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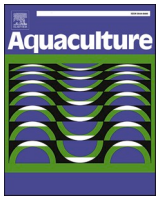

Short communication

Dermocistidiosis: A novel illness in *Pangasionodon hypophthalmus* in Brazil, and an alternative treatment

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ABSTRACT

The dermocistidiosys is a new fish disease in the Brazilian aquaculture. This is the second report occurred in *Pangasionodon hypophthalmus* in Brazil, but therapeutic alternatives to controlling it still are missing. The objective of this study was to characterize the *Dermocystidium* spp. infection in *P. hypophthalmus* fingerling and to evaluate different alternatives for controlling it. A total of 170 fish naturally infected with *Dermocystidium* spp. were used in this study. The fish were anesthetized and then submitted to blood and parasitological analysis. The glucose, total of erythrocytes, hematocrit percentage, hemoglobin concentration, hematimetric indexes, thrombocytes and differential of leukocytes were evaluated. In vitro tests were performed exposing *Dermocystidium* spp. cysts at to different therapeutic products: copper nanoparticles (CuNP), zinc sulphate ($ZnSO_4$), copper sulphate ($CuSO_4$), plant aqueous extract of *Costus spicatus* and *Schinus terebinthifolia*. The plasmodium viability (live/dead) were evaluated by fluorescent probes. Afterwards, an in vivo assay was carried out using the same therapeutic products plus one treatment represented by elevating temperature (32° C) during 96 h, evaluating the clinical signs and mortality. After 96 h, the surviving fish were monitored for more 60 days. The infected fish before experiments, presented discolored skin areas and a worm-like elongated cysts (plasmodium) within vesicles randomly distributed through the body, showing mean intensity of 80.70 ± 23.46 cysts/infected fish and the neutrophil was the most abundant cell in the blood. All therapeutic products caused mortality of spores within cysts in vitro assay. However, for in vivo test, despite the efficacy in vitro, fish died after exposure to treatments, except to elevating temperature. The fish into the controlled temperature strategy demonstrated 100% of survival at the end of 96 h and absence of clinical signs after 60 days.

1. Introduction

Mesomycetozoea is a group of fungus-like protists divided into two orders: Dermocystida and Ichthyophonida (Mendoza et al., 2002). Some species of the order Dermocystida (including the genus *Dermocystidium* Perez, 1908) have been reported to infect the skin, gills, and fins of the fish (Steckert et al., 2019). The main characteristic identification is the presence of cysts in the host tissues containing numerous spherical spores (Blazer et al., 2016). Currently, approximately 20 species of *Dermocystidium* have been described in freshwater and marine fish (Liu et al., 2021).

Dermocystidium spp. has been reported worldwide to cause mass mortality in tilapia (*Oreochromis niloticus*) (Steckert et al., 2019) and carp (*Cyprinus carpio*) (Sirri et al., 2020). In the Brazilian aquaculture, Fujimoto et al. (2018) reported a case of the hybrid fish tambatinga (*Colossoma macropomum* x *Piaractus brachypomus*); Steckert et al. (2019) observed the infection in tilapia fish species; and Cardoso et al. (2022) reported the first case of dermocistidiosys in *Pangasius hypophthalmus*.

P. hypophthalmus originated from Vietnam is produced worldwide (Khan et al., 2021) because of its rapid growth, large size, high survival

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rate, and market demand (Allam et al., 2020). Although *P. hypophthalmus* culture is recent in Brazil, prevalence of dermocistidiosis has been reported by Cardoso et al. (2022) declaring it as an emerging fish disease in the country.

Nonetheless, until this moment, demorcystidiosis has no cure or any alternative treatment. For these reasons, this study characterized an infection of *Dermocystidium* sp. in *P. hypophthalmus* fingerling and evaluated different alternatives for controlling it.

