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Malpighian tubules of the nymph of *Baetis rhodani* (Ephemeroptera, Baetidae)

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ABSTRACT

The ultrastructure of the Malpighian tubules of the final instar nymph of the mayfly *Baetis rhodani* is described. Numerous Malpighian tubules enter the alimentary canal at the transition from midgut to hindgut. Each of them is made up of a cylindrical distal portion and a proximal narrow duct emptying into the gut. At the junction between the two regions, the cylindrical portion enlarges and curves, to continue into the thin duct. The wall of the cylindrical portion consists of primary cells characterized by typical features of the secretory/absorbing epithelia. These cells show a large polytene nucleus, an apical microvillated border, a high number of mitochondria and basal infoldings. The narrow duct is formed of large cells joined by highly convoluted junctions, and their cytoplasm is filled with electron-translucent vesicles. The apical border of the cells of narrow duct lacks microvilli but bears several finger-like extensions. Each narrow duct empties into a canal interposed between groups of gut epithelial cells, which constitute the transition area between midgut and hindgut. The Malpighian tubules of *B. rhodani* represent an ancestral condition and their structural arrangement provides important information for tracing mayfly evolutionary trends.

KEY WORDS: Mayfly - Excretion - Phylogeny - Ultrastructure - SEM - TEM.

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INTRODUCTION

Mayfly adults are short-lived but nymphs are strictly aquatic and predominate the life cycle. Osmoregulatory mechanisms are of primary importance in aquatic insects since many spend most of their life cycle in fresh water. They are usually faced with the problem of loss of salts to the environment and dilution of the body fluids due to a continual diffusion of water through the body surface (Kapoor, 1994).

As far as mayflies are concerned, much attention has been devoted to the chloride cells that are specialized in absorbing inorganic ions from very diluted solutions (Wichard *et al.*, 1972; Komnick, 1977; Filshie & Campbell, 1984), but few data are available on the Malpighian tubules from either the physiological or ultrastructural perspectives. Grandi (1950) has reported some preliminary morphological aspects of Malpighian tubules of several species of the Italian mayfly.

The comparative anatomy and arrangement of the Malpighian tubules of Ephemeroptera nymphs, belonging to different families, have been the object of intense study by Landa (1969), Landa *et al.* (1980) and Landa & Soldán (1985). In the last paper, the authors consider the gross morphology of the Malpighian tubules on account of their phylogenetic relevance. In particular, the outlined trends are based on both the external shape of the tubules (degree of coiling of the distal portion) and their arrangement when entering the gut (connection to the gut with or without intermediate trunks). Ultrastructural investigation on mayfly Malpighian tubules is limited to that of Nicholls (1983) on the heptageniid *Ecdyonurus dispar*, in which the aim was to relate regional specialization to the function performed by the cells in each tract.

Baetis rhodani (Pictet, 1843) is a mayfly commonly present in various freshwater habitats. It is a typically univoltine species with considerable flexibility in its life cycle (Brittain, 1982), probably in relation with temperature variations (Humpesh, 1979). Some aspects of the biology of this species have previously been investigated by us and concern the ultrastructural details of the association with gregarines (Gaino & Rebora, 1998a), and of the organization and distribution of antennal sensilla (Gaino & Rebora 1998b), with particular attention to the taxonomic and phylogenetic relevance of these sensory systems (Gaino & Rebora, 1999).

The aim of the present work is to describe at ultrastructural level (SEM, TEM) the architecture of the Malpighian tubules as well as their emptying into the alimentary canal in the nymph of *B. rhodani*. This species belongs to Baetidae, a family that, according to Landa & Soldán (1985), has the most primitive tubules among Ephemeroptera.

