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TRYPANOSOMES, A BLOOD PARASITE OF RAJIDAE (ELASMOBRANCHII, RAJIFORMES) OFF THE ALGERIAN COAST.

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Abstract: - Trypanosomes were found from the blood of tow rajidean fishes (Raja miraletus and Raja asterias) off the central Algerian coast. We described shortly and illustrated this blood protozoan parasites, based on morphological characters. Light microscopy and Immunofluorescence were used for the observation and study of these parasites. Drawings were scanned and redrawn with Adobe Illustrator CS5.1. Specific characteristics such as and morphometric measurements are within the ranges given in previous description by Laveran and Mesnil 1904 of rajidean trypanosomes. Unfortunately, there is no vouchers available for comparison of rajidean Trypanosoma of the type host. Thus, as hosts are different, and localities are widely separated. This is the first record of Trypanosoma off Algerian cost.

KEY WORDS: -Protozoans, Parasites, Trypanosoma, Mediterranean Sea, Rajidae.

1. INTRODUCTION

The Mediterranean Sea, with its exceptional marine biodiversity, is home to numerous fish that are confronted with a diversity of parasites. Among these parasites the trypanosomes, transmitted by leeches and infecting marine and freshwater fishes worldwide (Lemos et al. 2015). The relationship between a host and its parasite can biologically extend from healthy carriage of

parasites by the host to chronic diseases, where leeches are both hosts and vectors of fish trypanosomes (Jones and Woo 1991).

Blood parasite diversity of fish of the Southern part of the Mediterranean Sea is still incompletely explored. Early works dealing with trypanosomes include those of Laveran and Mesnil, 1902 and Kudo, 1923 describing Trypanosoma rajae and Trypanosoma scylli based only on morphological data, hosts, and locality. Many Trypanosoma spp. were described with reference to their hosts e.g. Trypanosoma aeglefini from haddock Melanogrammus aeglefinus. Lebailly (1904) described two species, Lebailly, 1904 and Trypanosoma flesi Lebailly, 1904, respectively infecting plaice Pleuronectes platessa L. and flounder Platichthys flesus (L.) off France.

All descriptions of trypanosome species infecting rays available to date were based exclusively on morphology and host taxonomy criteria (Aragort et al. 2005; Laveran and Mesnil 1904; Aragort W. et al. 2002; Kudo 1923; Neumann 1909; Laird and Porter 1951). In general, the flagellum length is not consistent for many species of piscine trypanosomes, and it may vary according to the development of the parasite and methods of fixation or staining (Becker and Overstreet 1979). Specific identification cannot be accurately identified without molecular identification. The life cycle of Trypanosoma rajae may involve the epimastigote stage, the extraintestinal found in the hemocoel of the leech, metacyclic, infective stage, ingested by the insect vector during the blood meal, and the procyclic stage residing in the tissues of the vertebrate host (Povelones, Holmes, and Povelones 2023).

In the course of a parasitological study of blood parasites of Rajidae fishes off the Algerian coast, we collected trypanosomes consistent with the diagnosis of Trypanosoma rajae from the blood of Raja miraletus and the Mediterranean starry ray Raja asterias. The original description lacking details and poorly illustrated prompted a redescription of this species.

2. MATERIAL AND METHODS

- Fish and study site

A total of 37 Raja asterias and 17 Raja miraletus (Rajidae) were sampled from Bouharoune (36° 37′ 24.17″ N, 2° 39′ 17.38″ E) and Cap Djinet (36° 52’ 37” N, 30° 43’ 23” E) off the central basin of the Algerian coast (Figure 1), collected during the period from 2013 to 2016. Fish specimens were transported to the laboratory shortly after capture and identified using keys (Fischer, Bauchot, and Schneider 1987; Pollerspöck and Straube 2021, WoRMS 2023), measured, and sex determined.

