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Immature stages and re-description of *Henneguya suprabranchiae* (Myxosporea: Myxobolidae), an intestinal parasite of the catfish *Clarias gariepinus* in the River Nile, Egypt

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ABSTRACT: A new morphological description, supported by light microscopy photographs, is presented for various immature stages and for mature *Henneguya suprabranchiae* Landsberg, 1987 spores infecting the intestine of the Nile catfish *Clarias gariepinus*, Burchell, 1822 (Syn.: *C. lazera*). Large cysts of 2 to 4.5 mm diameter, containing immature and mature stages, were present in the outer layer of the intestine. They caused severe damage to the smooth muscle and atrophy due to the increased size and resultant pressure of the plasmodial mass. From September 2000 to April 2001, 21 infected fishes were detected, with a parasite prevalence of 21.2%. Nine immature stages were distinguished, and these have been measured, sketched and described. In addition, caudal process development was recorded. The mature spores are re-described and compared with previous descriptions of *H. suprabranchiae* spores. The main new morphological characteristics described are the number of polar filament coils, triangular thickening of the sporoplasm base, and a suture line visible only in lateral view.

KEY WORDS: Myxosporea · *Henneguya suprabranchiae* · Immature stages · Mature stages · Fish parasites · River Nile · Egypt

INTRODUCTION

Myxosporean parasites have received much attention in Africa and Egypt during the last decade. Investigators in various African countries have described about 100 myxosporean species from 9 genera, among them species from the genus *Henneguya* (Thelohan 1892, Paperna 1996, Fomena & Bouix 1997). A great number of myxosporean species still need to be investigated. In African freshwater fishes, 12 species of the genus *Henneguya* have been described, including *H. suprabranchiae*, which was first described by Landsberg (1987) and subsequently by Ashmawy et al. (1989), Abdel-Ghaffar et al. (1995) and Hegazy (1999). All these studies described only 1 stage. The present study provides a new description of the immature, pre-mature and re-description of mature spores in 10 stages.

The present study, describes morphological differentiation between juvenile (immature) and mature stages of *Henneguya suprabranchiae* spores, which will be useful for distinguishing between spores of related species, and re-describes the mature spores, introducing some new distinctive characteristics (number of polar filament coils, triangular thickening of the sporoplasm base, and a suture line). In addition, histopathological effects of the parasite on the intestine of the Nile catfish are described.

Henneguya species have been implicated as factors causing economic losses in catfish farms (McCraren et al. 1975) and tropical diseases in subtropical and ornamental fishes (Abolarin 1971, Paperna 1973, Shariff 1982, Kent & Hoffman 1984). In addition, *H. exilis*

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causes serious mortality in mature catfish (Current & Janovy 1978).

MATERIALS AND METHODS

The freshwater fish used in this study comprised 99 specimens of the catfish *Clarias gariepinus* Burchell, 1822 (synonym: *C. lazera*) of both sexes 33 to 36 cm in length and 244 to 279 g in weight. They were collected by trapping (beginning of September 2000 to end of April 2001) in the River Nile at Giza and the Cairo Governorates.

Immediately after collection, the fish were transported alive to the laboratory, where their length and weight were measured. They were then dissected and the intestine examined microscopically for myxosporean parasites under the stereo and light microscopes.

Different sporogonic stages were obtained from the cysts. An average of 45 spores was measured according to the methods recommended by Lom & Arthur (1989). Permanent preparations were made by placing some spores in glycerol-gelatine and mounting under a cover slip. The structure of immature and mature stages was studied using a light microscope. In addition, air-dried smears were fixed in absolute methanol and stained with Giemsa. For histological examination, infected portions of the intestine were fixed in Bouin's solution, embedded in paraffin wax, cut in 4 to 5 µm thick sections and stained with H&E (Conn et al. 1960, Geoffrey & Aimls 1978, Drury & Wallington 1980).

RESULTS

Of the 99 catfish examined, 21 (21.2%) were infected with *Henneguya suprabranchiae*. Within the plasmodia, immature stages dominated from September to January. Mature stages appeared in January and started to disappear by mid-April. They had completely disappeared by the end of April. These parasites formed large cysts embedded along the outer layer of the intestine. The plasmodia were grayish and ovoid in shape and measured 2 to 4.5 mm in diameter.

