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Hematodinium perezi (Dinophyceae: Syndiniales) in Morocco: The First Record on the African Atlantic Coast and the First Country Record of a Parasite of the Invasive Non-Native Blue Crab *Callinectes sapidus*

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Abstract: Dinoflagellates belonging to the genus *Hematodinium* are key parasites of marine crustaceans, primarily decapods. In this study, we document the first report of *H. perezi* Chatton & Poisson, 1930 on the African Atlantic coast. This is also the first parasite record in the invasive non-native Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 in Morocco. Specimens of *C. sapidus* were sampled in winter 2023 from two Ramsar sites on the Moroccan Atlantic, namely Merja Zerga and Oualidia Lagoons, and were screened to detect the presence of parasites in their hemolymph. Based on staining fresh hemolymph smears, we did not detect *Hematodinium* in any of the 36 investigated individuals (20 and 16 from Merja Zerga and Oualidia Lagoons, respectively), probably due to methodological artifacts. The PCR-based method was revealed to be more accurate in diagnosing the *Hematodinium* parasite. It showed that at Merja Zerga Lagoon, 13 individuals of *C. sapidus* were infected by the parasite (prevalence: 65%) in comparison to four at Oualidia Lagoon (25%). Genetic analysis, based on the ITS1 rDNA gene from *Hematodinium*, confirmed the sequences as being those of *Hematodinium perezi*.

Keywords: bioinvasion; parasites; ITS1 rDNA; coastal lagoon; North Africa

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1. Introduction

Biological invasions are considered to be a severe threat to marine biodiversity and the functioning of invaded coastal and marine ecosystems [1]. In addition, their ability to carry invasive and/or enhance native parasites can induce a loss of native biodiversity and an increase in disease and mortality in native species, which may pose risks to human health and the economy [2–4]. The establishment of introduced hosts and their parasites may also affect the life cycles of native parasites. On the other hand, native hosts can be infected by parasites associated with introduced species, i.e., 'host switching' (more specifically, spill-over) [3,5–8]. For example, the invasive nematode *Spirocamallanus istiblenni* Noble, 1966, was introduced to the Hawaiian archipelago along with *Lutjanus kasmira* Forsskål, 1775 from French Polynesia, and this introduction led to the spread of the nematode into native hosts [9]. The monogenean *Nitzschia sturionis* (Abildgaard, 1794) Krøyer, 1852 was co-introduced with *Acipenser stellatus* Pallas, 1771 from the Caspian Sea to the Aral Sea

and induced severe mass mortalities of the native bastard sturgeon *Acipenser nudiventris* Lovetsky, 1828. Once infested by the parasite in its gills, the fish ends up dying on the beach [10]. The eel nematode *Anguillicola crassus* Kuwahara, Niimi & Itagaki, 1974 has infected seven different eel species on four different continents via the global eel trade, causing severe damage to the swim bladder of the eels, resulting in significant mortality in most eel species [11–14]. The rhizocephalan barnacle *Loxothylacus panopaei* Gissler, 1884, which infects native flatback mud crabs *Eurypanopeus depressus* Smith, 1869, was probably co-introduced with infected mud crabs (species unknown) in batches of oysters transferred from the Gulf of Mexico to Chesapeake Bay (North America) [15].

The Atlantic blue crab *Callinectes sapidus* Rathbun, 1896, belonging to Portunidae Rafinesque, 1815, is native to the western Atlantic Ocean [16,17]. Its natural geographical distribution ranges from Nova Scotia, Canada, down the eastern coast of the United States, and as far south as Northern Argentina [16,18,19]. This species was accidentally (with water ballast being the most likely introduction vector [16,20]) or intentionally introduced into Asia, Europe and Africa, and this widened its current worldwide biogeographic distribution. The first record from European Seas occurred in 1900 along the Atlantic coast of France [21]. In the Mediterranean Sea, the first presence of *C. sapidus* was reported in Italy in 1949 [20]. Now, the species is present all over the Mediterranean and the Black Sea [16,22–24], where it is ranked among the most invasive species [25]. It was recorded in 2022 in a freshwater ecosystem in the Mediterranean area, in Sicilian inland waters [26]. In Morocco, *C. sapidus* was first reported on its Mediterranean coast in the Marchica Lagoon in 2017 [27], while its first record in the Moroccan Atlantic was in the Merja Zerga Lagoon in 2019 [28]. The species is now established in many localities along the Mediterranean and Atlantic coasts of Morocco [28–30]. In native habitats, *Callinectes sapidus* is known to host a wide range of pathogens, including viral, bacterial and microalgal agents [31], which have been implicated in causing diseases and mortalities or reducing fecundity [32]. While *C. sapidus* constitutes a relevant model for studying invasions related to the spread of microorganisms along invaded Mediterranean and north-eastern Atlantic coasts, scientific research on the species concerns mostly its population dynamics, structure, and fisheries [33]. The occurrence of parasites in the species has been explored very little, even though it is an appreciated shellfish product in many countries such as Greece, Turkey, Italy, and Egypt [34].

