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Electron microscopy studies on the *a*-bacteroids in the fat bodies of the lantern bug *Pyrops candelaria* Linn. (Homoptera: Fulgoridae)

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Abstract The ultrastructure of *a*-bacteroids in relation to the fat-body cells of the lantern bug *Pyrops candelaria* was described. The fat-body-cell cytoplasm contained numerous mitochondria, rough endoplasmic reticula, vacuoles, and storage granules. Its nucleus had scattered chromatin materials. The *a*-bacteroid was enveloped by three membrane layers, namely, the plasma membrane, the cell wall, and the membrane envelope. Its cytoplasm contained amorphous dense bodies. The bacteroid reproduced by binary fission. Tracheoles were also found among fat-body cells.

Introduction

Sap-ingesting homopteran insects constitute the type of sucking insects that are invariably found to possess symbionts because they are reasonably suspected of depending nutritionally on symbionts owing to the limited nutrient composition in the natural diet (Houk and Griffiths 1980). Waku and Endo (1987) have reported that homopteran insects harbor various symbiotic microorganisms of vital importance to their development and metabolism in specialized tissue called the *mycetome*.

Buchner (1965) categorized the homopteran insects' intracellular symbionts by letters of the alphabet. He described in the leafhoppers *a*-, *t*-, *h*-, and companion symbionts and in fulgorids *a*-, *b*-, *t*-, *f*-, *h*-, and companion symbionts in certain combinations.

Observations on the ultrastructure of homopteran insects' intracellular symbionts have been well documented, for example, in leafhoppers (Körner 1969; Chang and Musgrave 1972; Mitsuhashi and Kono 1975), in aphids (Lamb and Hinde 1967; Hinde 1971; Griffiths and Beck 1973; Fukatsu and Ishikawa 1992), in planthoppers (Noda 1977), and in Psyllidae (Chang and Musgrave 1969; Waku and Endo 1987).

Little research has been carried out on the symbiotic relationship of microorganisms with the insect host in Fulgoridae. Müller (1940) and Buchner (1965) reported that it was surprising that in several *Oliarus* species there were up to five different symbionts in one host. The symbionts of *Pyrops candelaria* are little known except at the light microscopy level. Investigations on their fine structure are fundamental for an understanding of their taxonomic position and significance.

The objective of the present investigation was to find out the fine structure of *a*-bacteroids in the fat-body cells of *P. candelaria* and to provide insight into the relationship between bacteroids and the host.

Materials and methods

Adults of the lantern bug *Pyrops candelaria* Linn. were collected from Lam Chuen and the Chinese University campus in the New Territories of Hong Kong. Fat bodies were dissected out in 0.2 *M* phosphate buffer, (pH 7.2, with glucose added), fixed in 2.5% glutaradehyde in 0.2 *M* phosphate buffer at 4 °C for 1 h, and postfixed in 1% osmium tetroxide in phosphate buffer. Tissues were dehydrated in ascending series of alcohol, infiltrated with acetone and Spurr resin (1:1), and embedded in pure Spurr resin. Sections were cut with a Reichert ultratome and were stained with uranyl acetate and lead citrate. Sections were observed under a Jeol-JEM 1200 EXII electron microscope.

Results

A fat-body cell in *Pyrops candelaria* appeared oblong or cuboidal in shape (Figs. 1–5). It had a round or oval nucleus with scattered patches of chromatin materials. Numerous mitochondria, rough endoplasmic reticula, vacuoles, and storage granules were found in the ground cytoplasm. Tracheoles were found between the fat-body cells.