Immature stages (Figs. 1 to 10 & 13 to 27)

Nine immature stages were distinguished as a function of size and caudal process (tail) development (Table 1).

Stages 1 and 2. Disporic pansporoblasts within a cyst may be separated or may remain attached to each other until maturity. In both stages the spores are spherical, with 2 well-developed polar capsules. A very short polar filament is extruded. The spore body is surrounded by a very thin transparent membrane with a spherical small mass of sporoplasm cells. These stages do not possess a tail (Figs. 1, 2, 13, 14, 15 & 17). In stage 2 an additional thin membrane surrounds the spore body (Figs. 3 & 16).

Stage 3. The spore body is slightly elongated. Two additional cell-like structures are visible at the base of the sporoplasm (Figs. 3 & 18).

Stage 4. Spore size increases due to elongation and division of the 2 cells at their base (Figs. 3 & 19).

Stage 5. This stage differs in shape from previous stages due to further elongation of the 2 cells at the spore base. These cells develop into a thin, short tail and triangular thickening of the spore base. The spore body decreases in size (Figs. 4 & 20).

Stages 6, 7 and 8. The tail is clearly visible and more developed. Polar capsules are similar in size to those at the fifth stage (Figs. 5 & 21). Spore size is larger (Figs. 7, 8 & 22) and the tail is longer (Figs. 8 & 23).

Juvenile stage (Stage 9)

Total spore length is 31.7 µm, the spore body is short, polar capsules are more developed, and polar capsule

Figs. 1 to 12. *Henneguya suprabranchiae* light microphotographs of fresh unfixed spores of immature and mature stages (host: *Clarias gariepinus*) ×1150. Fig. 1. Stage 1, disporic pansporoblast within a cyst (arrow). Fig. 2. Stage 1, free spherical spores with 2 well-developed polar capsules and mass of sporoplasm, the latter surrounded by a very thin transparent membrane (a). Two polar capsules seem to be oriented in opposite directions (b), short polar filament extrudes from a spore, and outer cyst is damaged (c). Fig. 3. Stages 3 to 5; in Stage 2, additional membrane is visible surrounding the spore body, and sporoplasm shape is more irregular (f); in Stage 3, 2 cells (arrow) are discernible at the base of the spore body (d); in Stage 4, elongation and division of 2 cells (arrow) are clearly visible (e). Fig. 4. Stage 5, characterized by very short tail (arrow) and 2 small polar capsules (g). Fig. 5. Stage 6, elongation of tail continues (h). Fig. 6. Spore body (arrow) is larger and ovoid in shape also sporoplasm is wider. Further elongation of the tail is clearly visible. Fig. 7. Shell opening (arrow) of sporulation stage (i). Fig. 8. Stage 8, showing separation of the symmetrical spore-shell valves (arrow) and further elongation of the spore body, sporoplasm and tail (j). Fig. 9. Lateral view of Stage 9 (juvenile) stage: 1 polar capsule and a well-developed long tail are clearly visible (k). Fig. 10. Stage 9, showing characteristic body shape of the spore; polar filament coils are further developed and tail is long. Fig. 11. Stage 10, showing pansporoblast with 2 mature spores lying parallel to each other, 1 spore appears slightly longer than the other. Fig. 12. Stage 10, spore in anterior view (bottom spore) with completely developed polar filaments; triangular thickening (arrow) is complete; in top spore central iodinophilous vacuole is enclosed in the sporoplasm (arrow). Lateral view (l) shows protruding suture edge and suture line (arrow)