Among pathogens of *Callinectes sapidus*, the parasite *Hematodinium perezi* (Dinophyceae: Syndiniales: Syndiniaceae) is an important disease-causing agent infecting over 40 species of crustaceans worldwide [35–37]. This parasite causes the so-called bitter crab disease in a number of species of crab such as *Chionoecetes opilio* Fabricius, 1788 and *Chionoecetes bairdi* Rathbun, 1924 [38], resulting in a crab meat flavour resembling bitterness with an aspirin-like taste due to biochemical alterations [35]. Also, the *Hematodinium*-infected hosts generally exhibit tissue or organ dysfunction or failure, as well as mortality in the later stages of infection, due in part to the enormous multiplication of parasites in the hemolymph of affected tissues [39–41]. In the Atlantic blue crab, *H. perezi* does not directly induce discernible biochemical alterations, but it can severely impact host health by inducing critical tissue or organ dysfunctions, ultimately resulting in high mortality rates [42].

Hematodinium perezi is currently the only confirmed *Hematodinium* species known to be infecting *Callinectes sapidus* [43]. The other representative of this genus, namely *Hemtodinium* sp., was reported as a parasite of *C. sapidus* from many coastal areas in USA) [44–53], and in Turkey [54]. This one, infecting boreal hosts, particularly *Chionoecetes opilio* and *Nephrops norvegicus* (Linnaeus, 1758), has not been identified at the species level yet (Shields J.D., *pers. comm.*).

Hematodinium perezi was first described from *Carcinus maenas* Linnaeus, 1758 and *Liocarcinus depurator* Linnaeus, 1758 on the French coastline [55]. Since then, records on the number of host species and distribution have notably increased [56]. Gallien [57] reported its spread in the French host *Portunus latipes* Pennant, 1777. In the Mid-Atlantic, it showed

rare infections in *Cancer irroratus* Say, 1817 and *Cancer borealis* Stimpson, 1859, and in *Ovalipes oceallatus* Herbst, 1799 from the New York Bight area of the Northeastern United States [58]. *Hematodinium perezi* was documented in *Callinectes sapidus* for the first time in coastal Maryland and Virginia, USA [59], and afterwards in many habitats in the USA [49,60–64]. In the Mediterranean and north-eastern Atlantic, regions where *C. sapidus* invaded, *H. perezi* was reported as an endoparasite of *C. sapidus* in the Eastern Mediterranean, Greece, [34] and now also on the African Atlantic coast (this study) (Figure 1).

