Chapter 2
Morphology of Human and Animal *Blastocystis* Isolates with Special Reference to Reproductive Modes

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Abstract *Blastocystis*, an anaerobic unicellular eukaryotic protozoon, is one of the most encountered microorganisms in fecal samples of various host animals including humans. The parasite is generally spherical in shape but shows a great variation in size from 5 to 50 μm. When the isolates from the feces were cultured in vitro, the size of the cell increases and some cells reach up more than 200 μm in diameter. Since a large central vacuole occupies the central part of the organisms, the cytoplasm containing nucleus, mitochondria-like organelle, and Golgi apparatus is pushed to the peripheral rim. The central vacuole possesses storage function and contains various substances such as carbohydrates, lipids, and basic proteins identified under the light and electron microscopy. These substances are accumulated in the vacuole via Golgi apparatus or crathrin-based endocytosis. There are various naming for morphologically different organisms, such as vacuolar (vacuolated), multivacuolar, avacuolar, granular, amoeboid, and cyst forms, which appear in fecal and in vitro samples. The vacuolar and granular forms are predominantly detected in the fresh feces or in vitro cultures, while some other forms, multivacuolar and avacuolar forms are rare and small in size and are mainly characterized by electron microscopy. The amoeboid (ameba) forms are also small in size and are generally more frequently observed in the samples of in vitro culture. Irregular shape of the amoeboid form is difficult to be distinguished from the amoebic forms of other intestinal protozoa, and locomotion of the amoeboid form of *Blastocystis* has not been recognized. The cyst forms are mainly seen in the fecal samples and rarely in in vitro cultures. The nuclei in vacuolar and granular forms can be clearly seen when the cells are stained with DAPI or Giemsa. In general, small cells show one or two nuclei, while bigger cells show many nuclei.

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When there were two nuclei in a cell, both the nuclei were usually located at the opposite pole of the cell. The life cycle of *Blastocystis* is still not clear. The most conflicting mode is the reproductive stages in the proposed life cycle. Binary fission is frequently observed in fresh fecal and cultured samples. However, other reproductive stages such as schizogony, plasmotomy (budding), endodyogeny, and sac-like pouches are not accepted generally for the true modes of *Blastocystis*. Schizogony-like and budding-like organisms, which possess many nuclei in the peripheral cytoplasm or have many daughter cell-like structures in the central vacuole, are observed especially in in vitro cultures. Many reproductive processes have been proposed for *Blastocystis*, however, to date, only binary fission, budding, or plasmotomy have been proven. Pseudopodal activity, on a few occasions, is seen in the amoeboid forms, and this form may be proposed as another alternative reproduction process except three identified modes of reproduction, binary fission, plasmotomy, and budding (or schizogony) under ultrastructural insights while locomotion of the amoeboid form has not still been confirmed.

2.1 Introduction

Blastocystis was first thought to be a fungus (nonpathogenic yeast), but it is now recognized as a human and animal parasitic protozoan inhabiting the cecum and large intestine (Yamada et al. 1987; Teow et al. 1992a; Boreham and Stenzel 1993; Stenzel et al. 1994; Stenzel and Boreham 1996; Abe et al. 2002; Tan 2004; Yoshikawa et al. 2004, 2007). Morphology of *Blastocystis* isolates has been extensively elucidated by light microscopy using the samples from fresh fecal materials and axenic or xenic cultures of human and animal isolates (Zierdt 1973, 1988, 1991; Yamada et al. 1987; Teow et al. 1992a; Boreham and Stenzel 1993; Zaman et al. 1993; Stenzel et al. 1994; Stenzel and Boreham 1996; Abe et al. 2002; Tan 2004; Yoshikawa et al. 2004, 2007). *Blastocystis* is strictly anaerobic eukaryotic unicellular organism, because the cells are quickly degenerated when they are exposed to the air. The most typical feature of this organism is polymorphic appearances, including a great variation in cell size and distribution of granules in the large central vacuole and polymorphic morphological shape from spherical to amoebic form. These polymorphic features are frequently observed not only within a single sample but also among isolates under conventional and phase contrast microscopy in fresh materials and in vitro cultures. Therefore, it is difficult to assign a standard morphology for diagnosis of clinical samples. Here we describe in detail, the morphological features of major forms of *Blastocystis*.

2.2 Morphology of *Blastocystis*

2.2.1 Light Microscopy

Although *Blastocystis* organisms have been isolated from a variety of animals including, mammals, birds, reptiles, amphibians, and insects, all isolates are morphologically indistinguishable (Yamada et al. 1987; Teow et al. 1992a, b; Zaman et al. 1993; Stenzel et al. 1994; Singh et al. 1996; Suresh et al. 1997; Abe et al. 2002; Yoshikawa et al. 2004). However, there are various terms for morphologically different *Blastocystis* organisms that appear in human and animal fecal samples based on the light microscopy. Namely, there are vacuolar (vacuolated), multivacuolar, avacuolar, granular, amoeboid (ameba), and cyst forms (Zierdt 1973, 1988, 1991). In general, the vacuolar and granular forms are the most predominant in the fresh fecal and in vitro culture samples. The amoeboid form is generally more frequently observed in the samples of in vitro culture than in fecal materials, while predominant excretion of the amoeboid form in stool samples of symptomatic patients has been recently reported (Tan and Suresh 2006a). Irregular shape of the amoeboid form is difficult to be distinguished from the amoebic forms of other intestinal protozoans, and locomotion of the amoeboid form of *Blastocystis* has not been recognized. The cyst form is mainly seen in the stool samples and rare in in vitro cultures. The cyst form is relatively small in size, 3–5 μm in diameter (Yoshikawa et al. 2003); hence it is difficult to identify it in the fecal samples by routine diagnostic examinations. Several cyst-concentration techniques to detect cysts from fresh fecal samples have been reported (Zaman and Khan 1994; Zaman et al. 1995).

The vacuolar and granular forms are generally round in shape and range in size from about 5–50 μm in fecal samples. When the organism is cultured, it can grow to a size of more than 50 μm and reaches up more than 200 μm in diameter. Under phase contrast and Nomarski differential interference microscopy, a large central vacuole can be clearly observed in the center of the organism. The cells having an empty vacuole are called vacuolar forms (Figs. 2.1 and 2.2). The function of the central vacuole is not yet fully confirmed. However, metabolic and storage functions of the central vacuole containing various substances such as carbohydrates and lipids have been demonstrated by light microscopy (Yoshikawa et al. 1995a, b; Yoshikawa and Hayakawa 1996a) (Fig. 2.3). Since the central vacuole occupies more than 80 % of the cell volume, the cytoplasm of the organism is pushed to the periphery and is lined thinly just under the cell membrane. During in vitro culture and also in fecal samples, some take the shape of a peanut, which indicates that the organism is in the process of cell division (binary fission) (Fig. 2.2). Many small sized or a mass of granules are sometimes observed in the central vacuole in fecal samples and cultures, and this stage is called the granular form. Granular forms in the process of binary fission can also be observed (Fig. 2.4). When *Blastocystis* organisms were stained with iodine–potassium iodide solution or Lugol's iodine, the central vacuole or containing granules can be stained yellow

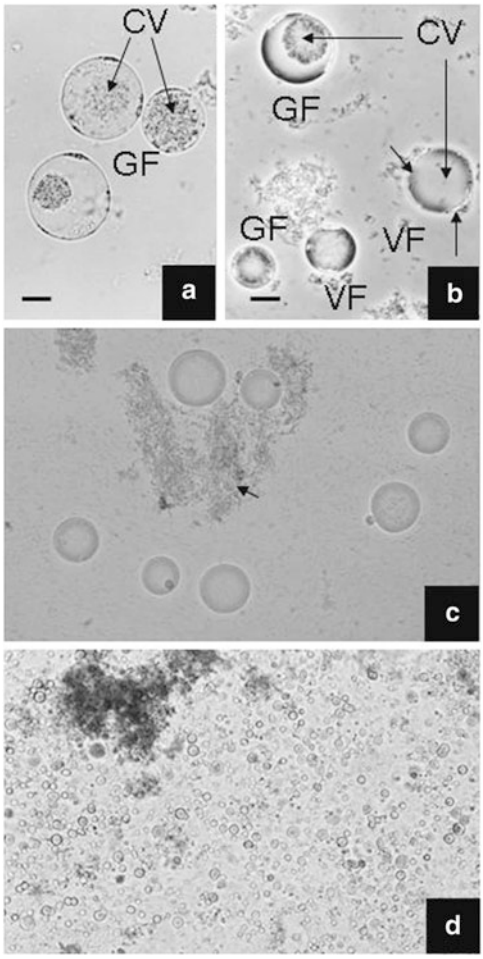

Fig. 2.1 Light microscopic images of the vacuolar and granular forms of *Blastocystis* (panels **a** and **b**). The cytoplasm (*arrows* in panel **b**) is located at the peripheral rim of the organisms due to the presence of the large central vacuole (CV). Great variations in size is evident in a culture sample and the smallest organism is observed in the center of this field (*arrow* in panel **c**, ×400). Low magnification view of the culture sample showing many morphologically different organisms (panel **d**, ×80). The conventional microscopy (panels **a**, **b**, and **c**) and phase contrast microscopy (panel **b**). Scale bar: 10 μm. *CV* central vacuole, *GF* granular form, *VF* vacuolar form

to brown, while in some cases, they are not stained at all. Staining features of Heidenhein's iron hematoxylin (HIH) are a little different to others and nonstaining areas surrounding the organisms are highlighted in the background as recognized unstained circles, due to the thick outer surface of the organisms (Fig. 2.5). This feature is also observed in the samples mixed with an India ink. Namely, circular transparent peripheral margin can be clearly seen demarcated from the surrounding

