Biodiversity and connectivity of the nematofauna for sustainable management of exploited macroalgal communities along the Brazilian coast

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A dissertation submitted to Ghent university in partial fulfillment of the requirements for the Degree of Doctor of Science, Biology

Academic Year 2016 – 2017

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This work was supported by a grant of the Flemisch Interuniversity Council – University Development Cooperation (VLIR-UOS)

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Acknowledgements :: Dankwoord :: Agradecimentos

Now I am coming to the end of a long journey which started almost 10 years ago. My taste for the unknown came at an early age, as it does for most children, but it persisted and hopefully will never cease to exist. First of all, I would like to thank my mother whom always allowed me to experience and experiment all (mostly) non-lethal elements in my surroundings, even when I accidentally set her couch on fire (sorry mom). But now, I am sure that she will understand that her nearly infinite patience was for a good cause. Because of that, all the support, love and many other things that I will not be able to list here, I would like to say "*Obrigado mãe*".

When I was offered this unique opportunity to come to Belgium and study at Ghent university, I grabbed it with all I had. The dream to study abroad however, comes with a bitter taste as one has to be far from family and friends, but the bitterness can be overcome and even enjoyable as I have learned by eating witlof (preferentially with cheese sauce!). More importantly, I have made very important friends, because especially the ones at university, I can no longer look at them simply as colleagues.

Many people came along in this journey such as Prof. Decraemer who is one of the kindest persons I had the pleasure to meet and from whom I obtained valuable knowledge both academically, as well as for life in general. Thank you Prof. Frieda. I also would like to thank Prof. Sofie Derycke for being there since the beginning of this academic adventure and for your patience. I have learned a lot from you. *Obrigado* Prof. Giovanni, for your support especially when I needed some extra strength. I am also grateful for all the insightful contributions of Prof. Tom Moens whom taught me how to critically improve my work. I would like to thank all jury members for the remarks that led to a better manuscript.

Thanks to all my friends/colleagues Hanne Steel, Prof. Wim Bert, Lidia Lins, Marjolein Couvreur, Alcides Sanchez-Monge, Toon Janssen, Yao Kolombia, Xue Qing, Dieter Slos, Nic Smol, Luana Monteiro, Tania Nara and many others whom were very important in my daily life. I really appreciate everything you have done for me and I will always be thankful. Thank you my sister Débora, niece Alice and all my family for existing in my life. I am also thankful to all my friends from Brazil Thiago, Zito, Rodrigo, Felipe for always being there to welcome me at the airport in Recife.

Thanks also to VLIR-UOS for funding this research.

I would like to dedicate this work to other people that believed in me but are not among us anymore such as my father José Maria, my grandmother Judite who is the first and a very unique biologist in my family, and Prof. Verônica.

Also I would like to dedicate this PhD thesis to my wife, who will always be my "biquinho" and was my Atlas, sharing the weight of my world on her shoulders whenever it became too heavy for me. Finally, I would like to dedicate my thesis to my daughter Iris who is my "supreme biological experiment", soothing every difficulty with her beautiful, still toothless smile and giving me the strength to keep on going forward. "*Painho lhe ama muito*".

I would like to apologize for names that were not mentioned here, but please know that I appreciated every single help you may have provided me and know that you have a place in my life.

Summary

Seaweed beds are diverse and productive ecosystems in shallow water coastal areas. They provide ecological services such as attenuation of currents, contribute for the formation of the adjacent sediment in the form of bioclast, sedimentation of particles suspended in the water column and are a natural cradle for marine organisms. Besides their ecological importance, many economically natural resources are valuable for industry from the seaweed itself to the fish and crustacean which reproduce and develop in the phytal ecosystem. Because of the industrial potential of seaweed beds, uncontrolled harvesting of seaweeds took place in the northeastern coast of Brazil, starting in the 1960's. This exploitation caused a massive transformation in the environment in at least one location known as Icapuí located in Ceará state – Brazil. As consequence, economically important fishes and lobsters disappeared from the region causing not only a negative ecological impact but also socially, as the local fishermen community relied on fishery resources for their own subsistence. Unfortunately, knowledge on the dynamics of seaweed beds and associated fauna is very limited especially in tropical areas such as along the South American coast. Small size metazoan associated with seaweeds such as the meiofauna are known to be food sources for higher trophic levels including economically important organisms. Also, because those small size organisms such as nematodes, graze on diatoms and cyanobacteria that grown on seaweeds surface competing for light and nutrients, they may play a role on controlling the populations of those microalgae. Studying the nematode communities, which are known for playing a key ecological role in marine ecosystems, could potentially provide insights on the dynamics of the phytal ecosystem.

We studied the spatiotemporal variation of the nematode communities at local scale. We used two seaweed species *Sargassum polyceratium* and *Halimeda opuntia*, measured the particle retention capacity of those macrophytes in regions more and less exposed to wave action by comparing two transects, 80 m apart and parallel to the beach line in Cupe Beach, Brazil over a five months period including rainy and dry seasons to: i) find temporal patterns of the nematode community; ii) look for differences in the nematode community between seaweeds; iii) test whether physical factors such as sediment accumulation and wave exposure would cause an effect in the abundances of the nematode community. We found that: a) nematode densities were higher during the rainy season (ANOVA) while the community composition was very similar with the genus *Euchromadora* being dominant in both seaweed species; b) the amount of sediment retained by the seaweed did not affect the total nematode abundances (ANOVA) but correlated positively with richness, showing in both seaweed species a positive correlation with the density of *Draconema* and *Euchromadora* (Spearman), two genera only found in seaweed and the spatial variation in the community appeared to be related to the level of exposure to the waves.

We expanded the sampling scale during rainy season to 8 beaches along the Brazilian coast along 3540 km coastline under the influence of the North Brazil and Brazil currents and looked to the nematode communities associated with seaweeds of the genera *Sargassum* and *Gracilaria* and in the adjacent bottom sediment, in exploited and non-exploited beaches to: iv) estimate the resemblance level between the nematode community of each substrate to understand whether the nematode community present in the sediment could simply recolonize and completely restore the diversity found on seaweeds; v) to look for changes in the nematode community which could have been caused by historical harvesting; vi) to look for latitudinal nematode

biodiversity patterns as two different sea currents. We found: d) that nematode assemblages present in the sediment may not completely restore the diversity found on seaweeds because the two communities were significantly different (PERMANOVA), meaning that by complete removal of seaweeds, the nematode genera richness loss can be substantial; e) although not conclusive, it is possible that seaweed harvesting decreases the abundances and diversity of the nematode communities depending on the intensity of the harvesting (ANOVA); and f) no latitudinal variation was observed but nematode communities from seaweeds were much more similar along the Brazilian coast compared with the ones found in the sediment (PERMANOVA and ANOVA).

Connectivity between populations is an important factor for communities to withstand negative impacts. We used a new nematode species (*Paracanthonchus gynodiporata* sp. n.) that only occurred associated with seaweeds in two beaches with and two without historical exploitation and which were further divided by two opposite sea currents to: vii) test (F_{st}) whether those populations have strong population structuring due to genetic breaks caused by great distance (>1000 km) as well as by the two main divergent sea currents; viii) to look for effects on genetic diversity caused by historical harvesting; ix) describe the new species using the integrative taxonomy approach by combining morphology and molecular analysis using mitochondrial gene COI and test whether the genetic diversity is congruent with the morphology. We found that: g) an overall low genetic structure was observed between the populations despite the distance and main sea currents; h) no evidence for an effect on the haplotype diversity was observed as a consequence of historical exploitation; and i) the genotypes were very conserved while the phenotypes varied significantly which is the opposite from many other nematode species where cryptic speciation is substantial.

A multiple species and molecular markers approach to create genetic libraries to better estimate diversity will be necessary in the future to understand better the connectivity and dynamics of seaweed beds in tropical areas and the effect of anthropogenic stress in phytal ecosystems.

Samenvatting

Zeewierbedden zijn diverse en productieve ecosystemen in de ondiepe wateren van kustgebieden. Ze zorgen voor belangrijke ecologische diensten zoals de afname van zeestromingen, dragen bij tot de vorming van aangrenzend sediment tot bioklasten, verzorgen de afzetting van deeltjes in de waterkolom en zijn een natuurlijke wieg voor mariene organismen. Naast hun ecologisch belang, zijn de zeewierbedden economisch waardevol voor het zeewier zelf en voor de vissen en schaaldieren die zich in dit fytale ecosysteem voortplanten en ontwikkelen. Omwille van het industriële potentieel van zeewierbedden, werd het zeewier ongecontroleerd geoogst in het noordoosten van Brazilië sinds de jaren 60. Deze exploitatie zorgde voor een aanzienlijke transformatie van de omgeving in tenminste één locatie gekend als Icapuí in de staat Ceará – Brazilië. Ten gevolge hiervan verdwenen economisch belangrijke vissen en kreeften uit de regio, wat niet alleen resulteerde in een negatieve ecologische impact, maar ook socio-economische gevolgen had voor de lokale vissersgemeenschap die voor hun eigen bestaansmiddelen afhankelijk zijn van de visserij. Jammer genoeg is de kennis over de dynamiek van zeewierbedden en de geassocieerde fauna beperkt, voornamelijk in tropische gebieden zoals de kust van Zuid-Amerika. Kleine Metazoa geassocieerd met zeewier, zoals meiofauna, zijn gekend als voedsel voor hogere trofische niveaus waaronder economisch belangrijke organismen. Daarnaast is het mogelijk dat deze Metazoa, zoals nematoden, grazen op diatomeeën en cyanobacteriën die groeien op het oppervlak van het zeewier en strijden voor licht en nutriënten, en zo een rol spelen bij de controle van de populatie van deze microalgen. De studie van de nematodengemeenschap, die gekend is om zijn ecologische sleutelrol in het mariene ecosysteem, kan een potentieel inzicht verschaffen in de dynamiek van het fytale ecosysteem.

Wij bestudeerden de ruimtelijke en tijdelijke variatie van nematodengemeenschappen op lokale schaal. We gebruikten twee zeewiersoorten, *Sargassum polyceratum* en *Halimeda opuntia* maten gedurende 5 maanden de partikelretentie capaciteit van deze macrofyten in regio's die min of meer blootgesteld zijn aan golven op het strand door twee transecten op 80m van elkaar en evenwijdig aan het strand te vergelijken gedurende vijf maanden die zich over droog- en regenseizoen uitstrekken om: i) tijdelijke patronen van de nematodengemeenschap te vinden; ii) te zoeken naar verschillen in de nematodengemeenschap tussen de zeewiersoorten; iii) te testen of fysieke factoren zoals de sedimentaccumulatie en blootstelling aan golven een effect zou veroorzaken in de abundantie van de nematodengemeenschap. We vonden dat: a) de densiteit van nematoden hoger was tijdens het regenseizoen (ANOVA) terwijl de samenstelling van de gemeenschap zeer gelijkaardig bleef met het genus *Euchromadora* dominant in beide zeewiersoorten; b) de hoeveelheid sediment vastgehouden in het zeewier heeft geen invloed op de totale nematodenabundantie (ANOVA), maar correleerde positief met de rijkdom en met de densiteit van *Draconema* en *Euchromadora* (Spearman), twee genera die inkel in zeewier voorkomen, en de spatiale variatie in de gemeenschap bleek gerelateerd te zijn tot het niveau van blootstelling aan de golven.

