1. Introduction

The Tunisian coast of the Mediterranean Sea is known to harbour a great richness of chondrichthyan fishes, with at least 62 described species (Bradaï et al., 2012). The Family Triakidae comprises four species, namely Galeorhinus galeus (Linnaeus, 1758), Mustelus mustelus (Linnaeus, 1758) M. asterias Cloquet, 1819, and M. punctulatus Risso, 1827 (Saidi et al., 2009). Both species are of great commercial and gastronomic value in Tunisia; as such, as a consequence of their extensive consumption, they became vulnerable and even threatened in some areas, according to the International Union for Conservation of Nature and Natural Resources (IUCN).

The body of a shark confers many attachment sites for other organisms (Dippenaar and Molele, 2015). However, the studies of their parasites are scarce and with a main focus on single taxonomic group, such as copepods (Essafi, 1975; Benmansour and Youssef, 2019; Youssef et al., 2019) and monogenea (Neifar, 2001), disregarding other phyla. The aim of the current work was to provide original data on the parasitic taxa found on Triakidae sharks from Tunisian coasts and their infection indices.

2. Material and methods

2.1. Prospected area and specimen examination

Between 2018 and 2020, a total of 480 fish belonging to two species of sharks from the Family Triakidae (Mustelus mustelus and M. punctulatus) were collected from the coasts of Tunisia and inspected for parasites. Six copepod taxa (Perissopus dentatus Steenstrup & Lütken, 1861, Eudactylinae alba Wilson, 1932, Kroeyer lineata Van Beneden, 1853, Nesippus orientalis Heller, 1865 and Lernaeopoda galei Kroyer, 1837, Kroeyer sp.), four isopod species (Anilocra physodes (Linnaeus, 1758), Emetha audouini (H. Milne Edwards, 1840), Ceratothoa parall Tele (Otto, 1828) and Ceratothoa oestroides (Risso, 1816)) and two monogenean species (Erpocotyle sp1. and Erpocotyle sp2) were collected. A large number of global host records was reported, including the occurrence of E. audouini on M. mustelus and of Erpocotyle sp2 on M. punctulatus. The study of the diversity of parasites per host species revealed that M. mustelus had a higher parasitic richness compared to M. punctulatus. In this study, it was provided for the first records on ectoparasites on Triakidae sharks from Tunisian coasts and their infection indices.

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for ectoparasites (Table 1). The fish were collected weekly from local Tunisian fishermen in Bizerte Bay, Gulfs of Tunis, Hammamet, and from Gabes (Sfax and Zarzis) (Fig. 1). The fish were transported for further laboratory analysis and identified according to Compagno (1984), Fischer et al. (1987) and Serena (2005). The host nomenclature followed data in Froese and Pauly (2019).

2.2. Parasitological investigations

For every fish individual, all body traits (i.e. skin, mouth, gills, fins, cloaca, and nasal cavity) were thoroughly examined. Gills were placed afterwards in seawater in Petri dishes and arches of each gill carefully examined. Information about sampling date, prospected areas, taxonomic nomenclature and shape and corpus- lence of the host fishes and their parasite's microhabitats were noted.

Copepods and isopods were extracted from hosts and fixed in ethanol (70%), afterwards cleared for 2 h in lactic acid before being examined observed undera 50× stereomicroscope (Model WildHeerbrugg M5A) and a Nikon DS-Fi2 camera coupled with a Nikon microscope (Image Software NIS Elements Analysis Version 4.0 Nikon 4.00.07–build 787–64 bit). The specimens were dissected and temporary mounted on slides in lactophenol (Yamaguti, 1963). The taxonomic identification of parasites was done based on taxonomic keys of Deets (1994), Kabata (2003), Ho and Kim (2004) and Boxshall and Halsey (2004).

The examination of the collected isopods comprises the observation of their morphologic features and of different appendages in order to identify them to species level. Gender, life stage and fixing site of collected specimens were also identified. The taxonomic identification of isopods was performed according to the keys of Trilles (1979), Bruce (1986), Keable and Bruce (1997), Horton (2000) and Charfi-Cheikhrouha et al. (2000).

