Aus der Professur für Aquakultur und Sea-Ranching

der Agrar- und Umweltwissenschaftlichen Fakultät

Parasites of the aquaculture candidate *Siganus canaliculatus* Park, 1797 (Siganidae: Perciformes) from Omani waters, including their potential to indicate environmental health

Dissertation

zur Erlangung des akademischen Grades

Doktor der Agrarwissenschaften (Dr. agr.)

an der Agrar- und Umweltwissenschaftlichen Fakultät

der Universität Rostock

vorgelegt von

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aus dem Sultanat Oman

Rostock, den 02.06.2019

https://doi.org/10.18453/rosdok_id00002783

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Jahr der Einreichung: 02. Juni 2019

Jahr der Verteidigung: 24. Januar 2020

Mammi and Tia

Zusammenfassung

Parasiten des Aquakulturkandidaten *Siganus canaliculatus* Park, 1797 (Percoformes: Siganidae) aus den Gewässern vor Oman und ihr Potenzial als Umweltindikatoren

In der vorliegenden Arbeit wurde die Parasitenfauna von marinen herbivoren Fischen aus Küstengebieten des Sultanats Oman (Persischer Golf, Golf von Oman, Arabisches Meer) untersucht. Der Weisspunkt-Kaninchenfisch *Siganus canaliculatus* (Park) ist von großer wirtschaftlicher Bedeutung, parasitologisch jedoch kaum erfasst. Im Rahmen dieser Studie wurde erstmalig eine umfassende parasitologische Untersuchung an *S. canaliculatus* (n = 245) durchgeführt, wobei eine besonders diverse marine Parasitengemeinschaft dokumentiert wurde. Insgesamt konnten 44 Arten (ein microsporider Hyperparasit, neun Myxosporea, vier Monogenea, 16 Digenea, ein Cestoda, vier Nematoda, vier Acanthocephala, ein Hirudinea und vier Crustacea) nachgewiesen werden. Die Ergebnisse ermöglichen einen Einblick in die Diversität der Arten in hiesigen Ökosystemen. Es konnten 16 neue Wirts- und sechs Gebietsnachweise erbracht aber vor allem vier neue Parasitenarten beschrieben werden.

Umfassende morphologische Analysen mithilfe von Licht-, Rasterelektronen- sowie der Transmissionsmikroskopie wurden durchgeführt, um eine neue Myxosporea Art der Gattung *Unicapsula* zu beschreiben. Dabei wurden erstmalig die einzigartige Cystenstruktur und die Entwicklungsstadien für einen Vertreter der Gattung beschrieben. *Unicapsula fatimae* Al-Jufaili, Freeman, Machkevskyi and Palm, 2015 ist die erste Art, die auf dem Oesophagusepithel des Wirtes vorkommt. Zudem konnten im Rahmen der vorliegenden Arbeit Vertreter zweier Gattungen ancyrocephalider Monogenea erstmalig in Küstengewässern des Omans festgestellt werden. Vergleichende morphologische Untersuchungen sämtlicher Vertreter der Monogenea *Glyphidohaptor* und *Tetrancistrum* führten zu neuen Artbeschreibungen dieser Gattungen. Zusätzlich wurden erstmalig neben DNA/RNA Sequenzen der kleinen und großen Untereinheit auch die ITS-1 Region sämtlicher Ancyrocephaliden, die *S. canaliculatus* infizieren können, untersucht. Der Digenea *Hysterolecithoides amurparuchini* n. sp. konnte durch morphologische Charakteristika, molekulargenetische Analysen und den Sitzes im Wirt sowie dem neuen Gebietsnachweis als eine neue Art beschrieben werden.

Geographische Variationen in der Komposition der Parasitenfauna von *S. canaliculatus* wurden mithilfe von Cluster-Analysen und Multidimensionaler Skalierung (MDS Plot) unter Verwendung des Bray-Curtis Index ausgewertet. Die zoogeographische Verbreitung der Parasiten von *S. canaliculatus* war deckungsgleich mit der Unterteilung der Küstengewässer des Omans in drei Ökoregionen. Es konnten beispielsweise Larvalstadien des digeneen Trematoden *Stephanostomum* sp. ausschließlich aus Fischen des Persischen Golfs isoliert werden. Andere Parasiten wie *Hysterolecithoides amurparuchini* n. sp., der Digenea *Preptetos* sp. sowie die Myxosporea *Kudoa* spp. waren auf das Arabischen Meer beschränkt.

Auf der Grundlage ihrer Zoogeographie wird die Nutzung dieser Parasitenarten als biologische Indikatoren vorgeschlagen, um unterschiedliche Populationen des Fischwirts *S. canaliculatus* in den Küstengewässern des Omans aufzeigen zu können.

Die vorliegenden Untersuchungen ermöglichen die Bewertung der Umweltbedingungen mariner Ökosysteme der Küstengewässern des Omans. Detaillierte ökologische Analysen ermöglichen es unter Nutzung ausgewählter parasitologischer Parameter und ökologischer Indices anthropogen belastete Küstengebiete gezielt aufzuzeigen. Es wird deutlich, dass neben bisher verwendeten, auf Zackenbarschen basierenden Parasiten-Wirt-Modellen, auch die Parasitenfauna von *S. canaliculatus* ein hohes Indikatorpotenzial für Gewässerverschmutzungen aufweist. Insbesondere die in den Kiemen parasitierenden ancyrocephalide Monogeneen der Art *Glyphidohaptor safiensis* n. sp. und *Tetrancistrum* spp., der polyopisthocotylee Monogenea *Polylabris* sp. und die Crustacea *Hatschekia* spp. eigneten sich als aussagekräftige Bioindikator-Arten. Der Nematoda *Hysterothylacium* sp. sowie der Acanthocephala *Sclerocollum* sp. wiederum konnten zum Monitoring der Gewässerverschmutzungen aufgrund ihrer Fähigkeit der Schadstoffakkumulation verwendet werden.

Summary

This study aimed to investigate the parasite fauna of a marine herbivorous fish inhabiting the coasts of the Sultanate of Oman (Persian Gulf, Gulf of Oman, Arabian Sea). Although the white spotted rabbitfish, *Siganus canaliculatus* (Park) is a commercially important demersal fish both locally and regionally, knowasaledge regarding its parasite fauna is limited. For the first time in the region, a comprehensive parasite fauna from a single host species (n = 245) was documented, revealing highly diverse and species rich marine parasite community. The parasite fauna of *S. canaliculatus* consisted of one microsporidian hyperparasite, nine myxosporeans, four monogeneans, 16 digeneans, one cestode, four nematodes, four acanthocephalans, one hirudinea and four crustaceans. The results of this study provide insight into the richness and diversity of the marine ecosystem in the waters of Oman. Several species were reported for the first time as new host (16) and locality records (six). Among these, four are described and identified as new species to science.

Comprehensive morphological analysis using light, scanning and transmission microscopy was conducted to describe a new species within the myxosporean genus *Unicapsula* Davis, 1924. Unique cyst structure and developmental stages were described for the first time from a member of the genus. *Unicapsula fatimae* n. sp. is the only species among its congeners to be reported from the epithelium of the host oesophagus. In the course of the present work, several members of two monogenean ancyrocephalid genera that are known to infect siganids were reported for the first time from Omani waters. Comparative morphological investigations of all known members of *Glyphidohaptor* Kritsky, Galli & Yang, 2007 and *Tetrancistrum* Goto & Kikuchi, 1917, resulted in the description of two new species one from each of the genera. In addition, DNA/RNA sequences of the small and large subunit as well as the Internal Transcribed Spacer 1 of all ancyrocephalids infecting *S. canaliculatus* were obtained for the first time. The comprehensive analysis of one of digenetic trematodes infecting *S. canaliculatus* resulted in the description of a new species within the lecithasterid genus *Hysterolecithoides*. *Hysterolecithoides amurparuchini* n. sp. is described as a new species based on its distinctive morphological and molecular characteristics, zoogeographical distribution and its site of infection.

Geographical variations in the composition and distribution of *S. canaliculatus* parasite fauna were evaluated using clustering and multidimensional scaling based on Bray-Curtis similarity measure. The zoogeographical distribution of parasites of *S. canaliculatus* was coherent with the separation of the Omani coasts into three ecoregions. For example, larval stages of the digenean trematode *Stephanostomum* sp. were only recorded from the gills of hosts collected in the Persian Gulf. While some parasites such as *H. amurparuchini* n. sp. and the digenean *Preptetos* sp. as well as the myxosporean *Kudoa* spp. were restricted to samples collected along the Omani coasts of the Arabian Sea. We suggest that these parasites could be useful as biological tags to discriminate different populations of *S. canaliculatus* in Oman.

Finally, the survey of the parasite communities of *S. canaliculatus* provided an excellent opportunity to assess the status of the marine ecosystems in Oman. By conducting a detailed ecological analysis using 12 parasitological descriptors and five ecological indices of the obtained parasitological data, it was possible to reveal areas of anthropogenic alterations along the coasts of Oman. The study proved that similar to previous parasite-host models, which were based on marine groupers, *S. canaliculatus* is also a good model for the detection and monitoring of environmental impact in these marine ecosystem. Also, certain ectoparasites of *S. canaliculatus* showed great potential to indicate water quality. These include the gill infecting ancyrocephalid monogeneans *Glyphidohaptor safiensis* n. sp. and *Tetrancistrum* spp., the polyopisthocotylean monogenean *Polylabris* sp. and the crustaceans *Hatschekia* spp. While other parasites infecting *S. canaliculatus* were identified as more suitable as a sentinel for the monitoring of environmental pollution as bioaccumulators (e.g. the nematode *Hysterothylacium* sp. and the acanthocephalan *Sclerocollum* sp.).

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Abbreviations

%	Percent
µm	Micrometre
A	Acanthocephala
ANOVA	Analysis of variance
AS	Arabian Sea
C	Cestoda
Cd	Cadmium
Cr	Crustacea
D	Digenea
DNA	Deoxyribonucleic acid
e.g.	Exempli gratia
FAO	Food and Agriculture Organization
G	Gram
GoO	Gulf of Oman
H	Hirudinea
I	Intensity
i.e.	id est
m	Meters
mA	Mean abundance
mI	Mean intensity
Mi	Microsporidia
Mo	Monogenea
MoAF	Ministry of Agriculture and Fisheries Wealth
My	Myxosporea
N	Nematoda
nMDS	non-metric Multi-Dimensional Scaling
ºC	Grad Celsius
p	p-value of statistical testing
P%	Prevalence
Pb	Lead
PG	Persian Gulf
ppt	Parts per thousand
RDA	redundancy analysis
RNA	Ribonucleic acid
SD	Standard deviation
SIMPER	Similarity Percentage Analysis
SoO	Sultanate of Oman
TL	Total length
Twt	Total weight
USD	The United State Dollars
vs.	Verses
WoRMS	World Register of Marine Species

List of papers

1. Al-Jufaili S.H., Freeman M.A., Al-Nabhani A., Machkevskyi V.K. & Palm H.W. (2015). Morphological, ultrastructural, and molecular description of *Unicapsula fatimae* n. sp. (Myxosporea: Trilosporidae) of whitespotted rabbitfish (*Siganus canaliculatus*) in Omani waters. Parasitology Research, 115(3):1173-84.

   (Will be referred to as Al-Jufaili et al., 2015)

2. Al-Jufaili S.H. & Palm H.W. (2017). Species of *Tetrancistrum* Goto & Kikuchi, 1917 (Monogenea: Dactylogyridae) from the gills of the whitespotted rabbitfish, *Siganus canaliculatus* (Park) (Perciformes: Siganidae) off Omani coasts, with a description of *Tetrancistrum labyrinthus* n. sp. Systematic Parasitology, 94(7):809-818.

   (Will be referred to as Al-Jufaili and Palm 2017)

3. Al-Jufaili S.H., Machkevskyi V.K., Al-Kindi U.H. & Palm H.W. (submitted). *Glyphidohaptor safiensis* n. sp. (Monogenea: Ancyrocephalidae) from the White-spotted rabbitfish *Siganus canaliculatus* (Park) (Perciformes: Siganidae) from Oman, with notes on its phylogenetic position within the Ancyrocephalidae (sensu lato) Bychowsky & Nagibina, 1968.

   (Will be referred to as Al-Jufaili et al. ….a)

4. Al-Jufaili S.H., Machkevskyi V.K. & Palm H.W. (in preparation). *Hysterolecithoides amurparuchinii* n. sp. (Lecithasteridae: Hysterolecithinae) from white spotted rabbitfish *Siganus canaliculatus* from the Arabian Sea, Sultanate of Oman. Manuscript.

   (Will be referred to as Al-Jufaili et al….. b)

5. Al-Jufaili S.H., Unger P.F., Machkevskyi V.K. & Palm H.W. (in preparation). Parasite communities of herbivorous *Siganus canaliculatus* (Perciformes: Siganidae) from the Sultanate of Oman and their potential to indicate marine ecosystem health. Manuscript.

   (Will be referred to as Al-Jufaili et al. ….c)

The authors' contribution to the single publication

1. Morphological, ultrastructural, and molecular description of *Unicapsula fatimae* n. sp. (Myxosporea: Trilosporidae) of whitespotted rabbitfish (*Siganus canaliculatus*) in Omani waters. (*Al-Jufaili et al., 2015*)

   AJSH: Study design, data processing and analyses, writing, editing
   FMA: Molecular analyses, writing, editing
   MKV: Study design, data processing and analyses, writing, editing
   ANA: Scanning and transmission electron microscopy
   PHW: Study design, data processing and analyses, editing

2. Species of *Tetrancistrum* Goto & Kikuchi, 1917 (Monogenea: Dactylogyridae) from the gills of the whitespotted rabbitfish, *Siganus canaliculatus* (Park) (Perciformes: Siganidae) off Omani coasts, with a description of *Tetrancistrum labyrinthus* n. sp. (*Al-Jufaili and Palm, 2017*)

   AJSH: Study design, data processing and analyses, writing, editing
   PHW: Study design, data processing and analyses, editing

3. *Glyphidohaptor safiensis* n. sp. (Monogenea: Ancyrocephalidae) from the White-spotted rabbitfish *Siganus canaliculatus* (Park) (Perciformes: Siganidae) from Oman, with notes on its phylogenetic position within the Ancyrocephalidae (sensu lato) Bychowsky & Nagibina, 1968. (*Al-Jufaili et al. submitted*)

   AJSH: Study design, data processing and analyses, writing, editing
   MKV: Study design, data processing and analyses, writing, editing
   AKU Study design, data processing and analyses
   PHW: Study design, data processing and analyses, editing

4. *Hysterolecithoides amurparuchinii* n. sp. (Lecithasteridae: Hysterolecithinae) from white spotted rabbitfish *Siganus canaliculatus* from the Arabian Sea, Sultanate of Oman. (*Al-Jufaili et al. in preparation A*)

   AJSH: Study design, data processing and analyses, writing, editing
   MKV: Study design, data processing and analyses, writing, editing
   PHW: Study design, data processing and analyses, editing

5. Parasite communities of herbivorous *Siganus canaliculatus* (Perciformes: Siganidae) from the Sultanate of Oman and their potential to indicate marine ecosystem health. (*Al-Jufaili et al. in preparation B)*

AJSH:	Study design, data processing and analyses, writing, editing
PFU	Study design, data processing and analyses, writing, editing
MKV:	Study design, data processing and analyses, writing, editing
PHW:	Study design, data processing and analyses, editing

1 Introduction

1.1 Nature of Parasitism

In nature, virtually all living organisms spend their lives or portions of it in different forms of relations between the same species or with organisms from other species (Gunn and Pitt 2012). Heinrich Anton de Barry (1879) defined **symbiosis** as a physiological relationship between two different organisms living in close association, commonly one living on another (Kinne 1980). Symbiotic relationships can take many forms depending on the nature of the interaction between the two participants in the relationship, whether it is beneficial, harmful, or neutral (Loker and Hofkin 2015). Accordingly, **parasitism** describes an antagonistic form of symbiosis in which one organism (the parasite) either harms or in some sense lives at the expense of another organism (the host) (Schmidt and Roberts 1977). The host receives no benefit from this association and is often actively injured by the parasite (Watson 1965). **Damage** is an essential component of parasitism, and it is one of the key aspects that sets it apart from other symbiotic associations such as mutualism and communalism (Bush et al. 2001). The forms of damage exerted by this association include withdrawal of life-supporting substances, modification or destruction of host functions, the aberration of host structure and the reduction of the ecological potential of the host (Kinne 1980).

Parasitism is one of the most successful forms of symbiotic associations on earth (Poulin and Morand 2000). Various estimations suggest that at least 50% of all plants and animals are parasites at some stage during their life cycle (Bush et al. 2001). Conforming to the definition of parasitism, a **'parasite'** is an organism living in or on another living organism and obtaining from it part or its entire organic nutrient or needs for existence while imposing a net of a detrimental effect on it (Loker and Hofkin 2015). Thereby, technically any living organism that is capable of leading a parasitic lifestyle can pass as a parasite. However, in its strict sense, parasitology as a field of science only focuses on **eukaryotic animal** parasites (Bush et al. 2001; Rohde 2005), particularly with three major groups of animals; parasitic protozoa, parasitic helminths**,** parasitic and vector arthropods (Bogitsh et al. 2005; Lucius et al. 2017). Eukaryotic animal parasites have **unique characteristics** that distinguish them from other pathogens such as physiological adaptations, reduction or loss of organs, an increase of reproductive capacity, modification of existing structures, development of new structure and modification of life history (Watson 1965). They are also capable of displaying a range of mechanisms to evade the immune responses of the host

unknown to other pathogens (Cox 1993). Furthermore, because they are highly sophisticated, reproduce slowly and have low genetic flexibility, animal parasites can establish long-standing connections using strategies different from the **"hit-and-run"** strategies used by many viruses and bacteria (Lucius et al. 2017).

1.1.1 Parasite ecology and life cycles

Parasites are taxonomically diverse and have evolved a variety of strategies and life history traits, to infect their hosts and ensure successful transmission and reproduction (Poulin 2011). Parasites can be defined based on their location on the hosts as ectoparasites, endoparasites and mesoparasites. According to Bogitsh et al. (2005) and Lucius et al. (2017), **ectoparasites** attach to the skin or other external surfaces or can be superficially embedded in the body surface. **Endoparasites** usually reside inside their hosts' bodies, in the organs or within tissues (Lucius et al. 2017). Mesoparasites are mainly recorded from the aquatic realm and describe species that are partly imbedded inside the host tissue with other body parts hanging outside (Rohde 2005).

Other definitions of parasites are based on the nature of the host-parasite interaction and the level of physiological dependency. **Facultative** parasites are free-living adult organisms that optionally adopt a parasitic lifestyle at some point in their lives (Bush et al. 2001; Bogitsh et al. 2005), but are independent of the host for survival or completion of their life cycle (Lucius et al. 2017). **Obligate** parasites are physiologically dependent on their hosts and cannot complete their life cycle without spending at least one developmental stage in a parasitic relationship (Schmidt and Roberts 1977; Bogitsh et al. 2005). They are usually parasites as adults, but their larvae could be either obligate or free-living (Bush et al. 2001). The term **permanent** parasites describe adult parasites that reside in their hosts throughout their development and must be transferred from one host to another to complete their life cycles (Chandler 1947; Schmidt and Roberts 1977). Whereas parasites that live on their hosts at certain stages of their development and can leave them at defined intervals for feeding, moulting or mating and lay eggs are known as **temporary** parasites (Chandler 1947).

Parasites life cycles differ significantly but can be generally described as monoxenous (direct) or heteroxenous (indirect). The **monoxenous** life cycle is where the parasites complete their developmental stages, reach maturation, and may reproduce within a single host (Loker and Hofkin 2015). Parasites utilising this life cycle have both parasitic and free-living life stages in

which the parasites are directly transmitted from one host to another host that is usually a member of the same host species (Lucius et al. 2017). This mode of lifecycle promotes high productivity, which in turn increases parasite dispersal and proliferation (Rohde 2005). **Heteroxenous** life cycles are comprehensive and intricate, require at least two hosts and are accomplished by an indirect transmission that involves switching hosts at different life stages (Lucius et al. 2017). The complex life cycles observed for many parasites that utilise multiple hosts are a result of complicated and integrated ecological relationships with their hosts (Bogitsh et al. 2005).

Hosts are an essential lifeline for the parasites; according to Combes (1991), they are the primary ecological environment in which the parasite takes shelter, feed and reproduce and without it, the parasite might perish (Loker and Hofkin 2015). They are usually categorised depending on the role they play in the parasite's life cycle (Gunn and Pitt 2012). All parasites require a **definitive host** in which they can reach maturity and undergo sexual reproduction. Parasites with complex life cycles require one or several **intermediate hosts** in which they complete various developmental stages with or without asexual reproduction, but they never develop to adults or reproduce sexually (Gunn and Pitt 2012). **Paratenic hosts** are not essential for the completion of a parasite life cycle. Instead, they merely act as a bridge between the infective stage/intermediate host and the definitive hosts (Gunn and Pitt 2012). Paratenic hosts provide a refuge for the infective stage of the parasite where it can persist and prolong its survival and consequently increase the likelihood of its transmission to a new host (Loker and Hofkin 2015). Unlike paratenic hosts, **reservoir hosts** are carriers of infective organisms, often tolerating the infection without showing any effect (Bogitsh et al. 2005). Reservoir hosts harbour parasites that are usually associated with human infections (zoonosis) (Schmidt and Roberts 1977). With such intricate, highly adaptive way of life, it is not surprising that parasites are incredibly successful, make up an essential component of the earth's biodiversity and play critical roles in functional ecosystems (Thomas et al. 1999; Gomez et al. 2012).

There is a general difference between terrestrial and marine ecosystems because the parasite transmission requires different pathways. In the terrestrial ecosystem, many parasitic stages are transmitted through insects, where the intermediate stages multiply before infecting the final host. A parasite **vector** is a micropredator that transmit infections from one host to another (Bush et al. 2001). In the aquatic ecosystem, insects as vectors are mainly absent and leeches (Hirudinea) serve

as vectors for some blood protozoans (Hemmingsen 2008) or the parasites disperse directly or through the marine food web (see below).

1.1.2 Marine parasitology

Marine parasites are an integral component of marine ecosystems as they play a significant role in marine biodiversity, infecting hosts at different trophic levels (Palm 2004). The aquatic environment provides the ideal conditions for the propagation, distribution, and maintenance of aquatic parasites life cycles (Barber et al. 2000). As a result, almost all groups of marine animals ranging from the various invertebrates and vertebrates are important hosts to parasites, often with high prevalence and intensities of infection (Rohde 2005). In fact, among the vertebrates, fishes have the highest rates of parasitic infection, which is facilitated by the aquatic environment (Cavalcanti et al. 2012), making them essential as hosts of parasites in the aquatic ecosystems (Barber and Poulin 2002).

There are estimates of the existence of more than 100,000 marine fish parasite species around the world, including both protozoans and metazoans (Palm 2011). So far it is not possible to overview such high biodiversity, especially because not all different ecosystems and regions have been studied for fish parasites. Parasite-host checklists assist the enrichment of the knowledge about marine parasite biodiversity and their host specificity at different localities (Palm and Bray 2014). Many researchers conducted exhaustive surveys of marine parasites in definite geographical regions resulting in the compilation of parasite-host checklists from different regions of the world (Palm 2011; Palm and Bray 2014). The high number of parasites registered in these lists emphasises the **ecological importance** of marine fish parasites in the oceans (Rohde 1993). Furthermore, such surveys enable the estimation of the number of parasites in their respective marine habitats. For example, following a series of parasitological surveys, approximately 20,000 fish parasite species were estimated infecting the fishes of Heron Island, Australia (Rohde 1993). Similarly, based on an average of 3-4 metazoan parasites per fish (calculated for 13, 500 known fish species), Klimpel et al. (2001) estimated around 20,000 to 40,000 marine fish parasites inhabiting brackish and marine waters.

Aside from their role in marine biodiversity, marine parasites have immense economic importance as evident by the increase of research in areas related to human health, mariculture and fisheries as well as ecology (Rohde 1993). Many fish parasites have been the subject of

investigation because of their zoonotic potential, (i.e. their ability to infect humans (Lucious and Poulin 2017). In recent years, fishborne zoonoses has been becoming more common due to several factors including the globalisation of the food supply (Chai et al. 2005) and climate changes (Overstreet 2013). Subsequently, numerous seafood-borne parasites have been reported from fresh and marine water fishes; these are mostly **helminths** that use humans as intermediate or definitive hosts (Overstreet 2013). Other marine fish parasites are of **aesthetic** importance for the fishery and aquaculture industry since they infect the musculature and body surface of many teleost and therefore reduce their marketability and value (e.g. the myxosporeans *Kudoa* spp., Moran et al., 1999; trypanorhynch cestodes, Palm 2004). These infections can severely distort the host appreance causing implications for the seafood processing companies from infected wild fish stocks (Silva et al. 2017) as well as the mariculture industry (Moran et al. 1999; Tamaru et al. 2006). In addition to the problems related to the safety and quality of marine fishes, several groups of marine parasites correlate directly to their hosts' health.

The continuous increase in the demand for fish and seafood as a source of protein coupled with the ongoing decline of natural fisheries stocks is enforcing the expansion of aquaculture industry (FAO 2015). As a result, the global finfish production industry is increasing each year proving aquaculture to be the fastest growing food production and the most reliable supplier of seafood in the world (Guo and Woo 2009). However, the rapid development of mariculture industry and the nature of open water facilities have caused the emergence of disease outbreaks in many fish farms (Timi and MacKenzie 2015). Diseases, and among them **parasitic disease,** are one of the critical factors threatening the aquaculture industry (Rohde 2005). The infection of the Norwegian salmon farms with the parasitic copeopde *Lepeophtheirus salmonis salmonis* Krøyer, 1837 alone has caused annual losses of hundreds of millions USD (Abolofia et al. 2017; Lafferty et al. 2015). Other representatives of various animal parasites phyla that are considered as crucial disease-causing agents in fish cultivation facilities include many species of ecto-protozoans (Basson and Van As 2006; Dickerson 2006; Buchmann 2015); Myxosporea (Feist and Longshaw 2006; Lom and Dykova 2006; Yokoyama et al. 2013); Monogenea (Ogawa 2002; Buchmann and Bresciani 2006; Ogawa 2014); Digenea, especially blood flukes (Paperna and Dzikowski 2006; Ogawa 2014) and some species of parasitic copepoda (Johnson et al. 2004; Lester and Hayward 2006).

1.1.3 Fish parasites as environmental indicators

Despite their adverse effect on fisheries and mariculture industry, fish parasites are attracting increasing interest as indicators for a wide range of biological and environmental applications (Palm and Bray 2014). For over a century, several studies have demonstrated the usefulness of fish parasites from different taxonomic groups as an early warning system for the assessment of environmental health (Sures et al. 2017). During the last decades, there has been a considerable amount of research articles and reviews dealing with parasites of aquatic organisms as **environmental indicators** (Sures et al. 1999; Sures 2001; Vidal-Martinez 2007). One of the reasons for considering parasites as environmental indicators is that they outnumber free-living organisms (MacKenzie et al. 1995) and display various adaptations to the parasitic way of life in different types of hosts and diverse environments (MacKenzie 1999; 2008). Also, many parasites have **heteroxenous life cycles** involving several vertebrate and invertebrate intermediate hosts (MacKenzie 1999; 2008; Palm and Bray 2014). Thus, alterations in the populations of these intermediate hosts due to environmental changes or pollution could adversely impact the abundance or availability of the parasites and accordingly reflect environmental disturbance (MacKenzie 1999).

Moreover, many parasites have delicate short-lived free-living developmental stages used for the parasite transmission which are highly sensitive to environmental changes (MacKenzie 1999; 2008). Ectoparasites with monoxenous life cycle (direct) are suitable as indicators of water quality because of their direct contact with their environment and consequently the contaminant in the aquatic environment which might affect their vitality or increase their mortality rates (Galli et al. 2001; Sures et al. 2017). Pollution can impact parasites in different ways; it can either increase or decrease parasitism (Sures 2006). In fact, many studies showed that parasites respond differently to the same pollutant (Mackenzie 2008). For this reason, fish parasites are also useful as environmental indicators because of the variety of ways they respond to anthropogenic pollution (Lafferty 1997; Sures et al. 1999; Sures 2006). Lastly, Parasites can be used as effective monitoring tools in environmental impact studies as they efficiently **accumulate** certain pollutants (e.g. heavy metals and trace elements) at levels much higher than those of their ambient environment and free-living sentinels (Sures and Nachev 2015).

Accumulation indicators provide valuable information about the chemical state of the environment through their ability to concentrate toxins within their tissues (Sures et la. 1999) and give insight into the **biological availability** of this pollutant in the investigated environment (Nachev and Sures 2015). Several fish parasites groups were investigated for their capacity to accumulate pollutants in their tissues. However, **intestinal helminths** such as acanthocephalan and cestodes were the most efficient in metal concentration (Sures 2006). Due to their specific biology and physiology (Nachev and Sures 2015), these parasites can accumulate metals at levels several thousand-folds higher in their tissues than in the tissues and organs of their hosts (Sures 2004). For example, it was established that the freshwater acanthocephalan *Pomphorhynchus laevis* Zoega in Müller, 1776 accumulated lead at mean concentrations that were 2700 and 400 times higher than their host *Leuciscus cephalus* (Bonaparte) (Sures et al. 1999). Similarly, the freshwater cestode *Monobothrium wageneri* Nybelin, 1922 had higher lead and cadmium concentrations in their tissues than in their hosts *Tinca tinca* (Linnaeus) from Ruhr River (Palm 2011). These studies suggested that freshwater acanthocephalans exhibit better accumulation capacity than any other group of parasites and even better than free-living sentinels (Nachev and Sures 2015).

With respect to the **marine fish-parasite** system, several studies demonstrated the usefulness of marine fish parasites as accumulation indicators. Sures and Reimann (2003) detected higher levels of pollutants in the marine acanthocephalan *Aspersentis megarhynchus* von Linstow, 1892 in comparison to their host *Nothotenia coriiceps* (Richardson) and the established free-living accumulation indicators the bivalve *Leternula elliptical* (King) (Nachev and Sures 2015). While studies involving marine fish-acanthocephala are limited, the majority of marine system studies explored fish nematodes and cestodes as potential accumulation indicators (Nachev and Sures 2015). Analysis of two heavy metals (lead (Pb) and cadmium (Cd)) in the tissue of the marine cestode *Bothriocephalus scorpii* (Müller, 1776) Cooper, 1917 and its host *Scophthalmus maximus* (Linnaeus) showed that the first accumulated higher levels of these two metals compared to the host muscle (Sures et al. 1997). Similarly, higher concentrations of lead and cadmium were established in cestodes infecting sharks and rays off Iranian coats of the Persian Gulf and Gulf of Oman (Malek et al. 2007; Golestaninasab et al. 2014). Research concerning marine fish-nematodes systems from the Gulf of Oman, Arabian Sea, Mediterranean Sea and the Atlantic Ocean indicated the suitability of nematodes (e.g. *Hysterothylacium,* Khaleghzadeh-Ahangar et al. 2011;

Paraphilometroides nemipteri Moravec & Shaharom-Harrison, 1989, Mazhar et al. 2014) for metal biomonitoring (Nachev and Sures 2015; Sures et al. 2017).

A further application for using fish parasites as environmental indicators is their use as effect indicators for environmental changes (Palm 2011). **Effect indicators** are organisms that provide information about the chemical, physical, biological and ecological state of the environment through their presence or absence (Sures 2001; 2004). Pollution can influences fish parasite directly (i.e. toxicity to the parasite itself) or indirectly (i.e. effect on the intermediate, paratenic and final host) (Sures 2004; MacKenzie 2006). The direct influence of pollution results in reducing **viability** or **survivability** of the affected parasites individuals (Sures et al. 2017). The indirect influence causes changes in parasites diversity and composition through reduction or elimination of hosts (Sures e al. 2017). Several hundred articles have established that aquatic parasites display changes at the individual, population and **community** levels in relation to pollution (Sures 2004; Blanar et al. 2009).

Effect indicators at the community level involves the examination of the entire parasite community (protozoan and metazoan) of a particular host combined with estimation of quantitative descriptors of parasite populations (e.g. prevalence, intensity and abundance) and communities (e.g. diversity, evenness and richness) to provide information on the health status of the environment (Palm and Bray 2014). Various researchers verified the usefulness of fish parasite communities as bioindicators for ecosystem health. The total abundance of parasites of apogonid fish hosts was investigated as a potential indicator of the environmental condition of the coral-reef lagoon, New Caledonia (Sasal et al. 2007). The authors established that parasite abundance correlated to the environmental conditions and that overall, encysted digenetic metacercariae in the pericardic cavity were significant indicators of the environmental conditions in the inner bays. Diamant et al. (1999) combined host tissue biochemical and histochemical tests with parasite community descriptors such as the ratio between heteroxenous and monoxenous parasites (H/M) and the the ratio of heteroxenous and monoxenous species richness (SH/SM) to compare the effect of pollution on the parasite composition and structure.

Other studies applied different **statistical multivariate analysis** to test the association between parasite communities and environmental variables (Discriminant analyses, Valtonen et al.

1997; Canonical correspondence, Marcogliese et al. 2006; Constrained ordination by redundancy analysis (RDA), Pech et al. 2009; Vidal-Martinez et al. 2014).

Palm and Rückert (2009) established a **visual integration system** to assess the health of a tropical marine ecosystem in Indonesia using fish parasites as bioindicators. Based on previous investigations of Rückert et al. (2008), three significant parasitological descriptors (prevalence of trichodinid ciliates (P%), ecto- to endoparasite ratio (Ec/En) and Shannon diversity index (H`) for endoparasite diversity) were calculated and transferred onto a positive-negative axis and were plotted into a star graph. The results suggested that Segara Ankan Lagoon is an impacted location as indicated by low endoparasite diversity, high trichodinid ciliates infections and high ecto- versus endoparasite ratio. The study also supports the application of star graph as an effective tool to **visualise** the variations in parasite composition of different fish hosts sampled at different locations in a tropical ecosystem.

Subsequently, the system was successfully implemented as a model to various aquatic habitats in Indonesia. For example, the metazoan parasites of the grouper, *Epinephelus fuscoguttatus* (Forsskål) were used to monitor **long-term changes** in a mariculture facility in Thousand Islands, Indonesia (Palm et al. 2011). In this study, the variations in the prevalence of the Tetraphyllidean larvae *Scolex pleuronectis* Müller, 1788 as well as the nematodes *Terranova* sp. and *Raphidascaris* sp. were used as additional indicators to reveal changes in mariculture management and to detect environmental alterations under mariculture condition. Following the same methodology, Kleinertz et al. (2014) used a combination of fish health indicator (e.g. hepatosomatic index) with specific parasitological and ecological parameters to evaluate the environmental condition in two different locations off the coasts of Indonesia. The obtained results demonstrated regional variations in parasite composition and suggest that anthropogenic conditions reduce the parasite richness and the diversity of endoparasites.

A novel environmental indicator system was designed by Neubert et al. (2016) to assess the environment of a heavily polluted site in Indonesia (Jakarta Bay). For this purpose, twelve parasitological and ecological descriptors were selected, normalised and assigned onto several star graphs to illustrate the environmental condition of the targeted location in comparison to the previously obtained data from Indonesian waters. The areas of the star graphs were then calculated

and plotted as a histograph that represent a "**pollution light**" with large areas indicating healthy ecosystems while small areas indicating impacted or polluted sites (Figure 1.1).

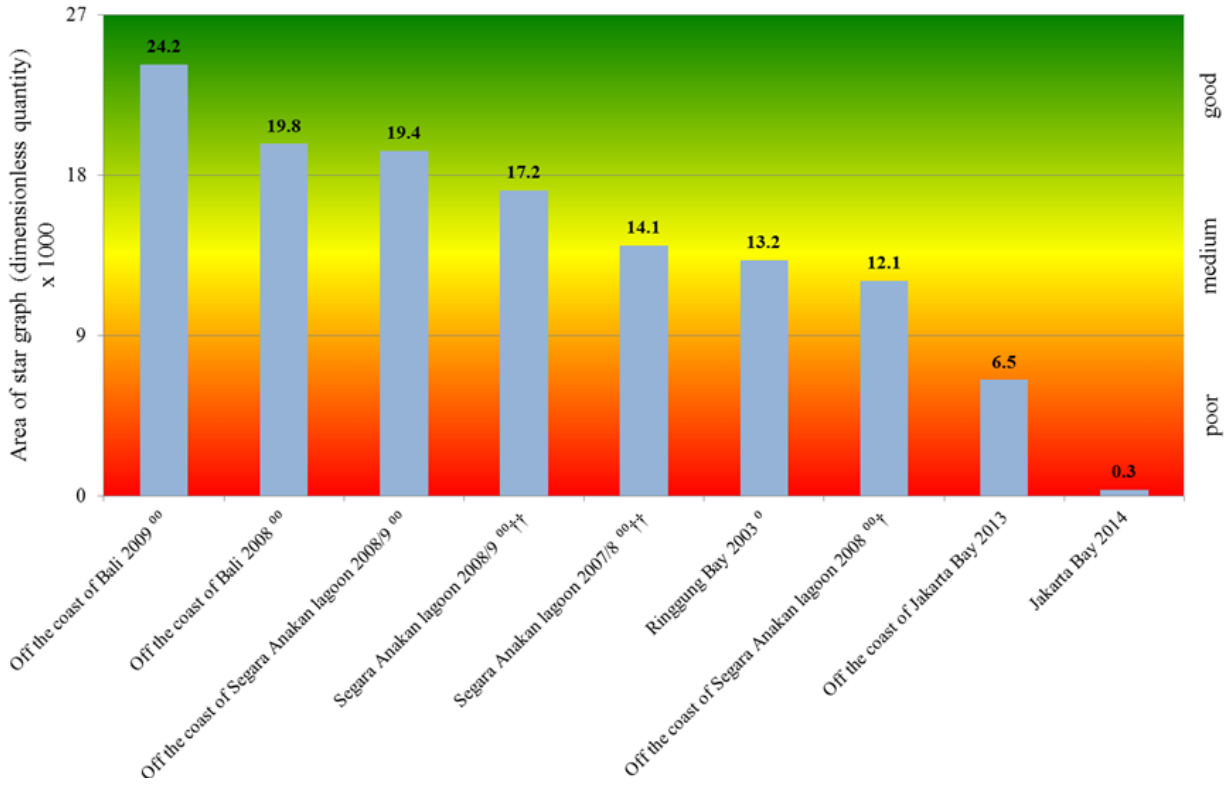

Figure 1. 1 Pollution light: Areas of star graphs calculated from normalised parasitological parameters of Epinephelus coioides. Analysed habitats sorted in a range from good (green), medium (yellow) and poor (red) to assess environmental conditions of sampled Indonesian coastal waters (Source: Neubert et al. 2016).

Recently, the above-mentioned methodology was effectively applied in Vietnam to estimate the influence of different mariculture facilities on the marine environment in the Gulf of Tokin (Truong et al. 2017) (Figure 1.2). Through the utilisation of protozoan and metazoan parasites of the grouper *Epinephelus coioides* (Hamilton), the study emphasises that epinephlid hosts could be excellent ecological models for estimation and monitoring of environmental health in various tropical marine systems. Also, the findings provided practical suggestion on how to reduce the impact of grouper mariculture facility in Vietnamese waters.

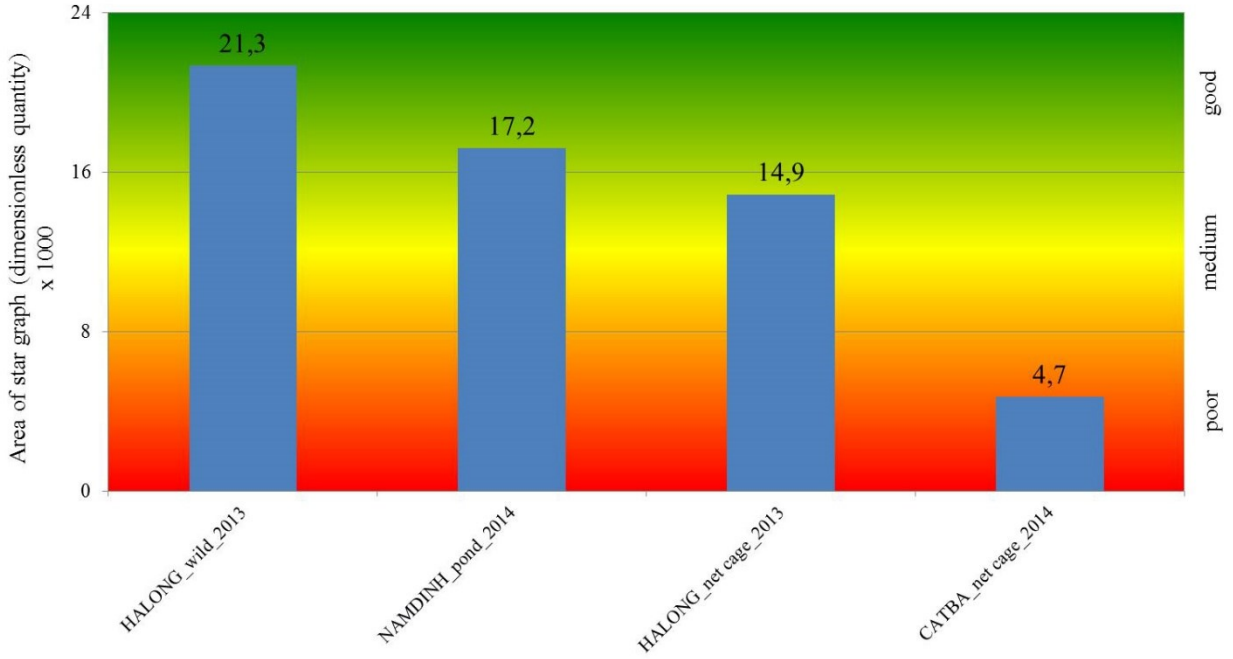

Figure 1. 2 Areas of star graphs calculated from normalized parasitological parameters of Epinephelus coioides, different aquaculture systems were compared with the natural environment, sorted in a range from good (green), medium (yellow) to poor (red) to assess aquaculture conditions of sampled facilities from Vietnamese coastal waters. (Source: Truong et al. 2017).

1.1.4 History of Marine parasitology in the Sultanate of Oman

The first parasitological investigation in the waters of Sultanate of Oman dated back to the 19th century through the visits of the Royal Navy ship H.M.S. c Cossack to different harbours in the Indo-tropical region (Bassett-Smith R.N.F.Z.S.F.R.M.S. 1898). Four species of crustacean copepods were described *Caligus platytarsis* Bassett-Smith, 1898, *Lepeophtheirus rotundiventris* Bassett-Smith, 1898, *Brachiella multifimbriata* Bassett-Smith, 1898 (= *Parabrachiella multifimbriata* Bassett-Smith, 1896) and *Pseudoclavella ovalis* Bassett-Smith, 1898 (= *Hatschekia ovalis* Bassett-Smith, 1898).

The earliest systematic marine parasitological investigations in the Sultanate of Oman was initiated in the late 1960s by the Russian parasitologists Mamaev Y.L. and Paruchin A.M. on board of the fishing research vessel "Skif". For more than a decade (1975-1989), the survey mainly covered the central part of Omani coasts off the Arabian Sea including Masirah Bay, Sawqarah Bay, and Kuria-Muria Bay (Figure 1.3). During their investigations, a total of 64 marine fish species belonging to 30 families were examined. A total of 104 parasite species were registered,

these parasites beloning to 5 major parasite taxa Monogenea (5), Digenea (72), Cestoda (1), Nematoda (16) and Acanthocephala (10). The results of this investigation were documented in two book and some articles (in Russian) (Mamaev and Paruchin 1975; 1976; Paruchin 1976; 1989).

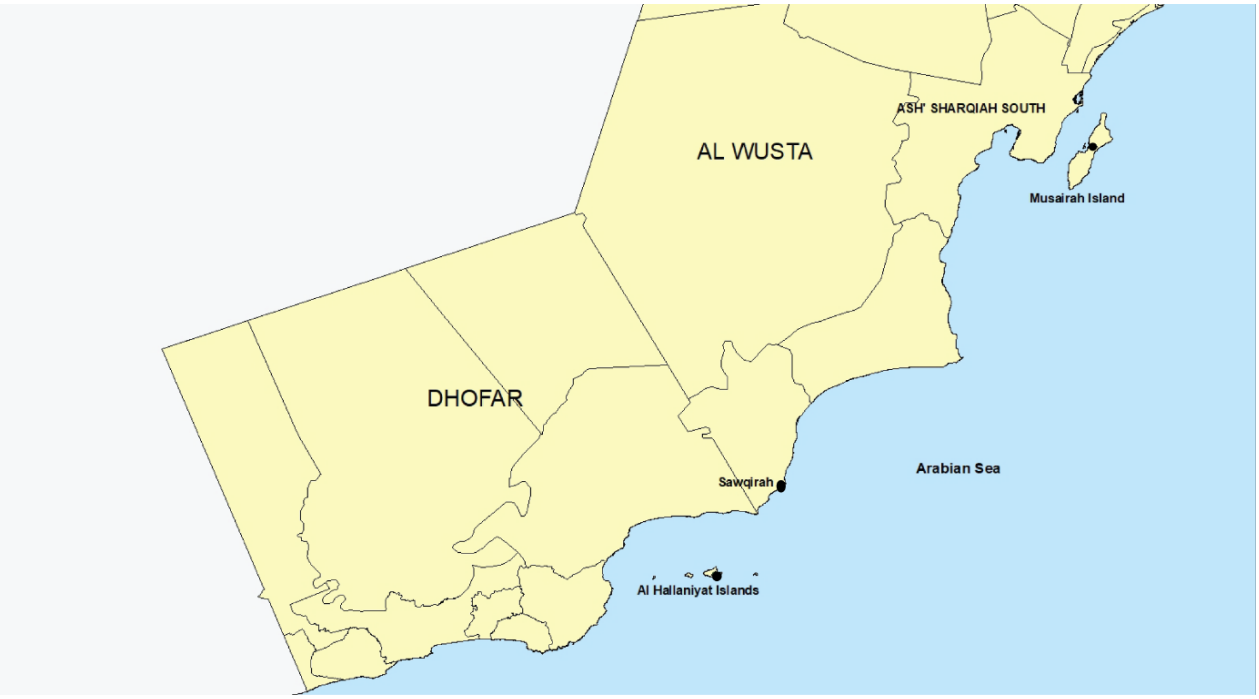

Figure 1. 3 Sampling locations along the Omani coasts of the Arabian Sea (Masirah Island, Sawqirah, Al Halaniyat Islands (Kuria- Muria), black solid circles) during the Russian expeditions in 1970s by Paruchin AM and Mamaev YL.

In the year 2009 marine parasitology investigations commenced again with the establishment of the Laboratory of Aquatic Parasitology (LoAP) at the Fishery Quality Control Centre, Ministry of Agriculture and Fisheries Wealth. For a duration of nine years (2009-2018) and through several research project and surveys, a total of 49 commercially important host species belonging to 22 families were examined for parasites. These surveys resulted in the registration of a considerable number of parasites belonging to different taxa (197 parasites). The new collection of parasites was composed of the following parasites, Microsporidia (3), Myxosporea (4), Monogenea (52), Digenea (16), Cestoda (28), Nematoda (36), Acanthocephala (4), Crustacean (52) and Hirudinea (2). These parasites species were mainly registered from the Omani coasts of Gulf of Oman.

During this period two new microcotylid monogenean were reported from Omani waters. *Omanicotyle heterospina* Yoon, Al-Jufaili, Freeman, Bron, Paladini & Shinn, 2013 was detected

and described from the gills of the King soldier bream *Argyrops spinifer* (Forsskål). This parasite was previously described as *Bivagina heterospina* Mamaev & Paruchin, 1974 from samples collected from Kuria Muria Bay, off Kuria Muria Islands in the Omani coasts of the Arabian Sea (Yoon et al. 2013). So far this monogenean is the only species reported from Omani waters although the host is widely distributed in the Indo-Pacific Ocean (including, Western Indian Ocean extending eastward to the Indo-Malayan archipelago and northern Australia, Froese and Pauly (2019) and was already investigated for monogenean gill parasites from other localities (e.g. Kritsky et al. 2000). An additional new species *Microcotyle omanae* Machkevskyi, Dmitrieva, Al Jufaili & Al Mazrooei, 2013 was described from the gills of the santer bream, *Cheimerius nufar* (Valenciennes) off the Omani coasts of the Arabian Sea.

The compilation of all marine parasites registered from Omani waters resulted in an updated host-parasite list including 305 parasites species reported from a total of 113 hosts. Counts of species in each major parasite group showed that the digeneans are the largest group in terms of species richness (100 species, 32.79%). This observation coincides with that fact that digenean parasites are the most speciose group of metazoan endoparasites (Rohde 2005). However, the proportion of the Digenea in Omani waters is lower than the proportion in Hawaii (Palm and Bray 214). This observation could be attributed to the low effort in investigation of this parasitic group in the beginning of marine parasitological investigation in Oman. The other parasite groups are represented by, in order of size: Crustacea (54 species, 17.70%), Monogenea (51 species, 16.72%), Nematoda (50 species, 16.39%), Cestoda (28 species,9.18%) and Acanthocephala (13 species, 4.26%).

The remaining parasite groups, Myxosporea (4 species, 1%), Microsporidia (3 species, 0.98%) and Hirudinea (2 species, 0.66%) are the least represented groups (Figure 1.4). It is noted that the proportion of the Nematoda and Cestoda was higher in Omani waters compared to Hawaii. The causes of this could be simply a representation of increased sampling efforts for these taxa because most of the nematodes registered in Omani waters were part of a survey that focused on the investigation of anisakid nematodes which also resulted in the detection of cestodes.

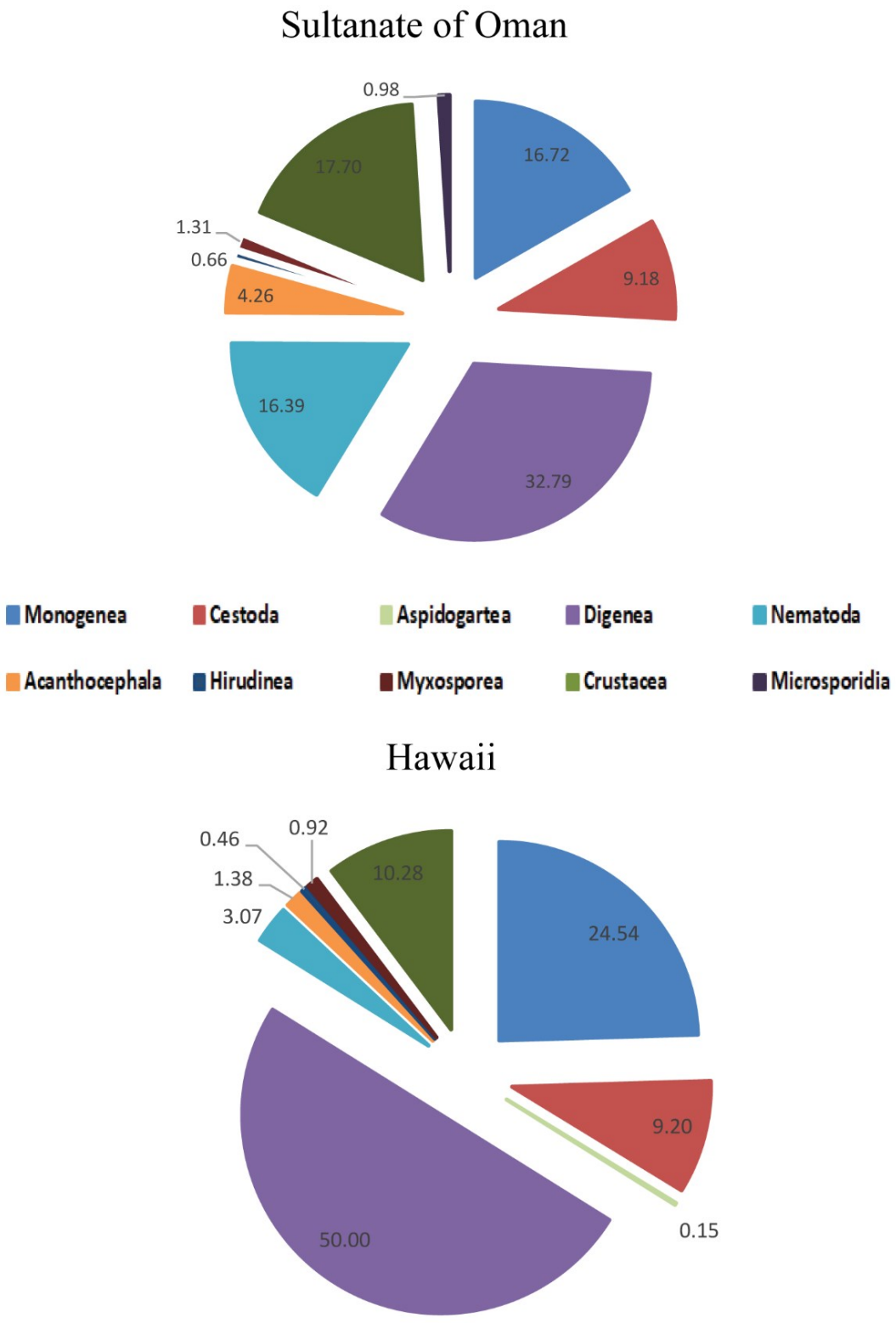

Figure 1. 4 Proportion (%) of the recorded 305 fish parasites taxa from the waters of Sultanate of Oman, coasts of Gulf of Oman and Arabian Sea (data compiled from available literature and recent surveys) and fish parasites taxa in Hawaiian waters according to Palm and Bray (2014).

The host species included in this list amounted to 107 marine fishes representing different habitats, the most investigated hosts were reef-associated species (56.8%) followed by demersal species (17.9%). Benthopelagic and pelagic-nitric hosts came third at 2.1% of the investigated hosts, while the least studies hosts were bathydemersal (2.1%). Considering that about 1070 fish species occur in the waters of Oman (Froese and Pauly 2019), only about 10% of the fishes were examined so far which does not demonstrate the actual parasite diversity in this locality. According to Palm and Bray (2014), an estimation of about 3-4 parasite species inhabit each fish (based on published host-parasite checklists). Thereby, parasite richness in Omani waters could be estimated at 3,210-4,280 metazoan parasites (excluding Protozoa). Since the complied list doesn't include all fish species and most parasite taxa are poorly represented (Microsporidia and Myxosporea), this list only provides a basic knowledge on the diversity of fish parasites and not a real estimation of parasite diversity in Oman.

1.2 Study area

The Sultanate of Oman (SoO) is the second largest country in the Arabian Peninsula; it is located in the southeastern corner of the Arabian Peninsula, northwest Indian Ocean (16.252 °N, 54.622° E) (Figure 1.5). Its coastal line extends 3,165 kilometres (in fine scale) from the Strait of Hormuz in the North to the borders of the Republic of Yemen in the South. The country overlooks three major water bodies: the Persian Gulf , the Gulf of Oman and the Arabian Sea. Each is characterized by a unique environmental and oceanographic features (Sheppard et al. 1992). Moreover, with a 200- mile exclusive economic zone, the SoO has about 340, 000 Km^2 of inshore and offshore waters which contains rich fishing grounds (Al-Hafidh 2006). Seven sampling sites were selected for this investigation based on their different geographical characteristics and suitability for the establishment of mariculture farms. Three sampling sites along the Arabian Sea; Raysut (Dhofar Governorate), Al Lakbi (Al-Wusta Governorate), Masirah Island (Al Sharqiya Governorate). Three locations facing the coasts of the Gulf of Oman; Muscat (Muscat Governorate), Sohar (Al Batinah Governorate) and Dabba (Musandam Governorate). The last location is Khasab (Musandam Governorate) which is the only location along the coasts of Persian Gulf.

Figure 1. 5 Map showing the Sultanate of Oman, its three water bodies and the seven locations investigated in the current study.

1.2.1 Arabian Sea

The Arabian Sea (AS) is one of the world's major Ocean basins, it is located at the north-western extension of the Indian Ocean (12.2502° N, 64.3372° E), bounded by Pakistan and Iran on the north, Somalia and Arabian Peninsula on the west and by India on the east. The total surface area of this sea is 3.862 million km^2 with two branches which connect it to two evaporation basins. One is on the south-west (Gulf of Aden) connecting the Arabian Sea to the Red Sea and another to the north-east (Gulf of Oman), connecting with the Persian Gulf (Carton et al. 2012). The maximum depth of the sea is reaching about 4,600 m and a maximum width of 2,400 km. it is characterized by significant shallow coastal waters, a moderate area of the continental shelf and a steep continental slope (Shallard et al. 2009).

The climate of AS is highly influenced by the south-west (SW) and north-east (NE) monsoon events that stimulate extreme weather fluctuations resulting in dramatic physical, chemical, and biological changes in the upper layers of the water column (Atlas 2010). These changes affect the productivity of the AS making it one of the most productive regions of the oceans (Madhupratap et al. 1996; Goes et al. 2005). The **SW monsoon** period takes place during the summer from May to September. In this period the primary productivity is greatly enhanced by the coastal and oceanic upwelling events that bring cool, low salinity and nutrient-rich water to the surface of the sea (Barber et al. 2001), encouraging active blooming of phytoplankton as indicated by elevated chlorophyll-a in the sea (Piontkovski et al. 2012) (Figure 1.6). During the events of the SW monsoon Sea surface temperature (SST) are low and variable with values ranging between 18-28 C° (Figure 1.7).

In the winter season, the **NE monsoon** occurs between November to February, in which the sea surface circulation is reversed from clockwise to anticlockwise (Gaye et al. 2018). Although not as substantial as the SW monsoon, the events of NE monsoon also promote phytoplankton growth and various biochemical processes because it influences the dynamics of mixed-layers and on various physical and oceanographic process (Goes et al. 2005). The temperatures during the NE monsoon are slightly higher and less variable than during SW monsoon (Figure 1.7). Another major feature which influences the productivity in AS is the seasonal development of one of the most prominent **oxygen deficient layers** in the world oceans at depths between 50 to 1000 m in which most fish cannot live (Shallard et al. 2009).

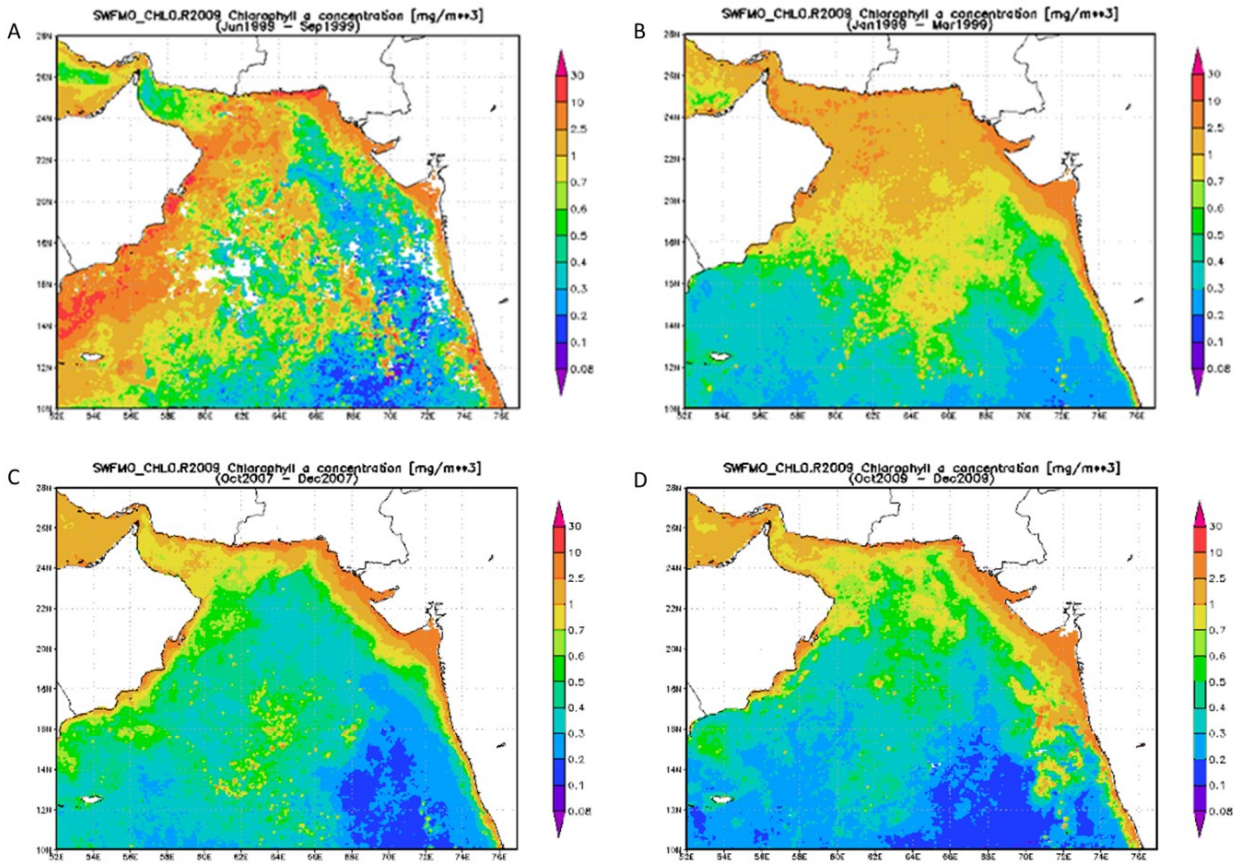

Figure 1. 6 Characteristic spatial patterns of chlorophyll-a concentration (in mg m-3) in the Arabian Sea, Gulf of Oman and Persian Gulf. Summer monsoon, (A), Winter monsoon, (B), Intermonsoon season, s (C) and (D) (source: Piontkovski et al. 2013).

1.2.2 The Gulf of Oman

The Gulf of Oman (GoO) is a semi-enclosed bathymetric triangular basin (Piontkovski et al. 2012) that is located in the subtropical zone of the Arabian Peninsula between 22° N and 26° N and 56° E and 62° E (Pous et al. 2004). It stretches from the Straits of Hormuz to the eastern tip of the Arabian Peninsula at Ras Al-Hadd (Al-Hashmi et al. 2012). GoO is about 480 km long and has a total surface area of 94,000 km^2. The narrowest point in GoO is at the eastern end of the strait of Hormuz (30 km) and the widest part is the end where it joins the Arabian Sea (370 km) (Walters and Sjoberg 1990). Bathymetry of GoO is characterized by a narrow continental shelf that has a gentle slope with increasing depths towards the south-eastern part reaching 3000 m (Piontkovski et al. 2012).

The climate in GoO is influenced by the hydrodynamics of the area which is driven mostly by monsoonal winds (SW and NE monsoon) and by the seasonal inflow and outflow of waters from the Persian Gulf (Persian Gulf Water mass) and the Indian Ocean (Indian Ocean Surface Water mass) (Piontkovski et al. 2012; Al-Azri et al. 2014) (Figure 1.8). During the summer, highly saline, oxygen-rich, and warm Persian Gulf Water mass (PGW) enters GoO through the Strait of Hormuz resulting in a coastal plume and formation of eddies and filaments of this plume (Al Azri et al. 2014). At the same time, a relatively fresh Indian Ocean Surface Water (IOSW) enters the Gulf of Oman on its northern side (Piontkovski et al. 2012). Another feature of the GoO during the summer monsoon is the formation of the Ras al Hadd Front, which acts as a liquid barrier between the GoO and AS. Furthermore, although the manifestation of SW monsoon is more pronounced in the southern region of GoO, its effect has been observed in GoO in the form of cool waters injected into the sea (Al Azri et al. 2010). Thus, in the summer the temperatures in GoO are cooler, ranging between 30 to 34 C°, while the productivity in GoO is minimal in comparison to those of the AS (Figure 1.6).

