Systematic Parasitology **22**: 25–31, 1992.

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Ceratomyxa hungarica n. sp. and *Chloromyxum proterorhini* n. sp. (Myxozoa: Myxosporea) from the freshwater goby *Proterorhinus marmoratus* (Pallas)

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Accepted for publication 23rd May, 1991

Abstract

When studying the parasite fauna of freshwater gobies *Proterorhinus marmoratus* collected from the reaches of the River Danube around Budapest, two species of Myxosporea were recovered, a renal and a gall-bladder form. Previously no myxosporeans had been reported from this fish species. The spores and pseudoplasmodia of the parasite described as *Ceratomyxa hungarica* n. sp. were found in the convoluted tubules of the kidney and in the cavity of Bowman's capsule. The pseudoplasmodia were loosely attached to the wall of the tubules, causing their distention. Within each pseudoplasmodium two spores were formed. In the case of *Chloromyxum proterorhini* n. sp. only spores floating freely in the contents of the gall-bladder were found. Since *Ceratomyxa* species are typically marine fish parasites, *Proterorhinus marmoratus*, a fish species which has adapted to freshwater, appears to have retained some of its marine myxosporean fauna.

Introduction

The decisive majority of the numerous myxosporeans belonging to the genus *Ceratomyxa* Thélohan are parasites of marine fishes. Shulman (1966) reported 14 species from fishes living in the seas surrounding the Soviet Union. The majority of these parasites are inhabitants of the gall-bladder, and only the species *Ceratomyxa caspia* Dogiel and *C. spectabilis* Dogiel were reported to occur in the urinary tract. *C. shasta* Noble, economically and pathologically the most important representative of the genus, parasitises the intestinal wall. With the exception of *C. shasta*, *Ceratomyxa* species are little studied parasites. At the same time, the literature contains considerable data on the development and morphology of *Leptotheca* and *Sphaerospora* spp., most of which parasitise the renal tubules and are both morphologically and phylogenetically closely related to *Ceratomyxa* spp.

The numerous and morphologically rather uniform representatives of the genus *Chloromyxum* Mingazzini parasitise both freshwater and marine fish species. The majority of them are inhabitants of the bile duct but, primarily in marine and anadromous fishes, they also frequently cause infection of the urinary tract. In some species, for example in the pholid *Polydapus dybowskii* and in the gadid *Lota lota* kidney and bile duct inhabiting species may occur concurrently (Shulman, 1966; Lom *et al.*, 1988).

The present paper describes two new myxosporean species from the freshwater goby *Pro-*

terorhinus marmoratus, a member of the family Gobiidae that has adapted to freshwater conditions. This fish is common in the Hungarian reaches of the River Danube. Of the two newly reported parasite species *Ceratomyxa hungarica* n. sp. parasitises the renal tubules, while *Chloromyxum proterorhini* n. sp. occurs in the gall-bladder.

Materials and methods

Freshwater gobies *Proterorhinus marmoratus* were collected with a close-meshed net from the reaches of the River Danube around Budapest and from small streams flowing into the Danube during 1989 and 1990. The 2–7 cm long fish were kept in the laboratory for 1–2 days, killed by decapitation and subjected to parasitological investigation. The gills and intestinal tract were examined under a stereomicroscope. The blood and the contents of the gall-bladder were checked for the presence of parasites under a coverslip, at 200–400× magnification. Pea-sized pieces of the parenchymal organs were squashed and examined under a microscope in a similar manner to scrapings taken from the gut and gills.

Measurements of the myxosporeans were taken from fresh preparations and drawings were made of unfixed parasites. The *Chloromyxum* spores were embedded in glycerine jelly or placed in ammonium picrate solution and preserved as slide preparations. For histological examination the kidneys were fixed in Bouin's solution, embedded in paraffin wax and sectioned. The 4 μm thick sections were stained with haematoxylin-eosin and by Farkas-Mallory's method.

Results

Four protozoan species were found in the 22 freshwater gobies examined. A *Ceratomyxa* species occurred in 14 fish specimens, a *Chloromyum* species in 10 fish specimens, and a *Goussia* (Apicomplexa) and a *Nosema* (Microsporea) species in one fish each. The two myxosporeans proved to be previously unknown species and are described as follows.

