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TWO MICROSPORIDIAN PARASITES FOUND IN MARINE FISHES IN THE ATLANTIC OCEAN

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Abstract. Two new species of microsporidia are reported from the Atlantic marine fishes: *Pleistophora duodecimae* sp. n. from skeletal musculature of the rat-tail, *Coryphaenoides nasutus* Günther and *Glugea capverdensis* sp. n. from the intestine, mesentery and ovary of the lantern fish, *Myctophum punctatum* Rafinesque. Formation of secondary xenomas was observed in the latter species. Both species may inflict serious damage upon their hosts.

Up to the present time, microsporidian infection has been found in 176 species of marine and freshwater fish; a total of 64 species were described and additional 39 microsporidians were recorded without any specific or, in some, even generic assignement — for the time being.

A great part of microsporidians known to infect fishes affects hosts important in commercial fisheries, e.g., *Glugea stephani* (McVicar, 1975) or in pet fish industry (Schubert 1978). This also is one of the reasons of the recent increase of interest in these parasites. The present communication presents a description of two new microsporidians found in species of Atlantic fishes exploited by the marine fish industry

MATERIAL AND METHODS

Host specimens fixed in 10% formalin were available. Fixed spores were examined unstained and after nuclear staining and PAS reaction. Histological sections were prepared for the study of parasite development and structure, and of host-parasite interaction.

RESULTS

Pleistophora duodecimae sp. n. Plate I, Fig. 1

Host: *Coryphaenoides nasutus* Günther, 1877 (Macruridae, Gadiformes), rat tail; marine.
Site of infection: trunk musculature.
Geographic distribution: Atlantic ocean, 20°32′02″ South and 12°03′04″ East, depth about 500 meters.
Prevalence: One out of 15 specimens found infected.

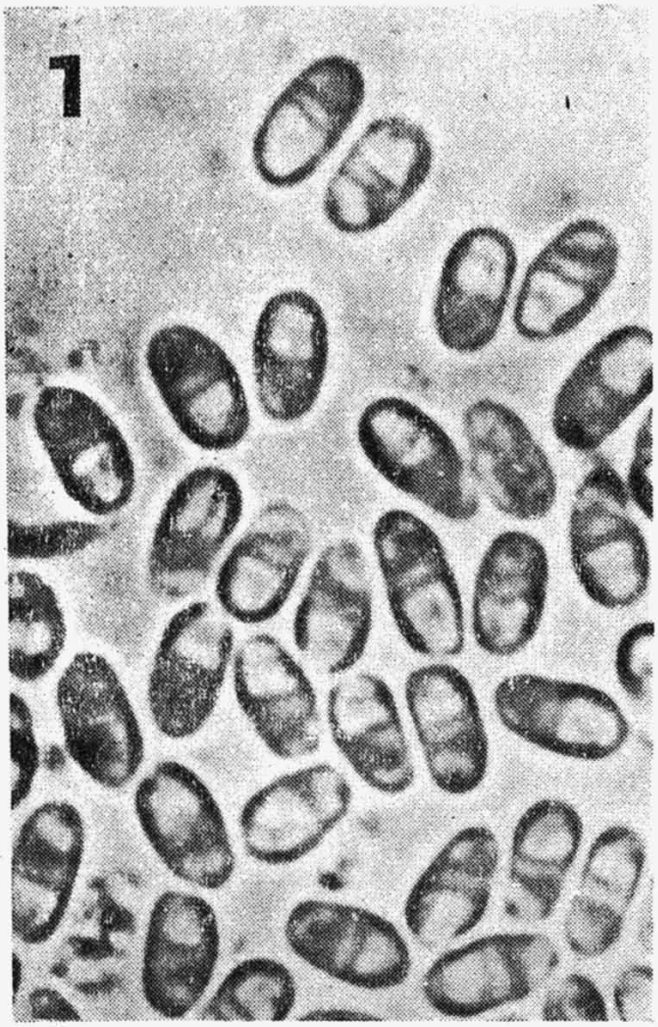
Structure and life cycle: The infected muscle fibers were found full of pansporoblasts with mature spores with only a few developmental stages interspersed among them. Sporogonial plasmodia with only a few nuclei were found to grow gradually to a size of 18 to 30 μm in diameter, at which stage they had numerous nuclei 0.4 to 0.6 μm in diameter. Such large sporonts were then fragmented into individual sporoblasts (Plate I, Fig. 1); however, nuclear division could be observed even during this fragmentation. Pansporoblasts with solid envelopes contained about 50 to 100 spores each. A very

small fraction only were smaller pansporoblasts with macrospores; their number did not surpass 8 to one pansporoblast. Sporonts developing into macrospores were distinguished by larger nuclei.

