

Myxozoan pathogens in cultured Malaysian fishes.

I. Myxozoan infections of the sutchi catfish *Pangasius hypophthalmus* in freshwater cage cultures

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ABSTRACT: Cage-cultured sutchi catfish *Pangasius hypophthalmus* (Sauvage, 1878), a favourite food fish in Southeast Asia, proved to be infected by 6 myxozoan species. Three species belonged to the genus *Hennegoides* (*H. berlandi*, *H.* , and *H. pangasii*), 1 to *Henneguya* (*H. shariffi malayensis*) and 2 to *Myxobolus* (*M. baskai*, and *M. pangasii*). Five myxozoans infected the gills and 1 was found on the spleen. Myxozoans infecting the gills were characterised by a specific site selection. *H. shariffi* sp. n. and *H. berlandi* sp. n. formed plasmodia in the multi-layered epithelium of the gill filaments. Of the 2 vascular species *H. pangasii* sp. n. developed in the gill arteries, while *M. baskai* sp. n. infected the capillary network of the gill lamellae. Plasmodia of *H. malayensis* sp. n. were found inside the cartilaginous gill rays of the filaments. Large plasmodia of *M. pangasii* sp. n. were located in a groove of the spleen but they affected only the serosa layer covering the spleen.

KEY WORDS: *Pangasius* · Myxozoan infections · *Henneguya* · *Hennegoides* · *Myxobolus* · Site selection · Histology

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INTRODUCTION

Freshwater fish culture in Southeast Asia is one of the fastest growing branches of agriculture. Traditional and endemic species including catfishes are cultured in ponds and cage systems (Csávás 1994). *Pangasius hypophthalmus* (Sauvage, 1878) is one of the most popular cultured species in Indo-Chinese countries. The original distribution of this fish, which is better known by its synonymous name *P. sutchi*, is the Mekong basin from where the fish was distributed to several Southeast Asian countries and China (Roberts & Vidthayanon 1991, Cacot & Lazard 2004), and more recently it was also introduced to Iran (Islam 2004). The technology of cage culture was developed by Cambodian fishermen. In addition, *Pangasius* fingerlings kept in aquaria are very popular among European pet fish hobbyists (authors' pers. comm).

Reports on the status of aquaculture in Malaysia, including catfish culture, were presented by Ang et al. (1989), Ang (1990), Khan et al. (1993) and Ong (1993). These authors reported that, in addition to *Clarias batrachus* and its hybrids, *Pangasius* spp. and a local catfish *Hemibagrus nemurus* (Valenciennes, 1840) (syn. *Mystus nemurus*) were the most commonly cultured fishes. Of these, *P. hypophthalmus* and *H. nemurus* proved to be the best adapted to cage cultures.

Little is known about parasitic infections of *Pangasius* spp. Data are mostly presented on their monogenean infections. Parisiella et al. (2002) described several *Thaparocleidus* species from the gills of *P. bo-courti*, *P. djambal* and *P. hypophthalmus*. These authors reported that the latter monogeneans were strictly host specific. No paper has reported about myxozoan infection of pangasiid fishes, although at a Hungarian local symposium in 2002 Baska (unpubl.) reported that

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fingerlings of *P. hypophthalmus* imported from Thailand to be kept in aquaria were heavily infected by several *Myxobolus* spp.

There is only a single published work from Malaysia on myxozoan infections (Shariff 1982). This author made a complex morphological and pathological study on a myxozoan of *Oxyeleotris marmoratus* and described the species found as *Henneguya shaharini*.

The number of known myxosporeans is very high. The genera *Myxobolus* and *Henneguya* are the most abundant species. Landsberg & Lom (1991) recorded 444 valid species of the genus *Myxobolus*, while Eiras (2002) mentioned 146 described species from the genus *Henneguya*. Little is known about myxozoan infections in Southeast Asia. Data on myxosporeans infecting fishes of the Indian fauna region (see Gussev 1969) are available mostly on several members of the genera *Myxobolus* and *Henneguya*, genera that have been described and recorded by Chakravarty (1939), Tripathi (1952), Lalitha-Kumari (1965, 1969), Sarkar (1985) and Sarkar et al. (1985). In addition to Shariff's (1982) work, myxozoan infections from Southeast Asian countries have been reported by Supamattaya et al. (1991), who described *Sphaerospora epinepheli* from the grouper *Epinephelus malabaricus* cultured in marine cages. Another *Sphaerospora* species, *S. ojiroveci*, was described by Lom & Dyková (1997) from the pet fish *Pangasius sutchi* originating from Thailand, and *H. longitudinalis*, a type species of the new genus *Hennegoides*, was described by Lom et al. (1991) from *Osphronemus gourami* in Thailand. Further data on myxosporeans are found in the work of Ky (1971) from fishes of northern Vietnam, which according to Gussev (1969) belongs to the Amur-Chinese zoogeographical zone while the southern part of Vietnam, together with other Southeast Asian countries and India, belongs to the Indian fauna region.

The present paper describes 6 new myxozoan species (3 *Hennegoides* spp., 2 *Myxobolus* spp. and 1 *Henneguya* sp.) from net-cage cultured *Pangasius hypophthalmus* and discusses their site selection in the infected organs and their pathogenic effect.

MATERIALS AND METHODS

Sutchi catfish were collected from a cage culture on the Terengganu River close to Kuala Terengganu, Terengganu State, Malaysia. Altogether 10 specimens of 16 to 19 cm and 10 specimens of 36 to 42 cm total length were dissected during a 2 wk period in late November and early December 2004. The investigations were aimed at studying myxosporean infections only. After surveying the skin and fins of the fish, the hemibranchia of the gills were excised, the abdominal cav-

ity opened, and the intestine, spleen, liver, gonads and the kidneys separated.