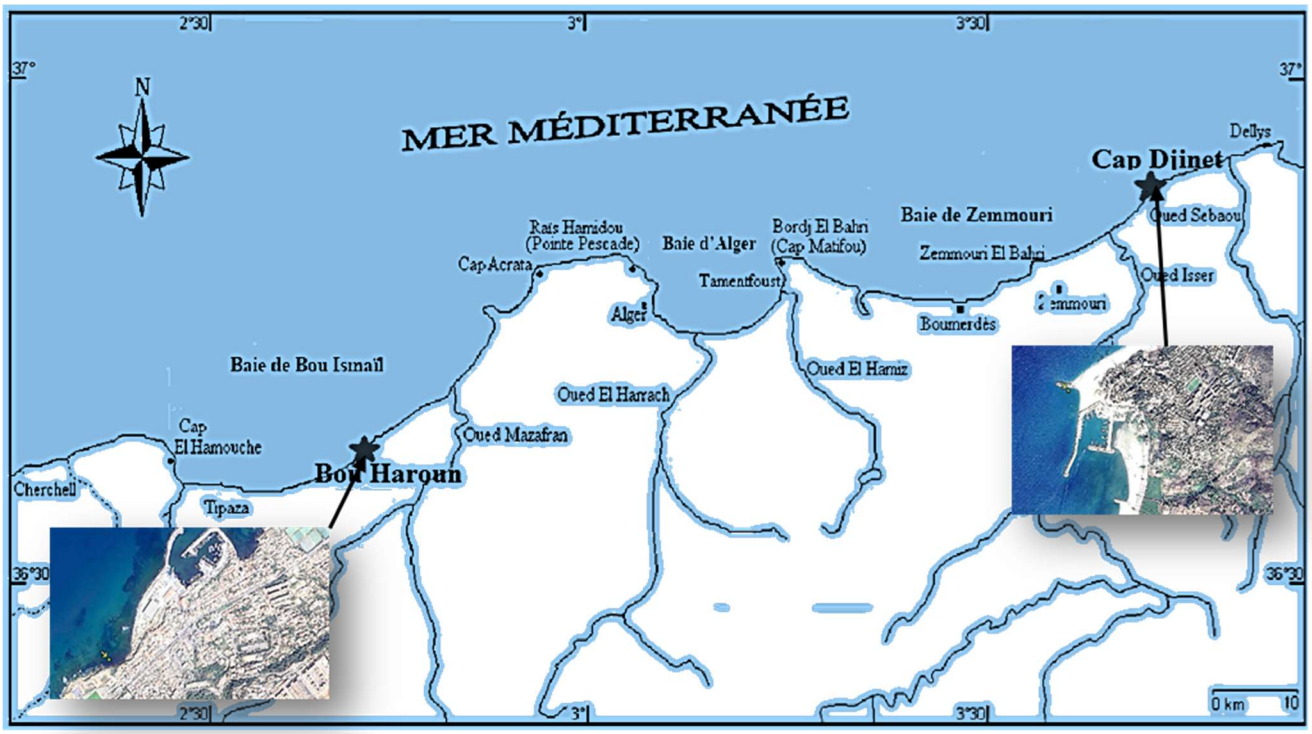

Figure 1. Geographic map of the location of the study regions.

- **Morphological methods**

Fish blood samples were collected by heart puncture in the laboratory and thawed aseptically. Thin and thick blood samples were prepared and examined for trypanosomes by using light microscopy. Thick smears were examined fresh, thin smears were air-dried, fixed with methanol, and stained with May-Grûnwald Giemsa and covered by a cover-slip with few drops of Eukitt mounting medium. Immunofluorescence microscopy was also performed to study the parasites (Ploubidou et al. 1999).

Microscopic images of trypanosomes were captured by using a Nikon optical microscope with a Nikon digital camera DXm1200C. Measurements were taken with the ImageJ software (http://imagej.nih.gov/ij/) (Schneider, Rasband, and Eliceiri 2012). Drawings were made with the help of an Olympus BH-2 microscope with a drawing tube. Drawings were scanned and redrawn on a computer with Adobe Illustrator CS5.1.

- **Statistical analysis**

Prevalences were calculated according to Bush et al. (1997) (Bush A. et al. 1997). A parametric test (Student's 't' test) was used to compare the data obtained for each fish species, sex, and sampling site. When the parameters for applying parametric tests were not met, the Mann-Whitney

U-test was utilized. The STATISTICA version 6.1 software was used to perform all statistical analyses.

