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Cymothoid Parasite, Nerocila Orbigni Inflicts Great Losses on Tilapia Zilli in Lake Qarun at El-Fayoum Province

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Abstract: *Cymothoid isopods are permanent ectoparasites of marine and freshwater fishes which causeserious problems to their fish hosts, either directly through damage of fish tissues or indirectly by acting as a portal of entry for other fish pathogens. In this study, a total of 150 Tilapia zilli were collected from Lake Qarun at El-Fayoum Province, Egypt during over a one year period (2014-2015).External and internal examinations of the isopod-infested fish were performed, with clinical signs, histopathological alterations at the attachment site, seasonal prevalence and the relationship with physico-chemical properties and heavy metal levelsin the water were documented. The infested fish displayed bulging of the opercula, sluggish movement, emaciation, severe erosions and hemorrhages of the gills, and a considerable mortality rate. It was revealed that 25% of the examined fish were infested with Nerocila orbignyi, with the highest seasonal prevalence occurring in the summer (65%). Moreover, the results revealed that ammonia (NH_3), nitrite (NO_2), sulphate $(SO_4)^{-2}$and organic matter, as well as Cadmium (Cd) and lead (Pb) levels were elevated over the permissible limits. It is possible that a higher prevalence of Nerocila orbignyican occur with higher NH_3, NO_2, SO_4, organic matter, Cd, Pb, and Hg levels in Lake Qarun.*

Keywords: *Nerocila orbignyi - Lake Qarun - attachment site-erosions - seasonal prevalence.*

1. INTRODUCTION

In recent years, crustacean ectoparasites are more frequently encountered in the aquaculture industry (**Tansel and Fatih, 2012**). It was found that about 25% of parasites infesting fish considered being crustaceans that areclassified into three main categories named copepod, brachiura and isopod (**Eiras *et al*., 2000 & Öktener and Sezgin, 2000**).Approximately 450 species of Crustacean isopod parasitize of marine and freshwater fish (**Varvaigos, 2008**).They didn't have naked carapace, only a cephalic shield covering the head is found (**Keable *et al*., 2002**).Isopods occur on the outer body surface, fins, insidethe mouth, the gills, nostrils, or occasionally burrowing in special tunnels in the musculature of their hosts (**Hoffman, 1998**).

Moreover, they were considered to be the largest ectoparasites that infest fishes worldwide (**Rhode, 2005**) and cause significant economic losses to fisheries, not only through mortalities, stunting growth or damaging tissues of the fish (**Bunkley-Williams *et al*., 2006 & Toksen, 2007**), but also by acts as vector for transmission of other fish pathogenic organisms (**Horton and Okamura, 2001**).

Lester and Hayward (2006), Ramadane *et al*. (2007) and Alas *et al*. (2008) documented that the life cycle of Cymothoidae is considered to be holoxenic cycle, that involve only one host (The final host).Additionally, these parasites take placeworldwide in various habitats, mainly in the seaside regions (**Sullivan and Stimmelmayr, 2008**).

According to **Woo (2006)**, this suborder contains approximately 500 species that parasitize fish, with about 46 species were recorded in Africa belong to families, Anilocrinae and Cymothoinae (**Trilles, 1986**). Morphologically, their mouth parts composed of two maxillipeds, four maxillae and mandible (**Rachael, 2004**).

Thorax is fused to the head and the last abdominal segment is fused to the telson, forming pleotelson (**Schotte *et al*., 2010**).

Some isopods release their fertilized eggs into a marsupium chamber where they keptprotected till they became mature enough to be released, while other species brood their eggs internally; yet isopods are never released as larva (**Rachael, 2004**).Cymothoid isopods are permanent ectoparasites

of marine and freshwater fishes causing serious problems to host fishes either directly or indirectly (**Ravichandran *et al*., 2009**).

They survive primarily on a hematophagus diet (blood and macerated tissues) (**Woo, 2006; Trilles, 1994 & Heckmann, 2003**), causing anemia and death in small fish (**Ravi and Raj kumar, 2007**).

Some species stay in mouth, while others live in gill chambers, causing gill damage, or on skin, including the fins (**Ravi chandran, 2007**).

