Biodiversity and community structure of free-living marine nematodes from intertidal areas in the Persian Gulf

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Biodiversity and community structure of free-living marine nematodes from intertidal areas in the Persian Gulf

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Thesis submitted in partial fulfilment of the requirements for the degree of Doctor in Science (Marine Sciences)

Academic year 2016-2017

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Dedicated to:

Hadi, Parmida, Amirhossein, my parents and Marianne for all their love and supports

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Acknowledgments

First and foremost of all, I would like to praise the Almighty God, who has given me all the blessings I have.

I would like to express my deepest gratitude to my promoter **Prof. Dr. Tom Moens** who accepted me as his student and for offering me a place in his working group to work on this topic and for all the supports he provided during my time in Ghent. From the beginning to the end of my PhD study, he was always patient with me, spent lots of time to look after my works, show me the way to go and correct my study. His guidance from the initial steps up to the final stages of this work enabled me to develop an understanding of the subject. On top of everything, Tom was not only a great promoter but also an understandable friend that sympathize and help me overcome difficulties in my life during stay in Belgium. I am really grateful for his excellent guidance throughout my doctoral study at Ghent University. It was a great honor to work with him and I am certainly looking forward for future cooperations.

I am extremely grateful to the members of the reading and examination committee: **prof. Dr. Ann Vanreusel, prof. Dr. Koen Sabbe**, **prof. Dr. Helena Adão**, **prof. Dr. Giovanni dos Santos, prof. Dr. Frederik Leliaert** and **Dr. Nele De Meester** for their excellent comments and suggestions that significantly improved the thesis quality.

I would like to express my sincere appreciation to **Dr. Ruth Gingold** of Sweep & More for a thorough proofreading and help with the structure of chapter 5.

Many years in the Marine Biology Research Group, allowed me to meet many fascinating people. I would like to thank all former and present colleagues at this group for creating a friendly atmosphere. Special thanks to **Jelle Van Campenhout**, **Frederik Leliaert**, **Nele De Meester** and **Sofie Derycke** for their helps and technical expertise. Thank you for sharing your knowledge, good advice, always being open to discuss results and ideas. My sincere gratitude to **Annelien Rigaux** for her assistance in molecular experiments. I would like to extend my appreciation to **An-Sofie D'Hondt** for the Dutch summary translation. Many thanks to my office-mate**, Joey Genevieve Martinez,** for his honest friendship. Thanks to **Jeroen De Caesemaeker** for all of his computer technical helps. Thanks also to **Bart Beuselinck, Niels Viaene** and **Isolde De Grem** for their assistances.

My grateful thanks are also expressed to **Mrs. Marianne Border** and her husband **Hans** for lots of helps during our staying in Belgium. I will never forget your kindness. Without them, those past couple of years, would have been much more difficult. Marianne shared many of the joyful and most memorable moments with us. I am most grateful for her extremely friendly and helpful attitude.

I wish you to thanks my all Iranian friends in Belgium. I consider myself very fortunate to share your friendship, thank you.

I wish to thank **Dr. Hossein Negarestan** for his helps in the preliminary step of my PhD works. **Dr. Keivan Ejlali Khanaghah** (Persian Gulf and Oman Sea Ecology Research Center) is also acknowledged for his effective assistances through my PhD work.

My sincere appreciations also go to **my parents** who have been pillar of strength and all of the things I have are from them. Thank to them for their prayers and great moral supports. Thanks to my sister **Nasim** and my two brothers **Hamed** and **Sadegh** for being always concerned about me and for turning the distance into a minor gap via their constant greetings through email and chat and phone calls. Without their attention life was difficult here.

Finally, I would like to express my special appreciation and gratitude to my beloved husband, **Hadi**. Undoubtedly without his helps, love, guidance, spiritual support and encouragement, this thesis would not have been easily completed. He patiently spent some years of his life abroad and shared every moment and giving me the strength. He always kept me going and optimistic. I would like to thank him deeply for his unwavering love and all valuable supports and encouragements.

Very special thanks to my lovely children, **Parmida** and **Amirhossein**, for creation of enjoyable moments and comfortable atmosphere to finish my thesis. Thank you for making me always smile and laugh, even during the hardest times. As always, it is difficult to mention everybody, I would like to thank all those who direct and indirect supported and helped me during the time being in Ghent and completing this dissertation.

Narjes Sahraean

March 2017

Summary

The Persian Gulf is a semi-enclosed marginal sea, connected to the Gulf of Oman through the Strait of Hormuz. About one third of the world’s oil is produced in this region. It is an important military, economic and political region due to its oil and gas resources and it is one of the most important waterways in the world. Moreover, the Persian Gulf is located in a subtropical, hyper-arid region with often extreme air temperatures, a high evaporation rate and hence a high water salinity. The Persian Gulf hosts a variety of marine habitats such as rocky shores, estuarine ecosystems, mangrove habitats, salt marshes, sandy beaches, mud flats and coral reefs. Intertidal mud flats and sandy beaches are among the most widespread coastal habitats in the area. Because of the intensive human exploitation, the Persian Gulf is considered as one of the world's most polluted bodies of water. In addition to often wide-ranging pollution from oil-related activities, industrialization, urbanization, local sewage discharges and the building of numerous piers and docks all pose potentially serious threats to the marine life in this strategic location.

In this PhD study, focus is on the northeastern part of the Persian Gulf, specifically the area of Bandar Abbas, the capital city of the Hormuzgan province, which is situated in the southeast of Iran. In the Iranian part of the Persian Gulf, only biodiversity and distribution of macrobenthos taxa or assemblages in intertidal marine habitats have hitherto received some attention. The meiobenthos has remained a completely neglected component of the benthos. This PhD therefore provides the very first study on the free-living marine nematode assemblages in coastal habitats of the Persian Gulf. It thus contributes substantially to the knowledge of the coastal marine biodiversity in the Iranian part of the Persian Gulf.

Since this is a pioneering study, we first investigate biodiversity of nematode assemblages with a focus on spatial patterns in relation to local (so non-overarching) point sources of pollution, asking the question whether in such a broadly stressed environment, local pollution sources would still have a measurable impact on nematode assemblages. For this purpose, we focus on four beaches, and within each beach location, we assign three stations at different distances from local point sources of pollution. We then repeat the same sampling design almost exactly one year later to get a first impression of the consistency of the obtained spatial patterns over time, more specifically year-to-year variability. Finally, we look into the population-genetic structure of the two most abundant nematode species across a somewhat broader stretch (52 km) of coastline with the aim of studying connectivity and gene flow between populations from beach locations at a scale at which in other population-genetic

studies of coastal nematodes, significant structuring has regularly been observed. Since both nematode species studied here tend to occupy different depth layers of the sediment, we also tested the hypothesis that the surface-dwelling species would show a lesser population-genetic structuring compared to the real endobenthic species.

More specifically, in **chapter 2**, we assess the structure and biodiversity of the nematofauna in intertidal soft sediment habitats along the coast near Bandar Abbas. In addition, we also assess effects of local pollution sources (mostly sewage and garbage disposal) on the nematode assemblages. A total of 39 genera from 17 families was recorded. This diversity is low but comparable to several other anthropogenically impacted beaches. Five genera (*Daptonema, Ptycholaimellus*, *Paramonhystera*, *Terschellingia* and *Promonhystera*) together comprised 75 % of nematode abundance. There were significant differences in abundance as well as genus diversity between locations, but these did not unequivocally correlate with known drivers of benthic assemblage structure like sediment granulometry and hydrodynamics/beach morphodynamics. The location exposed to the strongest pollution input had the lowest nematode diversity and a very low abundance, the latter, however, only at the station nearest the local pollution source. Distance from local pollution sources also significantly impacted genus diversity. Our data demonstrate that local sources of anthropogenic disturbance are a major driver of assemblage diversity and structure in this area; even though the overall low diversity of nematodes indicates that the entire area experiences substantial stress, the local sources of disturbance still have measurable impacts on benthic assemblage diversity.

The next chapter (**chapter 3**) demonstrates that the spatial pattern documented in the first manuscript is subject to substantial year-to-year variability. We repeated the sampling design of the previous study, i.e. four beaches, with three stations each along a distance gradient of 50, 100 and 150 m from a pollution point source, exactly one year later. We found strong changes in spatial patterns of nematode assemblages, where many of the between-location and between-distance differences observed in 2008 disappeared. Haghani remained the least diverse beach, consistent with the presence of the largest urban drainage of Bandar Abbas. Suro and Dolat Park exhibited decreased abundance and diversity, whereas Terminal showed the opposite pattern. Distance-to-pollution gradients found in 2008 were largely absent in 2009. We hypothesize that the nearly threefold higher precipitation in the weeks preceding the 2009 sampling compared to 2008 caused a larger sewage discharge rate, enhancing the local impacts at Suro and Dolat Park and spreading them over a larger beach area. The opposite pattern at Terminal is difficult to explain, although a sampling performed four years later demonstrated a substantial coarsening of the sediments at this location. If this had already initiated in 2009, it might explain the increase in diversity and abundance. The overall number of genera encountered in our samples was equally high in both years. Four out of the five dominant genera of 2008 together made up 80 % of nematode abundances in 2009, while 22 ‘unique’ genera, encountered only in 2009, together contributed only 6.5 %. Two thirds of the 62 genera in the total two-year dataset were only found in one year and were all rare. This demonstrates that a correct estimate of genus richness requires a large and repeated sampling effort specifically aiming at the tail of rare genera.

In **chapter 4**, particular attention is devoted to the population-genetic patterns of the two most abundant nematode species, *Terschellingia longicaudata* and *Ptycholaimellus pandispiculatus*. Based upon a mitochondrial COI gene fragment, 17 and 2 haplotypes were found for *P. pandispiculatus* and *T. longicaudata*, respectively. Analysis of molecular variance (AMOVA) did not reveal a significant population-genetic structure for either species. The absence of genetic structuring may indicate substantial dispersal and gene flow in our study area. In another part of this chapter, to assess the species structure of *T. longicaudata* at a larger geographic scale, we compare 18S rDNA and COI sequences from Iran and the Scheldt Estuary in The Netherlands in order to ascertain whether they truly belong to the same species. Our data confirm previous studies that *T. longicaudata* likely constitutes a complex of multiple cryptic species, with one of these species having a cosmopolitan distribution.

In the general discussion (**chapter 5**), we integrate and elaborate on some of the aspects from the different chapters, and present perspectives for future research on meiobenthos in Persian Gulf intertidal habitats. Despite differences in degree and type of local pollution and in sediment granulometry and beach morphodynamics, the nematode assemblages of beaches in the vicinity of Bandar Abbas all shared similar main features: they were characterized by a low point (= sample) and local genus richness and a very pronounced dominance of only four or five opportunistic and stress-tolerant genera. Local differences were significant in 2008 and appeared largely linked to local pollution sources; however, most of this spatial structure was erased in 2009, perhaps in relation to changes in environmental conditions. What remains when data from both years were analysed together, is a relatively homogeneous beach area where a combination of natural and anthropogenic stressors cause a low local diversity and high dominance of few genera, and where hydrodynamics and currents homogenize assemblages at a regional scale. The latter point (i.e. hydrodynamics and currents) is further supported by a complete lack of population genetic structure in two of the most dominant species, whilst the former (natural and anthropogenic stressors) is partly supported by the extremely low population genetic diversity in one of these two species. The overarching effects of broader-scale stressors and of homogenization of beach communities due to hydrodynamics, hampers to some extent the use of nematode assemblages as bio-indicators of local pollution effects in this area, as evidenced by the inconsistent spatial structuring of the assemblages in two subsequent years.

Samenvatting

De Perzische golf is een half-gesloten ‘marginal’ zee, verbonden met de Golf van Oman via de Straat van Hormuz. Ongeveer één derde van de globale olie productie wordt in deze regio geproduceerd. Het is een belangrijke militaire, economische en politieke regio dankzij haar olie- en gasbronnen en het is één van de belangrijkste waterwegen ter wereld. De Perzische Golf is gelegen in een suptropisch, hyper-aried klimaat met vaak extreem hoge temperaturen, sterke evaporatie en bijgevolg hoge watersaliniteiten. De Perzische Golf herbergt een verscheidenheid aan mariene habitats zoals rotskusten, estuaria, mangrove habitat, zoutmoerassen, zandstranden, wadden en koraalriffen. Intertidale zand- en modderplaten zijn de meest voorkomende kust habitats in de regio. Als gevolg van intensieve menselijke exploitatie, wordt de Perzische Golf beschouwd als één van 's werelds meest vervuilde wateren. Naast de vaak uiteenlopende verontreiniging door oliegerelateerde activiteiten, vormen industrialisatie, verstedelijking, de lozing van afvalwater en de bouw van talloze pieren en dokken, allen potentieel ernstige bedreigingen voor het mariene leven in deze strategische locatie.

De focus van dit doctoraat ligt op het noordoostelijk deel van de Perzische Golf, in het bijzonder op het gebied van Bandar Abbas, de hoofdstad van de provincie Hormuzgan in het zuidoosten van Iran. Tot noch toe kregen de biodiversiteit en de verspreiding van macrobenthos taxa en gemeenschappen in intertidal mariene habitats in het Iraanse deel van de Perzische Golf al enige aandacht. De meiobenthos gemeenschap werd daarentegen tot voor kort nog niet onderzocht. Dit doctoraat bevat de allereerste studie van de vrijlevende mariene nematodegemeenschap in kusthabitats van de Perzische Golf en draagt bijgevolg in grote mate bij aan de kennis van de mariene biodiversiteit in de kustgebieden van deze regio.

Vooreerst werd de biodiversiteit van de nematodengemeenschap onderzocht, met een focus op ruimtelijke patronen in relatie tot de lokale (niet-overkoepelende) puntbronnen van verontreiniging. We vroegen ons af of lokale verontreinigingsbronnen een meetbare impact hebben in een regio die reeds door verschillende factoren sterke stress ondervindt. Hiervoor richten we ons op vier stranden, en binnen elk strand werden drie stations op verschillende afstanden van lokale vervuilingsbronnen bemonsterd. Vervolgens hebben we hetzelfde bemonsteringsplan precies een jaar later herhaald om een eerste indruk te krijgen van de consistentie van de verkregen ruimtelijke patronen na verloop van tijd. Tot slot kijken we naar de populatiegenetische structuur van de twee meest voorkomende nematodensoorten, op een schaal waar in vergelijkbare studies significante verschillen werden waargenomen. Over een iets bredere strook (52 km) van de kust onderzochten we de genetische structuur om de connectiviteit en genetische uitwisseling te bestuderen. Aangezien beide nematodensoorten zich vaak op verschillende dieptes in het sediment bevinden, testten we de hypothese dat de aan het oppervlak levende soorten mindere populatiegenetische structuur zouden vertonen in vergelijking met de echte endobenthische soorten.

Concreet beoordelen we in hoofdstuk 2 de structuur en de biodiversiteit van de nematofauna in intertidale zachte sedimenthabitats langs de kust in de regio van Bandar Abbas. Daarnaast evalueren we ook het effect van lokale bronnen van verontreiniging (voornamelijk afvalwater en afvalphaling) op de nematode gemeeschap. Een totaal van 39 geslachten van 17 families werden geregistreerd. Dit is een lage diversiteit, hoewel deze vergelijkbaar is met andere, door de mens beïnvloede stranden. Vijf dominante genera (*Daptonema, Ptycholaimellus, Paramonhystera, Terschellingia* en *Promonhystera*) beslaan samen 75% van de nematode abundanties. De totale aantallen, alsook de gemeenschapssamenstelling waren significant verschillend tussen locaties, hoewel deze verschillen niet eenduidig correleren met gangbare factoren die benthische gemeenschappen structureren zoals sedimentkorrelgrootte en hydrodynamieken/strand morfodynamieken. Op de sterk verontreinigde stranden vonden we de laagste nematodendiversiteit- en zeer geringe abundanties. Dit laatste geldt echter alleen voor het station dichtst bij de plaatselijke verontreiniging. Onze gegevens tonen aan dat de lokale bronnen van antropogene verstoring belangrijke drijfveren zijn achter de diversiteit en structuur van de nematodengemeenschap in dit gebied. Hoewel de algemeen lage diversiteit van nematoden aangeeft dat het hele gebied aanzienlijke stress ervaart, hebben de lokale bronnen van vervuiling nog aanzienlijke gevolgen voor de benthische diversiteit.

Het volgende hoofdstuk (hoofdstuk 3) toont aan dat het ruimtelijke patroon gedocumenteerd in het eerste manuscript onderhevig is aan aanzienlijke jaar-op-jaarvariabiliteit. We herhaalden daarom de staalname van het vorige onderzoek; wat betekent dat we dezelfde vier stranden, met elk drie stations bemonsterden op een afstand van respectievelijk 50, 100 en 150m van een vervuilde puntbron, precies een jaar later. We vonden dat de ruimtelijke patronen van nematode samenstellingen sterk varieerden tussen beide staalnames. Heel wat van de verschillen tussen locaties en stations waargenomen in 2008, verdwenen in 2009. Haghani bleef het minst diverse strand, ingevolge de aanwezigheid van de grootste rioleringen van Bandar Abbas. Suro en Dolat Park vertoonden lagere abundanties en diversiteit, terwijl we voor Terminal het tegenovergestelde patroon vaststelden. Veranderingen in gemeenschapssamenstelling in correlatie met de afstand tot de vervuiling die in 2008 werden waargenomen, waren grotendeels afwezig in 2009. We veronderstellen dat dit een gevolg is van een nagenoeg verdrievoudiging van de neerslag in de weken die voorafgingen aan de staalname van 2009 in vergelijking met de neerslag in 2008. Het sterk verhoogde afvoerdebiet van de riolering heeft mogelijks een positieve invloed op de lokale impact voor Suro en Dolat Park, maar deze impact verspreidt zich over een grotere oppervlakte en afstand tot de verontreinigingsbron. Het tegenovergestelde patroon op Terminal is moeilijk te verklaren, hoewel een staalname vier jaar later aantoonde dat er een substantiële verhoging is in de mediane korrelgrootte van de sedimenten op deze locatie. Indien deze veranderingen in sedimenteigenschappen in 2009 reeds aanvingen, kan dit mogelijks de stijging van de diversiteit en abundanties verklaren. Het totale aantal genera aangetroffen in onze stalen was even hoog in beide jaren. Vier van de vijf dominante genera van 2008 beslaan in 2009 80% van de totale abundanties, terwijl 22 unieke genera, enkel in 2009 waargenomen, voor 6,5% bijdragen. Twee derden van de 62 genera die gedurende beide staalnames werden waargenomen, werd slechts in één jaar geobserveerd en alle waren zeldzaam. Dit toont aan dat een goede schatting van genusrijkdom en aanwezigheid van zeldzame soorten grote en herhaalde staalnames vereist.

In hoofdstuk 4 gaat de aandacht naar patronen binnen de populatiegenetica van de twee meest abundante nematodesoorten, *Terschellingia longicaudata* en *Ptycholaimellus pandispiculatus*. Op basis van een mitochondriaal CO-gen fragment, werden respectievelijk 2 en 17 haplotypes waargenomen. Analyse van de moleculaire variantie (ANOVA) toonde geen significante verschillen in de populatiegenetische structuur van beide soorten. De afwezigheid van genetische structurering is mogelijks een indicatie voor aanzienlijke geografische dispersie en de genetische uitwisseling in ons studiegebied. In een ander deel van dit hoofdstuk vergelijken we 18S rDNA en COI-equenties van *T. longicaudata* uit Iran en het Schelde-estuarium in Nederland om hun structuur te beoordeelen op een grotere geografische schaal en om zo ook na te gaan of ze echt behoren tot dezelfde soort. Onze gegevens bevestigen eerdere studies dat *T. longicaudata* waarschijnlijk een complex is van verschillende cryptische soorten, en dat één van deze soorten een kosmopolitische distributie heeft.

In de algemene discussie (hoofdstuk 5) integreren we een aantal aspecten van de verschillende hoofdstukken en gaan we hier verder op in. We doen hier ook een aantal suggesties voor toekomstig onderzoek naar het meiobenthos van intertidale habitats in de Perzische Golf. Ondanks duidelijke verschillen in de aard en mate van lokale vervuiling en in de granulometrie en morfodynamiek van de onderzochte stranden, vertoonden de nematodengemeenschappen van de stranden in de nabijheid van Bandar Abbas allemaal enkele zeer gelijkaardige hoofdeigenschappen: ze werden alle gekarakteriseerd door een lage genusdiversiteit op het niveau van individuele stalen en van stranden (= lokale diversiteit), en hadden ook alle een zeer uitgesproken dominantie van slechts vier of vijf opportunistische genera die tolerant zijn tegen allerhande omgevingsstress. Lokale verschillen (= tussen stranden) waren significant in 2008, in relatie tot lokale vervuilingsbronnen. Deze lokale structuur was evenwel grotendeels afwezig in 2009, mogelijk als gevolg van andere klimatologische omstandigheden. Wanneer de data van beide jaren samen worden geanalyseerd, komt een beeld naar voor van een relatief homogene strandgemeenschap, waar een combinatie van natuurlijke en anthropogene stressoren een lage diversiteit veroorzaken op het niveau van individuele stalen en stranden, en waar hydrodynamiek en stromingen de gemeenschappen op een regionale schaal homogeniseren. Dit wordt verder bevestigd door het complete gebrek aan enige populatiegenetische structuur bij twee van de meest dominante soorten in het gebied. Het overwegende effect van natuurlijke en anthropogene stressoren wordt deels ondersteund door de extreme lage populatiegenetische diversiteit van één van beide soorten. Dit effect, en het homogeniserende effect van de hydrodynamiek en stromingen in de regio, bemoeilijkt het gebruik van nematodengemeenschappen als indicatoren van lokale vervuilingseffecten, zoals moge blijken uit de inconsistente ruimtelijke structuur van de nematodengemeenschappen in twee opeenvolgende jaren.

Chapter 1

General introduction

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General introduction

This PhD research provides, at least to our knowledge, the very first study on free-living marine nematode communities in coastal habitats of the Iranian part of the Persian Gulf. In this introduction, four main topics are dealt with. First, a brief general overview of the study area is given, including 1) geographical and physical features, 2) geo-political and industrial importance, 3) different marine ecosystems and 4) environmental threats to the Persian Gulf marine environment. Secondly, we narrow down our focus to sandy beaches, and hence provide a definition, information on characteristics, and classification of sandy beaches. In the third part, we introduce the benthic organisms and their ecological significances with emphasis on the meiobenthic fauna and specifically marine nematodes. At the end, the specific objectives of the PhD study and their rationale will be addressed.

1. Study area: the Persian Gulf

1.1 Geographical, physical and climatological features

The Persian Gulf (henceforth abbreviated as PG), located in the Southwest Asian region, formed by rising sea levels from the Indian Ocean (Kassler 1973; Lambeck 1996). Earth history indicates that it has been subject to various periods of **glaciation**. At the end of the last ice age, the sea level rose gradually and about 6,000 years ago it reached its current depth. Consequently, the present shape of the PG formed during the last glacial period (Lambeck 1996; Taghizade et al. 2012) (Fig. 1-1).

The PG covers an area of 239,000 km^2 and spans a length of 990 km; it has a volume of 8780 km^3. It is a semi-enclosed and young marginal epicontinental sea, connected to the deep (1000 m) Oman Sea through the 56 km wide Strait of Hormuz (Chao et al. 1992). As such, **it forms an extension of the Indian Ocean (Oman Sea) through the Strait of Hormuz**.

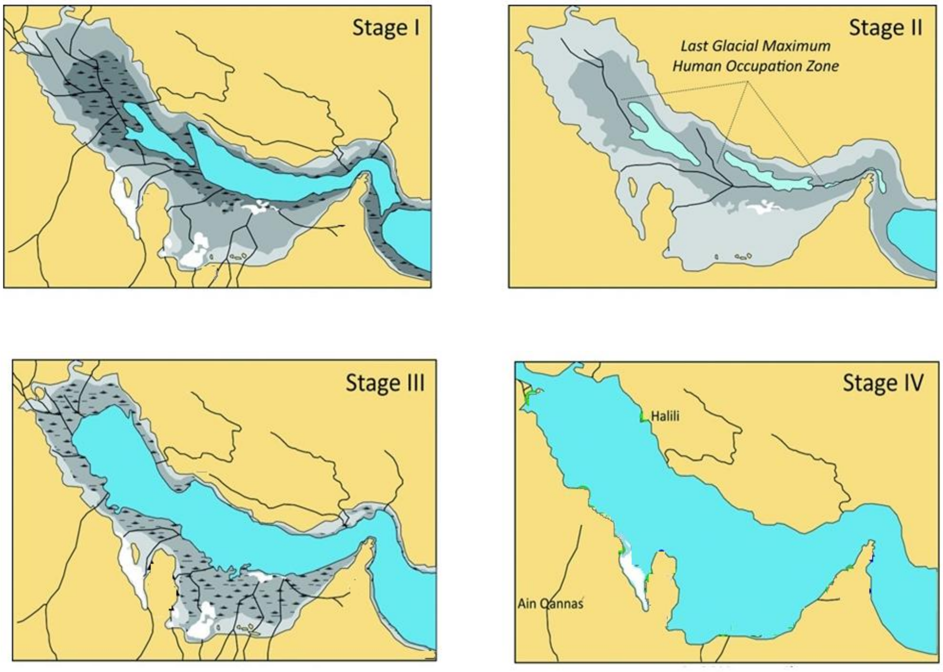

Fig. 1-1. A history of the Persian Gulf over the period from 74,000-24,000 years ago (stage I), 24,000-14,000 years ago (stage II), 14,000-8,500 years ago (stage III), until the present situation, which formed 8,500- 6,000 years ago (stage IV). Adapted from (Rose 2010).

The PG is surrounded by several countries including Saudi Arabia, the United Arab Emirates (UAE), Qatar, Bahrain, Kuwait and Iraq on the southwestern side and Iran on the northeast (Fig. 1-2). As its coastline dominates the whole northern shore of the PG, Iran is a very important country in the PG region. Its western end (northwestern shoreline) is marked by the major river delta of Arvand Rud, which carries the freshwaters of the Euphrates and the Tigris (Fig. 1-2). This is the major freshwater input into the PG and has its origins in both Iraq and Iran. Tigris, Euphrates and Karun river which together form the Arvand Rud have a seasonally variable discharge, with an annual mean of 1400 m^3/s (18 cm/year) (Pous et al. 2015).

Fig. 1-2. Map of the Persian Gulf region with surrounding countries.

The PG has an **average water depth of only 36 m**, comprising a broad shallow southern margin (< 20 m deep) along the coasts of Qatar, Bahrain and the UAE, and a relatively narrow and deep north-eastern margin along the coasts of Iran (Kämpf and Sadrinasab 2006). The deepest areas are in front of the Iranian coast, reaching from 60 m to about 100 m at the entrance to the Strait of Hormuz (Sheppard et al. 1992).

Highest **carbonate concentrations** are found in the shallow waters of the western and southern Gulf. Sediments of terrestrial origin are limited to the northwest, where the waterway of the Arvand Rud discharges into the PG, and the eastern Iranian shoreline where terrestrial fluvial sediments from the Zagros mountains are occasionally accumulated in the nearshore region (Barth and Khan 2008).

The PG is located between 24-30°N latitude and 48-57°E longitude. In those latitudes, descending dry air produces **arid conditions**. The largest continuous sand desert on Earth, namely the Rub’al-Khali desert (Mughal 2013), which covers 650,000 km^2 including parts of Saudi Arabia, Oman, the UAE and Yemen (*the Empty Quarter*), surrounds the PG. It is one of the largest continuous bodies of sand on Earth and has one of the largest oil reserves in the world. The hyper-arid climate causes a high evaporation rate and, as a consequence, a **high salinity**, as evaporation is greater than the combined rainfall and river discharge within the Gulf (Reynolds 1993). More specifically, evaporation rate is 1.4–2.1 m/year (per unit surface area), whereas total river runoff is 0.15–0.46 m/year and precipitation is very limited (0.07–0.1 m/year) (Sugden 1963; Reynolds 1993; Johns et al. 2003). Water exchange with the Oman

Sea through the Strait of Hormuz is the main source of the water balance for the PG. Owing to the high evaporation and small precipitation, the PG exhibits an **inverse estuary-like circulation** (Fig. 1-3) in which surface water flows into the PG in the northern part of the Strait of Hormuz as a wedge of less saline water that penetrates deep into the PG along the Iranian coasts, increasing in salinity and exiting at depth through the Strait of Hormuz into the Oman Sea (Kampf and Sadrinasab 2006). The effect of the high evaporation rate is further enforced by the rather narrow connection to the open ocean, together leading to the formation of the saline, dense water mass known as the "Persian Gulf Water" (PGW). Generally, salinity is minimal (39.3) in summer and maximal (40.8) in winter; in tidal pools and lagoons it may reach 70-80 (Fig. 1-4). However, salinity gradients can result either from river fluxes, precipitation and evaporation or from exchanges between the PG and the Oman Sea (John et al. 1990).

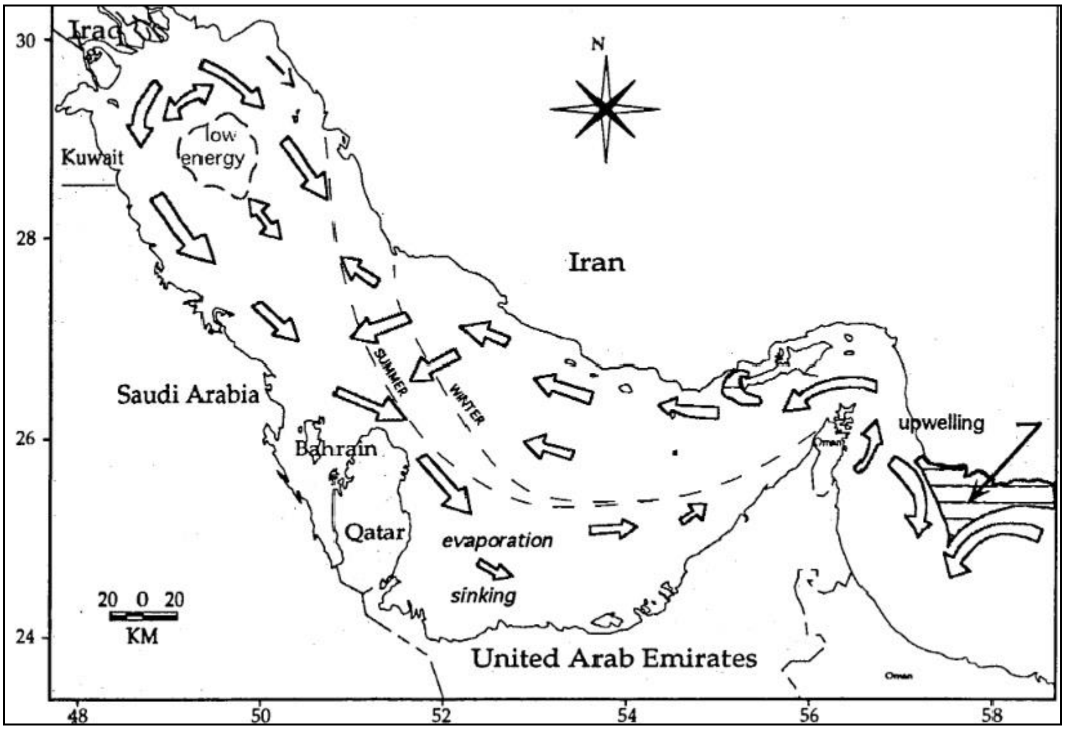

Fig. 1-3. Diagramatic representation of water circulation in the Persian Gulf (PG). The arrows show inflow of marine water from the Oman Sea to the PG at the sea surface. As a result of evaporation, this water becomes gradually more saline and therefore also more dense as it is moving further inward. Near the head of the Gulf, the now very dense surface seawater sinks down and outwells back to the Oman Sea near the bottom. Adapted from (Yao 2008).

During summer, **regional air temperatures are among the hottest in the world** and maximum air temperature can frequently reach in excess of 49 °C. Summer sea water temperature (SST) averages 33 °C, but can reach > 35 °C in shallow waters (Chao et al. 1992). Winter SSTs average 22 °C near the Strait of Hormuz and decrease to 16 °C near the head of the Gulf, resulting in an annual temperature range of ca. 20 °C across the PG, which is one of the largest annual fluctuations of seawater temperature in the world (see Fig. 1-5) (Chao et al. 1992). Due to its location near the tropical zone, the difference between SST and land temperature in the PG does not vary much throughout the year, allowing for the sea breeze to occur in all months (Eager et al. 2008). The winds in the PG are predominantly northwesterly throughout the year. Indeed, the **Shamal wind** is the best known weather phenomenon in the PG. It is a northwesterly wind which occurs year round (Thoppil and Hogan 2010). During winter (November-February), the winds are slightly stronger (5 m/s) than those during summer (June-September) (3 m/s).

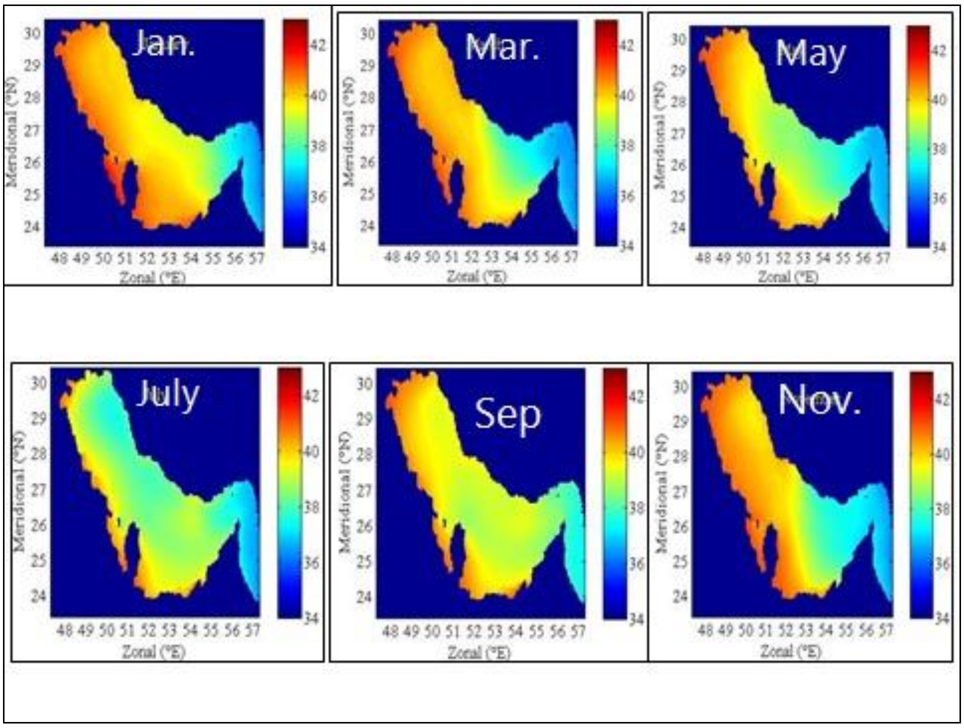

Fig. 1-4: Sea-surface salinity in the Persian Gulf in different times of the year, averaged over the period 2005-2012. Adapted from (Yan 2015).

The **tidal range** varies from about 1 m at Dubai and 1.2-1.5 m around Qatar to 3.0-3.4 m in the northwest (Kuwait), and to a maximum of 5 m in the Clarence Strait[1] between Qeshm island and the mainland (Schwartz 2005). Tidal currents are strong (up to 8 km/h) at the entrance of the gulf, but elsewhere rarely exceed 1 to 3 km/h, except between islands or in estuaries and lagoon entrances.

The southern part of Iran (north of the PG) is bounded with around 1000 km of subtropical coastal area. Along this rather long coastline, three coastal provinces are located (Fig. 1-2), Khuzestan in the northwest, Hormuzgan in the northeast (largest province), and Bushehr in the central. In this PhD study, focus will mainly be on the northeastern part of the PG, specifically the area of Bandar Abbas, the capital city of the **Hormuzgan province**, situated in the southeast of Iran, at ca. 1335 km from Tehran. Due to its geographical location, the Hormuzgan province plays a vital role in fisheries, petroleum and industrial activities, international trade and marine transportation in southern Iran. During 2003-2013, Hormuzgan province accounted for 60 % of total landings in Iranian waters of the PG and was the biggest fishery area of the region (IFO 2014). The human population of Bandar Abbas has increased from 87,000 in 1977 to around 500,000 in 2010; it is now considered the biggest city in the south of Iran. Since 1980, Bandar Abbas has been rapidly developed as an oil export pole of Iran. This city is located in a hot and humid region. The city possesses some small creeks, known as Khoor, which function as drainage systems for flash floods as well as sewages of residential districts.

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[1] The Clarence Strait is a narrow strait between Qeshm island and the mainland. The native name for the strait is Khuran (http://en.wikipedia.org).

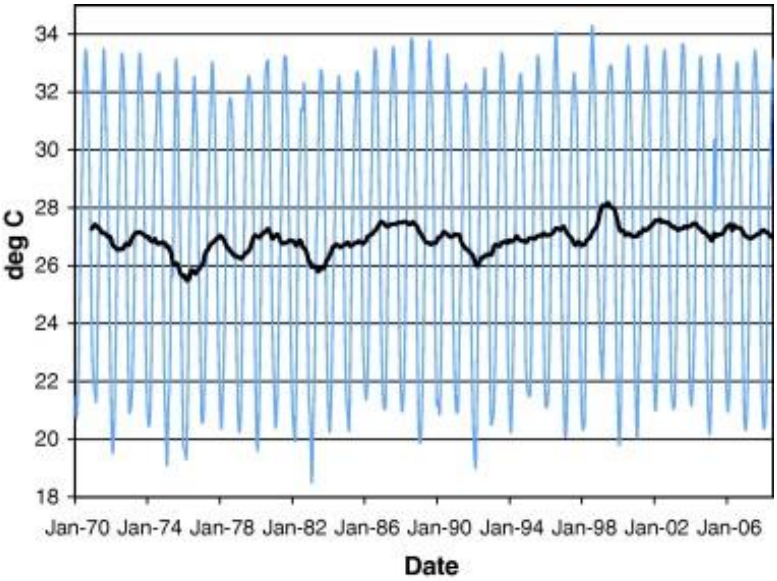

Fig. 1-5. Sea-surface temperature from the central part of the Persian Gulf. 1×1° lat/long block whose top left corner is 57°N, 52°E. The central black line is the running mean temperature per year, while the blue line depicts yearly maxima and minima. Figure from (Sheppard et al. 2010).

1.2 Geo-political and industrial importance

The name "Persian Gulf" dates back to about 500 years BC when the first Persian Empire (the Achaemenids) ruled over most of central and western Asia (Potter 2009). Some Arab governments refer to it as the "Arabian Gulf" or "The Gulf", but neither term is accepted internationally. The official name "Persian Gulf" is used by the International Hydrographic Organization.

The Middle East and its subregion, the PG region, have long been among the economically most important regions of the world and among the major centers of world affairs; they are strategically, economically, politically, culturally and even religiously sensitive areas. The PG and its coastal areas are **the world's largest source of crude oil/gas**; consequently, related industries dominate the region. Due to their huge oil and gas resources, the regional states play a significant role in global economy. Their natural wealth and geographical location have made the region very important from a geostrategic point of view. Nearly 50 % of the world's total oil reserves are estimated to be in the PG. Also, the PG is the richest area in gas worldwide, holding 40 % of global gas reserves. Iran and Qatar hold nearly 30 % of global gas deposits or 75 % of Middle East reserves (Moussavi and Aghaei 2013). For example, the

South Pars gasfield (offshore southern Iran) and its extension within Qatari territorial waters (North field) comprise the world’s largest offshore gas accumulation. As energy has always been regarded as a matter of security (Bahgat 2011; McGowan 2011), the oil and gas reserves present in the PG have been taking an increasingly critical role in the economies of the developed and industrialized countries.

The PG has always been a key international trade route connecting the Middle East to India, East Africa, Southeast Asia and China. The Strait of Hormuz is considered one of the world’s strategic passages for oil and its by-products which, if disrupted, would negatively affect the global economy. According to the U.S. Energy Information Administration, in the year 2011, an average of 14 tankers per day passed out of the PG through the Strait of Hormuz, carrying 17 million barrels (2,700,000 m^3) of crude oil. This represents 35 % of the world's seaborne oil shipments and 20 % of oil traded worldwide. The report stated that more than 85 % of these crude oil exports went to Asian markets, with Japan, India, South Korea and China being the most important destinations (Houshialsadat 2013).

Fisheries represent the second most important natural resource after oil/gas in the PG, and the most important renewable natural resource (Carpenter 1997). Accordingly, fisheries make a significant contribution to food provision in PG countries and play an important role in their economies (Sheppard et al. 2010). In the past, the PG has also been famous for its precious pearl oyster fisheries.

1.3 Marine Ecosystems of the Persian Gulf

Several papers have recently been published about fisheries, biodiversity, ecosystem health and sustainability of PG ecosystems (Khan and Al-Ajmi 1998; Nadim et al. 2008; Hamza and Munawar 2009; Sheppard et al. 2010; Feary et al. 2011; Sale et al. 2011; Burt 2013). Generally, the natural environment of the PG is very rich and **a wide variety of marine habitats** exists in the region (Fig. 1-6).

Despite the harsh environmental conditions resulting from a high salinity, high summer temperatures and large yearly temperature fluctuations, the PG supports a range of coastal and marine ecosystems which contribute to the biological and habitat diversity in the marine environment and provide valuable ecological sites for a variety of commercially and/or biologically important marine organisms. For example, *Portonus segnis* is the most commercially important of all true crabs[2] in the coastal habitat of Hormuzgan province (Safaie et al. 2013). The coasts also provide the main nesting habitats of species of sea turtles, i.e. the hawksbill turtle (*Eretmochelys imbricata*) and the green turtle (*Chelonia mydas*) (Mobaraki 2004). Several marine protected areas in the Iranian part of the PG, such as the Hara Biosphere Reserve, are among the most visible management steps taken to conserve specific habitats and/or species. In addition, the Strait of Khuran is a Ramsar site, providing habitat to two globally threatened species: a wintering habitat for the Dalmatian pelican *Pelecanus crispus*, and a regular feeding place for the green turtle *Chelonia mydas*.

The **main marine coastal ecosystems** of PG include rocky shores, coral reefs, seagrasses, mangrove habitats, estuaries, salt marshes, sandy beaches and mud flats (Sheppard et al. 2010) (see Fig. 1-6). In the following sections, we will briefly introduce those important coastal ecosystems of the PG.

- Rocky shores: Because of bed stability, presence of cracks and fissures in rocks and spaces available under stone fragments, rocky shores are considered as one of the most biologically rich environments in the PG (Sheppard et al. 2010). Rocky shores occur mostly in the north and have largely been formed as extensions of the Zagros mountains near the coastal areas. They are present mainly in the Oman Sea and the Strait of Hormuz (Nouri et al. 2010).

- Coral reefs: Although over the past two decades, research on coral reefs in the PG has grown exponentially, the PG's reefs are in fast decline. Over 70 % of regional reefs have by now been effectively lost (Wilkinson 2008), and the prognosis for the future of the remaining PG reefs is not bright. Generally, due to the extreme temperature and salinity, diversity of corals in the PG is low and comprises only ca. 55-60 species or about 10 % of the coral species that occur in the wider Indo-Pacific region (Sheppard et al. 2010). According to Riegl and Purkis (2009), coral communities in the PG also experience bleaching and frequent temperature-related mass mortality events. For a recent comprehensive study of coral communities in PG, we refer to (Bauman et al. 2013; Burt 2013).

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[2] Among decapod crustaceans, true crabs (Brachyura) are by far represent the most species-rich distinct decapod morphotype (with approximately 7,000 extant species). Because of their high nutritional value (proteins and minerals), they are used as a valuable food for livestock and poultry in the world (Naderlo et al. 2011)

- Estuaries: There are many definitions for estuaries (Mitra and Zaman 2016); one of the more commonly used states that an estuary is "*a semi-enclosed coastal body of water which has a free connection with the open sea at least intermittently and within which salinity of the water is measurably different from the salinity in the open ocean*" (Pritchard 1967; Dyer 1997). In the Iranian part of the PG, most of the estuaries are riverine. The largest and the most important riverine estuarine system is the Arvand Rud delta located in Khuzestan province, where the Tigris and Euphrates enter the northern PG. Bahmanshir is another important estuary in the southwest of Iran (Khuzestan province), which supplies the agricultural, irrigation and drinking waters for the large cities of Abadan and Khorramshahr (Etemad-Shahidi et al. 2015).

- Seagrasses: Seagrasses perform a variety of functions within ecosystems and have both economic and ecological value (Costanza et al. 1997). As a habitat, seagrasses offer food, shelter and essential nursery areas to fish species and to the invertebrates that live within, or migrate to seagrasses. Seagrasses also play important roles in water flow, nutrient cycling, and food web structure. Only three species of seagrass occur in the PG, namely *Halodule uninervis, Halophila stipulacea* and *Halophila ovalis* (Sheppard et al. 2010). These species are generally tolerant to salinity and temperature extremes. They are particularly prevalent along southern and western shores (Price and Coles 1992). According to (Erftemeijer and Shuail 2012), around 7000 km^2 of seagrass habitat have been mapped in the PG to date. However, seagrass habitats in the PG, like in other places in the world (Orth et al. 2006), are suffering a significant threat as a consequence of increasing anthropogenic stresses (Erftemeijer and Shuail 2012).

- Mangroves: Mangroves are coastal vegetations composed of shurbs and trees that grow mostly in saline or brackish water in tropical or subtropical areas. Mangrove habitats are ecologically important coastal ecosystems that provide food, shelter and nursery areas for a variety of terrestrial and marine fauna. The PG coastlines are mostly dominated by only one species of mangrove, *Avicennia marina* (13,000 ha). A second species, *Rhizophora mucronata*, is also found at Sirik in the Strait of Hormuz. They occur principally in lagoons and on leeward sides, islands and shoals along the Iranian coast as well as in Saudi Arabia, Bahrain, Oman, Qatar and the U.A.E. (Spalding et al. 2010). High salinity is believed to be responsible for the generally small stature of mangrove trees in the PG. Mangrove forests in Iran cover more than 15,000 ha distributed from the Oman Sea to the Mond protected area in the western part of the PG (Mehrabian et al. 2009).

- Sandy beaches: Sandy beaches are one of the dominant coastal habitats in the Iranian part of the PG. According to one estimate, the length of sandy shores in the Iranian part of the PG is about 577 km, 65 % of which are located in Hormuzgan province and the rest in Bushehr province (Naser 2014). Since we chose intertidal areas of sandy beaches in the northern part of the PG as the study area for this thesis, we will comprehensively introduce general features and more detailed information on sandy beach habitats in the following pages (*§2 Intertidal areas and sandy beaches*).

- Mudflats: due to the sedimentary nature of the PG, sandy and muddy substrata are the most widespread habitats. Mudflats are especially dominant intertidal habitats along the coastline of the western Gulf, where water movements are less turbulent. For example, as a result of the discharge of silt from the Tigris and Euphrates rivers, 57 % of the Kuwait coastline is characterized by mudflats (Al-Zaidan et al. 2003). The total area of mudflats in the Iranian part of the PG is estimated to be about 7345 km^2, 76 % of which in Khuzestan Province, 9.7 % in Bushehr province and the rest in Hormuzgan province (Etemad-Shahidi et al. 2015). In the Strait of Hormuz, three sites with a portion of intertidal mudflats occur, all being protected by the Ramsar convention (Matthews 1993).

Fig. 1-6. Habitat diversity along the Iranian coast of the Persian Gulf. a) "artificial" mangrove in Qeshm Island; b) pneumatophore zone of *Avicennia* mangroves in Mahshahr in Khuzestan; c) mangrove of Tiab Protected Area in Hormuzgan; d) a reflective sandy beach in Hormuzgan; e) mudflat in Khuzestan; f) a shore between Bandar Kangan and Asaluyeh in Bushehr; g) rocky/cobble shore between Bandar-Lengeh and Bandar Khamir in Hormuzgan; h) and j) rocky cliffs at the border of Hormuzgan and Bushehr. Adapted from (Naderloo and Tuerkay 2012).

1.4 Environmental threats to the Persian Gulf marine environment

General information

As mentioned before, the PG is a semi-enclosed sea with a high-latitude geographical position and is characterized by naturally extreme environmental conditions. Despite these harsh environmental conditions, the PG supports a range of coastal and marine ecosystems. On the other hand, this area is a major route for oil exploitation and export (Emmerson and Stevens 2012). This is one of the main reasons why the PG represents a stressed ecosystem and is considered one of the most polluted water bodies in the world (Elshorbagy 2005; Khan 2007).

In April 1978, eight governments, i.e. Bahrain, Iran, Iraq, Kuwait, Oman, Qatar, Saudi Arabia and the UAE, developed the Regional Organization for the Protection of the Marine Environment (ROPME). The objective of the ROPME is to coordinate the member states' efforts towards protection of the marine water quality, the environmental systems as well as marine life, and to diminish the pollution caused by the economical development activities of the member states. In addition, ROPME requested the members to exert their maximum efforts to protect the marine environment. There are four main components in the scope of the ROPME: environmental assessment, environmental management, legal component, and institutional and financial arrangements (Abuzinada et al. 2008). During the past years, ROPME has made significant contributions to the environmental health of the PG region (Nadim et al. 2008).

Environmental stressors

Marine organisms are subject to many stressors. Environmental threats to marine ecosystems can be generally attributed to multiple natural and anthropogenic stressors and the interactions between them (Halpern et al. 2008). It is sometimes difficult to clarify exactly whether the stressor is natural or anthropogenic in origin, as anthropogenic stressors are indeed superimposed on stress caused by natural environmental factors (Raffaelli and Hawkins 2012).

Natural stressors in marine environments can come in a number of forms and from a variety of sources. Environmental extremes represent stressors that interfere with normal functioning of marine ecosystems (Breitburg and Riedel 2005; Khan 2007). This is clearly the case for the PG where naturally extreme environmental conditions occur (high salinity, variation in SST > 20 °C). Predictions of the long-term salinity balance in the PG using a 3D numerical model actually indicate that the salinity will increase continually if no mitigation measures are taken (Yan, 2015) (Fig. 1-7). Consequently, these environmental variables, and the interaction between them, can have pronounced effects on the physiology of marine organisms in the PG, as well as on their overall abundance, community composition, biodiversity, and spatio-temporal distribution (Sheppard 1993; Sale et al. 2011; Erftemeijer and Shuail 2012). An increasing body of literature indicates that some animal and plant communities are substantially affected by the climatic extremes in the PG (Abuzinada et al. 2008; Sheppard et al. 2010; Bauman et al. 2013; Burt 2013; Al Shehhi et al. 2014). This is for instance the case for fish assemblages (Feary et al. 2010) and coral reefs (Coles 1997; Riegl and Purkis 2009).

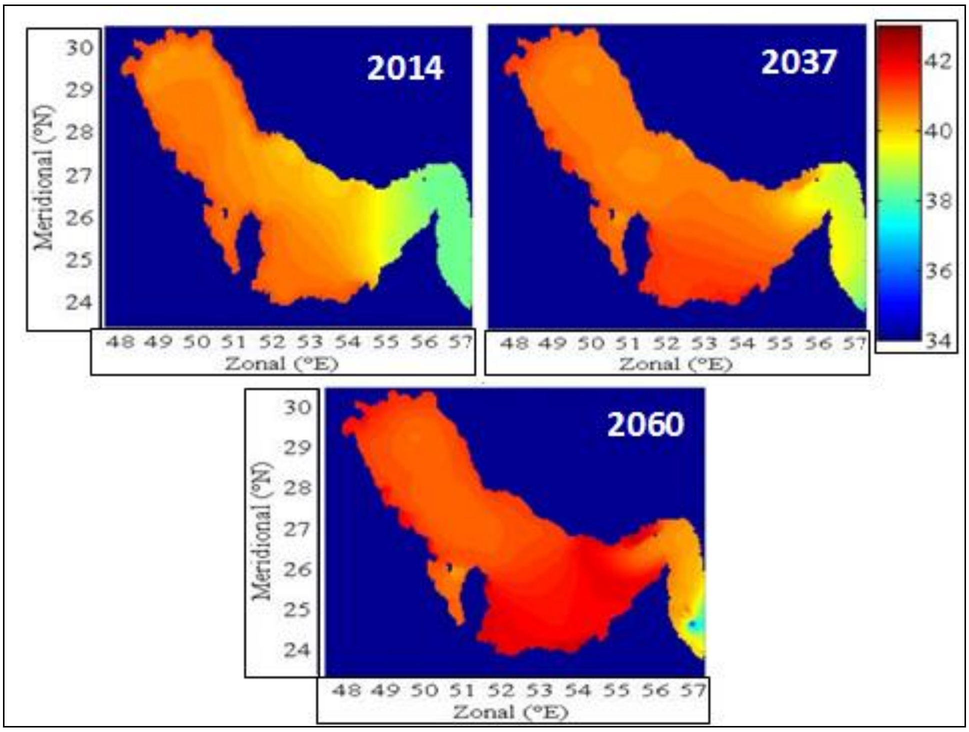

Fig. 1-7: Results of a numerical simulation of the long-term balance of salinity in the Persian Gulf, from January 2014 to December 2060. Adapted from Yan (2015).