MATERIALS AND METHODS

Mature nymphs of *B. rhodani* were collected in the Lemme stream (Alessandria, Piedmont, Italy) and in the Piediluco lake

(Terni, Umbria, Italy). Specimens were dissected under a stereomicroscope to remove the gut with the Malpighian tubules (MT). The arrangement of the MT was investigated by means of both light and scanning electron microscopy (SEM), whereas the fine architecture was highlighted by transmission electron microscopy (TEM). Immediately after dissection, selected material was fixed in Karnovsky's medium (1965) in cacodylate buffer, pH 7.2, for 1 h, repeatedly rinsed in the same buffer and postfixed in 1% osmium tetroxide for 1 h.

For SEM observations, the specimens were dehydrated using an ethanol gradient, followed by critical-point drying in a CO_2 Pabisch CPD apparatus. Specimens were mounted on stubs with silver conducting paint, sputter-coated with gold-palladium in a Balzers Union Evaporator and observed with a Philips EM 515 scanning electron microscope.

For TEM analysis, the tissue dehydrated in the graded ethanol series was embedded in Epon-Araldite mixture resin. Thin sections, obtained with a Reichert ultramicrotome, were collected on formvar-coated copper grids, stained with uranyl acetate and lead citrate. They were examined with Philips EM 400 and EM 208 electron microscopes.

RESULTS

The Malpighian tubule system of *B. rhodani* consists of numerous tubules (Fig. 1a) entering the midgut in a transition region close to the junction with the hindgut. A schematic reconstruction of a single Malpighian tubule is given in Figure 2a, which shows the sequence of the different regions of the tubule and its emptying into the gut. The squared areas (I-V) indicate the regions examined under TEM.

Each tubule shows a smooth surface and consists of two regions: a distal cylindrical portion and a proximal narrow duct (Fig. 1b). The distal portion slightly enlarges, thus acquiring a bag-like appearance, and curves to continue into the narrow duct (Fig. 1b), which enters the gut individually (Fig. 1c).

Ultrathin sections of the cylindrical region in its distal part (corresponding to the squared region I of Fig. 2a) show that the epithelial wall is formed by microvillated cells facing an eccentric lumen and characterized by numerous, short infoldings in their basal region (Fig. 2b). The cells tend to be fusiform in shape, each extends between the neighbouring ones (Fig. 2b), and shows a polytene nucleus occupying the expanded part of the cell body. The cell apex protrudes towards the lumen with numerous microvilli differing in length and including long, thin mitochondria (Fig. 2b and inset). In the microvilli, these have the same shape as those filling the cell cytoplasm. Moving towards the basal region (corresponding to the squared region II of Fig. 2a), the lumen becomes larger, and flocculent material accumulates above the cell microvillar border (Fig. 2c). In this area, microvilli of uniform length are devoid of mitochondria (Fig. 2d). However, mitochondria are interposed among electron-translucent vesicles in the cytosol (Fig. 2d).

The connection between the basal end of the cylindrical portion and the narrow duct (corresponding to the squared region III of Fig. 2a) shows an abrupt change in the cell morphology (Fig. 3a). The cells in this area lack apical microvillated luminal border and contain less mitochondria as compared to the cells of region II of the cylindrical portion of the Malpighian tubules (Fig. 3a,b). The walls of the tubule (squared region IV of Fig. 2a) are lined by large cells delimiting a central lumen, and show nucleolated nuclei (Fig. 3b) and basal infolds, which tend to be less extended than those of the cylindrical portion. The cells are joined by extensive highly convoluted junctions, the morphology of which is consistent with their attribution to smooth septate junctions (Fig. 3c). The apical cell border is thrown into a series of finger-like extensions, making the apical surface uneven (Fig. 3c). The cytoplasm is filled with electron-translucent vesicles of various size, giving the cells a spongelike appearance (Fig. 3d). Well-developed rough endoplasmic reticulum is present in the cytoplasm (Fig. 3d).

