International Journal for Parasitology Vol. 18, *No.* 2, *pp.* 191–196, 1988.
Printed in Great Britain.

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0020–7519/88 $3.00 + 0.00
Pergamon Press plc

SCANNING ELECTRON MICROSCOPE OBSERVATIONS ON THE ANTERIOR THORACIC AND POST-ABDOMINAL SPIRACLES OF *GASTEROPHILUS* LARVAE (DIPTERA: GASTEROPHILIDAE)

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(*Received* 5 *June* 1986; *in revised form* 10 *June* 1987)

Abstract—Principato M. and Tosti M. 1988. Scanning electron microscope observations on the anterior thoracic and post-abdominal spiracles of *Gasterophilus* larvae (Diptera: Gasterophilidae). *International Journal for Parasitology* **18**: 191–196. Comparative scanning electron microscope (SEM) photographs and drawings of the anterior thoracic spiracles of third instar larvae of the six Italian *Gasterophilus* species are presented for the first time. There are pointed out differences in size and shape of the spiracles and in number and distribution of their papillae. The trabecular tissue observed in the anterior thoracic spiracles becomes thicker towards the bottom of the spiracle felt-chamber, forming a plug that makes the anterior thoracic spiracles in larvae non-functional. The post-abdominal spiracles, that are functional, contain serrate-sided openings through which is visible some trabecular tissue very similar to that observed in the anterior thoracic spiracles. The protective systems of the respiratory apparatus, permitting *Gasterophilus* larvae to survive in the horse's digestive apparatus, are discussed.

INDEX KEY WORDS: *Gasterophilus*; anaerobiosis; anterior thoracic spiracles; post-abdominal spiracles; spiracular papillae; trabecular tissue; metapneustic; amphipneustic; felt-chamber; tracheal cells; peritremes.

INTRODUCTION

Gasterophilus larvae live in the gastro-intestinal tract of horses, where the oxygen tension is very low. Survival is made difficult by other adverse environmental conditions such as the high internal concentration of carbon dioxide, the gastric juices and the food swallowed by the animal tending to overwhelm the larvae. Their respiratory system has therefore been modified, to prevent extraneous material from entering it, and to take in and store the maximum quantity of oxygen. Oxygen was shown in fact to be released by the presence of intracellular haemoglobin (Keilin & Wang, 1946).

The respiratory system of *Gasterophilus* larvae has been the object of some light microscopy studies (Principato, in press), and it has been compared with those of some other dipterous larvae (Tatchell, 1960). A few scanning electron micrographs and drawings of some of the structural details of the post-abdominal spiracles were presented in another paper (Faucheux, 1976).

In the present investigation, both the post-abdominal spiracles and the anterior thoracic spiracles were examined in the scanning electron microscope. Particular attention was given to a comparison of the anterior thoracic spiracles from larvae of the six Italian *Gasterophilus* species. The details that in our opinion were important for the structure and functioning of respiratory spiracles were also pointed out.

MATERIALS AND METHODS

The following species of *Gasterophilus* larvae present in wild horses in Umbria (Principato, Piergili Fioretti & Moretti, 1984; Principato, Piergili Fioretti & Galeote, 1985; Principato, Piergili Fioretti, Moretti & Polidori, 1986) were used: *G. intestinalis*, *G. pecorum* (removed from horse stomachs), *G. nasalis*, *G. haemorrhoidalis*, *G. meridionalis* (from horse duodena), *G. inermis* (from horse recta). Larvae taken in the months of April–May at the third instar were identified according to the presently used larval keys (Zumpt, 1965; Grunin, 1969; Draber-Monko, 1978). The post-abdominal spiracles were removed by dissecting larvae with a microscalpel and the anterior thoracic spiracles, previously wetted with a drop of lactic acid, were extroverted under a stereomicroscope using the tip of a pin and very fine pointed forceps. The removed anterior thoracic spiracles and the post-abdominal spiracles were washed rapidly in normal saline and were dehydrated through a graded series of ethanol from 40% to 100%. Once dried, they were mounted on aluminium stubs with double-stick cellophane tape, coated with 20 nm gold in a sputter coater (Polaron Equipment, SEM Coating Unit E 5.100) and examined and photographed in a Philips 501/B scanning electron microscope.

