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ORIGINAL PAPER

Microsporidian parasites: a danger facing marine fishes of the Red Sea

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Abstract Out of 600 marine fish from the Red Sea belonging to three different species that were collected and examined for microsporidian parasites, 87 (14.5%) fish were found to be infected. The infection was recorded as cysts or xenomas embedded in the gut epithelium and the peritoneal cavity of the three fish species. The highest percent of infection with microsporidian parasites was recorded in *Saurida tumbil* 19.5% (39/200) followed by *Pagrus pagrus* 15% (45/300) and the lowest percent of infection was recorded in *Epinephelus chlorostigma* 3% (three out of 100). After rupture of the cysts, the spores were released and examined by light microscopy. Each spore was elongated to ellipsoidal in shape and possessed a posterior vacuole which is characteristic to phylum Microspora. They measure 1.6±0.5 μm (1.5–2.4 μm)×1.3±0.1 μm (1.3–2.0 μm) in *Saurida tumbil* and *Pagrus pagrus*, respectively. The spores of *Pleistophora* sp recorded from *E. chlorostigma* were ovoid to pyriform in shape and measure 1.9±0.5 μm (1.8–2.7 μm)×1.6±0.4 μm (1.5–2.4 μm).

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Introduction

Members of the primitive eukaryotic protozoan phylum Microspora (Sprague 1977) are obligate parasites infecting most invertebrate and some vertebrate groups, including bony fish (Canning and Lom 1986; Vossbrinck et al. 1987; Lom and Dykova 1992; and Sprague et al. 1992). Many microsporidians provoke severe disease in wild and farmed fish populations causing major losses (Lom and Dykova 1992; Grabda 1978; Estevez et al. 1992). Microsporidian infection has been described from many marine and freshwater fish in Egypt (El-Deep 2002).

Identification of microsporidians is based largely on ultrastructural features of the spores and/or on the characteristic cell structure of the developmental stages (Lom and Dykova 1992; Vavra et al. 1981; and Leiro et al. 1996). The spore is the most conspicuous and morphologically distinctive stage in the life cycle of microsporeans. The spore wall is composed of several layers and possesses a coiled polar filament whose basal part is joined with the polar cap at the anterior pole of the spore. Furthermore, the spore contains an anteriorly swelling organelle, a polaroplast, a fluid-filled vacuolated area at the posterior end of the spore, and the sporoplasm (Lom and Corliss 1997). The growth and proliferation of the microsporidian protozoan fish parasites always result in the complete destruction of the infected fish (Lom and Dykova 1992). Most studies of fish parasitic disease in Egypt have been conducted on freshwater fishes, thus it is important from the economic point of view to increase knowledge on parasitic diseases in marine fishes especially on fishes of the Red Sea.

The present investigation reports results on microsporidian species parasitizing some economically important marine fishes from the Red Sea.

Materials and methods

Six hundred Red Sea fishes representing three species (*Saurida tumbil*, *Pagrus pagrus*, and *Epinephelus chlorostigma*) were investigated randomly from September 2007 to April 2009. Freshly caught live small and medium-sized fish samples were collected from boat landing sites and sometimes from the markets in the Suez and Hurghada Red Sea. Fishes were immediately transported to the laboratory at the Zoology Department, Faculty of Science, Cairo University using special boxes with good aeration and cooling or when necessary living fish were kept in an aquarium. Fishes were identified according to Randall (1992). Macroscopic examination of the different organs was carried out to detect any visible myxosporidian or microsporidian cysts. Gross microscopic examinations of all organs for myxosporidian and microsporidian infections were done. Also squash samples of tissues and fluids of the different organs were examined.

Fresh plasmodia were taken from each infected fish and teased on glass slides to release their spores. Spores were examined by using an oil immersion objective with a critically adjusted illumination and measured with the help of a calibrated ocular micrometer. Only mature and undamaged spores were chosen for measurements or preserved as semi-permanent mounts as described by Lom and Dykova (1992). Highly infected organs were fixed in 10% phosphate-buffered formalin for histological examination. Paraffin sections of 5–7 μm were stained with haematoxylin and eosin by procedures outlined by Luna (1960). Light micrographs were taken using a Zeiss Axiovert 135 microscope equipped with a Canon digital camera. The measurements are presented as mean±SD (range) of at least 50 samples.