2. Material and method

A total of 170 *P. hypophthalmus* (3.22 ± 0.37 g and 6.91 ± 0.31 cm), naturally infected with *Dermocystidium* spp., were evaluated. Fishes from a fish farm in Sergipe, Brazil, distributed into two ponds (pond 1 and 2) were sampled, and the mortality was estimated in 60% of fish. 40 fishes were sampled from pond 1 for blood analysis and another 130 fishes were sampled from pond 2 for in vitro and in vivo assays. To count and collect the cysts, 25 fishes were selected and anesthetized with eugenol (60 mg/L) according Hoseini et al. (2015). The area from the pectoral to the caudal fin, on the left side of the fish was used to count the cysts and determine the mean intensity of the parasite (Fig. 1).

2.1. Hematological analysis

Blood samples (100 μL) were collected by puncturing the caudal vessels, and the amount of glucose (g/dL), total erythrocytes (cell/μL), hematocrit percentage (%), hemoglobin concentration (g/dL), and hematimetric indexes (MCV: mean corpuscular volume; HCV: hemoglobin corpuscular volume; and MCHC: mean corpuscular hemoglobin concentration) were determined (de Paiva et al., 2013). Additionally, blood smears were also used to determine the total thrombocytes and differential leukocytes.

2.2. In vitro assay

For in vitro assay, five therapeutic treatments against *Dermocystidium* spp. cysts were evaluated, which included copper nanoparticles (CuNPs), zinc sulfate ($ZnSO_4$), copper sulfate ($CuSO_4$), and *Costus spicatus* and *Schinus terebinthifolia* aqueous extracts. The plant extracts were prepared via static maceration of leaves in distillated water for 60 h at 60 °C (concentration 25 g/L) and then lyophilized. Approximately, 0.5 mg/L of CuNPs were synthetized using the chemical procedure of Din and Rehan (2017). $ZnSO_4$ and $CuSO_4$ were dissolved in water at a concentration of 0.5 and 1.0 mg/L, respectively, according to Surjawidjaja et al. (2004).

Cysts of *Dermocystidium* spp. were collected and placed in petri dishes (10 cysts per dish at the final volume of 2 mL). Randomized tests were performed with six treatments and three replicates (**T1**: Control without therapeutic product; **T2:** CuNP 0.5 mg/L; **T3:** ZnSO4 0.5 mg/L; **T4:** CuSO4 1 mg/L; **T5:** *Costus spicatus* 800 mg/L; and **T6:** *Schinus terebinthifolia* 800 mg/L). After 24 h, all the cysts were transferred to Kline plates (at the final volume 200 μL) and stained with SYBR-Green (SG - 5 μL) and propidium iodate (PI - 5 μL) according to the adapted methodology of Fujimoto et al. (2015). Subsequently, the viability of cysts was observed using Zeiss AxioPhot Fluorescent Microscope. Cyst viability was determined based on fluorescent color (**Green**: live cysts; **Red**: dead cysts).

2.3. In vivo assay – long therapeutic baths

For in vivo assay, approximately, 105 infected fishes were placed in polyethylene tanks (2 L) with a stocking density of 5 fish per experimental unit. The assay was arranged in a completely randomized design with six treatments and three replicates (**T1**: Control without therapeutic product; **T2:** CuNP 0.5 mg/L; **T3:** ZnSO4 0.5 mg/L; **T4:** $CuSO_4$ 1 mg/L; **T5:** *Costus spicatus* 800 mg/L; **T6:** *Schinus terebinthifolia* 800 mg/L; and **T7:** Controlled temperature 32 °C). The experiment was conducted for 96 h to determine mortality and clinical signs such as darkening skin, skin edema, and presence worm-like cysts were observed (Mahboub and Shaheen, 2020). Cysts were counted and cell viability was determined according to Fujimoto et al. (2015). After 96 h, the surviving fish were also monitored for more 60 d; their clinical signs were observed and survival determined.

3. Results

The fish were apathetic, presenting discolored areas through the skin, and a myriad of worm-like elongated cysts within vesicles randomly distributed along the skin, fins and gills (Fig. 2). The mean intensity of cysts per infected fish was 80.70 ± 23.46. These cysts were distributed sub-epithelially within vesicles all over the skin but were more intense at the base of the fin and gills (Fig. 3).

The mean intensity was 80.70 ± 23.46 cysts/ infected fish. These cysts were distributed through the skin subepithelially within vesicles, but more intensely at the base of the fin and gills (Fig. 3).

