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ORIGINAL PAPER

Ultrastructure and host parasite relationships of *Kudoa pagrusi* (Myxozoa) infecting the heart muscles of sea bream *Pagrus pagrus* (L.) from the Red Sea

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Abstract The present study is a part of a continuous investigation of myxosporean parasites-infecting fish of the Red Sea using light and electron microscopy. Out of 120, 80 (67%) *Pagrus pagrus* fish were found to be naturally infected with *Kudoa pagrusi*. The infection was intensive and appeared as clusters of ovoid to ellipsoidal plasmodia being restricted to the cardiac muscles. Histological studies elaborated tissue distortion at the sites of infection and the adjacent layers. The development of the plasmodia reduced the functional area of the heart muscle. Ultrastructural analysis showed that the plasmodia were surrounded by single-unit membrane with numerous projections and pinocytotic channels extended toward the host cell. The generative cells and the different developmental stages were arranged at the periphery of the plasmodia while immature and mature spores were centrally arranged. The present study showed the main criteria of this genus: the spores possess four polar capsules with four shell valves.

Introduction

Myxozoans are economically important fish parasites with more than 2,180 described species (Lom and Dyková 2006). The genus *Kudoa* Meglitsch, 1947 comprises myxosporean parasites with four valves, each of which contains one polar capsule. Species belonging to this genus are typically histozoic parasites of marine teleosts. However, since the establishment of the genus, a few coelozoic species have been described, too (Moran et al. 1999). There are currently 64 described species belonging to genus *Kudoa* (Myxozoa: Myxosporea : Multivalvulida), all parasitizing marine or estuarine fish (Diamant et al. 2005). Particular economic importance is attributed to muscle invading *Kudoa* species that release proteolytic enzymes causing flesh liquification which reduces considerable fish marketing value (Tsuyuki et al. 1982; Moran et al. 1999; Whipps et al. 2003). Some of the recorded species were proven to cause adverse effects on the fish population as causative agents of severe diseases to marine water fishes (Kostoingue et al. 2001; Adriano et al. 2005; Cuadrado et al. 2007). Due to their economic importance, myxosporean parasites infecting Egyptian fish received much attention during the last decades (Abdel-Ghaffar et al. 1995, 2005, 2008; Ali 1999; Abdel-baki 2009).In the present study, a detailed description is given for the plasmodia and sporogenesis of *Kudoa pagrusi* and its host parasite relationships by means of light and transmission electron microscopy.

Materials and methods

Fresh fish specimens were purchased from fishermen at Gulf of Suez. The fish samples were collected during regular visits between March 2008 and March 2009. A total of 120 sea bream (*Pagrus pagrus*) were grossly examined for myxosporean infection. The highly infected heart muscles were fixed in 10% neutral-buffered formalin for

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histological studies. For electron microscopy, small parts of the highly infected heart muscles were isolated and fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4), washed in the same buffer, and postfixed with 2% OsO_4 in the same buffer. The tissues were dehydrated in graded ethanol and embedded in araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and finally examined with a Zeiss 902 A electron microscope. Non-fixed spores were measured and photographed using a Zeiss Axiovert 100 microscope with C80 camera. Descriptions and measurements of spores followed the guidelines of Lom and Arthur (1989).

Results

Light microscopy

Plasmodia

The infection was detected as large clusters of plasmodia all over the heart (1 and 2 in Fig. 1). The prevalence was very high, 67% of the fish were infected (80 out of 120), and the intensity was very high. More than 100 cysts were counted per infected fish. Plasmodia were whitish in color, elliptical to ovoid in shape, measuring 1.73±0.5 (1.0–2.7) mm in length and 0.65±0.7 (0.63–0.80) mm in width (1 and 2 in Fig. 1).

Spore

In apical view, spores appeared quadrate with slightly rounded corners, and the suture line was slightly discriminated. The spore length was 5.3±0.8 (5.0–7.2) μm when seen laterally, while the width was 5.0±0.5 (5.4–6.8) μm. The four polar capsules were pyrifom in shape and were equal in size. They measured 1.8±0.5 (1.5–3.7) μm in length and 1.1±0.3 (1.0–2.0) μm in width. The windings of the polar filament and their pattern were not clearly encountered. The length of the polar filament reached 10–12 μm and was nearly four times longer than the length of the polar capsule when released (3, 4, 5, and 6 in Fig. 1).

