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Morphology of the Digestive System in the Wood-Feeding Termite *Nasutitermes takasagoensis* (Shiraki) [Isoptera: Termitidae]

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ABSTRACT—The morphologies of epithelial cells throughout the alimentary canal of the wood-feeding termite *Nasutitermes takasagoensis* (Shiraki) were examined. The digestive tract consists of four principal portions, which are the foregut, the midgut, the mixed segment and the hindgut. The midgut epithelium is primarily composed of columnar cells and degenerative cells. Most columnar cells have one or more autophagic vacuoles at cell apexes, suggesting a rapid turnover of the midgut cells. In the mixed segment, the mesenteric epithelium occupies half of the gut wall and the proctodeal epithelium covers the remaining wall. Extensive invaginations of the basal membrane are characteristic of the mesenteric columnar cells, suggesting active transport of an ionic fluid. The hindgut can be divided into five segments, the first of which is a simple tube lined with a thick cuticle, termed the first proctodeal segment. The epithelium of the third segment, the paunch, consists of cuboidal cells, which are covered by multiple cuticular layers. The apical membrane of these epithelial cells forms regular invaginations, suggesting that they have an absorptive function. In the anterior paunch, numerous spirochetes are found adhered to the gut wall. Our observations indicate that termites such as *N. takasagoensis* appear to have developed structures that enable more efficient interactions with intestinal microorganisms, particularly by the elongation and differentiation of the hindgut and the creation of the mixed segment.

INTRODUCTION

It is widely recognized that the midgut plays a central role in the digestion of food and in nutritional transport processes in most insects (Dow, 1986; Terra, 1988). In Isoptera (termites), the hindgut has traditionally been considered as the primary site of digestion, due to the presence of cellulolytic protozoa in 6 of 7 recognized termite families (Inoue *et al.*, 2000). Termites belonging to the remaining family (Termitidae) generally harbor only symbiotic bacteria and archaea in their hindguts, and these were initially assumed to play a major role in cellulose digestion (Noirot and Noirot-Timotheé, 1969). More recent biochemical studies though have suggested that only a trace of cellulase activity is present in the hindgut of Termitidae, as evidenced by *Nasutitermes takasagoensis* (Tokuda *et al.*, 1997), *N. walkeri* and *N. exitiosus* (Hogan *et al.*, 1988). In addition, it has been clearly shown by a molecular study that cellulase is endogenously produced in the midgut of *N. takasagoensis* (Tokuda *et al.*, 1999). In contrast, large amounts of cellulase activities were detected both in the salivary glands and the hindgut of the rhinotermitid *Reticulitermes speratus* (Yamaoka and Nagatani, 1975; Inoue *et al.*, 1997), and endogenous cellulase has been shown to be produced in the salivary glands of this termite (Watanabe *et al.*, 1997, 1998). These biochemical and molecular data suggest that the termite digestive system has undergone significant changes during evolution of Termitidae, which is widely accepted as one of the most recently evolved lineage of termites (Miura *et al.*, 1998). Such changes have been accompanied by considerable modifications in the morphology of the digestive tubes of Termitidae compared with those of other termites (Noirot and Noirot-Timotheé, 1969, 1977; Czolij *et al.*, 1984; Cruz-Landim and Costa-Leonaldo, 1990; Bignell, 1994; Costa-Leonaldo, 1995; Noirot, 1995).

Pioneering descriptions of the gut structure of the subfamily Nasutitermitinae were made by Kovoor (1969), who examined the gut anatomy of 21 genera by light microscopy. In the present paper, we report on the detailed morphology of the gut epithelium, especially in the midgut, the mixed segment and the hindgut of the wood-feeding termite *N. takasagoensis* [Isoptera, Termitidae, Nasutitermitinae], with the goal of improving understanding of the digestive physiology of termites.

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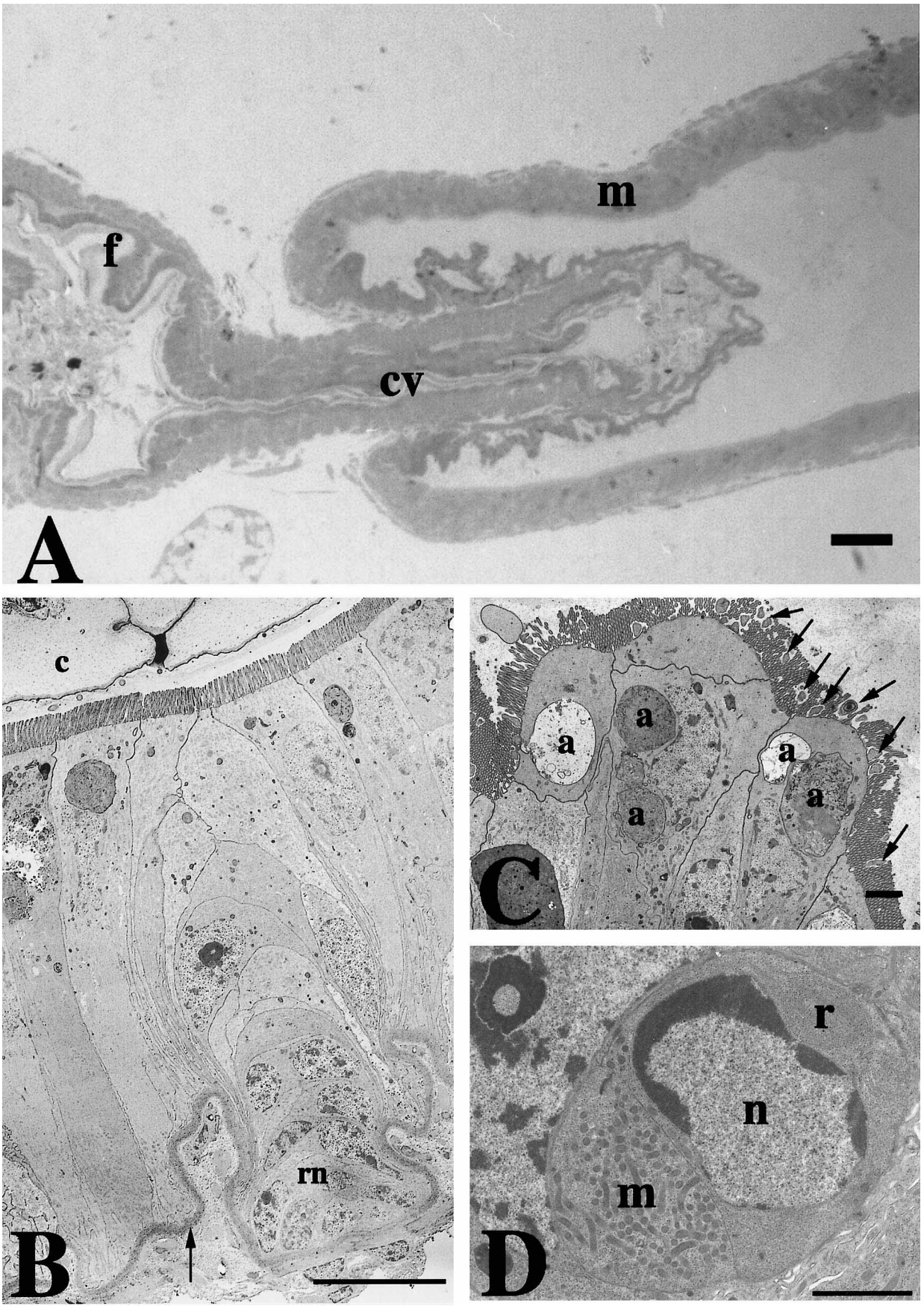