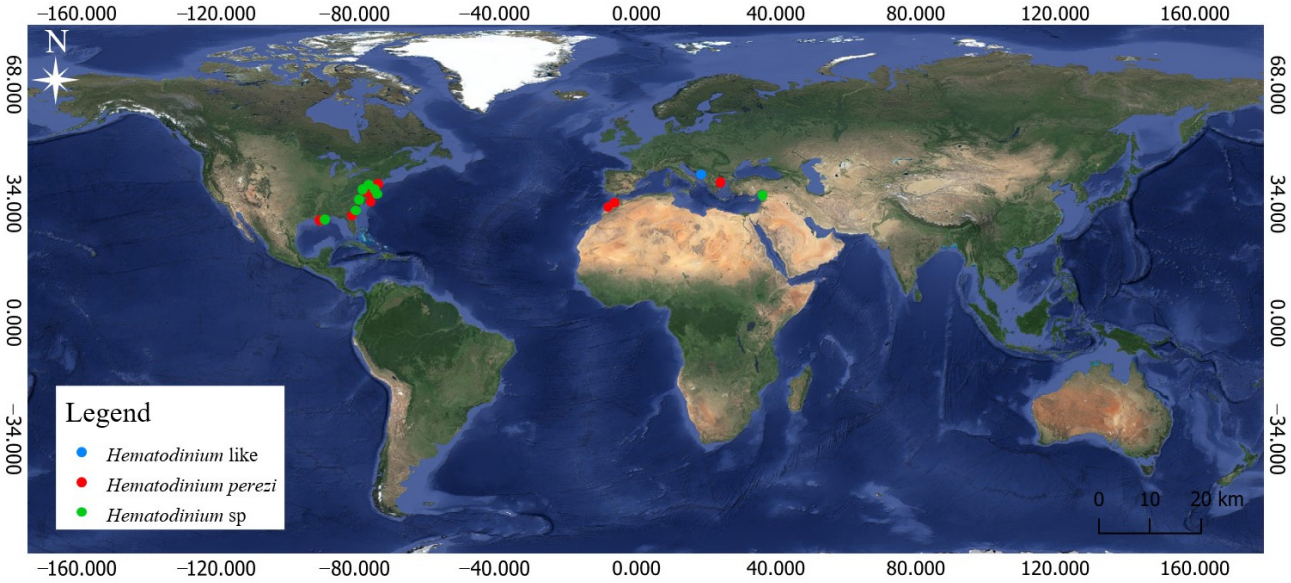

Figure 1. Map showing the distribution of reported representatives of *Hematodinium* infecting *Callinectes sapidus* (see also [34,65]).

In this study, the hemolymph of *Callinectes sapidus* specimens from Merja Zerga and Oualidia Lagoons, located on the Atlantic coast of Morocco, was screened to detect the presence of parasites. Here, we document the first detection of dinoflagellates belonging to *Hematodinium* in *C. sapidus* on the African Atlantic coast.

2. Materials and Methods

2.1. Study Sites

Merja Zerga and Oualidia Lagoons are two semi-enclosed coastal systems (SECS) situated along Morocco's Atlantic coast (Figure 2). Both sites are recognized as Sites of Biological and Ecological Interest (SIBEs) [66] and Ramsar sites as Wetlands of International Importance.

The Merja Zerga Lagoon (34°47′ N, 6°13′ W) is an elliptically shaped lagoon that is 9 km long and 5 km wide, with a depth from 0.50 to 1.50 m and a total surface of 35 km^2 [67]. The lagoon is connected to the ocean through a relatively deep gully (up to 6 m), and the circulation of sea water during flood and ebb is ensured by shallow subtidal channels. The freshwater supply is provided by Oued Drader and Canal of Nador [68]. Tides are semi-diurnal, with an average amplitude of 0.15 to 1.50 m [69]. Salinity in this lagoon fluctuates between 8 PSU and 36 PSU, with the mean water temperature varying between 14.6 °C and 24.15 °C [68].

The Oualidia Lagoon (32°74′ N, 9°03′ W) is over 7 km long and 1 km wide, with a mean depth of 2 m and a total surface of 3 km^2 [67]. This coastal basin takes the form of an elongated depression oriented east–north-west, bordered by a coastal consolidated dune ridge and a continental cliff [70]. Tides are semi-diurnal, with amplitudes ranging from 0.8 to 3.6 m [71]. The average water temperature ranges from 16.1 °C to 21.1 °C, and the lagoon's salinity varies between 20 PSU and 35 PSU at low tide, while at high tide, it can reach 30 PSU to 36 PSU throughout the year [72].

