# Hyperparasitism by *Myxidium giardi* Cépède 1906 (Myxozoa: Myxosporea) in *Pseudodactylogyrus bini* (Kikuchi, 1929) Gussev, 1965 (Monogenea: Dactylogyridae), a parasite of the European eel *Anguilla anguilla* L.

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**Abstract**

In a study of the parasite fauna of the European eel (*Anguilla anguilla* L.) in northwest Spain, we found frequent mixed infection of branchial tissues by the myxosporidian *Myxidium giardi* (prevalence 95%) and by two monogeneans (*Pseudodactylogyrus anguillae* and *P. bini*; joint prevalence 56%). All three species are common parasites in this host. In one of the 323 eels examined, hyperparasitism of *P. bini* by *M. giardi* was detected. This eel contained 281 individuals of *Pseudodactylogyrus* spp., of which 94% were *P. bini*. Spores of the myxosporidian were detected in 30% of the *P. bini* individuals, while corpuscles of unknown origin were detected in all of the *P. bini* individuals (but not in any *P. anguillae* individuals, or in *P. bini* individuals in other eels).

## Introduction

There have been numerous studies of the parasites of the European eel (*Anguilla anguilla* L.), in both marine and freshwater environments (Orecchia et al., 1987; Køie, 1988a, 1988b; Orecka-Grabda & Wierzbicka, 1994; Saraiva, 1994; Schabuss et al., 1997; Kennedy et al., 1998; Borgsteede et al., 1999). These parasites include the generalist *Myxidium giardi* Cépède, 1906, and the eel-specific *Pseudodactylogyrus bini* (Kikuchi, 1929) Gussev, 1965, which often occur together in branchial tissue. During morphometric studies of monogeneans infecting the European eel, we have detected evidence of hyperparasitism, i.e. infection of *P. bini* by *M. giardi*. We also found corpuscles of unknown origin in *P. bini* tissues. The findings are reported herein.

## Materials and methods

In 1999 and 2000, we obtained monthly samples of European eel from the River Ulla basin in Galicia (northwest Spain). A total of 323 eels were captured, and transferred alive

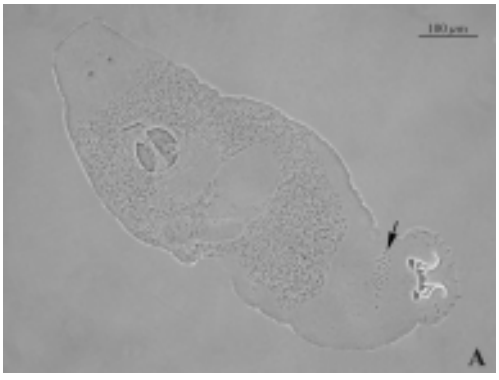

**Figure 1A.** *Pseudodactylogyrus bini*, general view showing *M. giardi* spores (arrow).

**Figure 1B.** *P. bini*, general view showing corpuscles (arrows).

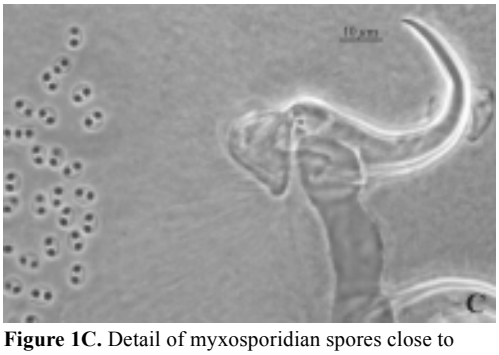

**Figure 1C.** Detail of myxosporidian spores close to the monogenean's opisthaptor.

to the laboratory, where they were maintained in freshwater aquaria with aeration and recirculating flow until sacrifice and necropsy (maximum 5 days).

At necropsy, the head was first removed, the gills extracted, the 8 branchial arches separated (4 right, 4 left), and each arch divided into three regions (dorsal, medial and ventral) (Buchmann, 1989; Dzika, 1999), which were placed in separate receptacles containing physiological saline, for examination under a stereomicroscope (3x). Individual filaments were also examined by high-magnification light microscopy (40x).

