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VERSITA

Redescription of *Myxidium sphaericum* Thélohan, 1895 and *Ceratomyxa beloneae* Lubat *et al*., 1989 from the gall bladder of the garpike, *Belone belone* in the Adriatic Sea

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Abstract

The garpike *Belone belone* (L.) is an epipelagic fish abundant in the Mediterranean, parasitized by two coelozoic myxosporidians in the gall bladder – *Myxidium sphaericum* Thélohan, 1895 and *Ceratomyxa beloneae* Lubat, Radujković, Marques et Bouix, 1989. In order to redescribe these intriguing and taxonomically complex species, whose original descriptions are limited only to line drawings based on light microscopy, we studied the morphology and ultrastructure of their vegetative and sporogenic stages. *M. sphaericum* has an oval to sigmoid or mild crescent shape in the frontal view, large conical polar capsules, opening at the spore end, in opposite direction of the spore length, with 8–9 coils of polar filament. *C. beloneae* has elongated and slightly arched valves; symmetrical and smooth, with the posterior surface almost straight and anterior surface mildly inclinated, having 5 coils of the polar filament.

Keywords

Fish, garpike, *Belone belone*, Myxozoa, *Myxidium sphaericum*, *Ceratomyxa beloneae*

Introduction

The garpike *Belone belone* (L.) is an epipelagic teleost restricted to the north eastern Atlantic, Mediterranean and the Black Seas, considered an economically valuable species especially for Mediterranean's countries. Three subspecies have been recognized (Collette and Parin 1986); *B. belone belone* restricted to the north-eastern Atlantic; *B. b. euxini* Günther found in the Black Sea, and *B. b. gracilis* Löwe mainly distributed in the Mediterranean. Especially great abundance of the garpike has been found around the sea cage fish farms, attracted by the structures in the pelagic environment and the uneaten portion of feed that falls through the cage nettings (Dempster *et al*. 2002).

Myxosporea represent a major group of fish parasites and their impact on wild and cultured fish is significant (Kent *et al*. 2001). Only two myxosporean parasites have been reported from the gall bladder of *B. belone* in the Adriatic Sea, namely *Myxidium sphaericum* Thélohan, 1895 and *Ceratomyxa beloneae* Lubat *et al*., 1989. The former was considered as a species new to the Adriatic, while the later was described for the first time. In both cases, however, no detailed morphological and ultrastructural data were provided. This led to an entangled taxonomy of *M. sphaericum*, which Lom and Dyková (1992) suggested was inadequately described from *B. belone*, being present mainly in the gadid fish (Noble 1957). Moreover, Kabata (1963, 1967) considered the myxozoan as a biological tag for gadid stock identification. Only later on, MacKenzie and Kalavati (1995) pointed out the differences between their description of *M. sphaericum* from the whiting (*Merlangius merlangius* L.) and the original description by Thélohan (1895) and Lubat *et al*. (1989), both from the pelagic garpike. Authors suggested that the type species *M. sphaericum* parasitizes pelagic belonid fish, while the forms reported from demersal fish belong to at least one other species (MacKenzie *et al*. 2005).

In respect to *M. sphaericum*, *C. beloneae* was isolated and identified for the first time in the gall bladder of the Adriatic Sea *B. belone* (Lubat *et al*. 1989). To our knowledge, it is the only record of this species, limited to a short description and

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illustration based on the isolation from three of the total five fish examined.

During the parasitological investigation of the *B. b. gracilis* sampled near the sea cages of an Adriatic Sea tuna farm, we have found high prevalence and abundance of these two myxosporean species in the *B. belone* gall bladder. In order to redescribe these intriguing and taxonomically complex species, whose original descriptions are limited only to line drawings based on light microscopy, we studied the morphology and ultrastructure of their vegetative and sporogenic stages.