Gill filaments of each hemibranchium were checked for myxozoan plasmodia under a Zeiss Stemi DV4 stereomicroscope. Plasmodia in filaments, lamellae or gill arches were differentiated according to Molnár (2002) to epithelial, vascular and chondroid locations. Plasmodia were carefully removed from the tissues and opened with a fine needle on a slide. A part of spores obtained from mature plasmodia were studied in fresh smears under a Nikon Optiphot compound microscope. Another part was saved for examination as permanent preparation, placed on slides and carefully covered by a coverslip containing a drop of melted glycerol-gelatine. Some of the plasmodia containing morphologically identical spores were fixed in 50 or 70 % ethanol in vials for molecular biological examinations. The intestine was opened lengthwise, and after removal of the faeces the epithelial lining was surveyed under a stereomicroscope and scrapings were examined under a coverslip. The inner organs were inspected *in toto*, and pieces from them were studied in squash preparations under a compound microscope at a magnification of $\times 200$. Digital photos of fresh spores were taken with a Nikon Optiphot microscope connected to a computer. Measurements were taken from 25 fresh spores according to the guidelines suggested by Lom & Arthur (1989). Additionally, photos of 25 spores were measured using the IMAGO® program.

Heavily infected hemibranchia of gills or some selected filaments were fixed in Bouin's solution for 4 h, washed in 80 % ethanol several times, embedded in paraffin wax, cut into 5 to 8 μm thick sections and stained with haematoxylin and eosin. Microphotographs from the histological sections were taken with an Olympus DP-10 digital camera mounted on an Olympus BH-2 light microscope.

RESULTS

No myxosporean infection was found in fishes of the younger age group. All the 10 *Pangasius hypophthalmus* specimens examined measuring 36 to 42 cm in length harboured myxosporean plasmodia. All the gills of the large-sized fish were infected, while 6 of them showed infection in the spleen. No infection was found in other organs.

On microscopic examination, spores of 6 different myxozoans (3 *Hennegoides* spp., 2 *Myxobolus* and 1 *Henneguya* sp.) were differentiated morphologically. Five species infected the gills while 1 *Myxobolus* sp. was found on the spleen. Most species had a characteristic location in the gills. All the species found proved to be new for science. Their description is as follows:



Henneguya shariffi sp. n.

Host: Sutchi catfish *Pangasius hypophthalmus*

Location: Terengganu River

Site of infection: Gill filaments

Prevalence of infection: 7 out of 10 fish 36–42 cm total length

Intensity: Moderate to intensive

Type material: Spores in glycerol-gelatine are deposited in the parasite collection of the Hungarian National History Museum. Collection number: HNHM-69907

Etymology: The species is named after M. Shariff, the well-known Malaysian fish pathologist who first described a *Henneguya* sp. in Malaysia

Trophozoites: Ellipsoidal or round plasmodia of varying size up to 0.2 × 0.5 mm in diameter were found at the

tip and the lateral edge of the gill filaments inside the multi-layered epithelium.

Spores: Spores are typical for the genus *Henneguya* (Figs. 1A & 2A). They are symmetric, elongated in shape with 2, relatively short caudal appendages, and with equal-sized polar capsules of elongated shape. The spore wall is thin and smooth and composed of 2 equal valves. The oral end of the spore body is blunt; the caudal end tapers and joins the caudal appendages. In a fresh state the length of the spore body is 10.3–11.9 (11.7 ± 1.26) µm, the width is 3.6–3.8 (3.71 ± 0.07) µm, and the thickness is 3.6–3.8 (3.7 ± 0.100) µm. The 2 polar capsules are elongated, blunt at the posterior end and taper anteriorly. Their length is 5.1–6.6 (5.48 ± 0.57) µm and their width is 0.9–1.2 (1.05 ± 0.15) µm. The polar capsules open at the blunt end of the spore body. The polar filaments are coiled in 7 turns perpendicular to the long axis of the polar capsules. The length of the extruded filaments is 13.2–21 (15.35 ± 3.99) µm. The sporoplasm has a small iodophilous vacuole. Caudal extensions are straight, continuously tapering and relatively short, 5.9–10.3 (7.54 ± 2.0) µm in length.

Remarks: Spores of this species resemble the spores of *Henneguya mystusia* Sarkar, 1985, but differ from this species due to their shorter caudal appendages.

Histology: Plasmodia of this species started their development at the tip or at the edges of the gill filaments inside the multi-layered epithelium and formed relatively small plasmodia (Fig. 3A). Plasmodia were directly bordered by epithelial cells without a connective capsule. Developing plasmodia had a layer of sporogonic developmental stages in the ectoplasm and elongated spores in the centre.

Hennegoides pangasii sp. n.

Host: Sutchi catfish *Pangasius hypophthalmus*

Location: Terengganu River

Site of infection: Large plasmodia in the gill filaments

Prevalence of infection: 6 out of 10 fish

Intensity: Moderate

Type material: Spores in glycerol-gelatine are deposited in the parasite collection of the Hungarian National History Museum. Collection number: HNHM-69905

Etymology: The species name is derived from that of its host.