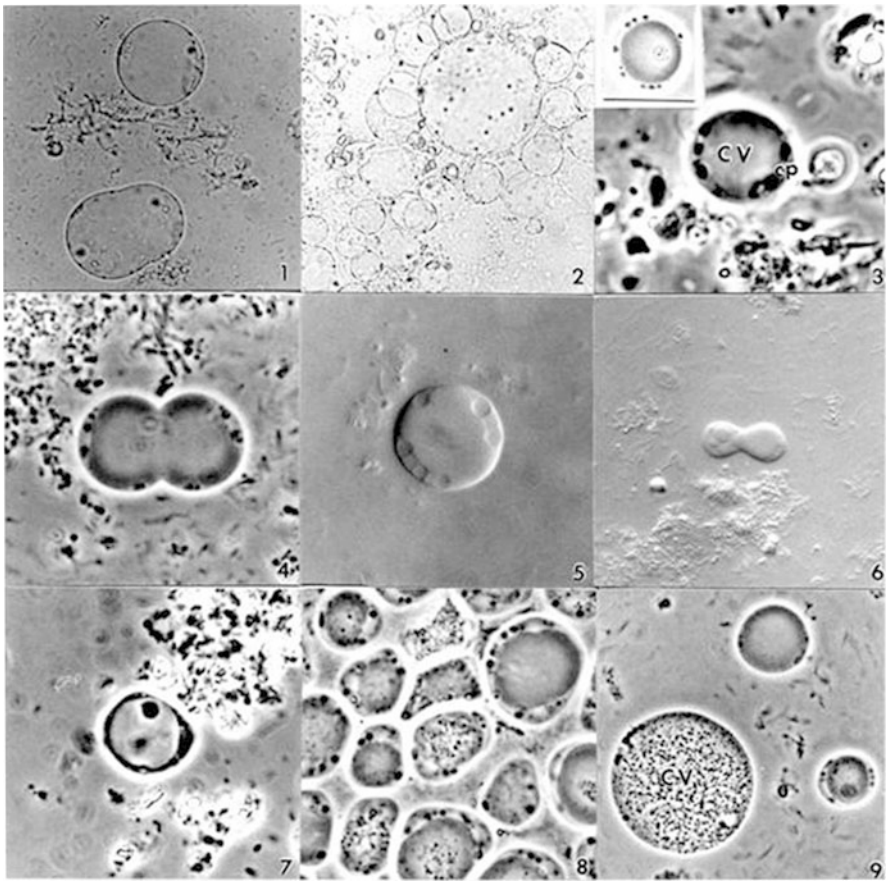

Fig. 2.2 Light microscopic images of *Blastocystis* observed conventional (panels 1 and 2), phase contrast (panels 3, 4, and 7–9), and Nomarski (differential interference) microscopy (panels 5 and 6). Panels 1 and 4, and probably 6, show organisms in progress binary fission (1, 3–5, ×800; 2, ×400; 6, ×200; 7, 8, ×600; 9, ×500)

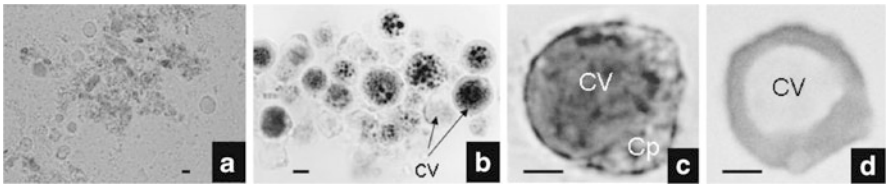

Fig. 2.3 Light micrographs of *Blastocystis* cells stained with various histochemical staining methods to detect lipids (panel **b**) and carbohydrates (panels **c** and **d**). Unstained fresh material (panel **a**) and *Blastocystis* organisms stained with Sudan black B (**b**), PAS (**c**) and Alcian blue at pH1.0 stain (**d**) Scale bars: 10 μm

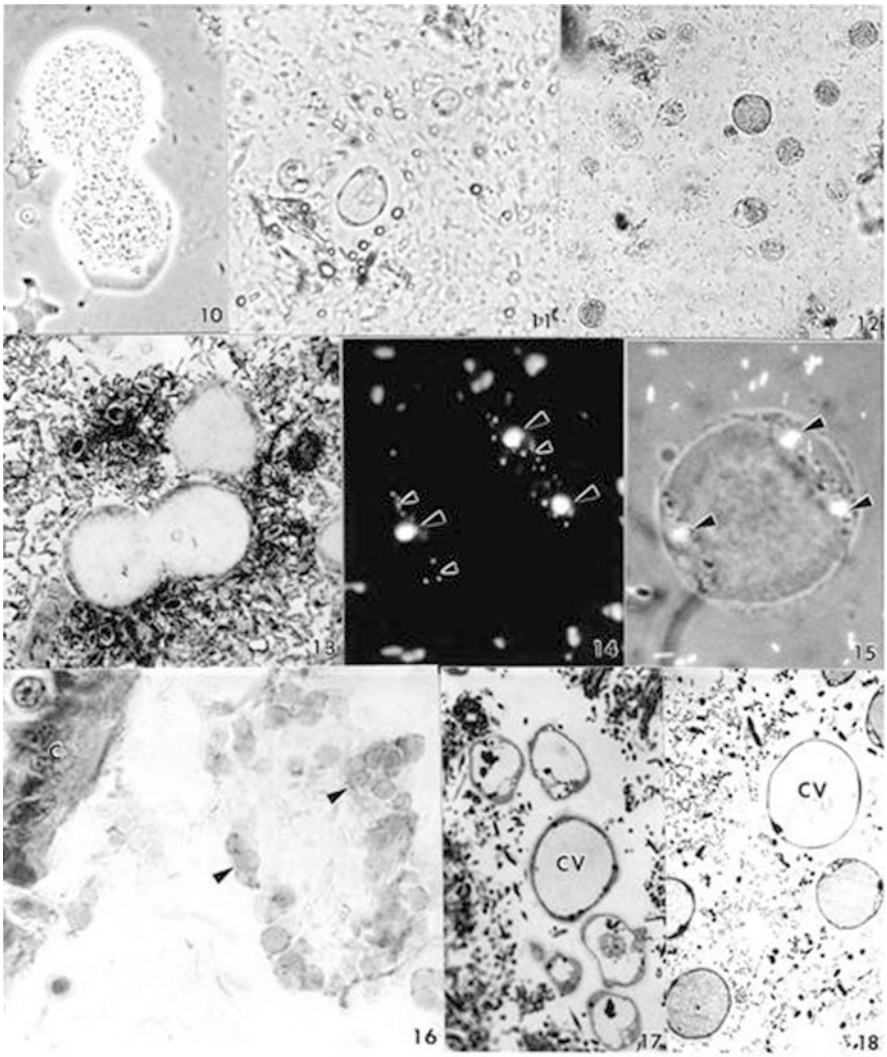

Fig. 2.4 Light microscopic appearances of *Blastocystis* organisms observed with various staining. A granular form in progress binary fission observed by the phase contrast microscopy (panel 10). Fresh materials with iodine-potassium iodide stain (Lugol's iodine) (panels 11 and 12). Note that vacuolar cells are not stained, while granular forms are well stained in the central vacuole. A diving cell stained with Giemsa (panel 13). A same organism stained with DAPI showing three nuclei (large arrowheads in panels 14 and 15) with many small dots of the DNA of mitochondria-like organelle (MLO) (small *arrowheads* in panel 14). It is evident that the thickened part of the cytoplasm contains the nucleus and MLO. A tissue section of the cecum (*c* in panel 16) stained with HE shows many *Blastocystis* in the rumen (*arrowheads* in panel 16). Semi-ultrathin sections of resin-embedded samples stained with Giemsa (panels 17 and 18) showing morphological variation of the central vacuole (panel 10, ×600; 11, 12, 16, ×200; 13, 17, 18, ×500; 14, 15, ×1,000)

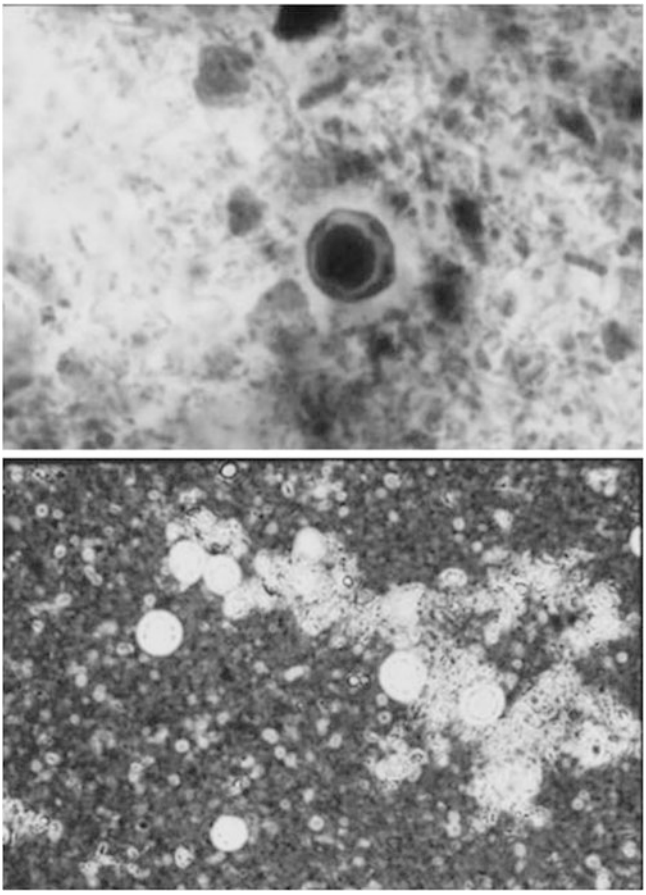

Fig. 2.5 *Blastocystis* organisms stained with Heidenhein's iron hematoxyline (HIH, *upper*) and India ink (*lower*) showing a circular transparent peripheral margin of the cells (*Upper*, ×300; *lower*, ×80)

materials (Fig. 2.5). These characteristics of *Blastocystis* organisms are hallmark for identification of the organisms correctly in fecal samples. On the other hand, organisms stained with HIH are difficult to recognize the nuclei, while the parasites stained with Giemsa can easily identify the eosinophilic nucleus and basophilic cytoplasm. This staining feature is also useful for differentiation of the parasite from other intestinal protozoan and stool debris (Fig. 2.4). *Blastocystis* isolates from monkeys usually possessed a large granule in the central vacuole under Giemsa and HIH stains (Fig. 2.6). HIH stain also shows a large granule (probably the nucleus or a volutin granule) in the peripheral cytoplasm.