The hematological parameters (erythrogram and leukogram) evaluated in the infected fish are presented in Table 1. Neutrophil was the most abundant defense cell (9.05 ± 0.85) followed by lymphocyte (3.20 ± 0.69).

In vitro tests revealed that all the treatments caused mortality in all the cysts within 24 h. Dead cysts were indicated by red color (Fig. 4A), and live cysts were indicated by light green color (Fig. 4B) during the viability test.

For in vivo experiment, the water quality parameters were as follows: temperature, 24.98 ± 0.22 °C; dissolved oxygen, 5.10 ± 0.28 mg/L; pH, 7.48 ± 0.04; electric conductivity, 125.06 ± 4.42 μS/cm; total

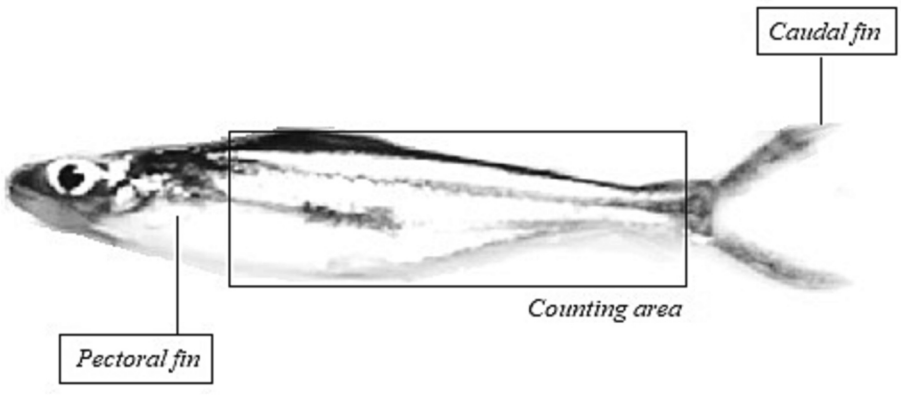

Fig. 1. Cysts counting area in the left side of *Pagasianodon hypophthalmus*.

Fig. 2. Fish *Pangasius hypophthalmus* naturally infected with *Dermocystidium* spp. with vesicles along the skin.

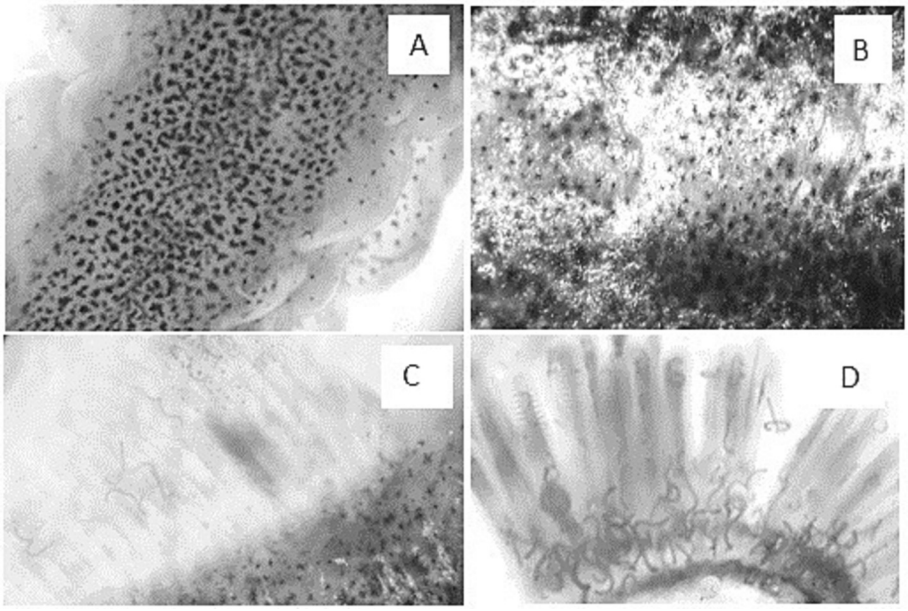

Fig. 3. Clinical signs of *Dermocystidium* infection. (A): Dorsal view of skin, details showing the vesicles and cysts; (B): Lateral view of skin detail with vesicles; (C): Fin detail presenting worm-like cysts; (D): gill detail showing a myriad of worm-like cysts in the base of lamella.