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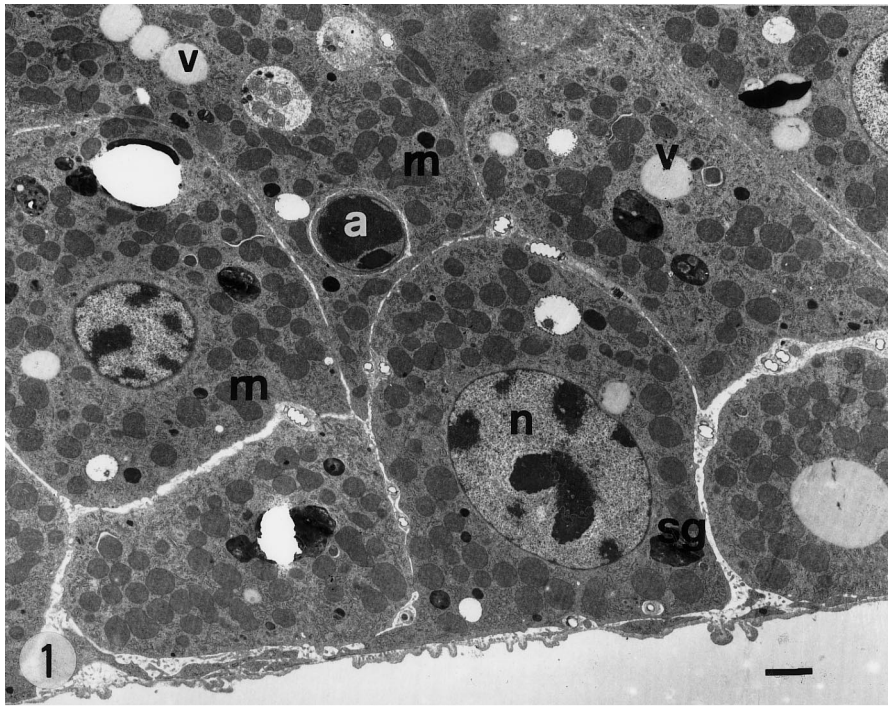


Fig. 1 Transmission electron micrograph (TEM) of fat-body cells, showing the nucleus (*n*), mitochondria (*m*), vacuoles (*v*), storage granules (*sg*), and an *a*-bacteroid (*a*). *Bar* 2 μm.

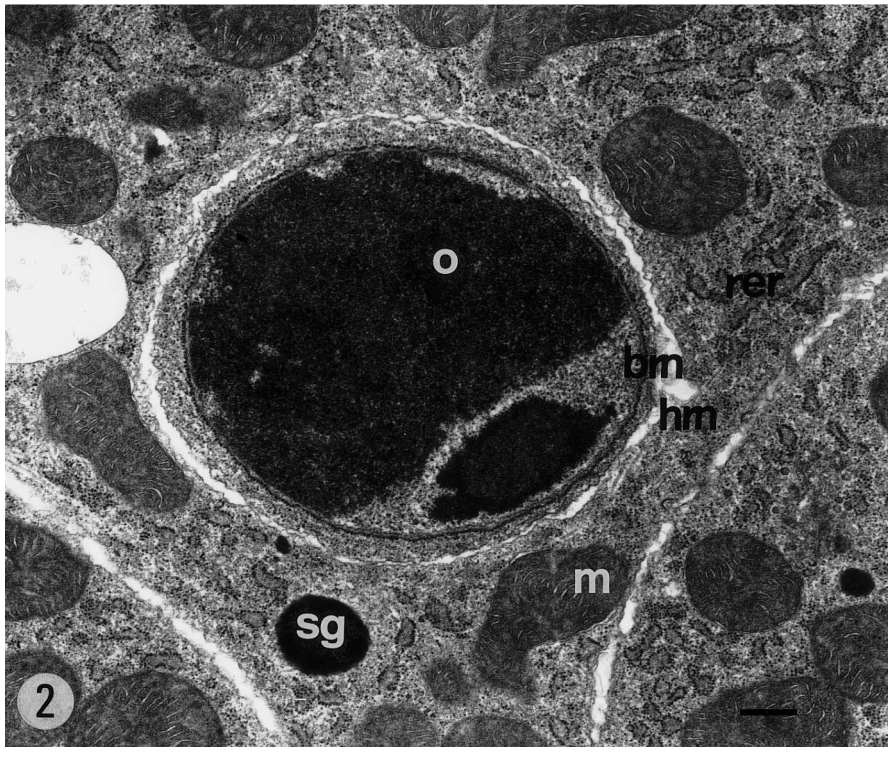
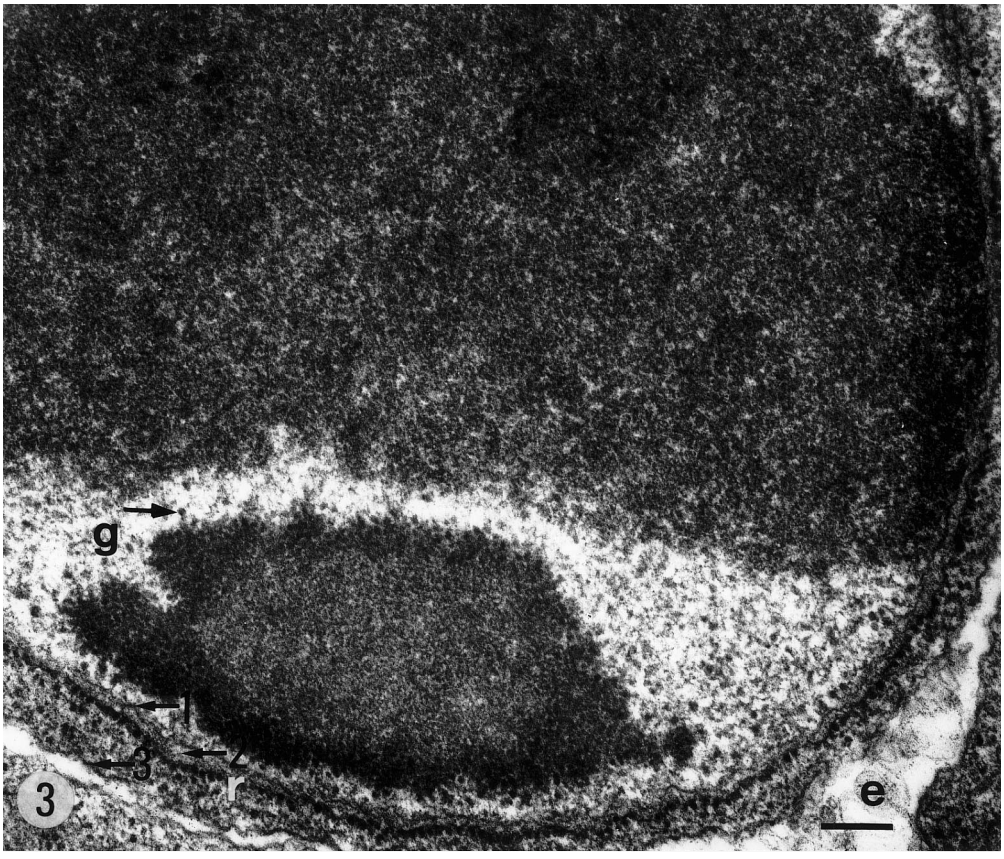