During the NE monsoon the Oman coastal current (the East Arabian Current) moves to a south-eastward flow and seasonal upwelling occurs in the Iranian coasts of GoO (Piontkovski et al. 2012). With the reversal of the above-mentioned current, the Ras Al Hadd Front becomes poorly pronounced or decays entirely and numerous eddies are formed instead (Piontkovski et al. 2011). These eddies aid in transporting of phytoplankton blooms to the coasts of GoO (Al Azri et al. 2014). Additionally, convective mixing in this season leads to decrease in SST and a well-mixed water column (Al Azri et al. 2010). The productivity of GoO is therefore significant during the winter due to the influence of NE monsoon events (Piontkovski et al. 2012; Al Azri et al. 2014) (Figure 1.6).

1.2.3 Persian Gulf

The Persian Gulf (PG), is an “L” shaped shallow and narrow, semi-enclosed marginal basin. It is located in an arid region of the Middle East between the Arabian Peninsula and Iran (L'Hégaret et al. 2013) extending between 24° N and 30° N and 48° E and 56° E (Pous and Carton 2004). The PG is bounded to the north by flatlands (the delta of Iranian and Iraqi rivers), to the north-east by the Zagros mountains, and to the south-west by the desert of Saudi Arabia (Pous et al. 2012). The surface area of the PG is 239,000 km2 with its broadest region of shallow water off the coast of the

United Arab Emirates (UAE) (Reynolds 1993). The maximum width of the PG is 338 km, and the length to its northern coast is 1000 km with a maximum depth of 120 m near the Straits of Hormuz (L'Hégaret et al. 2013). The Persian Gulf is separated from the GoO by the Strait of Hormuz, which is 56 km wide at its narrowest point (Reynolds 1993).

The climate and marine environmental conditions in the PG are among the most extreme on the planet (Naser 2013). The basin is characterized by low hydrodynamic energy, relatively shallow depths, high evaporation rates, extremely fluctuating surface temperatures, high salinities; and minimal water exchange (Khan 2007). Sea surface temperatures exceed 34 ºC in summer and can be less than 15 ºC in winter (Rezai et al. 2004), while salinities can be as great as 45 ppt reaching up to 60 ppt in some parts of the PG (Rezai et al. 2004). As a result of these extreme conditions, it is reported that flora and fauna of the PG are living close to the limits of their environmental tolerance (Price et al. 1993). The main water masses that influence the PG are the oceanic water flowing from the GoO and the outflow from the rivers located in the north-western end of the Iranian coast (Bjerkeng 2000). The other major event in the PG is the formation of one of the most saline water masses in the world, the PGW. The movement of this water mass affects the stability of the Indian Ocean's thermocline and introduces oxygen-rich water into GoO (Figure 1.7) at depths that are marked by extreme oxygen-depletion caused by the decay of surface layer primary production (Swift and Bower 2003).

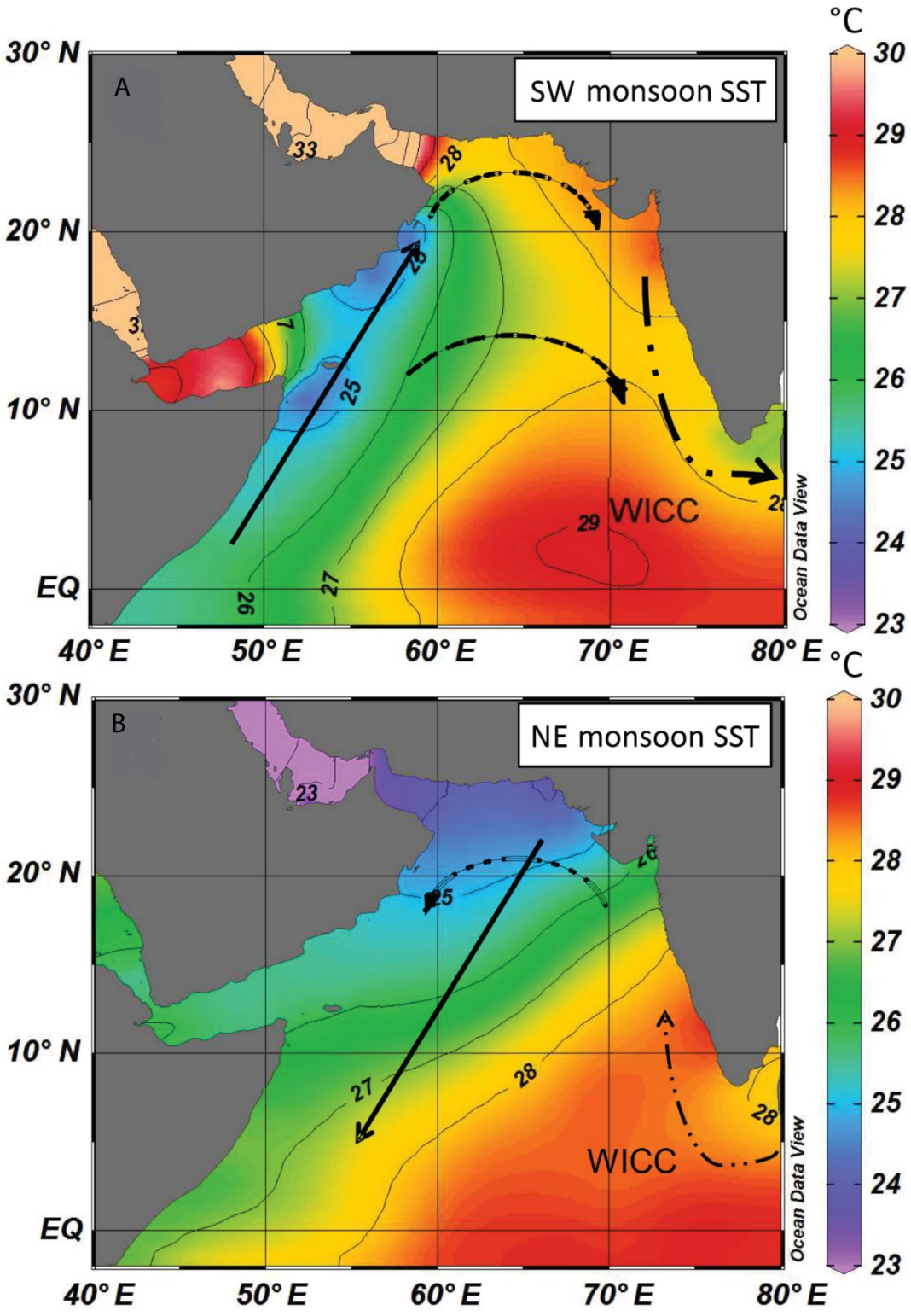

Figure 1. 7 Variations of sea surface temperature in the three water bodies during the two main seasons. South-West monsoon, (A). North-East monsoon, (B). (Source: Gaye et al. 2018).

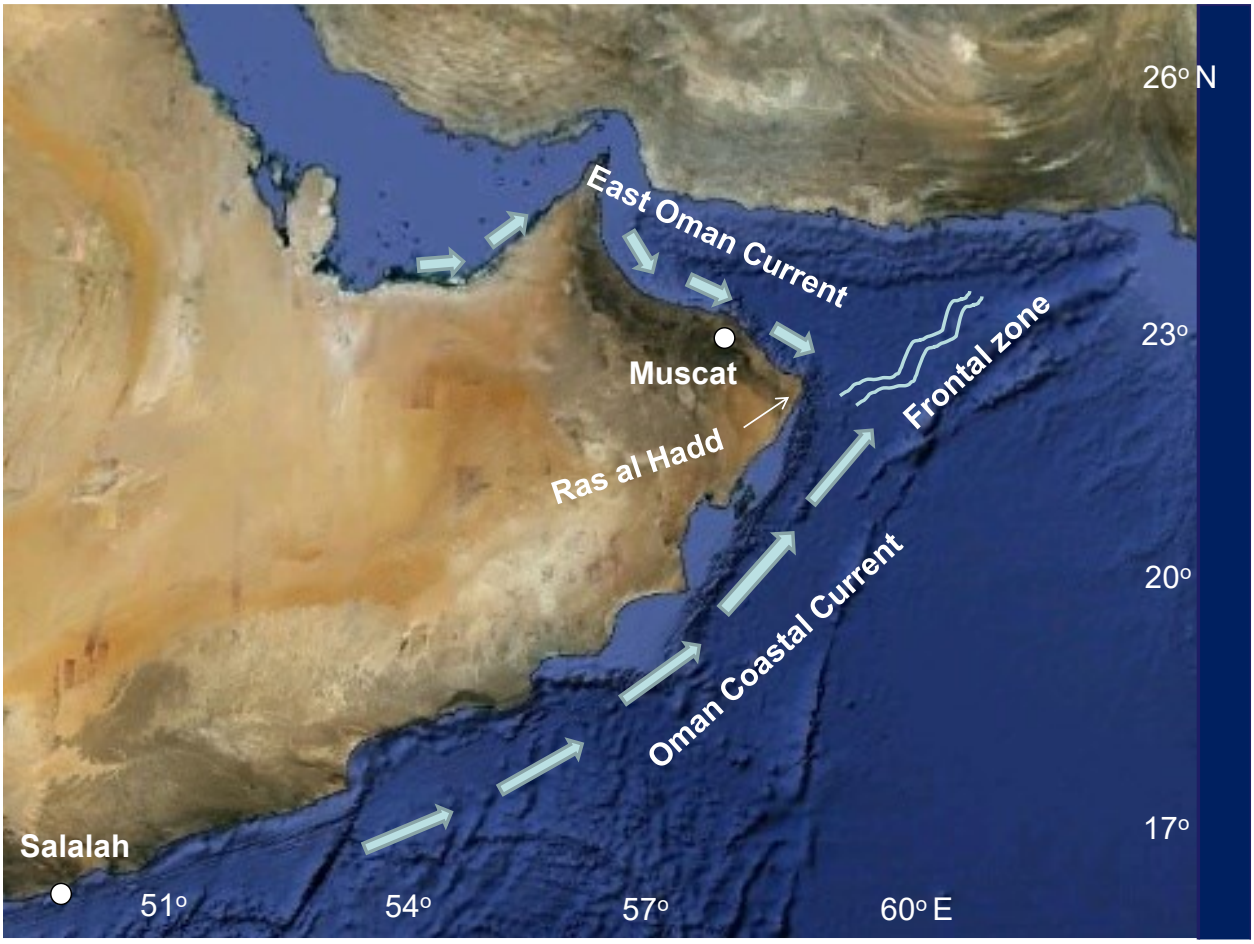

Figure 1. 8 The system of currents and water mass transport along the Omani coast. Two parallel lines (1) demarcate the location of the Ras Al Hadd frontal zone formed by the confluence of currents (3 and 4). Arrows (2-4) indicate the direction of the main currents (in summer through fall period). (2): inflow of the Indian Ocean Water mass, (3): outflow of the (Arabian Gulf) Persian Gulf Water mass, and (4): Oman Coastal Current (East Arabian Current) (Source: Piontkovski et al. 2012).

1.3 Rabbitfishes (Family: Siganidae Forsskål, 1775)

1.3.1 Taxonomy

Class Actinopterygii

Order Perciformes

Suborder Acanthuroidei

Family Siganidae Forsskål, 1775

Siganidae is a family of marine herbivorous fishes that are commonly known as rabbitfishes or spinefoots. They belong to the suborder Acanthuroidei (Actinopterygii: Perciformes) and are

closely related to three families within this suborder, the Luvaridae, Zanclidae and Acanthuridae (Pitt 1997; Tang et al. 1999). Formerly, siganids were regarded as members of the genus *Teuthis* (Linnaeus), however, this genus was suppressed by Woodland (1972, 1973) in favour of the genus *Siganus* (Forsskål) (Lam 1974; Duray 1998). Woodland (1983), discussed the zoogeographical distribution and species richness of siganids, reporting 27 species of siganids belonging to one genus *Siganus* and two subgenera *Siganus* and *Lo* (Seale). The latter was previously assigned to accommodate five species of siganid that have prominent, tubular snouts (Borsa et al. 2007). However, in his revision of the family, Woodland (1990) considered that the differences in snout shape between *Lo* and *Siganus* were not sufficiently clear to recognize them as two different genera (Borsa et al. 2007) and thus regrouping all known siganids into a single genus. Currently, with the addition of new species to the genus, *Siganus* consists of 29 nominal species (Woodland and Anderson 2014).

1.3.2 Morphology

Siganids exhibit uniformity in their morphological characteristics, such as the number of fin spines and rays, teeth shape and teeth count (Randall and Kulbicki 2005; Woodland and Anderson 2014). They all have XIII **dorsal** fin spines (ten soft rays) and VII **anal** fin spines (nine soft rays) (Woodland 1990). The spines of all fins are strong with a groove on each side bearing venom glands (Randall and Kulbicki 2005). The pelvic fins have two spines (one inner and one outer, with three soft rays in between), a character unique to this fish family (Woodland 1990). These fishes are also distinctive among other marine fishes for possessing two spines on their pectoral fins, which are separated by three soft rays (Jaikumar 2012). All known siganids have small, identical, compressed incisiform teeth on a single row (Woodland 1990; Jaikumar 2012). The snout is rounded or tabulate, jaws not protrusible, the mouth is small and terminal (Woodland 1990). Their skin is leathery with very small cycloid scales, giving the appearance of a scale-less surface (Duray 1998).

The colours of siganids species vary from drab, without patterns to bright with complex, ornate pattern (Randall and Kulbicki 2005). The drab-coloured siganids are known for their resistance to variations in salinity and temperatures, and the brightly coloured siganids are sensitive to physio-chemical changes (Duray 1998; Gorospe and Demayo 2013). Furthermore, fishes belonging to this family can be grouped into three clades based on their body shapes; deep bodied

species, slender-bodied species and streamlined, spindle-shaped species (Woodland and Anderson 2014). Like many marine fishes, siganids have separate sexes; though, sexual dimorphism is not obvious. However, there are some reports indicating that adult females are usually larger than males (Darsono 1993).

1.3.3 Habitat and distribution

Siganids are **exclusively marine** occurring in the littoral and sublittoral zones of the oceans (Gorospe and Demayo 2013) where they are associated with all types of coastal habitats that are known to support herbivorous fishes (Hoey et al. 2013). These include coral reefs, the surrounding grass flats, and other algae-rich environments, such as mangroves and rocky shores (Tyler and Bannikov 1997; Borsa et al. 2007). Two siganids, the orange-spotted rabbitfish, *Siganus guttatus* (Bloch) and the vermiculated rabbitfish, *S. vermiculatus* (Valenciennes), are an exception since they have been reported to enter freshwater rivers and lakes (Darsono 1993). Adult and juvenile siganids are demersal occupying shallow waters, while larvae are pelagic inhabiting the waters beyond the out reef (Duray 1998).

Originally, siganids are native to the western Indian and Pacific oceans. The highest siganids species richness was recorded in the Indo-Malayan area and the lowest was from French Polynesia, while East Africa occupies an intermediate position (Woodland 1983). The natural geographical distribution of siganids extends from the Persian Gulf (Grandcourt et al. 2007; Al-Qishawe et al. 2014) through the Arabian Sea (Al-Marzouqi et al. 2009), Red Sea (Mehanna and Abdallah 2002), East Africa to Polynesia (Quinitio and Castor-Saan 2008), southern Japan (Houque et al. 1999) and northern Australia (Pitt 1997; Fox et al. 2009, Hoey et al. 2013). However, there are no records of siganids from Hawaii and Eastern Island (Woodland 1983). About a century ago, two species, namely the marbled rabbitfish *S. rivulatus* (Forsskål) and the dusky rabbitfish *S. luridus* (Rüppell) invaded the eastern Mediterranean Sea from the Red Sea through the Suez Canal where they became successfully established among other invasive marine species (Bariche 2006; Shakman 2008). The expansion of the geographical distribution of these fishes in the Mediterranean Sea is an ongoing process, with the latest accounts reaching as far as the western Mediterranean, further widening the geographical range of siganids (Ounifi–Ben Amor et al. 2016).

1.3.4 Life cycle and feeding ecology

Siganids are known to grow moderately quickly, reaching sexual maturity at one year of age when their sizes reach 160-200 mm in length (Woodland 1990). Most siganids have a fixed spawning season (Lam 1974) with one or two peaks per year (Pitt 1997). Only *S. guttatus* was reported to spawn year-round (Hara et al. 1986). Spawning usually follows the lunar periodic cycle some siganids spawning either on the new moon (e.g. the whitespotted rabbitfish *S. canaliculatus* Park, 1797, the streamlined rabbitfish *S. argenteus* (Quoy & Gaimard), *S. luridus* and *S. rivulatus*) or the full moon (the golden-lined rabbitfish *S. lineatus* (Valenciennes) and little rabbitfish *S. spinus* (Linnaeus) (Houqe et al. 1999; Soliman and Yamaoka 2010). Other studies that are related to the spawning behaviour showed that siganids have specific spawning grounds such as tidal and marine flowering plants flats, fringing reefs and near mangrove areas (Duray 1998). Siganids breed in aggregations, where large females release up to 500 000 transparent, eggs at one spawning (Darsono 1993). Except for the eggs of *S. argenteus* which are floating and non-adhesive, the eggs of the majority siganids species are demersal, strongly adhesive, small, and spherical with many oil globules (Duray 1998).

Siganids are diurnal herbivores, feeding actively during the day and hiding in reef cervices at night (Kamukuru 2009). Hoey et al. (2013), differentiated four types of siganids based on their **dietary compositions**: (1) browsers that mainly feed on brown, leathery macroalgae; (2) croppers that feed on red and green algae; (3) mixed feeders that feed on mixed algal material, cyanobacteria, detritus and sediment, and (4) sponge feeders. Extensive investigations exploring the natural diet and feeding habits of siganids have been conducted to determine their potential for mariculture and to understand their ecological impact as invasive species (Bos et al. 2016). In summary, siganids feed on a wide range of macroalgae, such as seaweed of the phylum Chlorophyta (green algae), Rhodophyta (Red algae), Phaeophyceae (brown algae) and less frequently on seagrass of the phylum Magnoliophyta (Westernhagen 1973; Pitt 1997; Sabour and Lakkis 2007; Azzurro et al. 2007; Al Marzouqi et al. 2009). Moreover, they are also reported to feed on cyanobacteria (blue-green algae) (Bos et al. 2016) and Heterokontophyta (golden and brown algae) (Azzurro et al. 2007). The factors influencing feeding preferences in siganids are; the morphological characteristic of the macroalgae, the type of defensive chemicals of the macroalgae, the specific feeding behaviour of species, its jaw morphology and the nutrient assimilation mode of the siganid species (You et al. 2014).

Non-macrophytic taxa were also reported from the stomachs of several siganids, including an array of organisms such as epiphytic diatoms, hydrozoans, and detritus from the stomach of *S. rivulatus* (Karagitson et al. 1986), Euphausiaceae and gastropods from *S. luridus* (Stergiou 1988). Dowidar et al. (1992) also reported bryozoans, crustaceans, polychaetes, and molluscs from *S. rivulatus* collected off the Egyptian coast (Bariche 2006). The stomach of *S. luridus* in Italy contained amphipods and foraminiferans (Azzurro et al. 2007). In addition, sponges, fish larvae, crustacean larvae and siliceous spicules were also reported from the stomachs of some siganids (Lam 1974). Sand was reported in considerable amounts from specimens collected in Egypt and Greece (Bariche 2006).

1.4 Siganidae fisheries and aquaculture

1.4.1 Global fisheries overview

Several species of schooling siganids are excellent fishes for human consumption and are regarded as valuable traditional food for locals due to their delicacy and high nutritional value (Xu et al. 2011). They are considered an important source of income for local fisheries in many Indo-Pacific countries (Lam 1974; Tseng and Chan 1982; Darsono 1993) and in some parts of the eastern Mediterranean (Bariche 2004; El-Dakar et al. 2011), where they are commercially exploited providing significant contributions to the artisanal fisheries in these countries (Bariche 2004). Siganids are captured by a variety of fishing methods, such as seining (Lam 1974), fish corrals and intertidal fence nets (Grandcourt et al. 2006), trammel and gill nets (Bilecenoglu and Kaya 2002), dome-shaped wire traps (Grandcourt et al 2006; Jaikumar 2011), basket traps (Wambiji et al. 2009) and bagnets (Soliman and Yamaoka 2010). In Guam and Palau, fishermen capture siganids at night by spearing individual fish (Lam 1974).

According to the latest fishery statistics from The Food and Agriculture Organization (FAO), the total global Siganidae fisheries production from the four major fishing areas established by FAO (Western Indian Ocean (WIO), Eastern Indian Ocean (EIO), Mediterranean and Black Sea (MBS) and Western Central Pacific Ocean (WCP) was more than for the year 2017 was 116,112 tonnes accounting to an increase of 18% from the previous year (98,388 tonnes tonnes) (Figure 1.9). In general, throughout the period between 1950-2017 the global fishery production of Siganidae can be characterised as irregular with periods of decline and growth in total catches and trends of yearly increments. The yearly global Siganidae catches remained low and stable and not

exceeding 1000 tonnes between 1957 to 1967 (except for the year 1963). This period was followed by a steady increase in the global fishery production reaching almost 10 folds in the year 1975 in comparison to 1965. Within three decades the global Siganidae fisheries production reached three times the total catches of the year 1975. Since then, there has been a steady growth in the catch production with an average increment of about 20% between 2010 and 2017 (Figure 1.9).

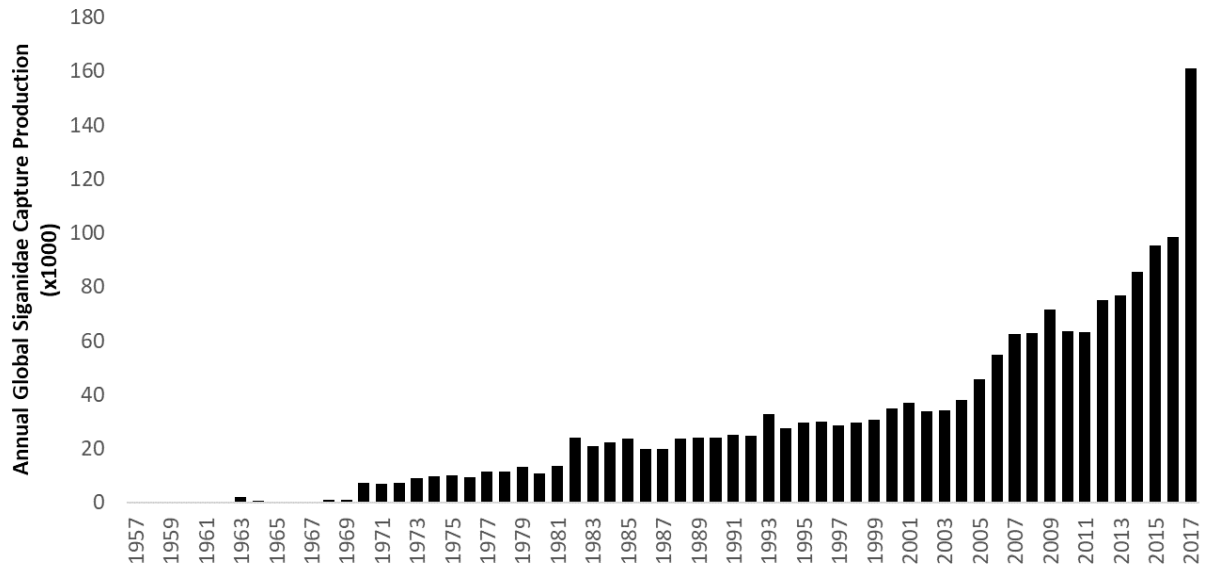

Figure 1. 9 Global annual fisheries total production of Siganidae from 1957-2017 (Source: FAO 2019).

Based on fishing areas, the majority of the world's siganids are caught in WCP (more than 70% of the cumulative global fisheries capture production (1957-2015)), followed by WIO (about 25%). The least catches were recorded from EIO (about 2%) and MBS (less than 2% of the cumulative global fisheries capture production) (Figure 1.10). The total catches from each region mirrored the zoogeographical distribution of siganids in the world's oceans. The biggest quantity of cumulative catches from WCP area was recorded from the Philippines accounting to more than 70% of the area's total global fishery captures. In the Philippines, *S. rivulatus* is the main target for the commercial fishery in Pujada Bay due it is immense abundance in this location (Nanual and Metillo 2008). In Lagonoy Gulf, three species, *S. canaliculatus*, *S. spinus* and *S. argenteus* constitute about 90% of the total siganid catch (Soliman et al. 2009). These catches contributed to approximately 10%-15% of the total fish catch of the Gulf's annual fishery production of 23,000 mt/yr (Soliman et al. 2009).

The main siganid fishery contribution from the WIO region originated from the United Republic of Tanzania, with a cumulative total catches exceeding 100,000 tonnes since 1950 (about 27% of the total catch in the region) (FAO 2017). In East Africa, wild siganids are an integral component of the artisanal fishery along the African coasts (Kamukuru 2009). Together with lethrinids, siganids comprised about 31% of the total reef fish landings along the Kenyan coast over the last five years of the 1990's (Wambiji et al. 2009). Moreover, during the 2004-2005 period, siganids constituted 85% of total catches from basket traps placed in the Dar es Salaam Marine Reserve (Kamukuru 2009). In western Kenya, *S. sutor* ('tafi') is the most abundant and important catch in the artisanal fishery (Agembe, 2012), contributing to 180 tonnes of artisanal fishery landings (Wambiji 2013).

The lowest siganid fishery production was recorded from countries in the eastern and western Mediterranean seas. According to the latest FAO fisheries statistics (FAO 2017), the cumulative capture quantity of siganids from the MBS region was about 27,000 tonnes. The highest production was recorded from the Mediterranean waters of Egypt, amounting to 60% of total cumulative fishery capture production from MBS (16,772 tonnes). In the eastern Mediterranean, siganids have been successfully integrated into the local fisheries becoming one of the main components of commercial catches (Papaconstantinou 1990; Bilecenoglu and Kaya 2002; Cicek and Avsar 2015). Furthermore, in some eastern Mediterranean countries, siganids have been successfully introduced to local markets where they have become commercially valuable (Saoud et al. 2008; Shakman et al. 2009). In contrast, although siganids are very abundant in Foumi Island, Greece, they are considered as low value and are often discarded as bycatch (Pennington et al. 2013). Similarly, the same aversion to this species occurs among local consumers in Cyclades Island off Italy (Giakoumi 2013).

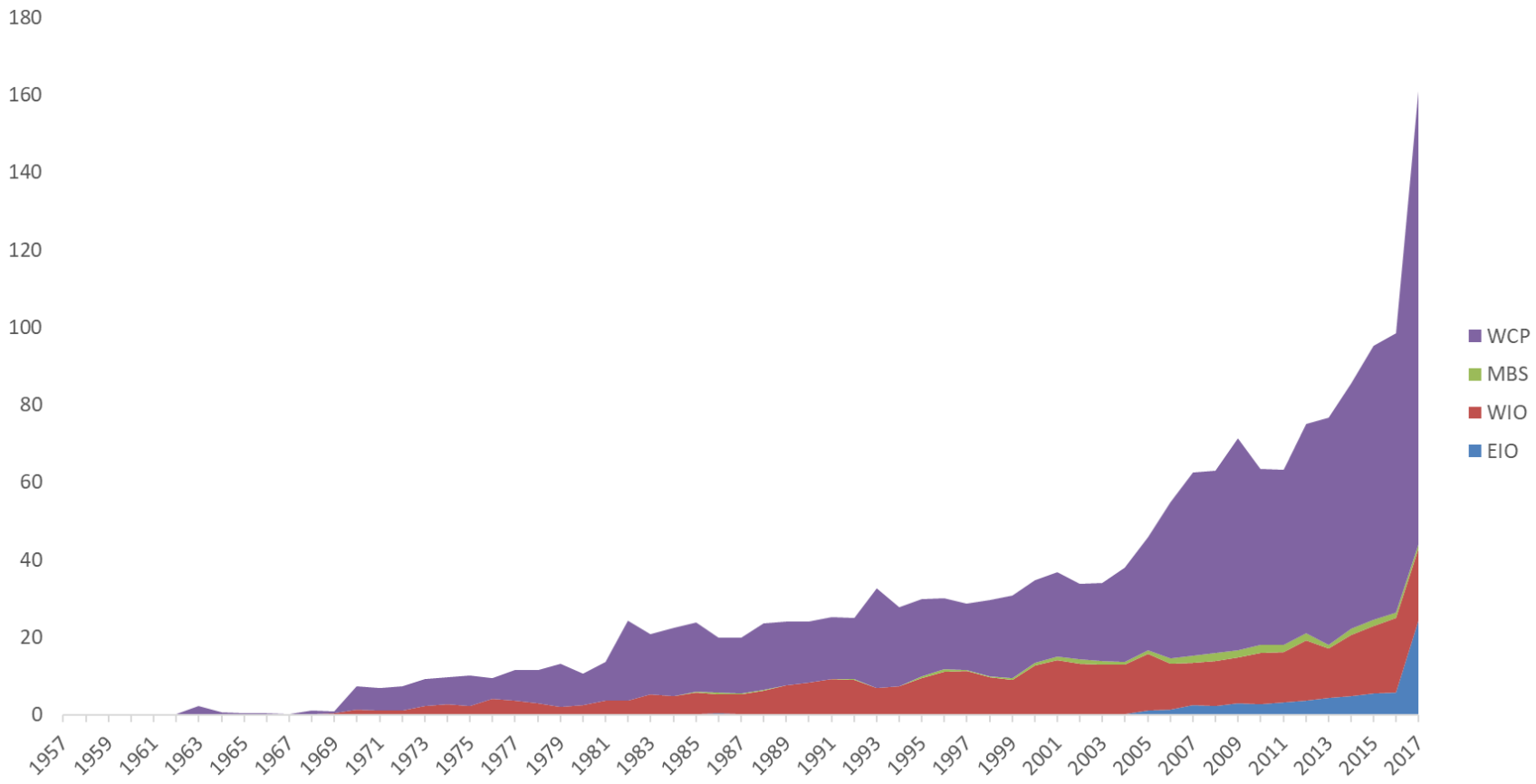

Figure 1. 10 Regional cumulative Siganidae production from the four major fishing areas from 1950-2015 (Source: FAO 2019).

1.4.2 *Siganus canaliculatus* fisheries in the Sultanate of Oman

The siganid fishery in Oman is represented by two species, *S. canaliculatus* which dominates the catches and the streaked rabbitfish *S. javus* (Linnaeus) (Al-Marzouqi et al. 2009). Both species are part of the artisanal and coastal fisheries both in the GoO and AS (Al-Marzouqi 2013). The majority of siganid catch production comes from the traditional artisanal fisheries with contributions reaching 97% of the total catch (MoAF 2015). In the coasts of Oman, artisanal fishermen harvest siganids using gillnets, traps, beach seines and by industrial trawlers (Al-Marzouqi et al. 2013). Although siganids are not as popular as other demersal fishes, such as lethrinids and sparids, their landings and production value have exhibited a yearly increase, indicating that they are becoming more targeted by fishermen (Figure 1.11). Within a decade, the total catches of siganids increased from 1155 tonnes in 2005 to more than 3000 tonnes in 2015 with an estimated value of 1. 956 million RO (~ 5 million USD) (MoAF 2015).

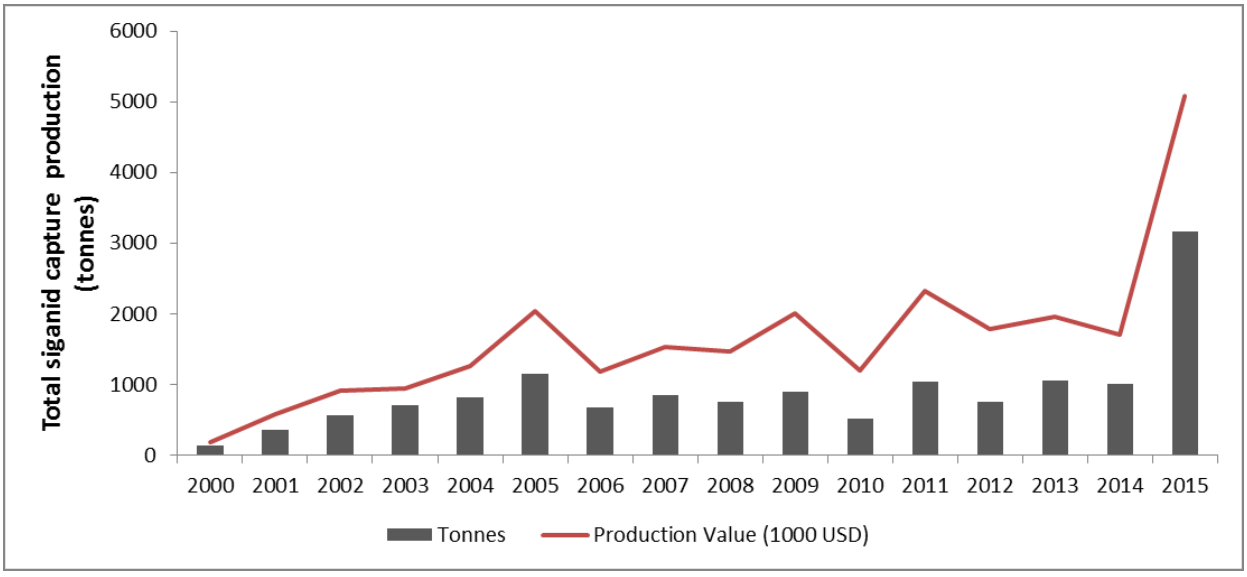

Figure 1. 11 Siganid fisheries capture production and value (USD $) in Oman from 2000-2015 (sources: MoAF 2015).

The main siganid fishing grounds in Oman are located in the AS with contributions amounting to 66% (2066 tonnes) and 26% (815 tonnes) of total landings of the year 2015, respectively. However, it has been reported that the catches of siganids exceeded the maximum sustainable yield in some seasons during the 2005-2014 period, which indicates overexploitation of this fishery (El-Barr 2016). Thus, better resource management and mariculture will likely be required to prevent overexploitation of this valuable fishery resource.

1.4.3 Global Siganidae mariculture

The feasibility of farming siganids was first accepted in the 1960s (Lam 1974; May and McVey 1977; Duray 1998). However, only after the 'Siganid Mariculture Implementation Conference,' which was held at the Hawaii Institute of Marine Biology in 1972, the potential to develop siganid mariculture was acknowledged (Bryan 1975). Since then, a significant number of studies have been conducted on various aspects of siganid rearing and propagation. Siganids attracted the attention of mariculturists and investors around the globe because they possess key attributes that facilitate raising them in captivity. Primarily, they are highly esteemed as an excellent fish for seafood consumption in many counties in the Pacific, the western Indian Ocean and eastern Mediterranean (Lam 1974; May and McVey 1977; Darsono 1993; Gorospe and Demayo 2013). Siganids are also regarded as desirable candidates for mariculture because they can easily shift from herbivory to omnivory in captivity (El-Dakkar et al. 2011). Many experimental studies have shown that siganids can tolerate cultivation at high stocking densities and that they have excellent tolerance to high temperature and salinity (Lam 1974; Ghanawi et al. 2010). Also, the fast growth rate of some siganid species, such as *S. guttatus* and *S. vermiculatus*, makes them ideal for mariculture (Shirinabadi et al. 2013; Gorospe and Demayo 2013).

The earliest attempts to breed siganids initiated from the Philippines by Manacop (1937) for *S. canaliculatus* (Juario et al. 1985). Since then, researcher steadily experimented on siganid breeding and mariculture (Von Westernhagen 1974; Von Westernhagen and Rosenthal 1976; Duray 1998). For instance, *S. guttatus* from the Philippines, has been frequently investigated concerning its potential for mariculture (Alcala and Luchavez 1980; Juario et al. 1985; Hara et al. 1986; Avila and Juario 1987; Ayson 1989; Parazo 1990; Quinitio and Sa-An 2008; Rabia 2016). This siganid is currently regarded as a good alternative to the milkfish *Chanos chanos* (Forsskål) for grow-out culture because it is as efficient as the milkfish in converting plant material into animal protein (Abalos 2015) and it commands a higher market price than milkfish (Ayson et al. 2014). Furthermore, because *S. guttatus* is euryhaline, it can be easily reared with the giant tiger prawn *Penaeus monodon* (Fabricius) instead of tilapia, which is less valuable in the Philippines than *S. guttatus* (Ayson et al. 2014). Besides, under captivity *S. guttatus* is able to spawn repeatedly all year round with the application of hormonal treatments (Hara et al. 1986).

Thus, *S. guttatus* is currently grown in at commercial scales in polyculture-pond systems (Angeles et al. 2014) with *P. monodon* or in floating net-cages (due to their natural tendency to clean algae from cage surfaces) (Ayson et al. 2014). This characteristic also makes *S. guttatus* favourable for culturing with milkfish because their algae cleaning ability helps improve water circulation inside the cages (Rabia 2016). According to the latest fisheries statistics, in 2017 maricultural production of siganids in the Philippines reached 148.8 tonnes in brackish-water-pond polyculture systems and 45.5 tonnes in floating-net cages (FAO 2019).

Research on siganid mariculture was conducted in Palau in the 1970s (Tsuda and Bryan 1973; Bryan et al. 1974; Bryan and Madraisau 1977), resulting in the establishment of a successful, large-scale hatchery production facility at the Micronesia Mariculture Demonstration Centre, which produced thousands of siganid fry for Palauan fish farmers (May and McVey 1977). However, factors such as high production costs, low local market prices for siganids and high transportation costs have probably been responsible for hindering the development of a viable commercial *Siganus* mariculture industry in Palau (May and McVey 1977).

Other species of siganids have also been considered as suitable candidates for mariculture (e.g. *S. rivulatus*) in Lebanon (Saoud et al. 2008a; 2008b), Cyprus (Stephanou and Georgiou 1999), Israel (Ben-Tuvia et al. 1973; Popper 1973; Popper and Gundermann 1975; Popper et al. 1979), Egypt (El-Dakar et al. 2007; 2010) and Syria (Ibrahim et al. 2008). Also, *S. canaliculatus* was regarded to be suitable for mariculture by researchers in the United Arab Emirates (Yousift et al. 1999; 2005a; 2005b), Tanzania (Bwathondi 1982) and Indonesia (Tacon et al. 1990). However, there are currently no commercial siganid farms in any of these countries.

1.4.4 Potential of *Siganus canaliculatus* mariculture development in Oman

The Sultanate of Oman with its long beaches, distinctive environmental features and geographical structures is ideal for developing various types of mariculture facilities. The Omani government recognised this and organised a strategic plan to help develop and establish regulations to manage the industry. Thus far, several sites along the Omani coast have already were chosen, and various fish and shellfish species were selected as potential candidates for mariculture (Atlas 2010). The selection of these species is based on scientific research that studied both local and international demands for various fishes, their biological efficiencies, marketability, and hardiness. Potential candidate species included gilthead seabream, *Sparus aurata* (Linnaeus), European

seabass *Dicentrachus labrax* (Linnaeus), the thinlip grey mullet *Liza ramada* (Risso) and to a lesser extent, the orange-spotted grouper *Epinephelus coioides* (Hamilton) and the yellowfin seabream *Acanthopagrus latus* (Houttuyn). More recently, additional species were considered as candidates for mariculture, including the santer seabream *Chemerius nufar* (Cuvier & Valenciennes) and the sandfish sea cucumber, *Holothuria scabra* (Jaeger). Moreover, the country is considering promoting the development of shrimp, tilapia, and abalone aquaculture industry.

Although not initially included among mariculture species candidates for Oman, *S. canaliculatus* should also be considered. Unlike those mentioned above, non-native species, *S. canaliculatus* is already a popular food fish in Oman, is in high demand in local markets, and has a good market value (Al-Marzouqi et al. 2011). Furthermore, although siganids are herbivorous, they exhibit omnivorous feeding habits in captivity and can be fed a wide variety of food (El-Dakkar et al. 2011). For example, studies have shown that laboratory-reared *S. canaliculatus* feed on a wide variety of feed, such as chicken pellets, cooked rice, dried shrimp and even fish scraps (Darsono 1993). Additionally, *S. canaliculatus* has been proven to be hardy, in that it tolerates drastic changes in salinity and temperature (Duray 1998). It also tolerates environmental stresses, frequent handling by humans and crowded conditions (Saoud et al. 2008a). *S. canaliculatus* is relatively fast-growing, reaching maturity within one year (Al-Ghais 1993). In fact, it is suggested that it mature more rapidly in captivity than in the wild (Duray 1998).

1.5 Parasites of the Siganidae

The literature on parasites of siganids is patchy and scattered, with the majority of it being of taxonomic or descriptive nature. Many of the available literature focuses on a certain taxon or are limited to few taxa that are known from siganids. To date, extensive parasite fauna investigations were conducted on few species of siganids (Diamant and Paperna 1986; Martens and Moens 1995; Geets and Ollevier 1996; Geets et al. 1997), while the parasite fauna of many siganids is largely unknown. The present thesis aims to describe the parasite fauna of the whitespotted rabbitfish, *S. canaliculatus* from the coasts of the Sultanate of Oman, focusing on the Myxosporea, Monogenea and Digenea. For the purpose of this thesis, only these major fish parasites groups will be discussed in this chapter. For the other major groups, it is referred to (Palm 2004; Cestoda), (Anderson 2000; Nematoda), (Taraschewski 2005; Amin 2013; Acanthocephala),

(Moodie 2005; Microsporidia), (Boxshall 2005; Copepoda and Isopoda), (Govedich et al. 2005; Hirudinea (leeches)).

1.5.1 The Myxosporea

The myxosporeans are a diverse and widely distributed parasitic members of the phylum Cnidaria, including more than 2000 registered species distributed among 60 genera (Bartošová et al. 2009). The history of taxonomic classification of these parasites has been controversial. Previously they were classified as protozoans; however, extensive molecular studies confirmed that myxosporeans are in fact, metazoans (Yokoyama et al. 2012). Myxosporeans are considered as metazoans because their development involves multicellular differentiation of the valvogenic, capsulogenic and sporoplasmic cells, which does not conform to the unicellular definition of Protista (Rohde 1995).

The majority of myxosporeans are reported from marine and freshwater fishes (Lom and Dykova 2006) with some exceptional occurrences from amphibians and aquatic birds (Longshaw et al. 2005; Bartošová et al. 2009). Most species of myxosporeans are harmless to their hosts. Nevertheless, some are regarded as serious pathogens to cultured and wild fish populations, causing considerable problems for mariculture and fishery industries worldwide, including mass mortality (Sterud et al. 2007; Feist and Longshaw 2008; Yokoyama et al. 2012), degradation of host marketability due to external deformation and muscle liquification (Feist and Longshaw 1995; Kent 2001; Yokoyama et al. 2012), reduction in productivity (Alvarez-Pellitero and Sitjà-Bobadilla 1993; Adlerstein and Dorn 1998; Al-Jahdali and El-Hassanine 2010) and abnormal host behaviour (McElroy et al. 2015). Based on the number of valves present, myxosporeans are divided into Bivalvulida, which are mature spores that exhibit two valves, and Multivalvulida, with three or more valves (Lom and Dykova 2006). Myxosporeans are further divided into coelozoic and histozoic myxosporeans (Feist and Longshaw 2006). Coelozoic species are those that infect the body cavity, gallbladder, bile ducts and the urinary tract. In contrast, histozoic species live in a variety of intercellular and sometimes intracellular tissues (Lom and Dykova 2006).

To date, species belonging to four genera of myxosporeans have been recorded from siganids. Diamant and Paperna (1986) reported species of *Zschokkella Auerbach, 1910, Ceratomyxa* Thélohan, 1892, *Ortholinea* Shulman, 1962, and *Kudoa* Meglitsch, 1947 from three siganids (*S. argenteus*, *S. rivulatus* and *S. luridus*). Subsequently, the highly pathogenic species, *Zschokkella*

icterica Diamant & Paperna, 1992 was described from the hepatic ducts and gallbladder of *S. luridus* caught off the Gulf of Eilat (Diamant and Paperna 1992). The histozoic species, *Zschokkella helmii* Abdel-Ghaffar, Ali, Al Quraishy, Entzeroth, Abdel-Baki, Al Farraj & Bashtar, 2008, was described from the gallbladder walls of *S. rivulatus* caught off the Saudi coasts of the Red Sea (Abdul-Ghaffar et al. 2008). Recently, the histozoic myxosporean, *Ortholinea saudii* Abdel-Baki, Soliman, Saleh, Al-Quraishy & El-Matbouli, 2015, was described from the kidneys of the above-mentioned host (Abdel-Baki et al. 2015). The only multiuvalid myxoporean reported from siganids is *Kudoa iwatai* Egusa & Shiomitsu, 1983 a systemic myxosporean that is known to infect a variety of host tissues and organs, including muscle, brain, eye and visceral organs (Diamant et al. 2005; Diamant et al. 2010).

1.5.2 The Monogenea

Monogenean flatworms are among the most problematic and pathogenic metazoan parasites in fish farms (Thoney and Hargis 1991). Monogeneans are hermaphrodites and are mostly ectoparasites of marine and freshwater fishes. They infect their host's outer surfaces, including gills, skin, and fins and, less commonly, the buccal, branchial and nasal cavity linings (Ogawa 2014). A few monogenea are endoparasites; occurring in organs, such as the oesophagus, cloaca, urinary tract, and heart (Buchmann and Bresciani 2006). Because monogenea have a monoxenous cycle, which does not require intermediate hosts, they tend to be highly host-specific (Perkins et al. 2009). Fifty-three families of monogenean are presently recognised (Whittington 2004) with an estimated species richness of 25000 species (Buchmann and Lindenstrøm 2002). These worms attach to their hosts using the opisthaptor. This is a posterior attachment organ consisting of hooks, clamps, suckers, friction pads, surface spines, cement glands, or a combination of these organs (Rohde 1993). Based on the morphology of the opisthaptor, monogeneans are classified into two main subclasses: the Monopisthocotylean and the Polyopisthocotylean. The Monopisthocotylean feed on host epithelium cells with an attachment organ consisting of a single, symmetrical attachment unit (Whittington 2006). Polyopisthocotyleans are exclusively blood feeders possessing attachment organs that bears numerous sclerotized clamps (Whittington 2006; Feist and Longshaw 2008; Perkins et al. 2009).

The importance of these parasites lies in their ability to propagate rapidly in systems where hosts are held in captivity at high densities, such as in fish farms and aquaria (Thoney and Hargis

1991). This group of parasites usually lead to high morbidity and mortality of cultured fish stocks, which in turn cause major economic losses in commercial aquaculture farms (Buchmann and Bresciani 2006). Monogenean parasites can cause mortality directly or indirectly by triggering secondary infections associated with bacterial and viral pathogens introduced by these flatworms (Rubio-Godoy 2007). It has been documented that the feeding and attachment modes of monogenea can have an adverse effect on the tissues of infected hosts, including erosion of skin epithelia, thinning of the epidermis, vacuolar degeneration and infiltration of mononuclear cells, hypertrophy and cell proliferation (Buchmann and Bresciani 2006; Rubio-Godoy 2007).

The first account of a monogenean parasite registered on a siganid was of the genus *Tetrancistrum* Goto & Kikuchi, 1917 to accommodate a species of a monopisthocotylean monogenean collected from the gills of *S. fuscescens* (Goto and Kikuchi 1917). Since then, seven members of this genus were reported from other siganids from various localities (Paperna 1972; Young 1986; Martens and Moens 1995; Kritsky et al. 2007b). More recently, two additional ancyrocephalid genera were reported exclusively from siganids (Lim 2002; Kritsky et al. 2007a): *Glyphidohaptor* Kritsky, Galli & Yang, 2007 and *Pseudohaliotrema* Yamaguti, 1953. Several members of the Polyopisthocotylean monogenean genus, *Polylabris* Euzet & Cauwet, 1967, were also described from gills of siganids: *Polylabris sigani* Dillon, Hargis & Harrises, 1983 from *Siganus oramin* (= S. *canaliculatus*) and *P. mamaevi* Ogawa & Egusa, 1980 from *S. stellatus* (Tingbao et al. 2007). *P. bengalensis* Sailaja & Madhavi, 2011 was recently described from *S. canaliculatus* and *S. javus* (Sailaja and Madhavi 2011). In addition, unidentified gyrodactylids species were reported from the Red Sea (Diamant and Paperna 1984). A new genus of viviparous marine gyrodactylid, *Acanthoplacatus* Ernst, Jones & Whittington, 2001, was erected to accommodate seven species of gyrodactylids detected on the fins and skin of siganid fishes collected from the Great Barrier Reef, Australia (Ernst et al. 2001).

1.5.3 The Digenea

Digenea is a subclass of the phylum Platyhelminthes, comprising the most speciose group of metazoan endoparasites, consisting of more than 2500 nominal genera and 18 000 registered species, according to a database compiled by the Natural History Museum in London (Cribb 2001; Gibson 2006). Digenea are taxonomically divided into three groups, based on the morphology of their cercariae: Strigeida, Echinostomatidae and Plagiorchiidae (Gibson 2006). The majority of

digeneans inhabits the gastrointestinal tract of their fish hosts, but may also occur in various other body cavities, organs, and tissues (Gibson 2006). These worms have a complex life cycle that typically involves a mollusc, a second intermediate host being either an invertebrate or a vertebrate, and a final, definitive host which is always a vertebrate (Cribb et al. 2001). An exception to this rule are species of the genus Aporocotyle, which require a polychaete annelid for larval development (Paperna and Dzikowski 2006).

Adult intestinal digeneans are generally considered harmless to their hosts even when they are encountered in high numbers (Paperna and Dzikowski 2006). On the other hand, extra-intestinal digeneans, such as blood flukes, are potentially highly pathogenic and might cause high mortalities to their hosts (Paperna and Dzikowski 2006; Ogawa 2014). Moreover, some digenetic infections can affect the marketability of fish by producing obvious damage to their host's external surface or by producing cysts in the muscles and skin, which both render the fish undesirable to consumers (Paperna and Dzikowski 2006). Digeneans are the most extensively investigated group of marine parasites infecting siganids. To date, 27 species of digenea in 18 genera and nine families have been reported from siganids (Yamaguti 1953, Madhavi 1972; Diamant and Paperna 1986; Barker et al. 1993: Bray and Cribb 1996; Arthur and Lumanlan-Mayo 1997; Bray and Cribb 2000; Bray and Cribb 2001; Hall and Cribb 2004; Shih et al. 2004; Hassanine and Gibson 2005; Al-Jahdali and Hassanine 2012).

1.6 Objectives

1. The investigated siganid *Siganus canaliculatus* harbours a rich parasite fauna, including species new to science.
2. The parasite fauna of *Siganus canaliculatus* from Omani waters is similar to the parasite fauna of the siganids from other regions.
3. Importance of *S. canaliculatus* in the life cycle of aquatic parasites
4. New host and locality records from the Sultanate of Oman extend the range of distribution of Indian Ocean parasites into the Persian Gulf, Gulf of Oman and the Arabian Sea.
5. The parasite infracommunity of *Siganus canaliculatus* is influenced by the three different water bodies, Persian Gulf, Gulf of Oman, and the Arabian Sea.
6. Parasites of *Siganus canaliculatus* in Omani waters can be used as biological indicators for environmental health in the region

7. Several parasites species from *Siganus canaliculatus* are of fisheries and mariculture importance.

1.7 Thesis structure

This thesis consists of seven chapters. **Chapter one** introduces the concept of parasitism in nature and discusses general terminology and important definitions concerning Parasitology. This is followed by an overview into the field of marine parasitology and the different aspects that are intertwined within this multifaceted field of science. The history of marine parasitology in the Sultanate of Oman is briefly discussed. Information about the oceanographical condition of the study area are provided. General information regarding the marine teleost family Siganidae are given including, geographical distribution, importance to fisheries, feasibility for mariculture both globally and locally. This is followed by a summary on some fish parasites reported from siganids, with an emphasis on three major parasite groups. **Chapter two** deals with the description of a new myxosporean parasite belonging to the genus *Unicapsula*. Morphological, ultrastructural, and molecular methods are used to identify *Unicapsula fatimae* n. sp. within the genus. In **chapter three** two species of the ancyrocephalid monogeneans are reported from the investigated host, one as a new species to science and the other as a new host and locality records from the waters of Oman.

Comparative morphological analysis using light and laser confocal microscopy were used to identify and describe *Tetrancistrum labyrinthus* n. sp. infecting the gills of *S. canaliculatus*. In **chapter four**, a combination of morphological and molecular methods was used to describe the ancyrocephalid monogenean *Glyphidohaptor safiensis* n. sp. infecting the gills of *S. canaliculatus*. The chapter provides new molecular data on ancyrocephalid monogenea and sheds light on the phylogenetic relationships of siganid ancyrocephalid and other closely related marine ancyrocephalid. The digenean fauna of siganids is discussed in **chapter five** with a taxonomic description of a new digenean species *Hysterolecithoides amurparuchinii* n. sp. together with molecular data of the small subunit DNA region. The new species is the third species to be reported from a siganid host. In **chapter six**, ecological analysis of parasitological data obtained from *S. canaliculatus* and multivariate statistical methods were used to evaluate the marine environment in Oman. For the first time star graphs based on 12 parasitological descriptors were constructed to visualize environmental condition of the investigated localities.

A general discussion is provided in chapter seven which includes the diversity and composition of *S. canaliculatus* parasite fauna, insights into the zoogeographical distribution of the parasites reported from *S. canaliculatus* and other siganids. **Chapter seven** also discusses the potential risk of some parasites as a threat in the sustainability of *S. canaliculatus* mariculture industry. In **chapter eight** the future prospect of marine parasitology and obstacles hindering its development were discussed. Possible solutions to overcome these obstacles are also suggested. In summary, the work conducted in the present thesis is one of its kind in the region which provides detailed information on parasite fauna of an herbivorous marine host and establishes a baseline for a host-parasite database on the country. The findings of this thesis will contribute in the ongoing monitoring of marine environment in Omani waters and will aid in the detection of potential pathogens that could impact the development of the mariculture industry in the country.

2 Morphological, ultrastructural, and molecular description of *Unicapsula fatimae* n. sp. (Myxosporea: Trilosporidae) of white-spotted rabbitfish (*Siganus canaliculatus*) in Omani waters

Abstract

Investigations regarding the parasite fauna of wild white-spotted rabbitfish (*Siganus canaliculatus*) Park, 1797 revealed white, spherical, loosely attached cysts measuring 896 (375-1406) µm in diameter in the inner endothelial wall of the oesophagus and stomach. Mature spores inside these cysts corresponded to the original description of spores belonging to the genus *Unicapsula* Davis, 1924. *Unicapsula fatimae* n. sp. spores were 6.23 (5.60-6.60) µm in length and 6.80 (6.12-7.39) µm in width. The length of large polar capsule was 2.62 (2.18-2.97) µm and width was 2.65 (2.32-2.90) µm, and the extended large polar capsule filament length was 15.50 (11.71-19.99) µm. Transmission electron microscope images of the plasmodia revealed a complex cyst structure that was unique among other *Unicapsula* spp. Ultrastructural details of the host-parasite interface and developmental stages of a species from the *Unicapsula* genus are described for the first time. Histology of an infected oesophagus revealed some abnormalities and changes in the host tissue around the infection site, including hypertrophy of host oesophagus epithelial cells and hyperplasia of host glandular tubules. The parasite presented here has been added to the genus *Unicapsula* using comparative morphological analysis and ultrastructural investigations supported by small subunit ribosomal DNA (SSU) molecular analysis.

[1]

[1] *This was published as: Al-Jufaili S.H., Freeman M.A., Al-Nabhani A., Machkevskyi V.K. & Palm H.W. (2015). Morphological, ultrastructural, and molecular description of Unicapsula fatimae n. sp. (Myxosporea: Trilosporidae) of whitespotted rabbitfish (Siganus canaliculatus) in Omani waters. Parasitology Research, 115(3):1173-84.*

2.1 Introduction

Myxosporea are a class of fascinating, microscopic, metazoan aquatic parasites belonging to phylum Cnidaria (Kent et al. 2001; Lom and Dykova 2006). Since their discovery, they have attracted much attention because of their mysterious and complex life cycles (Anderson et al. 2000; Marton and Eszterbauer 2011), enigmatic phylogeny (Whipps et al. 2004; Fiala 2006; Evans et al. 2010), and negative impact and pathogenicity on wild (Yokoyama and Itoh 2005; Burger et al. 2008; Dykova et al. 2011; Buchmann et al. 2012) and cultured (Katharios et al. 2014; Tossavi et al. 2015; Yuan et al. 2015) fish stocks. Although extensively investigated among various marine and freshwater fish hosts, there are few valid records of myxosporean parasites infecting wild and reared siganids worldwide. These are limited to few records from the Egyptian, Saudi Arabian, and Israeli coasts of the Red Sea. The highly pathogenic *Zschokkella icterica s* Diamant & Paperna 1992 was reported in the gallbladder of wild *Siganus luridus*, *Siganus rivulatus*, and *Siganus argenteus* in Israel (Diamant and Paperna 1986; Diamant 1992). *Zschokkella helmii* Abdel-Ghaffar, Ali, Al Quraishy, Entzeroth, Abdel-Baki, Al Farraj & Bashtar 2008 was recorded from the gall bladder of *S. rivulatus* from the Red Sea, Egypt (Abdel-Ghaffar et al. 2008).

Some unidentified ceratomyxids were observed from the gallbladder of *S. rivulatus* from Israel (Diamant 2010) and Egypt (Abdel-Ghaffar et al. 2008). An unidentified *Ortholinea* species from the urinary bladder of *S. rivulatus* caught off Israeli waters (Diamant 2010) and *Ortholinea saudii* Abdel-Azeem, Abdel-Baki, Soliman, Saleh, Al-Quraishy & El-Matbouli, 2015 was isolated from the kidney of *S. rivulatus* from the Kingdom of Saudi Arabia off the Red Sea (Abdel-Baki et al. 2015). To date, the only multivalvulid myxosporean reported from a siganid is *Kudoa iwatai* Egusa & Shiomitsu, 1983, a species known to cause systematic infection in cultured *S. rivulatus* from Israel (Diamant et al. 2005; Diamant 2010). Members of the genus *Unicapsula* Davis, 1924 are multivalvulids belonging to the family Trilosporidae, which accommodate myxosporean parasites that have three valves, each bearing a polar capsule (Lom and Dykova 2006). *Unicapsula* species are unique among other Trilosporidae because only one of the three polar capsules is fully developed and functional, whereas the remaining two are rudimentary and barely visible (Alama-Berjamo et al. 2009; Miller and Adlard 2013).

Since the description of the genus and the type species a total of 12 species of *Unicapsula* have been recorded from different localities and a wide range of marine host species (Naidjenova

and Zaika 1970; Schubert et al. 1975; Sarkar 1984; Sarkar 1999; Diebakate et al. 1999; Miller and Adlard 2013; Tomochi et al. 2014).

Similar to their closely related group, the Kudoidae, some members of *Unicapsula* have been associated with negative impact on their hosts mostly associated with aesthetic issues involving macroscopic pseudocysts or myoliquefaction (Lester 1982; Alama-Berjamo et al. 2009; Miller and Adlard 2013). Although the majority of species belonging to this genus has been detected from the musculature (Miller and Adlard 2013; Tomochi et al. 2014), some have been detected from other organs such as the gills (Diebakate et al. 1999), kidney (Sarkar 1999), and urinary bladder (Naidjenova and Zaika 1970). Although marine parasitological investigations in the Arabian Peninsula region dates to the 1980s, the investigation of myxosporean parasite fauna only started recently. This results in the description of several new species being recorded from various marine hosts, caught off the coasts of the Kingdom of Saudi Arabia (Red Sea and Arabian Gulf) (Zhang et al. 2014; Mansour et al. 2014; 2015a; 2015b). The present study describes a new species of *Unicapsula* using morphological, ultrastructural, histological, and molecular characterization, infecting the oesophagus and stomach endothelium of *S. canaliculatus*.

2.2 Material and methods

2.2.1 Host sampling

Fish were bought as live or moribund from local fish markets and landing sites along the coast of the Sultanate of Oman from November to December 2012. Thirty-five fish were obtained from Khasab landing site measuring 22.5-36.5 cm in total length and 140-562.2 g in weight, 35 fish from Dabba local fish market (24.1-37.4 cm total length, 169.6-660.8 g in weight), 35 fish from Sohar local fish market (24.5-42.5 cm total length, 233.5-976.5 g in weight), 35 fish from Muttrah local fish market (31-39 cm total length, 320.3-690.5 g in weight), 35 fish from Masirah landing site (29.5-40.7 cm total length, 355-963.6 g in weight), 35 fish from Lakbi landing site (25.1-34.4 cm total length, 207.6-465.5 g in weight), and 35 fish from Raysut local fish market (29.1-41.4 cm total length, 320.6-801.9 g in weight). Once obtained, individual fish were immediately placed in plastic bags, labelled, and transported to the laboratory on ice (4 °C) or as immediately frozen samples (-20 °C). Fish were either examined directly after arrival in the laboratory or stored at -40°C until further examination. Additional samples were obtained from

fresh fish from Salalah (year 2013) and Muscat (year 2014) and examined immediately for histology and EM analyses.

2.2.2 Parasitological examination and parasite collection

Thawed fish were dissected, and all organs and body fluids were examined for presence of ecto- and endoparasites (Palm and Bray 2014). For detection of myxosporean parasites, microscopic slides were prepared from smears of the brain, liver, kidney, spleen, contents of gallbladder, and urinary bladder, and were initially observed at ×200-400 magnification using a Zeiss Axio Scope. A1 compound microscope. The oesophagus, stomach, and intestine were cut open and examined for myxosporean cysts under Zeiss Stereo microscope (Discovery. V8). Gills were separated from the arches and observed under a stereomicroscope for cysts on the gill filaments. The operculum cover, buccal cavity, and abdominal cavity were examined through a magnifying daylight lamp at ×1.75 magnification (Daylight®). On the detection of cysts or free spores, their location, and numbers (for the cysts) were noted and their dimensions were obtained.

2.2.3 *Unicapsula* n. sp. spore morphology and measurements

Cysts that were detected from an infected oesophagus were photographed, and their diameter was measured using a Zeiss stereo microscope (Discovery. V8) equipped with an AxioCam HRc digital camera, using Axio Vision Rel. 4.8 software at ×1-×12 magnifications. Subsequently, individual cysts were separated from the infected tissues and a spore suspension was prepared by carefully disrupting the cysts using a sterile needle to release free spores in the physiological saline-filled small Petri dish (30 mm in diameter). A drop of prepared spore suspension was placed on a microscopic slide and was studied using an Olympus BX63 compound light microscope, equipped with an Olympus DP72 digital camera. Spores were observed using Nomarski differential interference contrasting illumination at magnification of ×200-1600, using oil immersion to study and describe the morphology of mature spores.

Several photomicrographs were obtained using Olympus CellDimension© imaging software to obtain measurements of mature spores according to Alama-Bermejo et al. (2009). In addition, spore apical length and width were obtained as shown in (Figure 2.1B). Measurements of polar filaments were obtained using the polyline function to obtain the most accurate full length of the polar filaments. Because the rudimentary polar capsules of *Unicapsula* spp. are difficult to observe

using light microscope, accurate measurements of the diameter rudimentary polar capsule were obtained from scanning electron microscopy (SEM) images only.

