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Myxidium biliare sp. n. (Myxozoa) from gall bladder of *Galaxias maculatus* (Osmeriformes: Galaxiidae) in Patagonia (Argentina)

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Abstract. *Myxidium biliare* sp. n., a new myxosporean species parasitizing the gall bladder of *Galaxias maculatus* (Jenyns), in Patagonia, is described. Its coelozoic plasmodia were floating free in the bile. Spores are fusiform 13.7 ± 0.9 μm long and 6.9 ± 0.6 μm wide, with rounded ends in frontal view and slightly pointed ends in sutural view; shell with ridges and sinuous sutural line. Both maximum prevalence and maximum percentage of immature plasmodia occurred in summer. In winter the prevalence and the percentage of immature plasmodia fell to their lowest values. Prevalence was independent of host sex but increased with host length. Prevalence in 15 Patagonian Andean lakes (situated from 39°25'S to 41°30'S) ranged between 4.2% and 70%.

Four species of *Myxobolus* Bütschli, 1882 have been recorded from *Galaxias maculatus* (Jenyns) throughout its distribution range. In New Zealand, Hine (1976) described *Myxobolus iucundus* Hine, 1976 parasitizing muscles. In Tierra del Fuego, Szidat (1953) described *M. magellanicus* Szidat, 1953 in gills and *M. galaxii* Szidat, 1953 in muscles and organs of the abdominal cavity. In Falkland Islands Kalavati et al. (2000) described *M. bartoni* Kalavati, Brickle et MacKenzie, 2000 in the trunk musculature. Viozzi (1996) recorded the presence of spores of an unnamed species of *Myxidium* Bütschli, 1882 in the gall bladder of *G. maculatus* in a Patagonian Andean lake. The aim of this study is to describe this new species of *Myxidium*, to provide information about its distribution range in north-western Patagonia and to describe the variation in its prevalence in relation to season and host sex and length from a Patagonian oligotrophic lake.

MATERIALS AND METHODS

The principal sampling locality was Lake Moreno (41°04'S, 71°33'W), an oligotrophic lake situated 764 m above sea level. It has a surface area of 5.4 km^2 with a maximum depth of 112 m. Its temperature varies throughout the year from 6°C to 20°C. The other 15 surveyed lakes are situated between 39°25'S, 71°20'W and 41°30'S, 71°40'W. They belong to the Araucanian Region and are also oligotrophic.

Galaxias maculatus is a widely distributed freshwater fish, known from Australia, Tasmania, New Zealand, South America and Falkland Islands. Both diadromous and landlocked populations can be found in South America.

Samples of *G. maculatus* were collected with baited traps. In Lake Moreno, they were captured monthly from November 1998 to November 1999; water temperature was also recorded monthly. One sample only in spring, summer or autumn was collected from the other lakes. Fish were transported to the laboratory and kept at a controlled temperature (6°C) until killed and examined between 24 and 72 hours after capture. Sex and total length of fish were recorded, the gall bladder dissected to obtain the trophozoites and the presence of mature spores recorded. In order to prepare and describe trophozoites and spores, Lom and Arthur's (1989) guideline was followed. Morphometric measurements were based on 90 fresh spores obtained randomly from different trophozoites. Spores were studied with a compound microscope and drawn with the aid of a camera lucida. Some gall bladders were fixed in 10% buffered formalin, dehydrated in a graded ethanol series, cleared in xylene and embedded in paraffin. Histological sections (7 μm thick) were stained with a Mallory's trichrome. Measurements are given in micrometres.

Monthly sample data were grouped by season according to water temperature as follows: winter samples under 10°C, spring and autumn samples between 10 and 15°C and summer samples over 15°C. Seven hundred and seven *G. maculatus* (mean length 49.9 ± 8.1 mm; range 23.3–85.5 mm) from Lake Moreno were examined. The sex of 599 specimens was determined, of which 327 were females and 272 males. To study the annual infection cycle, the presence of mature spores in plasmodia was recorded. The relationship between prevalence and season was tested by an R×C test of independence using the T-test ($P < 0.05$) (Conover 1980). The covariation of prevalence and host length was analysed using the Spearman rank correlation test ($P < 0.05$). A Chi-square test was carried out in order to examine the relation between prevalence and host sex ($P < 0.05$) (Conover 1980).