Ovoid microspores (formol-fixed material only was available) with broadly rounded anterior and a large posterior vacuole reaching to about midlength of the spore, 2.7 (2.4—3.7)×4.3 (4.0—4.8) μm in size. A single nucleus, 0.3×0.8 μm. Macrospores, 3.3 (3—3.7)×6.2 (6—7) μm in size, have a similar shape, being slightly more stubby. The posterior vacuole is extremely large, reaching anteriorly beyond the mid-spore length, with indications of polar filament's coils on its borders (Figs. 1, 3).

The infected muscle fibers are extremely enlarged. Since no early stages of infection could be observed, it is not clear whether they are hypertrophied single fibers or if they originated by confluence of several infected fibers. The sarcoplasma was completely filled with pansporoblasts and the fiber was encased with an envelope of fibroblasts, more compact internally and more loose externally. In some of the fibers, the pansporoblast envelopes persisted no more. There is a moderate degree of cell infiltration between the infected fibers. Alike in other muscle infections with *Pleistophora* (Dyková and Lom, in press) the infected fibers are target of the tissue reaction (Plate 1, Fig. 2).

It is not quite easy — due to the lack of comparative data in some instances — to distinguish *P. duodecimae* from the known species of fish *Pleistophora*. The presence of micro- and macrospores, among other things, differentiates it from *P. carangoidi*, *P. destruens*, *P. gadi*, *P. hippoglossoideos*, *P. hyphessobryconis*, *P. ladogensis*, *P.*



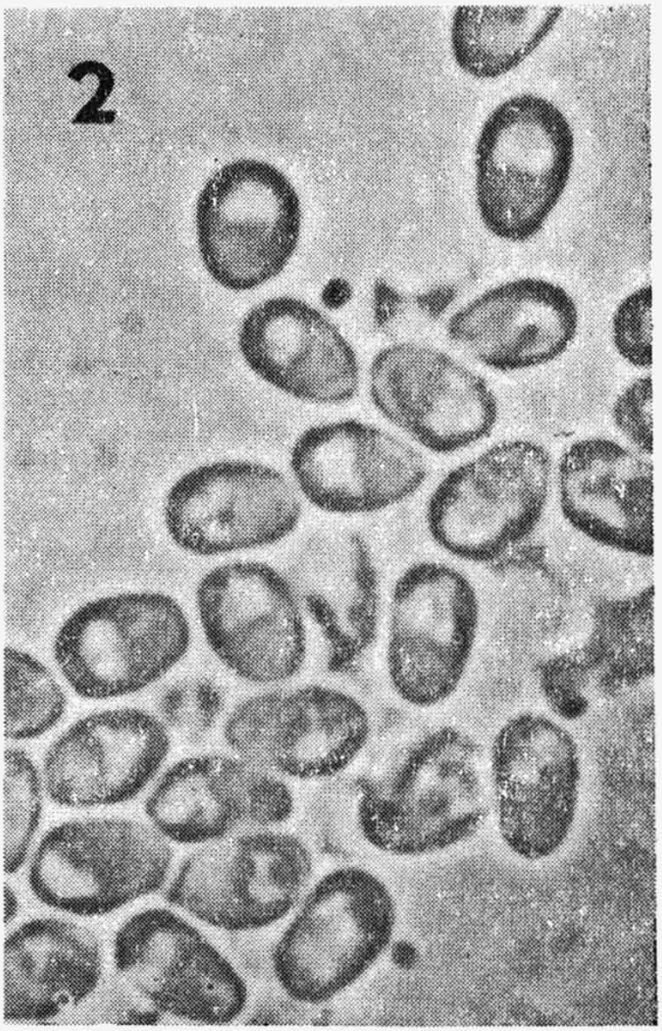


Fig. 1, 2. Formal-preserved spores of *Pleistophora duodecimae* sp. n. and *Glugea capverdensis* sp. n.

macrospora, *P. tahoensis*, *P. tuberifera* and *P. vermiformis*. The shape of spores differs from that in *P. anguillarum;* the shape of macrospores differs from those in *P. typicalis* and *P. littoralis*. There are two species which seem to be quite close to the present species, viz., *P. macrozoarcidis* and *P. ehrenbaumi*, both of which show spore dimorphism and infect the muscle bundles in the same way, i.e., replace their whole contents by a mass of pansporoblasts. However, in addition to taxonomically quite different hosts — which we do not feel is that important — some of their features are at variance with the present species. In *P. macrozoarcidis*, both micro- and macrospores are somewhat larger, and macrospores are sausage shaped rather than ovoid. In *P. ehrenbaumi*, the spores have a greater variability of shapes and much greater variability of spore number to one pansporoblast — which, however, is always much inferior to that in the present species — 4, 8 and 16!

