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Intraerythrocytic *Pirhemocyton*-Like Parasite of Walton's Mudskipper, *Periophthalmus waltoni* koumans, 1941 (Perciformes: Gobiidae) in the Coast of the Persian Gulf, Iran

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Abstract: Different kinds of pathogens may infect fish at various stages of their life and can be the immediate causes of their mortality. Very little are known regarding diseases occurring in fish of the genus *Periophthalmus*. By examining stained blood smears of *P. waltoni* under a light microscope, we encountered single or multiple rounds, clear to faint purple with dark ring inclusions within the cytoplasm which were similar to intraerythrocytic *Pirhemocyton* inclusion bodies that associated with albuminoid bodies. A case of the *Pirhemocyton*-like inclusions in *P. waltoni* in the Persian Gulf coast in southern Iran was reported here as the first time.

Key words: *Periophthalmus waltoni* · Intraerythrocytic · *Pirhemocyton* · Persian Gulf · Iran

INTRODUCTION

Walton's mudskipper, *Periophthalmus waltoni* Koumans, 1941, is an amphibious fish that can use their pectoral fins to "walk" on land [1, 2] (Fig. 1). This fish has numerous morphological, physiological and behavioral specializations for amphibious life style and spend extensive periods of time out of water [3, 4]. This species is found on open mudflats, in tidal mangrove forests and sandy-muddy shores of coastal inlets and estuaries of the Persian Gulf and Makran Sea including along Iran's southern coast [5, 6, 7]. Walton's mudskipper is reported to occur from the western part of Persian Gulf to Pakistan [8]. Walton's mudskipper has been considered in the order Perciformes (Perch-likes), family Gobiidae (Gobies) and subfamily Oxudercinae [9]. This speciesis not commercially valuable locally, but it is preyed upon by sea birds. *P. waltoni* may play an important role as a bio-indicator of anthropogenic impact on habitats in the intertidal areas and mangrove forests [10].

Several literatures reflect a large and diverse group of parasitic fauna in fish, amphibians and reptiles [11- 14]. Some of these parasites are intraerythrocytic parasites which affect ectothermic health, growth and survival of the hosts. One of intraerythrocytic parasites is *Pirhemocyton* which has been recorded from numerous

Fig. 1: Walton's mudskipper, *Periophthalmus waltoni*, from Nakhiloo Marine National Park, southwestern Iran.

additional ectothermic hosts in many parts of the world [15]. The form taken by *Pirhemocyton* in different hosts is variable, but in Wright stained blood films most appears as a rounded or irregularly shaped intraerythrocytic body. It measures l-5 µm diameter and stains purple. In most hosts, infected cells contain an albuminoid body which has no discernible structure and appear colorless or may stain blue-green [16].

Very little is known regarding diseases occurring in fish of the genus *Periophthalmus*. In most species, inadequate nutrition is probably the most important predisposing factor for disease [17]. Hosting of parasites such as *Myxobolus pfeifferi* and *Diplozoon* sp. (on gills), *Neoechinorhynchus* sp. (in intestine) and bacterial gill

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disease are recorded in *P. waltoni* from Iraq [18]. In this study, we investigated the possible occurrence of the parasites in the stained blood smears of *P. waltoni*.

MATERIALS AND METHODS

During the period from Nov to May 2019, *P. waltoni* samples (Fig 1) were collected on the mudflats of Nakhiloo Marine National Park (27°50' 48.5″ N 51°34'53″ E), eastern part of the Mond Protected Area, the western coast of the Persian Gulf, Bushehr Province, in southern Iran, (Fig. 2). External temperatures of the area range from about 10°C in winter to a maximum of about 38.1°C in summer, with very low precipitation of about 80 mm in winter to almost none during the summer (Figure 3 presents a climograph of Bushehr city, data from https://www.weather-atlas.com).

Fish were caught by hand and were transported live to the laboratory Zoology Laboratory of Farhangian University of Shiraz and kept in aquaria (75 × 45 × 35 cm). Blood samples from every specimen were obtained from the caudal fin, without killing specimens. They then were released at their collection site and monitored for approximately 2 h to see if caused any visible side effects, such as bleeding. The blood obtained directly was dripped onto a clean microscopic slide and were prepared smeared as a thin layer using push slide technique. The dried blood smears were stained with Wright's stain [19].