In preparations stained with DAPI (4,6-diamidino-2-phenylindole), a large, well-defined, and highly fluorescent body of the nucleus is clearly observed by a fluorescence microscope (Figs. 2.4 and 2.7), while the DNA of mitochondria-like

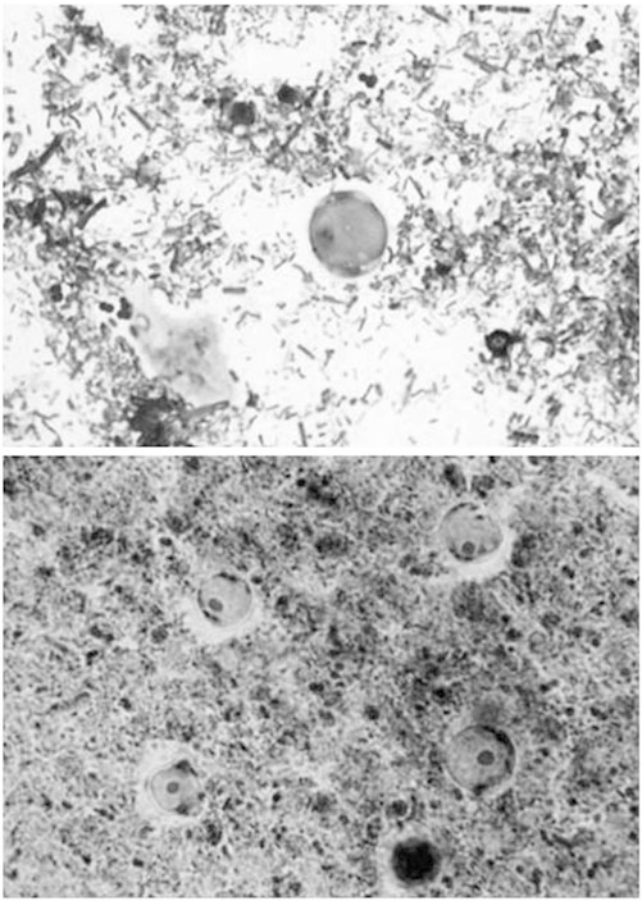

Fig. 2.6 Granular form of *Blastocystis* isolated from monkeys. Giemsa (*upper*) and HIH (*lower*) stains. A large granule can be seen in the central vacuole and unstained circular space is also observed on the outermost of the cells (*upper* ×800; *lower*, ×600)

organelle shows only small and weak fluorescence (Fig. 2.4). No fluorescence is observed within the central vacuole (Figs. 2.4 and 2.7), although schizogony and progeny in the vacuole has been reported (Zierdt 1973, 1991; Suresh et al. 1994; Singh et al. 1996).

The nucleus could also be clearly seen when fresh materials and semi-ultra thin sections are stained with Giemsa (Fig. 2.4). In general, small cells show one or two nuclei, while bigger cells show many nuclei. When there are two nuclei in a cell, both the nuclei are usually located at opposite poles of the cell (Fig. 2.1). The cytoplasm becomes thick on the side of the nucleus. The nucleus is sometimes found located in the equatorial region of the parasite.

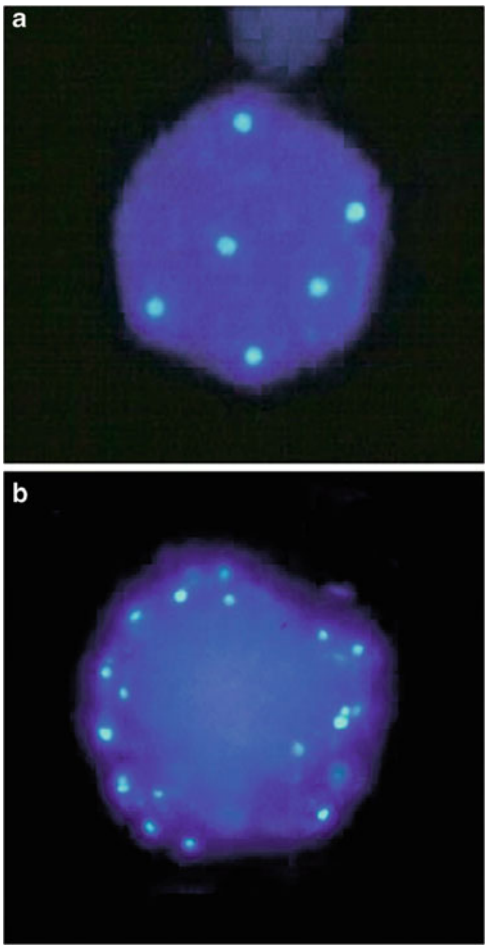

Fig. 2.7 The multi-nucleated organisms stained with DAPI observed by fluorescent light microscopy. It is evident that the nucleus of the large cell are existing on the peripheral rim of the organisms (panels **a** and **b**, ×2,000)

2.2.2 Transmission Electron Microscopy

Ultrastructure of *Blastocystis* has been extensively elucidated by transmission electron microscopy (TEM) (Zierdt 1988, 1991; Dunn et al. 1989; Stenzel et al. 1989, 1991, 1994, 1997; Stenzel and Boreham 1991, 1996; Yoshikawa and Hayakawa 1996a; Suresh et al. 1997; Pakandl 1999; Tan and Suresh 2006b; Yoshikawa et al. 2007). In general, basic morphology of *Blastocystis* organisms isolated from various host animals are very similar. However, morphological variation of the parasite is also observed within and between isolates among human origins (Dunn et al. 1989). Since a great variation of the central vacuole

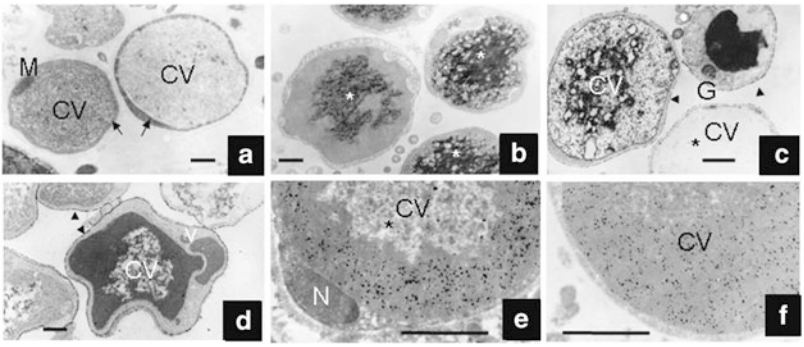

Fig. 2.8 Electron micrographs of *Blastocystis* stained with and without histochemical staining for detection of lipids, carbohydrates and basic proteins, respectively. Unstained material (panel **a**) and positive reactions with lipids (panel **b**, *asterisks*), carbohydrates (panels **c** and **d**), and basic proteins (panels **e** and **f**) are observed in the central vacuole, vesicles, or Golgi apparatus. *CV* central vacuole, *M* mitochondria-like organelle, *G* Golgi apparatus, *N* nucleus, *V* vesicles. Short *arrows* indicate the peripheral cytoplasm (panel **a**). *Arrowheads* show the filamentous layer on the surface (**c**, **d**). Each of *asterisk* in panels **c** and **f** shows no reaction with lipids and basic proteins in the central vacuoles, respectively. Scale bar: 1 μm

within a single isolate is observed in the density and distribution of the granules or materials containing the central vacuole, two distinctive vacuolar and granular forms cannot be clearly distinguished by TEM. Therefore, the terminology of vacuolar and granular forms is limited for identifying the organisms under the light microscopy. Interestingly, virus-like particles in the cytoplasm have been reported in the parasites isolated from *Macaca* monkeys (Stenzel and Boreham 1997). The presence of virus-like particles in the *Blastocystis* isolate from a seasnake, which was insensitive for DNase, while it was sensitive for RNase, was also reported (Teow et al. 1992b).

The most prominent feature of *Blastocystis* is having a large central vacuole; hence the cytoplasm is pushed in the peripheral rim. The function of the central vacuole is not yet conclusively confirmed. However, metabolic and storage functions of the central vacuole had been demonstrated by the trace of cationized ferritin added in culture medium and by the histochemical methods for lipids, carbohydrates, and basic proteins (Stenzel et al. 1989; Yoshikawa et al. 1995a, b; Yoshikawa and Hayakawa 1996a; Yoshikawa and Oishi 1997) (Fig. 2.8). Interestingly, pinocytosis via crathrin-based endocytosis on the surface of the organism clearly shows that nutrients are accumulated into the central vacuoles via small vesicles in the cytoplasm (Stenzel et al. 1989) (Fig. 2.9). It is suggested that the increasing number of the electron-dense pits on the surface membrane as the same phenomenon may indicate a change in the mode of nutrition or intensity of endocytosis (Pakandl 1999).

Blastocystis organism is surrounded by a thick outer surface coat, sometimes referred to as the fibrillar or filamentous layers or capsule. Interestingly, the thickness of the surface coat is thicker up to 1 μm among parasites freshly isolated

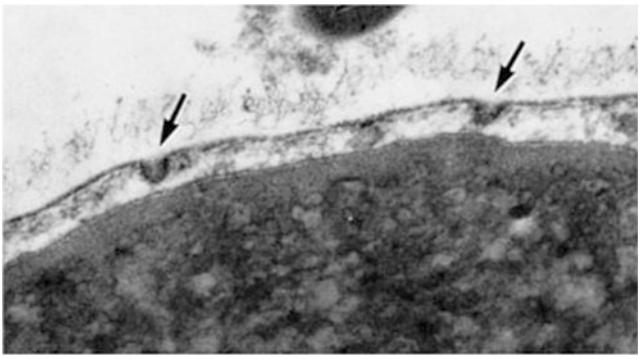

Fig. 2.9 Pinocytosis via crathrin-based endocytosis on the surface of the organism is clearly seen by electron microscopy (*arrows*) (×40,000)

from fecal samples, while it gradually becomes thin during laboratory cultures. The surface coat also contains various carbohydrates (Yoshikawa et al. 1995a; Lanuza et al. 1996). The function of the surface coat is unclear, while adherence of intestinal bacteria on the surface is frequently observed, suggesting a trapping purpose for nutrition (Dunn et al. 1989; Zaman et al. 1999).

