İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2025; 40(1): 37-41 • DOI: https://doi.org/10.26650/ASE20241594074

Short Communication

Efficacy of Hydroxychloroquine Sulphate for Treating Disease Caused by *Cryptocaryon irritans* Brown, 1951 in Marine Ornamental Fish

Pedro Henrique Magalhães Cardoso[1], Simone de Carvalho Balian[1], Herbert Sousa Soares[1,2], Beatriz dos Santos Neves Kahwage[2], Liura Sanchez Lauri[3], Maurício Laterça Martins[4]

Cite this article as: Cardoso, P. H. M., Balian, S. de C., Soares, H. S., Kahwage, B. dos S. N., Lauri, L. S., & Martins, M. L. (2025). Efficacy of hydroxychloroquine sulphate for treating disease caused by *Cryptocaryon irritans* Brown, 1951 in marine ornamental fish. *Aquatic Sciences and Engineering, 40(1)*, 37-41. DOI: https://doi.org/10.26650/ASE20241594074

ORCID IDs of the author:
P.H.M.C. 0000-0001-8387-0121;
S.C.B. 0000-0002-2211-9315;
H.S.S. 0000-0002-9887-5408;
B.S.N.K. 0009-0008-1867-0623;
L.S.L. 0000-0002-4623-6735;
M.L.M. 0000-0002-0862-6927

[1]Department of Preventive Veterinary Medicine and Animal Health, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

[2]Santo Amaro University, Postgraduate Program in One Health, São Paulo, SP, Brazil

[3]Department of Pathology, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

[4]AQUOS – Aquatic Organism Health Laboratory, Aquaculture Department, Federal University of Santa Catarina, Florianópolis, Brazil

Submitted: 02.12.2024

Revision Requested: 22.12.2024

Last Revision Received: 26.12.2024

Accepted: 27.12.2024

Online Published: 17.01.2025

Correspondence: Pedro Henrique Magalhães Cardoso
E-mail: pedrohenriquemc@yahoo.com.br

ABSTRACT

Cryptocaryon irritans is a ciliate protozoan parasite of wild and cultivated marine fish and causes the clinical signs of white spot disease. It results in significant losses for aquarists and commercial fishermen worldwide and infects various marine teleosts. This study reports on the efficacy of the administration of hydroxychloroquine sulphate as a treatment for white spot disease caused by *C. irritans*. Nine marine ornamental fish of different species showed behavioural changes such as flashing and had white spots on their skin and fins. Four fish with higher numbers of white spots were subjected to skin scraping to confirm the diagnosis under a microscope, through which the presence of *C. irritans* was confirmed. The aquarium containing these fish species was treated with 20 mg L^{-1} of hydroxychloroquine sulphate for fifteen days. One day after the addition of hydroxychloroquine sulphate to the tank, positive behavioural changes were observed among the fish. On the third day, none of the nine fish had signs of white spots visible, and no parasites were found on the skin scraping sample. In the study, it was concluded that hydroxychloroquine improved the clinical signs and prognosis of the disease caused by *C. irritans* in the fish species examined.

Keywords: Marine ornamental fish, Aquatic health, Parasite, Ciliated protozoan, Hydroxychloroquine

INTRODUCTION

The protozoan parasite *Cryptocaryon irritans* (Brown, 1951) belongs to the class Prostomatea. This ciliated organism infects marine fish, both in wild populations and aquaculture settings, and is responsible for a disease commonly referred to as white spot disease or cryptocaryoniasis. This disease leads to significant economic losses for aquarists and commercial fisheries worldwide and infects various marine teleost species (Chi et al., 2017). Through the infestation of epithelial tissues, it produces numerous tiny white spots (Li et al., 2022). The invasion of the epithelial tissues in the skin, gills, and fins can interfere with the host's osmotic balance and respiratory functions. Additionally, damage to these tissues may pave the way for secondary infections by bacteria, viruses, or fungi, which can cause significant mortality in various species of farmed marine fish (Gao et al., 2022; Colorni & Burgess, 1997).