We breidden de schaal van staalname uit tot 8 stranden langsheen de Braziliaanse kust over 3540 km kustlijn die wordt beïnvloed door twee tegenovergestelde zeestromingen, de Noord Braziliaanse en de Braziliaanse zeestromingen en keken naar de nematodengemeenschap geassocieerd met de zeewiergenera *Sargassum* en *Gracilaria* en in het aanpalende bodemsediment, zowel op geëxploiteerde als niet-geëxploiteerde stranden om: iv) het niveau van gelijkenis te schatten tussen de nematodengemeenschap tussen de substraten om te begrijpen of een

nematodengemeenschap simpelweg kan herkoloniseren en de diversiteit gevonden op het zeewier kan herstellen; v) te kijken naar veranderingen in de nematodengemeenschap die kunnen veroorzaakt zijn door historische oogsten; vi) te kijken naar latitudinale nematodenbiodiversiteitspatronen als twee verschillende zeestromen. We vonden dat: d) de nematodengemeenschap van het sediment de diversiteit gevonden in het zeewier wellicht niet volledig zou kunnen herstellen, omdat de twee nematodengemeenschappen significant verschillend (PERMANOVA) zijn. Dit betekent, dat bij de volledige verwijdering van het zeewier, het verlies van de genera rijkdom substantieel kan zijn; e) hoewel niet afdoend bewezen, is het mogelijk dat het oogsten van zeewier de abundantie en diversiteit van nematodengemeenschappen vermindert afhankelijk van de intensiteit van de oogst (ANOVA); f) er werd geen latitudinale variatie geobserveerd, maar nematodengemeenschappen van het zeewier waren veel meer gelijkaardig over de Braziliaanse kust in vergelijking met deze gevonden in het sediment (PERMANOVA en ANOVA).

Connectiviteit tussen populaties is een belangrijke factor voor gemeenschappen om negatieve impact te weerstaan. We gebruikten een nieuwe nematodensoort (*Paracantonchus gynodiporata* sp. n.) die enkel voorkomt in het zeewier op twee stranden met en twee zonder historische zeewierexploitatie, en dewelke ook gescheiden werden door de twee tegenovergestelde zeestromen om: vii) te testen (Fst) of deze populaties een sterke populatiestructuur hebben ten gevolge van genetische breuken veroorzaakt door afstand (> 1000 km) en de twee divergerende zeestromen; viii) te kijken naar de effecten op de genetische diversiteit veroorzaakt door historische oogsten; ix) de nieuwe soort te beschrijven met gebruik van de integratieve taxonomie methode door combinatie van morfologische en moleculaire kenmerken waaronder het mitochondriaal gen COI en te testen of de genetische

diversiteit overeenstemt met de morfologie. We hebben gevonden dat: g) er een totale lage genetische structuur werd geobserveerd tussen de populaties ondanks de afstand en de zeestromen; h) er geen bewijs is voor een effect op de haplotype diversiteit als gevolg van de historische zeewierexploitatie; en i) de genotypes waren zeer geconserveerd terwijl de phenotypes een significante variatie vertoonden, wat tegenovergesteld is aan vele andere nematodensoorten waar cryptische speciatie substantieel is.

In de toekomst zal het nodig zijn om genetische bibliotheken te creëren met een veelvuldige soorten en moleculaire markers methode, zodat we de connectiviteit en dynamiek van zeewierbedden in tropische gebieden en het effect van antropogene stress op fytale ecosystemen beter kunnen begrijpen.

Chapter 1: *General introduction*

1.1. Rationale

1.1.1. ***Biodiversity***

The alarming worldwide decline in biodiversity has strong consequences for ecosystem functioning because biodiversity often increases ecosystem process rates and resource use, and affects ecosystem stability (Loreau et al. 2001, Loreau 2010). The year 2010 was elected by the United Nations as the International Year of Biodiversity and the urgency to reduce biodiversity loss was emphasized. The reduction of biodiversity is associated with several environmental processes such as global warming and habitat fragmentation caused by anthropogenic activities (Sala et al. 2000, Duffy 2003, Fahrig 2003). In particular, an effort to detect biodiversity hotspots is fundamental to safeguard biodiversity on our planet. Biodiversity hotspots are generally defined as biogeographic regions that have undergone exceptional habitat loss and exhibit high concentrations of endemic species (Myers et al. 2000). Many of these hotspot areas are located pantropically in agreement with the general latitudinal diversity gradient hypothesis (Hillebrand 2004). The latter assumes an increase in biodiversity from higher to lower latitudes around the globe (see also under 1.1.2.).

1.1.1.1 Types of Biodiversity

The word "biodiversity" is a contraction of the words "biological" and "diversity" introduced by Lovejoy (1980) and formally described in article 2 of the Convention of Biological Diversity (https://www.cbd.int/convention/articles/default.shtml?a=cbd-02. Accessed in 06/11/2016) as "*the variability among living organisms from all sources including,* inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between*

species and of ecosystems". Biodiversity can further be subdivided into three major groups (Norse et al. 1986): (1) **genetic diversity** defined as any measure that quantifies the magnitude of genetic variability within a population (Hughes et al. 2008), (2) **species diversity** defined in many different ways but sometimes simply referred to as the number of species (Hamilton 2005) and (3) **ecological diversity.** The latter includes species diversity, niche width describing the resource availability to a species over a spatiotemporal scale (Magurran 1988), and habitat diversity which deals with the structural complexity of the environment (Mumby 2001).

1.1.1.2. Species Definition, Delimitation and Cryptic Speciation

Researchers often find it complex to precisely estimate species diversity (Hamilton 2005). This is partially due to the lack of agreement on the definition of "a species". Overviews of different interpretations are given and discussed in (Mayden 1997), (De Queiroz 1998), (Harrison 1998) and(Hey 2006). Over more than 52 species definitions have been proposed (Wheeler and Meier 2000), each emphasizing one or more biological aspects. Although they do have a certain level of overlap, all species concepts face some limitation at some point. For instance, the **biological species concept** (Mayr and Ashlock 1991) implies that a species is a group of interbreeding natural populations which is reproductively isolated from other groups. However, it is difficult to address the level of "reproductive isolation" in view of the existence of hybrids, and to apply this concept to organisms that reproduce asexually. The **evolutionary species concept** states that "*a species is a lineage of ancestral descendant populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate*" (Wiley 1978). However, individually diverging lineages which follow an evolutionary tendency now may

reticulate in the future (Adams 1998), indicating that they were not different species in the first place. The **morphological species concept** where species are the smallest groups of organisms that are consistently and persistently distinct and distinguishable by ordinary means (Cronquist 1978), and focuses on the morphological discontinuity between species or morphological distinctiveness whereby morphological differences are considered as surrogates for underlining genetic differences (Decraemer et al. 2008). The limitation in this case is the morphological plasticity as a result of environmental variation (Reed et al. 2011) which decreases intraspecific resemblance (Chapter 4) or is the result of convergent evolution which may cause different species to look similar (Givnish et al. 1999, Lindgren et al. 2012, Muschick et al. 2012), or the result of morphological stasis (lack of change in phenotype) while the genotypes are significantly distinct as observed in cryptic speciation.

Cryptic species are defined as two or more species which are erroneously classified under one species name (Bickford et al. 2006). Cryptic speciation illustrates that evolution of the genes is not always accompanied by morphological changes, which is particularly true for recently diverging species (Leliaert et al. 2014), and in organisms with well-developed chemosensory system in which pheromones may be more important for mating than morphology (nematodes - O'Halloran et al. 2006, Edison 2009). Cryptic species can in some cases reflect ecological differences in food source preferences (Derycke et al. 2016), environmental tolerance (De Meester et al. 2011, De Meester et al. 2015), and/or reflect on distinct evolutionary histories (Elmer et al. 2013, Glasby et al. 2013, Pérez-Portela et al. 2013). Consequently, there is an underestimation of species diversity which might be much higher than originally thought (Trontelj and Fišer 2009). With the increase of available molecular tools,

cryptic speciation has been observed in a wide range of taxa spread all over the globe (Pfenninger and Schwenk 2007).

Many species concepts are incongruent on how and at what point one should consider divergent lineages distinct species in the evolutionary history, also known as the "**grey zone**" (De Queiroz 1998, 1999, 2005)(Fig. 1). In an effort to unify the diverse species concepts, (De Queiroz 2007) focused on the congruence among the concepts making a distinction between species conceptualization and delimitation. The idea of species being a separately evolving metapopulation was categorized as a **primary defining property** of a species, and intrinsic reproductive isolation (biological concept) or morphological distinctiveness (morphological concept), as **secondary defining property**. As such, the definition of species (primary property) is separated from secondary property, used as evidence for lineage separation and delimitation.

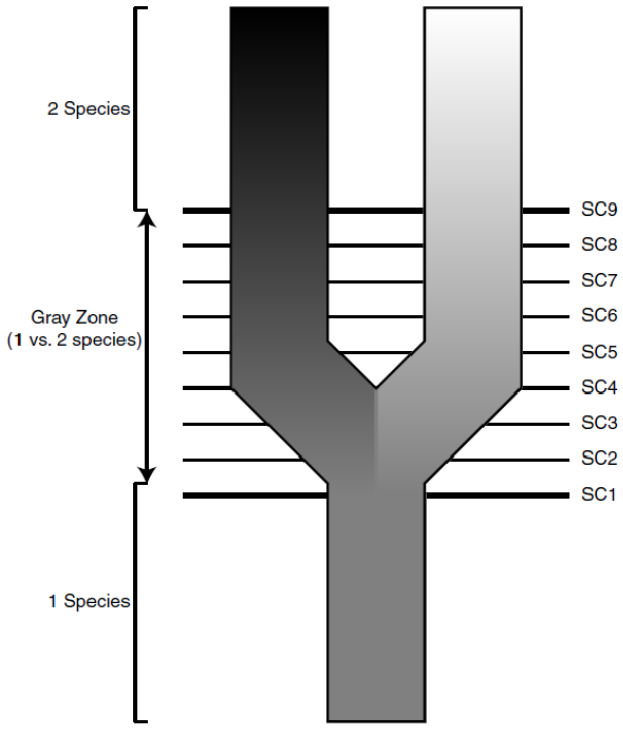

Figure 1. Independently evolving lineages and representation of the so called Gray Zone where different species concepts may conflict. Modified after De Queiroz (2007).