RESULTS

The two anterior thoracic spiracles are located in the anterior lateral aspects of the larval body. Each one consists of a chitinous tube, called felt-chamber (Keilin, 1944) that, at one end, extends into the larva as a large tracheal trunk and, at the other end, opens towards the outside, with the opening embedded in a

deep groove in the larval cuticle (Fig. 1). By folding back the cuticle, only the basal part of the spiracle is covered and the remainder is therefore in communication with the outside, by a great number of orifices called spiracular papillae (Figs. 1, 2, 4). Extrusion of the whole anterior thoracic spiracle occurs only when the larva pupates, so that both become clearly visible from outside as pupal "horns". The felt-chamber covered by the cuticle is pushed out of the larval body, and all the spiracular papillae are perfectly visible (Fig. 3). The spiracular papillae are prominent, spherical openings, looking like open mouths, variously ranged on the anterior part of the thoracic spiracle (Figs. 2, 4). Through these openings, a trabecular tissue can be observed, as a sort of narrow irregular reticulum, occupying the interior of the spiracle (Fig. 2). The anterior thoracic spiracles in larvae of the six Italian *Gasterophilus* species are of different sizes; the papillae also appear to be different in size and in their distribution at the spiracle apex from one species to the other (Figs. 5–10; Fig. 11). Their number is always higher on the lateral part of