Table 1
Glucose, red and white blood cells value of *Pagasianodon hypophthalmus* naturally infected by *Dermocystidium* sp.

Parameters	Values	Parameters	Values
Erythrocyte (cell x10^6)	1.35 ± 0.21	Thrombocyte	4.24 ± 1.23
Hematocrit (%)	40.67 ± 6.78	Leukocyte	26.99 ± 0.91
Hemoglobin (g/dL)	15.37 ± 2.21	Lymphocyte	3.20 ± 0.69
MCV (fL)	307.65 ± 72.73	Monocyte	2.04 ± 0.88
HCV (pg)	116.82 ± 27.27	Neutrophil	9.05 ± 0.85
MCHC (%)	38.30 ± 6.17	Basophil	1.27 ± 0.88
		Glucose (g/dL)	107.00 ± 23.63

Erythrocyte, thrombocyte, leukocytes, lymphocyte, monocyte, neutrophil and basophil (10^4 cell/μL), MCV- mean corpuscular volume, HCV – hemoglobin corpuscular volume, MCHC – mean corpuscular hemoglobin concentration.

ammonia, 0.21 ± 0.01 mg/L; and toxic ammonia, 0.01 ± 0.00 mg/L. Only the group with controlled temperature demonstrated different value (31.97 ± 0.25 °C). The clinical signs such as erratic swimming, darkened skin, lethargy, skin edema, accelerated opercular beating, and hemorrhagic points were observed. Skin edema was the most common clinical sign, and petechial hemorrhages were only observed in fish from the without therapeutic procedure exposition (Table 2).

At the end of 96 h, fish exposed to the controlled temperature (32 °C) did not show any mortality. However, other treatments recorded 100% mortality, indicating that CuSO4 was the most toxic causing total mortality within 24 h (Fig. 5).

After 96 h of exposure, despite high fish survival in the controlled temperature group, edema was still observed. Further investigation was performed by observing the fish in the laboratory under the same conditions (same stocking density and temperature 32 °C) for more 60 d, with feed offered twice a day ad libitum, and after this period, no edema was observed (Fig. 6).

4. Discussion

Dermocystidium is a group of parasites that infect fish, causing visible gill and skin damage (Liu et al., 2021), and was first reported in brown trout and *Salmo trutta* in 1914 (Rowley et al., 2013). Eiras and Silva-Souza (2000). reported the first case in wild catfish *Trichomycterus* sp. in Brazil. Nile Tilapia (*Oreochromis niloticus*) and tambatinga (*Colossoma macropomum* × *Piaractus brachypomus*) have also been included in the list of infected fish species (Fujimoto et al., 2018; Steckert et al., 2019). To the best of our knowledge, this study is the fifth report of *Dermocystidium* sp. and the second of *P. hypophthalmus* in Brazil.