White spots or nodules can be challenging to detect on fish with light-coloured scales, making it difficult to confirm the absence of *Cryptocaryon* spp. infection through visual inspection alone. This highlights the necessity of diagnostic tests for accurate identification. When fish are infected with *Cryptocaryon* spp., mortality rates can escalate quickly within a few days. The intensity of the outbreak is determined by several factors, including the salt water temperature, the strain's virulence, and the host previously exposed to the ciliated parasite (Yanong, 2009).

The parasite *C. irritans* completes its life cycle within 8 to 10 days at temperatures ranging from 24 to 25 °C. However, has also been observed to complete its cycle in as little as 7 days. The duration of the life cycle can significantly fluctuate, from a few days to several months, influenced by factors such as specific host species and water temperature (Yanong, 2009).

The *C. irritans* life cycle has four morphologically and physiologically different stages: parasitic trophont, protomont, reproductive tomont and infective theront (in the environment) (**Figure 1**). Trophonts are commonly found in marine fish. They appear as white spots present in the host's gills, fins, skin, and eyes. In this stage, the parasite penetrates the epithelial layer, forming a cavity that shields it from the host's immune defences while granting it access to body fluids, tissue particles, and whole cells, which serve as its food source. Microscopic can range in size from about 27 µm to 452 µm and have spherical to pear-shaped, ciliated and rolling in the slide. Mature trophonts leave the fish, becoming protomonts. After a few hours, protomonts reduce their movements, slowing down and encysting in the tissue, and then transform into tomonts, which are the reproductive stage. Tomonts undergo successive asymmetric binary fissions, dividing into numerous tomites that develop into pear-shaped theronts. The exact mechanism triggering the release of trophonts from the host remains unclear, but evidence suggests that the photoperiod plays a significant role, as trophonts are typically released into the water just before dawn. After fully developing, theronts are released from the tomonts, leaving the cyst through a small opening, thus beginning the infective free-swimming stage. Theronts actively search for a new fish host, swimming quickly until reaching their destination. They have a lifespan of approximately 24 h or more, but their infectivity quickly decreases after they leave their original host (Li et al., 2022; Yanong, 2009).

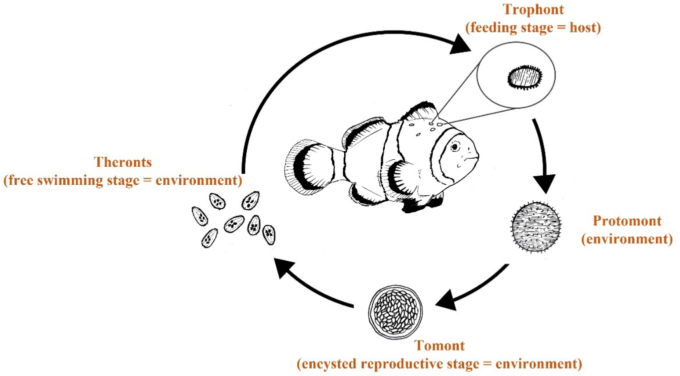

Figure 1. Life cycle of *Cryptocaryon irritans* (Yanong, 2009).

If not treated early, white spot disease can yield a mortality rate of 100% in a few days. Currently, the treatments available within veterinary practice are chloroquine and cooper prolonged immersion in the theronts phase. The use of copper sulphate ($CuSO_4$) at a therapeutic concentration of 0.2 mg L^{-1} for two to three weeks was found to help in removing *Cryptocaryon sp.* infections in marine hosts but presented high toxicity to fish (Noga, 2010). Hydroxychloroquine sulphate is another possible treatment, but its use is not very common in Brazil. This compound disrupts the processes of endocytosis and haemoglobin breakdown in vulnerable parasites; it also increases the pH of acidic cytoplasmic vesicles and, therefore, inhibits the activity of lysosomal enzymes and other organelles (Schlesinger & Krogstad, 1987; Al-Bari, 2017).