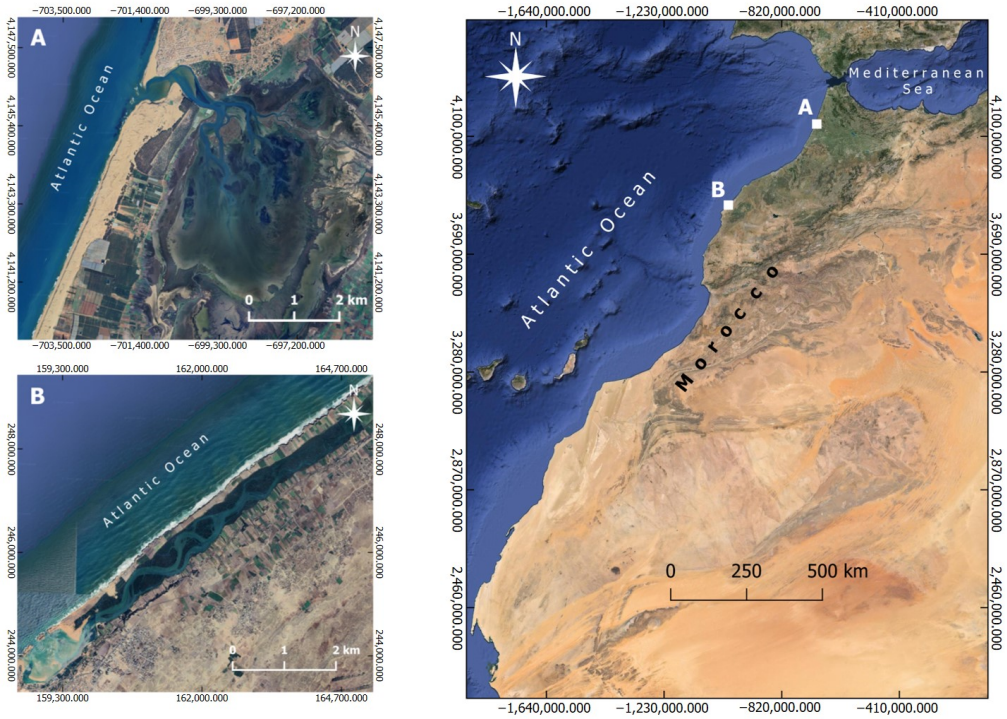

Figure 2. Map showing the localization of Merja Zerga (**A**) and Oualidia (**B**) Lagoons on the Moroccan Atlantic.

2.2. Sampling and Microscopic Analysis

Specimens of *Callinectes sapidus* were sampled from Merja Zerga (February 2023) and Oualidia (March 2023) Lagoons using a seine net. Collected crabs were transferred to the laboratory in refrigerated containers. Before dissection, we soaked each crab individually in ice water for about 15 to 20 min, depending on the size of the individuals, to induce anaesthesia. The captured crabs were identified based on morphological criteria (shape and colour of the carapace) according to Williams [18] and numbered. For each crab, sex and maturity were determined, and then, carapace length (CL), carapace width (CW) and fresh weight (W) were measured.

The hemolymph was extracted from each specimen (based on dorsal view) at the uncalcified joint of the right swimming leg near the carapace. For complete sterilisation, the leg was sterilized twice with a 70% ethanol-soaked cotton swab. A disposable 1 mL syringe coupled to a 26 g needle was inserted into the leg. The hemolymph of each specimen was analysed by the preparation of wet smears; one drop of hemolymph was mixed (1:1) with 0.3% neutral red solution on a glass slide and directly observed under an optical microscope Leica® DM 2500 (sourced from Leica Microsystems, Wetzlar, Germany). Hemolymph (0.1 mL) was also collected and placed in EDTA tubes containing 1 mL of 95% ethanol and frozen at −20 °C for DNA extraction to detect the presence of *Hematodinium* by polymerase chain reaction (PCR).

2.3. DNA Extraction, Amplification and Sequencing

Before extracting DNA, 200 µL of ethanol-preserved hemolymph was centrifuged at 1500× *g* for 1 min to eliminate excess ethanol [44]. In order to allow residual ethanol to evaporate, samples were dried for at least 30 min at 55 °C [44]. The Invitrogen TM Kit Blood and Tissue kit (sourced from Thermo Fisher Scientific, Waltham, MA, USA) was used to extract DNA as recommended by the manufacturer, with an overnight lysis of the hemolymph samples and two 5 min elution incubations and two 50 µL elutions.

For amplification, HITS1F (5′CATTCACCGTGAACCTTAGCC3′) and HITS1R (5′CTA GTCATACGTTTGAAGAAAGCC3′) primers that target the ITS1 rDNA were used according to Gruebl [45], with the expected length of 299 bp [46].

Each amplification was carried out in a final volume of 25 µL containing 5X standard *Taq* (Gquence) reaction buffer, 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 0.5 µM HITS1F, 0.5 µM HITS1R, 1 unit of Platinum™ *Taq* DNA Polymerase, 50 ng of extracted DNA [44] and 16.2 µL of ddH_2O water. Amplification reactions were performed in a thermal gradient PCR MultiGene OptiMax Thermal Cycler (sourced from Labnet International, Edison, NJ, USA) according to the following program: 95.0 °C for 10 min; 40 cycles of 94.0 °C (30 s), 56.0 °C (30 s), 72.0 °C (1 min), and a final extension at 72.0 °C for 10 min. Amplified products were separated by 1% agarose gel electrophoresis stained with ethidium bromide. Positive PCR products were sent to the National Centre for Scientific and Technical Research (CNRST) in Rabat for purification and sequencing. The sequencing was carried out using a Genomix sequencer (MGX) with identical primers as used in the initial PCR.

