

Myxidium volitans sp. nov., a parasite of the gallbladder of the fish, *Dactylopterus volitans* (Teleostei: Triglididae) from the Brazilian Atlantic coast - Morphology and pathology

Carlos Azevedo^{1,2/+}, Graça Casal^{1,3}, Sérgio Carmona São Clemente⁴, Leila Maria Silva Lopes⁴, Patrícia Matos⁵, Abdel Azeem Abdel-Baki², Elsa Oliveira¹, Edilson Matos⁶

¹Departamento de Biologia Celular, Instituto de Ciências Biomédicas e Laboratório de Patologia, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Largo Prof. Abel Salazar 2, P-4099-003 Porto, Portugal

²Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia

³Departamento de Ciências, Instituto Superior de Ciências da Saúde-Norte, Gandra, Portugal

⁴Laboratório de Inspeção e Tecnologia dos Alimentos, Faculdade de Veterinária, Universidade Federal Fluminense, Niterói, RJ, Brasil

⁵Laboratório de Animais Aquáticos, Universidade Federal do Pará, Belém, PA, Brasil

⁶Laboratório de Pesquisa Carlos Azevedo, Universidade Federal Rural da Amazônia, Belém, PA, Brasil

Myxidium volitans sp. nov. (Myxozoa: Myxidiidae) parasitizing the hypertrophied green-brownish gallbladder of the teleost *Dactylopterus volitans*, collected in the Atlantic coast near Niterói, Brazil was described based on ultrastructural studies. The spores were fusiform, sometimes slightly crescent-shaped on average $21.7 \pm 0.3 \mu\text{m}$ (mean \pm standard deviation) ($n = 50$) long and $5.6 \pm 0.4 \mu\text{m}$ ($n = 30$) wide. The spore wall was thin and smooth, comprising two equally-sized valves joined by a hardly visible sutural ridge. Spores containing two pyriform polar capsules (PC) ($5.0 \pm 0.4 \times 2.3 \pm 0.3 \mu\text{m}$) ($n = 30$) are situated in each extremity of the spore. The PC wall was composed of hyaline layer ($0.20\text{--}0.29 \mu\text{m}$ thick) and by a thin external granular layer. Each PC contains a polar filament (PF) with irregular arrangements that was projected from its apical region to the bases of PC and coiled laterally from bases to the tip of PC. Some regular striations and S-like structures in the periphery of the PFs with four-five irregular sections were observed. Based on the spore morphology, ultrastructural differences and the specificity of the host we describe this parasite as a new myxosporidian, named *M. volitans* sp. nov.

Key words: Myxozoa - *Myxidium volitans* sp. nov. - gallbladder - spore - ultrastructure

Beside the considerable information available on myxozoan species collected from different geographical areas (Lom & Dyková 2006), little is known about those from South America and in particularly about those from the Brazilian Atlantic coasts, where a diverse assemblage of several hundred species of fish live. Within the myxozoans the genus *Myxidium* is widely distributed infecting several marine and freshwater fishes, some amphibians and reptiles (Lom & Dyková 2006).

Some *Myxidium* spp infecting the fish gallbladders in the Atlantic ichthyofauna from different regions have been recorded (Lom & Dyková 1992, 2006, MacKenzie & Kalavati 1995, MacKenzie et al. 2005): *Myxidium incurvatum*, collected from several species of the French coasts (Lom & Dyková 1992), *Myxidium sphaericum* in *Belone belone* and other hosts, *Myxidium bergensis* in *Pollachius virens* and other hosts, *Myxidium oviforme* in *Pagon imberbis* from Mediterranean Sea and in other fish species in the Atlantic Ocean, *Myxidium gadi* in *Pollachius pol-*

ladius from French region (Mackenzie & Kalavati 1995), *Myxidium gigantissimum* in *Alepocephalus australis* off South African coast (Dubina & Isakov 1976), *Myxidium baueri* in *Patagonotothen sima* from Falkland Islands (Kovaleva & Gaevskaya 1982, Kalavati et al. 1996), *Myxidium trachinarum* in *Echiichthys vipera* from England (Canning et al. 1999) and *Myxidium finnmarkicum* in *Merlangius merlangus* from Norway (MacKenzie et al. 2010) (Table). A Brazilian myxosporidian check-list were published (Gioia & Cordeiro 1996) in which the great majority of the results were based on light microscopy, however, this record does not report any *Myxidium* sp. from the Brazilian coast. Until now, *Dactylopterus volitans* was not reported as a host of *Myxidium* sp.