Iron acetocarmine was necessary to stain monogeneans to be examined in permanent mounts, using Canada balsam (Bailenger and Neuzil, 1953). Monogeneans species identification was carried using the keys of Sproston (1946), Neifar et al. (1998), Tazerouti and Neuzil, 1953). Monogeneans species identification was carried out using the keys of Sproston (1946), Neifar et al. (1998), Tazerouti and Neuzil, 1953). Monogeneans species identification was carried out using the keys of Sproston (1946), Neifar et al. (1998), Tazerouti and Neuzil, 1953).

2.3. Data analyses

The infestation rates were assessed through prevalence (P = N/ H × 100) and mean intensity (MI = n/N) [N: number of infected hosts; H: total number of hosts examined; n: total number of collected individuals of one parasitic species] (see Margolis et al., 1982; Bush et al., 1997). The software Plymouth Routines In Multivariate Ecological Research 'PRIMER'v.5.0 was used to quantify the parasites' taxonomic diversity and evenness on their distribution on hosts, following the calculation of the indices Margalef's species richness (SR), Shannon index (H') and Pielou's evenness (E).

3. Results

3.1. Taxonomic diversity

A total of twelve parasites, comprising copepods, isopods and monogeneans were identified (Table 2). Copepods comprised the most diverse group, namely: Eudactylinella alba Wilson, 1932, Kroyeria sp., Kroyeria lineata Van Beneden, 1853, Perissopus dentatus Steenstrup and Lütken, 1861, Nesippus orientalis Heller, 1865 and Lernaeopoda galei Krøyer, 1837 (Table 2, Fig. 2). Kroyeria sp. could comprise a new species that awaits further investigations. It was also observed that M. mustelus had a higher number of parasites, namely six species (Fig. 3) compared to M. punctulatus, which was the hosted of a single copepod species, Lernaeopoda galei.

| Host              | Sampling location                        | Season      | Number of examined fish |
|-------------------|------------------------------------------|-------------|-------------------------|
| Mustelus mustelus | Bay of Bizerte 37°16'25"N-9°53'14"E20 to 100 m | Autumn 22   |                         |
|                   |                                          | Winter 12   |                         |
|                   |                                          | Spring 30   |                         |
|                   |                                          | Summer 36   |                         |
|                   | Gulf of Tunis 37°00'0.00"N-10°29’59.99"E20 to 100 m | Autumn 30   |                         |
|                   |                                          | Winter 30   |                         |
|                   |                                          | Spring 30   |                         |
|                   |                                          | Summer 30   |                         |
|                   | Gulf of Hammamet 36°04’60.00’N-10°44’59.99"E20 to 100 m | Autumn 30   |                         |
|                   |                                          | Winter 15   |                         |
|                   |                                          | Spring 47   |                         |
|                   |                                          | Summer 28   |                         |
|                   | Gulf of Gabes 34°00’0.00’N-10°30’0.00"E20 to 100 m | Autumn 30   |                         |
|                   |                                          | Winter 25   |                         |
|                   |                                          | Spring 25   |                         |
|                   |                                          | Summer 40   |                         |
| Mustelus punctulatus | Bay of Bizerte 37°16'25"N-9°53'14"E20 to 100 m | Autumn 7    |                         |
|                   |                                          | Winter 6    |                         |
|                   |                                          | Spring 9    |                         |
|                   |                                          | Summer 11   |                         |
|                   | Gulf of Tunis 37°00'0.00"N-10°29’59.99"E20 to 100 m | Autumn 8    |                         |
|                   |                                          | Winter 7    |                         |
|                   |                                          | Spring 10   |                         |
|                   |                                          | Summer 12   |                         |
|                   | Gulf of Hammamet 36°04’60.00’N-10°44’59.99"E20 to 100 m | Autumn 7    |                         |
|                   |                                          | Winter 7    |                         |
|                   |                                          | Spring 11   |                         |
|                   |                                          | Summer 9    |                         |
|                   | Gulf of Gabes 34°00’0.00’N-10°30’0.00"E20 to 100 m | Autumn 24   |                         |
|                   |                                          | Winter 28   |                         |
|                   |                                          | Spring 30   |                         |
|                   |                                          | Summer 30   |                         |
Both hosts were infected by four isopod species (Table 2). *Mustelus mustelus* was the host of three species (i.e. *Anilocra physodes* (Linnaeus, 1758), *Emetha audouini* (H. Milne Edwards, 1840) and *Ceratothoa parallela* (Otto, 1828)) whereas *M. punctulatus* of two (i.e. *Ceratothoa parallela* and *Ceratothoa oestroides* (Risso, 1816)).