Results

The present survey reported that 13.2% (87/658) of the examined fish were infected with microsporidian cysts located in the peritoneal cavity of the three fish species. The highest infection rate was recorded in *Saurida tumbil* 19.5% (39/200) followed by *Pagrus pagrus* 15% (45/300) while the lowest percentage of infection was found in *E. chlorostigma* 3% (three out of 100). Concerning the seasonal prevalence of the microsporidian parasites, it was noted that it generally increased during winter with a mean of 19.6% (78/397) and decreased to 3.4% (nine out of 261) during summer. Infection rate was 26.7% (32/120) during the winter fall to 8.7% (seven out of 80) in summer in *Saurida tumbil* while *Pagrus pagrus* showed 24.5% (43/175) in winter and only 1.6% (two out of 125) during summer. The lowest infection rate was recorded in *E. chlorostigma*, only during winter reaching 4.5% (three out of 67).

Microsporidian parasites recorded from *Saurida tumbil* fish

The examination of all organs revealed the presence of whitish macroscopic cysts embedded in the peritoneal cavity (Figs. 1, 2). In heavy infections, the parasites spread in many organs of the body, where they infected muscles, connective tissue of ovaries, and the intestinal epithelium. Early infections appeared as minute whitish cylinders about 0.6 mm in length that developed to tumor-like masses of often up to 2 cm in diameter. Fish microsporidia are embedded directly in the cytoplasm of the host cells which they destroy inducing an enormous hypertrophy. These formations transformed into xenoma within which the developing parasite and host cell components were included. Heavy infected muscles were hypertrophied and could easily become disrupted. Xenomas seen in semi-thin sections were spherical (Fig. 3). At high magnification, it was observed that the xenomas were covered by a wall encircling numerous spores in close contact with the cytoplasm of the hypertrophied host cell. Histopathological observations showed that parasitic foci were encapsulated by a fibrous layer produced by the host and was filled with mature spores. Rupture of cysts set free numerous ellipsoidal microsporidian spores (Figs. 3, 4).

Description of the spores

Spores of this microsporidian species were released from the infected fish by rupture of cysts. The spores were elongated ovoid or ellipsoidal in shape with a posterior vacuole reaching the midpoint of the spore (Fig. 4). They measured 1.6±0.5 μm (1.5–2.4 μm)×1.3±0.1 μm (1.3–2.0 μm) in size. Under pressure, fresh spores ejected their filament. The spores observed were situated within a sporophorous vesicle which was covered by a dense envelope (Fig. 5).

Microsporidian parasites recorded from *Pagrus pagrus*

The marine fish *Pagrus pagrus* had a high infection rate of 24.5% (43/175) while in summer the infection was low reaching 1.6% (two out of 125). During the course of the present study, a mixed infection of microsporidia with *Kudoa pagrusi* (Myxosporea) was detected. It occurred only in winter at a rate of 2.2% (four out of 125). Studies of