Materials and methods

From October 2007 till May 2008, 142 samples of garpike (*B. b. gracilis*) were fished by hook and line near the sea

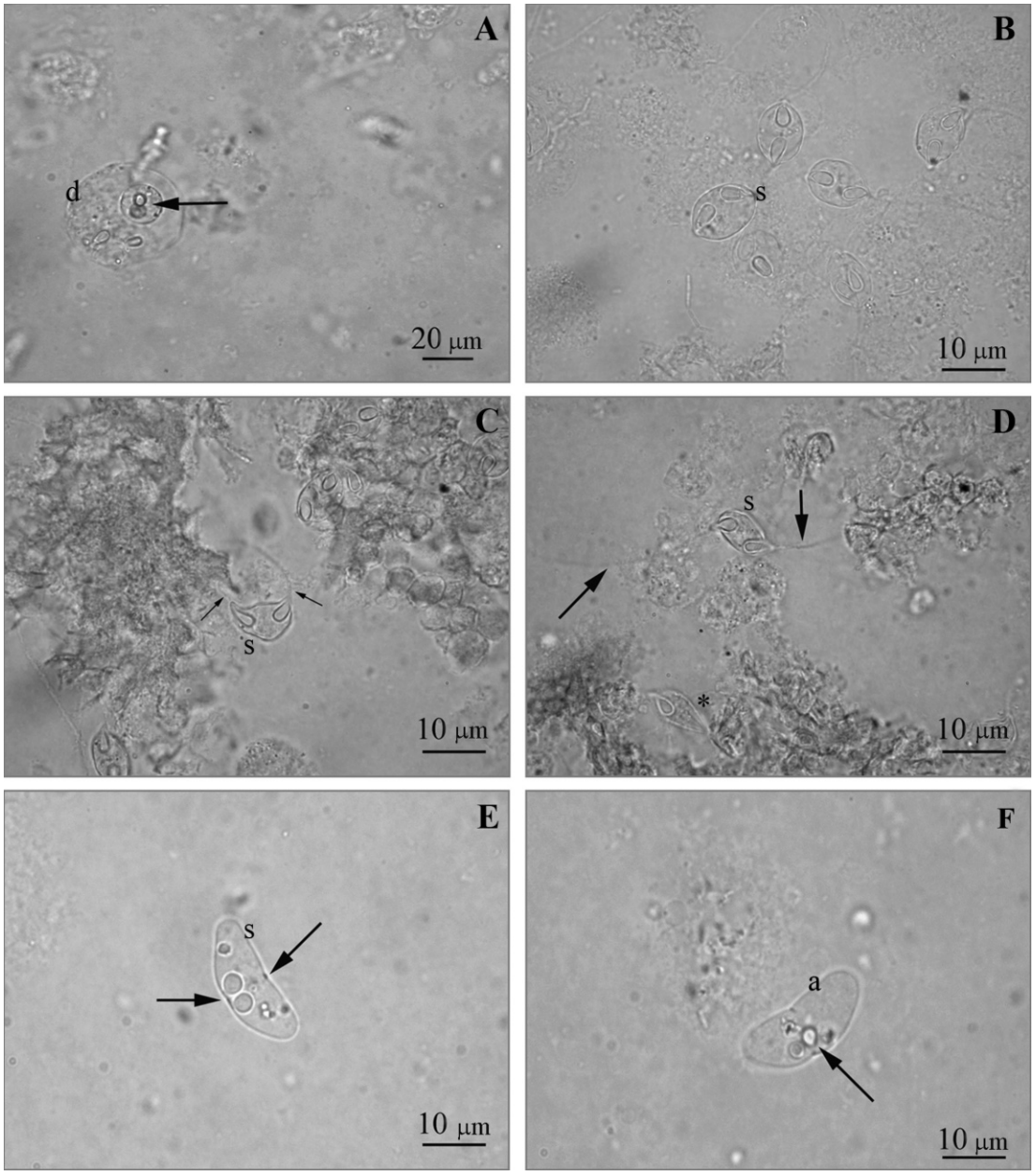


Fig. 1. A. Floating disporoblastic plasmodia (d) of *Myxidium sphaericum* in the gall bladder lumen. Two spores with an asynchronous development are floating freely in its cavity (arrow). **B.** Mature spores (s) of *M. sphaericum* in the gall bladder of the *B. belone*. **C.** Apical view of mature spore (s) of *M. sphaericum* in the gall bladder of the *B. belone*. Note the extruded long polar filaments (arrows). **D.** Lateral view of mature spore of *M. sphaericum* in the gall bladder of the *B. belone* (∗). Another spore (s) shows extruded long polar filaments (arrows). **E.** Mature spore (s) of *Ceratomyxa beloneae* in the bile of the garpike. Note the constriction of the suture (arrows). **F.** Mature aberrant (a) *C. beloneae* spore with three polar capsules (arrow)