Two monogenean species were also collected from both hosts: *Erpocotyle* sp. 1 and *Erpocotyle* sp. 2.

### 3.2. Parasitological indices of the studied parasite taxa

Data on the prevalence, mean intensity and infestation area are given in Table 2. Data analysis suggested that prevalence was low for most copepod and isopods but slightly higher for the monogeneans (Table 2). Among copepods, the species *Kroyeria lineata* found on *M. mustelus* presented the highest prevalence (P = 3.95%). Moreover, the species *Lernaeopoda galei* presented the second highest prevalence degree on *M. punctulatus* of two (i.e. *Ceratothoa parallela* and *Ceratothoa oestroides* (Risso, 1816)).

Two monogenean species were also collected from both hosts: *Erpocotyle* sp. 1 and *Erpocotyle* sp. 2.

#### Table 2

| Host            | NEF | Parasite taxonomic group | Parasite family | Parasite species | Microhabitat | P (%) | MI  |
|-----------------|-----|--------------------------|-----------------|------------------|--------------|-------|-----|
| *Mustelus mustelus* | 480 | Copepods                 | Eudactylinidae  | *Eudactylinella alba* | Gills        | 1.4   | 1.1 |
|                 |     |                          | Kroyeridae      | *Kroyeria lineata*  | Gills        | 3.95  | 1   |
|                 |     |                          |                 | *Kroyeria sp.*     | Gills        | 1.04  | 1   |
|                 |     |                          | Lernaeopodidae  | *Lernaeopoda galei* | Cloacae cavity | 2.50  | 1.2 |
|                 |     |                          | Pandaridae      | *Nesippus orientalis* | Mouth       | 0.41  |     |
|                 |     |                          |                  | *Perissopus dentatus* | Dorsal fin  | 0.83  | 1   |
|                 |     |                          |                  | *Anilocra physodes* | Body side   | 0.41  |     |
|                 |     |                          |                  | *Emetha audouini*  | Body side   | 0.41  |     |
|                 |     |                          |                  | *Ceratothoa parallela* | Cloacae cavity | 1.8   | 1.16|
|                 |     |                          |                  | *Erpocotyle sp. 1* | Gills        | 6.04  | 2   |
| *Mustelus punctulatus* | 216 | Copepods                 | Lernaeopodidae  | *Lernaeopoda galei* | Cloacae cavity | 3.24  | 1   |
|                 |     |                          | Ceratothoa oestroides | Gills        | 2.32  | 1   |
|                 |     |                          | Ceratothoa parallela | Cloacae cavity | 1.5   | 1.5 |
|                 |     |                          |                  | *Erpocotyle sp. 2* | Gills        | 5.55  | 1.5 |

On *M. mustelus* (P = 1.80%) and on *M. punctulatus* (P = 1.50%). Moreover, both parasites displayed a relatively high mean intensity, varying between 1 and 1.5 (Table 2).

The species *A. physodes* and *E. audouini* were found on *M. mustelus* and had the lowest parasitological indices (P = 0.41% and MI = 1) (Table 2).

