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Short Communications

HISTOPATHOLOGICAL EVALUATIONS OF PARASITIC INFECTIONS IN SPOTTED GREEN PUFFERFISH, *Dichotomyctere nigroviridis* (Marion de Procé, 1822) IN BAGAN LALANG, SELANGOR

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SUMMARY

The spotted green pufferfish (*Dichotomyctere nigroviridis*), commonly found in Malaysia's fresh and brackish waters, may carry parasites due to its diet of crustaceans and mollusks, posing potential public health risks. This study investigated ectoparasite and endoparasite infections and their histopathological effects in juveniles and adults sampled in Bagan Lalang, Selangor. Sixteen pufferfish were collected, with skin, fin, and gill samples examined for ectoparasites, which *Dactylogyrus* spp., was primarily found in the gills. Cellular degeneration was prevalent in adult gills. For endoparasites, myxozoans were detected in the kidneys of all fish, with no parasites in other organs. Histopathology revealed significant structural changes ($p<0.05$), including melanomacrophage deposition in the kidneys and spleen and mild congestion in various organs. These findings enhance our understanding of *D. nigroviridis* host-parasite interactions and highlight potential ecological and public health impacts of parasite imfections in wild fish populations.

Keywords: Dichotomyctere nigroviridis; spotted green pufferfish; Bagan Lalang; prevalence; parasite

INTRODUCTION

The spotted green pufferfish (*Dichotomyctere nigroviridis*), a member of the family Tetraodontidae, is widely distributed throughout Southeast Asia, including Malaysia. It inhabits various environments from freshwater streams, rivers, and floodplains to brackish water areas such as estuaries, mangroves, and coastal regions (Zhu et al., 2021; Froese and Pauly, 2022). This fish is notable for its unique appearance, characterized by a greenish-yellow upper body with black spots and a white abdomen. It can inflate its body when threatened and has been harvested for food and medicine. It is commonly used in the ornamental fish trade (Froese & Pauly, 2023). Increased interest in aquaculture and the aquarium trade raises awareness of parasites that may affect fish health (Blaylock & Bullard, 2014), and *D. nigroviridis* could serve as a sentinel species to understand these impacts. The study of parasite occurrence in *D. nigroviridis* is essential for monitoring environmental stressors and potential public health risks, as this species may harbour both ectoparasites and endoparasites. These parasites may cause histopathological alterations in fish tissues, affecting organs such as the kidneys and intestines (Feist & Longshaw, 2008).

This study aims to investigate the presence of both ectoparasites and endoparasites in *D. nigroviridis*, alongside associated histopathological changes, with implications for fish health and public safety. The findings may provide valuable insights into the effects of parasite infections on wild fish populations in the context of aquaculture and the aquarium trade. They could expand as a disease monitoring tool in surrounding aquaculture farms.

MATERIALS AND METHODS

Sixteen *Dichotomyctere nigroviridis* were collected from estuary sites in Pantai Bagan Lalang, Selangor, at two sampling times. The fish were obtained from local fishermen and were kept alive in an aerated container until dissection, conducted under IACUC approval (UPM/IACUC/AUP-U008/2023). Skin and fin scraping were performed to collect mucus for microscopic examination. Gills were collected for immediate microscopic observation, while the remainder was fixed in Davidson's solution for histological analysis. The fish were immobilised via decapitation and pithing of the central nervous system before further analysis. Gross examination was performed for signs of endoparasites and lesions. The gastrointestinal tract (GIT), liver, spleen, gonads, heart, kidney, and brain were removed for detailed gross and microscopic examinations. Parasite prevalence and intensity were calculated using formulas adapted from Muchlisin et al. (2014) and Shafiq et al. (2023). Organs were also fixed in 10% buffered formalin for histopathology or preserved in 97% ethanol for molecular studies. Squash smears were prepared for the kidney, brain, and muscle to detect parasite cysts and examined under a microscope. Microscopic examination of endoparasites was performed using a light microscope and identified through a pictorial

