

Myxozoan pathogens in cultured Malaysian fishes. II. Myxozoan infections of redbtail catfish *Hemibagrus nemurus* in freshwater cage cultures

K. Molnár^{1,*}, C. Székely¹, K. Mohamed², F. Shaharom-Harrison²

¹Veterinary Medical Research Institute, Hungarian Academy of Sciences, PO Box 18, 1581 Budapest, Hungary

²Kolej Universiti Sains & Teknologi Malaysia (KUSTEM), 2130 Kuala Terengganu, Terengganu, Malaysia

ABSTRACT: Cage-cultured Asian redbtail catfish *Hemibagrus nemurus* (Valenciennes, 1840), a popular food fish in Southeast Asia, proved to be infected by 3 myxozoan species. All the 3 species belonged to the genus *Henneguya*: 2 were identified as *H. mystusia* Sarkar, 1985 and *H. hemibagri* Tchang et Ma, 1993, while the other was described as *H. basifilamentalis* sp. n. All plasmodia were found in the gills and were characterised by a specific site selection. *H. mystusia* formed plasmodia in the multi-layered epithelium between the gill lamellae and in the non-lamellar edge of the gill filaments, while *H. hemibagri* developed in the capillary network of the lamellae. *H. basifilamentalis* sp. n. had large oval plasmodia located deep among the filaments just above the gill arch.

KEY WORDS: *Hemibagrus* · Redtail catfish · Cage culture · Myxozoan infections · *Henneguya* · Site selection · Histology · Pathology

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INTRODUCTION

Catfish species such as *Clarias batrachus* (Linneus), *Pangasius hypophthalmus* (Sauvage) or *Hemibagrus nemurus* (Valenciennes) are popular food fishes in Southeast Asia, and they are cultured in large volumes in freshwater cage systems in rivers. Data on their culture in Southeast Asia are summarised in a companion paper (Molnár et al. 2006, this issue). The Asian redbtail catfish *H. nemurus* (Valenciennes, 1840), which is better known by its synonymous name *Mystus nemurus*, is one of the endemic species cultured in Malaysia.

The parasitic infections of *Hemibagrus nemurus* are less well studied. More data are available on the monogenean infections of this fish species. Lim (1987) described 3 *Cornudisoides* spp. from this fish. Little is known about myxosporean infections of catfishes in Malaysia. Members of the genus *Henneguya* Thélohan are the best known myxosporean parasites of catfishes. Most of the species (*H. adiposa* Minchew, *H. diversis* Minchew, *H. exilis* Kudo, *H. ictaluri* Pote et al., *H. limatula* Meglitsch and

H. postexilis Minchew) have been described from the channel catfish *Ictalurus punctatus* in North America, but several species (*H. branchialis* Ashmawy et al., *H. claridae* Abolarin, *H. laterocapsulata* Landsberg, *H. suprabranchiae* Landsberg) were listed by Eiras (2002) from the African catfish *Clarias geariepinus* as well. Two species are known to occur in hemibagrid fishes (*Mystus* and *Hemibagrus* spp.). Sarkar (1985) described *H. mystusia* from the gills of a *Mystus* sp. in India, while Tchang & Ma (1993) described *H. hemibagri* from *H. macropterus* Bleeker, 1870 in China.

There is only a single study (Shariff 1982) concerning myxozoan infections of fish in Malaysia. This author performed a complex morphological and pathological study on the parasite of a cyprinid fish, *Oxyeleotris marmorata* (Bleeker), and described this species as *Henneguya shaharini*.

The present paper describes 2 known (*H. mystusia* Sarkar, 1985, *H. hemibagri* Tchang et Ma, 1993) and 1 new (*H. basifilamentalis* sp. n.) *Henneguya* species and highlights their site selection in the infected organs and their pathogenic effect.

*Email: kalman@vmri.hu

MATERIALS AND METHODS

Fishes were obtained from a cage culture of the Terengganu River close to Kuala, Terengganu, Terengganu Province, northeast Malaysia. Eight fish of 15 to 18 cm and 10 fish of 36 to 42 cm in total length were dissected during a 2 wk period in November 2004. The investigations were aimed at studying myxosporean infections only. For a detailed description of the techniques of parasitological dissections and the processing of the collected material see Molnár et al. (2006).