This study reports the results and efficacy of the administration of hydroxychloroquine sulphate as a treatment for the disease characterised by white spots and caused by *C. irritans* in nine marine ornamental fish that belonged to an aquarist in São Paulo, Brazil.

MATERIALS AND METHODS

Case history

In this case, nine marine ornamental fish of different species [*Acanthurus achilles* Shaw, 1803 (n=1), *Acanthurus nigricans* Linnaeus, 1758 (n=1), *Amphiprion ocellaris* Cuvier, 1830 (n=2), *Chelmon rostratus* Linnaeus, 1758 (n=1), *Pygoplites diacanthus* Boddaert, 1772 (n=1), *Siganus vulpinus* Schlegel & Müller, 1845 (n=1), *Zanclus cornutus* Linnaeus, 1758 (n=1), *Zebrasoma desjardinii* Bennett, 1836 (n=1)] showed behavioural changes such as flashing and had white spots on their skin and fins.

At the beginning of the present case, none of these ornamental fish had shown any clinical signs of disease. The translocation of *C. rostratus* and its introduction into the new habitat probably generated stress and changed the dynamics in the already established environment, causing imbalance and favouring the emergence of diseases. Thus, three days after the introduction of an apparently healthy specimen of *C. rostratus* to the existing population of the aquarium, all the fish began showing behavioural changes. In addition to behavioural changes, the species *A. achilles, C. rostratus, P. diacanthus, Z. desjardinii* (**Figure 2**) presented white spots on their bodies, and these fish were chosen to verify the diagnosis by microscopically identifying the presence of the parasite. The other fish *Acanthurus nigricans, Amphiprion ocellaris, Siganus vulpinus, Zanclus cornutus, and Zebrasoma desjardinii* did not show visible signs of white spot disease, like the previous ones, but because they were in the same environment they were considered to have the same disease.

Treatment practice

The fish were housed in a 0.3 m³ reef aquarium with a recirculation system and were fed three times daily with a commercial feed (Aloe large granules, Dr. Bassler Biofish-food®, Belgium) provided *ad libitum*. The water condition metrics of the aquarium were monitored weekly and maintained as follows: salinity at 33 g/L, temperature at 26 °C, alkalinity at 9 dKH, dissolved oxygen at 5 mg/L , pH at 8.3, total ammonia levels below 0.25 mg/L, nitrite at 0.25 mg/L, and nitrate at 10 mg/L. The aquarium was treated once with 20 mg L^{-1} of hydroxychloroquine sulphate (Reuquinol® Aspen) for 15 days (Dhayanithi et al., 2022). No water changes were made during the treatment: only the substrate was cleaned using a gravel vacuum twice a day, and an external filter was used. The temperature was controlled through a thermostat. Right at the end of the treatment, 100 g of Hw® activated charcoal was placed in the filter to remove residual hydroxychloroquine sulphate from the water.