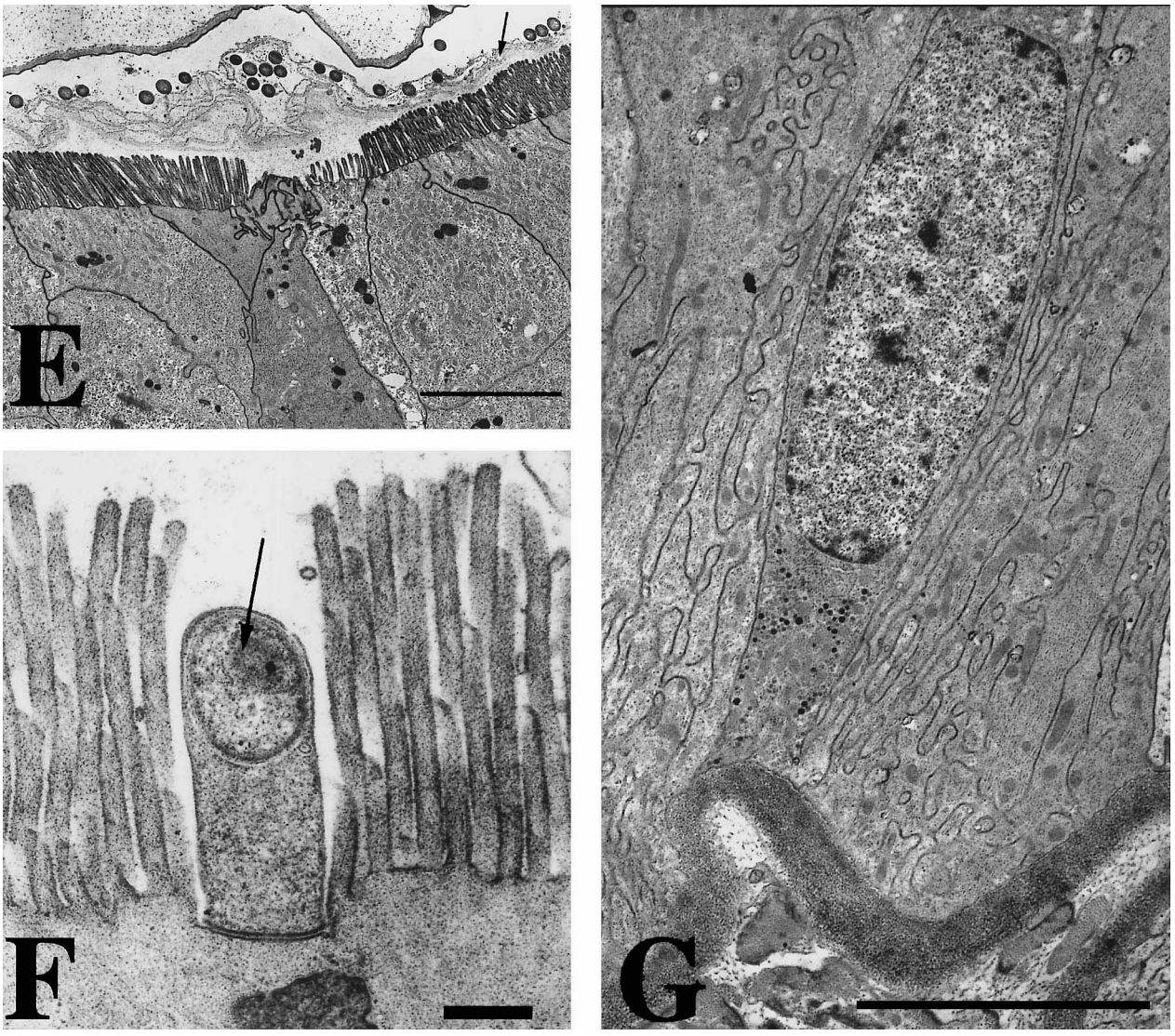

Fig. 1. Epithelial structure in the midgut. (A) Light micrograph of the longitudinal section of the foregut and the midgut walls. The posterior end of the foregut sinks into the midgut and forms the cardiac valve (cv). f, foregut epithelial cells; m, midgut wall. Bar, 50 μm. (B) Electron micrograph of the gut wall of the anterior midgut. Columnar cells showing low electron density are situated around the regenerative cells and those showing high electron density reside between the lucent cells. An arrow indicates the basal sheath. rn, regenerative nidus; c, foregut cuticle. Bar, 10 μm. (C) Autophagic vacuoles (a) are present in most columnar cells. Heterogeneous projections (arrows) observed among microvilli. Bar, 2 μm. (D) An autophagic vacuole, including a dense nucleus (n), mitochondria (m) and rough endoplasmic reticulum (r). Bar, 2 μm. (E) Bacteria present around and within the peritrophic membrane (arrow) of the most anterior part of the midgut. Bar, 5 μm. (F) High magnification of the heterogeneous projection. An inclusion body (arrow) is observed in the projection. Bar, 0.5 μm. (G) A small cell containing dense particles. The basal membrane of the cell does not form invaginations. Bar, 5 μm.

MATERIALS AND METHODS

Termite

Nasutitermes takasagoensis termites were collected and maintained as previously described (Tokuda *et al.*, 1997).

Electron microscopy

Guts were removed from mature worker-caste termites and dissected into the foregut, the midgut, the mixed segment and the hindgut in 2.5% glutaraldehyde in 0.05 M phosphate buffer, pH 7.4. The dissected guts were fixed in the same solution at 4°C for 2 hr. The samples were rinsed in the above phosphate buffer three times, and post-fixed in 1% osmium tetraoxide in the same buffer at 4°C for 1 hr. The fixed tissues were then dehydrated in an ordinal ethanol series and infiltrated by an acetone-Spurr resin series. In most cases, the embedded specimens in Spurr resin were cut by an ultramicrotome and stained by uranyl acetate and lead citrate. In some cases, semithin sections were placed on a slide glass and stained by 1% methylene blue, and were observed with a light microscope. Stained ultrathin sections were observed with a transmission electron microscope. (JEM-100C or JEM-1200EX; Jeol, Ltd, Tokyo, Japan).

RESULTS

An overview of the alimentary canal in this termite has been described elsewhere (Tokuda *et al.*, 1997, 2000). Briefly, the digestive tube of *N. takasagoensis* consists of the foregut,

the midgut, the mixed segment and the hindgut. The hindgut is further composed of five proctodeal segments as is the case of other termites belonging to the family Termitidae (Noirot and Noirot-Timotheé, 1969; Bignell, 1994). Because the general structure of the final proctodeal segment, the rectum, has been studied in detail (Noirot and Noirot-Timoteé, 1977) and because the rectum of *N. takasagoensis* is basically the same as that of other termites, we omit its description in the present report.

Foregut

The foregut is ovoid, and it occupies only 6% (approx. 0.9 mm long; excluding the posterior end of the foregut, which sinks into the midgut) of the total length of the whole gut (13.9 ± 0.3 mm; mean ± SD, n=5). The foregut wall is composed of several foldings, showing alternate grinding dentate plates (Fig. 1A). The posterior end of the foregut is buried into the midgut and forms the cardiac valve (Fig. 1A).

Midgut