2.4. Sequence Analysis

Using MEGA version XI [73], the obtained sequences were manually cleaned and aligned with the software ClustalW version 2.1 [74]. The resulting sequences were compared to genetic data previously published by the Basic Local Alignment Search Tool (BLAST) (sourced from the National Center for Biotechnology Information (NCBI), Bethesda, MD, USA) [75]. The uncorrected genetic p-distance between Moroccan sequences and all published sequences downloaded from GenBank were calculated using MEGA version XI (sourced from the MEGA Development Team, Tempe, AZ, USA). Using the same software, the optimal model of molecular evolution based on the Akaike information criterion (AIC) was the Jukes–Cantor model [76]. Maximum likelihood (ML) with Nearest-Neighbour Interchange (NNI) as a branch swapping algorithm and neighbour-joining (NJ) phylogenetic trees based on the unique haplotypes of ITS1 rDNA were constructed with 1000 bootstrap replicates using MEGA software version XI.

Sequences generated from this study were deposited in GenBank under accession numbers PP928476–PP928480 and PP933794–PP933803.

3. Results

3.1. Biometric Characteristics of Analysed Specimens of Callinectes sapidus

Overall, 36 specimens of *Callinectes sapidus* were collected in winter 2023. The twenty specimens from Merja Zerga Lagoon comprised five adult females, five adult males, five female juveniles and five male juveniles. Among the sixteen specimens from Oualidia Lagoon, there were four adult females, three adult males, five female juveniles and four male juveniles. Their biometric data are summarized in Table 1.

Table 1. Biometric measurements of specimens of *Callinectes sapidus* from Merja Zerga and Oualidia Lagoons on the Moroccan Atlantic. CL: carapace length; CW: carapace width; W: body weight; SD: standard deviation.

Lagoon	Sampling Period	Number of Individuals	W (g)		CW (cm)		CL (cm)	
			Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD
Merja Zerga	February 2023	20	4.6–237.5	88.9 ± 78.7	3.1–14.8	9.2 ± 4.1	2.2–6.6	4.6 ± 1.8
Oualidia	March 2023	16	18.8–207	57.0 ± 52.4	5.8–14.9	8.6 ± 2.6	3.1–7.4	4.5 ± 1.4

3.2. The Hemolymph Smear Assay with Neutral Red

Based on staining fresh hemolymph smears, we did not detect *Hematodinium* in any of the 36 investigated individuals (20 and 16 from Merja Zerga and Oualidia Lagoons, respectively) sampled in winter 2023.

3.3. PCR-Based Method and Sequence Analysis

Overall, 17 samples out of the 36 individuals investigated were successfully amplified, from which 13 specimens were revealed to be infected by the parasite at Merja Zerga Lagoon (prevalence 65%) and 4 at Oualidia Lagoon (25%).

The 15 ITS1 rDNA sequences that were generated (13 sequences from Merja Zerga Lagoon and 2 from Oualidia Lagoon) produced an alignment of a 295 bp long fragment. The ITS1 rDNA sequences compared, using BLAST search, to existing ones available in the GenBank database confirmed the identification of our parasites' sequences as being those of *Hematodinium perezi*.

The uncorrected p-distance between Moroccan sequences varied between 0% and 0.6%, and the uncorrected p-distances between Moroccan sequences and published sequences downloaded from GenBank [34,43,77] (Table 2) varied between 0.2% from *Hematodinium perezi* in *Callinectes sapidus* collected in Greece and 4% from *H. perezi* in *C. sapidus* from the United States of America (Table 3).

Table 2. List of *Hematodinium perezi* sequences used in the present study, their GenBank accession numbers, host species, localities and references.

GenBank Accession Number	Host	Locality	References
PP056127	*Callinectes sapidus* (Portunidae)	Greece	[34]
EF065716	*Liocarcinus depurator* (Polybiidae)	South Coast of England	[43]
EF065708	*Liocarcinus depurator*	South Coast of England	[43]
EF065711	*Liocarcinus depurator*	South Coast of England	[43]
KX244637	*Portunus trituberculatus* (Portunidae)	China	[77]
KX244644	*Portunus trituberculatus*	China	[77]
KX244641	*Portunus trituberculatus*	China	[77]
KX244634	*Callinectes sapidus*	USA	[77]

Table 3. Range of uncorrected pairwise genetic distances (p-distances in %) between ITS1 rDNA sequences of *Hematodinium perezi* infecting *Callinectes sapidus* collected from Morocco and all published sequences from GenBank.