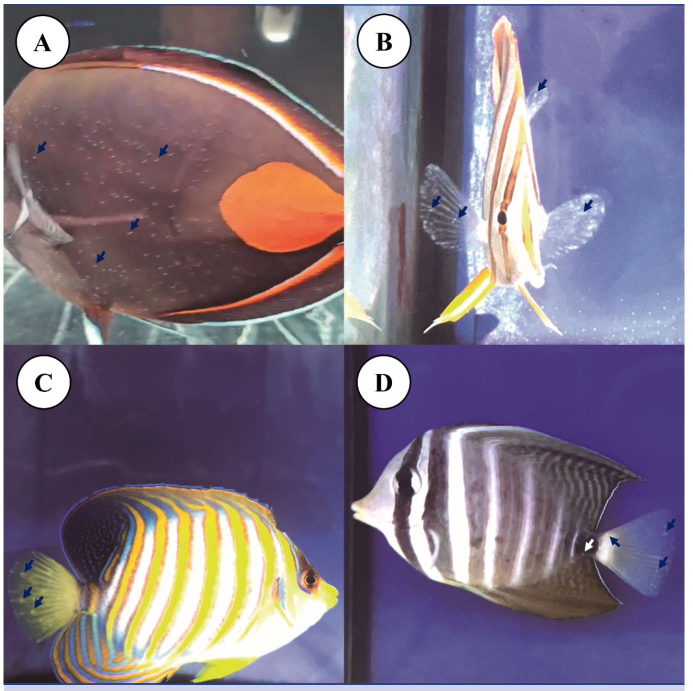

Figure 2. Clinical signs of white spots in fish: A. achilles (A), *C. rostratus* (B), *P. diacanthus* (C), and *Z. desjardinii* (D).

RESULTS AND DISCUSSION

After skin scraping, trophonts were observed in the microscopic had range in size from about 150 to 250 µm and have spherical to pear-shaped, ciliated and rolling in the slide which confirmed the presence of *C. irritans* (**Figure 3**) according to Yanong, (2009). One day (24 hours) after the addition of hydroxychloroquine sulphate to the tank, positive behavioural changes could be seen among the fish; also, another skin scraping was done, and the trophonts present in the sample showed less and slower movement than before. On the second day (48 hours) the trophonts did not move and had small vacuoles in the cell. On the third day (72 hours), no one of the nine fish expressed any manifestation of white spot visible in clinical inspection, in addition no parasites were found on a skin scraping sample. Nonetheless, the administration of the medication was continued until the end of the 15-day treatment cycle, without water change. Thus, the application of hydroxychloroquine for the treatment of *C. irritans* resulted in a significant reduction in the clinical signs of infection within 72 h, with improved fish behaviour and complete elimination of parasites observed under microscopic analysis. No apparent adverse effects were detected in the treated fish during the observation period, and mortality was reduced to zero. The 20 mg L^{-1} concentration demonstrated superior efficacy compared with alternative treatments reported in the literature, highlighting its potential as a safe and effective antiparasitic for ornamental marine fish.

The research conducted by Van and Nhinh (2018) demonstrated a significant difference in the frequency of *C. irritans* in distinct fish species. Certain species, such as *Chaetodon adiergastos* Seale, 1910; *Paracanthurus hepatus* Linnaeus, 1766; *Amphiprion frenatus* Brevoort, 1856; *Diodon holocanthus* Linnaeus, 1758; *Platax teira* Forsskål, 1775; *Pterois volitans* Linnaeus, 1758; *Plectorhinchus vittatus* Linnaeus, 1758; *Siganus guttatus* Bloch, 1787; and *Pygoplites diacanthus* Boddaert, 1772, show high susceptibility to the parasite, with infection rates reaching up to 70%. In contrast, other species like *Rhinecanthus aculeatus* Linnaeus, 1758, *Zanclus cornutus* Linnaeus, 1758, *and Zebrasoma veliferum* Bloch, 1795 exhibited much lower susceptibility, with infection rates under 50%. These insights are valuable for aquarists managing ornamental fish in controlled environments and addressing outbreaks of this parasite. Although the previous study evaluated the susceptibility of several species, we cannot state in this study that *A. achilles, C. rostratus, P. diacanthus, and Z. desjardinii*, which had white spots early, are less or more susceptible than the other inhabitants of the aquarium (*A. nigricans, A. ocellaris, S. vulpinus, Z. cornutus, Z. desjardinii*). We chose to perform skin scrapings only on the animals with the most evident clinical signs so as not to stress the other fish and to begin treatment as soon as possible to ensure that all of them survived this lethal parasite.