Unless future studies show the contrary, we consider the species found in *Coryphaenoides nasutus* a new one and propose to designate it as *P. duodecimae* sp. n.

Glugea capverdensis sp. n. Plate II, Fig. 1.

Host: *Myctophum punctatum* Rafinesque, 1810 (Myctophidae, Myctophiformes), lantern fish; marine.
Site of infection: intestinal wall, mesentery, ovary.
Geographic distribution: Atlantic ocean, region of Cap Verde.
Prevalence: one infected specimen, 5.8 cm in size, was found.

Structure and life cycle: Rounded or oval xenomas up to 2 mm in diameter have a structure typical of the large glugeas (Plate II, Fig. 1). The xenoma wall is up to 3.3 μm thick. The peripheral cytoplasmic layer (Plate II, Fig. 3) is full of vegetative, dividing

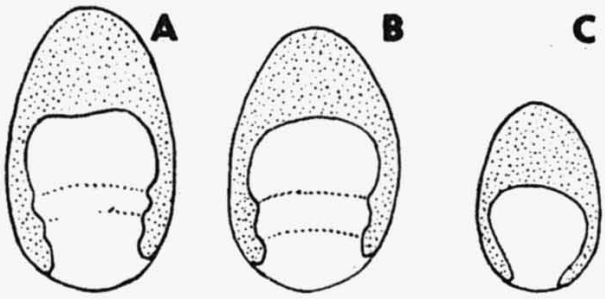


Fig. 3. Macrospores (A, B) and microspore (C) of *Pleistophora duodecimae* sp. n.

stages and elongated nuclear fragments of the host cell; centripetally, the cytoplasmic layer reveals numerous sporogonial vacuoles and the center is packed full with freely located mature spores.

Early developmental stages of the parasite are globular about 1.5 μm in diameter, or oval 1.5×2 μm, or spindle shaped surpassing the 2 μm length (Figs. 4A, B, C). They grow into multinucleate cells (Figs. 4E, F) and, finally, into cylindrical schizonts up to 20 μm long and about 2—3 μm wide (Fig. 4G). They fragment into uninucleate cells again, so that the peripheral cytoplasm of the xenoma harbours an array of schizonts with 1, 2, 3, 4 and more nuclei. Nuclei are all of about the same size, 0.8 μm. Sporogony sequence opens by formation of the sporogonic vacuole around multinucleate, elongated plasmodia (with an average of 14 nuclei) (Fig. 4H). They fragment into sporonts, rounded cells which, however, can arise from the sporogonial plasmodium also by radial segmentation (Fig. 2K). Sporonts divide in two sporoblasts — elongated cells with striking chromophil axis in the light microscope (Fig. 4N). They mature into spores.

Formol-fixed spores, the only material available, were elipsoidal or very slightly ovoid, both ends being almost equally rounded, 2.3 (1.8—2.6) × 4.2 (3.6—4.8) μm large. The large posterior vacuole reached to about mid-body of the spore. There is a single nucleus about 0.8 μm in its larger diameter (Fig. 2).

Large mature xenomas were extremely plentiful in the organs affected. Even at time when they only were encased with a thin, vascularized flat layer of connective tissue cells without eliciting other tissue reaction, because of their sheer number they caused a considerable pressure atrophy. At some places, only small islands of the original tissue are left unchanged. In the intestine, conglomerates of xenomas were so closely packed that they often bulged one into another. They were localized subepithelially reaching into the muscularis or were situated beneath the visceral sheet of the peritoneum. Many xenomas protruded into the intestinal lumen so that they seriously obstructed the passage through it.