3. RESULTS

This study was carried out with a sample of 69 skates belonging to Raja asterias (n = 39) and Raja miraletus (n = 30). We recorded trypanosome infection (Figure 2) with an overall prevalence of 24.64%, distributed according to the host species examined as 27.78% for Raja asterias and 21.21% for Raja miraletus (non-significant difference, P=0.5291). Prevalence comparison according between study sites (Cap Djinet 28.21% vs Bouharoun 20%) also indicates that there is a non-significant difference (P=0.4355).

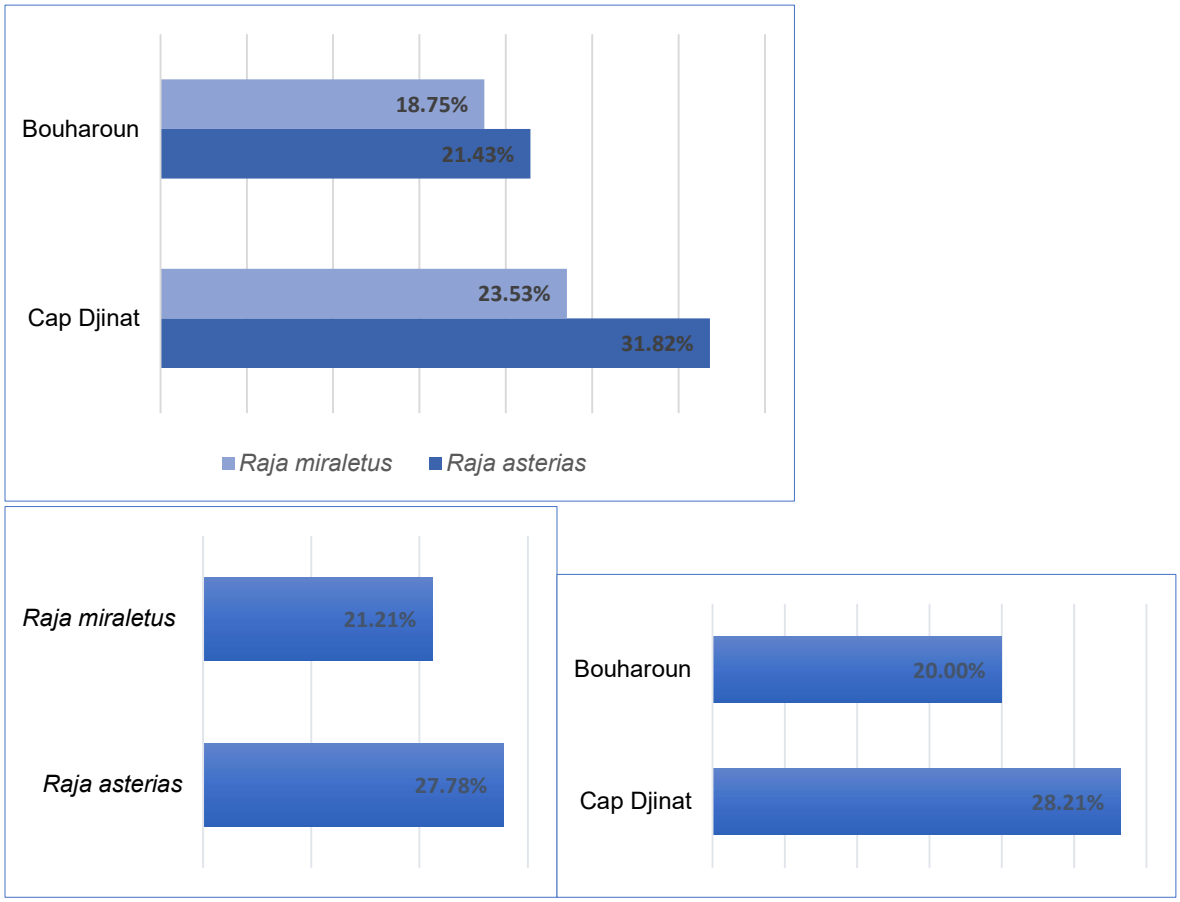

Figure 2. Parasite prevalences depending on the host species and the sampling site.