Ceratomyxa infection

Infected specimens were found only among fish examined in April and May, when 14 of 18 gobies examined were found to be infected by developmental stages and spores of a new species of *Ceratomyxa*. In April only the developmental stages filling the renal tubules and, occasionally, Bowman's capsule were found. These proved to be pseudoplasmodia corresponding to the sporogonic stages. In fish examined in May, the infected renal tubules contained, in addition to pseudoplasmodia, spores with two polar capsules. Using spore morphology the parasite could not be identified with any of the known species of *Ceratomyxa*.

Ceratomyxa hungarica n. sp. (Fig. 1a,b)

Type-host and locality: *Proterorhinus marmoratus* (Pallas), River Danube, Hungary.
Site of infection: Lumen of the renal tubules and Bowman's capsule.
Prevalence: 14 of the 22 fish examined were infected.
Type-material: Histological sections with paratypes, slides no. 1991.9.7.1, have been deposited in the collection of The Natural History Museum, London.

Description (based upon 50 mature spores from several host fish)

The pseudoplasmodia are spherical or elliptical structures. The younger of the spherical plasmodia are 11–14 um, while those which already contain primordia of spores are 15–17 μm in diameter. The elliptical plasmodia mostly contain spores and range between 21 × 13 and 26 × 9 μm in size. Within each plasmodia 2 spores are formed. The spores (Fig. 1a) are transversally elongate with a flattened elliptical shape and have a smooth surface. They are 10.1 (10.0–10.5) μm

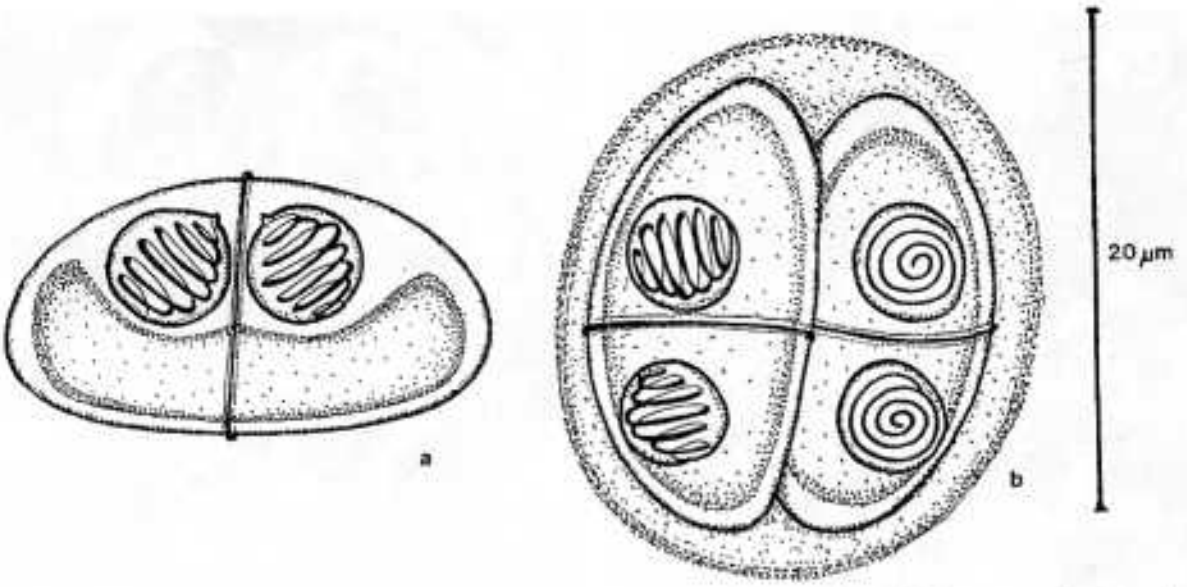

Fig. 1. Ceratomyxa hungarica n. sp. (a) Schematic representation of the spore in frontal view. (b) Bisporous pseudoplasmodium with two developing spores.

long and 18.8 (18.5–19.5) µm wide. Each spore contains 2 almost spherical polar capsules 4.8 (4.5–5.0) µm in diameter. The coiled polar filament perpendicular to the axis of the polar capsule rotates 6 times. The polar capsules are situated medially in the anterior part of the spore. The transversally elongate sporoplasm lies in the posterior part of the spore. The spore shell consists of 2 equal valves connected by a suture perpendicular to the plane of the polar capsules. No mucous envelope was seen around the spores.