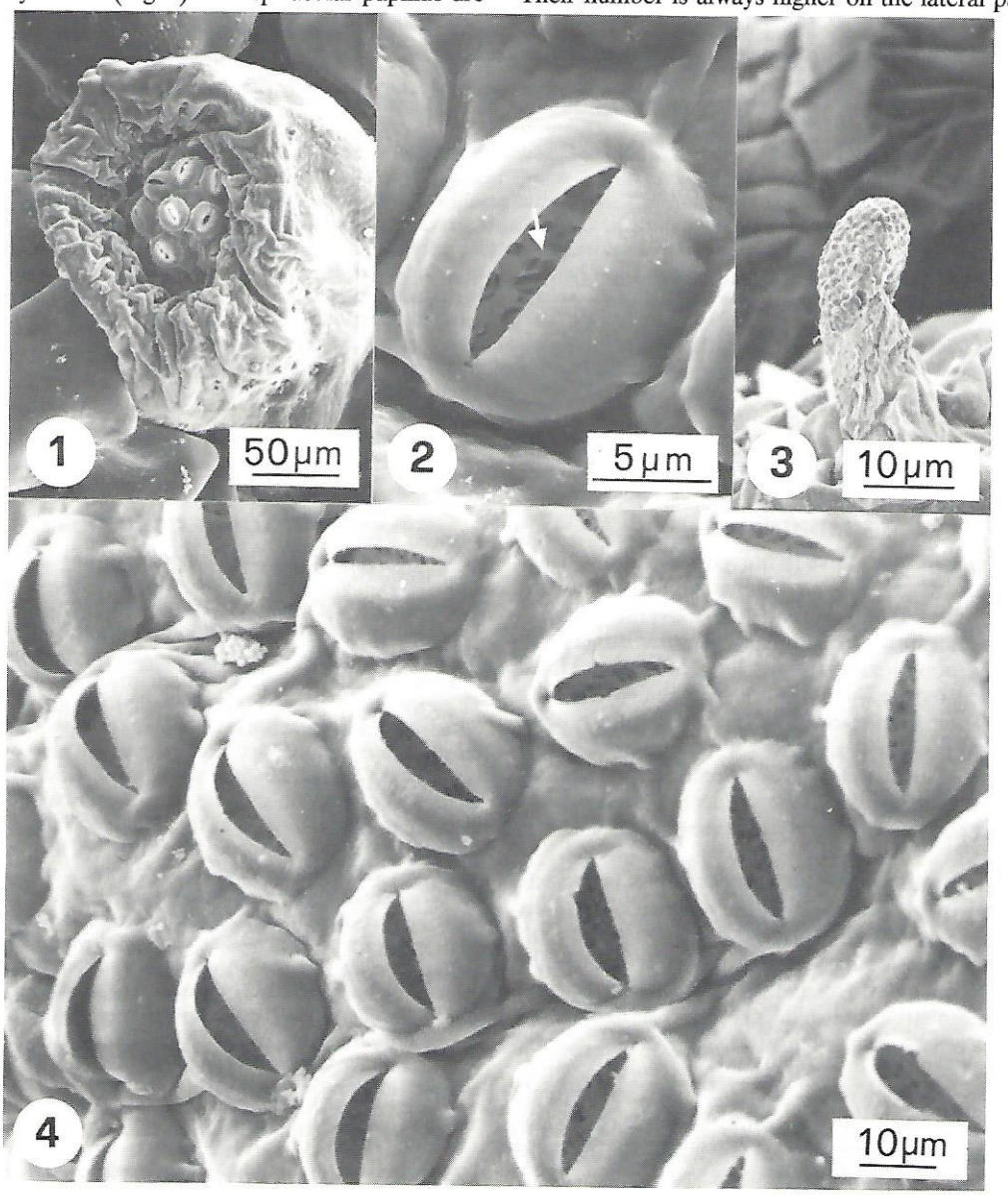
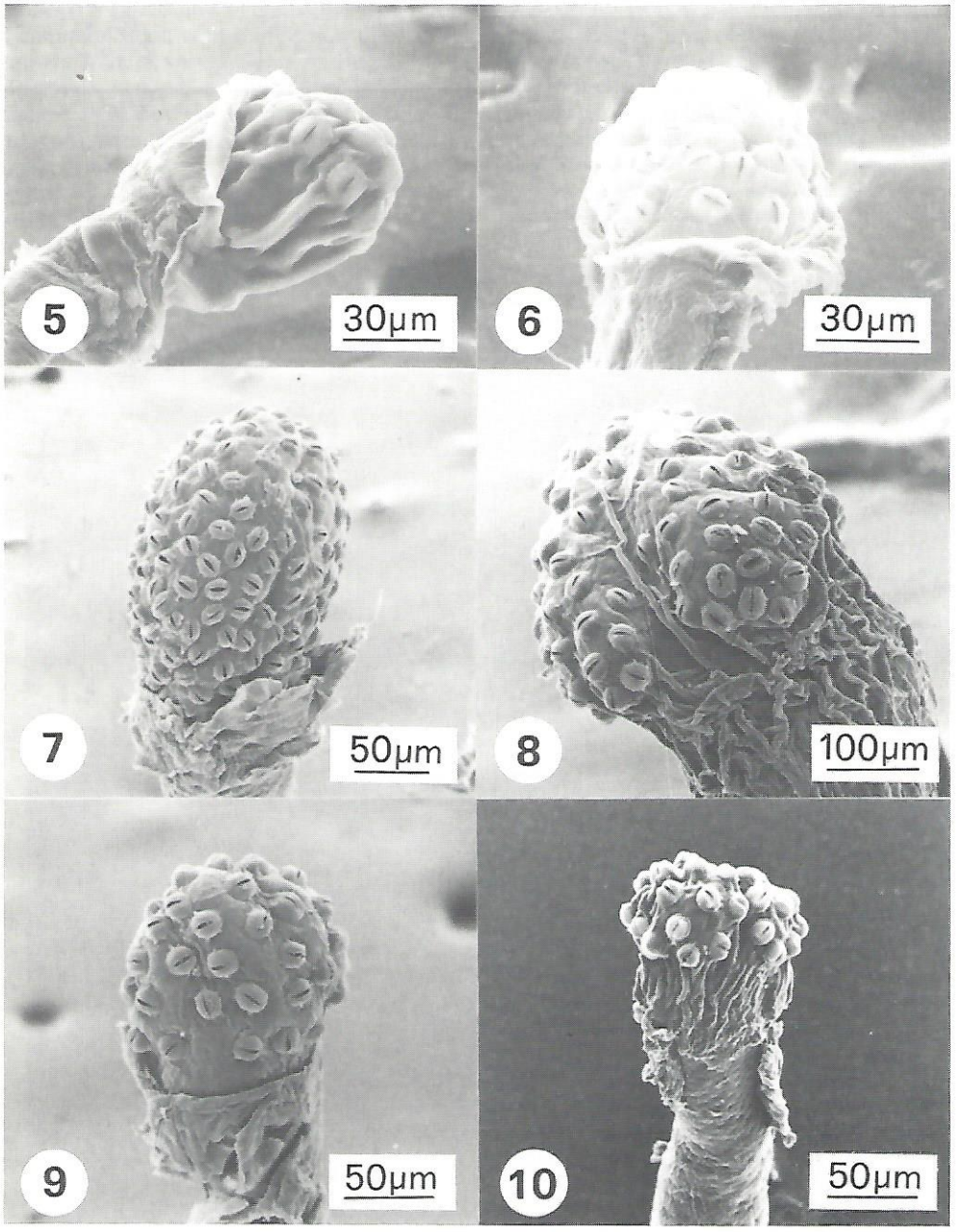


Fig. 1. An anterior thoracic spiracle surrounded by the larval cuticle.

Fig. 2. Trabecular tissue (arrow) in the interior of a papilla.