RESULTS

Myxozoan infection was found both in fishes of the younger age group and in those of the older generation. All examined *Hemibagrus* specimens harboured myxosporean plasmodia in the gills, but among the dissected specimens there were individuals infected by only a few plasmodia and there were heavily affected specimens, where the gills contained up to several hundred plasmodia. In most cases the gills were infected by 3 different *Henneguya* spp. No infection was found in other organs. Two species were identified as *H. mystusia* Sarkar, 1985 and *H. hemibagri* Tchang et Ma, 1993, while another species (*H. basifilamentalis* sp. n.) proved to be new to science. A redescription of *H. mystusia* and *H. hemibagri* and a description of *H. basifilamentalis* sp. n. are as follows:

Henneguya mystusia Sarkar, 1985

Host: Asian redtail catfish *Hemibagrus nemurus*

Location: Terengganu River

Site of infection: Gill filaments

Prevalence of infection: 16 out of 18 fish

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-69964.

Trophozoites: Ellipsoidal small plasmodia between the gill lamellae 100–120 × 80–95 µm in size and round or ellipsoidal plasmodia 220–700 × 180–240 µm in size at the edge of the gill filaments inside the multi-layered epithelium.

Spores: The spores (Figs. 1A & 2A–C) are elongated with 2 straight caudal

appendages and with elongated polar capsules located side by side. The spore wall is thin and smooth, composed of 2 equal valves. The suture is distinct in lateral view. The oral end of the spore body is blunt, slightly tapering; the caudal end is rounded but continues into the caudal appendages. In a fresh state the spore body is 11–13 (12.17 ± 0.61) µm, its width is 3–3.7 (3.35 ± 0.25) µm and its thickness is 2–2.5 (2.21 ± 0.22) µm. The 2 polar capsules are composed of 2 equal cylindrical rods opening at the anterior end of the spore body. Polar capsules measure 4–5 (4.5 ± 0.46) µm in length and 0.9–1.2 (1.01 ± 0.08) µm in width. The length of the anterior capsule with its elongated tubular part is 2.8–3.2 (3.0 ± 0.179) µm; the length of the posterior capsule is 4.5–5 (4.83 ± 0.25) µm. The polar filaments are coiled in 6 to 7 turns perpendicular to the long axis of the polar capsules. The length of the extruded filaments is 27–31 (29.4 ± 3.99) µm. The sporoplasm has a small round iodophilous vacuole. In frontal view the 15–20 (17 ± 2.0) µm long caudal appendages cover each other, while in lateral view they run straight and parallel to each other.

Remarks: This species has been identified as *Hemibagrus mystusia*, a species described from the gills of a *Mystus* sp. in West Bengal, India. Both the shape and the measurements of the spores of the latter species are about the same as those we found for *H. nemurus*.

Due to the mixed infection of the gills it was difficult to find uniform spores. After opening mature plas-

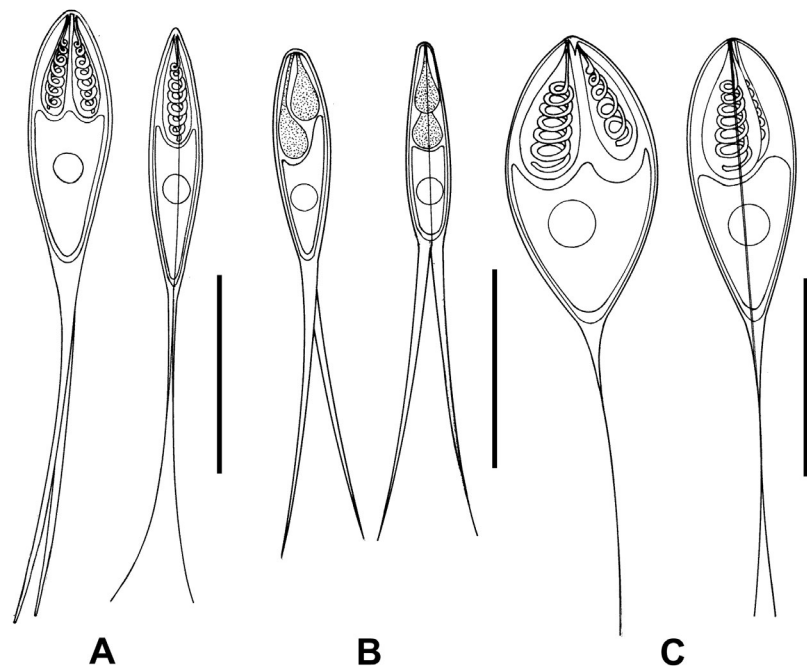


Fig. 1. *Henneguya* spp. spores infecting the gills of the Asian redtail catfish *Hemibagrus nemurus* in frontal and lateral views. (A) *Henneguya mystusia*. (B) *H. pseudobagri*. (C) *H. basilamellaris* sp. n. Scale bars = 10 µm