Histological examination

The plasmodia were tightly packed in the infected tubules but were only loosely attached to the tubular walls. The numerous plasmodia and spores situated in infected segments caused considerable distension of the tubules (Fig. 2) and flattening of the tubular epithelium. The most intensive infection was consistently found in the initial portion of the tubules, where they unite with the lumen of Bowman's capsule (Fig. 3). In such cases the pseudoplasmodia become jammed in Bowman's capsule and cause distension of the lumen, forming a ring around the glomerulus (Fig. 4). Spore maturation was not synchronous and both mature and young plasmodia occurred in the same tubular portion (Fig. 5). In some cases a given bisporous plasmodium contained spores of varying states of maturity at the same time. Young stages stained blue, developing spores purple and mature spores red using Farkas-Mallory's staining method.

***Chloromyxum* infection**

Spores only were found. In freshwater gobies examined in April, May and June the spores occurred freely in the gall-bladder. Based upon the characteristic morphology of the spores, the parasite proved to be a new species which is described as follows.

***Chloromyxum proterorhini* n. sp.** (Fig. 6a,b)

Type-host and locality: *Proterorhinus marmoratus* (Pallas), River Danube, Hungary.
Site of infection: Gall-bladder.
Prevalence: Ten of the 22 fish examined were infected.
Type-material: Slides with spores in ammonium picrate have been deposited in the collection of


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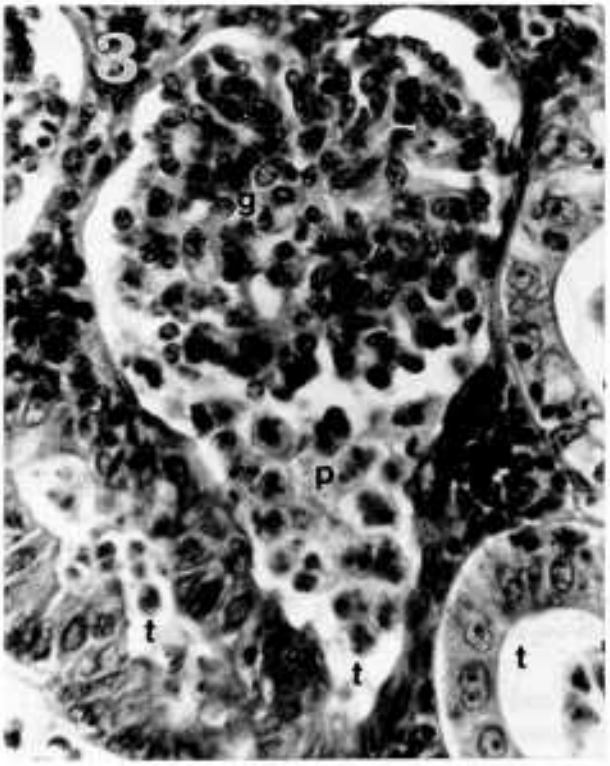
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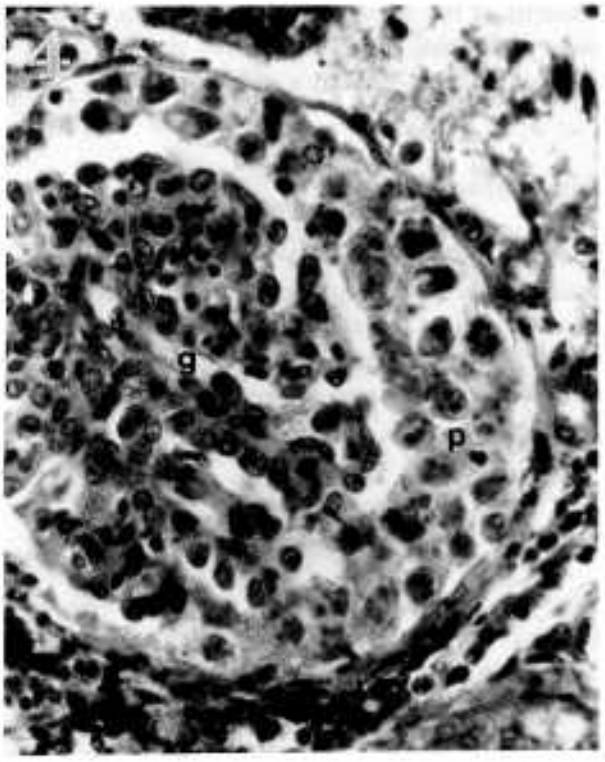
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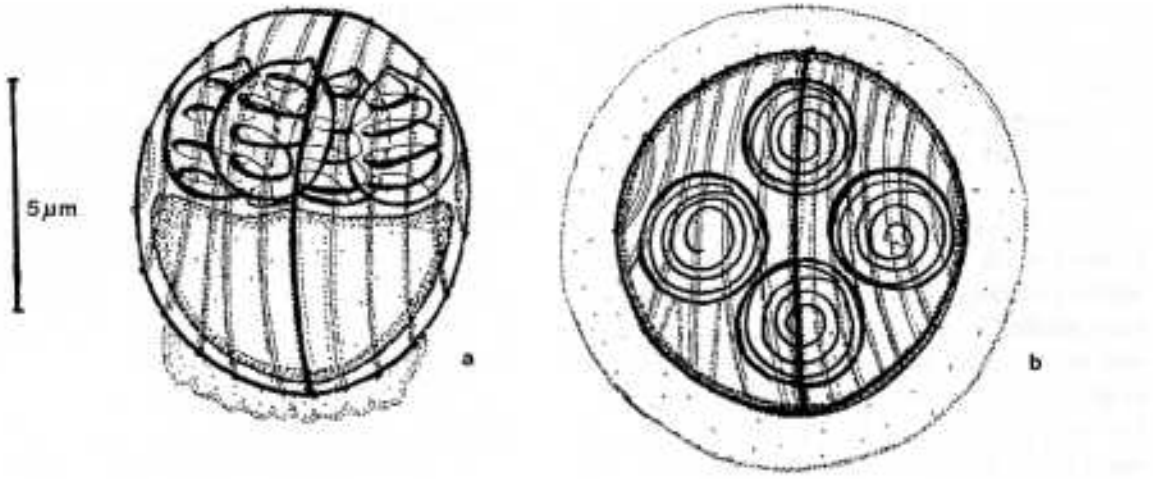

