FIRST RECORD OF MARTEILIOSIS IN *MYTILUS GALLOPROVINCIALIS* IN BIZERTE LAGOON

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ملخص

أول كشف عن مرض المرتليوز لدى بلح البحر *Mytilus galloprovincialis* ببحيرة بنزرت : "المرتليوز" أو مرض " Abers" هو مرض تسبّبه نوع من الطفيليات المُسَمَّى "*Martelia sp.* " والذي تمّ تصنيفه ضمن الأمراض الواجب الإبلاغ عنها حسب المنظّمة الدّوليّة للأوبئة الحيوانيّة. بلح البحر "*Mytilus galloprovincialis*" يمثّل إحدى أبرز حاملي هذا الطفيلي. تمثّل دراسة علم الأنسجة المقاربة الأوّليّة لإثبات النتائج العينيّة لتحديد مختلف أطوار نموّ الطفيليّ ". *Marteilia sp* " داخل نسيج بلح البحر. وقد تمّ هذا التّحليل إثر ظاهرة موت جماعي " *Mytilus galloprovincialis* " ببحيرة بنزرت. تظهر الدّراسة الحالية انتشارا منخفضا لهذه الطفيلي بنسبة 34% من عدد الكلي لبلح البحر.إن الكشف عن هذا النّوع من الطّفيليات لأوّل مرّة بتونس لدى بلح البحر *Mytilus galloprovincialis* يبرز نسبة الانتشار الجغرافي لهذا الطفيلي الذي يبدو أنّه قادر على إصابة مجموعة واسعة من الأنواع ذات الصدفتين".

كلمات مفاتيح: دراسة الأنسجة الطفيلي ,. *Marteilia sp* , بلح البحر, بحيرة بنزرت. المحار*Mytilus galloprovincialis*

RESUME

Première détection de la maladie « la marteiliose » chez la moule *Mytilus galloprovincialis* dans la lagune de Bizerte : La marteiliose, ou maladie des Abers est la maladie causée par l'agent parasitaire *Marteilia spp*. qui a été notifié par l'Office International des Epizooties (OIE) comme une maladie à déclaration obligatoire. La moule *Mytilus galloprovincialis* constitue l'hôte de ce parasite. Ce parasite n'a jamais été signalé dans les fermes mytilicoles en Tunisie. Dans la présente étude, une analyse histologique a été utilisée afin d'identifier le parasite *Marteilia sp*. dans les tissus de la moule *Mytilus galloprovincialis* après un phénomène de mortalité massive qui a été observé dans la lagune de Bizerte. L'étude de la prévalence d'infection montre un niveau d'infection faible dans la population infectée avec un taux de 34%. La détection du parasite *Marteilia sp*. en Tunisie et chez la moule, *Mytilus galloprovincialis* élargit la gamme d'hôtes et la répartition géographique de ce parasite qui semble être capable d'infecter un large éventail d'espèces de bivalves.

Mots clés : Histopathologie – *Marteilia sp.* – *Mytilys galloprovincialis* – Lagune de Bizerte.

ABSTRACT

Marteiliosis, a mollusc disease caused by the protozoan parasite *Marteilia spp.*, is one of the shellfish diseases listed by the World Organization for Animal Health (OIE). *Mytilus galloprovincialis* is sensible to this disease. Marteiliosis has never been notified in Tunisian mussel farming before this investigation. In the present study, histological approaches were used to identify the parasite *Marteilia* in the mussel *Mytilus galloprovincialis* after reports of massive mortality in Bizerte lagoon. 34 % of tested animals using histological method were infected by *Marteilia spp*. The detection of *Marteilia sp.* in a new location, (Tunisia) in *Mytilus galloprovincialis*, expands the geographical distribution of this parasite, which seems to be able to infect a wide range of bivalves.

Keywords: Histopathology - *Marteilia sp.* – *Mytilus galloprovincialis* – Bizerte lagoon.

