



UNIVERSITI PUTRA MALAYSIA

**STUDIES ON THE MORPHOLOGY OF *THELOHANELLUS SP.*, AND
THE EFFECTS OF WATER QUALITY AND RAINFALL ON ITS
PREVALENCE IN *PUNTIUS GONIONOTUS* (BLEEKER)**

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FPSS 1994 3



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by

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**Thesis submitted in partial fulfilment of the requirements for the Degree of
Master of Science in the Faculty of Fisheries and Marine Science,
Universiti Pertanian Malaysia.**

1994



ACKNOWLEDGEMENTS

I wish to express my deep sense of gratitude and appreciation to my esteemed chairman, Dr. Faizah Shaharom for her guidance, constructive criticism and constant support. My deepest gratitude is also extended to supervisory committee members, Professor Sheikh Omar Abdul Rahman and Dr. Hassan Mohd. Daud for their guidance and advice.

I also would like to express my sincere thanks to Mr. Liew Hock Chark (UPM- K. Terengganu) in assisting me with the statistical analysis of the data herein presented. Mr. Rosli Aslim, for making the schematic figures of *Thelohanellus* spores. Appreciation is extended to those at Electron Microscope Unit in the Faculty of Veterinary Medicine and Animal Science, UPM: Dr. Fauziah Othman, Mrs. Aminah Jusoh and Mr. Ho Oi Kuan for helping me with SEM and TEM.

My warmest regard is also extended to my colleagues Ms Rokiah Abdul Latif, Sukerman Sarbini and Thomas Antonimuthu for their technical assistance,



cooperation, encouragement and friendship. Similarly, my gratitude is conveyed to the Director General of Fisheries, Department of Fisheries for his permission to pursue this study and the Head of FFRC, En. Hambal Hanafi for his keen interest and support.

I would like to acknowledge the supporting bodies i.e NACA and UNDP for partial funding of this research.

I also would like to take this opportunity to express my utmost appreciations and thanks to my dear friends who have rendered assistance in every way throughout the accomplishment of this thesis.

Finally, my deepest gratitude to my husband Abu Hassan Mohd Ali, my sister Umi Kalsum and my dearest daughters, Siti Nadiah and Siti Akmal Izzati for their unfailing support, patience, encouragement and understanding during the course of the study.



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LIST OF ABBREVIATIONS

FAO	=	Food and Agricultural Organization of the United Nations
FFRC	=	Freshwater Fish Research Centre
UPM	=	Universiti Pertanian Malaysia
SEM	=	Scanning Electron Microscopy
TFCRI	=	Tropical Fish Culture Research Institute

Abstract of the thesis submitted to the Senate of Universiti Pertanian Malaysia in partial fulfilment of the requirements for the degree of Master of Science

STUDIES ON MORPHOLOGY OF *THELOHANELLUS SP.*, AND EFFECTS OF WATER QUALITY AND RAINFALL ON ITS PREVALENCE IN *PUNTIUS GONIONOTUS* (BLEEKER).

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A myxosporean parasite belonging to the genus *Thelohanellus*, Kudo 1933 found infecting the gills of lampam jawa *Puntius gonionotus* (Bleeker), was studied. The morphological features of this myxosporean cysts and spores were examined through light and electron microscope to identify the species of *Thelohanellus* and the results were compared with other known *Thelohanellus* species. Morphologically, the spore size is 25.8 μm X 10.5 μm X 9.0 μm . These spores were tear-shaped or pyriform, having a distinct straight suture. Both membranous and mucous envelopes were distinctly conspicuous; polar capsule size ranged from 11 - 17 μm (length) X 6-8 μm (width). Within the polar capsule were 8 - 12 coils of polar filament.



Plasmodial size was 0.5 - 2.0 mm. Scanning electron micrographs of the cysts of this *Thelohanellus sp.* showed that the cysts were sausage-shaped and perched on the surface of the gill filaments. They were enclosed within the epidermal layer of the primary lamella.