Anthropogenic stressors can originate from different sources (Fig. 1-8) including, among others, pollution from oil-related activities (oil exploration and production, oil spills), sewage treatment plants, power and desalination plants, chemically contaminated effluents from agricultural and industrial activities, coastal reclamation and destructive fishing practices (over-fishing) (Khan 2007). All these anthropogenic activities result in a number of marine pollution problems and a wide range of adverse effects that cause significant damages to the ecosystem.

In the last decades, the PG has experienced **coastal development** associated with the discovery and exploitation of oil. Coastal development in the area is often not properly planned and has caused environmental stress such as habitat fragmentation and destruction (Sale et al. 2011). PG has suffered from frequent oil spills. Serious spills have, for instance, occurred in 1983 (during the Iraq-Iran war) when 38 million gallons of crude oil were released from the Nowruz Field (Nadim et al. 2003). In 1991, the PG faced the First Gulf War. During this event, Kuwait oil fields were set on fire, resulting in a catastrophic risk to the marine environment of the PG region. Moreover, 500 tonnes of light crude oil were released in the Strait of Hormuz in 2005. In 2007, an area of 800 km^2 was contaminated near

Bandar Abbas as a result of oil spills along the Strait of Hormuz (Subanthore 2011). In the Iranian part of the PG, the pollution impact of these activities has been evaluated in several studies, all indicating that crude oil accumulated and remained for a long time in the coastal area (Emtiazi et al. 2009; Hassanshahian et al. 2010; Aein Jamshid et al. 2011; Hassanshahian 2014). In those studies, only biodiversity and distribution of macrobenthos communities in the marine habitat have been evaluated (see section 3-10); meiofauna have not been part of any monitoring approaches so far. Overall, there are persistently high levels of hydrocarbon pollution throughout the waters of the PG (see Table 1-1) (Gawad et al., 2008; Gevao et al., 2006 (Tehrani et al. 2012; Mohebbi Nozar et al. 2014).

Table 1-1: Concentrations of total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) in sediment samples in the Iranian domain of the Persian Gulf and in other coastal areas worldwide. For references see Table 3 in page 47 of "Aein Jamshid et al. (2011)".

Area	Compounds	Concentration (µg/g sediment dry weight)	Year
Iranian domain of the Persian Gulf	TPH	0.85-114.92	2001-2002
Iranian domain of the Persian Gulf	PAHs	0.00143 - 1.2677	2001-2002
Persian Gulf	TPH	50-1448	1992
Persian Gulf (Qatar & Saudi Arabia area)	PAHs	0.1-0.5	1992-93
Australia area	PAHs	Nd-4.50	1992-93
Mexico area	PAHs	0.01-3.208	1995-96
Coast of California	TPH	0.77-70.64	1996
Black Sea	PAHs	0.012- 2.4	1988

Various other pollution sources, including urban waste, agricultural and industrial discharges, hot and saline wastewaters from desalination plants, and discharge of ballast water from ships have been reported as pollution sources in the PG (Abuzinada et al. 2008; Khan 2007). Contamination of the marine environment by trace metals is another and rather critical form of pollution and usually associated with multipoint industrial effluents (see Table 1-2) (Khoshnood et al. 2010; Astani et al. 2012; Monikh et al. 2013; Rahmanpour et al. 2014; Sarhadizadeh et al. 2014). In addition, pesticides and other persistent organic pollutants (POPs) are among the most critical agrochemicals threatening the marine environment (Mohebbi Nozar et al. 2014).

Table 1-2. Range of heavy metals concentrations in sediments of the Persian Gulf and some other marine areas in the world (data are in µg/g sediment dry weight). N.d.: is not determined. For references see Table 6 in page 48 of "Aein Jamshid et al. (2011)".

Area / Guidelines	Cd	Pb	Ni	V
Iranian domain of the Persian Gulf	2.89	90.48	64.90	52.0
Global baseline values	0.30	19.0	52.0	
NOAA marine sediment guideline	1.20	46.70	20.90	
RSA guideline	1.20-2.0	15.0-30.0	70.0-80.0	20.0-30.0
Continental shelf of Pakistan	0.31	10.41	77.90	n.d.
Caspian Sea, Iran	0.10-0.24	11.30-24.60	29.40-67.80	76.50-145.0
USA, Snake River Basin	0.69	23.75	23.45	77.60

Cd: Cadmium; Pb: lead; Ni: Nickle; V: Vanadium

One further important problem related to marine ecosystems including the PG is **eutrophication**, defined as "the process of organic matter and nutrient enrichment (mainly nitrogen and phosphorus) of water leading to increased growth, primary production and biomass of algae; changes in the balance of organisms; and water quality degradation" (Smith et al. 1999). In developing countries, on average more than 90 % of wastewater and 70 % of industrial wastes are discharged into coastal waters (Creel 2003). Agricultural runoff and domestic sewage are rich in various nutrients and organics. All these pollutants may cause localized eutrophication and are considered another important threat to the sustainability of the PG ecosystems (Gawad et al., 2008). Studies have shown that this specific type of urban pollution can have potential influence in coastal marine environments and can change structural and functional attributes of biodiversity (Pearson and Rosenberg 1978; Balestri et al. 2004; Terlizzi et al. 2005).

Moreover, throughout the past decades, **climate change and global warming** have caused considerable shifts in marine ecosystems (Thomas et al. 2004; Barange et al. 2010). In terms of global climate change, environmental factors that affect the structure and functioning of marine systems are temperature (both increasing mean temperatures and a higher frequency of episodic extremes), sea-level rise, availability of water from precipitation and runoff, wind patterns, ocean acidification and storminess (Abuzinada et al. 2008; Bauman et al. 2013). As an extreme example, cyclone Gonu (in June 2007) was the strongest tropical cyclone hitherto recorded in the Strait of Hormuz and Oman Sea. It caused landfall in Oman and in southern Iran. The total amount of rainfall (> 600 mm) exceeded the average yearly rainfall by as much as tenfold, resulting in storm run-off and flash-floods in the ephemeral river beds. Because of strong surge and wave, Gonu caused severe coastal damage in the area. The effects of this disturbance event on the marine environment of the PG have been studied in several publications such as (Fritz et al. 2010; Abdalla and bin Yahya Al-Abri 2011; Amini Yekta et al. 2012). For example, phytoplankton blooms in the PG after Gonu have been attributed to strong storm-induced mixing and upwelling (Wang and Zhao 2008).

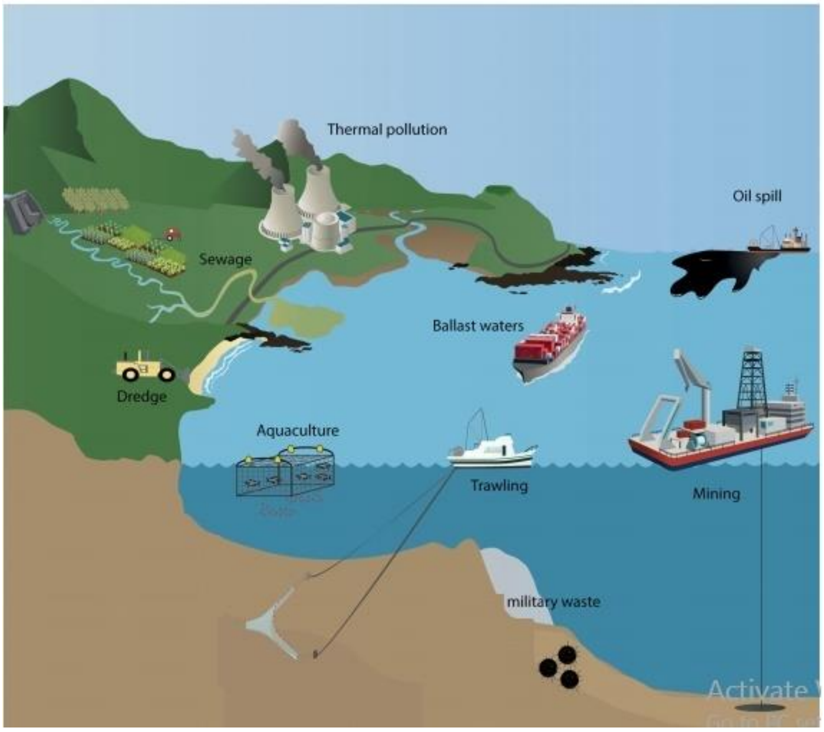

Fig. 1-8. A schematic drawing showing different sources of anthropogenic impacts on marine environments. Adapted from (Zeppilli et al. 2015).

The introduction of aquatic **invasive species** is another major threat facing the marine environment (Stachowicz et al. 2002; Pyšek and Richardson 2010). Hamza (2006) reported several exotic phytoplankton and zooplankton species in water samples collected from ballast water tanks of a gas tanker stopped along the UAE coastal area. The last decade has seen a noticeable increase in the frequency of harmful algae blooms (commonly referred to as "red tides" caused by the marine ichthyotoxic dinoflagellate *Cochlodinium polykrikoides* in the PG (Al Shehhi et al. 2014). For instance, massive blooms affected the PG from August 2008 to May 2009 (in which period our sampling campaign of 2008 (chapter 2) took place) causing widespread fish kills, damaged coral reefs, restricted fishing activities, impacted tourism and interrupted desalination operations (Richlen et al. 2010). Fig. 1-9 illustrates the progression of the 2008–2009 harmful algal bloom in the PG, which started in the south of the Strait of Hormuz in August 2008 (Hamzehei et al., 2012), then rapidly spread westward along a large part of the Iranian coast. Such blooms are obviously facilitated by the above-mentioned coastal eutrophication and episodic climate extremes.

Invasive species remain a major environmental problem in the world's oceans. Managing the vectors of introduction is the most effective means of mitigating this problem. Maritime vectors are diverse. The major vectors include ballast water. Other vectors are the biofouling of vessels by a community of sessile and associated mobile organisms that colonize and grow on any wetted surface of a vessel, such as hulls, anchors, storage lockers, and other colonizable locations; aquaculture; live seafood; live bait; the ornamental species trade and marine debris.

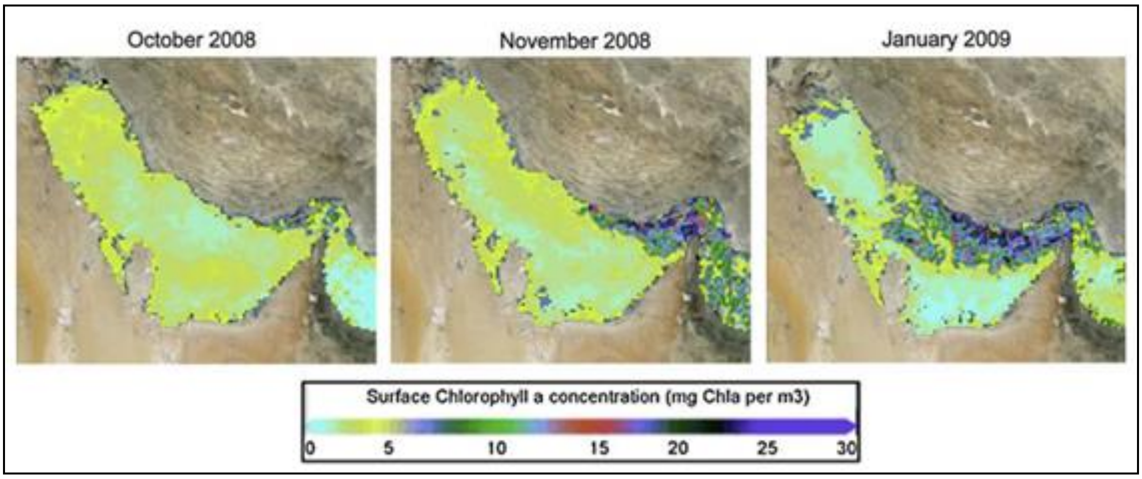

Fig. 1-9. Distribution of chlorophyll *a* concentration in October and November 2008, and January 2009, illustrating the initiation and progression of a massive toxic dinoflagellate bloom (Hamzehei et al., 2012).

Tourism and recreational activities are another important source of anthropogenic stress in the PG (Gladstone et al. 2013). In one study in Bandar Abbas city, it was found that tourism and recreational activities were responsible for more than 90 % of litter production on beaches (Sarafraz et al. 2016).

2 Intertidal areas and sandy beaches

2.1 Intertidal areas

In this study, we will focus on intertidal habitats and more specifically on sandy beaches. Here we briefly introduce some general features of intertidal areas and then elaborate more on sandy beaches.

The **intertidal zone**, also called **littoral**, is the area between low and high water marks and forms part of many landscapes that appeal to humans, allowing the formation of important recreational and tourist economies. This is an area of a foreshore and seabed which is exposed to air at low tide and submerged at high tide. Throughout the intertidal area, prominent **zonation** occurs. Zonation is evident from the occurrence of specific organisms in distinct bands. These distinct bands result from many complex physical and biological factors that affect marine organisms. In other words, the zonation of organisms is the reflection of their response to both physical and biological factors (McLachlan and Jaramillo 1996).

Based on the duration of submergence, the intertidal zone can be subdivided into three subzones: 1) the *high tide zone* (upper mid-littoral) is covered by water during spring high tide only, so it experiences dry periods daily; it is a highly saline environment and spends much of its time as a semi-terrestrial habitat; 2) the *middle tide zone* (lower mid-littoral) is regularly covered and uncovered (twice a day when tides are semidiurnal) by tides; 3) the *low tide zone* (lower littoral) which borders on the *shallow subtidal zone* and is mostly submerged and is dry only at the lowest tides (Doty 1946). This zone often harbours the highest biodiversity of macrobenthos (Armonies and Reise 2000). Intertidal animals experience physiological stress during low tide and species inhabiting the upper intertidal zone are often more tolerant to thermal and desiccation stress than those found in other zones (McMahon 1990; Short 1999).

Climatic factors (temperature, humidity and wind) and also other factors (such as desiccation and light penetration) all affect community structure in the intertidal zone. Consequently, the only animals to be found in areas regularly emerged by the tide, are those that have adapted to these more variable and extreme conditions. The adaptations may be behavioural (i.e. movements or actions), morphological (i.e. characteristics of external body structure), and/or physiological (i.e. internal functions of cells and organs) (Giere 2009).

2.2 Sandy beaches: Definition, characteristics and classification

Beaches form the border areas between the land and water bodies such as oceans and seas. This strip of nature formed by a deposit of sediment between land and sea represents one of the world’s most dynamic natural environments (Schwartz 2005; Pilkey et al. 2011). Beaches in many places of the world are of substantial touristic value, drawing millions of visitors worldwide. There is no single, agreed-upon definition of a beach. The term "sandy beach" can be used to describe a wide range of environments, *from high-energy open-ocean beaches to sheltered estuarine sand flats* (McLachlan 1983). One fairly broad definition refers to a beach as an "accumulation of wave-washed, loose sediment that extends between the outermost breakers and the landward limit of wave and swash action" (Leatherman 1988). Our use of the term (sandy) beaches roughly corresponds to this definition.

In comparison to other coastal ecosystems such as rocky shores and seagrass beds, sandy beaches (also called sandy shores) are among the most simple systems in terms of habitat complexity (Reise 2001). They are distributed worldwide in many temperate and tropical areas, where they constitute an important habitat for a variety of fauna. Due to their ecosystem services and economic benefits to mankind (e.g. harvestable natural resources, storm buffers, recreation and tourism), sandy beaches are considered an important element of the coastal ecosystems with economic, social and cultural importance to humans (Costanza et al. 1997; Costanza 1999; Pilkey et al. 2011). Besides, beaches function as natural filters responsible for the re-mineralization of substances, which then return to the sea as nutrients (Coull and Chandler 2001; McLachlan and Brown 2006).

Sandy beaches are commonly found in association with coastal dunes and occur along approximately 20 % of the world's coast. Marine sandy beaches are composed of a mixture of quartz and carbonate sands from terrestrial and marine origin, respectively (Masselink and Pattiaratchi 2001).

The beach is often divided into the following zones (Fig. 1-10): 1) *upper beach or back shore*: area between high tide line and primary dune; 2) *swash zone*: area where waves rush up the face of the beach and retreat seaward (usually remains saturated), and 3) *surf zone*: area between the low water line and the point where breakers form.

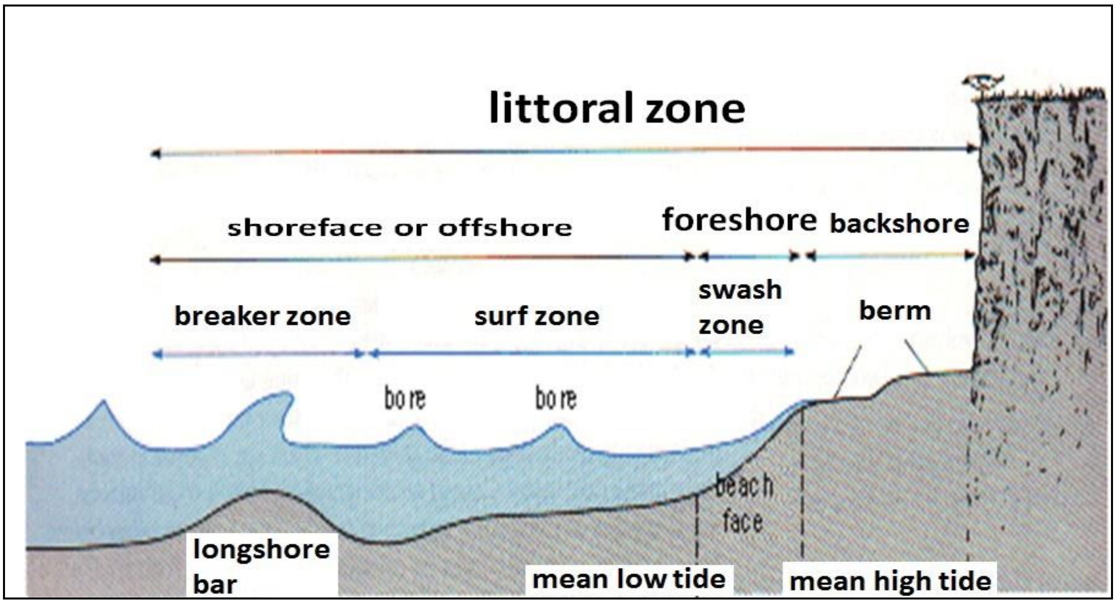

Fig. 1-10. Cross-section of an idealized beach showing the common beach zones and some other features at both high and low tide, adapted from: (http://www.marine.tmd.go.th/marinemet_html/lect20.html)

Sandy beaches are shaped principally by the interacting physical forces of *waves, tides and sediment movements*. In fact, the physical structure of sandy beaches can be defined in terms of sediment, waves and tides. The interaction of breaking waves, tides, slope and sediment texture determines the morphology of the beach as well as the circulation patterns of the surf zone (Wright and Short 1984; Masselink and Short 1993), collectively resulting in the so-called **"morphodynamic state of a beach"** (Carter 2013). Wright and Short (1984) proposed a morphodynamic continuum of beaches with two endpoint-type beaches and several intermediate types between them. These endpoints are **dissipative** and **reflective beaches**. The factors that determine the morphodynamic beach state or type include mainly the wave height, wave period and particle size of the beach sediment. Accordingly, beach state can be estimated by an index called Dean’s dimensionless fall velocity (also called parameter Ω). Ω reflects the interaction between wave height, wave period and sediment fall velocity of sand particles (Wright and Short 1984).

$$\Omega = H_b/W_s.T$$

where H_b is the average height of a wave (in cm) at the point where the wave starts to break, W_s is sediment fall velocity in cm/s, and T is wave period in seconds. When $\Omega < 1$, beaches are considered **reflective**, when $\Omega > 6$ they are **dissipative**, while in between ($1 < \Omega < 6$) they are **intermediate beaches**. Thus, coarser sediments (larger particles sink faster) and long wave periods contribute to low values of Ω and hence to more reflective beaches, whereas high waves with shorter periods, running over finer-grained sediments typically result in a dissipative morphodynamic beach state (Kaiser and Attrill 2011).

Dissipative beaches are a product of large waves moving over fine sands, resulting in a flat or low gradient beach face and wide surf zone. Waves start to break far from the shore in a series of spilling breakers that dissipate their energy along the broad surf zones. Dissipative beaches usually have rather stable morphologies, and exhibit minimal shoreline change with only a gentle slope (Short and Hesp 1982) (Fig. 1-11). Wave pressure on interstitial water is limited, hence organic matter can partly accumulate, leading to high microbial activity and steep geochemical profiles.

Intermediate beaches are located between the high energy dissipative and the lower energy reflective beaches. The two most distinguishing characteristics of intermediate beaches are 1) a surf zone and 2) a cellular rip circulation (rip currents) commonly associated with rhythmic bar and beach topography (Schwartz 2005). They are produced by moderate to high waves (0.5 - 2.5 m), fine to medium sands and longer wave periods (Short and Wright 1983) (Fig. 1-11).

Reflective sandy beaches lie at the lower energy end of the beach spectrum. These beaches are a product of lower waves and coarser sand. Their morphology consists of a steeper and narrower surf zone which is sometimes absent. Waves surge or break straight on the shore generating fast swashes with short periods. During the breaking process, part of the wave energy is "reflected" back to the sea by the very steep beach face (Fig. 1-11). Wave pressure on interstitial water is large, resulting in strong pore water flushing and very limited or no retention of organic matter. Geochemical profiles are therefore more homogeneous.

Sandy beaches have also been divided into three categories based on tidal range: microtidal, mesotidal and macrotidal. Areas with high tidal ranges (> 4 m) are referred to macrotidal, where waves reach further up the shore, while areas with lower tidal ranges are considered mesotidal (range = 2 – 4 m) or microtidal (range < 2 m) (Davies 1964). However, depending on wave energy and sediment particle size, even microtidal beaches can belong to any morphodynamic beach state (Wright and Short 1984; Masselink and Short 1993), illustrating nicely that tidal range is a poor criterion to properly classify beaches and reflect their true morphodynamic diversity.

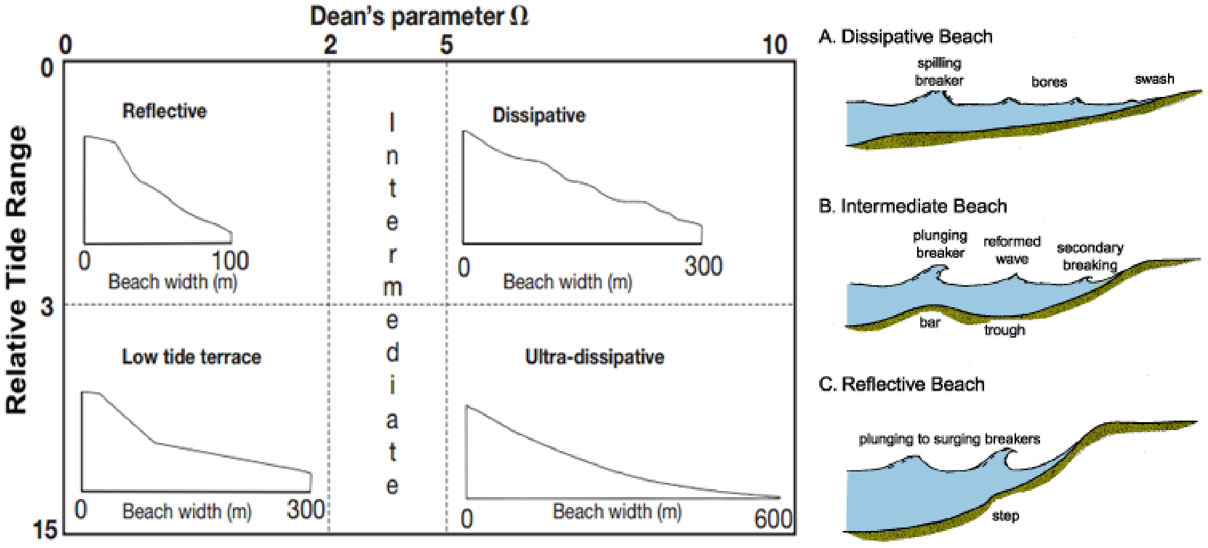

Figure 1-11 A schematic drawing showing the main beach morphodynamic types, Adapted from Short and Wright (1983).

3 Benthic communities of beaches

3.1 Definition, classification and ecological significance

Compared to other marine habitats such as mangroves, rocky shores or seagrasses, beaches may look like deserts without any life at first glance. However, a wide variety of organisms inhabit the interstitial spaces between the sediment particles in sandy beaches. The name "benthos" is derived from the Greek (βενθος), meaning "depth of the sea". The benthos is normally divided into three functional groups: the infauna or *endobenthos*, the *epibenthos* and *hyperbenthos*, i.e. those organisms living within the substratum, on the surface of the substratum and just above it, respectively (Coull and Chandler 2001). The term **benthos** thus refers to the community of organisms that live in, on, or near the seafloor. Generally, benthic communities are much more diverse in terms of species richness than those of the surface and mid-water layers. Almost 98 % of all marine species belong to the benthos (Peres 1982).

The benthos is further divided in subgroups, primarily defined by organism size, or more precisely by the pore size of sieves used to separate these size groups. **Microbenthos** pass through a sieve with mesh size ≤ 38 µm and include mostly unicellular organisms like flagellates, ciliates and diatoms, which are usually limited to the upper sediment layers in sheltered conditions, but can be mixed to deeper layers of sediment when subject to wave action (Giere, 2009). **Macrobenthos** are retained on sieves with a mesh size of 1 mm and are mainly represented by invertebrate animals such as crustaceans, molluscs, echinoderms and polychaetes. The term **meiobenthos** (or meiofauna), derived from the Greek word μειος meaning smaller, was introduced by Mare (1942) to define those benthic metazoans of intermediate size between macro- and microbenthos, i.e. they pass through a 1-mm sieve but are retained on a mesh with pore size of ~38 µm (small variations on the precise mesh size are common in the literature). As such, the distinction between micro-, meio- and macrobenthos is based on operational criteria and does not always follow clear taxonomic boundaries.

3.2 Intertidal meiobenthos: abundance and diversity

Meiobenthos are highly diverse and include organisms from a wide variety of higher taxa such as Nematoda, Copepoda, Turbellaria, Gastrotricha, Rotifera, Tardigrada, Kinorhyncha and several others (McLachlan, 1983; Giere, 2009). Importantly, more than 80 % of the meiobenthos in soft substrata is usually represented by nematodes. Harpacticoid copepods often constitute the second most abundant group. So, many studies on meiobenthic communities focus on one or both of these two taxa. Meiofauna are found in virtually all aquatic sediments and often abound in very large numbers, with densities of 10^6 ind./m^2 being typical of many marine soft sediments (Giere, 2009). Biomass varies between 0.1-10 g dry wt/m^2, with the highest values in muddy brackish-water sediments (Heip et al., 1985). Meiofaunal abundances also vary greatly according to the habitat. Highest abundances are typically measured in intertidal muddy estuarine habitats, whereas the lowest are typically encountered in the deep sea (Coull, 1988; 1999).

Densities and diversities of meiobenthos assemblages have been related to several abiotic environmental factors, such as sediment grain size, organic content, substratum porosity and water percolation, degree of exposure to wave action, oxygenation of the sediment, temperature, salinity and water depth (Gheskiere et al. 2005; Nozais et al. 2005), as well as to biological factors such as food quality and quantity, predator impacts and competition (Montagna et al. 1983; Rudnick et al. 1985; Gallucci et al. 2005).

3.3 Marine nematodes

In this section, we present a brief background and introduction to several aspects of marine nematode populations and assemblages which are key to our own work. These aspects include: 1) general characteristics, 2) biodiversity, 3) temporal and spatial patterns, including horizontal and vertical distribution patterns on beaches, 4) dispersal and population genetics, 5) feeding strategies, 6) functional roles in ecosystems, and finally 7) their role as bio-indicators.

3.3.1 General characteristics

The phylum Nematoda is an evolutionarily successful group of organisms and they are considered among the most ubiquitous, abundant and diverse animals of terrestrial and marine ecosystems (Giere, 2009). The phylum belongs to the major protostome "supertaxon" Ecdysozoa, the clade of molting invertebrates (Aguinaldo et al. 1997). Throughout the past years, the taxon Ecdysozoa has been an important group for understanding the basal phylogeny of animals and has become well-established in metazoan systematics (Edgecombe et al. 2011).

Nematodes most likely arose slightly prior to or during the Cambrian explosion (Poinar 2011). A trace fossil from the Hubei Province of China resembling the burrows of nematodes may well represent the oldest record of activity by marine nematodes, preceding known nematode fossils by 70 million years. This study indicates that marine nematodes originated no earlier than the mid Early Cambrian, around 470 million years ago (Baliński et al. 2013). However, because of the absence of hard structures, the fossil record of nematodes is very limited, and may not provide a very accurate estimate of the timing of the origin of the phylum Nematoda. Evolutionary studies have shown that marine nematodes of the order Enoplida or Dorylaimida represent the earliest lineages of nematodes (Meldal et al. 2007; Blaxter 2011; Smythe 2015).

The phylum Nematoda are amazing for their adaptation to a wide variety of habitats, including virtually all terrestrial, freshwater and marine soils and sediments as well as plant and animal hosts. Nematodes can even abound in more extreme marine environments such as deep-sea hydrothermal vents, cold seeps and sea mounts (Vanreusel et al. 2010). Hydrothermal vents are enriched with toxic chemicals such as hydrogen sulfide and heavy metals and harbor a limited number of taxa which can survive under these conditions.

The field of marine nematology in some countries remains underdeveloped. This is also the case for Iran, where until recently, virtually all nematologists work(ed) on plant parasitic nematodes. Requirement of taxonomic expertise, along with the time-consuming nature of morphological identification, have been a major challenge for assemblage studies of nematodes at genus or species-level resolution.

3.3.2 Nematode biodiversity

Estimates of the total number of nematode species vary from 100,000 (Coomans 2002) to 100,000,000 (Lambshead 1993). Approximately 30,000 species have been described for the phylum as a whole (Baldwin et al. 2000; Hugot et al. 2001). Overall estimates for the phylum thus suggest that no more than 0.3 % to 5.3 % of the world's nematode fauna has been described (Hugot et al. 2001). Free-living nematodes are one of the most diverse groups of metazoans in most marine sediments, with a wide distribution from pristine to extremely polluted habitats (Heip et al. 1985; Boucher and Lambshead 1995; Moreno et al. 2011).

Estimates of the global species diversity of marine nematodes also span a broad range. According to a recent conservative estimate, the number of free-living marine nematode species could be up to 50,000, with 86 % of the existing species remaining to be discovered (approximately 6,900 species of free-living marine nematodes have been described) (Appeltans et al. 2012), while previous studies have estimated marine nematode species number at 10,000-20,000 (Mokievsky and Azovsky 2002) at the lower extreme and in excess of 1×10^6 at the higher end (Lambshead 1993; Snelgrove et al. 1997; Lambshead and Boucher 2003). Such large discrepancies to a significant extent depend on how extrapolations are made from already inventoried to non-inventoried areas of the seafloor, particularly in the deep sea, where nematode assemblages can exhibit a very high local species diversity (Danovaro et al. 2010; Vanreusel et al. 2010). This, in large part, depends on estimates of species turnover, which are poorly supported in many marine habitats, particularly across larger geographical areas.

In any case, the described diversity of nematodes is only a small part of the true species diversity, and this is further aggravated by the potentially high cryptic species diversity, ignored in all the above estimates. Cryptic species are morphologically very similar but genetically distinct species. Indeed, recent genetic studies have revealed the presence of substantial cryptic diversity in marine nematodes belonging to different orders (Bhadury et al. 2008; Derycke et al. 2010a; De Oliveira et al. 2012; Palomares-Rius et al. 2014).

3.3.3 Temporal and spatial patterns in marine nematode assemblages

Understanding the spatial and temporal variations in communities is important to determine the pattern of distribution, abundance, maintenance of species diversity, and stability of communities (Koenig 1999; Ellingsen 2002; Thrush et al. 2010; Moens et al. 2013).

3.3.3.1. **Temporal patterns**

Marine nematodes exhibit temporal fluctuations on the scale of days to years (Alongi 1990b; Gourbault et al. 1998; Nicholas 2001; Riera et al. 2011b; Maria et al. 2013a; Ramalho et al. 2014), even though some studies have observed a lack of any temporal pattern (Warwick and Buchanan 1971; Juario 1975; Liu et al. 2007). Information on temporal variation in marine nematode assemblages has mostly focused on seasonal variations (Boaden and Platt 1971; McIntyre and Murison 1973; Platt 1977; McLachlan 1978; Blome 1982; Sharma and Webster 1983; Gourbault et al. 1998; Nicholas and Hodda 1999; Nicholas 2001; Albuquerque et al. 2007; Liu et al. 2008; Venekey et al. 2014a), whereas longer-term variation has received only limited attention (Coull 1985, 1986; Eskin and Coull 1987; Li et al. 1996; Riera et al. 2011a). Below, we mention the main causes for temporal fluctuations of marine nematodes.

Temperature is an important abiotic factor in marine environments. Even slight temperature changes can impact reproductive and metabolic activity (Moens and Vincx 2000). It can affect nematode abundances directly, e.g. *via* dehydration and/or effects on reproduction, and indirectly as well, e.g. by controlling the growth of food items such as bacteria and diatoms (Harris 1972a) and/or by affecting the depth of the redox discontinuity layer in sediments (Dye 1983). Temperature regime can also have a substantial effect on the interspecific interactions and life-history characteristics of marine nematodes (De Meester et al. 2015b; De Meester et al. 2015c).

Other climatic factors such as **precipitation** can also cause seasonal fluctuations in nematode assemblages. Some studies in tropical regions have, for instance, indicated that nematode density changes are affected by rain cycles (Pattnaik and Lakshmana Rao 1990; Ingole and Parulekar 1998; Venekey et al. 2014b). On a much shorter temporal scale, short episodes of rainfall, such as a heavy shower on an exposed intertidal flat, may cause vertical migrations of nematodes in sediments (Steyaert et al., 2001).

Salinity is another important factor affecting both the temporal and spatial distribution of marine nematodes. From a temporal perspective, salinity fluctuations are linked with seasonal cycles in precipitation (Paranhos and Mayr 1993). Within sandy beach habitats, there can also be considerable short-term variability in salinity, both in direct relation to the tides and indirectly through episodic events such as the above-mentioned episodes of heavy rainfall during low-tide exposure (Steyaert et al., 2001).

Food availability is another driver of temporal as well as spatial variation in nematode assemblages (Heip et al. 1985; Moens et al. 2013). For instance, the densities of certain nematode feeding guilds have been shown to be related to variations in the abundances of the food sources of those groups (Austen and Warwick 1995; Kendall et al. 1995; Ólafsson and Elmgren 1997).

3.3.3.2. **Spatial patterns**

Spatial distribution of marine nematodes can be viewed at large and small scales. Several factors have been proposed to explain these distributions, including physico-chemical (e.g. temperature, salinity, mean grain size of sediment and dissolved oxygen concentration) (Steyaert et al. 2003; Hourston et al. 2005; Nozais et al. 2005; Adão et al. 2009) as well as biological factors such as food quality and quantity, predator/competition impacts and reproductive behaviour (Montagna et al. 1983; Rudnick et al. 1985; Moens et al. 1999; Gallucci et al. 2005; dos Santos and Moens 2011; Maria et al. 2012; Urban-Malinga et al. 2016). It is often suggested that **physico-chemical factors** typically determine macro-scale (e.g. kilometre scale) patterns, whilst **biological factors** cause micro-scale (e.g. (sub)metre scale) heterogeneity (Heip et al. 1985; Moens et al. 2013). Marine nematodes are generally influenced by these complex and interacting physical and biological processes, leading to variation in their distribution at different spatial and temporal scales.

Salinity is a key factor with strong significance on nematode distribution, especially in estuarine habitats (Adão et al. 2009; Chen et al. 2012). A strong relationship between salinity gradients and meiofaunal/nematode assemblage structure has been reported (Austen and Warwick 1989; Barnes et al. 2008). Salinity has also been correlated with the distribution of particular nematode feeding types in estuarine habitats (Austen 1989). At high salinities, selective deposit feeders and epigrowth feeders tend to dominate, while at lower salinities, omnivores and non-selective deposit feeders often do so. These trends perhaps relate to the availability of organic matter in different reaches of an estuary. In addition to gradients in average salinity, the local range of salinity fluctuations varies along estuaries but also, for instance, across the intertidal zone; ranges of daily salinity fluctuations may have yet more pronounced effects on marine/estuarine organisms than average values per se (Kaiser and Attrill 2011). To what extent elevated mean salinity, as in the PG area, affects nematode abundance, diversity and assemblage composition is not properly documented.

As marine nematodes live constantly in the sediment, any changes in **sediment characteristics** (grain size, grain shape, sorting and sediment pore space) may have an effect on their assemblage structure (Giere 2009). Herman (1982) and Boyd et al. (2000b) reported that nematodes are more sensitive to shifts in sediment structure than macrofauna. For example, it has been frequently reported that coarser sediments have higher nematode species richness and diversity than finer sediments (Heip and Decraemer 1974; Steyaert et al. 1999; Vanaverbeke et al. 2002; Semprucci et al. 2010; Vanaverbeke et al. 2011). Similarly, nematode assemblage composition also changes according to the granulometric properties of the sediment (Wieser 1959; Heip et al. 1985; Vincx et al. 1990; Vanaverbeke et al. 2002; Vanaverbeke et al. 2011; Fonseca et al. 2014). In addition, nematodes from sandy habitats are often more slender as they have to move through the sediment, whereas nematodes from muddy habitats are generally more robust for burrowing through the sediment (Moens et al., (2013). Gheskiere et al. (2004) also found that grain size explained the horizontal nematode distribution at a Belgian beach (De Panne) (see 3.4.1).

Sediment characteristics also determine other aspects of the environment of the sediment, such as **organic matter availability** (Coull 1985; Danovaro and Gambi 2002). Generally, clay and silt substrata retain more organic matter than sand. It has been reported that the content of organic matter can partly explain the spatial patterns of distribution of free-living nematodes in some marine habitats (Ólafsson and Elmgren 1997; Schratzberger et al. 2006). (Sajan et al. 2010) reported that average biomass and density of nematodes were higher in silt/clay substrata than in sandy and mixed sand. The authors stated that fine particles may hold more labile organic matter and this may cause higher biomass and density of nematodes, supporting the general trend of higher nematode abundances found in finer sands (Adão et al. 2009). Comparison of sheltered versus exposed sandy beaches further indicates that meiofauna densities at the sheltered beaches are usually higher compared with exposed beaches (Ólafsson 1991; Gheskiere et al. 2002; Corgosinho et al. 2003; Urban-Malinga et al. 2004; Gheskiere et al. 2005; Hourston et al. 2005). In sheltered beaches, the sediment stability increases organic matter accumulation. However, some studies (Calles Procel et al. 2005) have reported contrasting trends, namely highest meiofauna densities in exposed beaches and lower densities in sheltered beaches, but these results were probably related more to anthropogenic impacts than to real granulometric effects. Organic matter availability, in turn, is an important determinant of sediment oxygenation because it stimulates microbial growth, which consumes most of the oxygen (Bickford 1996; Kristensen 2000; Steyaert et al. 2007). In addition, organic matter can bind pollutants, thus affecting their retention in sediments and availability to consumers (Philippe and Schaumann 2014; Mazzei and Piccolo 2015).

Pollution is indeed another important abiotic factor affecting the distribution pattern of marine nematodes; effects depend on pollutant type, exposure level and field conditions. Pollutants influence marine nematodes by changes in abundance and diversity, trophic group composition, reproductive ability etc. (Nanajkar and Ingole 2010; Balsamo et al. 2012; Kang et al. 2014).

Furthermore, marine nematode distribution patterns can result from **biological interactions**. Biological *interactions between nematodes and macrofauna* can occur and influence nematode distribution (Van Colen et al. 2009; Braeckman et al. 2011; Maria et al. 2011b; Van Colen et al. 2012; Urban-Malinga et al. 2016). According to Mirto et al. (2000), for instance, mussels induce changes in sediment characteristics, organic matter quantity and quality, and depth of oxygen penetration in the sediment.

Moreover, *competition*, both among individuals within a species and among species, can also play a major role in limiting faunal abundances and distribution (Gray and Elliott 2009; Moens et al. 2013). *Predation* among nematodes can also be responsible for controlling nematode densities and perhaps also diversity (Gallucci et al. 2005; dos Santos and Moens 2011).

Aggregative small-scale horizontal distribution (at scales of meters or less) of meiofauna is a well documented phenomenon (Ólafsson 1992; Blome et al. 1999; Giere 2009; Maria et al. 2013b; Urban-Malinga 2014). The causative factors for patchiness are multifactorial: biological factors, such as reproductive activities, predation and availability of, and competition for food have been reported as main drivers (Giere 2009). Nematodes are highly influenced by small-scale patches of food and by disturbance, both of which create microhabitats in space and time. The resulting spatial patchiness may be defined at the scales of cms (Findlay 1981; Blanchard 1990; Ólafsson 1992; Sandulli and Pinckney 1999), and due to such patchy distribution pattern, meiofauna density and assemblage composition may fluctuate over distances of a few centimeters. The spatial autocorrelation between patches and patch sizes of meiofauna and of microphytobenthos suggests that at least in intertidal sediments, *variations in food resources* may be the principal determinant of meiofauna small-scale patchiness (Findlay, 1981; Blanchard, 1990).

3.4 Zonation patterns of intertidal meiofauna/nematodes

Intertidal meiobenthic species inhabit specific zones according to their ecological requirements, life cycle, feeding habits and interactions with other organisms (Giere, 2009). Generally, the zonation pattern of meiofauna is a reflection of their responses to both physical and biological factors. More specifically, distribution patterns of meiofauna in sandy beach habitats can be divided into vertical and horizontal zonation (McLachlan and Turner 1994; McLachlan and Jaramillo 1995; Giere 2009; Moens et al. 2013; Urban-Malinga 2014). Below we briefly describe horizontal and vertical patterns of meiofauna distribution.

3.4.1 Horizontal zonation patterns

McLachlan and Jaramillo (1995) have described four different types of horizontal zonation of fauna on sandy beaches, namely: 1) no clear zonation; 2) two zones delimited by the driftline; 3) three zones of supralittoral, littoral and sublittoral, according to Dahl’s (1952) classification; and 4) four physical zones, a dry zone, a zone of water retention, a zone of resurgence, and a zone of saturation based on sediment moisture. These horizontal zonations are reflected in the distribution patterns of both macrofauna and meiofauna and can be important in planning of biodiversity surveys.

It has been reported that in sandy beaches, benthic zonation is predominantly controlled by *physical forces* related to wave exposure, which in turn influence other habitat parameters, including *granulometry* (sediment grain size and sediment structure) (McLachlan and Brown 2006; Schlacher et al. 2008). They also influence the fluctuations of other environmental variables such as water table level, temperature and salinity (Hinton 2000; Li et al. 2000; Urish and McKenna 2004).

As part of the physical forces mentioned above, the *tidal level* has been identified as one of the major factors influencing the horizontal patterns of meiofauna. Indeed, the concept that meiofauna assemblages change along a gradient perpendicular to the waterline has been well recognized (Warwick 1971; Hodda and Nicholas 1985). Several reports have found significant **differences in meiofaunal densities among intertidal positions**, showing that *densities increase from the upper towards the lower littoral zone* (Hodda and Nicholas 1985; Ólafsson 1991; Gheskiere et al. 2002; Kotwicki et al. 2005). In fact, nematode assemblages at the lower intertidal are often considered an extension of, and not fundamentally different from, subtidal nematode assemblages (Gheskiere et al. 2004; Maria et al. 2013b). However, some exceptions with highest densities in the upper beach zone exist (Rodriguez et al. 2001). The density pattern of marine nematodes resembles the usual pattern for macrofauna, but the diversity pattern does not: in macrofauna, density and diversity both tend to increase towards the lower intertidal, whereas in meiofauna diversity more commonly peaks at the mid-tidal level (see below) (Fig. 1-12).

Regarding the beach type, McLachlan and Turner (1994) have stated that *optimum conditions for the existence of a diverse and abundant meiofauna occur in intermediate beaches*. Their prediction was based upon the fact that beaches with intermediate morphodynamic characteristics represent an equilibrium state between organic inputs (which increase towards the dissipative beach state) and oxygenation conditions (which increase towards the reflective beach state) (McIntyre 1969; Ott 1972). On the scale of a single beach, essentially the same equilibria may explain the occurrence of diversity peaks of meiofauna around the mid-intertidal (Armonies and Reise 2000; Gheskiere et al. 2004; Gingold et al. 2010; Maria et al. 2013b) (see below for more info).

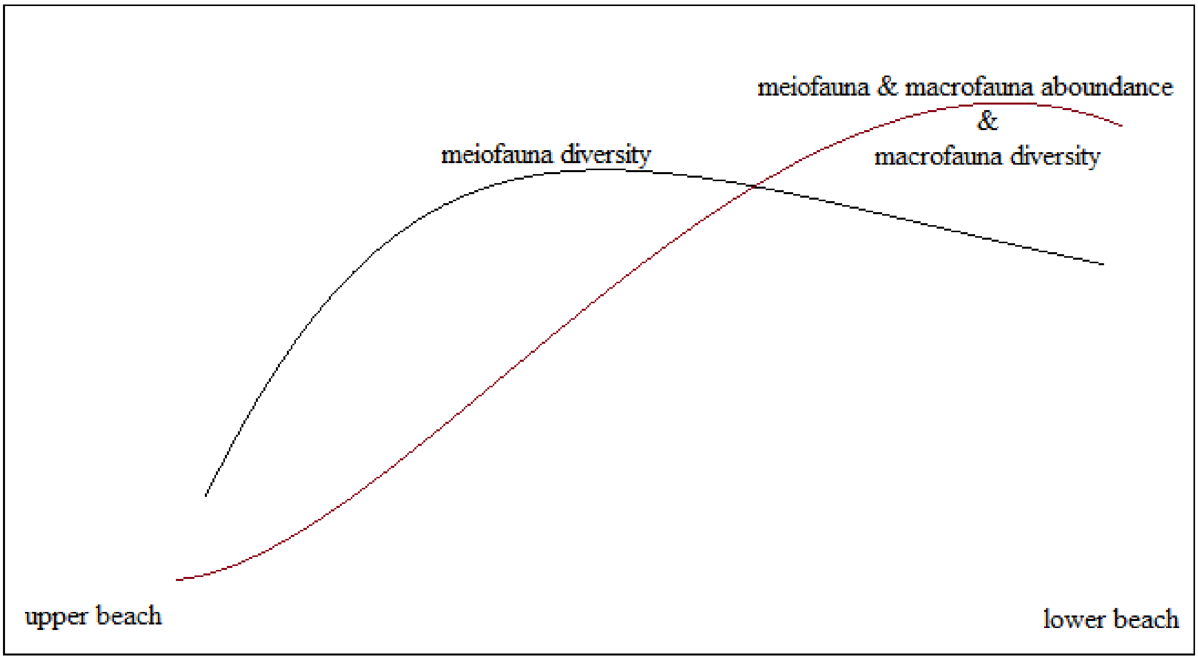

Fig. 1-12. Conceptual diagram of typical across shore gradient in meiofauna and macrofauna abundance and diversity.

Regarding the effect of tidal level on the **horizontal diversity pattern** of marine nematodes, it has been demonstrated that nematode species diversity often increases from lowest values at the upper littoral to a maximum around the mid-tidal level and then again decreasing – albeit not drastically – towards the low-water line (Armonies and Reise 2000; Gheskiere et al. 2004; Gingold et al. 2010). This is in contrast with macrobenthos diversity, which generally increases almost linearly from the upper to the lower intertidal. This trend may be explained by a direct dependence of feeding activity in many macrofaunal species on tidal submergence (Armonies and Reise 2000). The unimodal diversity pattern of meiofauna is why in the present study, we sampled from this mid-intertidal zone. Higher diversity at the middle beach can be attributed to the fact that an optimal balance among desiccation/temperature/salinity stress, hydrodynamic disturbance and sediment stability, food availability and oxygen concentration is usually reached somewhere in the mid-intertidal. As such, nematode diversity gradients across beaches may provide a good illustration of both the *intermediate disturbance hypothesis* (IDH) (Connell 1978) and the *dynamic equilibrium hypothesis* (DEM) (Huston 1979). The IDH predicts that species diversity will be highest at intermediate levels of disturbance (here the combination of hydrodynamic disturbance and physiological stress following from low-tide exposure), whereas the DEM predicts that the effect of disturbance or stress on diversity depends on productivity (here the availability of organic matter). Another point is that the middle beach is a transitional area, with a mixture of swash and surf zone processes, which allows the co-occurrence of species from both the upper beach and the subtidal (McLachlan and Brown 2006; Maria et al. 2013b). In the study of Maria et al. (2013b), 63 % of the nematode species occurring in the middle beach were also found in the upper beach and/or in the subtidal. Furthermore, biological factors, such as predation and competition for food, are also known to play a key role for the establishment and maintenance of meiofauna zonation on sandy beaches (Snelgrove and Butman 1995; Giere 2009; Maria et al. 2011b; Maria et al. 2012).

In addition, the presence of particular '**microhabitats**' on beach can interfere with the above-discussed horizontal diversity and density patterns of nematodes on sandy beaches. Gingold et al. (2010) demonstrated that beach microhabitats (runnels and sandbars) differed in environmental conditions and possessed significantly distinct nematofaunal assemblages. Runnels featured higher levels of taxonomic and functional diversity, while sandbars possessed a more homogeneous nematode community. In contrast with sandbars, in which food (organic matter and microbenthic algae) is scarcer, runnels remain submerged over a longer period of time and accumulate organic matter. Consequently, the runnel community with relatively calm conditions exhibited a higher degree of patchiness around food sources resulting in small-scale aggregations and clumped distributions, presumably owing to a predominance of active displacement under calmer conditions and sediment cohesion by algal films (Gingold et al. 2011). Maria et al. (2013b) also reported that nematode communities from runnel and sandbar habitats are significantly different at macrotidal ridge-and-runnels beaches in the North Sea.

3.4.2 Vertical patterns

Vertical zonation of the meiobenthos is generally controlled by oxygen and the position of the redox discontinuity layer, RPD (McLachlan and Jaramillo 1996; Steyaert and Vincx 1996; Steyaert et al. 2003). The RPD is a distinct redox-cline which marks the transition between oxidized and reduced conditions in the sediment (Gray 1981). The position of this layer is controlled by hydrodynamic forces, sediment properties (such as grain size) and organic content of the sediment. These abiotic factors are in fact variable in the different habitats and this is reflected in different vertical distribution patterns of meiofauna, which in turn exhibit "taxon" (e.g. Nematoda tend to be more tolerant to hypoxia than harpacticoid copepods (Modig and Ólafsson 1998; Moodley et al. 2000) and species-specific (see e.g. Steyaert et al., 2007) tolerances to reduced oxygen concentrations.

Both long and short-term effects of oxygen on vertical distribution of meiofauna can be important. Reports have indicated that long oxygen stress (longer than 2 months) can cause changes in meiofaunal community structure and vertical distribution patterns (Moodley et al. 1997; Wetzel et al. 2002). Yet, short-term exposure to hypoxia and/or anoxia can also profoundly affect nematode abundances and decimate populations of common species, including some which are generally considered fairly tolerant to reduced oxygen conditions (Steyaert et al., 2007). It is not always clear whether such impacts are the direct consequence of hypoxia or rather an indirect result through increased concentrations of toxic sulfides under low-oxygen concentrations (Wetzel et al. 2001; Wetzel et al. 2002; Steyaert et al. 2007).