	Merja Zerga	Oualidia	South Coast of England	China	USA	Greece
Merja Zerga Lagoon	-	-	-	-	-	-
Oualidia Lagoon	0–0.6%	-	-	-	-	-
South Coast of England EF065716, EF065708, EF065711	0.6–1.3%	0.6–1.3%	-	-	-	-
China KX244637, KX244644, KX244641	1.7–2.3%	1.7–2.3%	1–1.7%	-	-	-
USA KX244634	3.7–4%	3.7–4%	3.6–4%	3.7–4%	-	-
Greece PP056127	0.2–1.3%	0.2–1.3%	0.3–0.6%	1.3–1.7%	3.4%	-

Maximum likelihood (ML) and neighbour-joining (NJ) phylogenetic trees were topologically identical. Statistical support for most nodes was low, though the topology suggests that the Moroccan sequences were more related to those from Greece (Figure 3).

The measurements of specimens of *Callinectes sapidus* parasitized by *Hematodinium perezi* (as identified using PCR) from the Merja Zerga and Oualidia Lagoons of Morocco are reported in Table 4. All four groups (male adult, female adult, male juveniles and female juveniles) were parasitized. Moreover, females (adults and juveniles) were most likely affected in the Merja Zerga Lagoon, while in the Oualidia Lagoon, the infected crabs were mostly juveniles.

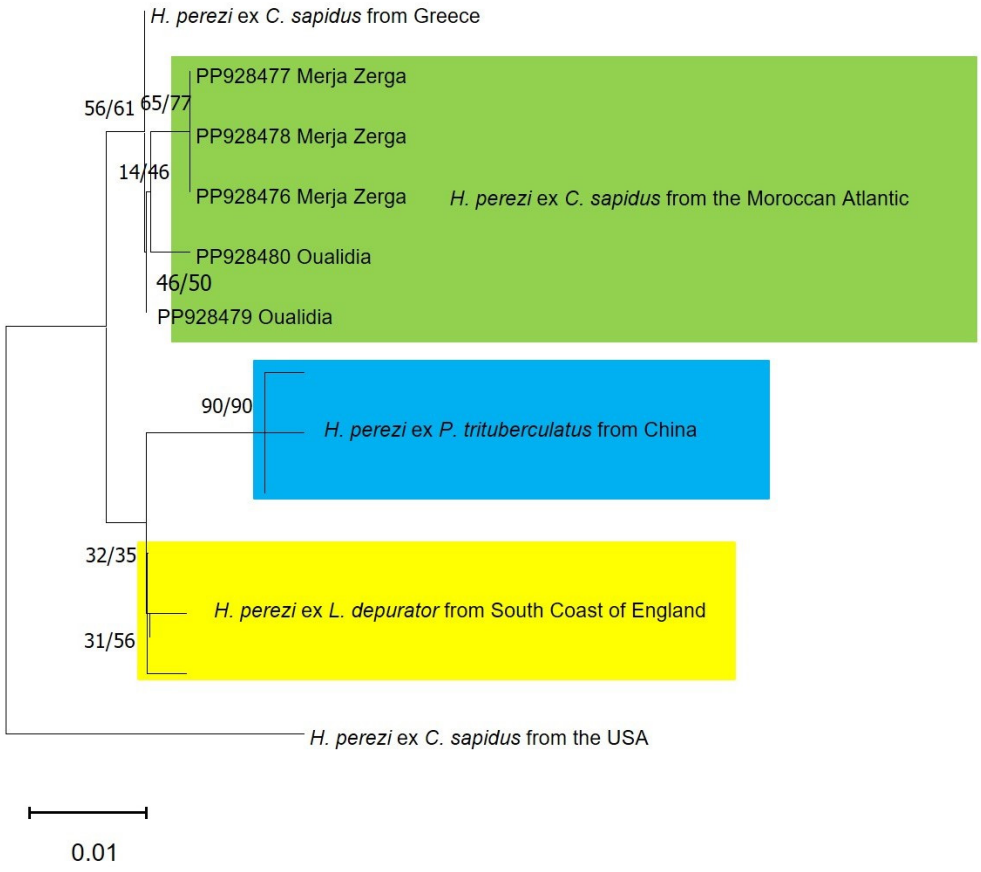

Figure 3. Phylogram constructed using maximum likelihood (ML) and neighbour-joining (NJ) methods based on ITS1 sequences of *Hematodinium perezi*. Bootstrap support from 1000 replicates is shown based on the ML method (before slash) and on the NJ method (behind slash). ML and NJ trees are topologically identical, and it is the ML tree that is shown here (midpoint rooted). The scale bar represents the number of expected substitutions per site.