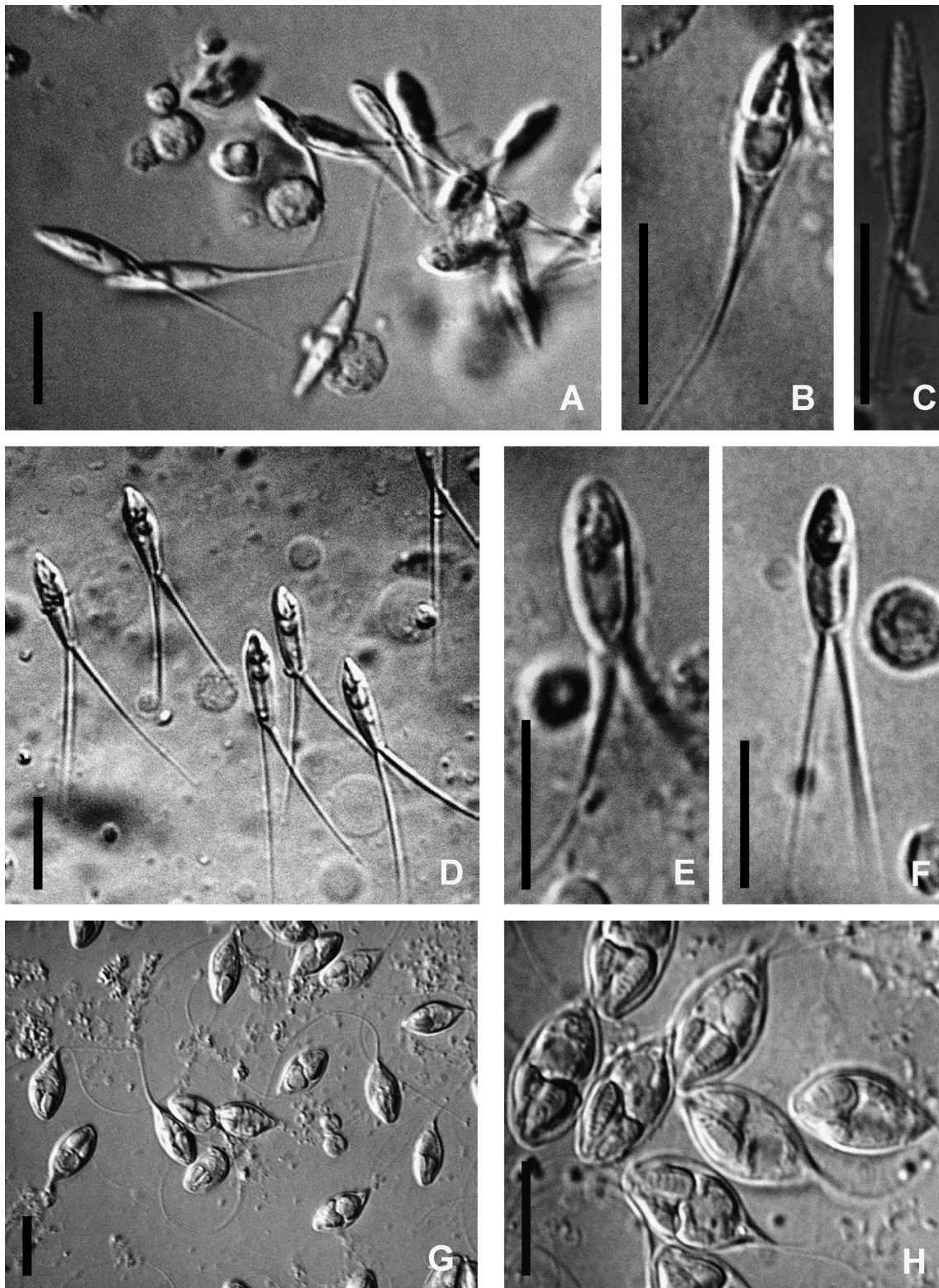


Fig. 2. Spores of *Henneguya* spp. infecting the gills of the Asian redbtail catfish *Hemibagrus nemurus*. (A) *H. pseudobagri* spores with strongly bifurcated caudal extensions and *H. mystusia* spores with elongated caudal extensions from a mixed infection. (B) *H. mystusia* spore in frontal view. (C) *H. mystusia* spore in lateral view. (D) *H. pseudobagri* spores with their strongly bifurcated tails. (E) *H. pseudobagri* spore in frontal view. Note the 'one behind the other' location of the polar capsules. (F) *H. pseudobagri* spore in lateral view. (G) *H. basifilamentalis* spores in frontal and lateral views. Only a short part is seen from the long but very thin caudal extensions. (H) Spore bodies of *H. basifilamentalis* in frontal view. See the polar capsules of different size. Scale bars = 10 μ m

modia, besides the typical *Hemibagrus mystusia* spores, some *H. hemibagri* spores were also found among the spores released.