INTRODUCTION

Marine ecosystems are of high ecological and economic importance, because they support vital habitats for organisms and sustain several anthropogenic pressures. Lagoon areas are very productive ecosystems with high biodiversity, and therefore considered to be of great ecologic and economic value (Costanza et al., 1997). Many lagoons in the Mediterranean sea suffer from degraded conditions, because of the anthropogenic pressures, including urban, agricultural and industrial waste (De Casabianca et al., 1997; Solidoro et al., 2005). This can ultimately lead to the decrease of natural resources (Cajaraville et al., 2000; Monserrat et al., 2007; Cravo et al., 2009). In the last few decades, progressive economic development along the Mediterranean coastline and in several lagoons has resulted in substantial changes especially in the north of Tunisia. In fact, Bizerte lagoon is located in a heavily industrialised area of northern Tunisia, has been used in shellfish production since 1964 (Beji, 2000). It is particularly important for fisheries, especially bivalves. This has led to a decrease in bivalves and fish production over the last few decades (ANPE, 1990). In 2014 mussel production, mainly originated from Bizerte lagoon, was evaluated over 160 tons (FAO, 2014). In this lagoon there are 2

breeding modules, which occupy 1200 m^2 of the surface and are located in the northern and estern areas. This production system is known as suspended nets using ropes of 2.5 m long and tables (15 tables with unit area of 650 m^2 each) (Béji, 2000). To put this into perspective, only 160 tons of mussels were produced in 2014 compared with 500 -600 tons estimated in 1998 (CRDA, 1998).In the Bizerte lagoon, a several of studies have investigated biological tissues (Ben Ameur et al., 2013a, 2013b), sediments (Derouiche et al., 2004; Trabelsi and Driss, 2005; Barhoumi et *al*., 2013), structure of the planctonic organisms (Hlaili et al., 2007), hydrobiological pattern (Bejaoui et *al*., 2008). Nevertheless, the sanitary monitoring of this impacted environment was restricted to the identification of fish parasites (Bahri et Marques, 1996; Ben abdalla et al, 2011; Antar et ben Abdallah, 2013), and did not provide information on the mollusc parasites (Livingstone et al., 1992).

Marteiliose has been responsible for recurrent mass mortalities of flat oysters in Europe over the last four decades (Grizel et al, 1974a; Berthe et al, 2004) and has thus been recognized by the World Organization for Animal Health as a significant pathogens of bivalve mollusks (OIE.Cod, 2011). *Marteilia sp*. is a protozoan parasite belonging to the phylum Cercozoa and the order of Paramyxida (Cavalier-Smith, 1998; Cavalier-Smith and Chao, 2003; Feist et al., 2009) which affects commercially important bivalve species including the flat oyster *Ostrea edulis*, the mussel *Mytilus edulis*, and *Mytilus galloprovincialis* (Grizel et al., 1974a,b ; Le Roux et al., 2001, Lopez-Flores et al., 2004, Novoa et al., 2005; Robledo et al., 1995a; Villalba et al.,1993b).

The developmental stages of *Marteilia spp*. in bivalves were described by Grizel et al. (1974), Perkins (1976), Franc (1980), Grizel (1987). Sporulation occurred within the sporangiosorus via a unique process of internal cleavages (endosporulation) to produce cells within cells (Fig. 2). The specific name of M.refringens was derived from these 'refringent' inclusion bodies. Infections by all Marteilia spp. are presumably initiated by a primary cell or stem cell (5 to 8 µm in diameter) in the epithelial cells of the gut (usually the stomach) and possibly the gills and labial palps (Kleeman et al., 2002a).

The primary uninucleate cell develops a secondary uninucleate daughter cell in a vacuole within its cytoplasm. The primary cell degenerates to release the daughter cells, which become new primary cells.

At the initiation of sporulation, uninucleate segments become delimited within the cytoplasm of the sporangiosorus to form the sporangial primordia (secondary cells).