The midgut is tubular and does not have a gastric caecum, which is seen in the midguts of most insects (Dow, 1986; Terra, 1988). The midgut occupies 18% of the total length of the whole gut, and its epithelium is composed primarily of columnar cells and regenerative cell masses (Fig. 1B). The lumen is regularly enfolded with the columnar cells, and crypts of the regenerative nidi - which are located at the base of the epithelium - rest on the basal sheath at regular intervals. The basic structure of the columnar cells is as follows: the nucleus is situated at the apical part of the cell, and a well-developed rough endoplasmic reticulum occupies the center of the cell (Fig. 1B). The basal plasma membrane infolds intensively into the cytoplasm, sometimes reaching the center of the cells, with mitochondria placed between the basal invaginated extracellular spaces thus formed (Fig. 1B). The apical cell surface is formed into microvilli, and each columnar cell possesses one or more large vacuoles that contain myelin structures (Fig. 1B, 1C) and/or degenerative organelles such as dense nuclei, mitochondria and/or the endoplasmic reticulum (Fig. 1D). These observations suggest that such vacuoles are autophagic vacuoles. Formation and distribution of autophagic vacuoles were reported in *R. speratus* (Yamaoka and Nagatani, 1980). In contrast to the restriction of autophagic vacuoles to the “phagocytes” (which are normally found around the glandular cells) in *R. speratus*, such autophagic vacuoles are present in almost all columnar cells in *N. takasagoensis*. This suggests that every midgut cell fulfills its function quickly and is then endocytosed and digested by another midgut cell. This phenomenon might be a strategy for the termite to recycle nitrogen to aid survival on nitrogen-poor diets. In the anterior midgut, the peritrophic membrane is being formed in the lumen, and several bacteria are distributed above and under the membrane (Fig. 1E). In addition, bacteria are also observed within the peritrophic membrane (Fig. 1E), suggesting that some bacteria can enter the ectoperitrophic space where peritrophic membrane synthesis is occurring. This observation contrasts with that in *N. walkeri*, which implied that the midgut is more or less devoid of microorganisms (Czolij *et al.*, 1985). Heterogeneous projections are often observed between microvilli (Fig. 1C, 1F). The height of the projections is almost the same as that of the surrounding microvilli, and they are distinctly separated from the midgut wall. An inclusion body is often observed within the projection (Fig. 1F). Small cells (~ 10 μm in diameter), which display a lucent cytoplasm and contain several dense particles, are sometimes observed within or near the regenerative nidi (Fig. 1G). According to their morphologies, such cells are predicted to be endocrine cells, and are similar to those reported in a cockroach (Nishiitsutsuji-Uwo and Endo, 1981). Midgut endocrine cells are thought to secrete small peptides, such as gastrointestinal hormones or neuropeptides (Sehnal and Zitnan, 1996). A role for such endocrine cells is not studied in termites, and such cells have not been discovered to date in the termite families except Termitidae. The endocrine cells were only found in a few species of the family Termitidae: *Grigiotermes bequaeri* (Apicotermitinae; Cruz-Landim and Costa-Leonardo, 1990) and *Odontotermes formosanus* (Macrotermitidae; Tokuda *et al.* unpublished data). Presumably, the representatives of Termitidae have a common mechanism to regulate their digestive physiology by the secretion of peptides from the endocrine cells. The secretory cells are almost non-existent in the middle part of the midgut, where the electron density of the cytoplasm is uniform in all cells. Every columnar cell in the middle midgut has a well-developed endoplasmic reticulum, but the Golgi apparatus is rarely observed in these cells, in contrast to the anterior midgut. Microvilli length gradually becomes shorter from the anterior (3 μm) to the posterior region of the midgut (1 μm).