***Henneguya hemibagri* Tchang et Ma, 1993**

Host: Asian redbtail catfish *Hemibagrus nemurus*

Location: Terengganu River

Site of infection: Gill lamellae

Prevalence of infection: 14 out of 18 fish

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-69965.

Trophozoites: Ellipsoidal or round plasmodia of 80–95 × 75–82 µm in size inside the gill lamellae.

Spores: The spores (Figs. 1B & 2A,D–F) are elongated with 2, strongly bifurcated, outside curving caudal appendages, and with polar capsules located one behind the other. Spore walls are thin and smooth, composed of 2 equal valves. The suture is clearly visible in lateral view. The oral end of the spore body is blunt, slightly tapering. The caudal end is rounded but continues into the caudal appendages. In a fresh state the spore body is 9–11.2 (10.03 ± 0.72) µm, the width of the spore body is 2–4 (2.91 ± 0.64) µm, and the thickness is 1.8–2.5 (2.06 ± 0.26) µm. The 2 polar capsules are composed of a round body and a thin tube opening at the anterior end of the spore body. The tubular part of the posterior polar capsule is much longer than that of the anterior one. The rounded parts of the 2 polar capsules measure 1.3–1.5 (1.35 ± 0.09) µm in length and 1.3–1.4 (1.35 ± 0.05) µm in width. The length of the anterior capsule with its elongated tube part is 2.8–3.2 (3.0 ± 0.17) µm. The length of the posterior capsule is 4.5–5 (4.83 ± 0.25) µm. Neither extruded polar filaments nor coils in the polar capsules were observable. The sporoplasm has a small, round iodophilous vacuole. In frontal view the 15–19 (17 ± 1.41) µm long caudal appendages cover each other; in lateral view, however, the outward curving and continuously tapering caudal appendages diverge from the posterior end of the suture.

Remarks: The morphology and size of the spores we found resemble the first description and illustration of *Hemibagrus hemibagri*, and differ from all other species by the fact that the polar capsules are located one behind the other. The spore body of the spores we collected from *H. nemurus* was a bit smaller and the caudal extension longer than those of the type material described by Tchang & Ma (1993); the rounded parts of the apical and distal polar capsules were of about equal size, although Tchang & Ma (1993) recorded a larger apical and a smaller distal capsule in their original description. Despite these small morphological differences and the different host

species, until more detailed investigations are made, we regard the species found in Asian redbtail catfish as *H. hemibagri*.

Spores of *Hemibagrus hemibagri* were mostly found in small plasmodia, but uniform material free from *H. mystusia* spores was only rarely collected.

***Henneguya basifilamentalis* sp. n.**

Host: Asian redbtail catfish *Hemibagrus nemurus*

Location: Terengganu River

Site of infection: Basal crypts of the hemibranchia

Prevalence of infection: 12 out of 18 fish

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-69910.

Etymology: The species was named after its specific location at the base of the filaments.

Trophozoites: Large, ellipsoidal plasmodia 580–720 × 240–320 µm in size at the crypts of hemibranchia between 2 gill filaments.

Spores: The spores (Figs. 1C & 2G,H) are wide, tapering anteriorly and posteriorly. They have 2 very thin caudal appendages and 2 elongated polar capsules of different sizes. The spore wall is thin and smooth, composed of 2 equal valves. The oral end of the spore body is blunt; the caudal end is tapered and joins the caudal appendages. In a fresh state the spore body is 13–15 (14.06 ± 0.65) µm, its width is 6–7.5 (6.91 ± 0.56) µm, and its thickness is 4–5 (2.81 ± 0.33) µm. Caudal extensions are very thin and their length is about 26–38 µm. The 2 polar capsules are different in size, elongated, blunt at the caudal end and taper anteriorly. The length of the larger capsule is 5–7 (6.52 ± 0.8) µm and its width is 2–3 (2.81 ± 0.39) µm. The length of the smaller polar capsule is 4–5.6 (5.08 ± 0.56) µm and its width is 1.3–2 (2.6 ± 0.53) µm. The polar capsules open at the anterior end of the spore body. The polar filaments are coiled in 7 turns perpendicular to the longitudinal axis of the capsule. The length of the extruded filaments is about 10 µm. The sporoplasm has a large iodophilous vacuole.