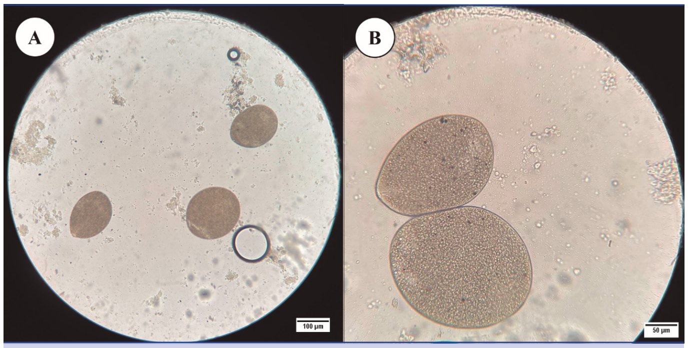

Figure 3. Cryptocaryon irritans on wet-mount preparations under a light microscope at magnifications of 10 x (A) and 20 x (B).

Prompt diagnosis of *C. irritans* infection in aquarium fish is crucial, as the disease can lead to significant mortality in a brief timeframe (Colorni & Burgess, 1997). Light-coloured fish are particularly vulnerable and may not survive without early detection. The challenge in identifying this infection often arises from the difficulty of observing white spots, which can go unnoticed even by experienced professionals who lack specific training in recognising these signs (Cardoso et al., 2019).

Hydroxychloroquine acts on *Plasmodium* spp (the protozoan that causes malaria) by accumulating in the acidic food vacuole, where it raises the pH and interferes with the digestion of haemoglobin, which is essential for the parasite. In addition, it inhibits the polymerisation of toxic haem into hemozoin, leading to the intracellular toxicity of the protozoan. This mechanism compromises the survival of the protozoan and the continuity of the infection. In addition, there are numerous other immunomodulatory effects of hydroxychloroquine that are still unclear (Ben-Zvi et al., 2012). There are no studies of this information in relation to the protozoan *C. irritans*, but taking into account the various positive effects against other comorbidities already described (Abdel-Aziz et al., 2022; Ben-Zvi et al., 2012) and the observation of the evolution of the disease in the present case study, it is suggested that hydroxychloroquine sulphate is a possible drug for the healing of this disease in ornamental marine fish. However, more advanced and robust studies should be conducted in the laboratory to confirm the clinical findings.

Picón-Camacho et al., (2011) conducted in vitro tests for the control of *C. irritans* theronts and found that hydroxychloroquine at 20 mg L^{-1} eliminated over 90% of theronts within 1.5 h, while doses of 50 mg L^{-1} and 80 mg L^{-1} eliminated more than 93% and 96%, respectively. Leethochavalit (2011) found that at a dose of 20 mg L^{-1} of hydroxychloroquine, 100% of the theronts were not moving after 30 min of treatment. For chloroquine at a dose of 10 mg L^{-1}, after 60 min, more than 96% of the theronts were not moving.

The use of a drug based on chloroquine to treat parasites of the phylum Ciliophora was found to be effective at doses of 10 and 20 mg kg^{-1} orally and presented low toxicity for the freshwater fish *Cyprinus carpio* Linnaeus, 1758 , *Ictalurus punctatus* Rafinesque, 1818 and *Ctenopharyngodon idella* Valenciennes, 1844. Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) was treated with a prolonged immersion bath at 10 mg L^{-1} for 24 h and the results indicated moderate efficacy, suggesting that longer exposure time or dose adjustments may be necessary for optimal results (Yevtushenko, 2019). The therapeutic concentration of chloroquine was used in the form of prolonged immersion for treating protozoan parasites in aquarium fish at doses of 10 to 20 mg L^{-1} for up to 21 days and is nontoxic to fish but highly toxic to micro-and macroalgae and to various invertebrates (Noga, 2010). A study using hydroxychloroquine phosphate at a dose of 10 mg L^{-1} demonstrated that this was effective for controlling the parasite *Amyloodinium ocellatum* Brown, 1931 in orange clownfish (*Amphiprion percula* Lacepède, 1802) and was also effective for increasing the survival of the affected fish when compared to traditional treatments, such as malachite green and formalin, which can be toxic at therapeutic doses. A single dose of 10 mg L^{-1} of hydroxychloroquine was applied for a period of 15 days, and no significant side effects or evident toxicity were observed in the treated fish during the study. The authors assert that the relative safety of hydroxychloroquine in this dose may be a viable therapeutic option for the management of parasites in ornamental fish (Dhayanithi et al., 2022).