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guide (Klimpel et al., 2019). The skin and gill samples were fixed in Davidson's solution while other the target organs (brain, gastrointestinal tract (GIT), liver, spleen, gonad, heart, and kidney) were carefully removed and fixed in 10% buffered formalin for standard histological processing. Scoring for gill histopathology was based on Mitchell et al. (2012). In brief, the histopathology scoring of the gills was done based on five criteria which are lamellar hyperplasia, lamellar fusion, cellular anomalies, lamellar oedema, and ancillary criteria. Other target organs histopathological scoring followed the criteria suggested by Jovanovic et al. (2014) which includes presence of interstitial pigment (melanomacrophages) and tubular epithelial degenration and necrosis. DNA extraction of the kidney was done using Zymo DNA extraction and purification kit (Zymo Research, USA) according to the manufacturer's protocol followed by conventional PCR reactions using *SphF* and *SphR* primers with Székely et al. (2009) protocol. All the data was tabulated by using Microsoft Excel and statistical analysis using t-test whereby Shapiro-Wilk test was done for normality and Mann-Whitney test to compare the total parasites between juvenile and adult. The data was analysed using GraphPad Prism version 10.

RESULTS

Dactylogyrus spp. were prevalent in 100% of juveniles and 84.62% of adults with wet mount morphological examination showing the presence of opisthaptor (Figure 1A) with four eye-spots (Figure 1B).

Myxozoan was observed from the squash smear slides prepared (Figure 2A) and the histological examination of the kidney (Figure 2B). No other parasites were observed in other examined organs. The myxozoan observed in the squash smear of the kidney showing the sporoplasm stage in the fish. In the histological section of the kidney, myxozoan can be seen in the lumen of the tubules with interstitial pigment melanomacrophage (MMC) seen

In Figure 3, cellular anomalies were the most prominent histopathological lesion in the gills of juvenile and adult fish with 15 out of 16 fish samples (92.3%) followed by ancillary criteria with 87.5% affected. From (Figure 4), all the fish (100%) showed interstitial pigment melanomacrophage (MMC) while 44% showed congestion and 19% showed tubular epithelial degeneration.

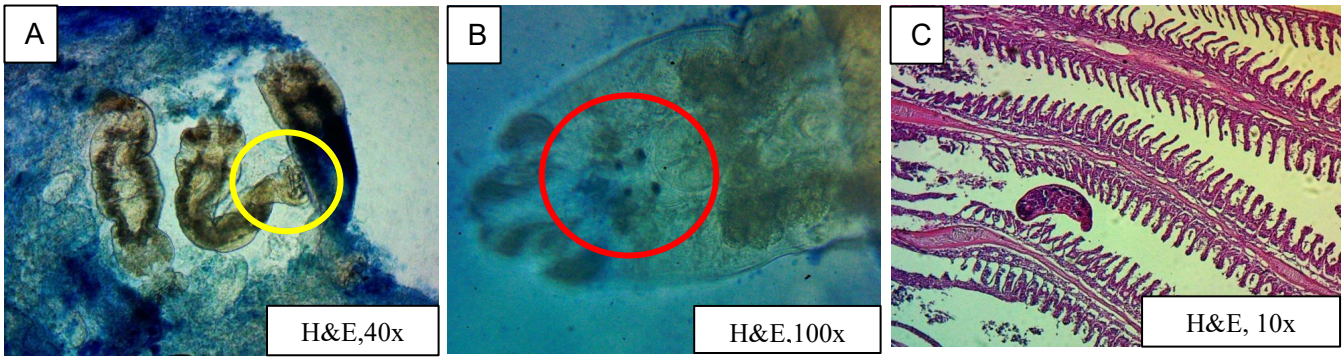

Figure 1. Wet mount examination of the gills of *Dichotomyctere nigroviridis* showing *Dactylogyrus* spp. identified by the presence of the opisthaptor (yellow circle) (A) and the four eye-spot (red circle) (B) and the histological slides showing adult *Dactylogyrus* spp. with lamellar hyperplasia (C).

