visible. *P. viator* ; TEM. × 26 100. Inset : the longitudinal section through the tracheolar intima. Below undulated dark cuticulinar layer exist remainders of exocuticular taenidial band (dark arrow). In the thinnest tracheoles (Fig. 14) the last are not observable. × 70 000 ; 14) A slightly oblique section through a very thin tracheole localized within a nerve bundle between axons (Ax). Note the circumferential folding of intima. *P. viator* ; TEM. × 33 000.

possess a markedly flattened nucleus, scarce mitochondria, free ribosomes and ER cisternae, as well as quite numerous microtubules arranged parallelly to the long axis of the tracheae or tracheoles. The tracheolar cells that surround tracheae form the mestracheons, while the tracheoles penetrate the tracheolar cells without it. Both cell membranes between the adjacent tracheolar celles as well as mestracheons coupled by septate desmosomes can be frequently observed.

FIG. 15 : A schematic drawing showing structure of a longitudinally sectioned trachel wall in insect (a) and in mesostigmatic mite (b). Cc) cuticulin ; Ex) exocuticle ; TC) tracheal cell.

DISCUSSION

The mites belonging to the family Parasitidae are characterized by a lively, predacious mode of life (MICHERDZIŃSKI, 1969), hence possessing a considerably high level of metabolism. Moreover, the integument covering those mites is thick. No wonder, that they have an efficient tracheal respiratory system. The stigmata, leading to the system do not possess any shutting device, allowing for the regulation of the respiration and transpiration. A similar situation occurs in other mesostigmatic acari, although in Uropodidae the stigmata can be probably tightly veiled by the third pair of legs (WOODRING and GALBRAITH, 1976).

The walls of stigmal chamber, similarly to the walls of peritremes are coated with very numerous cuticular minute spines, which frequently form in the proximity of the internal stigmal orifice a branched cuticular structures. These can prevent penetration into the tracheal system of parasites frequently occuring on the surface of mites (such as nematodes, fungal spores etc.). Similarly functioning filter structures occur in the stigmata of ticks (BABOS, 1964 ; WOOLLEY, 1972), myriapodans (CURRY, 1974), as well as in spiracles in many insects (EIDMANN and KÜHLHORN, 1970).

The elongated peritremes, lying at both sides of the idiosoma are characteristic feature of Mesostigmata. The exact role of peritremes is still not clear. The presented studies have not demonstrated a sensory function of these structures, since sensory cells are absent in the hypoderma beneath peritremes. Furthermore, the surface of cuticle which forms peritremes, as observed in SEM, does not possess pores or infoldings mostly encountered in the sensory organs of arthropodans (AXTELL et al., 1971 ; FOELIX and AXTELL, 1971 ; NOSEK et al., 1973).

The cuticle of peritreme has the same structure and thickness as that in other regions of

dorsal or ventral plate, hence it can not serve for cuticular respiration. Such process was described in water mites, their cuticle, however, in the places of gas exchange is thinner and devoid of the endocuticular layer (REDMOND, 1972).

HUGHES (1959) believed, that peritremes are the main way of the gas exchange for the tracheal system, assuming, that the stigmata were not open. Although the following studies (JAKEMAN, 1961 ; WOODRING and GALBRAITH, 1976) refuted that hypothesis by providing evidence, that stigmata are not closed, a role of peritremes as the sporadical way of gas exchange can not be excluded in case of blocking the external aperture of stigmata by dirt, or by legs (*Uropodina*).

The minute spines, densely coating the walls of stigmal chamber and peritreme, as well as the narrow fissure between the borders of peritreme indicate the possibility of plastron formation in peritremes and stigmata (WOODRING and GALBRAITH, 1976). In case of wetting the mite surface, e.g. during long-lasting rain or sudden decrease of the temperature of a wet forest bedding, the air stored in stigmata and peritremes enables the mite to survive during an unfavourable period of time. Besides the presence of minute spines, the antiwetting properties of the surface of those structures is enhanced by the cement (surperficial layer) and the wax layer (tectostracum). Precursor substance for these layers are probably secreted via pore canals in mites (BRODY, 1970 ; KRANTZ, 1971) as well as in insects (SMITH, 1968 ; GHIRADELLA and RADIGAN, 1974).

The considered above possible functions of peritremes are without doubt profitable for mites, on the other hand, some facts seem to lessen their importance, at least in Parasitidae. The arguments for the latter conclusion include the occurrence in the protonymphs of all species, as well as in adult individuals of several species belonging to the genus *Leptogamasus* of very short peritremes, whose capacity as emergence air stores is insignificant, as compared with the capacity of whole tracheal system. Even the fully developed peritremes probably do not exceed the 1/10 of the total capacity of that system. Further physiological investigations seem to be necessary for the elucidation of this problem.

The morphology of tracheae and tracheoles of the Parasitidae representative — *P. viator*, basicaly resembles the morphology of these structures in the insects. The tracheae possess a quite thick cuticular intima strenghtened by a taenidial fold. The latter structure was observed in light microscope in some Mesostigmata (WOODRING and GALBRAITH, 1976), whereas in other representatives of that suborder (JAKEMAN, 1961) and in Oribatids (HUGHES, 1959 ; WOODRING and COOK, 1962) its presence was not reported. The above observations remain in a full agreement with the HUGHES' (1959) hypothesis, proposing an independent development of the respiratory systems in various groups of mites. The ultrastructure of tracheal wall in *P. viator* in details differs from the ultrastructure of tracheae in insects. In insects (LOCKE, 1957 ; SMITH, 1968) and also in ticks (WOOLLEY, 1972 ; NOSEK and CIAMPOR, 1973) the taenidial fold occurs as a circularly running convexity protruding to the tracheal lumen. The exocuticular cores of folds are clearly separated and lie far from each other, while in *P. viator* the neighbouring coils of an exocuticle lie close to each other. Furthermore, the core of taenidial folds in *P. viator* is formed only with a part of coil of an exocuticular helix. The layer of cuticulin covers not only the internal surface of trachea, but also penetrates between the adjacent exocuticular coils. Comparison between the structure of tracheal wall in insects and in mites is given in fig. 15. The observations mentioned above also confirm Hughes' hypothesis.

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