This paper describes, based on light and ultrastructural data, a new *Myxidium* species parasite of a marine fish collected in the Brazilian Atlantic coast.

MATERIALS AND METHODS

Small fragments of parasitized gallbladder walls and free spores were removed from the bile of the economically important marine fish flying gurnard, *D. volitans* (Linnaeus, 1758) (Teleostei: Dactylopteridae) (Brazilian common name *coiô*). Sixty four fishes (39 males and 25 females) with an average total length of ~ 25 cm (range 21–29 cm) ($n = 64$), collected in the Atlantic coast ($22^{\circ}58'S$ $43^{\circ}00'W$), near the city of Niterói, state of Rio de Janeiro (RJ), Brazil, were examined and the spores were measured

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+Corresponding author: azevedoc@icbas.up.pt

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using Nomarski differential interference contrast microscopy equipped with a micrometric ocular and image software. Measurements were based on fresh spores and data are presented as the mean \pm standard deviation (range).

For transmission electron microscopy (TEM), the free spores and small fragments of parasitized gallbladders were fixed in 5% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.4) for 24 h at 4°C, washed overnight in the same buffer at 4°C and post-fixed in 2% osmium tetroxide buffered with the same buffer for 4 h at the same temperature. After dehydration in an ascending graded ethanol series and propylene oxide, the fragments of gallbladders and free spores were embedded in Epon. Semithin sections were stained with toluidine blue. Ultrathin sections, cut with a diamond knife, were double-stained with aqueous uranyl acetate and lead citrate and observed in a JEOL 100CXII TEM operated at 60 Kv.

RESULTS

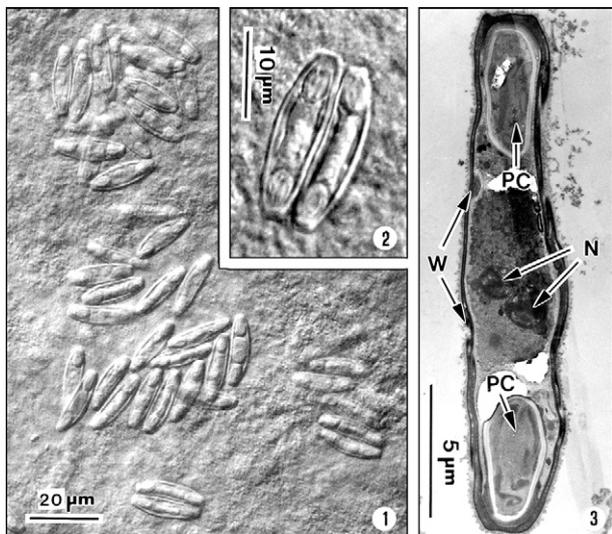
During a parasitological survey conducted to detect microparasites it was observed that some specimens of the marine fish *D. volitans* showed a hypertrophy of the gallbladder with a green-brownish colour compared with the light green colour of the non parasitized gallbladders. Several developmental life cycle stages and free spores were observed immersed in the bile and located within the epithelial cells of the gallbladder wall (Figs 1-5).

Based in the spore morphology, the parasite was identified as belonging to genus *Myxidium* Bütschli, 1882 and

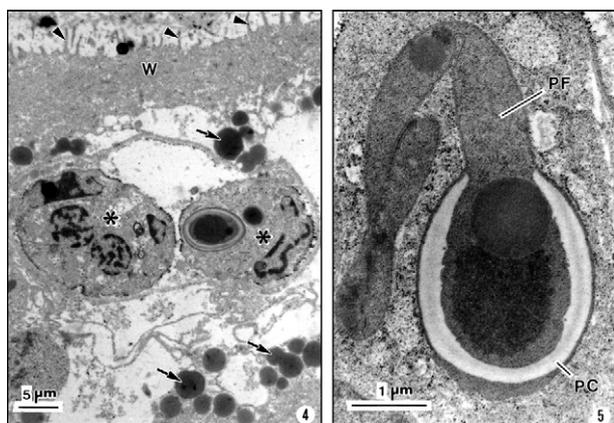
following Lom and Dyková (2006), we propose the establishment of a new species classified as follows: Phylum Myxozoa Grassé, 1970, Class Myxosporea Bütschli, 1881, Order Bivalvulida Shulman, 1959, Family Myxidiidae Thélohan, 1892, Genus *Myxidium* Bütschli, 1882.