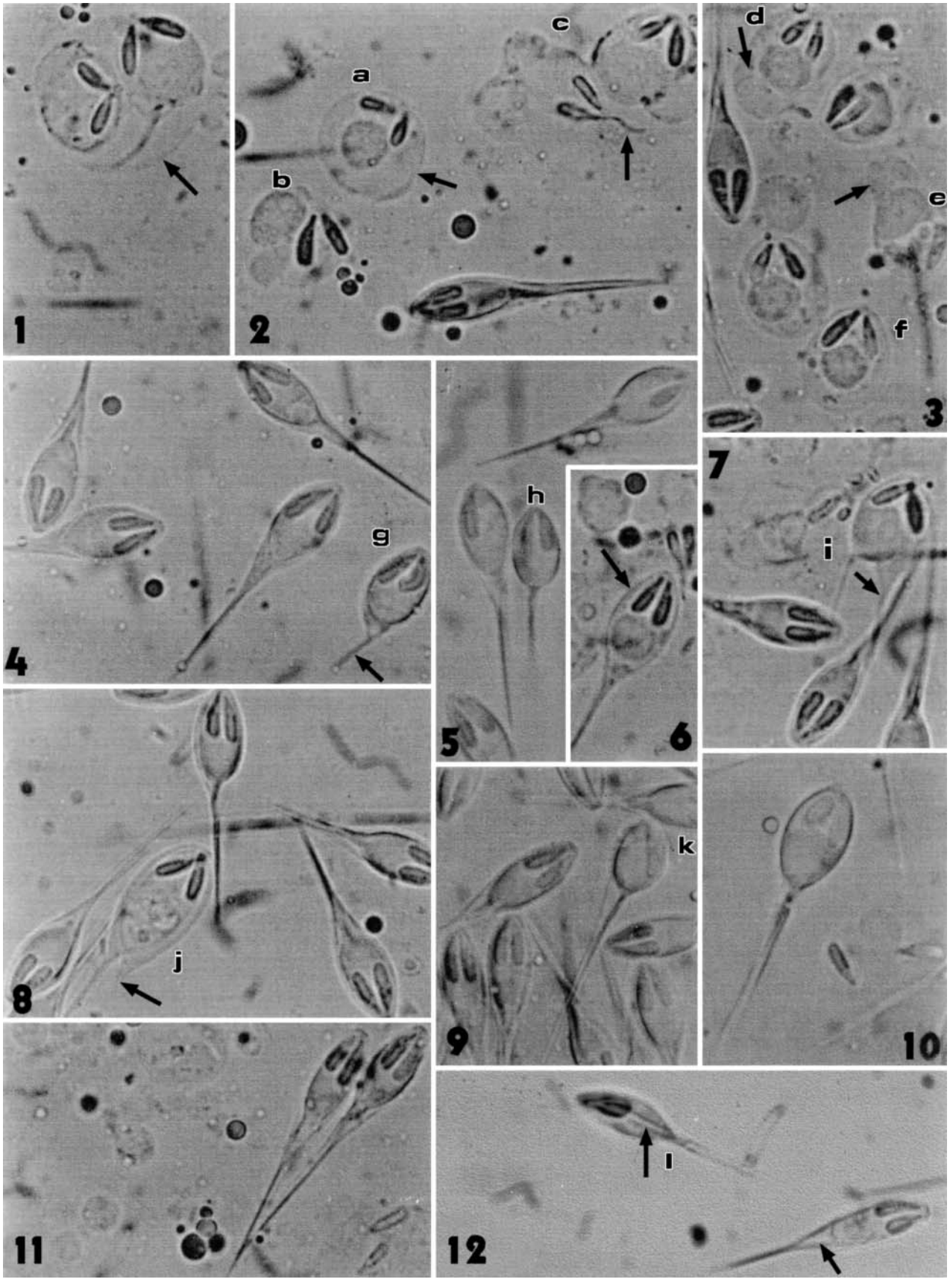
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Table 1. *Henneguya suprabranchiae*. Measurements (µm) of immature stages