Remarks: *Henneguya basifilamentalis* differs from most of the known *Henneguya* spp. by virtue of its wide spore body, its indistinct caudal appendages and its typical location in the gill. It resembles *H. lesteri* Hallet & Diamant, 2001, a parasite of sand whiting, due to the shape of the spores and its location in the gills, but differs from the latter by virtue of its larger spores, its polar capsules of different size and the number of filamental turns in the capsule. Both *H. basifilamentalis* and *H. lesteri* are typically located in the filamental crypts, which corresponds to the location of *Myxobolus basilamellaris* Lom & Molnár, 1983, a parasite of the common carp.

Histology

In some of the examined fish, especially in specimens of the younger age group, heavy *Henneguya* infections were found (Figs. 3A,C & 4C). In these cases the majority of gill filaments harboured developing and mature plasmodia of *Henneguya* spp. Of the 3 species identified by spore morphology only one, *H. basifilamentalis* sp. n., could easily be differentiated histologically from the other two as it had very large plasmodia at the base of the gills close to the cartilaginous gill arch (Fig. 4C). In most of the gill filaments small plasmodia were found in interlamellar position among the multi-layered epithelial cells filling the space of 2 neighbouring gill lamellae (Figs. 3A,D & 4B). These plasmodia usually harboured developing *Henneguya* sporogonic stages, but some of them were filled with mature spores. Some of these epithelial forms bulged toward the non-lamellar lateral edge of the filaments (Fig. 3B), resulting in an intrafilamental location. This intrafilamental location of spores was most frequently found at the tip of the gill filaments, where the occurrence of large plasmodia was common (Fig. 3C). In fresh preparations these large plasmodia usually harboured the spores of *H. mystusia*, but some *H. hemibagri* spores were incidentally found among them. In some cases inter- and intralamellar plasmodia developed in close vicinity (Fig. 3D). The latter stages could be easily differentiated from the interlamellar ones as they occupied the space inside the capillary network of the lamellae (Fig. 4A). Plasmodia of *H. basifilamentalis* sp. n. were always located at the base of the gills between 2 neighbouring gill filaments (Fig. 4C). The large ellipsoidal plasmodia were in close contact with the cartilaginous basal parts of the gill rays, and only a thin connective tissue separated the cartilage from the plasmodium. This specific location resembled the site of *Myxobolus basilamellaris*, a parasite of the common carp, but the plasmodia of *H. basifilamentalis* never entered the lumen of the cartilaginous gill arch.

Pathogenicity

No general alterations were found in the gills. In some fish with *Henneguya mystusia* infection whole filaments were occupied by growing and mature plasmodia (Fig. 3A,C), which deformed the lamellae. The multi-layered epithelium between the lamellae was replaced by plasmodia, and in these sections the apical parts of the lamellae were overgrown by plasmodia (Fig. 3A). In less heavily infected places the non-infected lamellae were also deformed by the compression exerted by plasmodia developing among the

neighbouring lamellae. At the tip of the filaments large plasmodia, supposedly formed by the fusion of small interlamellar ones, completely deformed the structure of the filaments (Fig. 3C). At the base of the hemibranchia the large plasmodia of *H. basifilamentalis* compressed the neighbouring filaments and no lamellae were found in this section. Above the plasmodia the basal epithelial layer was lifted up in an apical direction (Fig. 4C). At the time of examination only minor signs of recovery were observed. As a sign of past infection, granulomatous inflammatory reaction was observed in some filaments (Fig. 4D).

DISCUSSION

Dissections of cage-cultured *Hemibagrus nemurus* specimens showed that myxozoan infections are rather common and they cause intensive infections of the gills in this fish species. After dissecting 18 specimens of *H. nemurus* to find myxosporeans, only *Henneguya* infection caused by 3 species were found. The infection was restricted to the gills. *Henneguya* infections, especially those involving the gills, are rather common in fishes. Besides the genus *Myxobolus*, the genus *Henneguya* has the highest number of species. Eiras (2002) reported about 146 valid species in his synopsis. Most species infect catfishes in North America (Hoffman 1999), but several species are known from catfishes of the tropical zones of Asia and Africa (Abolarin 1971, Sarkar 1985, Landsberg 1987).