cages of a tuna farm in the Mid Adriatic Sea. A part of collected gall bladders was stored on ice until arrival in the laboratory, where scrapings of bladder mucosa and bile were examined under the light microscope. Fresh myxosporidians were photographed by Olympus digital camera C0404 and processed using data-processing software, DP-soft based on measurement guidelines of Lom and Arthur (1989).

Small fragments of gall bladder and bile infected by parasites were collected and fixed in 3.5% paraformaldehyde and 3% glutaraldehyde in 0.1 M PBS (phosphate buffer solution) immediately after catching the fish. The rest of the same tissue was examined prior further processing, in order to prepare TEM tissue blocks where only one myxosporidian species was present in the bile. Tissue was postfixed in 1% osmiumtetroxide for 1 h, then dehydrated in an ascending series of acetone and embedded in Durcopan resin. Semithin sections were stained with methylene blue and examined under light microscope Olympus BX41. The ultrathin sections (0.05 μm) were made from the chosen area of interest and stained with uranyl acetate and lead citrate. The electron microscope FEI Morgagni 268D was used for examination of the ultrathin sections.

Results

Myxidium sphaericum

Type host: *Belone belone gracilis*.

Location in the host: Coelozoic, in the bile.

Prevalence: 85%.

Type location: Adriatic Sea, 44°02′17.87″N, 15°10′57.52″E.

Type material: Infected bile fixed in 70 and 100% ethanol is deposited at the parasitological collection of Institute of Oceanography and Fisheries, Croatia, under accession number MY2008/014.

Vegetative stages: The youngest developmental stages observed in the lumen were free, large proliferating trophozoites, abundant with lipid droplets or β-glycogen granules (Fig. 2A). They had large eccentric nucleus, scarce mitochondria, and some empty vacuoles. In their cytoplasm, small and spherical secondary cells, enveloped by a double membrane, showed a small rime of granulated cytoplasm and condensed centrally located large nucleus. Older proliferating stages consisted of a primary trophozoite cell with eccentric vegetative nucleus, and embedded secondary cells (Fig. 2B). In the primary cell cytoplasm, scattered ribosomes, lysosomes and mitochondria were abundant. Floating disporoblastic plasmodia were in the gall bladder lumen, without pseudopodia, large, spherical, comprising two spores floating freely in its cavity (Fig. 1A). Asynchronous development in the disporoblast was observed.

Spores: In frontal view oval to sigmoid or mild crescent shape (Fig. 1B). In apical view crescent shape, slightly pointing backwards (Fig. 1C). In lateral view, conical, tear-like, sharply pointing backwards (Fig. 1D). Large conical polar capsules, opening at the spore end, in opposite direction one from the other. It had an inner thick electron-lucent and an outer electron-dense wall. Long polar filament had 8–9 coils, each coil spiralised on itself, perpendicular with the longitudinal axis of the polar capsule (Fig. 2H). Suture line was feebly visible, thin, vertical to the transversal spore axis, overlapping (Fig. 1C). Sporoplasm was binucleated, rich in ribosomes, mitochondria and vacuoles, occupying central part of the spore, with some sporoplasmosomes. No external ornamentation was present. Spore (n = 50) measured 12.95 ± 1.1 μm (length) × 13.16 ± 2.16 μm (width) × 8.17 ± 1.05 μm (thickness); polar capsules measured 2.83 ± 0.52 μm (width) × 5.89 ± 0.98 μm (length).

Ceratomyxa beloneae

Type host: *Belone belone gracilis*.

Location in the host: Coelozoic, in the bile.