Myxidium volitans sp. nov. (Figs 1-13)

Description - Developmental stages, including immature and mature spores, with morphological characters of the genus *Myxidium* Bütschli, 1882 were observed free and immersed in the bile and in the epithelial cells of the gallbladder wall. The development was asynchronous with all the developmental stages and immature spores inter cellular in the epithelial cells of the gallbladder wall (Figs 4-7). Some free mature spores were observed in the initial tract of the intestine. The spore bodies are fusiform, sometime slightly crescent-shaped with smooth surface and more or less rounded ends (Figs 1-3). The spore wall, is thin and smooth, comprised two equal-sized valves without ridges or projections (Figs 1-3). Sutural lines joining valves were hardly visible. Mature fresh spores have the following dimensions: $21.7 \pm 0.3 \mu\text{m}$ (range 21.3-22.0) ($n = 50$) in length and $5.6 \pm 0.4 \mu\text{m}$ (5.2-5.9) ($n = 30$) in width and contain two pyriform polar capsules (PC) that are 5.0 ± 0.4 (4.6-5.5) μm long and 2.3 ± 0.3 (2.0-2.5) μm ($n = 30$) wide, these are situated in each extremity of the spore (Figs 1-3). The PC wall measured 0.20-0.29 μm ($n = 30$) thick (Figs 7-11). The PC wall consists of a continuous external dense layer and an internal hyaline thick material which contains an apical pore without a visible stopper (Figs 7-9). The PC contains a polar filament (PF) with irregular arrange-



Figs 1-3: light and ultrastructural micrographs of a fish-infecting myxosporean *Myxidium volitans* sp. nov. found in the gallbladders of the marine fish *Dactylopterus volitans* collected in the Atlantic Brazilian coast. 1: several free spores observed by differential interference contrast microscopy (DIC); 2: DIC image of two free spores, showing the wall, two evident polar capsules (PC) located at each extremity of the spore and the binucleated sporoplasm cell; 3: longitudinal section of a spore observed with low magnification showing the spore wall (W), two PCs each at the extremity of the spore and two nuclei (N) of the sporoplasm.



Figs 4, 5: ultrastructural micrographs of *Myxidium volitans* sp. nov. infecting gallbladders of the marine fish *Dactylopterus volitans* collected in the Atlantic Brazilian coast. 4: ultrathin transverse section of the periphery of the epithelium of the gallbladder wall (W), showing two developmental stages (*) and several dense bodies (arrows), possibly the components of the bile. The epithelium of the gallbladders wall shows numerous microvilli (arrowheads) projected towards the lumen of gallbladders; 5: ultrathin section of the polar filament (PF) and polar capsule (PC) of a spore maturing process located at the wall tissues of gallbladders.

ments that is projected laterally from its apical region to the bases of PC and coiled from the bases to the tip of PC (Figs 7-11). Some regular and parallel longitudinal striations were observed at the periphery of the PF. At the matrix of the PC there are 1-2 S-like structures that are adherent to the periphery of the PF (Figs 8-11). Schematic drawings of the spore and PF based on ultrathin serial sections are shown in Figs 12, 13.

Type host - *D. volitans* (Linnaeus, 1758) (Teleostei: Dactylopteridae) (Brazilian common name *coiô*).

Site of infection - Different developmental stages and immature spores in the epithelial cells of the gallbladder wall and later developmental stages, immature and mature spores immersed in the bile.