RESULTS

Myxidium biliare sp. n. Figs. 1–3

Description

Coelozoic plasmodia numbering 1–6 were seen through the gall bladder wall, floating free in bile. They were not found in any other organs. Gross pathological

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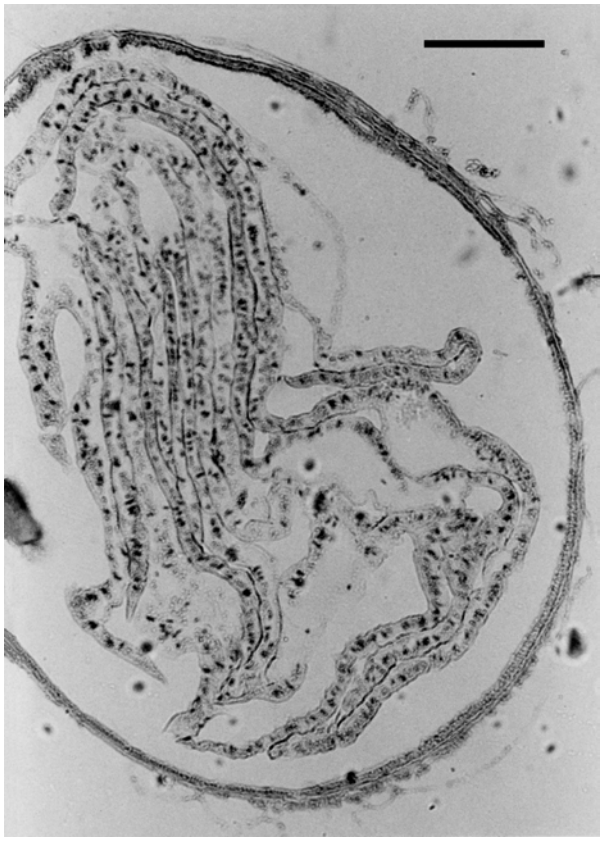

Fig. 1. Histological section of gall bladder with a folded plasmodium of *Myxidium biliare* sp. n. Scale bar = 150 µm.

signs in parasitized gall bladders were not observed. Plasmodia were 2,048 ± 555 (range 1,420–3,690; n = 20) long, flat, folded; they occupied most of the volume of the gall bladder (Fig. 1). They contained numerous pansporoblasts and spores. Sporogenesis asynchronous, pansporoblast disporoblastic. Ectoplasm narrow.

Mature spores fusiform with rounded ends in frontal view and slightly conical ends in sutural view. Shell with 7–9 ridges on the surface of each valve. Sutural line slightly sinuous. Fresh spores 13.7 ± 0.9 (12–15) long, 6.9 ± 0.6 (6–8) wide and 6.9 ± 0.6 (6–9) thick. Polar capsules at opposite ends of spores, equal in size, 5.7 ± 0.5 (5–6) long, pyriform, rounded in proximal zone, with a narrow, slightly curved distal end and openings at tips of spores. In some specimens polar capsules slightly angled to one another. Polar filaments with 5–7 coils. Single sporoplasm located between polar capsules (Figs. 2, 3).

Taxonomic summary

Type host: *Galaxias maculatus* (Jenyns, 1842) (Osmeriformes, Galaxiidae).

Type locality: Lake Moreno (41°04'S, 71°33'W).

Site of infection: Gall bladder.

Overall prevalence in type locality: 37% (260/707).

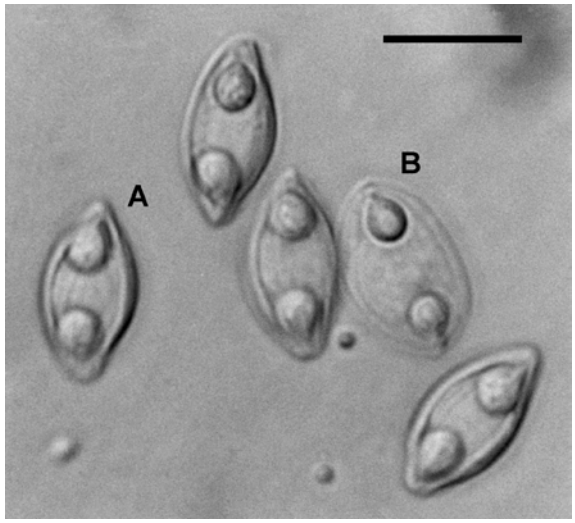

Fig. 2. Spore of *Myxidium biliare* sp. n. in sutural view (A) and in frontal view (B). Scale bar = 10 µm.

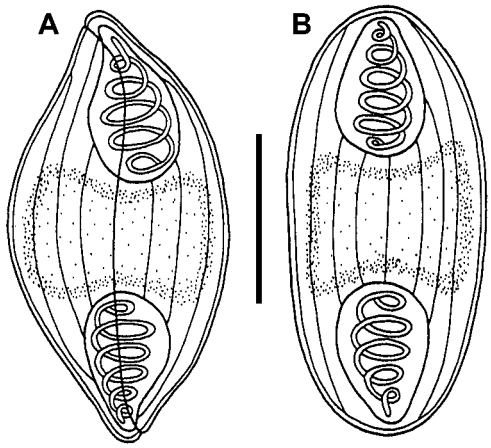

Fig. 3. Line drawing of spore of *Myxidium biliare* sp. n. in sutural view (A) and in frontal view (B). Scale bar = 5 µm.