Histopathological study

Histological study showed that the cysts were embedded partly or completely in the heart tissues (8 and 9 in Fig. 1). The growing cysts caused distortion at their site of infection and also in the adjacent layers. The host reaction was manifested by the encapsulation of the plasmodia with a thick layer of connective tissue (7, 8, and 9 in Fig. 1). Most of the heart muscles were substituted by numerous growing plasmodia which reduced the functional area of the heart muscles.

Ultrastructural study

Plasmodia

The plasmodia were bordered by a single-unit membrane which forms numerous projections and pinocytotic canals extending for various lengths into the host tissue (10 in Fig. 2). The plasmodial wall was covered by a fine granular coat preventing the direct contact between the parasite and the host tissue. Internal to the pinocytotic canals, there was a broad belt of ectoplasm containing a fine granular cytoplasm. Passing inward into the plasmodia, the endoplasm contained subspherical to ovoid mitochondria and various developmental stages of the parasite (10 in Fig. 2).

Sporogenesis

The earliest recognizable stages of sporogenesis were the generative cells. These cells were characterized by their large nuclei which usually occupied most of the cell volume (11 in Fig. 2). Sometimes, the nucleus lacked a distinct nucleolus. The homogeneous cytoplasm of these cells and of other developmental stages contained a number of subspherical mitochondria and numerous cytoplasmic ribosomes. Two generative cells lied close together. Then as first interaction among the cells during sporogenesis, one of them enveloped the other (12, 13, and 14 in Fig. 2). Subsequent developmental events involved divisions of the sporont and its progeny resulting in the pansporoblast stages. A pansporoblast consisting of three cells resulted from the division of the sporont (14 in Fig. 2). Cell differentiation as well as the arrangement of the differentiated cells seemed to occur rapidly in this myxozoan parasite (15 in Fig. 2). Sporont progeny of the pansporoblastic stages achieved some differentiation into their final positions for actual spore construction (16 in Fig. 2).

Capsulogenesis

Capsulogenesis was apparently the first process to be completed during spore formation. Capsulogenic cells were characterized by relatively large amounts of lacunes of the endoplasmic reticulum, by several mitochondria, a distinct nucleus, and polar capsules at various degrees of maturity within the cytoplasm (11 and 15 in Fig. 2).

The first recognizable stage was the capsular primordium-differentiated cells of which had not yet reached their final position (16 in Fig. 2). It appeared as a trilaminar structure with an outer electron dense zone, an adjacent electron lucent zone, and a fine granular cortex (23 in Fig. 4). The external tubules were seen in the cytoplasm of the capsulogenic cells either being surrounded (16 in Fig. 1) or connected with the capsular primordium (19 in Fig. 3).