Molecular tools are very useful to delimit species boundaries but are not free of inconsistencies. Threshold for genetic distance to establish species boundaries in some cases might look arbitrary (Avise and Walker 2000, Meier et al. 2006). For instance the interspecific threshold for the mitochondrial DNA cytochrome oxidase subunit I (COI) varies across species (Ferguson 2002, Will and Rubinoff 2004, DeSalle et al. 2005) and can be subjective as the intra- and interspecific variation values approach the threshold value.

Stochastic evolutionary processes which lead to lineage differentiation such as genetic drift (more details in 1.1.3.) play an important role on diverging lineages. The way in which alleles that are inherited and lost by diverging lineages may result in a non-monophyletic tree compared with the species tree, which is known as **incomplete lineage sorting** (Fujita et al. 2012). Among others, evolutionary processes such as hybridization, and trans-species polymorphism where recently diverging lineages share a number of alleles (Klein et al. 1998) can blur species delimitation. Fortunately, a growing number of statistical models based on the coalescent theory (Fujita et al. 2012) with single and multilocus data have been developed for species delimitation. In our work we agree with the species conceptualization proposed by De Queiroz (2007), and have used a combination of multiple independently evolving loci (nuclear and mitochondrial - Chapter 4) to support our species delimitation. The task of describing biodiversity involves different approaches, different disciplines and is of fundamental importance to understand ecological and evolutionary processes, and to estimate how far natural or anthropogenic changes in the environment will affect the fate of the ecosystems.

1.1.2. ***Latitudinal Biodiversity Gradient***

The latitudinal biodiversity gradient states that there is an increase in biodiversity from the poles to the tropics, symmetrically in both hemispheres, for active and passive dispersers in terrestrial and aquatic habitats around the world (Hillebrand 2004). There are three main hypotheses to explain this general pattern from an evolutionary angle (Mittelbach et al. 2007). (1) Higher diversification rates in the tropics largely based on the hypothesis that higher temperatures increase the speed of evolutionary processes (Rohde 1992, Allen et al. 2002) (2) Same diversification rate across the same latitude but diversification time in the tropical region is longer. This hypothesis which is in contrast with the first, states that tropical environments are just older with many clades originating from the tropical zone, (Wallace 1878, Fischer 1960, Futuyma 1998, Wiens and Donoghue 2004), and that dispersal of instant clades outside the tropics is recent and more limited (Farrell et al. 1992, Latham and Ricklefs 1993, Brown and Lomolino 1998, Futuyma 1998). (3) Different extinction rates deal with the hypothesis that environmental stability is higher in the tropics (Darwin Charles 1859, Wallace 1878, Fischer 1960) and combined with spatial capacity, the tropical region harbours larger population sizes and higher species diversity (Terborgh 1973, Rosenzweig 1995). The latter two features would lead to lower extinction rates in the tropics compared to temperate regions.

1.1.2.2. Latitudinal Patterns in Small-Size Metazoans

Despite the general global latitudinal pattern for biodiversity (an increase in biodiversity towards low latitudes), this pattern does not always apparent. For meiofaunal organisms, biodiversity patterns seem to be taxon and habitat related. A study

investigating the meiofauna from sandy beaches, ranging from higher (artic) to lower latitude (tropics), found no latitudinal pattern for the true meiofauna (Kotwicki et al. 2005). Biodiversity patterns in nematodes largely vary and can have (1) no latitudinal pattern: biodiversity is driven by food rather than latitudinal gradient (deep sea - Lambshead et al. 2000; 2002); (2) intercalation of biodiversity between zones, i.e. higher diversity in the tropics and temperate zone, and lower diversity in subtropical and polar zones are observed (estuaries - Fonseca & Netto 2015); and (3) higher species diversity in the tropics: biodiversity follows the general global latitudinal pattern (sandy beaches - Lee & Riveros 2012). However for the latter pattern, species richness may be higher at low latitudes, while from a phylogenetic point of view, diversity can be lower as low-latitude assemblages may be phylogenetically closer related compared to higher latitudes assemblages (wetlands - Wu et al. 2016). It has been demonstrated that specially for nematodes, biodiversity patterns are not always followed by the general latitudinal biodiversity gradient, and many times depend on the kind of environment they live in. There are some important environments which could receive more attention in this aspect. Information on latitudinal gradient pattern for epiphytic small metazoans are currently unclear, and studies on this ecosystem could contribute to our understanding of biodiversity in shallow water ecosystems across a latitudinal gradient, and the evolutionary processes which led to the current pattern.

1.1.3. ***Evolutionary Processes and Connectivity, and the Advance of Molecular Methods***

1.1.3.1 Evolutionary Processes

Mutation, genetic drift, selection and gene flow are all important processes involved in differentiating populations (Hartl 2000). Mutation is one of the fundamental phenomena underlying evolution by increasing genetic variability (Nei 1983, 1987). This genetic variability can be observed in different copies of a gene (alleles). Provided there is a barrier to gene flow between two populations, and that there is enough time, fixation of alternative selected alleles (divergent selection) can ultimately lead to profound genetic differentiation between lineages, culminating in speciation (Coyne and Orr 2004). Speciation can occur at different conditions: **Allopatric** – lineages are geographically separated e.g. by a vicariant event such as the emergence of a physical barrier preventing gene flow between populations; **Peripatric** – which can be considered a type of allopatric speciation where a small peripheric subpopulation is separated from the main population without gene flow between this subpopulation and the original population; **Parapatric –** occurs when speciation takes place between contiguous populations, i.e. populations are partially separated but still overlap at a certain level and; **Sympatric** – speciation occurs within the same area (overlap) of the offspring. It has some similarity with parapatric speciation but differs from the former because premating reproductive isolation takes place before a population shifts to a new niche (Mayr 1963, Bush 1975) (Fig. 2).

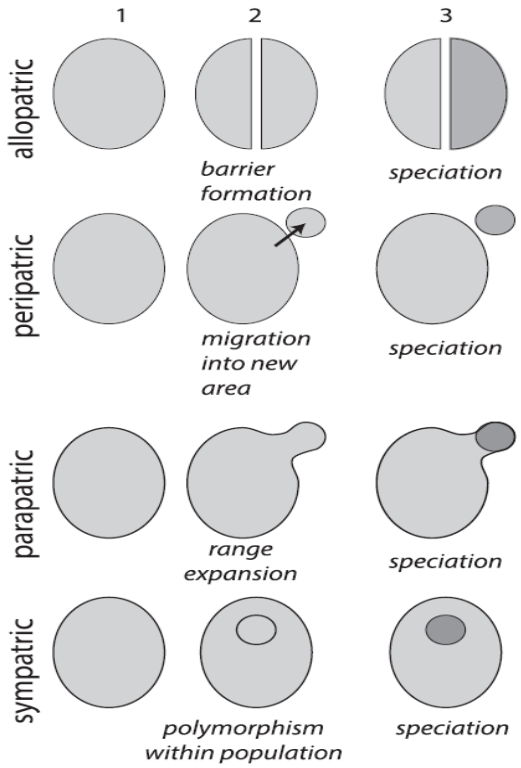

Figure 2. Allopatric, peripatric, parapatric and sympatric speciation. Modified after Renema 2015.

In contrast with mutation and divergent selection, there are important evolutionary processes that decreases genetic diversity. A significant decrease in population size caused by e.g. predation, resulting in a decrease of a population gene pool, characterizes a genetic **bottleneck** (Wright 1931, Nei et al. 1975, Chakraborty and Nei 1977, Nei 1977) . This pressure can be of anthropic origin, as for instance overfishing (Pinsky and Palumbi 2014), causing massive changes in population size, affecting allelic frequencies. The random loss of alleles over generations is known as **genetic drift,** a process that is always acting but is particularly important in small populations. Differences in allelic frequencies can also be a result of the **founder effect**, i.e. when a small fraction of a source population (underrepresenting the original gene pool)

colonizes a new area, allowing for rare alleles to reach higher frequencies compared with the source population (Mayr 1942, Matute 2013). In ephemeral habitats, the founder effect can play an important role in shaping the genetic diversity and structure of the populations as colonization dynamics are likely to be high (nematodes - Derycke et al. 2007). Despite the loss of genetic diversity after a deleterious event (e.g. bottleneck), some variety of alleles might persist in the gene pool for much longer than is expected by natural genetic drift as a result of **balancing selection** (Aguilar et al. 2004), which favours the maintenance of heterozygosity in populations (Hedrick 2007). This is important because it maintains a certain level of genetic diversity which may be crucial for populations to withstand adverse environmental conditions. **Gene flow** is the exchange of alleles between populations e.g. through migration (Wright 1943), leading to reduced differentiation between populations.

1.1.3.2. Environmental Variation and Genetic Diversity

Genetic diversity generates critical response diversity for species to adapt to changing environments (Ehlers et al. 2008, Wilkinson et al. 2010) and can even be increased by gene flow. In general, the higher the connectivity between populations, the higher the resilience to withstand negative impacts (Duffy et al. 2001, Loreau et al. 2001, Duffy 2003, Cook et al. 2007, Ehlers et al. 2008, Hughes et al. 2008). However, in some occasions, higher connectivity may also have a negative effect, allowing for non-native organisms to swamp the native populations with alleles that are locally less fit (**migration load** - Hu & Li 2003). Studies estimating connectivity are very important to understand how populations can respond to changes in the environment they are embedded in.

1.1.3.3. Connectivity and Estimation Methods

Connectivity can be seen as a broad concept which encompasses different aspects of physical factors and how the organisms interact in terms of behaviour, migration and reproduction capacity. One of the first concepts of connectivity was proposed by Taylor et al. (1993), who focused on the physical aspect in terrestrial ecosystems, stating that connectivity is the degree in which the landscape prevents the movement among resource patches. Later on, a concept that suits better with aquatic ecosystems was proposed, which also included hydrologic connectivity (Pringle 2003). Other authors introduced more biological details in which connectivity is the net result of transport, larval survival, settlement and post-larval survival (Pineda et al. 2007). Currently, demographic and genetic (Lowe and Allendorf 2010), together with the above mentioned aspects, are important aspects to directly or indirectly infer connectivity levels.

