

Morphology and ultrastructure of a specialized bacterial pouch in the digestive tract of *Tetraponera* ants (Formicidae, Pseudomyrmecinae)

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Abstract

The digestive tract in workers of some species of the pseudomyrmecine ant genus *Tetraponera* is characterized by a conspicuous pear-shaped diverticulum at the transition between the midgut and the intestine, that so far has not been found in any other ant species. As this organ is filled with a mass of bacteria, we propose to designate it as a bacterial pouch. Its distal wall is formed by a thin ectodermal epithelium, through which tens of tracheal branches penetrate into the bacterial mass that fills the pouch lumen. The proximal wall, in contrast, is formed by a cylindrical epithelium with a conspicuous microvillar differentiation of the apical cell membrane, but without a cuticular lining. The contact region between both epithelia occurs as a complex fold surrounding the pouch like a belt. The Malpighian tubules open into the pouch through the cylindrical epithelium adjacent to the belt fold. The functional significance of the bacterial pouch remains unknown, although the abundant presence of bacteria may indicate a symbiotic function. The conspicuous tracheolar supply illustrates the metabolic activity in the pouch, while the microvillar differentiation of the cylindrical epithelium may be interpreted in the uptake of metabolites from the pouch lumen. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: *Tetraponera*; Formicidae; Bacterial pouch; Digestive tract; Morphology; Ultrastructure

1. Introduction

The anatomical organization of the insect digestive system in general shows the same elements, albeit with eventual differences in the proportional development of particular parts, that can be related with the insects' dietary characteristics. A very peculiar situation is found in a number of insects that harbour a microcosmos of symbiotic microorganisms inside their digestive tract, mostly for the digestion of cellulose, for which the insect itself lacks the necessary enzymatic infrastructure (Dettner and Peters, 1999). A well-known example is found in the termites, that contain flagellates or bacteria in their hindgut for this purpose (Noirot and Noirot-Timothee, 1969). In many of the Termitidae, the bacteria are found in the so-called mixed segment that occurs at the junction between midgut and intestine, and that is characterized by the lumen being lined on one side by the midgut epithelium, and by the intestinal epithelium on the other (Kovoor, 1968; Noirot and Noirot-Timothee, 1969). Bacteria have equally been described in the digestive tract of overwintering honeybee

workers, where they occur in the lumen of the hindgut (Cruz-Landim, 1972). In the stingless Meliponinae, bacteria can be found in the entire gut system from oesophagus to rectum (Cruz-Landim, 1990).

Also in ants, especially in species belonging to the tribe Cephalotini, a variety of microorganisms has been reported in the digestive system, with bacteria and fungi the most common representants (Hölldobler and Wilson, 1990). The anatomical location of these endosymbionts may vary, but generally they occur in the lumen of the midgut and especially in the posterior part of the ileum (Caetano, 1989; Caetano and Cruz-Landim, 1985, 1987). The structural organization of the organ they are situated in usually is in agreement with the common appearance of the organ concerned, without obvious specializations. Also intracellular endosymbiotic bacteria may be found, such as in specialized bacteriocytes in the midgut of *Camponotus* species (Schröder et al., 1996).

We here report on the presence of a unique, hitherto unknown and structurally complex lateral pouch organ, filled with a bacterial mass, that occurs at the junction between the midgut and the intestine in *Tetraponera attenuata*, *Tetraponera binghami* and *Tetraponera nitida*, whereas it is lacking in some other species of this genus.