Coarser (permeable) sediments are generally more oxygenated with a deeper RPD, whereas in finer sediments or in non-permeable sediments, meiofauna can be largely restricted to the upper first cms or mms of the sediment (Coull, 1988). Numerous studies (McLachlan 1978; Ólafsson and Elmgren 1997; de Jesús-Navarrete and Herrera-Gómez 2002; Kotwicki et al. 2005) have reported highest meiofauna densities in the top 10 cm of beach sediments, while Martins et al. (2015) reported the highest meiofauna densities in the 10-30 cm strata in southeast/southern Brazilian reflective beaches. In the latter study, the higher abundance of meiofauna in intermediate strata (10-30 cm) was potentially caused by the migration of organisms in an attempt to escape the physical stress caused by the wave impact and the desiccation characteristic of the intertidal zone (Urban-Malinga et al. 2004). In addition, it has been reported that in well-oxygenated sandy beaches, meiofauna occasionally can be distributed to depths of 50 cm or deeper (McLachlan and Brown 2006). For example, Munro et al. (1978) recorded nematodes down to 105 cm at such well-oxygenated beaches. Such distances are too large to be explained by active downward migration alone. They could, however, partly relate to passive up- and downward migration of nematodes with the sediment water table during incoming and outgoing tides, a phenomenon which can be very pronounced particularly in coarser sediments near the upper littoral (McLachlan et al. 1977; Urish and McKenna 2004). Another factor which also affects the depth of the RPD zone is seasonal temperature variations. With increasing summer temperatures, the RPD layer will move upwards, resulting in change in meiofaunal assemblages. This factor can be particularly important on exposed sandy beaches, where seasonal temperature fluctuations can be considerable (Harris 1972b).

3.5. Nematode feeding strategies

For a more functional analysis of nematode assemblages, as well as a better understanding of the importance of resource availability for assemblage composition, nematodes are often classified according to feeding types (Wieser 1953; Jensen 1987; Moens and Vincx 1997). Several feeding-type or guild classifications for marine nematodes have been proposed, but only two are commonly used: the one by Wieser (1953) and the one by Moens and Vincx (1997).

Free-living marine nematodes utilize a broad range of resources, including bacteria, organic detritus, microalgae, fungi, protozoa and other meiofauna. Based on the morphology of their buccal cavity, marine nematodes have been classified into four feeding groups, two each with and without buccal armature (such as teeth, jaws, denticles or other cuticularized stoma structures) (Wieser 1953) (Figs. 1-13; 1-14).

1) Selective deposit feeders (1A-group): nematodes with a small buccal cavity that can only ingest small particles, mainly in the size range of bacteria.

2) Non-selective deposit feeders (1B-group): nematodes with a more spacious buccal cavity than 1A but still without teeth, being able to ingest bigger particles, including for instance certain diatoms and other protists.

3) Epigrowth feeders (2A-group); nematodes with one or more (relatively small) tooth or teeth in the buccal cavity that allow them to break or pierce cells and suck their content, or to scrape off particles from surfaces.

4) Predators or omnivores (2B group); often large nematodes with large teeth and/or mandibles that allow them to capture prey and ingest them, pierce them or tear them to pieces.

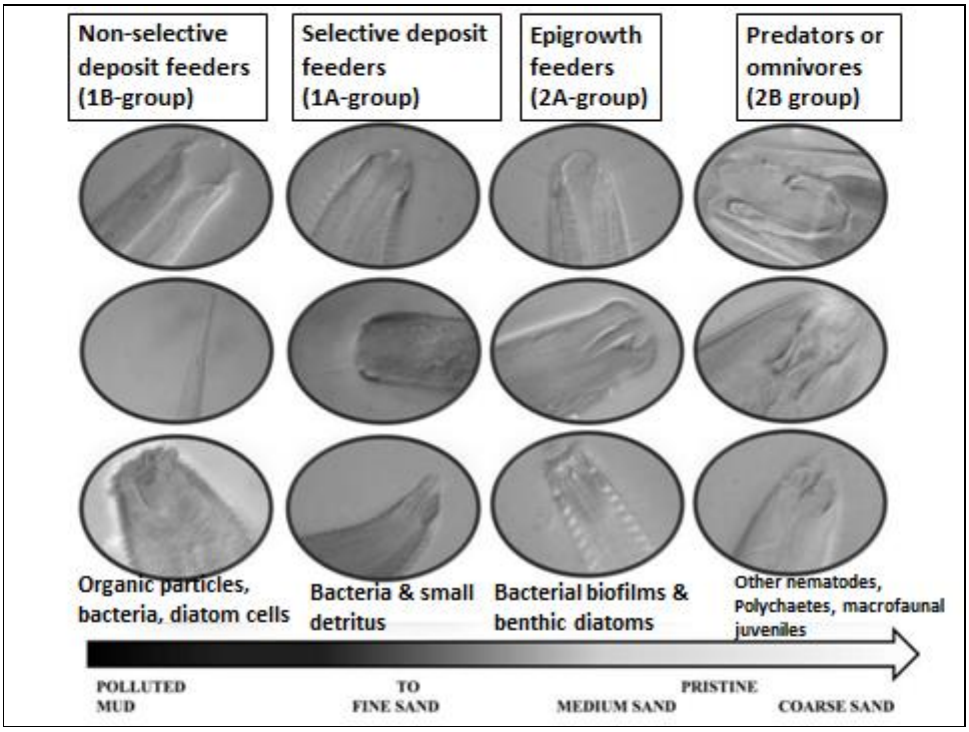

Fig. 1-13: Marine nematode trophic groups in relation with the ecological status of sediments and grain size. Adapted from Semprucci and Balsamo (2012).

This feeding type classification by Wieser (1953) suffers some shortcomings, when compared with empirical evidence. While the morphology of the stoma puts obvious constraints to the range of prey types that can be handled as well as to the way of obtaining prey, it can be thoroughly misleading (Moens et al. 2004). Moreover, nematodes may have flexible feeding strategies, being able to shift between different feeding modes and hence also feeding types/guilds. In this sense, Moens and Vincx (1997) proposed a modified classification based on observations of the feeding behavior of a variety of estuarine nematodes, establishing six trophic groups (Fig. 1-14): 1) microvores, feeding exclusively on bacteria and/or dissolved organic matter; 2) ciliate feeders, feeding mainly on ciliates but also on bacteria; 3) deposit feeders, feeding mostly on bacteria, diatoms and/or other microalgae, but occasionally also capable of predation on other meiofauna; 4) epigrowth feeders, that mainly feed on diatoms and other microalgae in the same way as explained above under epigrowth feeders; 5) facultative predators, that feed on several items, including detritus, but are all capable of predation on other nematodes and protists; and 6) predators, that feed mainly or strictly on other benthic invertebrates such as nematodes. Moens and Vincx (1997) also emphasize that many marine nematodes may have fairly flexible feeding strategies and may thus shift prey depending on their availability.

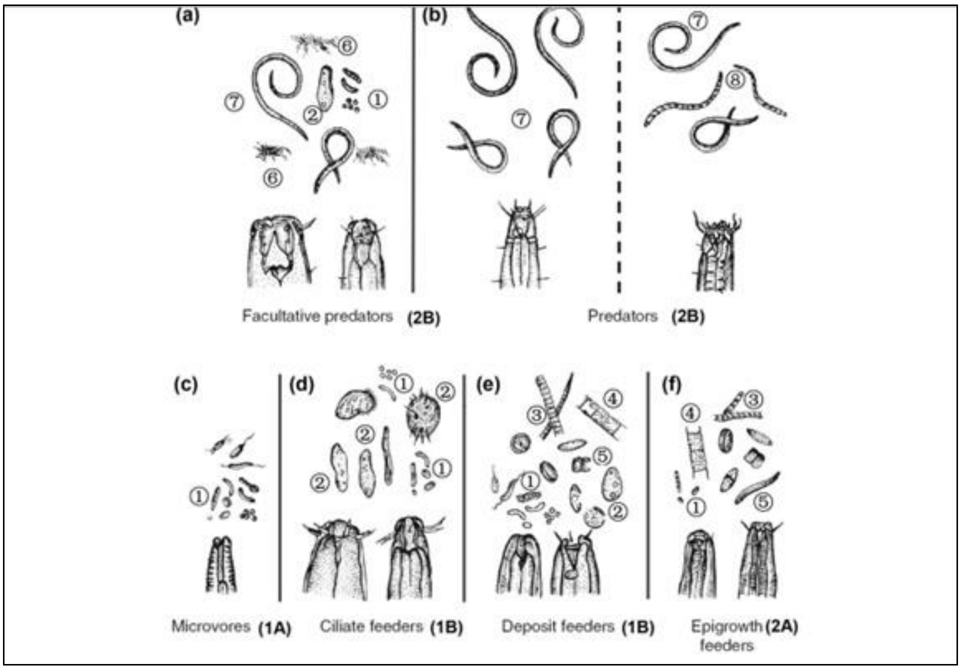

Fig. 1-14: Feeding type classification of Moens and Vincx (1997). Figure modified from Moens and Vincx (1997) and from Du et al. (2014). 1A, 1B, 2A and 2B refer to the feeding types from Wieser (1953). 1 = prokaryotes, 2 = ciliates and flagellated protozoans, 3 = filamentous cyanobacteria and microalgae, 4 and 5 = photoautotrophic unicellular protists, like diatoms and (some) chlorophytes, 6 = sediment and/or detrital particles, 7 = nematodes, 8 = other invertebrate metazoans like oligochaetes.

The study of nematode feeding ecology has recently gained momentum by the development and application of novel techniques. The use of bulk stable isotope measurements to pinpoint carbon sources and trophic level in particular has become well-established (Moens et al. 2002; Moens et al. 2005a), whereas the use of next generation sequencing to characterize gut contents of bacterivorous nematodes has only just been pioneered (Derycke et al. 2016). Stable isotope data and observations of living nematodes have shown that stoma-morphology based guild classifications do not always provide good predictions of nematode resource utilization and even trophic level (Moens et al. 2005a; Vafeiadou et al. 2014), and dedicated experiments have highlighted that even closely related species can have differential resource utilization (Derycke et al. 2016; Vafeiadou et al. 2014).

3.6. Functional roles of nematodes in ecosystems

The high abundances of nematodes in nearly all soft marine sediments can substantially affect many ecological processes such as regeneration of nutrients, transfer of energy to higher trophic levels in benthic food webs, and bioturbation of sediments. Our aim is not to review all those functions here; however some important roles in ecosystems will be touched upon in the next sections of this introduction.

3.6.1. Roles in benthic food chains

Meiofauna have been regarded as an important component in benthic ecosystems due to their high abundance and fast turnover rates compared to macrofauna (Heip et al. 1985; Coull 1999). Despite having a lower biomass, the meiofaunal biomass turnover rate is typically five times higher than that of macrofauna (Giere 2009) and as such may exceed the production of macrofaunal assemblages. Within the benthic food web, many nematodes and harpacticoid copepods take an intermediate trophic position between primary producers (microalgae) and primary decomposers (bacteria) on the one hand, and higher trophic levels on the other (Gee 1989). Meiofauna can thus establish an important link between smaller and larger organisms in marine sediments mainly through trophic interactions (Piot et al. 2014). Indeed, many meiofauna are food for the juvenile stages of many commercially important marine species at higher trophic levels, including fish and crustaceans (Warwick 1987; Coull 1999; Menn 2002; Schückel et al. 2013) (Gee 1989; Coull 1990; Feller and Coull 1995; Colombini et al. 1996). In a case study at the surf zone of a sandy shore habitat in Southern Korea, juveniles of the amphipod species *Synchelidium lenorostralum* fed mainly on copepod nauplii and nematodes (Yu et al. 2003). The importance of nematodes to higher trophic levels has been somewhat better studied in freshwater compared to marine systems. Among the more common predators of freshwater nematodes are other nematodes (Majdi and Traunspurger 2015). Besides, the importance of many flatworms of the Turbellaria class, as predators of freshwater nematodes has also been reported (Martens and Schockaert 1986; Majdi et al. 2014). However, their lack of hard structures results in a fast digestion once inside the gut of a predator, which hampers correct estimates of the true predation pressure by higher organisms on nematodes (Hofsten et al. 1983). Nevertheless, some studies have tried to model the energy flow through the benthic food web. For example, (Gontikaki et al. 2011) showed that at Faroe-Shetland Channel (UK), sub-surface deposit-feeding polychaetes obtained 35 % of their energy requirements from ingestion of nematodes. Together, these reports highlight the significant and important energy transfer in the benthic food chain through nematodes.

3.6.2. Roles in microbial decomposition and nutrient cycling

Marine nematodes have catalytic functions in organic matter decomposition and nutrient cycling (e. g. mineralization of nutrients) and organic matter decomposition. Decomposition is mainly a microbial process and in this regard, both stimulatory (Findlay and Tenore 1982; Alkemade et al. 1992) or inhibitory (De Mesel et al. 2003) effects of marine nematodes on bacterial activity have been reported.

A stimulatory effect of nematodes on microbial abundance may result from locomotory activity and *microbioturbation* by marine nematodes which can enhance fluxes of oxygen and nutrients, which is essential for microbially driven decomposition processes (Alkemade et al. 1992; Aller and Aller 1992). A recent study reported increased bacterial denitrification in marine sediments by meiofauna through stimulation of nitrifiers and denitrifiers (Bonaglia et al. 2014).

The effects of bacterivorous nematodes on the decomposition process (De Mesel et al., 2003) were shown to be *species-specific* and dependent on interactions between nematode species (De Mesel et al. 2006). Even at relatively low densities, nematodes can significantly impact bacterial community composition in a very species-specific way. This may result from differential food preferences and hence a *differential grazing* (De Mesel et al. 2004).

Another function of marine nematodes is their *impact on the secretion of extracellular polymeric substances* (EPS) by diatoms and/or bacteria (Hubas et al., 2010). In intertidal areas in which the ecosystem is subject to hydrodynamic forces, the sediment stability is very important. In this context, microbial and diatom secretion of EPS has been recognized as a major sediment-stabilizing force (Stal 2010). Hubas et al. (2010) showed that the presence of bacterivore nematodes had a positive impact on the abundance of bacteria and diatoms and on their EPS production.

However, nematodes also produce *mucus* of their own, mostly during locomotion. This mucus may have several important roles in stimulating or affecting microbial communities. First, this mucus agglutinates sediment particles and may serve as an energy source for microbes (Gerlach 1977; Riemann and Schrage 1978). Specific direct and indirect trophic roles of this mucus have been demonstrated. Warwick (1981) demonstrated that cells of the green alga *Tetraselmis* settled onto mucus tracks of the nematode *Praeacanthonchus punctatus* and formed round resting cells, which were readily grazed upon by this nematode, whereas it was unable to feed on the active cells. Moens et al. (2005b) demonstrated that the bacteria able to successfully colonize nematode mucus tracks were a highly specific subset of bacteria present in the environment. And Riemann and Helmke (2002) proposed the *enzyme sharing concept*, in which they hypothesize that nematodes secrete enzymes along with mucus. These enzymes would contribute to the decomposition of complex macromolecules and would thus facilitate the growth of microbes, which then continue the decomposition process. The nematodes would benefit mostly from the dissolved organic compounds released by the microbes during their organic matter digestion.

3.7. Marine nematode dispersal and population genetics

3.7.1. Dispersal

Movement and dispersal are fundamental to many ecological processes (Ronce 2007). Understanding dispersal rates can shed light on distribution patterns of organisms, as dispersal extends the range of a species and enables recolonization of sites following perturbations. Dispersal also enhances the genetic diversity of populations and is, amongst others, an important determinant of population genetic structure of species (see the next part of this section). Moreover, differential dispersal abilities can be a strong driver of the coexistence of closely related marine nematode species (De Meester et al. 2015a).

Marine nematodes are generally considered poor swimmers (Barstead and Waterston 1991). Moreover, they have an endobenthic life style and lack pelagic larvae or any other dispersal stages (Giere 2009). Considering these presumed dispersal limitations, cosmopolitan distribution of marine nematodes has previously been considered a paradox (Giere 2009), as is the case for other marine organisms without a pelagic larval phase (Winston 2012). However, nematodes can be dispersed through sediment, water and perhaps even air (Derycke et al. 2013).

Thus, there are several passive dispersal modes of marine nematodes. For instance, marine nematodes can be suspended in the water column by hydrodynamic forces such as currents and waves (Eskin and Palmer 1985; Fegley 1987; Palmer 1988; Commito and Tita 2002; Wetzel et al. 2002; Boeckner et al. 2009). Species living close to the sediment surface are more susceptible to erosion than deeper dwelling species. Passive transport, for instance with the ballast water of ships (Radziejewska et al. 2006) or on drifting algal mats (Arroyo et al. 2006) can occasionally lead to long-distance dispersal (Thiel and Gutow 2005; Derycke et al. 2008b).

Besides passive transport in the water column, there is also a possibility for active dispersal of marine nematodes, but only over small distances. Nematodes move through sediments in response to cues emanating from, among others, food sources (Schratzberger et al., 2004b; Gallucci et al., 2008). In addition, some nematode species actively emerge into the water column and swim over short distances (Jensen 1981), and they may partly select the locations where they will re-settle (Jensen 1981; Ullberg and Ólafsson 2003; Schratzberger et al. 2004b; Gallucci et al. 2008). As such, they may enhance intermediate-distance dispersal, by actively emerging into the water and subsequently being passively carried over larger distances with the water currents. Thomas and Lana (2011) demonstrated that nematode transport over scales of centimeters to tens of meters is directly influenced by their body morphology and swimming ability, and indirectly by their feeding strategies, which ultimately define their position in the sediment column (see also Committo and Tita 2002).

3.7.2. Population Genetics

Population genetics is the study of genetic variation within populations and provides valuable information on distribution patterns and connectivity between populations (Kartavtsev 2015). Indeed, population genetic data reflect a combination of historical, ecological, behavioural and environmental processes that determine rates and patterns of dispersal amongst populations (Grosberg and Cunningham 2001). Population genetics data can also be used to understand the ecological and evolutionary dynamics of species and to assess the evolutionary forces such as migration, mutation, genetic drift and natural selection (Avise 2000).

Published information about population structure and gene flow of marine nematodes worldwide is largely limited to intertidal species with high colonizer ability, which may not be fully representative of endobenthic species (Derycke et al. 2013; Kumar et al. 2015). These authors found significant population-genetic structuring at scales of (a few tens of) kms, despite many opportunities for passive dispersal linking the different subpopulations. A similar pattern was found for a species that, like the previous ones, lives in association with macroalgae, but contrary to the previous species has a very different, k-selected life strategy (Derycke et al., 2010). While we might intuitively expect endobenthic species to have more pronounced population genetic structuring, because they are less prone to passive dispersal, first data on endobenthic nematode species show both similar and lesser population genetic structure, depending on the species studied (Derycke et al. 2013; Kujundzic 2014). The effect of life history, habitat type and environment on population genetic structure of marine nematodes are not yet properly understood, and require information from different habitats (Hauquier et al. 2016) as well as from species with different life histories and colonizer abilities (Kujundzic 2014). Therefore, it is clearly necessary to perform research on the topic to get more information on the population genetic structure and cryptic variation of marine nematodes. One chapter of this thesis will be devoted to this topic.

3.8. Nematodes as bio-indicators

The term “bio-indicator” is used to refer to a species or assemblage of species that depicts the occurrence of pollutants on the basis of specific symptoms, reactions, morphological changes or concentrations (Markert et al. 2003). Many bio-indicator approaches have been developed to assess spatial and temporal patterns of coastal marine contamination. These include macrobenthos, such as mussels, clams and barnacles (Lee and Chin 2003; Bebianno et al. 2004), but also seabirds (Kushlan 1993; Furness and Camphuysen 1997) and fish (De Andrade et al. 2004). Within the benthic fauna, most investigations have used macro-infauna to monitor the effects of environmental change in the marine ecosystem, whereas the smaller meiofauna have been studied somewhat less in monitoring programmes because of the time-consuming analysis of their assemblages (Kennedy and Jacoby 1999). However, one of the most important and interesting features of marine nematode assemblages is their potential for environmental monitoring and assessment as bio-indicators of environmental stress (Boyd et al. 2000b; Schratzberger and Jennings 2002; Moreno et al. 2011; Semprucci et al. 2013; Semprucci et al. 2015b).

The theoretical and practical advantages and disadvantages of using nematodes as bio-indicators have been summarized in (Bongers and Ferris 1999; Schratzberger et al. 2000). One of the main reasons which make them potentially good indicators is that they are restricted to the sediments throughout their life and have a limited mobility, hence they cannot escape from stressful events or impacts. As a result, they are more consistently and intimately exposed to stressors in their environment. Due to their wide diversity and range of adaptations, they occur nearly everywhere in very high numbers. There is a high diversity in tolerances to various kinds of disturbance. Thus, alterations in their assemblage structure can often be related to environmental perturbations (Platt and Warwick 1980).

Both bio-indicator species and bio-indicator communities can be applied in environmental impact assessment studies, although community structure data is most frequently used, rather than single species as bio-indicators (Wilson and Khakouli-Duarte 2009). This is partly due to the fact that most marine nematode species are hard to culture under controlled conditions in the laboratory, and those species which are more amenable to culturing and dedicated pollution testing are often not the most representative taxa of real benthic nematode assemblages (Moens and Vincx 1998).

There are many examples of bio-indicator species or genera, such as *Chromaspirina*, *Hypodontolaimus*, *Onchalaimellus*, *Paracanthonchus*, (*Seto)sabatieria* and *Xyala* which have been used as bio-indicator of petroleum pollution (Semprucci and Balsamo 2012). *Terschellingia*, *Molgolaimus* and *Ptycholaimellus* are tolerant to metal contamination (Somerfield et al. 1994), while *Eleutherolaimus* is sensitive to heavy metals (Millward and Grant 1995). However, whereas the information on species or genus level often remains rather anecdotal, studies of marine nematode assemblages have increasingly been used to determine the health of the coastal environment (Kennedy and Jacoby 1999; Danovaro et al. 2009; Semprucci and Balsamo 2012).

Nematodes are often the only remaining taxon in extremely (organically) polluted conditions, because they are more tolerant to anoxic conditions than macro- and other meiofauna (Moodley et al. 1997). For example, high loads of organic matter can lead temporarily to anoxic and sulfidic conditions. Certain marine nematode species can survive and even thrive in these conditions (Wilson and Khakouli-Duarte 2009). Moreover, they have comparatively short life cycles. Their short generation times enable them to respond more quickly than macrofauna to changes in environmental conditions and alterations in food supply (Heip et al. 1985). Another advantage of using nematodes as an ecological indicator is the existence of readily identifiable functional guilds (Bongers and Ferris 1999), although empirical evidence to properly classify species or genera into these guilds is often lacking (Moens et al., 2013).

All together, these characteristics make nematode assemblages a potential tool for detecting a more rapid and unequivocal reaction to environmental changes than macrofauna (Balsamo et al. 2010). Platt and Warwick (1980) concluded that any general assessment of the ecology of intertidal habitats is incomplete if the nematode fauna is not taken into consideration.

Nevertheless, an argument against the use of nematodes as ecological indicators is that their morphological identification at species level is really difficult and time-consuming and is hard for non-specialists. However, it has been suggested that for bio-monitoring purposes, nematode identification to genus level is usually sufficient and a more detailed taxonomic resolution did not necessarily improve the final outcome of environmental quality assessments (Warwick 1988; Moreno et al. 2008b; Moreno et al. 2011). Still, recent research highlights that even closely related species can substantially differ in their tolerance to various kinds of pollution (Hoss et al. 2011; Monteiro et al. sub.), so species-level identification is still warranted.

3.9. Diversity measures to monitor impact

As described under section 3.8, nematode assemblages potentially hold important information based upon which the recent disturbance history of a habitat can be assessed. In addition to analyses of differences in genus or species composition, a variety of indicator indices have been developed to capture assemblage-level information in a number, the change of which can indicate a disturbance or, the opposite, a recovery from disturbance. In terrestrial soils, the **maturity index** (MI) is the most frequently used nematode-based indicator of environmental status. It is based on a division of nematodes in five categories defined along a gradient of extreme *colonizers or opportunists* to "*persisters*", species which reproduce slowly, produce few progeny, have a long generation time and hence cannot easily re-establish after having been perturbed (Bongers 1990; Bongers et al., 1991; Bongers and Ferris, 1999). Colonizer potential (cp) is in turn linked to life history and to pollution tolerance. Hence, various kinds of disturbances favour species with a high colonizer ability and cause decrease or loss of persister species, resulting in a decrease in MI. A number of other, related indices have later been proposed (Bongers and Bongers 1998; Bongers and Ferris 1999; Ferris et al. 2001), some of which merge cp with feeding-type information. These indices have become very successful in soil nematology, but much less so in aquatic nematology, among other reasons because of the all-too-limited information on nematode life histories and feeding ecology, and because of the sometimes weak link between cp and disturbance tolerance (Wilson & Kakouli-Duarte, 2009).

In addition to these specific nematode-based indices of soil condition, **measures of biodiversity** may be very useful to indicate changes in assemblages resulting from pollution or other disturbances (Moreno et al. 2008a; Moreno et al. 2008b; Moreno et al. 2011). Biodiversity indices are many, however some of them are widely used. Here we briefly introduce those biodiversity measures which were used in this PhD study. They can be subdivided into the following categories: richness measures, evenness and dominance measures, and measures of phylogenetic relatedness.

Richness measures provide an estimate of the mere number of taxa present in a sample/habitat/community. We have used N_0, (Hill index) (Hill 1973) which is merely the number of taxa (in our case genera) identified from each sample, Margalef's d and expected number of genera. N_0, like most diversity measures, is heavily dependent on sampling size/effort, an issue which can greatly impact on the reliability of richness comparisons among samples, habitats, studies.

Margalef's d is calculated as:

$(S - 1) / \ln N$

where S and N are the number of taxa and of individuals in a sample, respectively, and is supposed to lower the impact of the actual sample size on richness estimates, but it is actually very poor at doing so. Hence we also used the rarefaction index "expected number of genera" in a hypothetical sample of 50 individuals (EG(50)). Rarefaction is a permutation technique which allows to interpolate from a richness measure of a larger sample to a richness measure of a smaller (sub)sample. It thus yields the number of taxa one would have expected to find if a smaller set of individuals had been identified. That expected number of taxa is the ET(x) (expected number of taxa in a sample of x individuals) and is fully independent of the actual sample size.

In addition to richness, we estimated *evenness and dominance*, two mutually linked properties of an assemblage. Evenness determines the spread of the individuals in an assemblage over the taxa, whereas dominance does essentially the same, but with a prime focus on the most dominant taxon or taxa of an assemblage. We calculated "**Pielou's J**" as an evenness index (Pielou 1977):

$J' = H' / \ln S$

where S is the number of species in each location or station.

We also calculated **Simpson's** index, which measures the probability that any two randomly sampled individuals belong to the same species. This index varies between 0 (infinite diversity) and 1 (assemblage composed of a single species), and provides a measure of the dominance structure in an assemblage.

$\lambda = \sum (N_i/N)^2$

where N is the total number of individuals, and N_i is the number of individuals of the *i* genus (*i* from 1 to S).

The Shannon–Wiener diversity index (***H'***) is another index that is commonly used to characterize species diversity in a community (Shannon and Weaver 1949).

$H' = -\sum(N_i = N) \ln(N_i = N)$

This index integrates aspects of species richness and evenness.

Indices of phylogenetic relatedness (Clarke & Warwick, 1994) estimate the phylogenetic relatedness of taxa in an assemblage. The rationale is that disturbance will select for tolerant taxa, and that tolerance or sensitivity is related to physiological and other characteristics of organisms which have an evolutionary basis. Hence, closely related taxa are on average expected to have more similar sensitivities than more distantly related taxa. Two assemblages with the same richness and evenness may still differ in their diversity, whereby a phylogenetically more distinct assemblage is considered more diverse than an assemblage with phylogenetically more related taxa. We calculated taxonomic distinctness ($\Delta*$) as a measure of the average taxonomic distance among all pairs of genera in an assemblage, and taxonomic diversity (Δ) as the average weighted path length between every pair of individuals in a phylogenetic tree.

Besides, biodiversity can be measured and monitored at several **spatial scales**. For example, *alpha diversity* is local diversity, which can be defined at the level of a sample or a station, but is sometimes also reported at the level of a site or a habitat. Site would correspond to a beach location in thus PhD, but each beach location comprises different stations, each with their own local diversity. *Beta diversity* reflects the differences in community composition between sites and gamma diversity is the diversity of entire landscape. Indeed, the *gamma diversity* is a product of both beta- and alpha-diversity (diversity at a single site) (Gray 2000).

3.10. Benthic fauna of the PG

Generally, the meiofauna of the Iranian part of the PG is poorly studied. Indeed, no comprehensive data from any marine habitat are available on meiofauna in this important area. Studies of benthic assemblages in the Iranian part of the PG have hitherto focused on macrobenthos. Macrofauna have traditionally and routinely been the most used group for biological assessment of marine environmental health worldwide (Rosenberg and Resh 1993; Ogbeibu and Oribhabor 2002; Parr and Mason 2003; Ogleni and Topal 2011). This has also been the case in the northern part of the PG.

Published data on the macrobenthos of the Iranian part of the PG can be divided thematically into two main subjects, "Biodiversity and community structure" and "pollution monitoring". However, studies are very diverse in terms of areas, habitats, taxa, contexts… studied. An overview and integrated discussion of the most important results is therefore not straightforward to make, and falls beyond the scope of this work.

4. Research aims and objectives

As outlined in the previous sections, the Persian Gulf provides both a wealth of resources to humans (oil as well as fisheries) and a large diversity of marine habitats. However, while a substantial number of studies have been performed on the benthic macroinvertebrates in this region, the meiobenthos has so far been largely neglected. Accordingly, the main objective of this thesis was to study, for the first time, the free-living marine nematode communities in a coastal habitat of the Iranian part of the Strait of Hormuz. This will not only provide critical baseline information, but will also contribute substantially to the knowledge of marine biodiversity and ecology in the area, and of the impacts of anthropogenic pollution on coastal benthos biodiversity. This main objective is extended by studying different aspects of marine nematode assemblages such as spatial and temporal variability and population genetic structure of two selected species.

After the current introductory chapter, in chapter 2 we assess the structure and biodiversity of the nematofauna in intertidal soft sediment habitats. Since this is a pioneering study, we first investigate the biodiversity of nematode assemblages and compare the observed diversity with that of other beaches across the world. In doing so, we try to assess to what extent the naturally (high salinity, large temperature fluctuations with often extreme maxima) and anthropogenically (for instance hydrocarbon pollution related to the role of the Persian Gulf as the world’s largest transportation route of crude oil) stressful conditions translate into a low biodiversity in the area. At the same time, however, we focus on spatial patterns in relation to local (so non-overarching) point sources of pollution, asking the question whether in such a broadly stressed environment, local pollution sources still have a measurable impact on nematode assemblages. For this purpose, we focus on four beaches, and within each beach location, we assign three stations at different distances from local point sources of pollution. This chapter has recently been conditionally accepted (‘minor revision’) for publication in *Hydrobiologia* as Sahraean et al. (2017a) “Nematode assemblage structure and diversity in intertidal beaches of the northern Persian Gulf”.

In chapter 3, we then repeat the same sampling design almost exactly one year later to get a first impression of the consistency of the obtained biodiversity estimates and spatial patterns over time, more specifically year-to-year variability. Given the often substantial between-year variability in climatological conditions, we want to see to what extent the biodiversity and the between and within-beach location differences observed in 2008 and linked there in large part to the effect of local pollution sources, would be consistent or be overruled by broader-scale phenomena such as differences in precipitation and related environmental conditions. This chapter is in preparation for later publication as Sahraean et al. “Year-to-year variability in beach nematode assemblage structure and biodiversity in the northern part of the Strait of Hormuz”.

In chapter 4, we investigate the connectivity and gene flow between populations from beach locations at a scale at which in other population-genetic studies of coastal nematodes, significant structuring has regularly been observed. Given our conclusion from chapter 2 that local pollution point sources can substantially impact diversity and structure of nematode assemblages, and given the temporal variability observed in chapter 3, we wanted to assess to what extent we can expect recolonization after a major local disturbance event by individuals from nearby habitats. To this end, we looked into the population-genetic structure of the two most abundant nematode species from our study area across 52 km of coastline. Moreover, since both nematode species studied here occupy different depth layers of the sediment, we also tested the hypothesis that the surface-dwelling species would show more gene flow and a lesser population-genetic structuring compared to the real endobenthic species. In another part of this chapter, we compare 18S rDNA and COI sequences of one of these species, *Terschellingia longicaudata*, from Iran and the Scheldt Estuary in The Netherlands in order to ascertain whether they truly belong to the same species. This chapter has been accepted for publication in the journal *Marine Ecology* as Sahraean et al. (2017b) “Lack of population genetic structure in the marine nematodes *Ptycholaimellus pandispiculatus* and *Terschellingia longicaudata* in beaches of the Persian Gulf, Iran”.

Finally, in chapter 5, an overview and integration of the results from the different chapters is provided, and perspectives for future research on meiobenthos in Persian Gulf intertidal habitats are presented.

Chapter 2

Nematode assemblage structure and diversity in intertidal beaches of the northern part of the Strait of Hormuz

Chapter 2

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This chapter is adapted from:

Sahraean, N., T. Nara Bezerra, K. Ejlali Khanaghah, H. Mosallanejad, E. Van Ranst & T. Moens, 2017a. Nematode assemblage structure and diversity in intertidal soft shores of the northern Persian Gulf. Hydrobiologia accepted. (pending minor revisions)

Abstract

This study is the first to present data on local and regional diversity of beach nematode assemblages from the Persian Gulf. We investigated four beaches near the city of Bandar Abbas, Iran. On each beach, we sampled three stations with increasing distance (50, 100 and 150 m) from a local pollution source, mostly domestic sewage. A total of 39 genera from 17 families was recorded. This diversity is low and suggests that the entire area experiences substantial stress. Five genera together comprised 75 % of nematode abundance. There were significant differences in abundance as well as genus diversity between locations, but these did not unequivocally correlate with known drivers of benthic assemblage structure like sediment granulometry and hydrodynamics. The location exposed to the strongest local pollution input had the lowest nematode diversity and a very low abundance at the nearest distance to the pollution source (50 m). Distance from local pollution sources also significantly impacted genus diversity, but this pattern was only pronounced in two of the four beaches. Our data demonstrate that local sources of anthropogenic disturbance are a major driver of assemblage diversity and structure in this area, despite an overarching effect of natural (salinity, temperature) and anthropogenic stressors in the area.

Keywords: marine; benthic assemblages; local diversity; regional diversity; anthropogenic impact; sewage discharge

1 Introduction

The Persian Gulf is one of the most important waterways in the world in view of its role in oil and gas transport and of its geostrategic position (Mojtahed-Zadeh 1999). It is an extension of the Indian Ocean, connected to the Oman Sea through the narrow Strait of Hormuz. The Persian Gulf is a subtropical, hyper-arid region, located between Iran and several Arabian countries. The Gulf's marine environment is of special interest because of its shallowness, high salinity and limited interaction with oceanic waters (Reynolds 1993; Kämpf and Sadrinasab 2006). The PG has an average water depth of ca 36 m; there is a broad shallow southern margin (< 20 m deep) along the coasts of Qatar, Bahrain and the UAE, and a relatively narrow and deep north-eastern margin along the coasts of Iran (Kämpf and Sadrinasab 2006). The deepest areas are in front of the Iranian coast, reaching from 60 m to about 100 m at the entrance to the Strait of Hormuz (Sheppard et al. 1992). Salinity generally ranges from ≥ 39 in summer to ≤ 41 in winter; however, in tidal pools and lagoons it may reach much higher values, up to 70-80 (John et al. 1990). Among the most important coastal habitats of the Persian Gulf are intertidal areas and estuaries (Sheppard et al. 2010). Major human impacts on these habitats include oil pollution, solid and liquid waste disposal, coastal development and recreational activities, which can synergistically affect the biodiversity and abundance of the benthos.

As a result of its high salinity and sea-surface temperature (Chao et al. 1992), accompanied by low primary productivity resulting from high turbidity (Nezlin et al. 2010), and of its intensive human exploitation, the Persian Gulf area may be expected to harbor comparatively low local and habitat-specific biodiversity. There are, for instance, persistently high levels of hydrocarbon pollution throughout the waters of the Persian Gulf (Gevao et al. 2006; Gawad et al. 2008), while agricultural runoff and domestic sewage may cause localized eutrophication (Gawad et al. 2008).

Free-living nematodes are the most numerous and diverse group of marine benthic metazoans, and usually by far the most abundant meiofaunal taxon in soft sediments. They play important roles in benthic ecosystems (De Mesel et al. 2003, 2006; Bonaglia et al. 2014). Within the benthic food web, nematodes take an intermediate trophic position between primary producers (microalgae) and primary decomposers (bacteria) on the one hand, and higher trophic levels on the other (Gee 1989; Schuckel et al. 2013). Nematodes are also useful tools for environmental monitoring and assessment as bio-indicators of environmental stress (Lambshead 1986; Bongers and Ferris 1999; Balsamo et al. 2010; Moreno et al. 2011; Losi et al. 2013; Semprucci et al. 2015a).

In sandy beaches, nematodes typically show a pronounced horizontal zonation, with increasing abundances from high to low-water line (Hodda & Nicholas, 1985; Gheskiere et al., 2002, 2005; Kotwicki et al., 2005), and with an often unimodal diversity pattern, with highest diversity in the middle part of the beach (Armonies and Reise 2000; Gheskiere et al. 2004; Gingold et al. 2010; Maria et al. 2013b). Higher diversity at the middle beach has been attributed to the fact that an optimal balance among desiccation/temperature/salinity stress, hydrodynamic disturbance and sediment stability, food availability and oxygen concentration is usually reached somewhere in the mid-intertidal.

Hitherto, most research on the benthic assemblages in the northern part of the Persian Gulf has focused on macrobenthos and has generally found relatively low diversity and abundance of macrofauna in intertidal sediments, which has mainly been attributed to a combination of natural and anthropogenic stressors (see above) (Ejlali Khanaghah et al. 2010; Naderloo and Tuerkay 2012; Safahieh et al. 2012; Pourjomeh et al. 2014). Here, we present the first study on free-living marine nematode assemblages in intertidal soft sediment habitats of the northern part of the Persian Gulf. The main aim of our study is to assess the structure and diversity of the nematofauna on beaches in the Persian Gulf and compare it to those from beaches elsewhere in the world. Our study was designed to assess impacts of local pollution sources, mostly in the form of sewage and garbage disposal, on the nematode assemblages. We focused on four intertidal locations in the northern part of the Persian Gulf. In each location, we sampled three stations along a gradient of increasing distance to an anthropogenic point source of pollution. We tested the null hypotheses that the genus diversity of the nematofauna and the dominance of stress-tolerant genera would not differ among beach locations nor depend on distance towards local pollution sources. Additionally, we tested the hypothesis that nematode diversity at these Persian Gulf sandy beaches would not profoundly differ from that of beaches in other geographical areas.

2 Materials and Methods

2.1 Sampling sites and design

Focus in this study was on the northern part of the Persian Gulf, particularly the coast near Bandar Abbas, the capital of the Hormuzgan Province of Iran. Four intertidal locations were selected based on the presence of local point sources of pollution (Fig. 2-1). Haghani is situated next to the largest sewage drainage canal of the city, and visually appears by far the most impacted location. The second location, Suro, is an isolated site receiving untreated sanitary sewage of a residential complex through a small estuary. The types of input are similar to those in Haghani, but the amounts are considerably smaller. The third station, Terminal, is located next to a canal of urban sewage effluents constituting mainly of urban run-offs; however, there is little information on the amounts of sewage that are discharged here and it is therefore difficult to assess its pollution status relative to, e.g., Suro. Data on macrobenthic assemblage structure indicate only moderate to low levels of pollution impact at Terminal (Negarestan et al. 2007). Finally, Dolat Park is considered a comparatively less impacted site, receiving only small amounts of litter from people using the coastal park for recreation, but no local sewage inputs. Both quantitative and qualitative information on the actual (amounts of) inputs and hence on the degree of pollution at these locations is, however, lacking.

All sampling stations were located in the mid intertidal zone, on ridges (in any case, runnels were not prominently present). Tidal amplitude in the area ranges from 1.5 to 4 m; the width of the intertidal zone is typically in between 500 and 600 m, except at Haghani, where it is on average just less than 30 m. The intertidal zones vary from a moderately reflective beach at Haghani to more sheltered tidal flats at the other three locations (Mohebi 2007; Salehi 2007; Samadi 2007; Alimomohammadi 2009).

Our sampling was designed to assess local and regional diversity of the nematofauna in beaches at Bandar Abbas, while at the same time evaluating effects of anthropogenic pollution point sources. For this purpose, at each location, we sampled three stations at 50-m intervals with increasing distance from the pollution point source. Given that there was no visible sewage input at Dolat Park, the most impacted station at this site was taken as the station nearest the Park, which is the zone most affected by recreation and with visibly more garbage on the beach. Thus, the stations at each location are labeled as 1, 2 and 3, station 1 being closest (50 m) to the pollution and station 3 most distant (150 m) from the pollution point source. At each distance to pollution, we collected three replicate samples for nematode assemblage analysis at ca. 1-m distances from each other. Admittedly, the close proximity of the replicate samples within a station entails the risk of pseudo-replication. At the same time, substantially increasing the distances between replicates would have increased the risk of significant variability in relation to beach (micro)profile. Given that patchiness in intertidal nematode assemblages tends to be very pronounced already at scales of centimeters (Blanchard 1990; Hodda 1990), we consider replication at the station level as sufficiently independent.

Fig. 2-1. Map of the study area with indication of the four beaches sampled. A: map of Iran showing the Persian Gulf in the south; B: map of Strait of Hormuz showing Bandar Abbas location; C: our four sampling locations along the coast of Bandar Abbas.

2.2 Sample collection, elutriation and analysis

Sampling was performed in December 2008. Three replicate samples were collected using 3.5-cm diameter PVC cores that were pushed in the sediment down to a depth of 5 cm. One additional sample was taken to determine the sediment granulometry using a Malvern Hydro 2000G Particle Size Analyzer. Sediment fractions in the particle size range of 38 to 1000 µm were defined according to the Wentworth scale (Buchanan 1984) and expressed as volume percentages. The fraction < 38 µm was negligible in all locations, and the measured silt fraction was hence composed of particles with sizes in between 38 and 63 µm. At the time of this sampling, several logistical constraints prohibited the collection of more samples for the measurement of additional environmental parameters. At a later sampling occasion in August 2013, we collected sediment samples with proper replication for every distance to pollution, for total organic carbon and total nitrogen and for total element analysis, all of which could potentially inform on the relative importance of sewage inputs. It is, however, important to remember that these data do not stem from the same moment as the nematode assemblage data.

Total organic carbon (TOC) and nitrogen (TN) were thus analysed on sediment samples collected in the same way as in 2008, but in August 2013, with three replicates per distance to pollution. TOC and TN analysis was performed using a FLASH 2000 CHN elemental analyser on dried sediment aliquots after prior removal of inorganic carbon through the use of dilute HCl (Nieuwenhuize et al. 1994).

The dried beach sediment samples from 2013 were further characterized by total element analysis of bulk samples following fusion with 2 g lithium metaborate powder in a platinum crucible for 15 min at 950 °C in a preheated muffle furnace. The flux that is thus formed is allowed to cool and then dissolved in 100 ml of 4 % HNO_3 (ISO 14869). Contents of major and trace elements were measured with a Varian 720-ES Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) at the Laboratory of Soil Science of Ghent University. Loss upon ignition (LOI) was determined by heating the samples at 1000 °C.

Samples for nematode analysis were preserved immediately with 4 % buffered formaldehyde and transferred to the laboratory. There, they were rinsed thoroughly with tap water and decanted over a 38-µm sieve. The fraction retained on that sieve was then elutriated by centrifugation with the colloidal silica gel Ludox HS40 (density = 1.18) (Heip et al., 1985). The supernatant was again passed through a sieve of 38 µm. This procedure was repeated three times, and the fractions retained on the sieve were then pooled.

For each sample, all meiobenthic animals were counted after staining with Rose Bengal. Subsequently, at least 100 nematodes were picked up randomly from each sample and gradually transferred to glycerol through a series of ethanol-glycerol solutions. This procedure renders the nematodes more transparent, facilitating examination of both external and internal structures; the gradual transfer prevents the animals from collapsing (Seinhorst 1959). When samples contained less than 100 nematodes, all available specimens were transferred to glycerol. Then, nematodes were mounted on permanent slides with anhydrous glycerin for identification to genus level. Genus identification was done using pictorial keys (Platt and Warwick 1983; Warwick et al. 1998) and the NeMys online identification key (Vanaverbeke et al. 2015).

2.3 Data analysis

Nematode assemblage structure was described by a number of univariate descriptors, including abundance and different diversity measures such as number of genera (N_0), Margalef's genus richness, expected number of genera in a sample of 50 individuals (EG50), Shannon-Weaver's diversity (H'), Simpson's diversity index (Si), Pielou's evenness (J), and taxonomic diversity (Δ) and distinctness ($\Delta*$). N_0 is merely the number of genera identified from each sample, whereas Margalef's richness lowers the impact of the actual sample size on richness estimates. Nevertheless, even the latter index is not independent of sample size; hence we also used the rarefaction index 'expected number of genera' in a hypothetical sample of 50 individuals (EG50). Shannon-Weaver's diversity combines aspects of richness with evenness, whereas Pielou's J is a specific evenness index. Finally, Simpson's index measures the probability that any two randomly sampled individuals belong to the same species. This index varies between 0 (infinite diversity) and 1 (assemblage composed of a single species), and provides a measure of the dominance structure in an assemblage. Taxonomic distinctness ($\Delta*$) provides a measure of the averaged taxonomic distance among all pairs of genera in an assemblage, whereas taxonomic diversity (Δ) is the average weighted path length between every pair of individuals in a phylogenetic tree (Warwick and Clarke 1998, 2001). All these diversity indices were calculated in PRIMER 6.0 (Clark and Gorley 2006).

We further calculated the frequency of occurrence of nematode genera according to Arasaki et al. (2004). Genera were classified as constant if they occurred in at least half of the samples ($F \geq 50$ %), common (25 % $\leq F \leq 50$ %) or rare ($F \leq 25$ %). Nematodes were also grouped into feeding types according to the feeding type classification of (Moens and Vincx 1997), which is based on a combination of observations and on the assumption that nematode stoma morphology is an important determinant of food selection. This classification recognizes six feeding types: microvores, deposit feeders, ciliate feeders, epigrowth feeders, facultative predators and predators.

Differences in each of these univariate assemblage descriptors between different locations and distances to pollution were assessed by analysis of variance (ANOVA) in the software Statistica 7 (Statsoft). Prior to analysis, data were tested for normality by means of the Kolmogorov-Smirnov test, and homogeneity of variances using Levene's test. If the data did not conform to these assumptions, they were log(x+1) transformed; if this data transformation did not solve the issue of normality and/or homoscedasticity, data were analyzed using permutational multivariate analysis of variance (PERMANOVA) (Anderson et al. 2008) with location and distance to pollution as fixed factors (with four and three levels, respectively) using 999 permutations. A Euclidian distance based resemblance matrix was used for PERMANOVA on univariate (i.e. total number of nematode, diversity indices….) data. Homogeneity of multivariate dispersion was assessed using PERMDISP.

ANOVA followed the same two-way factorial design as for PERMANOVA. Tukey's HSD test was used for pairwise a posteriori comparisons between locations, distances to pollution and their interaction in case of a significant factor or interaction effect in ANOVA. Pairwise comparisons following a significant PERMANOVA result used Monte Carlo permutations of residuals under a full model (if the number of permutations was lower than 150, the Monte Carlo permutation p was used). The same statistical approach was used to test for differences in heavy metal or TOC and TN concentrations among locations and distances to pollution for the 2013 dataset. Sediment granulometry data of 2008 were analysed for differences between locations only using one-way ANOVA.

Non-metric multidimensional scaling (nMDS) based on Bray-Curtis similarities was applied to visually explore differences in nematode assemblage structure between locations and distances to pollution. Nematode assemblage composition was further compared between locations, distances to pollution and their interaction using the same PERMANOVA design as for the univariate assemblage descriptors, but using Bray-Curtis similarities. Genera abundances were square root transformed prior to analysis for a better weighting of the contributions of dominant and rare genera. In addition, the genera contributing most to the dissimilarities between distances to pollution and locations were investigated using the similarity percentages procedure (SIMPER) in PRIMER 6.0, again on square root transformed nematode abundance data.

3 Results

3.1 Sediment granulometry and total element analysis

Table 2-1. Sediment granulometry (measured on samples from December 2008), total organic carbon (TOC) and total nitrogen (TN) (measured on samples from August 2013) and genus richness (number of genera, N_0, determined on samples from December 2008) of the four beaches studied. Data on granulometry are means ± 1 standard deviation of three stations per beach, with one sample per station. Data on TOC and TN are means ± 1 standard deviation of three stations per beach, with three samples per station. All statistically significant differences indicated by different letters.

	Suro	Haghani	Dolat Park	Terminal
median grain size (µm)	155±8.9 b	175±18.6 b	177±17.5 b	119±9.4 a
mean grain size (µm)	185.6±28.3 b	194.9±23.4 b	198.1±11.1 b	175.0±2.6 a
% silt	0.4±0.1 b	1.0±1.0 b	5.9±3.9 b	17.8±3.0 a
%very fine sand	31.4±2.8	23.9±7.0	19.6±5.5	34.8±1.2
%fine sand	53.6±4.0 b	53.2±2.1 b	48.2±8.3 ab	29.5±4.2 a
%medium sand	11.4±3.4	20.3±7.1	25.0±3.5	12.61±1.3
%coarse sand	1.9±1.9	1.6±1.2	1.2±0.8	3.4±0.8
%very coarse sand	0.6±0.6	0.0±0.0	0.0±0.0	0.9±0.3
%TOC	0.1±0.0 b	0.2±0.1 ab	0.1±0.0 b	0.3±0.1 a
%TN	0.03±0.0	0.02±0.0	0.03±0.01	0.03±0.0
genus richness	10.44±1.59 a	5.6±0.28 bc	9±0.78 ad	8.1±0.9 cd

The sediment of the four locations was mainly composed of very fine sand and fine sand throughout. Significant differences in median grain size between locations were observed (df = 3, pseudo-F = 3.57, p = 0.05), Terminal having a lower median grain size and higher silt fraction than the three other locations (Table 2-1 and Table S2-3).

Total nitrogen (TN) concentrations were always < 0.1 % and did not differ depending on the location-by-distance interaction (df = 6, pseudo-F = 0.99, p = 0.50), nor between locations (df = 3, pseudo-F = 1.04, p = 0.7) or with distance from pollution (df = 2, pseudo-F = 0.61, p = 0.57). Total organic carbon (TOC) concentrations varied from 0.1 to 0.7 % and were not affected by the interaction of location and distance (df = 6, pseudo-F = 1.68, $p = 0.16$); however, they differed significantly between locations (df = 3, pseudo-F = 4.2, $p < 0.02$) as well as between distances from pollution (df = 2, pseudo-F = 4.46, $p < 0.02$). Terminal showed a significantly higher TOC concentration than Suro ($p = 0.01$) and Dolat Park ($p = 0.02$) (Table 1), and stations nearest pollution sources and at 100 m away both had significantly higher % TOC than the most distant station (both $p < 0.05$). The significant distance-to-pollution effect was largely due to Haghani and Terminal, where the station nearest the pollution point source had, respectively, more than and nearly threefold higher TOC concentrations than the two more distant stations.

The elements As, Be, Bi, Cd, Co, Hg, Ni and Pb were present at concentrations < 10 ppm throughout our study area (data not shown). Ba, Cr, Cu, Sr, Zn, Y and S were obtained in higher concentrations and varied among locations, distances to pollution and/or their interaction (supplemental material – Table S2-1), except Cu. However, no single location or distance to pollution had clearly higher or lower levels of multiple elements. Haghani had significantly higher concentrations of Cr than all other locations, while both Haghani and Suro had significantly higher concentrations of Sr, which could be considered in line with the expected higher pollution at Haghani and to a lesser extent Suro, but other metals did not always follow those trends. Likewise, elemental concentrations did not exhibit clear trends with distance to a pollution source (Table S2-1). Cu, which is often linked to sewage and other anthropogenic pollution sources, did not vary significantly between locations and distances to pollution.