The polychromatic staining solutions such as Wright stain contain methylene blue and eosin. These basic and acidic dyes induce multiple colours when applied to cells. Methanol acts as fixative and also as solvent. The fixative does not allow any further change in the cells and makes them adhere to the glass slide. The basic components of cells (i.e. cytoplasm) are stained by acidic dye and are described as eosinophilic or acidophilic. The acidic components (e.g. nucleus with nucleic acid) take blue to purple shades of the basic dyes and are called basophilic [20]. The neutral components of the cell are stained by the both dyes.

All blood smears were generally examined and photographed under a digital camera microscope (ToupView 3.7), allowing for simultaneous comparison and facilitating the analysis of possible infections. Twenty fields of view per slide were examined and the numbers of erythrocytes with inclusions for each fish were recorded. Parasites were identified using manuals of Davies and Johnston [15], Johnston [21] and Stehbens and Johnston [22] *Pirhemocyton* infections were diagnosed based on the presence of the inclusion body (I) and albuminoid body (A) [20, 23].

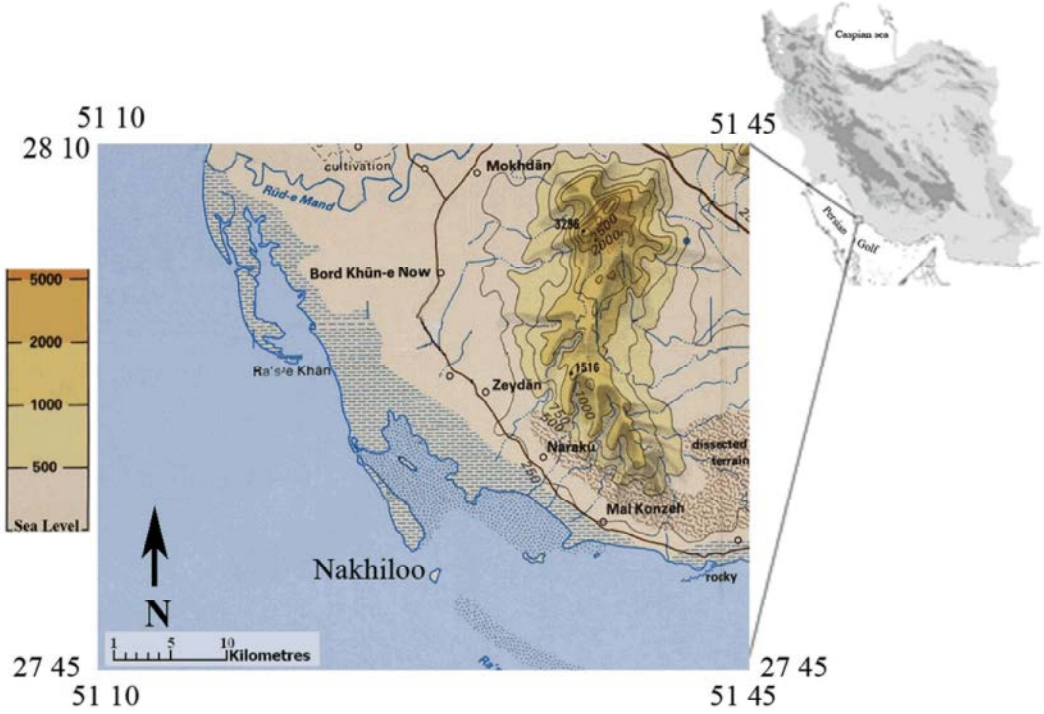

Fig. 2: Geographic position of sampling locality of *Periophthalmus waltoni*, Nakhiloo, in the western coast of the Persian Gulf, in southern Iran.

