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Light and Scanning Electron Microscopy (SEM) of *Ortholinea africanus* Abdel-Ghaffar *et al*., 2008 (Myxozoa: Myxosporea) Infecting Tilapia Fish *Oreochromis niloticus* (Osteichthyes: Cichlidae) with Description of Preparation of Coelozoic Myxosporea for SEM

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Summary. *Ortholinea africanus* Abdel-Ghaffar *et al*., 2008 is re-described using light and scanning electron microscopy (SEM) to reveal the external ornamentation of the spores. Present spores matched the original description in morphology but were larger in all dimensions. They measured 9.6 ± 0.8 (8.1–10.9) µm in length, 9.5 ± 0.6 (8.8–10.9) µm in width and 8.9 ± 0.4 (8.6–9.1) µm in thickness. Polar capsules were 3.9 ± 0.3 (3.0–4.3) µm in length with polar filaments turned 4–6 turns. The pattern of polar filament coils was different in each of the polar capsules. In the same spore, one capsule contained oblique filament turns to the longitudinal axis of the capsule, while the other capsule contained perpendicular turns. SEM revealed completely different shell ridge patterns than those described by light microscopy in the original description. This emphasizes the necessity of SEM in the reliable description of any myxosporean genera with external shell ornamentation like *Ortholinea* and *Chloromyxum*. The present species is considered the first (purely) freshwater *Ortholinea* described from a proper freshwater host. Three different methods of isolation and preparation of coelozoic spores for SEM were evaluated. The best combination was using a syringe filter holder with a membrane filter of 5 µm pore nucleopore filters.

Key words: Myxosporea, *Ortholinea*, *Oreochromis*, Protozoa, Parasite, Tilapia, Nile.

INTRODUCTION

The taxonomy of myxosporean species which have external ornamentation like *Ortholinea* and *Chloromyxum* requires the scanning Electron Microscopy (SEM) to reveal the pattern of these external structures. This prerequisite was concluded and encouraged by Lom & Dykova (1993), Ali (1998) and Ali (2000). However, only a few species were described using SEM, e.g., *Chloromyxum trijugum* Kudo and *C. catostomi* Kudo by Listebarger & Mitchell (1980); *Chloromyxum* spp., Lom & Dykova (1993), *Ortholinea australis* Lom *et al*. (1992), *Chloromyxum vanasi* Ali (1998); *Ortholinea basma* Ali (2000) and *Chloromyxum alii* Abdel-Baki (2007). Meanwhile, some species were described without the use of SEM like *Ortholinea striateculus* Su & White (1994), *Ortholinea fluviatilis* Lom & Dykova (1995) and the present species *Ortholinea africanus* Abdel-Ghaffar *et al*. (2008). The original description

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of *O. africanus* could not recognize the external shell structure and described the spores as showing "striations" which required the necessity of a more detailed description of the species.

The main obstacle in utilizing SEM for description of coelozoic myxosporean species is the difficulty in isolation and preparation of the spores. In many cases, the number of free spores in coelozoic infection is relatively low, compared to the histozoic species which contain a large number of spores. Also, the volume of the infected fluid like gall or urinary bladder is usually small, especially in small fishes. These two reasons make it hard to retrieve a reasonable number of intact and clearly visible spores for good description.

The present paper redescribes and reviews *Ortholinea africanus* of Abdel-Ghaffar *et al*. (2008) using the SEM as a prerequisite for the reliable classification of a new species. Some tools for the isolation and preparation of coelozoic myxosporean species for SEM observation are also tested.

MATERIALS AND METHODS

A total of 54 freshly caught tilapia fishes (*Oreochromis niloticus*) were obtained from the fishermen at Bahr Shebin, a tributary of the River Nile. The fish were immediately transported and examined. Fishes were collected during summer months of 2004 and 2007. Kidneys, uretra and urinary bladders were examined for the presence of any myxosporean infection. Positive specimens were preserved in 10% neutral buffered formalin. Fresh spores were first photographed and then measured. Spores were described according to Lom & Arthur (1989). Measurements are made on 30 spores and given in micrometers as: arithmetic mean ± standard deviation (range).

To prepare specimens for SEM: preserved urinary bladders were cut open and emptied in small plastic vial of 5 ml. The inner wall of the urinary bladder was gently scraped into the vial. To rinse the spores, the contents of the vial was diluted with distilled water to 4–5 ml, shaken, left to stand for 10 min. and then 2 ml of the supernatant containing foreign materials were discarded. The specimen was then processed in either two ways:

In the first method, part of the concentrated spores were treated as described by Lom & Dykova (1993). The second method: the specimens were filtered on membrane filters using a syringe filter holder (13 mm diameter). Two types of membrane filters were used in the holder: 0.45 µm pore Millipore filters (Millipore Corp., Bedford, Mass.), and 5 µm pore Nucleopore filters (Nucleopore Corp., Pleasanton, Calif.).