Fig. 3. An anterior thoracic spiracle naturally everted in a *Gasterophilus intestinalis* pupa.

Fig. 4. Papillae of the anterior thoracic spiracles.



FIGS. 5–10. Anterior thoracic spiracles of third instar *Gasterophilus* larvae:

FIG. 5. *G. pecorum.*

FIG. 6. *G. inermis.*

FIG. 7. *G. intestinalis.*

FIG. 8. *G. nasalis.*

FIG. 9. *G. haemorrhoidalis.*

FIG. 10. *G. meridionalis.*

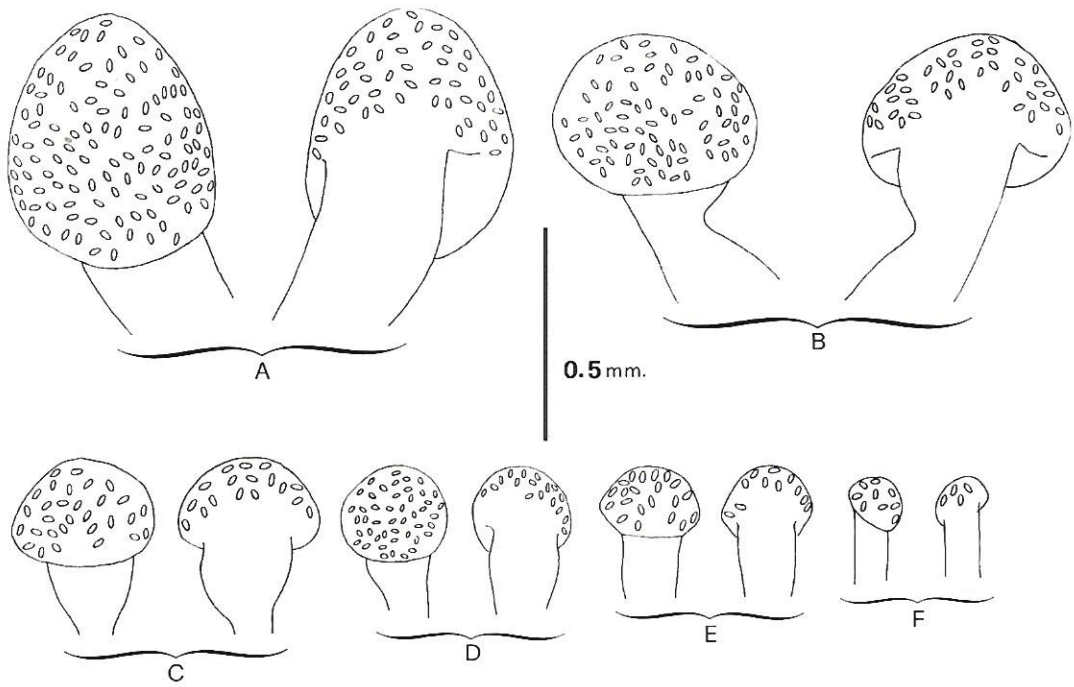


FIG. 11. Anterior thoracic spiracles of 3rd instar larvae of *Gasterophilus* species seen on their two different sides: (A) *G. intestinalis*; (B) *G. nasalis*; (C) *G. haemorrhoidalis*; (D) *G. inermis*; (E) *G. meridionalis*; (F) *G. pecorum*.