Hemibarbus nemurus specimens were in most cases simultaneously infected by 3 *Henneguya* species, of which *H. basifilamentalis* could easily be differentiated from the other two by its large fatty spores and the typical basilamellar location of its plasmodia. The exact location of *H. mystusia* and *H. hemibagri* was difficult to identify. Both species formed spores in the filamental part of the gills. When mature plasmodia were opened and examined, some spores from a different species were often found in the collected spore material. Histological studies, however, revealed that these parasites were present in 2 different locations. Besides intralamellarly developing small plasmodia there were more common infections with interlamellarly developing plasmodia, which often bulged away from an intraepithelial location into the non-lamellar edges of the filaments where large cysts developed, presumably after the fusion of small plasmodia. Based on the fact that spores of *H. hemibagri* occurred less frequently, and the large epithelial cysts contained mostly *H. mystusia* spores, we assume that it is the *H. hemibagri* species which develops in the intralamellar location inside the capillary network. The authors of the present paper consequently make a

distinction between gill lamellae and gill filaments. Some authors, e.g. Current & Janovy (1978), speak about primary and secondary lamellae; therefore, the meaning of the phrases 'intralamellar' and 'interlamellar' differs from that suggested by Molnár (2002).

Using this latter terminology, the epitheliophilic species *H. mystusia* might develop in interlamellar and intrafilamental epithelial locations, while *H. hemibagri* seems to be a vascular species developing in the lamellae, but never in the filamental arteries.