Stage No.	Spore body Length	Spore body Width	Polar capsules Length	Polar capsules Width	Tail	Total length
1	10.5 (9.75–11.25)	10.9 (9.8–12)	4.9 (4.5–5.3)	1.3 (1.1–1.5)	Absent	10.5
2	12.4 (12.0–12.8)	11.7 (10.5–12.8)	5 (3.75–6.0)	1.1 (0.75–1.5)	Absent	12.4
3	14.7 (14.3–15.0)	6.4 (5.3–7.5)	4.9 (4.5–5.3)	1.3 (1.1–1.5)	Development begins with 2 slightly elongated cells (3.8 µm)	14.7
4	15.4 (15.0–15.8)	7.9 (7.5–8.3)	4.9 (4.5–5.3)	1.1 (0.75–1.5)	Cell mass is more elongated and divided (4.5 µm)	15.4
5	10.2 (9.8–10.5)	5.7 (5.3–6.0)	4.9 (4.5–5.3)	1.7 (1.5–1.9)	Thread-like short tail visible (7.5 µm)	17.3
6	8.8 (8.6–9.0)	5.1 (4.9–5.3)	4.7 (4.1–5.3)	1.7 (1.5–1.9)	Tail more prominent (10.5 µm)	19.5
7	11.6 (11.3–12.0)	7.2 (6.8–7.5)	5.1 (4.9–5.3)	1.3 (1.1–1.5)	13.9 (13.5–14.3)	25.5
8	14.7 (14.3–15.1)	7.8 (7.5–8.3)	4.9 (4.5–5.3)	1.3 (1.1–1.5)	14.7 (14.3–15.0)	29.4
9	12.3 (12.0–12.8)	7.2 (6.8–7.5)	5.3 (4.5–6.0)	2.1 (1.9–2.3)	19.4 (17.4–21.8)	31.7

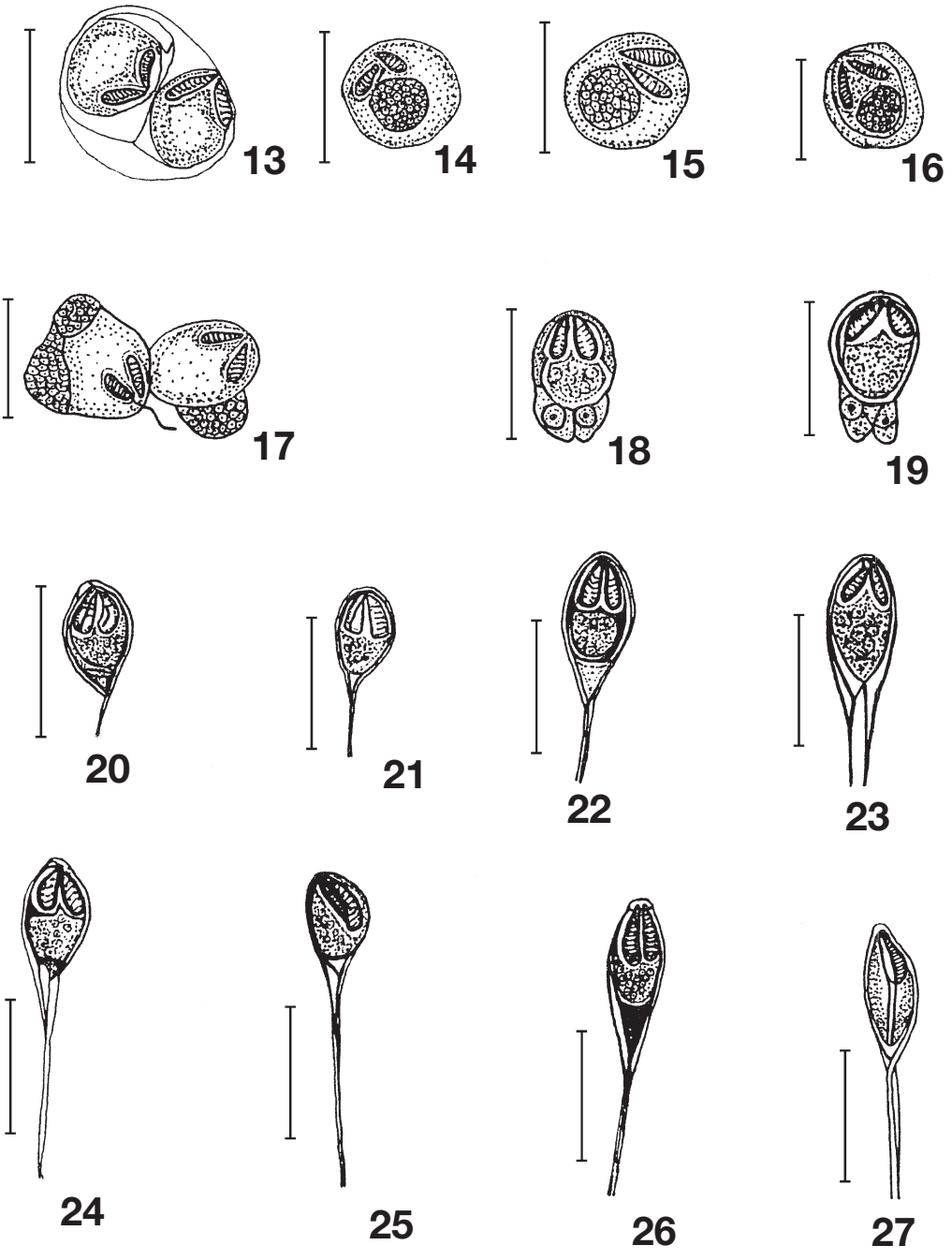
caps appear at the anterior end of the spore. The spore tail is longer than in the earlier stages (Figs. 9, 10, 24 & 25).

