

## An ultrastructural study on the merogonic stages of *Goussia senegalensis* (Faye, 1988) Diouf and Toguebaye, 1993 (Apicomplexa, Coccidia) from the liver of *Pagellus bellottii* (Pisces, Teleostei)\*

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**Abstract:** Little is known about the merogony of coccidians from fishes in Africa. To our knowledge, including both freshwater and marine fishes, developmental stages have been recorded for only *Goussia cichlidarum*, *Cryptosporidium* sp. parasitizing the stomachs of juvenile cichlid fish, and *Eimeria* (s. l.) *vanasi*. A survey of coccidians parasitizing marine fishes from the coast of Senegal (West Africa) was done between 1988 and 1992 and samples of infected organs were collected for further transmission electron microscopy observations. Merogonic stages were observed. *Goussia senegalensis* undergoes merogony in the hepatic cells of *Pagellus bellottii* as follows: meronts develop inside the cytoplasm of the hepatocytes and merozoites develop from them through endodyogeny and endopolygeny. Early meronts are uninucleate, but for most advanced meronts the single large nucleus is divided into 2 or more nuclei as the pellicle precursors of new merozoites arise. In this study, those associated with invaginations of the inner membrane were then arranged around portions of the cytoplasm, each including one nucleus. Mature meronts contained either 2 or several merozoites. At least 2 generations of merozoites developed.

**Key words:** Coccidian, ultrastructure, *Goussia senegalensis*, merogony, *Pagellus bellottii*

### 1. Introduction

The data on coccidians in Africa are particularly scarce and are available for only *Goussia cichlidarum* Landsberg and Paperna, 1985; *Cryptosporidium* sp. described in the stomachs of juvenile cichlid fish by Landsberg and Paperna (1986); and *Eimeria* (s. l.) *vanasi* Landsberg and Paperna, 1987 (Landsberg and Paperna, 1985, 1986, 1987). We have previously described *Goussia senegalensis* (Faye, 1988) Diouf and Toguebaye, 1993, a coccidian of 2 marine fishes of the Senegal coast, *Pagellus bellottii* (Sparidae, Perciformes) and *Apsilus fuscus* (Lutjanidae, Perciformes) (Diouf and Toguebaye, 1993), but many features of the development of merogony stages in this parasite remain unknown. In this paper, we present further findings describing how the parasite develops in hepatocytes.

### 2. Materials and methods

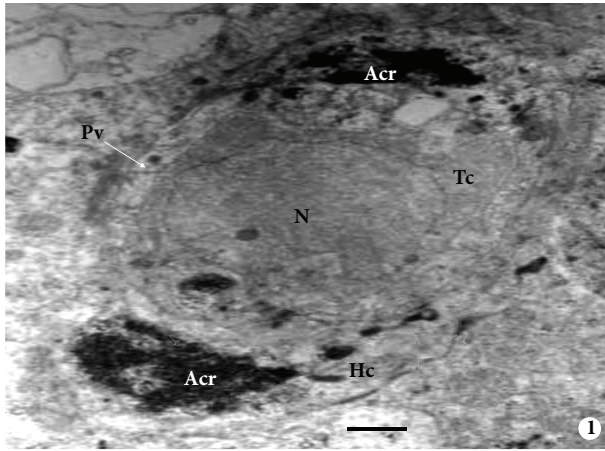
A total of 46 specimens of *Pagellus bellottii* Steindachner, 1892 (Teleostei) were collected from the coasts of Dakar (Senegal, West Africa). The livers were examined for coccidians. Infection was determined by observation of a fresh smear using light microscopy. For transmission electron microscopy, small fragments of infected organs

were fixed at 4 °C in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH = 7.2) for 24 h, and then post-fixed for 1 h in 2% osmium tetroxide in the same buffer at the same temperature. After dehydration through a gradual ethanol series and propylene oxide, organ fragments were embedded in Spurr resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined using a Hitachi H- 600 electron microscope.