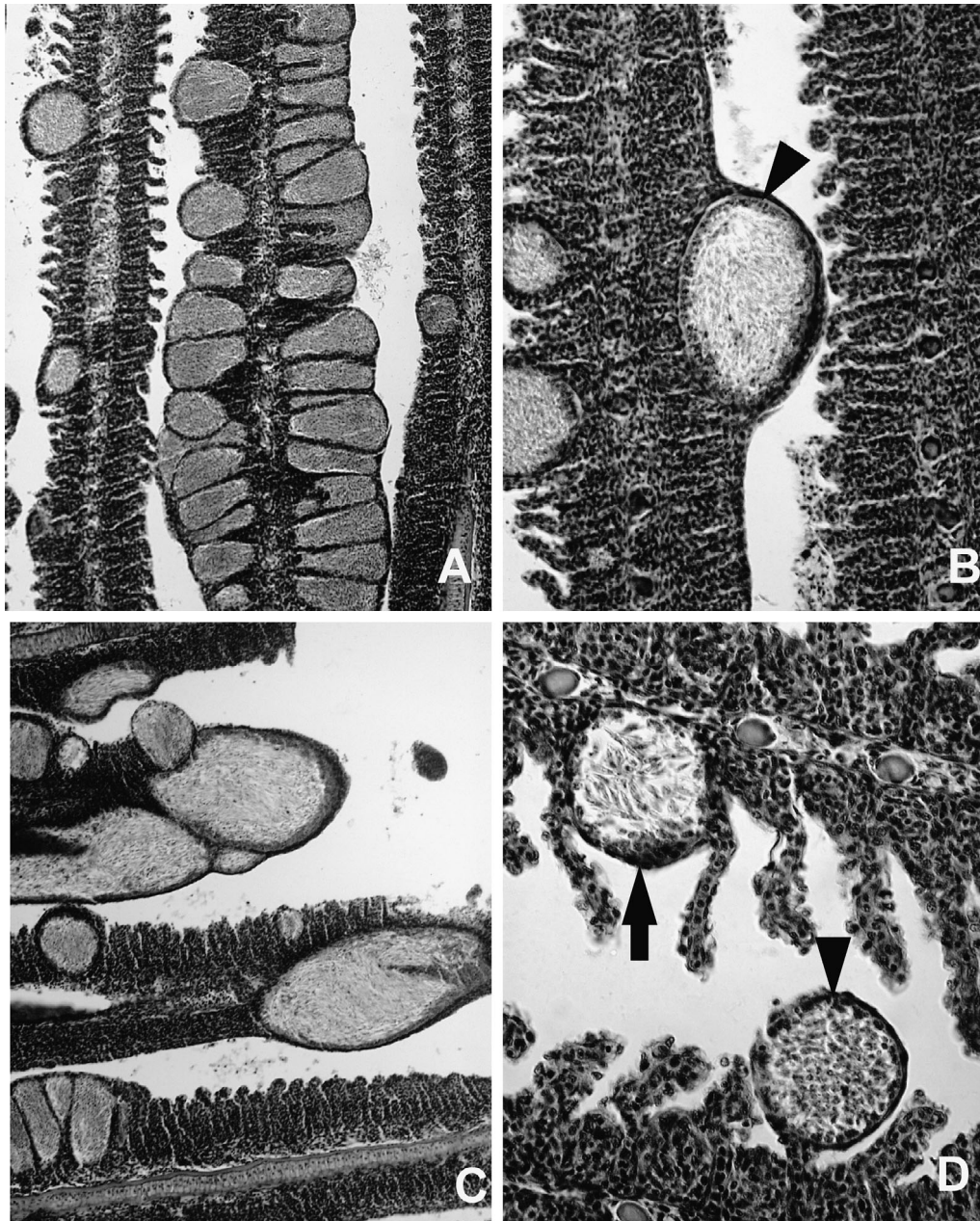


Fig. 3. *Hennequya* infections in the gills of the Asian redbtail catfish *Hemibagrus nemurus*. (H&E). (A) Heavy intralamellar *H. mystusia* infection in the gill filament. Plasmodia develop typically in the multi-layered epithelium between 2 lamellae. The tips of the gill lamellae are overgrown by plasmodia at the infected region. $\times 127$. (B) A *H. mystusia* plasmodium (arrowhead) bulging over the lamellar region of a gill filament. $\times 225$. (C) Large plasmodia of *H. mystusia* in the multi-layered epithelium of the tip of gill filaments. The plasmodia were possibly formed by fusion of the bulging parts of interlamellar plasmodia. $\times 127$. (D) An interlamellar (*H. mystusia*) plasmodium (arrow) between gill lamellae and an intralamellar (supposedly *H. hemibagri*) plasmodium (arrowhead) inside a gill lamella. $\times 450$

The spore of *Henneguya mystusia* is typical for this genus, and resembles several species found in catfishes and in other fish species in both its dimensions and morphology. We tentatively identified it as the species described by Sarkar (1985), since the

genera of the hosts *Mystus* and *Hemibagrus* are related. Further, presumably molecular, studies might differentiate the species in the 2 hosts. Little is known about the host specificity of *Henneguya* species. It is supposed that morphologically similar spores