Direct methods to infer connectivity involve mark-recapture techniques where the target organism is tagged in point A and the same tagged organism is recaptured in another moment in point B (Webster et al. 2002, Jacobson and Peres-Neto 2010). This method is precise and provides valuable information. Organisms connectivity can also be remotely estimated by means of tagging the target organism and tracking them using high frequency radio devices, weather radar and/or satellites (Millspaugh and Marzluff 2001, Rutz and Hays 2009, Randall et al. 2011). However, direct methods also have limitations, including organism size (for very small ones e.g. nematodes), large population size and distances (Kool et al. 2013). Moreover, direct methods are more limited to a demographic time-scale.

Indirect methods are very important tools to estimate connectivity at an evolutionary time-scale. Levels of connectivity can be indirectly estimated by measuring the allelic

frequencies between populations by means of a fixation index (F_{st}), which is based on Wright's F statistic (Wright 1921, 1949). It compares the average heterozygosity within a subpopulation with the total heterozygosity. This calculation assume the Hardy-Weinberg principle (Hardy 1908, Weinberg 1908) which states that the allelic frequencies should be constant in subsequent generations in the absence of evolutionary forces as previously described (mutation and selection). F_{st} values range between 0 to 1, where 0 is the total absence of differences in allelic frequencies between subpopulations and 1 implies that the allelic frequencies between subpopulations are completely different, i.e. completely fixed for alternative alleles. Wright (1978) categorized the degree of population genetic structure (F_{st}) as follows: little (0.0-0.05), moderate (0.5-0.15), large (0.15-0.25) and very large (above 0.25) genetic differentiation. Differently from diploid data where the level of heterozygosity is measured, haploid data uses the haplotype diversity to estimate genetic structure (Excoffier et al. 1992).

1.1.3.4. Advances in Population Genetic Study Methods

Early population genetics studies, included, among others, the use of blood cell antigens or serum proteins to estimate locus polymorphism. In 1966, Lewontin and Hubby introduced a molecular method using allozymes and electrophoresis to estimate polymorphisms. With the development of the Polymerase Chain Reaction (PCR) in the 1980's (Mullis et al. 1986), researchers quickly adopted the technique as observed in the steep growth of publications (Bartlett and Stirling 2003). Nowadays, this technique is still often used and is also applied in the current study (Chapter 4). With DNA sequencing, researchers were able to detect variations at a single base pair (bp), increasing the level of detail in genetic variation between populations. Currently, next

generation sequencing is providing an increasing amount of genomic data, allowing studies to test for specific environmental conditions (e.g. temperature), and their effect in the organism genome (Bank et al. 2014). This type of information is important because it provides insights on allelic selective forces underlying lineages differentiation. Finally, such approach could reveal microevolutionary mechanisms, allowing for more precise predictions of population genetic response and fate to changing environments.

1.1.3.5. Connectivity in Marine Environments

Fragmentation of the environment has a very strong negative effect on natural populations, such as in the Brazilian Atlantic Forest (Chiarello 1999), because it limits population connectivity, hampering gene flow among populations. Fragmentation also affects marine biota, and can be particularly harmful in regions recognized as biodiversity "hotspots", such as the Mediterranean Basin, the Philippines and also the Abrolhos reef Bank in Brazil (Ginsberg 1999, Myers et al. 2000, Francini-Filho et al. 2010). Information on biodiversity and connectivity is still scarce for Brazil's marine coastal ecosystems which represent about 60% of the Atlantic South American coastline (Angulo et al. 2006). Studies on this subject in Brazil are usually focused on large organisms such as coral reefs (D'Agostini et al. 2015), sea turtles (Gallo et al. 2006), wales (Wedekin et al. 2010), and on species that have a direct economic importance such as tuna (Paiva and Le Gall 1975). Nevertheless, studies on small size organisms are equally important to understand species interactions and ecosystem dynamics. Populations of marine biota were generally considered to have higher connectivity compared to the terrestrial environment because of their greater capacity of passive dispersal and the presumable higher homogeneity in the ocean (Kinlan and

Gaines 2003). Therefore, fragmentation was likely more harmful in terrestrial than marine environments (Carr et al. 2003). However, water currents and distinct biological attributes of larvae now show that population connectivity in the marine environment may be much lower than first thought (e.g. *Amphiprion polymnus* - Jones et al., 2005; *Lottorina* spp. - Hohenlohe 2004). Population genetic studies are highly suitable to understand connectivity because they describe the distribution and frequency of alleles and highlight potential gene flow between populations. More studies investigating the link between genetic diversity and ecosystem stability (Hughes and Stachowicz 2004, Reusch et al. 2005, Vellend 2006), are necessary to improve our knowledge on how communities respond to different impacts.

1.1.4. ***Biodiversity - Ecosystem Functioning***

As they provide benefits not only to society but also to ecosystem health as a whole, the importance of ecosystem services is more and more recognized (Loreau 2010). Ecosystem services can be divided into four categories: (1) **Supporting** - which is the basic service and involves nutrient cycling, soil formation and primary production, (2) **Provisioning** – which concerns the products from the ecosystem such as food and fresh water; (3) **Regulating** – is the service that balances the processes such as climate, flood, disease regulations and water purification and (4) **Cultural** – which involves human activities such as aesthetic, spiritual, educational and recreational ones (Millennium Ecosystem Assessment 2005). Those services are a result of complex ecological processes involving the interaction among organisms and between organisms and the environment such as nutrient cycling and energy transfer in the foodweb (Millennium Ecosystem Assessment 2005, McGregor et al. 2008).

Biodiversity seems to be a fundamental component of ecological processes. Many studies focused on experiments with plant primary production, as they are the basal component of most ecosystems (Tilman 1996, Tilman et al. 1996, Loreau et al. 2001). It has been observed that in general, the higher the species richness, the higher the productivity. There are two main mechanisms, that could be underlying the observed results: (1) the increase in primary productivity could be a result of **facilitation between species**, i.e. the higher the richness the higher the number of different niche complementary species which facilitate the acquisition of limiting resources, enhancing primary productivity (Hooper and Vitousek 1997, Hector et al. 1999, Tilman et al. 2001); and (2) there exists one key highly productive species that is fundamental to maintain the process. These two main mechanisms seem to be at two different ends of a rope, and some responses to increase/decrease of productivity, may lay in between this gradient as a combination of both facilitation and presence of a dominant highly productive key species (Loreau et al., 2001). In general terms, it appears that the higher the biodiversity the more enhanced ecological processes are. In marine ecosystems the same trend is observed. Worm et al. (2006) demonstrated, by doing a meta-analysis combining different studies (with plant and marine seaweed), that with the increase in species diversity, both primary and secondary productivity also increased, ranging from 78% to 80% . Moreover, growth in genetic diversity also increased the capacity of an ecosystem to withstand disturbance, and enhanced the capacity of the ecosystem to recover after a disturbance (Hughes and Stachowicz 2004, Reusch et al. 2005).

1.1.4.1. Biodiversity and Ecosystem Stability

Ecosystem stability can be defined as a temporal constancy of functions established by the community resilience, after a disturbance and resistance to environmental change (Worm and Duffy 2003).There are four main hypotheses concerning ecosystem stability (Fig. 3); (1) **Diversity-stability hypothesis**: it establishes a linear relationship between species diversity and ecosystem stability, where the removal of any species would increase the ecosystem susceptibility (MacArthur 1955); (2) **Rivet hypothesis**: proposes that an ecosystem can withstand the removal of some species without increasing the susceptibility of the ecosystem because some species may be functionally redundant. However, beyond a certain threshold of species loss, the ecosystem would suddenly and catastrophically collapse. This hypothesis establishes a non-linear but a positive relationship between species diversity and ecosystem stability (Ehrlich and Ehrlich 1981); (3) **Redundancy hypothesis**: organizes species in functional groups with species belonging to the same group being redundant. It creates a non-linear relationship between species diversity and ecosystem stability, and the increase of species diversity will reach an asymptote where the species diversity no longer enhances ecosystem stability (Walker 1992); and (4) **Idiosyncratic hypothesis**: proposes a null or indeterminate relationship between species biodiversity and ecosystem stability. The more complex the interactions between species are, the more difficult it is to estimate the relationship between diversity loss and ecosystem susceptibility to disturbance (Lawton 1994). Studies have demonstrated that higher species diversity often increases the ecosystem stability (Worm and Duffy 2003). However, other studies emphasize that the level of interaction between species can be a more important process playing a role in stability than

species diversity alone (Johnson et al. 1996, Loreau et al. 2001). Another important component of ecosystem stability is resilience.

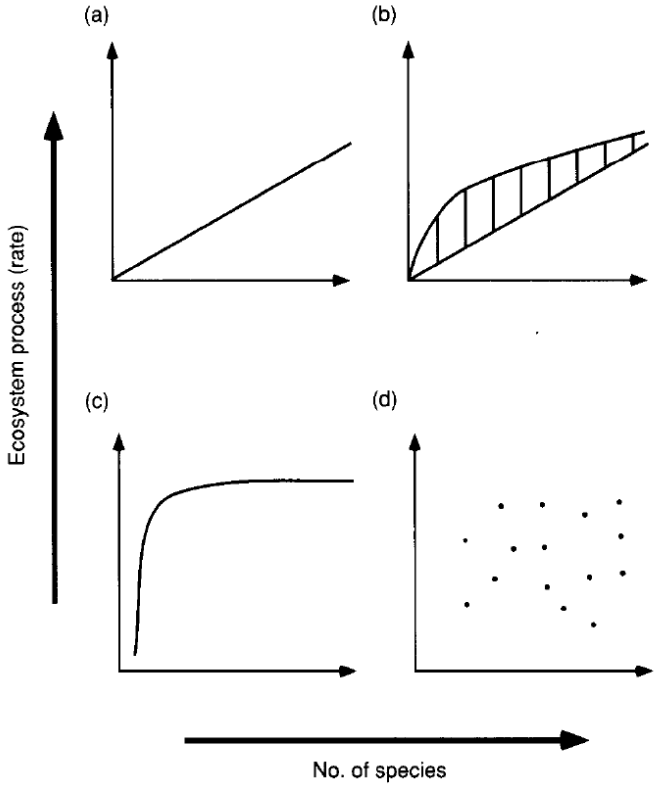

Figure 3. Relationships between ecosystem processes and species richness. (a) Diversity-stability hypothesls; (b) Rivet hypothesis; (c) Redundancy hypothesis; (d) Idiosyncratic hypothesis. Modified after Johnson et al. 1996.

Resilience can be defined as the capacity of an ecosystem to absorb disturbance and return to its original state (self-organize) in structure, function and feedbacks (Walker et al. 2004), which also has a direct relationship with biodiversity (Folke et al. 2004). Anthropic pressure can go beyond the resilience capacity of an ecosystem, causing shifts in the community structure. For instance, overfishing in the Caribbean has led to a succession of redundant herbivore species over time, but without generally affecting the primary producers (e.g. seaweed) population sizes. However, with the continuous

and intense anthropogenic pressure, those herbivores populations decreased dramatically, causing the ecosystem to shift from coral reef-dominated to algal-dominated (Knowlton 1992, Hughes 1994, Folke et al. 2004). Studies investigating the resilience of ecosystems are crucial to understand and predict the fate of ecosystems. Particular attention should be given to ecosystems that have direct contact with human activities, such as shallow water coastal areas, which provide important ecological services and can potentially harbour high levels of biodiversity such as phytal ecosystems.

