

GILL INFECTION OF *Leporinus macrocephalus* GARAVELLO & BRITSKI, 1988 (OSTEICHTHYES: ANOSTOMIDAE) BY *Henneguya leporinicola* n. sp. (MYXOZOA: MYXOBOLIDAE). DESCRIPTION, HISTOPATHOLOGY AND TREATMENT

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Received May 15, 1998 – Accepted October 21, 1998 – Distributed September 10, 1999

(With 4 figures)

ABSTRACT

Piaçus (*Leporinus macrocephalus*), were raised in 300 m² ponds (density of 10 fish/m²) presenting asphyxia signals and daily mortality of 27 fishes. Specimens with 8-cm total body length, were collected for necropsy. Mucus of body surface and pieces of organs were collected and examined microscopically, in wet mounts, stained or in histological sections. The smears examination showed the presence of several spores in the secondary lamellae of the gill filaments, identified as *Henneguya leporinicola* n.sp (Myxozoa: Myxobolidae). Histopatological study showed epithelial hyperplasia and fulfilling of the spaces between the secondary lamellae, congestion and teleangiectasia sinusoidal. It was also observed hyperplasia of the goblet cells and several cysts of parasite with 70.3 µm diameter. Such cysts were situated among the secondary lamellae, covered or not by the hyperplastic epithelium. With this diagnostic, three applications of formalin solution 10 ml/m³ were carried out. Fifteen days after that, fish were examined again to ascertain whether the treatment was efficient on disease caused by the protozoa. The tissue alterations present in the gills after the treatment were just a moderate sinusoidal congestion and a slight epithelial hyperplasia on the base of the secondary lamellae.

Key words: Myxozoa, *Henneguya leporinicola*, *Leporinus macrocephalus*, histopathology, description.

RESUMO

Infecção branquial de *Leporinus macrocephalus* Garavello & Britski, 1988 (Osteichthyes: Anostomidae) por *Henneguya leporinicola* n. sp. (Myxozoa: Myxobolidae). Descrição, histopatologia e tratamento

Piaçus (*Leporinus macrocephalus*) criados em viveiro de 300 m² (densidade de 10 peixes/m²) apresentavam sinais de asfixia e mortalidade diária de 27 peixes. Exemplares com 8 cm em média foram examinados quanto à presença de lesões e seus possíveis agentes. Muco da superfície corporal e fragmentos de órgãos foram colhidos e examinados à microscopia de luz, em esfregaços a fresco ou corados ou em cortes histológicos. O exame dos esfregaços mostrou a presença de inúmeros esporos entre as lamelas secundárias dos filamentos branquiais, identificados como *Henneguya leporinicola* n.sp (Myxozoa: Myxobolidae). À histopatologia verificou-se marcada hiperplasia epitelial com preenchimento dos espaços entre as lamelas secundárias, congestão e teleangiectasia sinusoidal. Observou-se ainda hiperplasia de células calciformes e inúmeros cistos do parasito, com até 70,3 µm de diâmetro, localizados entre as lamelas secundárias, recobertos ou não pelo epitélio hiperplásico. Estabelecido o diagnóstico foram realizadas três aplicações de formalina na dose de 10 ml/m³ de água. Após 15 dias da última dose do tratamento, os peixes foram novamente examinados, constatando-se a eficácia das medidas adotadas para interromper a mortalidade, a enfermidade e combater o parasitismo. As

únicas alterações teciduais presentes nas brânquias nesse último exame foram moderada congestão sinusoidal e discreta hiperplasia epitelial na base das lamelas secundárias.

Palavras-chave: Myxozoa, *Henneguya leporinicola*, *Leporinus macrocephalus*, histopatologia, descrição.

INTRODUCTION

Aquaculture in Brazil has become the main activity on the farms, instead of a secondary activity or a sort of hobby as it used to be some years ago. These transformations, which evidence the intensification of the culture systems, require from the fishfarmers the knowledge on the advanced techniques to improve the health conditions of the fish. The parasites take advantage of the stressful condition occurred on high stocking densities and the importance of their control increases.