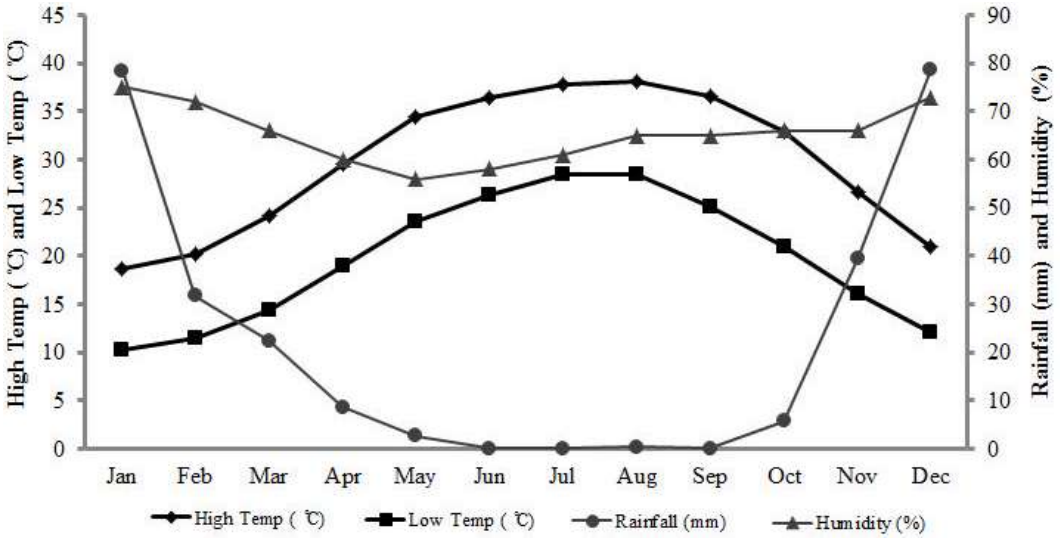

Fig. 3: Mean monthly high and low temperature values (°C), rainfall (mm) and humidity *(%)* values (mm) measured at Bushehr, the closest city to the Nakhillo. The values shown are means extracted from https://www.weather-atlas.com/en/iran/bandar-bushehr-climate#rainfall.

In the present study, we examined the stained blood smears with a light microscope. Inclusions and albuminoid bodies within the red blood cells was the key feature of *Pirhemocyton* identification. Each slide was individually examined and scored as infected or not. The characteristics of inclusions and albuminoid bodies which we described here were consistent with previous studies in other fish, amphibians and reptiles [22, 23].

RESULTS

The red blood cells of *P. waltoni* caught between Nov- Dec 2019 inclusive were infected with a parasite which closely resembled *Pirhemocyton* sp. In *P. waltoni*, the affected erythrocytes stained with Wright's method showed single or multiple small, circular structure, or inclusion bodies, which stains purplewith white ring within the cytoplasm and a pale vacuole, or albuminoid body, which is largely unstained (Fig. 4).

Purple inclusions could be detected, however, in the cytoplasm of some host cells in blood films stained. The inclusion bodies were randomly distributed within the cytoplasm. They measured 0.89-2.74 µm in diameter and were commonly, but not invariably. The size of the albuminoid bodies was approximately about 1.6-4.4 µm in diameter andwere displaced towards the cell's periphery (Table 1).

Intraerythrocytic inclusion bodies were found in 4 of 7 (57.14% of 100%) fish we examined. In parasitized fish the incidence of infected blood cells was between 9-18 percent. The average number of erythrocytes with inclusions was 41.75 % and erythrocytes with albuminoid body were 5.26 % in fields of view (Table 2). These fish did not exhibit recognizable external symptoms and the only indication of infection was the presence of parasitized red blood cells themselves. In 91.76 % of infected erythrocytes, one infectious particle is found (Table 3). The staining characteristics, the size and the nuclear position were similar in both affected and unaffected erythrocytes (Table 3).