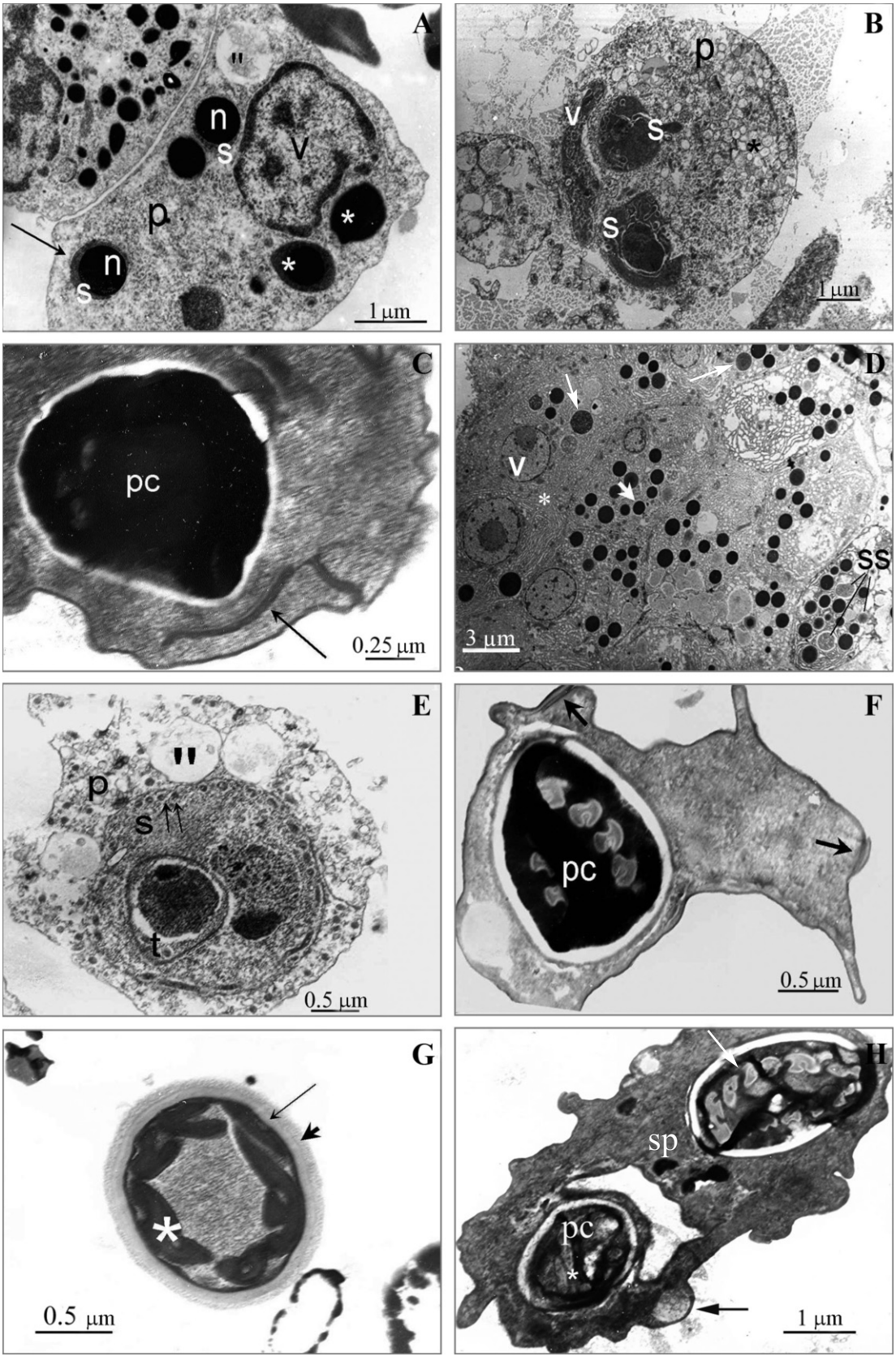
Prevalence: 62%.

Type location: Adriatic Sea, 44°02′17.87″N, 15°10′57.52″E.