Most interesting was the formation of secondary xenomas. They were observed in granulomas originating from xenomas destructed by the host tissue response in the ovary (Plate II, Fig. 2). Evidently, the spores ingested by the phagocytes discharged their germs which initiated the development of secondary xenomas inside — not out-

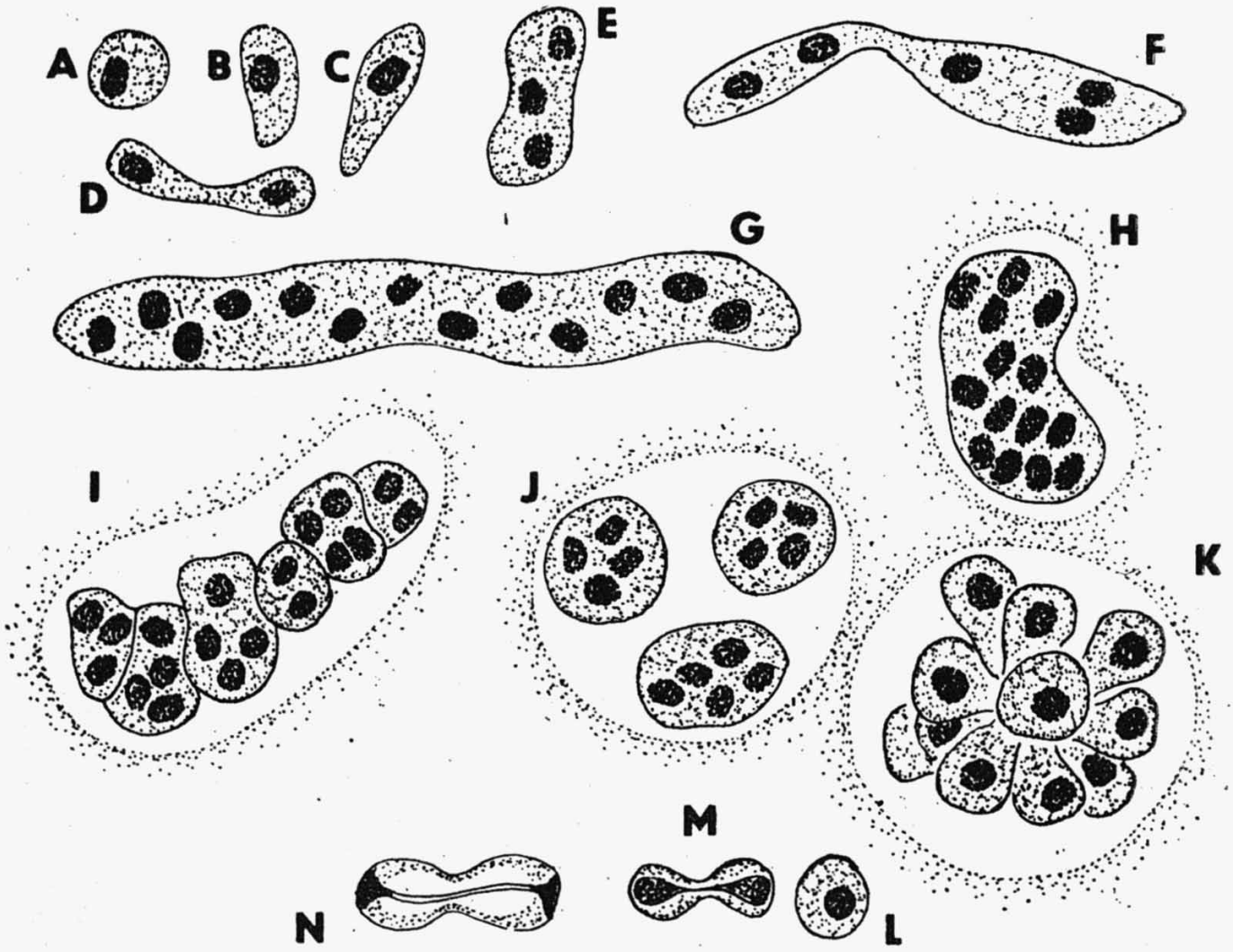


Fig. 4. Developmental stages of *Glugea capverdensis* sp. n. A, B, C — uninucleate schizonts; D — dividing schizont; E, F — multinucleate schizonts, F — dividing by plasmotomy; G — large multinucleate cylindrical schizont; H — sporogonial plasmodium within the sporogony vacuole; I, J — fragmentation of the sporogonial plasmodium; K — cleavage of the plasmodium directly into sporonts; L — sporont; M, N — less and more advanced sporont division into sporoblasts.

side! — the boundary of the "old" xenoma. Such phenomenon was recorded just once before, in *Glugea anomala* (Dyková and Lom, in press).