The cytoplasm and nucleus are located on the peripheral rim of the organism due to the presence of the large central vacuole. The cytoplasm of anaerobic *Blastocystis* organisms contains many organelles commonly observed in eukaryotic cells, namely, various-sized vacuoles, mitochondria-like organelle (MLO), Golgi apparatus, rough endoplasmic reticulum, microtubules, and ribosomes in the cytoplasm (Fig. 2.10). Binary fission of the vacuolar/granular form is frequently observed, while this division is rare in the multivacuolar cells. However, nuclear division with mitotic apparatus does not yet observed. Interestingly, two daughter nuclei are frequently seen separated by a divided inner membrane, the whole still enclosed within an intact outer membrane (Fig. 2.11). Same observation was also reported in *Blastocystis* isolates from pigs (Pakandl 1999) and by freeze-fracture electron microscopy of human isolates (Yoshikawa et al. 1988). This observation lends credence to the theory that nuclear division of *Blastocystis* begins with separation of the parent nucleoplasm by the inner membrane into two parts, followed by division of the outer membrane forming two discrete nuclei. This mode of nuclear division is reported to occur in the mitosis of *Eimeria necatrix* (Dubremetz 1973).

Blastocystis is strictly anaerobic organism, and it possesses many electron-dense organelles which morphologically resemble mitochondria (Figs. 2.10 and 2.11). Since it is known to lack cytochrome C complex genes (Wawrzyniak et al. 2008), this organelle is now called as MLO. The function of the MLO is not known, while it may play a similar role in energy metabolism as classic mitochondria (Hamblin et al. 2008). The MLO of *Blastocystis* are either spherical or oval in shape, 1–3 μm in diameter, and showed many tubular or sacculate cristae which are typical feature of eukaryotic protozoa. Interestingly, under high magnification, spiral structures consisting of intertwined coils are observed inside each tubular crista (Fig. 2.12).

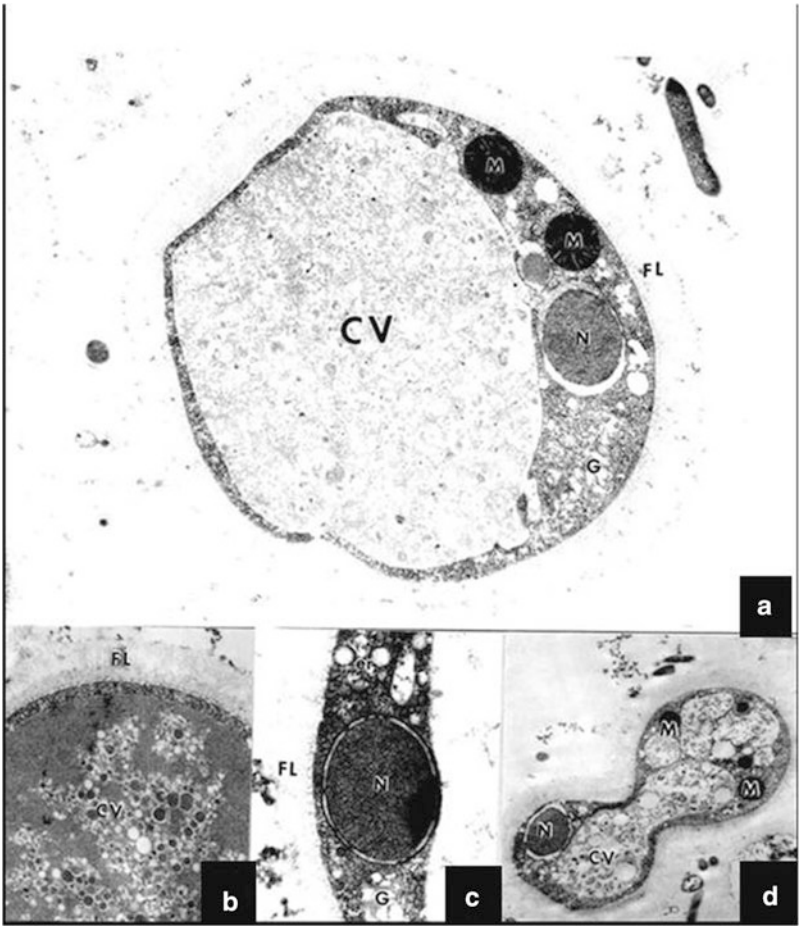

Fig. 2.10 Ultrastructure of *Blastocystis* organisms. The central vacuole (CV) showed various density and distribution of granules among the organisms (panels **a**–**d**). High magnification view of the cytoplasm showing a nucleus (*N*), Golgi apparatus (*G*), and endoplasmic reticulum (*er*) (panel **c**). A granular form in progress binary fission (panel **d**). *CV* central vacuole, *FL* filamentous layer, *G* Golgi apparatus, *M* mitochondria-like organelle, *N* nucleus (panel **a** ×1,000, **b** × 20,000, **c** ×13,000, **d** × 5,000)

The Golgi apparatus of *Blastocystis* is always found adjacent to the nucleus (Figs. 2.8 and 2.10). Small electron-dense secretory granules are often seen near the Golgi apparatus. As general function of this organelle, synthesis of carbohydrate has been demonstrated histochemically at ultrastructural level (Yoshikawa et al. 1995a) (Fig. 2.8).

Since the cyst form is relatively small in size, it is difficult to identify it by light microscopy; hence the cyst form has been examined by electron microscopy in human and animal isolates (Stenzel and Boreham 1991; Zaman et al. 1993, 1995; Stenzel et al. 1997; Chen et al. 1999; Moe et al. 1996; Yoshikawa et al. 2003).

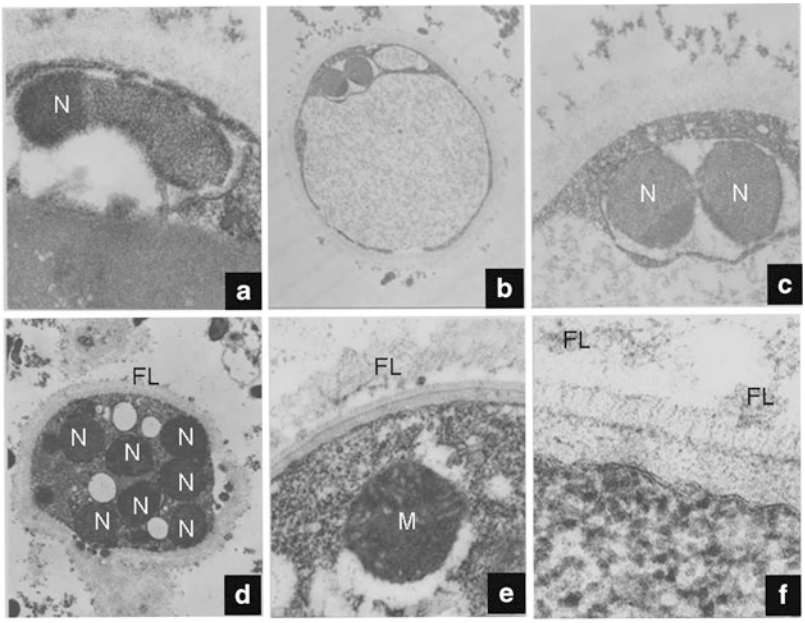

Fig. 2.11 Nuclear division and cyst form of *Blastocystis* of human isolates in cultures and fecal samples, respectively. Dividing nucleus or two daughter nuclei surrounding the outer nuclear envelope was frequently observed in the cultured samples (panels **a**–**c**). It is evident of the thick surface filamentous layer of the organisms after the short in vitro cultures. The multi-nucleate cell shows seven nuclei in a section of thickened cytoplasm (panel **d**). A cyst form observed in human fecal samples showed a three-layered thick cyst wall surrounding with filamentous materials on the outer surface (panels **e** and **f**) which may be corresponding to the outer filamentous layer existing on the surface of the vacuolar and granular forms (panels **a**–**d**). *FL* filamentous layer, *M* mitochondria-like organelle, *N* nucleus (panels **a**, **c**, **e** × 20,000; **b** × 5,000; **d** × 6,500; **f** × 95,000)

The morphological characteristics of the cyst form reveal a thick wall and a constant average size in diameter, 3–5 μm, except for the larger size of an isolate from a monkey (Stenzel et al. 1997) (Fig. 2.12). Although the size of the cyst form is relatively small, it contains one to 4 nuclei (Yoshikawa et al. 2003). Since a thick cyst wall is composed beneath the outermost surface coat, the outermost surface coat (filamentous layer) seems to be fuzzy (Figs. 2.11 and 2.14). In general, the cyst form is only detected in the fecal samples of human and animals, and rarely in in vitro cultures (Stenzel and Boreham 1991; Zaman et al. 1993, 1995; Stenzel et al. 1997; Chen et al. 1999; Moe et al. 1996; Yoshikawa et al. 2003). Interestingly, the central vacuole does not exist in this form, while a large mass of the glycogen granules in the cytoplasm is reported in a reptilian isolate (Yoshikawa et al. 2003) (Fig. 2.13). The number of nucleus is also variable among the isolates and it has been reported from a single nucleus to 4 nuclei (Stenzel and Boreham 1991; Zaman et al. 1993, 1995; Stenzel et al. 1997; Chen et al. 1999; Yoshikawa et al. 2003).