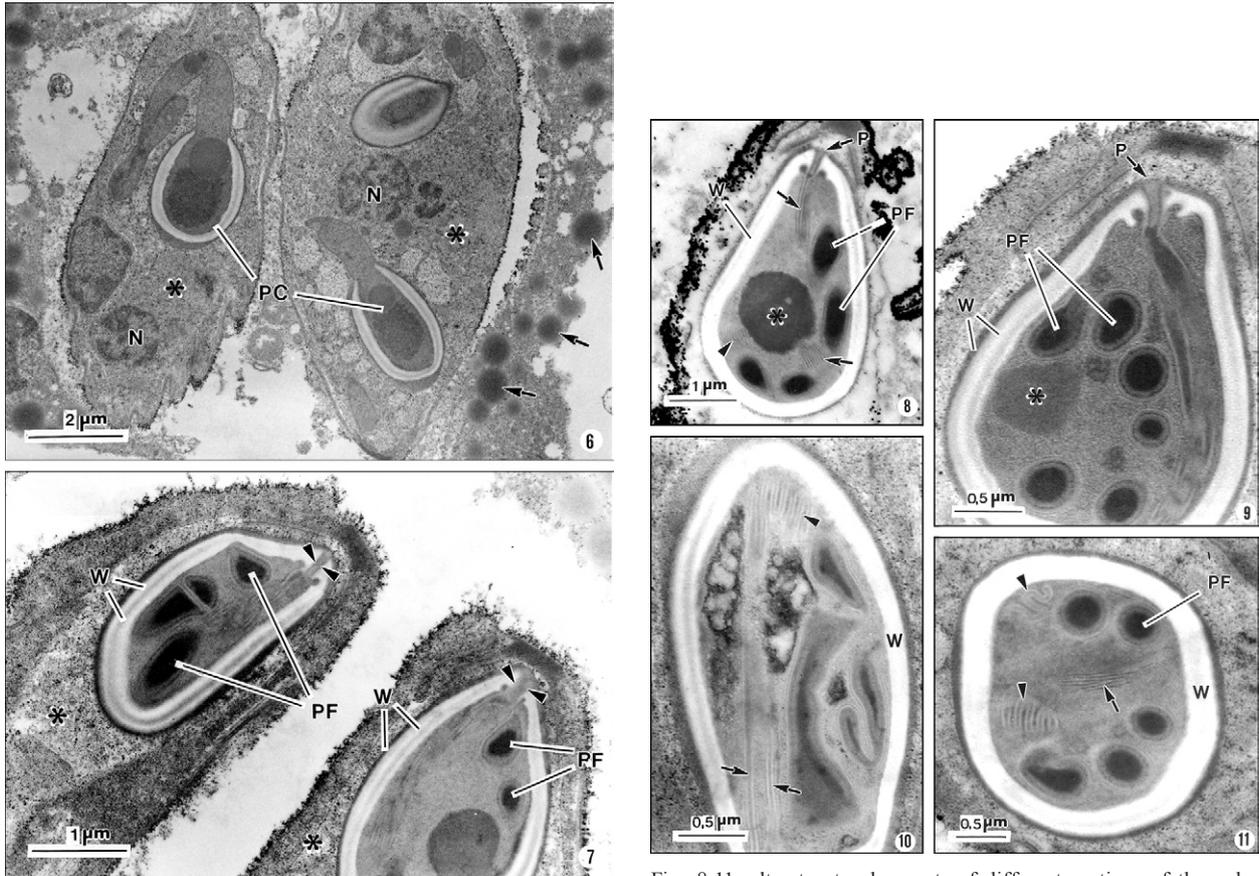
Type locality - Atlantic coast near Niterói (22°58'S 43°00'W).

Type data and depository - A glass slide with several semithin sections of the mature spores and some other developmental stages of the hapantotypes was deposited in the Myxozoa Type Slide Collection at the Instituto Nacional de Pesquisa da Amazônia - INPA, Amazonas, Manaus, Brazil, under acquisition 004/11.

Prevalence of infection - Twenty two out 64 (40.7%) of *D. volitans* specimens [15/39 (38.5%) for females, 7/25 (28%) for males].

Pathology signs - The signs of the infection were macroscopically observed by a well evidenced gallbladder hypertrophy and by the appearance of a green-brownish colour of the infected gallbladders in contrast to the light green colour in the non-infected specimens. Some spores were observed in the initial tracts of the intestine.

Etymology - The specific epithet (*volitans*) is derived from the name of the specific epithet of the genus of the host.



Figs 6, 7: ultrastructural aspects of the spores of *Myxidium volitans* sp. nov. infecting gallbladders of the marine fish *Dactylopterus volitans* collected in the Atlantic Brazilian coast. 6: two immature spores (*) observed in the peripheral epithelial cell wall of the gallbladder showing the polar capsules (PC) and the sporoplasm nuclei (N). Several dense droplet structures possibly the bile component (arrows) are located near the spores; 7: extremities of two spores (*) showing the PC wall (W), the longitudinal section of the PCs, some polar filament sections (PF) projected across the PC wall (arrowheads) and the apical pole of the PC without evident stopper covering the pore (2 arrowheads).