Fig. 2 High-magnification TEM showing an *a*-bacteroid with osmiophilic materials (*o*), the bacteroid membrane (*bm*), the host-cell membrane (*hm*), rough endoplasmic reticulum (*rer*), a storage granule (*sg*), and mitochondria (*m*) of the host cell. *Bar* 0.5 μm

Occasionally, *a*-bacteroids were found inside their cytoplasm. An *a*-bacteroid was almost oval in shape, measuring 4 × 5 μm (Fig. 2). Large osmiophilic patches and glycogen particles (Cheung, unpublished data) were found inside its cytoplasm (Fig. 3). The large osmiophilic patches had an oval inclusion body of 1-μm diameter and specks of granular or filamentous materials. An *a*-bacteroid was enclosed by three membrane layers: (1) the outer membrane envelope, (2) the cell wall, and (3) the internal plasma membrane of unit-membrane construction. Host ribosomes were sometimes seen on the outside of the cell wall.

An *a*-bacteroid sometimes appeared to divide by binary fission (Fig. 4). Initially, no cross-wall was laid down during division. Later, two daughter bacteroids appeared to have formed (Figs. 5, 6).



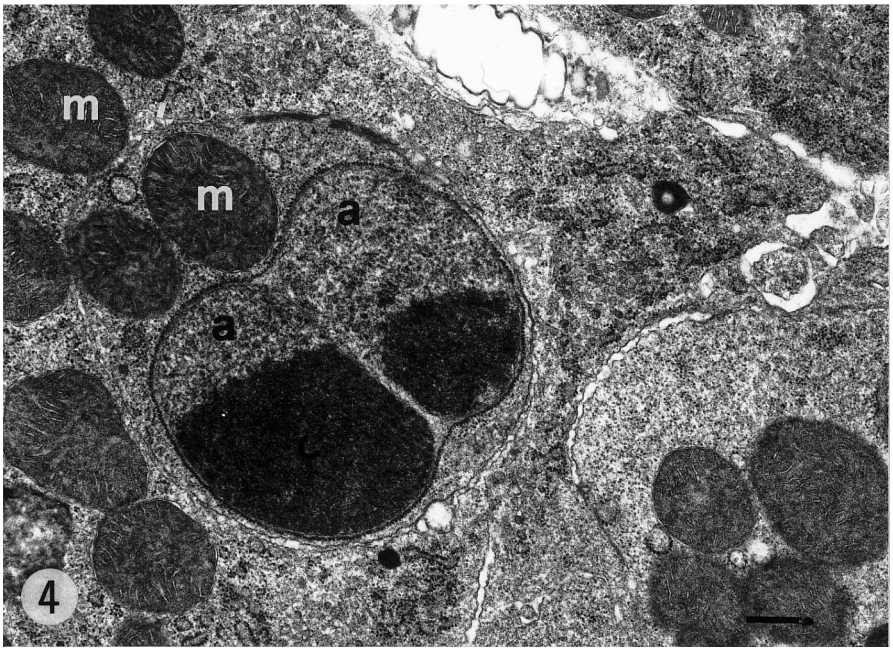


Fig. 3 Higher-magnification TEM showing the bacteroid plasma membrane (*1*), the cell wall (*2*), the membrane envelope (*3*), glycogen (*g*), the extracellular space (*e*), and a ribosome circle (*r*). *Bar* 0.2 μm. **Fig. 4** TEM showing a dividing bacteroid (*a*) and host mitochondria (*m*). *Bar* 0.5 μm

Discussion

Buchner (1965) reported that *a*-symbionts (or *a*-bacteroids) apparently occurred in all species of homopteran insects. These insects had di- or trisymbionts when they further acquired *h*- or *t*- and/or companion symbionts. In fat-body cells of *Pyrops candelaria*, *a*-bacteroids were found. Chang and Musgrave (1972) reported that *a*-symbionts appeared as irregular dark granular bodies in the leafhopper *Helochara communis*. Each symbiont was enclosed by three peripheral membranes. It also had ribosome-like particles and amorphous dense materials. Occasionally, dense bodies or

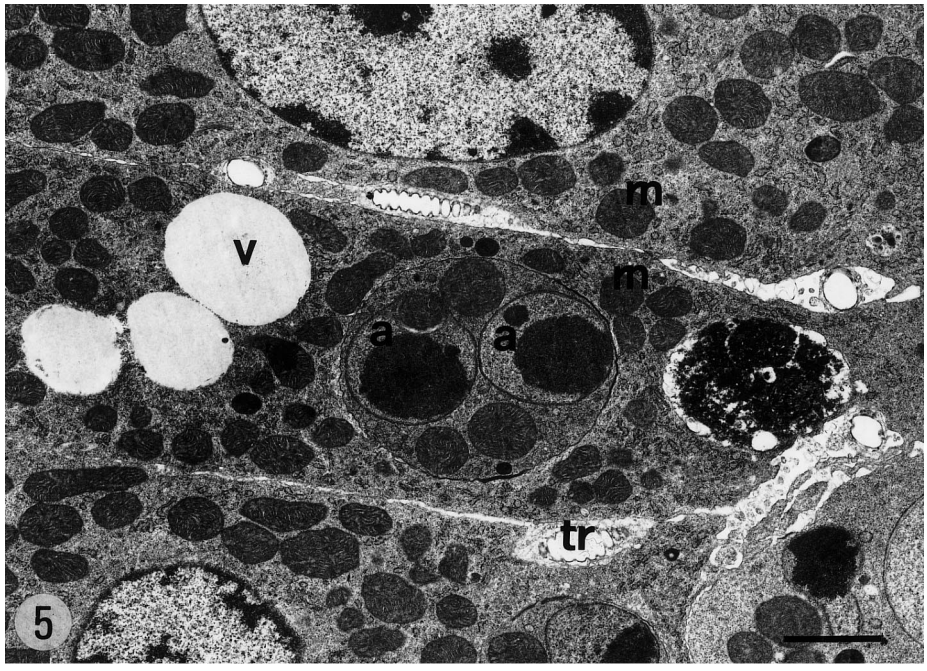


Fig. 5 TEM showing two daughter bacteroids (*a*) formed after cell division. Note the host mitochondria (*m*), vacuoles (*v*), and tracheoles (*v*). *Bar* 2 μm.