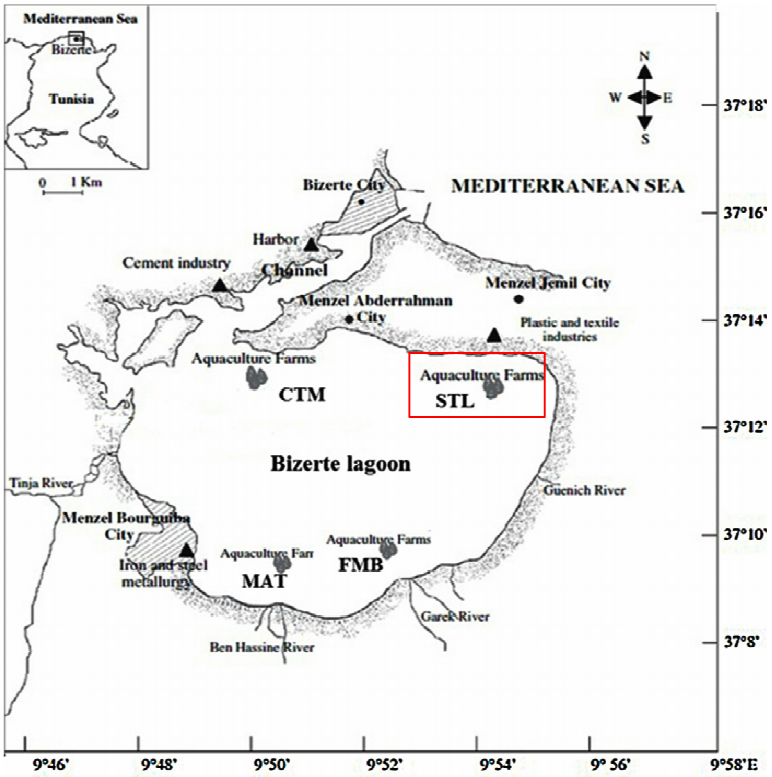

Figure 1. Geographical location of the Bizerte lagoon

Eventually, 8 to 16 sporangial primordia (each about 12 µm in diameter at maturity) form within the sporangiosorus that retains its nucleus and enlarges to about 30 µm in diameter. Each spore contains 3 uninucleate sporoplasms of graded sizes, with each of the smaller sporoplasms being enclosed within the

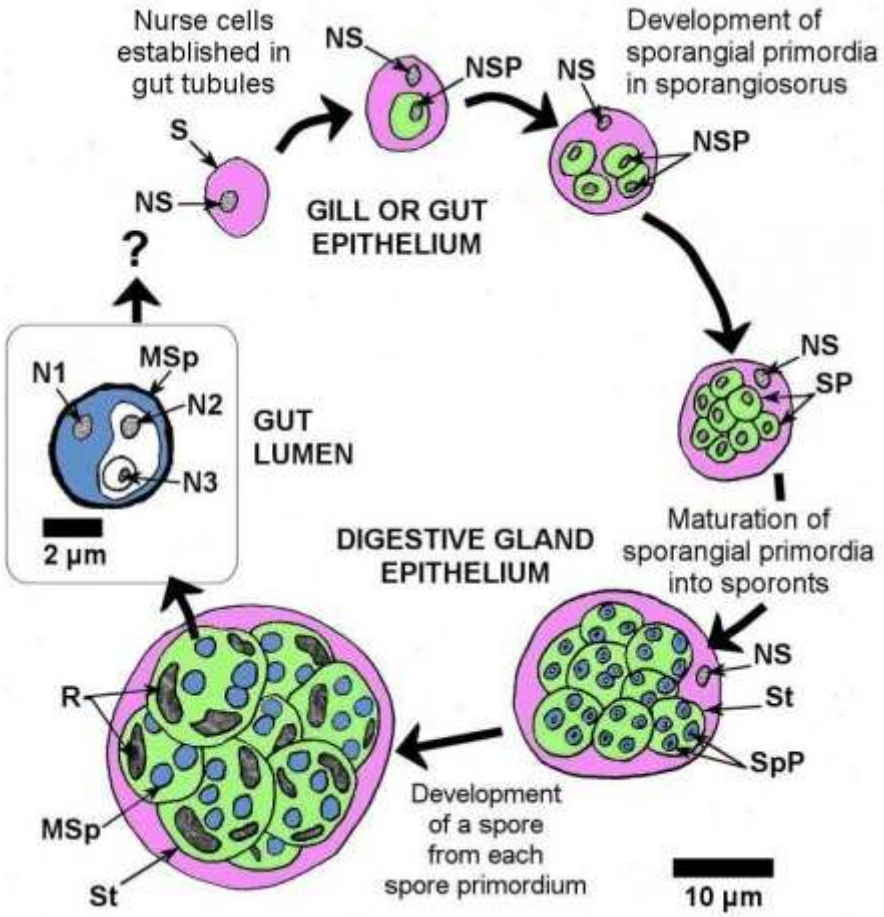

Figure 2. Schematic drawing to scale, of the sporulation process of Marteilia spp. with the cytoplasm of Each internship color coded for easy recognition. S = sporangiospores (or primary cell, pink colored cytoplasm), NS = nucleus of sporangiospores, SP = sporangial primordia That mature into a sporont (the secondary cell, green cytoplasm), DK = nucleus of sporangial primordia, St = sporont, SPP = That spore primordia mature into the spore (the tertiary cell, blue cytoplasm) = MSp mature spore, R = refracting bodies, N1 = nucleus of outer MOST sporoplasm, N2 = nucleus of middle sporoplasm, N3 = nucleus of inner MOST sporoplasm.