Mixed segment

The mixed segment is a unique organ present only in Termitidae. The length of the mixed segment is approximately 1.3 mm, and comprises 13% of the total gut. An overview of the mixed segment in *N. takasagoensis* has been described elsewhere (Tokuda *et al.*, 2000). The basic constitution of the mesenteric epithelium in the mixed segment is similar to that observed in the midgut. However, the structure of the columnar cells in the mixed segment is distinct from that in the midgut: the apical part of the cells is electron lucent and does not show any notable feature, except that most cells contain autophagic vacuoles similar to those of the midgut cells (Fig. 2A). The microvilli of these cells are very short (0.5 μm) and sparse, with the exception of those in the anterior portion of the mixed segment. Numerous bacteria are observed between the microvilli and in the ectoperitrophic space (see Tokuda *et al.*, 2000). The nucleus is situated at the cell center, and small Golgi complexes and several particles are distributed under the nucleus (Fig. 2B). Basal invaginations are much more extensive than those of the midgut, and mitochondria - well-developed along the long axes of the cells - are placed among the invaginations (Fig. 2C), which are not observed in the mixed segment of the soil-feeding termites (Bignell, 1983). Such in-

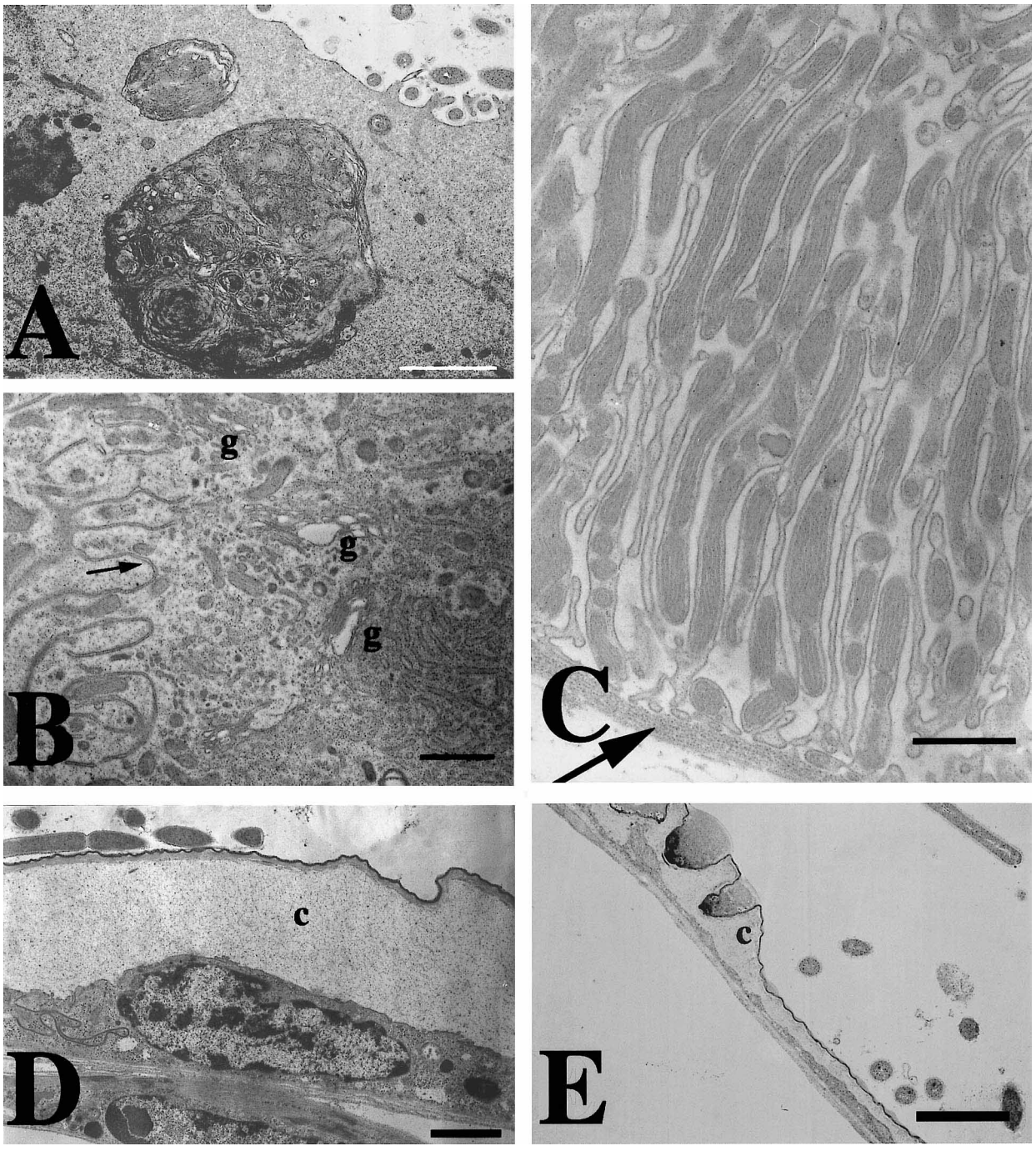

Fig. 2. Epithelial structure in the mixed segment. (A) Autophagic vacuoles at the apical part of the mesenteric columnar cell. Myelin structure is observed with in the autophagic vacuoles. Bar, 1 μm. (B) Intermediate part between the nucleus and the basal invaginations (arrow) of the mesenteric columnar cell. Small Golgi complexes (g) and particles are observed in the cytoplasm. Bar, 1 μm. (C) Extensive basal invaginations of the mesenteric columnar cell. Mitochondria are well developed along the long axis of the cell. An arrow indicates the basal sheath. Bar, 1 μm. (D) Proctodeal tissue of the mixed segment. Large cuticle (c) and thin epithelial cells are characteristic. Bar, 2 μm. (E) The proctodeal epithelium is gradually thinner toward the opposite site of the mesenteric tissue. c, cuticle. Bar, 1 μm.

vaginations though are not deep like those observed in the midgut, since they fold up to one third of the total length of the cells. The mesenteric epithelium of the mixed segment has a much thinner basal sheath (approx. 0.4 μm thick; Fig. 2C) compared to that of the midgut (approx. 1 μm thick; Fig. 1B). Small cuboidal cells mediate the connections between mesenteric and proctodeal tissues. Large cuticle layers and thin epithelial cells are characteristic of the proctodeal tissue of the mixed segment (Fig. 2D). The gut wall becomes gradually thinner towards the opposite side of the mesenteric tissue, reaching a minimum of 0.15 μm (Fig. 2E; comprising epithelial cells 0.05 μm and a cuticle 0.1 μm thick).

Hindgut

The hindgut of *N. takasagoensis* is divided into five proctodeal segments as previously described (Tokuda *et al.*, 1997, 2000).

The first proctodeal segment (ileum) is a simple tube, which occupies 20% of the total gut length. The epithelial cells are flat, vary in thickness from 0.25 ~ 1.5 μm, and are lined with a thick cuticle (~ 2.5 μm thick) (Fig. 3A). No significant features are observed in the cytoplasm, but several microtubule masses are recognized under high magnification (Fig. 3B). The posterior end of the first proctodeal segment is slightly folded into the paunch (third proctodeal segment) and forms