The narrow duct enters the gut in a transition region interposed between midgut and hindgut (squared region V of Fig. 2a). TEM images of this region show that the cells delimiting the narrow duct are closely adherent to those bordering the gut (Fig. 4a). The duct opens into a narrow canal interposed between groups of epithelial cells building up the transition region between midgut and hindgut (Fig. 4a). This region consists of an epithelium, which lies on a thick basal lamina, constituted of cells with deep infoldings of their basal membrane and with an apical border not lined by cuticle (Fig. 4b). The infolds penetrate more than halfway through the thickness of the cell, following a tortuous path and anastomose, resulting in a network of canaliculi opening into the space facing the basal membrane (Fig. 4b). The cells differ in length according to their position: cells delimiting the gut lumen are much more extended than those bordering each narrow canal. In addition, the cells bordering the narrow canal show an apical membrane with irregularly arranged microvillar protrusions that extend into the lumen (Fig. 4c).

DISCUSSION

Malpighian tubules (MT) have been investigated in depth in many insect groups (Wigglesworth & Salpeter, 1962; Berridge & Oschman, 1969; Sohal, 1974; Green, 1979, 1980; Ryerse, 1979; Meyran, 1982; Satmary & Bradley, 1984; Bradley, 1985; Hazelton *et al.*, 1988; Kukel & Komnick, 1989; Dallai *et al.*, 1991; Kapoor, 1994). MT are regarded as typical transporting epithelia involved in secretion and resorption processes. They occur along successive segments (Maddrell, 1977) and lead to the formation of the primary urine. In spite of the different physiological activity performed by MT in various insect species, there is a surprising morphological similarity in the primary cells: extensive membrane infoldings along their basal region, microvillar apical border, and a remarkable number of mitochondria.

The organization of the Malpighian tubules in *B. rhodani* is consistent with a different activity performed by