cytoplasm of the next largest one. Mature spores are shed into the tubule lumen for evacuation from the oyster and infected oysters may shed large numbers of spores before oyster death (Kleeman et al., 2002a).
According to our knowledge, no published work is available about the parasitological status of the mussel *Mytilus galloprovincialis* in Tunisia, furthermore, the socio-economic importance of this species in Tunisia. The present study aims to investigate of the parasitological status of *Mytilus galloprovincialis* in Bizerte lagoon using histology technique for the detection of parasites causing important diseases in mollusks.

MATERIAL AND METHODS

Sampling site

The Bizerte Lagoon is a Mediterranean transitional ecosystem located in north Tunisia (latitude: 37°8'–37°14'N, longitude 9°46'–9°56'E), with an extension of over 150 km^2 (the maximum width is 11 km and the maximum length is 13 km) and an average depth of 7 m (Fig. 1).
It connects with the Mediterranean Sea through a straight channel that is about 6 km long, 300 m wide, and 12 m deep. It communicates in the south with Ichkeul Lake via the Tinja stream. Furthermore, this lagoon has also been subjected to intensive maritime traffic and indirectly to several pollutants coming from oil and steel factories. Water temperature in the lagoon follows a seasonal cycle, ranging from 10 °C in winter to 28 °C in summer (Harzallah, 2002). The water salinity ranges from 30 in winter to 38 psu in summer (ANPE, 1990, Béjaoui et al., 2005).
This lagoon has been exploited for fishing activities for several centuries and for mussel farming since 1964. The mussel *Mytilus galloprovincialis*, the European flat oyster *Ostrea edulis*, and the Japanese oyster *Crassostrea gigas* have been intensively cultured in this lagoon for decades, and natural beds for the European clam *Ruditapes decussatus* are still present in the lagoon.

Sample collection and preparation

Sanitary monitoring of mussels in Bizerte lagoon was carried out in June 2012. 50 Specimens of *M. galloprovincialis* were sampled from an aquaculture farm in Bizerta lagoon, after reports of the occurrence of mortality in this station. All collected specimens were kept at a temperature of 4° C, and analysed by histological methods. For each sample, Mussels were washed, taking off deposited matters on shells, and opened carefully to avoid damage to the animals. Each sample was fixed in Davidsonl solution, in order to prepare histological materials. Standard sections were taken through the digestive gland, to include gills, mantel, gonads and palps. After being embedded in paraffin and sectioned, the slides were stained with haematoxylin-eosin (H&E) and observed under light microscopy. Histological analyses were then carried out, to investigate possible causes of these mortalities using optical microscopy.

RESULTS

The evaluation of 50 *M. galloprovincialis* sampled in June 2012 revealed that 17 mussels were infected with the parasite *Marteilia sp.* Infection rate was 34% of the total number of analyzed subjects. The table 1 shows the biometric measurement of the total mussels and the infected ones.

Different stages of *Marteilia sp*. biological cycle were identified by light microscopy examination: plasmodium, sporangial primordial (SII) and spore primordial (RS). The parasite is characterized by a basophilic cytoplasm and an eosinophilic nucleus. In case of high infection, most of the digestive gland appeared affected and the presence of parasites was observed (Fig. 3A). In advanced stage of infection, some primary stages ((SI) and (SII)) could be detected in stomach epithelium and more mature stages were observed in the epithelium of digestive tubules (Fig. 3B). Different parasite stages were observed in the epithelium of digestive tubules including zoosporangious (SI), sporangial primordial (SII) and spore primordial (RS) (Fig. 3C-3D).