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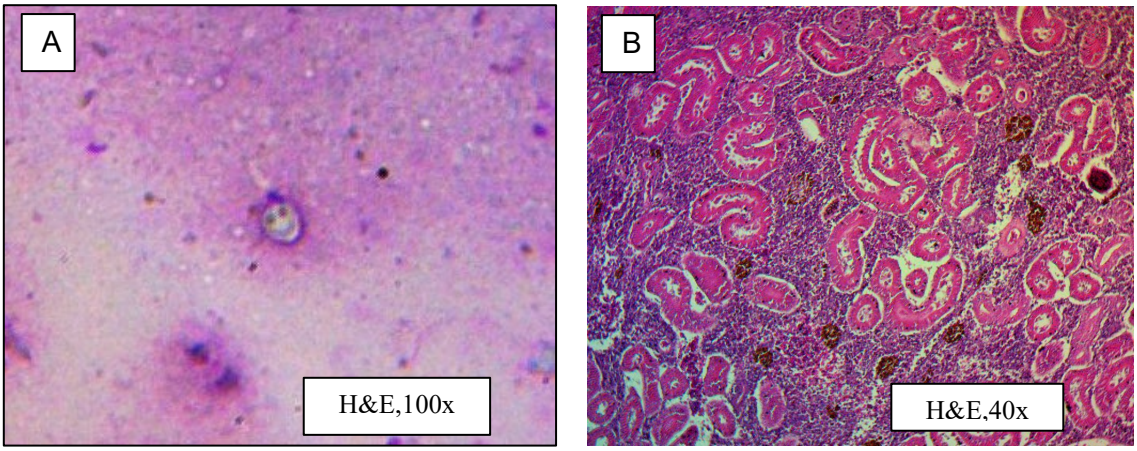

Figure 2. Myxozoan observed in squash smear (A) and from histopathology slides (B) of the kidney of *Dichotomyctere nigroviridis.*

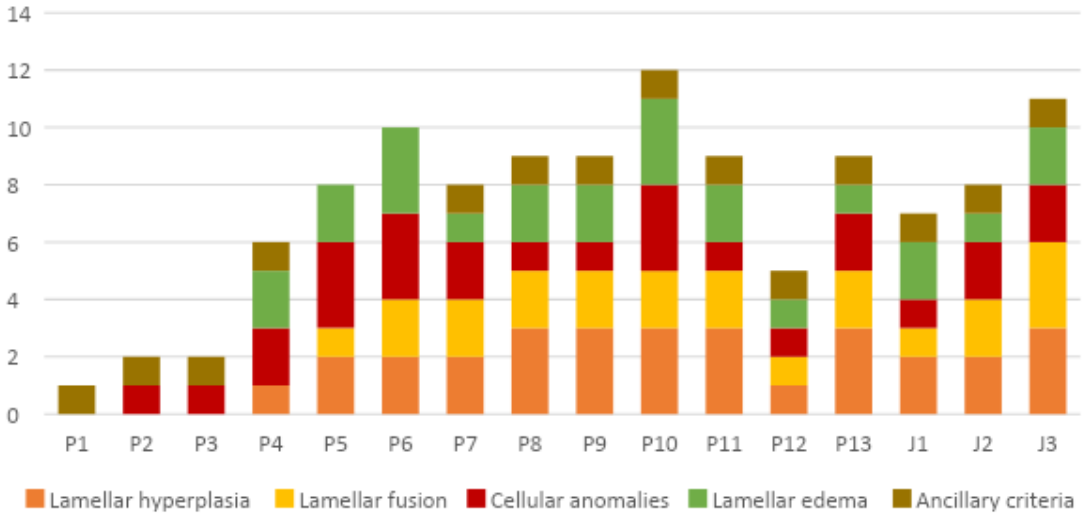

Figure 3. Bar chart showing the histopathology scoring of gills in adult and juvenile *Dichotomyctere nigroviridis* sample