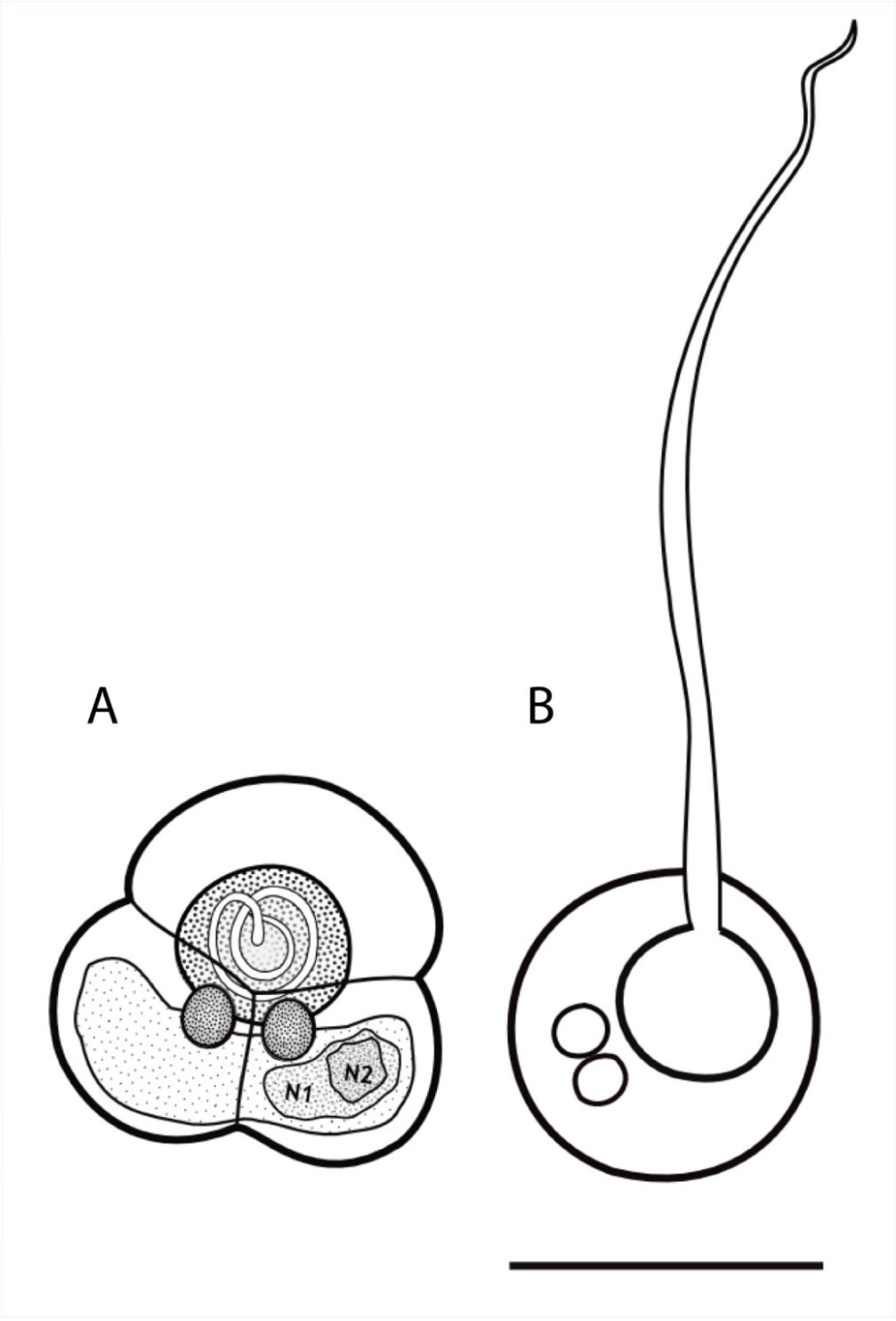

Figure 2. 1 Line drawings depicting mature spores of Unicapsula fatimae n. sp. frontal view, (A) and apical view, (B). Scale bars A and B=5 µm

2.2.4 Histology and host–parasite relationship

Infected and uninfected host oesophageal tissues with parasite cysts were fixed in either 10% buffered formalin or Bouin fixative for histological analysis of the host-parasite interactions. Tissues were processed using standard histological techniques and 5-µm thick sections were produced using a microtome. Sections were stained with haematoxylin and eosin (H&E) to study the host inflammation response, and cover slips were applied using the mounting medium DePex. Further slides were studied, and photomicrographs were obtained and investigated for cyst structure, histopathology, and host-parasite interaction.

2.2.5 Scanning electron microscopy imaging

For SEM, spore suspensions in physiological saline were centrifuged in 1.5 ml eppendorf tubes at 2000 rpm for 5 min to allow sedimentation of the spores to the bottom of the tube. After the supernatant was removed, pelleted spores were prepared for SEM as follows. The spore pellet was fixed in 2.5% glutaraldehyde for a maximum of 3 h and was briefly vortexed to mix with the fixative. The fixed spore suspension was transferred to a syringe attached to a membrane filter holder and passed through a 13-mm diameter, 0.4-µm Whatman® nuclepore track-etch membrane filter, followed by two rinses in 100mMsodium cacodylate buffer pH 7.2, each for 15 min. Further, the spores were post-fixed in 1% osmium tetroxide in 100 mM sodium cacodylate buffer for 30 min and were washed with distilled water for 15 min. The spores were dehydrated in an ascending series of ethanol (25, 50, 75, 95, and 100%), each for 5 min. The membrane was removed from the holder, critical point dried, mounted onto aluminium stubs, sputter coated with gold, and viewed with a Jeol JSM 5600 LV SEM microscope at 60 Kv.

2.2.6 Transmission electron microscopy imaging

Isolated cysts were fixed with 2.5% glutaraldehyde in 1.0 M phosphate buffer (pH7.4) and were washed several times with the same buffer. The washed cysts were post-fixed in osmium tetroxide in 1.0 M phosphate buffer and dehydrated in an ascending acetone series from 30 to 100 %. The cysts were embedded in epoxypropane by adding a 1:1 ratio of epoxy resin and acetone, 1:3 ratio of epoxy resin and acetone ratio, and full-strength epoxy resin three times. The cysts were transferred to fresh resin in molds and dried for 48 h at 60 °C. Semithin sections were obtained from the cysts and were stained with 1 % toluidine blue for 1 min and mounted. Once the desired region of the cysts was observed, ultrathin sections were cut, mounted on grids, and stained with

uranyl acetate and lead citrate. Grids were examined in a JEM 2100F field emission electron microscope (JEOL Ltd).

2.2.7 DNA analysis and phylogeny

Infected tissues and parasite cysts were fixed in 95% ethanol and given into DNA lysis buffer for molecular analyses. Total DNA was extracted using a GeneMATRIX DNA isolation kit (EURx, Poland) following the tissue protocol and used as templates in subsequent PCRs. Small subunit ribosomal DNA (SSU rDNA) of parasites was amplified using the general myxosporean primers according to the methodology described by Freeman et al. (2008) and the Kudoa-specific primers Kud-80f and Kud-730r (Kristmundsson and Freeman 2014), utilizing the same polymerase chain reaction (PCR) conditions. PCRs were conducted on parasite DNA from 4 fish and performed in triplicate. PCR products of the expected sizes were recovered using a GeneMATRIX PCR product extraction kit (EURx, Poland) and sequencing reactions were performed using BigDyeTM Terminator cycle sequencing chemistry, utilizing the same oligonucleotide primers that were used for the original PCRs.

DNA sequencing was performed in forward and reverse directions for all PCR products, and nucleotide BLAST searches were performed for each sequence read to confirm a myxosporean origin (Zhang et al. 2000). Contiguous sequences were obtained manually using CLUSTAL X and BioEdit (Thompson et al. 1997; Hall 1999). CLUSTAL X was used for the initial SSU rDNA sequence alignments of the novel sequence and 19 other histozoic marine myxosporean parasites. Phylogenetic analyses were performed using the maximum likelihood methodology in PhyML (Guindon et al. 2010) with the automatic smart model selection [selection criterion: Akaike Information Criterion (AIC)], running the general time-reversible substitution model (GTR+G6+I) with 1000 bootstrap repeats.

2.3 Results

Whitish, spherical, loosely attached cysts measuring 896 (375-1406) µm in diameter (n=50) were detected from the oesophageal and stomach inner lining of several *S. canaliculatus*, caught off Omani waters. The cysts contained myxosporean spores that had similarities to those from the genus *Unicapsula* Davis 1924 (Lom and Dykova 2006; Alama- Bermejo et al. 2009). The infection intensity ranged from 1 to 18 cysts per hosts (Figure 2.1A-C 2A-C), with numerous cysts are of a sample from Muscat Governorate collected in 2014). In some cases, several empty cysts were

detected, which probably represented ruptured mature cysts (Figure 2.1 C). The cysts were detected in hosts from 5 out of 7 assigned sampling locations. The highest prevalence was from Al- Lakbi landing site with 17 of 35 examined fish infected.

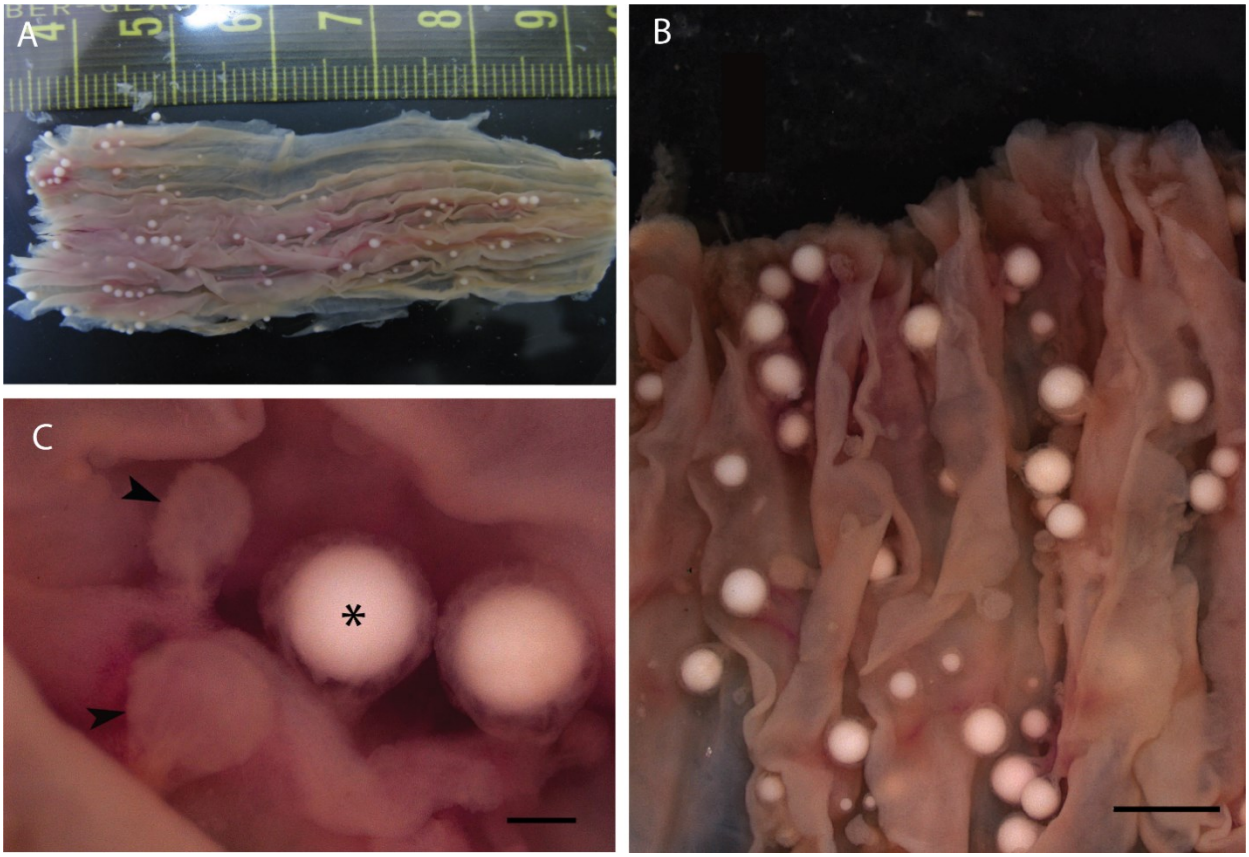

Figure 2. 2 A Heavily infected oesophagus of Siganus canaliculatus collected from Muttrah local fish market showing numerous Unicapsula fatimae n. sp. cysts (>100 cysts detected), (A). Close-up of a portion of the infected oesophagus showing the variable sizes of the cysts and several empty cysts, (B). C Magnified portion of the oesophagus showing two full cysts (asterisk) and two empty cysts (arrow heads), (C). Scale bar 3mm for B and 500 µm for C.

2.3.1 Taxonomical description

U. fatimae n. sp. from oesophageal and stomach endothelium of white-spotted rabbitfish *S. canaliculatus* (Park). Based on the information obtained from the shape of cysts, morphological, ultrastructural, molecular data of mature spores, site of infection, tissues tropism, host type, and geographical locality, we confirm that the *Unicapsula* species described herein is unique among the previously described *Unicapsula* spp.

Class Myxosporea

Order Multivalvulida

Family Trilosporidae Shulman, 1959

Genus *Unicapsula* David, 1924

Species *U. fatimae* n. sp.

Type host: *S. canaliculatus* Park, 1797 (Siganidae)

Type locality: Dhofar Governorate (Raysut fish market), Arabian Sea, The Sultanate of Oman

Other localities: Al-Wusta Governorate (Al-Lakbi landing site), Al- Sharqiya Governorate (Masirah Island), Muscat Governorate (Muttrah fish market), and Musandam Governorate (Dabba landing site)

Site of infection in the host: Myxosporean cysts attached to the endothelium lining of host oesophagus and stomach.

Prevalence: 11 out of 35 from Dhofar Governorate (31%), 17 out of 35 from Al-Wusta region (48.6%), 4 out of 35 from Al Sharqiya region (11%), 3 out of 35 from Muscat Governorate (8.6%), 0 out of 35 from Sohar city, 2 out of 32 from Dabba (5.7%), and 0 out of 35 from Khasab city off the Persian Gulf.

Material deposited: Glycerine–gelatine fixed spores on microscope slides MPM21011, MPM21012 and MPM 21013

Etymology: The species name, fatimae, is given in honour of my mother Fatima Al-Jufaili for her never-ending support and tireless care throughout my life.

Description

The description is based on 41 individual mature spores from thawed material. Spores trifolium with one large functional semi-spherical polar capsule and two smaller rudimentary polar capsules that are sometimes visible using light microscope (Figure 2.3B). Sutural lines, which are not easy to observe with light microscope, divide the spore into three valves, one slightly larger

than the other two. Mature spores 6.23 (5.60-6.60) µm in length, 6.80 (6.12-7.39) µm in width. The large polar capsule length was 2.62 (2.18-2.97) µm and width was 2.65 (2.32-2.90) µm. The length of the extended large polar capsule filament (n=26; Figure 2.3C) was 15.50 (11.71-19.99) µm. Polar filament tapering sometimes double turns at the anterior part (Figure 2.3D). Turns of the large polar capsule filament were partially visible at ×1000 magnification with oil immersion; however, the number of turns was difficult to detect. Additional measurements were obtained from the spore apical view (Figure 2.1B); the apical length was 5.11 (4.45-5.76) µm and apical width was 5.41 (4.45-6.26) µm.

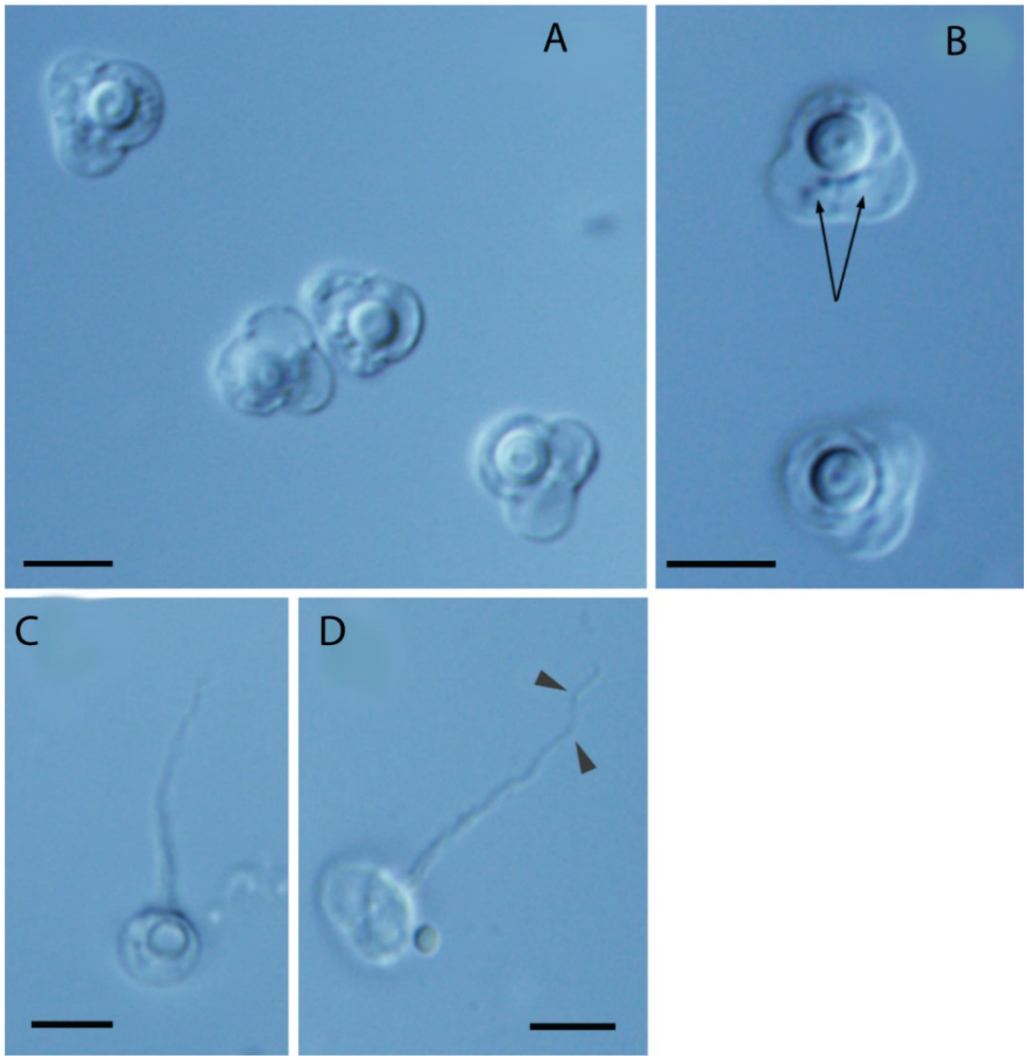

Figure 2. 3 Mature spores of Unicapsula fatimae n. sp., (A). Some mature spores of U. fatimae n. sp. with visible rudimentary polar capsules (arrows), (B). Apical view of U. fatimae n. sp. spores with extruded polar filament, (C). Extruded polar filament of U. fatimae n. sp. tapering to the anterior portion and with double turns (arrow heads), (D). Scale bar for all images =5 µm.

Remarks

Morphological data obtained from this study showed that *U. fatimae* n. sp. was morphologically comparable to what is termed as the sub-spherical *Unicapsula* species that include *Unicapsula andersenae* Miller & Adlard, 2013, *Unicapsula seriolae* Lester, 1982, *Unicapsula pflughfelderi* Schubert, Sprague & Reinboth, 1975, *Unicapsula galeata* Naidjenova & Zaika, 1970, *Unicapsula shulmani* Aseeva & Krasin, 2001, *Unicapsula pacifica* Aseeva & Krasin, 2001, *Unicapsula setoensis* Tomochi, Li, Tran, Yanagida & Sato, 2014 and *Unicapsula* chirocentrusi Sarkar, 1984, and was morphologically distinct from all other *Unicapsula* species. *U. andersenae* from *Argyrosomus japonicas* (Temminck & Schlegel), *Acanthopagrus australis* (Günther); *Eleutheronema tetradactylum* (Shaw), *Lutjanus russellii* (Bleeker) and *Sillago ciliata* (Cuvier) from Australia was genetically the most closely related species to *U. fatimae* n. sp. in the BLAST search with approximately 97% similarity. However, spore size, diameter of the large polar capsule, and length of the polar filament of *U. andersenae* were considerably smaller compared to *U. fatimae* n. sp.

In addition, the general shape of *U. andersenae* was more spherical compared to the trifolium shape of the new species. Although the general shape of *U. seriolae* is remarkably similar to *U. fatimae* n. sp., comparative morphological data revealed that the size of the polar capsule and polar filament of this species were much larger than those of *U. fatimae* n. sp. In addition, a comparison of the SEM images of the two parasites revealed that the rudimentary polar capsule of the former is located differently than in *U. fatimae* n. sp. Furthermore, molecular data, infection site, and type host differentiate *U. seriolae* from the new species. Spores of *U. pflugfelderi* were three fourth the size of *U. fatimae* n. sp. and the length of the extended polar filament was one half compared to *U. fatimae* n. sp. In addition, host type, infection site, and species locality further distinguish *U. pflugfelderi* from the new species. Because the description of *U. galeata* is poor, it was rather difficult to distinguish it from the new parasite species. However, superficial morphological comparison between the two species and site of infection for the new taxon (muscle tissue vs oesophagus tissue) and its host (*Parupeneus ciliatus* (Lacépède) vs *S. canaliculatus*) could be used to distinguish between the two species. Both *U. shulmani* and *U. pacifica* were excluded because of their larger spore size (*U. shulmani* 7.3-8.6 μm and *U. pacifica* 7.8-10.3 μm), type host

Albatrossia pectoralis (Gilbert), infection site (*U. shulmani*: urinary bladder and *U. pacifica*: muscles), and geographical locality.

The recently described *U. setoensis* had a slightly smaller spore size and shorter polar filament compared with *U. fatimae* n. sp. However, spore shape with the permanently extended polar filament and shell valve arrangement of *U. setoensis* separates it from all previously recorded *Unicapsula* species and from the new taxon described herein. Finally, *U. chirocentrusi* can be differentiated from the new species by the general shape of the mature spore in addition to its site of infection and type of host. With regard to the remaining *Unicapsula* species, they can be easily distinguished from *U. fatimae* n. sp. by their unique spore shapes, infection site, and geographical locality *U. pyramidata* (Naidjenova and Zaika 1970), *U. marquesi* (Diebakate et al. 1999), *U. muscularis* (Davis 1924) and *U. maxima* (Sarkar 1999). Because most of the *Unicapsula* species are very simple in their spore morphology with a few that exhibit unique features, there is a requirement to re-describe some *Unicapsula* species and to include ultrastructural and molecular data to better understand and differentiate previously described species and facilitate identification of new ones.

2.3.2 Scanning electron microscopy

The sutural line is clearly visible and forms a Y shape on the frontal and dorsal view, dividing the three valves almost equally (Figure 2.4A). Rudimentary polar capsules were visible as leaf-shaped protrusion structures immediately under the large polar capsule, measuring 0.8 (0.7-0.9) µm in diameter. Compared with the only available SEM images of two *Unicapsula* species, the rudimentary polar capsules were similar to those of *U. pflugfelderi* in their shape and size (Alama-Bermejo et al. 2009).

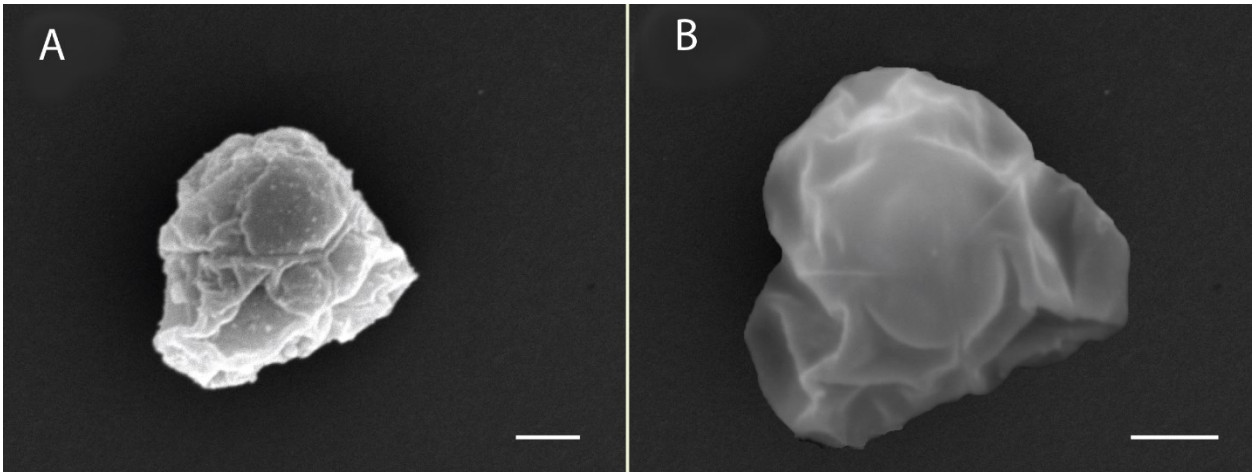

Figure 2. 4 SEM images of the apical pole view of a mature spore, showing the position of the large functional polar capsule and two rudimentary polar capsules immediately below it. The capsulogenic cells bearing the rudimentary polar capsule can be seen as two protrusions that take a leaf shaped form, A. The sutural lines form a Y shape on both the apical pole and basal pole view dividing the three valves equally, B. Scale bar for A, B= 1.0 µm, 2.0 µm and 1.0 µm, respectively.

2.3.3 Transmission electron microscopy

Semi-thin sections of the cysts revealed that they are divided into an endoplasm (EN; Figure 2.5A) and ectoplasm (EC; Figure 2.5A). A thin layer of darkly stained fibrous membrane was located outside the ectoplasm (arrows). A wall of host-originated connective tissue (CT; Figure 2.5A) is observed surrounding the plasmodia and separating it from host epithelial tissue (HT; Figure 2.5A); more details of the composition of host tissue complex surrounding the cysts is provided in the histology section. Ultrastructural details of the plasmodia observed using transmission electron microscope revealed more information regarding the composition of the membrane surrounding the ectoplasm (Figure 2.5B). The membrane was a multi-layered membrane unit that contained numerous branches or channels facing towards the ectoplasm. We assume that they could be pinocytotic channels (PiC) or passages that may aid in transporting nutrients into the plasmodia. These channels formed a web-like structure and contained several vacuoles of various sizes and shapes. In some areas, the host nucleus can be observed to be trapped in the complex membrane (Figure 2.5B). Immediately next to the complex membrane, the ectoplasmic region is observed containing several mitochondria and electron dense lipid droplets (Figure 2.5C).

Sporogenesis was asynchronous with both generative cells and presporogenic stages located at the periphery of the plasmodia and mature spores at the centre. The earliest stages of the parasites are single nucleated cells of various sizes (possibly generative cells). Pansporoblasts were also observed and young spores with capsular primordium and primordium of rudimentary polar capsules (Figure 2.5D) were seen in the ectoplasm region of the plasmodia. Mature spores were composed of three shell valves, one containing a large polar capsule possessing a fully functional polar filament and two contained two bodies, which were similar to the polar capsule; however, they were much smaller and had reduced polar filaments (Figure 2.6A). The polar capsule was composed of an inner lucent layer and an outer electron dense layer similar to other *Unicapsula* spp. and other myxosporean parasites.

Electron dense bodies were detected near the opening of the polar capsule and anterior to the rudimentary polar capsules. It was not easy to determine the exact number of turns of the polar filament for this species; however, after studying several images, the number of turns was estimated to be between two and one half and three turns. The sporoplasm of this parasite contained what appeared to be two adjacent nuclei in a single sporoplasm (Figure 2.6C). A closer look at the sporoplasm and the two nuclei revealed the presence of another membrane surrounding one of the nuclei, which we think could be the second sporoplasm, as mentioned by earlier authors (Schubert et al. 1975; Lester 1982; Alama-Bermejo et al. 2009) (Figure 2.6D).

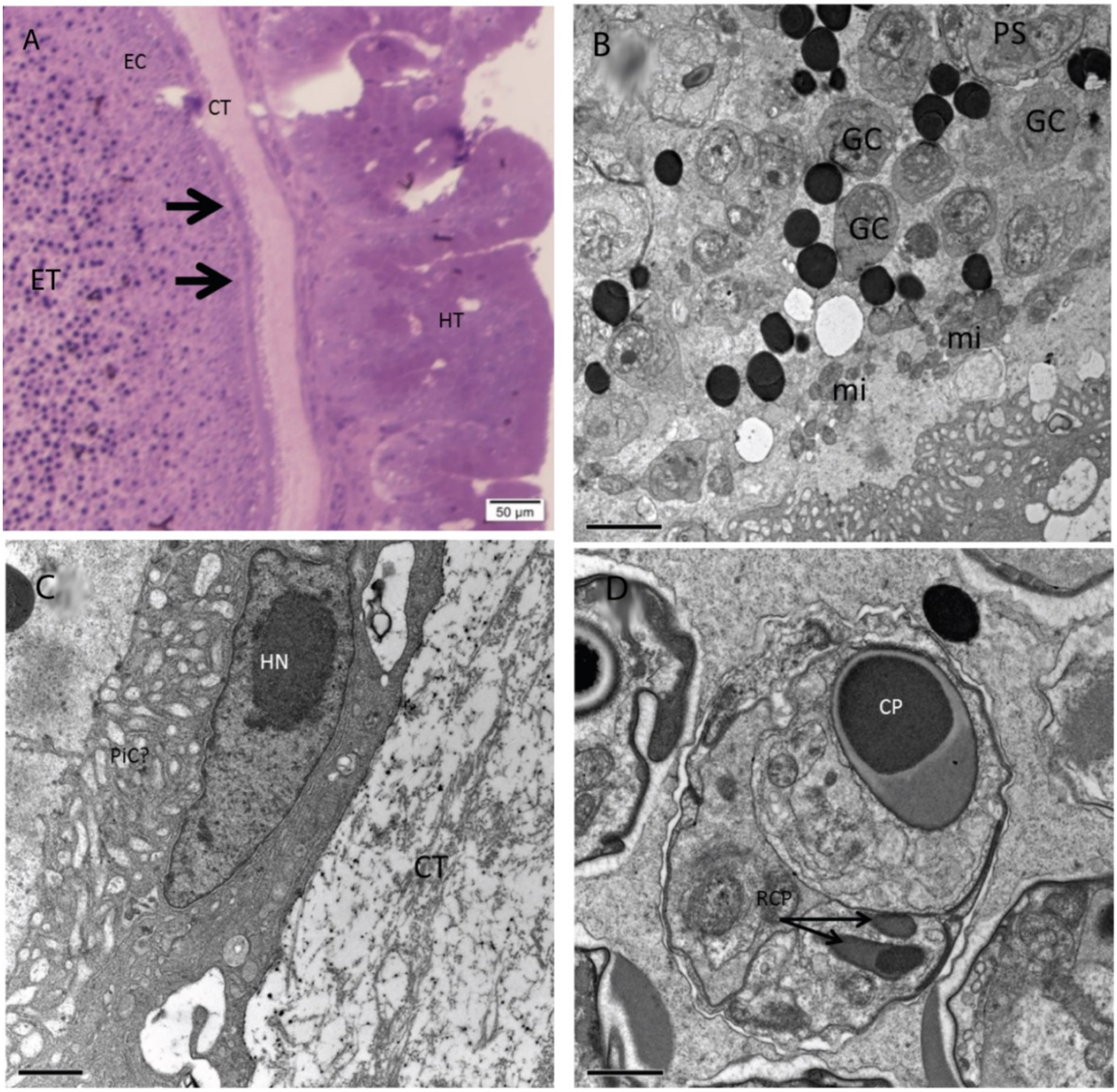

Figure 2. 5 Semi-thin section through a cyst showing the division of the cyst complex into several layers, the endoplasmic region (EN), ectoplasmic region (EC), peripheral membrane (arrows), connective tissue (CT), and host tissue (HT), (A). Ultrathin section of the plasmodia showing a close-up of the plasmodia and host interface with details of the peripheral membrane which is located between the ectoplasmic region and the connective tissue wall, (B); note the host cell (asterisk) with the nucleus (HN) trapped inside the membrane and the several arms or web-like structure which possibly could be pinocytotic channels (PiC), (C). Details of the ectoplasmic region with several single nucleus GC generative cells, PS Pansporoblasts, mi mitochondria, lipid droplets, and young spores. Young spore with a developing polar capsule or CP capsular primordium of the large polar capsule, (D). Furthermore, note the two structures (arrows), which appear to be primordium of the RCP rudimentary polar capsules, (D). Scale bar for B, C, D=1.0, 2.0, and 1.0 µm, respectively.

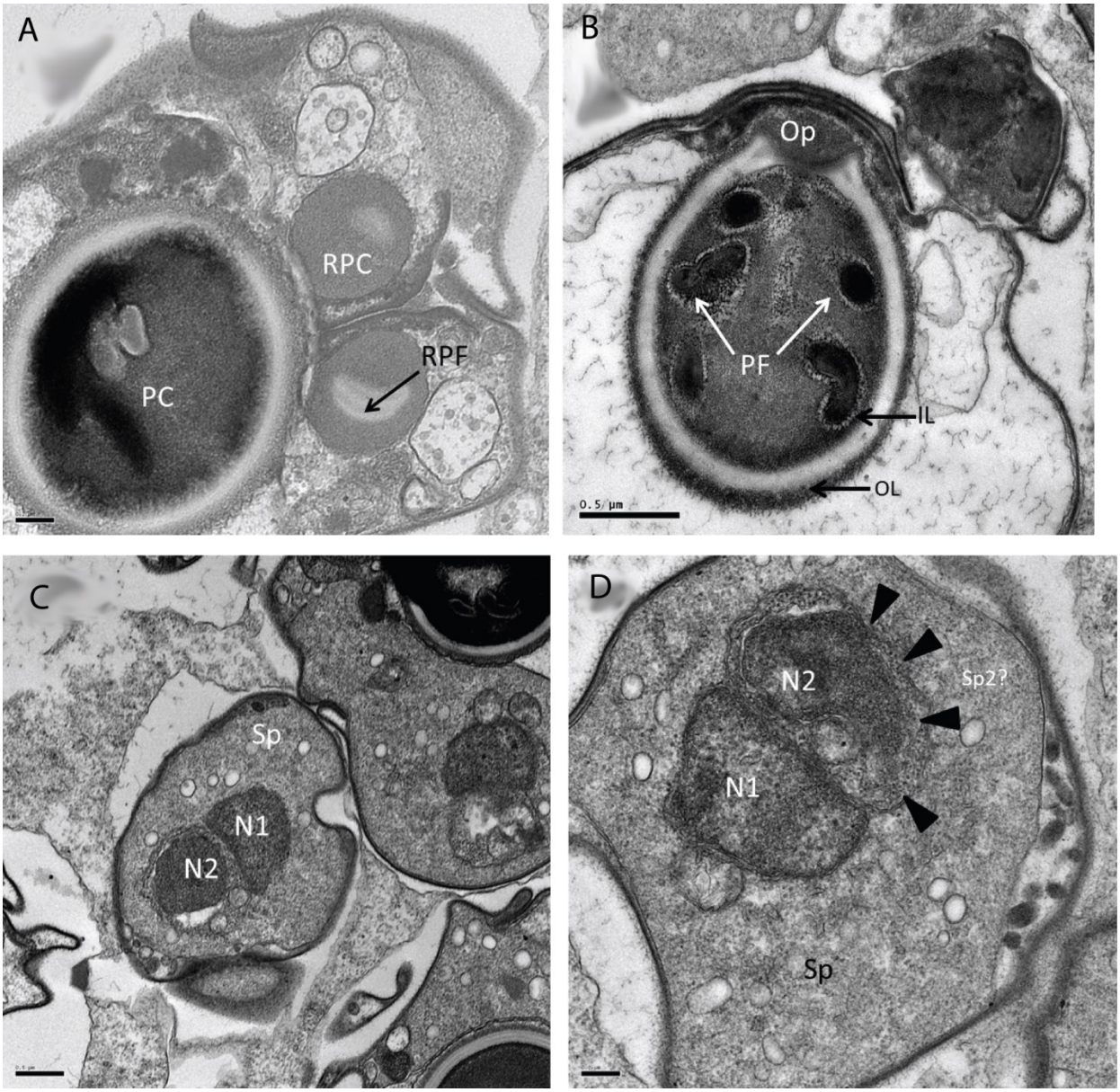

Figure 2. 6 Section through a mature spore showing the large polar capsule and the two rudimentary polar capsules with what appears to be a rudimentary polar filament, (A). Section through a mature polar capsule with the filaments showing 2 and half turns and the opening of the polar capsule, (B). Section through the sporoplasm of some mature spores showing the two adjacent nuclei, (C). Close-up of the sporoplasm showing the two nuclei and indicating the second membrane which could be a secondary sporoplasm (arrow heads) within the main sporoplasm, (D). Scale bars: A - D=0.2 and C= 0.5 µm.

2.3.4 Plasmodia gross morphology and histology

The cysts of *U. fatimae* n. sp. were localized on the mucosal surface of the oesophagus and occasionally on the gastric mucosa. The gross morphology shows that the cysts were attached to the host using a stalk that appears pedunculated (Figure 2.7A, white arrowhead). The cyst itself

was surrounded with a layer of host-derived tissue and appeared to be loosely attached to the inner lumen of the host oesophagus (Figure 2.7A). Histological sections of infected tissues with the cysts revealed that the cyst is composed of a multiple, complex structure consisting of a layer of folded hypertrophic host epithelial cells, a wall of host connective tissue, and a fibrous membrane separating the plasmodia from the host connective tissue. The function of the folded epithelial tissue layer is unknown, but its formation could be used by the parasite to protect itself and/ or to maintain a supply of nutrients to the plasmodia.

Histology of the stalk structure showed that it is composed of host epithelial cells, glandular tubules, and connective tissue. No inflammatory reaction could be detected in histological sections; however, some abnormalities were observed in the host tissue near the site of the infection. The mucosal tissue near the cysts was thicker than the normal tissue distant from the cysts and showed hyperplasia of glandular tubules and epithelial cells accompanied by hypertrophy of epithelial cells. In addition, it appears that the cysts begin developing within the submucosa and glandular tubule region of the host oesophagus tissue. This observation is supported by observation of epithelial cells formed inside the glandular tubule regions and in some sections by the appearance of an island of host originated tissues (epithelial and glandular cells) observed in the submucosal region of the host oesophagus.

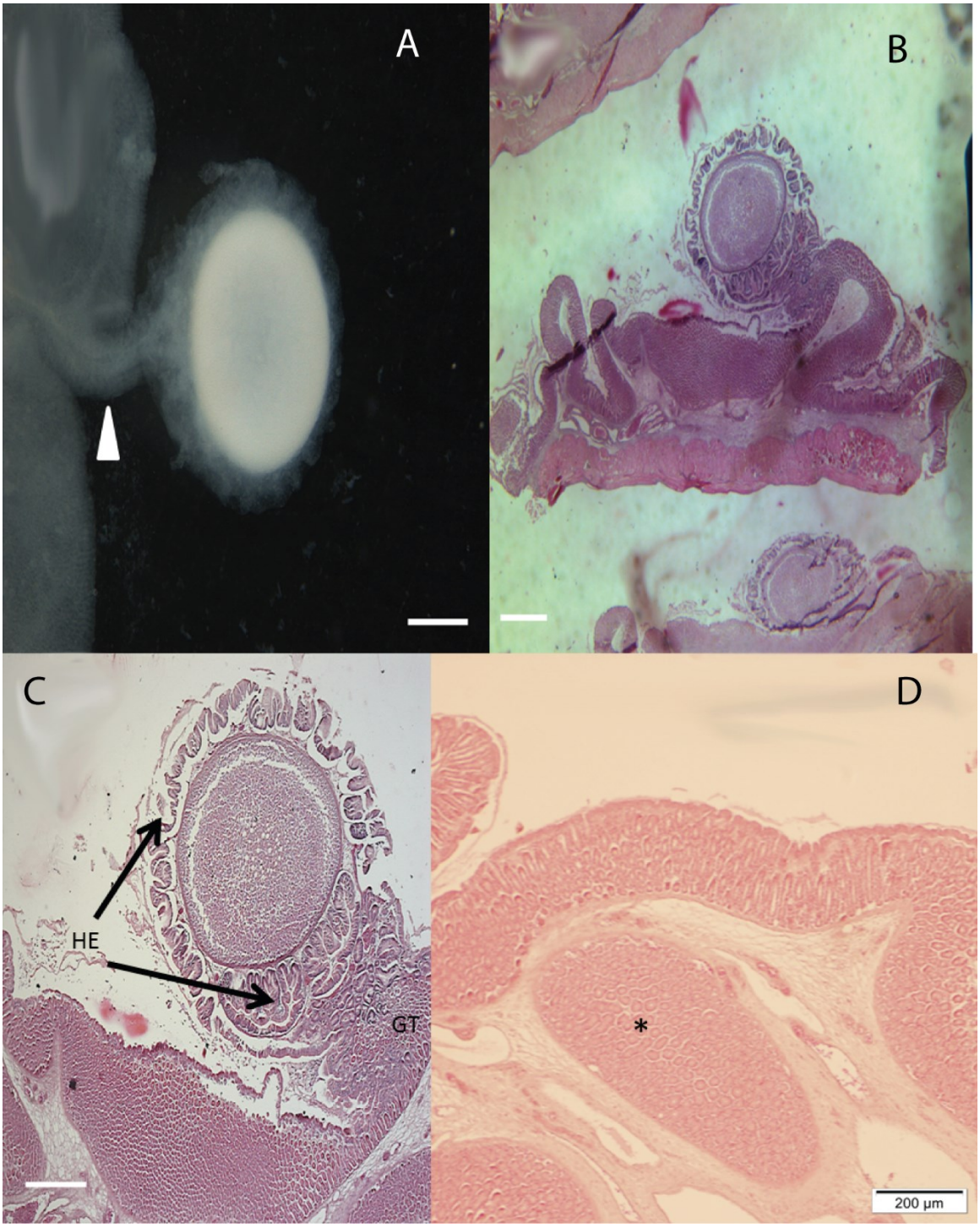

Figure 2. 7 Gross morphology of Unicapsula fatimae n. sp. Cyst showing part of the host oesophageal tissue, the stalk (peduncle) by which the parasite is attached on to the host tissue and the spherical plasmodia that is surrounded by host tissue (white arrow head), (A). Histological section through U. fatimae n. sp. host complex showing the structure of the oesophagus tissue near the infection site and the position of the stalk structure and cyst complex, (B). Close-up of the cyst complex showing the hypertrophic folded host oesophagus epithelial cells (HE) and glandular tubules (GT) that comprise the stalk formation, (C). The formation of an abnormal structure (asterisk) within the submucosal region of the oesophagus, (D). Scale bars: A= 500 µm, B=600 µm, and C= 400 µm.

2.3.5 DNA and molecular analysis

An almost complete SSU rDNA sequence of 1653 bp was obtained for the *U. fatimae* n. sp. and the sequence was deposited in GenBank (Accession Number: KT894108). The sequences of the four strains of *Unicapsula* were 100 % identical. Submission to BLAST searches of the contiguous sequence showed that the closest relative in the databases were other *Unicapsula* spp. with similarities ranging between 90 and 98 %. The closest match was a sequence for *Unicapsula* sp. infecting the muscle of *A. japonicus* from Australia (GenBank Accession No. AY302725). The SSU rDNA of 20 histozoic marine myxosporeans were used for phylogenetic analysis and the maximum likelihood topology was based on 1828 informative characters to produce a tree (Figure 2.8).

The resulting tree showed that the Trilosporidae are robustly supported from node A, as a monophyletic sister group to the Kudoidae and, together, they form the Multivalvulida. All available sequences of *Unicapsula* spp. are fully supported and form node B within Trilosporidae. The new taxon is placed in a fully supported sister clade with *Unicapsula fatimae* n. sp. and *U. andersenae* forming a monophyletic group. In addition, both *U. setoensis* and *U. pyramidata* formed another monophyletic sister group within the *Unicapsula*. Basal to the Multivalvulida are the Monomyxidae and Gastromyxidae, *Enteromyxum leei* was used as an outgroup.

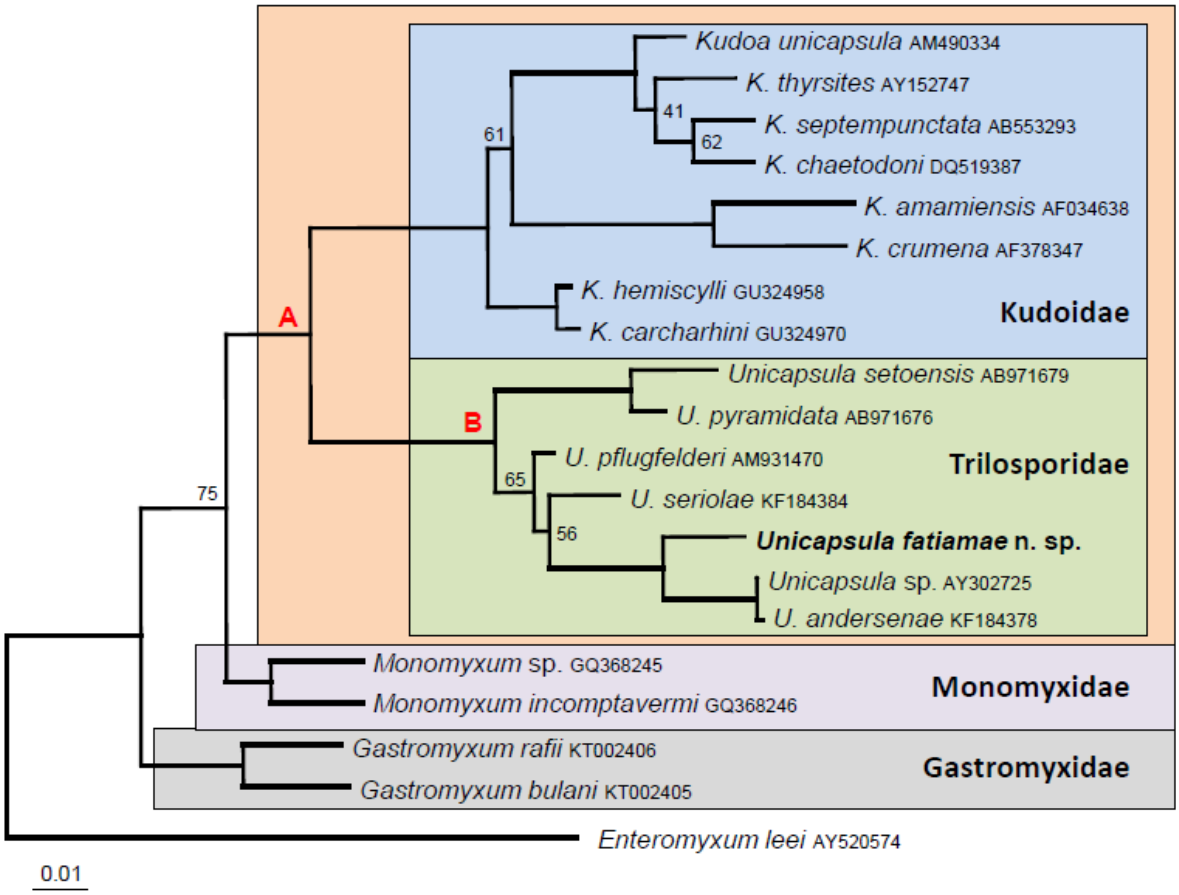

Figure 2. 8 Maximum likelihood topology of 20 histozoic marine myxosporean SSU rDNA sequences using PhyML. Tree shows the phylogenetic relationship between the species of Unicapsula based on the available Unicapsula and the closest matches of SSU rDNA sequences available on GenBank NCBI. Numbers at the nodes represent bootstrap support values; nodes with no numbers are fully supported.

2.4 Discussion

A comprehensive morphometric comparison of all described *Unicapsula* species supports the view that the species presented in this article is a novel addition to the genus. This is the first record of the genus *Unicapsula* in Omani waters. Compared with previously described *Unicapsula* species, *U. fatimae* sp. n. is the only species among them to be found infecting the smooth muscles of a fish and the only one that forms spherical cysts. These two features alone strongly distinguish the new taxon from all previously described species. Further details of morphological features obtained through light microscopy and electron microscopy distinguish the new parasite from its congeners. Some ultrastructural details regarding *Unicapsula* species were provided by Schubert et al. (1975), Lester (1982), and Alama-Bermejo et al. (2009), both Schubert et al. (1975) and

Alama-Bermejo et al. (2009) stated that the plasmodia of their respective *Unicapsula* species were divided into ectoplasmic and endoplasmic regions, whereas the ultrastructural description of Lester (1982) lacked such information. Similar to what has been described for the *Unicapsula* species and other histozoic myxosporeans (Ali et al. 2003; Azevedo et al. 2013), the plasmodia of *U. fatimae* n. sp. were divided into ectoplasmic and endoplasmic regions. However, unlike what is known from its congeners, it exhibited a more complex structure and contained cytoplasmic organelles and generative cells of the parasite, whereas Alama-Bermejo et al. (2009) described the ectoplasmic region of *U. pflugfelderi* as being smooth. In his description of *U. pflugfelderi*, Schubert et al. (1975) mentioned brief information with respect to the host–parasite interface, where he noted some channels near the periphery of the ectoplasm that were suggested to be pinocytotic channels.

Investigations regarding the host–parasite interface in myxosporean infections in fish were reported extensively for the genera *Myxobolus* (Ali et al. 2003; Milanin et al. 2010) and *Henneguya* (Matos et al. 2005; Lovy et al. 2015). These studies report the presence of a fibrous single or double membrane unit with some pinocytotic activity at the periphery of the ectoplasm, and the appearance of several vesicles or vacuoles within the membrane and observation of some host cells in the vicinity of the membrane. For the first time, the present study presents some details regarding the host–parasite interface membrane reported from the genus *Unicapsula*. Similar to what has been reported by these authors, *U. fatimae* n. sp. plasmodia had a fibrous membrane separating the plasmodia from the host tissue. The membrane of this parasite was complex and had a rather extensive network of web-like structures near the ectoplasmic region. Because many vesicles were observed on the membrane and supported by the occasional presence of host cells trapped in the membrane*, U. fatimae* n. sp. plasmodia may feed off host cells via the process of pinocytosis.

Ultrastructure studies of *U. fatimae* n. sp. plasmodia revealed information regarding the developmental stages of this new parasite that were similar to what is known to other myxosporean parasites (Ali et al. 2003; Adriano et al. 2009). This is the first description of developmental stages from a species in the genus *Unicapsula*. The earliest stages that could be seen were of single nucleated cells, which could be generative cells. From what was observed, it appears that sporogenesis was achieved by cell in cell development of generative cells to form a pansporoblast.

This form of spore development was similar to what is observed in other myxosporean parasites (Abdel-Baki et al. 2015). The capsular primordium of the large polar capsule was observed in young spores often without any signs of formation of the rudimentary polar capsule. This could indicate that the formation of the larger polar capsules precedes the formation of a rudimentary polar capsule. Details of mature spores agreed to what has been described for other *Unicapsula* species (Schubert et al. 1975; Diebakate et al. 1999; Lester 1982 and Alama-Bermejo et al. 2009). The aforementioned authors recorded the presence of two sporoplasms in the respective *Unicapsula* species in their articles. In the present study, two nuclei were observed to be adjacent to each other, with one of them included in a membrane within the sporoplasm. We think that this is similar to what was described in the abovementioned studies, although slightly different. This feature of the sporoplasm may be a genus specific feature, which is exhibited only in *Unicapsula* species.

Histological investigations of the infected oesophagus tissue did not reveal any inflammatory response induced by the parasite at the site of infection. However, the formation of the cyst complex and plasmodia peduncle induced some notable abnormalities in the host tissue; these changes could possibly impair the function of oesophageal tissue. The possible negative impact of *U. fatimae* n. sp. on its host could also be emphasized by the high intensity of parasites noted on some hosts with >100 cysts recorded in one host (samples collected from Muttrah city in 2014). In addition, the occurrence of several "empty" cysts indicates the release of mature spores into the lumen of the host oesophagus, suggesting a possibility of parasite dispersion within the same host in the case of a direct life cycle. Further histological investigations are required to confirm the impact of this parasite on the host. Because *S. canaliculatus* is intended as a suitable candidate for the aquaculture industry in the Sultanate of Oman, a high prevalence and intensity of this parasite could be a possible to the future development of sustainable aquaculture in Oman.

Acknowledgments We are incredibly grateful to the Ministry of Agriculture and Fisheries Wealth, Sultanate of Oman, and for the TRC council in Oman for funding this research through ORG-EBR_11-TRC grant.

3 Species of *Tetrancistrum* Goto & Kikuchi, 1917 (Monogenea: Ancyrocephalidae) from the gills of the white-spotted rabbitfish, *Siganus canaliculatus* (Park) (Perciformes: Siganidae) off Omani coasts, with a description of *Tetrancistrum labyrinthus* n. sp.

Abstract

Tetrancistrum labyrinthus n. sp. is described from the gills of the marine herbivorous fish *Siganus canaliculatus* (Park) found in the Western Indian Ocean (Sea of Oman and Arabian Sea). Comparative morphological analyses of all previously described species of *Tetrancistrum* Goto & Kikuchi, 1917 confirmed the distinct status of *T. labyrinthus* n. sp. The new species closely resembles *T. suezicum* Paperna, 1972 and *T. oraminii* Young, 1967 in the morphology of the male copulatory organ. However, it can be distinguished by possessing a thin handle-like anterior basal flange and a compound accessory piece that is composed of a tapering rod-shaped anterior part and a large cylindrical, elongated posterior part. The new species can be further distinguished from other *Tetrancistrum* species by its highly sclerotized and complex disc-shaped vaginal vestibule. This is the first record of *Tetrancistrum* from the Sea of Oman and Arabian Sea, and the fourth of nominal species of *Tetrancistrum* known to parasitize *Siganus canaliculatus* (Park). In addition, *T. indicum* Paperna, 1972 is re-described here with an updated locality record.

2

[2] *This was published as: Al-Jufaili S.H. & Palm H.W. (2017). Species of Tetrancistrum Goto & Kikuchi, 1917 (Monogenea: Dactylogyridae) from the gills of the whitespotted rabbitfish, Siganus canaliculatus (Park) (Perciformes: Siganidae) off Omani coasts, with a description of Tetrancistrum labyrinthus n. sp. Systematic Parasitology, 94(7):809-818.*

3.1 Introduction

The Siganidae is a small family of marine fishes commonly known as rabbitfishes (Perciformes: Siganidae) that inhabit coral reefs in the tropical and subtropical Indo-Pacific region (Woodland 1990). Species of three genera of ancyrocephalid monogeneans are known to infect rabbitfishes: *Tetrancistrum* Goto & Kikuchi, 1917; *Glyphidohaptor* Kritsky, Galli & Yang, 2007 and *Pseudohaliotrema* Yamaguti, 1953 (see Kritsky et al. 2007a). *Tetrancistrum* was the first genus of ancyrocephalid reported from a siganid host (Goto and Kikuchi 1917). The genus was erected to accommodate *T. sigani* Goto & Kikuchi, 1917 recorded from *Siganus fuscescens* (Houttuyn) off Japan and towards the Philippines (see Kritsky et al. 2007b). This was followed by the description of *T. nasonis* Young, 1967 and *T. oraminii* Young, 1967 from the gills of species of the Acanthuridae and Siganidae, respectively, in Australian waters, and the placement of *T. fusiforme* (Yamaguti 1953) [formerly *Pseudohaliotrema* (*Pseudohaliotrematoides*) *fusiforme* Yamaguti, 1953] to the genus (Young 1967). In their revision of the genus, Kritsky et al. (2007b) placed in *Tetrancistrum* four species of *Pseudancyrocephalus* Yamaguti, 1968. In addition, Kritsky et al. (2007b) elevated *Pseudohaliotrematoides polymorphus eilaticus* Paperna, 1972, *P. polymorphus indicus* Paperna, 1972 and *P. polymorphus suezicus* Paperna, 1972 all reported from off East Africa and the Red Sea to species rank and transferred them to *Tetrancistrum*.

Currently, the genus includes 16 species from Indian-Pacific waters, nine of which were isolated from siganids, five from acanthurids (Perciformes: Acanthuridae) and two from lutjanids (Perciformes: Lutjanidae) (Kritsky et al. 2007b). During a comprehensive investigation of the parasite fauna of *S. canaliculatus* from Omani waters, two species of *Tetrancistrum* were recovered from the gills. This is the first report of the genus *Tetrancistrum* from the waters of Oman and an expansion of the locality range for *T. indicum* Paperna, 1972. The present study aims to provide a morphological description of a new species of *Tetrancistrum* based on the general morphology and morphometric analysis of the male copulatory organ (MCO) and haptoral armaments. The information obtained within the framework of this study justifies the recognition of *T. labyrinthus n. sp.*

3.2 Materials and methods

3.2.1 Sample collection and processing

Between November and December 2012, 245 white-spotted rabbitfish, *Siganus canaliculatus*, were purchased alive from local fishermen along the coasts of the Sultanate of Oman. Thirty-five fish were purchased from each of seven locations: Khasab fishing harbour (26.1644°N, 56.2426°E); Dabba fish market (25.6365°N, 56.2538°E); Sohar fish market (56.7075°N, 24.3461°E); Muscat fish market (23.5859°N, 58.4059°E); Masirah Island fishing harbour (20.3173°N, 58.6916°E); Al-Lakbi fishing harbour (18.113°N, 56.3255°E); and Raysut fish market (16.9698°N, 53.9814°E). The fish were morphologically identified using the guidelines of Randall (1995) and FAO identification sheets (FAO 1983). The gills were excised from the fish, then gill arches were separated and placed in a small Petri dish filled with filtered seawater and examined under a dissecting microscope. Parasites were detached from the gill filaments using a fine needle and kept in filtered sea water at 4°C until fixation.

3.2.2 Morphological investigation

Entire ancyrocephalid were fixed (unflattened) using AFA (alcohol: formalin: acetic acid) or 4% neutral buffered formalin. Fixed specimens were stained overnight with Mayer's paracarmine, differentiated with drops of acid alcohol solution (70% ethanol with 3% HCL), dehydrated in a graded ethanol series (70-100%), cleared in clove oil and mounted in Canada balsam. To study sclerotized structures, whole mounts were prepared using compressed, unfixed samples stained with acetocarmine (Machkevskyi et al. 2013). Measurements are in micrometres and are given as the range followed by the mean and the number (n) of structures measured in parentheses. Body length includes the haptor. Measurements of the copulatory complex, anchors and hooks, as well as the description of the new species are according to Kritsky et al. (2007b). Illustrations were prepared with the aid of a camera lucida attached to an Olympus BX63 motorized compound light microscope with differential interference contrast (DIC) optics and were digitalized using Adobe illustrator CC 2015.3 and the program Inkscape 0.48.2.-1 (Scalable Vector Graphics 2011).

3.2.3 Comparative morphological analysis

Voucher specimens were obtained from the Natural History Museum, London, UK (*T. sigani*, BMNH 1992.7.28.90-92; *T. polymorphum* Paperna, 1972, BMNH 2007.1.3.46-48; and *T. suezicum* Paperna, 1972, BMNH 2007.1.3.43), Meguro Parasitology Museum, Tokyo, Japan (*T. sigani*,

MPM 18853; *T. polymorphum*, MPM 18851; *T. strophosolenus* Kritsky, Galli & Yang, 2007, MPM 18850; and *T. suezicum*, MPM 18854) and Queensland Museum Brisbane, Australia (*T. sigani*, QMG225792 and QMG227593). In addition, micrographs of parasites were obtained from the US National Parasite Collection, Beltsville, Maryland for comparison (*T. yamaguti* Kritsky, Galli & Yang 2007, MPM 23010), and *T. fusiforme* Yamaguti, 1953 (USNPC 99363).

3.2.4 Confocal microscopy

Several 95% ethanol-fixed specimens were examined using a confocal laser scanning microscope following the procedure described in Galli et al. (2006) and Marchiori et al. (2015). The images were obtained using a Leica TCS SP2 confocal microscope equipped with an inverted Leica DMIRE2 microscope and a PL APO 363 oil immersion objective (numerical aperture 5 1.4) at the Live Cell Imaging Centre, Department of Biology, Rostock University.

3.3 Results

Family Ancyrocephalidae Bychowsky & Nagibina, 1968

Genus *Tetrancistrum* Goto & Kikuchi, 1917

Tetrancistrum labyrinthus n. sp.

Type-host: *Siganus canaliculatus* (Park) (Perciformes: Siganidae), white-spotted rabbitfish. Type-locality: Sea of Oman, off Muscat City (23.0000°N, 58.0000°E), Sultanate of Oman. Other localities: Sea of Oman, off Khasab fishing harbour (26.1644°N, 56.2426°E), Dabba fish market (25.6365°N, 56.2538°E). Arabian Sea, off Masirah fishing harbour (20.4711°N, 58.8153°E), off Al-Lakbi fishing harbour (18.113°N, 56.3255°E); Raysut fish market (16.5500°N, 54.0100°E). Sultanate of Oman (November and December 2012).

Type-material: Berlin Natural History Museum (ZMBE. 7436: the holotype; ZMBE. 7438: five paratypes; ZMBE. 7439: seven paratypes). Meguro Parasitology Museum, Tokyo, Japan (MPM. Coll. No. 20960: three paratypes).

Site in host: Gills.

Prevalence: Khasab fishing harbour: 29% (10 out of 35 fish); Dabba fish market: 77% (27 out of 35 fish); Sohar fish market: 0% (0 out of 35 fish); Muscat fish market: 74% (26 out of 35 fish);

Masirah Island fishing harbour: 86% (30 out of 35 fish), Al-Lakbi fishing harbour: 88% (31 out of 35 fish); and Raysut fish market: 94% (33 out of 35 fish).

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Tetrancistrum labyrinthus* n. sp. is urn:lsid: zoobank.org:act:CC65584E-3721-46EC-8A0C-D5D 66806D4A1.

Etymology: The specific name (labyrinthus) refers to the disc-shaped, maze-like, complex vaginal vestibule that is unique to the species described herein.

3.3.1 Description (figures 3.1, 3.2, 3.3)

[Based on 23 specimens.] Body leaf-like; trunk broad; cephalic region and peduncle narrow, tapered. Body 1,142-2,190 (1,552; n = 23) long, with smooth tegument and greatest body width at base of germarium, 374-647 (465; n = 23). Cephalic lobes well developed; each head organ comprises several groupings of terminations of cephalic-gland ducts; large bilateral groups of cephalic glands present posterolateral to pharynx. Eyespots absent; accumulations of minute chromatic granules common; isolated granules scattered throughout cephalic region. Mouth midventral, subterminal at head organs, opens into buccal tube. Buccal tube extends posteriorly along midline to pharynx to form buccal cavity. Pharynx muscular, elongated, ovate, 58-113 x 48-71 (79 x 60) (n = 21). Intestinal caeca bifurcating posterior to pharynx, with diverticula, terminating blindly posterior to gonads (difficult to observe in most specimens).