About 1 ml of the specimen solution was drawn by a 5 ml syringe; the volume is diluted with another 2 ml of distilled water. Then a membrane filter holder was mounted to the syringe and the specimen solution is passed slowly. The rinsing is repeated twice, then the specimen was dehydrated with the ethanol gradient (30, 50, 75, 85, 95% and 2 × in 100%, for 5 min. each) using the same syringe. The filter is then critical point dried using CO_2, then sputter-coated with gold and examined with SEM Jeol Winsen JSM 6400 at 5kv.

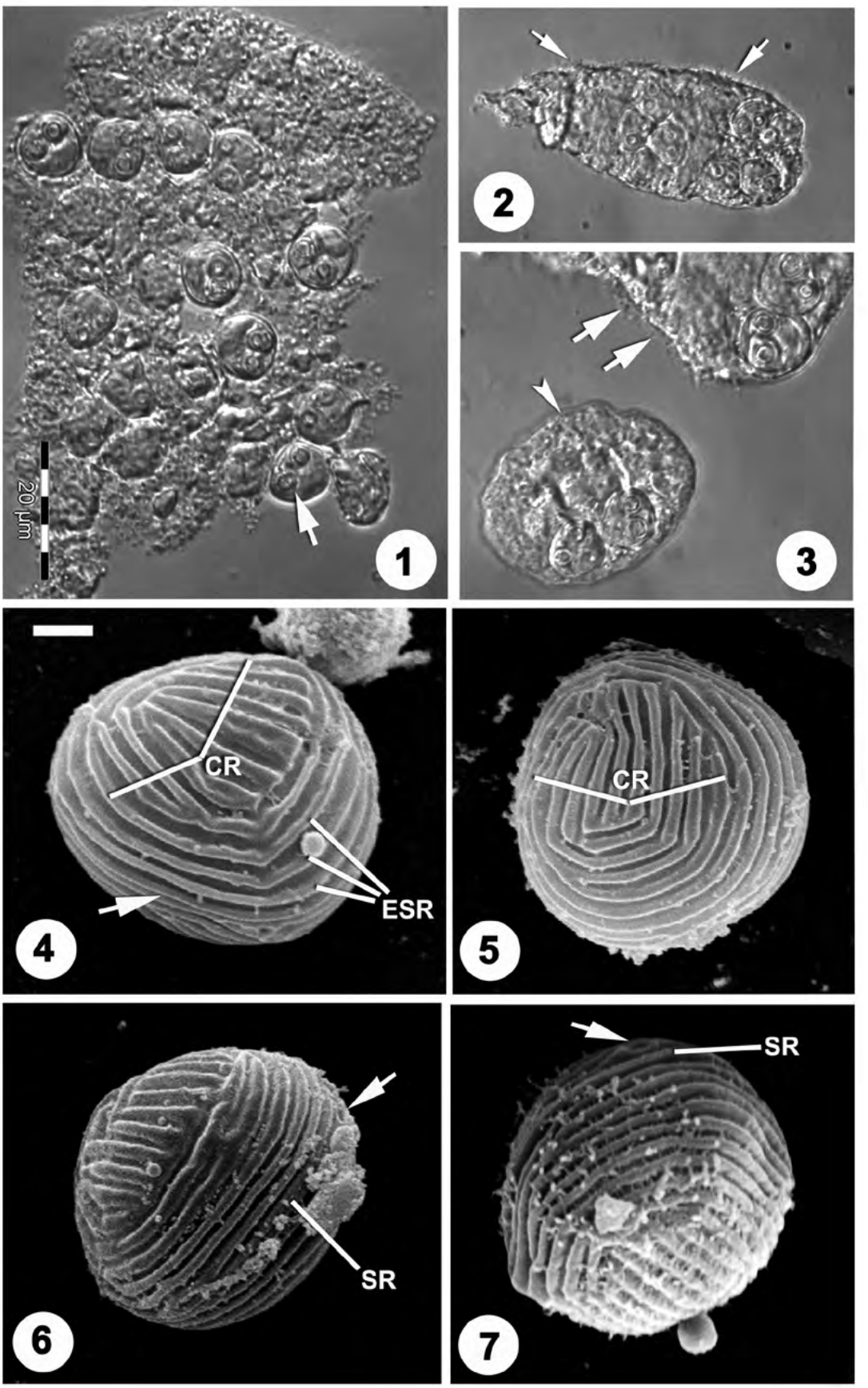
RESULTS

The degree of infection of the examined fishes was generally mild and spores were observed in moderate numbers. In the case of severe infection, the contents of the bladder appeared slightly turbid. Infection with *Ortholinea* spores was detected in 26 fishes accounting for 48%. Only urinary bladders were infected with these spores. Free spores and plasmodia were found in the urine of the infected fish. Plasmodia were mono- to polysporic and showed arbitrary shapes: large dense mat (Figs 1–3) and groups of endothelial cells with aggregates of spores. Some disporic round plasmodia were also recorded. Polysporic plasmodia showed hair-like projections at the periphery (Figs 2–3). Plasmodia had a distinct endo- and ectoplasm (Fig. 3). Defined polysporic plasmodia were irregular spherical in shape and measured 42.4 ± 12.3 (28.6–64.5) µm in diameter.

Light microscopy: The spores are round to triangular in frontal view with a wider and flat anterior end. Spore ridges appeared clearly as striations and the suture ridge was discernable. Spores measured 9.6 ± 0.8 (8.1–10.9) µm in length, 9.5 ± 0.6 (8.8–10.9) µm in width and 8.9 ± 0.4 (8.6–9.1) µm in thickness. Polar

▶▶

Figs 1–7. Mature spores of *Ortholinea africanus* Abdel-Ghaffar *et al*., 2008 from *Oreochromis niloticus*. **1** – light micrographs of fresh spores floating within a mat of sloughed endothelial cells in the fluid of urinary bladder with polar filaments clearly visible (arrow); **2**, **3** – polysporic pseudoplasmodia with hair-like projections (arrows) and clear ectoplasm (arrow head); **4–7** – SEM micrographs of typical mature spores showing sutural ridge (SR), surrounded by extrasutural ridges (ESR) and a central field of longitudinal ridges (CR) and polar filament pores (arrow). Scale bar: 1 µm (Figs 4–7), 20 µm (Figs 1–3).