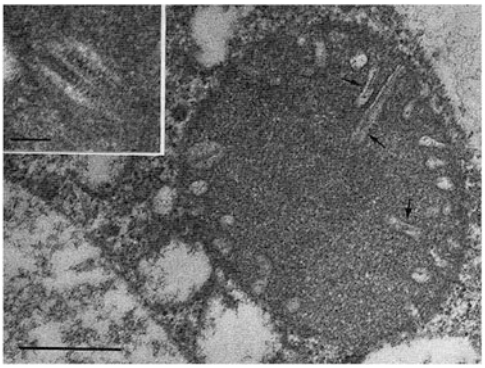

Fig. 2.12 High magnification view of the mitochondria-like organelle (MLO). It is evident a spiral structures consisted of intertwined coils in the rumen of each tubular crista of the MLO. Scale bars: 0.5 μm (**a**), 0.1 μm (**b**). This figure was cited from Fig. 2.13 in page 1490 in Yoshikawa T (1988) Fine structure of *Blastocystis hominis* Brumpt, 1912. J Kyoto Pref Univ Med 97:1483–1500

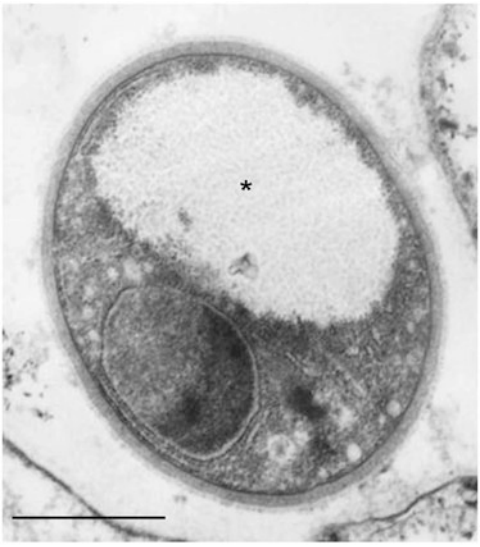

Fig. 2.13 A typical cyst form of *Blastocystis* reptilian isolate having a mass of glycogen granule (*asterisk*) Scale bar: 1 μm

2.2.3 Freeze-Fracture Electron Microscopy

Ultrastructure of a human *Blastocystis* isolate has also been elucidated by freeze-fracture electron microscopy (Yoshikawa et al. 1988; Yoshikawa and Hayakawa 1996b). The most remarkable feature of *Blastocystis* is the heterogeneous

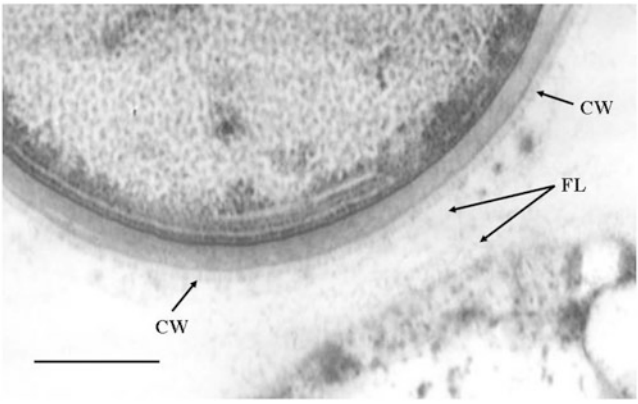

Fig. 2.14 High magnification view of the cyst form and vacuolar form of *Blastocystis* reptilian isolate. It is evident that cyst wall is composed of three layers, while the vacuolar form shows only filamentous outer layer on the surface. Scale bar: 0.5 μm

distribution of the intramembranous particles (IMPs) of the plasma membrane because IMPs, known to be integral membrane proteins, are generally distributed homogeneously in the plasma membrane (Yoshikawa et al. 1988). Similarly, membrane cholesterol is also distributed heterogeneously associated with membrane proteins (Yoshikawa and Hayakawa 1996b). In addition, different organization of the membrane proteins and cholesterol between the plasma membrane and central vacuole membrane demonstrate different organization of the two membranes (Yoshikawa et al. 1988, Yoshikawa and Hayakawa 1996b). A great variation of the distribution and density of the granules in the central vacuole was also observed. As TEM images, two daughter nuclei enclosed within an intact outer membrane are also clearly observed by freeze-fracture electron microscopy (Yoshikawa et al. 1988). When the cyst form of reptilian isolate of in vitro culture was observed by freeze-fracture methods, the protoplasmic face of the plasma membrane of cyst form showed extremely low density of the IMPs with many striated indentations (Fig. 2.15), while non-cyst form (i.e. vacuolar and granular form) showed many IMPs (Yoshikawa et al. 2003). Practically no IMPs in cyst form support the resting stage of this form because most of the IMPs have enzymatic activity or transport function of the membrane. The fractured cyst wall surrounding the small-sized cyst form show many fine granules (Fig. 2.15), suggesting protein constituents within filamentous materials observed by TEM (Fig. 2.11).

2.2.4 Scanning Electron Microscopy

A great variation in cell size among a human *Blastocystis* isolate and smooth surface and round shape of the organisms are also evident by scanning electron

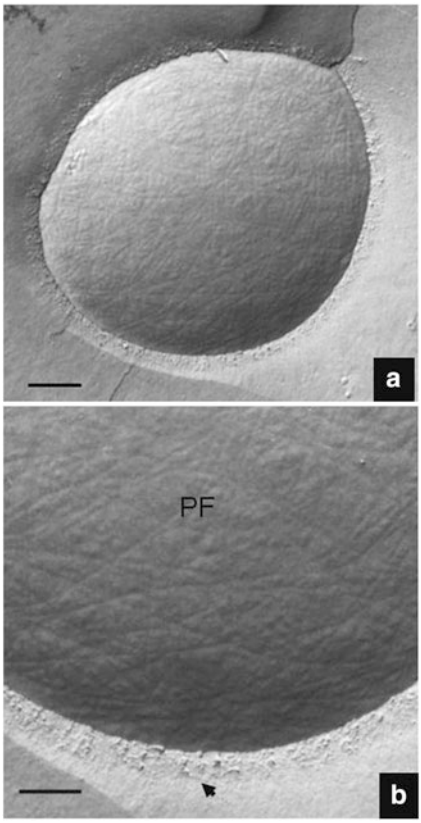

Fig. 2.15 Freeze-fracture images of *Blastocystis* cyst form from reptilian isolate in in vitro culture. The P-face (PF) of the plasma membrane of the cyst form of a reptilian isolate showed an extremely low density of IMPs and many striated indentations. Many fine granules are seen on the fractured cyst wall (*arrow*). Scale bar in panel **a** 1 μm, Scale bar in panel **b** 0.2 μm

microscopy (SEM) (Fig. 2.16) (Matsumoto et al. 1987). Most of the organisms in in vitro cultures show spherical shape, while binary fission is also observed (Fig. 2.16). The cell surface of the organisms from humans, monkeys, pigs, and chicken fecal samples has been compared with a cultured human isolate (Cassidy et al. 1994). It is apparent that the surface structures of *Blastocystis* isolates from different hosts are variable, and the organisms from culture are not typical of the fresh fecal samples. Namely, a cultured sample showed much thinner surface coat and smooth outer surface than those of the fecal samples. However, these morphological differences among the isolates are insufficient to differentiate speciation (Cassidy et al. 1994). Interestingly, *Blastocystis* organisms in a chicken fecal sample show surface coat projections. Similarly, fibrillar structure of the surface coat and individual fibrils extending up to 5 μm from the periphery of the organism are also observed in human *Blastocystis* isolates (Zaman et al. 1999). In some cells,

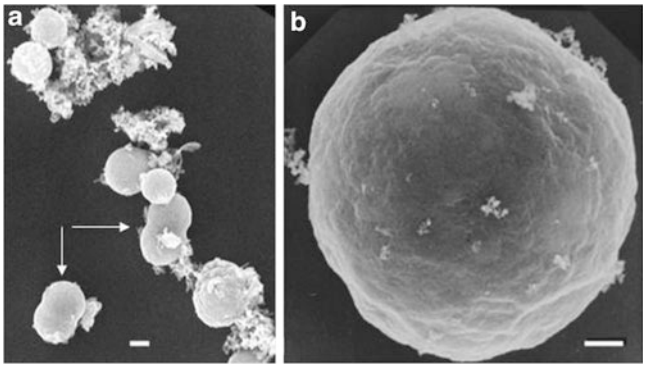

Fig. 2.16 *Blastocystis* organisms observed by scanning electron microscopy (SEM). Note the cell division (binary fission) (*arrows*) among spherical organisms in in vitro culture (**a**). The surface of the organism is uneven (**b**). Scale bars: 10 μm (**a**), 5 μm (**b**)

bacteria adhering to the surface are also observed. Although the function of the surface coat is not known, entrapment mechanism for bacteria for nutritive purpose and adherence to the epithelial lining of the gut are speculated (Zaman et al. 1999).

2.2.5 Reproductive Modes

2.2.6 Binary Fission

The life cycle of *Blastocystis* is not yet conclusively demonstrated. Many hypotheses for reproduction mode of *Blastocystis* have been put forward, though little supportive evidence exists (Suresh et al. 1994, 1997; Govind et al. 2002, 2003; Tan and Stenzel 2003; Windsor et al. 2003). The current only accepted mode of reproduction for *Blastocystis* is binary fission (Matsumoto et al. 1987; Dunn et al. 1989; Stenzel and Boreham 1996; Moe et al. 1996; Govind et al. 2002; Tan 2008). When DNA content of a single nucleus among various organisms in in vitro culture of human *Blastocystis* isolate was randomly measured by DAPI staining with fluorescence microscopy using the same method as reported in *Pneumocystis* organism (Yamada et al. 1986), two peaks corresponding to 1C and 2C was observed (Fig. 2.17). In addition, when DNA content of the each single nucleus was compared between a single- and two-nucleated cells, the lower or higher DNA content nuclei than 1C cells was observed among the two-nucleated organisms, respectively (Fig. 2.17). These results strongly support that some cells are in progress of nuclear division. Therefore, binary fission truly exists in the life cycle of *Blastocystis*.