The myxozoan is normally present on fishes, in nature or on captivity, without cause any problem if there is equilibrium between host and environment. When any kind of stress, handling, poor water quality or crowd, is present to the fish, the parasites take place and several kinds of diseases show up (Lom & Noble, 1984). Normally fishfarmers do not pay attention to the myxozoan, but they are present when there are mortality related to the presence of fungus and bacteria. This situation indicates that the injuries made by the myxozoan serve as an opening for other parasites to infect the animal. Ceccarelli *et al.* (1990) warn to the intense spread out of that parasite in the State of São Paulo. In Brazil, Pinto (1928 a,b) described *H. linearis* in the gills of *Rhandia sebae* and *Pseudoplatystoma fasciatum*, *H. occulta* in Callichthyidae fish, *H. wenyoni* in *Astyanax fasciatus* and *H. iheringi* in *Serrasalmus spilopleura*. Guimarães & Bergamim (1934) observed *H. santae* in *Tetragonopterus santae*. Azevedo & Matos (1989) have studied *Henneguya* infection in the gills of *Hoplosternum litorale* while Rocha *et al.* (1992) observed *H. amazonica* in *Crenicichla lepidota*. *H. adherens* was present in *Acestrorhynchus falcatus* (Azevedo & Matos, 1995) and *H. malabarica* in *Hoplias malabaricus* (Azevedo & Matos, 1996). Gioia & Cordeiro (1996) assembled Brazilian myxosporidians in a checklist. Martins & Souza (1997) related *H. piaractus* in the gills of *P. mesopotamicus*. Martins *et al.* (1997) have reported infections of *Henneguya* sp. in the gills of *Piaractus mesopotamicus* that caused

severe mortality. The histological studies showed petechiae, excessive mucus production, severe inflammatory foci and hyperplasia in the gill epithelium.

The objective of this study was to evaluate the pathogenic pattern of a new parasite on piauçu, *Leporinus macrocephalus* and to compare its morphological features with the previous descriptive studies done on other fish species in Brazil.

MATERIAL AND METHODS

In a fishfarm was happening a mean mortality of 27 fish/day. Piauçu were placed in a 300 m² pond, along with pacu *Piaractus mesopotamicus* Holmberg, 1887, totaling 3,000 fishes. Nevertheless, death was observed only among the piauçu, that remained congregated near the inflowing water. Ten piauçu *L. macrocephalus* with 8 cm length originated from a commercial fishfarm located in Capivari, SP, Brazil were examined. The organs were placed on Petri plates with 0.65% saline solution for microscopic observation. The organs fragments were compressed between a slide and a microslide for smear preparation. The smear was air-dried at room temperature, fixed by immersion in undiluted methylic alcohol and stained by 1:9 Giemsa solution for 10 minutes (Meyers *et al.*, 1977, slightly modified). The parasite identification was done according to Lom & Arthur (1989).

For the visualization of the iodophilic vacuole, the spores were fixed in a 10% buffered formalin solution and stained by Lugol's solution. Seventy-eight myxozoan spores were measured (μm) and drawn with a camera lucida and a light microscope. After fixed, the material was embedded in paraffin, slashed to 6 μm thickness and stained by hematoxylin-eosin for microscopic observation.

In the fishfarm, fishes were treated with 10 ml of formalin per m³. The treatment was done for three times, always at 9:00 am. The first two times was done in consecutive days and, the third one, 48 hours after the second one. Fifteen days after the end of the treatment, ten piauçu were

examined and the gill fragments were fixed for the histological and parasitological examination.

RESULTS

Macroscopic alterations or cysts were not observed on the skin of the fish, but the gills showed swelling and paleness. The microscopic gill examination showed some *Piscinoodinium pillulare* (Dinoflagellida) and *Ichthyobodo necator* (Protozoa: Kinetoplastida) among several round myxozoan cysts that measured 53.1 (23.4 to 70.3) of diameter (Fig. 1).

When the cysts were blown, several elongated spindle-shape spores appeared, provided from the caudal processes (Fig. 2). There were two polar capsules on the anterior extremity, each one with one polar filament. The sporoplasm and a iodophilic vacuole were found in the interior of the spore stained by Lugol. The spore characteristics were: body spore length 7.6 (5.5-8.7); body spore width 4.2 (3.6-4.9); polar capsule length 3.0 (2.0-3.6); polar capsule width 1.6 (1.2-2.0); distance of anterior extremity of the spore to the polar capsule 0.8 (0.0-1.2); caudal length 21.8 (12.9-32.2).