Nerocila has nearly about 65 species, harboring the skin or the fins of fish. The appendages of *Nerocila orbignyi* (Guérin-Méneville, 1832) are highly modified to strongly attach the body surface and tear the flesh of the infested fish (**Ramesh kumar *et al*., 2013**) and can also attach to the gills (**Mladineo, 2003; Alas *et al*., 2008 & Eissa *et al*., 2012**). It is commonly distributed throughout Egypt (**Trilles, 1994**).

Lake Qarun is a closed one, which instigated from lake Mories. It receives annually 400 million m^3 of agricultural wastewater drainage (**Egyptian Company for Salts and Minerals, 1995**). The worsening of water assets in the lake during summer is believed to beof dangerous risk to aqua cultured organisms (**Mansour and Sidky, 2002 & Fathi and Flower, 2005**).

Lake Qarun considered from the heavily affected water bodies in Egypt, with exposure to ecological variations. Other people have described fluctuations in the water quality of Lake Qarun (**Bishai and Kirollus, 1980 & Mansour *et al*., 2000**), as well as the presence of heavy metal pollution (**Mansour and Sidky, 2003 & Mohamed and Gad, 2008**).

The contaminants may disturb the intermediate hosts as well as the other stages in the parasite life cycle (**Sinder mann, 1990**). Pollution can impact the frequency of parasites by impairing the host's immune response, or the parasite possibly will lower the host endurance to impurities (**Khan and Thulin, 1991**).

The parasitism possibly will raise the fish vulnerability to pollutants or the impurities may result in eitherrise or lower in the occurrence of these parasites (**Kuperman, 1992**).

Therefore, the present study seeks to identify the devastating effects of *Nerocilasp* infesting Tilapia zilli at Lake Qarun and to determine the relationship of the prevalence of Cymothoid isopods with water quality parameters.

2. MATERIAL AND METHODS

2.1. Area of Study

Lake Qarun (**Photo 1**) is a closed salty lake in the western Egyptian desert, located 83 km southwest of Cairo. It is boundedon the northern side by the desert and by cultivated land on the southern and southeastern side (**Abdel-Satar *et al*., 2010**). The lake is profound, its depth of about 4.2 m and the majority of the lake ranging in depth from 5 to 8 meters. The water level of the lake varied between 5 to 8 meters (**Sabae and Ali, 2004**). Itgets the drainage water from the surrounding farmed land across twelve drains, with two primary drains: El-Batts drain (at the northeast corner) and El-Wadi drain (near midpoint of the southern shore).

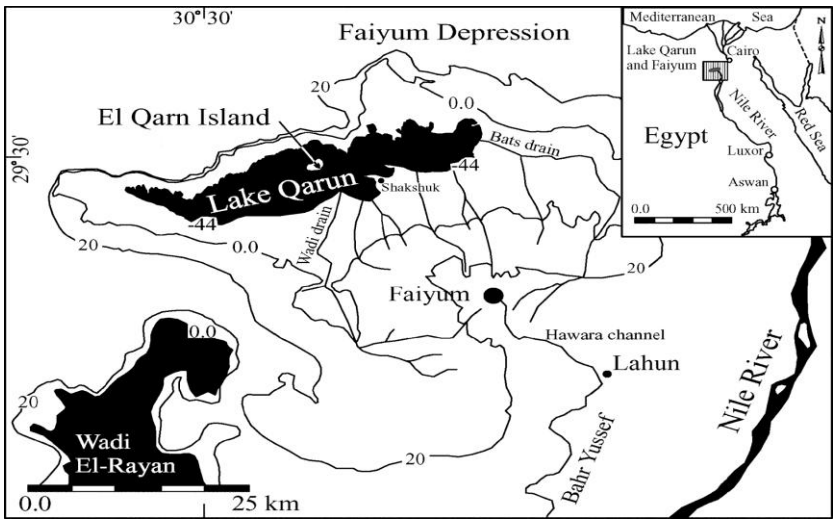


Photo1. *Lake Qarun and the Fayoum Depression, showing elevations in meters with respect to mean sea level. The Lake Qarun shoreline usually lies at 44 m below sea level (contour line − 44).*

2.2. Sampled Fish

Overall of one hundred and fifty (150) Tilapia zilli, with an average body weight of 50± 10 g, were collected from Lake Qarun at El-Fayoum Province, Egypt throughout a period of one year (2013-2014). Fish werethen transported to the laboratory alive in fiberglass tanks, with 2/3 of its volume occupied by water, and supplied with an aerator for oxygenation.