Fig. 6. Schematic view of the spore of *Chloromyxum proterorhini* n. sp. (a) in frontal view; (b) in anterior view. The spore is surrounded by a mucous envelope.

The Natural History Museum, London. Reg. no. 1991.9.7.2.

Description (based upon 50 mature spores from several host fish)

The spores are short elliptical in frontal and spherical in anterior view. They consist of two equal valves united longitudinally by a suture. The suture only slightly protrudes over the spore surface. The spores are 8.5 (8.0–9.0) µm long and 7.1 (6.5–7.5) µm wide. On the spore surface there are ridges, some of which run parallel to the suture while others are coiled or resemble a fingerprint. When the spores are examined in fresh preparations, a narrow, skirt-like projection can often be seen in their posterior region. The 4 normally elliptical, polar capsules are situated in the anterior part of the spore equidistant from each other. The polar capsules are 3.4 × 2.3 (3.0–3.5 × 2.0 × 2.5) µm in size. The polar filament has 4, less often 5, rotations in the polar capsule (the rotations are mainly perpendicular to the longitudinal axis of the capsules). The apertures of the polar capsules are orientated in the same direction, anteriorly. Some of the spores found in the bile were situated in a mucous envelope 10–14 µm in diameter.

Discussion

Few data are available on the parasite fauna of the freshwater goby. More is known about its helminth parasites, four species of which have been described by Vojtek (1959, 1974) and one by Ergens (1967). No information is available on the occurrence of protozoa.