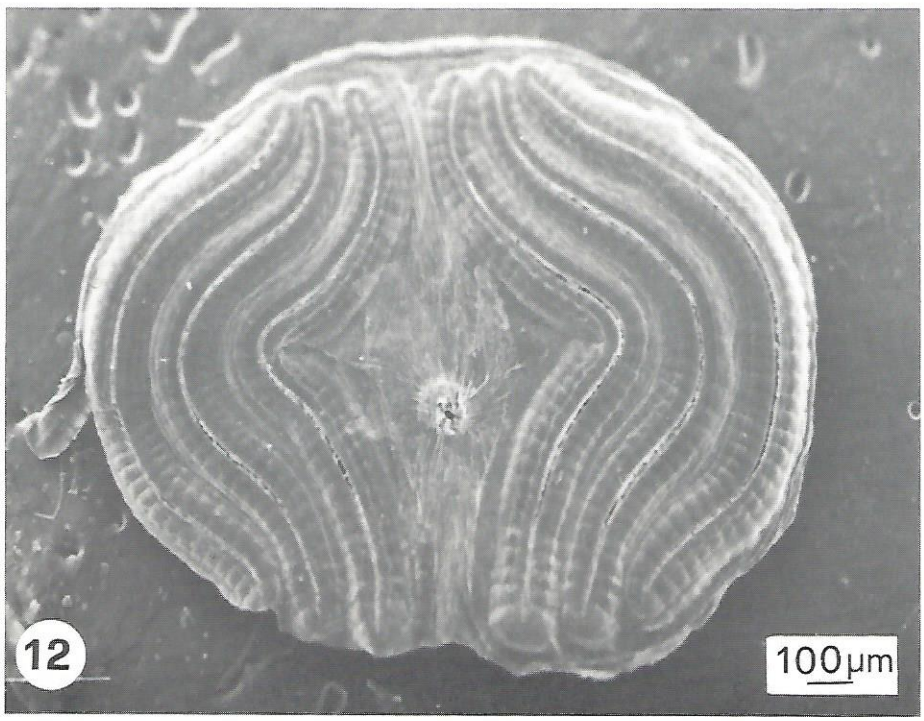


FIG. 12. The whole view of the post-abdominal spiracles.

the spiracle rather than medially. The area of the spiracle bearing papillae is clearly delineated from the adjoining larval cuticle and all the papillae, therefore, are in communication with the outside through the deep groove in the larval cuticle.

The presence of papillae open to the outside makes one assume that they are functioning parts of the respiratory system in the newly moulted third instar larvae. However, experimental evidence by Tatchell (1960) and by Faucheux (1976) who tried to inject cobalt naphthenate into the tracheal system of *Gasterophilus* larvae placed in a vacuum, showed that larvae are physiologically only metapneustic.

In the present investigation, sections cut at different points at the base of the felt-chamber showed a progressive internal closure. Near the joining point of the felt-chamber with the tracheal trunk, there appears to be a plug, crossed by a tiny tube, that disappears towards the felt-chamber wall, making the occlusion complete.

The post-abdominal spiracles are located at the posterior end of the larval body, in a hollow of the