This study is the first in vitro assay that evaluated different

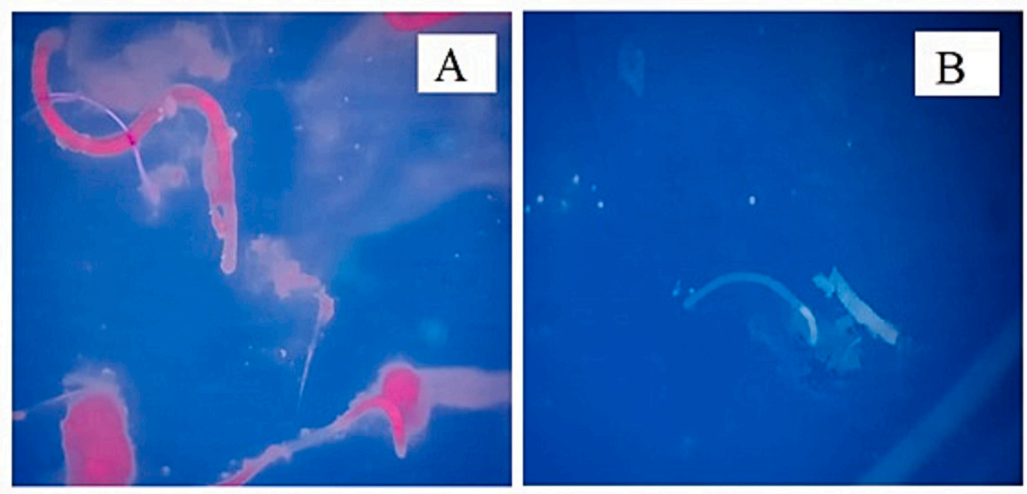

Fig. 4. *Dermocystidium* cysts stained with SYBR Green and Propidium iodate after in vitro test. A- Red color means dead cysts, B- light green means live cysts. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Clinical signs of *Pagasianodon hypophthalmus* naturally infected by Dermocystidium spp. submitted to different therapeutic long baths.

	ES	DS	LT	EOS	AOB	PH
CTRL	+	+	+	+	+	+
CuSO4	+	+	+	+	+	−
ZnSO4	+	+	+	+	+	−
CuNP	+	+	+	+	+	−
C. spic	−	+	−	+	+	−
S. tere	−	+	−	+	+	−
Temp	−	−	−	+	−	−

CTRL- control, CuSO4- copper sulfate, ZnSO4- Zinc sulfate, CuNP- Copper nanoparticle, C. spic- *Costus spicatus*, S. tere- *Schinus terebinthifolia*, Temp- temperature (32 °C). "+"means the presence of clinical sign and "-"means absence of clinical sign, ES- erratic swimming, DS- darkest skin, LT- lethargy, EOS- edema on skin, AOB- accelerated opercular beating, PH- petechial hemorrhages.

treatments used for controlling *Dermocystidium*. Furthermore, the therapeutic products used in this in vitro study recorded high efficacy. The intense fluorescence of propidium iodide indicated the presence of non-viable parasite spores and cysts. Moreover, the chemical compounds used probably damaged the parasite membrane or made it more permeable to the therapeutic product, impairing cellular osmoregulation and protein synthesis in the parasite (Mateus, 2017; Yap and Peng, 2019; Tavares-Dias, 2021).

Despite the positive in vitro results, the therapeutic products caused mortality in the infected fish in the in vivo assay. The infected fish were sensitive to all chemical treatments, probably because of the health impairments that increased the toxicity of some of the products (Tavares-Dias, 2021). Infected fish presented intermediate values of erythrocytes, hematocrit, hemoglobin, and leukocytes but presented high values of HCM, VCM, and CHCM compared to healthy *P. hypophthalmus* (Galagarza et al., 2017; Shahjahan et al., 2018; Manna et al., 2021). The present result indicated alterations in oxygen uptake