DISCUSSION

The infection discussed in the study is similar to part of studies related to *Pirhemocyton* which are done using light microscopy [15, 16, 21, 22]. *Pirhemocyton* has previously been recorded from reptiles and occasionally from amphibians and fish. Intraerythrocytic *Pirhemocyton*-like particles in this study are morphologically very similar to those found in the red blood cells that have been recorded from erythrocytes of *Blenniuspholis* [23], *Gehyra variegate* [22], *Neruda erythrogaster flavigaste* [20], *Carettacaretta* [15]. They are also reported to occur in kidney cells of

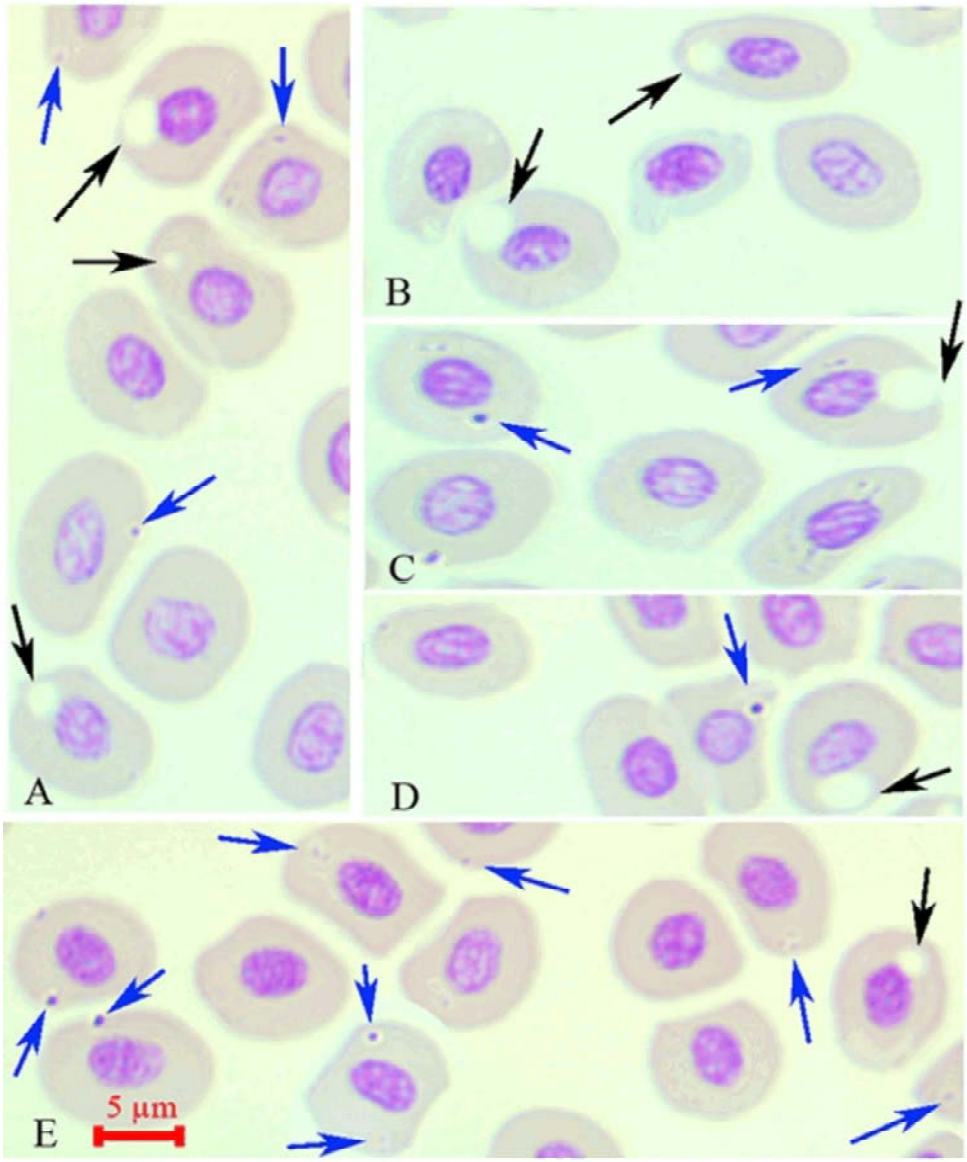

Fig. 4: Blood film, Wright's stain, *Pirhemocyton*-like infection from the mudskipper, *Periophthalmus waltoni*, magnification 6300 x. Note the inclusion body (blue arrow) and albuminoid body (black arrow), the large clear vacuole, in erythrocytes. Scale bar = 5 µm. A and B *Pirhemocyton*-infected erythrocytes containing small purple-staining bodies with white ring in the cytoplasm. C, one erythrocyte shows cytoplasmic granularity. D and E, erythrocytes containing single or more densely stained areas.