Table 4. Biometric measurements of specimens of *Callinectes sapidus* parasitized by *Hematodinium perezi* from Merja Zerga and Oualidia Lagoons on the Moroccan Atlantic. CL: carapace length; CW: carapace width; W: body weight; SD: standard deviation.

Lagoon	Groups	Total Individuals	W (g)		CW (cm)		CL (cm)	
			Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD
Merja Zerga	male adult	2	159.2–175.7	167.4 ± 11.6	11.7–12.1	11.9 ± 0.2	6.2–6.5	6.3 ± 0.2
	female adult	5	109.9–153.1	131 ± 18.2	12.5–13.3	12.9 ± 0.3	5.8–6.3	6.1 ± 0.2
	male juveniles	1	4.6		3.1		2.2	
	female juveniles	5	10.4–22.2	17.0 ± 5.0	4.3–6.4	5.4 ± 0.9	2.6–3.2	3.0 ± 0.3
Oualidia	male adult	1	70.1		9.7		5.0	
	female adult	0	-	-	-	-	-	-
	male juveniles	2	21.6–30.5	25.8 ± 6.6	6.2–7.1	6.6 ± 0.6	3.3–3.7	3.5 ± 0.3
	female juveniles	1	18.7		6.1		3.1	

4. Discussion

The present study documents the first detection of *Hematodinium perezi* (Dinophyceae: Syndiniales) on the African Atlantic coast and also represents the first report of this (or any) parasite in the invasive non-native crab *Callinectes sapidus* in Morocco, namely in the Merja Zerga and Oualidia Lagoons.

Seasonal, sex- and size-related relationships or correlations have been reported between *Hematodinium* species and their hosts ([78] and the references herein). In this study,

specimens of *Callinectes sapidus* were collected in winter 2023, and females and juveniles seemed more likely to test positive for *Hematodinium perezi*, respectively, in the Merja Zerga and Oualidia Lagoons. Notwithstanding, the sampling effort in this study was small in terms of both time and space. Our ongoing in-depth research will enable us to better define the key drivers of *C. sapidus* infection by *H. perezi* in its area of introduction, in particular on the Mediterranean and Atlantic coasts of Morocco.

Because of its wide host range and capacity to transition between different host species, *Hematodinium* is regarded as a generalist parasite [35,43,56]. This trait enables it to persist in the environment, even in situations when its preferred host may become rare. Positive infections of *Hematodinium* were reported in 13 crustacean species belonging to two orders, Decapoda and Amphipoda [65]. The epidemiology of *Hematodinium* is influenced by a number of variables, including environmental factors (salinity and temperature). It is well known that this parasite prefers to infect hosts in highly saline waters [79]. For example, in Europe (Wadden Sea), no detection of *Hematodinium* in 1252 individuals of eight crustacean species from six sites was reported due to lower salinity [65]. Epidemics of these parasites have damaged commercial stocks of *Nephrops norvegicus*, *Chionoecetes opilio*, *Chionoecetes bairdi* Rathbun, 1924, *C. sapidus* and *Necora puber* (Linnaeus, 1767) [35]. Moreover, their impact on fisheries and host populations is thought to be similar to that of viral diseases of crustaceans [43], resulting in significant mortality in the host [80]. In the present research, the diagnosis of infection by representatives of *Hematodinium* in *Callinectes sapidus* was performed using the fresh hemolymph smear essay with neutral red and molecular analysis (PCR-based method and sequencing). The hemolymph smear assay with neutral red was used as an initial assessment tool due to its cost-effective and time-efficient diagnostic method for the detection of members of *Hematodinium* [65] as well as its specificity and sensitivity [81]. *Hematodinium* lysosomes actively absorb neutral red, producing a distinctive stain that visually contrasts with host hemocytes [35]. In our case, based on staining fresh hemolymph smears, we did not detect *Hematodinium* in any of the 36 investigated individuals (20 and 16 from the Merja Zerga and Oualidia Lagoons, respectively), probably due to methodological artifact. Indeed, as smears are rated positive when abnormal cells (i.e., cells that cannot be identified as crab hemocytes, but have certain characteristics corresponding to those of *Hematodinium*) are observed, expertise in parasite identification is required [82]. Some pathogens of crustaceans such as parasitic dinoflagellates and rhizocephalans may be more difficult to identify for the non-specialist [83]. Moreover, certain stages of parasites belonging to *Hematodinium* can be very difficult to detect in fresh hemolymph smears because the trophic stages resemble hemocytes. The vermiform plasmodium (cf. filamentous trophont) is the most straightforward form to identify, while the most frequently observed form (the vegetative, amoeboid stage) is easily confused with a hemocyte by the uninitiated, and they may be present at relatively low densities, making microscopic diagnosis difficult [35,50].