The present study corroborated previous investigations, and the use of hydroxychloroquine sulphate at a dose of 20 mg L^{-1} for the treatment of *C. irritans* in nine species of ornamental fish demonstrated highly positive results. No deaths were recorded during the 15-day treatment period, and the parasites were completely eliminated within 72 h after the start of therapy. In addition, no clinical signs or side effects were observed in the treated fish throughout the study. These findings suggest that hydroxychloroquine, at the dose used, is a safe and effective option for the management of *C. irritans* in multispecies ornamental systems, providing improvement in fish behaviour and the absence of relapses until the end of the observation period.

Hydroxychloroquine degradation in aquariums occurs mainly due to microbial action in the biofilms in the pipes, where microorganisms use the drug as a source of carbon and nitrogen. This process can reduce the therapeutic concentration of the drug, compromising antiparasitic treatment and favouring the selection of resistant parasites. In addition, degradation increases operational costs due to the need for redosing and can impact water quality and animal health. Strategies such as biofilm management, water flow adjustments, and the development of more stable drugs are essential to mitigate these problems and ensure effective treatments (Hu et al., 2022). The researchers' previous findings led us to believe that a dose of 20 mg L^{-1} lasting more than 15 days is a good alternative to prevent the complete degradation of hydroxychloroquine.

Unfortunately, there is no test on the aquarium market that measures the concentration of hydroxychloroquine sulphate in aquarium water, as we have for copper sulphate, for example. However, considering that hydroxychloroquine sulphate is a promising drug for treating *C. irritans* and other possible parasitic protozoa, a test that measures the concentration gradient in the water to check whether the drug remains stable over the days of treatment would be very useful for professionals who treat these animals. Although chloroquine and hydroxychloroquine are widely used worldwide by aquarists, scientific publications are scarce; therefore, our findings are extremely valuable and contribute to increasing scientific and technical dissemination. Larcombe et al., (2024) highlighted that the ornamental fish trade represents a multi-billion dollar global industry, carrying significant responsibility for ensuring the welfare of countless fish species regularly traded across international borders. Disease-related mortality and morbidity pose serious threats to fish welfare and result in considerable economic losses for the industry, highlighting critical gaps in scientific knowledge that demand immediate attention.

As soon as there is any suspicion of a *C. irritans* infection, it is essential for aquarists to consult a qualified professional. This ensures accurate identification of the disease and timely implementation of the appropriate treatment. *C. irritans* is known to cause mortality rates of up to 100% in infected fish populations if left untreated, posing significant economic and ecological challenges to aquarists and the ornamental fish industry (Colorni, 1987; Yanong, 2009; Dickerson, 2006; Fridman, 2022).

Therefore, the prompt diagnosis and timely application of therapeutic strategies involving hydroxychloroquine-based treatments can enhance both the clinical outcomes and the overall prognosis of infections caused by *C. irritans*. The application of this therapeutic approach should be carefully tailored and monitored according to the specific fish species being treated.