With those morphometric data, the identification of the parasite as *Henneguya* Thélohan, 1892 (Myxozoa: Myxobolidae) was allowed. The comparison of those characteristics with those of species already described on fish gills, in Brazil, as showed on Table 1. The authors suggests the name *Henneguya leporinicola* n.sp. proposed from the name of the host.

Type host: *Leporinus macrocephalus*

Garavazzo & Britski, 1988.

Site of infection: gill filaments.

Locality: Capivari, SP, Brazil.

Histopathological study revealed the presence of hemorrhages and severe inflammatory foci in the gill epithelium, where the cysts were located. Occasionally, injuries was also noted on the primary and, more frequently, on the secondary lamellae. Moreover, it was observed that the cysts were involved by two layers of elongated cells, fibroblast-like-cells, and by intense inflammatory mononuclear infiltrate. The cysts pushed the lamellae, diminishing the respiratory efficiency. Hyperplasia and displacement of the respiratory epithelium were also observed (Fig. 3).

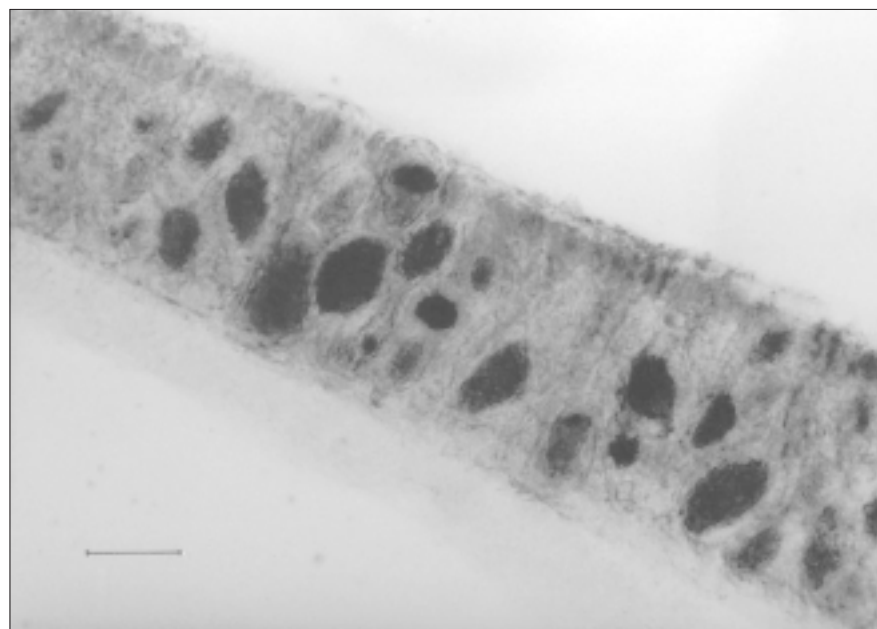


Fig. 1 — Wet mount of the gill filaments of *Leporinus macrocephalus* showing cysts of *Henneguya leporinicola*. Scale bar = 100 μ m.