Trophozoites: Trophozoites are large

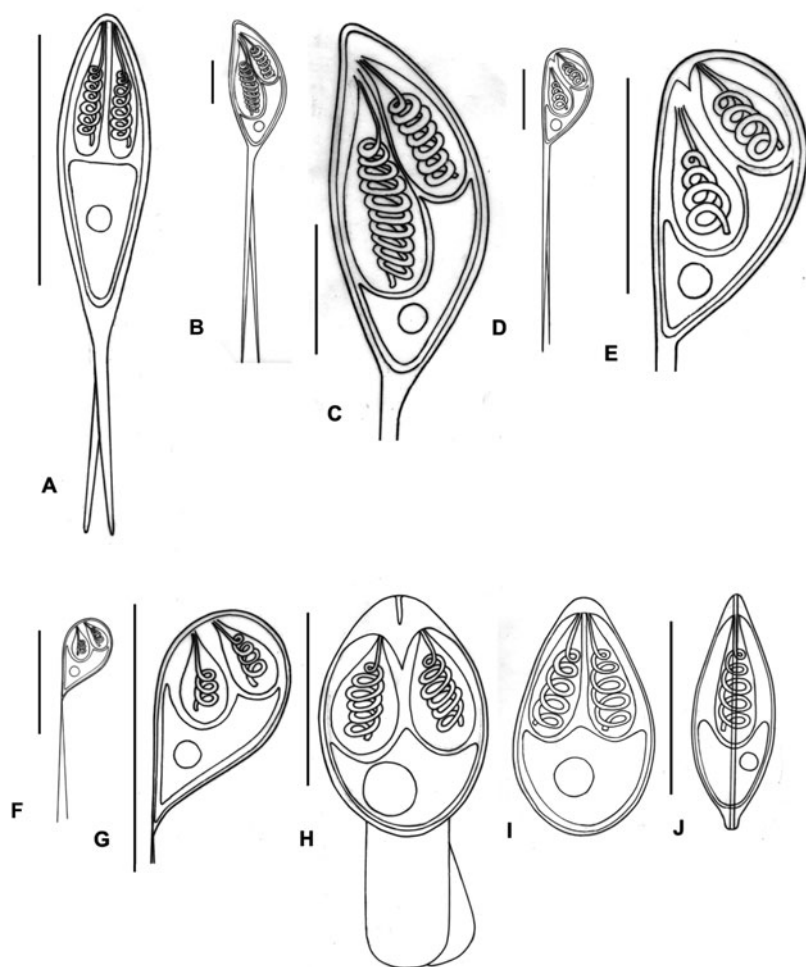


Fig. 1. *Henneguya*, *Hennegoides* and *Myxobolus* spp. found in *Pangasius hypophthalmus*. (A) *Henneguya shariffi* sp. n. (B) *Hennegoides pangasii* sp. n. spore. (C) Spore body of *H. pangasii* sp. n. spore. (D) *Hennegoides malayensis* sp. n. spore. (E) Spore body of *H. malayensis* sp. n. spore. (F) *Hennegoides berlandi* sp. n. spore. (G) Spore body of *H. berlandi* sp. n. spore. (H) *Myxobolus baskai* spore with 2 caudal extensions at the posterior end. (I) *M. pangasii* spore in frontal view. (J) *M. pangasii* spore in lateral view. Scale bars = 10 µm