1.1.5. ***Phytal Ecosystem***

The term “phytal” (Greek, Phyton = plant) was first proposed by Remane (1933) to designate a marine habitat dominated by macrophytes, such as seaweed (Coull et al. 1983) and seagrass (De Troch et al. 2001), coexisting with many other organisms constituting an important marine biocenosis in shallow water regions. Phytal ecosystems (Fig. 4) are known for being highly productive ecosystems that harbour thousands of associated organisms in seaweed beds (Coull and Wells 1983, Mineur et al. 2015, Takao et al. 2015), from vertebrates such as fish (Dubiaski-Silva and Masunari 2008) to many other invertebrates such as copepodes, nematodes and polychaetes (Coull et al. 1983), from megafauna (sea turtles – Bjorndal 1985), to microscopic non-metazoan organisms (Protozoa – Davidova 2010; Cyanobacteria – Bour et al. 2013), comprehending all trophic levels (Coull et al. 1983, Ferreira et al. 2001, Da Rocha et al. 2006, Dubiaski-Silva and Masunari 2008, Wahl et al. 2012). Economically important species from tropical regions, such as the commonly consumed lobster (*Panulirus argus*) which can have their whole life cycle associated

with calcareous and non-calcareous seaweeds (Bos et al. 2003), the fish *Hemiramphus balao*, different species of cuttlefish *Sepia officinalis* (Neves et al. 2009) and abalone *Haliotis rufescens, H. fulgens*, *H. discus* (Tenore 1976), use the seaweed beds as a nursery site (Arasaki and Arasaki 1978).

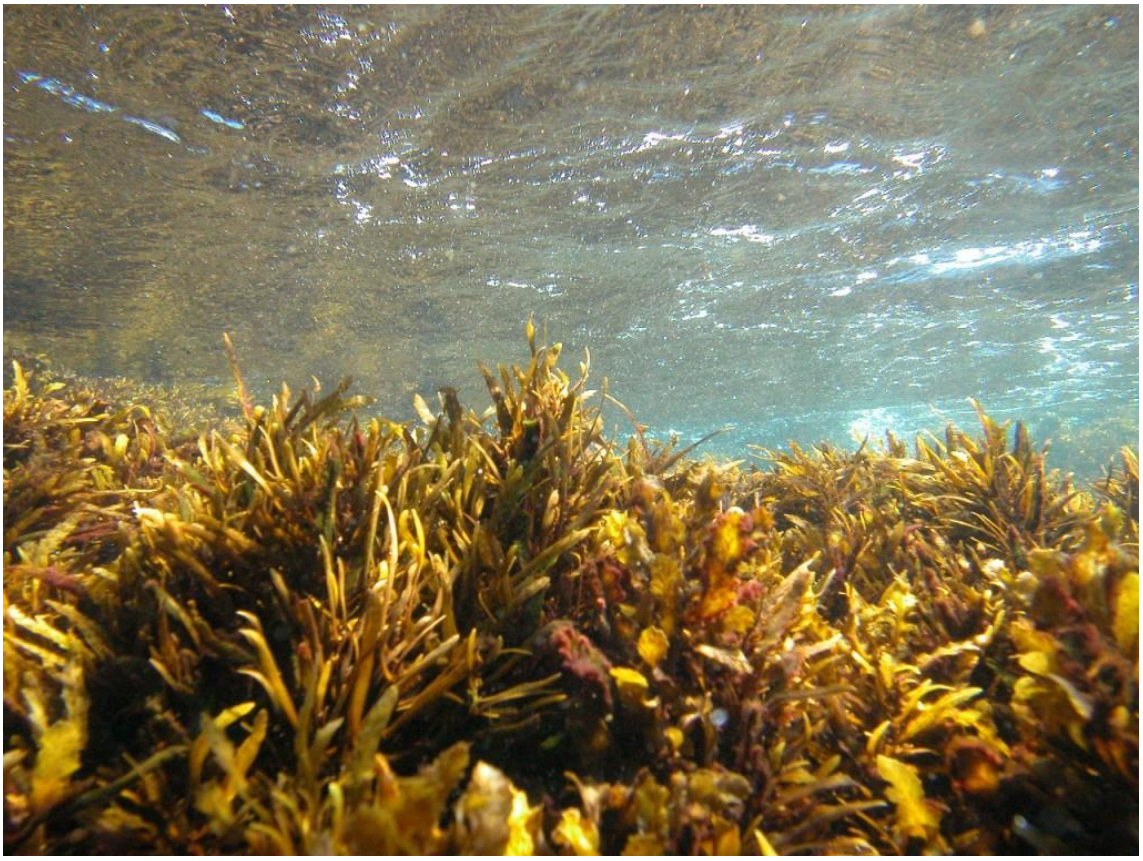

Figure 4. Seaweed bed dominated by brown macroalgae in the Australian coast. Image obtained from Sydney Institute of Marine Sciences. http://sims.org.au/foundation/sydney-harbour-research-project.

1.1.5.1 Seaweed Beds at Local scale

Seaweed beds are involved in many ecosystem processes. The presence of seaweed mats can significantly decrease currents velocity while increasing the sedimentation rate (Romano et al. 2003), and act as pH and dissolved oxygen buffers (Komatsu 1989,

Komatsu et al. 1990). Coralline seaweeds, which accumulate calcium carbonate within their cell walls, are important as reef builders in shallow water ecosystems; they increase habitat complexity and provide hard substrate for other organisms to attach and develop on (Björk et al. 1995, Brown and Taylor 1999). Individuals from the genus *Halimeda*, a calcified seaweed, are known to contribute to the sediment composition through thallus fragmentation (bioclast) (Carson and Peterson 2012). Moreover, thallus fragments from various seaweed species ultimately sink to the bottom, also contributing to the detritus food chain (Chung et al. 2013). Marine seaweeds provide microenvironments for shelter, food and reproduction of meiofauna organisms (Hicks 1977, Coull and Wells 1983, Song et al. 2010) which serve as food source for higher trophic levels (Hicks and Coull 1983). Frequently, copepods are the dominant meiofauna organisms followed by nematodes, and other less abundant taxa such as polychaetes, ostracodes and turbelarians (Coull et al. 1983, Curvelo and Corbisier 2000). The structural complexity of diverse seaweed thalli provides numerous microhabitats mitigating the effects of hydrodynamic forces and predation on the associated meiofaunal community (Coull and Wells 1983). In addition, the accumulation of sediment seem to increase the number of microhabitats, which in turn, provide a more suitable condition for meiofaunal colonization (Gibbons 1988). Exposure also appears to be an important factor for phytal meiofauna. On seaweeds occurring in sites more exposed to wave action, meiofaunal taxa which are dominant in the sediment, such as nematodes, are less abundant (Heip et al. 1985). In contrast, the more protected and closer to the sediment and detritus a portion of the seaweed thallus is (e.g. holdfast), the higher the abundance of interstitial meiofauna (Arroyo et al. 2004, Arroyo et al. 2007, Giere 2008). However, when fronds and holdfast are heavily loaded with silt-clay or detritus, nematodes tend to dominate (Coull et al. 1983).

Since copepods are usually dominant on macrophytes, it is possible that some species can develop more efficient adaptations, such as claws, for phytal substrate to better withstand higher hydrodynamic condition and thus depend less on sediment accumulation on the macrophyte compared to nematodes. In fact, phytal meiofauna found in seaweed beds often exhibit morphological and behavioral adaptations to the morphology of the algal substrate. Some adaptions are the development of claws, a sucker-like structure, a flattened body and mucous substances to improve adherence (Wieser 1959, Warwick 1977, Gee and Warwick 1994a, Gee and Warwick 1994b, Giere 2008). Further, meiofaunal organisms can behave differently depending on the taxon and period of the day. For example, nematodes and copepods are together on the seaweed during the day, while at night they separate: copepods swim towards the water column while nematodes migrate towards the bottom sediment; this different strategy was interpreted to avoid fish predation for both organisms (Kolesnikova et al. 1996).

Few meiofaunal phytal organisms feed directly from the cells of the algal substrate, possessing adapted mouth structures such as species from the genera *Halenchus* (nematode) and *Echiniscus* (tardigrade) (Giere 2008). Most of the meiofauna use the seaweed only as a substrate, feeding on the epigrowth biofilm, (e.g. feeding on diatoms - Athersuch, 1979; Jensen P. 1984; Arroyo, et al., 2007). Studies with phytal macrofauna showed that the grazing activity, mostly from amphipods, can have positive, neutral or negative effect on the macroalgae productivity, depending on whether the species feed exclusively on the epiphytic diatoms or feed also or exclusively on the host seaweed (Brawley and Adey 1981, Norton and Benson 1983, D'Antonio 1985, Duffy 1990). It is still unclear however, whether the specialized mouth apparatus of non-macroalgal feeding meiofauna allows to remove epiphytic diatoms

without damaging the host seaweed, and to what extend meiofaunal phytal organisms contribute to seaweed productivity. A mutualistic relationship between the associated fauna and the seaweeds is also present. While the seaweed provides grazing sites, they benefit from the nitrogen present in the ammonium excreted by the meiofauna, as observed for the seaweed *Cladophora* (Bracken et al. 2007). Overall, besides the complex interspecific interactions, it appears that exposure, tidal stress and the algal complexity are very important physical factors shaping the composition and the microdistribution of the littoral phytal meiofauna (Gibbons 1988, 1991, Atilla et al. 2005, Hooper and Davenport 2006).

1.1.5.2. Seaweed Beds at Medium Scale (between beds)

From a local to medium geographical scale (few to hundreds of km), population connectivity also plays an important role in seaweed bed dynamics. Reefs and seaweed beds are often coexisting habitats and studies demonstrated that connectivity enhanced the biomass of herbivore fishes, which control algal population and increase ecosystem resilience (Olds et al. 2012). It has been shown that higher levels of connectivity between ecosystems increase the larval recruitment of fishes that live in multiple habitats including seaweed beds (Berkström et al. 2012). Seaweed rafting is considered an important mechanism involved in dispersal and evolutionary processes for marine organisms (Thiel and Haye 2006), especially for organisms without pelagic larvae (Collin 2001, Porter et al. 2002, Sponer and Roy 2002, Colson and Hughes 2004). Although seaweeds are considered to be poor dispersers (Phillips 2013), studies have demonstrated that some seaweeds themselves are able to cross large distances via drift (Guillemin et al. 2014, Li et al. 2016). Epiphytic meiofauna that can

withstand such long seaweed dispersal, could potentially colonize far away but similar ecotopes.