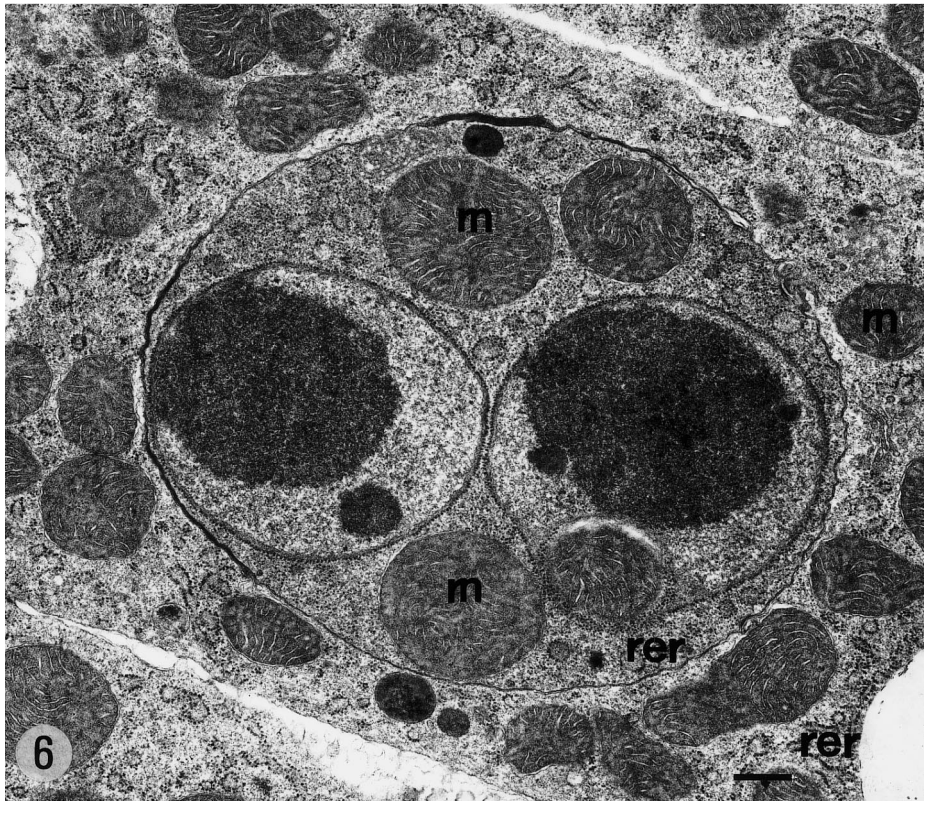


Fig. 6 TEM showing host mitochondria (*m*) and rough endoplasmic reticulum (*rer*). *Bar* 0.5 μm

spherical inclusions were present. There was never any evidence of DNA. The *a*-bacteroids in fat-body cells of *P. candelaria* are normally oval in shape. They are also enveloped by three membrane layers. Ribosomes are found on the outside of the cell wall. This feature was not reported by Chang and Musgrave (1972). Mitsuhashi and Kono (1975) reported in *Nephotettix cincticeps* that a small electron-dense inclusion was present in the *a*-symbionts. It was surrounded by two thin membranes. Besides a larger amorphous dense body in the matrix, a smaller one is sometimes seen in *a*-bacteroids of *P. candelaria*. In embryos of the leafhopper *Euscelis plebejas* (Körner 1969) the *a*-symbionts contained crystalline bodies and, sometimes, tubular membrane bodies in their cytoplasm. The *a*-symbionts in the cabbage aphid *Brevicoryne brassicae* were slightly oval in shape

and were bound by a pair of unit membranes (Lamb and Hinde 1967). In the center of the cytoplasm a large osmiophilic area like that seen in *P. candelaria* was found.

The *a*- and *t*-symbionts in the green rice leafhopper *N. cincticeps* had both infectious and vegetative forms (Mitsuhashi and Kono 1975). These two forms of symbionts were also found in *Anomoneura mori* (Waku and Endo 1987). Nasu (1965) had classified the mycetomal symbionts of *N. cincticeps* into L- and H-symbionts according to their electron density. From the morphological point of view, *P. candelaria a*-bacteroids appear to correspond to H-symbionts.

Noda (1977) stated that yeast-like symbionts were observed in the mycetocytes of the smaller brown planthopper *Laodelphax stritellus*. The symbionts were oval in shape and seemed to propagate by budding. The *a*-bacteroids of *P. candelaria* reproduce by binary fission. The symbionts of *L. stritellus* were Feulgen-positive, indicating the presence of DNA. The question as to whether any DNA exists in *a*-symbionts of *P. candelaria* has not been explored.

Almost all types of homopteran symbionts have two endogenous peripheral membranes and a third membrane of host origin (Houk and Griffiths 1980). This strict structural relationship between symbionts and host cells might also account for the great difficulty encountered in cultivating the bacteroids in vitro (Polver et al. 1986). Most researchers have postulated that this third membrane would be used as a protective device against host enzymes (Daniel and Brooks 1972).

Symbionts have an obligatory relationship with their hosts. Buchner (1965) argued that the homopteran symbionts provided essential nutritional factors, probably vitamins, found lacking in the diet of the host. However, such dependence on microorganisms would require a mechanism that would ensure transovarial transmission of symbionts from the parental generation to the progeny ad infinitum.

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