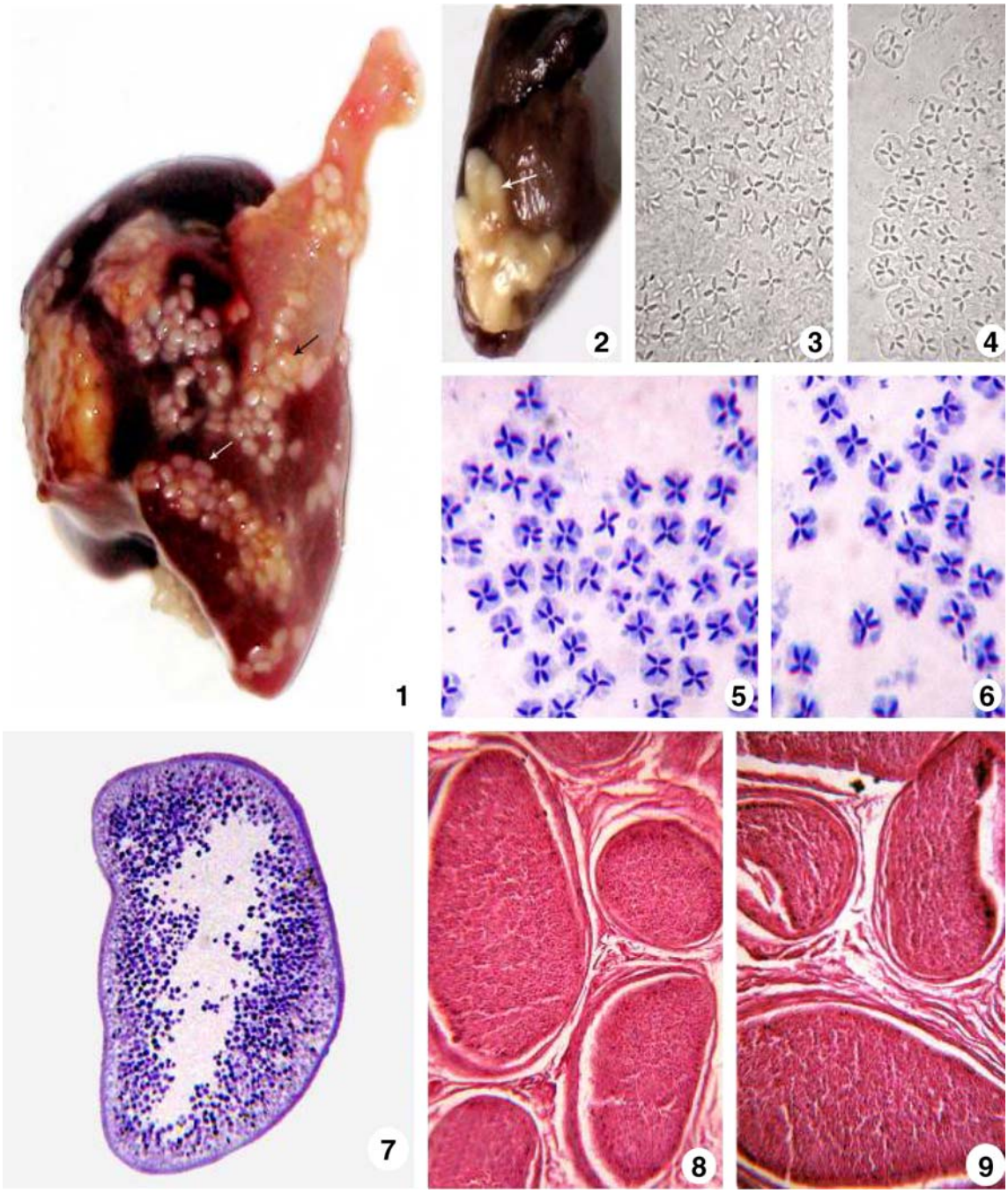
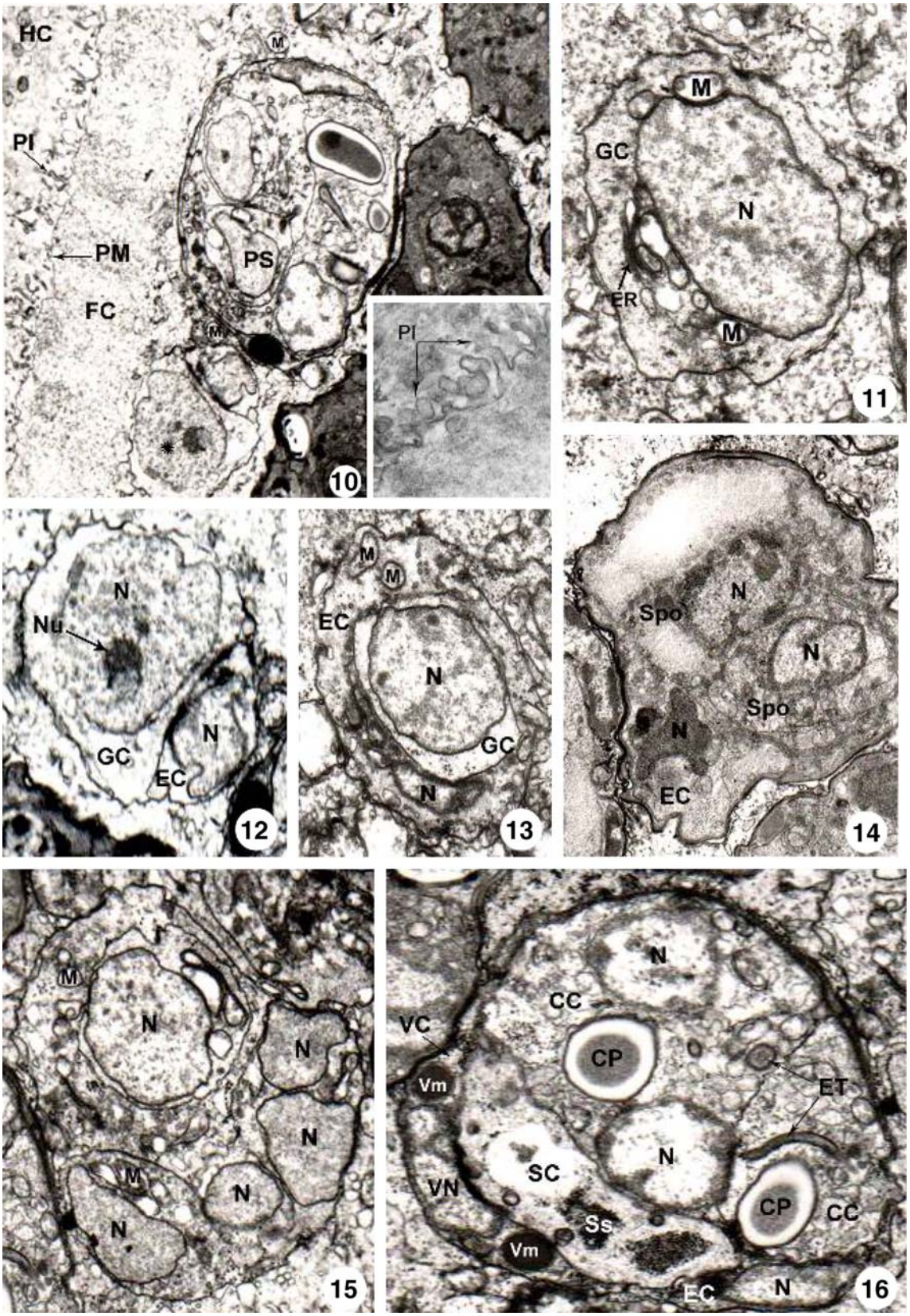