Of the *Ceratomyxa* species, only *Ceratomyxa caspia* Dogiel has previously been reported from the kidney of gobies (Shulman, 1966). The spores of this species, however, differ substantially from those occurring in the freshwater goby. The species described here bears the greatest resemblance

Figs. 2–5. 2. Developing pseudoplasmodia of *C. hungarica* n. sp. in the lumen of the renal tubules of a freshwater goby. The pseudoplasmodia distend and completely fill the lumen of the tubules. The tubular epithelium is flattened in the infected portions. Histological section. Farkas-Mallory's staining method, ×750. 3. Kidney of a freshwater goby. The lumen of the Bowman's capsule is filled with glomerulus (g) and pseudoplasmodia (p). Pseudoplasmodia can also be found in the portion of the convoluted tubules (t) joining Bowman's capsule. The pseudoplasmodia (p) are situated around the glomerulus (g) within the capsule (c). Histological section. Haematoxylin and eosin, ×750. 5. Pseudoplasmodia of *C. hungarica* n. sp. containing spores (arrow) in the lumen of a renal tubule. Histological section. Farkas-Mallory's staining method, ×750.

to the parasite reported by Shulman (1966) from the gall-bladder of the fish *Sphaeroides borealis*; however, the spores of *S. hungarica* n. sp. are less transversely elongate, and differences in host types and site also suggest that the two species are distinct.

In the structure of sporogonic stages and their location in the kidney, *Ceratomyxa hungarica* resembles various *Sphaerospora* species that have been studied in detail recently. The kidney-parasitic members of the genus *Ceratomyxa* develop, in all probability, similarly to renal sphaerospores, i.e. only the sporogonic developmental stages occur in the kidney and the presporogonic forms develop in some other part of the fish. The presporogonic development of *Sphaerospora renicola* takes place in the blood and swim-bladder of the fish (Csaba *et al.*, 1984; Molnár & Kovács-Gayer, 1986). Other sphaerospores are also characterised by multiplication in the blood during the vegetative stage (Lom *et al.*, 1985, 1989; Baska & Molnár, 1988). At the same time, in PKD of trout, which is also considered to be a sphaerosporosis, the presporogonic stages, the so-called PKX-parasites, are located in the renal parenchyma and in other organs (Hedrick *et al.*, 1988). It is an interesting feature of *C. hungarica* that pseudoplasmodia occur not only in the tubules, but also within Bowman's capsule surrounding the glomerulus. Such cases have already been reported by Baska (1990) in connection with *Sphaerospora colomani* Baska infection of the sterlet. Infection of the glomerulus is common also in *Myxidium rhodei* (see Dykova *et al.*, 1987) and *M. lieberkuehni* (see Lom *et al.*, 1989) infections. In these latter cases, however, not only the sporogonic but also the vegetative stages can be found, i.e. the existence of blood stages is not absolutely necessary. Blood stages have not been demonstrated in the present material either; however, they probably do exist and presumably it will be possible to demonstrate them if a larger number of blood samples from an earlier stage of infection are examined.

It is a fact of some faunistic importance that the goby, which was originally a marine species, has retained one of its original marine parasites following its adaptation to freshwater conditions. This parasite is the sole representative of the genus *Ceratomyxa* in Hungary.

Chloromyxum proterorhini n. sp., a species reported from the gall-bladder of the freshwater goby, rather closely resembles known species of *Chloromyxum* with a ridged spore surface, but differs from them in its more elongate spores and the skirt-like projection present at the posterior end of the spores. In addition to the morphological characteristics, its validity is also indicated by the well-known host specificity of *Chloromyxum* spp. and the fact that no *Chloromyxum* has previously been described in *Proterorhinus* or in fish species closely related to it.

Chloromyxum proterorhini, a parasite known only on the basis of its spore, is faunistically a less unusual parasite, since members of the genus parasitise both marine and freshwater fishes. Unfortunately, the rather low intensity of infection prevented the collection of data on the developmental stages. Although Mitchell *et al.* (1980) found the sporogonic plasmodia of *C. trijugum* attached to the gall-bladder wall, the early developmental stages should be looked for in the bile duct, as suggested by Davis (1985), who discovered plasmodia of the also gall-bladder parasite *Zschokkella russeli* in this site. In the case of parasites of this type, the presence of blood stages is unlikely, although Lom *et al.* (1988) did not preclude this possibility.

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