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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 supra-nuclear 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

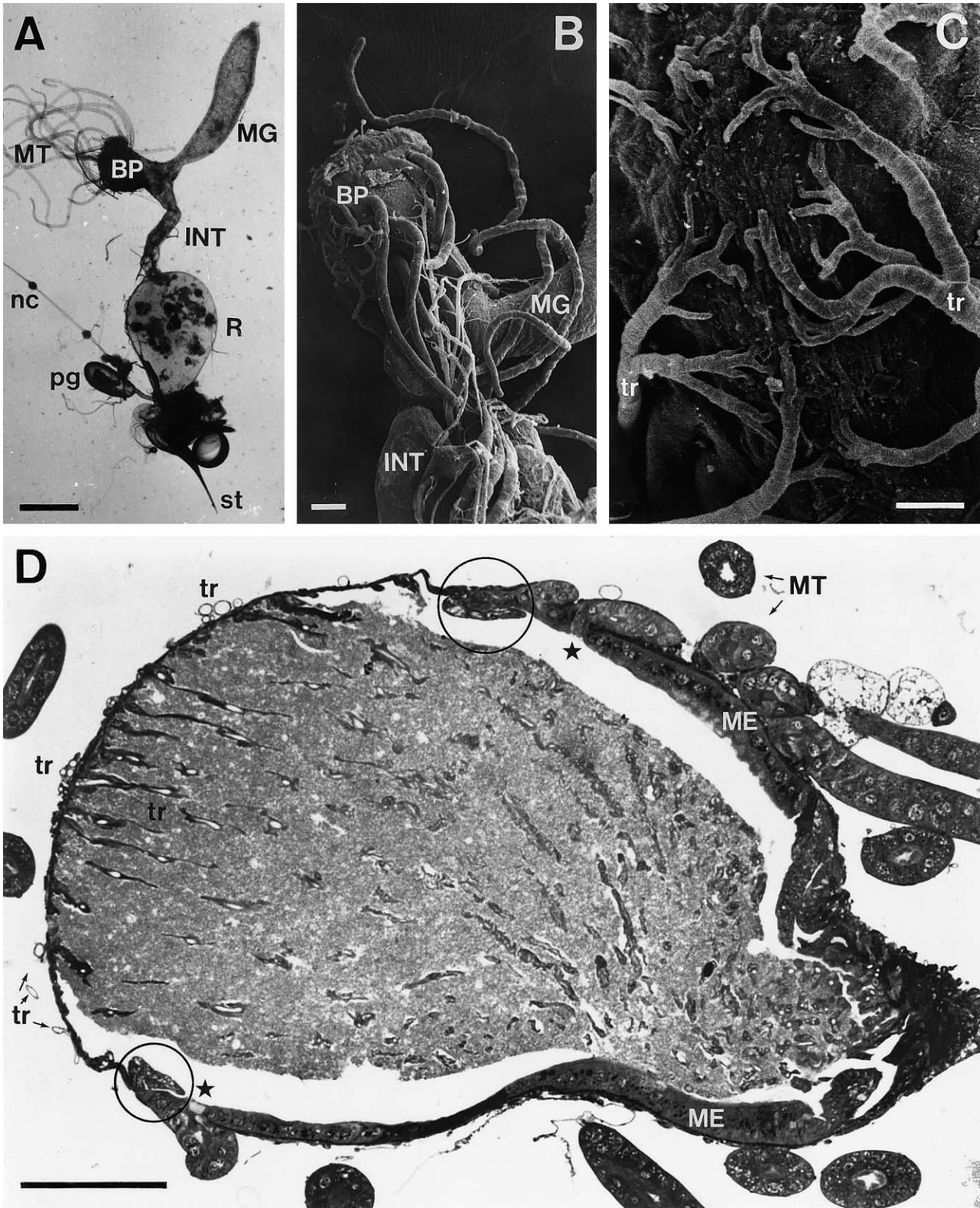


Fig. 1. (all *T. binghami* workers). (a) Dissection view of the sting (st) with attached alimentary tract. BP, bacterial pouch; INT, intestine; MG, midgut; MT, Malpighian tubules; nc, nerve cord; pg, poison gland; R, rectum (scale bar 1 mm). (b) Scanning micrograph of the bacterial pouch (BP) with connected Malpighian tubules. INT, intestine; MG, midgut (scale bar 100 μ m). (c) Scanning micrograph detail of finger-like branching of tracheae (tr) on surface of bacterial pouch (scale bar 50 μ m). (d) Longitudinal semithin section through bacterial pouch, showing tracheal penetration (tr) from distal part. Note transition zone with epithelial fold between thin and thick epithelial lining [encircled; see detail in Fig. 3(f)]. \star : region where Malpighian tubules (MT) open into bacterial pouch. ME, microvillar epithelium. Grey filling of pouch is formed by huge amounts of bacteria. Opening into gut is near lower right corner (scale bar 100 μ m).