Mature spores (Stage 10)

Mature spores were measured (Table 2) and are illustrated in Figs. 11, 12, 26, 27 & 31. The mature spores are elongated in anterior view, with a protruding, rounded, anterior end. In lateral view, a suture line is visible (Fig. 12). Two thin and equal caudal processes extend from the posterior end of the spore. At the base of the sporoplasm a triangular thickening is fully developed (Fig. 12). The polar capsules are elongated and parallel, and are tapered, with a cap-like structure. The number of polar filament coils is 8 to 12 upon release. A few sporoplasms contain a rounded central, iodinophilous vacuole (Fig. 31).

Table 2. *Henneguya suprabranchiae*. Comparison of measurements (mm) of mature spores. (Stage 10 based on 126 spores) with those of morphologically similar species

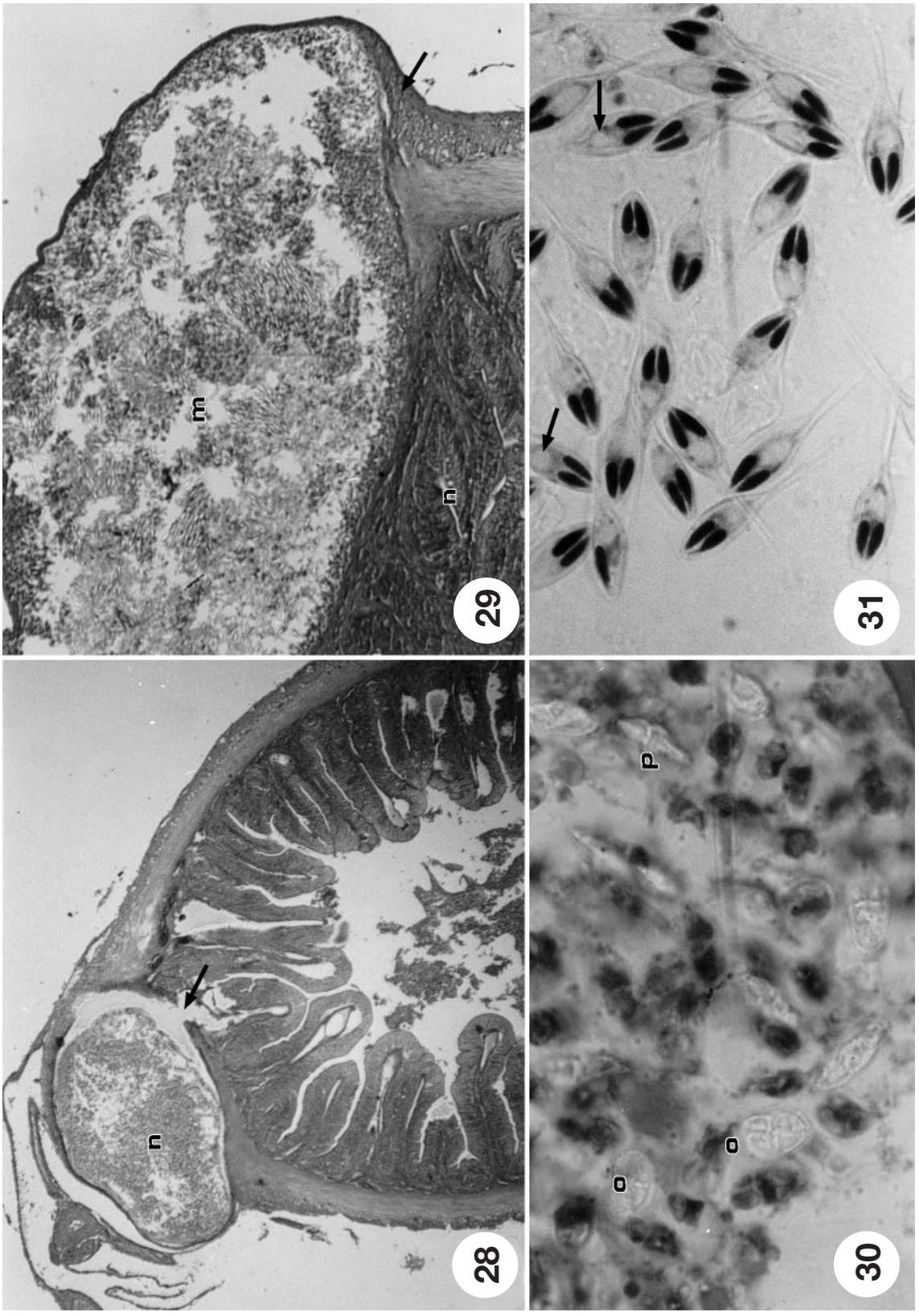
Species	Spore body Length	Spore body Width	Polar capsules Length	Polar capsules Width	Tail (caudal process)
H. bopeleti (Fomena & Bouix 1987)	17.2 (15.0–19.0)	6.35 (5.7–7.1)	8.01 (7.0–8.9)	2.0 (1.6–2.5)	27.43 (22.5–29.0)
H. branchialis (Ashmawy et al. 1989)	14.5 (13.3–15.4)	5.6 (4.7–5.6)	6.2 (5.5–7.7)	2.2 (2.15–2.58)	17.3 (14.6–25.8)
H. branchialis (Abdel Ghaffar et al. 1995)	14.7 (12.7–17.6)	5.0 (4.4–6.4)	6.9 (5.9–8.3)	2.1 (1.5–2.9)	19.7 (15.7–23.5)
H. nyongersis (Fomena & Bouix 1996)	12.6 (10.0–14.0)	5.4 (4.5–6.5)	6.2 (5.5–7.7)	2.3 (2.0–2.8)	21 (20.0–23.5)