When monogeneans were detected, they were separated from the underlying branchial tissue, intensively washed to remove mucus, then fixed in Berland's fluid (Berland, 1984) and conserved in 70% alcohol. For species-level identification, parasites were mounted in Hoyer's medium (Schell, 1969), which contains a clearing agent and thus facilitates visualization of key structures.

For detection of protozoan parasites, we obtained fresh contact smears of each branchial arch. Spores detected were measured with the aid of a calibrated micrometer.

Monogeneans with *M. giardi* or corpuscles were photographed for detailed morphometric study. To investigate the nature of the corpuscles, they were removed from the host, placed on glass slides and crushed under cover slip pressure to view their content by light microscopy (40x). For the morphometric study we randomly selected 61 individuals of *P. bini*, some with *M. giardi*, some not, and measured all corpuscles found (total 112).

## Results

Analysis of the eel branchial tissues indicated that *M. giardi* was present with a prevalence

of 95%, while the monogeneans *P. anguillae* (Yin & Sproston, 1948) Gussev, 1965 and *P. bini* were present with a joint prevalence of 56%. In one of the 323 eels, we observed hyperparasitism, i.e. the presence of *M. giardi* spores in *P. bini* tissues (Figure 1A). The total number of *Pseudodactylogyrus* individuals detected in this eel was 281, 94% of which were *P. bini*. In 30% of these *P. bini* individuals we observed *M. giardi* spores, and in all these individuals we observed corpuscles of unknown origin (1 - 5 corpuscles per individual; Figure 1B). Neither *M. giardi* spores nor corpuscles were observed in *P. anguillae*, nor were corpuscles observed in *P. bini* individuals from other eels, strongly suggesting that they are associated with the presence of *M. giardi*.

The *M. giardi* spores were in all cases free, never encysted, and were located in the ventral region of *P. bini*, close to the opisthaptor (Figure 1C). The corpuscles were located in the medial region, around the cirrus (Figure 1D), and were spherical in shape. They can be divided into three size classes, namely small, medium and large (Table 1). Light microscopy revealed that the corpuscles comprised an ochreous cuticle containing a homogeneous translucent substance (Figure 1E).

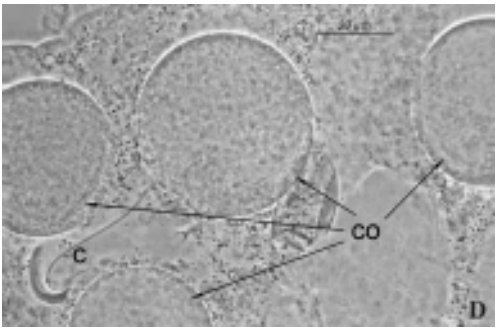


**Figure 1D.** Detail of corpuscles around the monogenean cirrus (c = cirrus, co = corpuscles).

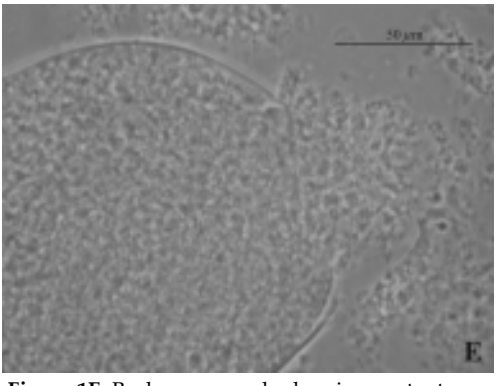


**Figure 1E.** Broken corpuscle showing content.

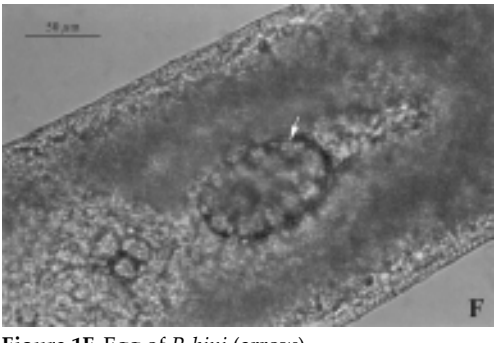


**Figure 1F.** Egg of *P. bini* (arrow).

| Size class | Mean ± SD* | Range (minimum-maximum) | N° of measured corpuscles |
|---|---|---|---|
| Small | 58.53 ± 10.56 | (40-70) | 49 (43.75%) |
| Medium | 91.66 ± 11.49 | (74-113) | 44 (39.28%) |
| Large | 157.9 ± 31.58 | (118-265) | 19 (16.96%) |