In the ovary growing xenomas caused also pressure atrophy reducing the number of oocysts and changing their shape without even any inflammatory reaction.

This species has to be assigned to the group of *Glugea*-species inducing the formation of large xenomas (i.e., larger than about 1 mm in diameter) encased with a definite light refractile wall, with host nucleus divided into numerous hypertrophied fragments and with solely peripherally located developmental stages. The development follows the pattern found in *G. anomala*.

This group includes *G. anomala* (with possibly synonymous *G. weissenbergi*), *G. jennica*, *G. hertwigi* and *G. plecoglossi* from among the freshwater hosts. All these species with the exception of *G. anomala* differ clearly in the shape of their spores from the present species. *G. anomala* has spores of about the same size range and of similar shape. However, we refrain from identifying the present species with *G. anomala* because of the different hosts, different salinity in which they live and different spectrum of organs infected.

The same group includes three marine species: *G. atherinae*, *G. shiplei* and *G. stephani*. The first two differ in the shape of spores to an extent sufficient for a clear-cut separation. However, *G. stephani* has spores which have an overlapping size range and the shape rather similar to the present species. If we do not identify the present species with *G. stephani*, it is because of the lack of positive evidence for the identity of both species: they have taxonomically different hosts; *G. stephani* infects the ovary rather exceptionally, only in cases of heavy infections, while in the present species it seems to be the normal site of infection, and the spores do reveal differences in shape and inner structure.

Rather than to designate it as *Glugea* sp. which would raise the number of undetermined *Glugea* species, we choose to establish it for practical reasons as a new species. Unless future findings will prove otherwise, we consider it a new species and designate it as *G. capverdensis* sp. n.

DISCUSSION

Fish are invaded by members of at least 7 microsporidian genera (Sprague 1976). It is very probable that their number will increase, since the *Glugea*-complex will quite certainly be split into several genera so that all genera might be dependably differentiated by their development and type of structural host-parasite interaction. Within the genera, however, a completely reliable morphological separation of species is sometimes difficult to achieve; the xenoma formation (in *Glugea*) or morphogenesis of pansporoblasts and their association in "cysts" (in *Pleistophora*) may be almost identical in many species. If they have distinctly different spores as, e.g., *Glugea hertwigi* vs. *G. anomala*, their specific independence seem to raise no doubts (although this is a rather fragile evidence according to Weissenberg (1968), author of the *G. hertwigi* description himself). Sometimes, however, the spore shape and size may be quite similar. In *G. stephani* and *G. anomala*, the developmental stages and the structure of xenomas (with the exception of slight differences in cortical layer of the hypertrophied cells on the ultrastructural level — see Weidner 1976 and Lom et al. 1979) are almost identical. Here, it is the hosts and organ preferences which determine the separatedness of both species. Other similar cases could be quoted, including members of the genus *Pleistophora*. In such instances, the lack of other differentiating characters (electron microscopical findings, electrophoretic and immunological analysis, multivariate analysis of morphological features) as well as the practical impossibility to perform cross-infection

is made themselves painfully evident. Future multi-approach research will perhaps be in position to solve the species-level taxonomical problems. Until then, the practice of establishing duly described microsporidians infecting different fish hosts and their organs as separate species cannot be generally contradicted.

ДВА НОВЫХ ВИДА МИКРОСПОРИДИЙ, ПАРАЗИТИРУЮЩИХ У МОРСКИХ РЫБ В АТЛАНТИЧЕСКОМ ОКЕАНЕ

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Резюме. Описаны два новых вида микроспоридий от морских рыб из Атлантического океана: *Pleistophora duodecimae* sp. n. из скелетной мускулатуры *Coryphaenoides nasutus* Günther и *Glugea capverdensis* sp. n. из кишки, мезентерита и яичника *Myctophum punctatum* Rafinesque. Наблюдали образование вторичных ксеномов у *Glugea capverdensis.* Оба вида могут вызывать важные повреждения хозяинов.