Fig. 1 *1*, *2* Photographs of *K. pagrusi* plasmodia (cysts) in the heart of sea bream fish *P. pagrus* (*arrow*). *3*, *4* Photomicrographs of fresh spores of *K. pagrusi*. ×1,000. *5*, *6* Photomicrographs of giemsa-stained spores with polar capsules of *K. pagrusi*. ×1,000. *7* Photomicrograph of a semithin section through *K. pagrusi* plasmodia surrounded with connective tissue and stained with toluidine blue. ×70. *8*, *9* Photomicrographs of histological sections through infected heart with cysts of *K. pagrusi*. The cysts were surrounded with connective tissue (H &E). ×70

Accumulations of granular materials were observed within the external tubule similar to the capsular primordium (19 in Fig. 3). Finally, four polar capsules were recognized within the fully developed mature spores (24 in Fig. 4). The development of the polar capsules was not always synchronized. One polar capsule might have been nearly fully developed, while the other was still in the process of polar filament formation (22, 23, and 24 in Fig. 4).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◀ **Fig. 2** Electron micrographs showing: *10* the plasmodial wall (*PM*), the adjacent host tissue (*HC*), the pinocytotic canals (*PI*), and the ectoplasm which contains a fine granular cytoplasm (F*C*). Note the presence of mitochondria (*M*), early enclosed state of development (*asterisk*), and pansporoblast stage (*PS*) in the outer region of the endoplasm. ×7,000; *11* unicellular stage (generative cell (*GC*)) with a large nucleus (*N*), nucleolus (*NU*). *M* mitochondria, *ER* endoplasmic reticulum. ×20,000. *12* Early enclosed state (one generative cell enveloping the other). Generative cell (*GC*), envelope cell (*EC*), and nucleus (*N*). ×20,000. *13* Two-celled pansporoblast originating from the envelopment of a sporont by an envelope cell (*EC*). Generative cell (*GC*), nucleus (*N*), nucleolus (*NU*), and mitochondria (*M*). ×20,000. *14* Three-celled pansporoblast apparently resulting from the division of the sporont within the envelope cell being composed of two daughter sporonts (*Spo*) within the envelope cell (*EC*), nucleus (*N*). ×12,000. *15* Early pansporoblast stage with five daughter sporonts (*Spo*) within the enveloping cell. ×12,000. *16* Young pansporoblast within enveloping cell (*EC*) containing two capsulogenic cells (*CC*) with two capsular primordium (CP), external tubules (ET), capsulogenic nucleus. The valvogenic cells (*VC*) containing valvogenic nucleus (*VN*), valve forming materials (*Vm*). The sporoplasm cell (*SC*) containing sporoplasmosomes (*Ss*). *N* nucleus. ×12,000