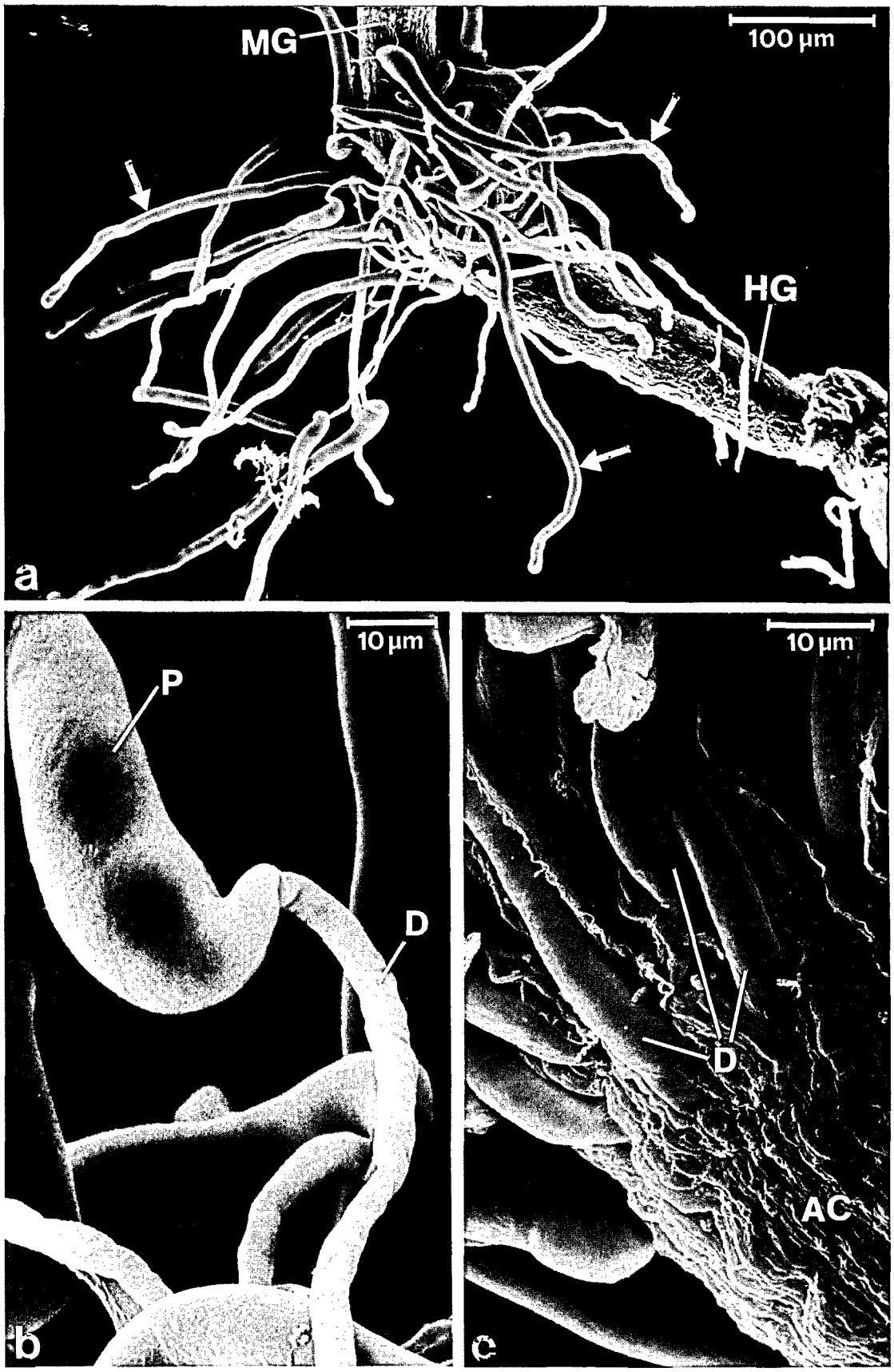

Fig. 1 - Scanning electron micrographs of the Malpighian tubules of *Baetis rhodani*. **a**, Malpighian tubules (arrows) entering the alimentary canal at the transition from midgut (MG) to hindgut (HG); **b**, A Malpighian tubule showing the connection between the distal portion (P) and the narrow duct (D). **c**, Narrow ducts (D) of some Malpighian tubules entering the alimentary canal (AC) individually.