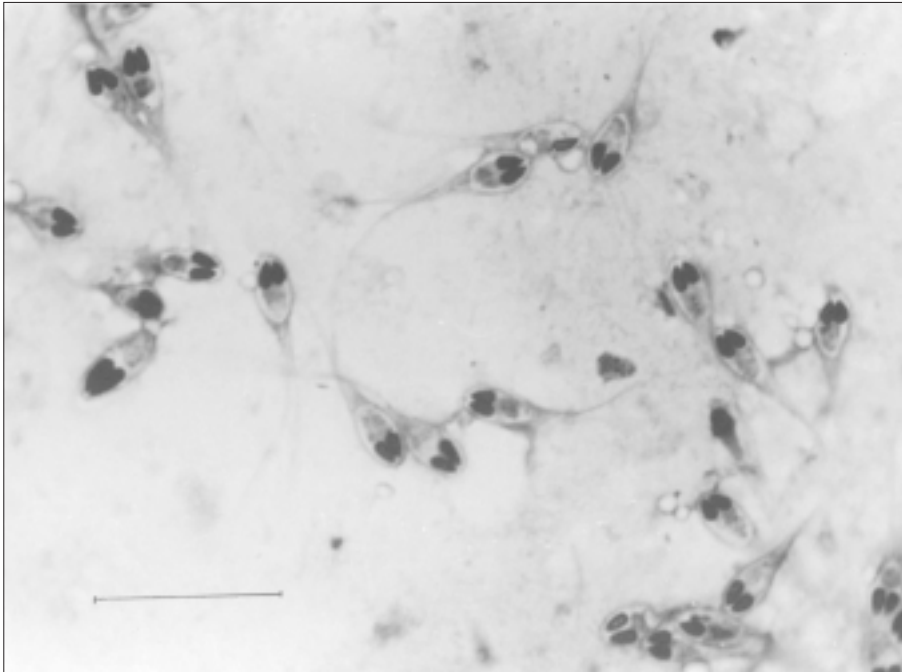


Fig. 2 — Spores of *Henneguya leporinicola* n.sp. from the gills of *Leporinus macrocephalus*. Scale bar = 20 μ m.

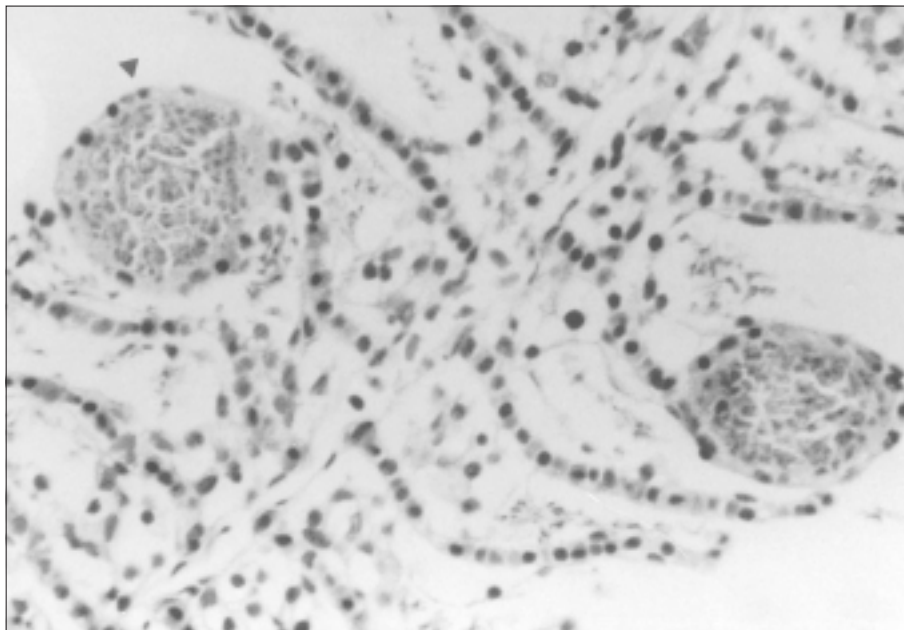


Fig. 3 — Hyperplasia and displacement of the respiratory epithelium of *Leporinus macrocephalus* infected with cysts of *Henneguya leporinicola* (arrow). H-E, x 400.

TABLE 1
Measures of several species of *Henneguya* described in fish, in Brazil. (Anterior Extrem. means distance from anterior extremity to polar capsule.)