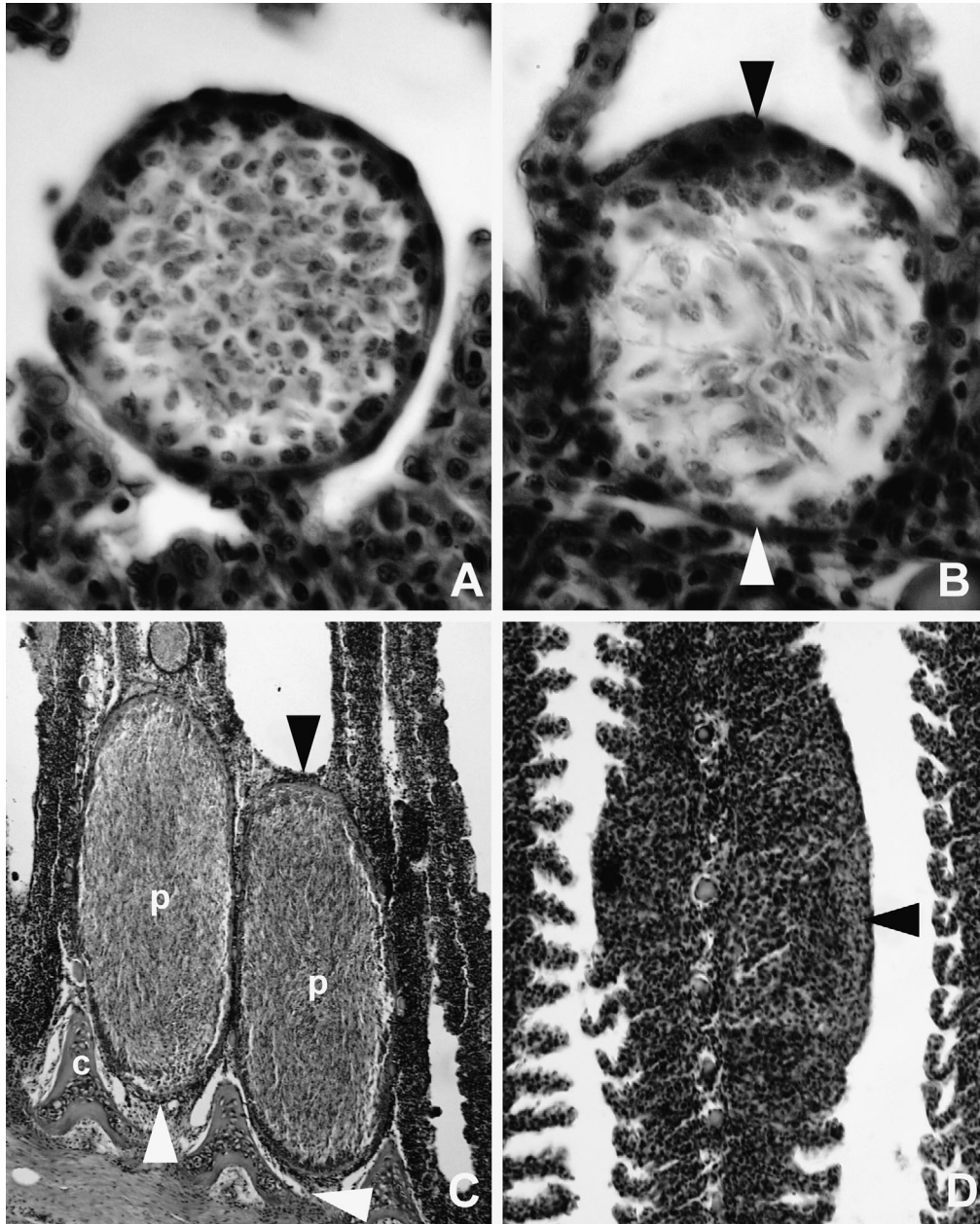


Fig. 4. *Henneguya* infections in the gills of the Asian redtail catfish *Hemibagrus nemurus*. (H&E). (A) An intralamellar *H. hemibagri* plasmodium inside the capillary network of a gill lamella. The relatively small plasmodium contains gamogonic developmental stages. $\times 1170$. (B) A semi-mature *H. mystusia* plasmodium in the epithelium between 2 lamellae. The remaining epithelial cells cover the developing cyst (black arrowhead) and are located at the base of the plasmodium (white arrowhead). In the centre of the plasmodium some spores have already formed, while sporogonic developmental stages are located at the periphery of the plasmodium. $\times 1170$. (C) *H. basifilamentalis* plasmodia (p) at the base of a hemibranchium between 2 neighbouring filaments. Plasmodia are attached to the base of the cartilaginous gill rays (c) by connective tissue (white arrowheads). The large plasmodia have lifted up the basal epithelium in apical direction (black arrowhead). $\times 127$. (D) Granulomatous inflammatory cells fill the place left by the released *M. mystusia* spores between and over gill lamellae (arrowhead). $\times 225$

infecting fishes of the same genera might be the same. This is why *Henneguya* species found in the Asian redbtail catfish were identified as *H. hemibagri* despite small morphological differences in the spores. The 'one behind the other' location of the polar capsules in the spore is rather unusual in the genus *Henneguya* and only the spores of *H. hemibagri* from *Hemibagrus macropterus* and *Hemibagrus nemurus* are characterised by this unusual location of the polar capsules. *Henneguya basifilamentalis* sp. n. is also a unique species. It also has several features different from those of the known species. For a long time, basillamellar location of the plasmodia was known only in the genus *Myxobolus*, where *M. basilamellaris* Lom & Molnár, 1983 formed its plasmodia at the base of the filaments and in the lumen of the gill arch. Recently, however, Hallett & Diamant (2001) have described *H. lesteri* from sand whiting, which had the same location among the crypts of hemibranchia as *H. basifilamentalis*. Despite some similarities, both *H. lesteri* and *H. basifilamentalis* differ from *M. basilamellaris*, as the plasmodia of the former 2 species never enter the lumen of the gill arches. Another characteristic of *H. basifilamentalis* is the extraordinarily thin caudal extension of the spores, which could only be observed by thorough microscopic examination of fresh material.

Henneguya spp. frequently cause heavy infections. Current & Janovy (1978) reported that *H. exilis* caused signs of disease in cultured channel catfish *Ictalurus punctatus* in the USA, and Current (1979) found similar severe changes in the fins due to *H. adiposa* infection. There are also reports of heavy infections in Europe. Dyková & Lom (1978) described heavy infection and damage caused in the gills of pike and 2 percid fishes by *H. psorospermica*, while Haaparanta et al. (1994) and Molnár (1998) found similar changes caused by *H. creplini* in the gills of perch and pikeperch, respectively. It is not yet possible to evaluate the economic importance of *Henneguya* species infecting *Hemibagrus nemurus*, but the intensity of infection in some of the dissected fish indicates that after the intensification of catfish culture these species could cause losses in channel catfish stocks similar to those caused by *Henneguya exilis*.

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