Figs 8-11: ultrastructural aspects of different sections of the polar capsules (PC) and polar filaments (PF) obtained at different levels of *Myxidium volitans* sp. nov. spores infecting gallbladders of the marine fish *Dactylopterus volitans* from the Atlantic Brazilian coast. In favourable sections was possible to observe the longitudinal striation (P) at the periphery of the PF (arrows) and some S-like structures (arrowheads) located within the PC matrix. The PC wall (W) composed of two layers: the external one is granulo-fibrillar and dense, while the internal is composed of light hyaline material. Internally the matrix contains some transverse section of the PF. A dense granulo-fibrillar mass (*) appeared frequently in the matrix of the PC.

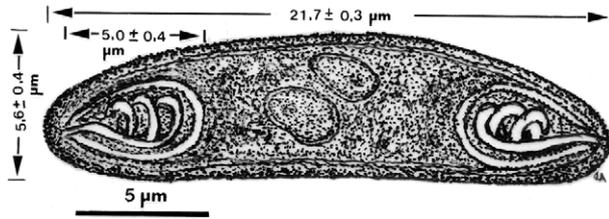


Fig. 12: semischematic drawing of the spore of *Myxidium volitans* sp. nov. parasite from gallbladders of the marine fish *Dactylopterus volitans* collected in the Atlantic Brazilian coast showing the morphology of the different structures and there of the marine fish *D. volitans* from the Brazilian coast.

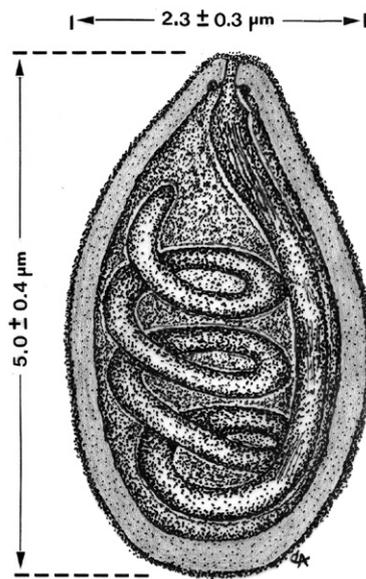


Fig. 13: semischematic drawing of the polar capsule of *Myxidium volitans* sp. nov. and the arrangements of the polar filament coils obtained by serial ultrathin section observations.

DISCUSSION

In the present paper we use the light microscopy and ultrastructural data to describe the spores of the myxosporean isolated from the gallbladders of the marine fish *D. volitans* which have widely geographic distribution in the Atlantic Ocean (Eschmeyer & Demoster 1990). The present study reveals a structural organization and similarities to genus *Myxidium* Thélohan, 1892 (family Myxidiidae) (Lom & Dyková 1992, 2006, MacKenzie & Kalavati 1995). This genus is characterized by having elongated spores, that are slightly crescent-shaped, with two PC in opposite ends, discharge terminally and a longitudinal curved sutural line (Lom & Dyková 2006).

Considering the separation of marine and freshwater myxosporean species into two major branches (or clades) in results of the recent molecular data using small subunit

TABLE

Comparative measurements (in µm) and other characters of the spores of different species of *Myxidium* spp infecting the gallbladders in the fish collected from Atlantic Ocean