CONCLUSION

In this context, it was concluded that the administration of hydroxychloroquine sulphate contributed to an improvement in clinical signs and the overall prognosis of the disease caused by *C. irritans* in the species studied *A. achilles*, *A. nigricans*, *A. ocellaris*, *C. rostratus*, *P. diacanthus* , *S. vulpinus*, *Z. cornutus* and *Z. desjardinii* in a home aquarium. Further studies are necessary to evaluate whether other species of marine ornamental fish might exhibit sensitivity to hydroxychloroquine sulphate at the dose used in this study. Additionally, future research should explore the broader application of this therapeutic approach on a larger scale while assessing its potential ecotoxicological impacts across diverse environmental contexts. Such investigations will provide critical insights into the safety and efficacy of hydroxychloroquine in managing parasitic infections in ornamental aquaculture.

Acknowledgments: The authors would like to thank the aquarist for permission to publish the case in a scientific journal in order to prevent epizootics from this parasite.

Conflict of Interest: The author states that there are no conflicts of interest to disclose.

Ethics Committee Approval: As no experimental animals were involved in this study, approval from an ethics committee was not necessary.

Financial Disclosure: We acknowledge the National Council for Scientific and Technological Development (CNPq) for providing a research grant to M.L. Martins (CNPq #303822/2022-8). This research was partially funded by the Coordination for the Improvement of Higher Education Personnel (CAPES) - Brazil, under Finance Code 001.

REFERENCES

Abdel-Aziz, A., Saadeldin, M,K., Salem, A.H., Ibrahim, S.A., Shouman, S,, Abdel-Naim, A.B. & Orecchia, R. (2022). A Critical Review of Chloroquine and Hydroxychloroquine as Potential Adjuvant Agents for Treating People with Cancer. *Future Pharmacology, 2*(4): 431–443. https://doi.org/10.3390/futurepharmacol2040028

Al-Bari A. (2017). Targeting endosomal acidification by chloroquine analogs as a promising strategy for the treatment of emerging viral diseases. *Pharmacology Research & Perspectives, 5*(1): e00293. https://doi.org/10.1002/prp2.293

Ben-Zvi, I., Kivity, S., Langevitz, P. & Shoenfeld, Y. (2012). Hydroxychloroquine: From Malaria to Autoimmunity. *Clinical Reviews in Allergy & Immunology, 42*(2): 145–153. https://doi.org/10.1007/s12016-010-8243-x

Cardoso, P.H.M., Soares H.S., Martins, M.L., Balian, S.C. (2019). *Cryptocaryon irritans*, a ciliate parasite of an ornamental reef fish yellowtail tang *Zebrasoma xanthurum*. *Revista Brasileira de Parasitologia Veterinaria,* 2019;28(4):750-753. https://doi.org/10.1590/S1984-29612019033.

Chi, H., Taik, P., Foley, E.J., Racicot, A.C., Gray, H.M., Guzzetta, K. E., Lin, H.Y., Song, Y.L., Tung, C.H., Zenke, K., Yoshinaga, T., Cheng, C.Y., Chang, W.J. & Gong, H. (2017). High genetic diversities between isolates of the fish parasite *Cryptocaryon irritans* (Ciliophora) suggest multiple cryptic species. *Molecular Phylogenetics and Evolution*, *112*: 47–52. https://doi.org/10.1016/j.ympev.2017.04.015

Colorni, A. &, Burgess, P. (1997). *Cryptocaryon irritans* Brown 1951, the cause of 'white spot disease' in marine fish: an update. *Aquarium Sciences and Conservation*, *1*(4): 217-238. http://dx.doi.org/10.1023/A:1018360323287

Colorni A. (1987). Biology of *Cryptocaryon irritans* and strategies for its control. *Aquaculture,* 67(1-2): 236-237. http://dx.doi.org/10.1016/0044-8486(87)90041-X

Dhayanithi, N. B., Sudhagar, A., Kumar, T. T. A., & Lal, K. K. (2022). Study on amyloodiniosis outbreak in captive-bred percula clownfish (*Amphiprion percula*) and improved control regimens. *Journal of Fish Diseases*, 46(4), 1103–1109. https://doi.org/10.1007/s12639-022-01530-1