The use of molecular analysis is increasingly widespread in disease diagnosis, pathogen identification and monitoring, as well as the detection of cryptic organisms such as dinoflagellates and parasitic stages [45,84–86]. The PCR-based method offers 1000 times higher sensitivity compared to histology approaches [45]. The sensitivity of PCR diagnosis has been estimated at 1 parasite cell in 300,000 crab hemocytes [45]. Use of the PCR test eliminates the need for the visual identification of cells with ambiguous characteristics. Overall, a combination of morphological and molecular characterization is often used to ensure the accurate detection and monitoring of *Hematodinium* infections in crustacean populations, because PCR results simply indicate the presence of parasite genetic material. To confirm active infection or disease, it would be necessary to detect live parasite cells or clinical signs of infection by morphological characterization.

The PCR assay, adopted in our study to detect the *Hematodinium* infecting *Callinectes sapidus*, based on the amplification of the parasite's first internal transcribed spacer region (ITS1), was developed by Small et al. [50]. The difference in parasite prevalence between the Merja Zerga (65%) and Oualidia Lagoons (25%) could be explained by environmental

factors [47,65,80] or biological factors [53,87]. According to Barbosa et al. [80], several studies have shown that temperature and salinity conditions favour the invasion of *Hematodinium*. The prevalence of *Hematodinium* decreases at lower temperatures [80], and lower salinity can also limit the distribution of the parasite [65]. For example, the highest prevalence (69%) of the parasite in *C. sapidus* collected from the USA was found at salinities of 26 PSU to 30 PSU and a water temperature >25 °C, and no infected crabs were found below 11 PSU salinities [45]. *Hematodinium* may also be more prevalent because of the wide range of environmental reservoirs and high densities of hosts [87], and the absence of host immunological response can also be the reason of the high prevalence of *Hematodinium* in crustaceans [87]. Furthermore, according to Parmenter et al. [53], additional factors showing temporal or geographical variation may contribute to varying levels of *Hematodinium* infection in *C. sapidus*.

Calculated pairwise uncorrected p-distances from sequences of *Hematodinium perezi* parasitizing *Callinectes sapidus* in Morocco and all published sequences of *H. perezi* obtained from GenBank show that Moroccan sequences are closely similar to that from the host *C. sapidus*, collected from Greece (A.N., PP056127), and to those from *Licocarcinus depurator* collected from the South Coast of England (A.N., EF065716, EF065711, EF065708), and differ more from the sequence from *C. sapidus* collected from the USA (A.N., KX758132). The mean uncorrected p-distances between Moroccan sequences and sequences having an A.N of KX244637, KX244644, or KX244641 from *Portunus trituberculatus* Miers, 1876 from China is 1.8% (Table 3). According to Small et al. [43], the mean interspecific genetic distances between *H. perezi* from *L. depurator* and the *Hematodinium* sp. infecting *P. trituberculatus* and *Scylla serrata* Forskål, 1775 is 2.5% and 4.6% between *Hematodinium* sp. from *C. sapidus* and *H. perezi* from *L. depurator*. In our study, the mean intraspecific genetic distances between *H. perezi* from *C. sapidus* collected from Morocco and all published sequences of *H. perezi* from GenBank is 1.1%. In comparison with the above-mentioned interspecific distances, this confirms that our sequences belong to parasites that are conspecific with *H. perezi*.

Ultimately, in-depth studies are desirable for understanding the interactions between the invasive non-native blue crab and its parasites in the coastal areas of Morocco and for assessing their effects on native biodiversity, associated marine diseases and risks to human health. In addition, whole genome-based analyses of native and introduced populations of the Atlantic blue crab, sampled at a large scale, along with its associated *Hematodinium* parasites, will contribute to understanding the invasion history of the Atlantic blue crab in Morocco.

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