3.2 Nematode abundance and dominant genera

The abundance of nematodes at the four locations ranged between 13 and 1371 ind. per 10 cm² (lowest and highest values, respectively, in single replicates). It was significantly affected by the interaction between location and distance to pollution (df = 6, F = 14.07, $p = 0.000001$). This interaction mainly reflected differences in nematode abundance in the stations nearest and furthest away from pollution point sources between different beach locations (Fig. 2-2). The station nearest the local pollution input at Haghani had significantly lower nematode abundances than any other station at any beach (Fig. 2-2). At Suro, by contrast, the station nearest the pollution point source had the highest nematode abundance. Dolat Park and Terminal exhibited less pronounced differences between stations (Fig. 2-2).

Table 2-2. Genus composition and relative abundances (%) of nematodes at the four sampling locations and average over all sampling locations. Data are means of 3 stations per location, with 3 replicates per station. Feeding types (FT) according to Moens & Vincx (1997) and Wieser (1953) are also listed. MV = microvore, DF = deposit feeder, CF = ciliate feeder, EF = epistrate feeder, FP = facultative predator, PR = predator; 1A = selective deposit feeder, 1B = non-selective deposit feeder, 2A = epigrowth feeder, 2B = predator or omnivore.

.	Suro	Haghani	Dolat park	Terminal	Average	FT
Daptonema	32.2	13.2	28.1	7.0	20.13	DF/1B
Ptycholaimellus	9.2	2.8	23.9	44.3	20.06	EF/2A
Terschellingia	1.1	1.7	23.0	30.6	14.10	MV/1A
Promonhystera	0.8	38.2	2.7	0.1	10.46	DF/1B
Paramonohystera	3.0	35.1	2.8	0.7	10.41	DF/1B
Paraethmolaimus	13.2	2.0	0.1	1.8	4.30	EF/2A
Eumorpholaimus	1.4	0.0	4.5	5.3	2.78	DF/1B
Oncholaimus	7.4	1.7	0.2	0.3	2.39	FP/2B
Theristus	3.6	2.5	2.7	0.5	2.32	DF/1B
Viscosia	6.5	1.8	0.0	0.0	2.09	FP/2B
Metoncholaimus	7.0	0.4	0.1	0.0	1.88	FP/2B
Eleutherolaimus	1.2	0.0	0.6	4.1	1.46	MV/1A
Metalinhomoeus	0.0	0.0	2.4	1.4	0.96	MV/1A
Rhynchonema	3.7	0.0	0.0	0.1	0.94	DF/1B
Prochromadora	0.9	0.0	1.5	1.4	0.92	EF/2A
Bathylaimus	0.7	0.0	2.7	0.2	0.89	CF/1B
Spilophorella	0.1	0.0	2.0	0.8	0.75	EF/2A
Onyx	1.7	0.0	0.4	0.1	0.57	FP/2B
Paracanthonchus	1.4	0.0	0.0	0.0	0.34	EF/2A
Synonchium	1.3	0.0	0.0	0.0	0.33	FP/2B
Cyatholaimidae sp.	1.2	0.0	0.0	0.0	0.30	EF/2A
Metachromadora	0.6	0.0	0.5	0.0	0.28	EF/2A
Nygmatonchus	0.5	0.1	0.0	0.1	0.18	EF/2A
Sabatieria	0.0	0.0	0.3	0.4	0.16	DF/1B
Chromadorina	0.4	0.1	0.1	0.0	0.16	EF/2A
Thalassomonhystera	0.0	0.0	0.5	0.0	0.12	DF/1B
Xyalidae sp.	0.0	0.3	0.0	0.2	0.12	DF/1B
Enoploides	0.0	0.1	0.2	0.0	0.09	PR/2B
Polysigma	0.0	0.0	0.0	0.2	0.06	EF/2A
Tripyloides	0.0	0.0	0.2	0.0	0.06	CF/1B
Chromadorita	0.2	0.0	0.0	0.0	0.06	EF/2A
Leptolaimidae sp.	0.2	0.0	0.0	0.0	0.06	MV/1A
Odontophora	0.23	0.0	0.0	0.0	0.05	FP/2B
Chromadorella	0.0	0.0	0.2	0.0	0.05	EF/2A
Chromadora	0.0	0.0	0.1	0.0	0.04	EF/2A
Phanoderma	0.1	0.0	0.0	0.0	0.03	PR/2B
Doliolaimus	0.0	0.0	0.0	0.1	0.03	FP/2B
Hypodontolaimus	0.1	0.0	0.0	0.0	0.03	EF/2A
Oxystomina	0.0	0.0	0.0	0.1	0.03	MV/1A

A total of 39 genera of free-living marine nematodes, belonging to 17 families and five orders, were identified. Only five genera occurred in 50 % or more of all samples (these genera are considered as 'constant'), and only *Daptonema* and *Ptycholaimellus* occurred in more than 80 % of samples (Fig. 2-3a). *Terschellingia*, *Promonhystera* and *Paramonhystera* were the other constant genera. *Daptonema* and *Ptycholaimellus* also had the highest overall abundance (ca 20 % for both), followed by *Terschellingia*, *Promonhystera* and *Paramonhystera* (Table 2-2). These five genera together on average accounted for 75 % of the nematode abundance in the sampling area (Fig. 2-3b). Only six other genera occurred in relative abundances of more than 2 % (Table 2-2).

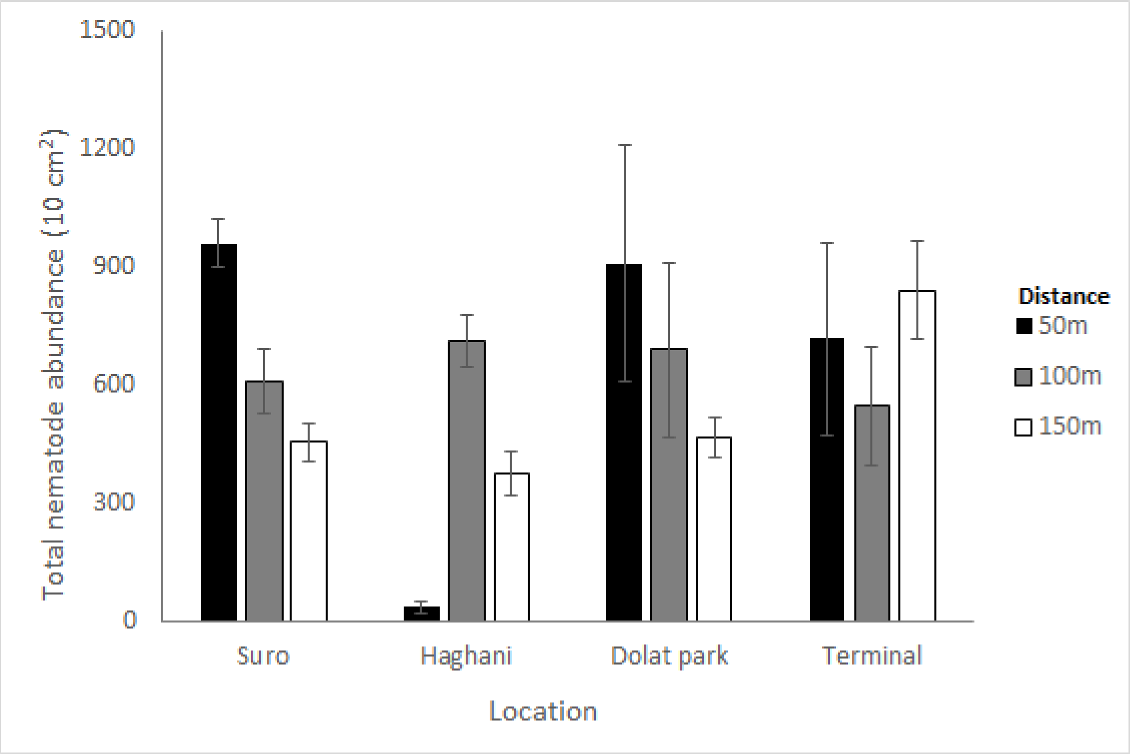
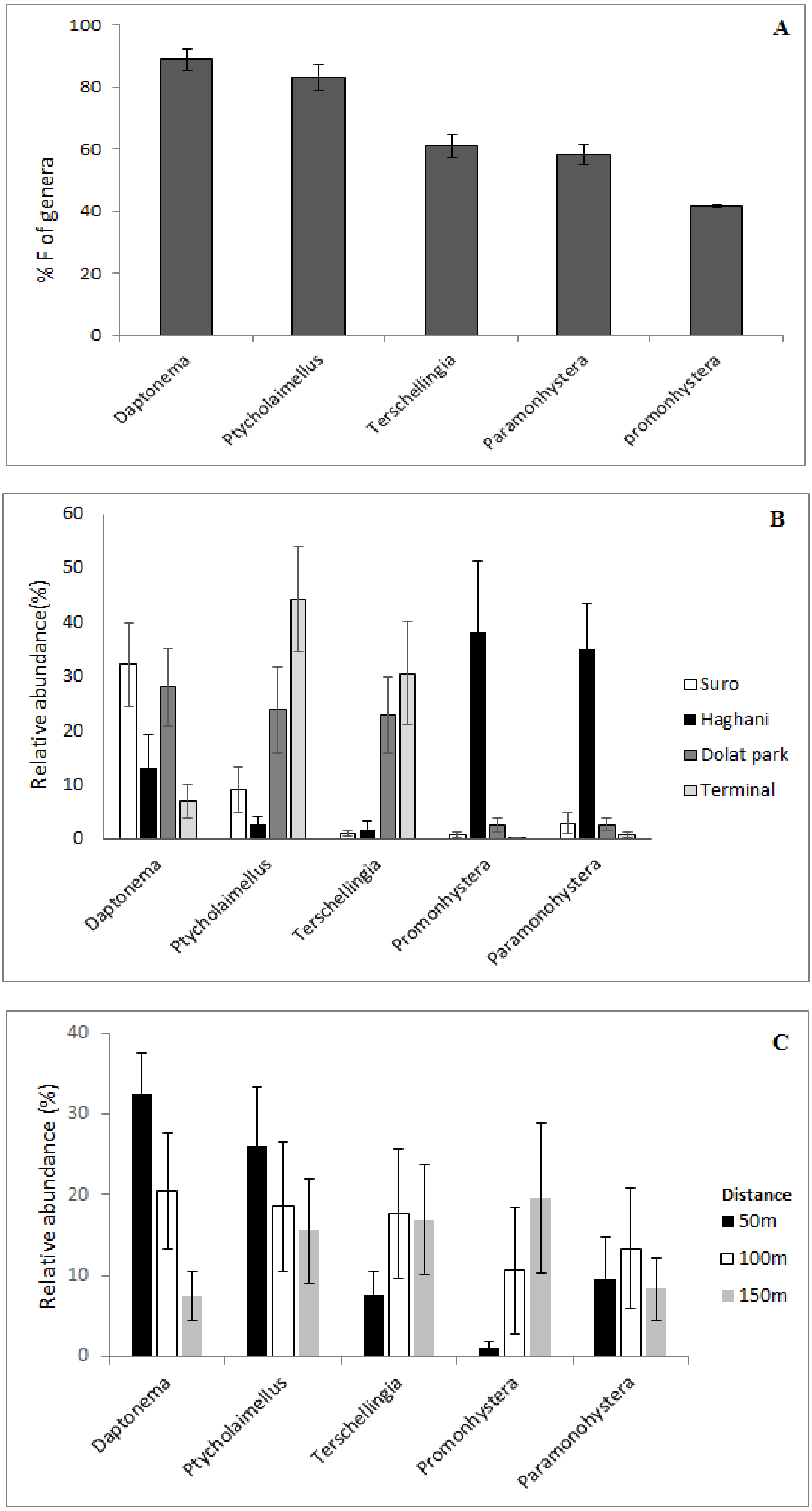


Fig. 2-2. Total nematode abundance per location and distance to pollution. Data are means ±1SE of three replicates for each station (50 m = closest distance to pollution source, 150 m = largest distance).

Fig. 2-3. A. Frequency of occurrence of the five most abundant genera at four intertidal sampling locations near Bandar Abbas; B. relative abundance of the five most abundant genera at the four sampling locations, and C. relative abundance of these same five genera as a function of station distance to local pollution sources (50 m = closest distance to pollution source, 150 m = most distant). Data are means ± 1SE of three stations, each with three replicate samples, per location.

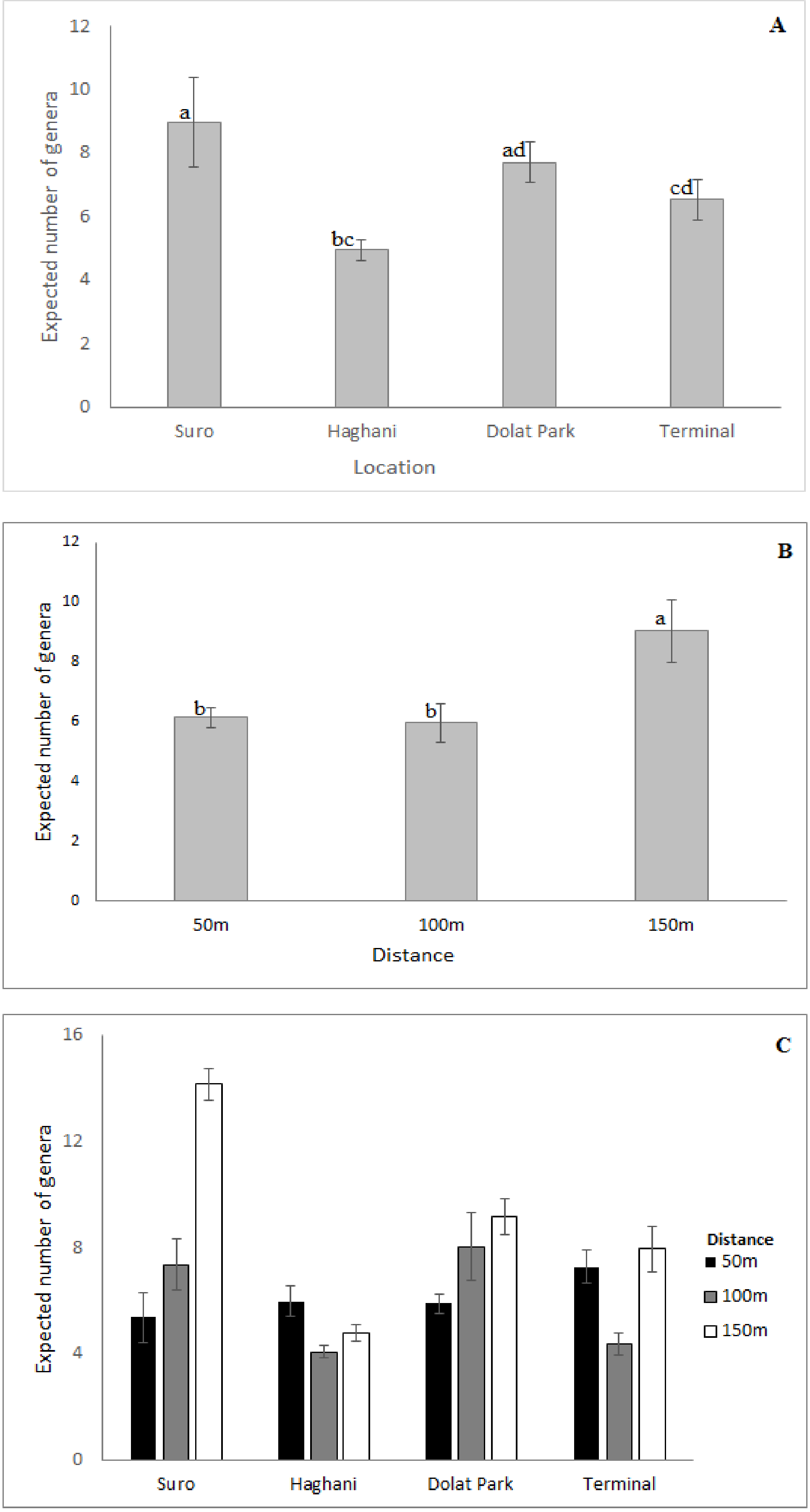

A
% F of genera
100
80
60
40
20
0
Daptonema
Ptycholaimellus
Terschellingia
Paramonhystera
promonhystera
B
Relative abundance(%)
60
50
40
30
20
10
0
Daptonema
Ptycholaimellus
Terschellingia
Promonhystera
Paramonohystera
☐ Suro
■ Haghani
▨ Dolat park
☐ Terminal
C
Relative abundance (%)
40
30
20
10
0
Daptonema
Ptycholaimellus
Terschellingia
Promonhystera
Paramonohystera
Distance
■ 50m
☐ 100m
▨ 150m

3.3 Nematode diversity

The interaction of location by distance to pollution significantly affected genus richness, and this for all three richness indices used: N_0 (i.e. number of genera per 10 cm²) (df = 6, F = 7.94, $p < 0.0001$); EG50 (Fig. 4C, df = 6, F = 0.00001) and Margalef's richness (df = 6, pseudo-F = 7.36, p = 0.001; but Permdisp indicated significantly heterogeneous variation for the factor location, p = 0.01). The number of constant genera per location was more or less a constant proportion of genus richness, with 9, 7, 6 and 4 constant genera at Suro, Dolat Park, Terminal and Haghani, respectively. Averaged across distances to pollution, richness was highest at Suro and lowest at Haghani (Fig. 4A). Averaged across all four beaches, the station most distant from a pollution point source had a significantly higher EG50 than stations closer to that point source (factor station, df = 2, F = 16.34, $p < 0.00005$) (Fig. 2-4B), but this difference was largely driven by Suro and to a lesser extent Dolat Park and was absent from the other beaches (Fig. 2-4C).

The interaction between the factors beach and distance to pollution also significantly affected Shannon-Weaver's diversity (df = 6, F = 3.49, $p < 0.05$) and Pielou's evenness (df = 6, F = 2.71, $p < 0.05$), but not Simpson's index (df = 6, F = 1.39, p = 0.26) (Fig. 2-5). Averaged across distances to pollution, Shannon-Weaver's diversity was significantly higher at Suro than at Haghani ($p < 0.001$) and Terminal (p = 0.01), and at Dolat Park compared to Haghani ($p < 0.05$). However, while the highest Shannon-Weaver diversity at Suro was found furthest away from the local pollution source (distance 50m vs distance 150m, $p < 0.05$), no such differences between stations were found for Haghani, Terminal or Dolat Park. No significant pairwise location-by-distance differences were found for Pielou's evenness, despite the significant overall interaction effect. The Simpson index differed significantly between locations (df = 3, F = 4.05, $p < 0.02$), Haghani having a significantly lower Simpson diversity than Suro ($p < 0.03$). Evenness did not significantly differ between locations and distances to pollution, nor did dominance differ between distances.

Fig. 2-4. A. Genus diversity (expressed as expected number of genera in a sample of 50 inds.) for the four sampling locations. Data shown are means ±1 SE of three distances to pollution, each with three replicate samples, per location. Different letters indicate statistically significant differences at $p < 0.05$. B. Genus diversity per 10 cm² as a function of distance to pollution sources (50 m = closest to pollution source, 150 m = most distant). Data shown are means of four locations each with three replicates per distance. C. Genus diversity per location and distance. Data are means ±1SE of three replicates for each distance.


A
Expected number of genera
12
10
8
6
4
2
0
a
bc
ad
cd
Suro
Haghani
Dolat Park
Terminal
Location
B
Expected number of genera
12
10
8
6
4
2
0
b
b
a
50m
100m
150m
Distance
C
Expected number of genera
16
12
8
4
0
Suro
Haghani
Dolat Park
Terminal
Distance
■ 50m
▨ 100m
☐ 150m

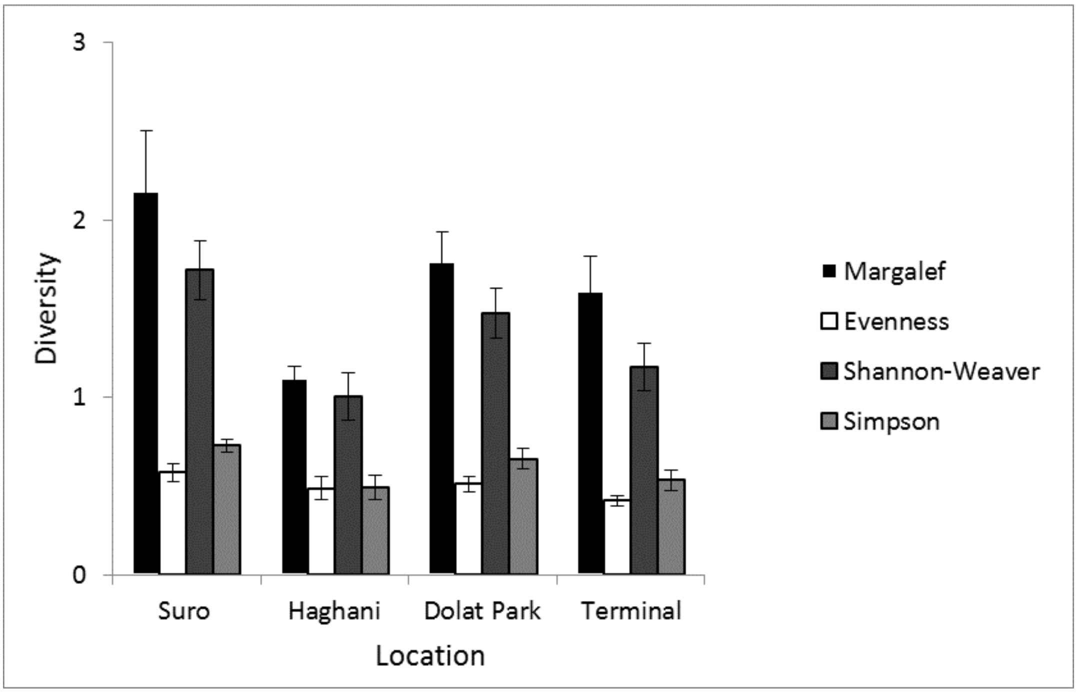


Fig. 2-5. Nematode diversity expressed as Margalef's index (richness), Pielou's evenness, Shannon-Weaver's index (H') and Simpson's dominance index, at four sampling locations. Data shown are means ± 1SE of three distances to pollution, each with three replicate samples, per location.

Taxonomic distinctness was significantly affected by the interaction between locations and distances to pollution (df = 6, pseudo-F = 4.42, p = 0.002; Permdisp: $p > 0.05$ for both factors) as well as by the separate effect of location (df = 3, pseudo-F = 17.87, p = 0.001). Suro had significantly higher taxonomic distinctness than Terminal (Fig. 2-6), except at the smallest distance to pollution. Suro also had a higher taxonomic distinctness than Haghani, except at the intermediate distance. Dolat Park had a higher taxonomic distinctness than Terminal and Suro, but only at the intermediate distance to pollution (100 m). Taxonomic diversity only differed significantly between locations (df = 3, F = 7.67, $p < 0.001$). Much like for taxonomic distinctness, Suro had significantly higher taxonomic diversity than Haghani ($p < 0.001$) and Terminal ($p < 0.002$) (Fig. 2-6).

Fig. 2-6. Nematode taxonomic distinctness and taxonomic diversity at four sampling locations. Data are means ± 1SE of three distances to pollution, each with three replicate samples, per location.

3.4 Nematode assemblage structure

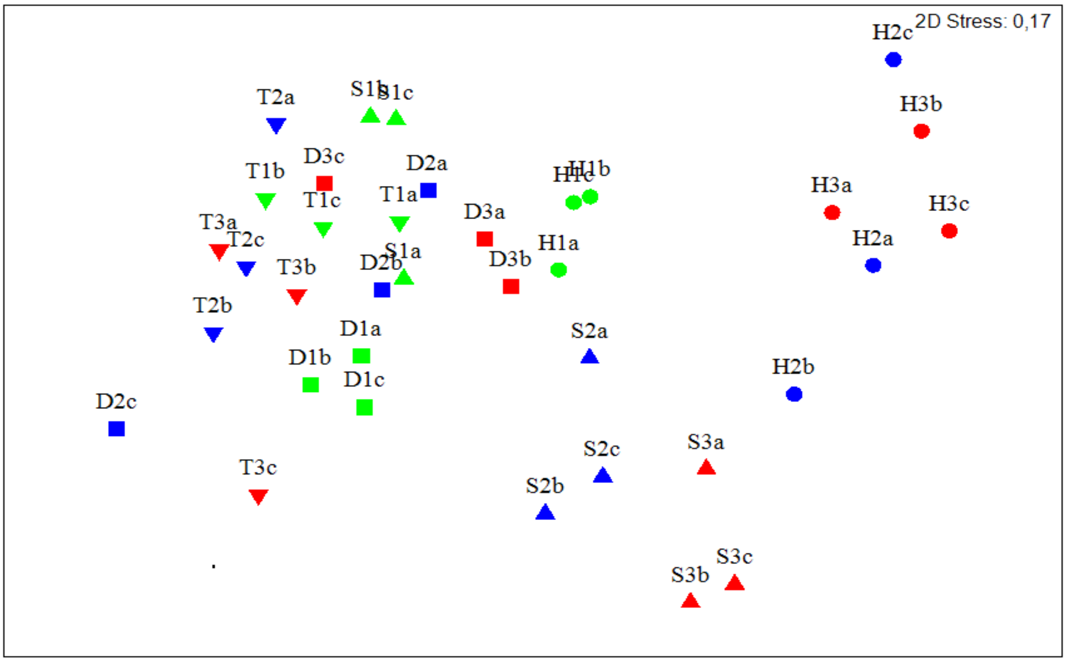


Fig. 2-7. Non-metric multidimensional scaling (nMDS) of nematode assemblages based on square-root transformed genus abundance data. Sample codes are as follows: first (capital) letter indicates location (S=Suro, H=Haghani, D=Dolat Park, T=Terminal), numbers indicate different stations (1 = closest to pollution source (50 m), 3 = most distant (150 m)), letters (A, B, C) indicate different replicates. Locations are indicated by different shapes (▲= Suro,●= Haghani, ■ = Dolat Park, ▼ = Terminal), and distances to pollution by different colours

(green= closest to pollution source (50 m), blue= intermediate distance(100m) and red= most distant (150 m)) .

The nMDS analysis separated stations at the intermediate and largest distance to pollution at Haghani, as well as at the intermediate and largest distance to pollution at Suro, into two distinct groups (Fig. 2-7). All other samples formed a loosely defined cluster where some further structuring was visible (e.g. station nearest pollution of Haghani, station nearest pollution of Suro) but not pronounced. The results of this nMDS are reflected in a highly significant location x distance-to-pollution effect on the nematode assemblage composition (df = 6, pseudo-F = 3.72, $p = 0.001$). This effect demonstrates that differences in nematode assemblage composition did not merely follow location or distance boundaries, but ran across locations and distances to pollution. Hence, significant differences existed within locations: at Haghani, Terminal and Dolat Park, significant differences were found between the smallest and largest distance to the local pollution source (all $p \leq 0.05$), while at Suro, all distances to pollution differed significantly from each other (all $p < 0.05$). Vice versa, with a single exception (150 m at Dolat Park and Terminal, $p = 0.072$), all pairwise comparisons of nematode assemblage composition between locations for a given distance to pollution yielded significant differences (all $p < 0.05$). Nevertheless, the location x distance effect on assemblage composition should be interpreted with caution, because Permdisp indicated significantly heterogeneous variances for the factor distance to pollution ($p = 0.002$).

SIMPER analysis comparing locations and distances to pollution indicated that the strongest dissimilarity between locations (81 %) was between Haghani and Terminal. This was largely due to much higher relative abundances of *Ptycholaimellus* and *Terschellingia* at Terminal and of *Pro-* and *Paramonhystera* at Haghani (Fig. 2-3 and Table 2-3). The lowest dissimilarity between locations (55 %) was found between Dolat Park and Terminal. *Ptycholaimellus* and *Terschellingia* contributed to this dissimilarity with higher relative abundances in Terminal, whereas *Daptonema* was more abundant in Dolat Park (Fig. 2-3 and Table 2-3). In addition, some low-abundant genera like *Eumorpholaimus* and *Metalinhomoeus* also contributed to the dissimilarity between Dolat Park and Terminal (Table 2-3). Differences between different distances from a pollution point source indicated the strongest dissimilarity (73 %) between the smallest (50 m) and largest (150 m) distance. *Daptonema* and *Ptycholaimellus* were on average more abundant nearest the pollution source, whereas *Promonhystera* and to a lesser extent *Terschellingia* reached their highest abundances furthest away from local pollution sources (Fig. 2-3 and Table 2-4). However, these distance-to-pollution trends were not consistent at all locations. For instance, *Daptonema* and *Ptycholaimellus* did not exhibit higher relative abundances near pollution point sources at the least disturbed beach, i.e. Dolat Park, and *Promonhystera* had a considerably higher abundance at the most distant station only at Haghani, not at the other beaches.

Table 2-3. Results of pairwise SIMPER (Similarity Percentages) analysis showing percentage dissimilarity between nematode assemblages of the four beach locations, as well as the genera contributing most to the observed dissimilarity. Average abundances have been square-root transformed.

Genera	**Av.**	**abundance**	**Contrib%**	**Cum.%**
Suro & Haghani	Average	dissimilarity	=63.36	
Paramonhystera	1.11	5.40	15.37	15.37
Promonhystera	0.51	4.80	13.90	29.27
Daptonema	5.23	2.63	10.91	40.17
Viscosia	2.00	0.83	5.63	45.81
Paraethmolaimus	2.08	0.89	5.56	51.37
Suro & Dolat Park	Average	dissimilarity	=65.01	
Terschellingia	0.59	4.34	11.14	11.14
Ptycholaimellus	2.44	4.42	8.41	19.56
Daptonema	5.23	4.81	8.36	27.92
Paraethmolaimus	2.08	0.13	7.75	35.67
Viscosia	2.00	0.00	5.35	41.02
Odontophora	2.10	0.15	5.15	46.17
Oncholaimus	2.10	0.15	5.15	51.32
Suro & Terminal	Average	dissimilarity	=72.30	
Ptycholaimellus	2.44	6.24	11.52	11.52
Terschellingia	0.59	4.83	11.29	22.82
Daptonema	5.23	2.23	9.38	32.19

Paraethmolaimus	2.08	1.05	8.49	40.68
Viscosia	2.00	0.00	5.34	46.02
Odontophora	2.10	0.24	5.26	51.28
Haghani & Dolat Park	Average	dissimilarity	=71.83	
Paramonhystera	5.40	1.17	14.00	14.00
Promonhystera	4.80	1.14	13.18	27.18
Terschellingia	0.44	4.34	12.96	40.14
Ptycholaimellus	1.06	4.42	10.88	51.02
Haghani & Terrminal	Average	dissimilarity	=81.37	
Ptycholaimellus	1.06	6.24	15.89	15.89
Paramonhystera	5.40	0.40	14.91	30.80
Promonhystera	4.80	0.11	14.73	45.53
Terschellingia	0.44	4.83	14.59	60.12
Dolat Park & Terminal	Average	dissimilarity	=54.64	
Ptycholaimellus	4.42	6.24	14.45	14.45
Terschellingia	4.34	4.83	11.58	26.02
Daptonema	4.81	2.23	10.87	36.90
Eumorpholaimus	1.32	1.64	8.18	45.08
Metalinhomoeus	0.86	0.54	5.41	50.48

Table 2-4. Results of pairwise SIMPER (Similarity Percentages) analysis showing percentage dissimilarity between nematode assemblages of the three distance to local pollution sources (50 m = closest distance to pollution source, 150 m = most distant), as well as the genera contributing most to the observed dissimilarity. Data of all four beach locations have been grouped per distance.

Genera	**Av.Abund**	**Av.Abund**	**Contrib%**	**Cum.%**
50m & 100m	Average	dissimilarity	=61.58	
Daptonema	5.46	3.63	12.63	12.63
Ptycholaimellus	4.63	2.99	10.91	23.53
Terschellingia	2.19	2.72	8.98	32.51
Paraethmolaimus	2.19	0.38	8.70	41.21
Promonhystera	0.39	1.65	6.90	48.11
Paramonhysrera	1.66	2.17	6.57	54.68
50m & 150m	Average	dissimilarity	=72.64	
Daptonema	5.46	2.09	11.15	11.15
Ptycholaimellus	4.63	3.01	9.55	20.70
Promonhystera	0.39	2.88	9.13	29.83
Paraethmolaimus	2.19	0.54	7.50	37.33
Terschellingia	2.19	2.74	7.38	44.70
Paramonhysrera	1.66	2.23	5.91	50.61
100m & 150m	Average	dissimilarity	=57.08	
Daptonema	3.63	2.09	8.95	8.95
Promonhystera	1.65	2.88	8.33	17.28
Paramonhysrera	2.17	2.23	8.15	25.43
Ptycholaimellus	2.99	3.01	8.06	33.49
Terschellingia	2.72	2.74	6.73	40.22
Theristus	1.35	0.80	5.35	45.57
Metoncholaimus	0.23	1.31	4.61	50.17

4 Discussion

Free-living nematodes may play an important role in the functioning of marine benthic ecosystems, among other things because of their high abundance and generally fast turnover, their role as a food source for higher trophic levels, and their interactions with microbiota through which they can affect decomposition processes (Snelgrove et al. 1997; Coull 1999; De Mesel et al. 2006; Gray and Elliott 2009), and constitute a major component of the meiobenthos in soft bottoms (Heip et al. 1985; Higgins and Thiel 1988; Moens et al. 2013). Despite their importance, we are unaware of published data on marine nematodes of the Persian Gulf. The present study is therefore the first on nematofauna of this area, and a comparison of both diversity, abundance and assemblage composition with beaches elsewhere in the world is thus important. Moreover, effects of local (point) sources of pollution in a generally stressed environment (high salinity, large temperature fluctuations, anthropogenic pollution) on nematode assemblage structure and diversity have hitherto received little attention, rendering the present study relevant beyond the focal study area.

4.1. The study area

Intertidal areas in the Persian Gulf are particularly harsh environments given the large daily variations in temperature and interstitial salinity which occur in the area. They are also subject to many of the above-mentioned anthropogenic impacts. We could therefore expect an overall low diversity of beach interstitial fauna, including nematodes, in our study area. This was indeed the case, with low values of local and regional beach nematode richness and assemblages characterized by a pronounced dominance of a few (very) abundant genera (see below). Despite the fact that the harsh environmental conditions and considerable region-wide anthropogenic disturbance may largely account for the observed low diversity, the spatial patterns demonstrated in this study reveal substantial local effects, which at least in part appear linked to very localized pollution sources, mostly in the form of sewage outflows which discard on, or immediately in front of, the beach, supporting the claim of Gawad et al. (2008) about the significant threat of such localized pollution sources to the sustainability of Persian Gulf coastal ecosystems. While Haghani was visibly the most sewage-impacted location, which was reflected in both abiotic (e.g., % organic carbon) and biotic (lowest nematode diversity overall and lowest nematode abundance at the station nearest the sewage drainage) variables, our organic matter and heavy metal concentration data did not support Suro but rather Terminal as the second most impacted location (cf. highest % organic carbon, low diversity, no effect of distance to local pollution on nematode diversity and abundance at Terminal in contrast to Suro). Terminal is the most sheltered location and accumulates more inputs, as evidenced by its significantly smaller grain size and higher silt and organic matter content compared to all other locations.

4.2. Nematode diversity and abundance

In this study 39 genera belonging to 17 families were found in a single inventory of four beaches. All these genera have been reported from elsewhere in the world, so endemism, if at all present, will be restricted to the species level. Although we have not identified to species level, we have no indications that endemism would be prominent.

4.2.1. Comparing diversity patterns of beach nematofauna across studies

When looking within the same latitudinal zone, beach studies with low(er) local nematode genus richness tend to be impacted by anthropogenic disturbance. Anthropogenic disturbance and pollution may thus be important causes of low nematode diversity on sandy beaches. Our nematode data appear to support the conclusion of local studies in the Persian Gulf performed on macrobenthos: a generally low biodiversity and abundance, probably as a consequence of both natural stressors and anthropogenic impacts (Ejlali Khanaghah et al. 2010; Naderloo and Tuerkay 2012; Safahieh et al. 2012; Pourjomeh et al. 2014). A comparison of our data with those of other beach studies worldwide does not, however, support a clear latitudinal diversity pattern, in line with the general conclusion of Mokievsky and Azovsky (2002) for marine nematode assemblages.

Caution is, however, due when comparing diversity estimates from different studies because of differences in sampling strategies (number of replicates, position on the beach, sediment depth stratum etc…), sample sizes and numbers of stations and beaches between studies. We should ideally be able to compare average diversity per sample in each station at each beach as a measure of local or sample diversity for each of the four beaches in our study. We should then also look at aspects of richness, evenness and dominance. However, several of the studies with which we compare do not allow calculation of such an average sample diversity and/or of evenness and dominance components. So here, we have opted to consider beach locations (i.e. individual beaches) as the scale on which to determine local diversity, and restrict this part of our discussion to taxon richness, mostly expressed as the number of genera (N_0). It is important to note that sampling effort differs tremendously between studies and therefore greatly impacts comparisons. The cumulative diversity over different beaches can be referred to as gamma diversity. Comparison of gamma diversity, then, is mostly affected by the number of beaches studied and perhaps by the distance between them, but also by habitat diversity when different beach types are being considered (Barnes et al. 2011; Lee and Riveros 2012; Maria et al. 2016).

Local as well as gamma diversity in our study were within the wide range of values reported for other beaches worldwide, but nevertheless in the lower part of that range. Several factors such as climate, sediment granulometry, beach morphodynamics and pollution are all potential drivers of variations in abundance, diversity and taxonomic composition of beach nematode assemblages, but there is no comprehensive understanding of what driver(s) have a predominant impact.

4.2.2. Effects of climate and latitude on beach nematode diversity

The overall low genus richness in our study area may in part relate to the climatic conditions and relatively high salinity of the Persian Gulf and to broad-scale anthropogenic impacts (see above; Kämpf and Sadrinasab, 2006; Reynolds 1993). Nevertheless, published literature on the nematofauna of beaches from very different geographical areas does not yield clear patterns of diversity with climatic region/latitude, with the notable exception of beaches along the Chilean and Australian coastlines, where diversity decreased with increasing latitude (Nicholas and Trueman 2005; Lee and Riveros 2012). Within a similar latitude, for instance, considerably higher local diversity than in our study was obtained in the Gulf of California, with up to 96 genera per beach (Mundo-Ocampo et al. 2007; Gingold et al. 2010), and at Guanabara Bay, Rio De Janeiro, with 62 genera (Maria et al. 2008b). However, sampling effort considerably exceeded that in our study, since both aforementioned studies sampled transects across the entire high-to-low water gradient. In addition, Gingold et al. (2010) specifically incorporated ridges and runnels (the latter holding a higher diversity), whereas a majority of other studies, including ours, have focused solely on ridges. Ridges and runnels harbor partly different nematode assemblages and the horizontal zonation of nematodes across the beach intertidal differs between ridges and runnels (Gingold et al., 2010; Maria et al., 2013b). The beaches we studied do not exhibit a clear ridge-and-runnel morphology and we did not incorporate horizontal zonation. A better comparison between these studies and ours is therefore possible when looking at sample diversity rather than beach diversity. Average sample diversity in the studies of Maria et al. (2008b) and Gingold et al. (2010) ranged from 9 to 43 genera in the former and from 6 to 27 in the latter, compared to 5 to 16 in our study. The lowest genus diversity in the study by Gingold et al. (2010) was found at the upper beach and highest diversity in the lower and middle parts of the beach. Our samples were taken from the mid intertidal, hence the average genus richness per sample in our study can be considered comparatively low. Local (28-29 genera) and gamma diversity (40 genera) at two Ecuadorian beaches were more similar to those in our study (Calles Procel et al. 2005). These beaches were more influenced by human activity, i.e. high recreational use on one, and fishing activities on the other. While impacts of tourism per se can be significant, they are mostly restricted to the upper beach (Gheskiere et al. 2005). Only 21 genera were found at a beach along the central west coast of India, encompassing five stations along a gradient of sewage pollution (Nanajkar and Ingole 2010). It therefore appears that within the same latitudinal zone, local nematode genus richness is affected by anthropogenic disturbance.

4.2.3. Effects of pollution on beach nematode diversity

The degree and type of environmental pollution are well known to affect nematode assemblages (Heip et al. 1985; Somerfield et al. 2003). Sewage discharges influence nematode assemblage structure through changes in several environmental parameters, especially organic matter enrichment and associated pollutants (Pinto and Bemvenuti 2006). Total organic carbon concentration showed a clear distance effect in Terminal and Haghani, but much less so in the other two beaches. The low genus richness at Haghani, combined with the extremely low nematode abundance at the station nearest the sewage canal at this beach, support a prominent local pollution impact. Haghani was situated next to the largest sewage drainage canal of the city, and we assume that the influence of the sewage inputs on nematode diversity extended over all stations on this beach, thus resulting in an overall lower genus richness. In addition, there was also a significantly higher richness in the beach station furthest from the local pollution source at Suro. The latter is concordant with Pinto and Bemvenuti (2006), who also reported the highest diversity away from the sewage discharge point. At the other beaches, a clear gradient of nematode richness with distance was not detected. Dolat Park does not receive sewage inputs and pollution is mainly restricted to limited amounts of local deposition of garbage from recreational beach use, the influence of which is unlikely to extend much into the surrounding sediments. Terminal, however, had the finest sediments and highest % TOC, suggesting prominent deposition of organic detritus, but this probably results more from its sheltered position rather than from local sewage inputs, and was not clearly reflected in the nematode assemblage.

4.2.4. Influence of granulometry and beach morphodynamics on nematode diversity

Sediment granulometry is well known as one of the most, if not the most important factor in determining meiobenthic assemblage abundance, structure and diversity (Heip et al. 1985; Coull 1988; Vanaverbeke et al. 2000). Marine nematode diversity often tends to increase with an increasing sand fraction (Tietjen 1984; Moens et al. 2013). In our study, however, the differences in granulometry between beaches were limited and there was no clear relationship between nematode abundance and diversity on the one hand and sediment granulometry on the other. The location with the highest nematode diversity and abundance (Suro) had the second lowest median grain size of all beaches investigated, while the location with the lowest nematode densities and diversity (Haghani) had slightly coarser sediment.

Sediment granulometry relates to beach hydro- and morphodynamics. In our study, Haghani had a moderately reflective profile and slightly coarser sediments (except when compared to Dolat Park), whilst the other beaches were more sheltered and characterized by very gentle slopes. Nematode abundances did not consistently differ between beaches, but rather between stations across beaches. Nematode genus richness did differ between locations. The low genus richness at Haghani, combined with an extremely low abundance at the station nearest the pollution source, suggest that local pollution sources rather than morphodynamics, physical disturbance or related factors contribute to this pattern, which contrasts with expectations based on literature: higher meiofauna density and/or diversity at more reflective beaches compared to on dissipative beaches (McLachlan et al. 1977; McLachlan and Jaramillo 1996; Rodriguez et al. 2003). Reflective beaches typically have coarser sediments with a high permeability, low organic matter concentrations, and deep penetration of oxygen and nutrients, a combination of factors which may benefit a higher diversity (Moens et al. 2013). The somewhat higher permeability of sediments at Haghani may in part explain why this most polluted location did not accumulate higher concentrations of most heavy metals than the other three locations, and why the visibly strong sewage inputs yielded organic carbon concentrations which did not exceed those at Terminal.

4.2.5. Different diversity indices yield similar patterns

The genus richness trends were generally well reflected in other diversity measures. Most indices showed similar trends, confirming the conclusions of Simboura et al. (1995). On the other hand, our data do not confirm the contention that taxonomic distinctness or diversity outperform richness and evenness in detecting impacts of disturbance (Warwick and Clarke 1995, 1998, 2001; Salas et al. 2006), perhaps even the contrary: richness indices revealed more significant differences among locations and distances to pollution than taxonomic distinctness or diversity, in line with findings of Nicholas and Trueman (2005) for Australian beach nematofauna. Taxonomic distinctness at the most polluted location, Haghani, was only surpassed by that at Suro and exceeded that at Terminal. Taxonomic diversity only revealed significant differences between locations but not distances to pollution.

4.3 Nematode assemblage composition

In our whole study area, five genera (*Daptonema*, *Ptycholaimellus*, *Terschellingia*, *Promonhystera* and *Paramonhystera*) together comprised 75 % of the overall nematode abundances. Hence, all significant differences in nematode assemblage structure could be largely attributed to differences in the relative abundances of these dominant genera (Table 2-2). *Daptonema*, *Paramonhystera* and *Promonhystera* are (non-selective) deposit feeders (Moens and Vincx, 1997), a feeding type which in intertidal habitats probably derives most of its nutritional requirements from diatoms and other microalgae and from bacteria. *Daptonema* is widely distributed in organically enriched marine sediments and has a high tolerance to a variety of pollution types (Vanreusel and Vincx 1989; Boyd et al. 2000a; Schratzberger et al. 2006). Nanajkar and Ingole (2010) reported it as dominant in anoxic, degraded and polluted habitats, even though dedicated lab experiments have shown its tolerance to hypoxia/anoxia to be much weaker than expected (Steyaert et al. 2007). Such contradictory outcomes may in part relate to species-specific differences within a genus, but may also reflect behavioural strategies such as vertical migrations in sediments which limit the duration of exposure to the actual anoxia under field conditions and/or serve to avoid predation (Steyaert et al., 2001; (Maria et al. 2012). While *Daptonema* and *Ptycholaimellus* were abundant throughout our study locations and in most cases had their highest abundances nearest sewage inputs, *Promonhystera* and *Paramonhystera* had their highest relative abundances at the most polluted beach (Haghani), but *Promonhystera* had lower abundance in the stations nearest sewage inputs, rendering interpretation of distribution patterns far from straightforward. The high abundances of *Daptonema*, *Promonhystera*, *Paramonhystera* and, to a lesser extent, *Theristus*, at our sampling locations do corroborate previous reports that beaches composed of fine to medium sands tend to show a high abundance of Xyalidae (Gheskiere et al. 2004; Calles Procel et al. 2005; Hourston et al. 2005; Moreno et al. 2006; Moens et al. 2013).

Beaches with medium to very coarse sands, in contrast, are often more dominated by Chromadoridae (Sharma and Webster 1983; Urban-Malinga et al. 2004; de Jesus-Navarrete 2007; Maria et al. 2013a).

The most abundant chromadorid nematode on Persian Gulf beaches was *Ptycholaimellus*. *Ptycholaimellus* is an epigrowth feeder (2A) which uses a tooth to pierce or crack diatoms, other microalgae and filamentous cyanobacteria (Moens and Vincx, 1997). Members of this genus are often particularly prominent in the surface layer (upper 1cm) of intertidal muds and sands covered with microphytobenthic biofilms (Commito and Tita 2002; Steyaert et al. 2003; Van Colen et al. 2009). They appear to respond rapidly to microphytobenthos blooms (Van Colen et al. 2009), and we therefore tentatively attribute their higher relative abundance in the stations nearest sewage inputs to a stimulatory effect of the concomitant nutrient inputs on microphytobenthos. The same may hold for *Daptonema*.

Terschellingia is a microvore with a tiny buccal cavity, suggesting it feeds mainly or exclusively on bacteria-sized particles (Moens and Vincx, 1997). Recent stable isotope evidence demonstrates that *T. longicaudata* obtains most of its nutrition from chemoautotrophic bacteria which utilize methane as a carbon source (Moens et al. 2011; Vafeiadou et al. 2014). This genus, and particularly the species present in our samples (*T. longicaudata*), has been reported worldwide from often organically enriched or otherwise polluted sediments (Heip et al. 1985; Somerfield et al. 2003; Pinto and Bemvenuti 2006; Schratzberger et al. 2006; Moreno et al. 2008a). It has a remarkable tolerance to hypoxic and anoxic conditions (Hendelberg and Jensen 1993; Modig and Olafsson 1998; Steyaert et al. 2007), but in our study, *Terschellingia* had its highest abundance at the least organically polluted beach, Dolat Park, and at Terminal. Terminal, and to a lesser extent Dolat Park, are the two least exposed beaches and have higher silt content than the other two locations. In the absence of data on redox profiles and other relevant sediment characteristics, we can only speculate that its preference for physically little disturbed, fine sediments is a more prominent driver of its occurrence than organic matter availability.

Because the same few nematode genera dominated all beaches, but exhibited different spatial patterns between beaches as well as between stations within a beach, it is not surprising that the nematode assemblage structure differed significantly as a function of the interaction between beaches and distance to local pollution sources.

5 Conclusions

Nematode assemblages at four beaches in the Persian Gulf near Bandar Abbas showed abundances and a genus composition that are fairly typical of fine-grained beaches elsewhere in the world. Genus richness was overall low, probably in relation to the harsh environmental conditions and broad-scale pollution effects in the Persian Gulf, but also appeared substantially affected by local anthropogenic inputs rather than by beach morphodynamics and sediment composition. Hence, local anthropogenic impacts have pronounced effects on assemblages, even in habitats which naturally experience strong environmental fluctuations and broad-scale anthropogenic disturbance.

Supplementary Table S2-1. Heavy metal concentrations (in mg/kg) at the four beaches, with three distance to local pollution sources (50 m = closest distance to pollution source, 150 m = most distant). Data are means ±1SE of three replicates per distance x beach combination.

	Distance	Ba	Cr	Cu	Sr	Zn	Y	S
Suro	50m	85.1±6.3	646.7±82.1	25.5±8.6	1815.4±59.8	31.9±5.7	12.7±1.8	768.4±41.5
	100m	113.7±5.8	559.1±124.1	17.6±1.9	1695.1±54.6	23.9±1.2	10.0±0.1	783.9±70.1
	150m	128.1±15.9	703.0±139.5	18.9±1.6	1655.7±22.8	27.7±0.3	10.2±0.3	708.1±20.3
Haghani	50m	121.2±10.7	755.7±167.9	17.9±1.7	1406.9±164.1	27.2±2.0	10.8±0.5	785.2±27.6
	100m	109.0±15.8	779.1±43.7	20.8±1.0	1636.2±173.3	27.7±1.7	10.7±0.3	760.1±14.6
	150m	103.8±2.6	1295.2±102.7	28.4±1.0	1579.1±50.4	30.1±1.4	12.2±0.2	723.6±9.6
Dolat Park	50m	144.2±4.6	413.9±28.7	21±1.9	731.9±37.8	24.4±0.4	11.6±0.6	962.2±57.3
	100m	143.9±1.3	492.1±18.1	19.5±2.6	706.9±13.7	24.3±0.7	11.1±0.08	907.7±7.7
	150m	142.0±6.6	413.0±20.1	18.8±1.1	810.6±72.0	34.5±1.6	13.4±0.6	910.6±19.2
Terminal	50m	179.8±9.0	533.3±86.1	15.4±1.2	639.6±47.7	30.0±2.3	10,7±0.1	758.3±78.4
	100m	150.4±3.6	660.2±105.2	19.9±1	1141.5±83.1	27.9±1.8	12.1±0.5	990.3±139.7
	150m	168.5±2.8	758.7±75.1	22.3±0.8	1077.3±19.4	51.4±6.2	12.7±0.3	1016.8±122.3

Supplementary Table S2-2. PERMANOVA results for different heavy metals at different locations and distances to pollution. Results are showing differences of each heavy metal with location, distance to pollution and their interaction. Significant pseudo-P-values obtained by Monte Carlo permutation are indicated in the last column.