### 3. Results

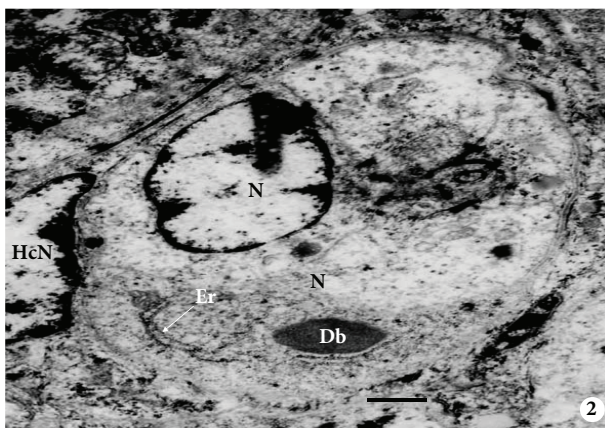
A survey of coccidians parasitizing marine fishes off the coast of Senegal (West Africa) was done between 1988 and 1992. The fishes were systematically screened and from 46 specimens of *Pagellus bellottii* Steindachner, 1892 (Teleostei), 30% were parasitized by *Goussia senegalensis*. In order to understand the developmental stages of coccidians, an exhaustive microscopic analysis was performed on the livers of the fishes, and the parasites were found located in the hepatocytes. The youngest meronts we observed possessed a centrally located nucleus surrounded by a distinct parasitophorous vacuole in the cytoplasm of the cell. The rest showed an apical complex that disappeared progressively (Figure 1). Then the

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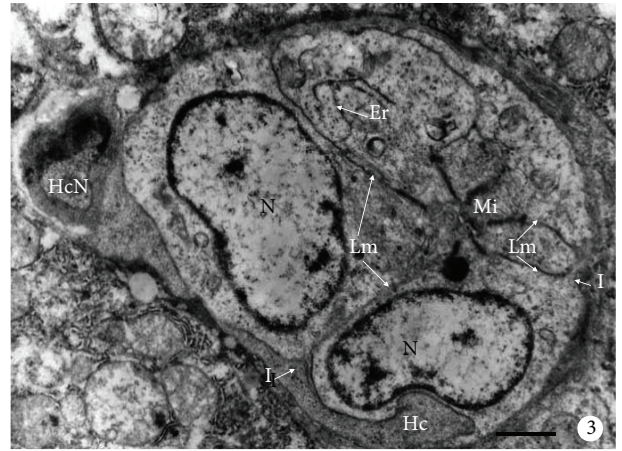


**Figure 1.** Trophozoite or early meront. Acr = apical complex residuum, Hc = host cell cytoplasm, N = nucleus, Pv = parasitophorous vacuole, and Tc = trophozoite cytoplasm. Scale: 2.8  $\mu$ m.

meronts increased in volume. They contained electron-dense bodies and abundant endoplasmic reticula (Figure 2). Limiting membranes arose. Merozoites formation started with lengthening of the limiting membranes. They stretched and slimmed (Figures 3 and 4). Nuclear divisions led to meronts containing several nuclei (Figure 3). At the same time, invaginations of the inner membrane appeared. Slimming membranes and invaginations arranged themselves around portions of the cytoplasm, each including one nucleus. Several invaginations also took place along the limiting membrane (Figure 4). Then new merozoites formed their pellicle. A double membrane started surrounding them: the inner membrane by invagination of the limiting membrane of the merozoites and the outer membrane was from the inner membrane of the meront (Figure 5). Meronts containing only 2



**Figure 2.** Advanced meront showing a nucleus (N). Db = dense body, Er = endoplasmic reticulum, and HcN = host cell nucleus. Scale: 2.6  $\mu$ m.

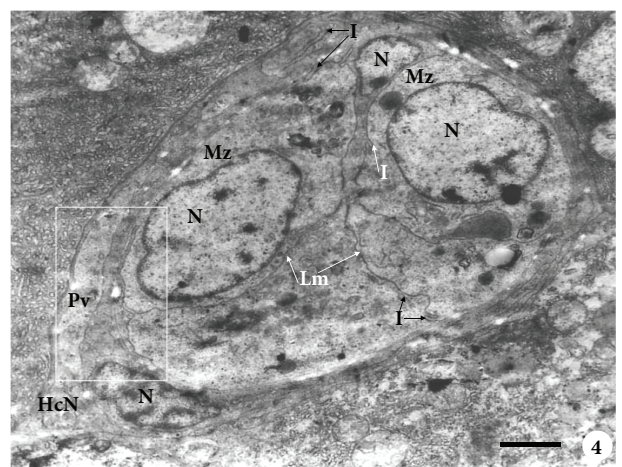


**Figure 3.** Meront showing the limiting membranes of merozoites (Lm). Er = endoplasmic reticulum, Hc = host cell cytoplasm, HcN = host cell nucleus, I = invagination, Mi = mitochondrion, and N = nucleus. Scale: 2.3  $\mu$ m.

merozoites were also observed. The components of their apical complex progressively disappeared. Only a small amount of residual cytoplasm and peripheral mitochondria remained. The pellicle of the merozoites originated from invaginations of the inner membrane of the meront (Figure 6).