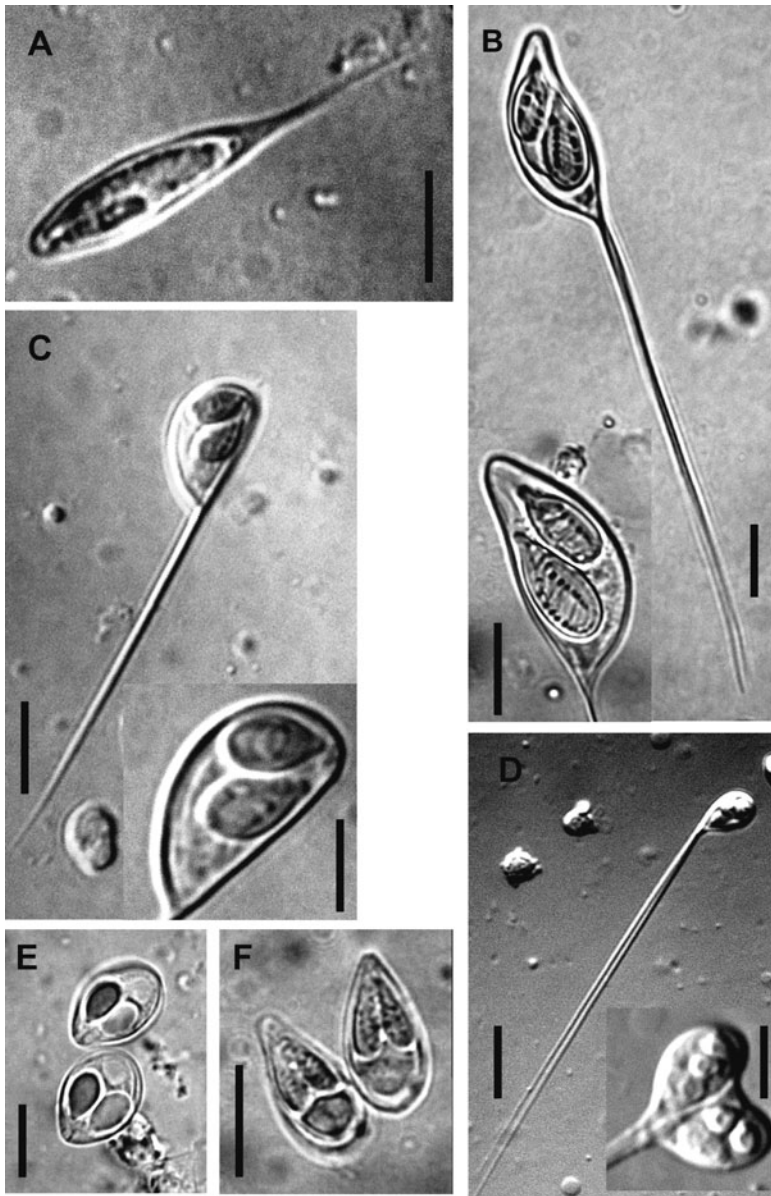


Fig. 2. *Henneguya*, *Hennegoides* and *Myxobolus* spp. spores found in *Pangasius hypophthalmus*. (A) *Henneguya shariffi* sp. n. Scale bar = 5 μ m. (B) *Hennegoides pangasii* sp. n. Scale bar = 10 μ m (inset: spore body of *Hennegoides pangasii* sp. n. Scale bar = 10 μ m). (C) *Hennegoides malayensis* sp. n. Scale bar = 10 μ m (inset: spore body of *Hennegoides malayensis* sp. n. Scale bar = 5 μ m). (D) *Hennegoides berlandi* sp. n. Scale bar = 10 μ m (inset: still attached developing spore bodies of *H. berlandi* sp. Scale bar = 5 μ m). (E) Fixed spores of *Myxobolus baskai* sp. n. without caudal appendages. Scale bar = 10 μ m. (F) *Myxobolus pangasii* sp. n. Scale bar = 10 μ m

ellipsoidal plasmodia 0.2–0.5 \times 0.7–1.3 mm in size which are located in the central and apical region of the gill filaments.

Spores: The mature spores are asymmetric in frontal view (Figs. 1B & 2B). One side of the spore body is slightly vaulted while the other is strongly vaulted. The

caudal appendages extend axially. The spore body tapers both in oral and caudal directions but at the anterior end it is rounded (Figs. 1C & Fig. 2B inset). The length of the spore body is 24–30 (27.3 ± 1.73) μ m, the width is 12–13.8 (12.6 ± 0.76) μ m and the thickness is 10.2–12 (13.6 ± 1.3) μ m. The 2 polar capsules are elongated and drop-like, different in size and have different locations in the cyst. The larger capsule located in the caudal part of the spore body measures 11.4–15 (13.9 ± 1.26) μ m in length and 6–7.2 (6.5 ± 0.45) μ m in width. The smaller capsule located close to the anterior end of the spore body at the more vaulted side of the spore wall measures 10–12.2 (11.5 ± 0.89) μ m in length and 4.8–6 (5.1 ± 0.46) μ m in width. The openings of the polar capsules do not extend to the anterior end of the spores. The threads of the polar filaments inside the larger polar capsule have 9 to 10 coils perpendicular to the longitudinal axis of the capsule; the number of coils in the smaller polar capsule is 7 or 8. The extruded polar filaments are curled, measuring 48 to 54 μ m in length. The relatively small sporoplasm is located at the more vaulted part of the spore between the 2 polar capsules extending partly to the caudal edge. The straight, continuously tapering caudal extensions are 48–72 (64.5 ± 9.24) μ m long.

Remarks: The main features of this species bears the characteristics of the genus *Hennegoides* described by Lom et al. (1991). *H. pangasii*, however, differs from the type species *H. longitudinalis* due to its larger spore body and its long caudal extensions. In addition, in the polar capsule of *H. pangasii* the filamental turns are perpendicular to the long axis, while in the polar capsules of *H. longitudinalis* the threads are parallel to the long axis.

Histology: Elongated ellipsoidal plasmodia containing spores and sporogonic stages were located inside the afferent artery attaching closely to the cartilaginous gill rays of the filaments (Fig. 3B). The plasmodium was surrounded only by a single layer of host origin (presumably by the endothelium of the artery) which delimited the plasmodium from the cartilage on one side and from the subepithelial layer on the other side.