H. suprabranchiae (Landsberg 1987)	13.5 (12.2–14.3)	6.4 (5.6–6.9)	7.6 (7.0–8.1)	2.1 (1.8–2.3)	24 (18.5–29.0)
H. suprabranchiae (Hegazy 1999)	12.06 (9.24–15.0)	5.03 (3.85–6.93)	6.36 (3.85–9.0)	1.34 (1.0–2.5)	23.81 (20.02–30.0)
H. suprabranchiae (present study; Stage 10)	12.4 (12.1–12.7)	5.3 (4.5–6.0)	5.7 (5.3–6.0)	1.3 (1.1–1.5)	23.7 (22.9–24.4)



Figs. 13 to 27. *Henneguya suprabranchiae*. Schematic illustration of immature (Figs. 13 to 25) and mature (Figs. 26 to 27) spores infecting intestine of *Clarias gariepinus*. Figs. 13, 14, 15 & 17. Stage 1; Fig. 16. Stage 2; Fig. 18. Stage 3; Fig. 19. Stage 4; Fig. 20. Stage 5; Fig. 21. Stage 6; Fig. 22. Stage 7; Fig. 23. Stage 8; Figs. 24 & 25. Stage 9; Figs. 26 & 27. Stage 10. (Scale bars = 15 µm)

Histology

Cysts comprising immature and mature spores were found in the outer layer of the gut wall (Figs. 28, 29 & 30). The cysts developed in the smooth muscle were covered by the tela subserosa and the serous membrane. Because of their large size, the cysts induced severe alterations such as atrophy of the muscle layer. Thus, the plasmodium was covered by a layer of degenerated smooth-muscle cells. The ectoplasm of the plasmodium consisted of a layer containing the dark-staining nuclei of generative cells, while the plasmodium center was filled with mature spores.