2.3. Clinical Examination

All fish were totally inspected for any clinical abnormalities and any ectoparasitic infestations according to **Woo (2006)**.The infested fish were carefully dissected at the site of isopod infestation to determine its target organ in the host. The abundance and sites of infections were also recorded.

2.4. Parasitological Examination

2.5. a. *Macroscopic Inspection*

Macroscopic inspection was done for detection of any irregularities in fish body by naked eyes and hand lens. Skin, fins, gills, eyes and opercula were dissected and examined for presence of parasitic crustaceans.

2.6. b. *Microscopic Examination*

Parasites were collected by a fine brush, special needle or eye dropper, washed for several times in fresh water until the specimens had died and left in refrigerator at 4 ^{0}C to be completely relaxed. The crusta ceans then fixed in70% alcohol glycerin, passed through ascending grades of alcohol (70, 80, 90, 95% and absolute) cleared in xylol, mounted in Canada balsam or by clearing in lactophenol and mounted in glyceringelatin (**Lucky, 1977**). Crustacean parasites were identified according to **Kabata (1979), Brusca (1981), Bowman and Tareen (1983) & Al-Zubaidy and Mhaisen (2013).**

2.7. Histopathological Examination

For histopathological examination, normal and infested fish gill tissues were obtained from the site of attachment by mouthparts and appendages and immediately fixed in alcoholic Bouin's solution for 24 hours. These specimens were dehydrated in ascending concentrations of ethyl alcohol, cleared in xylol and embedded in paraffin wax. Vertical sections were cut at 5 to 7 microns, and stained with Harri's Hematoxylin and subsequently counter stained with eosin. Finally, the slides were microscopically examined and photographed using camera mounted on light microscope and described (**Carleton *et al.*, 1967**).

2.8. Collection of Water Samples

2.9. a. *Assessment of physico-chemical water properties*

The water physico-chemical properties measured were: dissolved oxygen (D.O) (measured by a dissolved oxygen meter), percent of water salinity (measured by a Salinometer), pH values (measured by a pH meter), and unionized ammonia and sulphate measured by special kits (**USA, Virginia Company, lot. No. 201134**).

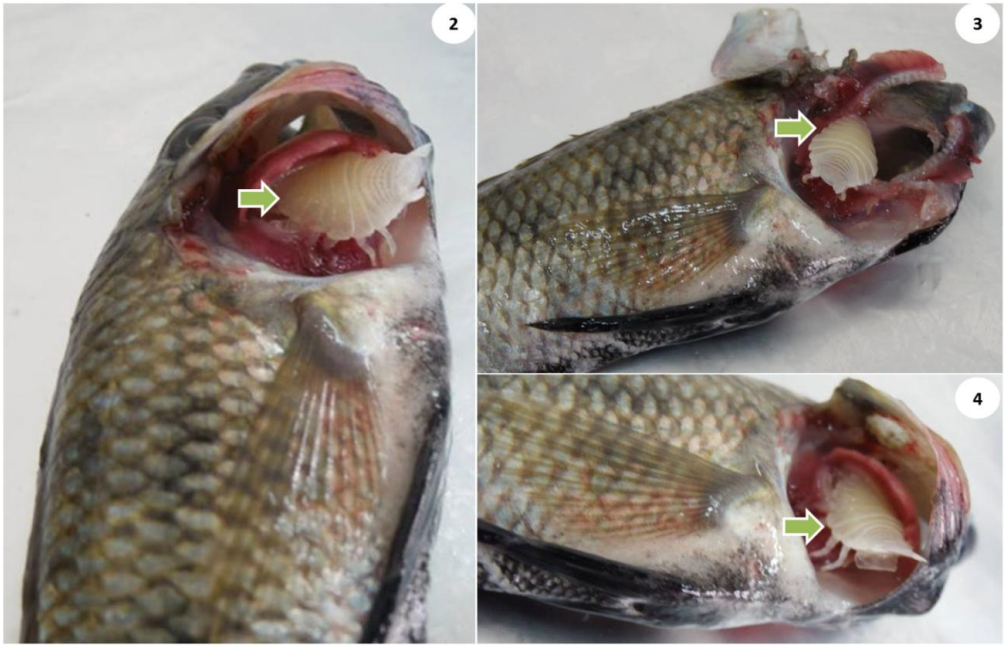
2.10. b. *Water samples for heavy metal measurements*