1.1.5.3. Seaweed Beds at Global Perspective

Seaweed beds are also an important at global level. Considering the current scenario of a significant increase of greenhouse gases, seaweed beds contribute to CO_2 fixation by converting it into biomass via photosynthesis. It is has been estimated in a study in Japan that about 32.000 tons of carbon are fixed per year just by seaweed cultivation, which corresponds to 1.2% of the annual macrophyte production along the Japanese coast (Muraoka 2004). Although marine habitats vegetated with macrophytes accounts for less than 2% of the sea surface area, it contributes for about 50% of the carbon fixation in the global coastal oceans (Duarte et al. 2005). Because of their potential for removing CO_2 from the atmosphere, seaweed cultivation has been suggested as a way to mitigate the effects of global warming (Chung et al. 2013). However, some seaweed species are more susceptible to the acidification of the sea as a result of the increase in CO_2 levels, especially the ones in which the development involves calcification of the thallus (*Halimeda* species - Price et al., 2011).

Despite the importance of the phytal ecosystem in coastal areas, knowledge on the biodiversity and the dynamics of the associated organisms is still very limited, especially in tropical areas such as in Brazil (Da Rocha et al. 2006, Venekey et al. 2008). Considering the fact that molecular advances have revealed the presence of cryptic species (Blaxter 2004, Derycke et al. 2008a, Derycke et al. 2010a, Apolônio Silva De Oliveira et al. 2012), the biodiversity of such ecosystem is likely to be underestimated.

1.1.6. ***Historical Background of Seaweed Exploitation***

1.1.6.1. Human Interactions with Seaweed Beds

Seaweed beds are suffering from anthropogenic pressure (Mineur et al. 2015). Domestic pollution in coastal areas contributes to eutrophication by increasing P and N levels causing a tremendous increase in harmful microalgae. The latter intoxicate several forms of life including economically important fishery resources (Imai et al. 2006). The increase of nutrients makes epiphytic seaweeds e.g. *Enteromorpha* and *Ulva* reach high densities, thereby, outcompeting other seaweeds, blocking sunlight and as the seaweeds die and decompose, decrease the level of water dissolved oxygen (Lotze et al. 1999, Schramm 1999) Schramm, 1999). Tourism is also an activity which has a direct impact on seaweed beds e.g. by trampling (Sarmento and Santos 2012). This is particularly true for phytal ecosystems on rocky bottom substrates and reefs. Tourists tend to step on those hard substrates, causing damage and decrease of the abundances and diversity of the associated fauna (Brown and Taylor 1999). Overexploitation of seaweeds, because of economically important algal products (Câmara Neto 1987) also strongly impact phytal ecosystems, causing habitat loss and affecting coastal fisheries' resources such as shrimps, lobsters (Miller et al. 1971) and fishes especially in early developmental stages. Phycocolloids (e.g. carrageenan, alginate and agar) are industrially very important due to their stabilizing and thickening properties. Those colloids are extracted from red (Rhodophyta) and brown (Phaeophyta) seaweeds and are used in a variety of products. About 5.5 to 6 billion US$ per year in products are used mostly for human consumption (McHugh, 2003). Phycocolloids are also used in pharmacological (cosmetics) and agricultural industry (fertilizers, pesticides and pH soil buffers) (Aitken and Senn 1965). Seaweeds

usage dates from the 6th century in China and currently they are used in many continents (McHugh 2003) including South America.

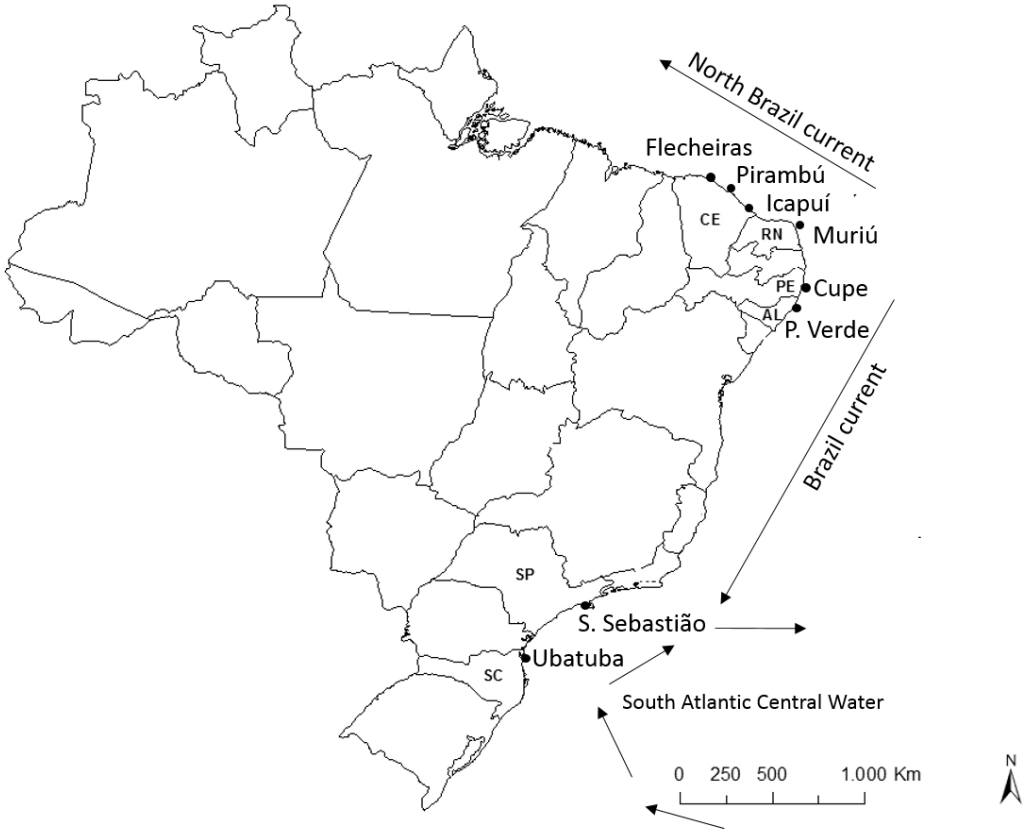

Figure 5. Map of Brazil and sampling sites of the current study in the northeastern (Flecheiras (CE); Pirambu (CE); Icapuí (CE); Muriú-RN; Cupe (PE); Ponta Verde (AL)), southeastern (São Sebastião (SP)) and south (Ubatuba (SC)) Brazilian coast, indicating the main sea currents.

1.1.6.2. Start of Seaweed Exploitation in Brazil

In Brazil (Fig. 5), seaweed exploitation dates from the early 1960's along the northeastern coast (de Paula et al. 2015, Marinho-Soriano 2016). Most of those activities were developed informally which made it impossible to gather substantial

data. The information presented here is obtained from few published studies, interviews of local fisherman (audio files), and online text and videos about the cultivation background, which allowed to pinpoint some relevant events in specific beaches along the NE Brazilian coast. Moreover, due to the temporary nature of some projects in collaboration between the traditional fishermen community and developmental organizations and institutes, some online references are no longer available and are indicated by a "*".

In the 1960's, a Japanese company observed the industrial potential of the seaweed beds in the NE Brazilian coast, and made an informal agreement with the traditional fisherman community to extract the seaweeds (De Paula et al. 2015, and local communication). Exploitation started in the coast of the states of Ceará (CE), Rio Grande do norte (RN) and Paraíba (PB) (Marinho-Soriano 2016). Although seaweeds from the genera *Gracilaria*, *Gracilariopsis*, and *Hypnea* are the most relevant seaweeds to exploit, seaweeds were, at the beginning, indiscriminately extracted. In Icapuí-CE, seaweeds were initially collected on the beach after they became detached from the rocky substrate from the intertidal zone. However, as the traditional fisherman community saw seaweed exploitation as a potential extra source of income, seaweeds were directly extracted from the natural bed, and in many cases, even bringing along the hard substrate to which the seaweeds were attached to (De Paula et al. 2015). In the 1970's, exploitation of the natural seaweed bed started in Flecheiras-CE, also in a similar uncontrolled manner as observed in Icapuí-CE (Rocha 2013).

1.1.6.3. Production Estimation, Socio-ecological Impact

Records of seaweeds exploitation extended to several other beaches in the NE of Brazil, as for instance the beaches of Baleia (CE) and Muriú (RN) *(http://www.nutrialgas.com.br/index_arquivos/ProjetoAlgasMarinhas.htm, and fisherman local communication). It is estimated that the overall peak production in Brazil was between 1973-1974, exporting about 2000 tons per year to Japan (UNCTAD/GATT 1981), nevertheless the year of peak production varied from place to place. Exploitation intensified as a result of increasing demand (Marinho-Soriano, 2016), and the need for logistic improvement to process the seaweeds and improve the quality of the exported product came to light. In 1977, the first factory was installed to process the seaweeds in Paraíba State (PB), a state more to the south of Ceará State (CE) but yet in the northeast region *(http://www.nutrialgas.com.br/index_arquivos/ProjetoAlgasMarinhas.htm, http://www.altaneirafm.com/2013/10/projeto-transforma-algas-marinhas-em.html and fisherman local communication, and audio file). Locally, a remarkable intensification of the exploitation in Flecheiras-CE and in Icapuí-CE in the 1980's was observed as more families joined the activity *(http://www.ventura.org.br/noticias/page/830/ , Rocha 2013). In Flecheiras-CE in the same decade it was estimated that between 12 to 17 tons per month (Rocha 2013) of seaweed were extracted from the natural bed, while in Icapuí-CE exploitation was even more intensive with estimation up to 45 tons per week (de Paula et al. 2015). In the latter, villagers reported that in 1990's seaweeds nearly disappeared along with lobsters (e.g. *Panulirus argus, Panulirus laevicauda*), shrimps (e.g. *Penaeus subtilis, Xiphopenaeus kroyeri*) and fish (e.g. *Lutjanus analis*), significantly affecting the main source of income of the fishermen community *(http://www.ventura.org.br/noticias/page/830/,

http://www.pesca.sp.gov.br/noticia.php?id_ not=2093, Costa et al. 2011, Costa et al. 2012). This disappearance of seaweeds followed by associated fauna suggests that anthropogenic pressure went beyond the resilience threshold of that particular phytal ecosystem.