Prevalence and intensity of the *Thelohanellus sp.* were examined in fry (2-4 wk), juveniles (5.1 - 10g) and adults (10.1-40g) in FFRC ponds including fry from two government breeding stations in Pahang and Kedah. This yet unreported species infected all stages, about 50% of the *P. gonionotus* examined, with mean intensity of two and five (cysts) in fry and juveniles respectively. All were found on the gills, hence showing tissue specificity.

Examination of wild fish caught from Sungai Melaka, was also conducted to determine the possible source of infection of all fish examined, 31.25% were infected by myxosporea with a prevalence ranging from 31.2 to 100%. However this study was not conclusive as to whether the *Thelohanellus sp.* found originated from Sungai Melaka.

The relationship between the prevalence of *Thelohanellus sp.* variations with water quality and rainfall was also studied. The prevalence showed no seasonal variations with respect to temperature and rainfall, and also independent of dissolved oxygen, pH, ammonia, conductivity and alkalinity to the infected pond water.



It is proposed that this *Thelohanellus* be named *Thelohanellus puntii* based on the comparative morphological difference of the spores. This is the first report on the genus *Thelohanellus* infecting *P. gonionotus* in Malaysia.



Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia, sebagai memenuhi sebahagian daripada keperluan untuk mendapat Ijazah Master Sains.

**KAJIAN MORFOLOGI *THELOHANELLUS SP.*, DAN KESAN MUTU
AIR DAN HUJAN TERHADAP PREVALENS DALAM
PUNTIUS GONIONOTUS (BLEEKER)**

Oleh

SITI ZAHRAH ABDULLAH

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Sejenis parasit myxosporea tergulung dalam genus *Thelohanellus*, Kudo 1933 yang menjangkiti insang lampam jawa *Puntius gonionotus* (Bleeker) telah dikaji. Ciri morfologi sista dan spora myxosporea ini telah diperiksa menggunakan mikroskop cahaya dan elektron untuk mengenalpasti spesies *Thelohanellus* serta membandingkan keputusan dengan spesies *Thelohanellus* lain yang telah diketahui. Secara morfologi saiz spora adalah $25.8 \mu\text{m} \times 10.5 \mu\text{m} \times 9.0 \mu\text{m}$. Spora ini mempunyai bentuk titisan air mata, piriforma, dengan sutur jelas yang lurus. Kedua-dua penutup membran dan mukus kapsul kutub mempunyai saiz julat $11 - 17 \mu\text{m}$ (panjang) dan $6 - 8 \mu\text{m}$ (lebar). Di dalam kapsul kutub terdapat $8 - 12$ lingkaran filamen kutub. Saiz plasmodium adalah $0.5 - 2.0 \text{ mm}$. Mikrograf skan elektron mikroskop sista *Thelohanellus sp.* ini menunjukkan bahawa



Thelohanellus berbentuk sosej dan tersembul di permukaan filamen insang yang tersimpan dalam lapisan epidermis lamela primer.

Prevalen dan keamatan *Thelohanellus sp.* telah diperiksa dalam fri (2-3 minggu), juvenil (5.1 - 10g) dan dewasa (10.1 - 40g) di kolam FFRC. Pemeriksaan juga dilakukan ke atas fri dari dua stesen kerajaan di Pahang dan Kedah. Spesies ini didapati menjangkiti ke semua peringkat, hampir 50% *P. gonionotus* yang diperiksa, dengan purata keamatan masing-masing dua dan lima (sista) dalam fri dan juvenil. Ke semua ditemui pada insang, yang mana menunjukkan pengkhususan tisu jangkitan.

Pemeriksaan ikan liar yang ditangkap di Sungai Melaka, juga dijalankan untuk menentukan kebarangkalian sumber jangkitan ke semua ikan yang diperiksa, 31.25% telah dijangkiti oleh myxosporea dengan prevalen berjulat antara 31.2 ke 100%. Kajian ini walau bagaimanapun tidak lengkap untuk menentukan sama ada *Thelohanellus sp.* yang dijumpai adalah berasal dari Sungai Melaka.

Perhubungan di antara variasi prevalen *Thelohanellus sp.* dengan mutu air dan hujan juga dikaji. Prevalen menunjukkan tiada perubahan musim terhadap suhu dan hujan, dan juga bebas daripada oksigen terlarut, pH, amonia, konduktiviti dan alkaliniti pada air kolam yang dijangkiti.