Valvogenesis

Two valvogenic cells usually surrounded each diplokaryotic sporoplasm and two capsulogenic cells (19 in Fig. 3), finally giving rise to two valves surrounding each spore. There was an accumulation of electron dense bodies ("valve forming material") in the cytoplasm of the valvogenic cells in the mature spore (20 in Fig. 3). Also, numerous microtubules were observed parallel to the ridge (20 in Fig. 3 and 21 in Fig 4). Immature and mature spores had highly electron dense valves which were composed of an outer and an inner wall being continuous except at the sutural zones where the valves appeared thickened and overlapping (20 in Fig 3 and 24 in Fig. 4).

Sporoplasm

During the maturation of sporoplasm, it showed one or two nuclei. The sporoplasm contained the electron-dense sporoplasmosomes (17 and 19 in Fig. 3) and the normal constituents such as mitochondria, cytoplasmic ribosomes, lacunae of the endoplasmic reticulum (17, 19, and 20 in Fig. 3), and glycogen-like material (20 in Fig. 3).

Discussion

The class Myxosporea includes two orders, the Multivalvulida and Bivalvulida (Lom and Dyková 1992). The genus *Kudoa* has been assigned to the species of the Multivalvulida group that have four shell valves and with four polar capsules (Whipps et al. 2003). Since the early establishment of the genus *Kudoa* Meglitsch, 1947, about 45 species were recognized (Moran et al. 1999). Since then, several other *Kudoa* spp. have been described, *Kudoa aegyptia* (Koura 2000), *Kudoa dianae* (Dyková et al. 2002), *Kudoa quadricornis*, and *Kudoa permulticapsula* (Whipps et al. 2003), and others that have been transferred from genera synchronized with *Kudoa* (Whipps et al. 2004).

Spore measurements of the present species were more or less different from the majority of the previously described species. However, only *K. aegyptia* (Koura 2000) could be compared to the current myxosporean. *K. aegyptia* can be distinguished from the present species in having quite longer and wider spores. The measurements of the present species coincide with those described by Al Quraishy et al. (2008). Therefore, the present species was also identified as *K. pagrusi*. The detection of *K. pagrusi* spores in the cardiac muscles of the sea bream *P. pagrus* was of interest because it is the only recorded *Kudoa* specie that heavily infects this important vital host site.

Regarding histopathological effects, the present study showed that the parasite *K. pagrusi* is a pathogenic species. The site of infection described was restricted to the cardiac muscles. Most other members of the genus *Kudoa* form macroscopic cysts in the muscles and cause tissue degradation in a broad range of hosts (Egusa 1986; Moran et al. 1999).The plasmodia contained immature spores and sporogenic stages at the periphery, while the center of the plasmodium was filled with mature spores, a finding in agreement with Ali (1999) and El-Mansy and Bashtar (2002). The growing cysts caused distortion at their site of infection and also in the adjacent layers. These observations are also in agreement with those of Kabata and Whitaker (1981) and Al Quraishy et al. (2008). Inflammations were also recorded by other authors as a principle of defense mechanism utilized by fish to combat myxosporidian infections. Histozoic plasmodia of *Kudoa* and *Henneguya* spp. often elicit the formation of an envelope originating either from host connective tissue or from a compression of the adjacent host cell (Moran et al. 1999). Such observations were recorded in the present study, too.

Ultrastructural studies showed that the host parasite interaction is species-specific and depends on the developmental stages of the parasite (Voelker et al. 1978; Lom et al. 1983; Moran et al. 1999)

Plasmodium

The ultrastructural studies of the plasmodial wall of myxosporeans are of major importance to show the characteristic features of this dangerous group of parasites (Current and Janovy 1976). The plasmodia of the present