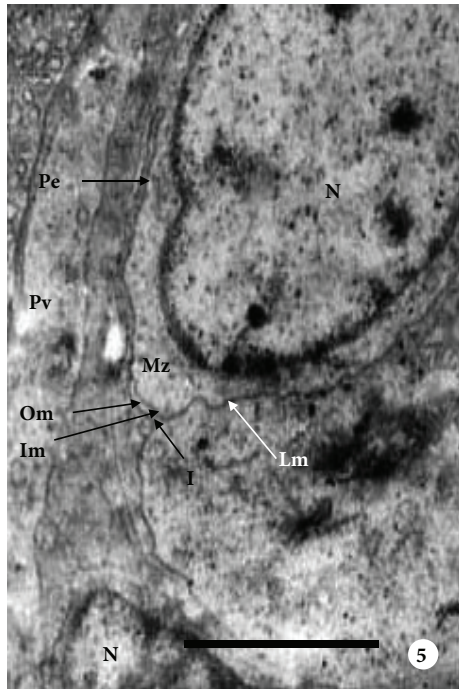
#### 4. Discussion

*Goussia senegalensis* forms its merozoites by endomerogony. The same process was observed in *G. carpelli* parasitizing *Cyprinus carpio* (Steinhagen, 1991). However, in the family Cryptosporidiidae, including the genera *Cryptosporidium* and *Epieimeria*, members form their merozoites by



**Figure 4.** Enclosure of merozoites (Mz) by endomerogony. The limiting membranes (Lm) elongate to surround each nucleus (N) and a part of the cytoplasm. Many invaginations (I) can be seen, some of them forming the pellicle (boxed area). HcN = host cell nucleus, and Pv = parasitophorous vacuole. Scale: 2  $\mu$ m.

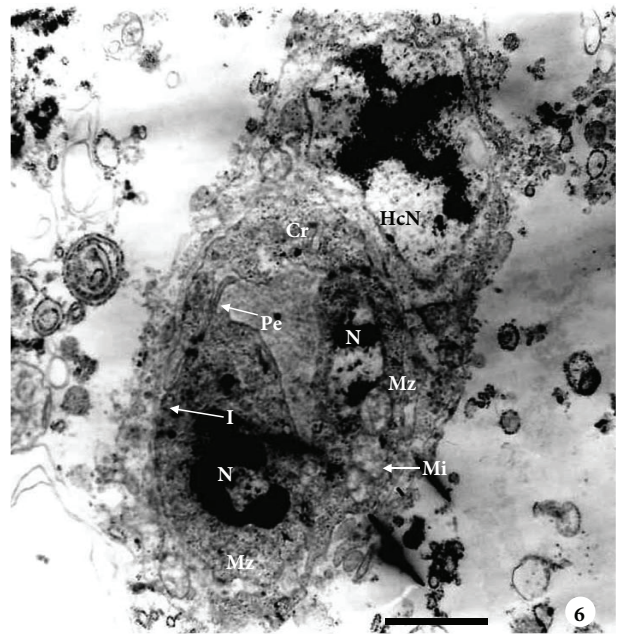




**Figure 5.** Detail of formation of the pellicle (Pe) shown in the boxed area of Figure 4. A double membrane forms around the new merozoites (Mz). The inner membrane (Im) is from the limiting membrane (Lm) of the merozoite by invagination (I), and the outer membrane (Om) is from the inner membrane of the meront. N = nucleus, Pv = parasitophorous vacuole. Scale: 2  $\mu$ m.

ectomerogony as was the case for *Cryptosporidium* sp. parasitizing Cichlid fish *Epieimeria anguillae*, *E. isabellae*, and *E. puytoraci* (Landsberg and Paperna, 1986; Molnár and Baska, 1986; Daoudi, 1987). This type of formation is similar to that reported for *Eimeria* (s. l.) *vanasi*, *E. funduli*, *E. sinensis*, and *Goussia aculeati* (Hawkins et al., 1984; Landsberg and Paperna, 1987; Baska and Molnár, 1989; Jastrzebski, 1989). Both forms of merogony were found in the *Eimeria iroquoiana* life cycle; the first and second generations of merozoites formed by ectomerogony while the third formed by endomerogony (Paterson and Dessser, 1981).