<i>Myxidium</i> spp	Hosts (countries)	SpL (µm)	SpW (µm)	PCL (µm)	PCW (µm)	PFc (n)	References
<i>M. incurvatum</i> Thélohan, 1892	Several species (France)	8-20	4.0-8.2	3.0-5.5	2.4-2.7	5-7	Lom and Dyková (2006)
<i>M. sphaericum</i> Thélohan, 1895	<i>Belone belone</i> ^{a,b} (England)	15-20	5-10	3-5	2.5-5.0	3-4	MacKenzie and Kavalati (1995)
<i>M. bergensis</i> Auerbach, 1910	<i>Pollachius virens</i> ^{a,b} (Norway)	17.5-20.0	6.2-7.5	5.4	-	5-6	MacKenzie and Kavalati (1995)
<i>M. oviforme</i> Parisi, 1912	<i>Apogon imberbis</i> ^{a,b} (Italy)	11.75-16.25	7.50-9.50	4.25-5.0	-	4-5	MacKenzie and Kavalati (1995)
<i>M. gadi</i> Georgévitch, 1916	<i>Pollachius pollachius</i> (France)	12-15	5.0-7.5	2.5-4.5	2.5-4.0	5-6	MacKenzie and Kavalati (1995)
<i>M. gigantissimum</i> Dubina & Isakov, 1976	<i>Alepocephalus australis</i> ^a (South Africa)	97.5	8.5	~32	-	13-14	Dubina and Isakov (1976)
<i>M. baueri</i> Kovaleva & Gaevskaya, 1982	<i>Merluccius australis</i> (Falkland Islands) ^b	19-24	9-11	4.5-5.5	3-4	-	Kovaleva and Gaevskaya (1982)
<i>M. trachinorum</i> Canning et al., 1999	<i>Echiichthys vipera</i> (England)	17.2	8.8	6.8	3.7	Up to 82 ^c	Canning et al. (1999)
<i>M. fimmarchicum</i> MacKenzie et al., 2010	<i>Merlangius merlangus</i> (Norway)	17.6-22.4	6.4-9.6	4.8-6.4	3.2-4.8	4-5	MacKenzie et al. (2010)
<i>M. volitans</i> sp. nov.	<i>Dactylopterus volitans</i> (Brazil)	21.7 (21.5-22.0)	5.6 (5.3-5.8)	5.0 (4.7-5.3)	2.3 (2.0-2.5)	2-3 Rarely 4	Present study

a: type species; b: several species; c: extruded polar filament; PCW: polar capsule width; PCL: polar capsule length; SpL: spore length; SpW: spore width.

DNA sequences (Kent et al. 2000, 2001, Fiala 2006), we consider for this discussion only *Myxidium* spp hosted in the marine Atlantic fishes. On the other hand, taking in consideration the role of tissues and organs preferences in differentiating between closely related species for identification of species (Lom & Dyková 2006), we establish spore comparison only infecting fish gallbladders from hosts collected from Atlantic areas.

So, comparing the characteristics of *M. volitans* sp. nov. (morphology and dimensions of the spore and PCs, number of coils and arrangements of the PFs, and host species), none of these characters were simultaneously equals when compared with those reported for *Myxidium* spp infecting fish collected in the different geographical areas of the Atlantic Ocean (Table). The spores of *M. volitans* sp. nov. are substantially smaller ($21.7 \pm 0.3 \mu\text{m}$ long) than *M. gigantissimum* ($97.5 \mu\text{m}$ long) and larger than *M. oviform* spores ($11.75\text{-}16.25 \mu\text{m}$) and than *M. trachinarum* spores ($17.2 \mu\text{m}$).

All other spores of the different *Myxidium* spp reported in the Table have similar ranges and mean of dimensions, however, with different PC arrangement and polar capsule coils (PFC) number.

While the spores of *M. volitans* sp. nov. have PF coiled 2-3 times, in all other species the PFC are coiled more times, excepted the PC of *M. sphaericum* that have a similar number of PFC (3-4). These two species have, however, different morphology of spores: *M. volitans* have fusiform slightly crescent-shaped with more or less round end, while *M. sphaericum* have fusiform spores with broad at the centre and a relatively blunt extremity in valvular view (MacKenzie & Kalavati 1995).

Moreover, the ultrastructural analyses showed the PC and the arrangement of the PC tapering from its tip to the bases and an irregularly fold from the bases to tip of the PF, and this structural organization is different of those of the previously described species. Additionally the presence of the longitudinal striation on the periphery of the PC and the presence of the some S-like structures in the PC matrix were never reported in the previously described species. And finally, the species here described differs from the previously reported *Myxidium* spp (Table) in terms of its host specificity (Lom & Dyková 1992).

The confusion in the literature regarding the validity and host specificity among some of these *Myxidium* spp recorded in the Table, have been reported some years ago. Considering the similar morphology of the spores of *M. sphaericum* and *M. bergensis*, it was suggested that two of these species may be conspecific (Noble 1957, Moser et al. 1989). Recently was referred and discussed by MacKenzie et al. (2010) the existence of a great confusion and doubt regarding the validity and host specificities of *M. incurvatum*, *M. phaericum*, *M. bergensis* and *M. gadi* which need detailed investigation on the morphological and molecular data.

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