	Source	df	SS	MS	Pseudo-F	Pseudo-P
Ba	Location	3	23.49	7.83	30.86	0.001
	Station	2	0.28	0.14	0.55	0.59
	Lo x Sta	6	5.13	0.85	3.37	0.017
	Total	35	35			
Cr	Location	3	16.35	5.44	14.35	0.002
	Station	2	4.05	2.02	5.34	0.014
	Lo x Sta	6	5.482	0.91	2.40	0.061
	Total	35	35			
Cu	Location	3	1.75	0.58	0.67	0.608
	Station	2	1.60	0.80	0.92	0.428
	Lo x Sta	6	10.85	1.80	2.08	0.064
	Total	35	35			
Sr	Location	3	29.47	9.82	93.34	0.001
	Station	2	0.79	0.39	3.77	0.032
	Lo x Sta	6	2.20	0.36	3.49	0.016
	Total	35	35			
Zn	Location	3	7.15	2.38	6.84	0.005
	Station	2	9.45	4.72	13.56	0.001
	Lo x Sta	6	10.02	1.67	4.79	0.005
	Total	35	35			
Y	Location	3	3.45	1.15	1.79	0.18
	Station	2	3.88	1.94	3.02	0.06
	Lo x Sta	6	12.25	2.04	3.17	0.011
	Total	35	35			
	Location	3	12.64	4.21	6.56	0.001

S	Station	2	0.51	0.25	0.40	0.66
	Lo x Sta	6	6.44	1.07	1.67	0.16
	Total	35	35			

Supplementary Table S2-3. Sediment granulometry (measured on samples from December 2008) of three distance to local pollution sources (50 m = closest distance to pollution source, 150 m = most distant) of each of four beaches studied. Data on granulometry are with one sample per distance to a local pollution source.

Location	Distance	Median grain size(µm)	Mean grain size(µm)	%Silt	%Very fine sand	%Fine sand	%Medium sand	%Coarse sand	%Very coarse sand
Suro	50m	172.53	242.07	0.63	26.14	45.78	18.03	5.63	1.75
	100m	149.32	160.65	0.16	32.26	58.59	8.98	0	0
	150m	143.27	153.96	0.54	35.87	56.41	7.16	0	0
Haghani	50m	204.53	232.04	1.98	14.09	49.09	30.86	3.95	0
	100m	180.85	201.02	0	20.05	55.88	23.13	0.92	0
	150m	140.73	151.52	1.05	37.39	54.62	6.91	0	0
Dolat park	50m	193.90	205.49	0	11.51	63.66	24.81	0	0
	100m	195.53	212.48	4.82	17.29	45.77	31.03	1.07	0
	150m	142.37	176.36	13.02	30.08	35.26	19	2.62	0
Terminal	50m	134.11	170.47	13.39	32.53	36.33	14.81	2.17	0.26
	100m	121.09	176.80	16.40	35.32	30.51	12.60	3.18	0.95
	150m	101.81	177.61	23.55	36.60	21.67	10.40	4.87	1.30

Supplementary Fig. S2-1. Our sampling locations in an aerial photograph taken from Google Earth (http://earth.google.com). S=Suro; H= Haghani; D= Dolat Park; T= Terminal


مسجد غدیر
مسیر دوچرخه سواری و عابر پیاده
Dolat Park
Google


Bus Terminal
Velayat Park
Terminal

Chapter 3

Year-to-year variability in beach nematode assemblage structure and biodiversity along the northern coast of the Strait of Hormuz, Persian Gulf

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This chapter is adapted from:

Sahraean et al. “Year-to-year variability in beach nematode assemblage structure and biodiversity in the northern part of the Strait of Hormuz”. In preparation.

Abstract

Intertidal areas in the Persian Gulf, particularly in the Strait of Hormuz, experience a combination of natural and anthropogenic stressors, resulting in overall moderate to low diversity of meio- and macrobenthic assemblages. Despite the overarching salinity, climate and hydrocarbon-pollution effects on benthic biodiversity, impacts of local, small-scale anthropogenic pollution sources are significant. We recently demonstrated that local pollution sources, mainly sewage discharge points, determine spatial differences in nematode assemblages between and within beaches along the coast near Bandar Abbas, the capital of the Hormuzgan province. Here we assess whether the spatial patterns obtained in that study were consistent over time. We repeated the sampling design of the previous study, i.e. four beaches, with three stations each along a distance gradient of 50, 100 and 150 m from a pollution point source, exactly one year later. We found strong changes in spatial patterns of nematode assemblages, where many of the between-location and between-station differences observed in 2008 disappeared. Haghani remained the least diverse beach, consistent with the presence of the largest urban drainage of Bandar Abbas. Suro and Dolat Park exhibited decreased abundance and diversity, whereas Terminal showed the opposite pattern. Distance-to-pollution gradients found in 2008 were largely absent in 2009. We hypothesize that the nearly threefold higher precipitation in the weeks preceding the 2009 sampling compared to 2008 caused a larger sewage discharge rate, enhancing the local impacts at Suro and Dolat Park and spreading them over a larger beach area. The opposite pattern at Terminal is difficult to explain, although a sampling performed four years later demonstrated a substantial coarsening of the sediments at this location. If this had already initiated in 2009, it might explain the increase in diversity and abundance. The overall number of genera encountered in our samples was equally high in both years. Four out of the five dominant genera of 2008 together made up 80 % of nematode abundances in 2009, while 22 ‘unique’ genera, encountered only in 2009, together contributed only 6.5 %. Two thirds of the 62 genera in the total dataset were only found in one year and were all rare. This demonstrates that a correct estimate of genus richness requires a large and repeated sampling effort specifically aiming at the tail of rare genera.

Keywords: meiobenthos, intertidal, biodiversity, assemblage structure, spatial variability, temporal variability.

1 Introduction

Free-living marine nematodes are the most abundant and diverse metazoan fauna in marine benthic systems (Heip et al. 1985; Moens et al. 2013). Their activity may affect ecosystem processes such as organic matter decomposition, nutrient recycling and biofilm formation (De Mesel et al. 2003; Chinnadurai and Fernando 2007; Hubas et al. 2010), and their biomass may serve as food for benthic and hyperbenthic macrofauna and fish (Gee 1989; Coull 1990; Schuckel et al. 2013). Nematode assemblages are also potentially very well suited for environmental impact studies, due to, among others, their limited mobility and lack of larval dispersion, their high abundance, their relatively short generation times, differential and species-specific sensitivity to different types of disturbance, and the presence of different feeding modes and trophic levels (Sandulli and De Nicola 1991; Bongers and Ferris 1999; Moreno et al. 2011; Patrício et al. 2012).

The Persian Gulf is an important military, economic and political region due to its strategic position and its oil and gas resources. It is intensively utilized by men, mainly as a transport route. Its environment has been subject to rapid changes and diverse pressures such as oil pollution, solid and liquid waste disposal (including sewage), coastal development and recreational activities, many of which may act profoundly on benthic systems (Doustshenas et al. 2009; Dehghan Madiseh et al. 2012; Ejlali Khanaghah et al. 2015; Farsi et al. 2015). However, studies on the benthos in the northern part of the Gulf are scanty and have mostly been restricted to macrofauna (Samadi et al. 2010; Saledhoust et al. 2011). We know of only one study on meiofauna in this area, which dealt with the abundance, diversity and genus composition of nematodes at four sandy beaches near Bandar Abbas, the capital of the Hormuzgan province in Iran (Sahraean et al. 2017a , chapter 2 of this PhD). That study found an overall low to moderate genus diversity, but also substantial effects of local sewage inputs. However, it was based on a single sampling event in December 2008, and nematode assemblages can be highly variable in both space and time. Such variability depends on several climatological, physical and chemical (e.g. temperature, salinity, mean grain size of sediment and dissolved oxygen) (Hourston et al. 2005; Nozais et al. 2005; Moens et al. 2013) as well as on biological factors such as food quality and quantity, macrobenthic infaunal activity and predator impacts (Gallucci et al. 2005; Braeckman et al. 2011; dos Santos and Moens 2011; Maria et al. 2011b).

A majority of studies which have assessed temporal variability of coastal intertidal nematode assemblages have looked at seasonal variability (Boaden and Platt 1971; McIntyre and Murison 1973; Platt 1977; McLachlan 1978; Blome 1982; Sharma and Webster 1983; Gourbault et al. 1998; Nicholas and Hodda 1999; Nicholas 2001; Albuquerque et al. 2007; Liu et al. 2008; Venekey et al. 2014a). Fewer studies have considered longer-term (i.e. across years) variability (Coull 1985, 1986; Eskin and Coull 1987; Li et al. 1996; Riera et al. 2011a; Riera et al. 2011b). Moreover, a majority of studies have only considered total abundances of nematodes and other higher meiofauna taxa. Coull (1985, 1986) reported variability of meiofauna abundance at higher-taxon level from two Southern Californian estuarine sites (one muddy and one sandy) over an eleven-year study period. Year-to-year variability in meiofauna abundance exceeded seasonal variability in both sites, but no recurrent patterns other than seasonal ones were found. Predation by juvenile spot was suggested as a significant factor controlling meiofauna abundances in the muddy sediment (Smith and Coull 1987). Li et al. (1996) in turn concluded that variability in nematode biomass over a one-year cycle with monthly or fortnightly samplings was strongly impacted by macrobenthos, albeit that this mostly affected predatory and omnivorous nematodes, whereas biomass of primary consumers was more dependent on food availability. Riera et al., (2011a) reported seasonal and long-term (six years) variability of a meiofaunal community under the influence of a fish farm (Canary Islands, NE Atlantic Ocean). These authors observed a lack of any seasonal pattern in all meiofaunal taxa except harpacticoid copepods. Year-to-year variability of meiofauna abundance was similar throughout the study period except the last year, which showed a significant increase in overall meiofauna abundance. Riera et al. (2011b) investigated the meiofauna at an intertidal beach on a monthly basis for one year. Temporal fluctuations were species-specific, but only a very small portion of this temporal variability could be assigned to sediment granulometry, organic matter content or nitrogen concentration. Finally, Materatski et al. (2015) studied nematode assemblages pre and post collapse of seagrass (*Zostera noltii*) meadows in the Mira estuary, Portugal; despite significant changes in overall abundance and diversity, they also found a surprisingly high resilience of nematode assemblages, particularly in terms of diversity and trophic composition.

The main objective of the present study was to investigate year-to-year variability of nematode assemblage structure and its spatial patterns on beaches of the northern part of the Strait of Hormuz, Persian Gulf. For this purpose, we repeated the sampling design used in 2008 (Sahraean et al. 2017a, chapter 2 of this PhD) in December 2009, at the same four locations and using the same stations at each location. In view of substantial differences in climatological conditions (mainly a threefold higher precipitation in the weeks preceding the 2008 and 2009 sampling events, as well as higher temperatures in 2009 compared to 2008), we assessed whether the spatial patterns in nematode assemblage structure and diversity would also differ between both years.

2 Materials and Methods

2.1. Sampling sites and design

Nematode samples were collected at four intertidal beaches, characterized by different degrees of local anthropogenic pollution, in the northern part of the Strait of Hormuz, Persian Gulf, near Bandar Abbas, Iran. These beaches are: Haghani (most polluted), Suro and Terminal (intermediate pollution), and Dolat Park (least polluted). Sewage inputs are the main source of pollution at the first three locations, whereas Dolat Park is relatively ‘pristine’, with garbage spills from recreational tourism as the main local source of anthropogenic disturbance. However, it is important to note that this area of the Persian Gulf is overall impacted by anthropogenic effects such as elevated hydrocarbon levels (Gevao et al. 2006; Gawad et al. 2008), and in addition offers a naturally stressful environment (for instance high salinity and very large seasonal differences in surface-water temperatures (Chao et al. 1992)). These four beaches also differ in morphodynamics, from a narrow (ca 30 m) and moderately reflective beach at Haghani to more sheltered and much wider (500 – 600 m) tidal flats at the other three locations. Nevertheless, sediment granulometry exhibited only moderate differences, Terminal presenting finer sediments and a higher silt fraction than the other three beaches. More detailed information on the study sites is provided in (Sahraean et al. 2017a, chapter 2), and a summary of the most important sediment characteristics and nematode abundance and diversity data from that study in 2008 is provided in table 3-1.

At each beach, a transect parallel to the water line was established with three stations at 50-m intervals along a gradient of increasing distance from a local sewage input (Sahraean et al., 2017a, chapter 2). Given the absence of sewage inputs at Dolat Park, the gradient at this site started at the side of the beach which receives most garbage left behind by tourists. At each station at each beach, three replicate sediment cores, with interdistances of ca 1 m along a line perpendicular to the water line, were taken using PVC hand corers with an inner diameter of

3.5 cm down to a depth of 5 cm. Samples were immediately preserved in 4 % buffered formaldehyde. This sampling design, using the same sampling locations and distances to pollution, was exactly the same as in 2008 (Sahraean et al. 2017a., chapter 2).

2.2. Nematode quantification and identification

In the laboratory, nematodes were extracted using centrifugation with Ludox© HS40 at a specific density of 1.18 (Vincx 1996) and collected over a 38-µm mesh. The nematodes retained on the 38-µm sieve were counted, and 100 nematodes were picked out randomly, transferred through a graded series of ethanol-glycerol solutions, and mounted on glass slides prior to identification to genus level using the pictorial keys of (Platt and Warwick 1980; Platt and Warwick 1983) and Warwick et al. (1998), as well as the Nemys online identification key (Vanaverbeke et al. 2015). When less than 100 nematodes were found, all specimens were mounted on slides. This is again exactly the same procedure as for the 2008 sampling.

	Suro	Haghani	Dolat Park	Terminal
median grain size (µm)	155±8.9	175±18.6	177±17.5	119±9.4
mean grain size (µm)	186±28.3	195±23.4	198±11.1	1756±2.3
% silt	0.4±0.1	1.0±1.0	5.9±3.9	17.8±3.0
nematode abundance (ind. 10 cm^{-2})	674.6±81.5	373.7±100.6	688.1±125.6	700.4±99.7
nematode genus richness	10.4±1.6	5.6 ± 0.3	9.0±0.8	8.1±0.9
Shannon-Weaver diversity	1.7±0.2	1.0±0.1	1.5±0.1	1.2±0.1

Table 3.1. Sediment granulometry (mean and median grain size and % silt) and nematode abundance, nematode genus richness and Shannon-Weaver diversity of the four beaches studied, based on the data collected in 2008. Data are means ± 1 SE of three (nematode data) and one (sediment data; values are thus the mean of the values for each distance) replicates taken from each of three distances to pollution per beach. Diversity data thus represent sample diversity (α diversity).

2.3. Data analysis

We calculated nematode genus richness in terms of rarefied (expected number of genera in a sample of 50 nematodes, EG50) and absolute richness. In addition, we calculated Shannon-Weaver's diversity (H'), Simpson's diversity index (Si) as a measure of dominance, and taxonomic diversity (Δ) and distinctness (Δ*), using the DIVERSE routine in PRIMER 6.0 (Clarke and Gorley 2006). Taxonomic distinctness is the average taxonomic distance between any two specimens in the assemblage, whereas taxonomic diversity is the average taxonomic distance between pairs of taxa, in this case genera (Warwick and Clarke 1998). The nematodes were classified into six feeding categories according to Moens and Vincx (1997): microvores, deposit feeders, ciliate feeders, epigrowth feeders, facultative predators and predators.

Differences in total nematode abundances and all diversity indices between different locations and stations and between years, as well as their interactions were tested. Prior to analysis, data were tested in the software Statistica 7 for normality by means of the Kolmogorov-Smirnov test, and homogeneity of variances using Levene's test. If the data did not conform to these assumptions, they were log(x+1) transformed. In many cases, this data transformation did not solve the issue of normality and/or homoscedasticity; hence for reasons of consistency, we decided to analyze all data using permutational multivariate analysis of variance (PERMANOVA) (Anderson et al. 2008), Location, distance to pollution and year were included as independent fixed factors. A pairwise test was applied to assess the significance of main and interaction effects under a full model. If the number of permutations was lower than 150, the Monte Carlo permutation p was used. Since a PERMANOVA test can show differences between groups, but not distinguish between a difference due to the factor effect or to data dispersion, homogeneity of variances was tested with PERMDISP, using the distance among centroids. A Euclidian distance based resemblance matrix was used for univariate (i.e. total number of nematode and diversity indices) measurements.

Nematode assemblage composition for both years was visualized using non-metric Multidimensional Scaling (nMDS) on fourth-root transformed data using Bray-Curtis similarity. The assemblage composition data were further analysed using PERMANOVA with the same three-way design as for the abundance and diversity data. The genera contributing most to the dissimilarities between different locations, distances to pollution and years were identified by two-way crossed SIMPER analyses with factors location x year or distance x year.

3 Results

3.1 Nematode abundance

Nematode abundance varied greatly, with a minimum of 13 and a maximum of 2909 ind. per 10 cm^2 in single replicate cores. A highly significant location x distance x year interaction (Table 3.2; df = 6, F = 3.86, p = 0.004) demonstrates that the spatial pattern of nematode abundances differed between years (Fig 3.1). Although lower-order effects need to be interpreted with caution, only location x year (df = 3, F = 3.42, p < 0.02) and location (df = 3, F = 7.84, p = 0.001) had significant effects on nematode abundances. Haghani harboured significantly lower nematode abundances than all other beaches except Suro in 2009 (all p < 0.005) (Fig. 3-1A). Nevertheless, all these effects need to carefully interpreted, since Permdisp demonstrated significant dispersion effects for all factors (all p < 0.05), even after log transformation of the data.

Total nematode abundance	Source	df	SS	MS	F-value	Pseudo-p-value
	Lo	3	3.5589E6	1.1863E6	7.8424	**0.001**
	Dis	2	7.3581E5	3.6791E5	2.4322	0.102
	Ye	1	40565	40565	0.26817	0.599
	LoxDis	6	1.6351E6	2.7251E5	1.8015	0.114
	Lo x Ye	3	1.5517E6	5.1723E5	3.4193	**0.017**
	Dis xYe	2	2.8604E5	1.4302E5	0.94549	0.422
	Lo x Dis x Ye	6	3.5106E6	5.8509E5	3.8679	**0.004**
	Res	48	7.2608E6	1.5127E5		
	Total	71	1.8579E7			

Table 3-2. Results of a three-way PERMANOVA on total nematode abundances with factors location, distance to pollution, year and their interactions. Significant interactions and factors are highlighted in bold.

The location x year interaction largely reflects the opposite temporal trends in Suro and Terminal (Fig. 3.1A). Much of this was, however, restricted to opposing trends in the station closest to the local pollution source (Fig. 3-1C), hence the significant three-way interaction and the absence of a significant distance x year effect (Fig. 3-1B). Indeed, nematode abundances at a distance of 50 m from local pollution at Suro were dramatically lower in 2009 than in 2008 ($p < 0.001$), whereas the opposite was true for the same distance at Terminal ($p < 0.02$) (Fig. 3-1C).

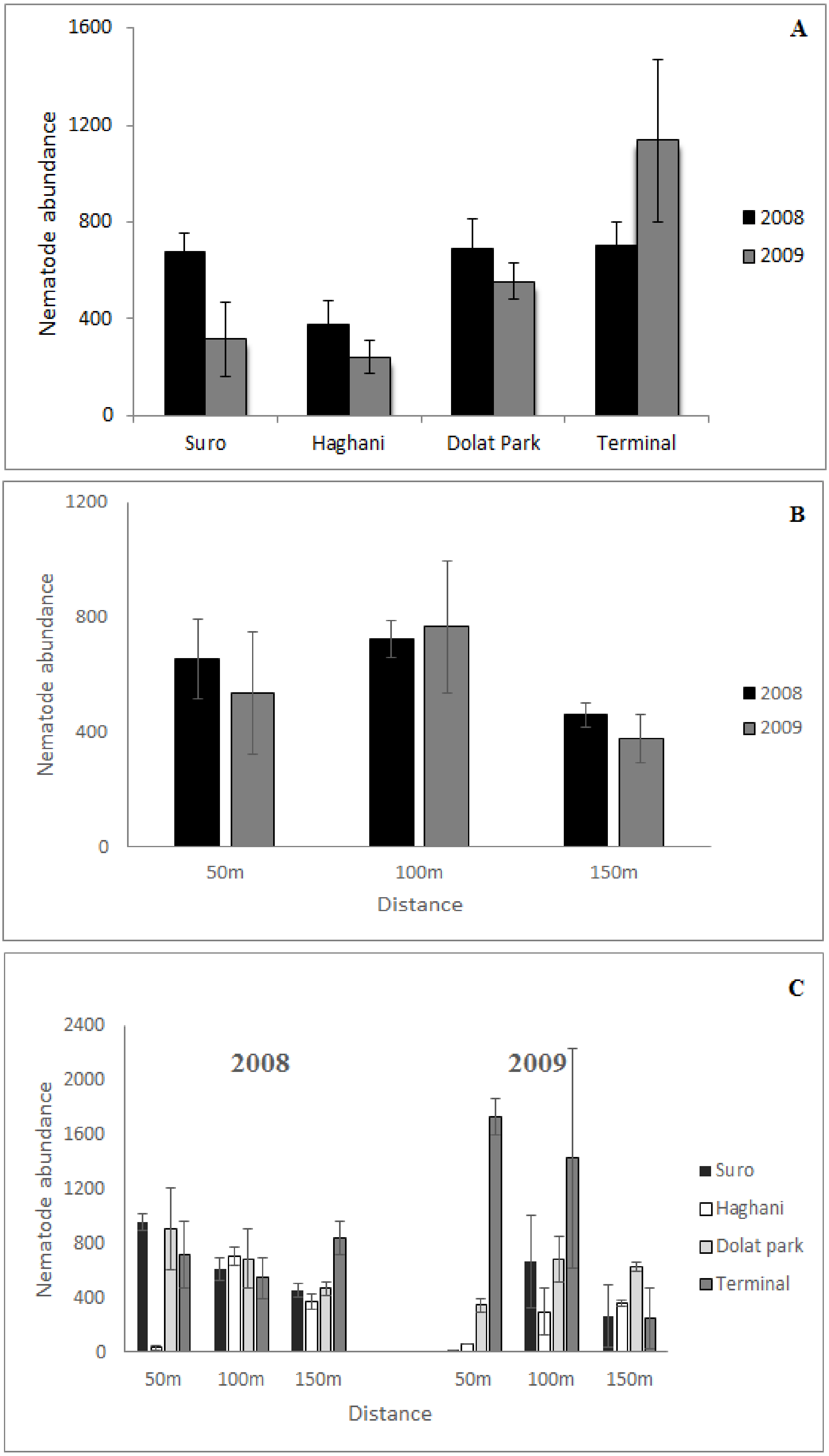

A
Nematode abundance
1600
1200
800
400
0
Suro
Haghani
Dolat Park
Terminal
■ 2008
■ 2009
B
Nematode abundance
1200
800
400
0
50m
100m
150m
Distance
■ 2008
■ 2009
C
Nematode abundance
2400
2000
1600
1200
800
400
0
2008
2009
50m
100m
150m
50m
100m
150m
Distance
■ Suro
☐ Haghani
☐ Dolat park
■ Terminal

Fig. 3-1 Total nematode abundance across four beach locations (A), three distances to pollution (B) and four locations x three distances (C) in December 2008 vs 2009. Data are means ±1SE of three replicates for each distance x beach x year combination. 50 m = closest distance to pollution source, 150 m = largest distance.

3.2 Nematode diversity and dominant genera

A total of 62 genera belonging to 24 families were identified over the two years (Table 3-3 and S3-1): 39 genera belonging to 17 families in 2008 *vs* 41 genera from 21 families in 2009. The turnover between both years was substantial. In 2008, there were 20 unique genera accounting for 9.4 % of total nematode abundance. In 2009, only 6.5 % of total nematode abundance was made up by no less than 22 unique genera. 20 genera were found in both years. In 2008 and 2009, only five and four genera, respectively, occurred in 50 % or more of all samples. These were *Terschellingia*, *Daptonema*, *Ptycholaimellus* and *Promonhystera* in both years and *Paramonhystera* in 2008 only. Together, these dominant genera accounted on average for 75 and 80 % of the total nematode abundance in 2008 and 2009, respectively (Fig. 3-2A,B). Over the whole study period, only one other genus (*Paraethmolaimus*) occurred in a relative abundance of more than 2 % (Table 3-3 and S3-1). General trends in abundance between years were usually not very pronounced, but *Terschellingia* generally increased while *Paramonhystera* decreased in 2009 compared to 2008 (Fig. 3-2).

Table 3-3. Genus composition and relative abundances (%) of nematodes at the four sampling locations and averaged over all sampling locations. All data are means of 2008 and 2009, and of 3 stations (i.e. distances to local pollution) per location per year, with 3 replicates per distance. Feeding types (FT) according to Moens & Vincx (1997) and Wieser (1953) are also listed. MV = microvore, DF = deposit feeder, CF = ciliate feeder, EF = epistrate feeder, FP = facultative predator, PR = predator; 1A= selective deposit feeder, 1B= non-selective deposit feeder, 2A= epigrowth feeder, 2B= predators or omnivore.

	Suro	Haghani	Dolat Park	Terminal	Average	FT
Terschellingia	22.3	9.1	30.9	28.1	22.6	MV/1A
Ptycholaimellus	15.7	9.7	16.3	36.0	19.4	EF/2A
Daptonema	19.1	13.2	24.6	11.1	17.0	DF/1B
Promonhystera	4.2	40.1	6.9	1.8	13.3	DF/1B
Paramonohystera	1.9	20.3	5.7	0.4	7.0	DF/1B
Paraethmolaimus	7.7	1.1	0.1	2.5	2.8	EF/2A
Eumorpholaimus	0.8	0.7	2.3	3.5	1.8	DF/1B
Oncholaimus	4.3	0.9	1.2	0.5	1.7	FP/2B
Sabatieria	3.5	1.7	0.1	1.0	1.6	DF/1B
Viscosia	3.9	1.0	0.6	0.2	1.4	FP/2B
Bathylaimus	0.4	0.0	4.7	0.5	1.4	CF /1B
Monhystrella	0.0	0.0	0.0	5.2	1.3	DF/1B
Odontophora	3.7	0.8	0.1	0.1	1.2	FP/2B
Theristus	1.8	1.2	1.4	0.3	1.2	DF/1B
Metoncholaimus	3.5	0.2	0.4	0.0	1.0	FP/2B
Eleutherolaimus	0.6	0.2	0.4	2.1	0.8	MV/1A
Desmodora	2.2	0.0	0.0	0.0	0.6	EF/2A
Metalinhomoeus	0.0	0.0	1.2	0.7	0.5	MV/1A
Rhynchonema	1.8	0.0	0.0	0.1	0.5	DF/1B
Spilophorella	0.1	0.0	1.0	0.7	0.5	EF/2A
Prochromadora	0.4	0.0	0.7	0.7	0.5	EF/2A
Onyx	0.9	0.0	0.2	0.1	0.3	FP/2B
Oxystomina	0.2	0.0	0.0	0.8	0.3	DF/1B
Dichromadora	0.0	0.0	0.0	0.9	0.2	EF/2A
Sphaerolaimus	0.4	0.0	0.0	0.3	0.2	FP/2B
Paracanthonchus	0.7	0.0	0.0	0.0	0.2	EF/2A
Synonchium	0.7	0.0	0.0	0.0	0.2	FP/2B

Calyptronema	0.3	0.0	0.0	0.3	0.2	PR/2B
Cyatholaimidae sp.	0.6	0.0	0.0	0.0	0.2	EF/2A
Metachromadora	0.3	0.0	0.3	0.0	0.1	EF/2A
Deontolaimus	0.0	0.0	0.0	0.6	0.1	DF/1B
Symplocostoma	0.6	0.0	0.0	0.0	0.1	FP/2B
Camacolaimus	0.0	0.0	0.0	0.4	0.1	EF/2A
Haliplectus	0.0	0.0	0.0	0.4	0.1	MV/1A
Nygmatonchus	0.3	0.1	0.0	0.1	0.1	DF/1B
Chromadorina	0.2	0.1	0.1	0.0	0.1	EF/2A
Axonolaimus	0.0	0.0	0.0	0.3	0.1	DF/1B
Thalassomonhystera	0.0	0.0	0.2	0.0	0.1	DF/1B
Xyalidae sp.	0.0	0.2	0.0	0.1	0.1	DF/1B
Pomponema	0.0	0.0	0.2	0.0	0.1	FP/2B
Tripyloides	0.0	0.0	0.2	0.0	0.1	CF/1B
Enoploides	0.0	0.1	0.1	0.0	0.0	PR/2B
Phanoderma	0.1	0.0	0.0	0.1	0.0	EF/2A
Chromadorella	0.0	0.0	0.2	0.0	0.0	EF/2A
Marylynnia	0.2	0.0	0.0	0.0	0.0	EF/2A
Prochromadorella	0.0	0.1	0.0	0.0	0.0	EF/2A
Spirinia	0.0	0.1	0.0	0.0	0.0	EF/2A
Tubolaimoides	0.0	0.1	0.0	0.0	0.0	MV/1A
Polysigma	0.0	0.0	0.0	0.1	0.0	DF/1B
Pseudolella	0.1	0.0	0.0	0.0	0.0	FP/2B
Chromadorita	0.1	0.0	0.0	0.0	0.0	EF/2A
Leptolaimidae sp.	0.1	0.0	0.0	0.0	0.0	MV/1A
Longicyatholaimus	0.0	0.0	0.0	0.1	0.0	EF/2A
Cyatholaimus	0.1	0.0	0.0	0.0	0.0	EF/2A
Hopperia	0.1	0.0	0.0	0.0	0.0	DF/1B
Araeolaimus	0.0	0.0	0.0	0.1	0.0	MV/1A
Camacolaimus	0.0	0.0	0.0	0.1	0.0	EF/2A
Halichoanolaimus	0.0	0.0	0.0	0.1	0.0	FP/2B
Chromadora	0.0	0.0	0.1	0.0	0.0	EF/2A
Doliolaimus	0.0	0.0	0.0	0.1	0.0	FP/2B

Hypodontolaimus	0.1	0.0	0.0	0.0	0.0	EF/2A

Expected number of genera (genus richness) was significantly affected by the interaction of location x distance x year and by all lower-order effects (Table 3-5). However, Permdisp demonstrated significantly heterogeneous variances for the factors location ($p = 0.01$ after log transformation) and distance ($p = 0.029$ after log transformation), but not for year ($p = 0.33$ after log transformation). Richness in Suro ($p < 0.03$) and Dolat Park ($p = 0.002$) was significantly lower in 2009 than in 2008, but only at the largest distance to pollution, while the opposite trend was observed at 50 and 100 m away from local pollution at Terminal (both $p < 0.01$) (Fig. 3-3A). Overall, genus richness was significantly highest in the station most distant from pollution, but only so in 2008 (all $p < 0.001$) (Fig. 3-3B). Fig. 3-3C shows that the absence of such a distance effect in 2009 was largely attributable to a lower richness in the stations at 150 m from a local pollution source at Suro and Dolat Park in 2009 compared to 2008 ($p = 0.025$ and 0.002, respectively). No other pairwise differences in richness between distance x year combinations were observed (Table 3-5, all $p > 0.05$).

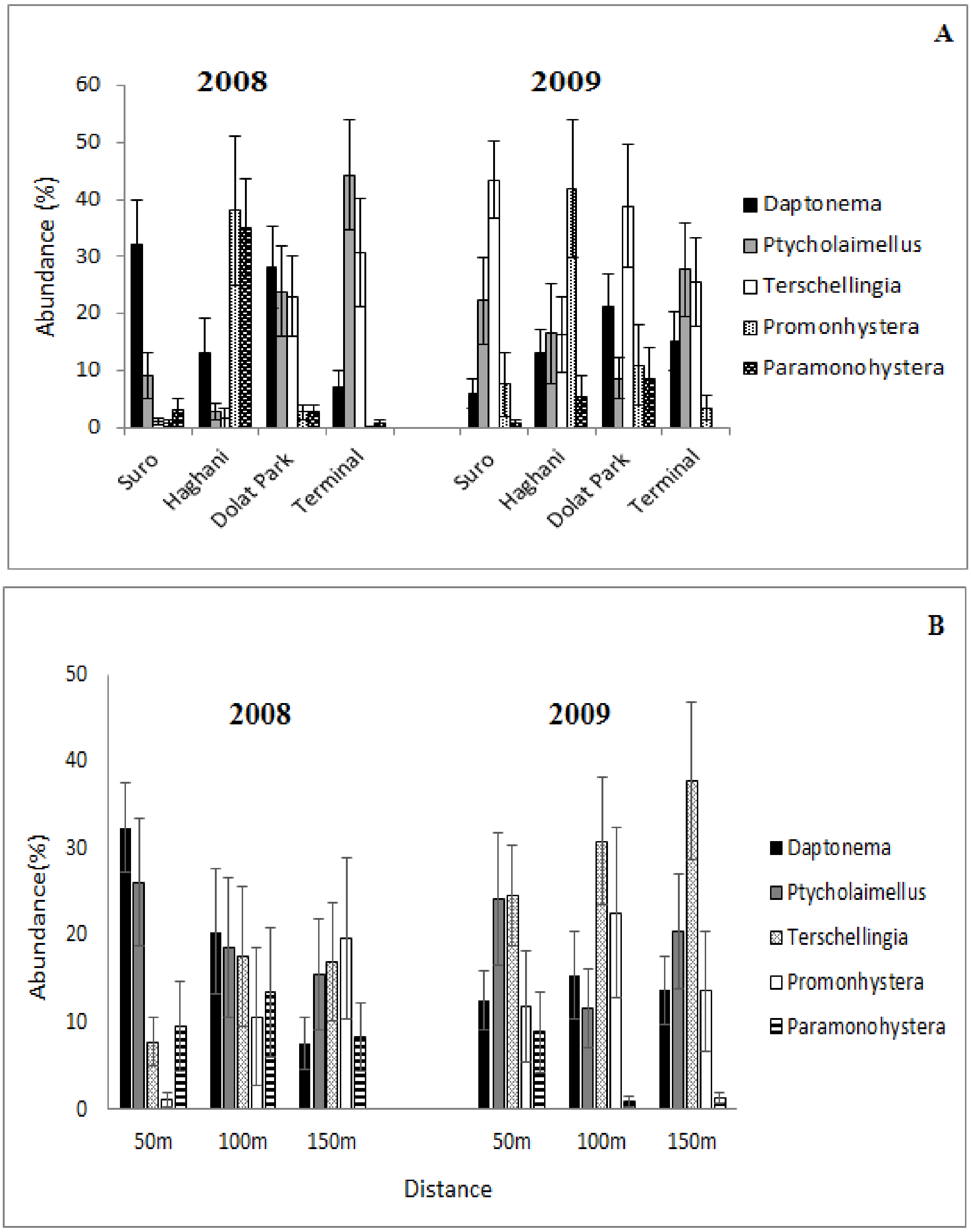


Fig. 3-2. A. Relative abundances of the five most abundant genera at the four sampling locations, and B. relative abundance of these same five genera as a function of distance to a local pollution source (50 m = closest distance to pollution source, 150 m = largest distance) in two subsequent years. Data are means ± 1SE of three distances, each with three replicate samples, per location.

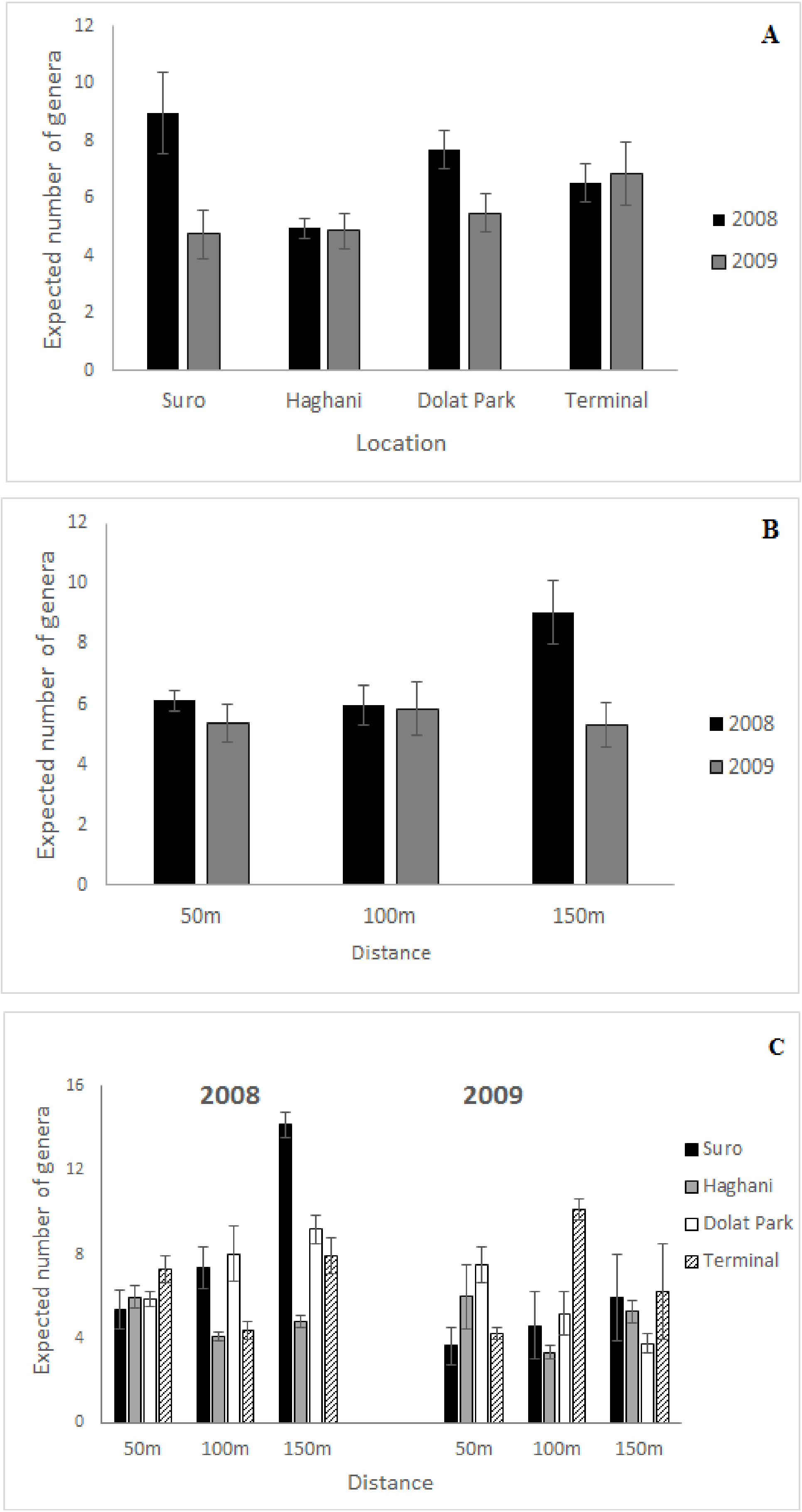

A
Expected number of genera
0
2
4
6
8
10
12
Suro
Haghani
Dolat Park
Terminal
Location
■ 2008
■ 2009
B
Expected number of genera
0
2
4
6
8
10
12
50m
100m
150m
Distance
■ 2008
■ 2009
C
Expected number of genera
0
4
8
12
16
2008
2009
50m
100m
150m
50m
100m
150m
Distance
■ Suro
■ Haghani
□ Dolat Park
▨ Terminal

Fig. 3-3. Nematode genus richness (expressed as expected number of genera in a sample of 50 inds.) across four beach locations (A), three distances to local pollution (B) and four locations x three distances (C) in December 2008 vs 2009. Data are means ±1SE of three replicates for each beach x distance x year combination. 50 m = closest distance to a local pollution source, 150 m = largest distance.

	Source	df	SS	MS	F-value	Pseudo-p-value
Expected number of genera	**Location**	3	44.81	14.937	5.0462	**0.005**
	Distance	2	29.326	14.663	4.9539	**0.009**
	Year	1	42.895	42.895	14.492	**0.001**
	Lo x Dis	6	93.516	15.586	5.2657	**0.002**
	Lo x Ye	3	59.996	19.999	6.7564	**0.001**
	Dis x Ye	2	43.877	21.939	7.4119	**0.002**
	Lo x Dis x Ye	6	100.05	16.675	5.6335	**0.001**
	Res	48	142.08	2.9599		
	Total	71	1.5504E5			
Shannon-Weaver index	Location	3	1.7114E7	5.7047E6	0.96944	0.402
	Distance	2	1.8256E7	9.1281E6	1.5512	0.215
	Year	1	1.3527E8	1.3527E8	22.987	**0.001**
	Lo x Dis	6	6.4736E7	1.0789E7	1.8335	0.112
	Lo x Ye	3	1.7124E7	5.7078E6	0.96997	0.418
	Dis x Ye	2	1.8257E7	9.1285E6	1.5513	0.249
	Lo x Dis x Ye	6	6.4755E7	1.0792E7	1.834	0.125
	Res	48	2.8246E8	5.8845E6		
	Total	71	6.1797E8			
Simpson index	**Location**	3	4232.1	1410.7	2.0088	**0.026**
	Distance	2	1274.8	637.42	0.90766	0.499
	Year	1	1.5054E5	1.5054E5	214.37	**0.001**
	Lo x Dis	6	4930.6	821.76	1.1702	0.256
	Lo x Ye	3	3558.1	1186	1.6889	0.071
	Dis x Ye	2	1611.4	805.72	1.1473	0.307
	Lo x Dis x Ye	6	4980.4	830.07	1.182	0.289
	Res	48	33709	702.27		
	Total	71	2.0484E5			
Taxonomic diversity	**Location**	3	12.825	4.2749	7.4056	**0.001**
	Distance	2	1.4928	0.7464	1.293	0.282
	Year	1	3.1928	3.1928	5.531	**0.025**
	Lo x Dis	6	11.575	1.9291	3.3419	**0.008**
	Lo x Ye	3	10.883	3.6278	6.2846	**0.002**
	Dis x Ye	2	0.47385	0.23692	0.41044	0.674
	Lo x Dis x Ye	6	6.6325	1.1054	1.915	0.094
	Res	48	27.708	0.57725		
	Total	71	74.782			
Taxonomic distinctness	**Location**	3	7.823	2.6077	6.3907	**0.001**
	Distance	2	0.30405	0.15203	0.37258	0.656
	Year	1	8.5619	8.5619	20.983	**0.001**
	Lo x Dis	6	5.6321	0.93868	2.3004	0.056
	Lo x Ye	3	16.823	5.6076	13.743	**0.001**
	Dis x Ye	2	1.1719	0.58597	1.4361	0.267
	Lo x Dis x Ye	6	8.3226	1.3871	3.3994	**0.006**
	Res	48	19.586	0.40804		
	Total	71	68.224			

Table 3-5. Results of three-way PERMANOVA's on five different nematode diversity indices at four beaches, with factors location, distance to pollution, year and their interactions. Significant interactions and factors are highlighted in bold.

Taxonomic distinctness was the only other diversity measure with a significant location x distance x year interaction effect. Significant lower-order effects included location x year and the separate factors location and year (Table 3-5). However, Permdisp demonstrated significant dispersion effects for the factors location and year (both $p < 0.04$), calling for a cautionary interpretation of the observed effects. Taxonomic distinctness was generally higher in 2008 than in 2009, except at Terminal where the opposite effect was observed (Fig. 3-4A). A significantly lower taxonomic distinctness at Terminal compared to the other three beach locations was observed in 2008 (Fig. 3-4A; all $p \leq 0.003$). In that same year, Suro also had a significantly higher taxonomic distinctness than both Haghani ($p = 0.03$) and Dolat Park ($p = 0.006$). In 2009, Haghani harboured a significantly lower taxonomic distinctness than the three other beaches (all three $p < 0.04$), while Terminal had the highest (Terminal vs Dolat Park, $p < 0.05$), albeit not significantly higher than Suro ($p = 0.438$). In 2008, the only significant distance effect on taxonomic distinctness was at Suro, where the 50-m station had a significantly lower taxonomic distinctness than the 100-m and 150-m stations (both $p \leq 0.002$). By contrast, no significant distance effect was observed at Suro and Dolat Park in 2009, whereas such an effect was now visible at Haghani and Terminal (between the 50-m and 150-m station, $p = 0.021$ and 0.006, respectively).

Taxonomic diversity differed significantly between location x year and location x distance, as well as between locations and years (Table 3-5). Permdisp values were non-significant for all factors (all $p > 0.08$). In 2008, Suro harboured a significantly higher taxonomic diversity than Haghani and Terminal (both $p < 0.002$, Fig. 3-4A). In 2009, taxonomic diversity had significantly decreased at Suro ($p = 0.004$) and Haghani ($p = 0.035$) and increased at Terminal ($p < 0.025$) (Fig. 3-4A). Both Suro and Terminal now had significantly higher taxonomic diversity than Haghani (both $p < 0.004$).

Only the factor year had a significant effect on the Shannon-Weaver index (Table 3-5). Unfortunately, this was also the only factor for which Permdisp indicated significantly heterogeneous variation ($p = 0.001$). Fig. 3-4B suggests that a higher Shannon-Weaver diversity was present in 2008, but that this effect was largely driven by Suro and Dolat Park, even though the interaction term location x year was not significant (Table 3-5). The Simpson index was significantly affected by the factors year and location (with non-significant Permdisp values for all factors (all p > 0.07)), while the interaction of these two factors yielded a borderline non-significant effect (Table 3-5). Still, fig. 3.4B indicates no consistent differences between years across all stations. Simpson diversity rather followed the trend of abundance, richness and taxonomic diversity, where Suro and to a lesser extent Dolat Park decreased in 2009 and Terminal increased.

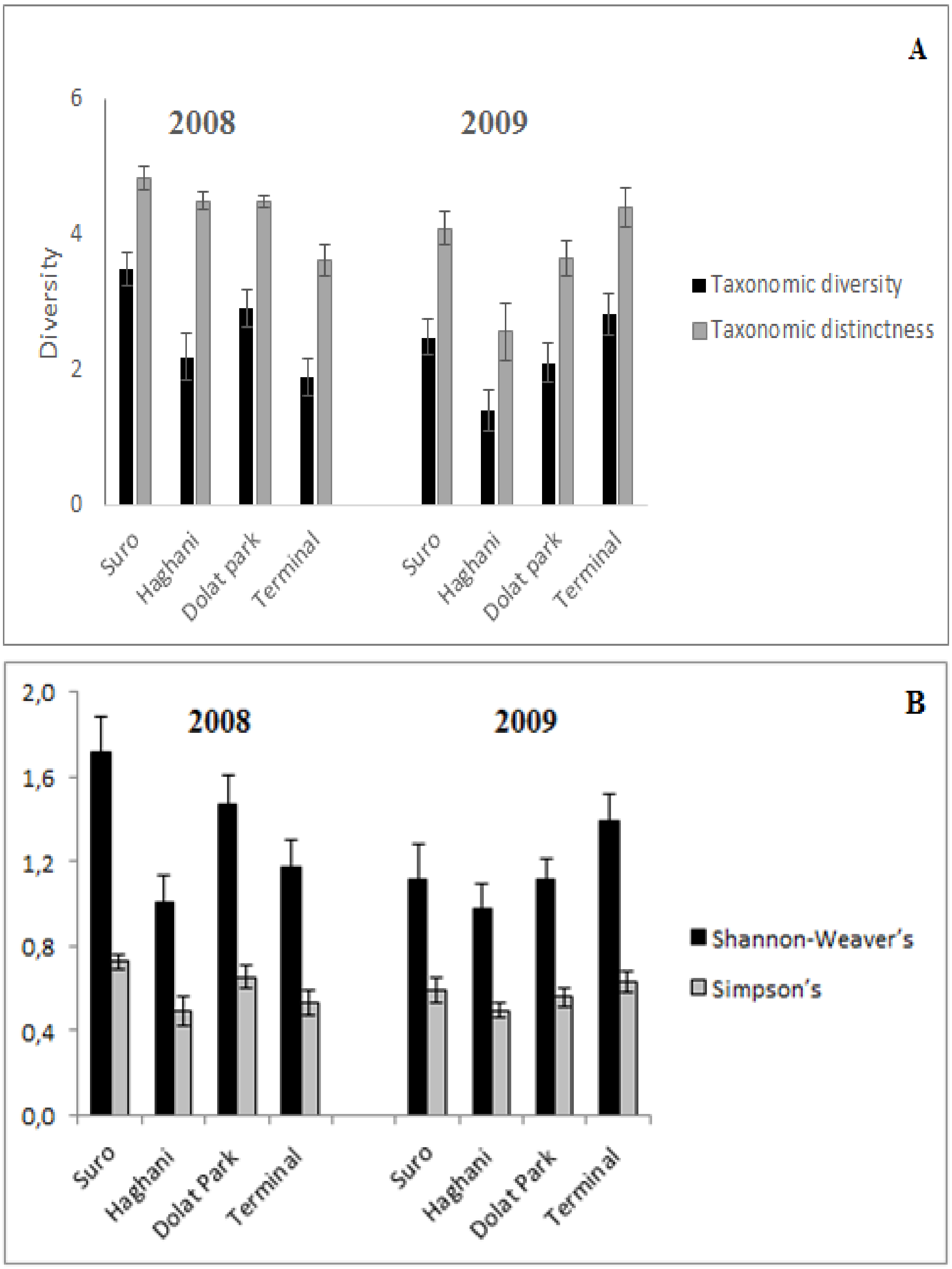


Fig. 3-4. Taxonomic distinctness and taxonomic diversity (panel A) and Shannon-Weaver and Simpson index (panel B) of nematodes across four beach locations in December 2008 vs 2009. Data are means ±1SE of three replicates of three stations at different distances to pollution per beach and per year.

3.3 Nematode assemblage structure

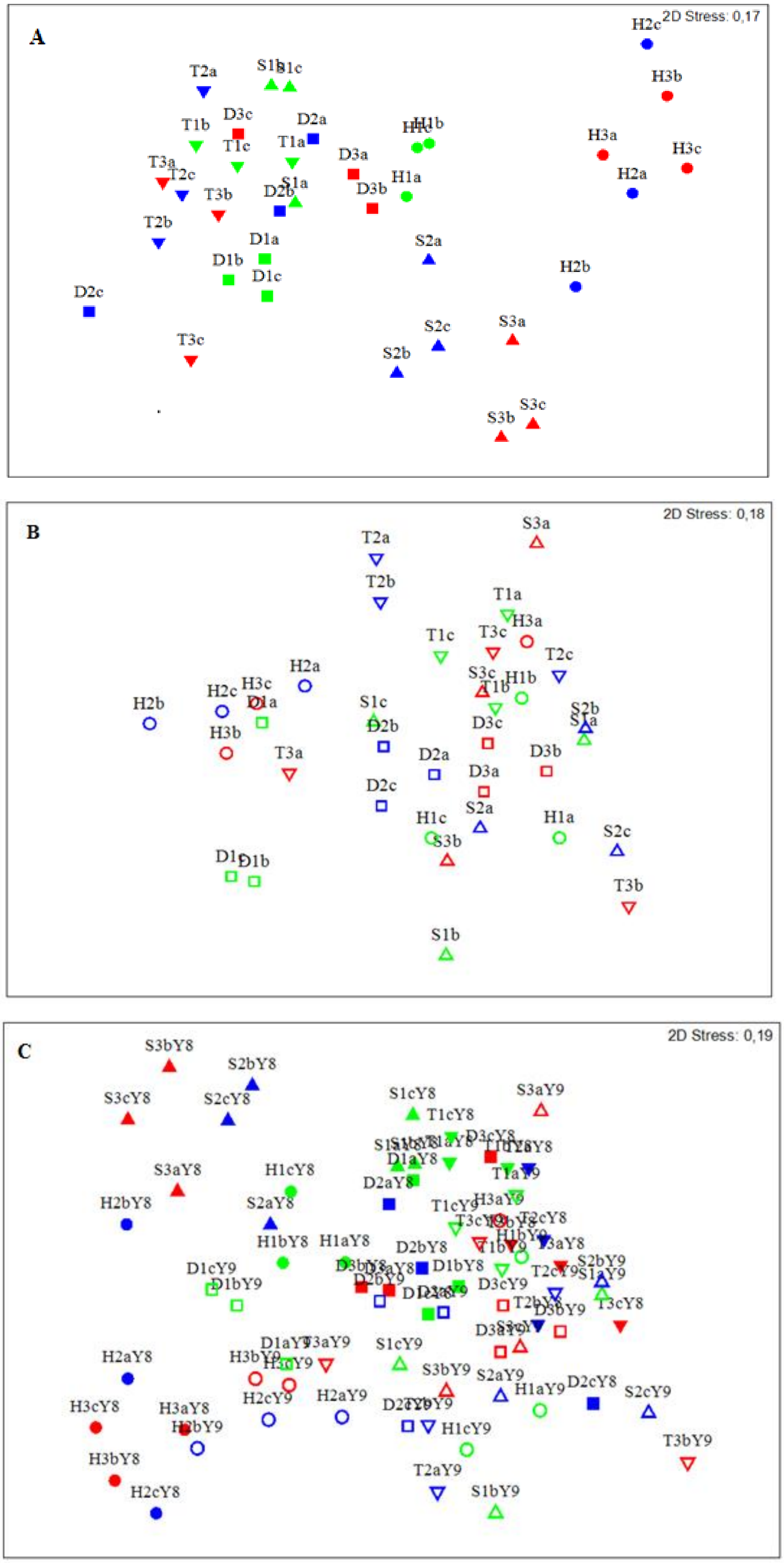
nMDS illustrates that nematode assemblage structure differed between both years. Samples of 2008 clustered in three main groups: stations 2 and 3 of Haghani formed a first group, stations 2 and 3 at Suro formed a second group, and all remaining samples formed a loosely clustered third group (Fig. 3-5A). In 2009, no clear structure was evident from the nMDS (Fig. 3-5B). The nMDS of both years together also yielded no clear structure (Fig. 3-5C).