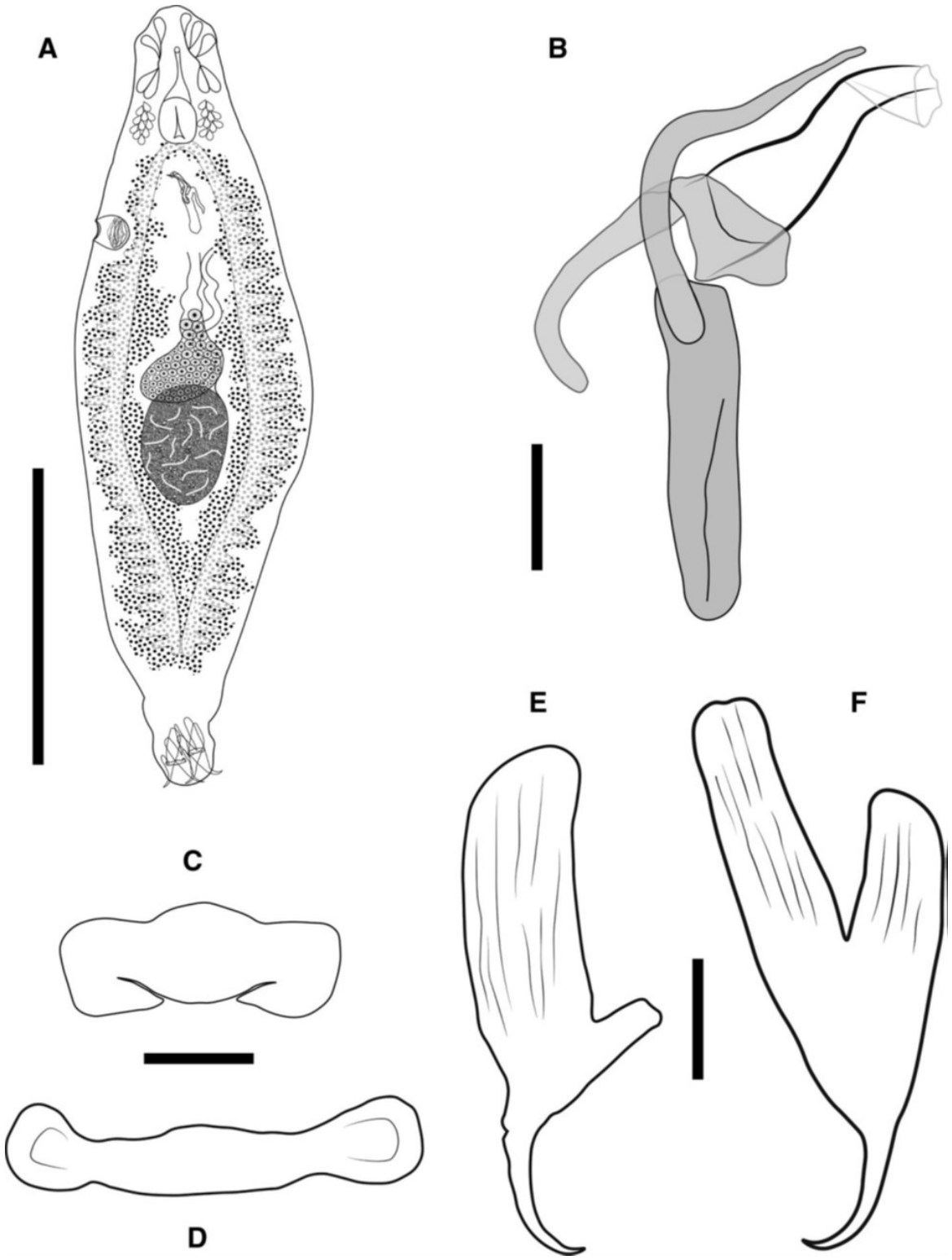

Figure 3. 1 Tetrancistrum labyrinthus n. sp. ex Siganus canaliculatus. (A), Holotype, ventral view; (B), Male copulatory organ, dorsal view; (C), Ventral anchor; (D), Dorsal anchor; (E), Ventral bar; (F), Dorsal bar. Scale-bars: A, 500 µm; B, E, F, 20 µm; C, D, 10 µm

Germarium pretesticular, relatively large, flask shaped, 91-210 (154; n = 23) long, 116-210 (163; n = 23) wide at base. Ootype receives vaginal duct and bilateral common vitelline ducts; uterus expands distally. Vaginal vestibule disc-shaped, heavily sclerotized, 36-62 (44; n = 23) wide. Vaginal vestibule composed of complicated, irregular network of rows (5-6) of stiffened, pointed structures (this sclerotized structure supports the opening of the vaginal aperture, which is a prominent cup-shaped muscular sack; Figure 3.3). Vaginal duct straight, slightly dilated in some specimens; vitellarium dense. Testis single, large, subspherical, 112-260 x 134-264 (223 x 197) (n= 23). Vas deferens leaving anteromedial region of testis, turning left to germarium; prostatic reservoirs large; seminal vesicle forming inverted ‘‘J’’ towards left side of copulatory complex; 2 bulbous prostatic reservoirs dorsal to copulatory organ, each emptying into base of MCO via individual ducts. Copulatory complex comprising sigmoid MCO tube, a thin handle-like anterior basal flange (Figure 3.2), accessory piece marginally hinged with MCO comprising 2 connected parts, a tapering rod-shaped anterior part and a large cylindrical, elongated posterior part. Copulatory complex 75-111 (95; n = 23) long.

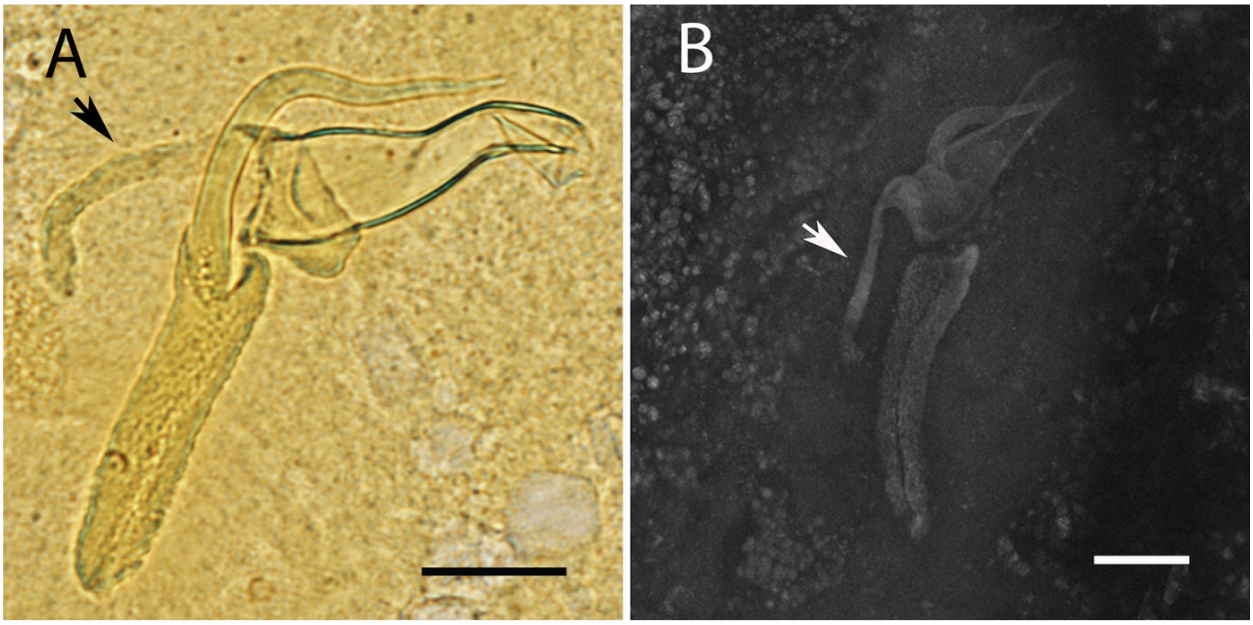

Figure 3. 2 Photomicrographs of Tetrancistrum labyrinthus n. sp. ex Siganus canaliculatus. Male copulatory organ, dorsal view. Light microscope image;(A). Confocal microscope image, (B). Arrows indicate the handle-like anterior basal flange. Scale-bars: 20 µm

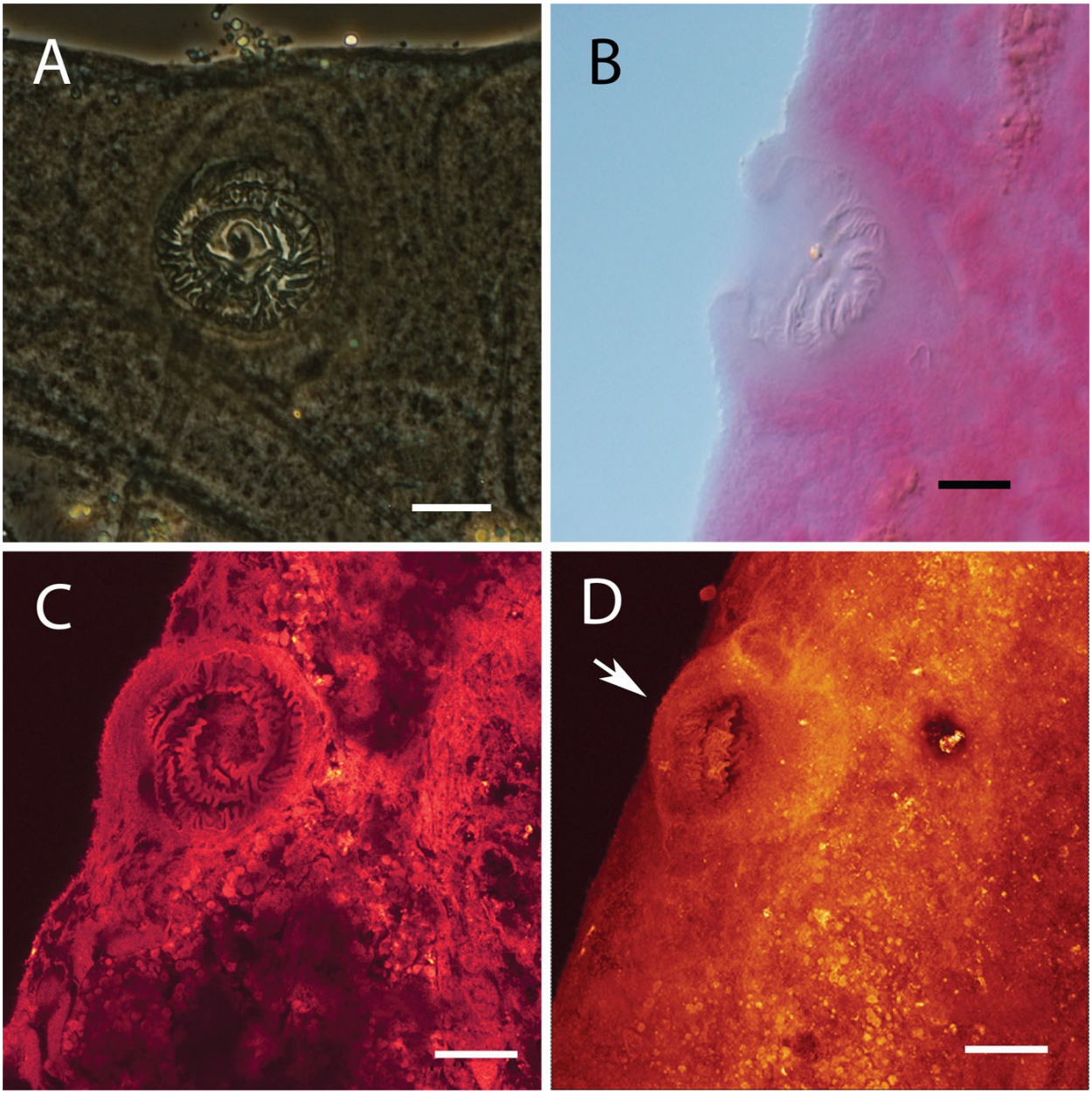

Figure 3. 3 Photomicrographs of Tetrancistrum labyrinthus n. sp. ex Siganus canaliculatus. Vaginal vestibule, ventral view. (A), Phase contrast image showing the disc shape of a flattened vaginal vestibule; (B), Carmine-stained specimen showing the rows (5-6) of the stiffened structures that make up the vaginal vestibule; (C), Confocal microscope image showing the complexity of the vaginal vestibule; (D), 3D-reconstruction of the vaginal vestibule showing the prominent cup-shaped vaginal pore. Scale-bars: 20µm.

Haptor 54-216 (108; n = 23) long, 41-196 (66; n = 23) wide. Haptoral hooks absent. Ventral and dorsal anchors typical of the genus; ventral anchor 77-108 (91; n = 21) long, base 26-35 (33; n= 13) wide, dorsal anchor 75-115 (103; n = 20) long, base 21-32 (27; n = 15) wide. Dorsal anchor

with superficial root tip and delicate shaft. Ventral bar 18-33 (26; n = 22) long, stout, with inwardly pointed tips and protruding middle part, forming a ‘‘crab-shape’’. Dorsal bar straight, grooved with terminal expansions 20-39 (31; n = 23) long.

Remarks

Comparative analyses of available descriptions of *Tetrancistrum* spp. showed that the morphometric data of the newly described species are similar to those of its congeners. In addition, some features of the copulatory complex and haptoral structures in *T. labyrinthus* n. sp. are similar to those of both *T. suezicum* and *T. oraminii.* The major differences in the copulatory complex which sets *T. labyrinthus* n. sp. apart from *T. suezicum* and *T. oraminii*, are the shape of the MCO tube and the anterior basal flange, and the shape and composition of the accessory piece. The MCO of *T. labyrinthus* n. sp. closely resembles that of *T. oraminii*, with both having a flared and ruffled anterior opening. However, the MCO of *T. labyrinthus* n. sp. is sigmoid and shorter in comparison to the slender ‘‘J-shaped’’ MCO of *T. oraminii*. In addition, the copulatory complex of the new species can be distinguished from that in both *T. oraminii* and *T. suezicum* by possessing an accessory piece that is composed of two connected parts. Further, the shape of the anterior basal flange of the male copulatory organ of the new species and its unique vaginal vestibule are two additional features that separate *T. labyrinthus* n. sp. from all previously described congeners. Comprehensive analyses of all previous descriptions of *Tetrancistrum* spp., examination of several voucher specimens and laser confocal microscope images obtained in this study confirmed that this unique composition of the vaginal vestibule is only exhibited by *T. labyrinthus* n. sp.

Tetrancistrum indicum Paperna, 1972

Host: *Siganus canaliculatus* (Park) (Perciformes: Siganidae), white-spotted rabbitfish. Localities: Sea of Oman, off Khasab fish harbour (26.1644°N, 56.2426°E); Dabba fish market (25.6365°N, 56.2538°E); Muscat fish market (23.0000°N, 58.0000°E); Sohar fish market (56.7075°N, 24.3461°E). Arabian Sea, off Masirah fishing harbour (20.4711°N, 58.8153°E); off Al-Lakbi fishing harbour (18.113°N, 56.3255°E); Raysut fish market (16.5500°N; 54.0100°E), Sultanate of Oman (November and December 2012). Voucher material: Berlin Natural History Museum (ZMBE. 7437: three non-type specimens; ZMBE. 7440: five non-type specimens); Meguro Parasitology Museum (MPM. Coll. No. 20961: six non-type specimens).

Site in host: Gills.

Prevalence: Khasab fishing harbour: 83% (29 out of 35); Dabba fish market: 71% (25 out of 35 fish); Sohar fish market: 3% (1 out of 35 fish); Muscat fish market: 49% (17 out of 35 fish); Masirah fishing harbour: 89% (31 out of 35), Al-Lakbi fishing harbour: 97% (34 out of 35 fish); Raysut fish market: 100% (35 out of 35 fish).

3.3.2 Description (Figures 3.4, 3.5, 3.6)

[Based on 20 specimens.] Body fusiform in some specimens; trunk broad with distinct indent found in most specimens near vaginal vestibule opening; cephalic region and peduncle narrow, tapered. Body 1,571-2,600 (2,013; n = 20) long, tegument smooth. Greatest body width above level of germarium, 315-581 (462; n = 20). Cephalic lobes well developed; each head organ comprises several groupings of terminations of cephalic gland ducts. Mouth midventral, subterminal at level of head organs, opens into buccal tube. Buccal tube large, with large opening extending posteriorly along midline to pharynx, forming buccal cavity. Pharynx elongate, ovate, 82-153 x 53-101 (115 x 76) (n = 20). Intestinal caeca bifurcating posterior to pharynx, with diverticula, terminating blindly posterior to gonads. Single pair of eyespots seen in some specimens; accumulations of minute subovate chromatic granules common; isolated granules scattered throughout cephalic region.

Germarium pretesticular, conical, forming a cap anterior to testis, 222 (140-350; n = 20) long, 87-217 (87; n = 20) wide at base. Ootype receives vaginal duct and bilateral common vitelline ducts; uterus expanded distally; vaginal vestibule elongated, slightly sclerotized tube with meandering vaginal duct; vitellarium dense. Testis small, subspherical, 216-290 x 139-231 (250 x 200) (n = 20) wide; vas deferens leaving anteromedial region of testis, passing left to germarium; prostatic reservoirs small; seminal vesicle forming inverted ‘‘J’’ towards left side of copulatory complex; 2 bulbous prostatic reservoirs dorsal to copulatory organ, each emptying into base of MCO via an individual duct. Copulatory complex simple, comprising MCO, anterior basal flange and accessory piece, 103-165 (139; n = 20) long. Haptoral hooks absent in adults. Ventral and dorsal anchors typical of the genus; ventral anchor 93-104 (98; n = 17) long, base 22-34 (28; n = 17) wide. Dorsal anchor 102-115 (108; n = 17) long, base 20-35 (29; n = 16) wide. Ventral bar short, 23-38 (29; n = 17), with inwardly pointed tips and protruding middle part forming a crab-like shape. Dorsal bar straight, 24-44 (36; n = 17) long, with terminal expansions.

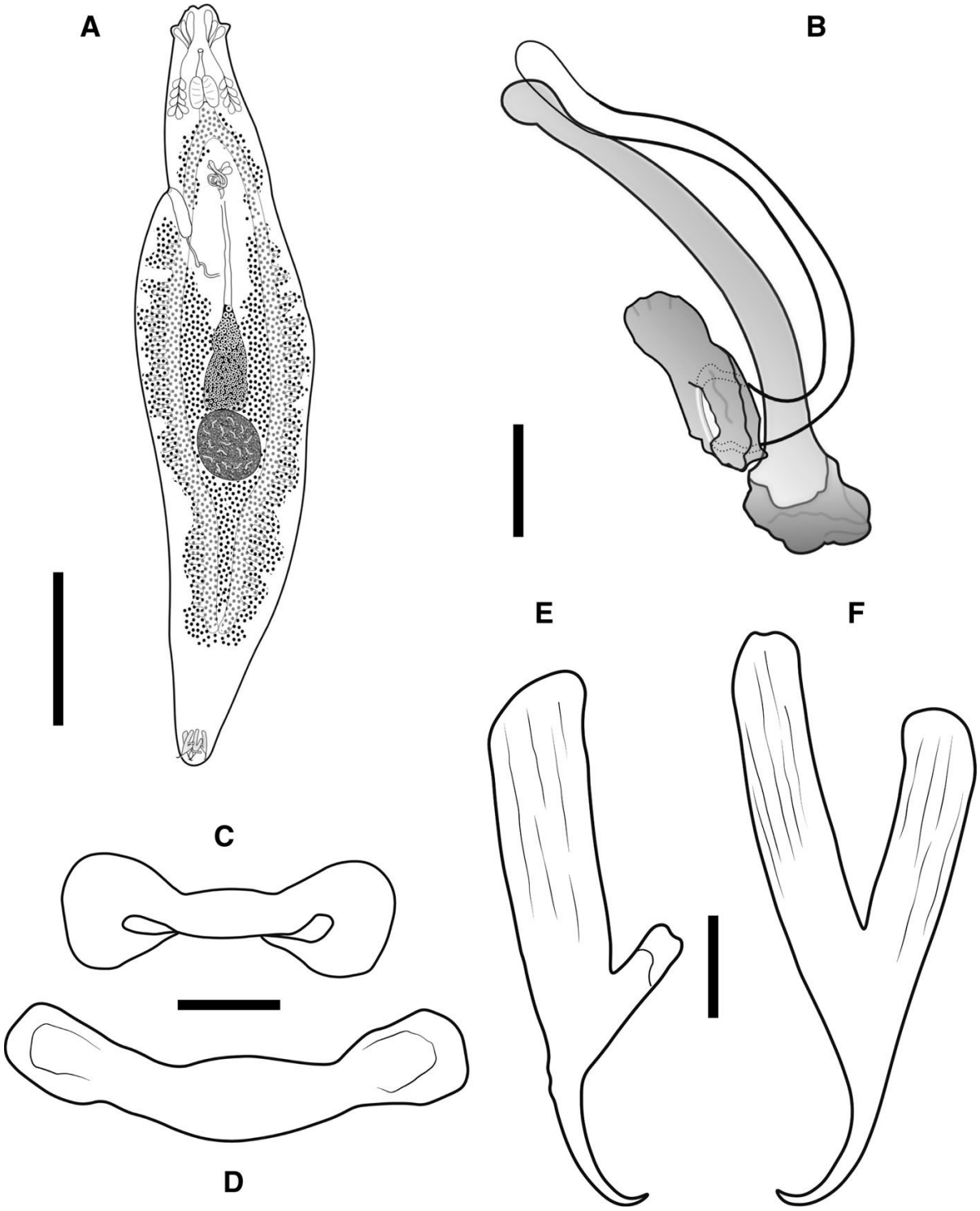

Figure 3. 4 Tetrancistrum indicum Paperna, 1972 ex Siganus canaliculatus. (A), Whole mount, ventral view; (B), Male copulatory organ, ventral view; (C), Ventral anchor; (D), Dorsal anchor; (E), Ventral bar; (F), Dorsal bar. Scale-bars: A, 500 lm; B, E, F, 20 µm; C, D, 10 µm

Remarks

The second *Tetrancistrum* species isolated from the same hosts in this study was the largest of all previously described *Tetrancistrum*. Paperna (1972) described *T. indicum* as ‘‘very large worms’’ and provided a single length measurement (1,690 μm). In contrast, the measurements of the voucher specimens provided by Geets et al. (1997) were smaller (see Kritsky et al. 2007b). These differences could be due to the limited number of voucher specimens or the different method of whole-mount preparation used by the authors of the voucher slides. Thus, comparative morphological analysis was only possible for the male copulatory organ of both specimen sets. Specimens of both sets, i.e. the voucher specimens by Geets et al. (1997) and the specimens obtained in the present study, possess a heavily sclerotized ‘‘J-shaped’’ MCO tube, curved distally towards the anterior part of the tube. The anterior basal flange of the specimens from both sets is a foot-like structure engulfing the MCO tube. The accessory piece is composed of two connected pieces, a thick rod-shaped anterior piece and a short subquadrate posterior piece. In addition, flattened specimens prepared in this study showed some variations of the MCO tube (terminal flare of anterior portion) and in the posterior portion of the accessory piece that are similar to those depicted by Paperna (1972).

This observation confirms the conclusion of Kritsky et al. (2007b) that variations of the *T. indicum* MCO is caused by different methods used for preparation of the whole mounts (Figure 3.5). In conclusion, the description of *T. indicum* by Paperna (1972) and the analysis of the copulatory complex in the voucher specimens of Geets et al. (1997) (deposited as *T. sigani* and then considered as *T. indicum* by Kritsky et al. (2007b)) alongside the MCO of the present samples confirm that the specimens investigated herein are *T. indicum*.

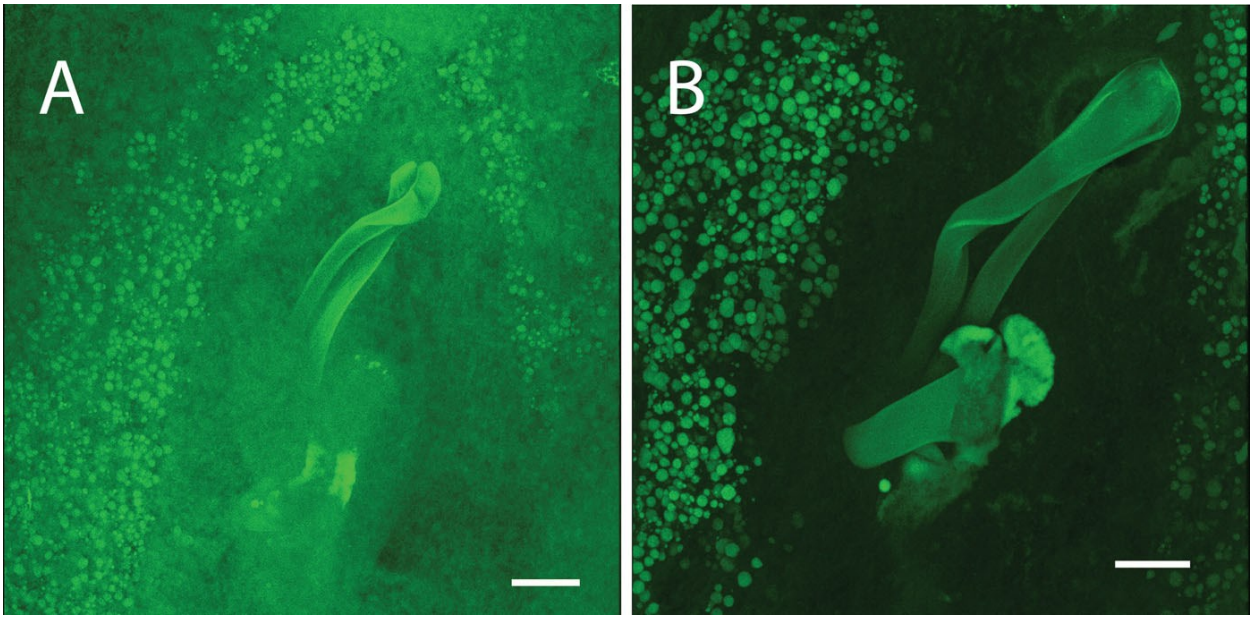

Figure 3. 5 3D reconstructions of confocal microscope images of the male copulatory organ (MCO) of Tetrancistrum indicum showing variations of the MCO tube. Narrow MCO tube; (A). Wide and flared MCO tube, (B). Scale-bars: 20 µm.

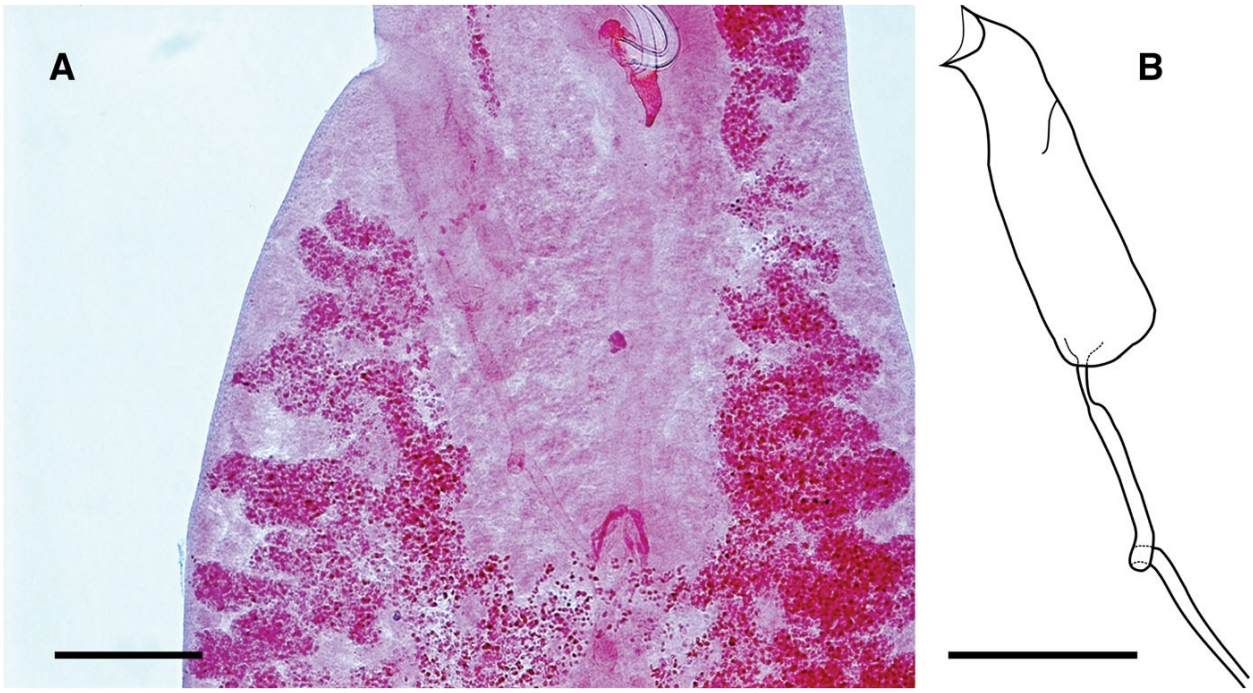

Figure 3. 6 Vaginal vestibule of Tetrancistrum indicum, ventral view. Photomicrograph of a stained specimen;(A). Line drawing showing the simplicity of the vaginal vestibule of T. indicum in comparison to the one depicted in T. labyrinthus n. sp., (B). (Figure 3. 3). Scale-bars: 100 µm.

3.4 Discussion

Including the new species described in this study, *Tetrancistrum* currently contains 17 species. Although members of *Tetrancistrum* share many similarities, they also exhibit great variations in their copulatory complexes, thus the description of new species relies heavily on the morphology of these structures. *Tetrancistrum labyrinthus* n. sp. described in the present study can be easily distinguished from its congeners by the shape and composition of the copulatory complex and by the unique vaginal vestibule. These two features of *T. labyrinthus* n. sp. remained constant in all specimens studied here, regardless of the method used for preparation of the whole mounts. Examination of various voucher specimens and the present material confirmed that these two features of *T. labyrinthus* n. sp. are unique to the new species and not a result of artefact, aberration, or misinterpretation of the current material. In addition, although the previous authors (Kritsky et al. 2007b) did not provide further details about the structure of the vaginal vestibule of other *Tetrancistrum* species, the vaginal vestibule of the species described in this study is a key feature that distinguishes it from all previously recorded *Tetrancistrum* spp. This conclusion is supported by the comparative examination of various voucher specimens and by laser confocal images obtained of the vaginal vestibule of the species described herein (Figure 3.5).

Aside from two unconfirmed records of *Tetrancistrum* parasitizing lethrinids (*Tetrancistrum lutiani* Tubangui, 1931 and *T. lebedevi* Gupta & Sharma, 1982), members of *Tetrancistrum* seem to be limited to two teleost families, the Siganidae and Acanthuridae (see Kritsky et al. 2007b). These host families are closely related; both are assigned to the suborder Acanthuroidei, along with four other families (Tang et al. 1999). Siganidae are distributed in the Indo- Pacific region, and the family consists of a single genus and two subgenera (Woodland 1983; Randal and Kulbicki 2005) with a total of 29 known species (Froese and Pauly 2016). In comparison, the Acanthuridae is a larger family which includes eight genera and more than 80 known species (Sun et al. 2011). Young (1967) proposed that *Tetrancistrum* might be restricted to Siganidae and is only occasionally found on acanthurids. However, Kritsky et al. (2007b) suggested that *Tetrancistrum* likely originated from the Acanthuroidei. It is noteworthy that at least 29 species of acanthurids (mainly from family Zanclidae and genus Acanthurus) have been previously investigated for monogeneans, yielding 25 species of *Haliotrema* Johnston & Tiegs, 1922 and no records of *Tetrancistrum* spp. (Sun et al. 2007; Sun et al. 2011; 2015). Yet, the examination of only three members of the acanthurid genus

Naso (Lacépède) (see Young 1967; Kritsky et al. 2007b) resulted in the description of five species of *Tetrancistrum*.

Similarly, the detailed host parasite checklist from Hawaiian fish lists three *Naso* species hosting five *Tetrancistrum* species while the other 15 sampled acanthurids were free of these monogeneans (Palm and Bray 2014). This might suggest that members of *Tetrancistrum* are restricted to the genus *Naso* within the acanthurids. However, the current knowledge of the host range and geographical distribution of *Tetrancistrum* is not sufficient to draw conclusions on the evolutionary history, geographical distribution, or host specificity of members of this ancyrocephalid genus. For example, some species of *Tetrancistrum* were reported from unidentified siganid hosts (Kritsky et al. 2007b), synonymy exists within the siganids (Froese and Pauly 2016) and only few members of the acanthurid genus *Naso* were investigated for ancyrocephalid monogeneans (Young 1967; Kritsky et al. 2007b; Palm and Bray 2014). Thus, examination of more *Tetrancistrum* from siganid hosts as well as from hosts of the genus Naso sampled from additional localities, revision of hosts that were reported to harbour *Tetrancistrum* and phylogenetic analyses of *Tetrancistrum* species is warranted. In addition, the fact that members of *Tetrancistrum* seem to be restricted to only two teleost genera (*Siganus* and *Naso*) from two different families, makes them of great interest for the study of host-parasite co-evolutionary relationships.

Acknowledgements

We are grateful to the Ministry of Agriculture and Fisheries Wealth, Sultanate of Oman, for the TRC Project ORG-EBR_11-TRC Grants, and to Laura Machkevska for help with the illustrations. Also, we would like to extend our thanks for Professor Kazuo Ogawa (Meguro Parasitological Museum), Mrs Eileen Harris (British Natural History Museum), Mrs Patricia Pilitt, (US National Parasite Collection), Dr Mal Bryant (Queensland Museum) for the voucher specimens loans and worm micrographs. In addition, we would like to thank Dr Sergei A. Kuznetsov (Institute of Biological Sciences Department of Animal Physiology Light Microscopy Centre) for Confocal Laser Microscope facility.

4 *Glyphidohaptor safiensis* n. sp. (Monogenea: Ancyrocephalidae) from the White-spotted rabbitfish *Siganus canaliculatus* (Park) (Perciformes: Siganidae) from Oman, with notes on its phylogenetic position within the Ancyrocephalidae (sensu lato) Bychowsky & Nagibina, 1968

Abstract

A new ancyrocephalid monogenean is described from the gills of wild White-spotted rabbitfish *Siganus canaliculatus* (Park) based on morphological and molecular analyses. *Glyphidohaptor safiensis* n. sp. can be distinguished from its congeners by its body size, the size and composition of its male copulatory organ (MCO) and the shape of ventral and dorsal anchors. The new species presents the largest body length and width among its congeners. Also, the MCO is the largest among *Glyphidohaptor* spp., though it most closely resembles that of *Glyphidohaptor phractophallus*. In comparison to *G. phractophallus* Kritsky, Galli & Yang, 2007, the MCO tube of the new species is less curved and equipped with a longer and narrower basal flange. The ventral and dorsal anchors of *G. safiensis* n. sp. have shorter roots compared with the congeners. Partial large subunit (LSU), small subunit (SSU) and complete internal transcribed spacer region 1 (ITS1) rDNA of the new species and two species of *Tetrancistrum* Goto & Kikuchi, 1917 from the same host and locality were sequenced and phylogenetically analysed. Comparison of the ITS1 rDNA sequences obtained for *G. safiensis* n. sp. with the only available sequence of another member of *Glyphidohaptor* yielded 99% similarity to *G. plectocirra* Paperna, 1972, confirming the generic identity of the species described herein. The LSU rDNA analysis grouped it with *Tetrancistrum* sp. from the gills of *Siganus fuscescens* from Australia, indicating a possible misidentification of the latter. Sequences of the SSU rDNA of the new species were most similar to *Pseudohaliotrema sphincteroporus*, demonstrating the close relatedness of these genera within the Ancyrocephalidae. This is the first record of the genus *Glyphidohaptor* from *S. canaliculatus* and from the Persian Gulf, the Gulf of Oman and the Arabian Sea.

3

[3] This article was submitted as: Al-Jufaili S.H., Machkevskyi V.K., Al-Kindi U.H. & Palm H.W. (submitted). *Glyphidohaptor safiensis* n. sp. (Monogenea: Ancyrocephalidae) from the White-spotted rabbitfish *Siganus canaliculatus* (Park) (Perciformes: Siganidae) from Oman, with notes on its phylogenetic position within the Ancyrocephalidae (*sensu lato*) Bychowsky & Nagibina, 1968.

4.1 Introduction

The Siganidae is a monotypic family of inshore and reef-associated tropical and subtropical fishes consisting of 29 valid species, all from the Indo-Pacific region (Froese and Pauly 2018). Several authors have investigated the parasite fauna of siganids reporting high parasite diversity (e.g. Diamant and Paperna 1986; Martens and Moens 1995; Geets and Ollevier 1996; Geets et al. 1997; Shih and Jeng 2002; Aloo et al. 2004; Hassanine and Al Jahdali 2007). To date, a total of 18 siganid species were investigated for monogenean parasites, resulting in identification of the species belonging to the polyopisthocotylean genus *Polylabris* Euzet & Cauwet, 1967 (Yang et al. 2006; Sailaja and Madhavi 2010) and four monopisthocotylean genera, including three genera of ancyrocephalids (Goto and Kikuchi 1917; Young 1968; Paperna 1972; Lim 2002; Kritsky et al. 2007a; 2007b; Kritsky and Galli 2007) and one viviparous gyrodactylid (Ernst et al. 2001). The ancyrocephalid genus *Glyphidohaptor* Kritsky, Galli & Yang, 2007 was erected by Kritsky et al. (2007a) to accommodate *Glyphidohaptor plectocirra* Paperna, 1972 which was initially described as *Pseudohaliotrema plectocirra* Paperna, 1972 (= *Tetrancistrum plectocirra* Lim, 2002) from *Siganus luridus* (Ruppell) and *Siganus rivulatus* (Forsskål) from the Gulf of Aqaba (Eilat, Red Sea). Two further species were described from the Great Barrier Reef from five siganid hosts, *Glyphidohaptor phractophallus* Kritsky, Galli & Yang, 2007 from *Siganus fuscescens* (Houttuyn) and *G. sigani* Kritsky, Galli & Yang, 2007 from *S. doliatus* (Guérin-Méneville), *S. lineatus* (Valenciennes), *S. punctatus* (Schneider & Forster) and *S. corallinus* (Valenciennes) (Kritsky et al. 2007a). Other reports include an unidentified *Glyphidohaptor* sp. from the gills of an unknown *Siganus* sp. from Macassar, Sulawesi (Indonesia) (Kritsky et al. 2007a).

The class Monogenea has been subject of several major molecular phylogenetic studies (Justine et al. 2002). Molecular-based phylogenetic relationships of the Platyhelminthes, including the Monogenea, were carried out utilising RNA and DNA gene sequences (Mollaret et al. 1997; Campos et al. 1998; Littlewood et a. 1999; Olson and Littlewood 2002). Mollaret et al. (2000a) investigated the phylogenetic relationships between the two main subclasses of the Monogenea (Polyopisthocotylea and Monopisthocotylea) using the large subunit region (LSU) of rDNA. Subsequently, several authors analysed the phylogenetic position of families within their representative subclasses (Jovelin and Justine 2001; Chisholm et al. 2001a; Justine et al. 2002; Simkova et al. 2003; Simkova et al. 2006; Perkins et al. 2009). Other researchers explored the origin and evolution of monogeneans (Bentz et al. 2001; Bentz et al. 2003; Mendlova and Simkova

2014; Theisen et al. 2017; 2018). Furthermore, a number of authors evaluated the phylogenetic position of the ancyrocephalid genera (e.g. *Haliotrema* Johnston & Tiegs, 1922, *Euryhaliotrema* Kritsky & Boeger, 2002, *Haliotrematoides* Kritsky, Yang & Sun, 2009 and *Pseudempleurosoma* Yamaguti, 1965) through analyses of the small subunit SSU and LSU regions of the rDNA (Plaisance et al. 2005; Wu et al. 2007; Dang et al. 2010; Garcia-Vasquez et al. 2015; Mendoza-Palmero et al. 2015; Theisen et al. 2017; 2018). The internal transcribed spacer regions 1 (ITS-1) region was often exploited to explore the intraspecific speciation and variability within species (Simkova et al. 2004; Kaci-Chaouch et al. 2008; Simkova et al. 2013; Kmentová et al. 2016). Additionally, some studies were devoted to address more specific issues related to monogenean phylogenetic such as hybridization and confirmation of morphologically based phylogenetics (Barson et al. 2010; Chisholm et al. 2001b; Fehlauer-Ale and Littlewood 2011; Poisot et al. 2011; Schoelinck et al. 2012; Marchiori et al. 2015; Rozhkovan and Shedko 2015; Theisen et al. 2017; 2018).

Although the identification and description of new species of monogeneans is traditionally made through morphological characterization (Desdevises et al. 2000), the combination of molecular data with morphological analysis for the description of new species is becoming more common (e.g. Freeman and Ogawa 2010; Bullard et al. 2015; Soo et al. 2015; Theisen et al. 2017; 2018). To date, only a few numbers of sequences are available from ancyrocephalid infecting siganids. The first gene sequence from a monogenean infecting siganids was provided by Mollaret et al. (1997). The authors analysed the LSU rDNA sequence of *Tetrancistrum* sp. obtained from *S. fuscescens* caught off Heron Island, Queensland, Australia, without providing any morphological information of this species. Later on, the SSU and LSU rDNA sequences of *Pseudohaliotrema sphincteroporus* Yamaguti, 1953 were obtained by Littlewood & Olson (2001) and used to evaluate the molecular phylogenetic relationships of families within the Monogenea. In addition, sequences of the SSU rDNA region of *Tetrancistrum nebulosi* Young, 1967 (= *T. sigani* Goto & Kikuichi, 1917) were deposited in GenBank by Wang et al. (2014) and Ummey et al. (2015). Furthermore, a comparison of the genetic variations in populations of *G. plectocirra* was investigated by utilising the Cytochrome c oxidase I (Cox1) and the ITS-1 gene regions (Stefani et al., 2012).

During a survey of the parasite fauna of *Siganus canaliculatus* (Park) from Omani waters, a new species of *Glyphidohaptor* was recovered. The objectives of the present study were to provide

a morphological description of the new species and to explore the phylogenetic relationships within the Ancyrocephalidae (*sensu lato*) Bychowsky & Nagibina, 1968 based on the LSU, SSU, and ITS-1 regions of ribosomal DNA, focusing on *Glyphidohaptor*, *Pseudohaliotrema* Yamaguti, 1953 and *Tetrancistrum* Goto & Kikuchi, 1917.

4.2 Material and methods

4.2.1 Sample collection and examination

A total of 245 White-spotted rabbitfish, *Siganus canaliculatus* (Perciformes: Siganidae), were purchased alive from local fish markets from seven locations along the coasts of the Sultanate of Oman (see Table 1). Fish were morphologically identified by using the guidelines of Randall (1995) and FAO identification sheets (1984). Fish specimens were transported on ice to the facility of the Laboratory of Aquatic Parasitology at the Fishery Quality Control Centre, Sultanate of Oman. The hosts were either immediately subjected for parasitological examination or frozen at -40 °C for subsequent investigation. Upon dissection, gills were excised; arches were separated, placed in a petri dish with filtered seawater and examined under a dissecting microscope. Parasites were detached from the gill filaments by means of fine needles and kept in filtered seawater at 4 °C prior to fixation. Monogenean whole mounts were prepared from unflattened AFA (alcohol: formalin: acetic acid) or 4% neutral buffered formalin-fixed worms, stained with Mayer's paracarmine, cleared in clove oil and mounted onto slides with Canada balsam.

Measurements, all in micrometres, are given as the mean followed by the range and number (n) of structures measured in parentheses. Body length includes that of the haptor; measurements of the copulatory complex, anchors and hooks and the description of the new species are according to Kritsky et al. (2007a). Illustrations were prepared with the aid of a camera lucida attached to an Olympus BX63 motorised light microscope with Nomarski differential interference contrast optics (DIC) and digitalised using Adobe illustrator CC 2018. and the program Inkscape 0.92.2 (Scalable Vector Graphics, 2).

Table 4. 1 Sampling localities and coordinates

Locality	Coordinates	Water Body
Khasab fishing harbor	26.1644°N; 56.2426°E	Persian/Arabian gulf
Dabba fish market	25.6365°N; 56.2538°E	Gulf of Oman
Sohar fish market	24.3783274, 56.7398163	Gulf of Oman
Muttrah fish market	23.0000°N; 58.0000°E	Gulf of Oman
Masirah Island	20.4711°N; 58.8153°E	Arabian Sea
Al-Lakbi fish harbor	18.113°N; 56.3255°E	Arabian Sea
Raysut fish market	16.5500°N; 54.0100°E	Arabian Sea

4.2.2 Comparative morphological analysis

Voucher slides were obtained from the British Natural History Museum (G. phractophallus BMNH 2006.8.8.13-14, *G. sigani* BMNH 2006.8.8.11-12), Meguro Parasitology Museum (*G. phractophallus* MPM 18828, *G. sigani* MPM 18829) and the Queensland Museum (*G. phractophallus* QMG227443, QMG227444, QMG227445, QMG227446 and QMG 227447), *G. sigani* (QMG227453, QMG 227454, QMG 227457, QMG G227460, QMG G227461) respectively. In addition, micrographs of selected parasites were obtained for comparison purposes (*Glyphidohaptor* sp. MPM 22839 and MPM 22837 and *G. plectocirra* USNPC 98587, USNPC 98588, USNPC 98590, USNPC 98591).

4.2.3 Confocal microscopy

Several 95% ethanol preserved specimens were stained with one step Gomori's Trichrome kit (Morphisto, Frankfurt, Germany), mounted in Histochoice (Amresco, Solon, OH, USA), and imaged using a Carl Zeiss LSM780 confocal fluorescence microscope and a PL APO 63 × 1.4 oil immersion lens following the procedure of Marchiori et al. (2015). Three-dimensional (3D) stacks of the diagnostic features were acquired with a typical voxel size of 66 × 66 × 500 nm (XYZ). The samples were excited using a DP55 561 laser (AOTF 3%) and a main beam splitter 488/561. Spectral emissions (566 to 694) were detected using the internal PMT detector (grain 800). Three dimensional images were obtained from a z-stack of 300 planes and the 3D rendering option in ZEN software.

4.2.4 DNA extraction and PCR amplification

Monogeneans infecting *S. canaliculatus* were preserved in 95% ethanol for molecular analysis. Total genomic DNA was extracted from individual worms using QIAamp mini DNA kit (Qiagen) according to the manufacturer's instructions with some modification. Due to the small size of the worms investigated in this study and in order to increase the DNA yield, for the final step in the DNA extraction protocol, the extracted DNA was eluted with 200 µl of elution buffer and incubated for 10 minutes at room temperature before final spin and collection of genomic DNA. The obtained DNA was then completely dried using a Concentrator plus/Vacufuge®Plus (Eppendorf), re-suspended with 40 µl elution buffer, then stored in a -20 ˚C freezer. This helped to increase the yield of extracted DNA.

The concentration of the obtained DNA was quantified (i.e., ng/µl) using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham Massachusetts). Polymerase chain reaction (PCR) amplification for partial SSU rDNA (18s region) were performed in 20 µl reaction volume containing 10 pmol of forward 390f (5′-AGA GGG AGC CTG AGA AAC G-3′) and reverse 870r (5′-GTT GAG TCA AAT TAA GCC GCA-3′) primers, and ~10 ng of DNA template using illustra™ puReTaq Ready-To-Go PCR beads (0.2 ml tubes, 96 reactions). After initial denaturation at 98 ˚C for 5 min, samples were subjected to 40 cycles of amplification (denaturation at 95 ˚C for 30 s, primer annealing at 55 ˚C for 45 s, and extension at 72 ˚C for 1 min, followed by 7 min terminal extension at 72 ˚C (Freeman et al. 2013). For amplification of the D1-D2 domain of the LSU rDNA the primers C1 (5′-ACCCGCTGAATTTAAGCAT-3′) and D2 (5′-TGGTCCGTGTTTCAAGAC-3′) were used, following Mendlova et al. (2010) with some modification (annealing temperature was changed to 60 ˚C to avoid formation of pseudogenes).

All PCR reactions were performed in 30 µl reactions containing 5 pmol of each primer and ~30 ng of concentrated genomic DNA in illustra™ puReTaq Ready-To-Go PCR beads. The following amplification condition was utilised: an initial denaturation at 94 °C for 2 min, followed by 40 cycles of amplification (denaturation at 94 °C for 20 s, primer annealing at 60 °C for 30 s, and extension at 72 °C for 1 min 30 s, followed by final elongation at 72 °C for 10 min). Furthermore, partial SSU rDNA and entire ITS1 regions were amplified in one round using the primers S1 (5′-ATTCCGATAACGAACGAGACT-3′) and IR8 (5′-GCTAGCTGCGTTCTTCATCGA-3′) that anneal to the 18S and 5.8S rDNA genes, respectively

(Simkova et al. 2003). Each amplification reaction was performed in a final volume of 35 µl containing 10 pmol of each primer and ~30 ng of genomic DNA using illustra™ puReTaq Ready-To-Go PCR beads (0.2 ml tubes, 96 reactions). The amplification conditions followed Simkova et al. (2013). All obtained PCR products (1 µl) were viewed on a 0.8% agarose gel stained with ethidium bromide.

4.2.5 Phylogenetic analyses

Contiguous sequences of the investigated worms were obtained manually using BioEdit (Hall 1999). Sequences were aligned using Clustal W (Thompson et al. 1994) implemented in MEGA v.7 (Molecular Evolutionary Genetics Analysis version 7; Kumar et al. 2016). After alignment, the ends of aligned sequences were trimmed to reduce excessive data. Newly obtained sequences were subjected to a Blast search in GenBank, and ancyrocephalids sequences that showed the highest similarity to the recently acquired data were retrieved from GenBank and used for the phylogenetic analysis (see Table 2). Phylogenetic trees were reconstructed utilising three different datasets. The first analysis used the partial LSU (D1-D2 domain) dataset containing 20 ancyrocephalid ingroup taxa and *Thaparocleidus asoti* Yamaguti, 1937 (Wu et al. 2007) as the outgroup.

The second analysis used the SSU data from 15 ancyrocephalid species, and *Lamellodiscus donatellae* Aquaro, Riva & Galli, 2009 as an outgroup. The third dataset was the combined partial SSU and the complete ITS1 rDNA sequences of the species investigated plus *G. plectocirra* sequences (accession numbers: HE601931, HE601932 and HE601933), while *Parancyrocephaloides daicociused* Yamaguti, 1938 was used as an outgroup. Phylogenetic analyses were performed based on best fit model in MEGA version 7 (Kumar et al. 2015). The robustness of the inferred phylogeny was assessed using a bootstrap procedure with 1,000 replications (Wu et al. 2005).

Table 4. 2 List of monogenean species used for phylogenetic analysis in this study with their GenBank Accession numbers.

Species included	**SSU**	**LSU**	**ITS-1**
Acolpenteron ureteroecetes			EF650054
Bravohollisia gussevi	KJ571007	DQ157665	
Bravohollisia maculatus SYSU20060429-3	KJ571018	KJ571008	
Bravohollisia parvianchoratus		KJ571009	
Bravohollisia plectorhynchus SYSU20060502-2	KJ571019	KJ571010	
Bravohollisia rosetta		KJ571011	
Bravohollisia tecta SYSU20060429-4	KJ571020	KJ571012	
Caballeria intermedius		KJ571013	
Euryhaliotrema johnii	EU836214	EU836193	
Euryhaliotrema perezponcei	JN054405	HQ615996	
Euryhaliotrema sp. 3 YS-2008	EU836215	EU836194	
Euryhaliotrema sp. HBDD	DQ537346	DQ537374	
Euryhaliotrematoides annulocirrus	EU836216	EU836195	
Euryhaliotrematoides microphallus	AY820606	AY820617	
Euryhaliotrematoides sp. 2 YS-2008	EU836218	EU836197	
Euryhaliotrematoides sp. LSJ-2011	JF938069	HQ615997	
Glyphidohaptor plectocirra			HE601933
Glyphidohaptor plectocirra			HE601932
Haliotrema cromileptis	EU523144	EU523146.	
Haliotrema platycephali		FJ767866	
Lamellodiscus donatellae	FN296214		
Parancyrocephaloides daicoci			LC176447
Protogyrodactylus amacleithrium	FM251947		
Protogyrodactylus hainanensis		DQ157653	
Pseudodactylogyroides apogonis	AB065115		
Pseudohaliotrema sphincteroporus	AJ287568	AF382058	
Tetrancistrum nebulosi	HM545910		
Tetrancistrum nebulosi	KT267177		
Tetrancistrum sp.		AF026114	
Thaparocleidus asoti		DQ157669	
Thylacicleidus sp.			AJ490169

Ancyrocephalidae (*sensu lato*) Bychowsky & Nagibina, 1968

Genus *Glyphidohaptor* Kritsky, Galli & Yang, 2007

Glyphidohaptor safiensis n. sp.

Type host: White-spotted rabbitfish, Siganus canaliculatus (Park) (Siganidae).

Type locality: Sea of Oman, off Muscat City (23.6249° N, 58.5624° E)

Other localities: Sea of Oman, off Khasab fishing harbour (26.1644°N; 56.2426°E), off Dabba fish market (25.6365°N; 56.2538°E); Arabian Sea, off Masirah Island (20.4711°N; 58.8153°E), off Al-Lakbi fish harbour (18.113°N; 56.3255°E), and off Raysut fish market (16.5500°N; 54.0100°E), Sultanate of Oman (November and December 2012).

Type-material: Berlin Natural History Museum (ZMB Monogenea 7434: one specimen, the holotype; ZMB Monogenea 7435: five paratypes). Meguro Parasitology Museum, Tokyo, Japan (MPM. Coll. No. 20959: nine paratypes).

Site in host: Gills.

Prevalence: Khasab fishing harbour: 29 out of 35 fish (83%); Dabba fish market: 33 out of 35 fish (94%); Muscat fish market: 34 out of 35 fish (97%); Masirah Island: 34 out of 35 fish (97%); Al-Lakbi fish harbour: 34 out of 35 fish (97%); Raysut fish market: 35 out of 35 fish (100%).

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Glyphidohaptor safiensis* n. sp. is urn:lsid:zoobank.org:act: (XXXX).

Etymology: The specific name (safiensis) recognises the Arabic local name of the host (Safi).

4.3 Description (Figures 4.1, 4.2)

[Based on 23 adult specimens]. Body fusiform, slightly flattened dorso-ventrally, 926-1326 (1113; n = 21) in total length (Figure 4.1A); greatest width 217-313 (266; n = 21) at level of gonads, tegument smooth. Cephalic lobes well developed; each head organ consisting of groupings of terminations of cephalic-gland ducts posteriolateral to the pharynx. Pharynx a muscular, glandular

bulb 43-67 (54; n = 21) long and 37-49 (43; n = 21) wide, spherical to subovate. Eyespots absent; chromatic granules scattered throughout the cephalic region and interior trunk. Mouth subterminal, midventral at level of head organs, opening into buccal tube. Buccal tube extending posteriorly along

body midline to pharynx. Intestinal caeca bifurcating posterior to pharynx, confluent, lacking diverticula. Testis fusiform, 173-258 (217; n = 21) long and 35-78 (61; n = 21) wide. Germarium pyriform, 64-132 (96; n = 19) long and 21-43 (32; n = 19) wide. Vaginal vestibule (Figure 4.1B) slightly sclerotized, sub-oval; vaginal opening 39-52 (45; n = 16) long, 26-33 (30; n = 6) wide. Vaginal tube sinuous or coiled, with a frayed opening at the end, 44-70 (54; n = 6) long. Male copulatory organ (MCO), tubular, slightly curved, enclosed in heavy sheath, with an arched sub-basal flange (Figure 4.2A), 58-76 (64; n = 21) long. Accessory piece rod-shaped, anteriorly flattened, with reniform plate-like projection. Haptor 41-58 (51; n = 20) long and 84-131 (118; n = 20) wide. Ventral anchor 48-57 (53; n = 11) long, base 29-36 (32; n = 11) wide (Figure 4.1D); dorsal anchor 54-63 (58; n = 10) long, base 24-33 (28; n = 10) wide (Figure 4.1C); ventral bar 34-46 (40; n = 10) long (Fig. 1E); dorsal bar 37-48 (40; n = 8) long (Figure 4.1F); and hooks 11-12 (11; n = 18) long (Figure 4.1G).

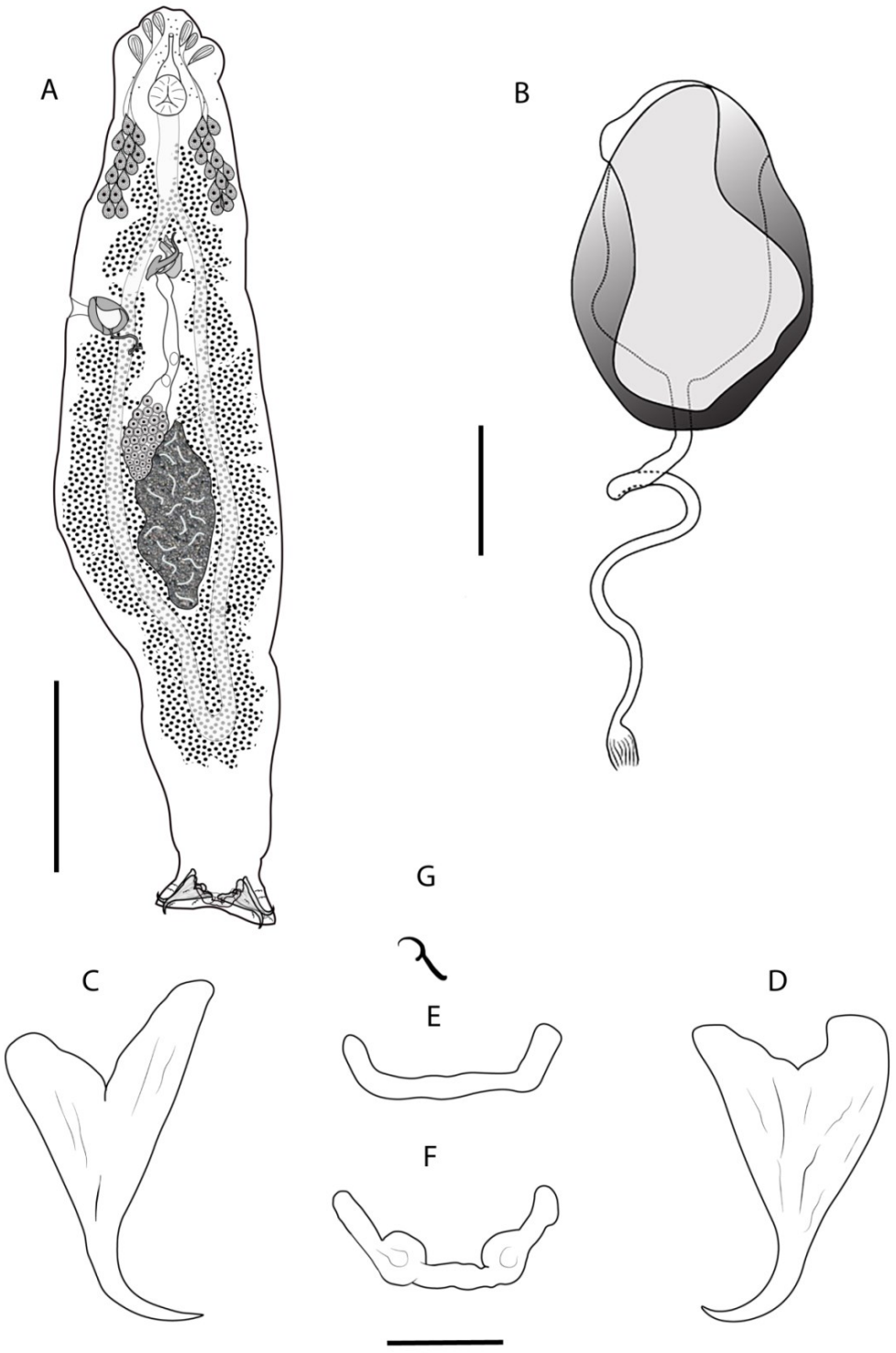

Figure 4. 1 whole mount drawing of Glyphidohaptor safiensis n. sp. ex Siganus canaliculatus. Holotype, ventral view, (A). Vaginal Vestibule, ventral view;(B). Dorsal anchor; (C), Ventral anchor; (D). Ventral bar; (E). Dorsal bar; (F). Hook, (G). Scale-bars: A, 200 µm; B-G, 20 µm

Remarks

G. safiensis n. sp. can be differentiated from all known species of *Glyphidohaptor* by its slightly curved MCO and the presence of a semi-circular structure distal to the basal flange (Fig. 2B). This structure is unique to the new species described herein and was not observed in any other species of the genus. The accessory piece further distinguishes the new species from its congeners. Similar to *G. plectocirra* and *G. sigani* Kritsky, Galli & Yang, 2007, the accessory piece of *G. safiensis* n. sp. was rod shaped. However, the accessory piece of *G. safiensis* n. sp. is distally expanded and flattened. Furthermore, the shape of ventral and dorsal anchors of *G. safiensis* n. sp. is different by having shorter roots in comparison to those of other species within the genus (Figure 4.3).

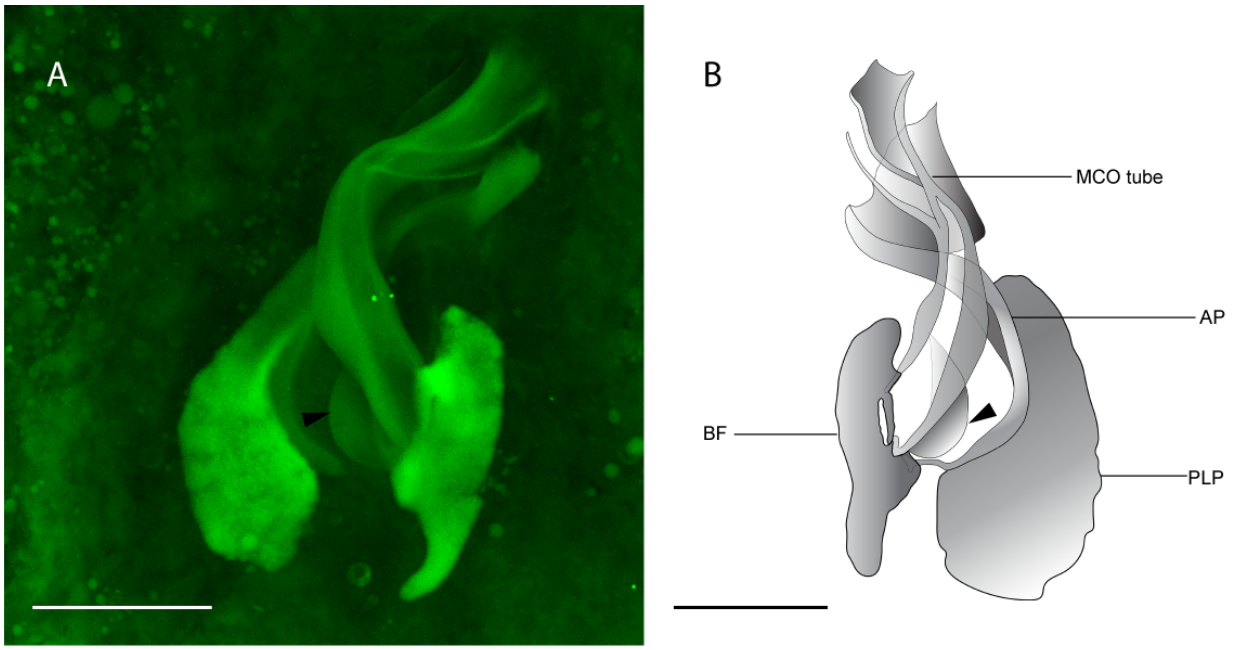

Figure 4. 2 The male copulatory complex of Glyphidohaptor safiensis n. sp. ex Siganus canaliculatus.Confocal microscope image showing the semi-circular structure positioned distally to the fan-shaped basal flange (arrow), (A). A drawing of male copulatory organ, dorsal view. BF, Basal flange; PLP, Plate-like projection; AP, Accessory, arrow head showing the semi-circular structure distal to the basal flange, (B). Scale-bars: A and B 20 µm.

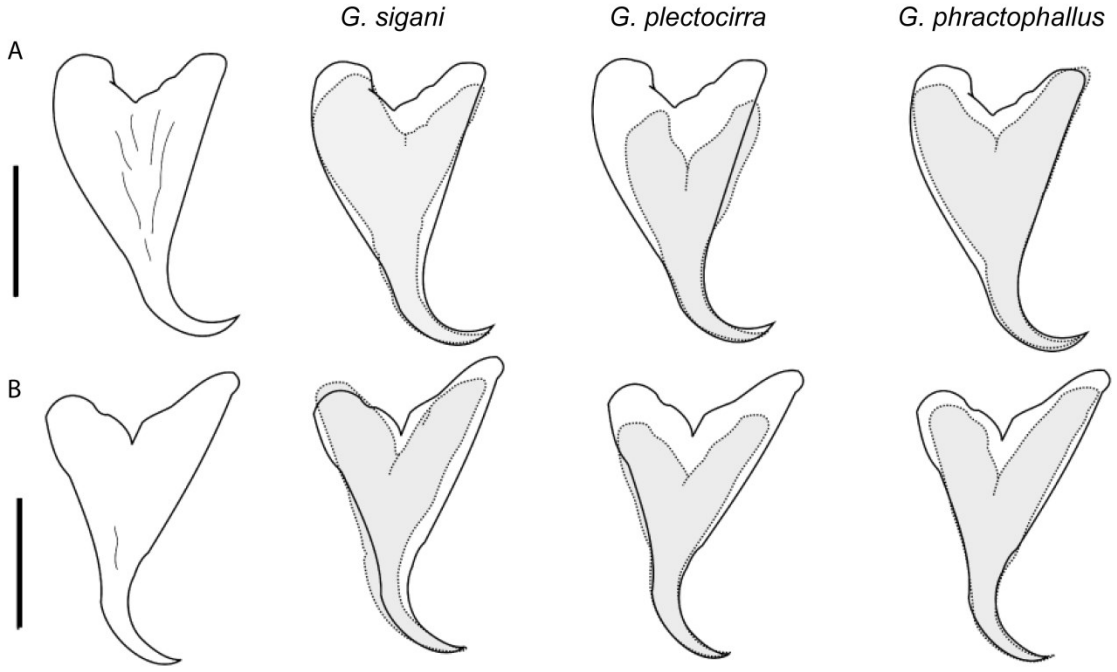

Figure 4. 3 Comparison of the dorsal and ventral anchors of Glyphidohaptor safiensis n. sp. ex Siganus canaliculatus. Ventral anchor overlay with ventral anchors of G. sigani, G. plectocirra and G. phractophallus, (A). Dorsal anchor overlay with dorsal anchors of G. sigani, G. plectocirra and G. phractophallus, (B). Scale-bars: A and B, 20 µm.