- **Description**

- **Trypanosoma from thick blood smears**

The body is elongated with very fast movements. Specimens often curl up on themselves, rapidly changing forms; sometimes taking on an S curves shape. The undulating membrane is bent from the anterior end to the posterior end. No nucleus or discernible granules were observed (Figure 3).

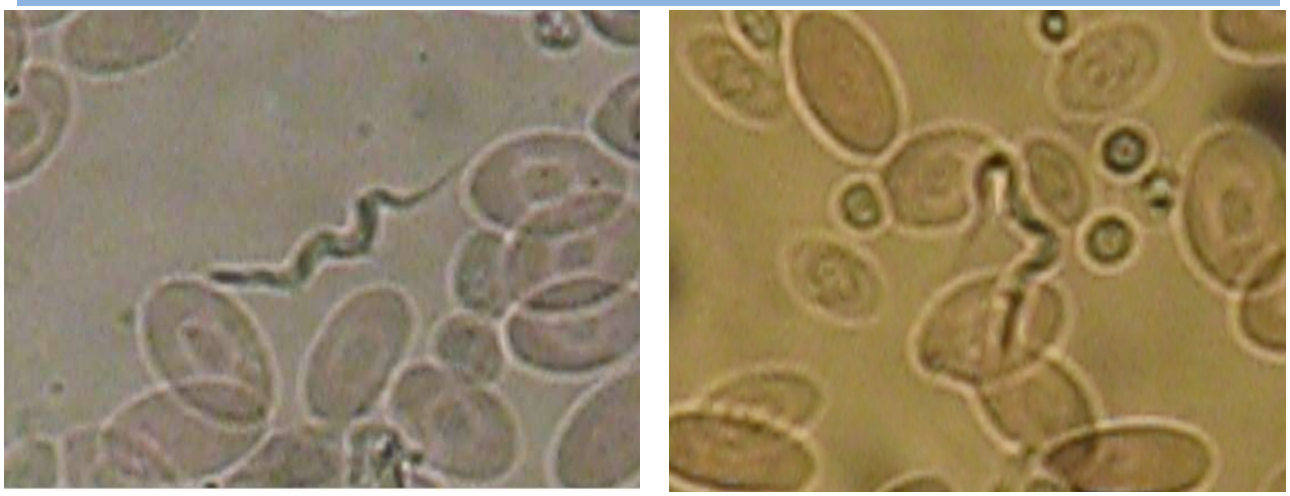

Figure 3. Living Trypanosoma observed in the thick drop under light microscope X40.

- **From stained blood smears**

The body appears thin to thick (Figures 4-5), elongated with a long flagellum, often coiled in spring-like form (Figure 5 A-B) and sometimes knotted or flexed. The cytoplasm is uniformly stained and contains fine chromatic granulations. The nucleus is rounded or oval, colored in purplish-pink, and sometimes occupies the entire width of the body. It is always located at the junction of the anterior and middle thirds parts of the body (Figures 4-5). The kinetoplast is small, rounded or stick-shaped, strongly colored, and located in the posterior third of the body near the posterior end. The posterior end exhibits variable shapes, tapering to conical (Figures 5-6). Although the posterior region is less stain than the rest of the body, a flagellar pocket is found anteriorly ahead of the kinetoplast. The flagellum borders the entire undulating membrane and ends with a free portion at the anterior end (Figure 6), being very thin in its free part as well as in the part bordering the undulating membrane.

Figure 4. Micrographic images of Trypanosoma from blood smears fixed with methanol and stained with MGG, in Raja asterias and Raja miraletus.