DISCUSSION AND CONCLUSION

The presence of *Marteilia sp.* in mussel farming in Bizerte lagoon was confirmed by this research. The percentage of infection was evaluated by histological analysis, and resulted in 34% of the total number of examined mussels. It is very important to plan sanitary monitoring, related to mollusc diseases, in suitable months, when diseases are mainly manifested. In general, outbreaks of Marteiliosis occurred at the end of spring (Berthe et al., 2004). For that reason, studies to evaluate the presence of *Marteilia sp.* are usually realized in spring and summer (Balseiro et al., 2005). In mussels, Marteiliosis infection is related to climatic changes, production zone conditions, pollution levels, water movement, geographic conditions and other unknown factors (Robledo & Figueras, 1995). The infection rate evaluated in this study is comparable to the infection rate of other mussel production areas (the Ebre Delta in Spain range from 1.67% to 26.67% (Carrasco et al. 2007, 2008), for Galicia from 5.5% to 38.5% (Robledo & Figueras 1995), 5% for Croatia (Zrnčić et al. 2001), 14.6% (Rayyan et al. 2006) and 21.25% (Virvilis, Angelidis & Photis 2003) for Greece, from 16.6% to 22.2% for the Adriatic Sea in Italy (Ceschia et al. 1992), 19.3% for Albania (Pëllumb, Ceschia & Kapllan 2006), and from 37% to 70% for northern Brittany (Auffret & Poder 1983).

Marteiliosis due to *Marteilia refringens* is a disease that causes serious recurring mortalities of the European flat oyster *Ostrea edulis* (Grizel et al., 1974a). The diagnosis of mollusc diseases usually relies on histology and cytology (including tissue imprints) but molecular tools are of the utmost importance, especially for species confirmation. We present herein the presence of marteiliosis, for the first time, in a new study site. Histological examinations of infected mussels revealed the presence of different parasite stages previously described in the literature in flat oysters *Ostrea edulis* (Grizel et al. 1974a) and mussels *Mytilus galloprovincialis* (Figueras et al. 1991; Villalba et al. 1993*). Mytilus galloprovincialis*, *Marteilia spp*. displays a digestive tropism like in other bivalve hosts. Early stages were observed in the ciliated epithelium of the stomach, while more mature stages containing refringent bodies, occurred in the epithelium of the digestive tubules. High infection level appeared associated with high haemocytic infiltration.

Marteilia refringens has previously been reported in different European countries and in the Mediterranean basin including Spanish, Morrocan, Greek, Croatian, Slovenian, Italian and French coasts. The presence of *M. refringens* and *M. maurini* in *M. edulis* was notified in France, Spain and Italy (Le Roux et al., 2001). These two species of *Marteilia* were also detected in *M. galloprovincialis* in Spain and Italy (Le Roux et al., 2001; Lopes-Flores et al.,

Table I. *Mytilus galloprovincialis*. **Biometric measurements**

Sex	Number of shells	Shell length (mm)	Shell height (mm)	Shell width (mm)	Total weight (g)
Female	31	53.21 ± 6.89	28.36 ± 3.87	16.56 ± 2.21	11.13 ± 3.24
Male	19	48.40 ± 7.67	25.86 ± 5.48	14.97 ± 1.89	9.90 ± 3.61
Average	50	50.80 ± 7.28	27.11 ± 4.67	15.76 ± 2.05	10.25 ± 3.43