4.4 Phylogenetic position of *Glyphidohaptor safiensis* n. sp. using the SSU dataset

The length of the partial SSU sequences of the investigated monogeneans in the present study was 890 bp for *Glyphidohaptor safiensis* n. sp. and about 900 bp for both *Tetrancistrum labyrinthus* Al-Jufaili & Palm, 2017 and *T. indicum* Paperna, 1972. The Basic Logical Alignment Search Tool (BLAST, HTTP:// www.ncbi.nlm.nih.gov/BLAST) results showed that *G. safiensis* n. sp. is 96% similar to *P. sphincteroporus* Yamaguti, 1953, and both sequences of *Tetrancistrum* spp. investigated in this study showed 98% and 99% similarity to *T. nebulosi* Young, 1967 (=*T. sigani* Goto & Kikuchi, 1917) (accession number: KT267177) and 98% similarity to *T. nebulosi* (accession number: HM545910). The Maximum-likelihood tree based on Kimura two parameters distance, gamma distributed with invariant sites constructed using the SSU data set revealed that *G. safiensis* n. sp. and *P. sphincteroporus* formed a separate clade clustering as sister taxa with a bootstrap value of 69%. The newly generated SSU rDNA sequences of the two *Tetrancistrum* spp. and the available SSU rDNA sequence of *T. nebulosi* (only one sequence was used, accession

number: HM545910) formed a well-supported monophyletic clade within the marine Ancyrocephalids. Whereas the clade containing *Glyphidohaptor* and *Pseudohaliotrema* was sister to the clade comprising members of *Bravohollisia* Bychowsky & Nagibina, 1970. In the *Tetrancistrum* clade, *T. labyrinthus* clustered with *T. nebulosi* as sister species, while *T. indicum* was basal to the two species.

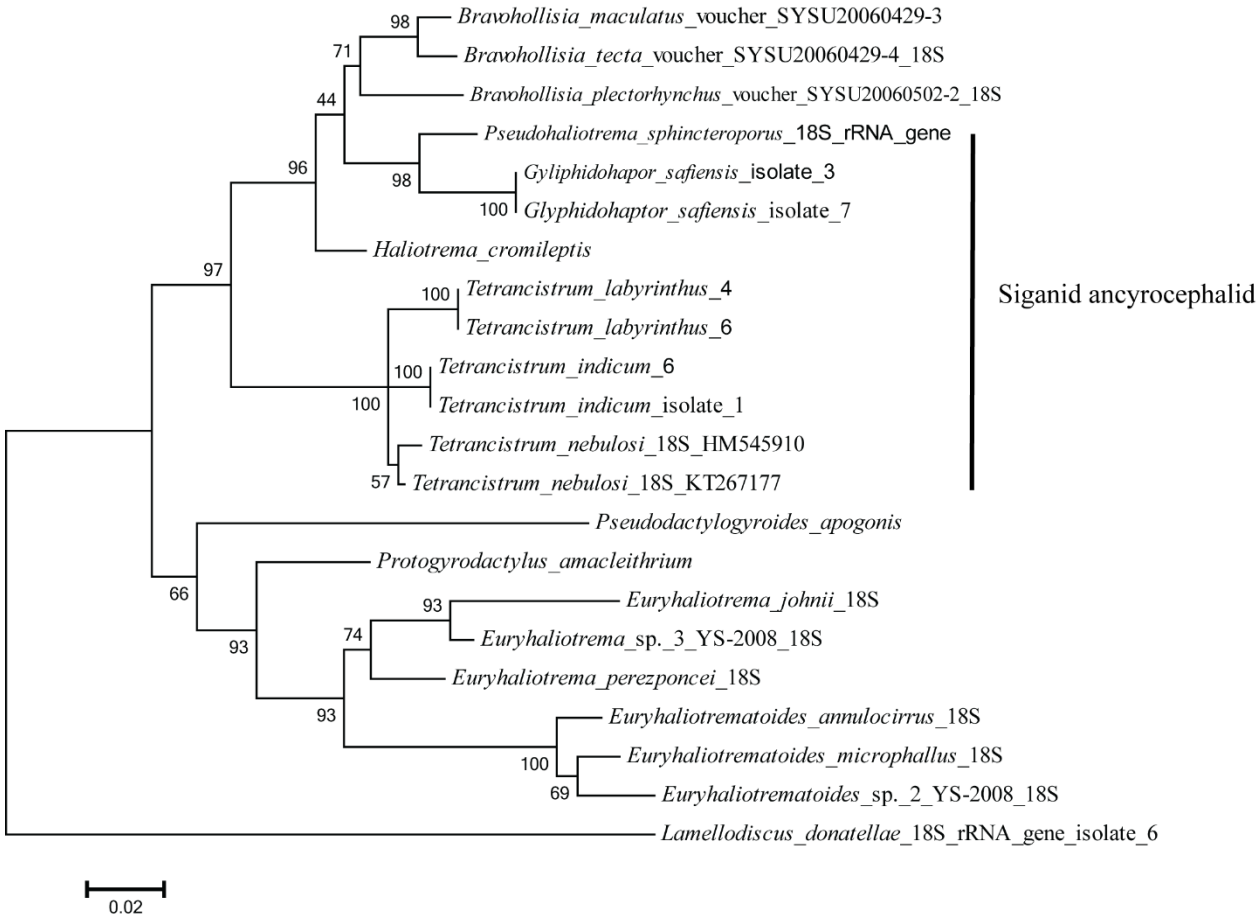

Figure 4. 4 Maximum-likelihood tree based on Kimura two parameters distance, gamma distributed with invariant sites inferred from analysis of SSU rDNA sequences of 15 species of ancyrocephalid monogeneans.

4.5 Phylogenetic position of *Glyphidohaptor safiensis* n. sp. using the LSU dataset

For the LSU rDNA gene, sequences reaching about 851 bp were successfully generated from *G. safiensis* n. sp., and 856 bp and 863 bp sequences were generated for *T. indicum* and *T. labyrinthus*, respectively. The highest similarity to *G. safiensis* n. sp. was with the LSU rDNA sequence of *Tetrancistrum* sp. collected from *S. fuscescens* from Australia (97% similarity), which was deposited by Mollaret et al. (1997). As for the *Tetrancistrum* spp. investigated in this study,

the closest match generated by BLAST search was with species of *Euryhaliotrema* Kritsky & Boeger, 2002, especially to *Euryhaliotrema perezponcei* Garcia-Vargas, Fajer-Ávila & Lamothe-Argumedo, 2008. The resulting neighbour joining tree that was constructed using the LSU gene data (Fig. 5) showed that *G. safiensis* n. sp. formed a well-supported sister species relationship with *Tetrancistrum* sp. (100% bootstrap value), while the two species *G. safiensis* n. sp. and *Tetrancistrum* sp. showed a close phylogenetic relationship to *P. sphincteroporus*, forming a sister taxon (bootstrap value of 89%).

The species of *Tetrancistrum* from the Gulf of Oman formed a well-supported monophyletic clade within the Ancyrocephalidae. The *Tetrancistrum* spp. clade was a sister group to the clades comprising *Euryhaliotrema* and *Euryhaliotrematoides* Plaisance & Kritsky, 2004. Phylogenetic position of *Glyphidohaptor safiensis* n. sp. using a partial SSU + ITS1 dataset Approximately 994 bp were generated for the partial 18s rDNA, 5.8s, and complete ITS-1 region of rDNA of *Glyphidohaptor safiensis* n. sp. This sequence was 99% similar to the only available sequence from another *Glyphidohaptor* member in GenBank, *G. plectocirra* (accession number: HE601931, HE601932 and HE601933). Sequences of the same rDNA region obtained from *Tetrancistrum* species showed 98% similarity to the SSU sequence of *T. nebulosi*.

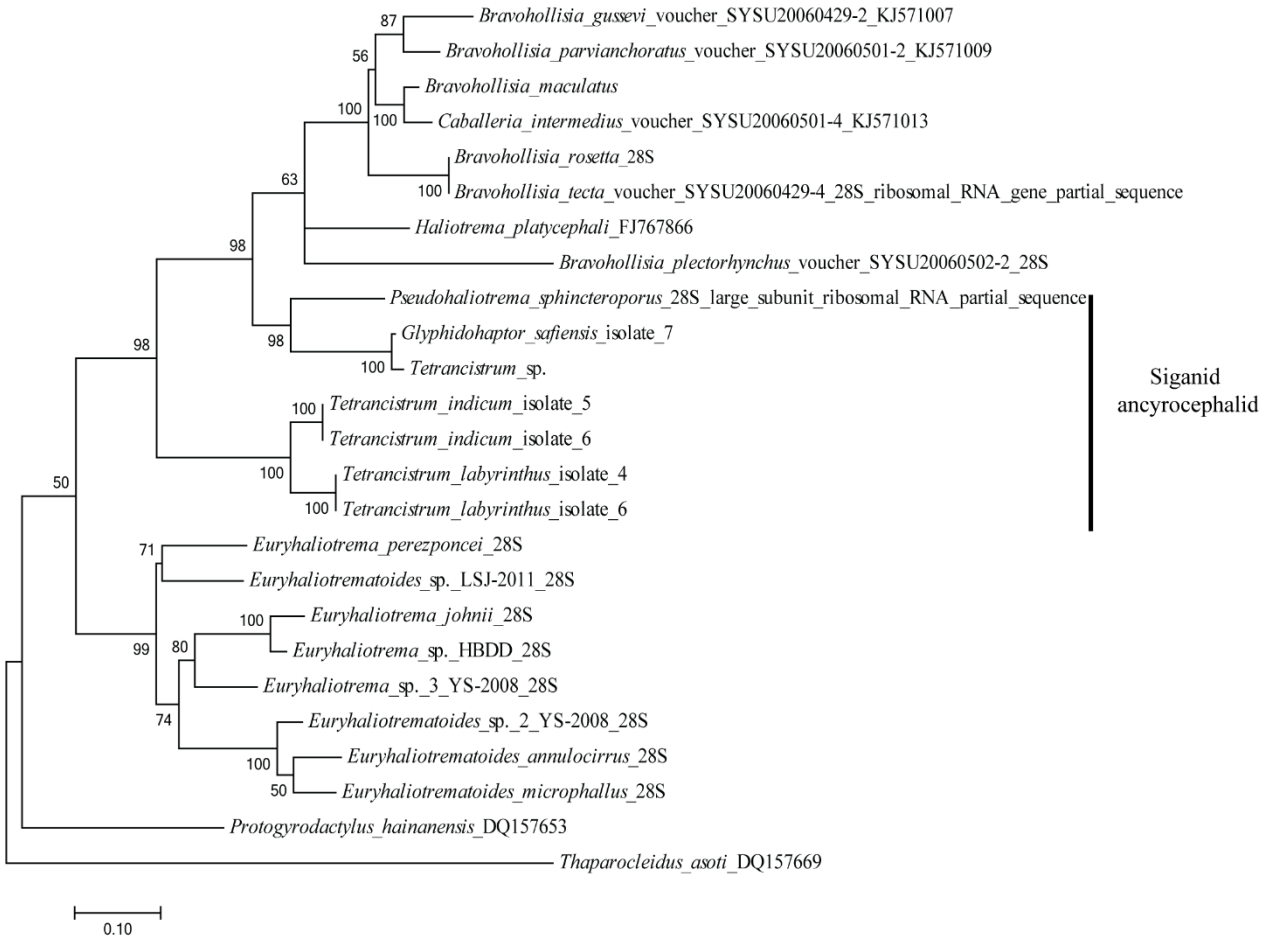

Figure 4. 5 Phylogenetic tree inferred from 28s rDNA based on General time reversible model using 20 species of ancyrocephalid monogeneans.

4.6 Discussion

4.6.1 Differential diagnosis

The three formerly described species within the genus *Glyphidohaptor* share many similarities in their morphological characters and in the features of their sclerotized organs (haptoral sclerites and MCO). The new species described in the present study also exhibited considerable morphological resemblance to its congeners. However, it displayed variations that support the designation of a new species. Primarily, the body length of *G. safiensis* n. sp. was larger than of *G. phractophallus* and double the body length of both *G. plectocirra* and *G. sigani*. The body of the new species was twice wider than its congeners. The haptor length of *G. safiensis* was in the range of other members of the genus while the width was slightly wider. Except for *G. plectocirra* which exhibited the smallest dorsal and ventral anchors, the measurements of the

remaining *Glyphidohaptor* spp. were overlapping and comparable. Thus, size independent comparative analysis of the anchors shape and form was found to be helpful in differentiating species that show no clear variations in the size of the haptoral sclerites. Illustrations of anchors overlay of all known *Glyphidohaptor* spp. proved that size alone isn't sufficient to distinguish between different members of *Glyphidohaptor*.

The MCO of the new species was the largest among its congeners. The Male copulatory complex of all members of *Glyphidohaptor* is composed of a basally articulated MCO that is equipped with a basal flange and an accessory piece with a plate-like projection (Kritsky et al. 2007). The shape of the MCO is variable among species of the genus. Both *G. phractophallus* and *G. sigani* have an arced MCO, *G. plectocirra* displays a straight MCO and *G. safiensis* n. sp. exhibits a slightly curved MCO tube. The sheath surrounding the tube is heavy and robust in all *Glyphidohaptor* species (including the new species) except for *G. sigani* which is thin and slightly sclerotized. The basal flange of all *Glyphidohaptor* species is fan-shaped, with some species-specific variations among members of the genus. Though, this structure is relatively elongated in *G. safiensis* n. sp. The accessory piece of *Glyphidohaptor* spp. appears to be the most suitable structure to differentiate between species of the genus. This structure of the accessory piece is unique to each *Glyphidohaptor* species. In *G. phractophallus* it is flat and blade-like with the distal part wrapping around the MCO tube. *G. sigani* displays a rod-shaped accessory piece with a bifid proximal end while the accessory piece of *G. plectocirra* is distally pointed. *G. safiensis* n. sp. has a rod-shaped accessory piece that is distally expanded and flattened. Finally, all *Glyphidohaptor* species possess a plate-like projection that is positioned along the proximal half of the accessory piece. The plate-like projection of *G. safiensis* n. sp. is large and reniform.

4.6.2 Diversity of *Glyphidohaptor* spp.

Prior to this study *Glyphidohaptor* spp. was only reported from the Great Barrier Reef and the Red Sea. Several micrographs of *Glyphidohaptor* sp. from an unknown *Siganus* sp. from Macassar, Celebes, deposited by Yamaguti (1953) were obtained for comparative analysis. It was noted that this species is unlike any of the previously described members of *Glyphidohaptor* and does not resemble the new species described herein. Thus, it is possible that this undescribed species could be another new species of *Glyphidohaptor*, thereby extending the geographical distribution of these worms. Geets et al. (1997) reported an unidentified species of

Pseudohaliotrema from the gills of *Siganus sutor* (Valenciennes) off East Africa. It is noteworthy that two of the three ancyrocephalid genera recorded from siganids, *Glyphidohaptor* and *Pseudohaliotrema*, are restricted to this fish family (Kritsky et al. 2007a).

However, *Glyphidohaptor* exhibits a wider geographical range and has been registered from both, reef-associated and coloured siganids (e.g. *S. corallinus*, *S. doliatus*, *S. lineatus* and *S. punctatus*) and drab coloured off-reef siganids (e.g. *S. luridus*, *S. fuscescens* and *S. rivulatus*). On the other hand, members of *Pseudohaliotrema* are so far geographically restricted to Eastern Indian Ocean and Western Pacific and seem to be limited to the deep-bodied reef-associated siganids (Lim 2002; Kritsky et al. 2007a). Given that the misidentification of *Glyphidohaptor* and *Tetrancistrum* species as members of *Pseudohaliotrema* was a common misconception among researchers (Kritsky et al. 2007b) and based on the geographical restriction of members of *Pseudohaliotrema*, we believe that the registration of *Pseudohaliotrema* sp. from *S. sutor* by Geets et al. (1997) from East Africa is probably another misidentification of a *Glyphidohaptor* species. Collection of new material from East Africa is deemed necessary to confirm this statement.

4.6.3 Molecular characterisation

The current study provides the first phylogenetic analyses of three marine ancyrocephalids genera that are known to infect siganids. These three genera show affinities to each other and affinities to other monogeneans within the marine Ancyrocephalidae. The analysis of the SSU rDNA dataset revealed that *Glyphidohaptor* and *Pseudohaliotrema* formed a separate clade within the marine Ancyrocephalidae and clustered as sister taxa. Kritsky et al. (2007a) stated that within the order Ancyrocephalidae, only *Glyphidohaptor* and *Pseudohaliotrema* displayed a germarium lying to the right of the anterior portion of the testis and suggested that this feature is apomorphic and that they are phylogenetically related. In addition, the geographical distribution and natural occurrence of these two genera further support this finding (Kritsky et al. 2007a). Future investigations including the molecular analysis of all known members of the two genera, in addition to their relationships with their representative hosts, could provide some fascinating insight into the host-parasite phylogenetic relationships and co-evolution.

On the other hand, *Tetrancistrum* species, which are found on both siganids and members of the Acanthuridae genus *Naso* (Lacépède), formed a distinct monophyletic group within the group of Ancyrocephalidae that includes *Pseudohaliotrema*, *Haliotrema* and *Glyphidohaptor* spp.

Tetrancistrum species formed a sister group with the clade containing *Pseudohaliotrema* and *Glyphidohaptor* as proposed by Kritsky et al. (2007a). All of the *Tetrancistrum* species included in this tree are from siganids; they were divided into two groups, one composed of *T. nebulosi* (= T. *sigani*) and *T. labyrinthus* and a separate group containing *T. indicum*. At the current state of knowledge, it is not clear what main characters are responsible for this phylogenetic division within members of *Tetrancistrum*. Obtaining additional molecular data from other members of *Tetrancistrum* from siganid and nasoid hosts will help to clarify the factors influencing the formation of these molecular divisions. The findings of the current study suggest that the SSU rDNA is a reliable marker to understand phylogenetic relationships between Ancyrocephalids.

The data obtained from the LSU rDNA region of the worms investigated in the current study revealed that *G. safiensis* sp. n. disclosed highest similarity to the sequence of *Tetrancistrum* sp. deposited by Mollaret et al. (1997). Since molecular divergence among species representing different genera is usually higher than that among species representing the same genera within the family (Wu et al. 2007), it can be suggested that the *Tetrancistrum* sp. analysed by Mollaret et al. (1997) is actually a species belonging to *Glyphidohaptor* rather than *Tetrancistrum*. However, re-evaluation of the species analysed by Mollaret (1997) will require confirmation before drawing any conclusions. Subsequently, the obtained sequences of *Tetrancistrum* species were always clustering as a separate monophyletic group regardless of the type of data set used in the molecular analyses. This observation supports the notion that *Tetrancistrum* sp. analysed by Mollaret et al. (1997) is a member of *Glyphidohaptor*, and most probably it is *G. phractophallus* since it is the only species reported from *S. fuscescens* so far.

This finding needs to be further investigated and confirmed by morphological and molecular analysis of *Glyphidohaptor* and *Tetrancistrum* type specimens from *S. fuscescens* in Australia. The tree constructed from the ITS-1 dataset was not very informative since only a few sequences were available for comparison. However, the sequences obtained of this region and the resultant tree confirms the validity of the new species described herein as a member of *Glyphidohaptor*.

Acknowledgements We are grateful to the Ministry of Agriculture and Fisheries Wealth, Sultanate of Oman, for the TRC Project ORG-EBR_11-TRC grants, to Laura Machkevska for her help with the illustrations, to the owner of the YouTube channel (Chiworld1234) for his beneficial adobe illustrator tutorials which helped me to achieve the digital drawings for this paper. Also, we would

like to extend my thanks to Prof. Kazuo Ogawa (Meguro Parasitological Museum), Mrs Eileen Harris (British Natural History Museum), Mrs Patricia Pilitt, (US National Parasite Collection), Dr Mal Bryant (Queensland Museum) for the voucher specimens loans and worm micrographs. In addition, I would like to thank Dr. Juan Luis Ribas of the Servicio de Microscopía Centro de Investigación, Tecnología e Innovación at the Universidad de Sevilla, for his outmost help and kindness with obtaining the Confocal microscopy images.

5 *Hysterolecithoides amurparuchinii* n. sp. (Lecithasteridae: Hysterolecithinae) from white spotted rabbitfish *Siganus canaliculatus* from the Arabian Sea, Sultanate of Oman.

Abstract

Worms affiliated with the genus *Hysterolecithoides* Yamaguti, 1934 were collected exclusively from the oesophagi of the white spotted rabbitfish *Siganus canaliculatus* (Park) (Siganidae) off the Omani coasts of the Arabian Sea. Comparative analyses of all valid species of *Hysterolecithoides* reveal that the new species is morphologically distinct from their congeners based on general morphological features, definitive host(s) and geographical locality. They also differed from species reported from other siganids (e.g. *H. epinepheli* and *H. frontilatus*) in the body length (mean length = 5350 µm vs 1919 µm - 4494 µm (*H. frontilatus*) and 4192 µm (*H. epinepheli*); and body width (mean width = 1633 µm vs 532 µm - 1294 µm (*H. frontilatus*) and 1166 µm, (*H. epinepheli*), forebody as % of length (46.5% = 30.3% -36.4vs 40%) and the distance of testes from the ventral suckers (mean distance of testes to ventral sucker = 136 µm vs 29 µm (*H. frontilatus*) and 46 µm (*H. epinepheli*). Newly obtained molecular data of the highly conserved small subunit (SSU) rDNA matched these worms to *Hysterolecithoides guangdongensis* Wu, 2000 sequences obtained from *S. fuscescens* off Chinese waters with 99% similarity. The large subunit (LSU) rDNA revealed 98% similarity to *H. frontilatus* and *H. epinepheli* and 87% similarity to *Machidatrema chilostoma* (Machida, 1980) León-Règagnon, 1998. This is the third record of a member of *Hysterolecithoides* from the Western Indian Ocean region, and the first registration exclusively from the oesophagi. From a phylogenetic point of view, molecular data obtained in this study supports the morphological description of *Hysterolecithoides amurparuchinii* n. sp. as a new species within the genus.

Key words: digenea, *Hysterolecithoides*, *Siganus canaliculatus*, Oman, Arabian Sea.

[4] This article will be submitted as: Al Jufaili Sarah Hamoud, Machkevskyi Vladimir, Palm Harry Wilhelm (20..) *Hysterolecithoides amurparuchinii* n. sp. (Lecithasteridae: Hysterolecithinae) from white spotted rabbitfish *Siganus canaliculatus* from the Arabian Sea, Sultanate of Oman.

5.1 Introduction

Digenean trematodes are the most frequently investigated group of fish parasites known to infect siganid hosts. The review of available literature indicates that siganids harbor a diverse assemblage of digeneans which infect various organs. To date, thirty species of digenean trematode belonging to nine families have been reported from siganids (Madhavi 1972; Diamant and Paperna 1986; Barker et al. 1993; Nahhas and Wetzel 1995; Arthur and Lumanlan-Mayo 1997; Bray and Cribb 2000; Bray and Cribb 2001; Nahhas and Sey 2002; Hall and Cribb 2004; Hall and Cribb 2005; Shih et al. 2004; Hassanine and Gibson 2005; Al-Jahdali and Hassanine 2012). *Hysterolecithoides* Yamaguti, 1934 (Lecithasteridae Odhner, 1905) a trematode genus infecting the intestine and stomachs of tropical marine fishes is also reported from siganid hosts. Like other hysterolecithins, members of *Hysterolecithoides* are characterized by having a ventral sucker in the middle of body, gut-caeca ending blindly near posterior extremity. Testes two, pre-ovarian, in anterior half of the hindbody. Seminal vesicle tubular, occasionally elongate saccular in forebody. A vitellarium with 2-7 subglobular, entire or irregular masses (Bray and Cribb 2000; Gibson et al. 2006).

The genus was created to accommodate *H. epinepheli* Yamaguti, 1934 infecting the Hong Kong Grouper *Epinephelus akaara* (Temminck & Schlegel). Subsequently, several species were described from marine fishes belonging to different teleost families. In their revision of the genus, Bray and Cribb (2000) validated and recognized five nominal species in addition to the type-species; *H. frontilatus* (Manter, 1969) Yamaguti, 1971; *H. pseudorosea* (Bravo-Hollis 1956) Yamaguti, 1971; *H. manini* Yamaguti, 1970; *H. zebrasomatis* Yamaguti, 1970 and *H. multiglandularis* Tang, Shi & Guan in Tang, Shi, Cao, Guan & Pan, 1983. Members of the genus are widely distributed in the Indo-Pacific region occurring from the Indian coasts of the Arabian Sea, Hawaii to the eastern Pacific coasts of Mexico (Bray and Cribb 2000).

The taxonomic history of species infecting siganids is complex. For example, *H. frontilatus* which was originally described as *Theletrum frontilatum* Manter, 1969 and was later placed in *Hysterolecithoides* by Yamaguti (1971). Shen (1982) established a new genus *Oligolecithoides* with its species *O. trilobatus* which was later synonymized with *H. epinepheli* by Bray and Cribb (2000). Both *H. epinepheli* and *H. frontilatus* were registered from several siganid hosts, some of which are reported only by their generic name. Furthermore, some of the siganid hosts attributed

to these worms do not exist in the respective geographical locality. For example, *H. frontilatus* reported from Moreton Bay, Australia was registered from *S. rivulatus* which according to Woodland (1990) is limited to East Africa and the Red Sea.

During a survey of the parasite fauna of the white spotted rabbitfish *S. canaliculatus* from three water bodies along the coasts of the Sultanate of Oman, members of the digenean genus *Hysterolecithoides* were isolated from the oesophagi of *S. canaliculatus* sampled from localities situated along the Arabian Sea. The present study aims to identify the species of *Hysterolecithoides* of *S. canaliculatus* of Omani waters based on comparative morphological analysis and molecular data of the small subunit (SSU) and large subunit (LSU) rDNA.

5.2 Materials and methods

One-shot *S. canaliculatus* sampling was carried out in 2012, at the seven sampling localities along the coasts of the Sultanate of Oman; Raysut, Al-Lakbi, Masirah, Muscat, Sohar, Dabba, Khasab. Freshly frozen hosts were subjected to parasitological analyses according to Palm and Bray (2014). Obtained trematodes were fixed with either 10% formalin or 70% ethanol for morphological investigations and in molecular grade 96% ethanol for molecular analysis. The collected parasites were stained by Mayer's paracarmine and a modified aceto-carmine method (Machkevskyi et al. 2013). For comparative morphological analysis several slides of *H. epinepheli* and *H. frontilatus* were obtained from Meguro Parasitology Museum, Tokyo, Japan (22850SY6026; 23558SYB108) and from Natural British History Museum (BMNH 1999. 1. 25. 15; BMNH 1999. 1. 25. 11). Description is based on the Bray and Cribb (2000). Additional measurements were obtained in the present study including, distance of sinus-sac to ventral sucker, distance of posterior of ventral sucker to anterior of testes, distance of testes to ovary, distance of posterior of ventral sucker to ovary, distance of ovary to posterior extremity and distance of vitellarium to posterior extremity (Table 5.3).

Further histological analysis of the obtained worms was carried out according to standard procedure for tissue samples. Histological sections and whole mount preparations were examined using the Olympus U-LHEAD microscope, equipped with digital camera Olympus DP73 and Olympus cellSens Dimension software.

For molecular analysis, total genomic DNA was extracted from individual worms using QIAamp mini DNA kit (Qiagen) according to the manufacturer's instructions. The concentration of the obtained DNA was quantified (i.e., ng/µl) using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham Massachusetts). Partial SSU rDNA was amplified using the primers WormA (5′-GCG AAT GGC TCA TTA AAT CAG-3′) and WormB (5′-CTT GTT ACGACT TTT ACT TCC-3′) according to Palm et al., (2009). To amplify the D1-D2 domain of the LSU rDNA, the primers C1 (5′ ACCCGCTGAATTTAAGCAT-3′) and D2 (5′-TGGTCCGTGTTTCAAGAC-3′) were used following Mendlova et al. (2010). Purified PCR products were sent to Apical Scientific (Selangor, Malaysia) for sequencing using the same amplification primers. Generated sequences manually edited and contigs were assembled using BioEdit 7.2.

Sequences that displayed the highest matches on BLAST (http://www.ncbi.nlm.nih.gov/blast) were downloaded to infer the phylogeny of the worms obtained in the present study. For the SSU tree, 30 sequences of digenean belonging to the families Hemiuridae (14 species) and Lecithasteridae (11 species) were used for phylogenetic analyses. In this tree the digenean *Hemiperina manteri* Crowcroft, 1947 (Gonocercidae) was used as an outgroup (Sokolov et al. 2018). For the construction of the LSU phylogenetic tree sequences of 39 digeneans were used. These include members of three subfamilies within the family Lecithaseridae; Hysterolecithinae (four species), Quadrifoliovariinae (six species) and Lecithasterinae (nine species). Seven subfamilies within the family Hemiuridea; Bunocotylinae (three species), Opisthadeninae (one species), Lecithochiriinae (two species), Plerurinae (one species), Dinurinae (one species), Elytrophallinae (one species) and Aphanurinae (one species).

The selected Maximum likelihood model estimated for the SSU tree was Kimura-2-paramaters with Gamma distribution and for the LSU tree the General Time Reversible model was estimated as the best fit model to infer phylogenetic relationships within lecithasterids. The branch support was estimated using 1000 bootstrap replicates.

Table 5. 1 List of hemiuroid taxa and their accession code in GenBank that were incorporated into the phylogenetic analysis.

Species	Subfamily	GenBank accession number	
		SSUrDNA	LSUrDNA
Family: Hemiuridae			
Aphanurus mugilus	Aphanurinae	LT607804-LT607806	LT607807-LT607808
Bunocotyle progenetica	Bunocotylinae	DQ354369	DQ354365
Dinurus longisinus	Dinurinae	AJ287501	AY222202
Lecithocladium cristatum	Elytrophallinae	MF539756	
Lecithocladium excisum	Elytrophallinae	AJ287529	AY222203
Hemiuridae sp.		KM401885	
Merlucciotrema praeclarum	Plerurinae	AJ287535	
Opisthadena sp.	Opisthadeninae	AJ287549	AY222198
Saturnius sp.	Bunocotylinae	DQ354370	
Saturnius gibsoni	Bunocotylinae		KJ010542
Robinia aurata	Bunocotylinae	DQ354371	DQ354367
Family: Lecithasteridae			
Aponurus laguncula	Lecithasterinae	KY471301	
Aponurus sp. DTJL-2006	Lecithasterinae	DQ354372	DQ354368
Aponurus sp. AK-2010	Lecithasterinae		HQ713441
Bilacinia australis	Quadrifoliovariinae		AY897568
Hysterolecithoides epinepheli	Hysterolecithinae	MH625963	MH625962-MH625964
Hysterolecithoides frontilatum	Hysterolecithinae	AF029813	MH628310
Hysterolecithoides guangdongensis	Hysterolecithinae	HM545901	
Hysterolecithoides amurparuchinii n. sp.	Hysterolecithinae	Present study	Present study
Hysterolecithoides amurparuchinii n. sp.	Hysterolecithinae	Present study	Present study
Hysterolecithoides amurparuchinii n. sp.	Hysterolecithinae		Present study
Hysterolecitha nahaensis	Hysterolecithinae	AF029811	
Lecithaster gibbosus	Lecithasterinae	AJ287527	AY222199
Lecithophyllum botryophoron	Lecithasterinae	AY222107	AY222205
Lecithaster salmonis	Lecithasterinae		MH625979, MH625980
Lecithaster gibbosus	Lecithasterinae	AJ287527	AY222199
Lecithaster mugilis	Lecithasterinae	LN865007	LN865016
Lecithaster sudzuhensis	Lecithasterinae	LN865013	LN865022
Lecithaster sp.	Lecithasterinae		MH625978
Lecithaster sayori	Lecithasterinae		MH625977
Lecithaster confusus isolate	Lecithasterinae		MH625973, MH625975

Table 5.1 (continued.)

Species	Subfamily	GenBank accession number SSUrDNA	LSUrDNA
Machidatrema chilostoma	Hysterolecithinae	AY222106	AY222197
Quadrifoliovarium maceria	Quadrifoliovariinae		AY897566
Quadrifoliovarium pritchardae	Quadrifoliovariinae		AY897567
Quadrifoliovarium quattuordecim	Quadrifoliovariinae		AY897565
Quadrifoliovarium simplex	Quadrifoliovariinae		AY897564
Thulinia microrchis	Hysterolecithinae	AF029812	
Unilacinia asymmetrica	Quadrifoliovariinae		AY897569
Outgroups			
Family: Gonocercidae			
Hemiperina manteri	Gonocercinae	AY222105	
Family: Azygiidae			
Otodistomum cestoides	Azygiinae		AY222187

5.3 Results

Digeneans, identified as *Hysterolecithoides* sp., were detected exclusively in the oesophagi of *S. canaliculatus* from three sampling locations along the Omani coasts of the Arabian Sea.

Family Lecithasteridae Odhner, 1905

Subfamily Hysterolecithinae Yamaguti, 1958

Genus *Hysterolecithoides* Yamaguti, 1934

Hysterolecithoides amurparuchinii n. sp.

Type-host: *Siganus canaliculatus* (Park) (Perciformes: Siganidae), white-spotted rabbitfish.

Type-locality: Arabian Sea, Off Raysut fish market (16.5500°N, 54.0100°E). Sultanate of Oman (November and December 2012).

Prevalence: from Raysut fish market (63%), from Al-Lakbi, and Masirah (60%). No *Hysterolecithoides* sp. were detected from sampling sites located in the Gulf of Oman (Muscat, Sohar and Dabba) and Persian Gulf (Khasab).

ZooBank registration:

Etymology: The specific name (*amurparuchinii*) is in honor of Dr. Amur Paruchin who initiated systematic investigation of fish parasites in the Sultanate of Oman.

5.3.1 Description (Figure 5.1)

Based on (31) whole mounts and serial histological sections obtained from seven adult specimens. Body massive, spindle-shaped (Figure 5.3A). Tegument unarmed. Maximum body width anterior to ventral sucker. Oral and ventral suckers globular or slightly transversely oval. Oral sucker subterminal, 1.55-1.97 (1.72) times smaller than the ventral sucker. The anterior lobe distinct. Ventral sucker in posterior half of body (it's center is post-equatorial by 4.2% of body length). Prepharynx absent. Pharynx small, close to base of oral sucker. Oesohagus distinct. Caeca reach well into hindbody; termination asymmetrical, but usually obscured by eggs when uterus is fully formed. Testes 2, small, subglobular, asymmetrical, below posterior edge of ventral sucker. Two thin, transparent vasa efferentia extend from testes, entering separately into seminal vesicle.

Seminal vesicle elongate, tubular, sinuous, overlapping ventral sucker, narrows posteriorly to sinus-sac to form pars prostatica. Pars prostatica, tubular, arcuate, shorter than seminal vesicle, runs dorsally to sinus-sac. Sinus-sac oval, thick-walled, with obliquely intersecting muscular fibers, posterior region thicker than anterior (Figure 5.3B). Internal hermaphroditic duct bipartite, joining inside sinus sac. Ductus ejaculatorius enters sinus-sac and merges with metraterm to form hermaphroditic canal. Sinus organ funnel-shaped, turned inward or protruding outward, depending on parasite physiological state, everted organ smooth, telescopic. Excretory pore opens subterminally 1.5% from posterior extremity. Excretory bladder cylindrical, relatively narrow, widens proximally, it extends to anterior margin of ventral sucker, divides into two lyre-shape arms that end blindly at level of midpoint of pharynx, distal part thick, ensheathed by cluster of glandular cells most noticeable on laterally positioned worms and histological sections (Figure 5.1A and B).

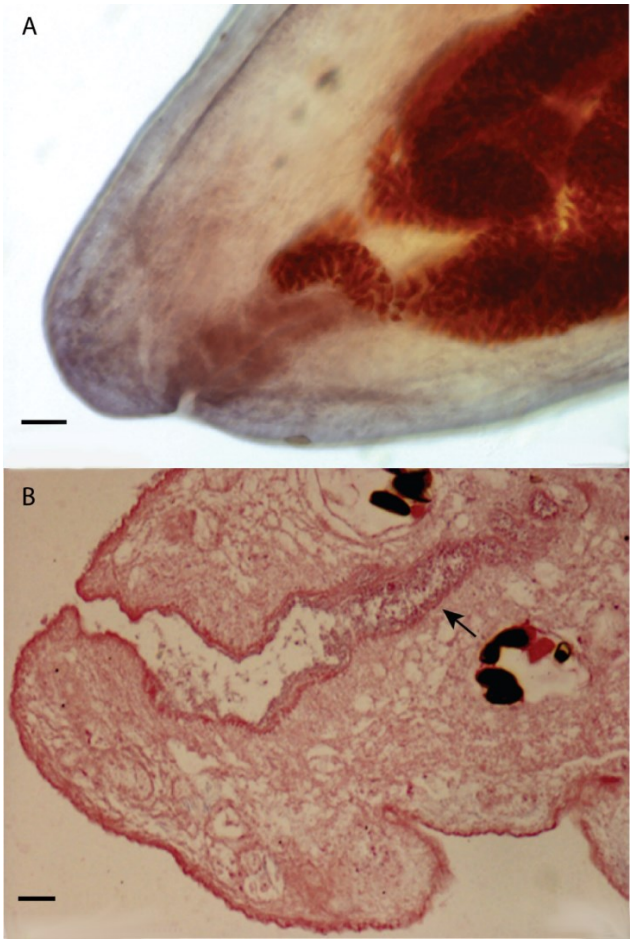

Figure 5. 1 Laterally positioned Hysterolecithoides amurparuchinii n. sp., ex Siganus canaliculatus showing the proximally thickened wall of the excretory pore, (A). Histological section of laterally positioned worms showing the structure of the distal portion of the excretory bladder, (B). Scale bars: A= 50, B=20 µm.

Ovary, oval slightly larger than testes, distinctly separated from testes, submedian. Vitellarium consists of 2-7 rounded follicles (most often 3-4) massed together ventrally, adjacent behind ovary. Uterine seminal receptacle and Mehlis' gland's not observed. Uterus posterioventral, posterior to testes. Uterus reaches end of caeca in most individuals, not reaching posterior end of body, such that terminal part of excretory vesicle visible. Of 100 gravid specimens, only one had its uterus completely filling hindbody. Eggs oval, slightly flattened, with clearly discernible embryo. Genital atrium funnel-shaped. Genital pore median, slightly protruding. Anterior to atrium, a disc shaped aperture is observed (Figure 5.2).

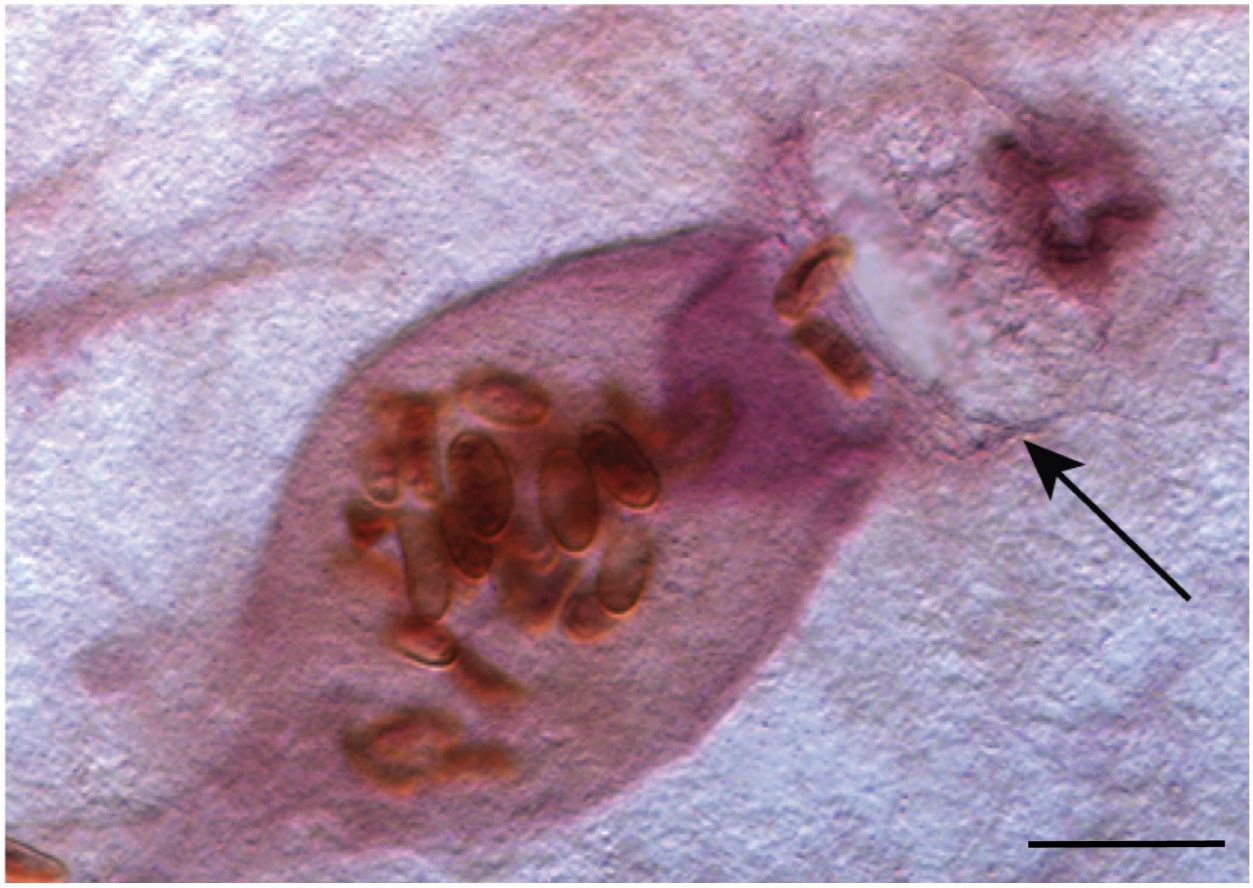

Figure 5. 2 The oval, disc shaped aperture anterior to the atrium of Hysterolecithoides amurparuchinii n. sp., ex Siganus canaliculatus, arrow head. Scale bar: 50 µm.

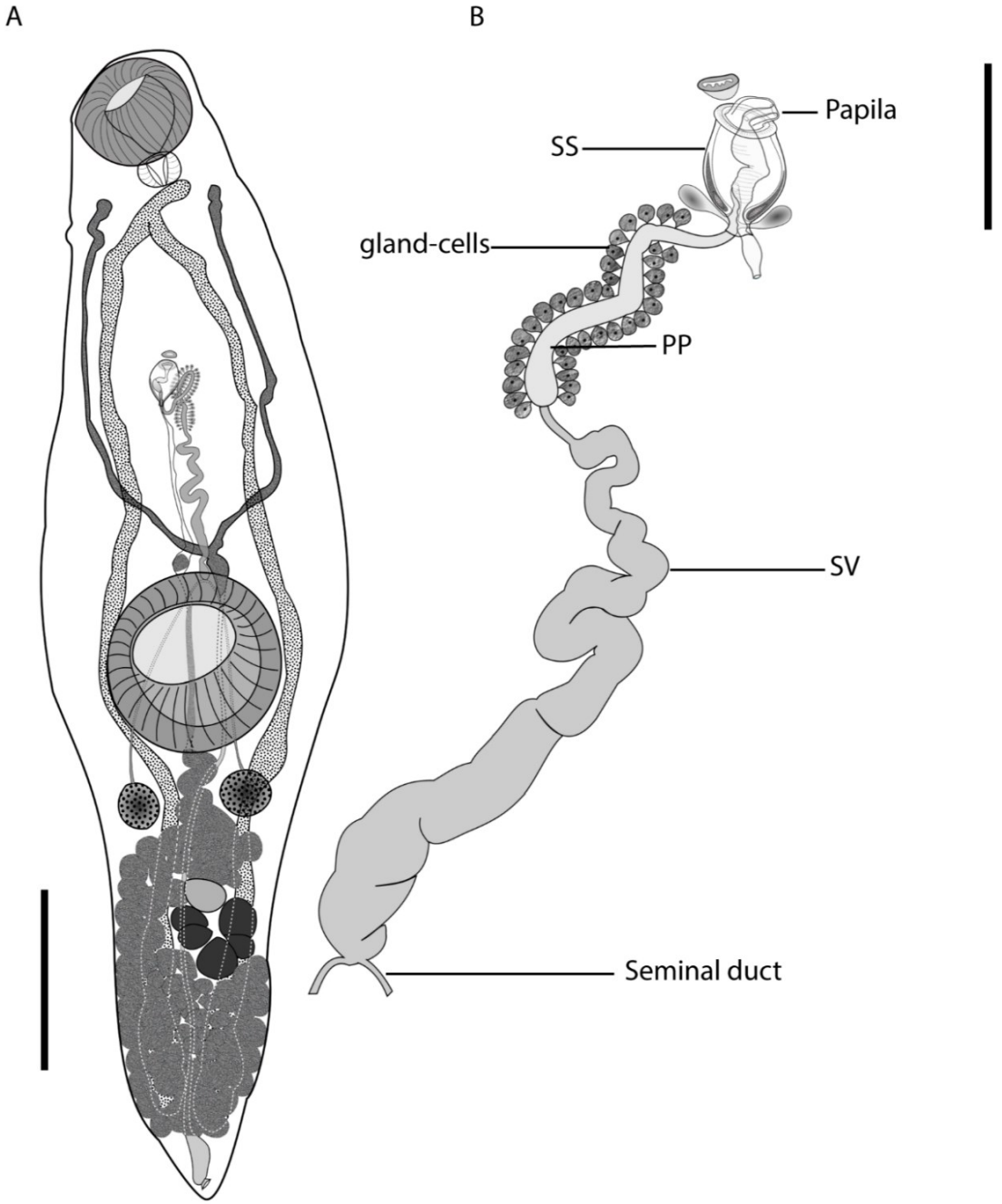

Figure 5. 3 Hysterolecithoides amurparuchinii n. sp., ex Siganus canaliculatus. Holotype, (A), Ventral view; (B), Terminal genitalia, SS, Sinus sack, PP, Pars prostatica, SV, Seminal vesicle. Scale bars: A= 1mm, B= 200 µm.

Remarks

Comparative morphological analyses of the material obtained in the present study with all known *Hysterolecithoides* species indicates that *Hysterolecithoides amurparuchinii* n. sp. is most similar to those reported from other siganids (i.e. *H. epinepheli* and *H. frontilatus*). They share several common features. Namely, the body width of all three species is about one-third to body length. Also, unlike other species within the genus (e.g. *H. manini* and *H. zebrasomatis)* the testes of these three species are asymmetrical and bilateral, and the ventral sucker is located in middle body. However, these species differ in many aspects concerning their morphology (Table 5.2), which is based on the data from Yamaguti (1953), Manter (1969), Bray and Cribb (2000) as well as loaned voucher specimens from museums.

The body length of *H. amurparuchinii* n. sp. is 1.22-2.04 and 1-1.28 times larger than *H. frontilatus* and *H. epinepheli*, respectively. Body width of our worms is 1.33-2 and 1.13-1.48 times wider than *H. frontilatus* and *H. epinepheli*, respectively. The forebody length of the species described herein is 2.12-2.57 times longer than *H. frontilatus*. Although the minimum forebody length of our worms is 0.84 times shorter than the minimum forebody length of *H. epinepheli*, the maximum forebody length is 2.32 longer. Pre-Oral lobe length of *H. amurparuchinii* n. sp. is 1.38-2.24 times longer than in *H. frontilatus*. In comparison to *H. epinepheli* only the maximum Pre-oral lobe length of *H. amurparuchinii* n. sp. is longer (150 µm vs 69 µm). Oral sucker length of *H. amurparuchinii* n. sp. is 1.38-1.56 and 1.06-1.57 times bigger than *H. frontilatus* and *H. epinepheli*, respectively. The oral sucker width of our worms is 1.41-1.49 times wider than *H. frontilatus*. However, only the maximum width is 1.51 times wider than *H. epinepheli*.

The distance of the intestinal bifurcation to ventral sucker of our species is 2.35-2.58 and 1-1.88 times longer than *H. frontilatus* and *H. epinepheli*, respectively. The distance of the genital pore to the ventral sucker of *H. amurparuchinii* n. sp. is 2.39-2.97 times longer than in *H. frontilatus*. Only the maximum distance of the genital pore to ventral sucker of our worms is longer than *H. epinepheli* (1.58 times longer). The Sinus-sac of *H. amurparuchinii* n. sp. is 1.37-1.39 x 1.01-1.10 times bigger than *H. frontilatus*. The maximum size of the Sinus-Sac of *H. amurparuchinii* n. sp. is 1.10 x 1.39 times larger than *H. epinepheli*. The size of the ventral sucker of our species is also larger than *H. frontilatus* (1.30-1.98 times longer and 1.25-1.75 times wider). In the case of *H. epinepheli* the maximum size of ventral sucker was smaller than in our species.

The vitellarium of *H. amurparuchinii* n. sp. is 1.08-1.26 larger and 1.06-1.51 wider than *H. frontilatus*. In comparison to *H. epinepheli* the vitellarium of *H. amurparuchinii* n. sp. is 1.05-1.56 longer and 1.22-2.78 wider.

Furthermore, the number of vitellarium masses in our species varied from 2-7 (most predominate 4-5). In *H. frontilatus*, species with 3 vitelline masses were most dominant (Bray and Cribb 2000). In *H. epinepheli* specimens available at our disposal (ex *Siganus fuscescens*, Japan), the vitellaria had a distinct "flower" shape. In five out of six specimens, it consisted of 7 vitelline mass. It was also noted that the value of width as % of body length of our species is bigger than *H. frontilatus* (1.20-1.29 times). The minimum width as % of the percentage of body length of our worms was comparable to that of *H. epinepheli*, but the maximum was larger (1.37 times). The forebody of *H. amurparuchinii* n. sp. is distinctively longer than *H. frontilatus* (1.21-1.82 times) and *H. epinepheli* (1.37 times larger for maximum value). The sucker width ratio of our worms is smaller than *H. frontilatus* by 0.29-0.72 times. The PVR as % of the length of our worms is 0.55-0.68 times smaller than of *H. frontilatus*. The PUR as % of body length of *H. amurparuchinii* n. sp. is smaller in comparison to *H. frontilatus* by 0.39-0.75 times. The Excretory pore to posterior extremity as % of length in *H. amurparuchinii* n. sp. is shorter than *H. frontilatus* (0.48-0.65 times) and *H. epinepheli* (0.20-0.91 times).

The additional measurements that were obtained from our material and the loaned museum slides show further differences between the three species (Table 5.3). For example, the distance of the Sinus-sac from the ventral sucker of *H. amurparuchinii* n. sp. was 699-1346 µm in comparison to 357-802 µm in *H. epinepheli* and 110-189 µm in *H. frontilatus*). The ovary of *H. amurparuchinii* n. sp. were positioned (300-964 µm) away from the ventral suckers compared to *H. epinepheli* (131-488 µm) and *H. frontilatus* (48-250 µm). Finally, the comparative construction of some members of all three species to the same scale shows the extent of the variations in organ ratios, especially the position of the ventral sucker to the body equator, distance of the testes to the ventral sucker, distance of the testes to the ovary and the relative overall size of all three species (Figure 5.4).

Table 5. 2 Comparative measurements of all Hysterolecithoides spp. that are known to infect siganid hosts based one data obtained in the present study and those of Manter (1969), Yamaguti (1953), Bray & Cribb (2000) and loaned slides from MPM.

	Hysterolecithoides amurparuchinii n. sp.			*Hysterolecithoides frontilatus*									*Hysterolecithoides epinepheli*						
	Siganus canaliculatus			*Siganus nebulosus*			*Siganus doliatus*			*Siganus doliatus*			*Siganus sp.*		*Siganus sp.*		*Siganus fuscescens*		
	Arabian Sea Oman			Morten Bay Australia			Lizard IS, Australia			New Caledonia			New Caledonia		Makassar		Japan		
	Present study, n= 31			Bray & Cribb (2000)									Manter 1969		Yamaguti 1953		Yamaguti 1938		
	Min	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Mean**
Body Length	2509	6983	5350	1232	3275	1919	4457	4531	4494	2528	3895	3458	2489	5719	2500	4400	3160	5457	4192
Body Width	733	2206	1633	366	827	532	1272	1315	1294	715	1248	966	627	1653	650	1250	868	1489	1165
Forebody length	1039	5125	2512	489	1224	696	1439	1507	1473	795	1272	1047	855	1995			1239	2208	1674
Pre-oral lobe length	13	150	54	12	52	27	102	109	106	6	103	52					26	69	45
Oral sucker length	223	625	462	161	325	231	367	386	377	277	400	333			210	340	288	397	346
Oral sucker width	241	705	509	162	316	228	380	399	390	280	388	342	301	502	260	380	347	468	406
Pharynx L	77	213	143	63	116	84	161	163	162	103	155	134	107	147	65	110	102	154	127
Pharynx W	96	373	184	63	116	83	147	148	148	99	155	135	134	167	84	120	113	157	135
Intestinal bifurcation to ventral sucker	620	2463	1637	264	791	428	902	954	928	393	734	638					620	1309	927
Genital pore to ventral sucker	377	1632	1122	158	473	241	483	549	516	213	411	308					468	1030	660
Sinus sac length	105	323	244	77	167	104	126	155	141	155	232	188	96	208	110	160	112	226	167
Sinus sac width	59	212	141	58	115	81	109	123	116	122	181	158	88	192	110	150	84	145	110
Ventral sucker length	470	1066	830	238	490	347	684	689	687	580	818	691					519	763	642

Table 5.2 continued

	Hysterolecithoides amurparuchinii n. sp.			*Hysterolecithoides frontilatus*										*Hysterolecithoides epinepheli*					
	Siganus canaliculatus Arabian Sea Oman			*Siganus nebulosus* Morten Bay Australia			*Siganus doliatus* Lizard IS, Australia			*Siganus doliatus* New Caledonia			*Siganus* sp. New Caledonia		*Siganus* sp. Makassar		*Siganus fuscescens* Japan		
	Present study, n= 31			Bray & Cribb (2000)									Manter, 1969		Yamaguti 1953		Yamaguti 1938		
	Min	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Mean**
Ventral sucker width	473	1096	873	270	535	393	752	779	766	599	869	701	536	874	460	750	565	796	673
Left testis length	80	356	224	71	206	135	180	232	206	96	230	144			90	210	291	343	321
Left testis width	83	344	218	97	219	146	238	245	242	103	232	175			75	180	178	287	239
Right testis length	89	358	242	71	200	141	193	251	222	97	206	146					255	306	280
Right testis width	118	294	211	84	225	147	238	263	251	126	245	167					200	251	225
Right testis to ovary	16	667	305	0	22	1	97	90	94	0	225	70					46	551	254
Ovary length	85	273	197	70	206	121	238	251	245	121	232	156			90	150	167	282	242
Ovary width	97	323	212	86	225	151	277	328	303	131	277	177			90	200	233	374	314
Vitellarium length	117	538	354	109	406	213	354	426	390	174	290	239			75	150	272	512	411
Vitellarium width	184	511	371	122	431	219	444	483	464	182	354	237			66	200	242	418	344
Post-vitalline region length (PVR)	453	1612	1004	277	1081	521	1402	1510	1456	599	1404	1205					647	1473	1030
Post-uterine region length (PUR)	102	908	252	145	274	220	206	258	232	245	684	476					240	575	337
Short caecum (left) to posterior extremity	125	468	250	193	313	254	451	586	519	341	882	522					171	297	262
Long caecum (right) to posterior extremity	107	313	214	133	261	203				232	521	372					219	383	290
Excretory pore to posterior extremity	21	152	83	23	90	45	39	58	49	24	88	55					58	179	109

Table 5.2 continued

	Hysterolecithoides amurparuchinii n. sp.			*Hysterolecithoides frontilatus*													*Hysterolecithoides epinepheli*		
	Siganus canaliculatus			*Siganus nebulosus*			*Siganus doliatus*			*Siganus doliatus*			*Siganus* sp.		*Siganus* sp.		*Siganus fuscescens*		
	Arabian Sea Oman			Moreton Bay Australia			Lizard IS Australia			New Caledonia			New Caledonia		Makassar		Japan		
	Present study, n= 31			Bray & Cribb (2000)									Manter, 1969		Yamaguti 1953		Yamaguti 1938		
	Min	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Mean**
Egg length	20	33	27	19	25	22	16	23	20	22	28	25	24	25	21	27	22	32	26
Egg width	10	18	15	10	13	12	13	14	14	12	15	13	12	13	12	15	12	16	13
Width as % of length	25.92	42.70	30.66	21.60	32.80	27.90	28.50	29.00	28.80	25.10	33.20	27.90					26.57	31.26	27.90
Forebody as % of length	32.39	75.69	46.48	31.90	41.70	36.40	31.80	33.80	32.80	26.80	34.60	30.30					38.01	42.73	39.94
Suckers width ratio	0.44	1.97	0.59	1.51	1.88	1.73	1.95	1.98	1.97	1.78	2.72	2.05	1.62	2.00			1.58	1.72	1.66
Oral sucker: pharynx ratio	1.00	4.88	2.90	2.47	3.43	2.57	2.57	2.71	2.64	2.28	2.94	2.55					2.65	3.50	3.02
PVR as % of length	10.81	27.65	18.81	19.80	33.50	26.70	31.50	33.30	32.40	23.70	40.90	34.70					19.76	26.99	24.05
PUR as % of length	1.79	15.14	4.88	5.04	16.30	12.00	4.62	5.69	5.16	7.04	20.10	13.70					4.40	14.59	8.56
Excretory pore to posterior extremity as % of length	0.34	3.00	1.54	1.25	2.75	2.37	0.88	1.28	1.08	0.70	2.47	1.60					1.66	3.29	2.54

Table 5. 3 Additional measurements obtained from slides of Hysterolecithoides amurparuchinii n. sp. and the slides of H. epinepheli and H. frontilatus obtained from museums.

Additional measurements	***H. amurparuchinii* n. sp.**			***H. frontilatus***			***H. epinepheli***		
	min	**max**	**Mean**	**min**	**max**	**mean**	**min**	**max**	**mean**
Distance of sinus-sac to ventral sucker	699	1346	974	110	189	150	357	802	521
Distance of posterior of ventral sucker to anterior of testes	0	296	136	0	111	29	0	104	46
Distance of Testes to ovary	0	421	290	0	208	66	0	247	85
Distance of Testes to posterior body	1339	2118	1812	850	1628	1288	1098	1418	1245
Distance of posterior of ventral sucker to ovary	300	964	625	48	250	162	131	488	322
Distance of ovary to posterior extremity	974	1593	1352	745	1520	1128	851	1306	1080
Distance of vitellarium to posterior extremity by	738	1306	1038	556	1323	941	645	1023	829

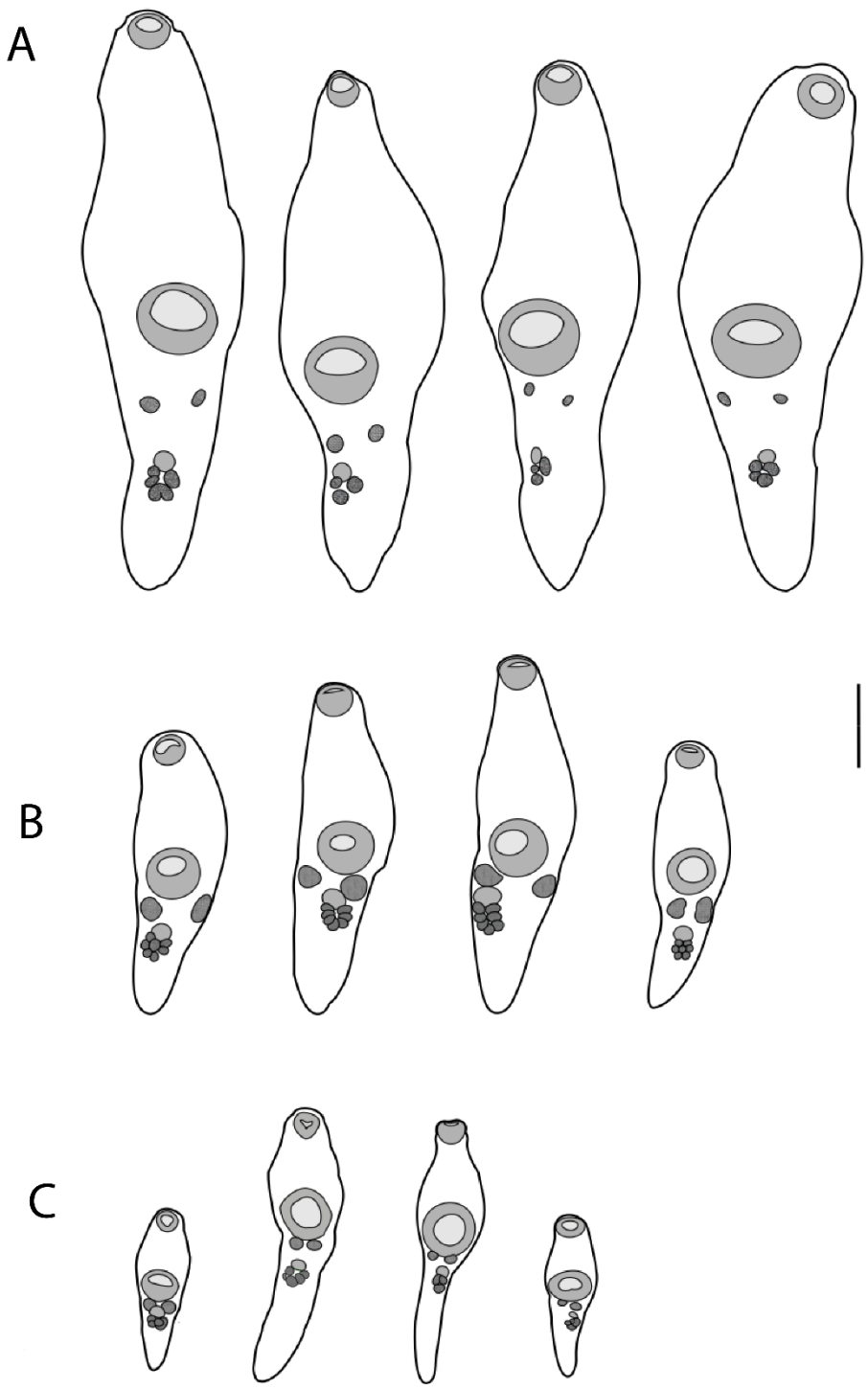

Figure 5. 4 A size invariant comparison of, (A); H. amurparuchinii n. sp., B; H. epinepheli and C; H. frontilatus illustrating the variations in the arrangements of some body organs (ventral sucker, testes, ovary and vitellarium) in the three species. Scale bar: 500 µm.

5.3.2 Molecular analysis

Contiguous sequences of the SSU and LSU regions of *Hysterolecithoides amurparuchinii* n. sp. were generated from three adult worms isolated from the oesophagi of the investigated hosts. The partial SSU was 1753 bp long, and the partial LSU was 930 bp long. The BLAST analyses of the generated sequences revealed a similarity of 99% to the SSU sequence of *H. guangdongensis* (accession code: HM545901) obtained from *S. fuscescens* off Chinese waters. The LSU sequence generated in the current study matched to *H. frontilatus* (accession code: MH628310) and *H. epinepheli* (accession code: MH625962-MH625964) both with 98% similarity, differing by 14 bases. The obtained sequences also matched to another hysterolecithin trematode *Machidatrema chilostoma* (Machida, 1980) León-Règagnon, 1998 (accession code: AY222106) with 86% similarity.