Memandangkan spora secara perbandingan morfologi adalah berbeza, oleh itu disarankan *Thelohanellus* ini dinamakan *Thelohanellus puntii*. Ini merupakan laporan pertama genus *Thelohanellus* yang menjangkiti *P. gonionotus* di Malaysia.



CHAPTER I

INTRODUCTION

Puntius gonionotus (Bleeker) or lampam jawa, previously known as *Puntius javanicus* is one of the most important freshwater carp species cultured in earthen ponds by Malaysian fish farmers. Since its introduction into Peninsular Malaysia, the number of fry produced and distributed by the main fish breeding centres in Malaysia has increased from 3.4 million in 1963 to 8.1 million in 1991. However, there was a dramatic decrease in production between 1974 and 1978 (Fig. 1) (Annual Statistics 1963-1991).

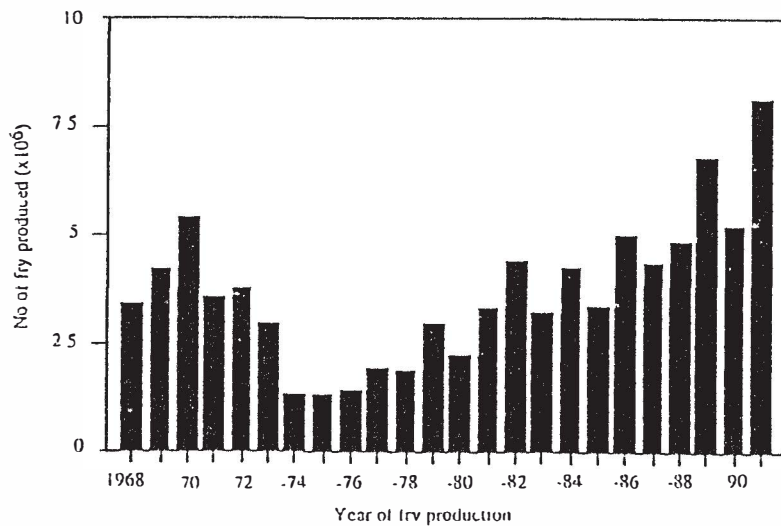


Fig. 1: Histogram showing the annual production of *Puntius gonionotus* fry by government stations (Malaysia)

Puntius gonionotus was introduced into Peninsular Malaysia in 1953 from Indonesia by the Fisheries Department (Soong, 1963). Out of the total number of *P. gonionotus* imported, ten fish survived. The ten fish that survived, spawned and between July 1958 and May 1959, 100 and 2000 *P. gonionotus* fry respectively were donated to the Tropical Fish Culture Research Institute (TFCRI), now known as Freshwater Fish Research Centre (FFRC) at Batu Berendam, Melaka (Tropical Fish Culture Research Institute Report, 1957-1959). Soong (1963) also mentioned a similar fry distribution to other fishery stations in East Malaysia, Singapore and public stocking of fish in Bukit Merah Reservoir and Cenderoh lake in Perak. *Puntius gonionotus* fry were also reported sold to fish culturists in southern Thailand by local farmers in 1962 (Soong, 1963). By 1985, fry production of *P. gonionotus* in Freshwater Fish Research Centre (formerly TFCRI) was easy and the increasing demand of its fry led to the yearly target production of one million fry for local distribution . In 1991 Malaysia produced 1790 metric tonnes of *P. gonionotus* (FAO Fisheries Statistical year book 1992) (Table 1).

Since then, a number of studies on *P. gonionotus* culture, nutritional requirement and viability have been carried out in Tropical Fish Culture Research Institute,(TFCRI Annual Report 1959-1962). In contrast, there were very few reports, during the early years of culture, on *P. gonionotus* disease-related problems. In 1963, Soong reported that attacks by *Lernea sp* and *Argulus sp* were common in *P. gonionotus*, protozoan infections namely *Trichodina sp.*, *Chilodonella sp* and *Ichthyobodo sp.* were also reported to have occurred in ponds

from 1981-83 (MARDI Annual Report, Unpublished). Shariff (1984), Shariff and Sommerville (1986) and Shariff and Vijiarungam (1986) also reported parasitic infections and treatment thereof on various species of carps including *P. gonionotus*. In Indonesia, its country of origin *P. gonionotus* is known to be susceptible to many parasites including myxosporea (Kabata, 1985). Even though similar infection problems have been observed in Malaysia, *P. gonionotus* has remained one of the favoured cultured carps in the country.