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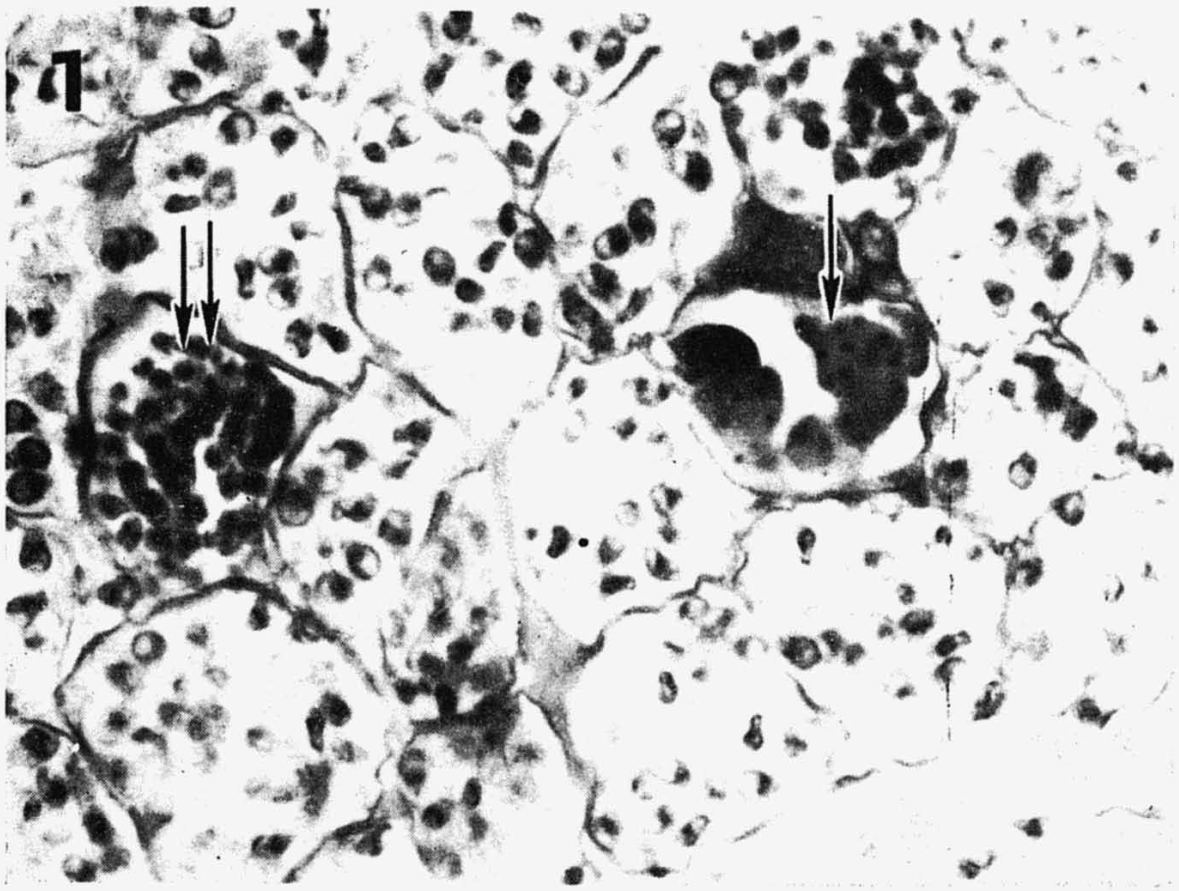