Six groups can be observed in the constructed SSU tree, the first group (A), consisted of representative of several hemiurid subfamilies (Aphanurinae, Elytrophallinae, Dinurinae, Lecithochiriinae and Plerurinae). The lecithochiriin *Lecithochirium caesionis* Yamaguti, 1942 clustered with the plerurin *Plerurus digitatus* (Looss, 1899) Looss, 1907. The plerurin *Merlucciotrema praeclarum* Manter, 1934 was distant from *P. digitatus*. The dinurin *Dinurus longisinus* Looss, 1907 clustered with the elytrophallin *Lecithocladium* Lühe, 1901. In group (B) the lecithasterin genus *Lecithaster* Lühe, 1901 formed a monophyletic clade, while the lecithasterin *Lecithophyllum botryophoron* Olsson, 1868 was distant. The relationship between these two genera is poorly resolved with low statistical nodal support. The hemiurid *Opisthadena* sp. was clustered with the lecithasterid *Aponurus* Looss, 1907 in group (C). in group (D), the lecithasterid genera *Hysterolecitha* Linton, 1910 and *Thulinia* Gibson & Bray, 1979 formed a sister to the hemiurid bunocotylin (*Robinia aurata* Pankov, Webster, Blasco-Costa, Gibson, Littlewood, Balbuena & Kostadinova, 2006), *Saturnius* sp. and *Bunocotyle progenetica* Chabaud & Buttner, 1959. Group (E) consisted of two hysterolecithin lecithasterid genera *Hysterolecithoides* and *Machidatrema*. These two were closely related and were clustered together as sister taxa. All species of *Hysterolecithoides* were clustered as a monophyletic group that was basal to *M. chilostoma* with high nodal support (90), group (E). Also, *H. amurparuchinii* n. sp. was basal to *H. frontilatus* and *H. guangdongensis*. Didymozoid trematodes formed a monophyletic cluster, group (F).

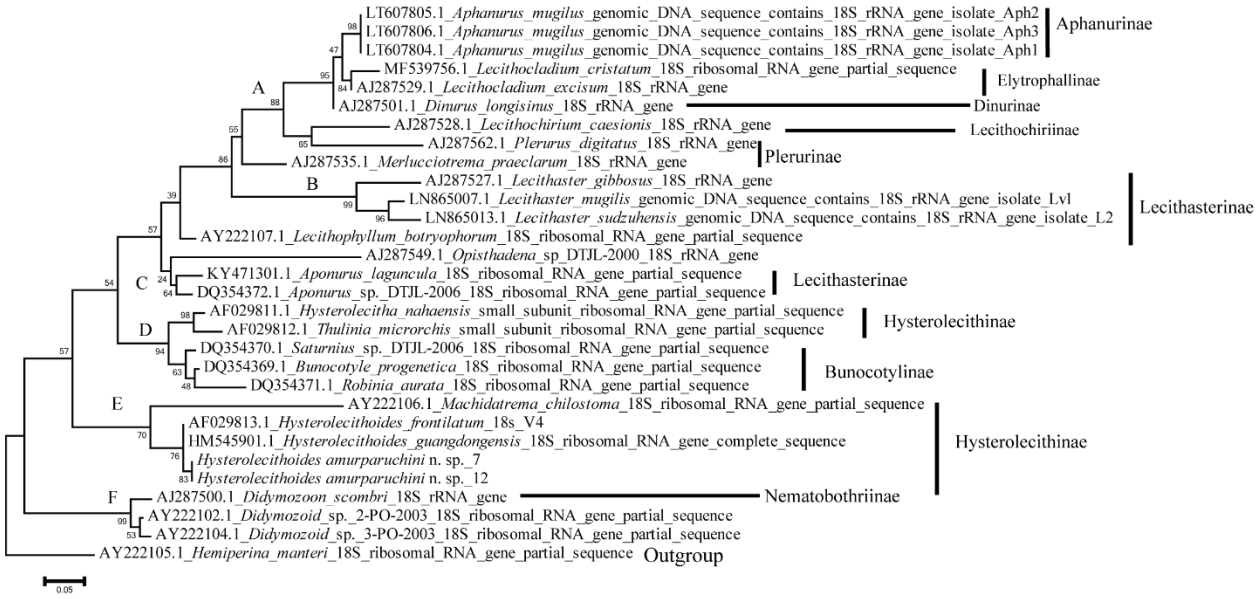

Figure 5. 5 The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model for SSU data set. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.5754)). The analysis involved 30 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 180 positions in the final dataset.

The LSU based phylogeny tree is divided into three major clades. Clade one is further subdivided into three groups (A), (B) and (C). Species of *Hysterolecithoides* formed a strongly supported monophyletic group. Sequences of *H. amurparuchinii* n. sp. were phylogenetically distant from those of *H. epinepheli* and *H. frontilatus*. Also, *M. Chilostoma* is a sister taxon to *Hysterolecithoides* in group (A). Group (B) consisted of all bunocotylid species. In group (C) the monophyletic subfamily Quadrifoliovariinae with its genera *Quadrifoliovarium* Yamaguti, 1965, *Bilacinia* Manter, 1969 and *Unilacinia* Manter, 1969 formed a monophyletic clade and *Bilacinia* was a sister taxon to it. Group (D) was composed of two lecithasterin genera *Aponurus* and *Lecithophyllum* Odhner, 1905. They were closely related and formed sister taxa with a strong statistical support. Group (E) contained several subfamilies within the family Hemiuridea. Using the LSU dataset memebers of the genus *Lecithaster* formed a monophyletic clade that was distant from other lecithasterids in group (F).

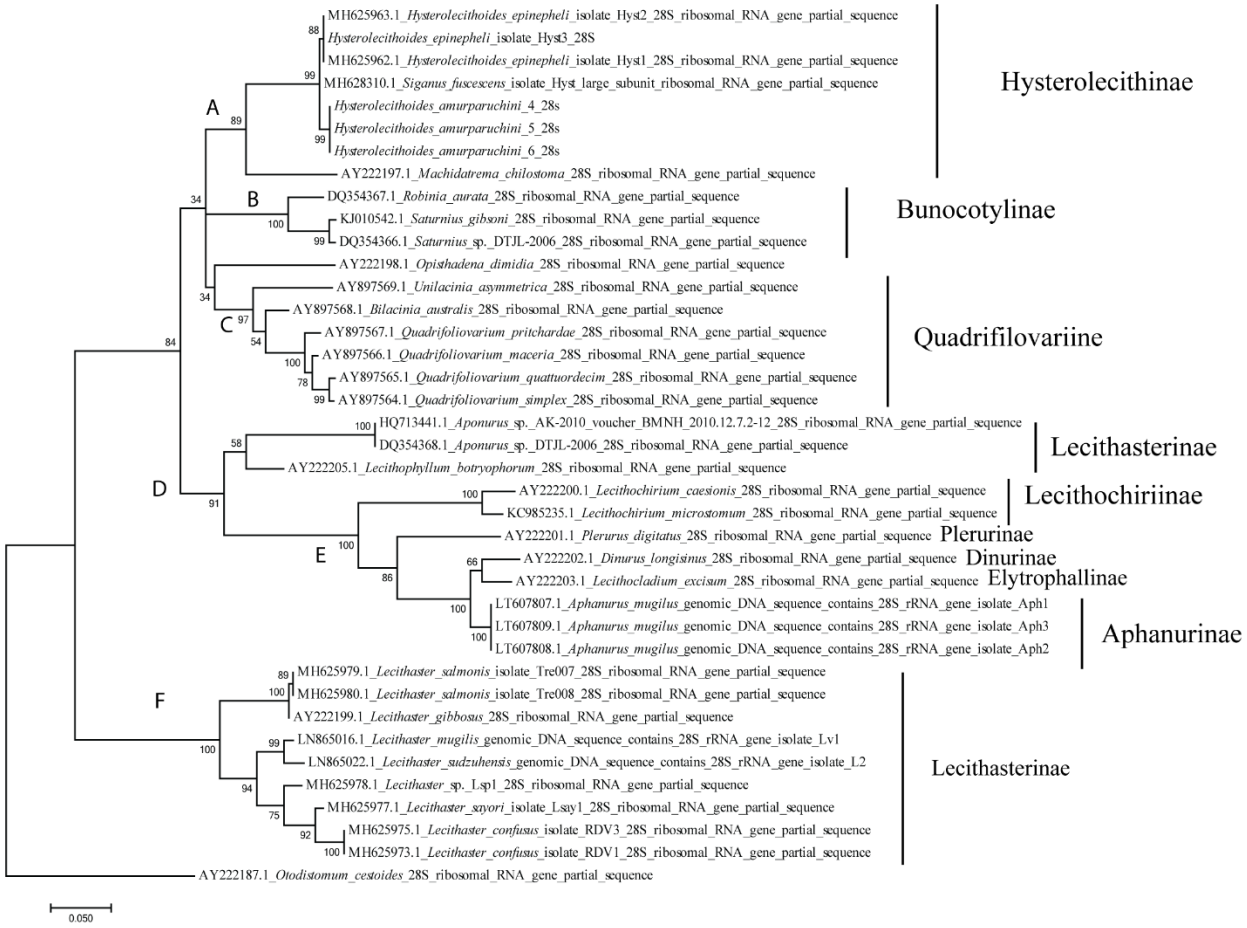

Figure 5. 6 The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model for the LSU data set. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.6817)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 21.9904% sites). The analysis involved 39 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 576 positions in the final dataset.

Table 5. 4 measurements of all species of Hysterolecithoides spp. infecting non-siganid hosts

Species name	*Hysterolecithoides pseudorosea*		*Hysterolecithoides manini*		*Hysterolecithoides zebrasomatis*		*Hysterolecithoides multiglandularis*		*Hysterolecithoides yamaguti*	
Host	*Cirrtitus virulatus*		*Acanthurus sandvicensis*		*Zebrasoma veliferum*		*Leiognathus brevirostris*		*Serranus flavocaeruleus*	
Author	Bravo-Hillis, 1956		Yamaguti, 1970		Yamaguti, 1970		Tang et al., 1983		Gupta and Dwivedi, 2006	
Locality	Mexico		Hawaii		Hawaii		southern Fujian, China		Ernakulam coast, Kerala	
	n= 1		n= 2		n= 2				n= 2	
		Min	Max	Min	Max	Min	Max	Min	Max	
Body Length	1860	2100	3350	1000	2700	813	1189	3200	4360	
Body Width	500	300	400	200	400	256	391	1020	1140	
Forebody length	500							1400	1480	
Pre-oral lobe length										
Oral sucker length	138	110	120	50	100	70	98			
Oral sucker width	165	120	150	80	130	45	78			
Pharynx L	47	70		50	70	24	30	90	110	
Pharynx W	62			60	90	24	30	110	130	
Intestinal bifurcation to ventral sucker										
Genital pore to ventral sucker										
Sinus sac length		80		116				630	770	
Sinus sac width		58		58				100	200	
Venral sucker length	545	240	300	150	240	195	240	550	650	
Ventral sucker width	165					180	231	580	680	
Left testis legnth		120	160	90	210	150	162	200	250	
Left testis width		100	120	70	180	108	144	110	130	
Right testis length						144	159	120	270	

Table 5.4 (continued.)

Species name	*Hysterolecithoides pseudorosea*		*Hysterolecithoides manini*		*Hysterolecithoides zebrasomatis*		*Hysterolecithoides multiglandularis*		*Hysterolecithoides yamaguti*	
Host	*Cirrtitus virulatus*		*Acanthurus sandvicensis*		*Zebrasoma veliferum*		*Leiognathus brevirostris*		*Serranus flavocaeruleus*	
Author	Bravo-Hillis, 1956		Yamaguti, 1970		Yamaguti, 1970		Tang et al., 1983		Gupta and Dwivedi, 2006	
Locality	Mexico		Hawaii		Hawaii		southern Fujian, China		Ernakulam coast, Kerala	
			n= 2							
		Min	Max	Min	Max	Min	Max	Min	Max	
Right testis width						105	117	120	160	
Ovary length		100	210	80	170			140	180	
Ovary width		100	150	70	160			180	200	
Vitellarium length		220	340					100	140	
Vitellarium width		150	270					100	180	
Post-vitalline region length (PVR)										
Post-uterine region length (PUR)										
Egg length	27-29	21	31	21	26	27	30	20	30	
Egg width	14-17	12	19	10	14	12	15	40	50	
Width as % of length		14.29	11.94							
Forebody as % of length								31.88	26.15	
Suckers width ratio	1:2.92	1:2		1.2						
Oral sucker: pharynx ratio										
PVR as % of length										
PUR as % of length										
Excretory pore to posterior extremity as % of length										

5.4 Discussion

The morphological characteristics that are used to distinguish between *Hysterolecithoides* species are limited. However, based on the morphological and molecular data that were obtained in the present study it is recognized that the species investigated herein is different from all known members of *Hysterolecithoides*, including those which are known from other siganid hosts. For instance, many morphological parameters of *Hysterolecithoides amurparuchinii* n. sp. were different from *H. pseudorosea* which was described from the giant hawkfish *Cirrhitus rivulatus* (Valenciennes) off the Pacific coast of Mexico. The most distinguishing feature was the enormous ventral sucker of the latter which separates it from *H. amurparuchinii* n. sp. and from other members within the genus. The two species that were reported from Hawaiian waters (i.e. *H. manini* and *H. zebrasomatis*) can be differentiated from the species presented herein by their definitive host species (both reported from acanthurids) and their geographical locality (Hawaii, where siganids are absent). Further, the bodies of these two species are narrower and shorter, while the shape was different compared to our worms (cylindrical vs spindle shape). In addition, the position and arrangement of testes was also different (tandem, *H. manini* and *H. zebrasomatis* vs parallel, *H. amurparuchinii* n. sp.).

As for *H. multiglandularis*, it has a vitellarium that consists of multiple globular masses (about 17) which distinguishes this species from all members of the genus and the species described herein. Furthermore, the ratio of suckers width is larger in *H. multiglandularis* in comparison to our worms (2.35-2.57 vs 1.55-1.96). Lastly, although *H. amurparuchinii* n. sp. most closely resembles *H. epinepheli* and *H. frontilatus* which are both registered on siganid hosts, comprehensive morphological analysis revealed that it is noticeably different from these two species. Various morphological characteristics were useful to separate the species described herein from the aforementioned species (Table 5.2).

Given that our finding is from the western Indian Ocean, it is important to discuss other occurrences in this region. Such records include that of Hafeezullah and Dutta (1980) who reported *H. frontilatus* from an unidentified fish host as well as from *S. oramin* (=*S. canaliculatus*) from Coromandel Coast, Gulf of Mannar. The worms described by the above mentioned authors are considerably larger than both *H. frontilatus* and *H. epinepheli*, while many of the body parameters overlap with our worms (e.g. body size, sucker width ratio, width as a percentage of body length

and forebody as a percentage of body length). It is observed from the illustrations that the arrangement of the main body organs such as the position of the testes and ventral sucker is also similar to our worms. Thereby, it is possible to theorize that the worms reported by Hafeezullah and Dutta (1980) are the same as the species obtained in the present study. Unfortunately, the description of Hafeezullah and Dutta (1980) is insufficient to confirm the accurate identity of the worms reported in their study. Thus, new material will be required to confirm this speculation and preferably molecular data should be obtained to support the taxonomical status of this worm.

Additionally, information regarding another species of the genus were published by Gupta and Dwivedi (2006). *H. yamagutii* was described from another epinephelid host *Serranus flavocaeruleus* (=*Epinephelus flavocaeruleus*, Lacépède) from Kerala, Indian Ocean. The description of this species is based on only two specimens, using limited morphological justifications (Table 5.4) and the type-slides were unattainable for comparative analysis (Argawal, personal communication). The above mentioned authors distinguished *H. yamagutii* from its congeners by its large Sinus-sac, number of vitelline masses and the presence of dermal gland in the anterior half of the body. The body size (length and width) of *H. yamagutii* overlapped with our worms. However, we didn't notice any dermal glands in our worms and the vitelline masses were more than in *H. yamagutii* (*H. amurparuchinii* n. sp., 2-7 masses). Based on this, we tentatively consider *H. yamagutii* as species inquirendum because there isn't sufficient justification for this species.

Some members of *Hysterolecithoides* exhibit wide geographical and host range, while some are more restricted. For example, *H. epinepheli* was registered in hosts of the Serranidae family (1 species), Carangidae (1 species) and Siganidae (3 species). In contrast, *H. frontilatus* is found exclusively in the family Siganidae (4 species). *H. pseudorosea* occurs in the family Cirrhitidae (1 species). Both *H. manini* and *H. zebrasomatis* are registered on Acanthuridae. Aside from siganids and acanthurids, all remaining host families are taxonomically and ecologically distant. The available literature suggests that hysterolecithin lecithasterids are commonly reported from acanthurid, siganids, and pomacentrids. Thus, the occurrence of *Hysterolecithoides* species in serranid and carangid hosts might be accidental. This confirmation of this proposition will require the knowledge of the infection levels and intensities in these hosts.

Geographically, *H. epinepheli* is the most widely distributed species of the genus. It was recorded from the Temperate Northern Pacific region (Mie and Hyogo Prefecture, Japan) and from the Central Indo-Pacific region including Hainan Island, Guangdong Province (China), Sulawesi (Indonesia) and most recently from Halong Bay (Vietnam). *H. frontilatus* is more restricted occurring only in the Central Indo-Pacific region (Queensland, Australia, New Caledonia, Bray and Cribb (2001)) and recently South China Sea off Vietnam, Atopkin et al., (2018) (also in the Central Indo-Pacific region). The remaining 4 species were registered from single localities in the Temperate Northern Pacific (*H. multiglandularis* from southern Fujian, China), Eastern Indo-Pacific (*H. manini* and *H. zebrasomatis* from Hawaii) and Tropical Eastern Pacific (*H. pseudorosea* from Mexico). However, their respective hosts exhibit a wider geographical range which might result in registration of new locality records for these worms and consequently the expansion of their geographical range.

For example, *Acanthurus sandvicensis* (= *A. triostegus* Linnaeus) has a wide range of geographical distribution which includes the entire Indo-Pacific region but excluding the seas around the Arabian Peninsula (Froese and Pauly 2018). Likewise, *Zebrasoma veliferum* (Bloch) has a distribution range spanning through the Western Indian Ocean and the Pacific Ocean (Froese and Pauly 2018). In case of our worms, the geographical distribution of its host *S. canaliculatus* includes the Western and Central Indo-Pacific region. However, of all the investigated localities in the present study, *H. amurparuchinii* n. sp. was limited to the southern region of Oman (coasts of Arabian Sea). Moreover, despite the fact that several siganids were previously investigated for parasites in the region of Arabian Sea and Red Sea (e.g. Diamant and Paperna 1986; Martens and Moens 1996; Geets and Ollevier 1997; Hassanine and Gibson 2005; Al-Jahdali 2013), none of these authors recorded any members of *Hysterolecithoides* from their hosts. Thus, considering the limited distribution of *H. amurparuchinii* n. sp., it can be assumed that these worms are geographically isolated and are endemic to the waters of Oman.

Interestingly, the results of our molecular analysis revealed that the SSU rDNA of *Hysterolecithoides amurparuchinii* n. sp. was 99% similar to the SSU sequence of a digenean species referred to as *H. guangdongensis* in NCBI GenBank database. These sequences were submitted by Wang et al. (2010) of worms obtained from *S. fuscescens* sampled from Zhanjiang sea area off Chinese waters. The original description of *H. guangdongensis* indicates that it was

formerly placed in the genus *Oligolecithoides* (Wu 2000). However, there is no taxonomic justification supporting the transfer of *O. guangdongensis* to *Hysterolecithoides* (Dr Bray, personal communication). In comparison to the description of Wu (2000), our worms are larger (maximum length reaching 5085 µm in *H. guangdongensis*), the width as a percentage of body length is larger (20.40-24.94% in *H. guangdongensis*), and the sucker width ratio is smaller (2.14-2.94, in *H. guangdongensis*).

The morphological distinction between the two species is further supported by the molecular and phylogenetic information obtained in the present study. Although usually highly conservative, the phylogenetic analysis of the small subunit sequences obtained from our worms indicated no inter-specific variations within members of the same species (all obtained sequences were 100% identical). However, a difference of 14 bases was detected between our worms and *H. guangdongensis*. It was observed that the alignment of the V4 region of all available SSU sequences of *Hysterolecithoides* species (Figure 5.5) shows that the sequence of *H. frontilatus* ex *S. nebulosus* which was submitted by Blair et al. (1998) was only identical to *H. guangdongensis*. Atopkin et al. (2018) concluded that *H. frontilatus* ex *S. nebuloses* and *H. guangdongensis* are both synonyms of *H. epinepheli* by comparing their SSU sequences.

However, using the LSU data the sequences of *H. frontilatus* which were submitted by Sokolov et al (2018) showed that it is slightly distant from *H. epinepheli*. This could propose that the LSU sequences of *H. epinepheli* ex *S. fuscescens* and *H. frontilatus* ex *S. fuscescens* are obtained from two distinct species. However, in their article, Atopkin et al. (2018) provided morphological evidence proving that the location of the distal part of the pars prostatica can be variable within the same species. It is noteworthy that our comprehensive morphological comparisons with all available data of *H. frontilatus* and *H. epinepheli* as well as the comparative measurements obtained from voucher slide and the additional measurements indicate that there are some morphometric variations between *H. epinepheli* and *H. frontilatus*.

These variations are most noticeable in the space between the two testes, position of ventral sucker relative to body length and the length of forebody as a percentage of body length (in *H. epinepheli* it is 1.41-1.02 times longer than *H. frontilatus*) (Figure 5.4). This observation might indicate that the trajectory of the distal part of the par prostatica alone isn't sufficient to differentiate between species of *Hysterolecithoides*. Thus, it is suggested to reconfirm these observations by

conducting a morphological analysis of these two species incorporating the additional measurements used in this study which further distinguish the three species as well as using molecular analysis to discern these speculations.

In conclusion, the morphological information obtained in the present study is consistent with the molecular data in justifying the consideration of *H. amurparuchinii* n. sp. as a new species to the genus *Hysterolecithoides*.

Acknowledgment

We are grateful to the Ministry of Agriculture and Fisheries Wealth, Sultanate of Oman, for the TRC Project ORG-EBR_11-TRC Grants, and to Laura Machkevska for help with the illustrations. Also, we would like to extend our thanks for Professor Kazuo Ogawa (Meguro Parasitological Museum), Mrs Eileen Harris (British Natural History Museum), for the voucher specimens loans.

6 Parasite communities of herbivorous *Siganus canaliculatus* (Perciformes: Siganidae) from the Sultanate of Oman and their potential to indicate marine ecosystem health

Abstract

For the first time, the parasite fauna of the herbivorous white-spotted rabbitfish, *Siganus canaliculatus* (Park) was used to study ecosystem health. Parasitological data of 210 host specimens from six sampling sites along the coasts of the Sultanate of Oman were analysed. Geographical variations in the parasite community along the Indian Ocean (Arabian Sea) until the Persian Gulf were detected by using multidimensional scaling (nMDS) and analyses of similarity (ANOSIM). Selected parasitological parameters and ecological indicators were utilised as biological descriptors, visualising the status of the marine environment. The resulting star graph identified Sohar inside the Gulf of Oman with its extensive industrial zone (including petrochemicals industry), as an affected location with less parasite diversity and mainly negative parasite descriptors, demonstrating environmental disturbance at this sampling site. The best environmental conditions based on the parasite fauna of *S. canaliculatus* were detected in two locations from the southern Omani coast in the Arabian Sea (Raysut and Masirah), followed by (Muscat) in the Gulf of Oman and (Khasab) in the Persian Gulf. This result demonstrates the effectiveness of the parasite community of an herbivorous marine fish as a bioindicators to evaluate the environmental conditions in the Sultanate of Oman. It provides the first baseline data for future studies regarding the use of fish parasites as bioindicators for ecosystem health in this less sampled region.

Keywords: Biological indicators, *Siganus canaliculatus*, Gulf of Oman, pollution, fish parasites

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[5] This article will be submitted as: Al Jufaili Sarah Hamoud, Unger Patrick Fabian, Machkevskyi Vladimir, Palm Harry Wilhelm (20..) Parasite communities of herbivorous *Siganus canaliculatus* (Perciformes: Siganidae) from the Sultanate of Oman and their potential to indicate marine ecosystem health

6.1 Introduction

The coastal zones are among the most dynamic regions in the world Oceans, although they only occupy 20% of the planet´s surface. They provide valuable resources by contributing to roughly 25% of the global biological production and supporting major important fisheries grounds (Norse 1993). About 70% of the world cities are located along the coasts with the inhabitants amounting to more than 40% of the global population and 75% of the world's largest urban agglomerations (Choudri et al. 2016). Consequently, the coastal ecosystems can be considered as the most vulnerable systems on earth due to constant exposure to anthropogenic stress and environmental degradation (Palm et al. 2011).

The Sultanate of Oman is situated in the south-eastern tip of the Arabian Peninsula. It has a vast coastline of 3,165 km (in fine-scale) extending from the entrance of the Persian Gulf (Strait of Hormuz) to the south of the western Arabian Sea near the borders of the Yemen Republic. The shores of Oman are surrounded by three water bodies from north to south: The Persian Gulf, the Gulf of Oman and the Arabian Sea. The Omani coastal waters are known for their high productivity and biodiversity due to their various habitats including intertidal mudflats, seagrass, algal beds, mangroves and coral reefs (DGNC 2010). However, recent developments and urbanisation combined with the unsustainable use of coastal resources have put substantial pressure onto the Omani coastal zones resulting in notable degradation of the marine ecosystems and a decline in natural resources and biodiversity (Choudri et al. 2016).

So far, most marine pollution monitoring programs in the country focused on direct analyses of chemical and physical parameters in sediment and water samples (e.g. Fowler et al. 1993; de Mora et al. 2003; 2004; 2005; Tolosa et al. 2005; Al-Rashdi and Suliman 2013). However, aquatic ecosystem health can also be indirectly assessed by using bioindicators (Palm et al. 2011). These organisms (free-living or parasitic) react sensitively to environmental conditions and change, leading to a wide range of applications, such as fish stock separation, feeding ecology, migration as well as environmental health indication (Palm 2011). Considering that aquatic parasites respond to anthropogenic pollution and environmental changes in a variety of ways, they are used to monitor aquatic ecosystem health (Sures 2001). Many studies have demonstrated their effectiveness to detect trace elements, heavy metals, eutrophication, pesticides and hydrocarbons in the environment (see Sures 2001). Several authors combined the changes in the structure of

parasite communities with physical, chemical, biochemical and histochemical analyses to assess environmental health (e.g. Barker et al. 1994; Diamant et al. 1999; Galli et al. 2001a; Chapman et al. 2015).

Palm and Rückert (2009) developed a method to visualise environmental differences between habitats using an array of selected fish parasitological descriptors and ecological parameters. A series of subsequent publications emended and successfully applied this method and utilized grouper parasites as biological indicators for different natural habitats (see Palm and Bray 2014), polluted sites (Neubert et al. 2016) and aquaculture localities in Indonesia (Palm et al. 2011) and Vietnam (Truong et al. 2017), respectively. The present study aims to apply this method for the assessment of six sampling sites along the coasts of Oman. For the first time this methodology is applied for the parasite community of a marine herbivorous fish, *Siganus canaliculatus*, of the family Siganidae. Non-metric multidimensional scaling (nMDS) analyses as well as the composition of the infra- and component parasite communities (see Holmes and Price (1986) and Bush et al. (1997)) were applied to demonstrate zoogeographical variation. With the help of 12 parasitological descriptors, diversity parameters and the star graph, the environmental status of each sampling site could be visualised.

6.2 Materials and methods

6.2.1 Host collection and examination

A total of 210 siganids were collected from six sampling sites along the coasts of Oman (Figure 6.1, Table 6.1). Samples were divided into two major geographical regions: North (Khasab, Dabba, Sohar and Muscat) and South of Al-Hadd cape (Masirah and Raysut). Furthermore, samples were subcategorised into three water bodies from north to south: Persian Gulf (PG), Gulf of Oman (GoO) and Arabian Sea (AS). These waters bodies correspond to three ecoregions according to the Marine ecoregions of the worlds (MEOW) system of Spalding et al. (2007).

Figure 6. 1 Sampling localities for whitespotted rabbitfish, Siganus canaliculatus along the coast of Oman.

Table 6. 1 Siganus canaliculatus collected from six localities off Oman.

Sampling site	Code	Coordinates	Sampling zone	Water body	Total length (cm)	Total weight (g)	Mean parasite abundance
Khasab	PG-1	26.1644°N; 56.2426°E	North	Persian Gulf	30±0.51	308±15.47	81±34.15
Dabba	GoO-1	25.6365°N; 56.2538°E	North	Gulf of Oman	31±0.51	331±16.96	159±81.00
Sohar	GoO-2	24.3461°N; 56.7075°E	North	Gulf of Oman	34±0.86[a]	536±15.62	63±45.18
Muscat	GoO-3	23.5859°N; 58.4059°E	North	Gulf of Oman	34±0.34[a]	485±15.62	619±264.08
Masirah	AS-1	20.3173°N; 58.6916°E	South	Arabian Sea	35±0.61[a]	593±30.28	552±198.14
Raysut	AS-2	16.9698°N; 53.9814°E	South	Arabian Sea	32±0.53	418±19.19	777±246.69

Hosts were transported to the laboratory in a thermal box, where they were deep frozen at -40°C. Upon examination, the total length (TL) and total weight (TW) of each fish were measured prior to dissection. Body surface, gills, branchial and body cavities, viscera (oesophagus, stomach, intestine, liver, gallbladder, spleen, heart, gonads and mesenteries), kidneys and musculature were examined with the aid of a ZEISS Stemi DV4 Stereo Microscope following Palm and Bray (2014). Due to the inability to quantify the abundance of microparasites (Microsporidians and Myxosporeans), only the incident and infection site of these parasites were noted.

Isolated parasites were collected, quantified and preserved in either 70% ethanol, 4% formalin or 95% ethanol for molecular identification. Helminths were stained with Acetic carmine or Mayer's paracarmine, differentiated, dehydrated, cleared in clover oil and mounted in Canada balsam. Nematodes were dehydrated in gradual ethanol series and transferred in 100% glycerine. Crustaceans were dehydrated and transferred onto Canada balsam. Obtained parasites were identified to the lowest taxonomic level possible, following available literature (Diamant and

Paperna 1986; Schmidt and Paperna 1978; Baker et al. 1993; Amin and Nahhas 1994; Martens and Moens 1995; Nahhas and Wetzel 1995; Bray and Cribb 1996; Geets and Ollevier 1996; Geets et al.1997; Bray and Cribb 1998; Bray and Cribb 2000; Bray and Cribb 2001; Sey et al. 2003; Yuniar et al., 2007; Tingbao et al. 2007; Kritsky et al. 2007a; Kritsky et al. 2007b; Amin and Van Ha 2011).

6.2.2 Parasitological and ecological parameters

For each sampling site, a variety of diversity parameters were calculated. These included the Shannon index of species diversity (Shannon 1948), Pielou index of evenness (Pielou 1966) and Berger-Parker index of dominance (Berger and Parker 1970). The ecto- to endoparasite ratio which was calculated according to Rückert et al. (2009a), included microsporidian and myxosporean parasites as present/absent data (Palm and Rückert 2009). The diversity indices of the metazoan parasites fauna were calculated from the abundance data of both ecto- and endoparasites (excluding microparasites such as Microsporea and Myxosporea). To investigate geographical variations among sampling sites, parasitological parameters (prevalence (P), mean intensity (mI) and mean abundances (mA)) for both infracommunity (parasites in individual hosts) and component communities (parasites in host population) were calculated according to Bush et al. (1997). Uninfected fishes as well as parasites with less than 10% prevalence, were omitted from the subsequent statistical multivariate analyses to remove possible satellite parasites (Carvallho et al. 2015; Pereira et al. 2014; Henriquez and Gonzalez et al. 2012).

6.2.3 Statistical analyses

To compare the characteristics of the parasite communities of *S. canaliculatus*, multivariate analyses was performed on the abundance data for the infracommunity level and on the prevalence data for the component community level using Primer program (release 7, Primer-E Ltd. Ivybridge, Devon, UK). Nonmetric multidimensional scaling (nMDS) was performed to visualize geographical variations in the composition of the *S. canaliculatus* parasite fauna. For multivariate analyses at the infracommunity level, microsporidian and myxosporean data were excluded from the analyses because it is not possible to calculate their abundance (Kleinertz and Palm 2015). Prior to the analyses, infracommunity abundance data were log-transformed (log (x+1)) to reduce the impact of the dominant species on those with low abundances. To compare geographical variation in parasite communities, a similarity index was constructed using the Bray-Curtis similarity

measure. Furthermore, a hierarchical agglomerative clustering was applied to the infra- and component communities using group-average linking.

One-way analyses of similarity (ANOSIM) were conducted on infracommunity abundance data with Bray-Curtis similarity measure to identify the differences in parasite species composition between the zones, water bodies and sampling sites (Table 6.1). In addition, routine similarity percentage analyses (SIMPER, Clarke 1993) was applied to test which parasite species contributed most to the differences in parasite infracommunities of *S. canaliculatus* with Bray-Curtis analyses, according to Bell and Barnes (2003). For the component community level, prevalence data of all recorded parasites (including microparasites) were used for the similarity index analyses, and nMDS was performed by using untransformed prevalence data with Bray-Curtis similarity index.

6.2.4 Visual integration

Visual integration of the obtained data sets was calculated using selected diversity and parasitological descriptors following Palm and Rückert (2009), Kleinertz and Palm (2015), Neubert et al. (2016) and Truong et al. (2017). To illustrate the star graphs, the prevalence of seven different parasite taxa (The myxosporeans *Ceratomyxa* spp., the monogenean *Glyphidohaptor* sp., the digeneans *Opisthogonoporoides* sp. and *Gyliauchen* spp., the acanthocephalan *Scllerocullom* sp., the nematode *Hysterothylacium* sp. and the copepod *Hatschekia* sp.) were used. The total Berger-Parker dominance index, total Shannon diversity index and Shannon diversity index for endoparasites, Pielou's Evenness index and ecto/endoparasite ratio were normalized onto a range of zero to 100 (Neubert et al. 2016). This is based on the assumption, that high values indicate natural environmental conditions and are oriented to the outer circle of the star graph and low values indicate affected environmental conditions and are oriented to the inner circle of star graph. For this reason, the Berger-Parker index was inversed to reflect the above assumption (Neubert et al. 2016).

6.3 Results

6.3.1 Parasite fauna and composition

Including the parasites with less than 10% prevalence, the parasite fauna of *S. canaliculatus* examined in the present study consisted of a total of 48 species. These belonged to the following taxa: one hyperparasite microsporidian, 13 myxosporeans, four monogeneans, 15 digeneans, one

cestode, four nematodes, four acanthocephalans, one species of Hirudinea and five crustaceans. Of the myxosporeans detected in the present study, *Ceratomyxa* spp. were the most predominant with a prevalence ranging from 20-69%. The ancyrocephalid monogeneans *Glyphidohaptor safiensis* Al-Jufaili, Machkevskyi, Al-Kindi & Palm, (unpublished) and *Tetrancistrum* spp. were the most predominant ectoparasites, with high prevalence ranging between 83-100% and 80-100 respectively. Among the endoparasites, the digenean *Opisthogonoporoides* sp. was the most prevalent with infection levels ranging between 46-66%. The highest total parasite abundance was recorded from Raysut sampling site (mean = 778.6 ± 246.7) and the lowest from Sohar (mean = 63.7 ± 45.2; Table 6.1). The richness varied across the sampled sites, the highest richness was recorded from Raysut and the lowest from Sohar with almost half of the value of the Raysut (Table 6. 2). All examined hosts were infected with at least one parasite taxa, except for some specimens from Sohar (n = 17).

6.3.2 Diversity indices

The Shannon diversity index varied between sampling locations, ranging from (0.83) Sohar to (2.19) Raysut. Similar results were obtained for the Shannon diversity index for endoparasites, with the value recorded from Sohar being less than half of Raysut (0.82 vs 1.79). The Pielou index was also lowest in Sohar (0.36), while Khasab and Raysut had the highest value (0.72). The Ec/En ratio was highest in Dabba and lowest in Sohar (0.50 and 0.0, respectively). The Berger-Parker index of dominance recorded in Sohar was about three times higher than from Raysut (0.65 vs 0.19; Table 6. 2).

Table 6. 2 Prevalence % (P), mean intensity (mI), mean abundance (mA) and diversity indices of whitespotted rabbitfish, Siganus canaliculatus parasites that were used for the multivariate statistical analyses collected from six locations off Oman (excluding parasites with 10% prevalence). Parasite used as environmental descriptors are marked with asterisk.

Water Body	**Persian Gulf**			**Gulf of Oman**									**Arabian Sea**					
Sampling site	**Khasab**			**Dabba**			**Sohar**			**Muscat**			**Masirah**			**Raysut**		
Parasite/Diversity index	**P%**	**mI(I)**	**mA**	**P%**	**mI(I)**	**mA**	**P%**	**mI(I)**	**mA**	**P%**	**mI(I)**	**mA**	**P%**	**mI(I)**	**mA**	**P%**	**mI(I)**	**mA**
Microsporidia sp.1 (hyperparasite) (Mi)	3	-	-	11	-	-	3	-	-	51	-	-	6	-	-	11	-	-
Zschokkella sp. (My)	3	-	-	29	-	-	11	-	-	51	-	-	23	-	-	17	-	-
Ceratomyxa spp. (My)*	63	-	-	31	-	-	40	-	-	20	-	-	37	-	-	69	-	-
Unicapsula fatimae (My)	0	-	-	6	-	-	40	-	-	9	-	-	11	-	-	31	-	-
Ortholinea spp. (My)	29	-	-	14	-	-	11	-	-	29	-	-	49	-	-	40	-	-
Kudoiidae indet. (My)	0	-	-	0	-	-	0	-	-	0	-	-	60	-	-	49	-	-
Glyphidohaptor safiensis (M)*	83	9(1-54)	7.80	94	49(1-373)	47.00	0	-	-	97	135(5-524)	131.00	97	52(2-282)	50.10	100	74(2-255)	75.00
Tetrancistrum spp. (M)	89	5(1-28)	4.37	94	17(1-126)	16.00	0	-	-	80	42(1-257)	34.00	97	48(2-197)	47.90	100	92(15-315)	94.60
Polylabris sp. (M)	11	4(1-8)	0.50	6	1(1)	0.10	0	-	-	11	4(1-8)	0.50	31	2(1-4)	0.50	49	2(1-6)	0.90
Hexangium spp.(D)	0	-	-	3	4(4)	0.1	6	2(1-2)	0.1	54	59(8-215)	9.40	26	14(2-76)	6.8	11	10(2-27)	0.20
Gyliauchen spp.(D)*	11	4(1-11)	0.40	0	-	-	0	-	-	80	17(1-112)	47	80	112(12-366)	102.80	80	92(2-357)	81.60
Opisthogonoporoides sp.(D)*	49	43(2-119)	21.80	0	-	-	46	70(6-279)	32.00	49	332(56-1327)	161.00	54	35(4-87)	25	66	169(7-673)	103.10
Schikhobalotrema sp. (D)	6	12(8-15)	0.70	0	-	-	0	-	-	20	24(4-110)	4.80	60	54(6-237)	47.8	94	115(6-1100)	77.80
Hapladena ljadovi (D)	0	-	-	0	-	-	0	-	-	6	6(1-10)	0.3	43	10(2-41)	4.70	23	6(2-12)	1.50
Thulinia sp. (D)	3	1(1)	0.03	0	-	-	0	-	-	34	4(1-8)	1.3	20	4(1-6)	0.90	63	14(1-112)	9.20
Aponurus sp. (D)	0	-	-	0	-	-	3	1(1)	0.00	17	5(2-15)	0.8	26	5(2-13)	1.50	37	6(2-31)	2.00
Hysterolecithoides sp. (D)	0	-	-	0	-	-	0	-	-	0	-	-	60	10(1-29)	6.20	63	5(1-28)	3.60

Table 6.2 (continued)

Water Body	Persian Gulf							Gulf of Oman						Arabian Sea				
Sampling site	**Khasab**			**Dabba**			**Sohar**			**Muscat**			**Masirah**			**Raysut**		
Parasite/Diversity index	**P%**	**mI(I)**	**mA**	**P%**	**mI(I)**	**mA**	**P%**	**mI(I)**	**mA**	**P%**	**mI(I)**	**mA**	**P%**	**mI(I)**	**mA**	**P%**	**mI(I)**	**mA**
Preptetos sp.(D)	0	-	-	0	-	-	0	-	-	0	-	-	40	3(1-6)	1.10	31	3(1-8)	1.00
Unisaccus sp. (D)	0	-	-	0	-	-	0	-	-	0	-	-	31	58(6-326)	16.70	9	5(1-11)	0.10
Stephanostomum spp. (D)	43	20(1-110)	8.8	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
Sclerodistomidae indet. adult (D)	0	-	-	17	3(1-7)	0.4	6	1(1)	0.1	0	-	-	0	-	-	0	-	-
Otobothrium sp.(C)	3	1(1)	0.90	3	19(19)	0.50	0	-	-	40	90(10-316)	36.10	0	-	-	11	2(1-6)	0.30
Hysterothylacium sp. (N)*	14	10(3-22)	1.40	6	35(4-66)	2.00	3	3(3)	0.10	66	12(2-59)	8.00	14	2(1-7)	0.60	20	11(1-31)	2.00
Capillaria sp.(N)	0	-	-	0	-	-	0	-	-	14	113(49-280)	16.2	23	11)1-55)	3.80	17	8(1-32)	1.60
Procamallanus sp. (N)	0	-	-	3	1(1)	0.00	3	3(3)	0.10	26	31(2-113)	8.10	26	14(2-44)	4.20	91	62(3-420)	48.80
Nematode indet. Larvae (N)	31	8(2-23)	0.00	6	5(3-6)	0.30	20	2(1-4)	0.40	6	2(1-2)	0.10	0	-	-	0	-	-
Sclerocollum sp.(A)*	66	20(2-186)	13.30	66	65(3-218)	42.50	14	108(4-280)	15.50	29	19(2-44)	5.50	26	14(2-44)	4.20	34	27(3-84)	13.70
Acanthocephala sp. 2 (A)	0	-	-	23	13(1-46)	3.10	11	8(3-12)	0.90	40	16(2-45)	6.30	0	-	-	40	34(8-100)	14.90
Acanthocephala indet. cystacanth	23	4(1-11)	0.90	37	24(1-129)	9.00	0	-	-	0	-	-	0	-	-	0	-	-
Acanthocephala sp. 3 (A)	20	3(1-10)	0.50	29	6(1-21)	1.8	6	1(1)	0.10	0	-	-	0	-	-	0	-	-
Hatschekia spp.(Cr)*	29	2(1-6)	0.70	14	3(1-10)	0.50	0	-	-	77	9(1-47)	6.90	14	14(7-24)	2.30	100	34(1-175)	36.00
Caligus spp.(Cr)	0	-	-	0	-	-	0	-	-	3	1(1)	0.00	94	114(9-670)	90.90	40	2(1-5)	1.00
Gnathiidae indet. sp.(pranzia larvae) (Cr)	11	1(1-2)	0.10	6	2(1-2)	0.10	0	-	-	3	17(17)	0.50	11	1(1-2)	0.20	14	1(1-2)	0.20
Ecto-to endoparasite ratio (Ec/En)*		0.36			0.50			0.10			0.44			0.44			0.41	
Shannon index of species diversity H` (total)*		1.90			1.50			0.83			1.93			2.08			2.19	

Table 6.2 continued

Water Body	**Persian Gulf**																	
Sampling site	**Khasab**			**Dabba**			**Sohar**			**Muscat**			**Masirah**			**Raysut**		
Parasite/Diversity index	**P%**	**mI(I)**	**mA**	**P%**	**mI(I)**	**mA**	**P%**	**mI(I)**	**mA**	**P%**	**mI(I)**	**mA**	**P%**	**mI(I)**	**mA**	**P%**	**mI(I)**	**mA**
Shannon index of species diversity H` (endoparasites)*		1.48			1.00			0.82			1.61			1.67			1.79	
Berger-Parker index of dominance d`(total)*		0.34			0.38			0.65			0.34			0.25			0.19	
Pielou index of evenness J` (total)*		0.72			0.58			0.36			0.63			0.71			0.72	

6.3.3 Multivariate analyses

The multidimensional scaling and ANOSIM of infracommunities revealed a slight separation of northern and southern samples (ANOSIM, R = 0.36, P = 0.001) (Figure 6.1A). The stress value of 0.2 indicates a reliable observation. When considering the three water bodies, samples obtained from AS aggregated and clustered together with a clear separation from the other sampling sites. Samples collected from both GoO and PG showed a more scattered and irregular distribution (ANOSIM, R = 0.42, P = 0.001) (Figure 6.1B), while PG was not different from GoO (R = 0.074, P = 0.37) but both PG and GoO were significantly different to AS (R = 0.91, P = 0.001 and R = 0.33, P = 0.001, respectively). As for individual sampling locations (Figure 6.1C), samples that were obtained from Raysut and Masirah (AS- southern region) were clustered with each other. Samples obtained from Muscat showed more similarity to samples from the southern region than to the samples collected from the same water body (Sohar and Dabba from GoO). Khasab samples which are the only samples obtained from PG, showed a more dispersed distribution while samples collected from Sohar were completely separated from all other samples. Overall, ANOSIM indicates a significant difference between all studied sampling sites (R = 0.68 at P= 0.01).

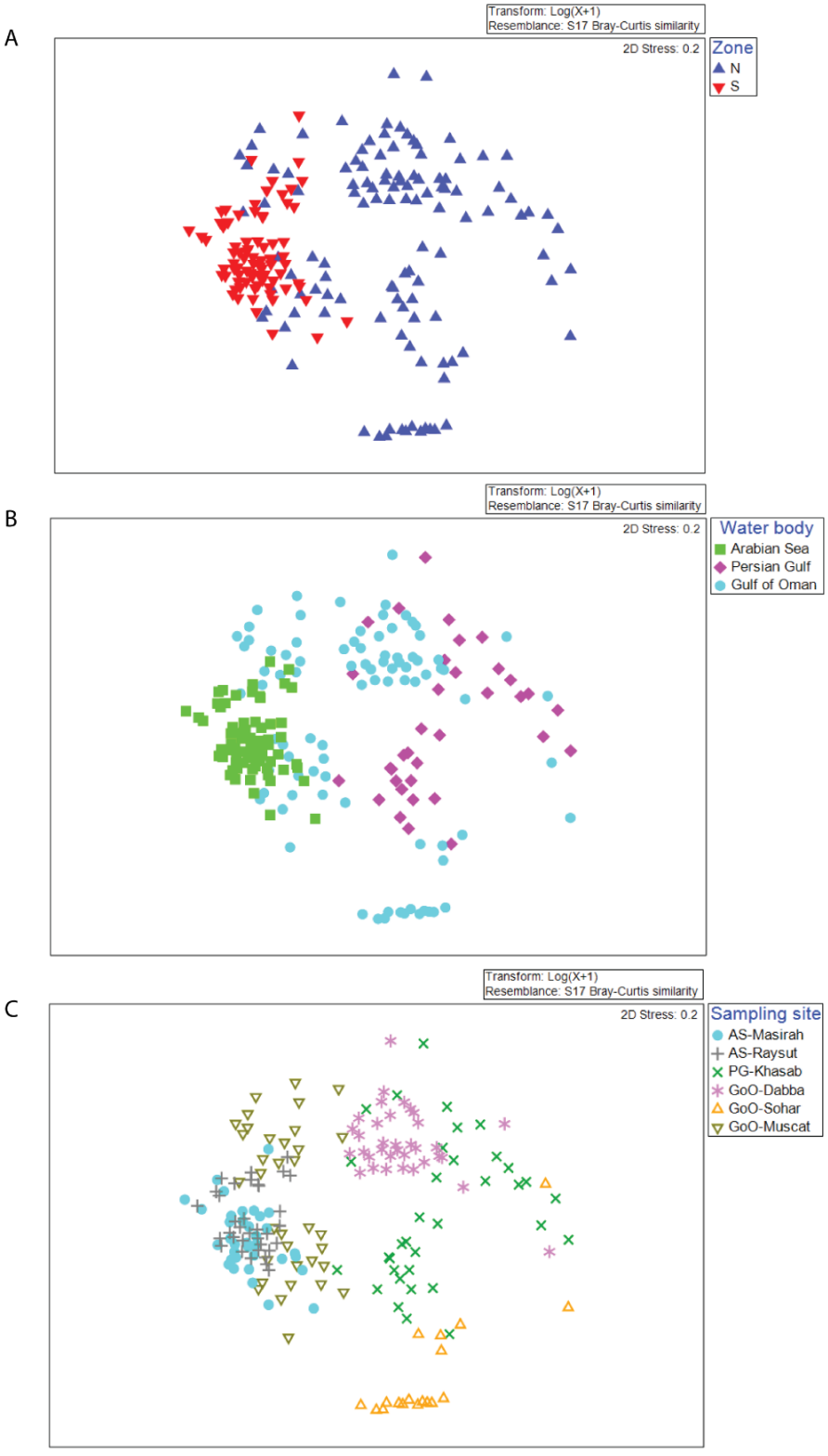

Figure 6. 2 Nonmetric multi-dimensional scaling plot of the parasite infracommunity of 193 specimens of Siganus canaliculatus from Omani waters using Bray-Curtis similarity index, North and South,(A), water bodies (PG- Persian Gulf, GoO- Gulf of Oman and AS- Arabian), (B), sampled locations, (C).

The nMDS and cluster analyses based on the total parasite prevalence data of *S canaliculatus* at the component community level using Bray-Curtis (untransformed) similarity measure (Figure 6. 2) revealed similar observations with the separation of the sampling sites into four clusters. Fish sampled from Raysut and Masirah sampling sites (South, AS) were clustered together showing strong association with 80% similarity. Samples from Muscat (North, GoO) were closest to both samples from the southern region with 60% similarity. Samples obtained from Dabba (North, GoO) were not similar to any of the other two samples that were obtained from the same water body (Sohar and Muscat, both North, GoO). Instead, samples obtained from Dabba showed higher similarity to those obtained from Khasab (North, PG) at 60% similarity level. Sohar samples were noticeably separated from all other samples. The stress levels of 0.0 on the figure indicate a parasite community composition that is substantially different from random.

With regard to SIMPER analyses, parasites contributing most to the dissimilarities did not reach very high percentage, indicating that several of the parasite species contributed similarly to the differences between the zones and habitats. The parasite species contributing the most to the geographical and regional differences at the infracommunity level was *Schikhobalotrema* sp. with 11.21% contribution at the zone level (north vs south). Between the different waterbodies, *Glyphidohaptor* sp. contributed with 14.75% to the dissimilarities between PG and GoO. *Hatschekia* spp. contributed with 11.59% to the dissimilarities between PG and AS. Lastly, *Schikhobalotrema* sp. contributed to the dissimilarity between GoO and AS at 11.16%. At the component community level, *Glyphidohaptor* sp. contributed to 16.61% of the dissimilarity between the two zones (north and south), it also contributed with 26.95% to the dissimilarity between PG and GoO. *Gyliauchen* spp. contributed with 18.38% and 15.91% to the dissimilarity between PG and AS, GoO and AS, respectively.

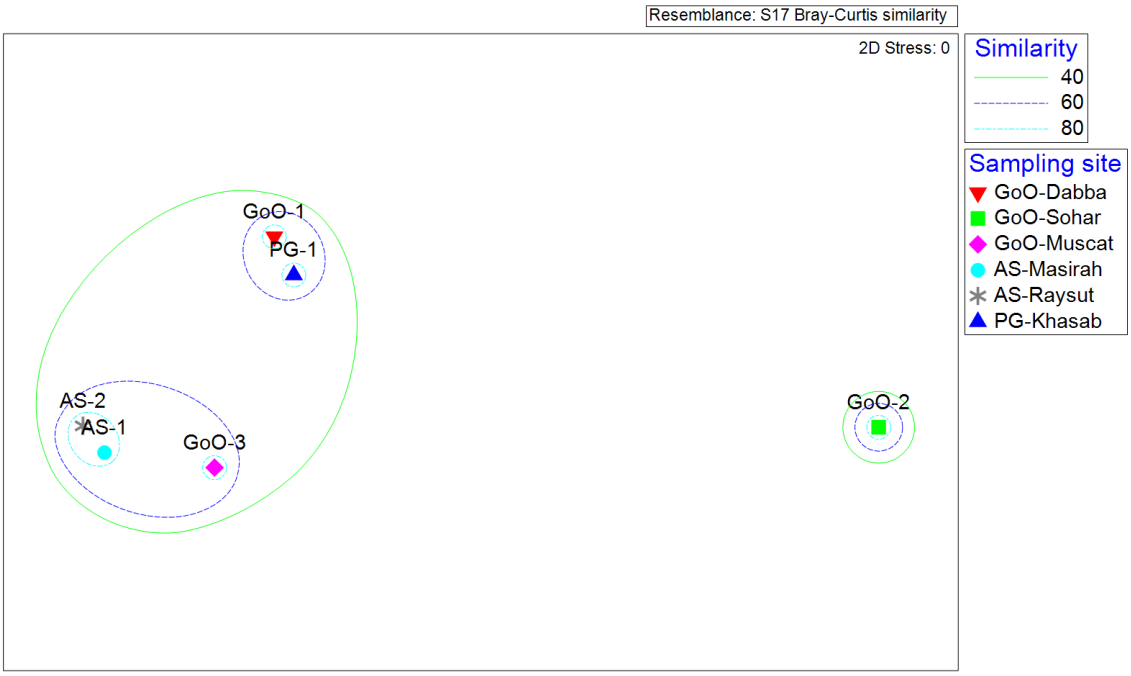

Figure 6. 3 Nonmetric multi-dimensional scaling plot based on the prevalence data of the component parasite communities of Siganus canaliculatus from six locations using Bray-Curtis similarity measure on untransformed data.

6.3.4 Biological descriptors and visual integration

Twelve descriptors from *S. canaliculatus* were evaluated as potential bioindicators for ecosystem health in Omani waters. The prevalence of seven parasite taxa and five diversity indices were chosen to visualize the obtained results in a star graph (Table 6.3). The largest star graph area was calculated for Raysut sampling site (25.4) followed by Masirah sampling site (19.2) and Muscat (16.4). Sohar sampling site had the smallest star graph area calculated in this study (0.9), (see Figure 6.3). To illustrate the environmental conditions based on the parasitological descriptors between the sampled locations, a pollution light following Neubert at al. (2016) is presented in figure 4 (x-axis range until 30 instead of 27). Analysed habitats assigned in a range from good (green), medium (yellow) and poor (red) to assess environmental conditions of studied Omani coastal waters.

Table 6. 3 Parasitological and diversity descriptors of Siganus canaliculatus *selected as bioindicators for the assessment of marine ecosystems in the Sultanate of Oman. Prevalence (%) data are followed by the normalized data in brackets.*

Sampling site	Khasab (PG-1)	Dabba (GoO-1)	Sohar (GoO-2)	Muscat (GoO-3)	Masirah (AS-1)	Raysut (AS-2)
Water body	Persian Gulf		Gulf of Oman		Arabian Sea	
Ceratomyxa spp. (p%)	63 (88)	31 (24)	40 (41)	20 (0)	40 (41)	69 (100)
Opisthogonoporoides sp. (p%)	49 (57)	0 (0)	46 (53)	49 (57)	86 (100)	66 (77)
Gyliauchen spp. (p%)	11 (14)	0 (0)	0 (0)	54 (68)	80 (100)	80 (100)
Sclerocollum sp. (p%)	66 (100)	66 (100)	14 (0)	29 (28)	26 (22)	51 (72)
Hysterothylacium sp. (p%)	14 (18)	6 (5)	3 (0)	66 (100)	17 (23)	20 (27)
Glyphidohaptor safiensis (P%)	83 (83)	94 (94)	0 (0)	97 (97)	97 (97)	100 (100)
Hatschekia spp. (p%)	29 (29)	14 (14)	0 (0)	77 (77)	94 (94)	100 (100)
Ecto- to endoparasite ratio (Ec/En)	0.36 (66)	0.50 (100)	0.10 (0)	0.44 (84)	0.44 (84)	0.41 (78)
Shannon index of species diversity H` (total)	1.90 (79)	1.50 (49)	0.83 (0)	1.93 (81)	2.08 (92)	2.19 (100)
Shannon index of species diversity H` (endoparasites)	1.48 (68)	1 (18)	0.82 (0)	1.61 (81)	1.67 (88)	1.79 (100)
Berger-Parker index of dominance d`(total)	0.34 (66)	0.38 (58)	0.65 (0)	0.34 (67)	0.25 (85)	0.19 (100)
Pielou index of evenness J` (total)	0.72 (100)	0.58 (62)	0.36 (0)	0.63 (77)	0.71 (96)	0.72 (100)

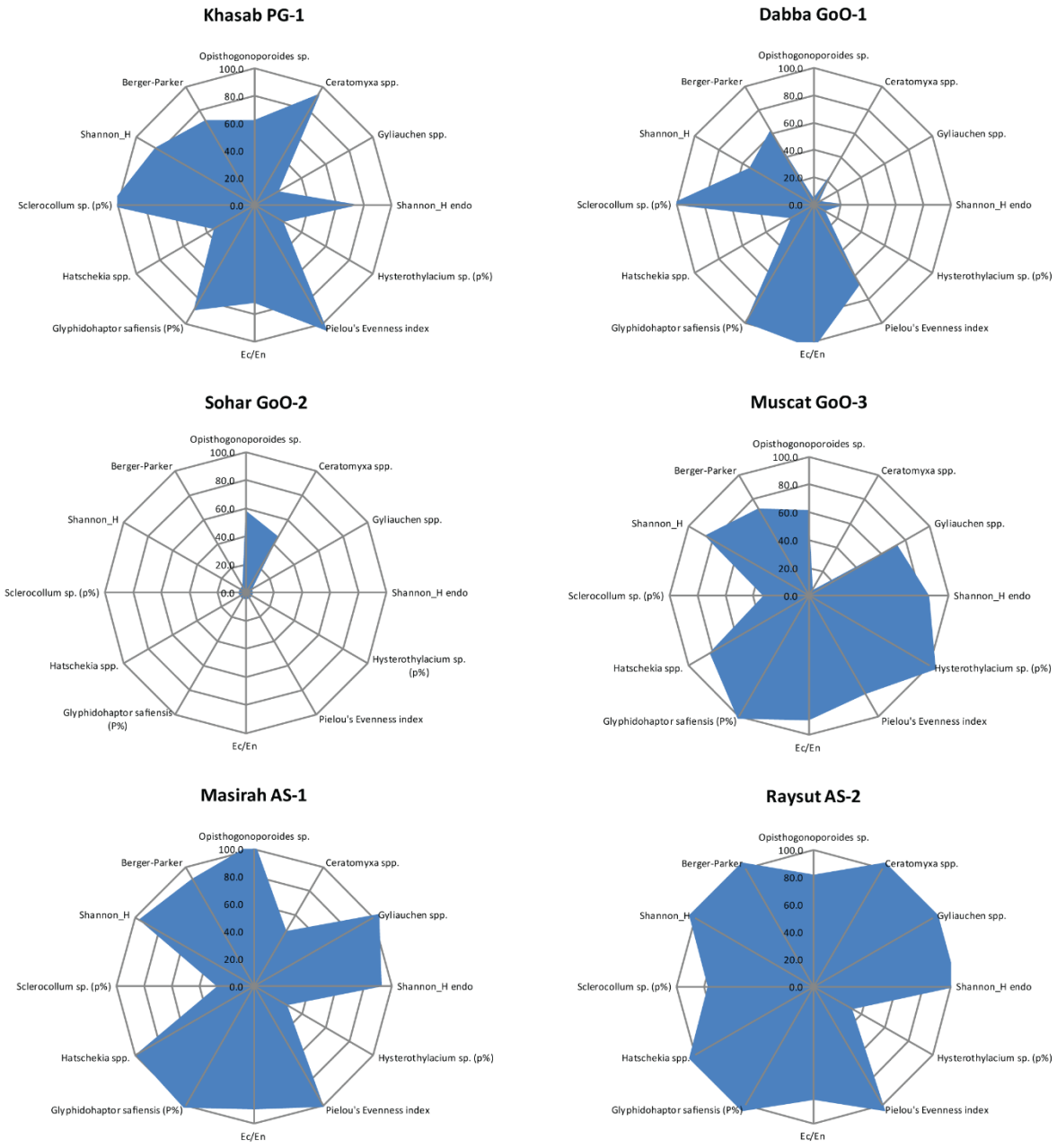

Figure 6. 4 Visual integration of seven parasitological and five diversity descriptors that were selected as environmental indicators of ecosystem status in Sultanate of Oman.

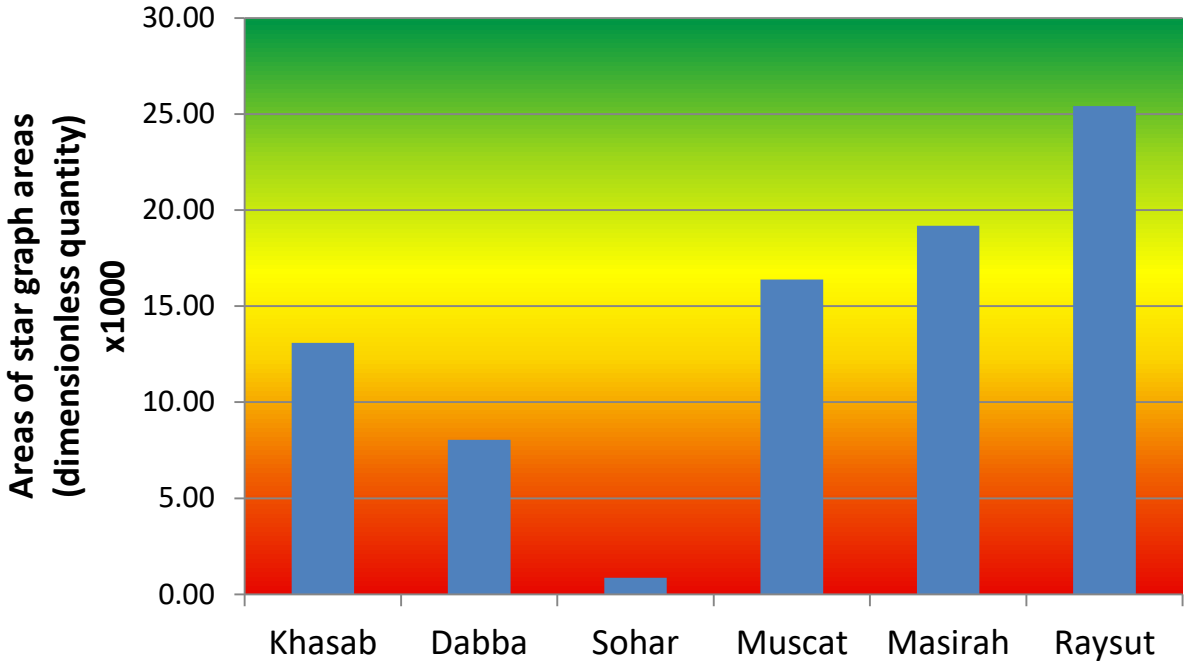

Figure 6. 5 Pollution light: the histogram represents calculated star graphs areas that were obtained from normalized parasitological and diversity parameters of Siganus canaliculatus parasite communities from six locations. The colour scheme reflects the status of the environment based on the parasite descriptors.