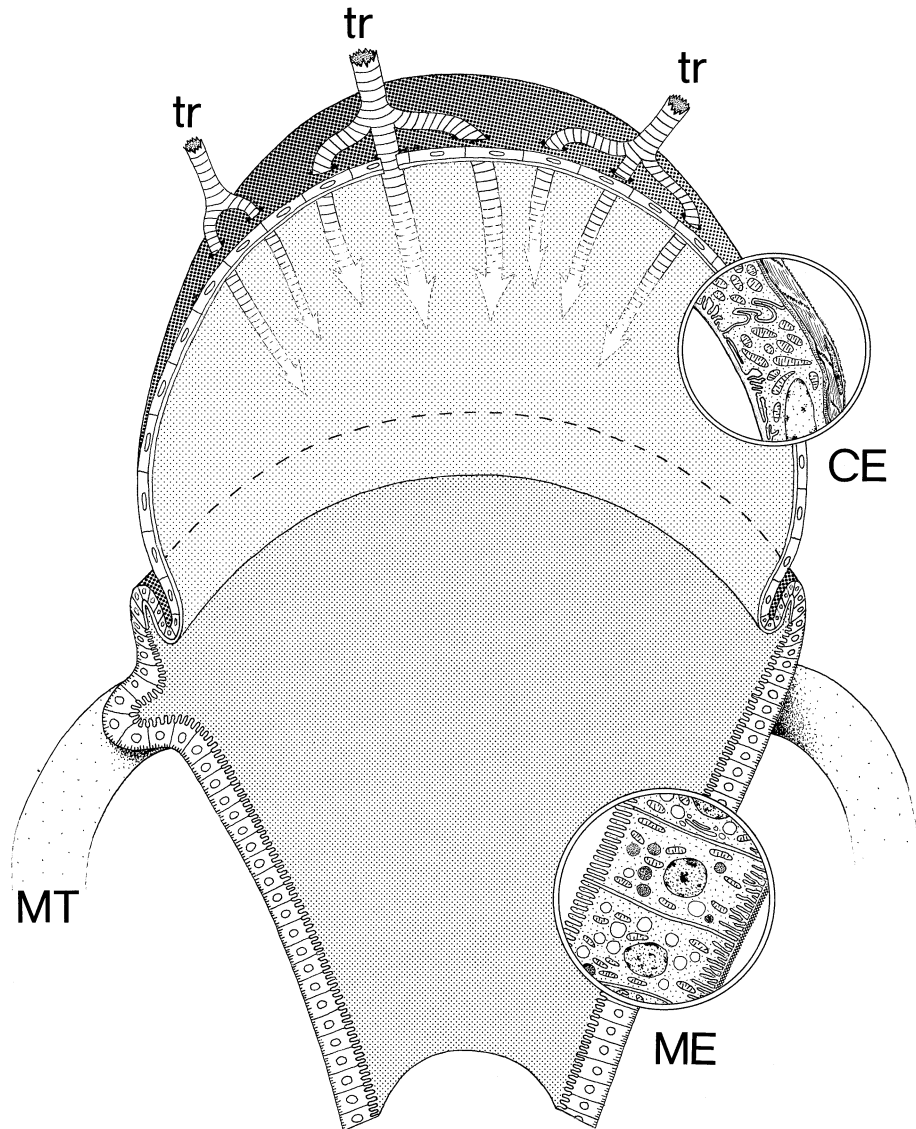


Fig. 2. Schematic survey showing structural organization of bacterial pouch with belt-like occurrence of fold at transition between thin distal cuticle-lined epithelium (CE) and thick proximal microvillar epithelium (ME). MT, Malpighian tubules; tr, tracheoles.