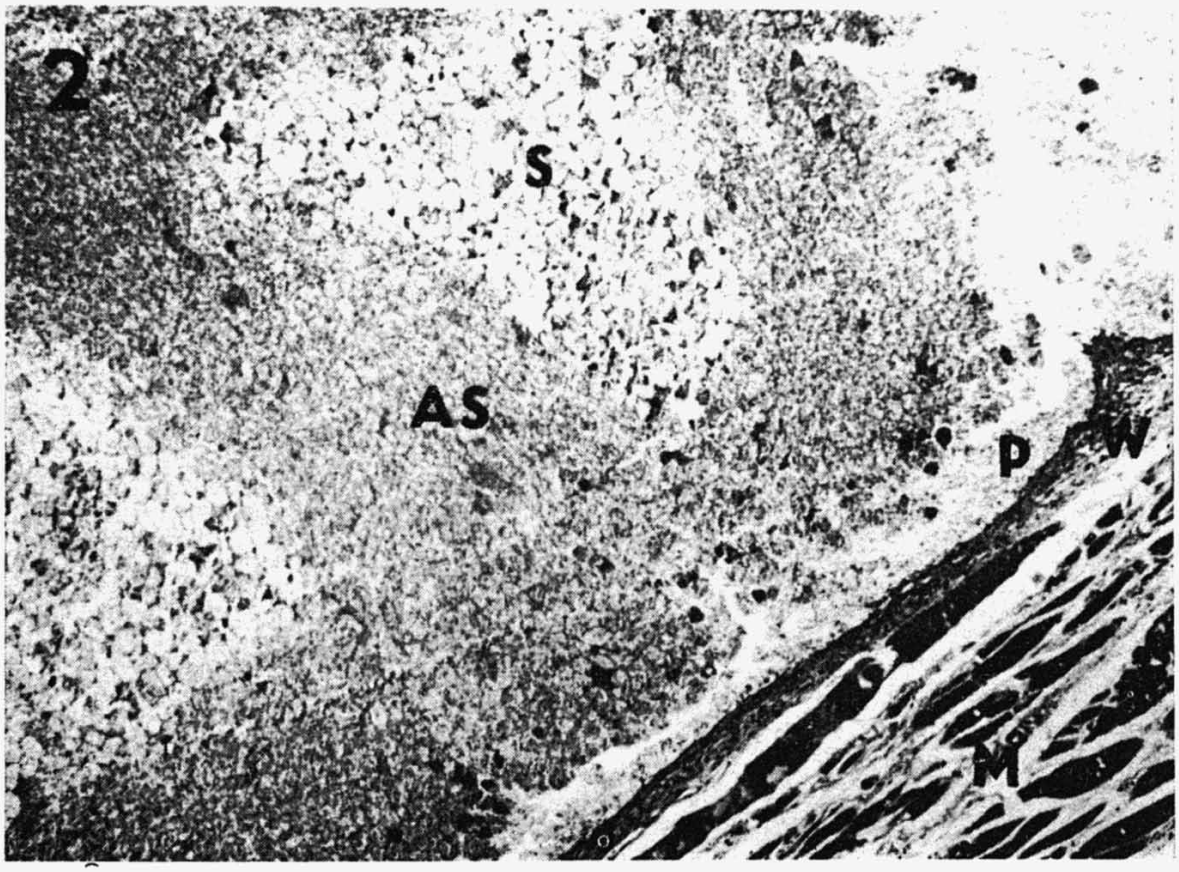
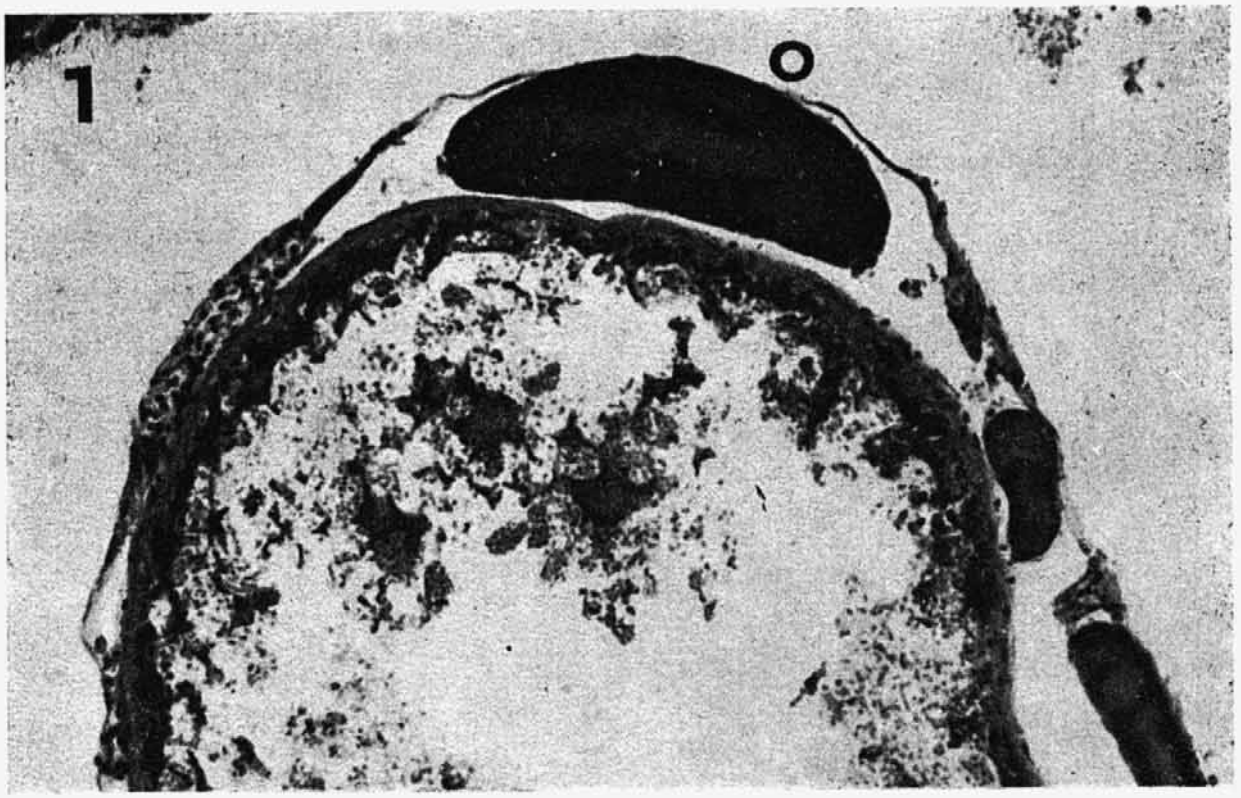