Table 1: Production of *Puntius gonionotus* in Malaysia

Year	Production (metric tonnes)
1986	969
1987	747
1988	1930
1989	1754
1990	1790
1991	1790

(Source: FAO Fisheries Statistical year book 1992)

In the course of a routine examination of *P. gonionotus* fry heavily infected with *Piscinoodinium pillulare*, at the FFRC, Batu Berendam, Melaka, myxosporean spores were observed in the fresh smears of the *P. gonionotus* gills. The observed spores belonged to the genus *Thelohanellus* Kudo 1933. The observed spores were found not to resemble any other *Thelohanellus* spores

previously described in the literature. Observation of this myxosporea, *Thelohanellus sp.* in *P. gonionotus* has been the basis of this present investigation.

The objectives of this work are therefore:-

1. To describe the morphological characteristics and identify the myxosporea *Thelohanellus sp.* detected in *P. gonionotus* in Malaysia.
2. To determine the source of *Thelohanellus sp.* infection on *P.gonionotus* cultured in ponds of the FFRC Batu Berendam Melaka.
3. To determine the prevalence and intensity of *Thelohanellus sp.* in *P. gonionotus* in other government breeding stations.
4. To determine relationship between the prevalence of *Thelohanellus sp.* in *P. gonionotus* in the infected pond, and variations in the water quality and rainfall.

CHAPTER II

LITERATURE REVIEW

In the late 1986 and throughout 1987, epizootics of *Piscinoodinium pillulare* infection in *Puntius gonionotus* were observed throughout Peninsular Malaysia, including fish breeding stations in Kong Kong (Johor) which recorded 100% mortality (Shaharom-Harrison *et. al*, 1990). Similar mortalities of *P. gonionotus* infected by *P. pillulare* in Freshwater Fish Research Centre ponds were reported by Siti and Rokiah (1988a). During this period, *P. gonionotus* fry were closely examined and as a control measure were treated with CuSO_4 (Siti and Rokiah 1988b) before they were supplied to farmers. Gill smears of the fry were found to be heavily infected with *P. pillulare* together with spores and cysts of *Thelohanellus sp.* attached to the gill filaments (Plate 1).

The smears contained spores having a single polar capsule. These spores were identified as those of myxosporea *Thelohanellus sp.* and were observed for the first time on *P. gonionotus* gill preparations. The mortality rate of infected *P. gonionotus* at this time, increased at a rate of five to ten and reached a maximum of almost 70%. The observed high mortality

rate of *P. gonionotus* could possibly be due to a combination of stress factors as a result of harvesting and gill infections caused by *P. pillulare* and the myxosporea, *Thelohanellus sp.*.

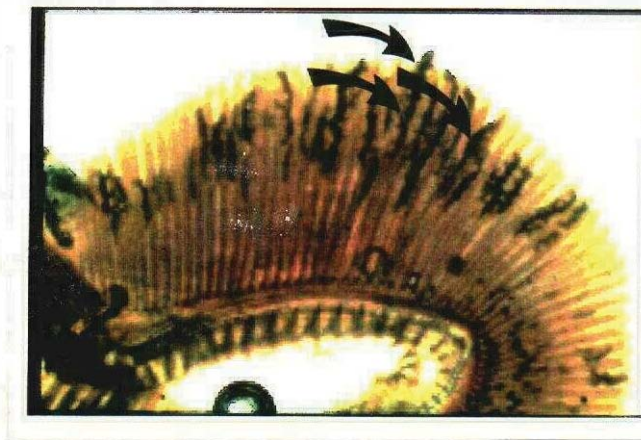


Plate 1 : Gill filaments heavily infected with the *Thelohanellus sp.* cysts (X100)

Lom (1987) reported that myxosporea, had been known since the early 19th century. To date, approximately 1100 myxosporea species were described from fish (Lom & Arthur, 1989) that belong to 46 genera. These myxosporea constitute a class of Protistan phylum Myxozoa. They are mostly fish parasites (Shul'man 1990). Histo-zoic species live in the tissues, either in intercellular spaces, in blood vessels or within cells, while coelozoic species infect cavities mainly within the gall and swimbladder (Lom, 1987). A lot of information is available on myxosporea infection in temperate countries and USSR (Shul'man, 1984).