1.1.6.4. Remediation Measures and Sustainable Cultivation

In 1998, remediation measures, such as seaweed cultivation, were taken in partnership with the Federal University of Ceará (UFCE) and in 2000 the Developmental Community Association of Flecheiras (ADCF) was created based on this partnership supported by the Terramar Institute (Algas Marinhas Project). Due to the high extend of degradation of the seaweed beds in Icapuí (CE), the successfully cultivated seaweeds from Flecheiras (CE) were transported to Icapuí (CE), using the same seaweed cultivation method of Flecheiras (CE), and in 2002 another project started at the site using *Gracilaria*, a red seaweed (Mulheres de Corpo e Alga Project). The cultivation was initially based on a method where the seedlings were attached to a long floating rope (25 m) or with a combination of materials used to cultivate mussels (net-cylinder) (Fig. 6). An amount of 200-250 kg seaweed can be obtained after 45-60 days. From those 200-250 kg only 15% remains as dry weight. During the period of destructive exploitation (mostly between 1970's-80's), the companies paid per kg from R$ 0,10 (about 0,03 euros at present) to R$ 1,00 (about 0,28 euros). Nowadays, it raised up to R$ 10 per kg (2,82 euros).

Figure 6. Illustration of the two methods used for seaweed cultivation on floating ropes at the Flecheiras beach, Ceará – Brazil.

1.1.6.5. General Characteristics of the studied Area and Seaweeds

The sampling sites of this study (Fig. 5) were grouped into two Brazilian geopolitical regions: the northeast (NE) and the southeast-south (SE-S). The **NE coast** includes the locations Flecheiras (CE), Pirambu (CE), Icapuí (CE), Muriú (RN) which are under the influence of the North Brazil Current, and Cupe (PE), and Ponta Verde (AL) which are under the influence of the Brazil Current. Those locations have similar climatological conditions such as the division of the year into two seasons, rainy and dry, and little temperature variation, with an average minimum air temperature of 22 ˚C during the rainy and average maximum of 32 ˚C during the dry season (Dantas 2004, Almeida 2010, Bastos et al. 2011, Barros et al. 2012, Diniz and Pereira 2015). Pluviosity increases towards the south, with rainy periods progressively shifting between January – June to March –September (Almeida and Barbirato 2004, Araujo 2006, Bastos et al. 2011, Peres 2012, Moura et al. 2015). Water temperature and salinity also vary little over the year reaching minimum values during the rainy season (26 ˚C; 32°C respectively) and maximum values during the dry season (34 ˚C; 40°C

respectively) (Araujo 2006, Vieira Hazin et al. 2008, Araujo and Rodrigues 2011, Bastos et al. 2011, Veras 2011). The locations are generally sand beaches with mostly medium to very fine grain sizes, but percentages of gravel and bioclast can also be high (Cupe (PE)) (Table 1; Chaves 2012; Lima 2013; Marino et al. 2013; Santos et al. 2014). The beaches in the NE of Brazil typically have sandstone reef formations along the coastline, (Leão 1994, Castro 2000). Those formations provide hard substrate and high habitat complexity for many taxa to reproduce and develop.

The **SE-S coast** includes the locations São Sebastião (SP) and Ubatuba (SC), which are under the influence of the Brazil Current but also the colder South Atlantic Central Water mass, causing the upwelling phenomenon, increasing both nutrients in the water column and primary productivity (Coelho-Souza et al. 2012). Differently form the locations in the NE, the four seasons of the year are more defined in the SE-S. Average air temperature ranges from 16 ˚C in winter to 25 ˚C during the summer when pluviometric values are the highest, which is between December and February (Migotto et al. 1993, Zular 2011). Salinity is generally lower compared to the NE coast ranging from a minimum of 24 to a maximum of 36 (Migotto et al. 1993, Carvalho et al. 1998). Water temperature can reach much lower values during the summer in the SE-S (16 ˚C) compared with the same period (dry season) at the NE coast (26 ˚C) (Migotto et al. 1993; Carvalho et al. 1998). Grain size of the area of the sampling sites are similar to the ones in the NE ranging from medium silt to medium sand, and rock bottoms in the intertidal zone are also present (Table 1; Barcellos & Furtado 1999; Horn Filho 2003).

1.1.6.6. Algal species used in Current Study

In both regions hard substrates are available for the attachment and development of macrophytes, including three commonly occurring seaweeds representing the three main seaweed divisions, Phaeophyta (brown), Chlorophyta (green) and Rhodophyta (red), which were collected in the intertidal zone near water surface, for the current study:

Sargassum Agardh (1820). Figure 7

Brown seaweed. **Habitat**: *Sargassum* is a genus of marine macrophytes which is vastly species rich and is distributed worldwide in tropical and inter-tropical regions. Grows attached to hard substrates such as rocks in the intertidal zone, forming a submarine dense forest. **Morphology**: Can range from few centimetres in exposed and several meters in sheltered habitats. Overall thallus shape more or less linear or bushy. Perennial thallus subdivided into a discoid, conical or rhizoidal holdfast which do not penetrate the substratum, in some free-floating forms holdfast is absent; one to many main axis ramified into “brunches” or more, cylindrical or flattened, which can differentiate to secondary axes with smooth or spiky surfaces. Branches can differentiate in a foliar structure. Shape of the leaves highly diversified with smooth, undulated, finely serrate or deeply dentate margins. Laterals branchlets modify into air vesicules or aerocysts with spherical, pyriform or ovoid shape. These are globular or spherical, air filled structures. They help in floating of the seaweed by increasing buoyancy. **Cell wall**: Double with inner layer composed of cellulose, and outer layer with a gummy consistence composed mostly by align acid but is also present in the inner layer. Not calcified. **Reproduction**: diplontic without alternation of generation.

Asexual reproduction by fragmentation. In dioecious species, sexual dimorphism in the thallus may be observed. Sexual reproduction is oogamous (http://www.algaebase.org/search/genus/detail/?genus_id=L9c77b3161969c937 , Sharma 1986, Engelen et al. 2005, Mattio and Payri 2011).

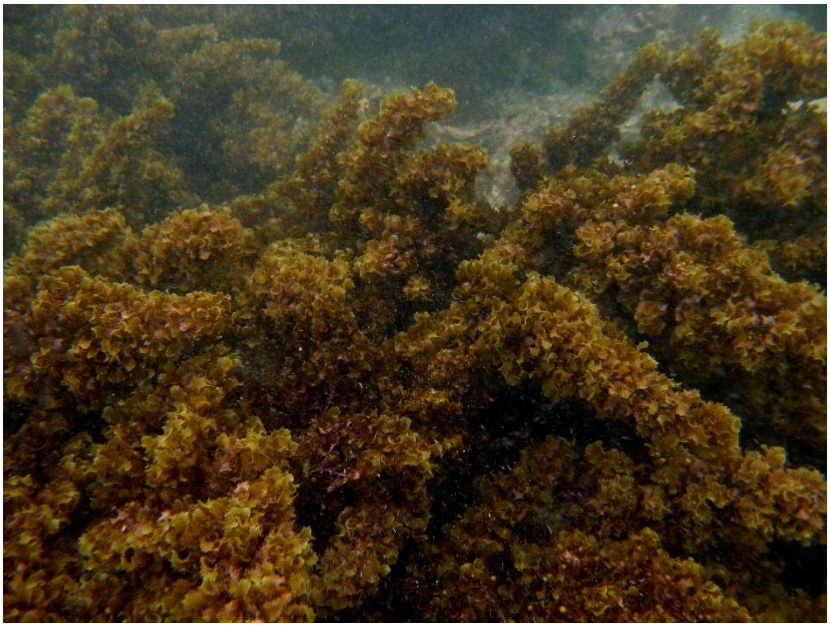

Figure 7. *Sargassum polyceratium Montagne* (1837). Courtesy Olga Camacho and Jimena Samper.

Halimeda Lamouroux (1812) Figure 8

Green seaweed. **Habitat**: *Halimeda* is widely distributed in warm waters over the tropical region. Occurs from the intertidal zone to deeper reef slopes in muddy, sandy and hard substrate depending on the species. **Morphology**: Perennial thallus constructed of articulated sequences of flattened calcified segments, shape vary according to the species, alternating with non-calcareous joints (nodes). Growth form erect, pendant, or sprawling, from few centimeters to more than a meter; holdfast can be a single large bulbous, typically 1 cm to about 13 cm long which is used to anchor

to mud-sandy substrate; or a single small, discrete holdfast of matted filaments up to a cm long; or several diffuse and inconspicuous patches of rhizoids arising from segments or nodes; branching, cohesion and fusion of siphons of segments produces a complex microstructure consisting of 2 main regions, a multiaxial or an uniaxial core of medullary siphons surrounded by a cortex of 2-5, rarely 6 layers of utricles (modified branches). **Cell wall**: calcified, deposition of aragonitic calcium carbonate begins after about 36 hours of segment development. Extent of calcification varies with age, species and environment. **Reproduction**: Asexual reproduction by fragmentation or by development of new thalli growing, either from segments or from filaments of the holdfast. In sexual reproduction biflagellated gametes. Gametes anisogamous (Hillis et al. 1998, Vroom et al. 2003, http://www.algaebase.org/search/genus/detail/?genus_id=he3ec1d1bb502b230).

Figure 8. *Halimeda opuntia* (Linnaeus) Lamouroux (1816). Courtesy Denis-Ader.

Gracilaria Greville 1830 Figure 9

Red seaweed. **Habitat**: Occur from temperate to tropical waters around the globe. Generally found in the intertidal zone, in pools on rocky substrate. *Gracilaria* is of high industrial importance due to their high agar content and is currently been cultivated in the locations of Flecheiras (CE) and Icapuí (CE). **Morphology**: Much branched perennial thalli, terete to flattened, branching subdichotomous to irregular. Some species form articulated fronds composed of cylindrical or irregularly shaped units. Can reach up to 60 cm in length; holdfast a disc or crust giving rise to one to many erect axes. Thalli red, olive, green to purple, cartilaginous or soft, smooth, fimbriate or dentate. **Cell wall**: three layers, decklamelle, agar matrix outer layer and fibrillar inner layer. Not calcified. **Reproduction**: Triphasic isomorphic life history, females with obvious swellings (cystocarps) with thick pericarp, ostiolate, and the presence of traversing tubular nutritive cells. Spermatangia in pits or shallow depressions. Sporophytes with tetrasporangia scattered in the outer cortex, cruciately divided (Bellanger et al. 1990, Dawes et al. 2000, Hoyle 1978, Iyer et al. 2004, http://www.algaebase.org/search/genus/detail/ ?genus_id=Xa8251fbe185f6f28 , accessed in 08/11/2016).

Figure 9. *Gracilaria* Greville 1830.

1.1.7. ***Nematodes: Diversity and Communities associated with Seaweeds and Sediments***.