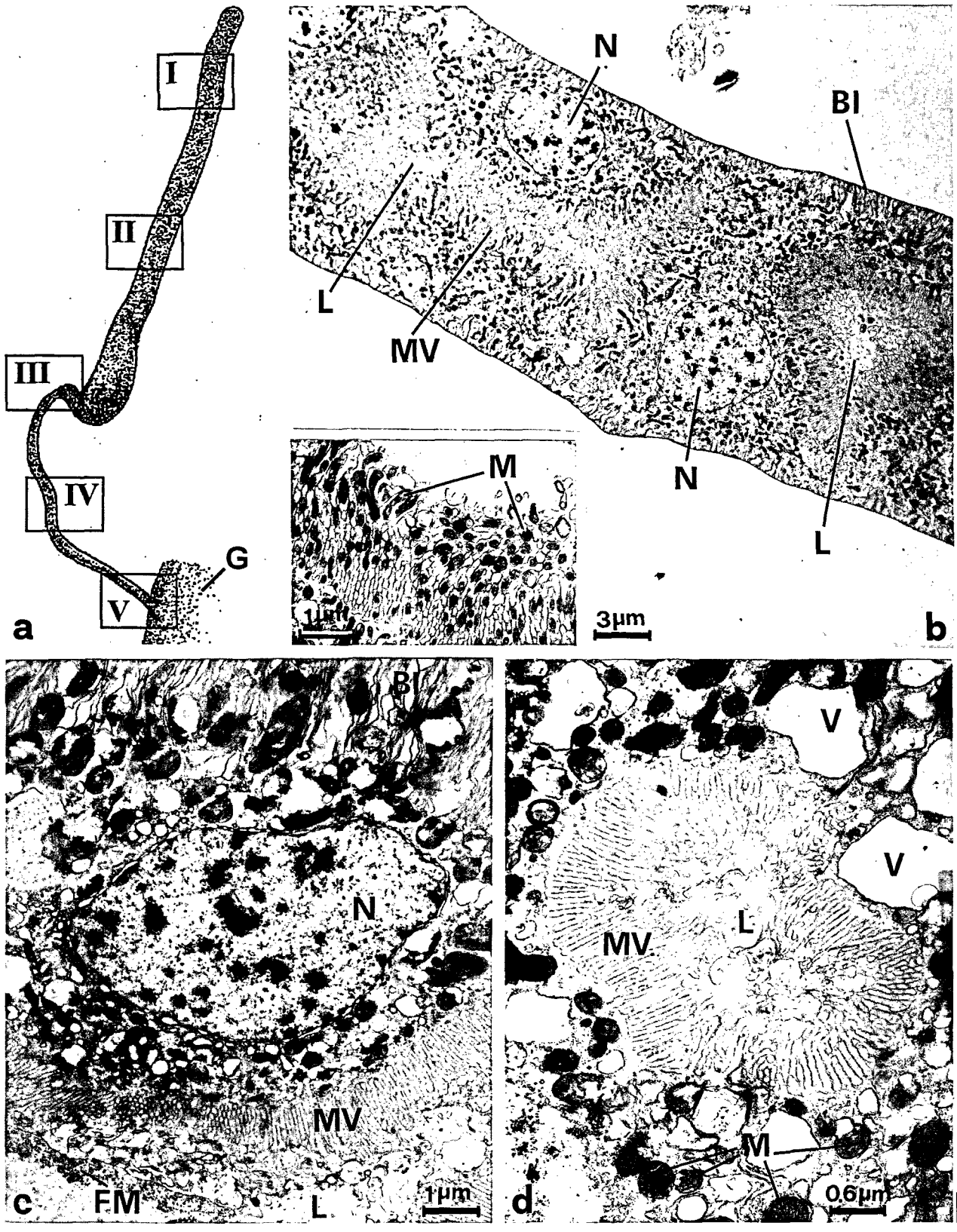

Fig. 2 - Schematic reconstruction (**a**) and transmission electron micrographs (**b-d**) of a Malpighian tubule of *Baetis rhodani*. **a**, Malpighian tubule showing the distal cylindrical portion and the narrow duct entering the gut (G). The squared areas (I-V) indicate the regions examined under TEM; **b**, Longitudinal section of the cylindrical portion in its distal part (corresponding to squared region I), showing epithelial fusiform cells with large polytene nuclei (N), basal infoldings (BI) and microvilli (MV) facing the lumen (L), and containing mitochondria (M) (zoomed in the inset); **c**, Cross section of the cylindrical portion (corresponding to squared region II).; **d**, Detail of squared region II showing that mitochondria (M) in the cytosol do not extend into the microvilli (MV) delimiting the lumen (L). V, electron-translucent vesicles; BI, basal infoldings; FM, flocculated material; L, lumen; MV, microvilli; N, nucleus.