Species	Spore Length	Spore Width	Anterior Extrem.	Polar Capsule Length	Polar Capsule Width	Presence Iodophil Vacuole	Caudal Length	Polar Filament Length	Host	Locality	Author
<i>H. adherens</i>	12.4	5.8	–	3.1	1.2	–	20.5	–	<i>Acestrotrichynchus falcatus</i>	Belém (PA)	Azevedo & Matos (1995)
<i>H. amazonica</i>	13.9	5.7	–	3.3	1.5	no	45.4	–	<i>Crenicichla lepidota</i>	Amazonas River (AM)	Rocha <i>et al.</i> (1992)
<i>H. cesarpinioi</i>	13.5	4.2	–	2.6	0.8	no	–	–	<i>Asyanax fasciatus</i> (Iambari)	Água Funda (SP)	Guimarães (1931)
<i>H. iheringi</i>	22.0	6.0	–	3.4	2.0	yes	–	–	<i>Serrasalmo spilopleura</i>	Turvo River, Pirangi (SP)	Pinto (1928 a)
<i>H. malabarica</i>	12.6	4.8 x 3.6	–	3.7	1.8	yes	17.1	–	<i>Hoplias malabaricus</i>	Amazonas River (AM)	Azevedo & Matos (1996)
<i>H. occulta</i>	18.0	9.0	–	8.0	–	–	20.0	17.0	<i>Loricaria</i> sp. (tambaotá)	Rio de Janeiro	Pinto (1928 b)
<i>H. piaractus</i>	12.7	3.6	1.6	6.7	1.2	yes	41.2	–	<i>Piaractus mesopotamicus</i> (pacu)	Jaboticabal (SP)	Martins & Souza (1997)
<i>H. pisciforme</i>	20.4	6.1	5.6	4.3	1.7	yes	10.6	–	<i>Hypessobrycon anistisi</i> (Iambari)	Campinas (SP)	Cordeiro <i>et al.</i> (1983/84)
<i>H. psorospermica</i>	–	–	–	–	–	–	–	–	Carp. Iambari and tilapia	Irati (PR)	Schönhofen <i>et al.</i> (1983)
<i>H. santae</i>	9.6	5.3	–	3.0	–	yes	11.8	3.0	<i>Tetragonopterus santae</i> (Iambari)	Pinheiros River (SP)	Guimarães & Bergamin (1934)
<i>H. striolata</i>	15.8	5.3	–	6.8	1.2	no	25.9	–	<i>Serrasalmus striolatus</i>	–	Casal <i>et al.</i> (1997)
<i>H. travassosi</i>	10.6	4.3	–	3.6	–	no	16.7	–	<i>Asyanax fasciatus</i> and <i>Leporinus copei</i> (Iambari)	São Paulo	Guimarães & Bergamin (1933)
<i>H. wenyoni</i>	10.2	5.2	–	3.7	1.5	yes	10.8	–	<i>Asyanax fasciatus</i> (Iambari)	São Paulo	Pinto (1928b)
<i>Henneguya</i> sp.	13.5	5.3	–	–	–	–	45.2	–	<i>Hoplosternum littorale</i>	Rio Amazonas (AM)	Azevedo & Matos (1989)
<i>Henneguya</i> sp.	–	–	–	–	–	–	–	–	<i>Mugil liza</i> and <i>M. curena</i>	São Paulo	Godinho <i>et al.</i> (1988)
<i>Henneguya</i> sp.	14.2	5.0	–	5.8	1.8	–	35.7	–	<i>Pimelodus maculatus</i>	São Paulo	Cordeiro <i>et al.</i> (1989)
<i>H. leporinicola</i>	7.6	4.2	0.8	3.0	1.6	yes	21.8	–	<i>Leporinus macrocephalus</i> (piaçu)	Jaboticabal (SP)	Present work

Based on diagnostic, three applications of 10 ml of formalin/m³ were effective in this case. Fifteen days after that, fish were examined again and parasite cysts were not present. Tissue altera-

tions present in gills after treatment were just a moderate sinusoidal congestion and a slight epithelial hyperplasia on the base of the secondary lamellae (Fig. 4).