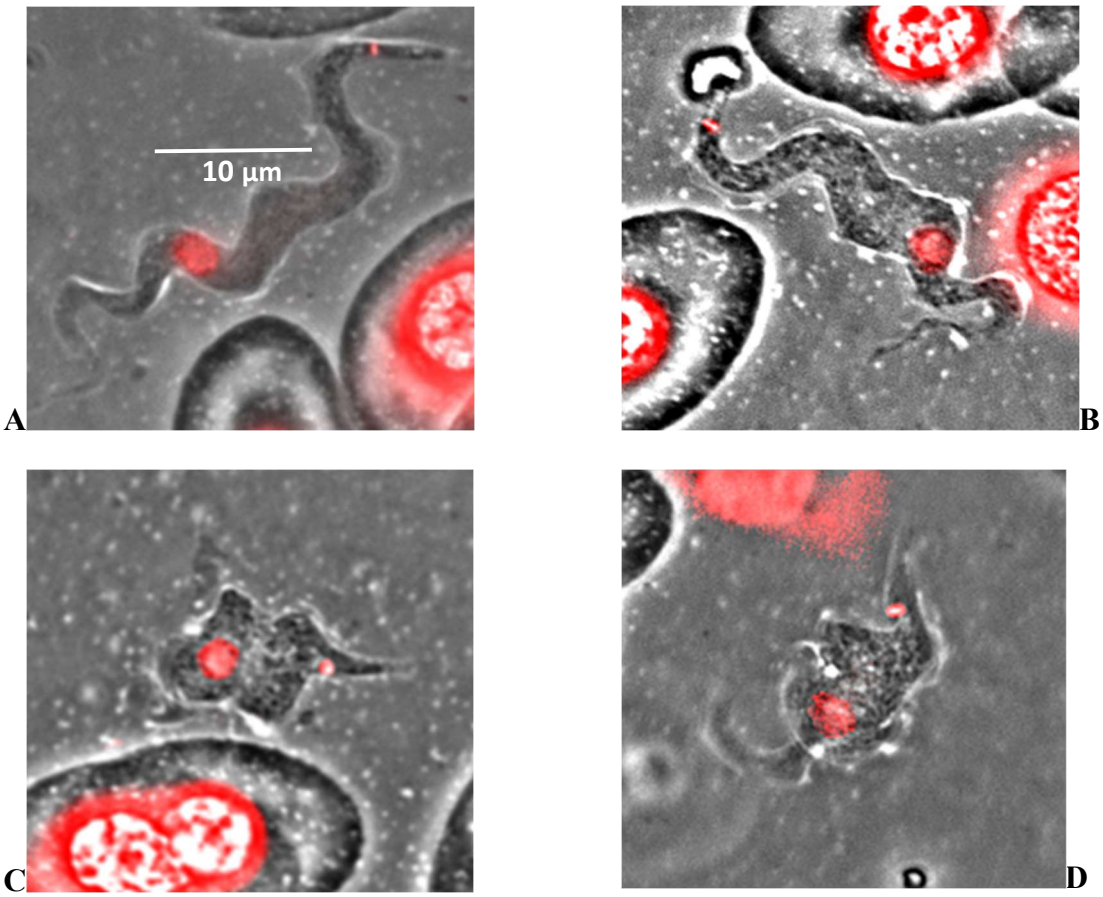

Figure 5. Phase contrast and DAPI fluorescence micrographs of Trypanosoma in blood of Raja asterias and Raja miraletus.

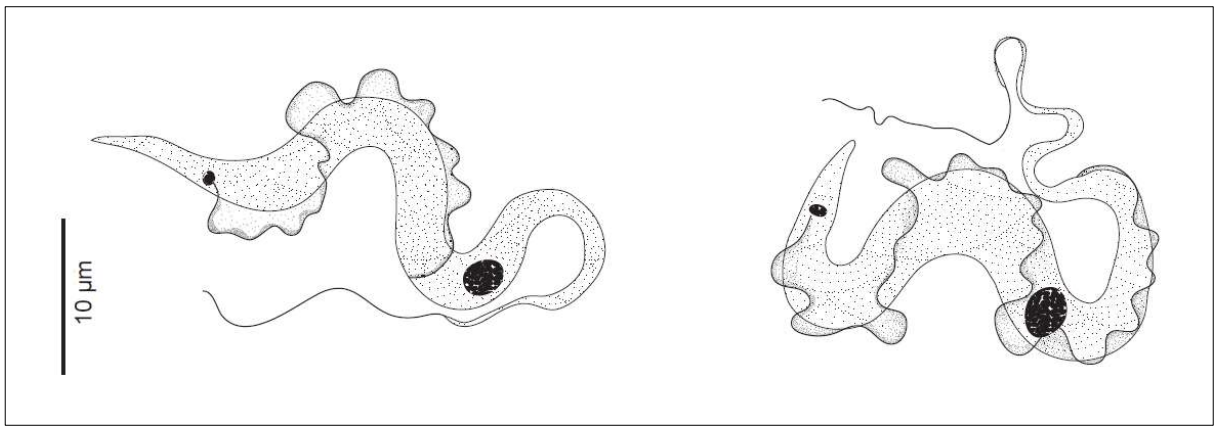

Figure 6. Trypanosomes from Raja miraletus and Raja asterias, drawn from thin blood smears stained with MGG.