Dykova & Lom, (1987) described *Thelohanellus pyriformis* in tench (*Tinca-tinca*) and other myxosporea species namely *Sphaerospora sp.* (Lom *et al.*, 1983a, Arthur and Lom, 1985, Lom *et al.*, 1985; Dykova and Lom, 1982), *Myxobolus sp.* (Lom and Molnar, 1983; Lom *et al.*, 1989a; Molnar *et al.*, 1986), *Hoferellus sp.* (Lom *et al.*, 1986) and *Myxidium sp.* (Dykova *et al.*, 1987; Lom and Dykova, 1989, Lom *et al.*, 1989b). *Kudoa sp* was reported by Lom *et al.*, (1983b), but was found in marine flatfish of genus *Arnoglossus*. Dykova and Lom, (1988) presented a review of pathogenic myxosporeans in intensive culture of carp *Cyprinus carpio* in Europe. These authors combined the results of their own investigations related to the distribution, life cycle, morphology, taxonomy and pathogenicity with other existing data on seven myxosporean species including *Thelohanellus sp.* which had a real pathogenic potential for the intensive carp culture in Europe. The same authors in their review indicated that the damage caused by the seven species they reported on, could range, from almost no damage to serious growth impairment or mortalities, depending on the intensity of the parasite infection, on fish condition and environmental factors. Myxosporea are also prevalent in cultured fish in China, where the number of species already described amount to 500 species (Hsieh Shing-Ren, personal communication). Some of the reported species are *Zschokkella sp.*, *Myxidium sp.*, and three new genera of *Spirosuturia*, *Triangula* and *Laterocaudata* (Chen, 1984). Studies on sporozoa of *Ophiocephalus sp.* in China was reported by Chen and Hsieh, (1960). A brief description of some *Thelohanellus sp.* important in freshwater fish was also reported by Chen and Hsieh, (1960) and Hsieh and Chen, (1989).

In contrast, information on myxosporea in the South East Asian region is limited. A recent report by Lumanlan *et al.*, (1992) noted that *Thelohanellus sp.* were recovered from only a number of host species imported into the Philippines. Tonguthai and Chinabut (1987) mentioned the occurrence of *Henneguya sp* on the gills, *Myxidium sp* in the gall bladder and *Myxosoma sp* in the gonad of *Clarias batrachus*. Dana (1988) reported 10 different species of myxosporean from cultured fishes in West Java. Dana and Maskur (1986) found that *Thelohanellus callisporis* could infect common carp as early as two days after hatching whereas *Myxobolus koi*, *Myxobolus toyamai* and *Myxosoma sp.* infect older fry around 6-8 days after hatching. Dana (1990) found that *Myxobolus koi* and *Thelohanellus callisporis* had significantly higher prevalence at higher temperatures whereas *Myxobolus artus* had not differed significantly at the different temperatures tested. Studies on myxosporea are still going on in many countries. To minimise ambiguous classification and taxonomy and to better understand myxosporea, Lom and Nobel (1984) reviewed the classification of two myxosporean genera. Following this, Lom (1987) reviewed myxosporea as parasites of fish based on taxonomical and their pathological effects on the host. Such information is essential for identification of new emerging myxosporean species.

A number of *Thelohanellus sp.* have been described mainly in temperate countries. Shul'man (1984) reported nine previously described species of *Thelohanellus* namely *Thelohanellus catlae*, *Thelohanellus dogieli*, *Thelohanellus oculileucisci*, *Thelohanellus nemachili*, *Thelohanellus misgurni*, *Thelohanellus carassii*, *Thelohanellus pyriformis*, *Thelohanellus otebike* and *Thelohanellus*