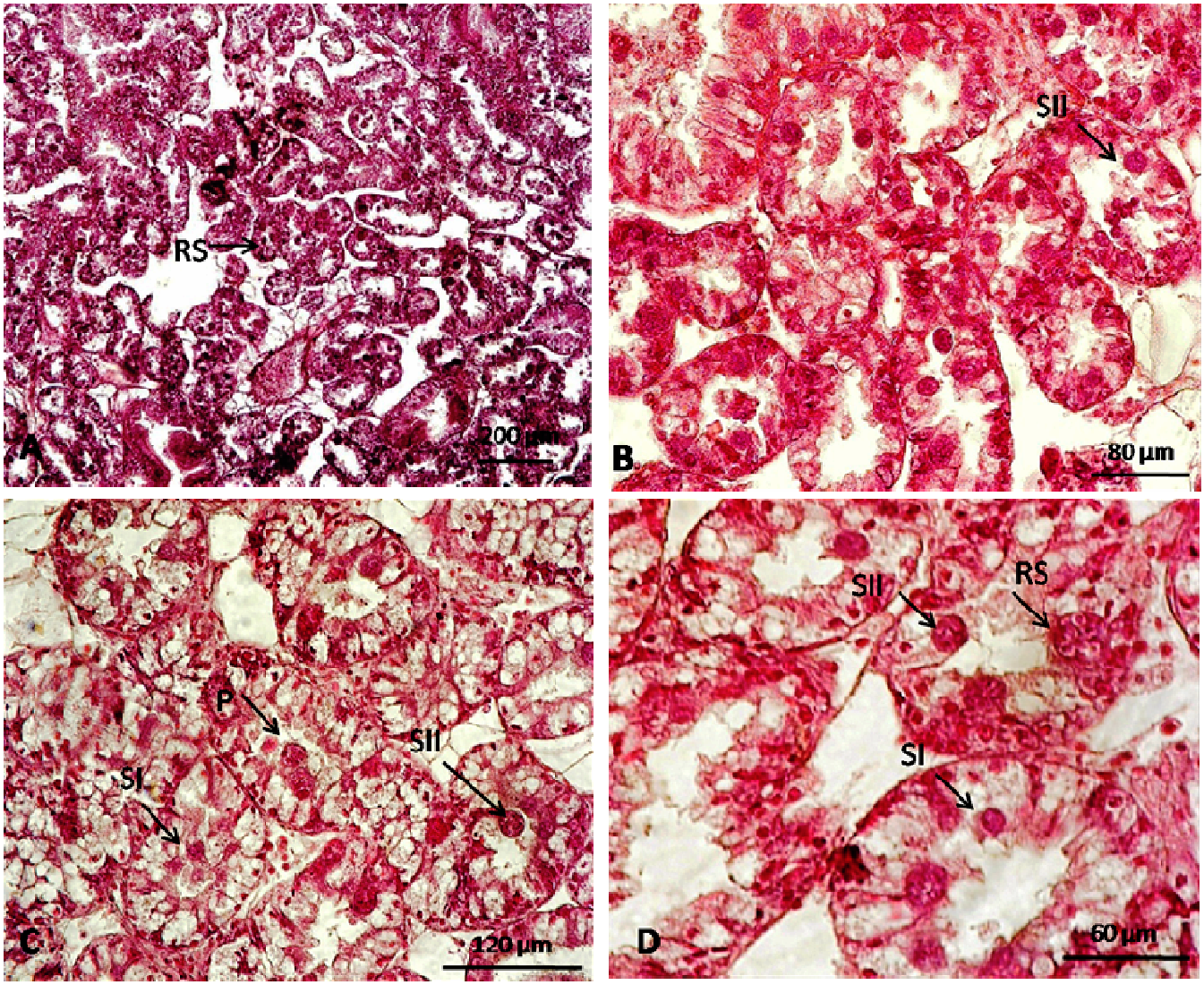

Figure 3. Hematoxylin and Eosin stained histological sections of *Mytilus galloprovincialis* infected with *Marteilia sp.*, showing various life cycle stages including primary stage (SI), secondary stage (SII), pansporoblaste (P) and refringens stages (RS). (A) Digestive tubules infected with Marteilia sp., (B) primary stages in the ciliated epithelium of the stomach, (C) and (D) different parasite stages in the epithelium of digestive duct canals

2004; Balseiro et al., 2005; Novoa et al., 2005). The first record of *Marteilia refringens* in Tunisia and in a new oyster species, *Ostrea stentina* was reported in 2013 (Elgharsalli et al. 2013). *O. sentina* enlarges the host range of this parasite, which seems to be able to infect a wide range of bivalve species in Tunisia. Thus, this study presents the first detection of *Marteilia sp.* infected *Mytilus galloprovincialis* Tunisian population. Considering that *M. galloprovincialis* and *O. edulis* are sympatric in some areas (Leal 1984; Lapegue et al. 2006) and presented the commercial bivalve species especially in Tunisia,

it is important that the survey of marteiliosis includes these two species whenever they cohabit.

Based on our results, *Mytilus galloprovincialis* of Bizerte lagoon should be included in the list of susceptible species to the infection with *Marteilia spp*.. However further studies are required firstly to determine the species of this parasite based on molecular analysis and secondly to determine the actual spread of the parasite in other locations of Tunisian seawaters.

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