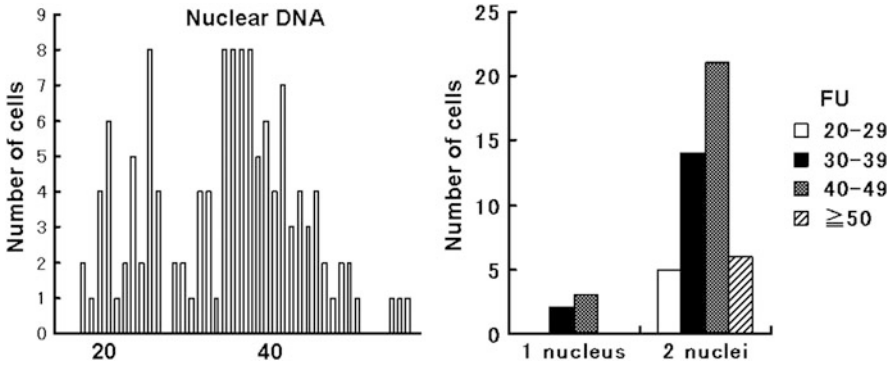

Fig. 2.17 DNA contents of *Blastocystis* nucleus measured by fluorescence microscopy with DAPI staining. *Left* Distribution of a single nuclear fluorescence intensity among *Blastocystis* organisms containing various number of the nucleus. It is evident of two peaks of the DNA contents showing nuclear division. Abscissa: fluorescence intensity in arbitrary unit. Ordinate: number of organisms. *Right* Distribution of a single nuclear fluorescence intensity among *Blastocystis* organisms containing a single nucleated cells and two nuclei cells. Most of the DNA contents of a each nucleus are equal among the both *Blastocystis* organisms, while a few number of the nucleus of the organisms having two nuclei show a lower or higher DNA contents than the two peaks of the organisms having a single nuclei. Abscissa: organisms containing a nucleus or two nuclei. *Ordinate* number of organisms. *FU* fluorescence intensity in arbitrary unit

2.2.7 Other Reproductive Modes

The additional reproductive modes of *Blastocystis* have also been reported. These are plasmotomy, budding, schizogony, endodyogeny, endogeny, and sac-like pouches (indicating the production of progeny) (Zierdt 1988; Suresh et al. 1997; Govind et al. 2002; Windsor et al. 2003; Tan and Suresh 2007). However, these reproductive modes are not generally accepted as truly present in the life cycle of *Blastocystis*. The schizogony (progeny reproduction) has been reasoned under phase-contrast microscopy by Zierdt (1991) and multiple fission or schizogony (plasmotomy-like) has been also characterized (Zaman 1997), but under the level of light microscopy, it is difficult to determine this mode clearly. Tan and Stenzel (2003) suggested that such progeny as a model of reproduction must be shown to contain genetic material and to develop into viable adult cells. In any case, care should be taken when describing reproductive processes in *Blastocystis* using light microscopy alone and these modes should be necessary to support electron microscopy (Windsor et al. 2003), while in in vitro cultures five modes of reproduction, namely, binary fission, endodyogeny, plasmotomy, budding, and schizogony are frequently observed (Zhang et al. 2007) (Figs. 2.18, 2.19, 2.20, 2.21, 2.22, 2.23, and 2.24). Tan and Suresh (2007) also confirmed that plasmotomy, leading to the formation of multiple nuclei, was the other reproductive mode of *Blastocystis* other than binary fission. Under Giemsa and HIH stains, fresh samples of the feces and in vitro cultures frequently show polymorphic budding organisms

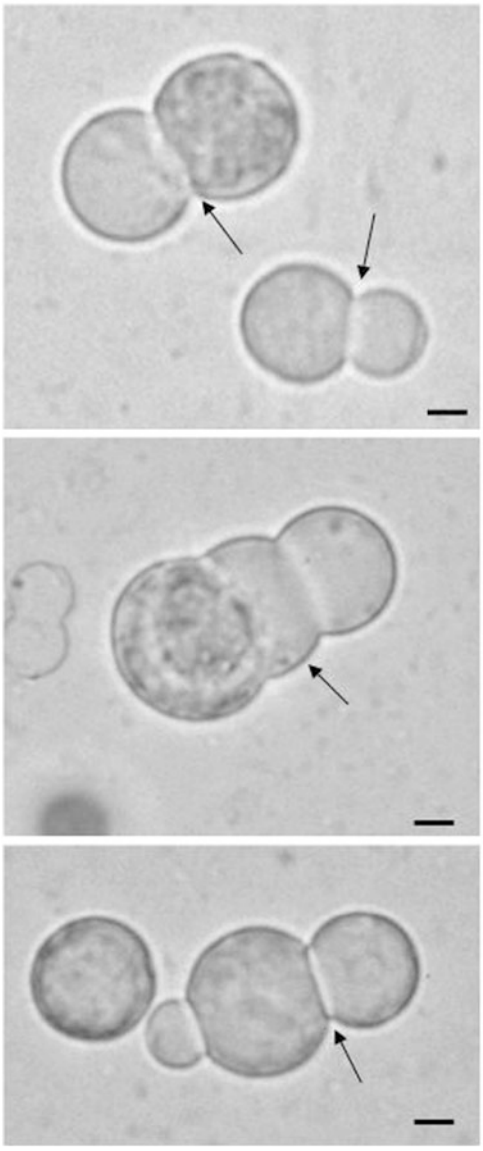

Fig. 2.18 Various reproductive modes of *Blastocystis* organisms (Figs. 2.18–2.23). A typical binary fission and budding form of vacuolar and granular forms. In vitro cultures, two or three dividing or budding organisms are frequently seen (*arrows*). Scale bar: 10 μm

(three-dividing organisms) and schizogony-like organisms showing multi-nuclei in the central part of the organisms (Figs. 2.18, 2.19, 2.20). Scanning electron microscopy showed that the cell division with binary fission is the main reproductive stage but budding-like organisms are rarely observed (Fig. 2.22). When observing cells

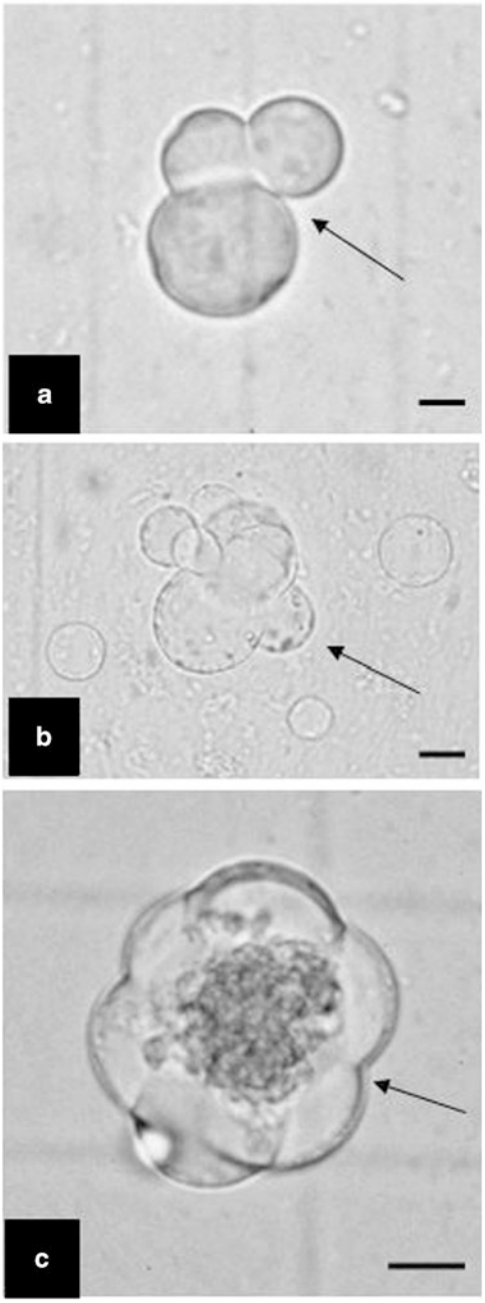

Fig. 2.19 An amoeba-like or budding-like organism (panel **a**), multi-budding (panel **b**), and schizogony-like (panel **c**) organisms are observed in vitro cultures (*arrows*). Scale bar: 10 μm

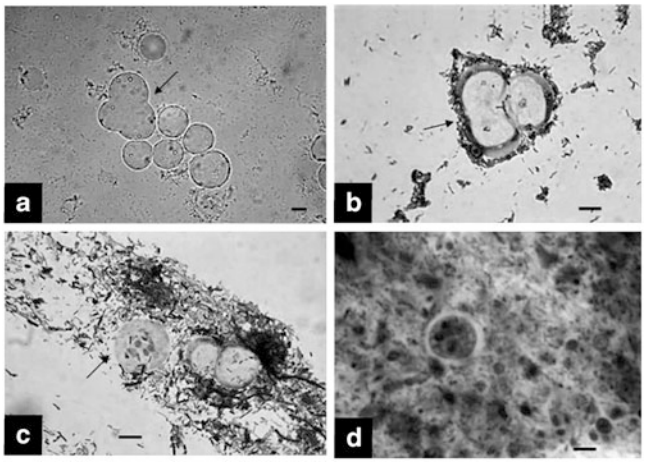

Fig. 2.20 Triple-budding-like organisms (*arrows*) observed in fresh specimen (panel **a**) and Giemsa stain (panel **b**) in culture samples. Schizogony-like organisms (*arrows*) stained with Giemsa (panel **c**) and HIH (panel **d**) in fresh human fecal samples. It is evident of multi-nuclei in the central part of the organisms (panels **c** and **d**). Scale bar: 10 μm

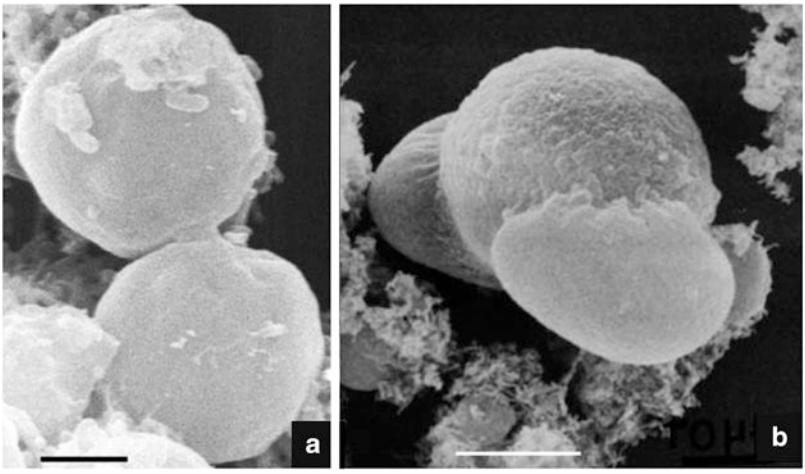

Fig. 2.21 *Blastocystis* organisms observed by scanning electron microscopy (SEM). The surface of the organism is uneven (panel **a**) and a budding-like organism is also observed (panel **b**). Scale bars: 5 μm (**a**), 10 μm (**b**)

in vitro cultures, schizogony-like organisms, which have many daughter cell-like structures in the central vacuole, are found (Fig. 2.22). Schizogony-like organisms, which possess many daughter cells in the central vacuole, are rarely seen in human fecal samples (Fig. 2.21c, d). In addition, under in vitro culture, budding-like organisms sometimes show many nuclei in each dividing cytoplasm (Fig. 2.24).