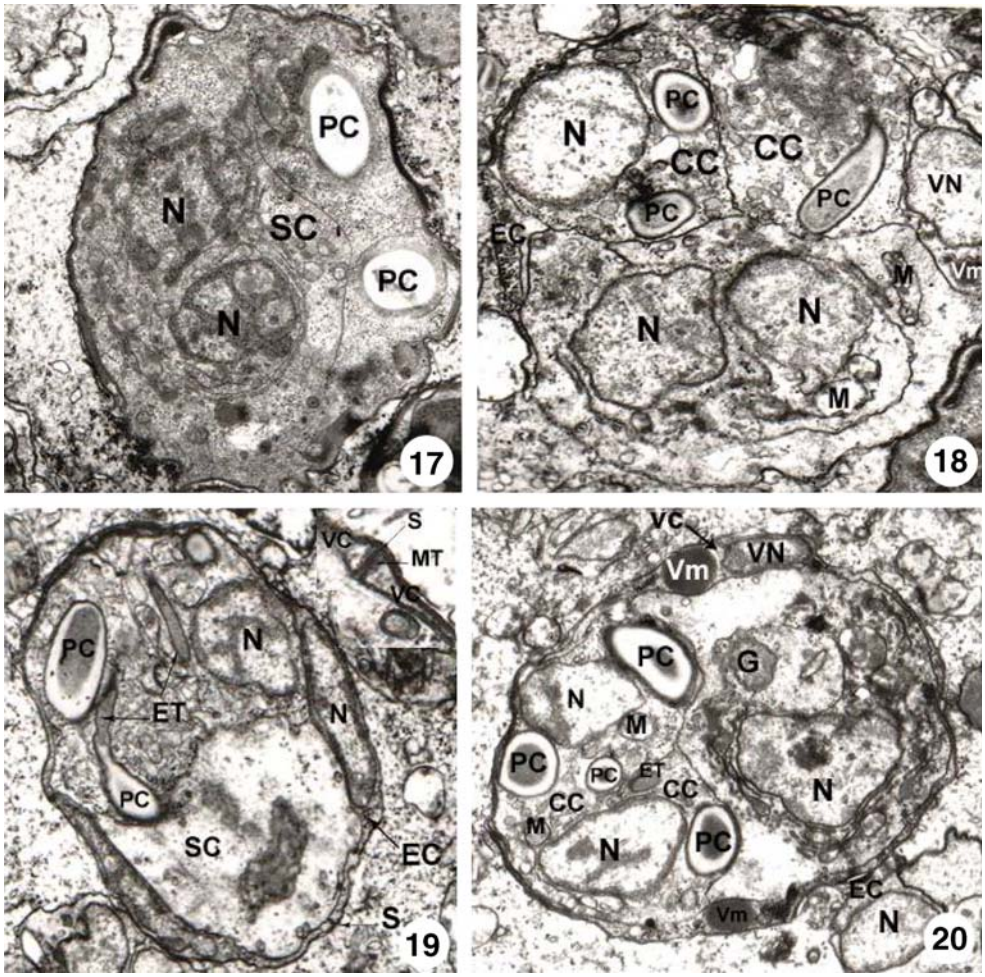

Fig. 3 *17* A pansporoblast containing two polar capsules (*PC*) and a sporoplasm cell (*SC*). Two nuclei (*N*) of the diplokaryotic sporoplasm are seen. ×20,000. *18* A pansporoblast within an envelope cell (*EC*). The capsulogenic cells (*CC*) containing polar capsules (*PC*) and a nucleus (*N*). On one side of the pansporoblast, the valvogenic cell (VC), its nucleus (VN) and valve forming material (Vm) are situated, mitochondria (M). ×20,000. 19 Electron micrographs showing: Section through capsulogenic cell showing the polar capsules (PC) with external tubule (ET). One of the polar capsules is attached to the external tubule while the other not, indicating an asynchronous development. Note the suture between two valvogenic cells (arrow). Inset: ultrastructural detail of the two equal valves, the sutural line (S), envelope cell (EC), microtubules (MT), nucleus (N). ×12,000. 20 Electron micrographs showing: Section through a developing sporoblast, showing four polar capsules (PC) with an external tubule (ET). Envelope cell (EC), valvogenic cell (VC), its nucleus (VN) and valve forming material (Vm), glycogen material (G), mitochondria (M). ×12,000

species were bordered by a single-unit membrane which was continuous with numerous pinocytotic canals extending for a various length into the host tissue. The plasmodial wall was covered by a fine granular coat preventing the direct contact between the parasite and the host tissue. This is in agreement with the findings in other myxosporeans described by Current and Janovy (1976), Desser and Paterson (1978), and Abdel-Aziz (1990). The plasmodium wall can supply various developing stages obtaining suitable nutrients necessary for growth via the pinocytotic canals which may incorporate host cell cytoplasm by pinocytosis. These findings are in agreement with other studies (Schubert 1968; Current and Janovy 1976; Desser and Paterson 1978; Abdel-Ghaffar et al. 2005).