6.4 Discussion

6.4.1 Parasite descriptors for environmental health

The parasite fauna of the whitespotted rabbitfish *Siganus canaliculatus* was speciose, with 48 different ecto- and endoparasites. Of these, seven were considered useful to characterize the faunistic differences between the sampled locations and habitats. The use of myxosporean parasites as bioindicators is not as common as other endoparasites because they seem to show contrasting reaction to pollution or contaminants. Also, their occurrence depends on the considered host species, life cycle, and the type of contamination (Marcogliese and Cone 2001). For example, Khan and Thulin (1991) reported an increase of the gallbladder infecting myxosporean *Ceratomyxa acadiensis* Mavor, 1916 influenced by elevated petroleum aromatic hydrocarbons (PAH) levels (Palm 2011). The enrichment of oligochaete alternate hosts induced by high levels of faecal coliforms influenced an increase of prevalence of myxosporean infecting the cyprinid *Notropis hudsonius* (Clinton) (Marcogliese and Cone 2001). Similarly, the prevalence of *Henneguya guanduensis* Thelohan, 1892 infecting the catfish *Hoplosternum littorale* (Hancock) was higher in

polluted areas in comparison to less polluted sites (Dias et al. 2017). Recently, Truong et al. (2017) recorded an increase of *Ceratomyxa* infection levels from hosts sampled at a poorly managed mariculture site. The findings of Diamant et al. (1999) contrasted the above observations. These authors noted that the prevalence of *Ceratomyxa* spp. infecting the gallbladder of *S. rivulatus* was lower in polluted areas. In the present study the prevalence of *Ceratomyxa* spp. was relatively high at all investigated locations (highest in AS (Raysut) and PG (Khasab)), possibly distinguishing these water bodies.

Some ectoparasites can show parallels to free-living stages of endoparasites such as cercariae and coracidia, since they are in direct contact with the environment and toxic substances might affect their vitality or increase their mortality rates (Galli et al. 2001b; Sures et al. 2017). This makes them suitable effect indicators for water quality (Sures 2001). Several studies suggested a negative relation between monogenea and environmental disturbance. Among these Diamant et al. (1999) and Dzikowski et al. (2003) reported a significant reduction of monogeneans in polluted sites. Similarly, Sanchez-Ramirez et al. (2007) noted a decrease of the abundance of the monogenean *Cichlidogyrus sclerosus* Paperna & Thurston, 1969 at high concentrations of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls, and heavy metals. In the present study, complete disappearance of all monogenean (including *Glyphidohaptor safiensis*) were noted on hosts collected from Sohar even though these parasites were relatively abundant in the remaining sampling areas. This is unusual observation suggests high levels of environmental disturbance at Sohar sampling site, enabling the use of the monogeneans *G. safiensis* as a bioindicator in Omani waters.

Palm (2011) and Sures et al. (2017) stated that the prevalence of digeneans in their intermediate and definitive hosts are inversely related to the degree of pollution and disturbance of the aquatic ecosystem. Siddal et al. (1994) reported a reduction of the digenean *Zoogonoides viviparus* (Olsson, 1868) Odhner, 1902 prevalence in snails due to sewage sludge (Lafferty 1997). Diamant et al. (1999) noted the absence of all gut parasites from *Siganus rivulatus* (Forsskål & Niebuhr) sampled from disturbed sites. Galli et al. (2001b) noted that high levels of eutrophication limited the existence of the digeneans *Asymphylodora tincae* Modeer, 1790 and *Diplostomum spathaceum* Rudolphi, 1819. Furthermore, Vidal-Martínez et al. (2003) found a significant negative correlation between DDT concentrations and the intensity of a digenean metacercaria

Mesostephanus appendiculatoides Price, 1934. In the present study, the Digenea *Opisthogonoporoides* sp. showed relatively high mean abundance and high prevalence throughout the sampling sites. However, it was absent from Dabba sampling site. As for *Gyliauchen* spp. it was absent from both Dabba and Sohar sampling sites. These observations could be linked to unfavourable water conditions, reduction or absence of the required intermediate host(s) or changes in the food web dynamics at both sites. Consequently, we chose *Gyliauchen* spp. and *Opisthogonoporoides* sp. as bioindicators, since they were recorded frequently with relatively high abundances from most of the other sampling sites.

Lafferty (1997) described a positive relation between nematodes and eutrophication, thermal effluent and crude oil. The meta-analyses conducted by Vidal-Martinez et al. (2010) also revealed a positive relation between the nematode populations and eutrophication, but reported a negative impact due to pulp-mill and crude oil. In contrast, Blanar et al. (2009) reported no significant relationship between any form of pollutants and nematode populations. However, Diamant et al. (1999) noted that nematodes infecting *S. rivulatus* were limited to undisturbed sites. Similarly, the nematode *Cucullanus heterochrous* Rudolphi, 1802 infecting the midgut of the European flounder *Platichthys flesus* L. (Linnaeus) had significantly higher prevalence in an unpolluted site off Helgoland in the German Bight (Broeg et al. 1999). In a tropical region, Kleinertz et al. (2014) and Neubert et al. (2016) reported significant reduction in endoparasitic helminths such as the nematode *Raphidascaris* sp. from polluted sites. Our findings support this observations, where the nematode *Hysterothylacium* sp. had lowest incidence at Sohar (both prevalence and intensity) and highest records at Muscat. In order to interpret the variations in abundance and prevalence of *Hysterothylacium* sp. between the sampling sites, further information on the diversity and distribution pattern of the potential intermediate hosts in Omani waters will be required.

The infection levels of acanthocephalans were previously used as bioindicators for anthropogenic alterations. Kussat (1969) observed that the infection levels of two acanthocephalans *Octospinifer macilentus* Van Cleave, 1919 and *Neochinorhynchus cristatus* Lynch, 1936 in the freshwater catfish *Catostomus commersoni* (Lacépède) increased with higher concentrations of industrial and domestic waste (Galli et al. 2001b). Galli et al. (2001) reported that the prevalence of the acanthocephalan *Acanthocephalus anguillae* Müller, 1780 was highest in heavily polluted sites, while the acanthocephalan *Pomphorhynchus laevis* Müller, 1776 from the

same host was highest in non-polluted areas. Schludermann et al. (2003) found that the prevalence of *P. laevis* was lower from heavily polluted sites. Similarly, both Kleinertz et al. (2014) and Neubert et al. (2016) reported the reduction or complete elimination of acanthocephalans in the investigated parasite communities under polluted conditions. It is obvious that acanthocephalans can react positively or negatively to the changing environment. In the present study, *Sclerocollum* sp. was chosen as a useful bioindicators because it was detected from all sampling sites with different prevalence, the highest being in Khasab and Dabba and lowest from Sohar. The exact cause of this variation in *Sclerocollum* sp. populations remains unclear and might be connected to the abundance and distribution of benthic amphipods which act as intermediate hosts (Taraschewski 2005). Also, *Sclerocollum* sp. can be used as accumulation indicators because it fulfils the criteria required for sentinels as suggested by Sures (2004).

Ectoparasitic copepods are also sensitive to changes in water quality, often with negative relation to different types of pollution (Galli et al., 2001a). The copepods *Achtheres percarum* Nordmann, 1832 and *Caligus lacustris* Steenstrup & Lütken, 1861 were completely absent from sites that were closest to the point of pulp mill effluent discharge (Overstreet and Thulin 1991). The prevalence of three species of crustacean ectoparasites *Lerneocera branchilais* Linnaeus, 1767, *Lepeophtheirus pectoralis* Müller, 1776 and *Acanthochondria* sp. infecting *P. flesus* L. were consistently lower in the most polluted sites (Elbe estuary, German Bight) (Broeg et al. 1999). Unger and Palm (2016) noted the absence of ectoparasites from cultured rainbow trout *Oncorhynchus mykiss* (Walbaum) in the western Baltic Sea, indicating the strong influence of the variable and changing conditions in this mesohaline waters on ectoparasites.

Members of the copepod genus *Hatschekia* Poche, 1902 are also useful as bioindicators. For example, Carreras-Aubets et al. (2011) reported the absence of the copepod *Hatschekia* mulli Van Beneden, 1851 from its host, the red mullet *Mullus barbatus* (Linnaeus) sampled from an anthropo-impacted site. Neubert et al. (2016) reported the complete absence of *Hatschekia* sp. from polluted site off Jakarta Bay. In the present study *Hatschekia* spp. (applied for the first time to the star graph system) were recorded from all investigated sampling sites (unpolluted) with moderate infection levels, but were absent from the polluted site Sohar showing a clear influence of water quality on this parasite.

6.4.2 Ecological descriptors

The Shannon diversity indices (excluding microparasites), Berger-Parker index, Evenness and the ecto- to endoparasite ratio index were used to indicate environmental disturbance at the sampling sites, following Neubert et al. (2016) and Truong et al. (2017). Many researchers recorded correspondence between low parasite species richness and diversity with environmental disturbance or pollution such as low pH levels (Cone et al. 1993; Marcogliese and Cone 1996; Halmetoja et al. 2000), organic pollution (Dusek et al. 1998; Galli et al. 2001b) and anthropogenic pollution (Rückert et al. 2008; Palm and Rückert 2009). In the present study, both total and endoparasite Shannon diversity indices were used as bioindicators, assuming that a healthy, functioning and resilient ecosystem is a system that is diverse and rich in parasite species (Sures et al. 2017). The index values were lowest at Sohar and highest at Raysut. This was most likely related to the low parasite richness and abundance that was observed in Sohar compared with the other locations.

Galli et al. (2001b) and Schludermann et al. (2003) reported high Berger-Parker index values from non-polluted sites. Contrasting observations were recorded by Kleinertz and Palm (2015) reporting highest Berger-Parker index value from a polluted site (Segara Ankan Lagoon) and the lowest from the healthy site (Bali). Similarly, Neubert et al. (2016) reported high Berger-Parker index value of (0.99) for endoparasites from the polluted area (Jakarta Bay) and lowest value from the less polluted (0.57). In the present study, total Berger-Parker index of dominance were highest at Sohar (0.65) and lowest at Raysut sampling site (0.19). This is reflected by the lower diversity and the predominance of few parasite species that was observed in Sohar. This finding is in agreement with the original definition of the index where a low diversity is associated with a disturbed environment, resulting in high Berger-Parker index value (Caruso et al. 2007).

Low evenness indicates an increase in the predominance of generalists or more tolerant species in a parasite community (Johnson and Roberts 2009). Thus, a parasite community with high evenness values is rich in species and exhibits an even distribution of parasites. Galli et al. (2001b) investigating the parasite fauna of chub *Leuciscus cephalus* (Linnaeus) reported a high evenness value at the river site with the highest eutrophication. Similarly, Schludermann et al. (2003) reported the highest evenness from the cyprinid barbel *Barbus barbus* (Linnaeus) sampled from river sites with the highest level of heavy metal pollution. Contrasting findings were reported

from several species of groupers off Indonesian waters, where consistently higher evenness index values (Pielou evenness index for endoparasites) were reported from non-polluted areas (Kleinertz et al. 2014; Kleinertz and Palm 2015; Neubert et al. 2016). In the current study, the highest total evenness index was recorded from Khasab and Raysut (0.72) followed by Masirah (0.71). While Sohar sampling site had the lowest recorded evenness index (0.36). This could be interpreted by the overall low species richness and the domination (uneven distribution of abundance) of few parasites (e.g. *Sclerocollum* sp.) in the parasite community of hosts sampled from Sohar.

The ecto- to endoparasite ratio index indicates that a parasite fauna with low endoparasites and consequently high Ec/En ratio represents an unnatural parasite infracommunity in a predatory fish species, such as groupers (Palm and Rückert 2009). Findings of Kleinertz and Palm (2015) and Neubert et al. (2016) both reported high Ec/En ratios in heavily polluted areas for the grouper *Epinephelus coioides* (Hamilton). Similar observations were obtained on some non-predatory hosts from the same region. Rückert et al. (2008) reported a low Ec/En value of (1.8) from the omnivorous *Scatophagus argus* (Linnaeus) sampled from non-polluted areas compared to a high value of (5) from polluted sites. Also, Palm & Rückert (2009) recorded high Ec/En ratio from the herbivorous *Mugil cephalus* (Linnaeus) (4.5 and 5.7) sampled from a polluted site (Segara Anakan Lagoon). In comparison to the observations above, the Ec/En parasite index value were generally lower in most of the sampling areas investigated in the present study. It was drastically reduced in Sohar (0.0), linked to the complete absence of all ectoparasites. In *S. canaliculatus* the naturally diversity and abundance of endoparasites is high. Bray & Palm (2014) listed Ec/En ratios for different fish families and demonstrated that beside the environment, the fish species itself strongly influences this index. Because we had an herbivorous fish with a unique feeding ecology, different results concerning its Ec/En ratio compared with the groupers from earlier studies are evident.

6.4.3 Geographical variation in parasite communities along the Omani coast

Geographical variations were detected for the infection indices and diversity parameters between the zones, water bodies and sampling sites. Different geographical and oceanographical conditions occur along the coasts of Oman. The coast is hydrologically divided into the North-east (including GoO and PG) and the South-west (AS) region by the Ras/Cape Al-Hadd frontal zone. This frontal zone acts as a boundary that separates the water masses between the two regions (North and South) (Piontkovski et al. 2011). However, the frontal zone is a seasonal phenomenon and is

strongly pronounced especially during summer and gradually decays in fall (Piontkovski et al. 2011). This causes interaction between the two zones and may explain the low correlation value that was detected by ANOSIM and an overlap of the parasite fauna of some hosts from GoO with samples from AS.

We observed that the waterbodies PG and GoO clustered together in the cluster analysis and MDS, with no significant difference in the parasite composition (ANOSIM). This is caused by their geography (relatively shallow semi-enclosed basins subject to extreme conditions) and connectivity between both systems. The GoO is a subtropical basin that has variable depths ranging from 70 m (towards the Strait of Hormuz) until 3,000 m (in its oceanic part) (Piontkovski et al. 2012). During Northeast Monsoon in winter, cooler water masses coming from the AS oceanic regions pass through the GoO towards the PG (cooling effect) (Piontkovski et al. 2011). This event causes the formation of a conventional up-welling and mesoscale eddies as a result of decreased water temperatures. The occurrence of the conventional up-welling enriches the surface water and aids flourishing phytoplankton blooms and growth of macrophytes (Jupp 2002). In contrast, the Southwest Monsoon during summer induces outflow of a high saline water mass coming from PG, which results in high water temperatures and low oxygen levels, low primary production and decreasing fishery catches (Piontkovski et al. 2012; Wang et al. 2013). The PG is both shallow and narrow with an average depth of 36 m (Cavalcante et al. 2016) and is considered as one of the harshest marine environments in the world due to prevailing natural stressors, with high salinity levels, temperatures, UV exposure, and reduced levels of pH (Naser 2011; de Mora et al. 2003; 2004). These extreme conditions are accompanied by weak development of phytoplankton and macrophytes (Jupp 2002) and consequently lower biodiversity.

There was high significant difference between the water bodies from the PG and GoO compared with the AS. The Arabian Sea is influenced by the event of one of the most powerful up-welling in the world during the South-west (summer) Monsoon (Jupp 2002), lowering the salinity and water temperatures below 20˚C, enriching the surface waters with nutrients followed by increased primary production of phytoplankton and high levels of chlorophyll-A (Piontkovski et al. 2012). Consequently, the AS has high productivity and higher marine biodiversity of e.g. macrophytes (Jupp 2002), mollusks (Al-Siyabi pers. comm.) and fish (MoAF 2015). This is

reflected by the highest parasite species richness and biodiversity in the AS, and might also explain the site "exclusive" species *Hysterolecithoides* sp., *Preptetos* sp. and Kudoiidae indet.

6.4.4 Visualization of environmental health

Neubert et al. (2016) linked the star graph area to the environmental conditions of the respective location. In their study, the lowest star graph area was calculated from Jakarta Bay (0.3) which was described as a highly disturbed environment and the highest star graph area was calculated from Balinese water (24.2) as a relatively healthy environment according to Kleinertz et al. (2014). Since Sohar (GoO) had the smallest star graph area while Raysut (AS) had the largest area, it is suggested that Sohar is under the strong influence of environmental disturbance. This assumption is supported by the information resolved from the above discussed parasitological parameters and ecological indicators. It is worth mentioning that Sohar is has one of the largest industrial areas in Oman known as the Sohar Industrial Zone (SIZ) (Al-Sawai 2015; Al-Wahaibi and Zeka 2015). SIZ is a coastal industrial estate that operates a wide range of petrochemical, metal based and agricultural industries (Al-Wahaibi and Zeka 2015; Jupp et al. 2017).

Some studies have indicated the availability of trace elements and heavy metals in the environment at SIZ and the surrounding areas (Al-Shuely et al. 2010; Al-Rashdi and Sulaiman 2013). Furthermore, a recent study revealed elevated levels of several heavy metals such as Cd, Cr and Pb from sediment samples collected from SIZ and adjacent areas (Al-Sawai 2015). However, no information is available regarding the distribution and levels of heavy metals and contaminants in the fishing grounds in Sohar. Thus, it is advisable to conduct a multidisciplinary investigation in Sohar fishing harbor and fishing grounds to evaluate the physical and chemical characteristics of the environment as well as the collection and analyses of different organisms (also fish parasites) to test their potential as bioindicators in the bioassay.

6.4.5 Conclusions

The present study highlights the usefulness of selected parasites of *S. canaliculatus* as bioindicators for the assessment of environmental health along the coasts of Oman. The suitability of applying the star graph method as a tool to visualize differences and possibly changes in the ecosystems is supported. Thereby, combining regular monitoring programs of the Omani coastal areas with the presented methodology is recommended in order to evaluate and observe the environmental status in the different regions. Furthermore, the additional use of the same

endoparasites from *S. canaliculatus* (e.g. *Sclerocollum* sp. and *Hysterothylacium* sp.) as potential bioaccumulators for long-term monitoring programs is also advised. We herewith emphasize the importance of ecological parasitology in order to evaluate environmental quality and changes in marine ecosystems. This method can be applied for environmental monitoring programs to support management measures by different stakeholders, contributing to governmental initiatives on coastal zone management, national legislation and marine ecosystem protection regulations in the Sultanate of Oman.

7 General Discussion

The main objectives of the present thesis were to investigate the parasite fauna of an herbivorous reef-associated fish belonging to the teleost family Siganidae from the waters of the Sultanate of Oman, describe the less known parasite species and apply its parasites as bioindicators for marine ecosystem health. The White-spotted rabbitfish *Siganus canaliculatus* was chosen, based on its high local commercial value and promising prospect for the development and diversification of the mariculture industry in the country. Carried out at seven localities along the entire Omani coast, this study is the first comprehensive investigation of fish parasites from the three water bodies surrounding the Sultanate of Oman. The selection of these localities was based on their potential as future aquaculture sites as well as their geographical and oceanographical differences. More than 40 different taxa of marine parasites were identified from *S. canaliculatus*, including several new locality (6), host records (16) as well as novel species to science (4). Together with the previously obtained parasite-host data (see chapter 1, section 1.6), the results of the present study indicate a highly diverse parasite fauna in the waters of Oman. Additionally, ecological analyses of the parasitological data revealed geographical variations in the composition and structure of *S. canaliculatus* parasite communities. This is in agreement with the documented different geographical and oceanographic conditions of the three investigated water bodies. Finally, parasites of *S. canaliculatus* have the potential to be utilized as biological indicators for the host stock populations and the environmental health status. Therefore, the present study strongly emphasizes the importance of fish parasitological investigations in the region.

7.1 Parasite community of *Siganus canaliculatus* in Omani waters.

In comparison to other siganids which have been previously investigated (e.g. *S. rivulatus*, *S. luridus*, *S. argenteus*, *S. sutor* and *S. doliatus*) (Diamant and Paperna 1986; Geets et al. 1996; Geets and Ollevier 1997; Martens and Moens 1995; Kleeman 2001), the parasite fauna of *S. canaliculatus* from Omani waters is more diverse and is characterized by the predominance of myxosporeans and digenetic trematodes. The parasite community of *S. canaliculatus* in the present study consisted of one microsporidian hyperparasite, 13 myxosporeans, four monogeneans, 15 digeneans, one cestode, four nematodes, four acanthocephalans, one species of Hirudinea and five crustaceans. Prior to this investigation, only five species of metazoan parasites were known infecting *S. canaliculatus* in Omani waters. The first marine parasite reported from *S. canaliculatus* is the microcotylid monogenea *Polylabris gerres* Mamaev & Paruchin, 1976 (=*P.*

mamaevi Ogawa & Egusa, 1980) from the Gulf of Masirah, coast of Arabian Sea (Mamaev and Paruchin 1976; Tingbao et al. 2007). Paruchin (1978) reported the digenean *Hapladena ljadovi* Paruchin, 1978 from Souqarah Bay, coast of Arabian Sea. This digenean is only reported from *S. canaliculatus* inhabiting the waters of Oman. Other records included the digeneans *Hysterolecitha sigani* Manter, 1969 (=*Thulinia microrchis* (Yamaguti, 1934) Bray, Cribb & Barker, 1993), *Gyliauchen papillatus* Goto & Matsudaira, 1918 and the camallanid nematode *Procamallanus annulatus* Yamaguti, 1955 all from Souqarah Bay (Paruchin 1989).

In the present study several other species were reported for the first time from this host. An unidentified microsporidian hyperparasite invading the plasmodia of *Zschokkella* sp. was detected from the gallbladder of *S. canaliculatus* with prevalence ranging between 3-51% (see chapter 6). To our knowledge, so far only three species of microsporidian hyperparasites are known to infect myxosporeans. Diamant and Paperna (1986) reported the microsporidian *Nosema ceratomyxae* Diamant & Paperna, 1985 invading the plasmodia of *Ceratomyxa* sp. in the gallbladder of *S. rivulatus* from the Red Sea. Two unidentified microsporidian hyperparasites were detected from two myxosporean species (*Leptotheca fugu* Tun, Yokoyama, Ogawa & Wakayabashi, 2000 and *Enteromyxum fugu* Tun, Yokoyama, Ogawa & Wakayabashi, 2002) infecting cultured tiger puffer, *Takifugu rubripes* (Temminck & Schlegel) (Freeman 2005). Preliminary molecular investigation of our microsporidian hyperparasite suggests that it is different from the above mentioned species and might be a new species within the microsporidian genus *Pleistophora* Gurley, 1893 or *Glugea* Thélohan, 1891.

Among the 13 species of myxosporeans recorded in *S. canaliculatus* one belonged to the genus *Zschokkella*, four to *Ceratomyxa*, three to *Ortholinea*, three to *Kudoa* and one to *Latyspora* Bartošová, Freeman, Yokoyama Caffara & Fiala, 2011. Species belonging to the first four of these myxosporean genera were already reported from siganids of the Red Sea (Diamant and Paperna 1986; Diamant and Paperna 1992; Diamant et al. 2005; Abdel-Ghaffar et al. 1998; Abdel-Baki et al. 2015). Myxosporean parasites resembling members of the currently monospecific genus *Latyspora* were detected in the kidney parenchyma of *S. canaliculatus*. *Latyspora scomberomori* Bartošová, Freeman, Yokoyama Caffara & Fiala, 2011 was described from the kidney tubules of the scombrid host the Indo-Pacific mackerel, *Scomberomorus guttatus* (Bloch & Schneider) sampled from Malaysia (Bartošová et al. 2011). The species recorded in the present study showed

some morphological differences (such as, spore shape and size) to *L. scomberomoi* indicating that it might be a new species in the genus. In addition, the present finding represents the first registration of a member of this genus from a siganid host.

Also, within the present study, a new myxosporean species of the genus *Unicapsula* Davis, 1924 was described. *Unicapsula fatimae* Al-Jufaili, Freeman, Machkevskyi & Palm, 2015 is the 13th member described since the erection of the genus. Unlike other members of the genus which mostly infect the musculature (striated muscles) of their hosts (excluding *U. maxima* which infects the kidney parenchyma and *U. marquesi* infecting the gill filaments), the new species is the only member of the genus infecting the oesophagus epithelium lining (smooth muscles) of its host. In addition, with its spherical shape that is attached to the inner lining of the host's oesophagus with a peduncle (see chapter 2), the cyst structure and composition was also unique to *U. fatimae* among all other members of the genus. So far this species is the only member of the genus which is reported from an herbivorous marine fish.

Myxosporeans are categorized as heteroxenous parasites with complex life cycle that involve annelid worms (polychaetes and oligochaetes in marine waters) and byrozoans (in freshwater) as intermediate hosts (Eszterbauer et al. 2015). The life cycle of marine myxosporeans is achieved through the infection of fish hosts with actinospores as a result of ingesting annelid worms (Alexander et al. 2015). Although the life cycles of myxosporeans infecting *S. canaliculatus* are unknown, it is can be assumed that the richness of myxosporean parasites in *S. canaliculatus* is linked to the abundance and availability of oligochaete or polychaete in Omani waters. Also, this observation might also suggest that *S. canaliculatus* inhabiting Omani waters is actively preying on these worms as part of its diet.

Digenean trematodes are the most extensively investigated group of parasites in siganids from various localities in the Indo-Pacific region (Yamaguti 1953, Madhavi 1972; Diamant and Paperna 1986; Barker et al. 1993: Bray and Cribb 1996; Arthur and Lumanlan-Mayo 1997; Bray and Cribb 2000; Bray and Cribb 2001; Hall and Cribb 2004; Shih et al. 2004; Hassanine and Gibson 2005; Al-Jahdali and Hassanine 2012). They exhibit two or three-host life cycles that involve molluscs as obligatory first intermediate host, crustaceans as optional second intermediate host and a vertebrate definitive host (Cribb et al. 2001). As an additional finding, while sampling the fish parasites from the stomach, a range of different amphipods and copepods as prey items were

detected in the stomach contents (Figure 7.1). Among the detected digeneans from *S. canaliculatus*, members of the digenean family Lecithasteridae (e.g. *Aponurus*, *Thulinia* and *Hysterolecithoides*) exhibit lifecycles that involve a gastropod as the first intermediate host and a copepod as the second intermediate host to reach the teleost definitive host (Bray et al., 1999). The occurrence of adult lecithasterid digeneans in *S. canaliculatus* is justified by the various copepods in its stomach.

The remaining species include representatives of the digenean families Haplopridae (e.g. *Unisaccus* Martin, 1973) and Haplosplanchnidae (e.g. *Schikhobalotrema* Skrjabin & Guschanskaja, 1955) as well as members of the genera *Gyliauchen* and *Hexangium*, which conduct life cycles involving the ingestion of encysted metacercaria that are attached to aquatic vegetation (Williams 1994; Al-Jahdali and El-Said Hassanine 2012; El-Said Hassanine et al. 2016; Huston et al. 2016). Thereby, the richness of digenean parasites in *S. canaliculatus* can be linked to the diverse prey items found in the host diet of the sampled fish as well as the evolutionary adaptation of the parasites to the host´s feeding ecology.

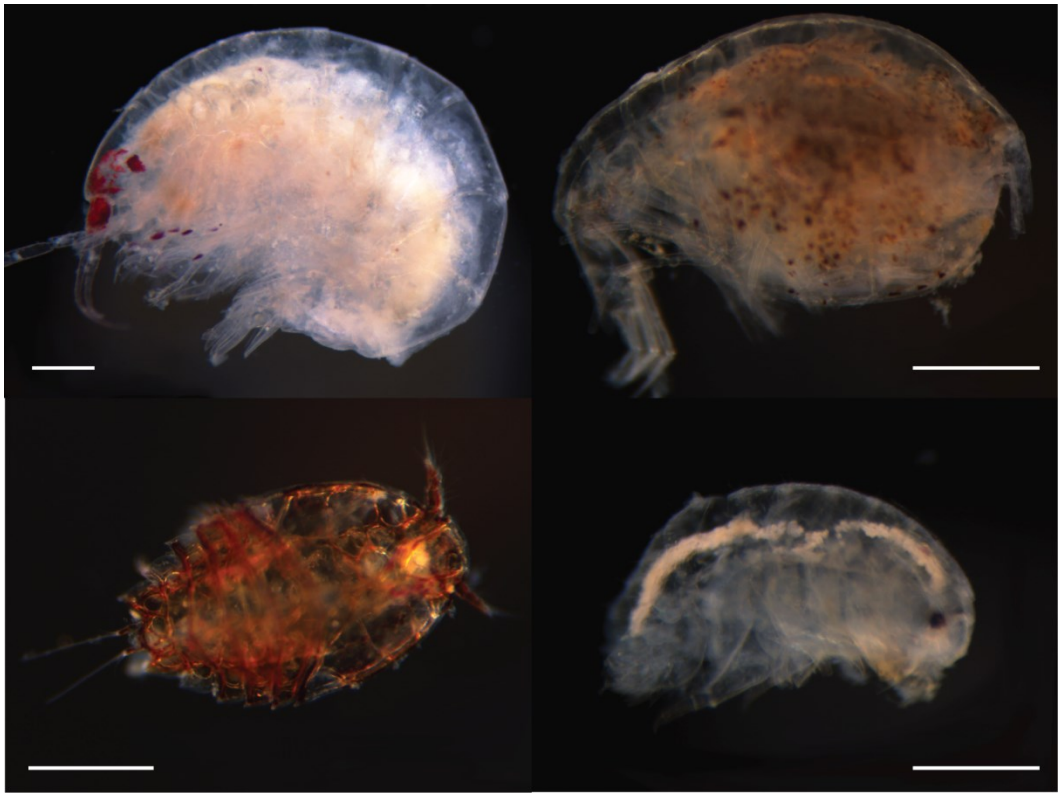

Figure 7. 1 Examples of some species of benthic crustaceans detected as prey items in the stomachs of Siganus canaliculatus from Omani waters (additional findings). Scale bar for all figures = 500 µm.

A new digenean trematode, *Hysterolecithoides amurparuchinii* n. sp. (Al-Jufaili et al., b), is also described within the present study. Members of the genus *Hysterolecithoides* were reported from different siganids hosts (e.g. *S. fuscescens*, *S. doliatus* as well as *S. canaliculatus*) (Yamaguti 1934; Yamaguti 1953; Hafeezullah and Dutta 1980; Hafeezullah 1990; Bray and Cribb 2000; Gupta and Dwivedi, 2006). However, comparative morphological analyses supported with molecular data indicated that the species detected from Omani waters is different from those reported from other localities (Al-Jufaili et al., b). Furthermore, although different siganids were also investigated from Persian Gulf (UAE coasts, El-Naffar et al. 1992), Red Sea (Diamant and Paperna 1986; Hassanine and Gibson 2005; Hassanine and Al-Jahdali 2007; Al-Jahdali 2013) as well as from Kenyan waters (Geets and Ollevier 1996; Martens & Moens, 1995; Aloo, 2004), this large digenean was not reported from these localities. This indicates that the distribution of members of the genus *Hysterolecithoides* does not extend to the Persian Gulf, Red Sea and Eastern coasts of Africa. This suggestion is further emphasized by the limited distribution of *H. amurparuchinii* n. sp. along the coasts of Oman (it was regionally restricted to the southern region of Oman, Arabian Sea).

Likewise, the availability of **non-macrophytes** prey items in the diet of *S. canaliculatus* favours the occurrence of other endoparasites such as nematodes and acanthocephalans, which also utilize benthic and pelagic crustaceans such as amphipods as intermediate hosts. For example, the raphidascaridid nematode *Hysterothylacium aduncum* Rudolphi, 1802, which is globally distributed and exhibit a wide host range, has been reported to use various benthic and pelagic crustaceans (e.g. calanoid or harpacticoid copepod, amphipods and euphausiids) as first intermediate and teleosts as definitive hosts (Køie 1993; Gonzalez 1998; Marcogliese 2002; Klimpel and Rückert 2005). Although the identity of the *Hysterothylacium* species detected in the present study is not yet confirmed it can be postulated that its lifecycle follows the same pattern as other *Hysterothylacium* spp. Similarly, the life cycle of the camallanid nematode *Procamallanus* sp. requires copepods as intermediate hosts (Anderson 2000). The first moult of this parasite takes place inside the copepod intermediate host and the last two moults take place in the fish (Akinsanya and Otubanjo 2005).

Earlier workers reported three acanthocephalan genera from siganids. *Diplosentis* Tubangui & Masilungan, 1937, *Neorhadinorhynchus* Yamaguti, 1939 and *Sclerocollum* Schmidt & Paperna,

1978. *Diplosentis amphacanthi* Tubangui & Masilungan, 1937 is only known from *S. canaliculatus* from the Philippines (Arthur and Lumanlan- Mayo, 1997). Members of the genus *Neorhadinorhynchus* were reported from Fiji Island (*S. vermiculatus,* Amin and Nahhas 1994), Taiwan (*S. fuscescens*, Shih et al. 2010) and Vietnam (*S. fuscescens*, Amin and Van Ha 2011). Acanthocephalans belonging to the genus *Sclerocollum* have been reported from various siganids from different regions (Diamant and Paperna 1986; Geets and Ollevier 1996; Martens and Moens 1995; Hassanine and Al Jahdali 2007; Pichelin et al. 2016). In the present study four species of acanthocephalans (three adults and one cystacanth larvae) were registered in *S. canaliculatus*.

Acanthocephalans also develop through heteroxenous life cycles involving amphipods or copepods as intermediate hosts and a vertebrate definitive host (Taraschewski 2005). For example, recently the life cycle of the acanthocephalan *Sclerocollum saudii* was found to involve the gammarid amphipod *Megaluropus agilis* Hoek, 1889 as an intermediate host. The infection occurs when *S. rivulatus* ingests the infected amphipod, which is abundant on algae and seagrass (Al Jahdali et al. 2015). Thus, the richness of acanthocephalans in *S. canaliculatus* emphasizes the role of crustaceans in the transmission pathways of endoparasites and the influence of host feeding behaviour on parasite diversity.

The gill parasite community of *S. canaliculatus* consisted of seven taxa of ectoparasites. Members of three monogenean genera, were registered from *S. canaliculatus* in the present study. Among them, two new ancyrocephalid monogenean belonging to the specialist genera *Glyphidohaptor* and *Tetrancistrum* were described based on morphological and molecular analyses. The prevalence and intensities of members of these two genera was consistently high along the coasts of Oman (except for one sampling site, see chapter 6), compared to other studies (e.g. Martens and Moens 1995; Geets et al. 1997; Diamant et al. 1999). Such high prevalence and intensities indicate that these parasites are typical for this host. Although *S. canaliculatus* was previously examined for these monogeneans from several localities in the Indo-Pacific region (e.g. Kritsky et al., 2007a; 2007b), *Glyphidohaptor safiensis* n. sp. (Al Jufaili et al., xxxx) and *Tetrancistrum labyrinthus* Al Jufaili & Palm, 2017, are so far only reported from the waters of the Sultanate of Oman indicating that they are endemic.

The remaining ectoparasites taxa included *Hatschekia* spp., *Caligus* spp., *Gnathia* sp. and unidentified Hirudinea species. *Hatschekia* spp. were the most abundant ectoparasites in the

parasite fauna of *S. canaliculatus* with moderate infection levels ranging between 14-100% (see chapter 6). This high infection level is a common phenomenon among members of this genus (Hermida et al. 2012). It is also worth mentioning that members of this copepod genus are so far only detected from *S. sutor* (Kenyan waters of Indian Ocean), *S. luridus* sampled from Red Sea, Gulf of Eilat and *S. canaliculatus* (Sultanate of Oman, present study). *Caligus* species are widely distributed in the Indo-Pacific region. However, prior to this study, they were only recorded from siganids sampled from Kenyan waters (Martens and Moens 1995; Geets et al. 1997), the Philippines (Arthur and Lumanlan- Mayo 1997) and from Indonesia (Yuniar et al. 2007).

In *S. canaliculatus* sampled from Omani waters, caligids were mainly reported from the southern region (Raysut and Masirah, Arabian Sea). This might be linked to the availability of favourable environmental conditions (such as water temperature and quality) for the occurrence of these parasites in these regions. The pranzia larvae of the isopod *Gnathia* sp. were encountered in *S. canaliculatus* with moderate infection levels (P% 3-14) and low intensities (see chapter 6). They were also detected from *S. sutor* from Kenyan waters (Martens and Moens 1995; Geets et al. 1997; Aloo 2004) and from *S. luridus*, *S. rivulatus* as well as *S. argenteus* from the Red Sea (Diamant and Paperna 1986) with moderate intensities. Hirudinea were only reported from siganids sampled from the Red Sea (*S. rivulatus* and *S. argenteus*, Diamant and Paperna 1986), from Indonesia (*S. javus*, Rückert et al. 2007) and from *S. canaliculatus* from Omani waters.

With the high number of parasite taxa recorded from *S. canaliculatus* in Omani waters (n= 48), at least four of them new to science, it can be concluded that the unique environmental conditions of the water bodies of the Sultanate of Oman (see section 1.2) and its highly diverse and rich marine ecosystems harbour many unexplored and less known fish parasite species. This supports the **Working hypothesis 1 that "the investigated siganid *Siganus canaliculatus* harbours a rich parasite fauna, including species new to science".**

7.2 Composition of *Siganus canaliculatus* parasite fauna

The number of parasites taxa recorded during the present study was significantly higher than any known report from other siganids. Diamant & Paperna (1986) reported a total of (27) protozoan and metazoan parasites from *S. rivulatus*, (24) from *S. luridus* and (18) from *S. argenteus*. The investigations conducted on *S. sutor* off the Kenyan coasts resulted in the registration of (16) metazoan ecto- and endoparasites (Martens and Moens1995; Geets and Ollevier 1996; Geets et al.

1997; Aloo 2004). Kleeman (2001) reported a total of (26) metazoan parasites infecting *S. doliatus* off the coasts of eastern Australia, of which 19 were ectoparasites (Rohde 2005). From the data obtained in the present study and the available literature of siganid parasite fauna, the ecto-endoparasites ratio of siganids (including microparasites) ranged between 2.71 (*S. doliatus,* 19 ectoparasites vs seven endoparasites) to 0.20 (*S. argenteus,* three ectoparasites vs 15 endoparasites) with a mean value of 0.86 (calculated from previously investigated siganids, including the present study) (Figure 7.2).

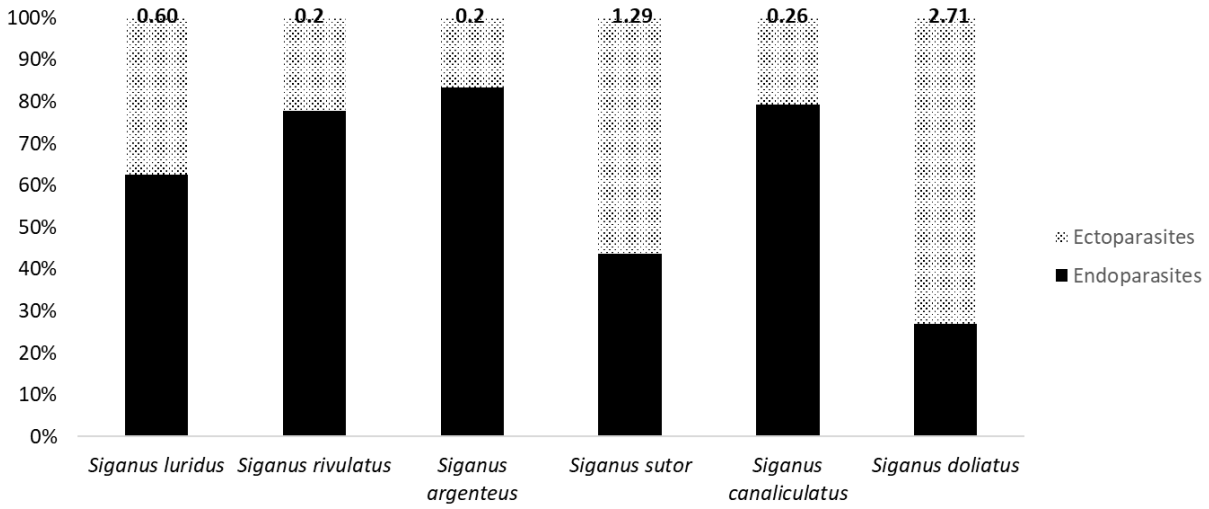

Figure 7. 2 Percentage of ecto- and endoparasites calculated from five species of siganid hosts. Ecto- endoparasite ratio values (Ec/En) of each host are presented on each stacked bar.

According to Palm & Bray (2014), stationary, common coral reef fishes have high Ec/En ratio as a result of high diversity of specialized ectoparasites. Thus, the unusually high ecto-endoparasite ratio (Ec/En) observed in *S. doliatus* could be linked to its habitat preference (coral-rich areas of lagoons and seaward reefs, Froese and Pauly, 2019) as well as low latitude gradient (Rohde 2005). The Ec/En ratio of *S. sutor* was also high (1.29) probably because of the low diversity of endoparasites in this host (7 taxa). For the remaining siganids, the values of ecto-endoparasite ratio (ranging between 0.2-.0.49) were within the values of other reef-associated marine herbivores inhabiting Hawaiian water such as mugilid (0.33), blennid (0.67) and kyphosids (0.50) (Palm and Bray 2014). Herbivorous reef-associated kyphosid hosts sampled from the Great Barrier Reef harboured 21 species of highly specialised digeneans (Manter 1966). Similarly, mullets are known as hosts for numerous digeneans and other endoparasites (Paperna and

Overstreet 1981). This suggest that siganids, as well as other tropical marine herbivores, do not follow the general notion that herbivorous hosts have an impoverished endoparasite fauna (Diamant 1989).

From the available data obtained in the present study (see Figure 7.3) it is established that *S. canaliculatus* has the highest proportion of myxosporean parasites (27.1%, 13 species) in comparison to the other siganids. Among the investigated siganids, only four species were reported to harbour myxosporean parasites (*S. punctatus*, *S. rivulatus*, *S. luridus* and *S. argenteus*) (Diamant and Paperna 1986; Lester and Sewell 1989; Abdel-Ghaffar et al. 2008; Abdel-Baki et al. 2015). Information on the parasite fauna of other siganids were compiled from available literature and check-lists (Jones and Hine 1983; Arthur and Lumanlan-Mayo 1997; Ho et al. 2004; Anshary et al. 2013; Nahhas and Wetzel 1995; Bray and Cribb 2000; Yuniar et al. 2007; Lester and Sewell 1989) which mainly focused on metazoan macroparasites (excluding protozoans, microsporidians and myxosporeans). These data do not demonstrate the actual diversity of the myxosporean parasite fauna in these hosts. Thus, future comprehensive investigations on the parasite fauna of siganids with a focus on microparasite (protozoans, microsporidians and myxosporeans) could reveal a myxosporean fauna that is comparable to that of *S. canaliculatus*.

Although the richness of monogenean parasites is mostly the same among most siganids (*S. rivulatus* (4), *S. luridus* (4), *S. sutor* (3) and *S. canaliculatus* (4)), the highest proportion of monogeneans was recorded in *S. sutor* (26.3%). This high value of monogenea proportion in *S. sutor* could be caused by the overall low richness of parasites and the limited number of taxonomic groups (6 groups) recorded in this host.

The predominant parasite taxa in all siganids were the digeneans, with *S. sutor* and *S. canaliculatus* showing the highest proportion (31.6 and 31.3%, respectively) of the total parasite fauna. The proportion of digeneans was also high in the parasite fauna of siganids sampled from the Red Sea (except for *S. luridus*). The high proportion of digeneans in these hosts could be related to the abundance of benthic organisms that act as intermediate hosts (Machado et al. 1996). Also, all above mentioned species (excluding *S. luridus*) are categorized as siganids that are associated with off-reef shallow waters (Woodland, 1990). Thus, similarities in the habitats of these fishes could reflect similar feeding behaviour, which in turn explains the resemblances in the proportion of digenean parasites in these three species (Sasal et al., 1999). The relatively low proportion of

digeneans in *S. sutor* could be attributed to its limited habitat range and its close association with the coral shelters (Diamant 1989).

The proportion of acanthocephalans was highest in *S. canaliculatus* (8.3%). With a richness of 4 species of acanthocephalans (one as a cystacanth), it is considered as the only siganid known to harbour such diversity of acanthocephalans. Since acanthocephalans utilize amphipods as intermediate hosts in their life cycles, the richness of acanthocephalans in *S. canaliculatus* could be linked to the abundance of amphipods in Omani waters. In addition, it can be linked to its feeding behaviour and habitat preference.

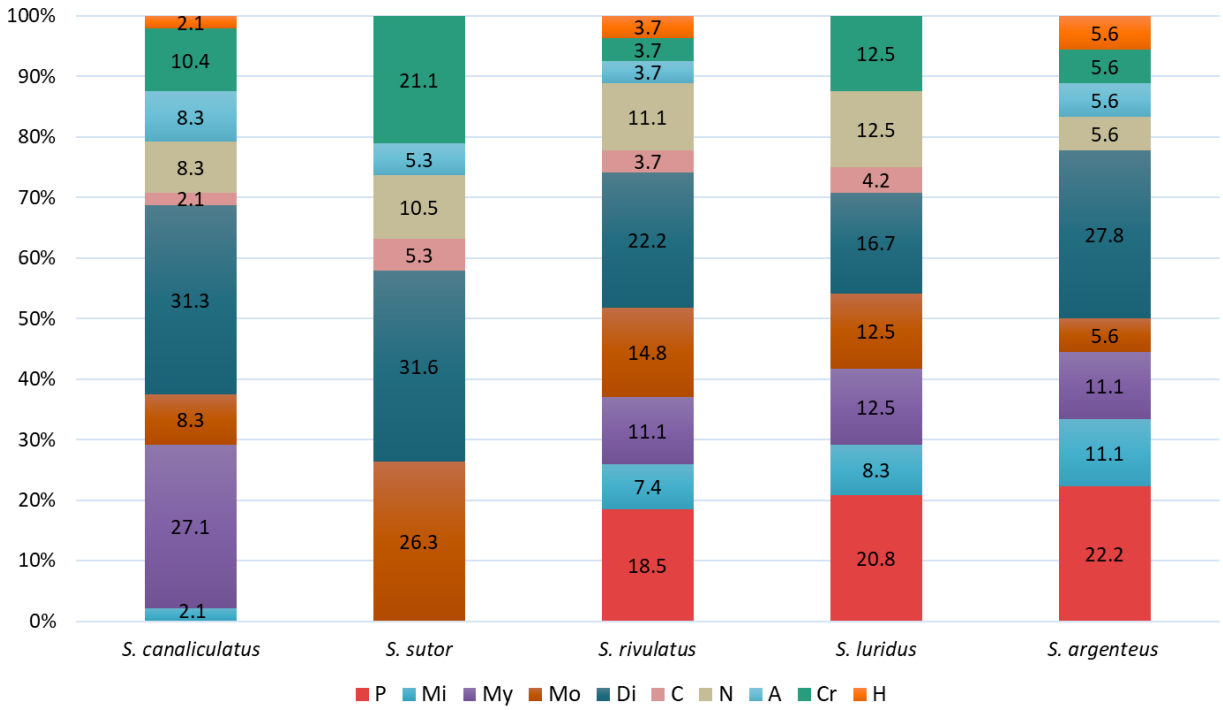

Figure 7. 3 Relative proportions (%) of the main parasite taxa making up the parasite fauna of five siganids. The results are based on parasite richness in each taxon. Mo (Monogenea), Di (Digenea), C (Cestoda), N (Nematoda), A (Acanthocephala), Cr (Crustacea), H (Hirudinea).

The composition of the parasite fauna of *S. canaliculatus* is similar to other siganids from other localities because they share several genera of fish parasites. Among these are the digeneans *Gyliauchen, Opisthogonoporoides*, *Hexangium, Schikhobalotrema*, *Thulinia* and *Aponurus* (Madhavi 1972; Diamant and Paperna 1986; Lester and Sewell 1989; Geets and Ollevier 1996; Martens and Moens 1995; Arthur and Lumanlan-Mayo 1997; Geets et al. 1997). Furthermore,

many of these genera are also shared with other reef-associated fishes such as acanthurid (*Opisthogonoporoides, Hexangium* and *Gyliauchen*), scarid (*Schikhobalotrema*) and chaetodontid (*Aponurus*), indicating phylogenetic relatedness and ecological similarities. The monogeneans *Glyphidohaptor, Tetrancistrum* and *Polylabris* genera were reported on other siganids from different regions of the world (Goto and Kikuichi 1917; Young 1967; Diamant and Paperna 1986). This indicates that the generic composition of the parasites fauna in siganids is reflected by similarities in the feeding spectrum (mainly herbivorous with occasional ingestion of various microbenthos (see section 1.3.4), habitat of these fishes (shallow water, reef associated), zoogeographical distribution (Indo-Pacific region) as well as host phylogeny. Consequently, **the parasite fauna of *Siganus canaliculatus* from Omani waters is similar to the parasite fauna of siganids from other regions (Working hypothesis 2).**

7.3 Importance of *Siganus canaliculatus* in the life cycle of aquatic parasites

Because of the presence of helminths larval stages (cestodes, digeneans and acanthocephalan cystacanths), *S. canaliculatus* also act as an intermediate or paratenic host. According to Alves & Luque (2001), fish hosts harbouring helminths larval stages are categorized as intermediate trophic level in the marine food web. The obtained results also suggest that *S. canaliculatus* plays an important role in the transmission of endoparasites to several definitive hosts, including piscivores fishes, seabirds, and elasmobranchs. For example, the gill arches of several *S. canaliculatus* were infected with yellowish, spherical cysts that harboured metacercaria of the digenean *Stephanostomum* Looss, 1899 (Figure 7.4). Metacercaria of this worm are commonly localized in the musculature, fins and skin of their intermediate hosts (Al-Zubaidy 2011). To our knowledge, the occurrence of the larval stages embedded in the bony gill arches is unusual and prior to this study it has been only reported from the gills of the bream *Dentex dentex* (Linnaeus) from the Mediterranean Sea (Gonzales et al. 2004). Adult *Stephanostomum* spp. are known to infect the intestine of warm water marine teleost such as carangids or scombrids (Bray and Cribb 2008) suggesting that *S. canaliculatus* is an intermediate host and might be a prey item for these large fishes.

Larval trematodes were also extracted from white spherical cysts localized on the skin, fins and gill filaments of several specimens of *S. canaliculatus*. Inside these cysts were larval trematodes resembling members of the digenean genus *Scaphanocephalus* Jägerskiöld, 1903 with

their wing-like anterior expansion (Figure 7.5). Species belonging to this genus, which currently comprises three species that are specialists and mature inside the small intestine of ospreys (fish-eating birds of the family Pandionidae) (Tubangui 1933; Foronda et al. 2009). *Scaphanocephalus* spp. are widely distributed with records including North America, Asia, Africa and Europe (Hoffman, 1953; Schmidt & Huber, 1985; Foronda et al., 2009). This is the first report of this digenean from the Omani coasts and the first from a marine herbivorous fish, proposing that *S. canaliculatus* is a prey item for these sea birds.

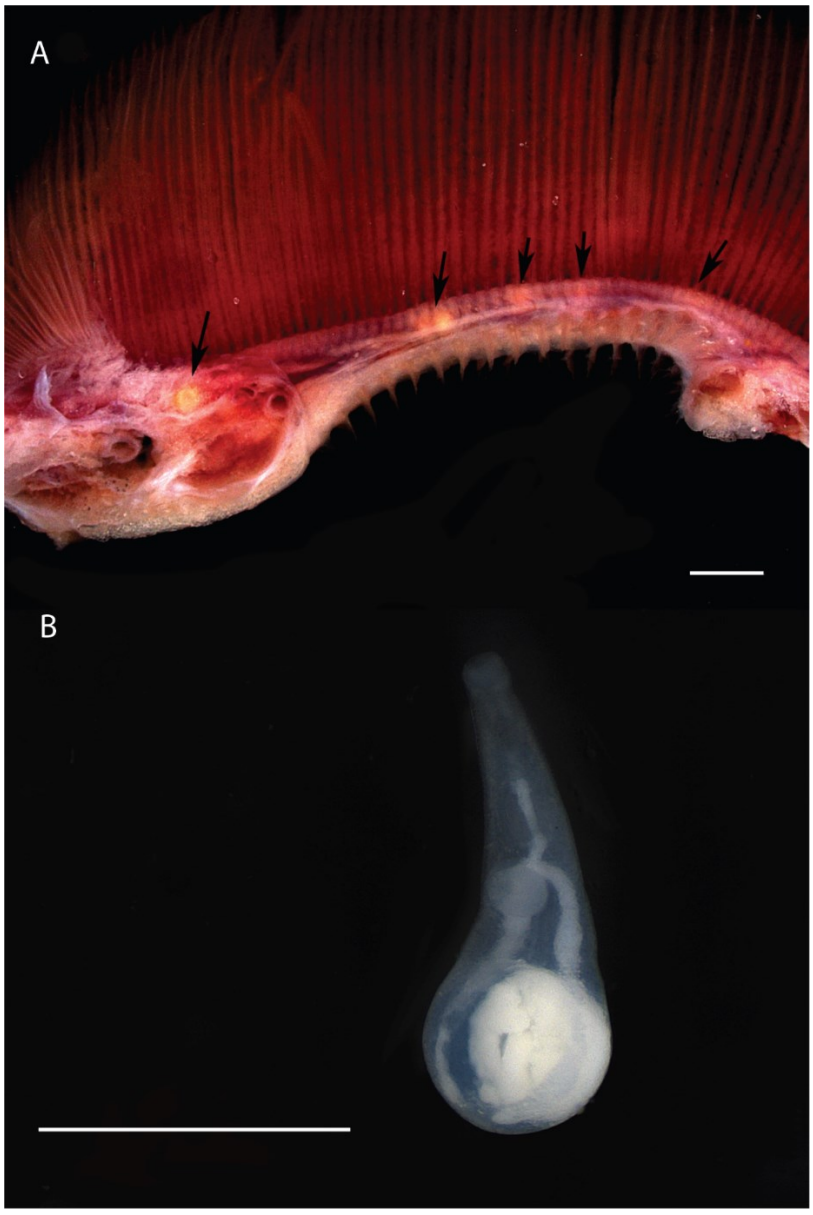

Figure 7. 4 Gill arch of Siganus canaliculatus infected with yellowish cysts of Stephanostomum sp. metacercaria, (A). A specimen of Stephanostomum sp. extracted from the cysts, (B). Scale bar, A=1000µm and B= 500 µm.

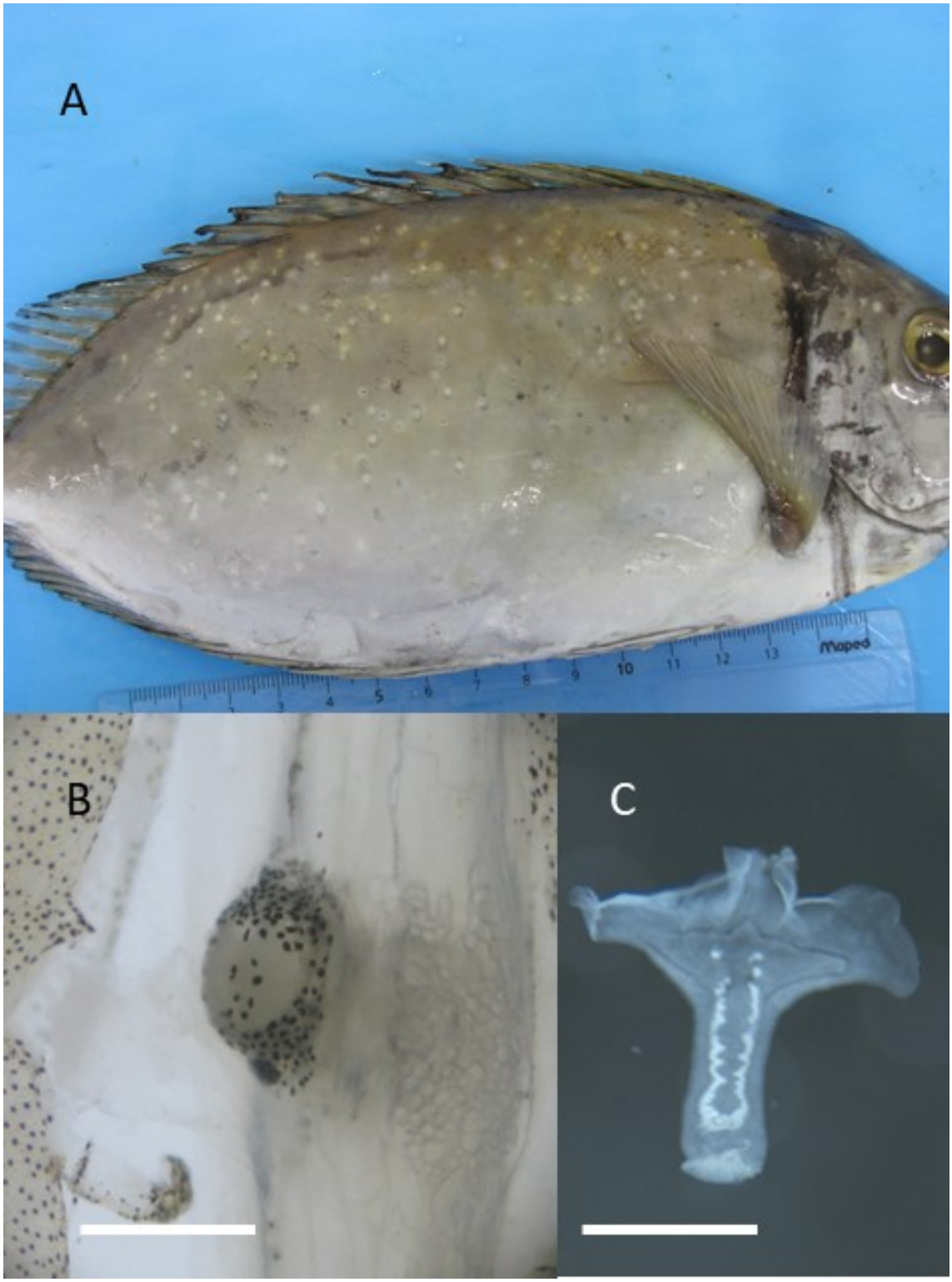

Figure 7. 5 White spot disease, with whitish cysts covering the body surface of Siganus canaliculatus sampled from Al Wusta region, (A). Magnification of one of the cysts on the fins of the infected host, (B). An extracted metacercaria of Scaphanocephalus sp., (C). Scale bars, B 500 µm; C 200 µm.

Larval stages of a species of trypanorhynch Cestoda were detected from *S. canaliculatus* in the present study. The presence of trypanorhynch cestode *Otobothrium* sp. plerocercoids in the intestinal wall of *S. canaliculatus* indicate that this it is an intermediate host for these worms and that it is targeted by elasmobranches. The typical life cycle of Trypanorhyncha involves two intermediate hosts including a copepod as first intermediate host, euphausiids or schooling fish as second intermediate hosts and elasmobranchs as final hosts (Palm 2004). Final hosts of these small *Otobothrium* species are especially sharks belonging to the families Carcharhinidae and Sphyrnidae (Beveridge and Justine 2007). The plerocercoids of these trypanorhynchs are known to parasitize the musculature of several teleosts, elasmobranchs, and squids causing undesirable changes (Palm and Overstreet 1999). In the present study, the infection site was exclusive to the lining of the digestive tract of *S. canaliculatus*.

Numerous cystacanth were isolated from the mesenteries of *S. canaliculatus*. These are larval stages of acanthocephalans that do not undergo further development into adults suggest that *S. canaliculatus* is a paratenic host for these worms. Since it was not possible to identify the cystacanth during this study, the definitive host for this acanthocephalan remains unknown. However, it is worth mentioning that this is the first documented registration of cystacanth from a siganid host. This underlines the working hypothesis three **Importance of *S. canaliculatus* in the life cycle of aquatic parasites and consequently, its importance for the marine ecosystem of the coasts of Oman.**

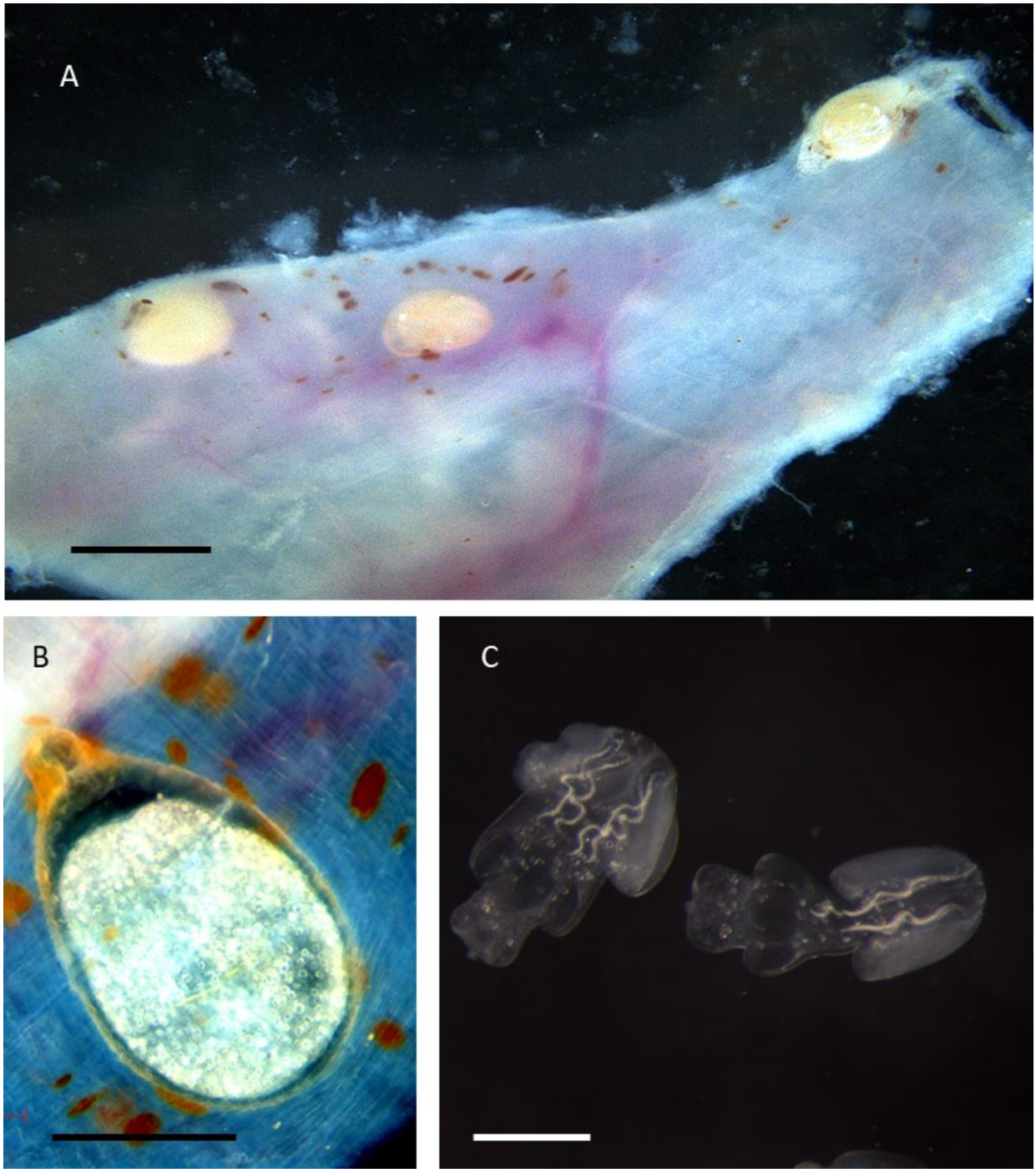

Figure 7. 6 Intestinal epithelium of Siganus canaliculatus infected with plerocercoids of the trypanorhynch cestoda Otobothrium sp., (A). Magnification of the tear-drop shaped blastocyst, (B). Plerocercoids extracted from blastocysts, (C). Scale bars, A= 500 µm, B= 200 µm, C= 100 µm.

7.4 Zoogeography of the parasites of *Siganus canaliculatus*

Marine zoogeography deals with the distributional patterns of aquatic organisms in the world's Oceans. The earliest attempt to summaries the zoogeographical distribution of marine organisms was that of Forbes and Godwin-Austin (1859) (Hedgpeth 1957). Since then, several efforts have been made to investigate the zoogeographical distribution of various marine organisms, including fishes, invertebrates and corals. However, most of these investigations neglected the ecological and historical distribution of marine fish parasites (Rohde 1984). One of the earliest works dealing with the zoogeographical distribution of marine fish parasites were those of Manter (1940), focusing on the marine trematodes of the tropical American Pacific. His work was followed by many comparable investigations on marine fish digenea, monogenea and Nematoda (Manter 1955; Szidat 1961; Manter 1967; Lebedev 1969; Fischthal 1972; Campbell 1990).