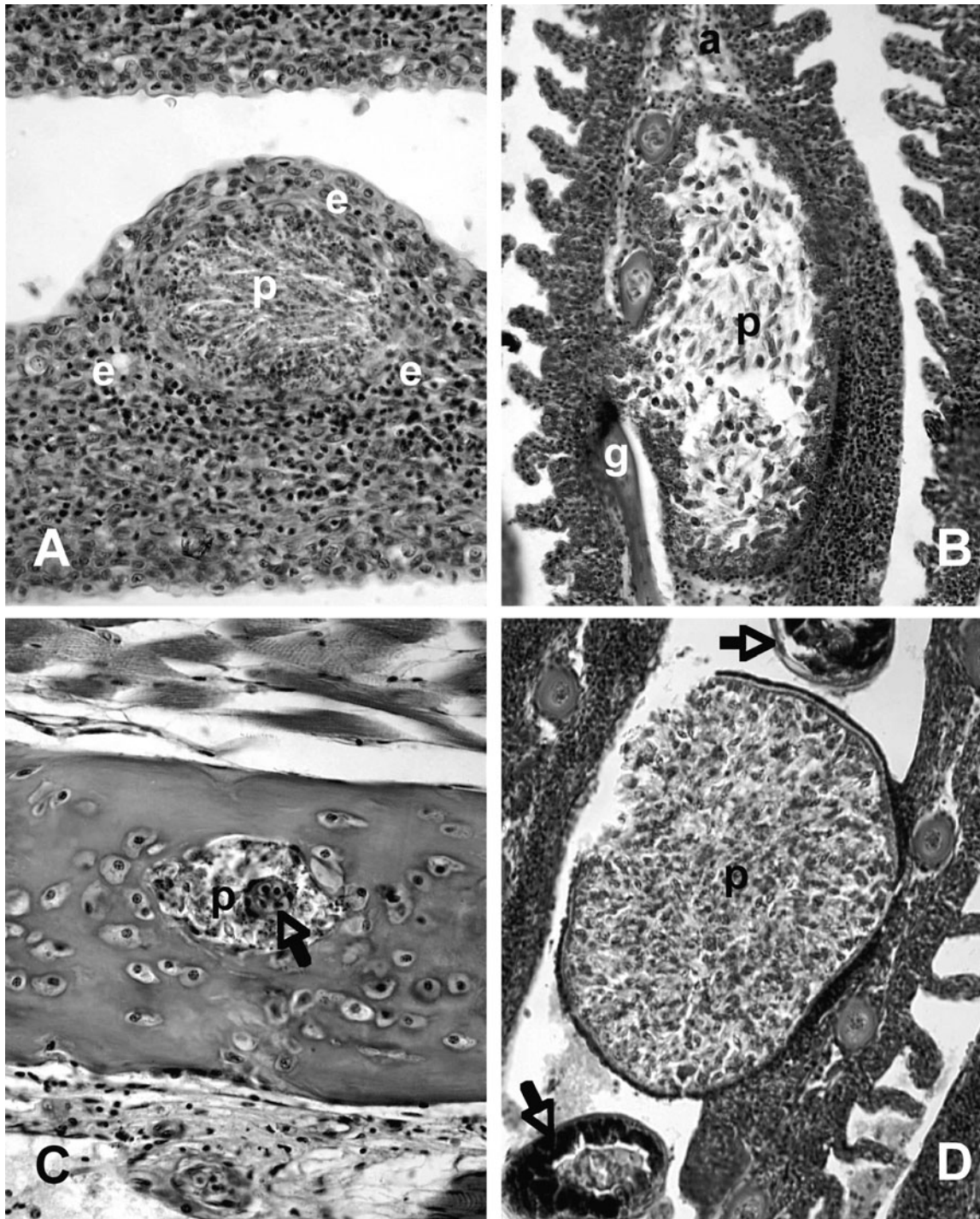


Fig. 3. *Haematoxylin* and eosin stained histological sections of the gills of *Pangasius hypophthalmus*. (A) Developing *Henneguya shariffi* sp. n. plasmodium (p) in the multi-layered epithelium (e) of the edge of a gill filament. The plasmodium contains sporogonic stages at the periphery and elongated spores in the centre. $\times 350$. (B) *Hennegoides pangasii* plasmodium (p) inside a gill artery (a). Inner part of the plasmodium is in close contact with the cartilaginous gill ray of the filament (g); the other side is covered by the epithelium. $\times 220$. (C) *Hennegoides malayensis* plasmodium (p) in the cartilaginous gill ray of the filament. In the centre of the plasmodium there is an island of cartilage cells (arrow). $\times 480$. (D) *Hennegoides berlandi* plasmodium (p) with mature spores originating from the non-lamellar filament edge. On both sides of the plasmodium there are cross-sectioned *Thaparogyrus* monogenean specimens (arrows). $\times 220$

Hennegoides malayensis* sp. n.*Host:** Sutchi catfish *Pangasius hypophthalmus***Location:** Terengganu River**Site of infection:** Small plasmodia inside the cartilaginous gill rays**Prevalence of infection:** 6 out of 10 fish**Intensity:** Moderate**Type material:** Spores in glycerol-gelatine are deposited in the parasite collection of the Hungarian National History Museum. Collection number: HNHM-69904**Etymology:** The species name is derived from the name Malaysia**Trophozoites:** Trophozoites are either $40 \times 60 \mu\text{m}$ elliptical or $40 \times 300 \mu\text{m}$ elongated plasmodia in the posterior part of the cartilaginous gill rays.**Spores:** The mature spores are asymmetric in frontal view (Figs. 1D & 2C). One side of the spore body (Figs. 1E & 2C inset) is strongly vaulted; the other one is straight. The oral end is rounded; the caudal end is slightly conspicuous. The length of the spore body is $12.5\text{--}14.4$ (13.7 ± 0.55) μm width its body is $6.4\text{--}7.5$ (6.83 ± 0.33) μm and its thickness is $4.2\text{--}6.3$ (5.1 ± 0.69) μm . The 2 polar capsules are drop-like and different in size; one of them located slightly behind the other. The anterior smaller capsule, located at the vaulted side of the spore, measures $5.1\text{--}6.2$ (5.66 ± 0.39) μm in length and $3\text{--}3.3$ (3.1 ± 0.13) μm in width. The posterior capsule located at the straight side of the spore measures $5.7\text{--}6.3$ (5.95 ± 0.22) μm in length and $3.1\text{--}3.5$ (3.3 ± 0.14) μm in width. Between the openings of the polar capsules there is a triangular intercapsular appendix with a length of about $1 \mu\text{m}$. The polar filamental threads are coiled in 4 turns perpendicular to the longitudinal axis of the capsule. The extruded polar filaments measure $17\text{--}20 \mu\text{m}$ in length. The relatively small sporoplasm is located at the vaulted and basal part of the spore. The caudal appendages which follow the line of the straight side are $36\text{--}40.5$ (39.1 ± 2.07) μm long.