Three water samples were collected for physical, chemical, and trace metal evaluations during the study period. The first sample (I) was collected at the northeast region of the lake (between the Shakshok Research Station and EL-Batts drain), the second sample (II) was collected at the middle east region of the lake(in front of the Shakshok Research Station),while the third sample (III) was collected from the southwest area of the lake.Water samples were kept at -20 ^{0}C and transferred to the laboratory. The physical-chemical and trace metals analysis of water were measured according to **APHA (1992).**The levels of cadmium (Cd), lead (Pb), and mercury (Hg) were determined at central lab of National Research Center using an atomic absorption spectrophotometer (**Model Thermo, AA spectrometer, S series, type s4**).

3. RESULTS

3.1. Results of Clinical Examination

It was found that 32of the 150 examined fish were infested with the isopod *N. orbignyi*, with a prevalence of 25 %. Additionally, summer displayed the highest seasonal prevalence (65%).The clinical examination of infested fish showed respiratory distress, surface swimming, bulging oopercula with unilateral infestation with *N. orbignyi* (**Photos2 –4**), sluggish movement, emaciation, severe erosions and hemorrhages of the gills, and a considerable mortality rate.



Photos 2–4*: Photographs of unilateral Nerocila infestations in the gills of Tilapia zilli from Lake Qarun, El-Fayoum province, Egypt. Scale bars: Figs. 2, 3, 4 = 4 cm.*

3.2. Results of Parasitological Examination (Morphological Description)

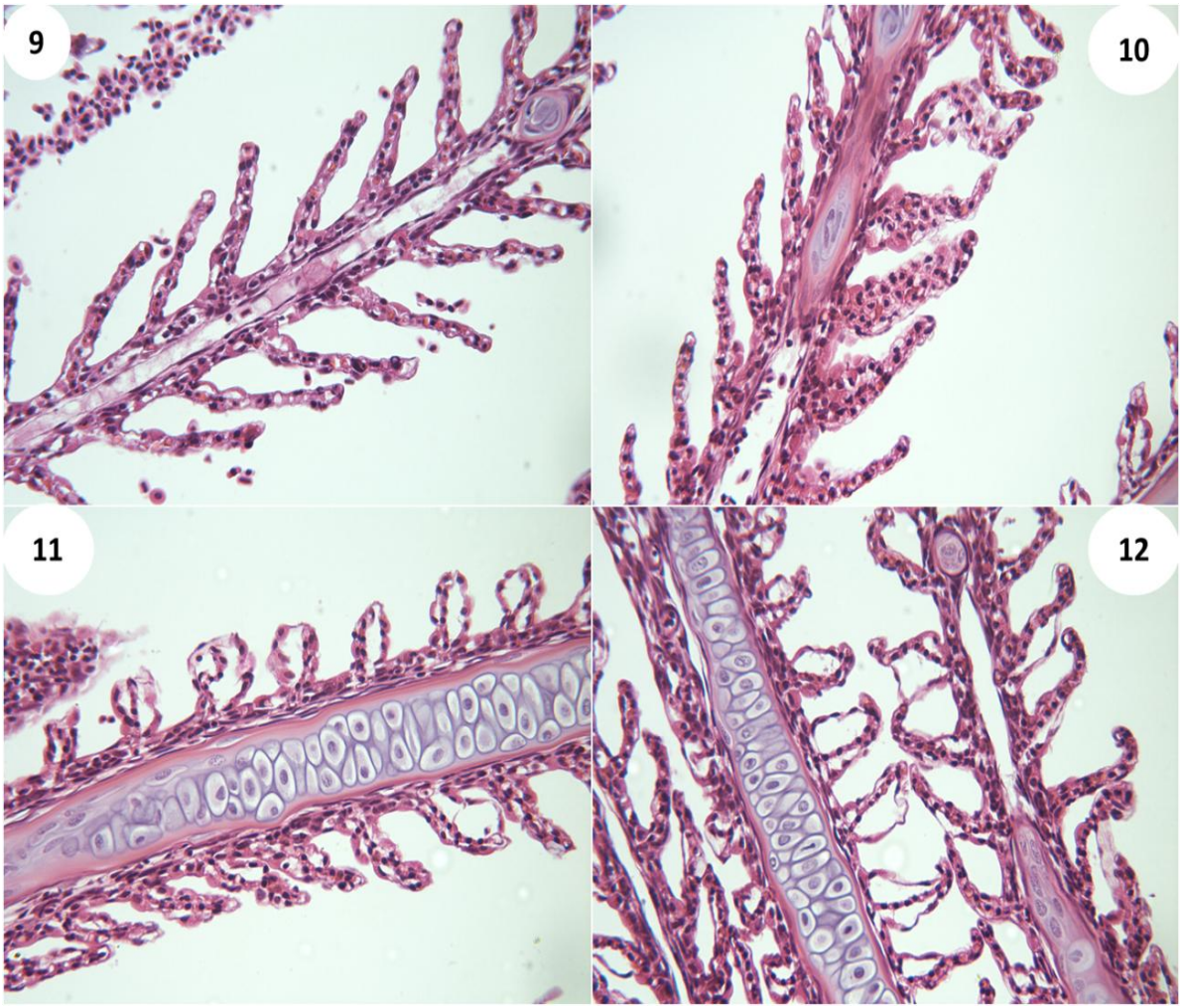
N. orbignyi was attached unilaterally to the bottom of gill arch and operculum of the infested fish (**Photos5–8**). The parasite was dorsoventerally flattened, with a symmetrical body and measured up to 2.4 mm. The mouthparts are often styli form. The head is not embedded in first segment of the peraeon.