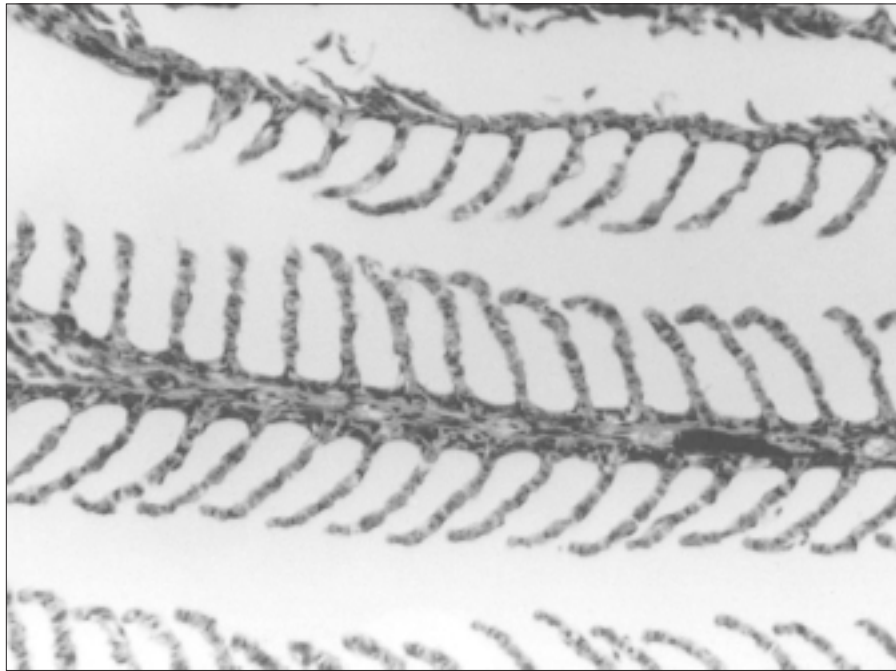


Fig. 4 — Fifteen days after formalin treatment showing a moderate sinusoidal congestion and a slight epithelial hyperplasia on the base of the secondary lamellae of *Leporinus macrocephalus*. H-E, x 200.

DISCUSSION

The occurrence of mortality from April to July, when the water temperature drops from 27-32°C to 17-24°C, has been a common fact observed in Northeast of São Paulo State, where the fishfarm is located.

The results of the parasitological examination of fishes suggest that the more important parasite found in this case was a non described species. Comparing measurements with the other described species, body length is shorter than the others (Table 1) but similar to *H. santae* (Guimarães & Bergamim, 1934). Spore width resemble the description of *H. cesarpinto* (Guimarães, 1931). The distance of anterior extremity to polar capsule was significantly different to *H. piaractus* (Martins & Souza, 1997) and *H. pisciforme* (Cordeiro *et al.*, 1983/84). Despite of similarity with *H. santae*, the

present description was different in body spore length, caudal process length and fish host. Nevertheless, the comparison of present parasite allowed us to suggest a new species for the gills of *L. macrocephalus*, a fish of economic importance in Brazilian fishfarm. By these characteristics the authors suggests the name *Henneguya leporinicola* n. sp. proposed from the name of the host.

In this case, the mass mortality was attributed to the pathogenic action of the *Henneguya* cysts in gill tissue. The inter and intralamellar presence of cysts, associated to hyperplasia and inflammation increased the adherence between secondary lamellae. This fact and the hyperplasia of the goblet cells associated with an increase in the mucus production caused respiratory distress syndrome and suffocation of fishes (Dyková & Lom, 1978; Current & Janovy, 1978; Shariff, 1982; Bowser & Conroy, 1985; Kalavati & Narasimhamurti,

1985; Martins *et al.*, 1997). The lesions were similar to those observed in *Henneguya* infections in perch, *Perca fluviatilis* (Dyková & Lom, 1978) and in *P. mesopotamicus* (Alexandrino *et al.*, 1995; Ferraz de Lima *et al.*, 1995).

The aggregate behavior of fishes near the inflowing water, caused by the infection of *Henneguya* was also observed by Martins & Souza (1997) and Martins *et al.* (1997) working with *P. mesopotamicus*. That behavior is also in agreement with Post (1987) and Eiras (1994) for myxosporidiosis outbreaks.

The efficacy of formalin treatment showed that in some cases may be an alternative against myxosporidians parasites of fishes. However, pond characteristics, water quality and number of parasites, must be observed. Fumagillin and malachite green were among efficient therapy against sphaerosporosis of the common carp and proliferative kidney disease (Molnár *et al.*, 1987, and Alderman & Clifton-Hadley, 1988). Székely *et al.* (1988) related efficacy of fumagillin against *Myxidium giardi* in European eel, *Anguilla anguilla*.

Severe damages caused in developmental stages of *Henneguya* sp. when tapir fish, *Gnathonemus petersi* was treated with triazinone, was observed (Schmahl *et al.*, 1991). Nevertheless, the authors commented that the mature spores were not affected, then the treatment has to be repeated within several months.

Under the conditions described in this report the formalin treatment may be an alternative for the control of myxosporidiosis like fumagillin and malachite green.

Acknowledgments — We would like to thank to Drs. Waldener Garutti and Francisco Langeani Neto (Departamento de Zoologia, UNESP, São José do Rio Preto) for specific identification of fish and to Dr. Ubirajara Ribeiro Martins (Museu de Zoologia, Universidade de São Paulo, SP) for suggestions about the name of parasite species.

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