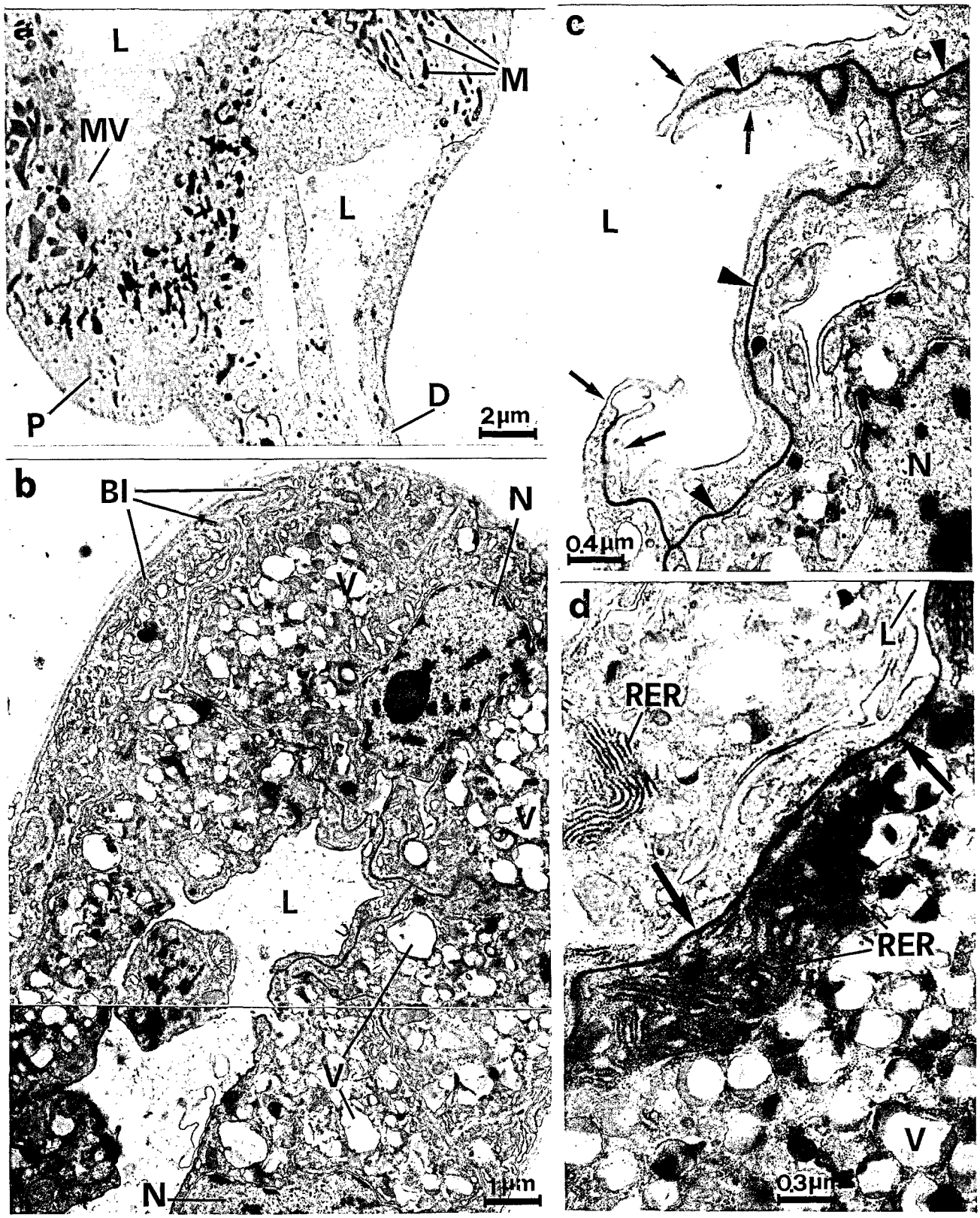

Fig. 3 - Transmission electron micrographs of a Malpighian tubule of *Baetis rhodani*. **a**, Squared region III of Figure 2a; note the different cell morphology between the narrow duct (D) and the enlarged cylindrical portion (P). **b**, Squared region IV of Fig. 2b; morphology of the cells lining the narrow duct in cross section. Note the sponge-like appearance of the epithelium due to the electron-translucent vesicles (V). **c**, Detail of the apical border of the cells that delimit the narrow duct and show their apical border in a series of finger-like extensions (arrows). Note the extensive convoluted junctions (arrowheads) between the cells. **d**, Enlarged view of the junction (arrows) between two adjacent cells (arrows). Note the electron translucent vesicles (V) filling the cell cytoplasm and the abundant rough endoplasmic reticulum (RER). BI, basal infolds; FM, flocculated material; L, lumen; M, mitocondria; MV, microvilli; N, nucleolated nucleus.