The Monogeneans presented the highest parasitological indices recorded during this survey. The prevalence of *Erpocotyle sp. 1* on *M. mustelus* was P = 6.04% and of *Erpocotyle sp.2* on *M. punctulatus* P = 5.55%. The mean intensity of both monogeneans was also high and varied between 1.5 and 2 (Table 2).

### 3.3. Parasitic richness per host taxa

The parasitic richness on both hosts revealed that *M. mustelus* was the richest (SR = 10), by hosting six copepods species, three species of isopods and one monogenean species (Table 3), whereas *M. punctulatus* had a lower parasitic richness (SR = 4), with one copepod, two isopods and one monogenean species (Table 3).

The maximum value of Shannon index (H = 1.69) of the parasite assemblage was found on *M. mustelus*. A value of 0.89 for Shannon-Weaver index was found for *M. punctulatus* (Table 3), confirming that the former host possessed the highest parasitic richness.

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Fig. 1. Sampling sites, indicated by red circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Table 2

| Host            | NEF | Parasite taxonomic group | Parasite family | Parasite species | Microhabitat | P (%) | MI  |
|-----------------|-----|--------------------------|-----------------|------------------|--------------|-------|-----|
| *Mustelus mustelus* | 480 | Copepods                 | Eudactylinidae  | *Eudactylinella alba* | Gills        | 1.4   | 1.1 |
|                 |     |                          | Kroyeridae      | *Kroyeria lineata*  | Gills        | 3.95  | 1   |
|                 |     |                          |                 | *Kroyeria sp.*     | Gills        | 1.04  | 1   |
|                 |     |                          | Lernaeopodidae  | *Lernaeopoda galei* | Cloacae cavity | 2.50  | 1.2 |
|                 |     |                          | Pandaridae      | *Nesippus orientalis* | Mouth       | 0.41  |     |
|                 |     |                          |                  | *Perissopus dentatus* | Dorsal fin  | 0.83  | 1   |
|                 |     |                          |                  | *Anilocra physodes* | Body side   | 0.41  |     |
|                 |     |                          |                  | *Emetha audouini*  | Body side   | 0.41  |     |
|                 |     |                          |                  | *Ceratothoa parallela* | Cloacae cavity | 1.8   | 1.16|
|                 |     |                          |                  | *Erpocotyle sp. 1* | Gills        | 6.04  | 2   |
| *Mustelus punctulatus* | 216 | Copepods                 | Lernaeopodidae  | *Lernaeopoda galei* | Cloacae cavity | 3.24  | 1   |
|                 |     |                          | Ceratothoa oestroides | Gills        | 2.32  | 1   |
|                 |     |                          | Ceratothoa parallela | Cloacae cavity | 1.5   | 1.5 |
|                 |     |                          |                  | *Erpocotyle sp. 2* | Gills        | 5.55  | 1.5 |
The study of the Pielou index of evenness revealed that *M. mus- telus* was the host of a highly diverse community of parasites ($E = 0.73$), followed by *M. punctulatus* ($E = 0.64$).

### 3.4. Parasites microhabitats

The parasites presence was intimately related to distinct microhabitats, as described in Table 2. Most parasites were collected...
from hosts’ gills. In turn, few other species were collected from cloaca (i.e. L. galei) or externally, from skin (i.e. A. physodes and E. audouini). The species N. orientalis was the only parasite collected from the mouth of M. mustelus.

4. Discussion

The current report revealed a high taxonomic diversity of ectoparasites on both species of sharks along the Tunisian coast, comprising a total of 12 species. The results obtained are in accordance with those of Raibaut et al. (1998), which confirmed the fact that the sharks are associated with Families Carcharhinidae (i.e. Priounga glauca (Linnaeus, 1758)) and Triakidae (i.e. M. mustelus) were frequently associated with the maximum richness for copepods among elasmobranch taxa in the Mediterranean Sea.

The copepods collected from both host species were the dominant taxonomic group. Indeed, the copepods comprised the highest diversity, with six species, followed by isopods with four species and monogeneans with two species. According to Carrier et al. (2012), among the ectoparasites of elasmobranchs, the subclass of copepods presented the highest diversity.