capsules are round, thick walled, equal and set widely apart at the anterior end of the spores. They measured 3.9 ± 0.3 (3.0–4.3) µm in length with polar filaments turned 4–6 turns. The pattern of polar filament coils was different in each of the polar capsules. In the same spore, one capsule contained oblique filament turns to the longitudinal axis of the capsule, while the other capsule contained perpendicular turns.

SEM: Spores appeared as walnut or almond seed in shape. Spores measured 6.7 ± 0.4 (6.1–7.5) µm in length and 6.2 ± 0.4 (5.5–7.0) µm in width and 6.0 ± 0.7 (5.9–6.2) µm in thickness. Scanned spores showed a variable pattern of shell valves. The shell valves usually had deep, well defined ridges. Two main shell patterns were identified with some transitional forms between the two main forms. The first spore pattern represented the majority of the examined spores. It showed a well defined sutural ridge followed by 3 to 12 circular ridges. These concentric ridges may be bent sharply around a central field of short ridges which are often fused from both ends (Figs 4, 5, 10, 11, 14c). This central field consisted of 4–11 ridges and may be displaced to one side of the shell valve (Figs 7–8). The central ridges may be whorled in the same direction of the outer circular ridges or transverse to them (Figs 4–11). The polar filament pores are conspicuous, elevated, oval and set widely apart. They are located at the first circular ridge or in between the sutural and first circular ridge (Figs 4, 6, 7, 8, 10, 12, 14b). Some spores showed different ornamentation on the two shells and also different shell sizes.

In the second spore pattern, which is almond seed shaped, the ridges were running longitudinally along the shell valve and may merge with a perisutural circular ridge (Fig. 9).

Spore preparation: Large numbers of spores prepared using the method of Lom & Dykova (1993) were lost and not detected on examination with SEM. The remaining spores were usually intact but incompletely washed from associated debris. The background appeared smooth and in good contrast with the spores.

Spores prepared using the syringe filter: The Millipore filters retained a large number of spores. All the observed spores were covered to a different degree with amorphous materials and debris.

Nuclepore membrane filters retained a high number of intact and clean spores. In many cases, the spore was lying over/or at the edge of a membrane pore, which made the spore somewhat unstable on examination with SEM. The background was smooth, clean and well contrasted.