[Fig. 1(d)]. The flattened ectodermal cells contain numerous dark mitochondria, and are characterized by folded intercellular contacts and an irregular microvillar differentiation of their apical plasmalemma. An obvious cuticular layer forms the luminal lining of the cells, and consists of a thin electron-dense outer epicuticle (approx. 30 nm) and an electron-lucid procuticle with a thickness of approx. 0.2 μm [Fig. 4(b)]. Numerous tracheal branches

penetrate the epithelium towards the pouch interior. The penetrating branches appear as naked tracheoles, that are surrounded by a thin layer of the cuticular epithelium [Fig. 4(a) and (c)].

3.5. Bacterial contents

The lumen of the pouch is almost entirely filled with

Fig. 3. Electron micrographs of proximal thick lining of bacterial pouch (A–E: *T. nitida*, F: *T. binghami*). (a) Microvillar epithelium, showing cylindrical cells with accumulations of clear (cv) and dark vesicles (dv), scale bar 10 μm . (b) Detail of apical region showing microvillar differentiation (mv), scale bar 1 μm . (c) Cytoplasm with clear (cv) and dark vesicles (dv), scale bar 1 μm . (d) Detail of Golgi apparatus (arrows) in cytoplasm, scale bar 0.5 μm . (e) Basal portion of thick epithelium showing abundant invaginations of basal plasmalemma. Note thick basement membrane and well developed muscle fibres (MF). Scale bar 1 μm . (f) Detail of epithelial fold in transition zone between thin cuticle-lined distal epithelium (towards upper left) and thicker proximal microvillar epithelium (lower right), scale bar 5 μm . M, mitochondria; mv, microvilli; N, nucleus.