- Systematic

Phylum: Euglenozoa Cavalier-Smith, 1981.

Class: Kinetoplastida Honigerg, 1963.

Order: Trypanosomatida Kent, 1880.

Family: Trypanosomatidae Dolflein, 1911.

Genus: Trypanosoma Gruby, 1843.

Trypanosoma rajae Laveran and Mesnil, 1902 (Laveran and Mesnil 1904)

Hosts: Raja punctate, Raja mosaica, Raja clavata and Raja microrynchus.

Infection site: peripheral blood and heart blood.

Vector: Pontobdella sp.

Voucher: not mentioned.

Type locality: Roscoff, France.

4. DISCUSSION

Trypanosoma rajae was first described by Laveran and Mesnil in 1904, based on observation of fresh blood and stained blood smears, it was rare or very rare, measuring 75µm to 80µm long, with a flagellum of about 20 µm in length. It is characterized by polymorphism of the tip of the posterior end (Figure 6). The cytoplasm is strongly stained in blue, containing fine chromophilic granulations, surrounding a rounded or oval nucleus, located in the anterior third of the body.

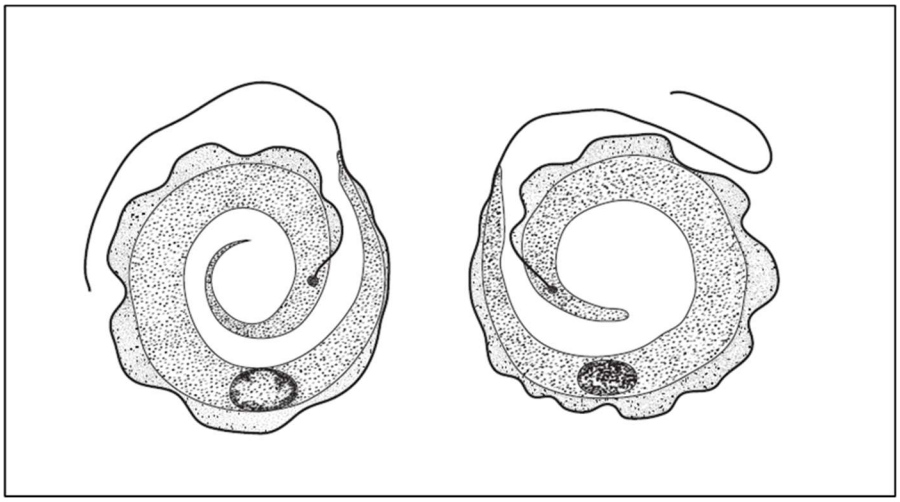

Figure 7. Trypanosoma rajae (Laveran and Mesnil, 1902) Laveran and Mesnil 1912 redrawn.

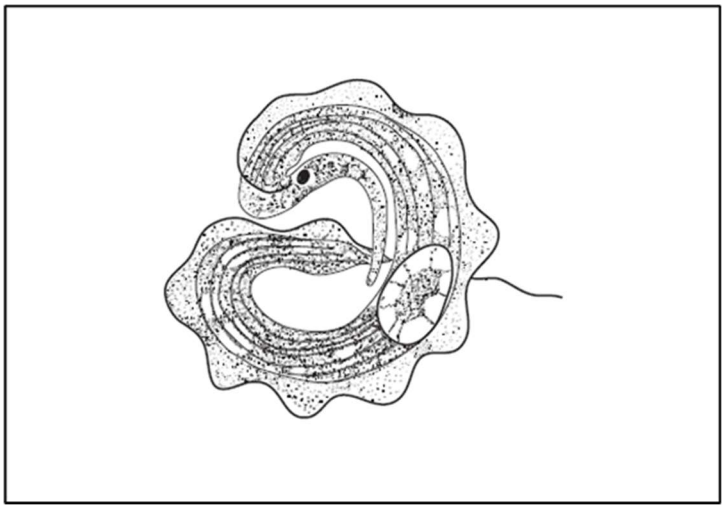

Figure 8. Trypanosoma rajae (Laveran and Mesnil, 1902) from Raia ocellata X 3200 (Kudo 1923) redrawn.