Pleon (abdomen) is markedly narrower and shorter than peraeon and consists of six segments; each of the first five segments carries a pair of bi-ramous natatory limbs (pleopods). The sixth segment is called the pleotelson, which is flanked by the bi-ramous uropods. Both appeared without marginal setation. Uropods with exopods titled so as not to be fully seen in dorsal aspect, slight to deep notch often present on medial margin. The peraeon, the largest part of the body is composed of the cephalothorax, where the head is un segmented and bears two pairs of antennae as well as two large black eyes. It consists of seven segments; each carries a pair of appendages (peraeopods). These can be prehensile or ambulatory. Such legs bearing segments are clearly separate from each other.

Photos 5–8: Photographs of *Nerocila orbignyi* infesting the gills of Tilapia zilli from Lake Qarun, El-Fayoum province, Egypt; dorsal view (Figs. 5, 6 and 7) and ventral view (Fig. 8). Scale bars = 1 cm.

3.3. Histopathological Findings

The gills, of infested fish, at the site of *Nerocila* attachment showed congestion of the lamellar vessels, congestion, edema and adhesion of most gill filaments (**Photos 9 – 12**).



Photos 9–12: *Photomicrographs of the histo pathological findings stained with H and E stain, of the gills of Tilapia zilli from Lake Qarun, El-Fayoum province, Egypt.* ***Photo 9,*** *showing gills from non-infected Tilapia zilli,* ***Photo 10,*** *showing congestion of the gill capillaries of Tilapia zilli infested unilaterally with Nerocila orbignyi, and* ***Photos 11 and 12,*** *showing gill edema. Scale bars (x40).*

3.4. Results of Water Quality Parameters

The parameters of water quality are detailed in Table 1.Noticeably, the ammonia, nitrite and organic matter levels were greater than the permissible limits.

Table1. *Physico-chemical parameters of water at different sites of Lake Qarun during the study period.*

Sites of Lake Qarun / Measured parameters	Site (I)	Site (II)	Site (III)	Permissible limits (WHO 1993)
Dissolved oxygen (D.O) (mg/l)	4.5	4.3	4.6	4 - 5
Ammonia (NH_3) (µg/l)	1450	850	990	50 - 500
Nitrite $(NO_2)^-$ (µg/l)	2.18	3.26	2.25	None
pH	7.9	7.31	6.95	7-8
Salinity (PPT)	13.1	27.8	25.6	_
Organic matter (mg/l)	4.79	5.25	6.23	2-3
Sulphate $(SO_4)^{-2}$ (mg/l)	1859	2606	2440	200 - 400
Temperature (^{0}C)	14-16	18	20	__

Moreover, the results of heavy metal analysis described in Table 2, revealed that lead (Pb), mercury (Hg) and cadmium (Cd) levels in Lake Qarun were also elevated over the permissible limits.