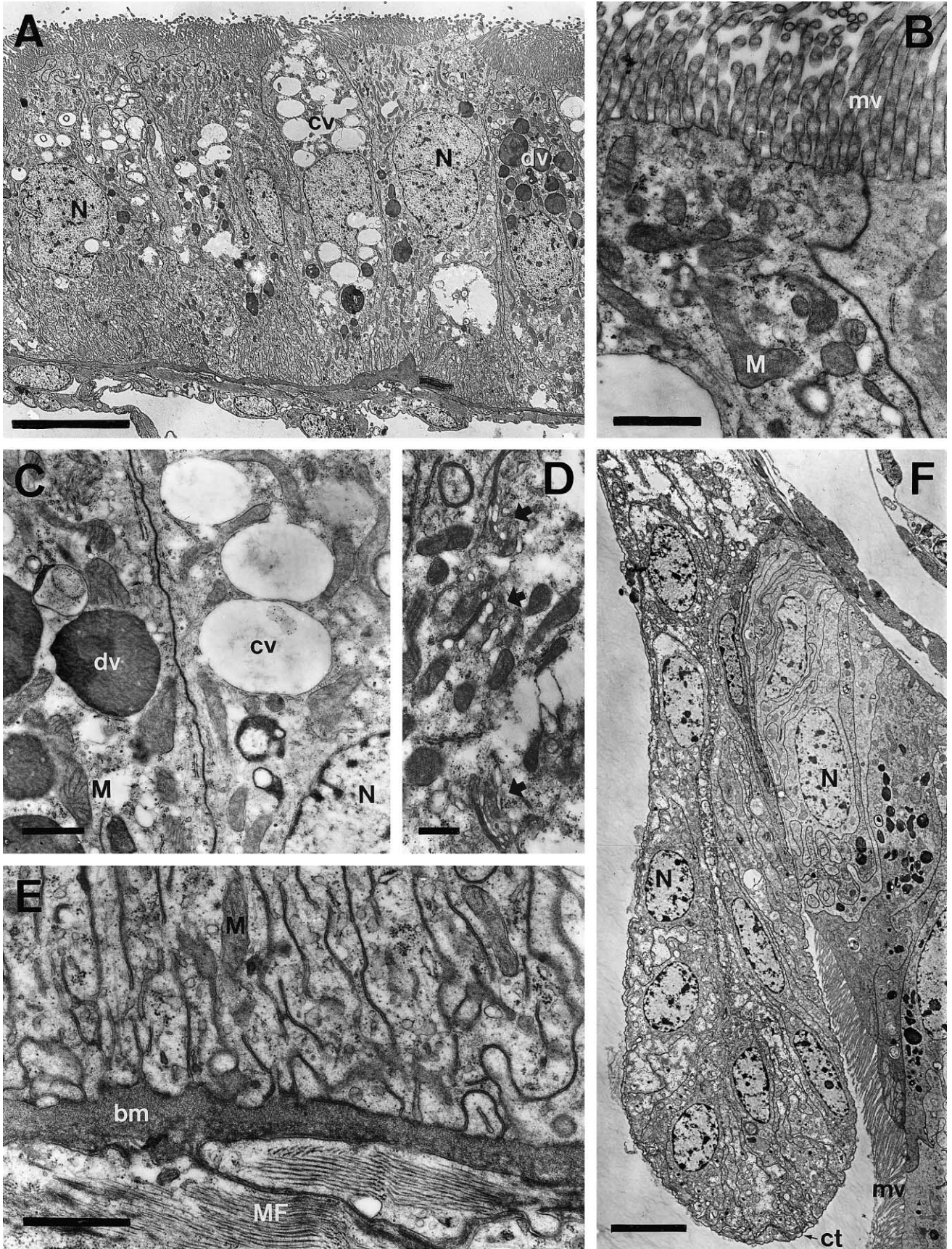


Fig. 3.