DISCUSSION

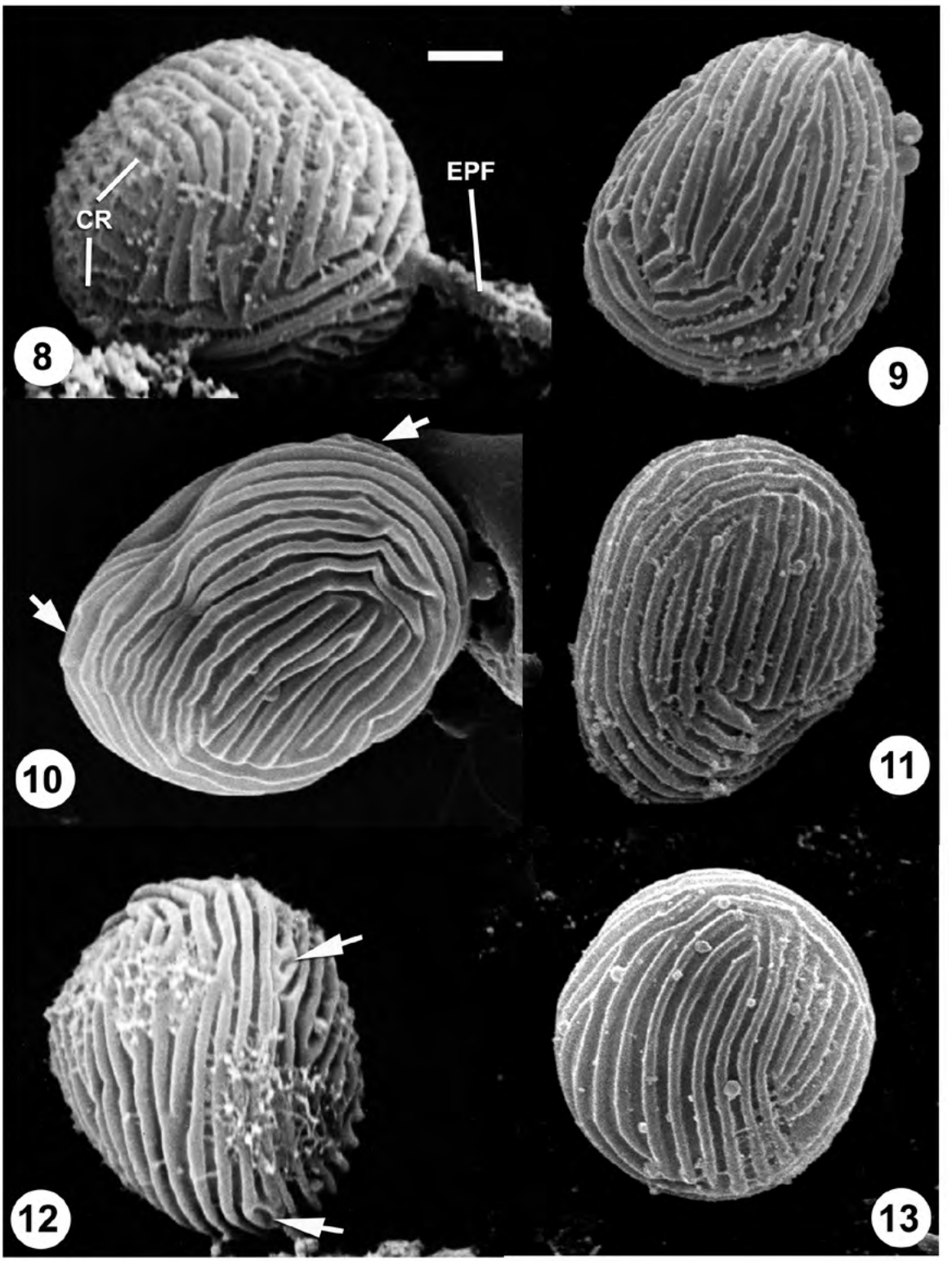
Results of the present description of *Ortholinea africanus* Abdel-Ghaffar *et al*., 2008 generally matched with the original description while some differences were observed. The size of the present spores in light microscopy is larger in all dimensions, while it is closer to the measurements taken from SEM spores. The shell ridge patterns depicted by Abdel-Ghaffar *et al*. (2008) from light microscopy are different than the pattern revealed here with SEM. This emphasizes the importance of the use of SEM in describing species with external ornamentation, particularly the genera of *Ortholinea* and *Chloromyxum*.

Only two species of *Ortholinea* were described using SEM; *O. australis* Lom *et al*., 1992 and *O. basma* Ali, 2000. The external ornamentation of the spore shell valves of the present spores is different than both compared species. In addition, the compared species belong to remote geographical origin and hosts.