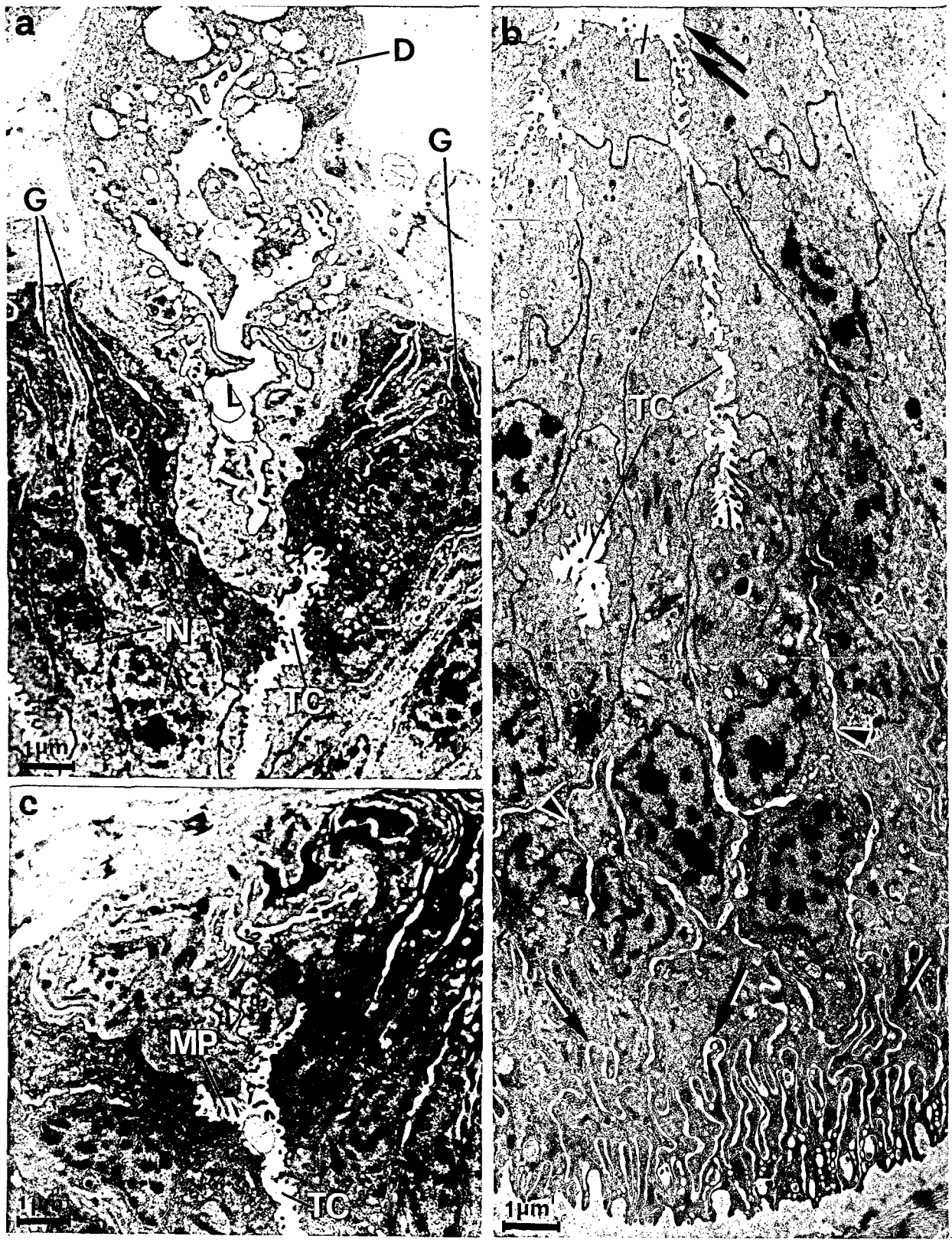

Fig. 4 - Transmission electron micrographs of the transition region between midgut and hindgut into which Malpighian tubules of *Baetis rhodani* empty (squared region V of Fig. 2a). **a**, Cross section of the narrow duct (D), which enters the gut close to a narrow canal (TC) interposed among gut cells (G). Note the different electron-density of the duct cells compared with that of the gut cells. **b**, Cross section of the transition region between midgut and hindgut into which Malpighian tubules open. Note the arrangement of the epithelial cells from the base towards the gut lumen (L). Narrow canals (TC) are interposed between the epithelial cells whose deep basal infoldings (arrows) form canaliculi (arrowheads). The luminal side of the cells is not lined by cuticle (double arrow); **c**, Detail showing the microvillar protrusions (MP) of the cells delimiting a narrow canal (TC). L, lumen; N, nucleus of the gut cells.

the distal portion compared with that of the narrow duct. The primary cells are the typical component of the distal part of the tubule of *B. rhodani*, where they must create concentration gradients and allow ion transport, as commonly observed in other insect groups (Berridge & Oschman, 1969; Oschman & Berridge, 1971; Pannabecker *et al.*, 1992; review in Nicolson, 1993). In particular, the insertion of mitochondria into microvilli, observed in the cylindrical distal portion of the tubule, is related to a rapid fluid transport (Bradley & Satir, 1977). In contrast, the cells of the lower part of the cylindrical portion, together with those of the slightly coiled tubule close to the junction with the duct, lack mitochondria in the microvilli. The different morphology presented by the cells of the tubule may be consistent with the different function performed along the tubule: secretive in the distal and absorptive in the proximal part of the cylindrical portion. The uptake of ions from the primary urine is particularly relevant in freshwater insects considering the loss of ions due to the external environment (Kapoor, 1994).