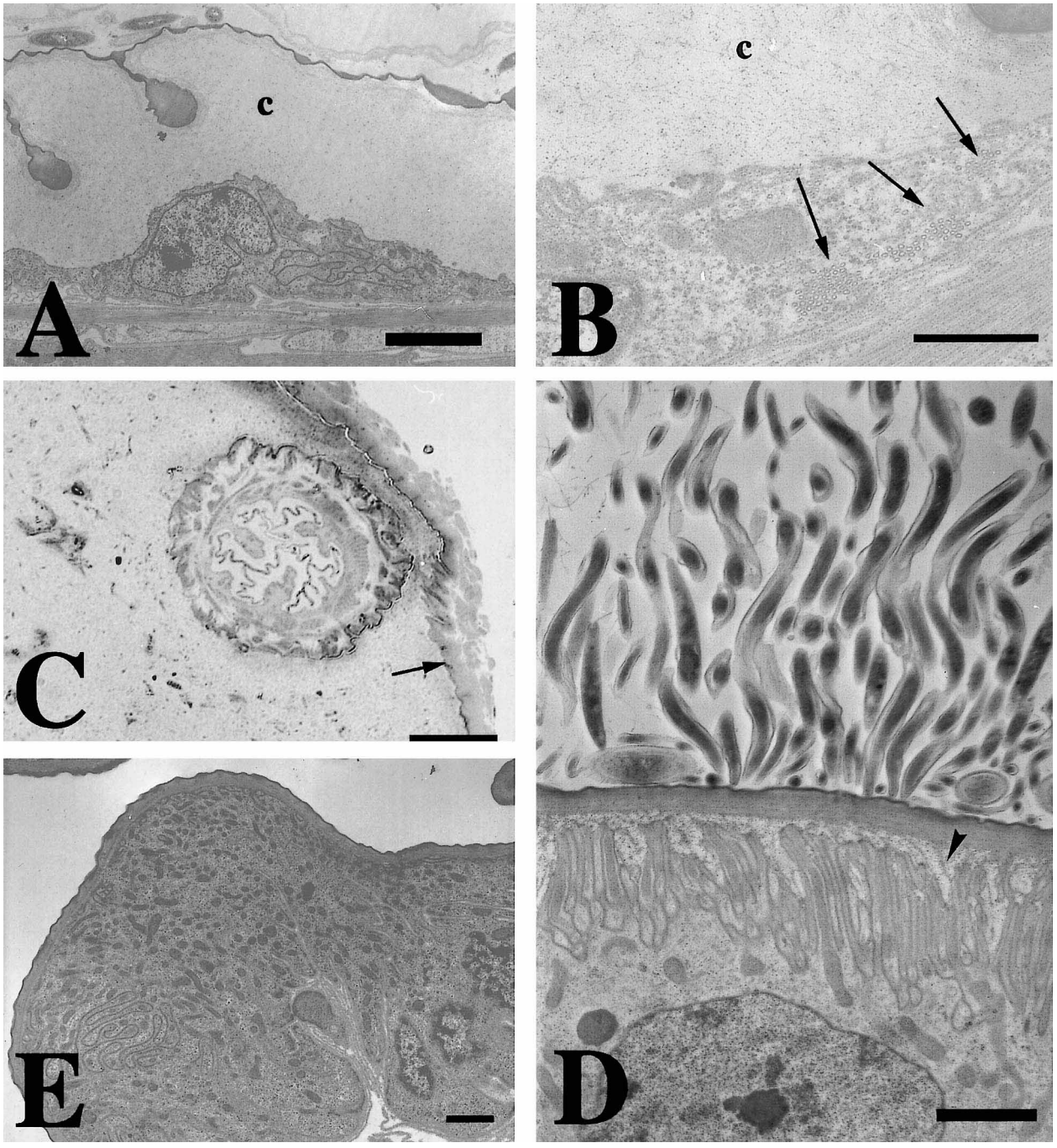

Fig. 3. Epithelial structure in the hindgut. (A) Epithelial cell in the first proctodeal segment. Some bacteria are present in the ectoperitorophic space. c, cuticle. Bar, 2 μm. (B) Masses of microtubules (arrows) present in the cytoplasm of the same cell. c, cuticle. Bar, 0.5 μm. (C) Light micrograph of a transverse section of the enteric valve. An arrow indicates the paunch wall. Bar, 50 μm. (D) Apical surface of the epithelial cell in the most anterior part of the paunch. Several bacteria, presumably spirochetes, are attached to the gut cuticle, which is composed of multiple cuticular layers. Regular invaginations of the apical membrane with mitochondria among them are observed under the sub-cuticular layer (arrowhead). Bar, 2 μm. (E) Epithelial cells in the colon. The sub-cuticular layer is not present and few invaginations of the apical membrane are observed under the cuticle. Bar, 1 μm.

the second proctodeal segment (the so-called enteric valve) (Fig. 3C).

The third proctodeal segment, termed the "paunch," is the most enlarged part of the hindgut, and numerous microorganisms inhabit this region. The length of the paunch is approximately 3.2 mm, which comprises 23% of the total gut length. The epithelium is composed of cuboidal cells that are covered with distinct multiple cuticular layers (epi-, ecto-, endo-, and sub-cuticular layers). The most significant feature observed in paunch epithelial cells is an extensive invagination of the apical cell membrane and the presence of mitochondria in these invaginations (Fig. 3D). Several bacteria, presumably spirochetes, are adhered to the gut wall of the most anterior part of the paunch (Fig. 3D).

The apical and basal invaginations gradually decrease from the middle to the posterior part of the paunch, and few invaginations of the cell membrane are observed in the fourth proctodeal segment, the so-called colon. In contrast to the paunch, the colon is a narrow tube, where large muscular layers enfold. It is approximately 2.1 mm long, and comprises 15% of the total gut length. The epithelium of the colon is wavy and irregular in shape (Fig. 3E). The nucleus is situated at the bottom of the cell and mitochondria are uniformly distributed in the cytoplasm. In contrast to more anterior regions of the hindgut, a sub-cuticular layer (a lucent area between endocuticle and apical invagination) is not present in the colonic epithelium (Fig. 3E).

DISCUSSION

Termites belonging to the family Termitidae show diverse feeding habits, consuming materials such as wood, fungi, moss, and humus. Among them, termites affiliated with the genus *Nasutitermes* are best known for their ability to digest wood, and they do this using their own enzymes (Tokuda *et al*., 1997, 1999). Information on the gut morphology of this genus can aid in the understanding of this process as well as other aspects of the digestive physiology of these termites.