Table 1: Comparative Table of Measurements of Trypanosoma rajae (Laveran and Mesnil, 1902).

	Trypanosoma (present study)	Trypanosoma rajae (Kudo 1923)	Trypanosoma rajae (Laveran and Mesnil 1904)
Body Length	55.69 µm ± 0.56	30 µm – 35 µm	55 µm – 60µm
Free Flagellum Length	21.9 µm ± 0.85	6	20

Trypanosoma rajae Laveran and Mesnil 1902, the trypanosome form just described, was morphologically similar to and fell within the size ranges of Trypanosoma rajae Laveran and Mesnil 1902 and also was considered to be Trypanosoma rajae Laveran and Mesnil 1902.

Distinct myonemes run longitudinally (Figure 8), five or six being the usual numbers (Kudo 1923). In the present description and the original description of Laveran and Mesnil 1904 (Figure 7), myonemes were absent from the specimens observed.

Minchin and wood cock (1910) believe that Trypanosoma variabile is identical with Trypanosoma rajae which shows marked polymorphism (Kudo 1923; Laird and Bullock 1969). Trypanosoma rajae is a pleomorphic species, which means it can adapt to various environments and host

organisms (Khan, Lee, and Whitty 1991). This adaptability likely allows it to play a diverse ecological role in its habitat. For example, it could serve as a food source for other organisms, contribute to nutrient cycling, or play a role in the transmission of diseases.

The main measurements of Trypanosoma rajae in our study proved to be quite similar to those given in the original description, though somewhat different from the data published by other authors (Table 1). The morphology of Trypanosoma rajae is not explicitly described in the search results and is known for its pleomorphism, which has created a false sense of its true biodiversity (Pretorius et al. 2021).

However, the question that remains is whether our specimens from Raja asterias and Raja miraletus from Algeria are Trypanosoma rajae, described from a distinct host from a different genus (Dipturus batis) and from a different locality (off Roscoff, France, North East Atlantic).In the western Mediterranean and the western Baltic Sea; Raja clavata is known from the East Atlantic, the South West Indian Ocean (from Iceland to Madagascar) and in the Mediterranean Sea; Raja miraletus is distributed in the East Atlantic as well (from the northern Portugal), throughout the Mediterranean to Madeira and South Africa and also in the southwestern part of the Indian Ocean; Raja asterias is found in East Atlantic. Although endemic to the Mediterranean, Raja asterias likely occurs in the Strait of Gibraltar, northern Morocco and southern Mauritania. This could facilitate a host switch of this Trypanosoma rajae. Moreover, leeches are known for their lack of host specificity. While it is known to inhabit marine, brackish, fresh, and terrestrial environments, the specific ecological role and impact of this species are not clearly defined (Baker 1982).

Research on the potential ecological impact of Trypanosoma rajae is limited, and there is a lack of information regarding its interactions with other organisms and its overall influence on the ecosystem.

Further research is needed to fully understand the life cycle of Trypanosoma rajae and its specific stages in different hosts and environments. The number of trypanosomes transmitted is of little importance for the further development of the parasitosis, because further multiplication of the parasites takes place in the fish (Foin 2005). In vitro cultivation of Trypanosoma rajae has been studied, as reported in a 1966 article by T.M. Preston (Preston 1966). This study aimed to develop a method for cultivating the species in the laboratory.

5. CONCLUSION

This study aimed to address the knowledge gap surrounding Trypanosoma rajae and its related trypanosomes. The specimens examined were initially categorized as Trypanosoma rajae pending further investigation. the reasons mentioned above until further investigation was conducted.

Our findings indicate that parasitism does not exhibit significant differences between sampling sites or host species. This underscores the importance of exercising caution when describing new trypanosome taxa, considering factors such as new hosts, geographical distributions, and unique

morphological characteristics. Further research is warranted to deepen our understanding of the diversity, ecology, and impact of trypanosomes in marine environments.

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