Other localities (lake name, coordinates, infected fish/examined fish): Quillén, 39°25'S, 71°20'W, (4/10); Lácar, 40°10'S, 71°30'W, (2/31); Villarino, 40°28'S, 71°35'W, (9/20); Falkner, 40°28'S, 71°30'W, (14/20); Filo Hua Hum, 40°30'S, 71°20'W, (8/20); Espejo, 40°41'S, 71°42'W, (1/24); Traful, 40°37'S, 71°35'W, (14/24); Correntoso, 40°44'S, 71°39'W, (3/28); Piré, 40°44'S, 71°43'W, (3/35); Nahuel Huapí, 40°48'S, 71°39'W, (4/25); El Trébol, 41°05'S, 71°30'W, (1/4); Gutiérrez, 41°11'S, 71°25'W, (2/40); Mascardi, 41°17'S, 71°34'W, (13/81); Roca, 41°21'S, 71°45'W, (8/25); Steffen, 41°30'S, 71°40'W, (3/27).

Voucher specimens deposited: Slides with histological sections (No. ZW 1500) and a whole parasitized gall bladder fixed in 10% buffered formalin (No. ZW 1501), in the Museum of New Zealand, Te Papa

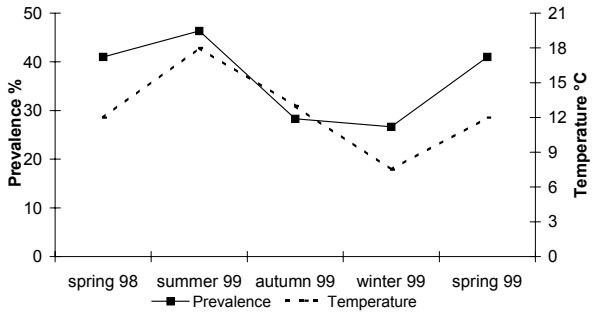

Fig. 4. Seasonal fluctuation of temperature and prevalence of *Myxidium biliare* sp. n. in *Galaxias maculatus* from Lake Moreno.

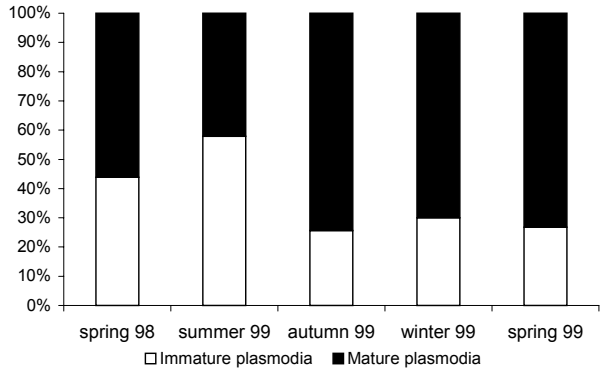

Fig. 5. Percentage of mature/immature plasmodia of *Myxidium biliare* sp. n. in relation to seasons in Lake Moreno.

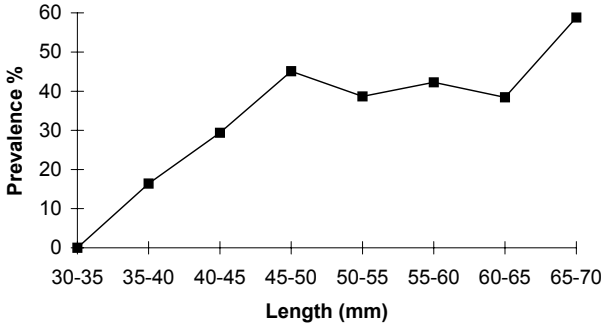

Fig. 6. Prevalence of *Myxidium biliare* sp. n. in relation to length of *Galaxias maculatus* in Lake Moreno.

Tongarewa, New Zealand; slides with histological sections (No. 408/1–3) and a whole parasitized gall bladder fixed in 10% buffered formalin (No. 408/4) in the Helminthological Collection of the Museo Argentino de Ciencias Naturales Bernardino Rivadavia (MACN), Buenos Aires, Argentina; slides with histological sections (No. 151/1–4) in the Colección Parasitológica de la Universidad Nacional del Comahue (Bariloche, Argentina), San Carlos de Bariloche, Argentina; a slide with histological section (No. H-PM-069) and two whole parasitized gall bladders fixed in 10% buffered formalin (No. H-PM-069m) in the Collection of the Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice.

E t y m o l o g y : The species name refers to its localisation in the gall bladder of the host.