**Remarks:** This species resembles *Hennegoides longitudinalis* and *H. pangasii*, but differs from *H. pangasii* by virtue of its more rounded anterior end and by the straight run of one side of the spore body. The size of *H. malayensis* is similar to that of *H. longitudinalis*, but it has longer caudal appendages, less elongated polar capsules and its filamental turns are perpendicular to the long axis.**Histology:** Round (Fig. 3C) or elongated shape were found inside the cartilaginous gill rays. The elongated Plasmodia plasmodia seemed to be composed of several ellipsoidal plasmodia. The plasmodia are not in contact with the chondrocytes; they are bordered directly by the cartilaginous material of the fin rays.***Hennegoides berlandi* sp. n.****Host:** Sutchi catfish *Pangasius hypophthalmus***Location:** Terengganu River**Site of infection:** Elongated plasmodia at the edge of the gill filaments**Prevalence of infection:** 6 out of 10 fish**Intensity:** Moderate**Type material:** Spores in glycerol-gelatine are deposited in the parasite collection of the Hungarian National History Museum. Collection number: HNHM-69906**Etymology:** The species is named after Dr. Björn Berland, the famous Norwegian fish parasitologist who actively promoted the work of this research team in KUSTEM.**Trophozoites:** Trophozoites are 2 to 4 mm long plasmodia located alongside the edge of the gill filaments, or 0.5–1 mm large round plasmodia bulging out from the lateral edges of the filaments.**Spores:** The mature spores are asymmetric in frontal view (Figs. 1F & 2D) One side of the spore is strongly vaulted while the other one is straight. The oral end is rounded and the caudal end is conspicuous. The length of the spore body (Figs. 1G & 2D inset) is $8\text{--}9$ (8.5 ± 0.54) μm . Its width is $2.5\text{--}3$ (2.8 ± 0.2) μm and its thickness is $2.8\text{--}3.2$ (2.98 ± 0.14) μm . The 2 polar capsules are drop-like and equal in size. The axis of the capsules runs at an angle of about 45° to the straight side of the spore body. The length of the capsules is $2.5\text{--}3$ (2.8 ± 0.27) μm and the width is $1.5\text{--}2$ (1.84 ± 0.23) μm . Between the openings of the polar capsules there is a very small round intercapsular appendix. The polar filaments are coiled in 4 turns perpendicular to the long axis of the polar capsules. The extruded polar filaments measure $14\text{--}18$ (15.14 ± 1.46) μm in length. The relatively small sporoplasm is located at the vaulted and basal part of the spore. There is a small round iodophilous vacuole in the sporoplasm. The caudal appendages, which follow the line of the straight side, are long, measuring $40\text{--}50$ (43.6 ± 4.13) μm .**Remarks:** *Hennegoides berlandi* differs from *H. malayensis* and *H. longitudinalis* by virtue of its smaller size, and its more rounded spore body and polar capsules.**Histology:** Both elongated and round plasmodia were located in the multi-layered epithelium of the non-lamellar edges of the filaments (Fig. 3D). Young trophozoites were mostly found at this location, but they also occurred in the lamellar part of the filaments in the multi-layered epithelium between 2 neighbouring lamellae (Fig. 4A).***Myxobolus baskai* sp. n.****Host:** Sutchi catfish *Pangasius hypophthalmus***Locality:** Terengganu River**Site of infection:** Gills**Prevalence of infection:** 4 out of 10 fish