To describe the biogeographical distribution pattern of marine organisms, different systems were established to divide the world's ocean into regions and provinces (Ekman 1953; Briggs 1974; Spalding et al. 2007). In the present study, the system of the Marine Ecoregions of the World (MEOW) established by Spalding et al. (2007) was used to discuss the zoogeographical distribution of the parasites of *S. canaliculatus*. This system divides the world's Oceans into 12 realms, 62 provinces and 232 ecoregions. According to this map, the Sultanate of Oman is included in the Western Indo-Pacific realm and is subcategorised into three ecoregions (Persian Gulf, Gulf of Oman and Arabian Sea). For the purpose of this section the patterns of the geographical distribution of *S. canaliculatus* parasites will be described at the level of the 12 realms as established by Spalding et al. (2007). This section will discuss the zoogeographical distribution of some members belonging to three parasite groups (Myxosporea, Monogenea and Digenea).

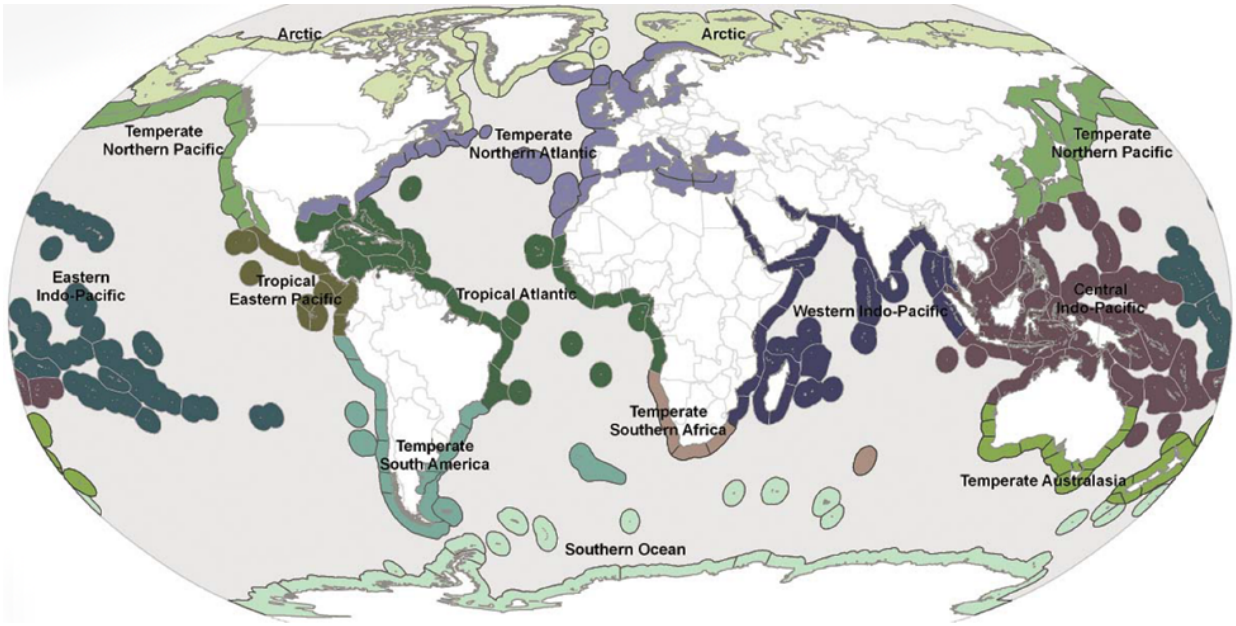

Figure 7. 7 The 12 realms of the world's oceans according to the (Marine Ecoregions of the World) after Spalding et al. (2007).

7.4.1 Myxosporea

Zschokkella species have been frequently recorded from fresh and marine hosts from various locations around the world (Mackenzie and Kalavati 2014). Most *Zschokkella* species have been reported from the Temperate North Atlantic (e.g. Yemmen et al. 2013; Rocha et al. 2013; Yurakhno and Ovcharenko 2014). Reports from the Western Indo-Pacific are limited to three species (Sarkar 1996; 2012), among them only one is registered from a Siganid host (Diamant and Paperna 1986). Prior to the present study, three species of siganids (*S. rivulatus*, *S. luridus* and *S. argenteus*) from the Red Sea. The present study extends both the host and locality range of the genus *Zschokkella*. So far, this is the first and only record of a member of this genus from the waters of Persian and the Gulf of Oman.

Ceratomyxa are well known from marine fishes in various regions of the world Oceans with most species being reported from the North Atlantic (Mackenzie and Kalavati 2014). Only one unidentified species of *Ceratomyxa* was reported from siganid of the Red Sea (Diamant and Paperna 1986; Abdel-Ghaffar et al. 2008). In the present study, *S. canaliculatus* harboured at least three different forms of ceratomyxid myxosporeans presenting the first registration of this genus from the Gulf of Oman and from *S. canaliculatus*. Other species of *Ceratomyxa* were already

reported from other localities along the Persian Gulf from non siganid host (Saudi Arabia, Mansour et al. 2015).

With the description of a new species within the genus *Unicapsula*, the present study reports the first registration of this genus from a siganid host, expanding the host family range and emphasizing on the wide host range exhibited by species of this genus. To date members of this genus have been reported from hosts belonging to three teleost orders; perciformes, gadiformes and pleuronectiformes. Twelve species of *Unicapsula* species are known from hosts belonging to the following marine teleost families; Nemipteridae, Scianidae, Carangidae, Centracanthidae, Sparidae, Polynemidae, Lutjanidae, Sillaginidae, Mullidae, Macrouridae, Gobiidae and Pleuronectidae (Miller et al. 2013; Tomochi et al. 2014). In addition of the new species from *S. canaliculatus*, the majority of *Unicapsula* species were reported from the Indo-Pacific (seven species from both Western Indo-Pacific and Central Indo-Pacific) and from the Temperate Northern Pacific (five species). The other regions, i.e. tropical Atlantic and Temperate northern Atlantic provide only one record of *Unicapsula* species, while no species were recorded from the Arctic and the Southern Ocean.

Among the species of the genus, *U. andersenae* is the most generalist species being reported from five different hosts belonging to five different families. However, it is only recorded from one region (CIP) so far. Two *Unicapsula* species exhibit wider geographical distribution. The first is *U. pyramidata*, which is currently known from two nemipterid hosts (*Nemipterus japonicus* (Bloch) and *Scolpsis monogramma* (Cuvier)). The first of the two hosts displays a distribution range that covers the entire WIP and some parts of the CIP (though it does not spread beyond northwestern Australia). The second one has a more restricted distribution that does not reach beyond the Andaman Sea (northeastern Indian Ocean). Unfortunately, the registration of *U. pyramidata* by Naidjenova & Zaika, 1970 does not specify the exact locality on the Indian Ocean. However, parasitological surveys conducted in the Gulf of Oman, reveal that the geographical range of this parasite extends to the western limit of the Indian Ocean (Al Jufaili, unpublished data).

The other wide spread specie is *U. seriolae* which displays a wider host and a greater distribution range than any of its congeners. This species is known from two hosts belonging to two distant families, the Yellowtail amberjack *Seriolae lalandi* (Valenciennes) and the Malabar grouper *Epinephelus malabaricus* (Bloch & Schneider) both of these hosts are known for their

wide distribution range that spans the entire Indo-Pacific Ocean. So far, it has been recorded off Australian and Japanese waters from tropical and temperate regions (CIP, Temperate Australia and TNP). The remaining species are more restricted in their geographical distribution and seem to coincide with the distribution of their hosts. For example, *U. pacifica* and *U. shulmani* were recorded from a one host species from Okhotsk Sea, while *U. marquesi* was only recorded from Tropical Atlantic region off of Senegal. In conclusion, only few records of *Unicapsula* exists to date, it can be postulated that the origin of this genus is the Indian Ocean and then it spread to the Pacific Ocean where the second highest richness was detected. Future descriptions of new species together with phylogenetic data of existing and less known species will give more insight into this interesting genus.

7.4.2 Monogeneans

The majority of the parasites infecting siganid follow the same distribution patterns as their hosts and are confined in the Indo-Pacific region (excluding the Eastern Indo-Pacific) and to the Northern Temperate Atlantic as a result of lessepsian migration. Among these parasites is the specialist ancyrocephalid monogenean genus *Glyphidohaptor* which is entirely restricted to siganid hosts (Kritsky et al. 2007a). These worms were registered from localities from the Central, Western Indo-Pacific region (including localities investigated in Omani coasts) to the Northern Temperate Atlantic, but were surprisingly absent from hosts sampled from the South China Sea (CIP) (Kritsky et al. 2007a) (see chapter 4). Members of the genus *Tetrancistrum* which are detected from both siganid and acanthurid hosts, are reported throughout the range of their respective hosts distribution which also includes Hawaii (Eastern Indo-Pacific) (Goto and Kikuichi 1917; Paperna 1972; Young 1986; Kritsky et al. 2007b).

Among the detected monogenean species in *S. canaliculatus*, an unidentified species of the polyopisthocotylean genus *Polylabris* was detected from the Omani coasts of Persian Gulf, the Gulf of Oman and the Arabian Sea. According to Hayward (1996) in his revision of the genus, the highest richness of members from the monogenean genus *Polylabris* was reported from the Temperate Australia marine realm (nine species). The second richest diversity came from the WIP (five species) from Kuwait, Oman and India. Both the EIP (Hawaii) and CIP regions have a single record of a representative of this genus. To date, only one member of *Polylabris* was registered from the Atlantic Ocean (both Temperate Northern Atlantic and Tropical Atlantic regions).

Although monogeneans are generally highly host specific either infecting one host or several members from the same genus, some members of *Polylabris* were recorded from different species of the same host genus. For example, *Polylabris sillaginae* was recorded from 11 out of the 34 currently known members of the family Sillaginidae (Hayward 1996). This species occurs mainly from the Australian coasts of the Central Indo-Pacific and Temperate Australia with in additional record from Thailand, Indonesia and New Caledonia. The distribution pattern of *P. sillaginae* is in accordance with the geographical distribution of its final hosts. Many of the sillaginids known to harbor *P. sillaginae* are endemic to Australian waters or are limited to the coasts of Temperate Australian marine ecoregion (e.g. *Sillaginodes punctate* (Cuvier), *Sillago bassensis* (Cuvier), *Sillago flindersi* (McKay)). The occurrence of *P. sillaginae* from the CIP marine realm might be a reflection of the distribution of *S. sihama*, which is the most widespread sillaginid, covering the entire WIP and CIP marine realm. This might propose that *P. sillaginae* have spread to the CIP with *S. sihama* and suggests a possibility of registration of new locality records along the geographical range of this host.

Similarly, *P. tubicirrus* was originally exclusively reported from closely related sparids that are known to occur in the Temperate Northern Atlantic and Tropical Atlantic ecoregion (Hayward 1996). However, with the spread of mariculture of the gilthead seabream *Sparus aurata*, expanded its natural host and geographical range (Silan et al. 1985). This indicates, that under mariculture facilities some species of *Polylabris* might have the potential to expand their host and geographical range. Other *Polylabris* specie which were reported from sparids have a wider distribution, crossing from the Japanese Temperate Northern Pacific region (*P. japonicas,* Ogawa and Egusa 1980) all the way to the Kuwaiti coasts of Persian Gulf in the WIP region (*P. angifer* and *P. acanthopagri,* Mamaev and Paruchin 1976).

To date, four members of the genus *Polylabris* have been recorded from siganids namely *P. virgatarum* Tubangui, 1931 which was described from *S. virgatarum* from the Philippines (Hayward 1996), *P. mamaevi* Ogawa and Egusa, 1980 originally described from *S. stellatus* from Omani coasts of Arabian Sea (Hayward 1996) and from *S. fuscescens* from Chinese waters (Yang et al. 2006); *P. sigani* Dillon, Hargis & Harrises,1983 was described from *S. fuscescens* in Australia waters (Hayward 1996). The latter was also previously reported from the Red Sea from *S. rivulatus* (Diamant et al. 1999), but was confirmed as *Polylabris* cf. *mamaevi* later on (Pasternak et al. 2007).

Recently *P. bengalensis* Sailaja & Madhavi, 2011 was described from *S. canaliculatus* and *S. javus* off Bay of Bengal (Sailaja and Madhavi 2011). The geographical distribution of the members of the genus known from siganids correlates with the distribution of their siganid hosts in the Indo-Pacific (CIP and WIP) regions and from the Temperate Northern Atlantic region of the Mediterranean Sea.

7.4.3 Digeneans

Several digenean parasites were previously registered from *S. canaliculatus* from different localities including the digeneans belonging to the genera *Opisthogonoporoides* Madhavi, 1972, *Hexangium* Goto & Ozaki, 1929, *Aponurus* Looss, 1907 and *Hysterolecithoides* Yamaguti, 1934 (Madhavi 1972; Arthur and Lumanlan-Mayo 1997; Bray and Cribb 2000; Nahhas 2002). Members of *Opisthogonoporoides* which was originally described from the Indian coasts of the Indian Ocean have been recorded from several localities in the WIP, from the Red Sea (Diamant and Paperna 1986), Kenyan coats of Indian Ocean (Geets and Ollevier 1996; Martens and Moens 1995; Aloo 2004) and from temperate Australian waters (TA) (Lester and Sewell 1989). The findings of the present study extend the host (*S. canaliculatus*) and locality range of (Arabian Sea, Gulf of Oman and Persian) of this genus.

Species belonging to the trematode genus *Hysterolecithoides* were reported from hosts species of different families and from various regions of the world Oceans (Yamaguti 1953; Bravo-Hollis 1956; Yamaguti 1971; Tang et al. 1983; Bray and Cribb 2000). Because these digeneans are found from other non siganid hosts families (e.g. Cirrhitidae, Acanthuridae, Serranidae and Carangidae), the distribution range is not confined to the Indo-Pacific region but extends to the Tropical Eastern Pacific, Temperate Northern Pacific as well as Eastern Indo-Pacific where siganids are absent (see chapter 1). With the identification of a new species within *Hysterolecithoides* in the present study, the geographical distribution of this genus is extended further into the WIO region.

The digenean genus *Schikhobalotrema* currently accommodates 26 species infecting the gastrointestinal cavity of fishes belonging to 14 families (Huston et al. 2017). The majority of the species were reported from shallow tropical and subtropical reef inhabiting teleost hosts such as Scaridae (nine species) and Acanthuridae (five species). Zoogeographical distribution of these worms follows a latitude gradient with an apparent trend of an increasing richness and host range

at lower latitude as proposed by Rohde (1984). The highest species richness and host diversity was recorded from the South American coasts of tropical Atlantic with 13 representatives of the genus registered from a total of 29 host species (WoRMS, 2019). The lowest richness of these worms was recorded from the Temperate Northern Atlantic region (two species from three hosts, Love and Manter 1983; Gibson and Costa; Keser et al. 1997). Furthermore, the geographical distribution of *Schikhobalotrema* species mirrors and conforms to the distributions of their hosts. For example, most of the host species harbouring members of *Schikhobalotrema* are endemic to their respective regions and are of neotropical origin (e.g. from the Tropical Atlantic the majority of the species were recorded from scarid hosts that are only known from their respective region). This indicates that this genus probably originated from neotropical scarid hosts. In the Indo-Pacific species of *Schikhobalotrema* are mainly known from acanthurid hosts (four species) that were sampled from Hawaiian waters.

So far, there are only two records of *Schikhobalotrema* from the WIP both from the Kuwaiti coasts of the Persian Gulf (Abdul-Salam and Khalil 1987; Nahhas and Sey 1998). The registration of an unidentified species of *Schikhobalotrema* from *S. canaliculatus* in the present study is regarded as the third record from the WIP and registration from a siganid host. It is likely that the increase in the investigation effort in the Indo-Pacific region and the examination of more acanthurid hosts will change the current state of knowledge on the zoogeographical distribution of members of this genus. In conclusion, for all these parasite taxa, "**new host and locality records from the Sultanate of Oman will extend the range of distribution of Indian Ocean parasites into the Persian Gulf, Gulf of Oman and the Arabian Sea, supporting working hypothesis four.**

7.5 Parasites of *Siganus canaliculatus* as biological indicators

Variations in the composition of parasites in terms of prevalence, abundance and richness in different geographical locations from different aquatic systems were documented (Cremonte and Sardella 1997; Bagge et al. 2004; Costa et al. 2009; Hutson et al. 2011; Mateu et al. 2014). According to many researchers, these variations can be explained by various biotic and abiotic factors such as oceanographic conditions (Rohde 1993; Bagge et al. 2004; Poulin 2007; Hutson et al. 2011). Oceanographic conditions such as temperature, salinity, depth and specific features of habitat may influence the composition of monoxenous ectoparasite fauna (Gonzalez et al. 2008;

Timi et al. 2010). The distribution of heteroxenous endoparasites also depends on the environmental conditions as well as the host distribution and density (Pereira et al., 2014). This is because the availability of suitable definitive and intermediate hosts that are required to complete the life cycle of these parasite is likely to be affected by these conditions (Poulin 2007; Timi et al. 2010). Likewise, by creating zoogeographical barriers, oceanographic conditions can restrict parasite dispersal (especially, infective larval stages), which in turn causes the differences in parasitic fauna between the geographical zones (Gonzalez et al. 2008; Timi et al. 2010; Jacobson et al. 2012).

Together with Analysis of similarities (ANOSIM), multidimensional scaling (nMDS) was used to graphically describe the qualitative (compositional) and quantitative (structural) difference in parasite infra- and component communities between the two sampling zones (north and south), the three water bodies (PG vs GoO vs AS) and each sampling sites along the coasts of Oman (Al-Jufaili et al. ….c). The analysis (which was based on parasite abundance data) showed a slight separation between the north and south zones which was supported with ANOSIM ($R = 0.36$, $P = 0.001$) with an average dissimilarity of 70.86% as calculated with similarity percentages (SIMPER). According to Lee et al. (2000), "the Findlater Jet in the atmosphere and the Ras Al Hadd frontal zone, both set up a boundary which makes the Gulf of Oman (GoO) dynamically isolated from the western Arabian Sea (AS)" (Piontkovski et al. (2011). Therefore, the separation between the two zones might be a consequence of an **oceanographic barrier** and differences in the physical-chemical condition between the two zones. This observation was similar to those of Gonzalez et al. (2008) who attributed the variations in the endoparasites fauna of hosts sampled from two regions along the Chilean coasts to the occurrence of differing oceanographic conditions and the formation of transitional zone caused by the Eastern South Pacific Intermediate Waters. Similarly, Vales et al. (2011), observed variations in parasite communities' descriptors and indices between two zones in Argentinian water as a consequence of a latitudinal gradient in oceanographic condition in the study area.

Physical, chemical and biological oceanographic conditions such as cyclonic eddy activities, upwellings, currents, nutrient abundance and chlorophyll concentrations can influence the structure and composition of free-living organisms as well as marine parasites. In the present study, distinctive groupings of *S. canaliculatus* parasite communities could be assembled according to

waterbodies surrounding Oman. Several studies established that the three water bodies surrounding the Sultanate of Oman vary in terms of temperature, salinity, depth and productivity (Pous et al. 2004a; Pous et al. 2004b; Piontkovski and Al-Jufaili 2013). In fact, according to Spalding et al. (2007), the three waterbodies are categorized as three distinct ecoregions. However, the parasite community of hosts sampled from the Persian Gulf (PG) and the Gulf of Oman (GoO) were not significantly different (ANOSIM R= 0.074, P= 0.37). Both PG and GoO are less productive and are characterized with similar geographical and oceanographic features (see chapter 1, section 1.2).

Also, the seasonal inflow of Persian Gulf Water mass (PGWM) might facilitate the connectivity of these waterbodies and probably the sharing of aquatic biota which is reflected through the similarities in the parasite composition and the marked overlap between samples from these waterbodies (Figure 6.1B). In contrast, The Arabian Sea coasts of Oman which surrounds the southern zone (Masirah and Raysut) is more productive than the other two waterbodies due to the seasonal upwelling events (see chapter 1, section 1.2). This phenomenon influences the primary marine productivity in this water body and consequently the availability and occurrence of a diverse species of free-living organisms that could act as intermediate hosts for parasites (see Jacobson et al. 2012).

Site to site variations in parasite fauna of *S. canaliculatus* occurred in terms of ecological indices and parasitological parameters. The nMDS analysis of sampling sites (Figure 6.1C) showed that the parasite structure and composition between the sampling sites were significantly different (R= 0.678, P< 0.01, Stress= 0.2). The separation is probably a consequence of ecological habitat features between the sites and occurrence of site-specific parasites species. Among the registered parasites, certain endoparasites were limited in their distribution to the certain sites along the Omani coasts. For example, species of *Hysterolecithoides* sp., *Preptetos* sp. and *Kudoa* spp. were only reported from hosts sampled from the Arabian Sea coasts of Oman (see chapter 6, section 6.4.3). *Stephanostomum* spp. metacercaria were only registered from hosts sampled from the Persian Gulf (Khasab). This observation could imply, that there are more than one population of *S. canaliculatus* along the coasts of Oman. Thus, parasites of *S. canaliculatus* could be useful as biological tags to separate between siganid stocks. It is evident that "**The parasite infracommunity of *Siganus canaliculatus* is clearly influenced by the three different water**

bodies defined by (Persian Gulf, Gulf of Oman and Persian Gulf), supporting working hypothesis 5.

Numerous studies have examined the relationship between parasites and pollution proving that different groups of fish parasites are valuable tools to monitor environmental status in marine and freshwater ecosystems around the world. According to the results of these studies, fish parasites are a good choice as indicators for the detection and evaluation of environmental changes through fluctuations in their intensities, abundance and distribution patterns (Lafferty 1997; Sures 2004; Vidal-Martinez et al. 2003; Sasal et a. 2007). The usefulness of fish parasite as bioindicators is based on their sensitivity to biotic and abiotic factors in their hosts' environment. Also because they react to pollutants in different manners, fish parasites can be used as biomarker, effect or accumulation indicators (Sures 2004).

Previously, carnivorous epinephelid groupers were used for the assessment of environmental impact in tropical marine ecosystems through visual integration of the parasite parameters into a star graph (e.g., Kleinertz et al. 2014; Kleinertz and Palm 2015; Neubert et al. 2016, Thuong et al. 2017). Following Neubert et al. (2016) and Truong et al (2017), twelve parasitological descriptors were selected from the parasite fauna of *S. canaliculatus* to assess the condition of marine ecosystem along the coasts of Oman. These included the prevalence of seven parasites species (*Glyphidohaptor safiensis* n. sp., *Hatschekia* sp., *Gyliauchen* p., *Ceratomyxa* spp., *Opisthogonoporoides* sp., *Sclerocollum* sp. and *Hysterothylacium* sp.) and five ecological indices (Berger Parker index of dominance, Pielou index of evenness, Shannon diversity and ecto-endoparasites ratio). The prevalence of the selected parasites was previously used by several authors to indicate anthropogenic pollution (Diamant et al. 1999; Dzikowski et al. 2003; Kleinertz et al. 2014; Neubert et al. 2016). These authors demonstrated that the reduction of complete elimination of these parasites was directly linked to alterations and disturbance in the investigated localities.

Ecological indices measure changes in the parasite fauna structure and compositions through changes in richness and diversity. Generally, these indices are reduced under pollution conditions. With values ranging between 0 to 1, the Berger-Parker index of dominance characterizes the dominance of a respective parasite species within the sampled host population (Palm et al. 2011) by calculating the proportional abundance of only the most abundant species in the population

(Morris et al. 2014). Higher values correspond to lower diversity (Patrício et al. 2009), an observation that is usually associated with disturbed environmental conditions (Caruso et al. 2007).

Pielou's index of evenness is a measure of the level of numerical equality in the distribution of abundances of species that occur within a host population in a specific area (Pielou 1966). Thus, low evenness index value is an indicator of an increase in dominance of generalists or more tolerate species in a parasite community (Johnston and Roberts 2009) and consequently, disturbed environmental conditions. In the Gulf of Tokin, Vietnam, net cage mariculture facilities had the highest Berger-Parker index and the lowest evenness value in comparison to natural and pond mariculture facility (Truong et al. 2017). These authors linked the dominance of certain parasites species (e.g. the monoxenous monogenean *Pseudorhabdosynochus* spp.) with bad management and water quality in net cage farming sites. Similarly, the Berger-Parker index was highest and the Pielous index was the lowest in Sohar in comparison to other sites (attributed to the dominance of *Sclerocollum* sp.). This observation is linked to the reduction of parasite richness through the elimination of suitable intermediate hosts (highlighted with reduction of endoparasites diversity) and the absence of ectoparasites (related to high levels of contaminants).

All above mentioned parasitological descriptors were used for the visualization of environmental health in the marine ecosystem of Oman through star graphs and pollution traffic light. Large star graph areas were calculated from Raysut (25.42), Masirah (19.18) and Muscat (16.38). All parameters and indices values in these locations were the highest in comparison to other sampled localities. This might reflect the good water quality, high productivity, and suitable habitat for parasites of *S. canaliculatus* (seagrass beds, sheltered reefs and abundance of intermediate hosts). On the other hand, the localities located in the north zone had lower star graph areas which is linked to the naturally harsh marine environment (Khasab (13.09), Persian Gulf, see chapter 1 section 1.2), Dabba (8.03) (which is the nearest location to Sohar and probably impacted). However, the lowest star graph was calculated form Sohar because of depauperate diversity and richness and accompanied with reduced parasitological and ecological parameters. This observation can only be explained by extremely unnatural environmental conditions associated with high levels of pollution. Thus, the use of *S. canaliculatus* and not groupers as a model for environmental assessment in the Sultanate of Oman, emphasizes that **parasites of *Siganus***

***canaliculatus* in Omani waters can be used as biological indicators for environmental health (Working hypothesis 6).**

7.6 Risk assessment of parasites of *Siganus canaliculatus* for mariculture industry

Marine diseases are a natural part of ocean ecosystems, and many have significant economic consequences for fisheries or aquaculture (Murray and Peeler 2005; Lafferty et al. 2015). Parasitic outbreaks in mariculture facilities are a major restriction against expanding the aquaculture industry (Huston et la. 2007; Sanchez-Garcia et al. 2014). Protozoan and metazoan parasites have a serious impact on global finfish and shellfish aquaculture by constraining production, sustainability, and economic viability (Shinn et al. 2015). Financial losses on a farm can be direct (e.g. mortality, increased management costs, marketability issues) or indirect (through concerns over welfare, increase disease and legislative burdens, potential quarantine difficulties) (Paladini et al. 2017).

Several taxa of fish parasites can impose health problems in different mariculture facilities. Ectoparasites with direct monoxenous life cycles and high production rate (e.g. monogenean and parasitic crustaceans), would pose the greatest threat to the mariculture industry (Hemmingsen and Mackenzie 2001, Huston et al. 2007; Shinn et al. 2015). This is due to their rapid reproduction and ability to directly infect the hosts and the enhancement of direct transmission under mariculture conditions (Huston et al. 2007). Heteroxenous endoparasites with simple life cycles or those with water-borne infectious stage (species of myxosporeans and some digeneans), are also a threat to the mariculture industry (Paladini et al 2017).

In the present study, parasites of *S. canaliculatus* were evaluated for their potential as threats to the development of siganid mariculture industry in the Sultanate of Oman. According to Huston et al. (2007) and Sanchez-Garca et al. (2014), risk assessment involves **hazard identification**, the **probability** of parasite establishment and proliferation and the **consequence** of parasite establishment and proliferation in the farm. The consequence of parasite establishment for *S. canaliculatus* in mariculture facilities was estimated using the four categories indicating the degree of potential damage caused by the parasite. According to Sanchez-Garca et al. (2014), parasites presenting all four factors entail extreme consequence; three factors imply high consequence; two factors moderate consequence; one factor low consequence; no factors negligible consequence. Parasites which were considered of negligible consequences were not included in the table (see Appendix 1). Based on these criteria, *S. canaliculatus* that may become problematic in mariculture

belonged to the following taxa Myxosporeans (5), Monogenea (3), Digenea (2), Nematoda (2) and Crustacea (2).

The estimation of the probability of establishment is composed of combining the **level exposure** and **pathway assessment** (Huston et al. 2007; Sanchez-Garcia et al. 2014) (Appendix 2). The level of exposure of farmed fish to wild infected fishes depends on the geographical distribution of the parasites (Huston et al. 2007). The assessment of the infection pathways of *S. canaliculatus* parasites was based on the possible life cycles of these parasites. The final quantitative risk ranking was achieved by using a numerical risk matrix as shown in appendix 3. Only parasites exhibiting risk ranking from (6-25) will be discussed in the following section.

7.6.1 Myxosporean parasites

Several myxosporean species are of economic importance to the mariculture and fisheries industry due to their pathogenicity to their hosts and their ability to directly infect through fish-to-fish transmission (Alvarez-Pellitero and Sitjà-Bobadilla 1993; Rigos et al. 1999). With the continuous development of the mariculture industry, outbreaks of myxosporean origin are expected to become more frequent and therefore impend the industry. Representatives of certain genera are well recognized as serious disease causing agents in cultured and wild fish populations. For example, muscle infecting myxosporeans belonging to *Kudoa*, *Henneguya* Thélohan, 1892 and *Unicapsula* can cause the appearance of unpleasant white cysts or spoilage of the muscle texture (Moran et al. 1999b). Myxosporeans infecting organs such as the brain (*Myxobolus* Bütschli, 1882), gills (*Henneguya*), reproductive organs (e.g. species of *Sphaerospora* Thélohan, 1892) and intestinal epithelium (*Enteromyxum* Palenzuela, Redondo & Alvarez-Pellitero, 2002) are among some of the important pathogens in maricultured fish hosts around the world. Infections of the kidney and urinary tract of teleost hosts caused by myxosporeans are also common and in many cases are highly pathogenic (Feist 1997).

Table 7. 1 Potential parasite risk analysis for Siganus canaliculatus mariculture along the coasts of Oman

Parasite taxa	Exposure	Pathway	Probability	Consequence	Risk	Ability to treat
Myxosporea						
Zschokkella sp.	High	Moderate	Moderate	Low	6	No[a,b]
Ceratomyxa spp.	High	Extreme*	Extreme	Moderate	15	No[a,b]
Latyspora sp.	Low	Moderate	Negligible	Low	2	No[a,b]
Ortholinea spp.	High	Moderate	Moderate	Low	6	No[a,b]
Unicapsula fatimae	High	Moderate	Moderate	Low	6	No[a,b]
Kudoa spp.	Low	Extreme*	Moderate	Moderate	9	No[a,b]
Monogenea						
Glyphidohaptor safiensis	High	High	High	Low	8	Yes[a]
Tetrancistrum spp.	High	High	High	Low	8	Yes[a]
Polylabris sp.	High	Extreme*	Extreme	Moderate	15	Yes[a]
Digenea						
Preptetos sp.	Low	Low	Negligible	Low	2	Yes[a,b]
Stephanostomum spp.	Negligible	Low	Negligible	Moderate	3	Yes[a,b]
Nematoda						
Hysterothylacium sp.	High	Extreme*	Extreme	Moderate	15	Yes[a,b]
Nematode indet. Larvae	Moderate	Low	Negligible	Moderate	3	Yes[a,b]
Procamallanus sp.	High	Low	Low	Low	4	Yes[a,b]
Crustacea						
Caligus spp.	Moderate	Extreme*	High	Moderate	12	Yes[a]
Gnathiidae indet. sp.(pranzia larvae)	High	Extreme*	Extreme	Moderate	15	Yes[a,b]

*parasites genera/species that were reported from marine farm facilities

[a]Huston et al. 2007

[b]Sanchez-Garca et al. 2014

Members of the genus *Ceratomyxa* are mostly coelozoic, parasitizing the gallbladder of marine fishes (Gunter 2009). Some species of *Ceratomyxa* are reported as pathogenic causing histopathological damage to the gallbladder of farmed hosts (Alvarez-Pellitero and Sitjà-Bobadilla 1993; Alama-Bermejo et al. 2011). Also, *Ceratomyxa* species reported from wild siganid by Diamant and Paperna (1986) were pathogenic to their host causing acute desquamation of gallbladder epithelium and chronic congestion and distention of the hepatic biliary canaliculi. In addition, a case of high levels or mortality were reported from hormone treated farmed *D. puntazzo* (Katharios et al. 2007). Thus, *Ceratomyxa* species that were detected in the present study are considered as potential hazard to the development of *S. canaliculatus* mariculture in Oman. The risk imposed by these parasites is ranked as moderate.

Some species of *Zschokkella* are also known as pathogenic causing enlargement of hepatic ducts, lowering duct epithelium and pericholangitis (*Z. russelli*, Davies 1985). Similarly, Bucher et al. (1992) reported proliferation and considerable distension of the ducts as well as metaplastic flattening of the duct epithelium caused by *Z. nova* Klokacewa, 1914. One of the most pathogenic species of *Zschokkella* was reported from the gallbladder of wild *S. luridus* sampled from the Red Sea caused severe hepatic necrosis, ascites and jaundice (*Z. icterica*, Diamant and Paperna 1992). Other than congested hepatic ducts that was associated with *Zschokkella* sp. infection in wild *S. canaliculatus*, no apparent pathological signs were detected in the gallbladder or the infected hosts. Therefore, the risk imposed by this parasite is ranked as low, however further histopathological investigated should be carried out to discern any possible risk to the development of *S. canaliculatus* mariculture.

Members of the genus *Unicapsula* usually infect the musculature of their hosts and are associated with reduction of their quality and marketability (Al-Jufaili et al. 2015). Spores of *Unicapsula fatimae* detected in the present study were isolated from cysts located on the endothelium lining of *S. canaliculatus* oesophagus with localized histological changes at the site of cysts attachments (Al-Jufaili et al. 2015). Therefore, at this stage of the investigation and due to insufficient data on the biology of this parasite, *U. fatimae* are considered as low risk for the development of *S. canaliculatus* mariculture.

In the present study, several specimens of *S. canaliculatus* were presented with enlarged urinary bladders that were filled with opaque urine (Figure 7.8). Subsequently, an infection with

multiple species of *Ortholinea* was detected from these hosts. Although species of *Ortholinea* were previously reported from *S. rivulatus* (Diamant 2010; Abdel-Baki et al. 2015) none of these records report such apparent gross clinical observations from their hosts. Furthermore, while both male and female *S. canaliculatus* were infected with these myxosporeans, only male hosts exhibited abnormalities of their reproductive organs (Figure 7.7). To our knowledge, this is the first report of members of *Ortholinea* being associated with gross clinical signs and abnormalities of the reproductive organs. The incident reported herein indicates possible pathological changes caused by this infection and thus ranking this parasite as low risk to *S. canaliculatus* mariculture. In addition, the abnormalities of the male reproductive organs suggest that these parasites are sex selective in their pathogenicity and might cause damage to the male reproductive organs.

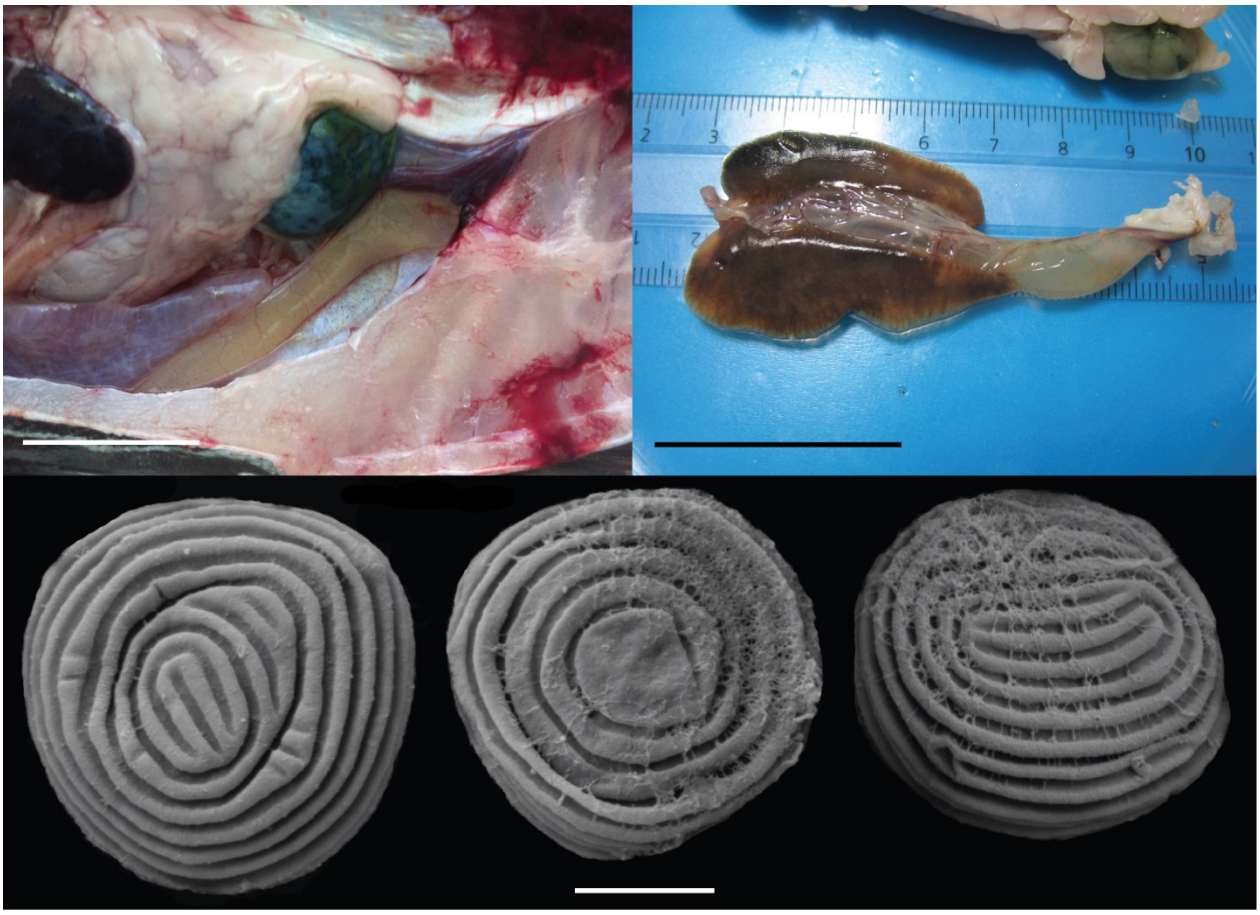

Figure 7. 8 A heavily infected urinary bladder of Siganus canaliculatus showing a swollen bladder filled with opaque urine, (A). Dissected infected urinary bladder of a male host exhibiting urine filled bladder and abnormally discoloured testes, (B). Scanning electron microscopy images of three different forms of Ortholinea spores detected from the infected urine of Siganus canaliculatus in the present study, (C). Scale bars, A= 2 mm, B= 4 mm and C= 2µm.

Since its description from the Red Sea bream *Pagrus major* (Temminck & Schlegel) (Egusa and Shiomitsu 1983), the multivalvulida myxosporean *Kudoa iwatai* Egusa & Shiomitsu, 1983 has been recorded from 19 wild and cultured fish hosts belonging to 13 families (Diamant et al. 2005; Burger and Adlard 2011). The low host specificity and production of cysts in the musculature makes this parasite of great importance for the fisheries and mariculture industry. In addition, the infection with *K. iwatai* is not restricted to the musculature but can establish in several infection sites (Diamant et al. 2005). *Kudoa* species with a systematic infection type (e.g. effecting several organs of the host) can be pathogenic to their hosts under mariculture conditions (*Kudoa lutjanus*, Wang et al. 2005). In the present study, white, spherical cysts belonging to *K. iwatai* were detected on the gill operculum of some *S. canaliculatus* specimens. Thus, although the infection level was relatively low, the occurrence of these parasites could be detrimental to the development of siganid mariculture industry as these parasites can negatively impact the quality and health of the infected hosts. The risk of this parasite to S. canaliculatus is estimated as moderate due to its potential pathology, ability to reduce marketability, and occurrence in mariculture facilities.

Future histological analyses are required to determine the presence or absence of pathological reactions induced by this parasite *in S. canaliculatus*. The registration of *K. iwatai* in Omani water is not limited to the current study, in fact this parasite has been reported from other local hosts including the gold-lined seabream *Rhabdosargus sarba* (Forsskål) (Al-Jufaili, unpublished data) indicating that these hosts could serve as natural reservoir for these parasites and may facilitate their transmission to mariculture facilities.

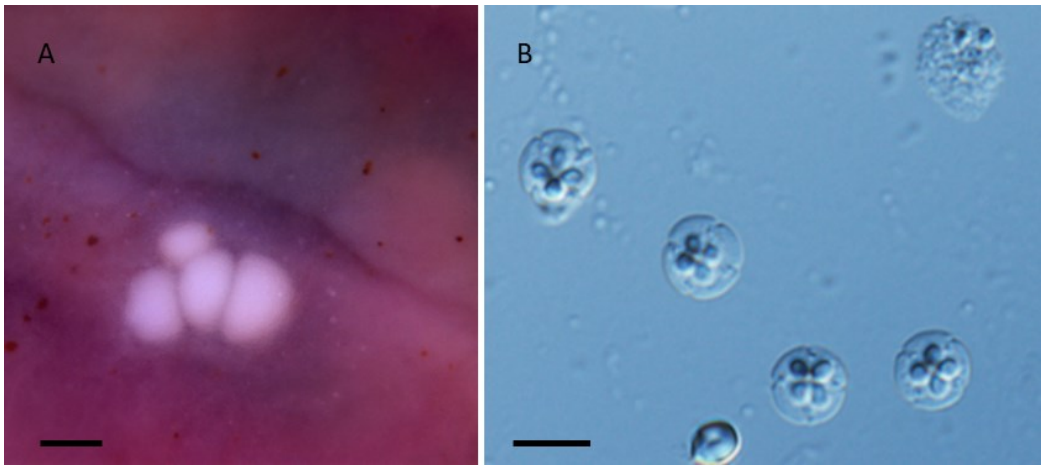

Figure 7. 9 White cysts of Kudoa iwatai infecting the muscles of Siganus canaliculatus, (A). Fresh preparations of Kudoa iwatai spores, (B). Scale bars, A 500 µm and B 10 µm.

7.6.2 Monogenean ectoparasites

Monogenean fish parasites are frequently associated with major economic losses in warm water mariculture facilities (Paperna et al. 1984; Gonzalez et al. 2004; Ernst et al. 2002) because farming conditions encourage the transmission and propagation of these ectoparasites (Diamant et al. 1999). Besides high prevalence, the infection with monogeneans is usually accompanied with viral and bacterial secondary infections (Rubio-Godoy 2007). In mariculture facilities, several monopisthocotylean and polyopisthocotylean monogeneans were the cause of severe mortalities under mariculture conditions. Among these, capsalids of the genera *Neobenedenia* and *Benedenia* in Japan and Australia (Ogawa 1995; Deveney et al. 2001; Ogawa 2006; Whittington and Chisholm 2008), *Sparicotyle chrysophrii* in wild and cultured Mediterranean sparids (Sitjà-Bobadilla et al. 2010; Antonelli et al. 2010), *Microcotyle sebastis* in cultured Korean rockfish, *Sebastes schlegeli* (Hilgendorf) (Kim et al. 1998; 2000; 2001).

Infections with Monopisthocotylea are commonly associated with secondary infections due to the damage caused by attachment method and feeding mechanisms (Rubio-Godoy 2007). Some of the pathology caused by infection with these worms include excessive mucus secretion, erosion of the epithelium, gill hyperplasia, impairment of gill respiratory function (Whittington 2006). Monopisthocotylean ancyrocephalid monogeneans infecting *S. canaliculatus* are regarded as low risk although they infected *S. canaliculatus* with relatively high infection levels and intensities (see chapter 6). This is because the infected hosts did not show any signs of pathological changes nor clinical symptoms associated with these worms. However, knowing that the prevalence and intensity of such monogeneans can increase under culture conditions, several aspects of the life and biology of these monogeneans should be investigated.

The blood-feeding polyopisthocotyleans are frequently linked to anaemia, gill hyperplasia, loss of lamella structure, clubbing of fusion of gill filaments and haemorrhage (Gonzalez et al. 2004; Rubio-Godoy 2007). Cases of mortalities caused by members of *Polylabris* were recorded in farmed *Sparus aurata* in France (Silan et al. 1985) and in cultured siganids in Israel (Paperna 1984). *Polylabris tubicirrus*, which naturally infect sparids of the genus *Diplodus* (Rafinesque), expanded its host range under culture conditions (Ogawa 2014). According to Paperna (1984), *Polylabris* cf *mamaevi* imposed a major problem to farmed *S. rivulatus* and *S. luridus* where infected hosts were emaciated and suffered from anaemia accompanied with low haematocrit

value. This species is well established in siganids inhabiting the WIO and Mediterranean Sea (Geets et al 1997; Martens and Moens 1995; Diamant and Paperna 1986, Pasternik et a l. 2007; Paperna 1972).

Wild *S. canaliculatus* investigated in the current study were infected with *Polylabris* sp. with relatively moderate infection levels (6-71%). Provided that members of the family Microcotylidae are known as blood-feeders and for producing filamented eggs will easily entangle in nets, the consideration of *Polylabris* sp. as a potential threat to the future siganid mariculture should not be neglected. Because of its possible pathogenicity, potential to cause mortality, its monoxenous life cycle and its establishment in mariculture facilities in other localities, *Polylabris* sp. risk to mariculture is ranked as moderate. Future investigations involving the identification of *Polylabris* sp., studies of its biology and experimental infection trials should be implemented to assess their role as possible disease-causing agents in Omani siganid mariculture facilities.

7.6.3 Nematode worms

Pathological alterations were observed in the flounder *Paralichthys isosceles* (Jordan) caused by infection with *Hysterothylacium* sp. (Knoff et al., 2012). Also, larval nematode of the genus *Hysterothylacium* caused massive necrosis and fibrosis of the liver of their siganid hosts (Diamant and Paperna 1986). These parasites which are usually trophically transmitted parasites have been reported from several mariculture farms which might indicate utilization of alternative transmission pathways (see Lima dos Santos and Howgate 2011). In addition, some species of *Hysterothylacium* have been reported as potential zoonotic (González-Amores et al. 2015). In the present study, *Hysterothylacium* sp. were detected in the mesentery of *S. canaliculatus* at prevalence ranging from 3-66% (Al Jufaili et al. …c). Based on the pathological and zoonotic effect of some species of this genus and their possibility to establish in mariculture facilities, *Hysterothylacium* sp. infecting *S. canaliculatus* poses moderate risk for development of its mariculture.

7.6.4 Crustacean ectoparasites

Many marine parasitic copepods, especially those belonging to families such as Ergasilidae, Caligidae, Sphyriidae and Pennelidae, are considered to be economically important, particularly those that parasitize on commercially important wild and cultured fish (Boxshall 2005; Lester 2005; Lester and Hayward 2006; Webber et al. 2010). These parasites can damage their hosts directly by their attachment mechanisms or indirectly by their feeding behaviours (Boxshall 2005;

Lester 2005). The mechanical damaged imposed by these crustaceans include noticeable pathological changes on the infected hosts' tissues, such as hyperplasia, local lesions, hypertrophy and pressure necrosis, etc. (Boxshall 2005; Lester 2005, Lester and Hayward 2006). In addition, feeding activities of these parasites might cause surface lesions, damage to epidermis, haemorrhaging (leading to anaemia), mortality (especially with fry and juvenile hosts), reduction of productivity, stunted growth and alterations in host behaviour (Boxshall 2005; Lester 2005). Furthermore, some skin infecting copepods exert an adverse impact on the value and marketability of infected fish (Boxshall 2005; Lester 2005; Lester and Hayward. 2006).

Infections with crustacean ectoparasites were registered on wild and captive siganid hosts. Ho et al. (2004) reported four caligid species (*Caligus epidemicus*, *C. quadratus*, *Pseudocaligus uniartus* and *Lepeophtheirus sigani*) infecting *S. guttatus* in the Philippines. Three species of crustacean ectoparasites were reported from wild *S. javus* off Indonesian waters with low infection intensities (*C. quadratus*, *C. epidemics* and *Ergasilus* sp.) (Yuniar et al. 2007). Vinobaba (2010) reported the occurrence of the ergasilid copepods on *S. lineatus* and *S. canaliculatus* caught off Batticaloa Lagoon in Sri Lanka. The histopathological investigations of this incident revealed extensive tissue damage due to the attachment and feeding behaviour of ergaslids. Such damages included hyperplasia, atrophy, and mucous cell proliferation, resulting in mass mortalities of these fishes (probably due to improper functioning of their gills). The impact of three caligids, *Caligus epidemicus* Hewitt, 1971, *Pseudocaligus uniartus* Ho, Kim, Cruz-Lacierda & Nagasawa, 2004 and *Lepeophtheirus sigani* Ho, Kim, Cruz-Lacierda & Nagasawa, 2004 on their host *S. guttatus* was thoroughly described by Cruz-Lacierda et al. (2011), which included severe erosion, haemorrhage of body surface and mortality. Anshary and Muyassar (2013) described the pathology of *P. uniartus* infecting *S. guttatus* cultured in Indonesia. In the present study, at least three different caligids species have been reported from *S. canaliculatus* off Omani waters. Considering the above-mentioned impact of caligids on their hosts, the identification, assessment and review of the biology and life cycles of caligids infecting *S. canaliculatus* is crucial to prevent threats to the future siganid mariculture industry in the Sultanate of Oman.

With more than 110 registered species (WoRMS 2019), Gnathia are ubiquitous marine and estuarine ectoparasites of teleosts and elasmobranchs (Jones et al. 2007). Several species are reported as harmful to wild and farmed hosts, causing mortalities due to skin lesions and anaemia

(Gonzalez et al. 2004). Grutter et al. (2011) reported reduced swimming ability, high rate of oxygen consumption and low survivability of juvenile damselfish *Pomacentrus amboinensis* (Bleeker) due to the infection with *Gnathia aureumaculosa* Ferreira, Smit, Grutter & Davies, 2009. In their risk assessment study, Sanchez-Garca et al (2014) listed *G. vorax* as posing moderate risk to *Diplodus puntazzo* (Walbaum) mariculture in western Mediterranean because of its low specificity and potential pathogenicity to cultured marine fish. Although Gnathia species were found with low infection level in the gills of *S. canaliculatus* (3-14%, Al Jufaili et al. …c), due to their known documented pathogenicity, ability to establish and proliferate in fish farms as well as causing mortality, Gnathia are ranked as moderate risk to *S. canaliculatus*. Because of their abundance in sheltered habitats (e.g. coral reefs and sponges, Smit and Davies 2004), more studies about the distribution of Gnathia larvae in Omani waters should be conducted before choosing sites for the development of mariculture farms. **Several parasites species from *Siganus canaliculatus* are of mariculture importance (Working hypothesis 7).**

8 Future prospect

The present thesis emphasizes on how poorly developed is the field of marine parasitology in the Sultanate of Oman. It also highlights the main challenges that are believed to hinder the progress of this field in the country. Some of the main perspectives that must be considered in the future are increase basic and applied research activities, training of highly skilled staff, enhancing outreach, implementation of biosecurity and issues related to seafood quality and safety.

8.1 Improving basic research activities

According to the available literature and data obtained in the present study, it is clear that the **biodiversity** of aquatic parasites (marine and freshwater) in Oman is largely unknown. So far only a fraction (about 10%) of the fish species known to inhabit the waters of Oman have been examined for parasites (mostly teleost fishes) while elasmobranches and shellfishes remain unstudied. Also, many landing sites, especially remote ones, are not included in parasitological surveys. Thus, future studies should focus on including more host species and from different marine habitats along the coasts of Oman (e.g. Deepwater fishes). Also, to have a better estimation of the actual biodiversity of aquatic parasites an updated **parasite-host checklist** should be established using the existing data and those which were obtained in the present study. The list should be published in peer-viewed journal to facilitate knowledge sharing. Furthermore, more attention should be given to poorly investigated fish parasite groups (e.g. protozoans, microsporidians and myxosporeans). In addition, **taxonomic description** of the parasites to species level using standardized morphological methods should be supported with **molecular identification** (fish parasite barcoding). For a better understanding the role of parasites in the ecosystems and their interaction with their hosts, special attention should be dedicated to investigations of the **life cycle** of parasites in Omani waters.

8.2 Human resources development

Based on the current status of marine parasitology in the country, there is a crucial need of skilled and qualified personnel to work in this field in Oman. Therefore, it is necessary to develop training plans catered for the development of students and employees (government and private sectors) in marine parasitology. This can be achieved through creation of a **university curriculum** directed towards marine parasitology as part of fisheries and marine sciences courses. The main goal of these courses is to ensure that the students obtain the required theoretical knowledge and practical experience in the basic aspects of marine parasitology. In addition, intensive internships

should be provided to individual university and college students. Also, special **workshops and training programs** should be developed for fishermen, aquarists, seafood inspectors, fish farmers and staff working in the fish processing companies on detection and identification of fish parasites. Focus on **capacity building** and creation of a team of local experienced professional on various aspects of marine parasitology.

8.3 Conducting Applied research

The field of marine parasitology is an interdisciplinary science since it encompasses different subjects of science (Marcogliese 2008). Among the applications for fish parasites are their use in environmental studies (as bioindicators for pollution) and fisheries (as biological tags for stock discrimination). Through the results obtained in the present study it was clear that fish parasites are good bioindicators for the evaluation and assessment of aquatic ecosystems and habitat in Omani waters (chapter 6). Further investigation should focus on applying the star graph method using other fish-parasites models (e.g. groupers). Also it is suggested to include other species of fish parasites as potential bioindicators and to expand the sampling localities to other regions along the coast of Oman, especially areas which are likely to be exposed to pollution. Future **marine pollution monitoring programs** in the Sultanate of Oman should include fish parasites as effect and accumulate indicators to monitor the environment on a regular basis. In addition, the **bioaccumulation** ability of various fish parasites should be tested for different pollutants (heavy metal, Polychlorinated biphenyls (PCBs) and pesticides) to establish marine host-parasite systems as bioaccumulation indictors. There is a need to conduct laboratory **toxicology experiments** to investigate the effect of pollutants on different fish parasites.

Fish parasites have been used as biological tags to provide information on various aspects of host biology including fish stock separation, fish recruitment migrations, fish diet and feeding behaviour, and host phylogenetics and systematics (Williams et al. 1992). The results obtained in the present study (chapter 6) indicate that at least two populations of *S. canaliculatus* exist in the waters of Oman, suggesting that some parasites of *S. canaliculatus* (e.g. *Hysterolecithoides* sp. and *Preptetos* sp.) can be used for **stock discrimination**. Before implantation of these parasites as biological tags, thorough multidisciplinary approach related to oceanography, fish morphology and biology should be considered. Also, additional fish parasite surveys should be carried out to identify more biological tags for **stock assessment** especially for commercially important hosts

(e.g. large pelagics such as the widely distributed and vulnerable Narrow-barred Spanish mackerel *Scomberomorus commerson* (Lacépède). The use of fish parasites together with other stock assessment techniques (e.g. biomarker, otolith microchemistry and artificial tags) in a **holistic approach** (Catalano et al. 2014) could be useful in the development of **sustainable fisheries policies** in Oman.

8.4 Mariculture

With the prospective expansion of the mariculture industry in the Sultanate of Oman there is an urgent need to investigate and identify local parasites species infecting potential mariculture candidates and determine which could pose as a threat to the development of this vital sector. Thus, the application of **qualitative and quantitative risk analyses** for mariculture candidate host species is crucial to recognize the risk associated with the occurrence of parasites of these hosts. With the implementation of **risk assessment** in Oman it is possible to identify parasites that might decrease profitability through mortality, morbidity and loss of marketability (Huston et al. 2007). Also, health **surveillance and monitoring programs** should be implemented to obtain information on the geographical distribution (for both parasites and their hosts), parasite life cycle and pathogenicity. Parasites that are registered as a result of these monitoring programs should be reported to World Organisation for Animal Health (OIE). Finally, to achieve sustainable mariculture production in Oman, **biosecurity measures** should be applied to mariculture facilities to improve diagnostic, detection and disease management methods.

8.5 Seafood safety and quality policies and legislations

Seafood borne parasitic infections are becoming an important public health problem due to the growing international markets, improved transportation systems, and demographic changes (such as population movements) (Chai et al. 2005). Wild fisheries capture production and regional exportation is one of the main economic contributors to the Sultanate of Oman (MoAF 2015). However, seafood quality and safety issues related to fish parasites still exist. Parasitological surveys that are focused on zoonotic parasites are lacking which is important for the identification and characterization of parasites that are **hazardous** for consumer health. These surveys Also, the current detection methods are slow and time consuming, more **rapid and improved detection methods** such as RT-PCR should be implemented in the routine screening of parasites in seafood

products. Establishing **maximum detection limits** for the different seafood safety and quality fish parasites in Omani fish species based on the data obtained through the surveys.

8.6 Science outreach

Although marine parasitology is an important field of science, the subject is linked with negative reputation and poor public perception in the Sultanate of Oman. Mainly due to ignorant of its role. To improve the situation, science outreach activities should be considered to showcase the findings of our research to the public. Some suggested examples of these outreach events include **science open day** with school children. Such event will bridge the gap between research and education. They can also play part as to motivate the young generation to consider science fields for future prospects and particularly marine parasitology.

With an increased trend to use **social media** platforms, increasing public awareness via various social media platforms (e.g. Twitter and Instagram) will help to familiarize the public about the subjects of marine parasitology. Creating interest amongst community members will attract the attention of the policymakers. This in turn might lead to a consideration of deploying funds for future projects in the field.

Further, knowledge sharing with counterparts and experts in the field is essential to ensure staying updated with any advancement in the field. Participation in **international conference** and **networking** with fellow researches helps to meet potential collaborators. The support from concerned government authorities is appreciated and essential to ensure that Oman is on the global map of parasitology studies.

Part of our science outreach is to publish a pictorial **marine parasitology book**. The book will serve as a reader-friendly parasite-host atlas which will help the public to appreciate the biodiversity and beauty of marine parasites in the waters of the Sultanate of Oman. It will also serve as a quick identification book to recognize fish parasites for students, aquarists, fishmongers and hobbyist. Moreover, we are intending to develop and launch a **website/Blog** to share our activities and findings with pictures and information about the different parasites species that we encounter as part of our research Work.

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Independence Declaration for the Dissertation

I hereby declare with my signature that I have written the dissertation on my own and that I have not used any sources other than those specified. The thoughts taken directly or indirectly from the sources are identified as such. The dissertation has not yet been submitted to any other examination authority in this form.

Hiermit erkläre ich durch eigenhändige Unterschrift, die vorliegende Dissertation selbstständig verfasst und keine anderen als die angegebene Quellen und Hilfsmittel verwendet zu haben. Die aus den Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht. Die Dissertation ist in dieser Form noch keiner anderen Prüfungsbehörde vorgelegt worden.

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2015	Morphological, ultrastructural, and molecular description of Unicapsula fatimae n. sp. (Myxosporea: Trilosporidae) of whitespotted rabbitfish (Siganus canaliculatus) in Omani waters. (Parasitology Research)
2014	*Lamellodiscus* aff. euzeti Diamanka, Boudaya, Toguebaye & Pariselle, 2011 (Monogenea: Diplectanidae) from the gills of *Cheimerius nufar* (Valenciennes) (Pisces: Sparidae) collected in the Arabian Sea, with comments on the distribution, specificity and historical biogeography of *Lamellodiscus* spp. (Systematic Parasitology)
2013	*Microcotyle omanae* n. sp. (Monogenea: Microcotylidae), a parasite of Cheimerius nufar (Valenciennes) (Sparidae) (Systematic Parasitology)
2013	*Omanicotyle heterospina* n. gen. et n. comb. (Monogenea: Microcotylidae) from the gills of *Argyrops spinifer* (Forsskål) (Teleostei: Sparidae) from the Sea of Oman (Parasites and Vectors)

Acknowledgment

First of all, I would like to thank my supervisor Professor Harry Palm for accepting me into his lab and for his assistence, support, encouragement throughout my study period. I appreciate all the guidance, constructive comments and the help through all my personal and research difficulties which I faced during my thesis preparation. Working with Prof. Palm made me gain tremendous experience and made me a better researcher. I promise to use all the skills that I have acquired to become a better marine parasitologist.

I would like to thank my family members back home who supported me and encouraged me throughout my 7 years of writing this dissertation. Each one of you made my journey less stressful and more meaningful. Thank you for your prayers, hopefulness and for believing in me. Also, thank you for taking time to read my manuscripts and doing your best to help me with proofreading.

I would like to extend my gratitude to all members of Department of Aquaculture and Sea-Ranching who warmly welcomed me from the first day I arrived to the department. Special thanks goes to Ekaterina Pikalov (I am indebted for your help and valuable friendship, words cannot describe how grateful I am for being there for me through it all), Patrick Unger (thank you for the good times and for your help with my manuscripts), Stefan Theisen (it was a pleasure meeting and collaborating with you, thank for your comments and editing of my manuscripts), Sylvia Witt (thank you for your kindness and help with administrative issues), Sonja Kleinertz, Adrian Bischoff-Lang, Björn Baßmann, Jaydipbhai Suthar, Hendrik Sörensen (thank you for the good times, interesting discussions and your encouraging words).

Special thanks goes to Cristin Boch (thank you for being there for me during my 7 years as a supportive friend, your friendship means so much to me). My deepest thanks to Nematodez (for your endless support, listening to my parasitological ramblings, for your encouragement and for formatting and reading this disstertation).

Appendix 1

Exposure level and Consequence of parasite establishment for Siganus canaliculatus in mariculture farms in the Sultanate of Oman.

	Exposure level							
Parasite taxa	**Zone (North/South)**	**Water bodies**	**Sampling site**	**Marketability**	**Pathology**	**Mass mortality**	**Consumer health**	**Consequence**
Myxosporea								
Zschokkella sp.	North and South	all waterbodies	all sampling sites	–	X	–	–	Low
Ceratomyxa spp.	North and South	all waterbodies	all sampling sites	–	X	X	–	Moderate
Latyspora sp.	North	GoO	2 sampling sites	–	X	–	–	Low
Ortholinea spp.	North and South	all waterbodies	all sampling sites	–	X	–	–	Low
Unicapsula fatimae	North and South	all waterbodies	5 sampling sites	–	X	–	–	Low
Kudoa spp.	South	AS	2 sampling sites	X	X	–	–	Moderate
Monogenea								
Glyphidohaptor safiensis	North and South	all waterbodies	all sampling sites	–	X	–	–	Low
Tetrancistrum spp.	North and South	all waterbodies	all sampling sites	–	X	–	–	Low
Polylabris sp.	North and South	all waterbodies	all sampling sites	–	X	X	–	Moderate
Digenea								
Preptetos sp.	South	AS	2 sampling sites	–	X	–	–	Low
Stephanostomum spp.	North	PG	1 sampling site	–	X	X	–	Moderate
Nematoda								
Hysterothylacium sp.	North and South	all waterbodies	all sampling sites	–	X	–	X	Moderate
Nematode indet. Larvae	North	PG, GoO	4 sampling site	X	–	–	X	Moderate
Procamallanus sp.	North and South	GoO, AS	5 sampling sites	–	X	–	–	Low
Crustacea								
Caligus spp.	North and South	AS, GoO	3 sampling sites	–	X	X	–	Moderate
Gnathiidae indet. sp.(pranzia larvae)	North and South	all waterbodies	all sampling sites	–	X	X	–	Moderate

Appendix 2

Qualitative probability estimation matrix (based on AFFA 2001)

Exposure						
	Extreme	Negligible	Low	Moderate	High	Extreme
	High	Negligible	Low	Moderate	High	Extreme
	Moderate	Negligible	Negligible	Low	Moderate	High
	Low	Negligible	Negligible	Negligible	Low	Moderate
	Negligible	Negligible	Negligible	Negligible	Negligible	Low
		Negligible	Low	Moderate	High	Extreme
		Pathway				

Appendix 3

Quantitative risk estimation matrix, Negligible risk (1-5), Low risk (6-10), Moderate risk (7-15), High risk (16-20), Extreme risk (25)

Probability						
	Extreme	5	10	15	20	25
	High	4	8	12	16	20
	Moderate	3	6	9	12	15
	Low	2	4	6	8	10
	Negligible	1	2	3	4	5
		Negligible	Low	Moderate	High	Extreme
		Consequence				