The variability of spore shell pattern of *Ortholinea africanus* as observed in SEM is commonly observed in the genus *Ortholinea*. This variability was observed in *O. polymorpha* Davis, 1917, *O. australis* Lom *et al*., 1992 and *O. fluviatilis* Lom & Dykova, 1995. Also, the different pattern of ridges on each of the two shell valves was reported by Lom & Dykova (1995) in *O. fluviatilis* and *Chloromyxum vanasi* Ali (1998).

All the described species of *Ortholinea* are from marine hosts, even *O. fluviatilis* is from a host from marine origin (*Tetraodon fluviatilis*) which is adapted to the freshwater habitat. Lom and Dykova (1995) pointed to the existence of species of the "marine genera" in freshwater habitats through the migration of fish hosts from brackish waters to rivers. However, the present *Ortholinea* represents the first purely freshwater species from a proper freshwater host; Nile Tilapia *Oreochromis niloticus.*

Spores prepared for SEM observation: The use of some different methods of separation and cleaning of the spores was carried out and the following was concluded. The method described by Lom & Dykova (1993) was found to be unsuitable for those with low infection and/or small volume of infected fluids like gall or urinary bladders. In summary, most of the spores were lost during rinsing and processing. Also, spores were not sufficiently cleaned enough with this method. However, when the degree of infection is high, the ren-



Figs 8–13. Mature spores of *Ortholinea africanus* Abdel-Ghaffar *et al*., 2008 from *Oreochromis niloticus*. **8** – spore with laterally displaced central ridges (CR) and extruded polar filament (EPF); **9** – almond seed shaped spore with the ridges running longitudinally along the shell valve; **10** – typical spore showing the wide openings of polar filament pores (arrows); **11** – spore showing the longitudinal central ridges merging at their ends; **12** – spore in sutural view with asymmetrical shell valves and polar filament pores (arrows) widely separated; **13** – atypical spore with a typical ridge pattern. Scale bar: 1 μm.

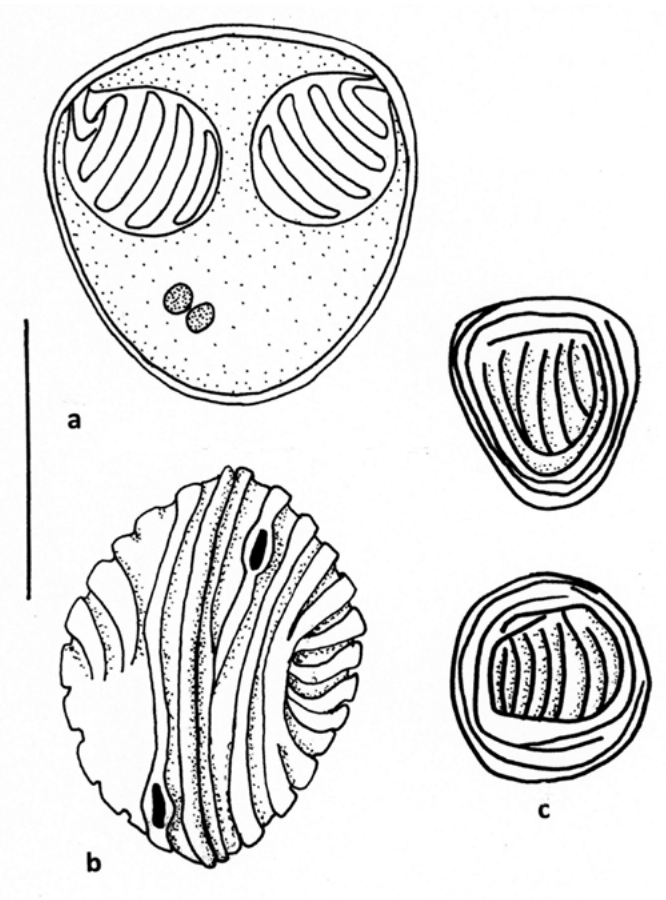


Fig. 14. Schematic drawings of *Ortholinea africanus* Abdel-Ghaffar *et al*., 2008. **a** – frontal view of typical mature spore; **b** – apical sutural view of spore showing the location of polar filament openings; Scale bar: 5 µm; **c** – different forms of central field ridges observed on spore shells. Not to scale.

dered spores are of good quality on a sharp contrasted background.

Using the syringe filter depended largely on the type of filter: in Millipore filters, almost all the spores were partially covered with amorphous materials and/or debris which rendered the spores useless for description. On the other hand, Nuclepore filters proved to deliver the best quality spores which were well cleaned and with a good background. When a spore lay on the edge of the membrane pore, it was unstable and difficult to assess under fine focus. The choice of the filter pore size can be selected according to the spore size.

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