The foregut of *N. takasagoensis* is very similar to that found in representatives of the family Rhinotermitidae, with a length much shorter than those of other families (Noirot, 1995). The presence of compact grinding dentate plates in the foregut is possibly an adaptation for efficient physical breakdown of wood particles. The formation of a typical cardiac valve is indicative of precise regulation of foregut fluid entering the midgut.

Although the overall characteristics of the midgut are similar to those reported in other termites, secretory granules are less frequent in the columnar cells of *N. takasagoensis* compared with other termites (Bignell *et al*., 1982; Czolij *et al*., 1984; Cruz-Landim and Costa-Leonardo, 1990; Costa-Leonardo, 1995). An experiment involving *in situ* hybridizaton has indicated an active production of cellulase in most midgut columnar cells (Tokuda *et al*., 1999), which is not compatible with a general lack of secretory granules in the middle midgut. Thus, at present, the mode of secretion of cellulolytic enzymes is not clear. Since the secreted enzymes from the midgut wall can act immediately on their substrates in the lumen and since cellulase produced in the midgut shows high specific activity (Tokuda *et al*., 1997), a large amount of production of cellulase might not be needed. Further studies using immunohistochemistry with an electron microscope are necessary to determine how these enzymes are secreted in this termite.

Similar structures to the heterogeneous projections present among microvilli in *N. takasagoensis* were reported in *R. flavipes* and *G. bequaerti*, and were originally assumed to be prokaryotic cells (Breznak and Pankraz, 1977; Cruz-Landim and Costa-Leonardo, 1990). However, the characteristics mentioned in these studies are distinct from our observations: they showed higher electron density and did not possess inclusion bodies. Precise information is not yet available on these structures, though molecular phylogenetic and *in situ* hybridization studies could be used to confirm whether or not they are prokaryotes.

The mixed segment is a unique organ in termites belonging to Termitidae, but its role in digestive physiology is still ambiguous. Some significant characteristics of the mixed segment are known and include an elevated pH (Brune *et al*., 1995) due to secretion of an alkaline fluid rich in potassium ions (Bignell *et al*., 1983), rapid decrease of oxygen concentration (Brune *et al*., 1995), and a close association between symbiotic clostridia and the mesenteric epithelium (Tokuda *et al*., 2000). Extensive basal invaginations observed in the mesenteric tissue of *N. takasagoensis* also suggest active transport of an ionic fluid. Small Golgi apparatus and particles are frequently observed under the nuclei but are rare in the apexes of the cells, indicating that certain unknown substances are synthesized and secreted into the hemolymph. Because only a trace of cellulase activity is present in this region (Tokuda *et al*., 1997), cellulolytic enzymes are most likely not produced in the small Golgi complexes of the mesenteric tissue. The presence of large autophagic vacuoles suggests a quick turnover of the mesenteric cells as observed in the midgut.