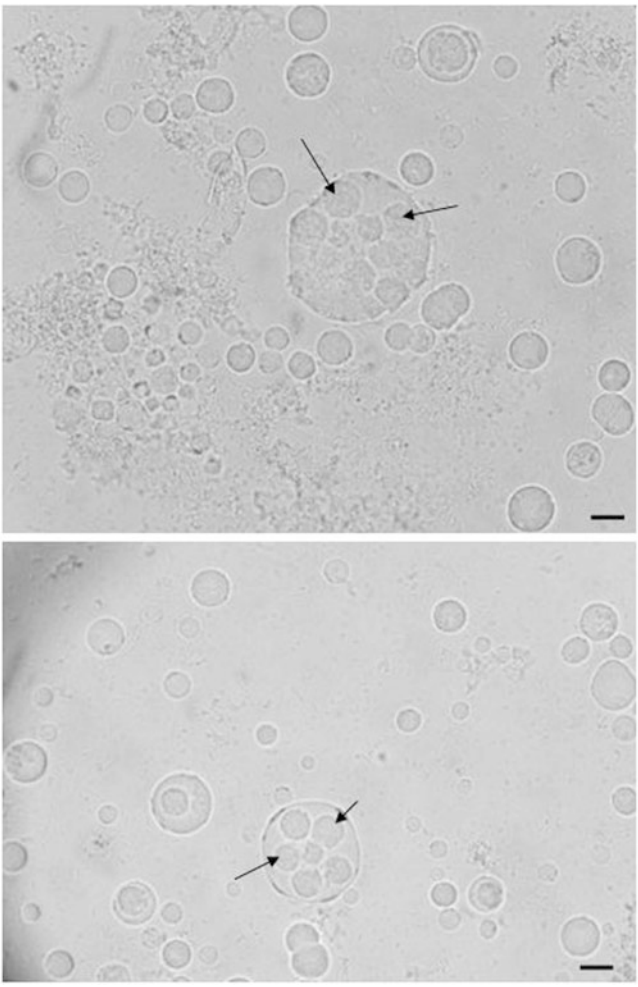

Fig. 2.22 Schizogony-like organisms observed in culture samples. Several daughter cell-like structures are seen in the central vacuole (*arrows*). Scale bar: 10 μm

Interestingly, however, these endodyogeny, plasmotomy, budding, and schizogony have not yet recognized by TEM.

The amoeboid forms of *Blastocystis* have been observed on a few occasions as the other alternative reproduction process (Tan and Suresh 2007) and they may indicate pseudopodal activity (Fig. 2.24). However, irregular shape is more likely artifact in the processing for TEM (Tan 2004) and disruption of this anaerobic parasite with exposing air is also concerned because locomotion of amoeboid form has not been yet observed.

Many reproductive processes have been suggested for *Blastocystis*, however, to date, only binary fission, budding, or plasmotomy have been proven. It is true that

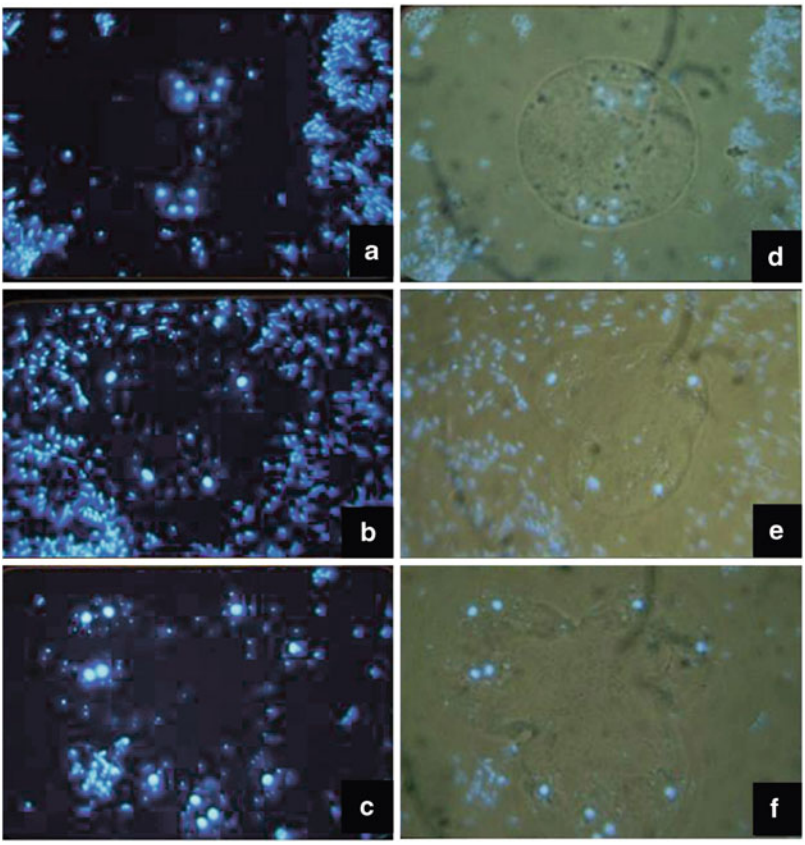

Fig. 2.23 Schizogony-like (panels **a** and **d**) and budding-like organisms (panels **b**, **c** and **e**, **f**) stained with DAPI observed by fluorescence microscopy (*left*, panels **a**–**c**) and conventional microscopy (*right*, panels **d**–**f**), respectively. It is evident that nuclei are localized in the central part of the cell showing a schizogony-like cell, while the several nuclei are separated into the budding cells (×2,000)

the reproductive modes of *Blastocystis* are binary fission, budding, and possibly schizogony. The mode of schizogony, supportive of the production of progeny, has been only confirmed with light microscope observations and has not been clearly determined with electron microscopy. The amoeboid form may be proposed as another alternative reproduction process (Fig. 2.22). Recently, three modes of reproduction, binary fission, plasmotomy, and budding were proposed under ultrastructural insights (Zhang et al. 2011). Since the available data for identifying the other reproductive modes, plasmotomy, schizogony, and division of amoeboid form are only limited at present, further detail research on the other reproductive modes will be required to clarify whether these modes are truly exist on the life cycle of *Blastocystis*.

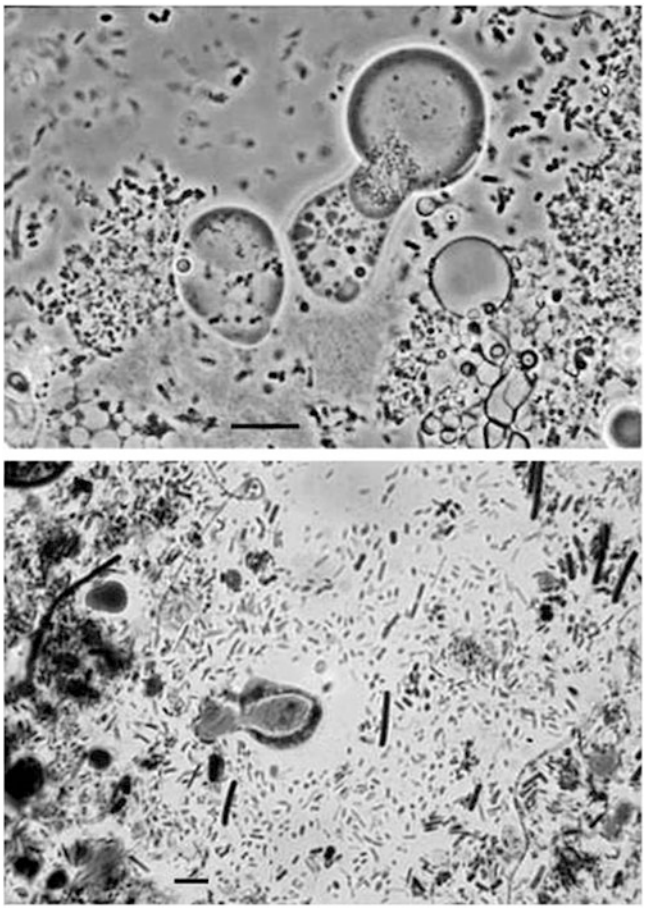

Fig. 2.24 The other alternative reproduction process of *Blastocystis* observed by phase-contrast microcopy and HIH stain. These morphologically irregular cells may show pseudopodal activity. Scale bar: 10 μm

2.3 Conclusions

Although *Blastocystis* is one of the common intestinal protozoa found in human and animal fecal samples, the taxonomy and reproduction modes of the organism are still not fully understood. Some of morphological appearances in light and electron microscopy are controversial surrounding this parasite is the lack of the uniformed consents. However, the recent accumulating evidences on the morphology and reproductive modes of various isolates promote that *Blastocystis* is more mysterious eukaryote and contribute to its biology gradually but steadily. Researches on the morphological approaches and identifications of the true reproductive stages or modes in the life cycle should be useful for the interpretation of a parasite infecting in the broad range of hosts from vertebrate to invertebrate animals.