Nematode assemblage composition was significantly affected by the interaction of location x distance x year and by all lower-order effects (Table 3-6), showing that differences between years were not consistent across locations and distances, and making it difficult to make strong statements about patterns between years. This is further aggravated by borderline significant Permdisp values for the factors year and distance (both $p = 0.05$) and a highly significant dispersion effect for the factor location ($p = 0.003$). Nevertheless, clear between-year differences could be observed from the pairwise comparisons of the three-way interaction factor. At Suro, for instance, assemblages differed significantly between years for all three distances to pollution (all $p \leq 0.035$), indicating a clear shift in assemblage structure accompanying the above-described differences in abundance and diversity at this beach. The same was true for the smallest and largest distance to pollution at Dolat Park (both $p < 0.04$, but only when running the pairwise comparisons under a reduced model; when using a full model, the differences between years were borderline non-significant, i.e. $p = 0.055$ for a distance of 50 m and 0.061 for 150 m), whereas at Haghani, only the station nearest to pollution had a significantly different assemblage composition between both years ($p = 0.04$). In contrast to Suro, the shifts in nematode abundance and diversity observed between years at Terminal were not reflected in significant differences in assemblage structure (all $p > 0.17$).

	Source	df	SS	MS	F-value	Pseudo-p-value
Nematode composition	**Location**	3	28727	9575.7	9.404	**0.001**
	Distance	2	6496.9	3248.5	3.1902	**0.001**
	Year	1	12060	12060	11.844	**0.001**
	Lo x Dis	6	20804	3467.3	3.4051	**0.001**
	Lo x Ye	3	15197	5065.5	4.9747	**0.001**
	Dis x Ye	2	5441.5	2720.7	2.672	**0.001**
	Lo x Dis xYe	6	17442	2907	2.8549	**0.001**
	Residuals	48	48876	1018.3		
	Total	71	1.5504E5			

Table 3-6. Results of three-way PERMANOVA on the nematode assemblage composition in four beaches, with factors location, distance to pollution, year and their interactions. Significant interactions and factors are highlighted in bold.

Fig. 3-5 Non-metric multidimensional scaling (nMDS) of nematode assemblages of four beach locations with three distances to a local pollution source each in two consecutive years (A = 2008, B = 2008, C =2008 and 2009 together) based on square-root transformed genus abundance data. Sample codes are as follows: first (capital) letter indicates location (S = Suro, H = Haghani, D = Dolat Park, T = Terminal), numbers indicate different distances to pollution (1 = closest to pollution source (50m), 3 = most distant (150m)), and letters (a, b, c) indicate different replicates. Locations are indicated by different shapes (▲= Suro,●= Haghani, ■ = Dolat Park, ▼ = Terminal), and distances to pollution by different colours (green= closest to pollution source (50 m), blue= intermediate distance(100m) and red= most distant (150 m)) . “filled symbols refer to 2008, open symbols to 2009.”


A
2D Stress: 0,17
H2c
H3b
H3a
H3c
H2a
H2b
T2a
S1b
S1c
T1b
D3c
D2a
H1c
H1b
T1c
T1a
D3a
T3a
T2c
S1a
D3b
H1a
T3b
D2b
T2b
D1a
S2a
D1b
D1c
D2c
S2c
S3a
T3c
S2b
S3b
S3c
B
2D Stress: 0,18
S3a
T2a
T2b
T1a
H3a
T1c
T3c
T2c
H2a
S3c
H1b
T1b
H2b
H2c
H3c
D1a
S1c
S2b
S1a
D2b
D3c
H3b
T3a
D2a
D3b
D3a
D2c
H1c
S2a
H1a
S2c
S3b
D1c
D1b
T3b
S1b
C
2D Stress: 0,19
S3bY8
S2bY8
S3cY8
S2cY8
S1cY8
S3aY9
T1cY8
D3cY8
D1aY8
T1aY9
S3aY8
H1cY8
D2aY8
H2bY8
S2aY8
T1cY9
H3aY9
T3cY9
T2cY8
H1bY8
H1aY8
D2bY8
T3aY8
D1cY9
D1bY9
D3bY8
D1bY8
D3cY9
T2cY9
S2bY9
S1aY9
T3cY8
D2bY9
T2bY8
D3bY9
D3aY9
S1cY9
D1aY9
T3aY9
H2aY8
H3bY9
H3cY9
S3bY9
S2aY9
H1aY9
D2cY8
S2cY9
H2cY9
H2aY9
H3cY8
H3aY8
H2bY9
H1cY9
T3bY9
H3bY8
T2aY9
S1bY9
H2cY8

SIMPER analysis indicated that dissimilarity in nematode assemblage composition between both years was high (66 %). This was mainly due to shifts in the relative abundances of the six most abundant genera, i.e. *Terschellingia, Ptycholaimellus, Promonhystera, Daptonema, Paramonohystera* and *Paraethmolaimus* between years.

The dissimilarity between locations across years ranged between 55 % (Dolat Park vs Terminal) and 72 % (Haghani vs Terminal) (Table 3-7). The dissimilarity between Haghani and Terminal was largely due to much higher relative abundances of *Promonhystera* at Haghani and of *Ptycholaimellus* and *Terschellingia* at Terminal (Table 3-7, fig. 3-2). *Ptycholaimellus* contributed to the dissimilarity between Terminal and Dolat Park with higher relative abundances in Terminal, whereas *Daptonema*, *Promonhystera* and *Terschellingia* were more abundant at Dolat Park (Table 3-7, fig. 3-2).

Clearly, most of the dissimilarities between locations could be attributed to combinations of genera belonging to the top-5 of most abundant genera overall. Other genera contributed occasionally. *Paraethmolaimus*, for instance, contributed to the dissimilarity between Suro and Terminal and to the dissimilarity between years, a fact which could be attributed to a more than fivefold higher abundance of this genus at the station furthest from pollution at Suro in 2008 compared to any other location or distance to pollution in 2008 and 2009, including the station furthest from pollution at Suro in 2009. *Bathylaimus* and *Eumorpholaimus* contributed to the dissimilarity between Dolat Park and Terminal, while *Oncholaimus* and *Viscosia* contributed both to the dissimilarity between Suro and Terminal and between Suro and Dolat Park (Table 3-7).

The dissimilarity between distances from a pollution point source was of a very similar magnitude in all pairwise comparisons (Table 3-7), indicating no clear gradient with distance. If such a gradient would have been prominent, we would have expected the largest dissimilarity to occur between the smallest (50 m) and largest (150 m) distance, but this was not the case. The higher abundances of *Ptycholaimellus* and *Daptonema* nearest the pollution sources, and of *Terschellingia* and *Promonhystera* away from pollution, were the most important contributors to the observed dissimilarities (Table 3-7).

Each of the five most abundant genera contributed to the 66 % overall dissimilarity between both years. *Terschellingia*, which was clearly more abundant in 2009 than in 2008, contributed slightly more than the other genera (Table 3-7).

Table 3-7. Results of pairwise SIMPER (Similarity Percentages) analysis showing percentage dissimilarity between nematode assemblages between the four beach locations across both years (location effect in a two-way SIMPER analysis with factors location and year), as well as between both years across locations (year effect in a two-way SIMPER analysis with factors location and year), and between distances to pollution across years (distance effect in a two-way SIMPER analysis with factors distance to pollution and year). The genera contributing most (in all cases, we list genera which together account for ≥ 50 % of the observed dissimilarity) to the observed dissimilarities are also listed. S = Suro, H = Haghani, D = Dolat Park and T = Terminal. 50 m = closest distance to pollution source, 150 m = largest distance). Average abundances have been square-root transformed.

Genera	**Av.**	**Abund**	**Contrib%**	**Cum.%**
Suro & Haghani	Average	dissimilarity	=66.75	
Promonhystera	0.95	4.99	17.47	17.47
Daptonema	3.46	2.89	10.83	28.30
Ptycholaimellus	3.08	1.99	10.29	38.59
Paramonhystera	0.70	3.44	10.16	48.74
Terschellingia	3.49	1.83	9.09	57.83
Suro & Dolat Park	Average	Dissimilarity	=60.20	
Terschellingia	3.49	4.89	11.94	11.94
Ptycholaimellus	3.08	3.42	10.73	22.66
Daptonema	3.46	4.54	10.43	33.09
Promonhystera	0.95	1.67	7.16	40.25
Paramonhystera	0.70	1.37	5.26	45.52
Oncholaimus	1.30	0.56	5.12	50.64
Viscosia	1.25	0.40	4.94	55.57
Haghani & Dolat Park	Average	Dissimilarity	=65.35	
Promonhystera	4.99	1.67	17.29	17.29
Terschellingia	1.83	4.89	14.81	32.10
Paramonhystera	3.44	1.37	11.75	43.85
Ptycholaimellus	1.99	3.42	11.11	54.96
Suro & Terminal	Average	dissimilarity	=63.70	
Ptycholaimellus	3.08	5.34	12.93	12.93
Terschellingia	3.49	4.78	11.62	24.55
Daptonema	3.46	2.79	10.54	35.09
Paraethmolaimus	1.28	1.14	6.85	41.94
Promonhystera	0.95	0.63	4.33	46.27
Oncholaimus	1.30	0.32	4.12	50.39
Viscosia	1.25	0.14	4.10	54.50
Haghani & Terminal	Average	Dissimilarity	=72.06	
Promonhystera	4.99	0.63	16.78	16.78
Ptycholaimellus	1.99	5.34	15.31	32.09
Terschellingia	1.83	4.78	12.79	44.87
Paramonhystera	3.44	0.20	10.92	55.80
Dolat Park & Terminal	Average	Dissimilarity	54.92	

Ptycholaimellus	3.42	5.34	13.30	13.30
Terschellingia	4.89	4.78	12.49	25.80
Daptonema	4.54	2.79	11.21	37.00
Promonhystera	1.67	0.63	6.77	43.78
Bathylaimus	1.34	0.30	5.30	49.07
Eumorpholaimus	0.71	1.22	5.25	54.32
2008 & 2009	Average	dissimilarity	=65.56	
Terschellingia	2.55	4.95	13.21	13.21
Ptycholaimellus	3.54	3.37	10.93	24.14
Promonhystera	1.64	2.48	10.86	35.00
Daptonema	3.73	3.12	9.60	44.60
Paramonhystera	2.02	0.83	7.79	52.39
50m & 100m	Average	dissimilarity	=60.30	
Ptycholaimellus	4.32	2.84	13.22	13.22
Terschellingia	3.33	3.80	11.50	24.71
Promonhystera	1.18	2.49	11.12	35.83
Daptonema	4.25	3.44	11.01	46.84
Paramonhystera	1.57	1.27	8.55	55.39
50m & 150m	Average	Dissimilarity	=62.27	
Ptycholaimellus	4.32	3.21	12.08	12.08
Promonhystera	1.18	2.50	11.10	23.18
Terschellingia	3.33	4.12	10.98	34.17
Daptonema	4.25	2.58	10.84	45.00
Paramonhystera	1.57	1.44	7.85	52.85
100m & 150m	Average	Dissimilarity	= 62.76	
Promonhystera	2.49	2.50	13.30	13.30
Terschellingia	3.80	4.12	12.33	25.64
Ptycholaimellus	2.84	3.21	11.55	37.18
Daptonema	3.44	2.58	9.55	46.73
Paramonhystera	1.27	1.44	6.12	52.85

4 Discussion

The between-year differences in total nematode abundances in our study ranged from 19 % at Dolat Park to 62 % at Terminal, which is in the same range as inter-annual variabilities in abundances of higher meiofaunal taxa in a muddy intertidal sediment in South Carolina over an 11-year study period (Coull 1985, 1986), where nematode abundances varied by 0 to 60 % and by 7 to 47 % in a nearby sandy sediment. In three of the four beaches at Bandar Abbas, nematode abundance decreased in 2009, but at Terminal, an increase by 62 % was noted in 2009 compared to 2008, hence the variability observed in our study was not consistent across even nearby locations. Changes in salinity, temperature, granulometry and presence and abundance of predators, as well as the occurrence of episodic extreme events are all potential causes for year-to-year variability in total nematode abundance (Coull 1985; Eskin and Coull 1987; Olafsson et al. 2000; Vanaverbeke et al. 2000; Schratzberger et al. 2004a; Riera et al. 2011a) and could have played a role here.

4.1 Sediment granulometry and inter-annual variation in nematode assemblages

Starting with granulometry, it is noteworthy that – like in the papers on the two above-mentioned Southern Carolina estuarine locations – temporal variability in nematode abundance was considerably larger in the site with the finest sediment. Terminal had a significantly lower mean grain size and higher mud fraction than the three other beach sediments, which all had a very comparable granulometry to the sandy Californian site (Coull 1985; Coull and Dudley 1985; Coull 1986; Eskin and Coull 1987). The aforementioned studies contend that assemblages in finer sediments are largely controlled by biological interactions, whereas those in sandier sediments are more constant because of a predominant hydrodynamic control. With few exceptions (e.g. *Sphaerolaimus, Calyptronema*), predatory/omnivorous nematodes had low abundances at Terminal compared to the other beach locations, whereas the herbivorous epistrate-feeding *Ptycholaimellus* reached its highest abundance at Terminal. Xyalidae, which are believed to ingest food particles, among which microalgae, in a rather non-selective way (Wieser 1953; Moens and Vincx 1997), had their lowest proportional abundance at Terminal. These results are not necessarily consistent with other studies, where, for instance, *Daptonema* and *Viscosia* have been shown to also abound in muddy sediments (Li and Vincx 1993). This indicates specific ‘local’ effects, but with the available data, it is not possible to ascertain whether these are caused by the abiotic environment or by biological interactions.

Terminal was the only beach which did not follow the overall trend of lesser abundances and diversity in 2009 compared to 2008. In fact, Terminal showed an opposite behaviour, with increases in abundance, richness, Simpson diversity, taxonomic distinctness and taxonomic diversity in 2009, most of these being the result of increases in the stations nearest (50 and 100 m) the local sewage outlet. In the absence of measurements of environmental factors, it is difficult to explain this contrasting trend between Terminal and the other three locations. At a later sampling in August 2013, Terminal had a substantially higher mean and median grain size and lower silt and very fine sand fractions, putting it amidst the other beaches in terms of granulometry. This might either reflect a long-term change or short-term fluctuations (see e.g. (Herman et al. 2001). In either case, if such a shift would have occurred between the 2008 and 2009 samplings, even if only partly, it could explain the observed 'positive' change in diversity and abundance of nematode assemblages (Gheskiere et al. 2005; Moens et al. 2013). Unfortunately, we have no sediment granulometry data from the 2009 sampling.

4.2 Climatological effects on inter-annual variation in nematode assemblages

Different climatological conditions in the weeks preceding the 2008 and 2009 sampling events could have caused differences in environmental factors which might in turn have interfered with biological interactions. Dominant genera such as *Ptycholaimellus* and *Daptonema* may benefit from the nutrient-rich inputs from the local sewage outlets (Sahraean et al. 2017a, chapter 2 of this PhD thesis). In 2009, the precipitation during the month of our sampling was 2.6 times higher than in 2008, whereas in the three months prior to sampling, rainfall was essentially zero in both years (supplementary table, S3-2, source: meteorological data from the official weather station in Bandar Abbas city, http://www.hormozganmet.ir). Increased precipitation could increase runoff from small rivers as well as sewage outlets into the beach and adjacent coastal area, and as such may lead to substantial increases in nutrient and organic matter availability, which in turn can stimulate more microphytobenthos and microbial productivity, leading to a higher food availability for algal- and bacterial-feeding nematodes (Somerfield et al. 2003; Pinto and Bemvenuti, 2006; Van Colen et al., 2009). If sewage runoff increased with the increased precipitation, we may expect (1) that sewage effects were more spread out over a larger beach area, thus overruling differences between stations at increasing distances from local sewage inputs; and (2) that differences between differently impacted beaches may have become smaller when higher amounts of sewage entered coastal waters and spread out over nearby beaches. Although this explanation cannot be unambiguously tested, it is thought-provoking that this was exactly the pattern we observed: spatial differences in nematode assemblages between beach locations and between stations within a beach were generally more pronounced in 2008 than in 2009 (Fig. 3-5).

More specifically, Haghani and to a lesser extent Suro and Terminal are beaches with pronounced local inputs of domestic sewage. Haghani not only harboured the lowest nematode abundances in the station nearest pollution in both years, it was also the least diverse location in terms of taxonomic diversity and distinctness and of Simpson diversity in both years. These data point at Haghani as a consistently impacted location in both years, whereas Suro became visibly impacted only in 2009. The dominant currents in the Strait of Hormuz run from south (in front of the northernmost tip of the United Arab Emirates) to north and then from east to west along this stretch of the Iranian coastline (Reynolds 1993). Given the small distances (< 5 km) between the beaches in this study (see fig. 1 in Sahraean et al. 2017a., chapter 2 of this PhD thesis), an impact of Haghani on its western ‘neighbour’ location Suro may also play a role. Such neighbor effects may also explain the trend of decreasing abundance and diversity in 2009 at Dolat Park, the westernmost of our sampling locations, and potentially under the influence of the sewage outlets of the three other beach locations.

Substantial differences in precipitation in the weeks preceding the two sampling events would likely have caused more pronounced fluctuations in salinity on intertidal beaches. Such episodic fluctuations may, for instance, trigger temporary downward migration of nematodes (Steyaert et al. 2001) or overall reductions in nematode abundance (Govindankutty and Nair 1966). The magnitude and frequency of such fluctuations likely vary as a function of precipitation frequency and intensity. Given that our faunal analysis was restricted to the upper 5 cm of the sediment, such downward shifts of nematode distribution in beach sediments could cause an ‘apparent’ decrease in abundance and/or diversity.

Short-term and longer-term vertical migrations of nematodes in sediments may also be caused by other climatological factors. Meteorological data from the official weather station in Bandar Abbas (http://www.hormozganmet.ir) indicate some differences in temperature between the two years of sampling. Although the average temperature of the 5 upper cm of the sediment during the month of sampling was identical in both years (23 °C), the average temperatures of the last, beforelast and third last month before our sampling in 2008 were higher in 2009 than in 2008 (S3-2). With average temperatures well in excess of 30 and even 35 °C, peak temperatures at the sediment surface during daytime low tides will have been even higher, and would likely have exceeded the upper tolerance limits of many nematode genera for at least several short episodes (Heip et al. 1985). Upper lethal temperatures of beach meiofauna may exceed the maximal temperatures experienced in their habitats by only a fraction (Wieser et al. 1974). Hence, even a relatively small difference in maximal average temperatures of 2 °C and accompanying higher peak temperatures may challenge the physiological tolerance limits of many species (Wieser et al. 1974; Wieser and Schiemer 1977), causing significant reductions in population abundance and/or shifts in, or contractions of their natural distribution. Wieser & Schiemer (1977) give the example of *Theristus floridanus* on a Bermudan beach which is found subtidally in summer but extends its range well into the intertidal in winter. Moens et al. (2006) demonstrated that even moderate peak temperatures at the sediment surface during low-tide exposure would force nematodes with a low tolerance for elevated temperatures to move deeper down in the sediment. Such phenomena may have contributed to the generally lower diversity in the top-5 cm of sediment in 2009. On the other hand, the total number of genera recorded in 2009 was not lower than in 2008, and nematode diversity decreases in 2009 were not consistent across all locations and stations, which contradicts the idea of temperature as a main driver of the observed differences in nematode assemblage patterns between 2008 and 2009.

4.3 Nematode genus composition and biological interactions

The observed shifts in nematode assemblages obviously reflect changes in genus composition. Resampling the same locations in 2009 yielded 22 additional genera which had not been encountered in 2008, but these 22 genera together comprised no more than 6.5 % of the total nematode abundance. This strengthens the picture of beach nematode assemblages characterized by a high dominance of a few (4 to 5) genera and a substantial ‘tail’ of rare genera, a pattern which may reflect the combined effects of natural and anthropogenic stressors (Vezzulli et al. 2008; Moreno et al. 2009; Sahraean et al. 2017a). Similar dominance patterns are not uncommon for the swash/breakers zone and have been explained in relation to the hydrodynamic disturbance in this zone of beaches (Gheskiere et al. 2005). However, none of the dominant genera in our beaches exhibit prominent morphological adaptations to hydrodynamic disturbance.

The same few genera strongly dominated assemblages in both years, but their relative abundances changed across locations, distances to local pollution and years. The most consistent pattern was obtained for *Terschellingia*, which generally increased in abundance in 2009 compared to 2008. *Terschellingia* is widely distributed in organically enriched sediments, showing high tolerance to a variety of pollution types as well as to hypoxic and anoxic conditions (Heip et al. 1985; Somerfield et al. 2003; Pinto and Bemvenuti 2006; Schratzberger et al. 2006; Moreno et al. 2008a). Its higher abundance in 2009 is consistent with the hypothesis of increased nutrient and organic matter inputs as a result of enhanced sewage discharge, and accompanying impacts on sediment oxygenation. In this context, the decrease of *Paramonohystera* in 2009 was unexpected, because at least the species *P. wieseri* is also very tolerant of hypoxic conditions (Wieser and Schiemer 1977). The other three dominant nematode genera exhibited rather inconsistent trends across locations, stations and/or years, although *Ptycholaimellus* generally displayed highest abundances in stations closest to local pollution sources. This could be explained by a stimulatory effect of nutrient inputs on the productivity of microphytobenthos, the prime food source of this genus (Moens and Vincx 1997; Van Colen et al. 2009). However, the same distance-to-pollution effect was not consistently observed for *Daptonema*, a genus which is also known to feed at least in part on microphytobenthos (Nehring 1992; Moens and Vincx 1997; Moens et al. 2014).

Microphytobenthos can be an important carbon source to sandy beach nematodes (Maria et al. 2011a; Maria et al. 2012). Different primary consumer species may occupy partly different vertical or horizontal niches to minimize competition and to escape from predatory nematodes living in the upper sediment layers (Maria et al. 2012). Hence, in addition to classical bottom-up effects determined by food availability, horizontal interactions among consumers may also contribute to the observed assemblage composition. Biological interactions of particular interest for intertidal nematode assemblages are, however, more top-down. Seasonal fluctuations in meiobenthic abundance at the muddy South Carolina tidal flat were explained at least in part by the yearly influx of juvenile spot which feed to a significant extent on meiofauna (Coull 1985; Eskin and Coull 1987; Smith and Coull 1987). Li et al. (1996) correlated nematode biomass fluctuations, particularly for larger-bodied species, to variations in the abundance of macrobenthos. Some studies on the macrobenthos from the beaches used in our work are available, but most are either non-quantitative or only focus on a specific group of macrobenthos at one or a few of our beach locations. Among the macrofauna which may affect meiobenthos through predation or physical interference are Glyceridae, Nephtyidae and Nereididae (Salehi Farsani 2007; Alimomohammadi 2009; Ejlali Khanaghah et al. 2015). Nephtyidae in particular were prominently present in Dolat Park and Terminal. *Nephthys* feed on smaller invertebrates and therefore this predation might be of importance to the observed nematode abundances and genus composition (Caron et al. 2004; Tue et al. 2012). In 2008, Glyceridae were most prominent in Terminal but absent from other beaches. Yet, data from 2009 are lacking. It is therefore currently not possible to determine a potential impact of macrobenthos on the variations in nematode abundance and assemblage structure.

4.4 Episodic extremes as a possible cause for inter-annual variability in nematode assemblages

Finally, episodic extremes or catastrophic events may be important drivers of short-term benthic assemblage structure (Thrush et al. 2003; Balthis et al. 2006; Negrello Filho and Lana 2013). Examples of extreme events with potential relevance to our results include oil spills, a major storm event, and the largest and longest-lasting dinoflagellate bloom ever recorded in the area. In 2007, an area of 800 km^2 was contaminated near Bandar Abbas as a result of oil spills along the Strait of Hormuz (Subanthore 2011). Whether this affected our sampling locations, and whether any such effects persisted into 2008 is unclear. However, levels of hydrocarbon pollution throughout the waters of the Persian Gulf are constantly high (Tehrani et al. 2012; Mohebbi Nozar et al. 2014) and may well contribute to the overall moderate to low meio- and macrobenthos diversity in the area. Phytoplankton blooms in the PG in the period of our research have been attributed to strong storm-induced water mixing (Wang and Zhao 2008). In addition, a massive bloom of the dinoflagellate *Cochlodinium polykrikoides* started in the south of the Strait of Hormuz in August 2008; by end of September, it had reached the coasts of Bandar Abbas, extending further and increasing in abundance for a period of over nine months (Hamzehei et al. 2012). Such blooms are mainly driven by industrial and urban sewage discharges into the coastal water (Hamzehei et al. 2012). As a consequence, highest chl *a* concentrations are typically recorded in front of the crowded industrial cities along the Strait of Hormuz such as Bandar Abbas, which is exactly at the heart of our sampling area. Whether, when and to what extent such events influenced the patterns we observed remains unclear.

4.5 Nematodes as environmental indicators in intertidal areas in the Strait of Hormuz

Whereas in terrestrial systems, the bio-indicator potential of nematode assemblages has been optimally exploited with the development of an array of indices (Bongers 1990; Bongers and Ferris 1999; Ferris et al. 2001) and indicator groups (e.g. the cp-1 nematodes as indicators of organic or nutrient enrichment), this is much less the case for aquatic and marine nematodes. This arguably has to do in part with a lesser knowledge, on average, of life-history information, feeding ecology and other aspects relevant to nematode responses to disturbance (Moens et al., 2013). Moreover, we should not overestimate the success of nematodes as bio-indicators in terrestrial soils, particularly when exiting agricultural soils: quite a few studies have published data which go against index-based expectations (Yeates et al. 1994; Heininger et al. 2007), and a detailed analysis of nematode assemblage composition may still provide the best information on environmental impacts, better than both the 'ecological indices' and diversity indices (Martinez et al. submitted).

Many marine studies using nematodes as indicators of environmental impacts have indeed provided detailed analysis of assemblage composition and in some cases compared these to, and found them superior to for instance the maturity index (e.g. Materatski et al. 2015). Based on the 2008 data of the present study, assemblage composition data allowed to discriminate the most and least impacted beach location, and at some locations also allowed to identify a distance effect from local pollution sources. Complementary information from total abundances and from diversity indices pointed to essentially the same structure in the dataset, and different diversity indices mostly pointed in the same direction, suggesting that abundance, diversity and assemblage composition data could all be successfully used to pinpoint local pollution effects.

However, the major differences between the spatial structure observed in 2008 and in 2009 casts serious doubts on the usefulness of nematode assemblages to pinpoint local pollution effects in beaches of the Strait of Hormuz. Haghani still came out as probably the most affected location in 2009, but other locations exhibited contrasting trends between 2008 and 2009. As a result, it is mainly the combination of a moderate local diversity with a (very) high dominance of a limited number of tolerant taxa which can be taken as a kind of bio-indicator. However, these features did not discriminate much among locations and distances to pollution, suggesting they are more informative about the overall stressful environment of beaches in the Strait of Hormuz than about specific local disturbances. The ecology and indicator value of these few dominant genera is discussed further in the general discussion (chapter 5).

5 Conclusion

The overall low diversity of nematodes indicates that the entire area experiences substantial stress, either from natural factors, anthropogenic influences or both. The spatial structure (between locations and between stations within locations) observed in 2008 largely disappeared in 2009. The large between-year turnover in rare genera has important repercussions for proper estimates of benthic biodiversity. At the same time, knowledge about temporal variability in assemblage abundance and structure is important for a proper use of (changes in) nematode community structure for bio-indicator purposes (discriminating natural from pollution-induced variability).

Supplementary tale S3-1. Genus composition and relative abundances (%) of nematodes at the four sampling locations of two different year (2008 and 2009). data are means ±1SE of 3 stations per location, with 3 replicates per station for each year separately.

	Suro 2008	Suro 2009	Haghani 2008	Haghani 2009	Dolat Park 2008	Dolat Park 2009	Terminal 2008	Terminal 2009
Terschellingia	1.08±0.5	43.5±6.6	1.7±1.7	16.4±6.6	23.01±7	38.8±10.8	30.6±9.5	25.5±7.7
Ptycholaimellus	9.2±4	22.2±7.6	2.8±1.3	16.5±8.7	23.9±7.9	8.6±3.5	44.3±9.5	27.7±8.1
Daptonema	32.2±7.7	6±2.6	13,2±6.1	13.1±4	28.05±7.2	21.1±5.6	7±3.1	15.2±5.2
Promonhystera	0.8±0.4	7.6±5.6	38.1±13	42±12.1	2.7±1.3	11±7	0.1±0.1	3.5±2.1
Paramonohystera	3.02±2	0.7±0.7	35.08±8.4	5.4±3.5	2.7±1.2	8.5±5.3	0.7±0.5	0±0
Paraethmolaimus	13.1±6.9	2.1±2.1	1.1	0.1±0.1	0.1±0.1	0.1±0.1	1.8±0.6	3±1.3
Eumorpholaimus	1.4±0.5	0.1±0.1	0±0	1.3±1.2	4.4±2.6	0.1±0.1	5.2±2.6	1.6±0.9
Oncholaimus	7.4±3.2	1.2±0.8	1,6±1	0.1±0.1	0.2±0.2	2.2±1.4	0.2±0.1	0.7±0.5
Sabatieria	0±0	7±3.6	0±0	3.4±1.9	0.2±0.1	0±0	0.4±0.1	1.6±1
Viscosia	6.5±2.3	1.3±1.1	1.8±1	0.2±0.2	0±0	1.2±0.4	0±0	0.3±0.2
Bathylaimus	0.65±0.3	0.2±0.1	0±0	0±0	2.66±1.5	6.7±4.4	0.2±0.1	0.7±0.5
Monhystrella	0±0	0±0	0±0	0±0	0±0	0±0	0±0	10.3±7.2
Odontophora	7.4±3.2	0±0	1.6±1	0±0	0.2±0.2	0±	0.2±0.1	0±0
Theristus	3.5±1.4	0±0	2.46±1	0±0	2.7±1.2	0±0	0.5±0.2	0±0
Metoncholaimus	6.9±3.7	0±0	0,4±0.2	0±0	0.1±0.1	0.6±0.4	0±0	0±0
Eleutherolaimus	1.1±0.7	0±0	0±0	0.5±0.4	0.6±0.6	0.1±0.1	4.07±1.8	0.2±0.2
Desmodora	0±0	4.4±4.4	0±0	0±0	0±0	0±0	0±0	0±0
Metalinhomoeus	0±0	0±0	0±0	0±0	2.4±1.8	0±0	1.4±1	0±0
Rhynchonema	3.6±2.2	0±0	0±0	0±0	0±0	0±0	0.1±0.1	0±
Spilophorella	0.1±0.1	0.1±0.1	0±0	0±0	2.04±1.3	0±0	0.8±0.5	0.6±0.6
Prochromadora	0,8±0.7	0±0	0±0	0±0	1.4±0.6	0±0	1.3±0.8	0±0
Onyx	1.7±0.8	0±0	0±0	0±0	0.4±0.2	0±0	0.1±0.1	0±0
Oxystomina	0±0	0.3±0.3	0±0	0±0	0±0	0±0	0.1±0.1	1.5±1.5
Dichromadora	0±0	0±0	0±0	0±0	0±0	0±0	0±0	1.8±1.8
Sphaerolaimus	0±0	0.8±0.8	0±0	0±0	0±0	0±0	0±0	0.6±0.4
Paracanthonchus	1.3±0.8	0±0	0±0	0±0	0±0	0±0	0±0	0±0

Synonchium	1.3±0.7	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Calyptronema	0±0	0.3±0.3	0±0	0±0	0±0	0±0	0±0	0.3±0.2
Cyatholaimidae sp.	1.2±1.2	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Metachromadora	0.6±0.4	0±0	0±0	0±0	0.5±0.3	0±0	0±0	0±0
Deontolaimus	0±0	0±0	0±0	0±0	0±0	0±0	0±0	1.1±0.7
Symplocostoma	0±0	1.1±1.1	0±0	0±0	0±0	0±0	0±0	0±0
Camacolaimus	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.8±0.8
Haliplectus	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.8±0.6
Nygmatonchus	0.5±0.3	0±0	0,1±0.1	0±0	0±0	0±0	0.1±0.1	0±0
Chromadorina	0.35±0.2	0±0	0,1±0.1	0±0	0.1±0.1	0±0	0±0	0±0
Axonolaimus	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.5±0.5
Thalassomonhystera	0±0	0±0	0±0	0±0	0.4±0.2	0±0	0±0	0±0
Xyalidae sp.	0±0	0±0	0.3±0.2	0±0	0±0	0±0	0.1±0.1	0±0
Pomponema	0±0	0±0	0±0	0±0	0±0	0.4±0.3	0±0	0±0
Tripyloides	0±0	0±0	0±0	0±0	0.2±0.1	0.1±0.1	0±0	0±0
Enoploides	0±0	0±0	0,1±0.1	0±0	0.2±0.2	0±0	0±0	0±0
Phanoderma	0.1±0.1	0±0	0±0	0±0	0±0	0±0	0±0	0.2±0.2
Chromadorella	0±0	0±0	0±0	0±0	0.2±0.2	0.1±0.1	0±0	0±0
Marylynnia	0±0	0.3±0.2	0±0	0±0	0±0	0±0	0±0	0±0
Prochromadorella	0±0	0±0	0±0	0.2±0.2	0±0	0±0	0±0	0±0
Spirinia	0±0	0±0	0±0	0.2±0.2	0±0	0±0	0±0	0±0
Tubolaimoides	0±0	0±0	0±0	0.2±0.2	0±0	0±0	0±0	0±0
Polysigma	0±0	0±0	0±0	0±0	0±0	0±0	0.2±0.2	0±0
Pseudolella	0±0	0.2±0.2	0±0	0±0	0±0	0±0	0±0	0±0
Chromadorita	0.23±0.2	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Leptolaimidae sp.	0.2±0.2	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Longicyatholaimus	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.2±0.2
Cyatholaimus	0±0	0.1±0.1	0±0	0±0	0±0	0±0	0±0	0±0
Hopperia	0±0	0.2±0.1	0±0	0±0	0±0	0±0	0±0	0±0
Araeolaimus	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.1±0.1

Camacolaimus	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.2±0.1
Halichoanolaimus	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.1±0.1
Chromadora	0±0	0±0	0±0	0±0	0.1±0.1	0±0	0±0	0±0
Doliolaimus	0±0	0±0	0±0	0±0	0±0	0±0	0.1±0.1	0±0
Hypodontolaimus	0.1±0.1	0±0	0±0	0±0	0±0	0±0	0±0	0±0

Supplementary tale S3-2 monthly amount of precipitation and mean temprature of 5 upper cm of the sediment measured at Bandar abbas of two different year (2008 and 2009)

	Precipitation (mm)		**Mean temp. of 5 upper cm of the sediment (°C)**	
	2008	**2009**	**2008**	**2009**
January	23.6	1.6	20.9	21.6
February	13.8	4.1	21	23.9
March	0	15.8	25.9	28
April	0	135.7	28.4	25.1
May	0	0	32.2	33.2
June	0	0	34.9	36.9
July	0	0	36.2	39.1
August	2.8	0	37.7	39
September	0	0	36.6	38.1
October	0	0	33.7	34.3
November	0	0	28.3	29.9
December	**10.9**	**28.7**	**23.1**	**23.1**

Chapter 4

Lack of population genetic structure in the marine nematodes *Ptycholaimellus pandispiculatus* and *Terschellingia longicaudata* in beaches of the Strait of Hormuz, Iran

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Lack of population genetic structure in the marine nematodes *Ptycholaimellus pandispiculatus* and *Terschellingia longicaudata* in beaches of the Strait of Hormuz, Iran

This chapter is adapted from:

Sahraean, N., J. Van Campenhout, A. Rigaux, H. Mosallanejad, F. Leliaert & T. Moens, 2017b. Lack of population genetic structure in the marine nematodes *Ptycholaimellus pandispiculatus* and *Terschellingia longicaudata* in beaches of the Persian Gulf, Iran. Marine Ecology accepted.

Abstract

We investigated genetic diversity and population genetic structure of two common benthic nematode species, *Ptycholaimellus pandispiculatus* and *Terschellingia longicaudata*, from sandy beaches in the area of Bandar Abbas (Iran), Persian Gulf. Based upon partial mitochondrial COI gene data, 17 and 2 haplotypes were found for *P. pandispiculatus* and *T. longicaudata*, respectively. Analysis of molecular variance (AMOVA) did not reveal a significant population-genetic structure for either species. The absence of genetic structuring indicates substantial dispersal and gene flow in our study area. To assess the species structure *of T. longicaudata* at a larger geographic scale, we compared 18S rDNA and COI sequences from Iran and the Scheldt Estuary in The Netherlands to ascertain whether they truly belong to the same species. Our data confirmed previous studies that *T. longicaudata* likely constitutes a complex of multiple cryptic species, with one of these species having a (near) cosmopolitan distribution.

Keywords: cosmopolitanism; cryptic species; dispersal; gene flow; marine nematodes; population genetics.

1 Introduction

Connectivity among marine populations is determined by the dispersal capacities of adults and/or larvae, as well as by the ‘permeability’ of the environment. Dispersal is important for persistence of species because it allows organisms to escape from unsuitable environmental conditions, avoid competition and increase their distribution range. Dispersal distances and directions have a profound effect on gene flow and genetic differentiation within species (Froukh and Kochzius 2007). Species with low dispersal capacities are expected to have a stronger population genetic structure than species with high dispersal abilities (Avise 2004). Both physical (e.g. ocean currents and habitat characteristics) and biological (e.g. spawning season, predation, larval and adult behavior) factors affect dispersal, and consequently also population genetic structure, of species in marine environments (Hohenlohe 2004; Derycke et al. 2013). Sandy beaches are dynamic and physically stressful environments, principally driven by the forces of waves, tides and sediment movements (McLachlan 1983; Short 1999; Rodil and Lastra 2004). These factors are important in shaping population structure, dynamics and connectivity of their inhabitants.

Free-living marine nematodes are the most abundant and species-rich metazoan fauna in sandy beaches and most other marine soft sediments, and are characterised by a wide variety of morphologies, life histories and feeding strategies (Heip et al. 1985; Giere 2009). Because most marine nematodes have endobenthic life styles, and because they lack planktonic or pelagic dispersal stages, marine nematodes are generally assumed to have limited dispersal capacity. On the other hand, they may be able to disperse passively through movement of sediments, currents, and ballast water of ships (Palmer 1988; Radziejewska et al. 2006; Boeckner et al. 2009). In addition, some nematode species may actively emerge into the water column and swim over short distances (Jensen 1981; Schratzberger et al. 2004b).

Many genera of marine nematodes have cosmopolitan or nearly cosmopolitan distributions. To some extent, this was also believed to hold at the species level (Bhadury et al. 2008), at least based on morphological criteria for species identification. More recently, however, considerable cryptic diversity has been uncovered in a number of coastal nematode species (Derycke et al. 2005; Derycke et al. 2007a; Derycke et al. 2010a; Armenteros et al. 2014), raising the question as to whether alleged cosmopolitan morphospecies in fact represent multiple cryptic species with more restricted geographical ranges. For example, a population-genetic study based on the mitochondrial COI locus of the nematode species complex

Litoditis marina revealed that only one out of ten cryptic species had a transatlantic distribution, whilst the remaining species had narrower geographical ranges (Derycke et al. 2008b). Since *L. marina* usually lives in association with macroalgae and has been shown to raft on drifting algae, its chances of long-distance transport are expected to exceed those of most benthic marine nematodes (Derycke et al. 2008).

Cryptic species diversity was also uncovered in the endobenthic nematode *Terschellingia longicaudata* based on nuclear 18S rDNA sequence data (Bhadury et al. 2008), with one species clade showing a very broad geographical distribution (including samples from Europe, the Atlantic coast of Mexico, and Malaysia). These results were not anticipated because *T. longicaudata* typically frequents hypoxic or anoxic (and hence deeper) layers of sediment and would, therefore, not be expected to rapidly emerge from sediments and passively disperse over larger distances. Since 18S rDNA sequences do not always show sufficient differentiation between closely related nematode species, the conclusion of Bhadury et al., (2008) should be confirmed using more variable marker genes. Nevertheless, the alleged cosmopolitan distribution of an endobenthic species with limited dispersal capacity is thought-provoking. Indeed, several recent studies on population-genetic structure in coastal and estuarine nematodes have invariably highlighted a significant population-genetic structure at local scales of 100 km and less in species which are considered more prone to passive dispersal (*Litoditis marina*, *Halomonhystera disjuncta* and *Thoracostoma trachygaster*) because of their association with macroalgae (Derycke et al. 2005; Derycke et al. 2007b; Derycke et al. 2010a; Derycke et al. 2013). The vast majority of marine nematode species are, however, (endo)benthic and may, depending on their habitat and position in the sediment, be more or less prone to erosion and thus passive dispersal.

In this study, we investigated the population genetic structure of two abundant benthic nematode species from sandy beaches in the area of Bandar Abbas (Iran), Persian Gulf: *Ptycholaimellus pandispiculatus* Hopper, 1961, and *Terschellingia longicaudata* de Man, 1907. *Terschellingia longicaudata* (Linhomoeidae) is common in intertidal and shallow subtidal sediments that are rich in organic matter and have sharp chemoclines (Heip et al. 1990; Schratzberger et al. 2006). Its peak abundances are typically reached below the top 2 cm of sediment, and the nematode can withstand hypoxia/anoxia (Steyaert et al. 2007). Carbon stable isotope ratios of *T. longicaudata* from seagrass, mangrove and estuarine tidal flat sediments have demonstrated a clear trophic link with chemoautotrophic bacteria (Vafeiadou et al. 2014). In contrast, species in the genus *Ptycholaimellus* (Chromadoridae) are epigrowth feeders that probably derive most of their nutrition from microalgae such as diatoms (Moens and Vincx, 1997). Members of this genus are particularly common in the surface layer (upper 1 cm) of intertidal sediments (Commito and Tita, 2002; Steyaert et al. 2003; Van Colen et al. 2009), where microphytobenthos tends to concentrate. Because of their nearly epibenthic life style, they can be expected to be more susceptible to resuspension and passive transport compared to the endobenthic *Terschellingia* (Commito and Tita 2002).

Here, we used the mitochondrial cytochrome oxidase c subunit 1 (COI) gene to tested if the following assumptions would hold: 1) population-genetic structure of both species would be limited on a scale of tens of kilometres because of the 'homogeneity' of the study area, lack of clear dispersal barriers, and strong hydrodynamics of the beaches, which may lead to regular erosion and passive transport, thus facilitating gene flow; 2) *Ptycholaimellus*, living at the surface of the sediment, would show less population genetic structure compared to the deeper-living species *Terschellingia*. In addition, 3) we compared 18S rDNA and COI gene sequences from *T. longicaudata* populations from the Persian Gulf and from an estuarine tidal flat of the Scheldt Estuary, The Netherlands, to ascertain whether they truly belong to the same species or, alternatively, their morphology is hiding cryptic diversity. Phylogenetic affinities of *T. longicaudata* from the Persian Gulf were assessed based on 18S phylogenetic analysis of published sequences sampled from a broad geographical range.

2 Materials and methods

2.1 Study area

Samples were collected at low tide in September 2012 from the mid-tidal level at 10 beach locations along the Strait of Hormuz, Persian Gulf, spanning 52 km of Iranian coastline (Fig. 4-1). Stations 1 to 4 are located immediately in front of the city of Bandar Abbas and are subject to variable types and degrees of anthropogenic disturbance (sewage inputs, tourism); they correspond to the beaches of Suro, Haghani, Dolat Park and Terminal, respectively (pers. obs.). The other beaches are located eastward of Bandar Abbas and – at least at first glance – are less subject to anthropogenic impacts.

For *T. longicaudata*, we also sampled the Paulina intertidal mudflat in the polyhaline reach of the Scheldt Estuary, The Netherlands, in July 2014. Details on the Paulina tidal flat are given in Gallucci et al. (2005); the sampling station corresponded to station H4 in (Cnudde et al. 2015).

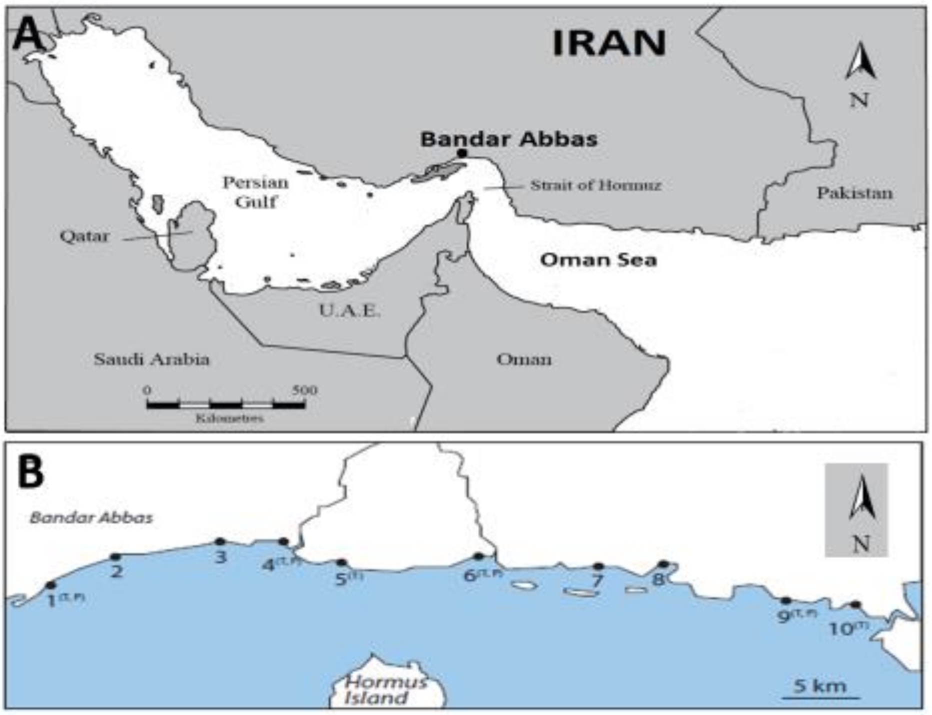


Fig 4-1. Map showing the sampling locations of *Terschellingia longicaudata* (six locations, indicated with "T") and *Ptycholaimellus pandispiculatus* (four locations, indicated with "P"). Insufficient individuals for population genetic analysis (less than 15 per species) were obtained from the other beach localities. Location 1=Suro, 2=Haghani, 3=Dolat Park and 4=Terminal as in chapters 2 and 3 of this PhD.

2.2 Sampling

From each sampling location, five samples were collected using 3.5-cm diameter PVC cores pushed into the sediment down to a depth of 5 cm. These samples were pooled into a single sample per location, preserved in DESS (Yoder et al. 2006). Nematodes were extracted from the sediments by vigorous washing of the samples with a jet of tap water, followed by decantation over a sieve with a mesh size of 38 µm. This procedure was repeated at least eight times, and the fraction collected on the sieve was subsequently centrifuged (3000 rpm, 12 min) in the colloidal silicagel Ludox™ at a specific gravity of 1.18. Supernatant was decanted over a 38 µm sieve and collected in a small amount of DESS. This procedure was repeated three times and the three supernatants of a sample were pooled. Sufficient individuals of *T. longicaudata* and *P. pandispiculatus* (at least 12 per species and sampling location) were obtained from six and four beaches, respectively (Fig. 4-1). 50 *T. longicaudata* were collected from the Paulina mudflat.

2.3 Morphological identification and vouchering of specimens

P. pandispiculatus and *T. longicaudata* were handpicked from each sample using a fine needle under a stereomicroscope. They were rinsed three times with sterile distilled water to remove DESS. Each specimen was mounted individually on a temporary slide and identified using diagnostic morphological characters (Armenteros et al. 2009b) under a LEICA DMR research microscope at high magnification. Diagnostic features of *P. pandispiculatus* and *T. longicaudata* specimens were photographed using LEICA Application software (Leica DLMB; objective 100X). Vouchered specimens were carefully removed from microscopic slides and then transferred individually into an Eppendorf tube containing 20 µL worm lysis buffer (WLB) (50 mM KCl, 10 mM Tris–HCl pH 8.3, 2.5 mM MgCl2, 0.45% NP40, 0.45% Tween 20) and frozen (-20 °C) till further processing.

2.4 Choice of marker sequences

The mitochondrial cytochrome oxidase c subunit 1 (COI) gene is one of the most widely used markers for species diversity assessment and population genetic analysis in animals, and for nematodes, it has proven useful for discriminating closely related species (Derycke et al. 2010b). It has also been well documented that species delimitation based on single gene data often falls short due to gene tree–species tree incongruence, and that multiple markers increase the accuracy of species delimitation (Dupuis et al. 2012; Leliaert et al. 2014), hence the inclusion of a second marker, the 18S rRNA gene, in our analyses of the phylogenetic relationships of *T. longicaudata* from the Persian Gulf. Even though 18S is known to have lower resolution than COI when it comes to distinguishing recently diverged species, 18S has been found useful for assessing species diversity in the genus *Terschellingia* (Bhadury et al. 2008).

2.5 DNA extraction, PCR amplification and sequencing

DNA extraction followed the protocol by (Williams et al. 1992). In short, proteinase K (1 µl, 10 g/ml) was added to each tube containing a single nematode in WLB, followed by incubation at 65 °C for one hour, and denaturation of the proteinase K at 95 °C for 10 minutes. Afterwards, tubes were centrifuged at 13,200 rpm for one minute at 20 °C and stored at 4 °C.

The mitochondrial cytochrome oxidase c subunit 1 (COI) gene was amplified with primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HC02198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (621 bp) (Folmer et al. 1994) for *T. longicaudata*, and primers JB3 (5'-TTTTTTGGGCCTGAGGTTTAT-3') and JB5 (5'-AGCACCTAAACTTAAAACATAATGAAAATG-3') (380 bp) (Bowles et al. 1992) for *P. pandispiculatus*. PCR-mix was prepared for each primer set separately in total volumes of 25 µL for *T. longicaudata*, containing 16.125 µL PCR grade water, 2.5 µL buffer, 2.5 µL dye, 2 µL $MgCl_2$, 0.5 µL dNTP (10 mM each), 0.125 µL of each primer (25 µM), 0.125 µL TopTaq polymerase (Qiagen, 5 U/µl) and 1 µL (*T. longicaudata*) or 2 µL (*P. pandispiculatus*) DNA. The PCR conditions for *T. longicaudata* were: initial denaturation of 1 minute at 94 °C, 5 cycles of (94 °C for 40 s; 45 °C for 40 s; 72 °C for 45 s), 35 cycles of (94 °C for 40 s; 51 °C for 40 s; 72 °C for 45 s) and a final extension of 5 minutes at 72 °C. The PCR conditions for *P. pandispiculatus* were: initial denaturation of 5 min at 94 °C, 35 cycles of (94 °C for 30 s; 50 °C for 30 s; 72 °C for 45 s) and a final extension of 10 min at 72 °C. 4 µl of each PCR product was loaded onto 1 % agarose gels (1 % agarose gel in 0.5 × TAE buffer) with a negative control to check the quality and reliability of the PCR and the size of the amplified product. PCR products were sequenced at Macrogen Europe (Amsterdam, The Netherlands) with the forward primer using the fluorescent dye terminator Sanger sequencing method. The newly generated sequences of *Terschellingia* and *Ptycholaimellus* have been deposited in the European Nucleotide Archive (EMBL-EBI/ENA) with accession numbers LT795763-LT795772 and LT795773-LT795788, respectively."