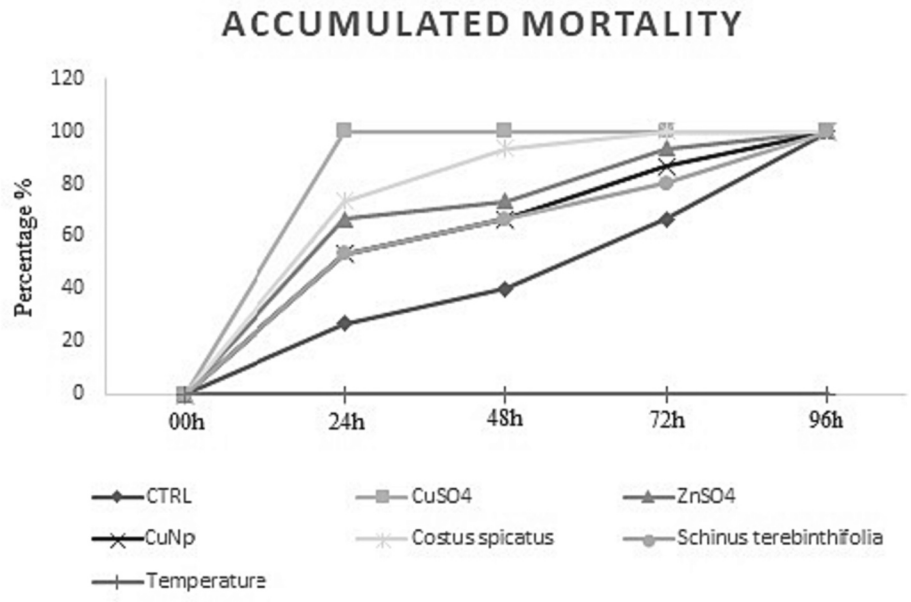

Fig. 5. Accumulated mortality *Pagasianodon hypophthalmus* naturally infected by *Dermocystidium* sp. submitted to different therapeutic long baths. CRTL- control, CuSO4- cooper sulphate, ZnSO4- zinc sulphate, CuNP- copper nanoparticle, *Costus spicatus*- aqueous extract, *Shinus terebinthifolia*- aqueous extract, Temperature- (32 °C).

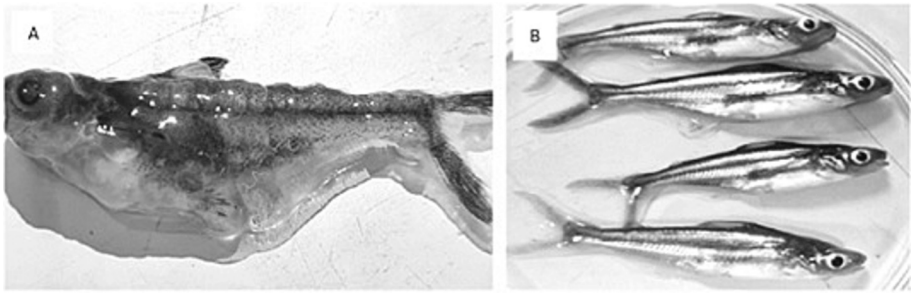

Fig. 6. (A) *Pangasius hypophthalmus* at the beginning of in vivo test presenting edema on skin, cysts into edema and petechial hemorrhages; (B) *Pangasius hypophthalmus* presenting normal coloration and skin without edema after 60 days over controlled temperature (32 °C).

during respiration and intense inflammatory processes, mainly related to the presence of cysts and edema; thus, corroborating the health impairment and sensitivity to chemical treatments.

Parasite mortality depends on the contact between the chemicals and cysts. However, a set of barriers, such as the skin, edema, and cyst wall, must be overcome before the chemical reaches the spores to cause a therapeutic effect. All these barriers are problem to the control of infection using chemical agents, despite their efficacy in vitro, making it unreliable as a treatment in vivo. Therefore, to use chemical products, a new protocol that considers these barriers should be developed.

Moreover, the controlled treatment with 32 °C was the only treatment that promoted 100% survival of the infected fish. This strategy has been used to control other protozoan parasite *Ichthyophthirius multifiliis* by interrupting its life cycle and to prevent disease progression (Mamun et al., 2020).

In addition, fish under high temperature treatment (above 32 °C) did not show further clinical signs after 60 d, which is the life cycle period of the parasite (Pekkarinen and Lotman, 2003P). This indicated that cysts were probably released from the host to complete their life cycle or that the fish defense system expelled dead cysts over time. Temperature control could be a potential alternative for fish recovery; therefore, the effects of temperature on parasites should be investigated in future studies.

The rearing of this fish species in Brazil has already led to discussions on the legality and usefulness of establishing new species in Brazilian aquaculture (Garcia et al., 2018). Concerns about the presence of this species and its diseases in native water bodies and their impact on national biodiversity can jeopardize the sustainability of rearing this species. Therefore, eco-friendly treatments should be used as handling practices to ensure the sustainability of *P. hypophthalmus* aquaculture in Brazil.

5. Conclusion

The infection by Dermocystidium sp. in *Pangasius hypophthalmus* fingerlings in Brazil caused high mortalities due alteration on respiratory physiology mainly in gill and blood parameters. Temperature over 32 °C throughout the 96 h can be used to ensure survival and after 60 days reduced the clinical signs.

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Ethical statement

All procedure were approved by an Animal Care Committee from Embrapa Tabuleiros Costeiros and Pio X (protocol numbers 024.2019 and 16/2018).

Declaration of Competing Interest

The authors have no any conflict of interest to declare.

Data availability

Data will be made available on request.

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