Since the pH of the luminal fluid in the first proctodeal segment is predicted to be more than 10 in the closely related *N. nigriceps* (Brune *et al*., 1995), the thick cuticle in *N. takasagoensis* may play a role in protection of the epithelium from such an alkaline fluid. The epithelium in the first proctodeal segment is notably thin and flat, and thus the masses of microtubules presumably strengthen the tissue against pressure from the luminal fluid. Owing to the presence of few notable features in the epithelium as well as the presence of a thick cuticle, no absorption or secretion is expected to occur in the first proctodeal segment. However, a morphological study of wood particles in the gut of *N. takasagoensis* has reported that significant modification and drastic down-sizing of the wood particles occur as they pass through the posterior midgut to the first proctodeal segment (Yoshimura *et al*., 1996). Presumably, enzymatic treatments in the midgut followed by a strong alkaline denaturing from the mixed segment to the first proctodeal segment efficiently break down the wood par-

ticles, grooming them as substrates for microbial metabolism in the paunch.

It has been suggested that the paunch epithelial cells of termites are differentiated for absorption (Noirot and Noirot-Timotheé, 1969) and that microbial metabolites such as volatile fatty acids are actively absorbed across the hindgut wall (Hogan *et al.*, 1985). In *N. takasagoensis*, the paunch epithelium also shows absorptive features such as intensive invaginations of the apical membrane and the presence of mitochondria among the invaginations. The ultrastructure of the epithelial cells differs from those of termites belonging to Rhinotermitidae (Breznak and Pankraz, 1977; Yamaoka and Nagatani, 1978), which are closely related to Termitidae (Miura *et al.*, 1998). Transmission electron microscopy has clearly shown that epithelial cells in the paunch of rhinotermitids are specialized to form an epithelial cup at the apex of each cell; apical invaginations and mitochondria are restricted to this region and active transport of ions has been proposed (Breznak and Pankraz, 1977; Yamaoka and Nagatani, 1978). Such structures were also clearly recognized at the surface of the gut wall of the same species by observations using a scanning electron microscope (Breznak and Pankraz, 1977), while no such structure was observed by scanning electron microscopy in the closely related species *N. exitiosus* (Czolij *et al.*, 1985). Our present study shows that the cup-like structure does not exist at the cell apexes and that apical invaginations are distributed regularly along the cell surface. This suggests that microbial metabolites are absorbed equally across the entire paunch wall in *N. takasagoensis*.

The presence of few invaginations in the apical membrane of the colonic epithelium suggests that there is weak or no transport of ions and/or nutrients. Special structures (e.g. cuticular pits) for the attachment of bacteria were reported in the colonic epithelium of a soil-feeding termite (Bignell *et al.*, 1980), but such specializations were not found in *N. takasagoensis*. It is known that the proportion of the colon in related to the whole gut is much higher in the soil-feeding termites than the wood-feeding termites (Bignell, 1994). The specialization of the colonic epithelium for an interaction with bacteria is probably an adaptation that has arisen in soil-feeding termites.

The number of studies examining the community and function of symbiotic microbes in termites has risen sharply in recent years (Brune and Friedrich, 2000). Our observations indicate that, despite the ability of *N. takasagoensis* to produce enzymes for cellulose digestion in the midgut (Tokuda *et al.*, 1997, 1999), it is still dependent on microbial metabolites in the hindgut for its overall physiology. This is probably due to the need to compensate for a lack of nitrogen in its diet and/or a lack or low activity of pyruvate dehydrogenase (Slaytor *et al.*, 1997; Itakura *et al.*, 1999). In the evolutionary transition to feeding on sound wood, termites such as *N. takasagoensis* appear to have developed structures that enable more efficient interactions with intestinal microorganisms, particularly by the elongation and differentiation of the hindgut and the creation of the mixed segment.

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REFERENCES

Bignell DE, Oskarsson H, Anderson JM (1980) Specialization of the hindgut wall for the attachment of symbiotic micro-organisms in a termite *Procubitermes aburiensis* (Isoptera, Termitidae, Termitinae). Zoomorphol 96: 103–112

Bignell DE, Oskarsson H, Anderson JM (1982) Formation of membrane-bounded secretory granules in the midgut epithelium of a termite, *Cubitermes severus*, and a possible intercelluar route of discharge. Cell Tissue Res 222: 187–200

Bignell DE, Oskarsson H, Anderson JM, Ineson P (1983) Structure, microbial associations and function of the so-called “mixed segment” of the gut in two soil-feeding termites, *Procubitermes aburiensis* and *Cubitermes severus* (Termitidae, Termitinae). J Zool Lond 201: 445–480

Bignell DE (1994) Soil-feeding and gut morphology in higher termites. In “Nourishment and evolution in insect societies” Ed by JH Hunt, and CA Nalepa, Westview Press, Inc., Boulder, Colorado, pp 131–158

Breznak JA, Pankraz HS (1977) In situ morphology of the gut microbiota of wood-eating termites [*Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki]. Appl Environ Microbiol 33: 406–426

Brune A, Emerson D, Breznak JA (1995) The termite gut microflora as an oxygen sink: microelectrode determination of oxygen and pH gradients in guts of lower and higher termites. Appl Environ Microbiol 61: 2681–2687

Brune A, Friedrich M (2000) Microecology of the termite gut: structure and function on a microscale. Curr Opin Microbiol 3: 263–269

Costa-Leonardo AM (1995) Morphology of the digestive tube in the termite *Serritermes serrifer* (Isoptera, Serritermitidae). Naturalia São Paulo 20: 31–44

