

2. Material and methods

Workers of *T. nitida* (F. Smith, 1860) were collected in Pak Chong, Thailand, while workers as well as males and females of the bamboo-dwelling *T. binghami* (Forel, 1902) were collected at the Ulu Gombak Field Studies Centre of the University of Malaya, Malaysia. Workers of two other species, *T. attenuata* F. Smith, 1877 and *T. PSW-71*, were collected from dry branches or from the vegetation in the same area. The bacterial pouch was lacking in *T. PSW-71*, but was present in the other species. Voucher specimens were sent to P.S. Ward (University of California, Davis, USA).

The alimentary tract was carefully removed from the abdomen and fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose. Postfixation was carried out in 2% osmium tetroxide in the same buffer. After dehydration in a graded acetone series, tissues were embedded in Araldite and sectioned with a Reichert Ultracut E microtome. Semithin 1 μm sections were stained with methylene blue and thionin and viewed in a Zeiss Axioskop microscope, double stained 70 nm thin sections were examined in a Zeiss EM900 electron microscope. Material for scanning microscopy was critical point dried in a Balzers CPD 030 instrument and examined in a Philips SEM 515 scanning microscope.

3. Results

3.1. General structure

Dissection of workers of *T. attenuata*, *T. binghami* and *T. nitida* revealed the occurrence of a very conspicuous pear-shaped lateral outgrowth of the digestive tract at the junction between the midgut and the intestine. The same structure also occurs in the queens and males of *T. binghami* that we had available, although the following description is based on workers. The lateral flanks of this organ form the region where the Malpighian tubules open [Fig. 1(a) and (b)]. It has a length between 0.7 and 1.0 mm and a diameter of approx. 0.4 mm. At its distal end, several tracheal tubes can be observed. Each of these shows extensive branchings over the surface, that penetrate into this pouch-like organ [Fig. 1(c)]. Microscopy sections confirm the penetration of tens of parallel tracheolar strands from the distal region of the pouch deep into its interior, which is filled with a mass of bacteria [Fig. 1(d)]. The epithelial wall of the pouch is very different in its distal and proximal part [Figs. 1(d) and 2]: the distal epithelium is formed by flattened cells with a clear cuticular layer apically, and more or less covers the distal quarter of the pouch. In contrast, the wall in the main proximal part is formed by a cylindrical epithelium without cuticular lining. The contact region between both parts is formed by a rather complicated inward epithelial fold, that occurs as a belt-like surrounding of the pouch (Fig. 2). The

Malpighian tubules open into the pouch through the cylindrical epithelium close to the proximal side of this folded belt [Figs. 1(d) and 2]. The lumen of the pouch continues into the main lumen of the digestive tract.

3.2. Proximal microvillar epithelium

The lateral as well as the neck region of the pouch is lined with a cylindrical epithelium that reaches a thickness of 30 μm [Figs. 1(d) and 3(a)]. The apparently endodermal epithelium is characterized by a very obvious microvillar differentiation of the apical plasmalemma, that forms the internal lining of the pouch lumen in this region, without any trace of cuticle [Fig. 3(a) and (b)]. The cylindrical cells have a centrally located ovoid nucleus, and have a cytoplasm with abundant rounded vesicles, that vary in diameter from 1–2 μm . Most cells contain electron-clear vesicles, some cells contain electron-dense vesicles, occasionally both vesicle types can be found in the same cell [Fig. 3(a) and (c)]. The vesicles can occur in a sub- as well as a supranuclear position. In the latter case they locally distort the regular microvillar pattern. Other cytoplasmic features include numerous mitochondria and a well developed Golgi apparatus [Fig. 3(d)]. Neither smooth nor granular endoplasmic reticulum are apparent, just some isolated ribosomes or polysomes are scattered in the cytoplasm. The basal cell membrane displays many irregular invaginations, and rests on a fairly thick amorphous basement membrane with a thickness of approx. 0.3 μm . A few muscle fibres occur underneath the basement membrane [Fig. 3(e)].

3.3. Transition region

The transition region between the proximal thick microvillar epithelium and the distal thin cuticle-lined epithelium occurs as a conspicuously folded lip, that forms a belt-like structure surrounding the pouch in its distal region [Figs. 2 and 3(f)]. Next to the most distal microvillar cell occur a number of slender cells with interdigitating apical cell contacts, but with their basal region still in contact with the pouch's external lining basement membrane [Fig. 3(f)]. More distally, the narrow cells lose their contact with the outer lining of the pouch as they suddenly invaginate into the pouch lumen as an obvious lip with a length of approx. 50 μm . In this region, the cells start to display a thin but clear cuticular layer at the lumen side. The epithelium then folds towards the distal region again, comes in contact with the outer lining of the pouch again, and continues as the thin cuticle-lined epithelium. The cells that form part of this lip structure have a cytoplasm with only few organelles. They lack an apical microvillar border, but have a cuticular lining instead.

3.4. Distal cuticle-lined epithelium

The distal quarter of the pouch surface is formed by a cuticle-lined epithelium with a thickness of approx. 1 μm