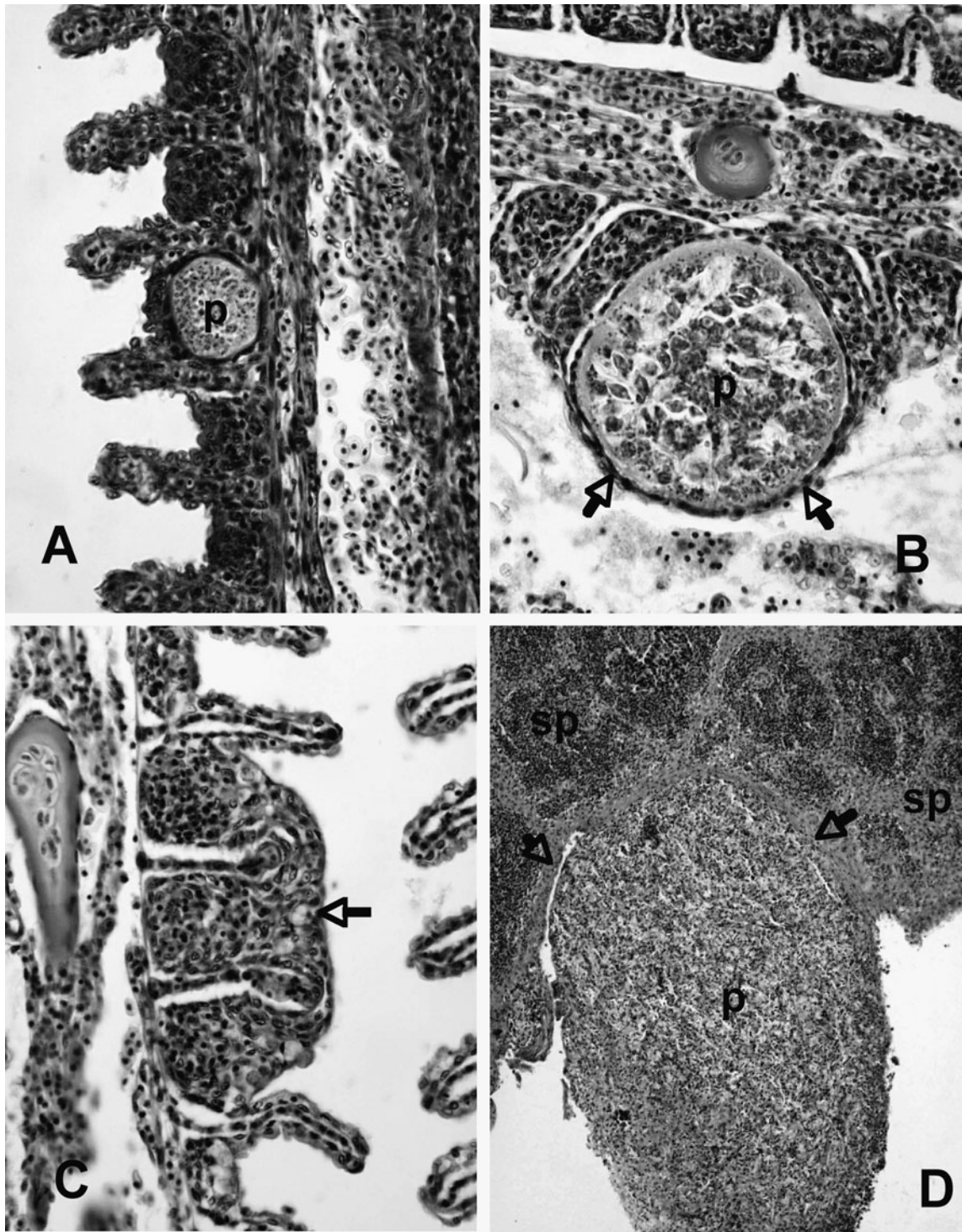


Fig. 4. *Pangasius hypophthalmus*. Haematoxylin and eosin stained histological sections of the gills. (A) Young *Hennegoides berlandi* trophozoite (p) developing in the multi-layered epithelium between 2 lamellae. $\times 480$. (B) *Myxobolus baskai* plasmodium (p) developing inside a gill lamella. The plasmodium is bordered by an endothelial and an epithelial layer of the lamellar capillary network (arrows). The neighbouring lamellae are compressed. $\times 480$. (C) Granulomatous inflammatory cells fill the place left by the released *M. baskai* spores between and over the gill lamellae (arrow). $\times 480$. (D) Large *M. pangasii* plasmodium (p) developing in the serosa (arrows) covering the spleen (sp). Part of the plasmodium sinks into the spleen; another part emerges over the surface of the organ. $\times 220$



Intensity: Moderate

Type material: Spores in glycerol-gelatine are deposited in the parasite collection of the Hungarian National History Museum. Collection number: HNHM-69908

Etymology: The species is named after the Hungarian fish pathologist Ferenc Baska

Vegetative stages: Round plasmodia 0.5–1 mm in diameter develop mostly in the non-separated basal part of the filaments and are located between the dorsal and ventral part of filaments routing from the same place of the gill arch.

Spores: The mature spores are ovoid in frontal view, rounded at the posterior end and slightly tapered at the anterior end (Figs. 1H & 2E). In lateral view the spores are lemon-shaped. Spore valves are relatively thin, symmetrical and smooth. The sutural line is indistinct. No sutural markings were found. The spores measure 13.5–15 (14.2 ± 0.59) μm in length, 10.5–11 (10.64 ± 0.37) μm in width and 6.5–7.2 (6.83 ± 0.39) μm in thickness. There is a relatively large, triangular intercapsular appendix 1.5–2 μm in length, residing on the thickened anterior part of the spore valve (Figs. 1H & 2E). The 2 polar capsules are drop-like in shape, equal in size and measure 5.5–7 (6.35 ± 0.52) μm in length and 3.5–5 (4.33 ± 0.5) μm in width. Polar capsules slightly converge toward the anterior end of the spores, and open at the base of the intercapsular appendix. Polar filaments are coiled obliquely with 5 or 6 turns in the polar capsule, at an angle of 45° to the longitudinal axis of the capsules. The extruded filaments measure 45–50 (47.83 ± 2.04) μm in length. Inside the relatively small sporoplasm the 2 nuclei and the iodophilous vacuole are well discernible. Attached to the posterior end of the spores there is 1 (in frontal view), in some spores 2 (in lateral view), approximately 10 μm long and 5 μm wide caudal appendages differing in nature from the spore valves, which are indiscernible in glycerol-gelatine preparations.

Remarks: Due to the presence of caudal appendages on the posterior end of the spores, this species could be classified to the genera *Unicauda* Davis, 1944 or *Dicauda* Hoffman and Walker, 1978, but the present authors think that the non-chitinous mucous structures characterise only the species. In addition to the presence of the appendages, this species differs from the known species by the thickened anterior part of the spores.

Histology: Plasmodia were located inside the capillary network of the gill lamellae surrounded by a single endothelial and epithelial layer of the lamellae (Fig. 4B).

***Myxobolus pangasii* sp. n.**

Host: Sutchi catfish *Pangasius hypophthalmus*

Locality: Terengganu River

Site of infection: Spleen

Prevalence of infection: 7 out of 10 fish

Intensity: Moderate

Type material: Spores in glycerol-gelatine are deposited in the parasite collection of the Hungarian National History Museum. Collection number: HNHM-69909

Etymology: The species name is derived from that of its fish host.

Vegetative stages: Round plasmodia or some closely attached plasmodia bulging above the surface by 1–3 mm were located in the serosa layer of the spleen.

Spores: The mature spores are of ovoid shape in frontal view, rounded at the posterior end and tapered at the anterior end (Figs. 1I & 2F). In lateral view the spores are lemon-shaped (Fig. 1J). Spore valves are relatively thin, symmetrical and smooth. The sutural line is distinct. No sutural markings were found. The spores measure 13.5–15 (14.3 ± 0.48) μm in length, 6–7.8 (7.03 ± 0.58) μm in width and 4–5 (4.6 ± 0.54) μm in thickness. No intercapsular appendix was found, but the spore walls thicken at the anterior part. The polar capsules are elongated, equal in size and measure 6–7.5 (6.42 ± 0.54) μm in length and 1.4–2 (1.68 ± 0.22) μm in width. Polar capsules slightly converge toward the anterior end of the spores, and open relatively far from the anterior end. Polar filaments are coiled with 5 turns in the polar capsule; the coiled threads are perpendicular or slightly oblique to the longitudinal axis of the capsules. The extruded filaments measure 24–27 (25 ± 1.09) μm in length. Inside the relatively small sporoplasm the 2 nuclei and the iodophilous vacuole are well discernible.

Remarks: Based on the shape of the spores *Myxobolus pangasii* resembles some common species such as *M. macrocapsularis* and *M. carassii* but differs from them by virtue of the anterior thickening of the spore valves.