Cruz-Landim C, Costa-Leonardo AM (1990) Functional adaptations of the epithelium from different gut segments of *Grigiotermes bequaerti* (Isoptera, Termitidae, Apicotermitinae). Revta Bras Entomol 34: 669–678

Czolij R, Slaytor M, Veivers PC, O’Brien RW (1984) Gut morphology of *Mastotermes darwiniensis* Froggatt (Isoptera: Mastotermitidae). Int J Insect Morphol Embryol 13: 337–355

Czolij R, Slaytor M, O’Brien RW (1985) Bacterial flora of the mixed segment and the hindgut of the higher termite *Nasutitermes exitiosus* Hill (Termitidae, Nasutitermitinae). Appl Environ Microbiol 49: 1226–1236

Dow JAT (1986) Insect midgut function. Adv Insect Physiol 19: 188–328

Hogan M, Slaytor M, O’Brien RW (1985) Transport of volatile fatty acids across the hindgut of the cockroach *Panesthia cribrata* Saussure and the termite, *Mastotermes darwiniensis* Froggatt. J Insect Physiol 31: 587–591

Hogan M, Veivers PC, Slaytor M, Czolij RT (1988) The site of cellulose breakdown in higher termites (*Nasutitermes walkeri* and *Nasutitermes exitiosus*). J Insect Physiol 34: 891–899

Inoue T, Murashima K, Azuma J-I, Sugimoto A, Slaytor M (1997) Cellulose and xylan utilisation in the lower termite *Reticulitermes speratus*. J Insect Physiol 43: 235–242

Inoue T, Kitade O, Yoshimura T, Yamaoka I (2000) Symbiotic associations with protists. In “Termites: Evolution, Sociality, Symbioses, Ecology” Ed by Abe T, Bignell DE, and Higashi M, Kluwer

Academic Publishers, Dordrecht, The Netherlands, pp 275–288
Itakura S, Tanaka H, Enoki A (1999) Occurrence and metabolic role of the pyruvate dehydrogenase complex in the lower termite *Coptotermes formosanus* (Shiraki). Insect Biochem Mol Biol 29: 625–633
Kovoor J (1969) Anatomie comparée du tube digestif des termites II. – Sous-Famille des Nasutitermitinae. Insectes Soc 16: 195–234
Miura T, Maekawa K, Kitade O, Abe T, Matsumoto T (1998) Phylogenetic relationships among subfamilies in higher termites (Isoptera: Termitidae) based on mitochondrial COII gene sequences. Ann Entomol Soc Am 91: 515–523
Nishiitsutsuji-Uwo J, Endo Y (1981) Gut endocrine cells in insects: the ultrastructure of the endocrine cells in the cockroach midgut. Biomed Res 2: 30–44
Noirot C, Noirot-Timotheé C (1969) The digestive system. In "Biology of Termites, Vol.1" Ed by K Krishna, and F Weesner, Academic Press, New York, pp 49–88
Noirot C, Noirot-Timotheé C (1977) Fine structure of the rectum in termites (Isoptera): a comparative study. Tissue Cell 9: 693–710
Noirot C (1995) The gut of termites (Isoptera). Comparative anatomy, systematics, phylogeny. I. Lower Termites. Ann Soc Entomol Fr 31: 197–226
Sehnal F, Zitnan D (1996) Midgut endocrine cells. In "Biology of the Insect Midgut" Ed by MJ Lehane, and PF Billingsley, Chapman & Hall, London, pp 55–85
Slaytor M, Veivers PC, Lo N (1997) Aerobic and anaerobic metabolosm in the higher termite *Nasutitermes walkeri* (Hill). Insect Biochem Mol Biol 27: 291–303
Terra RW (1988) Physiology and biochemistry of insect digestion: an evolutionary perspective. Brazilian J Med Biol Res 21: 675–734
Tokuda G, Watanabe H, Matsumoto T, Noda H (1997) Cellulose digestion in the wood-eating higher termite, *Nasutitermes takasagoensis* (Shiraki): distribution of cellulases and properties of endo-β-1,4-glucanase. Zool Sci 14: 83–93
Tokuda G, Lo N, Watanabe H, Slaytor M, Matsumoto T, Noda H (1999) Metazoan cellulase genes from termites: intron/exon structures and sites of expression. Biochim Biophys Acta 1447: 146–159
Tokuda G, Yamaoka I, Noda H (2000) Localization of symbiotic clostridia in the mixed segment of the termite *Nasutitermes takasagoensis* (Shiraki). Appl Environ Microbiol 66: 2199–2207
Watanabe H, Nakamura M, Tokuda G, Yamaoka I, Scrivener AM, Noda H (1997) Site of secretion and properties of endogenous endo-β-1,4-glucanase components from *Reticulitermes speratus* (Kolbe), a Japanese subterranean termite. Insect Biochem Mol Biol 27: 305–313
Watanabe H, Noda H, Tokuda G, Lo N (1998) A cellulase gene of termite origin. Nature 394: 330–331
Yamaoka I, Nagatani Y (1975) Cellulose digestion system in the termite, *Reticulitermes speratus* (Kolbe). I. Producing sites and physiological significance of two kinds of cellulase in the worker. (in Japanese). Zool Mag 84: 23–29
Yamaoka I, Nagatani Y (1978) Cellulose digestion system in the termite *Reticulitermes speratus* (Kolbe). III. Ultrastructure and function of the hindgut epithelium. (in Japanese). Zool Mag 87: 132–141
Yamaoka I, Nagatani Y (1980) Phagocytic cells in the midgut epithelium of the termite, *Reticulitermes speratus* (Kolbe). Zool Mag 89: 308–311
Yoshimura T, Tsunoda K, Takahashi M (1996) Degradation of wood in the digestive tract of a higher termite, *Nasutitermes takasagoensis* (Shiraki) (Isoptera: Termitidae). Mokuzai Gakkaishi 42: 1250–1257

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