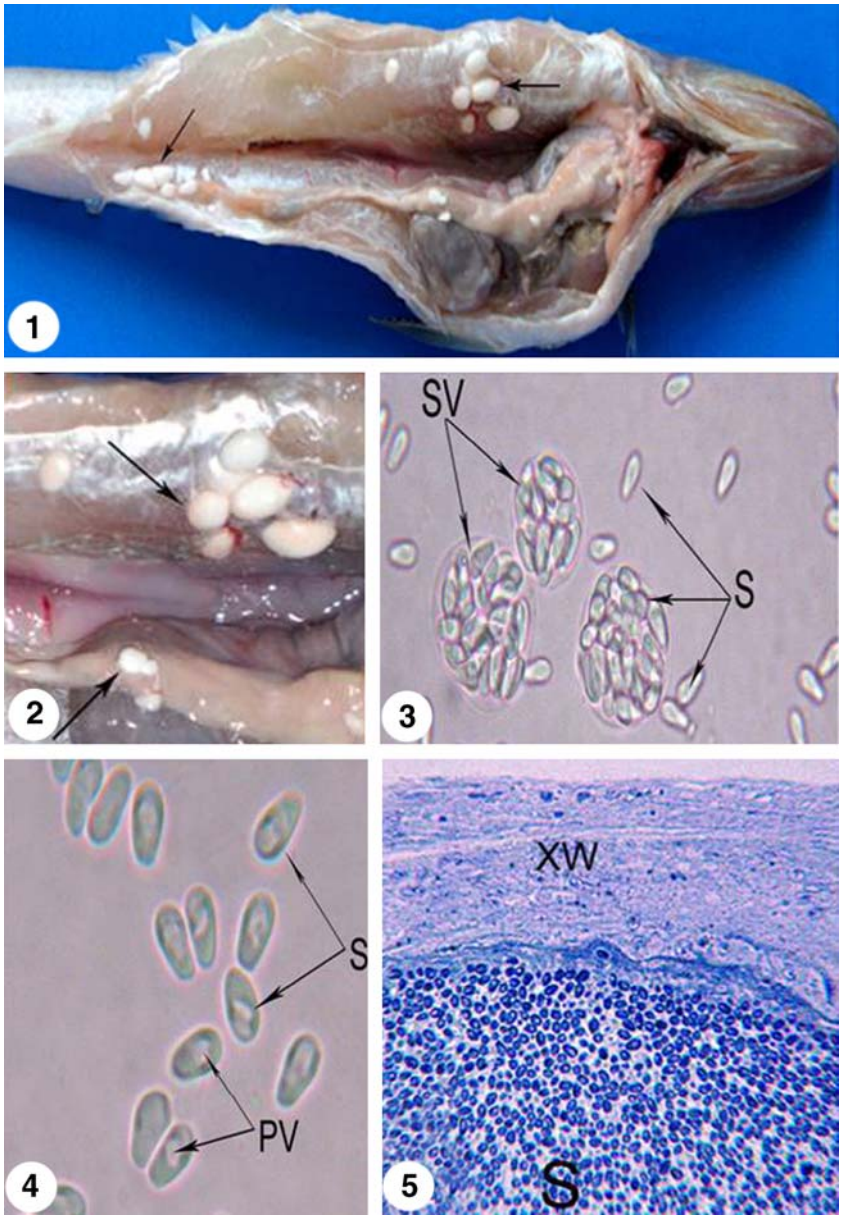

Fig. 1 Photograph of dissected *Saurida tumbil* fish infected with microsporidian parasites
Fig. 2 Infected *Saurida tumbil* fish. The infection is seen as whitish cysts (*arrows*) enclosing spores. The cysts are embedded in muscles as well as many organs
Fig. 3 Photomicrograph of spores (*S*) of the microsporidians infecting *Saurida tumbil* fish. The spores are mostly included within special structures known as sporophorous vesicle (*SV*). ×2,000
Fig. 4 High magnification of free spores (*S*) showing a posterior vacuole (*PV*) situated at the posterior pole. ×2,500
Fig. 5 Photomicrographs of semi-thin sections of the microsporidian cysts infecting *Saurida tumbil* stained with toluidine blue. Note the large number of spores (*S*) within the cysts. Each cyst or xenoma is limited by a wall (*XW*) which is species specific. ×2,200

fish organs revealed that microsporidiosis was spread in young and old fish. The infection could be identified as cloudy whitish macroscopic cysts along the surface of all inner organs of the peritoneal cavity and as tumor-like masses of up to 2.0 cm (Fig. 6). The infected organs became hyalinized and were destroyed. After rupture of the cysts, numerous spores appeared ovoid to pyriform in shape with a posterior vacuole (Figs. 7, 8). Unfixed spores measured 1.7±0.6 μm (1.5–2.7 μm)×1.5±0.3 μm (1.2–1.8 μm) in size. The coiled polar filament become spontaneously released or under pressure (Fig. 8). No sporophorous vesicles were detected in spores of this species. At high magnification, it was observed that the xenomas or cysts were encapsulated by a fibrous wall filled with fibroblasts encircling numerous spores which were studied in the cytoplasm of the hypertrophied host cell (Fig. 9).

Pleistophora sp. (Abdel-Ghaffar et al. 2009) recorded from *E. chlorostigma*

Only three fishes out of 100 (3%) were found to be infected with *Pleistophora* sp. during the winter season. Numerous macroscopic melanized black cysts (xenomas), ranging in size from 3 to 5 mm, were observed throughout the peritoneal cavity embedded in different organs (intestine, stomach, and muscles) (Fig. 10). These xenomas were surrounded by a thick melanized wall. Xenomas contained numerous spores in close contact to the cytoplasm of this hypertrophied host cell (Figs. 11, 12). Histological obser-