Similar to other myxosporeans, the peripheral portions of the plasmodia of the present parasite were usually filled with generative cells and young pansporoblasts while the later stages of the spore development were concentrated more centrally. On the other hand, young plasmodia contained relatively few mature spores, whereas in older (mature) plasmodia, immature and mature spores were observed. Mature spores of Myxosporea are identified as those exhibiting a very dense cytoplasm surrounded by extremely electron-dense valves with a completely formed shell and fully formed polar capsules containing completely formed and identifiable polar filaments with no capsulogenous or valvular nuclei. All other stages were considered immature (Lom and de Puytorac 1965; Schubert 1968; Lom 1969).

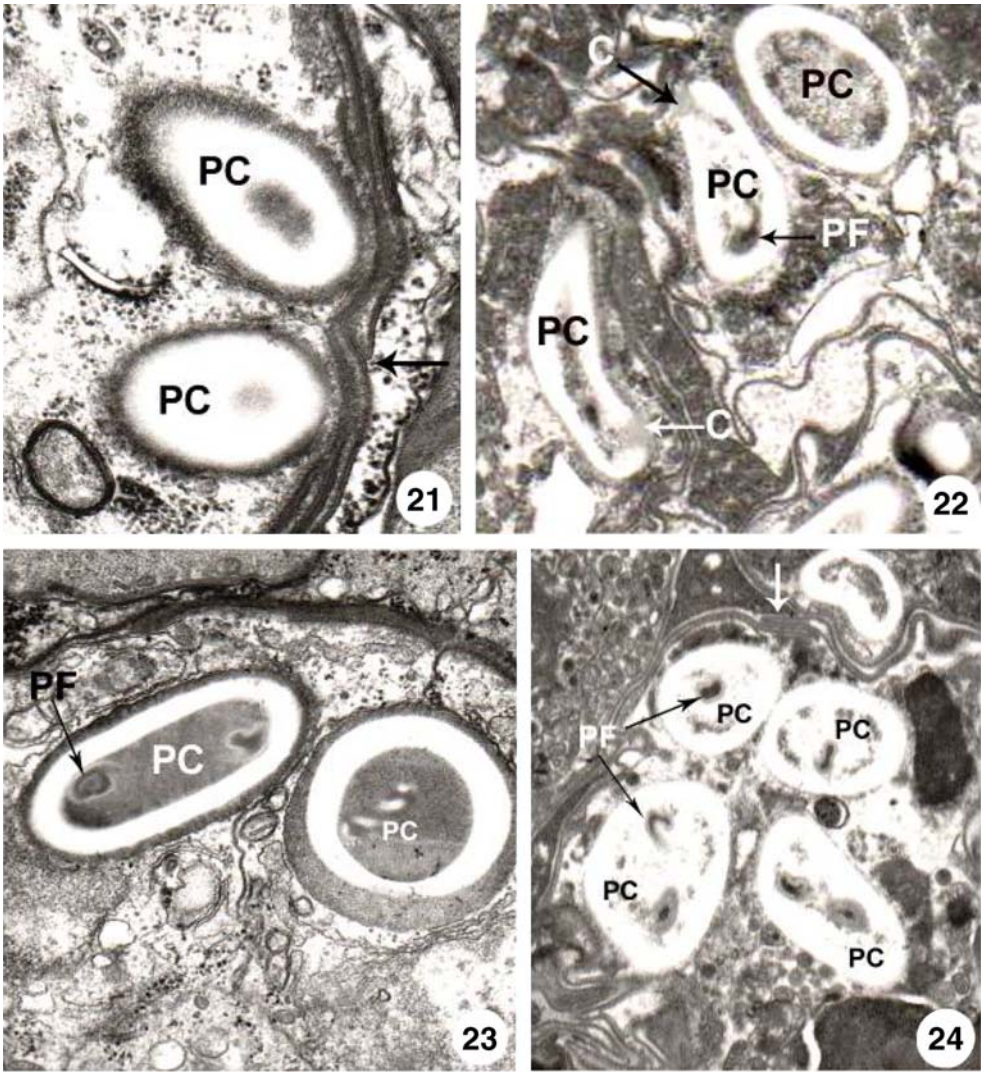

Fig. 4 . *21* Section through the spore apex showing details of desmosome-like valve junction at sutures (*arrow*) and two polar capsules (*PC*). Note the extensive overlapping of valves at sutures. ×30,000. *22* Cross-section through maturing spore showing the almost mature polar capsule (*PC*) with polar filament coils (*PF*). Note the presence of cap-like cover plugged the apex of the polar capsule (*C*). ×20,000. *23* Section through a capsulogenic cell showing two nearly mature polar capsules (*PC*) showing the asynchronous development. One of the polar capsules is seen within an early stage of polar filament formation while the other still has the shape of primordium. ×20000. *24* Cross-section through mature spore showing the four polar capsules (*PC*) with polar filaments (*PF*), note the valvular suture (*arrow*). ×20,000