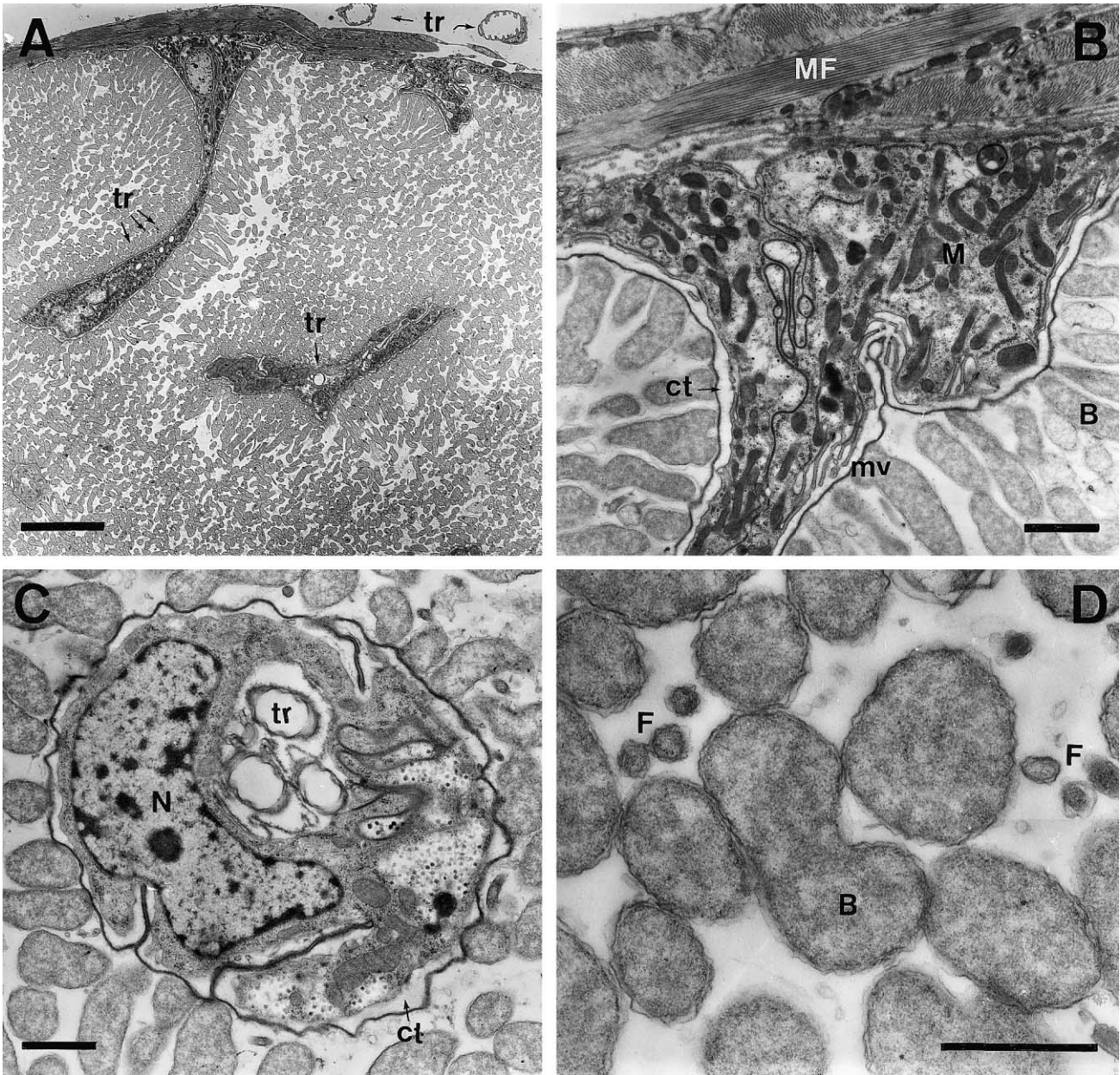


Fig. 4. Electron micrographs of distal thin epithelial lining and bacterial contents of pouch lumen (all *T. nitida*). (a) Thin epithelium lining distal part of bacterial pouch with penetrating strands containing tracheoles (tr), scale bar 10 μm . (b) Detail of thin cuticle-lined distal epithelium with irregular microvilli. Note bacteria (B) adhering to cuticle with their long axis perpendicular to cuticular surface. ct, cuticle; M, mitochondria; MF, muscle fibers, scale bar 1 μm . (c) Tracheoles (tr) in centre of bacterial pouch. Note surrounding cuticular lining (ct); N, nucleus, scale bar 1 μm . (d) Detail of bacterial pouch lumen with bacteria (B), showing double lining membrane. F: flagellar sections. Scale bar 1 μm .

massive numbers of rod-like bacteria with a length of approx. 2 μm and a diameter of approx. 0.6 μm [Figs. 1(d) and 4(a)–(d)]. In between the bacteria occur small thread-like structures with a diameter around 0.15 μm [Fig. 4(d)], that possibly correspond with flagellar tails. The bacteria are especially abundant near the apical border of the ectodermal epithelium and its tissue strands that accompany the penetrating tracheoles. These bacteria appear to adhere onto the lining cuticle with their long axis perpendicular to the cuticular surface [Fig. 4(a) and

(b)]. At higher magnification, the bacteria clearly show a double membrane lining their granular contents [Fig. 4(d)].

4. Discussion

T. binghami is a bamboo-dwelling species that lives in symbiosis with pseudococcid mealybugs (Coccoidea) within the internodes of the large bamboo *Gigantochloa scortechinii* it inhabits. The diet of these ants is not clearly

known, although most of the food requirements of the colony is probably provided by the symbiotic mealybugs in the form of honeydew (Buschinger et al., 1994). The ants rarely prey on insects outside the nests, whereas the other two species, *T. attenuata* and *T. nitida*, as well as *T. PSW-71* (the latter not having the bacterial pouch) were seen foraging on twigs and leaves for honeydew and small insects as is usual for most arboreal ants.

The reason for the presence of a very conspicuous culture of bacteria in the digestive system of these species is unknown. In general, bacterial microorganisms in social insects have been regarded as endosymbionts that play a role in the digestion of food compounds that the insect otherwise would not be able to use for its metabolism, or provide them with vitamins and other essential substances. We suppose that the bacteria could somehow recycle the nitrogen from uric acid which the Malpighian tubules release into the bacterial pouch, similar to the suggestions of Kovoov (1968) with respect to bacteria in the 'mixed segment' in certain termites. The well developed microvillar border as well as the presence of the intracellular vesicles may be indicative for a transportation function of this kind. Further studies on the precise dietary characteristics in these *Tetraponera* species will be needed.