For *T. longicaudata*, the 18S rRNA gene was amplified using two primers, MN18F (5’-CGCGAATRGCTCATTACAACAGC-3’) and Nem_18S_R (5’-GGGCGGTATCTGATCGCC-3’) (Bhadury et al., 2008). PCR-Mix was prepared in total volumes of 14.875 µl PCR grade water, 2.5 µL buffer, 2.5 µL dye, 2 µL $MgCl_2$, 0.5 µL dNTP (10mM each), 0.250 µL of each primer (25 µM) (forward and reverse primer), 0.125 µL TopTaq polymerase (Qiagen, 5U/µl) and 2 µL DNA. Final volume of the PCR-mix was 25 µl. The conditions for *T. longicaudata* from the Persian Gulf were: initial denaturation of 5 min at 95 °C, 40 cycles of (95 °C for 1 min; 54 °C for 1 min; 72 °C for 2 min) and a final extension of 10 min at 72 °C. PCR products were sequenced with forward and reverse primers.

2.6 Population genetic and phylogenetic analysis

Sequences were assembled using the SeqMan ProTM (Lasergene®, DNASTAR), and aligned using ClustalW in MEGA 6 (Thompson et al. 1994). Analysis of basis statistics (e.g. number of different haplotypes) and molecular variance (AMOVA) (Excoffier et al., 2005) was performed in ARLEQUIN (Version 3.1, 2005) (Schneider et al. 1996) to test the distribution of genetic variability among and within populations. The significance of the variance components was tested by permuting haplotypes among populations (Excoffier et al. 1992). A minimum spanning network was created using hapstar 0.7, and was adapted to incorporate the frequencies of haplotypes using Excel and PowerPoint. Uncorrected pairwise distances (p-distances) between haplotypes were calculated using MEGA 6.

Analysis of *T. longicaudata* population structure and cryptic diversity on a broader geographical scale was based on COI and 18S rDNA sequence datasets. To explore COI sequence conservation in *T. longicaudata*, amino acid sequences of the two *T. longicaudata* haplotypes were aligned and compared with COI sequences of other species of monhysterids, and representative species of the major clades of nematodes. Alignments were analyzed with Geneious v7 (Biomatters, www.geneious.com).

The COI dataset consisted of 101 sequences from the Persian Gulf and 50 sequences from the Scheldt Estuary, aligned using translation alignment in Bioedit (Hall 1999). The 18S dataset consisted of 128 sequences of *Terschellingia*, including 24 newly generated sequences from Iran, The Netherlands, and Vietnam, and 104 sequences from GenBank from various locations (United Kingdom, France, The Netherlands, Bahrain, Mexico, Malaysia and Taiwan) (Cook et al. 2005; Bhadury et al. 2006; Bhadury et al. 2008) (Supplementary table

S4-1). 18 sequences of *Cyartonema elegans*, *Daptonema procerus, D. setosum, D. normandicum, Daptonema* sp.*, Diplolaimelloides meyli, Halomonhystera disjuncta, Metadesmolaimus* sp.*, Monhystera* sp.*, Sabatieria celtica, S. punctata, Sphaerolaimus hirsutus, Theristus acer, T. agilis*, and *Theristus* sp. were selected as outgroup based on (Meldal et al., 2007). The 18S sequences were aligned using muscle (Edgar 2004). Phylogenetic trees were estimated using maximum likelihood (ML) and rapid bootstrap analysis with RAxML under the GTRCAT model via the RAxML BlackBox web-server (http://embnet.vital-it.ch/raxml-bb/) with default settings (Stamatakis et al. 2008).

3 Results and discussion

Recent molecular studies have indicated rampant cryptic species diversity in free-living marine nematodes, as well as different levels of genetic differentiation of populations at regional scales (reviewed in Derycke et al. 2013), comparable to the sampling scale of the present study (ca. 52 km of coastline). Here, we investigated genetic diversity and population genetic structure of two common benthic nematode species from sandy beaches in the Persian Gulf: *Ptycholaimellus pandispiculatus* and *Terschellingia longicaudata.*

Table 4-1. *Terschellingia longicaudata* and *Ptycholaimellus pandispiculatus*. Numbers of the different haplotypes at six beach locations in the Persian Gulf. Sufficient individuals for population genetic analysis (at least 15) of *T. longicaudata* and *P. pandispiculatus* were obtained from six and four beaches, respectively.

CO1	Haplotype	Loc1	Loc 4	Loc 5	Loc6	Loc 9	Loc 10	n
T. longicaudata	*Haplotype1*	1	0	1	0	1	2	5
	Haplotype2	16	15	18	14	16	17	96
P. pandispiculatus	*Haplotype1*	0	0	0	1	0	0	1
	Haplotype2	0	0	0	1	0	0	1
	Haplotype3	0	1	0	0	0	0	1
	Haplotype4	0	0	0	1	0	0	1
	Haplotype5	0	1	0	0	0	0	1
	Haplotype6	0	0	0	0	1	0	1
	Haplotype7	0	0	0	0	1	0	1
	Haplotype8	1	1	0	0	0	0	2
	Haplotype9	2	7	0	7	3	0	19
	Haplotype10	0	0	0	1	0	0	1
	Haplotype11	8	6	0	10	7	0	31
	Haplotype12	0	1	0	0	0	0	1
	Haplotype13	1	0	0	0	0	0	1
	Haplotype14	0	1	0	0	0	0	1
	Haplotype15	0	1	0	0	0	0	1
	Haplotype16	1	0	0	0	0	0	1
	Haplotype17	0	0	0	1	0	0	1

(n) Number of individuals.

3.1 Population genetic structure of *Ptycholaimellus pandispiculatus*

The DNA alignment of the mitochondrial COI gene fragment of the 66 individuals of *P. pandispiculatus* was 381 bp long and did not contain any insertions/deletions. 21 sites (5.5 %) were variable, six of which represented non-synonymous substitutions (i.e. resulting in amino acid changes). In total, 17 haplotypes were found (Fig. 4-2, Table 4-1). Uncorrected p-distances between haplotypes ranged between 0.003 and 0.018. Haplotypes 9 and 11 were considerably more frequent than the others, together comprising 75 % of all sequences. They were also the only ones present at all four locations. Most other haplotypes occurred as singletons or doubletons (Fig. 4-2). Location 4 had the highest amount of unique haplotypes (eight) and location 9 the least (four haplotypes). Analysis of molecular variance (AMOVA) did not reveal a significant population-genetic structure for *P. pandispiculatus* ($F_{ST} = 0.013$, $p > 0.2$) (Table 4-2). The genetic diversity found for *P. pandispiculatus* is comparable to the genetic diversity found in other marine nematodes on similar geographic scales. For example, along the Belgian North Sea coast and Scheldt Estuary (ca. 100 km), the number of COI haplotypes within single cryptic species of the *Halomonhystera disjuncta* complex ranged from 4 to 17, with intraspecific pairwise distances of 0.003-0.026 (with one exception due to a single highly divergent haplotype) (Derycke et al., 2007a). A similar number of haplotypes (15) was found within *Litoditis marina* cryptic species “PmI” along the Belgian coast, Western Scheldt and Eastern Scheldt (Derycke et al. 2005), while a higher haplotype diversity (33) was found in *Thoracostoma trachygaster* clade II, but this study covered a larger geographical scale (> 500 km) (Derycke et al. 2010a).

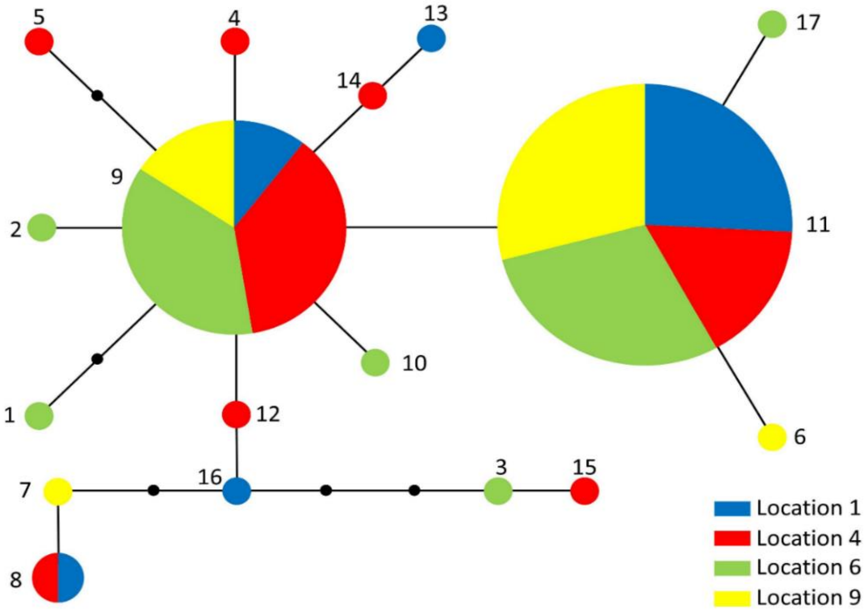


Fig. 4-2. Minimum spanning network for *P. pandispiculatus* from Persian Gulf beaches. Numbers indicate the different haplotypes, colours refer to the different locations, and the size of the circles is the frequency of sequences in different haplotypes.

We did not find indications of population genetic structuring among the four sampled locations in the Strait of Hormuz, which had interdistances of 14 to 52 km (Fig. 4-1). This indicates substantial dispersal and gene flow in our study area. Our findings contrast with some recent studies on free-living marine nematodes, where subtle to strong genetic differentiation of nematode populations was observed on similar geographic scales (Derycke et al., 2013). Similarly, low (but still significant) population genetic differentiation was observed in the Scheldt Estuary for *Bathylaimus assimilis*, which is characterized by an endobenthic life style (Derycke et al. 2013). Passive dispersal in endobenthic species is expected to be lower and population genetic structuring higher than in species associated with exposed and transient habitats such as seaweed wrack (*L. marina*, *H. disjuncta* and *T. trachygaster*) (Derycke et al. 2005, 2007a, 2010a). Rather than by its endobenthic life style, the absence of genetic structuring in *P. pandispiculatus* may be explained by passive dispersal of individuals through sediment resuspension and currents in the dynamic beach habitat (Boeckner et al. 2009; Derycke et al. 2013). Our study area represents a relatively non-interrupted environment of sandy beaches along a continuous stretch of coastline that lacks obvious dispersal barriers, and is characterized by a dominant eastward sea surface current (Reynolds 1993; Kämpf and Sadrinasab 2006). This contrasts with the study areas of some previous studies that included apparent differences in habitats such as coastal and estuarine environments (Derycke et al. 2005, 2007a). Only one prior study on nematode population genetic structuring has entirely focused on a beach habitat: *Thoracostoma trachygaster*, a species associated with macroalgae, exhibited strong population genetic differentiation along the southern Californian coast, but this coincided with well-known biogeographic barriers, such as Point Conception and the Los Angeles Region. In between these barriers, population genetic structuring was absent along large stretches of coastline (ca. 180 km) (Derycke et al. 2010a, 2013). Hydrodynamic forces of beaches result in erosion and re-suspension of sediments and enhance passive dispersal of endobenthic organisms (Palmer 1988; Gingold et al. 2010; White et al. 2010; Gingold et al. 2011; Derycke et al. 2013).

This is even more plausible for *Ptycholaimellus* species which are known as epigrowth feeders that live near the sediment surface, and are also frequently found in re-suspended sediment directly above the sediment surface (Eskin and Palmer 1985; Commito and Tita 2002).

Finally, active movement related to body morphology, swimming behaviour and feeding strategy may be an important dispersal mechanism for some free-living nematodes (Thomas and Lana 2011), for instance because nematodes which actively enter the water column have a higher probability of becoming passively transported over larger distances. *Ptycholaimellus* belongs to the same nematode family (Chromadoridae) and feeding guild as genera of which such active emergence into the water column has been observed (Jensen 1981).

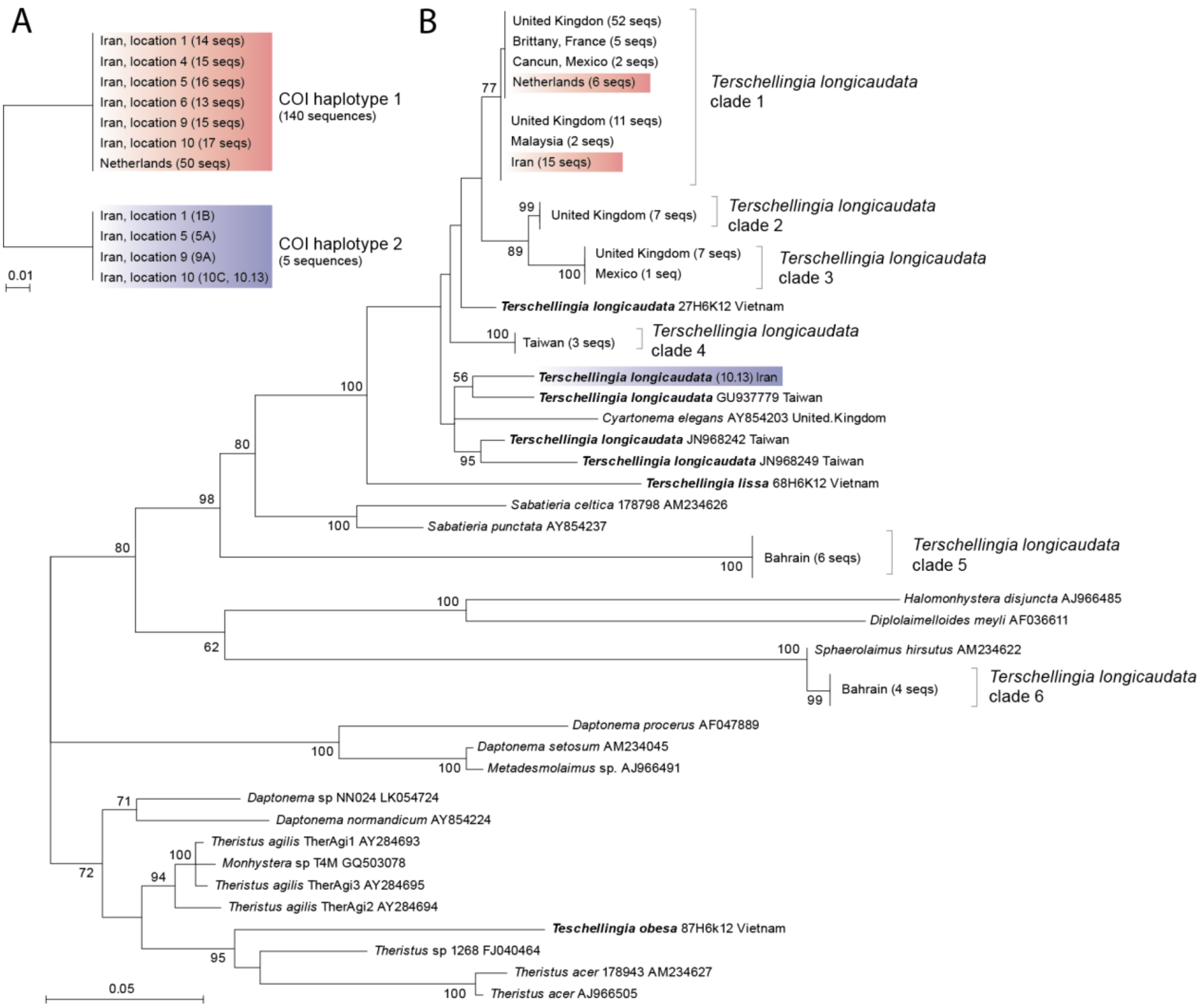


Fig 4-3. *Terschellingia longicaudata*, maximum likelihood trees inferred from (A) COI and (B) 18S rDNA sequences. ML bootstrap values (>50) indicated at the branches. The COI tree shows two divergent haplotypes: a common haplotype 1, including individuals from the Persian Gulf and the Netherlands, and a rare haplotype 2 including Persian Gulf individuals only. The 18S tree shows that both the genus *Terschellingia* and the species *T. longicaudata* are non-monophyletic, consisting of divergent clades. Sequences generated in this study are indicated in coloured background: red corresponds to specimens with the common COI haplotype 1 = 18S clade 1; blue corresponds to specimens with the rare COI haplotype 2, which formed a different branch in the 18S tree.

3.2 Population genetic structure and cryptic diversity of *Terschellingia longicaudata*

The COI alignment of the 101 individuals of *Terschellingia longicaudata* from Iran was 621 bp long and did not contain any gaps. These 101 individuals belonged to only 2 haplotypes, with 96 specimens (95.4 %) belonging to haplotype 1, and 5 specimens (4.95 %) to haplotype 2 (Fig. 4-3A, Table 4-1). The two haplotypes differed from each other in 36 nucleotides, 10 of which represented non-synonymous substitutions. Uncorrected pairwise distance (p-distance) between both haplotypes was 0.057. Haplotype 1 was found at all six locations, while haplotype 2 was found in four locations: 1, 5, 9 and 10. The sequences from the 50 individuals from The Netherlands were all identical to haplotype 1. Clearly, AMOVA did not reveal any genetic differentiation among the Persian Gulf populations ($F_{ST} = 0.027$, $p > 0.9$) (Table 4-2). The presence of the common COI haplotype in all sampled locations of the Persian Gulf suggests high gene flow, which is somewhat surprising given the fact that *T. longicaudata* lives endobenthic with peak abundances typically below the top 2 cm of sediment. This would suggest limited re-suspension in the water column and thus a low passive dispersal potential. However, beach hydrodynamics may regularly erode and resuspend sediments down to considerable depths (Bell and Sherman 1980).

The 18S rDNA alignment of *T. longicaudata* (including 12 outgroup taxa) was 951 bp long, including 465 (48.9 %) variable sites. The ML phylogenetic analysis was congruent with the published 18S phylogeny in Bhadury et al. (2008), and showed that the genus *Terschellingia*, as well as the species *T. longicaudata* (as traditionally circumscribed based on morphology) were non-monophyletic. At least six distinct *T. longicaudata* clades were recovered, in addition to five singleton sequences (Fig. 4-3B). One of these clades (clade 1 in Fig. 4-3B) included sequences from distant localities (Europe, Iran, Mexico and Malaysia). All individuals with the dominant COI haplotype from The Netherlands and the Persian Gulf also fall within that clade, which is consistent with the COI results which showed that the individuals from The Netherlands and the Persian Gulf belong to the same COI haplotype (haplotype 1). We were only able to obtain one partial 18S sequence from a Persian Gulf individual with the rare COI haplotype 2 (specimen 10.13). This sequence of 342 bp long differed from the common 18S sequences in 11 positions, corresponding with a p-distance of 0.033, and formed a different branch in the 18S tree that was more closely related to a *T. longicaudata* sequence from Taiwan (although strong bootstrap support was lacking) (Fig. 4-3B). The presence of two distinct clades in the Persian Gulf that were concordantly recovered by the COI and 18S data, suggests the presence of two different species in the Persian Gulf.

Although there are no universal threshold values of genetic distances for distinguishing species, the large genetic distances between the two COI haplotypes (p-distance 0.057) and between the two 18S ribotypes (p-distance 0.033) hint at the presence of two species in the Persian Gulf. The intraspecific COI variation reported for some nematode species by Derycke et al. (2005, 2010a, b), and the threshold of 5 % COI sequence divergence (Armenteros et al. 2014) supports our claim of two cryptic species of *T. longicaudata*. The genealogical concordance of the two unlinked loci provides further evidence for the existence of two distinct species (Derycke et al. 2007a; Leliaert et al. 2014).

Our data confirms the presence of one widespread cryptic species of *T. longicaudata* (18S clade 1), which has been collected from distant locations, including Europe, Iran, Mexico and Malaysia. The remaining cryptic species of *T. longicaudata* seem to have narrower geographic ranges, based on the data available. Several other cryptic species of free-living marine nematodes have been shown to have wide geographic ranges based on molecular data (Derycke et al. 2008b; Bik et al. 2010).

Admittedly, in the absence of a thorough morphological analysis of specimens of the different putative species of *T. longicaudata*, we cannot prove that they are cryptic species in the true sense of the word, meaning that they cannot be differentiated morphologically and/or morphometrically. In other marine morphospecies complexes, the discovery of substantial genetic divergence went ahead of the discovery of morphological differentiation. Using a reverse taxonomic approach, ‘cryptic’ species of the *Litoditis marina* and *Halomonhystera disjuncta* complexes could be differentiated based on a combination of morphometric measurements (Derycke et al. 2008a; Fonseca et al. 2008), whereas unique characteristics differentiated species within the *Thoracostoma trachygaster* complex (De Oliveira et al. 2012). Given the paucity of diagnostic characters and the high morphological plasticity of most species of *Terschellingia* (Armenteros et al. 2009), an integrative approach to the taxonomy of this genus, combining multilocus molecular and dedicated morphological tools, is warranted.

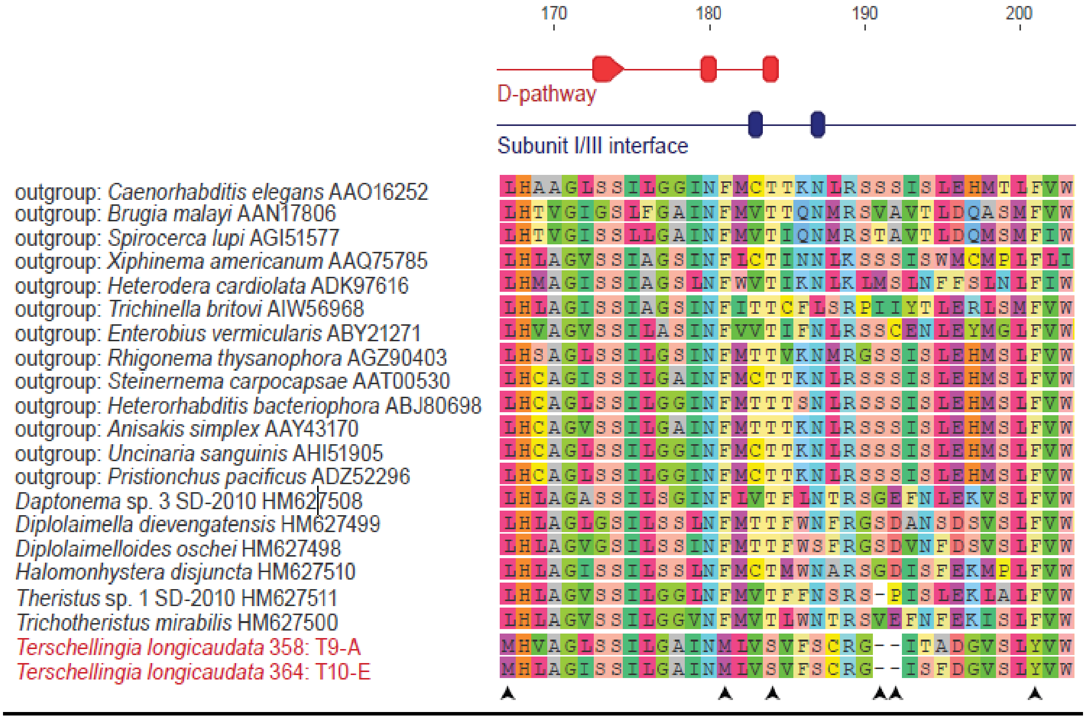
Table 4-2. AMOVA (analysis of molecular variance) table of the genetic variability among and within populations for *Ptycholaimellus pandispiculatus* and *Terschellingia longicaudata*.

Species		%	F_{ST}	p-value
P. pandispiculatus	among populations	1.30	0.013	NS
	within populations	98.70		
T. longicaudata	among populations	2.76	0.027	NS
	within populations	97.24		

NS: Not significant, P>0.1

In addition to the wide geographic range of a single COI haplotype, the lack of intraspecific genetic diversity in the COI gene could be indicative of a selective sweep, in which variation of the COI gene has been eliminated due to strong selective pressure on the COI gene itself, or due to strong natural selection on another genomic region, which eventually resulted in low genetic variation of the COI gene by genetic hitchhiking (Barton 2000). Selective pressure of the COI gene in *T. longicaudata*, a nematode that shows strong preferences for hypoxic environments, is a plausible scenario. This gene encodes the subunit 1 of the cytochrome c oxidase complex (a.k.a. respiratory complex IV), and is a key enzyme in aerobic metabolism. It is also the largest and most conserved subunit of cytochrome c oxidase (Michel et al. 1998). Subunit I contains two haem centres (haem a, which acts as an electron input device to the haem a3, and haem a3 which is part of a binuclear centre and is the site of oxygen reduction), in addition to two proton-conducting pathways (D- and K-pathway), and an electron transfer pathway (Dürr et al. 2008). The amino acid composition around these active sites may determine the affinity with oxygen, which may be more crucial for an organism living in hypoxic environments.

In order to find clues for possible positive selection of the COI gene in *T. longicaudata*, we constructed a COI amino acid alignment of the two *T. longicaudata* haplotypes, 11 other species of monhysterid nematodes, and thirteen outgroup species representing some of the main nematode lineages (Fig. 4-4, supplementary Fig S4-1). A region surrounding the end of the D-pathway (position 167-203) includes four amino acid changes and two deletions that seem to be unique in *T. longicaudata*. We did not find evidence for divergent amino acid composition of *T. longicaudata* in the two haem centres (supplementary Fig S4-1). It should be noted that taxon sampling in our alignment is low and that more extensive sampling, especially in the monhysterids, is needed to confirm these observations in order to find further indications for possible selection of COI linked to hypoxic environments.



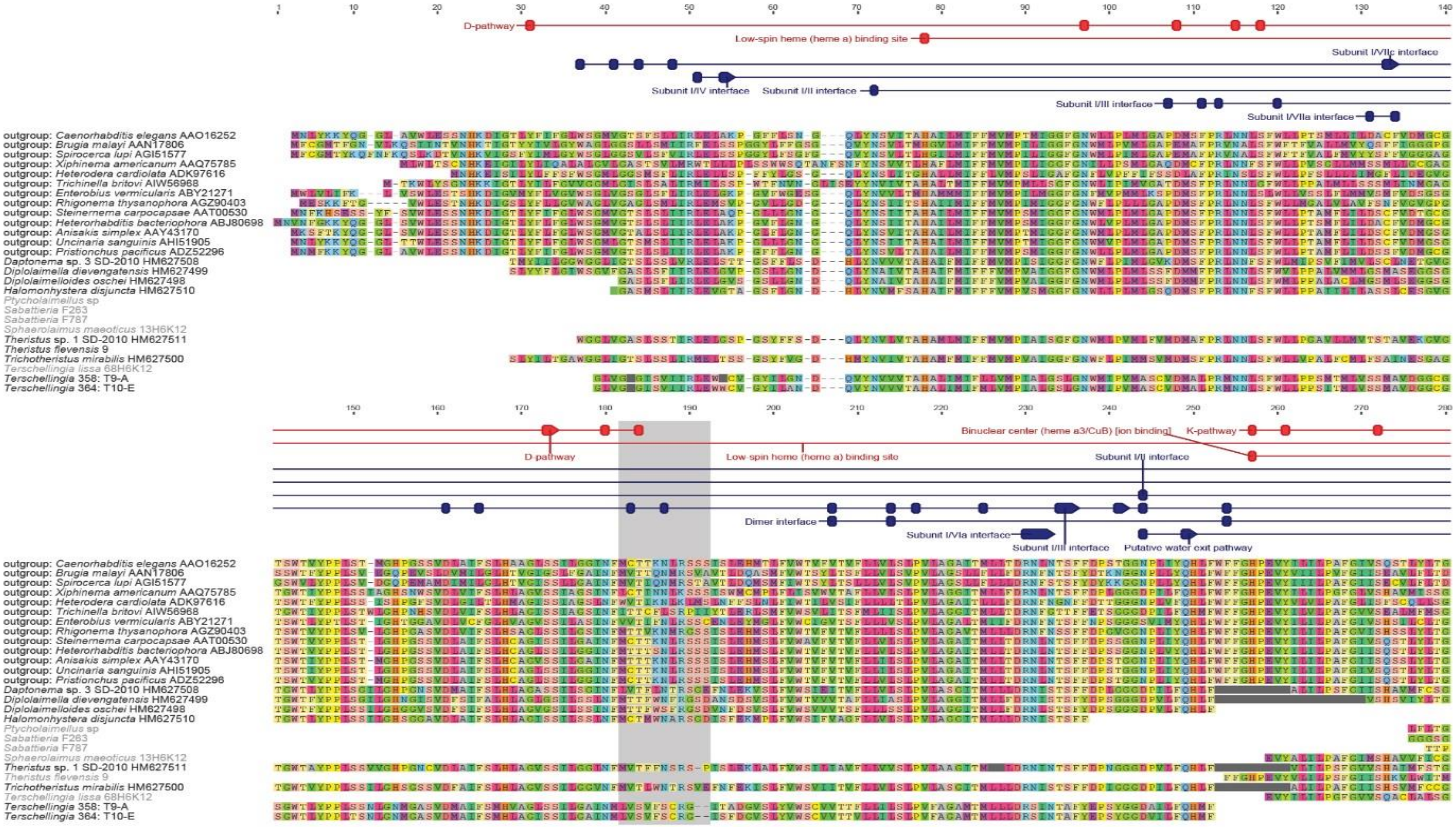
outgroup: *Caenorhabditis elegans* AAO16252	LHAAGLSSILGGINFMCTTKNLRSSSISLEHMTLFVW
outgroup: *Brugia malayi* AAN17806	LHTVGIGSLFGAINFMVTTQNMRSVAVTLDQASMFVW
outgroup: *Spirocerca lupi* AGI51577	LHTVGISSLLGAINFMVTIQNMRSTAVTLDQMSMFIW
outgroup: *Xiphinema americanum* AAQ75785	LHLAGVSSIAGSINFLCTINNLKSSSISWMCMPLFLI
outgroup: *Heterodera cardiolata* ADK97616	LHMAGISSIAGSLNFWVTIKNLKLMSLNFFSLNLFIW
outgroup: *Trichinella britovi* AIW56968	LHLAGISSIAGSINFITTCFLSRPIIYTLERLSMFVW
outgroup: *Enterobius vermicularis* ABY21271	LHVAGVSSILASINFVVTIFNLRSSCENLEYMGLFVW
outgroup: *Rhigonema thysanophora* AGZ90403	LHSAGLSSILGSINFMTTVKNMRGSSISLEHMSLFVW
outgroup: *Steinernema carpocapsae* AAT00530	LHCAGISSILGAINFMCTTKNLRSSSISLEHMSLFVW
outgroup: *Heterorhabditis bacteriophora* ABJ80698	LHCAGLSSILGGINFMTTTSNLRSSSISLEHMSLFVW
outgroup: *Anisakis simplex* AAY43170	LHCAGVSSILGAINFMTTTKNLRSSSISLEHMSLFVW
outgroup: *Uncinaria sanguinis* AHI51905	LHCAGLSSILGGINFMCTTKNLRSSSISLEHMSLFVW
outgroup: *Pristionchus pacificus* ADZ52296	LHCAGLSSILGGINFMCTTKNLRSSSISLEHMSLFVW
Daptonema sp. 3 SD-2010 HM627508	LHLAGASSILSGINFLVTFLNTRSGEFNLEKVSLFVW
Diplolaimella dievengatensis HM627499	LHLAGLGSILSSLNFMTTFWNFRGSDANSDSVSLFVW
Diplolaimelloides oschei HM627498	LHLAGVGSILSSINFMTTFWSFRGSDVNFDSVSLFVW
Halomonhystera disjuncta HM627510	LHLAGISSILSSLNFMCTMWNARSGDISFEKMPLFVW
Theristus sp. 1 SD-2010 HM627511	LHLAGVSSILGGLNFMVTFFNSRS-PISLEKLALFVW
Trichotheristus mirabilis HM627500	LHLAGVSSILGGVNFMVTLWNTRSVEFNFEKISLFVW
Terschellingia longicaudata 358: T9-A	MHVAGLSSILGAINMIVSVFSCRG--ITADGVSLYVW
Terschellingia longicaudata 364: T10-E	MHLAGISSILGAINMIVSVFSCRG--ISFDGVSLYVW

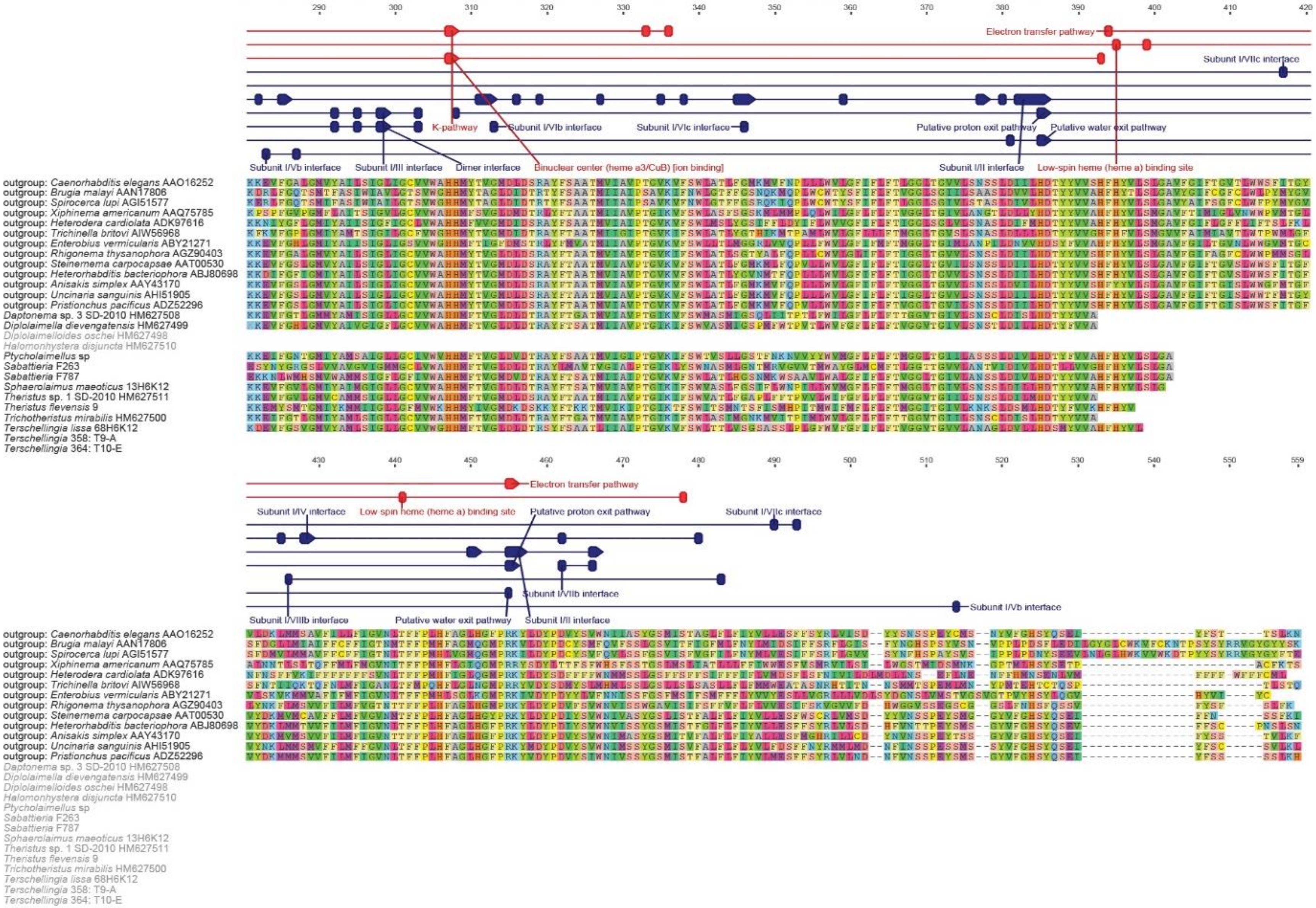
Fig 4-4. Part of COI amino acid alignment of the two *Terschellingia longicaudata* haplotypes, 11 other species of monhysterid nematodes, and thirteen outgroup species, showing four amino acid changes and a double deletion that are unique in *T. longicaudata*. A complete alignment is provided in supplementary Fig. S4-1.

4 Conclusions

Our data indicate the absence of genetic structure of both endobenthic nematode species (*T. longicaudata* and *P. pandispiculatus*), which probably reflects substantial passive dispersal and gene flow in our study area. As a result, both populations appear to be genetically homogenous. As such, our first hypothesis (limited population-genetic structure for both species) was confirmed, whereas our second hypothesis (less population genetic structure in *P. pandispiculatus*, living at the surface of the sediment, compared to the deeper-living species *T. longicaudata*) was rejected. Genetic diversity in *T. longicaudata* was very low with only two COI haplotypes recovered (one dominant and one rare). The COI data, combined with 18S rDNA sequences also confirmed previous studies that *T. longicaudata* likely constitutes a complex of multiple cryptic species, with one of these species having a wide geographical distribution.

Supplementary Fig S4-1. A complete COI amino acid alignment of the two *Terschellingia longicaudata* haplotypes, 11 other species of monhysterid nematodes, and thirteen outgroup species, showing four amino acid changes and a double deletion that are unique in *T. longicaudata*.




290
300
310
320
330
340
350
360
370
380
390
400
410
420
Electron transfer pathway
Subunit I/VIIc interface
K-pathway
Subunit I/VIb interface
Subunit I/VIc interface
Putative proton exit pathway
Putative water exit pathway
Subunit I/Vb interface
Subunit I/III interface
Dimer interface
Binuclear center (heme a3/CuB) [ion binding]
Subunit I/II interface
Low-spin heme (heme a) binding site
outgroup: Caenorhabditis elegans AAO16252
outgroup: Brugia malayi AAN17806
outgroup: Spirocerca lupi AGI51577
outgroup: Xiphinema americanum AAQ75785
outgroup: Heterodera cardiolata ADK97616
outgroup: Trichinella britovi AIW56968
outgroup: Enterobius vermicularis ABY21271
outgroup: Rhigonema thysanophora AGZ90403
outgroup: Steinernema carpocapsae AAT00530
outgroup: Heterorhabditis bacteriophora ABJ80698
outgroup: Anisakis simplex AAY43170
outgroup: Uncinaria sanguinis AHI51905
outgroup: Pristionchus pacificus ADZ52296
Daptonema sp. 3 SD-2010 HM627508
Diplolaimella dievengatensis HM627499
Diplolaimelloides oschei HM627498
Halomonhystera disjuncta HM627510
Ptycholaimellus sp
Sabattieria F263
Sabattieria F787
Sphaerolaimus maeoticus 13H6K12
Theristus sp. 1 SD-2010 HM627511
Theristus flevensis 9
Trichotheristus mirabilis HM627500
Terschellingia lissa 68H6K12
Terschellingia 358: T9-A
Terschellingia 364: T10-E
430
440
450
460
470
480
490
500
510
520
530
540
550
559
Electron transfer pathway
Subunit I/IV interface
Low spin heme (heme a) binding site
Putative proton exit pathway
Subunit I/VIIc interface
Subunit I/VIIb interface
Subunit I/Vb interface
Subunit I/VIIIb interface
Putative water exit pathway
Subunit I/II interface
outgroup: Caenorhabditis elegans AAO16252
outgroup: Brugia malayi AAN17806
outgroup: Spirocerca lupi AGI51577
outgroup: Xiphinema americanum AAQ75785
outgroup: Heterodera cardiolata ADK97616
outgroup: Trichinella britovi AIW56968
outgroup: Enterobius vermicularis ABY21271
outgroup: Rhigonema thysanophora AGZ90403
outgroup: Steinernema carpocapsae AAT00530
outgroup: Heterorhabditis bacteriophora ABJ80698
outgroup: Anisakis simplex AAY43170
outgroup: Uncinaria sanguinis AHI51905
outgroup: Pristionchus pacificus ADZ52296
Daptonema sp. 3 SD-2010 HM627508
Diplolaimella dievengatensis HM627499
Diplolaimelloides oschei HM627498
Halomonhystera disjuncta HM627510
Ptycholaimellus sp
Sabattieria F263
Sabattieria F787
Sphaerolaimus maeoticus 13H6K12
Theristus sp. 1 SD-2010 HM627511
Theristus flevensis 9
Trichotheristus mirabilis HM627500
Terschellingia lissa 68H6K12
Terschellingia 358: T9-A
Terschellingia 364: T10-E

Supplementary Table S4-1. Details of the voucher number, GenBank accession number, country and reference from where 18S of *Terschellingia longicaudata* was sequenced. For the new sequences from Iran, only unique sequences have received an accession number; the others (identical to one of the unique sequences) are indicated as LT*****.

Species (morphospecies)	**Voucher number**	**GenBank accession number**	**Country**	**Reference**
Terschellingia longicaudata	s.n.	AM234716	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	ArchTamar9	AM261967	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PB.2006.6	AM261974	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PT2005.1	AM941225	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PT2005.2	AM941226	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PT2005.3	AM941227	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PT2005.4	AM941228	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PT2005.5	AM941229	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PT2005.6	AM941230	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PT2005.7	AM941231	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PT2005.8	AM941232	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PT2005.9	AM941233	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PT2005.10	AM941234	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PT2005.11	AM941235	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PT2005.12	AM941236	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PT2005.13	AM941237	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PT2005.14	AM941238	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PT2005.15	AM941239	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PRH2005.1	AM941240	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PRH2005.2	AM941241	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PRH2005.3	AM941242	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PRH2005.4	AM941243	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PRH2005.5	AM941244	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PRH2005.6	AM941245	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PRH2005.7	AM941246	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PRH2005.8	AM941247	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PRH2005.9	AM941248	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PRH2005.10	AM941249	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PRH2005.11	AM941250	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PRH2005.12	AM941251	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PRH2005.13	AM941252	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PRH2005.14	AM941253	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PRH2005.15	AM941254	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PPL2005.1	AM941255	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PPL2005.2	AM941256	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PPL2005.3	AM941257	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PPL2005.4	AM941258	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PPL2005.5	AM941259	United Kingdom	Bhadury et al., 2008

Terschellingia longicaudata	PPL2005.6	AM941260	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PPL2005.7	AM941261	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PPL2005.8	AM941262	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PPL2005.9	AM941263	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PPL2005.10	AM941264	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PPL2005.11	AM941265	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PPL2005.12	AM941266	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PPL2005.13	AM941267	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PPL2005.14	AM941268	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PPL2005.15	AM941269	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PNM2005.1	AM941270	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PNM2005.2	AM941271	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PNM2005.3	AM941272	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PNM2005.4	AM941273	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PNM2005.5	AM941274	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PNM2005.6	AM941275	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PNM2005.7	AM941276	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PNM2005.8	AM941277	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PNM2005.9	AM941278	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PNM2005.10	AM941279	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PNM2005.11	AM941280	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PNM2005.12	AM941281	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PNM2005.13	AM941282	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PNM2005.14	AM941283	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PNM2005.15	AM941284	United Kingdom	Bhadury et al., 2008
Terschellingia longicaudata	PC2005.1	AM941285	Mexico:Cancun	Bhadury et al., 2008
Terschellingia longicaudata	PC2005.2	AM941286	Mexico:Cancun	Bhadury et al., 2008
Terschellingia longicaudata	PC2005.3	AM941287	Mexico:Cancun	Bhadury et al., 2008
Terschellingia longicaudata	PB2005.1	AM941288	France Brittany	Bhadury et al., 2008
Terschellingia longicaudata	PB2005.2	AM941289	France Brittany	Bhadury et al., 2008
Terschellingia longicaudata	PB2005.3	AM941290	France Brittany	Bhadury et al., 2008
Terschellingia longicaudata	PB2005.4	AM941291	France Brittany	Bhadury et al., 2008
Terschellingia longicaudata	PB2005.5	AM941292	France Brittany	Bhadury et al., 2008
Terschellingia longicaudata	PM2005.1	AM941293	Malaysia	Bhadury et al., 2008
Terschellingia longicaudata	PM2005.2	AM941294	Malaysia	Bhadury et al., 2008
Terschellingia longicaudata	PN2005.1	AM941295	Bahrain	Bhadury et al., 2008
Terschellingia longicaudata	PN2005.2	AM941296	Bahrain	Bhadury et al., 2008
Terschellingia longicaudata	PN2005.3	AM941297	Bahrain	Bhadury et al., 2008
Terschellingia longicaudata	PN2005.4	AM941298	Bahrain	Bhadury et al., 2008
Terschellingia longicaudata	PN2005.5	AM941299	Bahrain	Bhadury et al., 2008
Terschellingia longicaudata	PR2005.1	AM941300	Bahrain	Bhadury et al., 2008
Terschellingia longicaudata	PR2005.2	AM941301	Bahrain	Bhadury et al., 2008
Terschellingia longicaudata	PR2005.3	AM941302	Bahrain	Bhadury et al., 2008
Terschellingia longicaudata	PR2005.4	AM941303	Bahrain	Bhadury et al., 2008
Terschellingia longicaudata	PR2005.5	AM941304	Bahrain	Bhadury et al., 2008
Terschellingia longicaudata	Xx	AY854230	United Kingdom	Cook et al., 2005

Terschellingia longicaudata	Tamar5	DQ394729	United Kingdom	Bhadury et al., 2006
Terschellingia longicaudata	Tamar12	DQ394736	United Kingdom	Bhadury et al., 2006
Terschellingia longicaudata	Tamar13	DQ394737	United Kingdom	Bhadury et al., 2006
Terschellingia sp	Tamar15	DQ394739	United Kingdom	Bhadury et al., 2006
Terschellingia longicaudata	Tamar17	DQ394741	United Kingdom	Bhadury et al., 2006
Terschellingia longicaudata	Tamar23	DQ394747	United Kingdom	Bhadury et al., 2006
Terschellingia longicaudata	Tamar29	DQ394753	United Kingdom	Bhadury et al., 2006
Terschellingia longicaudata	Tamar37	DQ394761	United Kingdom	Bhadury et al., 2006
Terschellingia longicaudata	Tamar40	DQ394764	United Kingdom	Bhadury et al., 2006
Terschellingia sp	Plym7	DQ394771	United Kingdom	Bhadury et al., 2006
Terschellingia sp	Plym11	DQ394775	United Kingdom	Bhadury et al., 2006
Terschellingia longicaudata	Plym12	DQ394776	United Kingdom	Bhadury et al., 2006
Terschellingia sp	Plym39	DQ394803	United Kingdom	Bhadury et al., 2006
Terschellingia longicaudata	06Te03	GU937775	Taiwan	Hsiao & Shih, unpublished
Terschellingia longicaudata	06Te05	GU937776	Taiwan	Hsiao & Shih, unpublished
Terschellingia longicaudata	60106	GU937779	Taiwan	Hsiao & Shih, unpublished
Terschellingia longicaudata	0506G1	GU937781	Taiwan	Hsiao & Shih, unpublished
Terschellingia longicaudata	0506G2	GU937782	Taiwan	Hsiao & Shih, unpublished
Terschellingia longicaudata	CB1R09	JN968242	unknown locality	Fonseca et al. 2012
Terschellingia longicaudata	AH5R09	JN968249	unknown locality	Fonseca et al. 2012
Terschellingia sp	dna.T9.7	LT*****	Iran	This study
Terschellingia sp	dna.T10.8	LT*****	Iran	This study
Terschellingia sp	dna.T10.4	LT*****	Iran	This study
Terschellingia sp	dna.T10.5	LT*****	Iran	This study
Terschellingia sp	dna.T10.7	LT*****	Iran	This study
Terschellingia sp	dna.T10.7	LT*****	Iran	This study
Terschellingia sp	dna.T10.5	LT*****	Iran	This study
Terschellingia sp	dna.T10.4	LT795768	Iran	This study
Terschellingia lissa	dna.68H6K12	LT795763	Vietnam	This study
Terschellingia longicaudata	dna.27H6K12	LT795764	Vietnam	This study
Terschellingia sp	dna.3	LT*****	Netherlands	This study
Terschellingia sp	dna.48	LT*****	Netherlands	This study
Terschellingia sp	dna.2	LT*****	Netherlands	This study
Terschellingia sp	dna.47	LT*****	Netherlands	This study
Terschellingia sp	dna.4	LT*****	Netherlands	This study
Terschellingia sp	dna.6	LT*****	Netherlands	This study
Terschellingia sp	dna.T4.27	LT*****	Iran	This study
Terschellingia sp	dna.T4.8	LT*****	Iran	This study
Terschellingia sp	dna.T4.7	LT*****	Iran	This study
Terschellingia sp	dna.T1.3	LT795766	Iran	This study
Terschellingia sp	dna.T4.18	LT*****	Iran	This study
Terschellingia sp	dna.T4.25	LT795765	Iran	This study
Terschellingia sp	dna.T4.28	LT*****	Iran	This study

Teschellingia obesa	dna.87H6k12	LT795767	Vietnam	This study
Outgroup				
Cyartonema elegans	s.n.	AY854203	United Kingdom	
Daptonema procerus	s.n.	AF047889	unknown locality	
Daptonema setosum	178968	AM234045	United Kingdom	
Daptonema normandicum	s.n.	AY854224	United Kingdom	
Daptonema sp	NN024	LK054724	Cuba	
Diplolaimelloides meyli	s.n.	AF036611	unknown locality	
Halomonhystera disjuncta	s.n.	AJ966485	Netherlands	
Metadesmolaimus sp.	PDL-2005	AJ966491	Netherlands	
Monhystera sp	T4M	GQ503078	New Zealand	
Sabatieria celtica	178798	AM234626	United Kingdom	
Sabatieria punctata	s.n.	AY854237	United Kingdom	
Sphaerolaimus hirsutus	171327	AM234622	United Kingdom	
Theristus acer	Wester, Scheldt s.n.	AJ966505	Netherlands	
Theristus acer	178943	AM234627	United Kingdom	
Theristus agilis	TherAgi1	AY284693	unknown locality	
Theristus agilis	TherAgi2	AY284694	unknown locality	
Theristus agilis	TherAgi3	AY284695	unknown locality	
Theristus sp	1268	FJ040464	unknown locality	

Chapter 5

General discussion and future perspectives

Chapter 5

General discussion and future perspectives

5. General discussion

As reviewed in the introductory chapter, the Persian Gulf (PG) provides both a wealth of resources to humans (oil and fisheries) and a large diversity of marine habitats. Along the Iranian part of the PG coastline, much attention has been paid to the macrobenthos whereas studies on meiobenthos are totally lacking. The absence of information on meiobenthos, specifically on marine nematodes, of the sandy beaches of the northern PG prompted us to perform the present study.

In our study, we present, for the first time, data on local and regional diversity of beach nematode assemblages from the Iranian part of the PG. Nematodes are usually the most species-rich phylum in marine soft sediments, therefore our study contributes substantially to the science of marine biodiversity in the PG area. The current thesis establishes the baseline for a new research area in Iran, that is, marine nematology research. Our obtained data could pave the way for capacity building and future works on benthic ecology. In the present chapter, the results from this thesis are discussed in the context of beach nematode diversity worldwide, nematodes as pollution indicators and factors influencing the population genetic structure of marine nematodes. Finally, we present some perspectives for future research.

5.1. Marine biodiversity of the PG

Many scientists have been interested in marine biodiversity research in the PG region, especially to know whether this region is a "coldspot" or "hotspot" of biodiversity. At species level and for some benthic groups, such as corals, algae and echinoderms, low species richness at both large and smaller spatial scales has been reported (Price 1982; Sheppard et al. 1992). According to Sheppard et al. (2010), this is partly because of the relatively recent geological formation of the PG, but mostly because of the harsh environmental conditions. However, some researchers (see below) do not believe that the PG is a coldspot of biodiversity. For instance, Price and Iszak (2005) compared echinoderm datasets from the PG and Red Sea region. The authors suggested that the PG may not be the coldspot of biodiversity generally believed, especially if a broad set of measures is utilized, and they stated that any conclusions about biodiversity must be drawn after compilation and analysis of comprehensive datasets for many fauna and flora.

5.2. Nematode diversity in the PG compared to other sites worldwide

Biodiversity can be viewed at various spatial scales (Gray 2000; Poiani et al. 2000), and there is a long history of defining reference scales at which diversity should be reported (see Gray, 2000, for marine review). In our chapters 2 and 3, we have identified *diversity at four levels*: that of a sample, that of a station (= distance to pollution; this is the cumulative diversity over the replicate samples in a station, whereas the mean value of the replicates then provides an average sample diversity), that of a beach location (the cumulative diversity of all stations on a single beach) and a 'regional' diversity, which is the cumulative diversity across the four beaches studied here. Although our sample diversity as such corresponds to Whittaker's (1960) original definition of sample or α diversity, the other levels are less straightforwardly linkable to Whittaker's definitions.