Type material: Infected bile fixed in 70 and 100% ethanol is deposited at the parasitological collection of Institute of Oceanography and Fisheries, Croatia, under accession number MY2008/015.

Vegetative stages: The earliest vegetative stages were embedded intercellulary in the epithelium of gall bladder mucosa (Fig. 2D). They show large vegetative nucleus with large nucleolus, and small primordial secondary cells within their cytoplasm. Cytoplasm was rich in lipid or β-glycogen granules and extremely developed rough endoplasmatic reticulum. Few lysosomes were noticed as well as sporoplasmosome in very young stages. Free invading sporoplasm was ameboic with short pseudopodia, rich in sporoplasmosome, lipid or β-glycogen granules and large mitochondria. Older proliferating stages consisted of a more vacuolated primary trophozoite cell with eccentric vegetative nucleus, and embedded secondary cells. On few times, degenerative trophozoites were observed in the lumen, showing lysosomic vacuolization, mitochondria and chromatin degradation (Fig. 2E). Floating disporoblasts have 1–2 long pseudopodia.

Spores: Valves elongated and slightly arched, symmetrical and smooth (Fig. 1E). When observed from the frontal view, posterior surface of the spore is almost straight, slightly inclinated in some forms, while the anterior surface has a mild inclination downwards. Sporoplasm was in the central part of the spore, binucleate. Polar capsules were subspherical, equal in size, with foramina near the straight and conspicuous sutural line at the anterior part of the spore (Fig. 2F). It had 5 coils of polar filament, each coil spiralised on itself, perpendicular with the longitudinal axis of the polar capsule. Polar capsule wall had an inner homogeneous and electron-lucent layer and an outer granulous and proteinaceous (Fig. 2G). Rarely aberrant spores with three polar capsules were observed (Fig. 1F). Spore (n = 50) measured 11.88 ± 1.84 μm (length) × 28.4 ± 2.26 μm (width) × 4.77 ± 0.39 μm (thickness); polar capsules measured 4.61 ± 0.38 μm (width) × 4.77 ± 0.39 μm (length).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Discussion

Genus *Myxidium* Buetschli, 1882 comprises more than 149 species with a fusiform, straight or slightly crescent or sigmoid shape, and with more or less pointed ends (Lom and Dyková 1992). Among them, *M. sphaericum* isolated from Atlantic, Mediterranean, Florida and Australian waters, stands among those with the most complicated taxonomic history, being reported from 18 fish species distributed in 8 families. Noble (1957) believed, based on the spore morphology and size, that *M. bergense* and *M. sphaericum* isolated from the Atlantic whiting (*Merlangius merlangius*) were the same species. MacKenzie and Kalavati (1995) first raised the question concerning the validity of such geographical range, noticing that the parasite isolated from Atlantic gadids shows morphologically different features from the type species described from the garpike (Lubat *et al*. 1989), mainly reflecting in the number of filament coils (3–4 for the former *vs* 5 for the later). Another *M. sphaericum* type was recovered from Great Barrier Reef fish, showing same spore features as the previous types, except having 5–8 filament coils and a polysporous pansporoblast (Moser *et al*. 1989). This suggested that there is at least one additional species parasitizing demersal gadids different from the original type isolated from pelagic belonid fish (MacKenzie and Kalavati 1995). Our redescription gave new insight in the missing morphology of *M. sphaericum* vegetative stages, while the spore morphology is congruent with the description of the original type species from the garpike. The only difference was noticed in the number of filament coils (8–9 in our case) as evidenced by TEM. However, since most of *M. sphaericum* reports were substantiated based on line drawings and light microscopy, the interpretation of their numbers of filament coils must be considered with caution. Interestingly, Ali *et al*. (2006) recently described by light microscopy a new species, *Myxidium elmatboulii*, from another belonid *Tylosurus choram* Rüppell, 1837. They distinguished it from the garpike *M. sphaericum* based on the number of filament coils (9–10 in the former, respectively) and polar capsule size. This new *Myxidium* sp. differs from our redescription mostly in the appearance of the disporoblast, which in our case is large with two spores floating freely in its cavity, whereas in *M. elmatboulii* the spores are tightly packed together without any free space.