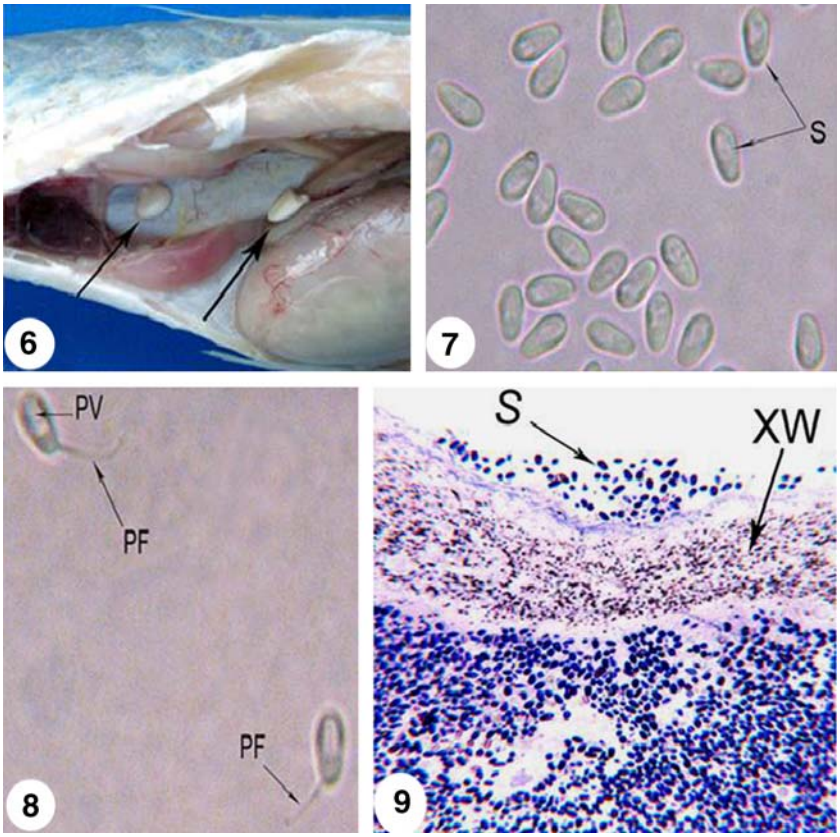

Fig. 6 Photograph of *Pagrus pagrus* infected with microsporidian parasites. The infection occurs in the form of xenomas enclosing spores (*arrows*) which appear as whitish cysts embedded within the interior of different organs (muscles, stomach) inside the peritoneal cavity of the fish

Fig. 7 Photomicrograph of freshly obtained pyriform spores (*S*) of the microsporidian parasites recorded from the *Pagrus pagrus*. The spores released after squeezing and rupture of cysts. Each spore contains a posterior vacuole (*PV*) which is considered as main constituent of mature spores. ×3,000

Fig. 8 Photomicrograph of freshly obtained microsporidian spores infecting *Pagrus pagrus*. The spore contains a posterior vacuole (*PV*) and an extruded polar filament (*PF*). ×3,000

Fig. 9 Photomicrograph of semi-thin sections of the microsporidian xenoma isolated from the peritoneal cavity of *Pagrus pagrus* showing the xenoma wall (*XW*) and the large number of spores (*S*). ×2,200

vations showed that the parasitic foci were encapsulated by fibrous layers and were filled with spores (Figs. 13, 14). Unfixed, fresh spores were mostly ovoid to pyriform in shape measuring 1.9±0.5 μm (1.8–2.7 μm)×1.6±0.4 μm (1.5–2.4 μm) in size and possessed a large vacuole at the posterior end (Fig. 12). Sporophorous vesicles containing a large number of mature spores were usually observed after rupture of the xenoma (Fig. 11). The polar filament was released spontaneously or under pressure (Fig. 12).

Discussion

In the present study, the infection rate with microsporidians was increased during winter and decreased in summer. These results agree with Fielding et al. (2005), who study the prevalence of *Pleistophora mulleri* infecting *Gammarus duebeni celticus* and found that the prevalence of this parasite was strongly seasonal, ranging from 8.5% in summer to 44.9% in winter. Also, Bcker and Speare (2004) claimed that number of microsporidian xenoma may be dramatically decreased when water temperature declines.

The three studied microsporidia destroyed their host cell and induced enormous hypertrophy of the infected fish cells and tissues. These results agree with Lom and Dykova (2005), who stated that microsporidia causing xenoma in fish offer an insight to cell pathology. The microsporidian parasites in the three fish species examined revealed the presence of whitish xenomas or cysts in *Pagrus pagrus* and *Saurida tumbil* or black xenomas in *E. chlorostigma* in the body cavity of which makes it unsuitable for sale (Fig. 10). Similar observations have been recorded by some authors (Canning 1976; Weissenberg 1976; Matos et al. 2005; Kent and Speare 2005; Lom and Dykova 2005; Lovy et al. 2007; Casal et al. 2008; Stephens 2009). All these authors stated that the type of host–parasite relationship involving microsporidia is often characterized by the production of xenomas. Similar results were documented also in *Pleisto-*