The narrow duct seems to be a conducting tubule. Nevertheless, the constitutive cells characterized by an abundant RER coupled with a rich amount of vesicles, which vary in size and include flocculent material, could express some specific metabolic activity. Similar vesicles with muco-substances were described in the cells of MT collecting duct of the nymphal dragonfly *Aeshna cyanea* by Kukel & Komnick (1989). Even though the mucous secretion was interpreted as a protective device, the actual function of these cells is still unknown. Apical finger-like protrusions together with extensive highly convoluted junctions between cells allow surface increase. This feature favours exchanges among cells and with the internal lumen. The surface increase is also evident in the cells located in the transition region where the narrow duct of the Malpighian tubule enters the gut. Indeed, only the apical border of the cells delimiting the narrow canals, each interposed between groups of gut cells, shows short, irregular microvillar protrusions. This feature may be consistent with the uptake of ions from the urine produced in the Malpighian tubules.

According to Landa & Soldán (1985), MT individually entering the gut represent the most plesiomorphic situation. Nevertheless, such an organization was considered by those authors as hypothetical, since they did not find it in any recent genus. Indeed, in their categorization, they included Baetidae among the mayfly families characterized by MT entering the alimentary canal in a narrow band or in lower buds. In contrast, our data on *B. rhodani* proved that tubules entering individually are not merely hypothetical. In addition, such an organization is not limited to *B. rhodani*, as Grandi (1950) observed a similar arrangement in *Acentrella sinaica* (cited as *Baetis atrebatinus*). It seems acceptable that this simple and plesiomorphic structure may be fairly common in Baetidae. The apomorphic condition is represented by the specialization of the coiled region of the Malpighian tubules together with the acquisition of an intermediate region connecting MT to the gut. This condition seems to be the most common in the remaining families of Ephemeroptera (Landa & Soldán, 1985). The slightly bent basal part of the cylindrical portion of the MT of *B. rhodani* may constitute the starting point for its further coiling and consequent complexity that took place during the evolution of the order.

In conclusion, the present investigation of the MT of *B. rhodani* proved the occurrence in present day Ephemeroptera of the hypothetical ancestral condition from which more complex MT organization found in this insect group seems to be derived.

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