Among the parasite copepods of M. mustelus, K. lineata had the highest prevalence (P = 3.95%), whereas N. orientalis had the lowest values (P = 0.41%). It is known that species from Kroyeria sp. genus are associated with a higher parasite load on their hosts compared to other copepods (Deets, 1994).

Among the six identified copepods, only the species L. galei was collected from both sharks (M. mustelus and M. punctulatus). This species is euryxenous (sensu Euzet and Combes, 1980) and is a common parasite among elasmobranchs. Moreover, this species was previously reported from various host taxa (Raibaut et al., 1998; Henderson et al., 2003; Dippenaar, 2004; Karaytug et al., 2004; Gaevskaya, 2012). Moreover, according to previous investigations, this species appears to have a preference for Triakids (see Raibaut et al. (1998); Dippenaar (2004) and Karaytug et al. (2004) for M. mustelus and Raibaut et al. (1998) for M. punctulatus). This parasite had a higher prevalence for M. punctulatus (P = 3.24%) compared to M. mustelus (P = 2.50%).

E. alba was found on the gills of M. mustelus. This copepod species is euryxenous, infesting numerous species of fish (Izawa, 2011). As previously described by Raibaut et al. (1998) from Mediterranean Sea and by Essafi (1975), it was also found on Dasyatis pastinaca from the Tunisian coasts. This is the first record of this parasite on M. mustelus in Tunisia as elsewhere.

K. lineata was another parasite found on the gills of M. mustelus. This species is cosmopolitan and was previously reported from various locations worldwide, such as Pacific Ocean (Izawa, 2008) and the Atlantic Ocean (Canadian coasts, see Deets, 1994; Benz, 1994). In Tunisia, K. lineata was found on M. asterias (Clouquet, 1918), and on M. mustelus (Essafi, 1975). This copepod species is stenoxenous, infesting mostly Triakidae species.

Kroyeria sp. was found on the gills of M. mustelus with low prevalence (P = 1.04%). The morphological study revealed that this species presented many differences compared to the other species known from the same genus from the Mediterranean Sea and the Atlantic Ocean. However, further studies are needed to support this hypothesis.

N. orientalis, found in the mouth of M. mustelus, is a well-known parasite of chondrichthysans (Cressey, 1967, 1970). This euryxenous species has a large geographical distribution and was reported in the South African coasts by Dippenaar and Jordaan (2012) and from the Mediterranean Sea by Raibaut et al. (1998) on Alopias vulpinus (Bonnaterre, 1788), M. mustelus and M. punctulatus. In Tunisia, this species was found on M. mustelus and M. punctulatus by Essafi (1975).

P. dentatus is euryxenous and was found in South African coast (Dippenaar and Jordaan, 2007). This copepod presents a large Mediterranean geographical distribution. P. dentatus was inventoried by Raibaut et al. (1998) and by Essafi (1975) in Tunisia water from M. mustelus.

Among isopods, it was observed that M. mustelus had a slightly higher isopod richness (three species) than M. punctulatus (two species). The latter host was exclusive for Ceratothoa sp. species, whereas the former was the host of a more diverse parasitic fauna (A. physodes, E. audouini and C. paralella).

The genus Ceratothoa sp. had the highest parasitological indices are known from a large number of chondrichthysans sharks (Trilles, 1994).

C. parallela has euryxenous specificity and was reported from various Mediterranean areas (Trilles, 1994; Papapanagioutou and Trilles, 2001; Ramdane et al., 2007; Oktener and Trilles, 2004; Ferri et al., 2008). In Tunisia, this parasite was observed by Charfi-Cheikhrouha et al. (2000) on teleostean fishes. According to Trilles (1994), this species was found on chondrichthyan fishes (i.e. Raja clavata Linnaeus, 1758 and R. asterias Delaroche, 1809) on both females and males. According to Pollerspöck and Straube (2018), this parasite has never been encountered before on M. mustelus and M. punctulatus in the world.