Dickerson, H.W. (2006). *Ichthyophthirius multifiliis* and *Cryptocaryon irritans* (phylum Ciliophora). In: Woo, P.T.K. (Ed.), *Fish Diseases and Disorders, Protozoan and Metazoan Infections*. 2nd ed., Vol. 1. CAB International, Wallingford, UK, pp. 116–153. https://doi.org/10.1079/9780851990156.0116

Fridman, S. (2022). *Cryptocaryon irritans* infection. In Aquaculture Pathophysiology (pp. 505–512). Elsevier. https://doi.org/10.1016/B978-0-12-812211-2.00078-0

Gao, M., Cui, H., Fang, W., Hu, H., Miao, L., Jin, S., et al. (2022). The effects of $CuSO_4$ on *Cryptocaryon irritans* tomonts and its potential mechanism. *Aquaculture*, 560. https://doi.org/10.1016/j.aquaculture.2022.738578

Hu, J., Hellgeth, N., Cabay, C., Clark, J., Oliaro, F.J., Van Bonn, W., Hartmann, E.M., (2022). Microbial degradation of hydroxychloroquine in public saltwater aquariums: challenges and management strategies. Science of the Total Environment, 807, 150532. https://doi.org/10.1016/j.scitotenv.2021.150532

Larcombe E, Alexander ME, Snellgrove D, Henriquez FL, Sloman KA. (2024). Current disease treatments for the ornamental pet fish trade and their associated problems. *Reviews in Aquaculture*, 17:e12948. https://doi.org/10.1111/raq.12948.

Leethochavalit, S. (2011). Characterization of *Cryptocaryon* sp. Isolated From Marine Fish in Thailand and In Vitro Treatment. https://www.researchgate.net/profile/Supannee-Leethochavalit/publication/256502202_Characterization_of_Cryptocaryon_sp_isolated_from_marine_fish_in_THAILAND_and_in_vitro_treatment/links/004635232a8e3d8ec1000000/Characterization-of-Cryptocaryon-sp-isolated-from-marine-fish-in-THAILAND-and-in-vitro-treatment.pdf

Li, Y., Jiang, B., Mo, Z., Li, A., & Dan, X. (2022). *Cryptocaryon irritans* (Brown, 1951) is a serious threat to aquaculture of marine fish. *Reviews in Aquaculture*, *14*(1), 218–236. https://doi.org/10.1111/raq.12594

Noga, E. J. (2010). *Fish disease: Diagnosis and treatment* (2nd ed.). Singapore: Wiley-Blackwell. https://doi.org/10.1002/9781118786758

Picón-Camacho, S.M., Ybáñez, M.R.R.D., Holzer, A.S., Arizcun, M., Muñoz, P. (2011). In vitro treatments for the theront stage of the ciliate protozoan *Cryptocaryon irritans*. *Diseases of Aquatic Organisms*, 94(2), 167–172. https://doi.org/10.3354/dao02323

Schlesinger, P. H., & Krogstad, D. J. (1987). The basis of antimalarial action: Non-weak base effects of chloroquine on acid vesicle pH. *American Journal of Tropical Medicine and Hygiene*, *36*(2), 213–220. https://doi.org/10.4269/ajtmh.1987.36.213

Van, K. V., & Nhinh, D. T. (2018). The prevalence of *Cryptocaryon irritans* in wild marine ornamental fish from Vietnam. *IOP Conference Series: Earth and Environmental Science*, *137*, 012094. http://dx.doi.org/10.1088/1755-1315/137/1/012094

Yanong, R. P. E. (2009). *Cryptocaryon irritans* infections (Marine White Spot Disease) in fish. *IFAS Extension, University of Florida*. https://doi.org/10.32473/edis-fa164-2009

Yevtushenko, A. V. (2019). Effectiveness of the drug based on chloroquine for ciliophorosis treatment in fish. *Veterinary Biotechnology*, 34, 32–39. https://doi.org/10.31073/vet_biotech34-04