Sporogenesis

In the present investigation, sporogenesis started with the formation of the pansporoblast stage: the enclosure of a generative cell by an envelope, following the pattern of other myxosporeans (Lom and de Puytorac 1965; Abdel-Ghaffar et al. 2005, 2008). Some authors stated that the spore development does not appear to start as a result of such "an engulfing process" (Stehr 1986). Subsequent developmental events with cellular divisions resulted in a pansporoblast giving rise to sporont progeny cells. The later divided until they reached the five-cell stage. The differentiation of the sporont progenesis into spore-producing units occurred as rapid as in several other myxosporeans (Lom and de Puytorac 1965; Abdel-Aziz 1990; El-Mansy and Bashtar 2002; Abdel-Ghaffar et al. 2005, 2008).

Capsulogenesis

Cheisin et al. (1961) showed that the polar capsule has a spherical or elongated shape and contained an inverted polar filament; the walls of which were continuous with the walls of the capsular primordium. The capsules appeared as membrane-bound, bulb-like, dense structures known as capsular primordium which were usually attached at their ends with a long external tubule. Microtubular participation in capsulogenesis of several myxosporidians was suggested by several authors (Lom 1969; Desser and Paterson 1978). They added that intracellular movements and contractions are involved in the polar capsule formation due to the fact that microtubules have been implicated in the movement of a wider variety of molecular and macromolecular components in the cells (Williams and Wolff 1970; Wessels et al. 1971).

In the present parasite, the filament appeared firstly in the external tubule while the capsular primordium still was filled with granules without any traces of a filament. Similar results were given by other investigators (e.g., Lom and de Puytorac (1965)). The rudimentary polar filament is formed within the capsular primordium and the external tubule possibly formed from the dense granular material present in both structures (Desser et al. 1983). On the other hand, it is generally thought that the external tubule invaginates into the polar capsule and undergoes reorganization to become the polar filament (Lom and de Puytorac 1965; Schubert 1968; Desser and Paterson 1978; El-Mansy and Bashtar 2002; Matos et al. 2005). The development of the four polar capsules was not always synchronized, a finding in contrast to the studies of Abdel-Aziz (1990) and Abdel-Ghaffar et al. (2005, 2008).

Valvogenesis

In most multivalvulid species including the present one, the plane of the junctions of valvogenic cells, is manifested as the sutural line and is set in a plane perpendicular or oblique to the spore circumference. A similar finding was recorded in other myxosporean studies (Abdel-Ghaffar et al. 2005, 2008). In Myxosporea, microtubules were not only involved in the formation of the polar capsule, but they were also observed in the valvogenic cells during sporogenesis. As valvogenesis proceeded, several microtubules appeared within the cytoplasm of the valvogenic cells, especially in the valve-suture-forming regions (Lom and Dyková 1988; Current and Janovy 1977; Current 1979; Current et al. 1979; Desser and Paterson 1978; El-Mansy and Bashtar 2002; Reimschuessell et al. 2003; Diamant et al. 2005).

Spore maturation

When sporoplasm maturation proceeded, an increase in the number of mitochondria and accumulation of glycogen particles has been reported for several myxosporidian species (Current and Janovy 1977; Current 1979; Current et al. 1979; El-Mansy and Bashtar 2002; Matos et al. 2005). These authors suggested that maturation of sporoplasm "germ cell" involved storage of metabolic reserves and acquisition of aerobic metabolism which, under the proper stimulus, could provide energy necessary for exsporulation and establishment of the sporont within a new host.

Conclusions

Dyková et al. (2002) stated that light and electron microscopic investigations are the best way to identify and classify the genus *Kudoa*. The presence of four sutures and four polar capsules in the spore of the present species supported the view that as important criteria, there are four shell valves with four polar capsules. This finding is in agreement with the results of Dyková et al. (2002) and Whipps et al. (2003).

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