Histology: Plasmodia were found in the serosa covering the spleen. Some of the plasmodia were sunken in the parenchyma of the spleen, while others grew over the surface of this organ. Despite the close contact, plasmodia were always delimited from the parenchyma of the spleen by the serosa (Fig. 4D).


Pathogenicity: No general alterations were found in the gills and the spleen. In the case of *Hennegoides pangasii*, local changes consisted of dilatation of the afferent artery of the filament, encapsulation of the plasmodium by an endothelial layer, atrophied respiratory lamellae around the site of the plasmodium and aneurysmal dilatation of the artery. In lamellar infection with *Myxobolus baskai* the function of the lamella was blocked by the growing plasmodium, and the large cyst compressed the neighbouring lamellae. At a later stage of repair, granulomatous inflammatory reaction was

seen at the site of invasion (Fig. 4C). In the case of epithelial infection with *Henneguya shariffi* and *H. berlandi*, local proliferation of the epithelium around the cyst and compression of the neighbouring lamellae and filaments were found.

Only degenerative changes were seen in the cartilaginous gill rays in *Hennegoides malayensis* infection, which resulted in the formation of holes around the spores inside the cartilage. In the case of splenic infection with *Myxobolus pangasii*, dilatation of the capillaries and an increased number of red blood cells in the serosa layer between the spleen and the large plasmodium were the most remarkable histological changes.

Co-infections: In addition to myxozoan infections, only a few parasites belonging to other groups were found, of which *Thaparocleidus* spp. were the most common (Fig. 3D).

DISCUSSION



Although an earlier report on pet fish specimens imported to Hungary (Baska unpubl.) has already indicated the frequent occurrence of myxozoan infection in *Pangasius hypophthalmus*, the investigations presented in this paper give further evidence that heavy myxozoan infections can develop in this favourite food and aquarium fish. Dissections of cage-cultured specimens have demonstrated that *P. hypophthalmus* is infected by at least 6 myxozoan species. Gills proved to be the most infected organ, in which 5 species were found. The occurrence of *Henneguya* spp. on the gills of tropical catfishes is rather common. In recent years, several new species have been described from Indian and African fishes (Sarkar 1985, Fomena & Bouix 1996, Kpatcha et al. 1997). Up to this time, however, the genus *Hennegoides* was represented only by a single species, *H. longitudinalis*, the parasite of the gourami (Lom et al. 1991). The finding of 3 new *Hennegoides* species in the gills of *P. hypophthalmus* indicates that further examinations could reveal the occurrence of several other members of this genus in Southeast Asia. These examinations also prove that a fish can be infected simultaneously with 6 different species belonging to 3 genera. Of the examined organs, only the gills and the spleen proved to be infected. All species found had a typical location in the infected organs. The site selection and specific locations of myxozoans infecting the gills have been studied in detail by Molnár (2002). This author differentiated species infecting blood vessels (vascular type), the epithelium, the connective tissue and the cartilage. Within these types he selected species that preferred the filaments, the lamellae or the gill arches as site. Most of the species

found during the present investigations could be assigned to the categories suggested by Molnár (2002). Out of the 5 new species infecting the gills, 2 species (*Henneguya shariffi*, *Hennegoides berlandi*) had a typical epithelial location, 2 others (*Hennegoides pangasii*, *Myxobolus baskai*) developed in the blood vessels, and *Hennegoides malayensis* selected the cartilage as typical location. *H. shariffi*, which was located in the multi-layered epithelium, infected mostly the tip and edges of the filaments. *H. berlandi* preferred the same sites but formed larger plasmodia than did *H. shariffi*. *H. berlandi* had characteristic elongated plasmodia running from the tip toward the base of the filaments inside the multi-layered epithelium of the filament edge. *H. malayensis* proved to be a chondrophilic species located inside the cartilaginous filament rays. *H. pangasii* had a vascular location inside the afferent artery. *M. baskai* was also characterised by a specific vascular location. The plasmodia of this latter species developed at the non-separated basal parts of filaments where an outer and an inner filament originating from the gill arch unite. Histological studies revealed that the young trophozoites started their development in the capillary network of the lamellae. *M. pangasii* was always found on the surface of the spleen and never in other locations in the abdominal cavity. Despite this specific location, *M. pangasii* cannot be regarded as a parasite of the spleen, as the plasmodia never came into contact with the parenchyma of the spleen but infected only the serous membrane covering the spleen.

The taxonomic position of *Myxobolus baskai* raises several questions. Due to the presence of caudal appendages on examination in a fresh state, this species differs from most of the known *Myxobolus* species and could be classified into the genera *Unicauda* or *Dicauda*. According to Lom & Dyková (1992), the members of these genera have *Myxobolus*-type spores with appendages adhering to the posterior part of the shell valves, but these appendages are made up of a different material than the spore valve. In our case, 1 or 2 appendages were seen at the caudal end, depending on the position of the spores. The presence of these appendages could not be seen in fixed preparations. Although there are more than 20 *Unicauda* and *Dicauda* spp., and Cone & Melendy (2000) listed 16 described *Unicauda* spp., the validity of the latter genera is still questionable because of the uncertain keys used for determination. We think that mucous envelopes, membranaceous envelopes and non-sclerotised appendages are useful tools for species identification but they must not be used for creating or accepting new genera. Therefore, we classified our species into the genus *Myxobolus* Buetschli.

The intensity of infection varied in the fish specimens examined in this survey. During the short **period examination** no disease symptoms were recorded and we cannot give data on the pathogenicity of the myxozoan infections. However, the large number of myxozoan species found, the intensive infection of some fish specimens, and the local changes seen around the parasites suggest the possibility that further intensification of the culture might produce evidence of more severe symptoms and losses.

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