Apart from the functional significance of the bacterial presence in various *Tetraponera* species is the very peculiar pouch organ in which these microorganisms occur. In contrast to all other social insects that contain bacteria in the digestive tract, the pouch in *Tetraponera* is a separate organ that can be considered a blind lateral extension of the main digestive tube, but that does not form part of the main trajectory the food follows. In cephalotine ants, the bacterial and fungal symbionts occur in the posterior region of the midgut and the anterior region of the intestine (Caetano and Cruz-Landim, 1985, 1987), where they are in direct contact with the passing food material. Even in many of the higher termites, that harbour their bacterial endosymbionts in a structurally complex mixed segment tube (Kovoov, 1968; Noirot and Noirot-Timothee, 1969), these microorganisms are still found in a part of the main lumen of the digestive tract.

The pouch in *Tetraponera* therefore represents a sheltered area, that moreover displays a fairly complex structural organization. A very obvious feature that is already externally visible is the extensive tracheal supply, of which histological observation reveals that tens of narrow tracheolar tubules penetrate into the lumen of the pouch, where they are entirely surrounded by the numerous bacteria. The penetrating branches appear to be formed by naked tracheoles that have lost their tracheolar cell lining, and become surrounded by the epithelium of the distal ectodermal epithelium only. The occurrence of such naked tracheoles has also been observed in other organs that are penetrated by tracheae, such as the rectum and flight muscles (Noirot and Noirot-Timothee, 1982). The close structural association between the bacterial mass and tracheoles is indicative for

an active metabolism that goes along with a high oxygen consumption. The bacteria show a very peculiar arrangement as they make contact with the tracheolar strands with their long axis perpendicular to the tracheoles, thus allowing a maximal number of bacteria to be in touch with the tracheoles. A similar organization of bacteria with a perpendicular orientation towards the rectal wall was also described in overwintering honeybee workers and in meliponine bees (Cruz-Landim, 1972, 1990), and towards the specialized part of the ileum in *Cephalotes* ants (Caetano, 1989).

The occurrence of a belt-like epithelial fold that connects the proximal and distal parts of the pouch to the best of our knowledge represents a unique structure in social insects. The 'mixed segment' in many of the higher termites equally contains two different epithelia (Kovoov, 1968), but the contact between these occurs in an oblique way and therefore is not as abrupt as in the *Tetraponera* pouch. The contact region between the two epithelia is the more interesting as the proximal microvillar epithelium has a mesenteric, endodermal origin, whereas the cuticle-lined distal epithelium is as an ectodermal formation. This ectodermal part appears as a distal cap that has no structural contact with any other ectodermal part. This peculiar organization inevitably must be the result of a complex ontogenesis, the understanding of which will require a study of the pouch development in the pupal or larval stage. Of special interest is the opening of the Malpighian tubules into the pouch at the proximal, endodermal part of the belt. Noteworthy in this regard is an early study of the digestive tract in ant larvae by Bonavita-Cougourdan and Poveda (1972). Although not dealing with any pseudomyrmecine ants nor with a pouch structure, this study reports on the complex junction of the endodermal midgut and the ectodermal hindgut during ontogenesis. In this case, the Malpighian tubules appear associated with the ectodermal hindgut part, which is in contrast to the unique situation in the *Tetraponera* pouch.

With respect to the occurrence of the bacterial pouch among the Pseudomyrmecinae, it is interesting that we found it in a couple of species belonging to a particular clade in the phylogenetic tree published by Ward (1991). It lacks in species belonging to other clades, such as *Tetraponera* PSW-71, *T. modesta* and *T. pilosa*, as well as in *Pseudomyrmex mexicanus* and *P. pallidus* (Buschinger et al., 1994), though as yet only very few species have been checked for its presence.

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