Fig. 1. *Pleistophora duodecimae* sp. n. Pansporoblasts with spores; only some of the spores are properly stained. Arrow points at a sporogonial plasmodium undergoing fragmentation; double arrow points at a pansporoblast with developing sporoblasts. (H&E, × 850.)

Fig. 2. Part of the cyst affected by the host tissue response. S — an islet of undisturbed pansporoblasts; AS — mass of sporoblasts with an altered appearance; W — wall of the cyst; P — zone of host's phagocytes; M — muscle fibers. (H&E, × 120.)



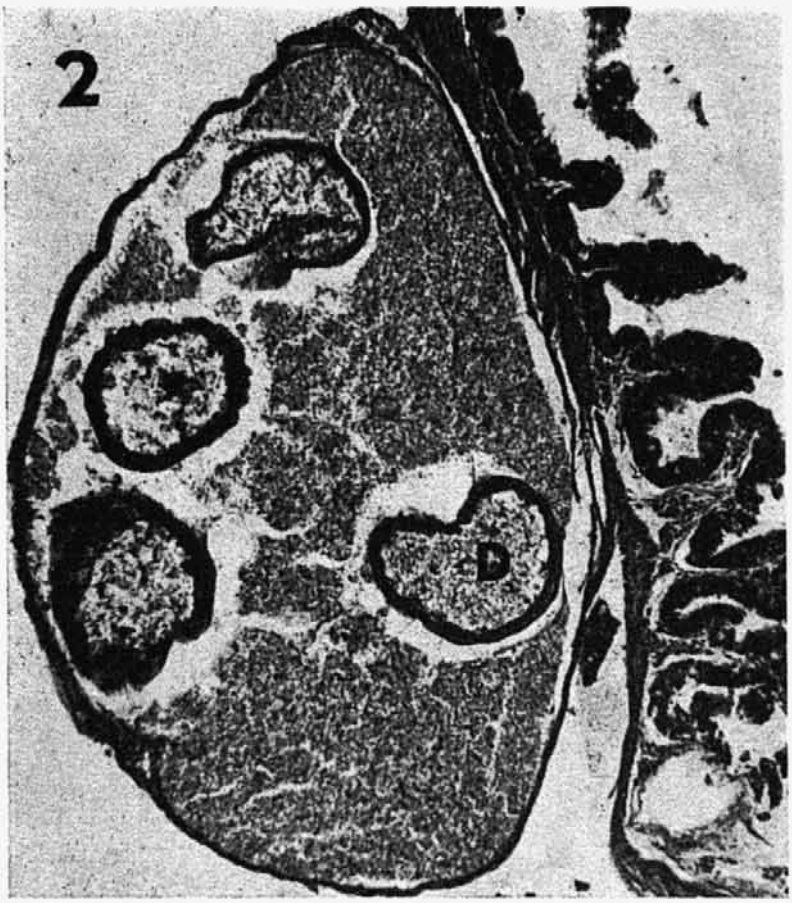


Fig. 1. *Glugea capverdensis* sp. n. Part of a xenoma in the ovary (empty central space is an artifact). O — flattened oocyte. Note the cortical layer with developmental stages. (H&E, × 295.)

Fig. 2. Four secondary xenomas (D) within a xenoma pervaded by host's phagocytes. The whole complex is attached to the lamina muscularis of the intestine. (H&E, × 130.)

Fig. 3. Cortical layer of a xenoma with developmental stages, encased with a refractile wall with a few adhering connective tissue cells. (H&E, × 780.)