In conclusion, it is hard to differentiate between *M. sphaericum* "complex" based on light microscopy data and the lack of ultrastructural evidences from all other types, moreover knowing that dimensions of trophic stages depend on many factors (Noble 1957). Therefore, we hope that molecular tools will aid in the correct definition of *M. sphaericum* species.

Among the coelozoic Myxozoa, the genus *Ceratomyxa* is widely distributed in marine habitats, showing broad spectra of hosts and geographical area. More than 147 isolated species were reported from the urinary tract or gall bladder (Lom and Dyková 1992, Eiras 2006). In the Adriatic Sea, ten different *Ceratomyxa* spp. were isolated, among these four were unidentified and unnamed (Lubat *et al*. 1989, Mladineo and Bočina 2006), showing both morphological and dimensional differences compared to *C. beloneae*. The type species *C. beloneae* was described as slightly curved in valves, with hemispheric valvular extremities, and polar capsules slightly elongated and inclinated one to the other. By comparing the original drawings of Lubat *et al*. (1989) to our redescription, there is a notable difference in the degree of the posterior spore inclination. While we observed almost straight posterior surface, curved in some forms, the authors drew spores being anteriorly convex and posteriorly concave, with slightly arched polar capsule. This incongruence might emerge as a result of the different microscopy techniques used (e.g. light microscopy in the later case), as well as the interpretation of the results, since the original description provides only line drawings. However, given the same type host and type location we argue that it is the same ceratomyxan species as described by Lubat *et al*. (1989). Similar straight posterior spore surface present in *C. beloneae* can be found in *C. buri* from the yellowtail *Seriola quinqueradiata* (Temminck et Schlegel, 1845), although they can be distinguished easily by the number of polar filament coils, which is only 3–4 in the later species (Yokoyama and Fukuda 2001). Another ceratomyxan *C. ghaffari* was recently described from a belonid fish, the Red Sea houndfish (*Tylosurus choram*), distinguish from the *C. beloneae* by size and in being more elongated, penniform and with slight inclination in both the dorsal and ventral surface (Ali *et al*. 2006).

Fig. 2. A. TEM micrograph of proliferating trophozoite of *Myxidium sphaericum*, abundant with lipid droplets or β-glycogen granules (∗). Note an eccentric large vegetative nucleus (v) of the primary cell (p), and small secondary cell (s) with condensed nucleus (n), enveloped by a double membrane (arrow). Lysosomes are also present (ıı). **B.** TEM micrograph of older proliferating trophozoite of *M. sphaericum* consisting of a primary cell (p) with eccentric vegetative nucleus (v), and embedded secondary cells (s). Note abundant mitochondria (∗). **C.** TEM micrograph of detail of the *M. sphaericum* spore below the polar capsule (pc), showing overlapping sutural line (arrow). **D.** TEM micrograph of early vegetative stages of *Ceratomyxa beloneae* embedded intercellulary in the epithelium of gall bladder mucosa. In some of them vegetative nuclei (v), small primordial secondary cells (arrows), cytoplasm rich in lipid or β-glycogen granules (white short arrow), developed rough endoplasmatic reticulum (∗) and sporoplasmosomes (ss with lines) are observed. **E.** TEM micrograph of degenerative trophozoite of *C. beloneae* with lysosomic vacuolization (ıı) inside the primary cell (p). Note chromatin degradation (arrows) in the secondary cell (s) and the tertiary cell (t). **F.** TEM micrograph of mature spore of *C. beloneae* with 5 coils of the polar filament in the polar capsule (pc) and the overlapping detail of the suture (arrows). **G.** TEM micrograph of a polar capsule of the mature *C. beloneae* spore enveloped by an inner (arrow) and outer layer (short arrow). Note the hollow coils of the polar filament (∗). **H.** TEM micrograph of mature spore of *M. sphaericum*, with a central sporoplasm (sp), 9 coils of polar filament (arrow), each spiralised on itself (∗) in the polar capsule (pc). Suture line is feebly visible and thin (white short arrows)

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