Figs. 28 to 31. *Clarias gariepinus* infected by *Henneguya suprabranchiae*. Cross-sections of infected part of catfish showing gut (Fig. 28) with plasmodium cyst (n) bulging out of the intestinal wall and degenerating submucosal layer (arrow). H&E (×100). Fig. 29. Atrophied (arrow) circular muscle layer (n) due to pressure of plasmodial mass (m) of *H. suprabranchiae*. H&E (×400). Fig. 30. High magnification of plasmodium showing immature (o) and mature (p) stages of the spores. H&E (×1000). Fig. 31. *H. suprabranchiae*. Giemsa-stained microphotograph of mature spores (arrows). (×1000)

DISCUSSION

Shape and size are important morphological characteristics in evaluating the degree of spore maturity, since they help to distinguish between variations in size between spores of related species. Therefore, the present study concentrated on documenting changes in shape and size throughout development in immature and mature stages of *Henneguya suprabranchiae*. The morphometric characteristics of the mature stage were compared to those of other *Henneguya* species, such as *H. branchialis* (Ashmawy et al. 1989) infecting the suprabranchial organ and gills of *Clarias gariepinus* in Egypt; *H. branchialis* infecting gills, the secondary respiratory organ and the outer wall of the small intestine of the *C. lazera* investigated by Abdel-Ghaffar et al. (1995) in Egypt; and *H. nyongensis* (Fomena and Bouix, 1996) infecting the gills and muscles of *Marcusenius moorii* in Cameroun (Table 2).

All the above-mentioned species are classified as Stage 9 in Table 1, since their characteristics, especially their measurements, seem to be most similar to this stage. Thus it would appear that stages of other species described by the cited authors as juvenile or pre-mature stages were in fact mature. Immature stages are mainly round in shape, with oval capsules and short caudal processes, and lack a suture line. Mature spores are elongated, with long, parallel polar capsules and long and slender caudal processes; they have a distinct suture line, and a rounded protrusion at the anterior end (this characteristic may be valuable for taxonomical purposes). Several characteristics do not appear in any of the immature and pre-mature stages (e.g. no suture line in lateral view), while other features are rudimentary or very weakly developed, e.g. the cap-like structure at the anterior pole and the triangular thickening of the sporoplasm base.

Mature spores of *Henneguya suprabranchiae* are redescribed and compared with those of other African species in Table 2. The spores of *H. bopeleti* (Fomena and Bouix 1987) infecting the gills of *Chrysichthys nigrodigitatus* in Cameroun differ from the spores in the present study by the larger size of the caudal processes. Despite a similar morphology to *H. suprabranchiae* (Landsberg 1987) infecting the suprabranchial organ of *Clarias gariepinus* in Israel and *H. suprabranchiae* isolated from the suprabrachial organ and intestine of the same host in Egypt (Hegazy 1999), the spores in the present study differed in the number of polar filament coils, the suture line and the triangular thickening of the sporoplasm base (e.g. Hegazy [1999] observed 9 to 10 coils vs 8 to 12 turns in the present study). Triangular thickening of the sporoplasm base was also noticed in *H. ghaffari* infecting the Nile perch *Lates niloticus* in Egypt by Ali (1999). A suture edge and line were also visible in the lateral view of the mature spores of other *Henneguya* species.

Spore maturity can be completed without cleavage of the pansporoplasm until the final stage is reached and the spores begin to separate (Fig. 11). In some cases such separation occurred at stage 1 resulting in 2 separate spores (Fig. 12).

Henneguya suprabranchiae appears to be a pathogenic species. It induced severe morphological changes in the intestinal wall of the infected portion of the host's gut, which becomes damaged and thickened. Similar symptoms were reported by Ali (1999) in *Lates niloticus*; he described intestinal plasmodia of *H. ghaffari* inducing atrophy of the intestinal muscularis and leading to significant loss of the muscular capacity of the intestine followed by peritonitis.

On the other hand, the present plasmodia did not invade the intestinal lumen, in contrast to *Myxobolus nodulointestinalis* described by Masoumian et al. (1996) in *Barbus sharpeyi*. The large plasmodium masses of *Henneguya ghaffari* observed by Ali (1999) caused a constriction of the intestinal lumen in *Lates niloticus*.

In the present study, establishment of the plasmodia in the outer layer of the gut caused severe thickening, damage, and atrophy of large areas of the smooth muscles that could lead to intestinal functional disorders.

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