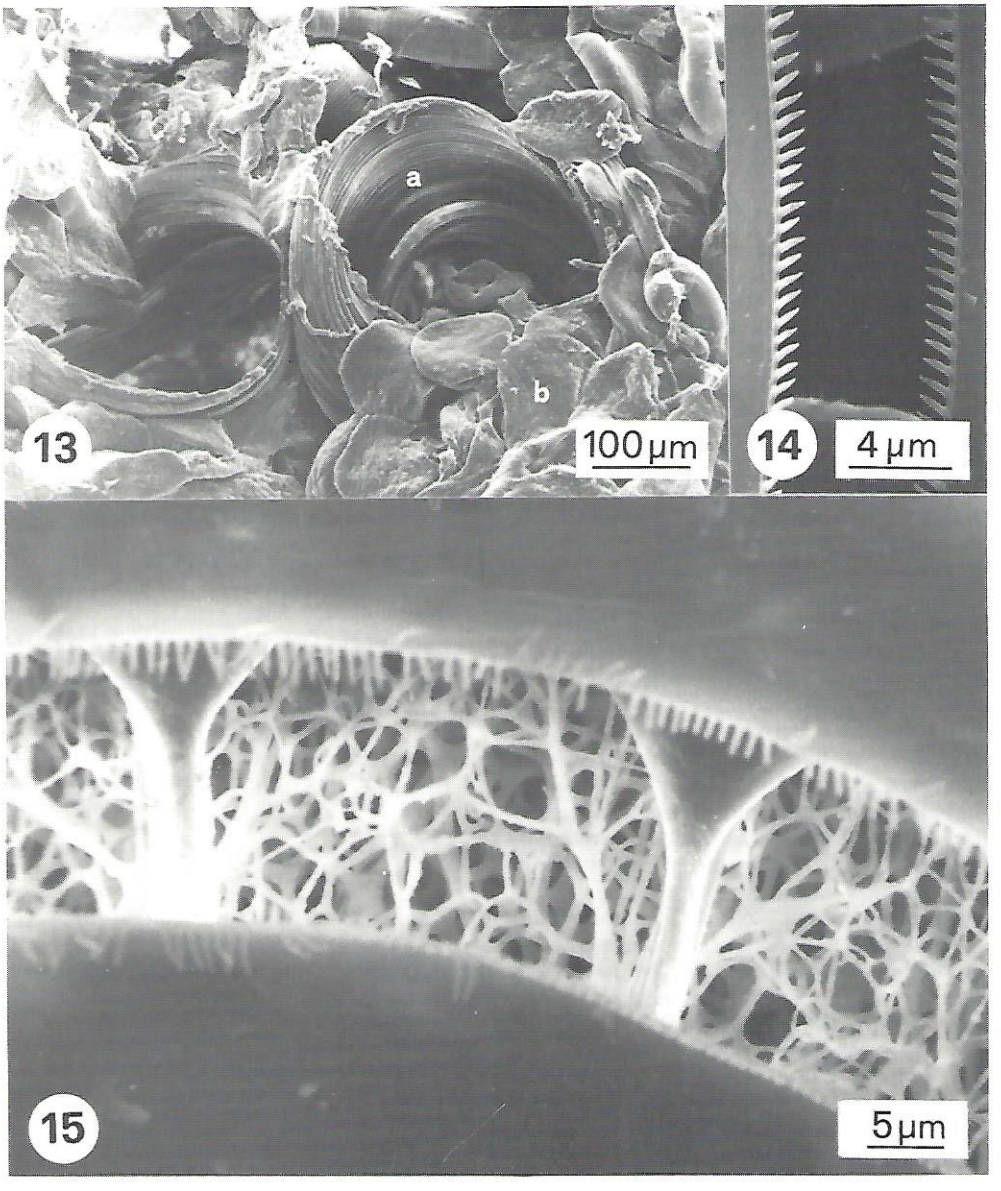


Fig. 13. Main tracheal trunks leading to the post-abdominal spiracles (a); groups of giant tracheal cells (b).

Fig. 14. Serrated sides of peritremes in the post-abdominal spiracles.

Fig. 15. Trabecular tissue in the interior of the post-abdominal spiracles.

cuticle that dorsally and ventrally forms two large lips, opening and closing over the spiracles, thus completely isolating them from the outside. The post-abdominal spiracles, in third instar larvae, consist of two chitinous plates joined to each other, both bearing three longitudinal narrow openings (Fig.12) and supported internally by a number of Y-shaped stigmatic sclerites. Those openings, called peritremes (Zumpt, 1965), communicate in the larval body with the main tracheal trunks and with the internal system of tracheal cells which are well developed in *Gasterophilus* larvae (Fig. 13). The peritremes in the SEM are narrow openings, the sides of which are serrate (Fig. 14). When they close, the serrate sides join to each other to form a hermetic seal preventing communication with the outside. Within the peritremes, an irregular trabecular tissue can be observed which is very similar to that present in the anterior thoracic spiracles (Fig. 15).

DISCUSSION

Although the anterior thoracic spiracles show some trabecular tissue and openings similar to those of the post-abdominal spiracles, their apparent internal occlusion means that *Gasterophilus* larvae are functionally metapneustic, even if they appear morphologically to be amphipneustic. Only when larvae pupate are the anterior thoracic spiracles functional.

Gasterophilus larvae have developed a mechanical system of protection for the respiratory spiracles that prevents the entry of extraneous material and helps them to survive in the gastro-intestinal tract. For the anterior thoracic spiracles, protection is given by their situation deep in a cuticular groove, by the narrow orifices of the spiracular papillae which may open and close and finally by the trabecular tissue within the spiracle which could have a filtering function and a supporting function to keep the spiracle open. As for the filtering function, actually intestinal contents are blocked by the first barrier represented by the cuticular groove, so they are unlikely to reach the trabecular tissue.

The supporting function of the trabecular reticulum to the spiracular walls is easily demonstrated by exerting pressure on the spiracle, which shows its elastic nature by still keeping its shape unchanged.

In the post-abdominal spiracles, a preliminary protection is exerted by the mobile cuticle covering and uncovering the spiracles and also by the opening and closing of the peritremes. The serrated sides of peritremes, while closing, function as a preliminary filter and once closed they secure the complete isolation of the whole respiratory system. The trabecular reticulum inside the post-abdominal spiracles could be an additional filter, as in the anterior thoracic spiracles, preventing material from entering the tracheal system.

In the adverse environmental conditions of the gastro-intestinal tract of horses where *Gasterophilus* larvae live, the low concentration of oxygen present in the stomach, swallowed by the animal with food, is absorbed through the post-abdominal spiracles and then stored in the very numerous large tracheal cells. These cells, real reservoirs of oxygen, give larvae a remarkable autonomy and resistance for many days, even in conditions of complete anaerobiosis (Keilin & Wang, 1946).

Tracheal cells, together with the protection system in respiratory spiracles, are therefore a perfect adaptation of larvae to their parasitic life.

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