References

Abe N, Nagoshi M, Takami K, Sawano Y, Yoshikawa H (2002) A survey of *Blastocystis* sp. in livestock, pets, and zoo animals in Japan. Vet Parasitol 106:203–212

Boreham PFL, Stenzel DJ (1993) *Blastocystis* in humans and animals: morphology, biology, and epizootiology. Adv Parasitol 32:1–70

Cassidy MF, Stenzel DJ, Boreham PFL (1994) Electron microscopy of surface structures of *Blastocystis* sp. from different hosts. Parasitol Res 80:505–511

Chen XQ, Singh M, Howe J, Ho LC, Tan SW, Yap EH (1999) In vitro encystation and excystation of *Blastocystis ratti*. Parasitology 118:151–160

Dubremetz JF (1973) Étude ultrastructurale de la mitose schizogonique chez la coccidie *Eimeria necatrix* (Johnson 1930). J Ultrastruct Res 42:354–376

Dunn LA, Boreham PFL, Stenzel DJ (1989) Ultrastructural variation of *Blastocystis hominis* stocks in culture. Int J Parasitol 19:43–56

Govind SK, Khairul AA, Smith HV (2002) Multiple reproductive processes in *Blastocystis*. Trends Parasitol 18:528

Govind SK, Anuar KA, Smith HV (2003) Response to Tan and Stenzel, and Windsor *et al*.: *Blastocystis* reproduction and morphology. Trends Parasitol 19:291–292

Hamblin K, Standley DM, Rogers MB, Stechmann A, Roger AJ, Maytum R, van der Giezen M (2008) Localization and nucleotide specificity of *Blastocystis* succinyl-CoA synthetase. Mol Microbiol 68:1395–1405

Lanuza MD, Carbajal JA, Borrás R (1996) Identification of surface coat carbohydrates in *Blastocystis hominis* by lectin probes. Int J Parasitol 26:527–532

Matsumoto Y, Yamada M, Yoshida Y (1987) Light-microscopical appearance and ultrastructure of *Blastocystis hominis*, an intestinal parasite of man. Zbl Bakt Microbiol Hyg Ser A 264:379–385

Moe KT, Singh M, Howe J, Ho LC, Tan SW, Ng GC, Chen XQ, Yap EH (1996) Observations on the ultrastructure and viability of the cystic stage of *Blastocystis hominis* from human feces. Parasitol Res 82:439–444

Pakandl M (1999) *Blastocystis* sp. from pigs: ultrastructural changes occurring during polyxenic cultivation in Iscove's modified Dulbecco's medium. Parasitol Res 85:743–748

Singh M, Ho LC, Yap ALL, Ng GC, Tan SW, Moe KT, Yap EH (1996) Axenic culture of reptilian *Blastocystis* isolates in monophasic medium and speciation by karyotypic typing. Parasitol Res 82:165–169

Stenzel DJ, Dunn LA, Boreham PFL (1989) Endocytosis in cultures of *Blastocystis hominis*. Int J Parasitol 19:787–791

Stenzel DJ, Boreham PFL (1991) A cyst-like stage of *Blastocystis hominis*. Int J Parasitol 21:613–615

Stenzel DJ, Boreham PFL, McDougall R (1991) Ultrastructure of *Blastocystis hominis* in human stool samples. Int J Parasitol 21:807–812

Stenzel DJ, Cassidy MF, Boreham PFL (1994) Morphology of *Blastocystis* sp. from domestic birds. Parasitol Res 80:131–137

Stenzel DJ, Boreham PFL (1996) *Blastocystis hominis* revisited. Clin Microbiol Rev 9:563–584

Stenzel DJ, Boreham PFL (1997) Virus-like particles in *Blastocystis* sp. from simian faecal materials. Int J Parasitol 27:345–348

Stenzel DJ, Lee MG, Boreham PFL (1997) Morphological differences in *Blastocystis* cysts—an indication of different species? Parasitol Res 83:452–457

Suresh K, Howe J, Ng GC, Ho LC, Ramachandran NP, Loh AK, Yap EH, Singh M (1994) A multiple fission-like mode of asexual reproduction in *Blastocystis hominis*. Parasitol Res 80:523–527

Suresh K, Mak JW, Chuong LS, Ragunathan T, Init I (1997) Sac-like pouches in *Blastocystis* from the house lizard *Cosymbotus platyurus*. Parasitol Res 83:523–525

Tan KSW (2004) *Blastocystis* in humans and animals: new insights using modern methodologies. Vet Parasitol 126:121–144

Tan KSW, Stenzel DJ (2003) Multiple reproductive processes in *Blastocystis*: proceed with caution. Trends Parasitol 19:290–291

Tan TC, Suresh KG (2006a) Predominance of amoeboid forms of *Blastocystis hominis* in isolates from symptomatic patients. Parasitol Res 98:189–193

Tan TC, Suresh KG (2006b) Amoeboid form of *Blastocystis hominis*—a detailed ultrastructural insight. Parasitol Res 99:737–742

Tan TC, Suresh KG (2007) Evidence of plasmotomy in *Blastocystis hominis*. Parasitol Res 101:1521–1525

Tan KSW (2008) New insights on classification, identification, and clinical relevance of *Blastocystis* spp. Clin Microbiol Rev 21:639–665

Teow WL, Ng GC, Chan PP, Chan YC, Yap EH, Zaman V, Singh M (1992a) A survey of *Blastocystis* in reptiles. Parasitol Res 78:453–455

Teow WL, Ho LC, Ng GC, Chan YC, Yap EH, Chan PP, Howe J, Zaman V, Singh M (1992b) Virus-like particles in a *Blastocystis* species from the seasnake, *Lapemis hardwickii*. Int J Parasitol 22:1029–1032

Wawrzyniak I, Roussel M, Diogon M, Couloux A, Texier C, Tan KSW, Vivares CP, Delbac F, Wincker P, EL Alaoui H (2008) Complete circular DNA in the mitochondria-like organelles of *Blastocystis hominis*. Int J Parasit 38:1377–1382

Windsor JJ, Stenzel DJ, Macfarlane L (2003) Multiple reproductive processes in *Blastocystis hominis*. Trends Parasitol 19:289–290

Yamada M, Matsumoto Y, Hamada S, Fujita S, Yoshida Y (1986) Demonstration and determination of DNA in *Pneumocystis carinii* by fluorescence microscopy with 4′,6-diamidino-2-phenylindole (DAPI). Zbl Bakt Microbiol Hyg Ser A 262:240–246

Yamada M, Yoshikawa H, Tegoshi T, Matsumoto Y, Yoshikawa T, Shiota T, Yoshida Y (1987) Light microscopical study of *Blastocystis* spp. in monkeys and fowls. Parasitol Res 73:527–531

Yoshikawa H, Hayakawa A (1996a) Morphological changes in the central vacuole of *Blastocystis hominis* during in vitro culture. Protoplasma 194:63–68

Yoshikawa H, Hayakawa A (1996b) Freeze-fracture cytochemistry of membrane cholesterol in *Blastocystis hominis*. Int J Parasitol 26:1111–1114

Yoshikawa H, Oishi K (1997) Ultrastructural localization of basic proteins of *Blastocystis hominis*. Protoplasma 200:31–34

Yoshikawa H, Yamada M, Yoshida Y (1988) Freeze-fracture study of *Blastocystis hominis*. J Protozool 35:522–528

Yoshikawa H, Kuwayama N, Enose Y (1995a) Histochemical detection of carbohydrates of *Blastocystis hominis*. J Eukaryot Microbiol 42:70–74

Yoshikawa H, Satoh J, Enose Y (1995b) Light and electron microscopic localization of lipids in *Blastocystis hominis*. J Electron Microsc 44:100–103

Yoshikawa H, Nagashima M, Morimoto K, Yamanouti Y, Yap EH, Singh M (2003) Freeze-fracture and cytochemical studies on the in vitro cyst form of reptilian *Blastocystis pythoni*. J Eukaryot Microbiol 50:70–75

Yoshikawa H, Morimoto K, Nagashima M, Miyamoto N (2004) A survey of *Blastocystis* infection in anuran and urodele amphibians. Vet Parasitol 122:91–102

Yoshikawa H, Wu Z, Howe J, Hashimoto T, Geok-Choo NG, Tan KSW (2007) Ultrastructural and phylogenetic studies on *Blastocystis* isolates from cockroaches. J Eukaryot Microbiol 54:33–37

Zaman V (1997) Phase-contrast microscopy of cell division in *Blastocystis hominis*. Ann Trop Med Parasitol 91:223–224

Zaman V, Ng GC, Suresh K, Yap EH, Singh M (1993) Isolation of *Blastocystis* from the cockroach (Dictyoptera: Blattidae). Parasitol Res 79:73–74

Zaman V, Khan KZ (1994) A concentration technique for obtaining viable cysts of *Blastocystis hominis* from faeces. J Pak Med Assoc 44:220–221

Zaman V, Howe J, Ng M (1995) Ultrastructure of *Blastocystis hominis* cysts. Parasitol Res 81:465–469

Zaman V, Howe J, Ng M, Goh TK (1999) Scanning electron microscopy of the surface coat of *Blastocystis hominis*. Parasitol Res 85:974–976

Zhang X, Qiao JY, Zhou XJ, Yao FR, Wei ZC (2007) Morphology and reproductive mode of *Blastocystis hominis* in diarrhea and in vitro. Parasitol Res 101:43–51

Zhang X, Zhang S, Qiao J, Wu X, Zhao L, Liu Y, Fan X (2011) Ultrastructural insights into morphology and reproductive mode of *Blastocystis hominis*. Parasitol Res 110(3):1165–1172

Zierdt CH (1973) Studies of *Blastocystis hominis*. J Protozool 20:114–121

Zierdt CH (1988) *Blastocystis hominis*, a long-misunderstood intestinal parasite. Parasitol Today 4:15–17

Zierdt CH (1991) *Blastocystis hominis*—past and future. Clin Microbiol Rev 4:61–79