Table 1: Size of inclusions and albuminoid bodies (mean ± SD) in erythrocytes *Periophthalmus waltoni* infected with *Pirhemocyton sp.*

	Inclusion body (I)	Albuminoid body (A)
Mean ± SD (rang)	1.81±0.47µm (0.89-2.74)	2.19±0.96 µm (1.6-4.4)

Table 2: Average number of erythrocytes with or without inclusion body (I) and albuminoid body (A) in infected fish

Erythrocytes with/ without I & A	RBC without inclusion body (I) and albuminoid body (A)	RBC with inclusion body (I)	RBC with albuminoid body (A)	RBC with inclusion body (I) and albuminoid body (A)
Percent (rang)	58.25 (42.65-72.73)	41.75 (27.27-57.35)	5.26 (2.51-10.71)	0.28 (0-0.69)

Table 3: Percent infected erythrocyte based on number of inclusion body in erythrocyte in infected *P. waltoni* with *Pirhemocyton* sp.

Number of inclusion body in erythrocyte	Erythrocyte with one inclusion body (I)	Erythrocyte with two inclusion body (I)	Erythrocyte with tree to up inclusion body (I)
Percent	91.76	7.31	0.91

frogs [24], in *Ranapipiens* [25], in *Octopus vulgaris* from Naples [26] and *Agama impalearis* [27].

The diameter of the inclusion body in *P. waltoni* varies from 0.89-2.74 µm. In *Gehyra australis* the inclusion body measured from 3-4 µm in diameter and contained 1or 2 inclusions that scattered in the cytoplasm of some infected cells with purple granules. Some infected cells, may have a relatively small albuminoid body [21]. In *Gehyra variegata* each infected cell contained at least one spherical body with 2-3 µm in diameter. This probably corresponded to the basophilic area. Two types of Albuminoid bodies have been reported by Stehbens and Johnston [22]. Some are surrounded by a yellowish green halo, others are empty and the halo is absent. Howse and Christmas [28] have stated that even within one group there is considerable variation in the size of parasites recorded from different hosts. This different size of the particles may be influenced by the use of different fixation procedures [23].

Stehbens and Johnston [22] and Alves and Paperna [27] provided ultrastructural evidences indicating that *Pirhemocyton* is a viral infection. The mode of transmission of *Pirhemocyton* among lizards has been demonstrated by tail to tail contact [29]. Daly *et al.* [20] transmitted *Pirhemocyton* by direct inoculation of blood from infected snakes. Recent reports have warned about the impact of the transmission of infectious diseases. However, due consideration must be taken of the role played by variables, such as the increase in international travel, migration and trade, with the risk of importing parasites with the goods [30].

Intraerythrocytic particles in this study are formed in the cytoplasm of the *P. waltoni* red blood cells and not in the nucleus. On the other hand, no particles were seen in this study to be undergoing binary fission as might be expected if the infection was caused by a rickettsia. Our direct comparison of light microscopic studies of this infection with the gecko parasite, *Tarentola mauritanica* [16] and the presence of intraerythrocytic inclusion accumulated with colorless globular body "albuminoid body", suggest that these infections appear to be closely resembled to *Pirhemocyton* sp.

CONCLUSION

A case of the *Pirhemocyton*-like inclusions in *P. waltoni* in the Persian Gulf coast in southern Iran was reported here as the first time. Presence of intraerythrocytic *Pirhemocyton* inclusions has not been reported in any species of ectotherms in Iran. Information on the extent of infection, distribution and prevalence of *Pirhemocyton* in any species of ectotherms which may be potentially susceptible to *Pirhemocyton* are important because of its potential threat to the populations of each of these species. Additional studies should be conducted to generate adequate ultrastructural evidences and for the control of the *Pirhemocyton*-like inclusions in this area.

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