For comparison with other published studies, we have therefore chosen to broadly/loosely follow Gray's (2000) definitions and terminology. We define the *richness encountered in the basal unit of a study*, i.e. a single sample, as the point (genus) richness, and redefine sample or α diversity as *resulting from "more sample units within a broader area"*. We define that area here as a *beach* or *location*. Note, therefore, that we do not define a particular 'station' diversity. The reason why we feel this is not useful for comparisons with other studies, is that the numbers of samples per station, and of stations per beach, undoubtedly exhibit the largest variation among studies. The *cumulative diversity across beaches*, then, yields gamma diversity and corresponds to 'large area diversity' as defined by Gray (2000). We do not discuss diversity at the epsilon or biogeographical province level, but in a few cases, table 5-1 does provide a measure of ε diversity.

Over the two sampling periods, the gamma diversity of the marine nematodes at the studied beaches was represented by 62 genera belonging to 24 families. Our study was restricted to only 4 beaches which were sampled twice with a 1-year interval, and, obviously, this may not be enough to identify the full set of genera present in the area. Consequently, the data are still incomplete and insufficient to draw firm conclusions regarding the biodiversity of marine nematodes in the region. Probably many nematode species remain uncovered, as indicated by the high proportion of genera obtained in only one of the two field samplings, and the true diversity is likely higher than the one observed in this thesis. In continuation, we compare the observed biodiversity of marine nematodes at our study sites with other sandy beach habitats of similar as well as dissimilar climatic and environmental conditions. The overview of beach studies with which we compare is arguably not complete, so it is not exhaustive in its literature review. It does, however, offer representative studies from a variety of geographical locations, beach types, human impacts etc.

In general, a comparison of beach meiofaunal diversity is extremely difficult due to the high variability in beach morphodynamics (gradients from reflective to dissipative), the tidal regime (macro- to microtidal), the nature of the substrate (volcanic, quartz), the seasonality (temperate, tropical) and the anthropogenic influence on the substrate. The variability of those factors is a potential driver of variations in abundance, diversity and taxonomic composition of beach nematode assemblages. We nevertheless present a comparative table (table 5-1) together with a discussion on local (= beach) and large-area (= gamma) diversity levels worldwide.

Under **similar climate conditions**, the overall observed nematode diversity is low but comparable to several other anthropogenically impacted beaches. Considerably higher local diversity than in our study was obtained in the Gulf of California, with up to 96 genera per beach (Mundo-Ocampo et al. 2007; Gingold et al. 2010), and at Guanabara Bay, Rio De Janeiro, with 62 genera (Maria et al. 2008b), compared to 21 (Haghani) to 38 (Suro) in the Strait of Hormuz, when accumulating the records of both years. However, despite the repeated sampling in our study, sampling effort in the three above-mentioned studies still exceeded that in our study, since in each of the aforementioned studies transects were sampled across the entire high-to-low water gradient. In addition, Gingold et al. (2010) specifically incorporated ridges and runnels, with higher diversity in runnels. The majority of other studies, including ours, have focused solely on ridges. A better comparison between these studies and ours is possible when looking at point diversity rather than local diversity. Average point diversity in the studies of Maria et al. (2008b) and Gingold et al. (2010) ranged from 9 to 43 in the former and from 6 to 27 in the latter. The lowest genus diversity in the study by Gingold et al. (2010) was found at the upper beach and highest diversity in the lower and middle parts of the beach. Our samples were also taken from the mid intertidal, but again, the average point genus richness in our study (5-16) can be considered substantially lower. By contrast, beach (28-29 genera) and gamma diversity (40 genera) at two anthropogenically influenced Ecuadorian locations were very similar to those in our study (Calles Procel et al. 2005). These beaches were more influenced by high recreational use and fishing activities. 21 genera, the same as in Haghani, were found at a beach along the central west coast of India, encompassing five stations along a gradient of sewage pollution (Nanajkar and Ingole 2010). Anthropogenic disturbance and pollution may thus be important causes of low nematode diversity on sandy beaches.

The average local diversity at beaches in **temperate regions** is comparable to, or slightly lower than, that in (sub-)tropical beaches in California and Rio (see above), but higher than in our study. At three Australian sandy beaches, ranging from sheltered with seagrass vegetation to highly exposed and bare, a local diversity range of 40-54 genera was recorded. The gamma diversity, however, was very similar to that in our study, with a total of 64 genera over the three beaches (Hourston et al. 2005). Local diversity in this study was based on three transects perpendicular to the water line. Average point richness per station was, however, low and never exceeded 5 species at any single sampling time. At an ultra-dissipative beach at De Panne, Belgian coast, Gheskiere et al. (2004) registered 65 genera. Sampling included four transects perpendicular to the water line with nine stations from the upper to the low tide level. Average point diversity ranged from 8 nematode species at the upper littoral to 34 species near the mid-intertidal. In other words, point diversity in these temperate beaches was similar as in the PG, and lower than in (sub)tropical beaches, whereas the higher local diversity in the temperate beaches compared to the PG probably reflects the sampling effort which better covered the whole beach. Hence, this brief overview of literature does not support the paradigm that benthic nematode diversity is lower in (sub-) tropical than in temperate areas (Alongi 1990a), at least with respect to beach nematofauna.

Data from **other climatic zones** are scant. Urban-Malinga et al. (2005) found a total of only 28 genera (gamma richness) in four Arctic beaches (two more exposed vs. two more sheltered), with sampling stations from two different tidal levels (the low-water line and closely below the drift line). In a similar sampling at two other Arctic beaches, one sheltered and one exposed, local genus diversities were even lower: from 8 at the sheltered beach to only three at the most exposed beach (Urban-Malinga et al. 2004). Such low numbers of genera may well be related to the harsh climatic and physical (ice cover in winter) conditions of the Arctic intertidal (Urban-Malinga et al. 2005).

Table 5-1: Overview of published studies providing information on local (= α), large-scale (= γ and (occasionally) biogeographic province (= ε) diversity of beach nematode assemblages. α, γ and ε diversity are expressed as genus richness, except for the study by Gheskiere et al. (2005), where species richness is given. α diversity is taken as number of genera per beach location, γ as the cumulative number of genera over different beaches in the same region, and ε diversity as the cumulative number of genera over a larger geographical scale. We provide information on beach type (n.d. = not described in original study), number of beaches and number of stations per beach, presence and nature of a gradient in the sampling design (HL = gradient from high to low water, PL = gradient parallel to water line, L = latitudinal gradient), and presence or absence of temporal data.

Reference and sampling location	Beach type	Number of beaches	Number of stations	gradient type	Presence of temporal data	α diversity	γ diversity	ε diversity
Similar climate conditions								
(Gourbault and Warwick 1994) At Gosier, Guadeloupe, West Indies	sheltered	1	6	Horizontal (PL)	Yes	61	_	-
Calles et al. (2005) Along Ecuadorian coast	sheltered & exposed	2	1	Pollution (PL)	Yes	28-29	40	_
Mundo-Ocampo et al. (2007) Two sites in the Gulf of California	One dissipative, one more exposed with coarser substratum	2	6	Horizontal (PL)	No	55-67	80	_
Nanajkar and Ingole (2010), Goa near the Panjim city, central west coast of India	sheltered	1	5	Horizontal (PL)	Yes	19	_	_
Sahraean et al. (2017a) Persian Gulf, Bandar Abbas	sheltered to reflective	4	3	Pollution (PL)	No	13-28	39	-

Gheskiere et al. (2005) northern coast of Poland and San Rossore Massaciuccoli Natural Park, Italy	dissipative and reflective	2	6	Horizontal (HL)	No	56-66		108
Moreno et al. (2006), Collelungo beach, in southern part of Tuscany, Italy	exposed	1	4	Horizontal (HL)	Yes	1 7	_	_
De Jesús-Navarette (2007) Socorro Island, Colima, Mexico	n.d.	3	1-2	Horizontal (HL)	No	2-25	26	_
Maria et al. (2008) Guanabara Bay, Rio De Janeiro, Brazil	sheltered	3	2	Horizontal (HL)	Yes	31-46	62	_
Gingold et al. (2010) upper Gulf of California	dissipative	1	10	Horizontal (HL)	No	96	_	_
temperate regions								
Lambshead (1986) Firth of Clyde, United Kingdom	fairly sheltered, estuarine beaches	5	1-2	Pollution (PL)	No	33- 54	71	_
Nicholas and Hodda (1999) East coast of Australia	exposed	1	7	Horizontal (HL, PL)	Yes	48	_	_
(Nicholas and Trueman 2005) Australian sandy beach	exposed	3	3-5	Horizontal (HL, PL)	No	44-82	_	100
Sharma and Webster (1983), two Canadian Pacific beaches	n.d.	2	1	-	Yes	24-56	59	_
(Nicholas 2001), East coast of Australia	exposed	1	1	_	Yes	40	_	_

Gheskiere et al. (2004), De Panne, Belgium	ultra-dissipative	1	9	Horizontal (HL)	No	65	_	_
Hourston et al. (2005), West Australian coast	sheltered, exposed and intermediate	3	3	Horizontal (HL)	Yes	40-54	64	-
Liu et al. (2008), Taiping Bay of Gingdan, China	n.d.	1	-	_	Yes	75	-	-
Maria et al. (2012), De Panne, Belgium	ultra-dissipative	1	10	Horizontal (HL)	No	60	-	-
Lee and Riveros (2012), Along coast of Chili	exposed (L)	66	1	_	No		99	
(Hua et al. 2016) Eastern coast of China	n.d.	3	1	-	No	15-24	-	34
Other climatic zones								
Urban-Malinga et al. (2004), two contrasting Arctic beaches on Bjornoya (Bear Island)	exposed and sheltered	2	3	Horizontal (HL)	No	3-8	8	_
Urban-Malinga et al. (2005) Kongsfjorden, Svalbard	sheltered and exposed	4	2	Horizontal (HL)	No	4-15	28	_

Usually, in non-impacted environments, grain size represents the most important factor in determining nematode community structure (Platt 1984). However, in our study there was no clear relationship between nematode abundance and diversity on the one hand and sediment granulometry on the other; else, Terminal – with the most ‘deviant’ sediment granulometry – should have had the most distinct nematofauna, which was not the case. As mentioned in the introductory chapter 1, the severe climatological conditions (e.g., large annual fluctuations of seawater temperature and high salinity) in the PG region are certainly co-responsible for the lower nematode diversity, and may be further aggravated by persistently high levels of contaminants such as hydrocarbons and metals (see chapter 1 for relevant literature). Finally, biological interactions (e.g., predation among nematodes) that were not measured during this study are also a potentially important driving force of changes in density and species composition (Steyaert et al. 2001; Gallucci et al. 2005).

5.3. Nematode assemblage composition in the context of environmental pollution

Intertidal organisms inhabit a transitional ecosystem between land and sea, and are therefore at risk from anthropogenic impacts, such as pollution, over-exploitation and sea-level rise resulting from global warming. Additionally, they are vulnerable due to the extreme fluctuations in environmental conditions (Levin et al. 2001). The PG is considered a highly stressful environment because of its high salinity, large seawater temperature fluctuations, extreme temperature maxima and high loads of anthropogenic pollutants (Abuzinada et al. 2008; Hamza and Munawar 2009; Hassanshahian 2014) and it is believed that most organisms living in the PG survive at the limits of their physiological tolerance (Price 1993; Beech 2004). Moreover, as mentioned in chapter 1, anthropogenic pressure is hypothesized to be a key factor influencing the community and structure of marine nematodes in our studied area. Even though we did not directly measure pollution or environmental disturbance, the overall low diversity of nematodes indicates that the entire area experiences substantial stress, and the local sources of disturbance had measurable impacts on benthic community diversity (see chapters 2 and 3).

One important observation from this thesis is that the same five genera (*Daptonema*, *Promonhystera*, *Paramonhystera*, *Terschellingia* and *Ptycholaimellus*) together accounted for more than 75% to 80% of the total nematode abundance at all beaches in 2008 and 2009, respectively. These genera have been reported from eutrophicated and/or polluted coastal habitats in other parts of the world. This may indicate that local hydrodynamics (currents, wave action) homogenized the beach nematode assemblages in this area, a hypothesis further supported by the lack of population genetic structuring in two of these dominant nematode species (see chapter 4), leading to an overall dominance of those species that are successful across a large range of pollution impacts. An excess of organic input into the marine environment can create an unbalanced ecosystem with high environmental stress (Austen and Warwick 1995). Increased input of organic matter (for instance directly from sewage, or indirectly as a result of coastal eutrophication caused by nutrient enrichment) generally allows fewer species to coexist as a result of competitive interactions as well as low-oxygen conditions. Susceptible species are replaced by more tolerant species (Schratzberger and Warwick 1998). In the present study, fewer dominant nematode genera existed at our studied area. The presence of stress-tolerant nematode genera appeared particularly informative, making the state of sediment contamination very evident. In continuation, we provide some information related to the ecology and feeding behavior as well as the stress tolerance of four out of the five dominant genera found at our study site.

Daptonema*, *Promonhystera* and *Paramonhystera are non-selective deposit feeders *sensu* Wieser (1953) and belong to the family of Xyalidae, a family of mostly free-living marine nematodes (Venekey et al. 2014a). Deposit feeders have a prismatic or conical spineless buccal cavity, and their food consists mainly of benthic diatoms (MPB), other microalgae, bacteria and perhaps non-living organic particles (Moens and Vincx 1997; Moens et al. 2004).

Daptonema likely feeds as an omnivore, obtaining carbon partly from MPB and partly from organisms that feed on MPB. In fact, many marine nematodes may be opportunistic feeders which may shift feeding behavior in response to food availability, and may complement preferred resources with other, more readily available ones (Moens et al. 2014). *Daptonema* seems to prefer the surface sedimentary layer, as the maximum abundances of, e.g., *Daptonema normandicum* were recorded in the uppermost 0-2 cm sediment section, probably due to a decreasing trend of dissolved oxygen, sediment organic carbon, and chl-*a* with depth (Singh and Ingole 2011). *Daptonema setosum* was reported to migrate upward at low tide and downward at high tide (Steyaert et al. 2001). Such type of behavior may be a part of the survival strategy of nematodes, mainly to utilize the epipelic diatoms, which are the main food source for deposit feeders, as well as to avoid predators. The reproduction rates of *Daptonema* may be high and its growth rapid growth; hence, *Daptonema* is considered an opportunistic nematode (colonizer) (Vanaverbeke et al. 2003).

Given the opportunistic feeding behavior and the rapid colonizer ability, *Daptonema* is considered to be relatively stress tolerant and able to live in many different habitats. Along the Italian coasts, for example, *Daptonema* is reported to be tolerant to hydrocarbon stress (oil spill), trace elements and fish farming (Vezzulli et al. 2008; Moreno et al. 2011; Losi et al. 2013; Mirto et al. 2014). In a subtropical humid region of south Brazil with intensive organic loads due to the biodeposition of mussel farms, assemblages consist predominantly of the opportunistic nematodes *Daptonema*, *Terschellingia*, and *Sabatieria* (Netto and Valgas 2010), probably because of the high microbial densities at the mussel culture bottoms (Mirto et al. 2000). *Daptonema normandicum* has been reported as the most dominant (> 67%) among the meiobenthic nematodes at a sandy beach in the Arabian Sea in the vicinity of Panjim market in India (Singh and Ingole 2011). As the sampling area was located in the vicinity of a municipal sewage dumping site, where organic carbon was abundant (promoting the increase of bacteria and protozoans in the sediment), it was concluded that these sources provide the main food for deposit-feeders. *Daptonema* is among the 15 genera that have been common to all environments along the Brazilian coast (Venekey et al. 2010). *Daptonema* o*xycerca* is one of the most abundant nematode species in the marine sediments of the tidal flat at Coroa Grande, a tropical region in Rio de Janeiro, Brazil (Esteves 2004). The spatio-temporal study of *D.* o*xycerca* in this region revealed that the density of *D. oxycerca* was negatively correlated with temperature, with a peak in August (the local coldest period) (Maria et al. 2008a). *D. oxycerca* was most abundant at the upper intertidal low-salinity zone, and none of the factors (organic matter, granulometry, and chlorophyll-*a*) could explain its distribution. A preference of *D. oxycerca* for the upper intertidal zone has also been shown at the Brouage mudflat (Marennes-Óleron Bay, France) (Rzeznik-Orignac et al. 2003). Again, none of the physical and biological parameters analyzed, such as chl-*a*, organic matter as well as heavy metals, was directly related to the spatial variation of *D. oxycerca*. The preference of *Daptonema* for organically enriched sediments seems at odds with experimental observations that both *D. setosum* and *D. tenuispiculum* were completely eliminated by one week of anoxia or even hypoxia (Steyaert et al. 2007). Similarly, the preference of *Daptonema* for the upper intertidal, where fluctuations in temperature and salinity can be large and rapid, seems hard to reconcile with its sensitivity to hyposmotic (= low-salinity) stress (Forster 1998), (Moens, unpubl. observations). Perhaps its vertical mobility (Steyaert et al. 2001) allows it to migrate to surface layers to obtain oxygen, and to deeper strata to escape stressful peaks in temperature and/or drops in salinity.

The genus ***Paramonohystera*** has been labeled "obligate anaerobic", indicating its survival under anoxic conditions (Wieser et al. 1974). The term “obligate anaerobic” may be rather inadequate; the near-exclusive occurrence of the genus under anoxia in the study by Wieser et al. (1974) may rather point at the genus being competitively stronger under conditions of low or no oxygen. It has also been demonstrated that *Paramonohystera* is tolerant to metal pollution (Gyedu-Ababio and Baird 2006) and resistant to glyphosate (Salem et al. 2016). In the latter study, the abundance of *Paramonohystera* sp. increased in the pesticide treatments up to 5 times compared to the untreated control. In any case, it is clear from the above that this genus thrives well in polluted or otherwise stressed environments.

Terschellingia longicaudata (Linhomoeidae) is a selective deposit feeder and is considered a bacterivorous nematode (Wieser 1953), which can be dominant in both sandy and muddy areas. Due to the small mouth cavity (3µm) it is unable to feed on protozoans and small metazoans, which makes bacteria the most probable food source (Rzeznik-Orignac et al. 2008). Alternatively, it may obtain part or all of its nutrition from bacterial endosymbionts which utilize methane as a carbon source (Vafeiadou et al. 2014; Moens, unpubl. observations). Methane can be a common carbon source in anoxic or hypoxic sediment strata, and *Terschellingia* is known to thrive in low-oxygen conditions (Steyaert et al. 2007) and live in anthropogenically disturbed and polluted habitats (Lambshead 1986; Schratzberger and Warwick, 1998; Liu et al. 2008), with the ability to tolerate metal and hydrocarbon contamination (Austen and Somerfield 1997; Armenteros et al. 2009a; Beyrem et al. 2010). The existence of *Terschellingia* in many habitats supports its cosmopolitan distribution and this was confirmed by our study. *T. longicaudata*, for example, occurs in a wide range of habitats, such as mangroves and mudflats (Hodda and Nicholas 1985), various subtidal habitats (Tita et al. 2002; Schratzberger et al. 2004b; Bhadury et al. 2005; Schratzberger et al. 2006), seagrass beds (Novak 1989) and lagoons (Villano and Warwick 1995). The presence of *T. longicaudata* in most of the marine habitats indicates its adaptability to different types of sediments.

Ptycholaimellus, which belongs to the family of Chromadoridae, is an epigrowth feeder (2A, Wieser, 1953) using a tooth to pierce or crack diatoms, other microalgae and filamentous cyanobacteria (Moens and Vincx, 1997). Epigrowth feeders may also ingest bacteria. *Ptycholaimellus* is considered a typical nematode belonging to the “surface” assemblage and has been reported to be abundant in several habitats, such as muddy intertidal flat sediments (Steyaert et al. 2003; Van Colen et al. 2009) and also hard substrates (Heip et al. 1985), which is probably related to their feeding on biofilms. In a Kenyan mangrove, the occurrence of *Ptycholaimellus* coincided with the increase in benthic microalgae and phytodetritus (Alongi 1990a), and a similar observation was made at an intertidal mudflat in the Scheldt Estuary, The Netherlands (Van Colen et al. 2009).

In contrast to *Terschellingia* with remarkable tolerance to hypoxic and anoxic conditions, the *Ptycholaimellus* species either migrate away from anoxic spots in the sediment (Franco et al. 2008) or may experience high mortality if their sensitivity to low-oxygen conditions would resemble that of other Chromadoridae, like *Chromadora macrolaima* (Steyaert et al. 2007). By contrast, *Ptycholaimellus* have been suggested to be opportunistic species after the contamination by hydrocarbons and trace elements (Austen and Somerfield 1997). This could perhaps again relate to peaks in microphytobenthos production resulting from a decimation of prominent MPB grazers (see Van Colen et al., 2009). Besides, *Ptycholaimellus ponticus* can tolerate a wide range of trace elements (Somerfield et al. 1994; Austen and Somerfield 1997). Moreover, in the intertidal zone of the Hichirippu shallow lagoon (Japan), *Ptycholaimellus* sp. seems to be very tolerant of elevated temperatures (Yodnarasri et al. 2008).

Although we consider natural and anthropogenic environmental stressors to be the main determinants of the PG nematode assemblage, other factors should also be considered. One important point worthy to mention is predation, a direct interaction that affects meiofaunal community structure (Coull 1985, 1999). In this context, some studies have addressed the predator-prey interactions using macrofauna as predators and meiofauna as prey (Olafsson 2003; O’Gorman et al. 2008). Additionally, many studies have also reported on the effects of meiobenthic predators controlling other meiofauna and/or their prey (Moens et al. 1999; Moens et al. 2000; Hamels et al. 2001; Gallucci et al. 2005; dos Santos and Moens 2011). Even at relatively low abundances, predatory nematodes can substantially impact abundance, species composition and/or diversity of their prey assemblages (Moens et al. 2000; Gallucci et al. 2005; Moens et al. 2013). Coarser sediments typically have a higher prominence of carnivorous and omnivorous nematodes, perhaps because of larger interstitial spaces that allow more efficient foraging on prey, potentially resulting in pronounced top-down impacts on prey abundance and assemblage composition (Gallucci et al. 2005). In turn, a higher predation impact may increase prey species diversity through a release of competitive interactions (Worm et al. 2002), hence a high relative abundance of predacious nematodes may positively affect prey species diversity. In our study, the location with the highest nematode diversity was indeed the one with the highest proportion of predacious nematodes

(23.9% at Suro in 2008). However, there was no clear relationship between nematode abundance and diversity on the one hand and sediment granulometry on the other. The location with the highest nematode diversity and abundance (Suro) had the second lowest median and mean grain size of all beaches investigated, while the location with the lowest nematode densities and diversity had slightly coarser sediment. Admittedly, the differences in granulometry between beaches were not very marked, exception made for Terminal.

Overall, nematode assemblages dominated by stress tolerant genera, as observed in the present study, are often indicative of a disturbed system. As reviewed in the first chapter, the marine ecosystems of the Iranian part of the PG have faced severe anthropogenic activities with great potential threats to the marine organisms. For example, massive harmful algal blooms during 2008-2009 (red tide) seen in the PG, Oman Sea and Strait of Hormuz originated from a phytoplankton named *Cochlodinium polykrikoides* (Richlen et al. 2010) and stayed for 9 consecutive months – alledgedly the longest-lasting algal bloom ever recorded across the world. It could be shown that there were higher algal densities around the crowded industrial cities along the Strait of Hormuz, such as Bandar Abbas and Qeshm (Hamzehei et al. 2012), supporting the importance of sewage outlets from major cities in eutrophication processes in our study area. High rates of organic matter in coastal waters in these two industrial zones, and the lack of a filtering system for the urban and industrial sewage, were considered the major causes of the high algal density. Another catastrophic event was the cyclone Gonu (in June 2007), which was the most intense tropical cyclone ever recorded in the PG. All together, these events may have had severe impacts on marine biodiversity and community structure in our studied area. Besides, the marine environment of Bandar Abbas has been subject to contamination by pollutants from a variety of other sources, such as oil-related pollution (Hassanshahian et al. 2012; Tehrani et al. 2012; Mohebbi Nozar et al. 2014), heavy-metal contamination (Khoshnood et al. 2010; Rahmanpour et al. 2014; Sarhadizadeh et al. 2014) and urban sewage effluents (Mansourri 2016) as a result of intense industrial activities and urban development. All the aforementioned environmental stressors together with our results—low nematode diversity, the dominance of a limited number of stress-tolerant genera—lead us to the conclusion that the studied area is an environmentally stressed region, continuously subject to a high degree of anthropogenic impact.

5.4. Population genetics

The population genetic structure of organisms not only informs about habitat connectivity and gene flow (Derycke et al. 2013), it also provides clues to the resilience of populations after local perturbations, because well-connected populations may re-seed individuals to patches after local extinction events (Cowen et al. 2007; Cowen and Sponaugle 2009). The fourth chapter of this PhD focuses on the population-genetic patterns of two of the five dominant nematode species in our study, *Terschellingia longicaudata* and *Ptycholaimellus pandispiculatus*. Previous studies have shown that marine nematode populations from several intertidal habitats show significant population-genetic structuring over spatial scales of 10–100 km (Derycke et al. 2005, 2007a, 2010a, 2013). We expected that *P. pandispiculatus* would have relatively little population–genetic structure, because, as an epigrowth-feeder, it tends to live at the sediment surface and hence is prone to passive transport. Furthermore, it has been reported that tube-construction is widespread in *Ptycholaimellus* species (Nehring 1993) which may facilitate transportation and dispersal of the species. By contrast, *T. longicaudata* dwells in deeper sediment layers and was expected to have a relatively more pronounced population-genetic structure. However, our data did not reveal a significant population-genetic structure for either species indicating substantial dispersal and gene flow in our study area. This corroborates the conclusion mentioned under 5.3 that local hydrodynamics (currents, wave action) homogenized the beach nematode populations and assemblages in this area. Given the relatively sheltered nature of some of our beaches, this suggests that gene flow in more exposed beach habitats is likely even more pronounced, and that barriers are required to produce population genetic structure (see under 5.4.1).

On the other hand, the two species showed very different patterns of genetic diversity: whereas *P. pandispiculatus* exhibited a genetic diversity comparable to that from other intertidal nematodes elsewhere, *T. longicaudata* showed very low genetic diversity (only 2 COI haplotypes, one of which comprising 95% of all individuals). Below we discuss some possible reasons for our results.

5.4.1. High dispersal, stress tolerance and high colonization ability may explain the lack of population genetic structure in *T. longicaudata* and *P. pandispiculatus*

One potential explanation for the observed pattern is that *T. longicaudata* and *P. pandispiculatus* may be characterized by high levels of dispersal and gene flow (i.e.

migration). The movement of individuals away from their natural environment is defined as dispersal and can lead to gene flow over different spatial scales. It also affects the rates of genetic changes and the processes of adaptation, speciation and hence the evolution of organisms (Froukh and Kochzius 2007). The lack of population-genetic structuring found in this study may be the result of the physically dynamic nature of the beaches along our study area, with lots of erosion and hence passive transport. Several studies have shown that local hydrodynamics can potentially influence passive dispersal and gene flow in the marine environment (Palmer 1988; Galindo et al. 2010; White et al. 2010; Derycke et al. 2013). At the studied PG beaches, there are no evident physical barriers (e.g., different marine habitats, buildings, rivers, harbors). In more energetic environments, nematodes become re-suspended with sediments and re-distributed at distances dependent on the prevalent hydrodynamic regimes. The constant connectivity among populations may thus lead to a constant gene flow, explaining the lack of population structure, at least at the spatial scale studied in the present work.

Another factor to be discussed is the recolonization potential of the marine nematodes. Considering that *Ptycholaimellus* and *Terschellingia* are probably quite stress tolerant, they will benefit from a competitive advantage resulting in higher colonizing abilities (Eskin and Palmer 1985). In order to be able to benefit from a dispersal mechanism, an individual that arrives in a new patch must be able to reproduce and colonize there. Soft sediments offer space for colonization in an always present, complex, 3-dimensional structure, and therefore colonization can begin immediately after a disturbance, provided the ‘propagule’ is fit for reproduction (Baden et al. 1998; Lee et al. 2001; Barnes and Conlan 2007). Accordingly, fast growth rates and the ability to reproduce before or faster than others do, will increase the benefits of dispersal. Fonseca-Genevois et al. (2006) reported that *Ptycholaimellus* colonized new habitats suspended above the ocean floor and attributed their arrival to periodic upwelling events. They also reported that passive long-distance dispersal through re-suspension in the water column can promote quick recolonization of more distant locations. Van Colen et al. (2009) found *Ptycholaimellus* and *Daptonema* among the fastest colonizers of intertidal muddy sediment which had experienced a major anoxic event. Both genera re-established at high abundances as soon as microphytobenthos biofilms started to recover and reach very high productivity, unhindered by the presence of major MPB grazers belonging to the macrofauna.

In order to properly understand the colonization potential of a species, and hence the biological factors determining the genetic structure of nematode populations, information on life-history traits is crucial (Duminil et al. 2007). Unfortunately, only limited information is available on the life history of most marine nematodes, including the dominant genera in our study area. Bongers et al. (1991) classified marine nematode genera into expected colonizers (c) and persisters (p) based on life-history traits. Nematodes with a cp score = 1 are extreme colonizers, with short generation times, high reproductive output, large population fluctuations, high dispersal abilities and are relatively stress tolerant, whereas those with a high cp (up to a max. of 5) are persisters, produce few offspring with long generation times and are sensitive to disturbance (Bongers and Ferris 1999). Significant population genetic structuring was found at small geographic scales, even for species with a cp score of 1 (Derycke et al. 2005 ; 2007a, 2013). According to Bongers et al. (1991), *T. longicaudata* and *P. pandispiculatus* both have a cp score of 3 (Patrício et al. 2012); therefore, we would expect them to show at best intermediate population genetic structuring. However, life-history information for both genera is lacking, and their cp classification is therefore at best tentative. Moreover, the fact that both species are stress-tolerant may outweigh other factors and render them competitive colonizers of anthropogenically impacted environments, contributing to the population-genetic structure observed in the present study.

5.4.2. Genetic diversity pattern suggests strong selection on CO I

Whereas both species, *P. pandispiculatus* and *T. longicaudata*, showed low population genetic structure, the genetic diversity pattern was very different with *P. pandispiculatus* exhibiting a much higher population genetic diversity than *T. longicaudata*. While *P. pandispiculatus* had 17 COI haplotypes, *T. longicaudata* had only 2, one of which comprising 95% of all individuals. Important ecological factors contributing to low genetic diversity are a small population size, lack of migration and hence inbreeding. The smaller the population, the more dramatic the fluctuation of allele frequencies, and the faster the loss of genetic variation. Small and isolated populations are vulnerable to inbreeding, that is, production of offspring from mating between close relatives. Given the high reproduction rate of marine nematodes, the high population abundances of both target species and the supposedly high migration rate at our study site, inbreeding is unlikely to be responsible for the low genetic diversity of *T. longicaudata*. Also, the comparison with other *T. longicaudata* specimens from the Scheldt Estuary, The Netherlands, revealed that all individuals there belonged to a single COI haplotype, actually the same as the dominant one in the PG. The fact that COI is so exceptionally little variable might indicate that this gene is under strong selection. Anthropogenic impact, such as pollution, can cause severe perturbations of the genetic structure. A reduced mitochondrial diversity under conditions of chemical pollution has been observed in marine nematodes (*Litoditis marina*) (Derycke et al., 2007b), meiobenthic copepods (Street and Montagna 1996; Street et al. 1998) and gastropods (Kim et al. 2003). Pollution may also induce demographic bottlenecks, which negatively affect the viability and fertility of individuals. Consequently, the effective population size will be reduced, leading to a reduced genetic diversity. Additionally, it has been reported that suboptimal salinity, pH and/or nutrient levels can potentially also cause genetic isolation of natural populations (De Wolf et al. 2001; De Wolf et al. 2004a; De Wolf et al. 2004b). COI is key in the respiratory chain and *Terschellingia* live in hypoxic sediments. Strong selection may thus be the key factor leading to the observed low COI diversity.

In conclusion, our results corroborate previous suggestions that genetic structure of marine nematodes, and marine invertebrates in general, are not dependent on a single factor but are more likely a result of multiple characteristics of the organisms and the environment, including morphology and life-history features, habitat characteristics and hydrodynamic forces (Derycke et al. 2013).

5.5. Future perspectives

As the current work was the first study dealing with marine nematodes in an important area of the PG, other important aspects of this neglected field of science need to be addressed through future research:

1) To accelerate studies on marine nematology in Iran, there is an urgent need to develop capacity building at the level of human resources through the academic training programs in universities and respective institutes. Current Iranian nematologists have actually all focused on identification, taxonomy, and management of plant parasitic nematodes in the agricultural sector. To develop the field of marine nematology in Iran, it is essential to motivate young scientists to pursue careers in marine ecology and nematology in particular.

2) Further research on other marine habitats is strongly needed to provide a comprehensive overview of marine nematode biodiversity in the PG region. In the present study, we worked only on marine nematodes inhabiting sandy beaches; however, the PG has a rich variety of other coastal habitats such as rocky shores, estuarine ecosystems, mangrove habitats, salt marshes and mudflats. This heterogeneous environment is of great ecological interest, as

environmental heterogeneity has been identified as a key factor for the maintenance of animal biodiversity in aquatic ecosystems (Levin et al. 2010).

3) The analysis of community structure by measuring the species diversity is a popular ecological technique during this era and widely employed by marine ecologists. With increasing interest in describing the relationship between species biodiversity and ecosystem functioning, this kind of research is of great importance. This is all the more relevant in organisms like nematodes, because their assemblages typically comprise multiple species with supposedly similar ecology and function. The degree of redundancy in nematode assemblages remains a matter of debate, and requires the development of novel experimental designs.

4) Given the high anthropogenic impact in the PG region, a pollution monitoring program should be established. This would allow to quantify pollution on the one hand and changes in the community state over time on the other. In line with many investigations, we would recommend that both macrofauna and nematodes should be used in pollution monitoring programs. Many studies have demonstrated the fundamental advantage of a multi-species approach in pollution monitoring. The inclusion of many taxonomic and functional groups that have a broad range of sensitivities to different environmental parameters may integrate different aspects of the system, revealing complementary aspects of the factors structuring the benthic ecosystem (Vanaverbeke et al. 2011; Patrício et al. 2012). Moreover, physico-chemical parameters (e.g., salinity, temperature, grain size, hydrocarbons, trace metals) should be measured on a regular basis. A sound monitoring program could be useful to establish guidelines for, e.g., sewage and industrial waste water filtering. Nevertheless, the results of the present PhD also demonstrate that the use of nematodes for environmental monitoring in coastal ecosystems has limits and may require optimization in order to better address local situations. Moreover, it may be difficult, if not impossible, to find ‘undisturbed’ or ‘pristine’ reference sites with which to compare the situation in perturbed areas; hence, monitoring may to a large extent have to remain restricted to a follow-up of changes over time and/or in response to specific disturbances or pollutions.

5) Whilst sandy beaches are relatively continuous over considerable stretches of coastline, and are physically dynamic and therefore more connected, other habitats in the PG are more discrete, less connected, and potentially much more vulnerable. Population-genetic studies on species from other habitats is therefore required to assess connectivity of these other habitats

among themselves and with other habitat types (for instance mangroves and bare tidal flats, beaches and shallow subtidal habitats like coral reefs, …), because this connectivity is an important aspect of resilience.

6) The present study has identified nematodes to the genus and occasionally to the species level. More systematic identification to the species level, and this not only based on classical morphological but also on molecular criteria, is required for a proper assessment of diversity, and of the presence and significance of species endemism (for which our present study did not find any evidence, but also did not take a proper approach).

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Conferences

1. **Sahraeian, N.**; Negarestan, H.; Bezerra, T.N. Moens, T., **2012**. Nematode assemblage structure and diversity in intertidal soft sediment shores of Bandar Abbas (Persian Gulf). *Poster presentation at VLIZ, Young Marine Scientists day, February 24th, 2012, Brugge, Belgium.*
2. **Sahraeian, N.**; Negarestan, H.; Bezerra, T.N. Moens, T., **2012**. Community structure and biodiversity of nematodes in intertidal soft sediment shores of the Persian Gulf in relation to local pollution sources. The 2nd International Symposium on Nematodes as Environmental Bioindicators. 5-6 July 2012, Ghent University, Belgium.

3- **Sahraeian, N.**, Mosallanejad, H, Negarestan, H and Moens, T. (**2012**). An investigation and identification of marine nematodes of intertidal beaches, Bandar Abbas. ^{17}th national and ^{5}th international Iranian biology conference. Kerman (Iran).

4- **Sahraeian, N.**, Mosallanejad, H, Negarestan, H and Moens, T. (**2012**). An investigation on fluctuation of marine nematodes in two intertidal beaches of Bandar Abbas, receiving different amount of urban pollution. ^{17}th national and ^{5}th international Iranian biology conference. Kerman (Iran).

5- **Sahraeian, N.**, Mosallanejad, H, and Moens, T. (**2013**). Community structure and biodiversity of marine nematodes of soft intertidal beaches of Bandar Abbas. National aquatic conference of animal science. 27-29 August 2013, Rasht, Iran.

6- **Sahraeian, N.**, Mosallanejad, H, and Moens, T. (**2013**). Effect of urban pollution on biodiversity of marine nematodes of soft intertidal beaches of Bandar Abbas. National aquatic conference of animal science. 27-29 August 2013, Rasht, Iran.

7- **Sahraeian, N.**, Mosallanejad, H, and Moens, T. (**2014**). Effect of urban pollution on community structure of marine nematodes of soft intertidal beaches of Bandar Abbas. First national conference of passive defense in marine sciences. 19-21 November 2014, Bandar Abbas, Iran.

Publication list

A1- peer reviewed articles

Sahraean, N., T. Nara Bezerra, K. Ejlali Khanaghah, H. Mosallanejad, E. Van Ranst & T. Moens, 2017a. Nematode assemblage structure and diversity in intertidal soft shores of the northern Persian Gulf. Hydrobiologia accepted. (pending minor revisions)

Sahraean, N., J. Van Campenhout, A. Rigaux, H. Mosallanejad, F. Leliaert & T. Moens, 2017b. Lack of population genetic structure in the marine nematodes *Ptycholaimellus pandispiculatus* and *Terschellingia longicaudata* in beaches of the Persian Gulf, Iran. Marine Ecology accepted.

Appendices

Appendix 1

Photographs of five most abundant marine nematode genera found in our study: *Daptonema*, *Ptycholaimellus*, *Terschellingia*, *Promonhystera* and *Paramonhystera.*

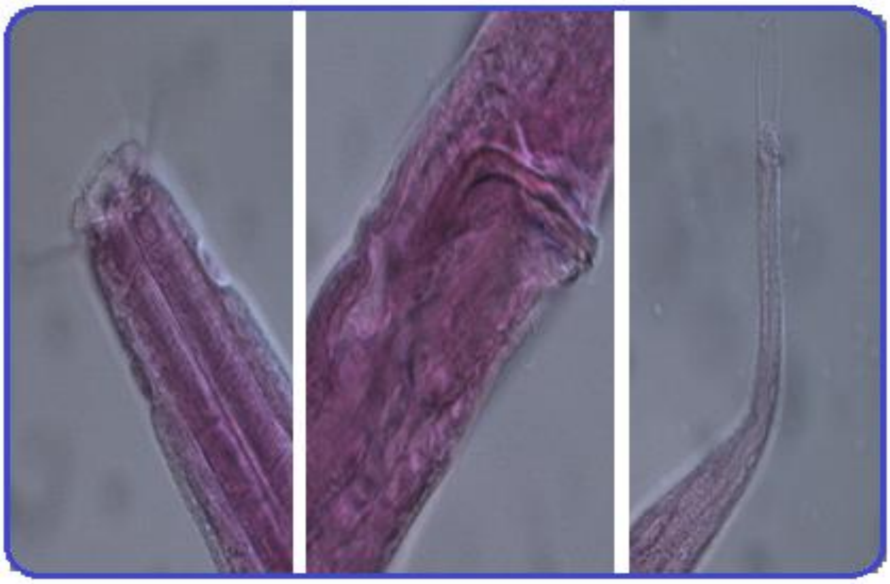

Fig. Ap.1) ***Daptonema sp.***; left: anterior part; middle: spicule apparatus and right: posterior part showing the tail; scale bars: 20 µm.

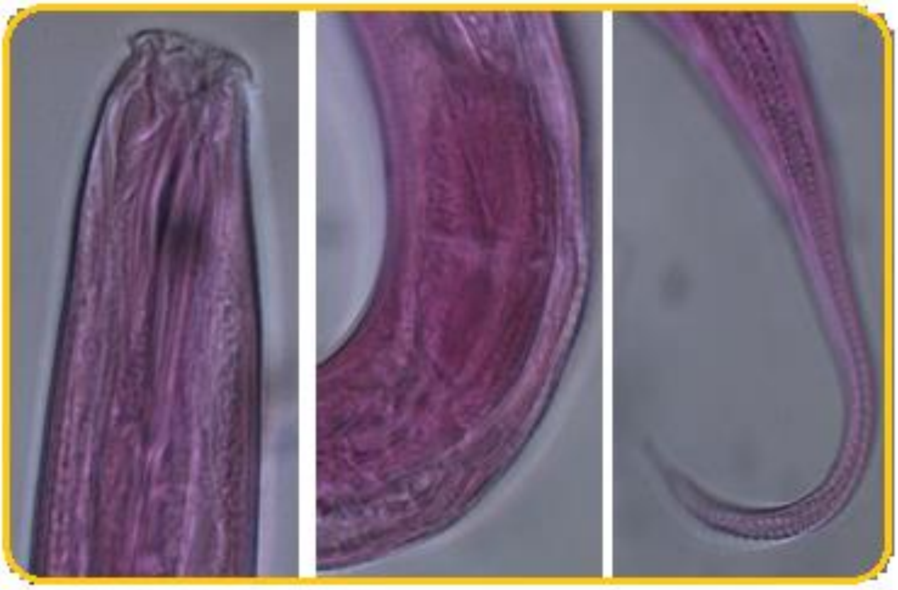

Fig. Ap.2) ***Ptycholaimellus pandispiculatus.***; left: anterior part; middle: pharyngeal bulb and right: posterior part showing the tail; scale bars: 20 µm.

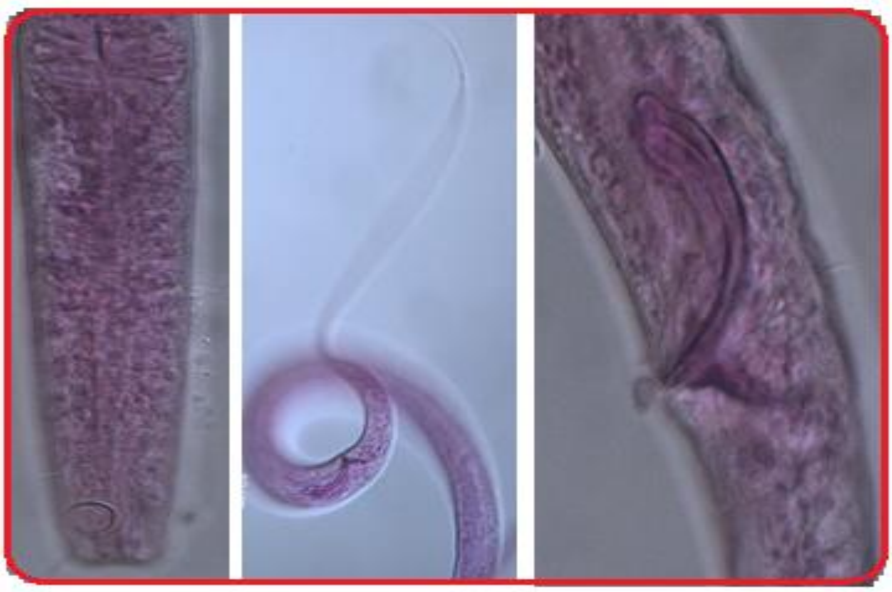

Fig. Ap.3) ***Terschellingia longicaudata***; left: anterior part with the pharyngeal bulb; middle: posterior part showing the tail and right: spicule apparatus; scale bars: 20 µm.

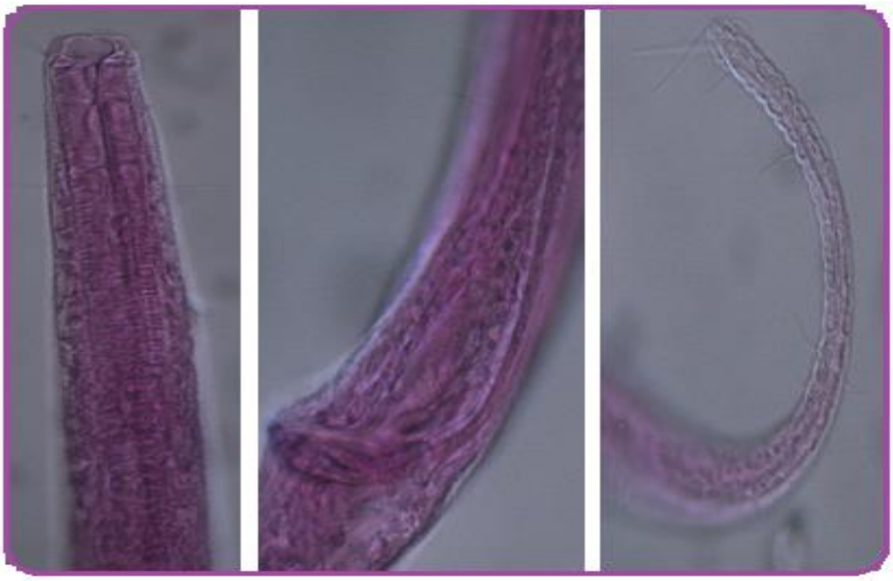

Fig. Ap.4) ***Promonhystera sp.***; left: anterior part; middle: spicule apparatus and right: posterior part showing the tail; scale bars: 20 µm.

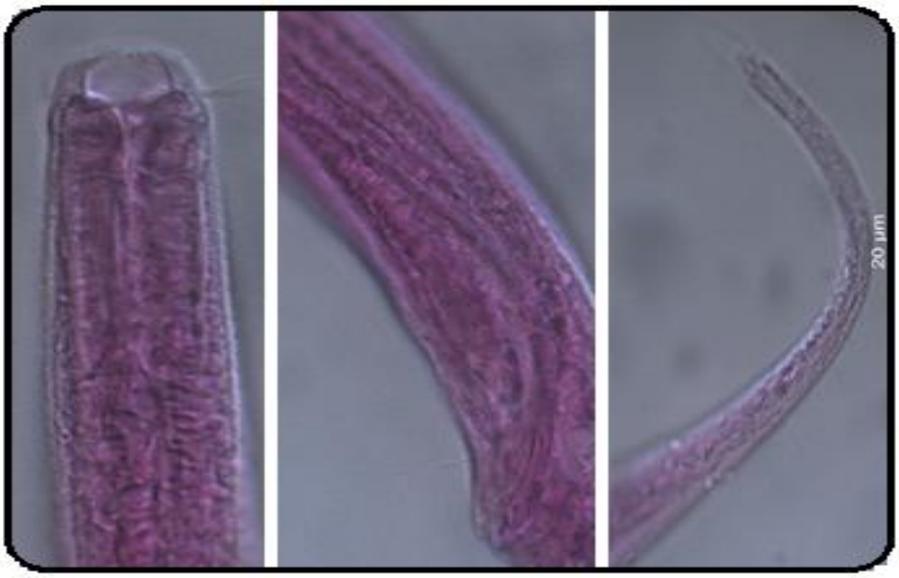

Fig. Ap.5) ***Paramonhystera spp.***; left: anterior part; middle: spicule apparatus and right: posterior part showing the tail; scale bars: 20 µm.

Appendix 2

The classification of nematodes found in the northern part of Persian Gulf. according to Deley et al. (2006) from phylum to family level and according to Lorenzen (1981, 1994) and Eyualem – Abbe et al. (2006) below family level.

PHYLUM NEMATODA Potts, 1932

Class ENOPLEA Inglis 1983
  Subclass ENOPLIA Pearse 1942
    Order ENOPLIDA Filipjev 1929

Family Enchelidiidae Filipjev 1918

Symplocostoma

Calyptronema Marion 1870

Family Oncholaimidae Filipjev 1916
Metoncholaimus Filipjev 1918

Oncholaimus Dujardin 1845

Viscosia de Man 1890

Family Oxystominidae Chitwood 1935
Oxystomina Filipjev 1921

Family Phanodermatidae Filipjev 1927

Phanoderma

Family Thoracostomopsidae Filipjev 1927

Enoploides Ssaweljev 1912

Family Tripyloididae Filipjev 1928

Bathylaimus Cobb 1894

Tripyloides de Man 1886

Class CHROMADOREA Inglis 1983
  Subclass CHROMADORIA Pearse 1942
    Order MONHYSTERIDA Filipjev 1929

Family Linhomoeidae Filipjev 1922

Eleutherolaimus Filipjev 1922

Eumorpholaimus Schulz 1932

Terschellingia de Man 1888

Family Monhysteridae de Man 1876

Monhystrella Cobb 1918

Thalassomonhystera Jacobs 1987

Family Sphaerolaimidae Filipjev 1918

Doliolaimus Lorenzen 1966

Metalinhomoeus de Man 1907

Sphaerolaimus Bastian 1865

Family Xyalidae Chitwood 1951

Daptonema Cobb 1920

Paramonhystera Steiner 1916

Promonhystera Wieser 1956

Rhynchonema Cobb 1920

Theristus Bastian 1865

Xyalidae *sp*

Class CHROMADOREA Inglis 1983
Subclass CHROMADORIA Pearse 1942
Order ARAEOLAIMIDA De Coninck & Schuurmans Stekhoven 1933
Family Axonolaimidae Filipjev 1918

Axonolaimus De Man 1889

Odontophora Butschli 1874

Pseudolella Cobb 1920

Family Comesomatidae Filipjev 1918

Hopperia Vitiello 1969

Sabatieria de Rouville 1903

Family Diplopeltidae Filipjev 1918

Araeolaimus de Man 1888*Araeolaimus*

Class CHROMADOREA Inglis 1983
- Subclass CHROMADORIA Pearse 1942
  - Order CHROMADORIDA Chitwood 1933
    - Family Chromadoridae Filipjev 1917
      - *Chromadora*
      - *Chromadorella* Filipjev 1918
      - *Chromadorita* Filipjev 1922
      - *Dichromadora* Kreis 1929
      - *Hypodontolaimus* de Man 1886
      - *Nygmatonchus*
      - *Prochromadora* Filipjev 1922
      - *Prochromadorella* Micoletzky 1924
      - *Ptycholaimellus* Cobb 1920
      - *Spilophorella* Filipjev 1917

    - Family Cyatholaimidae Filipjev 1918
      - *Cyatholaimus* Bastian 1865
      - *Longicyatholaimus* Micoletzky 1924
      - *Marylynnia* Hooper 1977
      - *Metacyatholaimus* Stekhoven 1942
      - *Paracanthonchus* Micoletzky 1924
      - *Pomponema* Cobb 1917
      - *Cyatholaimidae sp*

    - Family Ethmolaimidae Filipjev & Schuurmans Stekhoven 1941
      - *Paraethmolaimus* Jensen 1994

    - Family Selachinematidae Cobb 1915
      - *Halichoanolaimus* de Man 1886
      - Synonchium

Class CHROMADOREA Inglis 1983
- Subclass CHROMADORIA Pearse 1942

Order DESMODORIDA De Coninck 1965

Family Desmodoridae Filipjev 1922

Desmodora de Man 1889

Metachromadora Filipjev 1918

Onyx Cobb 1891

Spirinia Gerlach 1963)

Polysigma Cobb 1920

Order PLECTIDA Malakhov 1982

Family Haliplectidae Chitwood 1951

Haliplectus Cobb 1913

Class CHROMADOREA Inglis 1983
Subclass CHROMADORIA Pearse 1942

Order PLECTIDA Malakhov 1982

Family Leptolaimidae Örley 1880

Deontolaimus de Man 1880

Camacolaimus de Man 1889

Leptolaimoides Vitiello 1971

Leptolaimidae sp

Family Tubolaimoididae Lorenzen 1981

Tubolaimoides Gerlach 1963