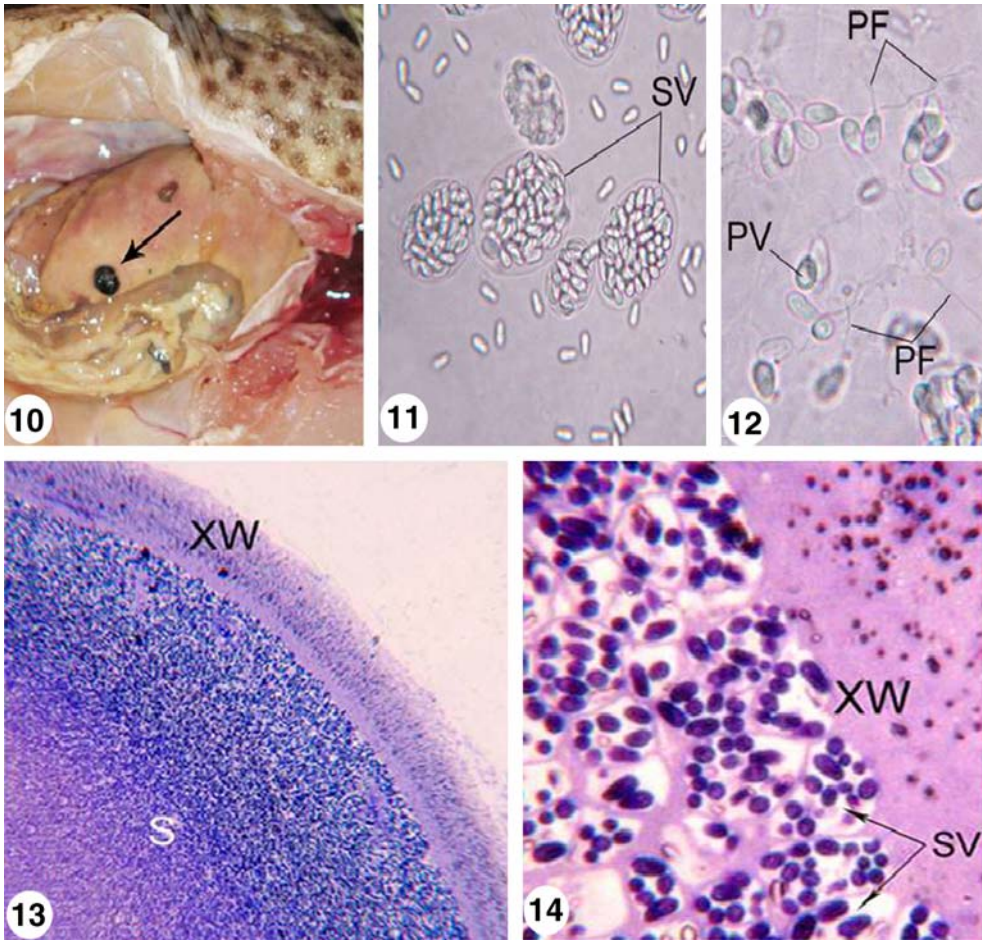

Fig. 10 Photographs of the marine *E. chlorostigma* fish infected with the microsporidian parasite *Pleistophora* sp. The infection appeared in the form of melanized black cysts or xenomas in a huge number embedded within the peritoneal cavity (*arrows*)

Fig. 11 Photomicrograph of unfixed, fresh spores of *Pleistophora* sp. after rupture of cysts. The spores (*S*) were contained within sporophorous vesicles (*SV*) which are surrounded by the sporophorous wall. ×2,500

Fig. 12 Photomicrograph of fresh unstained, unfixed preparations of *Pleistophora* spores showing the structure of the extrusion apparatus which is mainly composed of polar filament (*PF*) extruding from the spore. ×2,300

Fig. 13 Photomicrograph of a semi-thin section of parts of the *Pleistophora* sp. cyst from the peritoneal cavity of *E. chlorostigma* fish. The xenoma is surrounded by the xenoma wall (*XW*) that is formed by a layer of fibrillar material surrounding numerous mature spores (*S*) and sporonts. ×1,000

Fig. 14 Photomicrograph at higher magnifications of xenomas showing sporophorous vesicles (*SV*) ×3,000

Table 1 Comparative measurements of the present *Pleistophora* sp with the previously recorded species