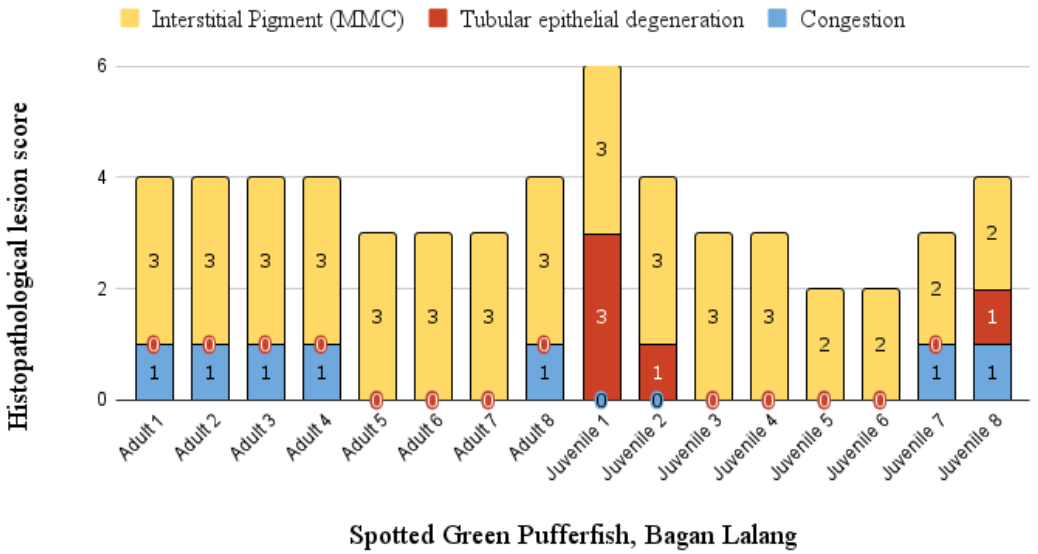

Figure 4. Kidney histopathological changes scoring in adult and juvenile *Dichotomyctere nigroviridis* sample

The sequencing of the myxozoan samples from the kidney using the 18S rDNA sequencing assay revealed NCBI BLAST results for *Triactinomyxon type sp.* strain TGR-2014 with the percentage of identity of 96.08% (accession no. was KF263540.1).

DISCUSSION

In the present study, significant histological changes include lamella hyperplasia and degeneration of cells in the gills as well as interstitial pigment melanomacrophage (MMC) in the kidney. Fish infected with *Dactylogyrus* can show gill hyperplasia and in severe cases, affect the respiratory functions and lead to death (Mozhdeganloo & Heidarpour, 2014). Cellular anomalies referring to degeneration or necrotic cells in the gills could be caused by the anchors of *Dactylogyrus* spp. arise from disruption in the cell integrity (Cheng et al., 2023). Histopathological changes associated with myxozoans infection in the kidney can be affect renal tubule epithelial cells as well as renal interstitial with inflammatory granulomatous reactions (Longshaw et al., 2005). The use of molecular techniques such as PCR and sequencing can be used to verify the species especially when the mature spores are not identifiable (Longshaw et al., 2005). From the NCBI BLAST analysis, *Triactinomyxon type sp.* strain TGR-2014 was identified. However, triactinomyxons are usually associated with annelids or segmented worms (Hallett et al., 2004) which maybe explained by the actinosporean stage involved in the annelid host. However, since the sequence

identity was 96.08%, DNA contamination could have occurred especially during the DNA extraction procedure. Alternatively, the detected parasite could be a variant or strain of the reference species, with genuine genetic divergence. Such divergence can reflect geographic strains, host-associated lineages, or cryptic species, not just lab contamination (Borkhanuddin et al., 2020). A 96.0% identity can reflect new or under-characterized species, common in parasite taxonomy where reference databases are incomplete (Samshuri et al., 2024). Previous reports of myxozoan infections particularly the *Myxidium* sp. has been reported in cultured fugu *Takifugu rubripes* with 100% prevalence in the intestine (Tun et al., 2000). To our knowledge, no *Dactylogyrus* spp. has been previously reported in pufferfish. Detecting myxozoan parasites in the kidneys and Dactylogyrus spp. in the gills raises concern about potential zoonotic transmission, particularly through handling or consuming undercooked or raw fish (Ziarati et al., 2022). Although these parasites are not always directly pathogenic to humans, their presence in wild fish populations highlights a potential reservoir for emerging zoonotic threats, especially in communities dependent on wild-caught fish for food or bait (Hochberg & Bhadelia, 2016).

This study reveals the presence of both ecto- and endoparasites in *Dichotomyctere nigroviridis*, with *Dactylogyrus* spp. and myxozoans causing notable histopathological changes. Significant tissue degeneration in gills and melanomacrophage accumulation in kidneys and spleen suggest compromised organ function. These findings underscore potential ecological consequences and highlight zoonotic risks posed by parasite-infected wild pufferfish. These findings also broaden the understanding of parasite-host interactions in wild pufferfish and highlight the necessity of continued surveillance for ecological health and zoonotic risk assessment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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