Seasonal occurrence and relation of prevalence to host's sex and length

The maximum prevalence of *M. biliare* was observed in summer (46.3%) and minimum prevalence (26.7%) in winter (Fig. 4). There were significant differences in prevalence between seasons (T = 13.31; df = 4; $P < 0.05$). The highest percentage of sporogenic plasmodia were observed from autumn to spring while the minimum percentage was in summer (Fig. 5).

The prevalence of *M. biliare* was 36.7% in females and 32.1% in males and was independent of host sex ($X^2 = 0.229$; df = 1; $P > 0.05$). There was a significant positive correlation between prevalence of *M. biliare* and total length of the host (rs = 0.786; $P = 0.021$) (Fig. 6).

DISCUSSION

Lom and Dyková (1992) listed 149 species of *Myxidium* Bütschli, 1882 in marine and freshwater fishes. The spores of *Myxidium* spp. are, as a rule, fusiform, straight or slightly crescentic or sigmoid, with more or less pointed ends, smooth or with ridges, with the sutural line bisecting the spore, and with two pyriform polar capsules. The capsular openings are situated in the sutural plane near the end of the spores and usually open in opposite directions. One sporoplasm is located between polar capsules (Lom and Dyková 1992). In comparison, the spores of *Zschokkella* Auerbach, 1910 are ellipsoidal in sutural view, slightly bent or semicircular in valvular view, with rounded blunt ends, straight, curved or sinuous sutural lines and with almost spherical polar capsules (Lom and Dyková 1992). Canning et al. (1999) pointed out that there is a considerable overlap in the characteristics of these two genera. Although the distinction between *Myxidium* and *Zschokkella* is arbitrary and still under discussion (Diamant et al. 1994), our species displays characteris-tics that fit more closely to the morphological features listed for *Myxidium* by Lom and Dyková (1992).

Pinto (1928) reported three species of *Myxidium* from the gall bladder of freshwater fishes in Brazil, but the ranges of the spore length and width do not agree with those of *M. biliare*. Moreover, the Brazilian species parasitize characiform and ophidiform fishes.

Myxidium biliare has a wide distribution in Patagonian Andean lakes (39°25'S–41°30'S), inhabiting landlocked *G. maculatus* populations of Atlantic and Pacific watersheds. This is the first species of *Myxidium* de-

scribed from galaxiid fishes and from any freshwater fish in Argentina.

Infection by spores of *M. biliare* is independent of host sex. This is the typical situation found in myxosporidiosis, and has also been described for species of the genus *Ceratomyxa* Thélohan, 1842 in *Dicentrarchus labrax* (Linnaeus) (Alvarez Pellitero and Sitjà Bobadilla 1993) and for *M. magellanicus* in *G. maculatus* (Flores and Viozzi 2001).

Cone (1994) and Molnár (1998) found clear evidence of seasonal cycles of *Henneguya doori* Guilford, 1963 and *H. creplini* (Gurley, 1894) parasitizing gills. They found sporogenic plasmodia with fully developed spores only from end of winter to spring. By contrast, immature and mature plasmodia of *M. biliare* were found in all seasons, so this myxosporean does not seem to have a clear seasonal cycle. However, the periods of recruit-ment and spore release exhibit some seasonality, as evidenced by the low prevalence and the higher percentage of mature plasmodia in winter and the high prevalence and the lower percentage of mature plasmodia in summer. From autumn to spring, a higher percentage of plasmodia would result in seasonal spore production and part of the fish population would shed them from the gall bladder through the intestine. Cone (1994) pointed out that marked seasonal fluctuation in the prevalence of myxosporideans appears to be common in those species having cysts on exposed surfaces of the host. Those species which infect internal tissues such as muscles, cartilage or nerves cannot have such cycles. Although the gall bladder is an internal organ, mature spores of *M. biliare* can easily reach water through the intestine, so there can be periods in which mature spores can be released.

Like the prevalence of *H. creplini* in *Stizostedion lucioperca* (Linnaeus) (Molnár 1998), the prevalence of *M. biliare* in *G. maculatus* increases with the length of the fish. The larval and young *G. maculatus* have planktonic habits and live in the limnetic zone, and the older fishes spend more time in the littoral, swimming in schools (Barriga et al. 2002). If the parasite employs an oligochaete worm as intermediate host, like *M. giardi* Cépede, 1906 (Benajiba and Marques 1993), the infection of larval and young fishes would therefore be rare. On the other hand, if the transmission of this species proceeds from fish to fish as in the case of *Enteromyxum leei* (Diamant, Lom et Dyková, 1994), (Diamant 1997), the chances of becoming infected would increase in the older and longer age class due to the longer time the hosts have been exposed to infectious spores.

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