C. oestroides is a ubiquitous and euryxenous species. It was reported in the Mediterranean area (Trilles, 1994; Horton, 2000; Oktener and Trilles, 2004; Bariche and Trilles, 2005; Ramdane et al., 2007). In Tunisia, this isopod was found on various species of teleostes (Bradaï, 2000); however, this is its first record on cartilaginous fish in the Mediterranean area, as elsewhere (Pollerspöck and Straube, 2018).

A. physodes and E. audouini are euryxenic species, with a wide host range and marked preference for teleostes (Trilles, 1979). The former species has also a marked preference for Sparids and Maenids (Trilles, 1979) and was reported from various locations in the Mediterranean Sea, such as in Algeria (Trilles, 1994; Ramdane et al., 2007), Turkey (Oktener and Trilles, 2004; Koc et al., 2018) and Lebanon (Bariche and Trilles, 2005). In Tunisia, Charfi-Cheikhrouha et al. (2000) reported the occurrence of this parasite on several species of Teleostes. The species E. audouini was found in several species of fish worldwide (Ramdane, 2007; Oktener and Trilles, 2004). The current record is the first for Tunisian coasts and on the shark M. mustelus.

The marked preference of both isopods for teleost fishes may explain their low prevalence (P = 0.41%) found in the current survey.

Chondrichthysans are known to host an extremely large number of monogeneans (Neifar, 2001; Boudaya and Neifar, 2016). However, it was only possible to collect two parasite species from the sharks M. mustelus and M. punctulatus. For M. punctulatus, a new species of Monogene, Triocoluotrema euzeti n.s.p. has been reported by Boudaya and Neifar (2016) in Tunisia. However, this parasite was found only in small host individuals in which the total length (TL) was less than 60 cm. In our study, all the fish examined had a length greater than 60 cm. For this reason, we did not find this monogenean.

Table 3

| Host            | Index | SR | H  | E   |
|-----------------|-------|----|----|-----|
| Mustelus mustelus | 10    | 1.69 | 0.73 |
| Mustelus punctulatus | 4    | 0.89 | 0.64 |
The two identified species belong to Erpocotyle genus. However, they appear to be different to E. catenulata (Guberlet, 1933) and E. laevis Van Beneden and Hesse, 1863, which are well-known parasites of M. mustelus (Lambert and Millard 1979; Llewellyn et al. 1984). Moreover, the current record of Erpocotyle species is the first for the shark M. punctulatus.

Host, microhabitat or site selections are displayed to various degrees throughout parasite taxas and groups (Kabata, 2003). Globally, all parasites exhibit high specificities in terms of attachment site and host (Kabata, 2003). Erpocotyle species are typically found on the gills of their host (Proston, 1946).

The Family Cymothoidae is known for its binding site rather than host specificity. This family comprises species with unique morphologic features, adapted to specific attachment sites on hosts and some genera with precise location (Fogelman and Grutter, 2008). Only E. audouini is known to be found in the mouth of its host (Ramdane, 2010). The current survey presents a new binding site, whereas the rest of other isopod species were found in their typical binding sites.

The physiologic and morphologic mechanisms that determine the selection of a given site by a specific copepod are still unidentified for most species (Kabata, 2003). However, most of copepods were encountered on gills on both hosts. It was noted that L. galei was found in the same microhabitat (cloaca) on both hosts, suggesting that it may be the preferential binding site to this anatomic part on Triakidae sharks.

5. Conclusions

In the current study it was observed that the parasite richness of M. mustelus and M. punctulatus differs, even if both host species have similar ecologic and ethologic characteristics. This is probably the cause of intrinsic characters of each host species.

The results could be related to the lower population abundance of M. punctulatus compared to M. mustelus off the Tunisian coasts. In fact, the density of both sharks is considered as one of the universal key components in driving the interspecific parasite diversity.

This current survey completed the known data on parasitic infections for sharks M. mustelus and M. punctulatus in Tunisian coasts, supporting the further use of parasites for a better understanding of the biology and ecology of their hosts.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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