Meronts containing only 2 merozoites and others with several merozoites either completely or not surrounded coexisted inside the liver. That means there are at least 2 generations of meronts in the *Goussia senegalensis* life cycle. More than one generation has been recorded for *Eimeria iroquoiana*, *E. funduli*, *E. sinensis*, *E. subepithelialis*, *Goussia cichlidarum*, and *G. carpelli* (Marinček, 1973; Paterson and Dessser, 1981, 1982; Hawkins et al., 1984; Landsberg and Paperna, 1985; Baska and Molnár, 1989; Steinhagen, 1991). *Eimeria dingleyi*, *E. variabilis*, and *Epieimeria anguillae* seem to develop only one generation of merozoites. (Davies, 1978; Molnár and Baska, 1986).



**Figure 6.** Endodyogeny: meront dividing into 2 merozoites (Mz). The pellicle (Pe) begins forming by invagination (I). Cr = cytoplasm residuum, HcN = host cell nucleus, Mi = mitochondrion, N = nucleus. Scale: 2.8  $\mu$ m.

Mature meronts containing only 2 merozoites have been also reported in *Eimeria* (s. l.) *vanasi*, parasitizing cichlid fish (Landsberg and Paperna, 1987). This is similar to some coccidians from the higher vertebrates and this type of merogony has been described in the genus *Frenkelia* (Scholtyssek, 1973). It is a particular type of endomerogony called endodyogeny, characterized by the formation of 2 daughter organisms within a mother cell. The other known method of merozoite formation by endomerogony is endopolygeny. In this case, merozoites (in a wide sense) begin forming after the second nuclear division of a mother cell; the term endopolygeny generally applies to the formation of single organisms called schizonts (in a strict sense) from a multinucleate parent organism, a schizont (in a strict sense) or a meront (in a wide sense). In the *Eimeria iroquoiana* infection, 8 merozoites were found in each schizont of the first merogonic generation, and 13 to 18 merozoites were found in the second and third generations (Paterson and Dessser, 1982). In *Eimeria subepithelialis*, the first generation meronts have 5 to 17 merozoites, the second have about 50, and the last have 8 to 17 (Marinček, 1973).

Schizonts multiply by repeated nuclear division and proper amounts of cytoplasm segment around each newly formed nucleus, producing merozoites in varying numbers according to species. This is the schizogony that is responsible for severe infections. The species we describe develops by schizogony and in a strict sense by merogony corresponding to endodyogeny. This is why hepatocytes

with 2 merozoites and hepatocytes with several merozoites were observed in the same samples.

The coccidian we describe here develops its meronts in the cytoplasm of the host cell, while sporocysts present a suture line joining the 2 valves. Moreover, it differs from a typical *Eimeria* due to lacking a Stieda body, and this is how we determined that it belongs to the genus *Goussia* (Diouf and Toguebaye, 1993). Because the life cycles of piscine coccidians are little known, we propose to continue considering morphological features valid for identification of genera. This system has met with little approval and some authors still consider these generic designations based on oocyst and sporocyst structure or site of infestation to be synonyms of the genus *Eimeria* (Dyková and Lom, 1981; Duszynski et al., 2000).

## 5. Conclusion

Data on the formation of the pellicle surrounding merozoites are scarce. The inner membrane in merozoites is synthesized de novo within the cytoplasm of the meronts in *Eimeria iroquoiana* while the external one is from the double membrane of the meront (Paterson and Desser, 1981). The basic processes of the merogony that we describe are similar to those of other coccidians: nuclear divisions

are the same type and the pellicle, a double membrane limiting the merozoites, is also present. However, they are inconsistent with the findings reporting that the external membrane of merozoites originates from the external one of the meront (Scholtyssek, 1973). In *Goussia senegalensis*, infection endomerogony begins by the limiting membrane clearly forming from the endoplasmic reticulum. This gives rise to the inner membrane of merozoites. The outer membrane is from invaginations of the inner membrane of the meront. Paterson and Desser (1982) pointed out that invertebrates play a role in coccidian infections of liver. Mainly for those of the genus *Goussia*, merogony may occur either in a facultative or an obligate intermediate host. The *Goussia senegalensis* merogonic stages we described have been found in the liver of *Pagellus bellottii*, between sporulated and unsporulated oocysts. This means that merogony also occurs in the liver of fishes.

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