	Host	Spore length	Spore width	Sporophorous vesicle (SV)	Type of spores
Pleistophora typicalis (Canning and Nicholas 1980)	*M. scorpius*	3	1.5	Spores within SV	Microspores and macrospore
Pleistophora schubergi (El-Garhy 1989)	*Choristoneura fumiferana*	2.5±0.5	1.5±0.5	Most spores are found free in host cytoplasm	Only microspore
Pleistophora sp (Abdel-Ghaffar et al. 2009)	*E. chlorostigma*	2±0.5	1.8±0.2	May be within SV or set free in cyst	Only microspores
Pleistophora sp (the present study)	*E. chlorostigma*	1.9±0.5	1.6±0.4	May be within SV or set free in cyst	Only microspores

phora longifilis infecting the testes of *Barbus barbus* and in *Pleistophora ovariae* infecting the ovary of *Pimephales promales* (Summerfelt 1964; Maurand et al. 1988). Moreover, a common reaction against invading microsporidia is the development of layers around the dividing parasites within the xenomas. In general, the original parasitized host cell becomes hypertrophied (Weissenberg 1976). In the cytoplasm of the hypertrophic host cell, the parasite divides repeatedly producing an enlarged xenoma containing numerous spores and other life cycle stages (Lom and Pekkarinen 1999; Matos et al. 2003; Kent and Speare 2005; Lom and Dykova 2005; Casal et al. 2008). There are numerous microsporidian species, but only a few form xenomas in fish (Lom and Pekkarinen 1999; Matos et al. 2003) (Fig. 14). Both parasite and host seem to benefit from the formation of a xenoma. The advantages of the parasite are good growth conditions and protection against host attacks by masking within host components. The host benefits by confining the parasite and limiting its spread (Lom and Dykova 1992a). After rupture of the xenoma, living spores have the ability to extrude their polar tubes as was reported by Ferguson et al. (2007).

Microsporidian parasites recorded from *Pagrus pagrus* and *S. tumbil* fish species

Whitish xenomas (cysts) were embedded in all body organs, including muscles, liver, intestine, and stomach. Similar results were previously recorded in other microsporidians (Weissenberg 1976; Matos et al. 2003; Kent and Speare 2005; Lom and Dykova 1992; Bcker and Speare 2004; Lovy et al. 2007; Casal et al. 2008).

Light microscopic studies of the spores revealed the presence of a small posterior vacuole as was recorded by Casal et al. (2008). Comparison between the size of the parasites in the present study with those obtained by other authors revealed that the dimensions obtained here were in agreement with those of Kabata (1959) and only slightly different from those obtained by Bossanquet (1910). These differences may be due to the changes that occurred during the preparation. The polar filament within the spore is extended posteriorly from the anterior mass (polar tube) and then laterally to form a coil just beneath the membrane occupying about two thirds of the spore. In cross sections, the coils appeared as a pair of beaded extensions along the two inner sides of the spore wall. The same observation was reported by Sprague (1966), Rodriguez-Tovar et al. (2003), and McGourty et al. (2007).

Pleistophora sp. (Abdel-Ghaffar et al. 2009) recorded from *E. chlorostigma*

Numerous macroscopic, black cysts (xenomas), ranging in size from 3 to 5 mm, were observed throughout the abdominal cavity of the infected fish being embedded in different organs. Similar observations were reported from other fish by Abdel-Ghaffar et al. (2009) and Stephens (2009). The black xenoma wall is a melanized layer where melanocytes are deposited around the tissues containing the parasite (Kabata and Whitaker 1981, 1989). This formation may be a type of host response against the parasite. The measurements of the present *Pleistophora* sp. are compared with the previously recorded species (Table 1). Measurements of the spore length and width were similar to the previous records of the genus *Pleistophora* as Abdel-Ghaffar et al. (2009) in *Pleistophora* sp. From *E. chlorostigma*, Canning and Nicholas (1980) in *Pleistophora typicalis* from *Myxocephalus scorpius* and El-Garhy (1989) in *Pleistophora schubergi* from the spruce budworm *Choristoneura fumiferana.* Spores could be enclosed within the SV and some are free, an observation agrees with the spores of *Pleistophora schubergi* (El-Garhy 1989) while the spores of *Pleistophora typicalis* (Gurley 1893) only present in the SV. Only one type of spores was observed as microspores, an observation agrees with spores of *Pleistophora schubergi* but spores of *Pleistophora typicalis* may exist as a two forms micro- and macrospores.

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