Table 2. *Trace metals concentration (µg/l) in water at different sites of Qarun Lake during the study period.*

Sites / Metals	Site (I)	Site (II)	Site (III)	Permissible limits (U.S.EPA 2006)
Lead (Pb)	170	128	150	100
Cadmium (Cd)	6	11	11	10
Iron (Fe)	300	150	225	1000
Copper (Cu)	18	10	12	90
Zinc (Zn)	24	84	60	120

4. DISCUSSION

Isopoda are prospective the leading group of the fish crustacean parasites (**Kabata, 1984**). Recently, they have been documented as an important constituent of worldwide biodiversity andresearch efforts directed toward identifying their diversity have been increased (**Poulin and Morand, 2004**). They are actually economically significant parasites as they have been identified to cause deleterious effects on cultured fish, such as stunted growth, anemia, and mortalities of fries and finger lings (**Ravi and Raj kumar, 2007 & Ravi chandran *et al*., 2011**).

Nerocila orbignyi, which was isolated from the gills of the fish in the study, are known to consume on gill tissues, which can causevarious harmsparticularly emaciation of affected fish. These results may be due to the adult isopods are hematophagus (i.e., feed on blood) and cause anemia. In addition, those parasites attached to the gills can seriously reduce the respiratory surface via atrophy of the gills on which they are attached (**Horton and Okamura, 2001**).

Concerning the seasonal occurrence of *Nerocila*, The greatest was in the summer, followed by winter. This agreed with the results gotten by **Eissa *et al*. (2012)** and **Noor El-Deen *et al*. (2013)**, who documented that summer was the maximum infestation rate, followed by autumn, while the lowest was in winter season. These results possibly will be accredited to the difference of geographical distribution of hosts and parasites.

The main clinical signs in naturally infested fish with *N. orbignyi* were respiratory distress, surface swimming, bulging of opercula, sluggish movement and hemorrhages of gills. These results may be endorsed to the reduced rate of respiration due to deteriorated gill epithelium initiated by the feeding activity, attachment, fixation, and movement of the crustaceans. Also, the emaciation recorded in fish infested with isopods may have been a result of a reduced appetite for food (**Nagasawa, 2004**) or due to reduced growth rates (**Costello, 2009**).

Concerning the histopathological findings, there were gill congestion, edema and necrosis, and these results may be accredited to damage of the efferent vessels by the infested crustacean isopods, the blood pressure is little and no widespread hemorrhages were produced and the tiny clotting carries about quick occlusions of the vessel then thrombus is made resulting in ischemia, followed in sequence leads to necrosis. These results were in concordance with that mentioned by **Tosken *et al*. (2008), Noor El-Deen (2007) & Noor El-Dean *et al*. (2012).**

Regarding the relationship between the levels of heavy metals and the abundance of the parasites, it has been shown that environmental change due to pollutants can influence parasitic-host interaction (**Khan and Thulin, 1991 & Khan, 2012**). In this study, *Nerocila* infestation was found to be positively related to heavy metals inwater of Lake Qarun. These results were not in concordance with **Lafferty and Kuris (1999), El-Seify *et al*. (2011)** and **Noor El-Deen *et al.* (2013),** who illustrated that the toxic effect of the heavy metals on the crustaceans may cut its life cycle.

5. CONCLUSIONS AND RECOMMENDATIONS

*Nerocila orbignyi*is harmful to fish by feeding on fry and finger lings,causing mortalities due to the tissue damage, stunting fish, retarding reproduction through nutritional drain from fish, and by causing secondary bacterial infections. This study showed that the prevalence of Nerocilasp in Tilapia zilli was elevated during the summer season and that there were higher than what is a permissible level of ammonia, nitrite, organic matter, cadmiumand lead in Lake Qarun.To be able to apply preventive practices and control measures in aquaculture facilities, it is necessary to determine the parasitic fauna in the aquatic environment.

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