* SD = standard deviation

**Table 1.** Measurements of the corpuscles observed in *Pseudodactylogyrus bini* individuals parasitized by *Myxidium giardi* (measurements in μm).

## Discussion

Hyperparasitism may involve parasite infection of either internal or external parasites. In a review by Dollfus (1946) numerous cases of hyperparasitism were reported, including the presence of the flagellate *Hexamita* sp. in uterus, eggs and other tissues of *Deropristis inflata* Moulin, 1859, a digenean intestinal parasite of *Anguilla chrysypa* Rafinesque.

Sey and Moravec (1986) described a case of hyperparasitism by the third larval stage of the nematode *Sprironoura babei* Ha Ky, 1971 in the caecum of the trematode *Amurotrema dombrowskajae* Akhmerov, 1959, an intestinal parasite of the cyprinid *Spinibarbichthys denticulatus* Oshima, 1926. These authors consider that hyperparasitism arises principally as a result of competition for space at high infection intensities. For example, Moravec (1979, cited in Sey & Moravec, 1986) observed massive mixed intestinal infections of the pike *Esox lucius* by the acanthocephalan *Acanthocephalus lucii* and the cestode *Triaenophorus nodulosus*; in these cases some *A. lucii* individuals were seen attached to *T. nodulosus* individuals, inserting their proboscis in the cestode's strobilus and causing severe damage. Similarly, Sey and Moravec report a case in which the nematode *Camallanus lacustris* was found attached to the strobilus of *Bothriocephalus claviceps* in the digestive tract of *A. anguilla*.

Several cases of hyperparasitism of monogeneans have been reported. Cable and Tinsley (1992) observed the presence of a microsporidian in *Pseudodiplorchis americanus*. Colorni (1994), in a study of the parasite fauna of the gilthead bream *Sparus aurata*, described the presence of the dinoflagellate *Amyloodinium ocellatum* in *Neobenedenia melleni*, finding trophonts closely adhered to the dorsal face of the opisthaptor. The most recent report of hyperparasitism of which we are aware is that of Mennie et al. (2000), who describe colonization of the haptor and body surface of *Gyrodactylus derjavini* by fungal hyphae.

In his 1946 review, Dollfus reported the observation by Dujardin (1845; see Dollfus, 1946) of corpuscles within free-living nematodes; these corpuscles showed a homogeneous appearance, like fat-storage inclusions and similar bodies, and were referred to by Dollfus as "crystaloids". Froelich (1789; see Dollfus, 1946) had erroneously identified those structures as host eggs. In the present study, there was no possibility of confusion with the eggs of the monogenean, which have very different morphometry (Figure 1F), and we initially thought that these corpuscles were cysts of the hyperparasitic myxosporidian. However, microscopic examination of the content of the corpuscles ruled out this possibility, and suggested a non-parasitic origin.

Given the frequency with which *M. giardi* and *P. bini* co-occur in branchial tissue of *A. anguilla*, it is interesting that we did not observe either cysts or free spores of *M. giardi* in the tissues of the eel in which hyperparasitism was observed: *M. giardi* cysts and spores were observed only within *P. bini*.

In conclusion, the present study found hyperparasitism of *P. bini* by *M. giardi* in a single eel of 323 eels examined. Hyperparasitism of *P. anguilla* was not observed.

In the eel showing hyperparasitism, *M. giardi* cysts or spores were not observed free in the eel branchial tissues. Corpuscles were clearly associated with *M. giardi*, since they were observed only in the eel showing hyperparasitism, and only in *P. bini*, not *P. anguilla*. This is the first report of hyperparasitism of *P. bini* by *M. giardi*.

**Acknowledgements**

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