1.1.7.1. Nematodes in Seaweed Beds

Nematodes are one of the most abundant and ubiquitous animal phyla on earth especially in marine environments (Lambshead 2004) with ca. 27 000 described species (Hugot et al. 2001). They have been successfully used as bio-indicators both in terrestrial and aquatic ecosystems because of their high abundance, high functional diversity and limited dispersal ability (Bongers and Bongers 1998). Nematodes are usually by far the dominant meiofaunal phylum in marine sediments reaching high densities (10^5–10^8 individuals/m^2, Heip et al. 1985), frequently in the top layer of the sediment (Moens et al, 2013) with a patched distribution (Hodda, 1999; Gallucci et al., 2009). The nematode aggregated distribution and community composition is driven by

microtopographic irregularities (Moens et al. 2014) such as the heterogeneity of the sediment, caused by waves and currents, sediment of different particle sizes (abiotic factors), and bioturbation (biotic), which in turn, affect food source aggregation (Reise 2002). Nematodes are important organisms involved in local ecological processes in the benthos. They play a role in carbon mineralization (Rysgaard et al. 2000) and bioturbation which increases the colonization surface for bacterial growth (Jensen, 1996). Nematodes feed on those bacteria regulating their population growth, and they also pray on equal and serve as food source for higher trophic levels (Schmid-Araya et al. 2002). An important food source for infaunal organisms is the organic matter that sinks to the bottom, as for instance seaweed fragments, that decompose and this way enters the heterotrophic/detritivorous food web (Kulkarni et al. 2003, Begon et al. 2006) .

Different from marine sediments, nematodes are usually the second most abundant meiofaunal phylum associated with macroalgae after copepods (Jensen 1979, Coull et al. 1983, Coull and Wells 1983, Hicks 1985). Chromadoridae is frequently the dominant family in the phytal habitat, followed by Cyatholaimidae and Monhysteridae (Jensen 1984a, Zhinan 1997, De Oliveira et al. 2016) and about 149 genera have been recorded in this habitat in the literature (data calculated from Santos et al., *in preparation*).

Epistrate feeders (nematodes feeding on epiphytic microalgae) are in general the most abundant feeding type as observed in *Gracilaria foliifera*, *Ulva Lactuca* (Coull et al. 1983), *Sargassum polyceratium*, *Halimeda opuntia* (Da Rocha et al. 2006, De Oliveira et al. 2016), *Colpomenia sinuosa* (Zhinan 1997) and *Laminaria hyperborean* (Moore 1971). When epistrate feeders are not the dominant feeding type, non-selective deposit feeders usually dominate as observed in *Sargassum confusum* (Kito 1982),

Enteromorpha prolifera (Zhinan 1997), and *Macrocystis integrifolia*, (Trotter and Webster 1983). Dominance of selective deposit feeders and predator/omnivores appear to be more rare (Coull et al. 1983, Da Rocha et al. 2006). Although phytal nematodes appear to be more dependent on the autotrophic food web (higher number of epistrate feeders), feeding type composition, might be affected by the amount of retained particles (detritus). Nematodes tend to reach high abundances in macrophytes which are heavily loaded with silt-clay (Coull et al. 1983). Those particles, increasing food input, habitat complexity (Gibbons 1988) and allow nematodes that are less fit for phytal habitat to colonize the seaweed. However, it has been demonstrated in the Baltic sea that the amount of silt content does not affect epiphytic nematode composition while salinity and food availability are the most important structuring factors (Jensen 1984a). Some nematode species can have as much microalgal uptake as 30 to 50 times their adult biomass along their development (Tietjen and Lee 1973, Jensen 1984b). Consequently, nematodes may contribute to controlling the densities of epiphytes, such as diatoms which compete for light and nutrients (Van Donk 1998, Ghobrial et al. 2007). However, whether nematodes can influence the development of the seaweed by removing competing diatoms is currently unknown. Furthermore, some studies have demonstrated that nematodes are also a food source for higher trophic levels, such as fish and especially for crustacean larvae (Brüggemann 2012). Hence, as seaweed beds are natural reproduction sites for many marine organisms, nematodes may serve as food source for organisms during the early stages of their life cycle.

1.1.7.2. Phytal Nematodes Dispersal and Colonization Dynamics

Recent genetic studies have indicated that dispersal of macroalgal associated nematode species is restricted to a scale of approximately 100 km (Derycke et al. 2005, Derycke et al. 2007a, Derycke et al. 2010a), but this may well be caused by substantial colonization dynamics of the ephemeral substrate (decomposing seaweed - Derycke et al. 2007b). Extreme colonizer nematodes with high reproduction rates such as *Litoditis marina* (Derycke et al. 2007c) are expected to colonize more effectively distant sites using seaweed drift compared to persisters such as *Thoracostoma trachygaster* (Derycke et al. 2010a), because the latter exhibit lower reproduction rates than the extreme colonizers. However, it has been demonstrated that nematodes that are considered to have distinct life histories appear to have similar levels of population genetic structure (Derycke et al. 2013). Only few studies have investigated nematodes associated with seaweeds along the Brazilian coast (Derycke et al. 2006, Venekey et al. 2008), and so far no population genetic studies using meiofaunal organisms in this ecosystem have been conducted. Considering the high diversity and abundances of nematodes in the sediment, and the accumulations of sediment on seaweeds increasing their microhabitat complexity, it is generally expected that nematodes can colonize the macroalgae (Gibbons 1988). Provided that such colonization behaviour is prevalent, nematodes from adjacent sediment could recolonize algal substrate following seaweed bed degradation and serve as source of biodiversity to and restoration of the nematode phytal community.

1.1.8. ***Nematode Integrative Taxonomy***

However because of the “taxonomy crisis” (Dayrat 2005) few young researchers are engaged in the time consuming and taxonomic complexity of morphologically based taxonomy especially after the introduction of the much faster molecular tools providing more objective features to determine diversity. For example, in 2012 and 2013 only 25 and 26 new free-living marine nematode species have been described (Decraemer and Backeljau 2015). Currently, both approaches have their weaknesses, and the combination of different types of data (morphological, morphometric and molecular) are considered important for species delimitation (Derycke et al. 2010a, Apolônio Silva De Oliveira et al. 2012, Decraemer and Backeljau 2015). This method is known as **Integrative Taxonomy**, which is based on multiple complementary perspectives to stablish species boundaries and is particularly suited for nematode communities (Fonseca et al. 2008, Apolônio Silva De Oliveira et al. 2012).

Because of the small size of free-living marine nematodes, the whole individual is usually necessary to obtain enough DNA for molecular analysis. Recent developments in vouchering (e.g. morphological digital video or photo vouchering) now allows the documentation of a specimen’s morphology prior to the destruction for DNA extraction (De Ley et al. 2005, Derycke et al. 2010a, Astrin et al. 2013). The use of molecular data, such as the small subunit 18S rRNA, to estimate biological diversity prior the morphological identification is called reverse taxonomy (Markmann and Tautz 2005) and has significantly increased the availability of molecular data in the last decade. Furthermore, the combination of multiple independently evolving genes such as Internal transcribed spacer (ITS) and mitochondrial cytochrome oxidase c subunit I (COI) have been used to look for congruence in the data set to stablish species boundaries (Apolônio Silva De Oliveira et al. 2012) because well-diverged species

lineages are expected to show genealogical congruence in neutral unlinked loci (Avise and Ball 1990, Rising and Avise 1993, Leliaert et al. 2014). This kind of approach often reveals cryptic diversity showing that biological diversity is significantly underestimated, especially in groups for which researchers require specialist morphological taxonomical skills, such as for nematodes. The (COI) has been used for barcoding, delineating cryptic species, population genetics and phylogeographic studies in marine nematodes (Derycke et al. 2008a, Derycke et al. 2008b, Fonseca et al. 2008). It is therefore the ideal starting point to address nematode diversity associated with macroalgae, both at the morphological and genetic level.

1.2. General aims

Considering the importance of seaweed bed ecosystems in shallow water areas, the limited knowledge on the coastline of Brazil (>7000 km) and the abundance of nematodes present in those environments, our **general aims** were:

(1) to reveal nematode biodiversity and species-specific relationships between nematodes, algal and sediment substrate, and to determine environmental processes which could be shaping nematode communities associated with seaweeds. To this end, comparisons between nematode communities from different seaweed species, spatial-temporal variation and the effect of sediment accumulation on nematodes in one beach at the NE coast of Brazil were assessed. We expected (i) nematode species preferences for a specific algal substrate according to differences in seaweed morphology; (ii) little seasonal variation in the nematode community because of very limited temperature variation throughout the year in the studied beach (local scale) and

(iii) to find higher nematode diversity and abundances on seaweeds which accumulate more sediment.

(2) By expanding our sampling area for seaweed and sediment over more than 3000 km coastline, we aimed to test whether there were differences between the two substrates over a latitudinal gradient to understand if nematodes from the surrounding sediment could serve as a source to recolonize impoverished seaweed beds after exploitation and restore their nematofauna. We expected i) to find differences in nematode communities between the substrates (seaweed and sediment) and between beaches along the latitudinal gradient, and; ii) higher similarity among seaweed communities compared with the sediment across the latitudinal gradient. It has been demonstrated that nematodes present on seaweeds are able to disperse via seaweed drift for at least 100 km while nematodes in the sediment can be suspended in the water column and dragged by the currents but are unlikely to remain suspended more than 2 hours, which would limit their dispersal capacity.

(3) By studying the genetic structure and diversity of nematode populations associated with seaweeds at different beaches, we aimed to (i) test whether the two dominant sea currents at the NE Brazilian coast function as biogeographical barriers to dispersal. By sampling locations with and without historical seaweed exploitation, we aimed to (ii) assess whether this anthropogenic activity is reflected in nematode genetic diversity. Additionally, we aimed to (iii) describe the biodiversity in an integrative way by comparing phenotypes and genotypes. We expected to find strong genetic structures between populations distributed over more than 1000 km coast line in view of the large distance and marine currents which could represent biogeographical barriers. As a result of historical seaweed exploitation, we expected to find lower genetic diversity in historically exploited areas because a seaweed-exploitation bottleneck effect may

have caused shifts in haplotype frequencies. We also expected to find nematode species with conserved morphology but with distinct genetic differences because cryptic speciation is substantial in marine nematodes.

1.3. Thesis Outline

In **Chapter 1**, we provided a general introduction, the background about importance and biodiversity of seaweed beds, the historical exploitation and general impacts on the ecosystem. We detail the particular case of Brazilian historical seaweed harvesting which were the motivation of the current work and provide the thesis outline.

In **Chapter 2**, we studied the nematodes associated with the seaweeds *Halimeda opuntia* and *Sargassum polyceratium,* which were abundant and present throughout the year, during 5 months covering the dry and rainy seasons, over two transects parallel to the beach line. Those transects differed in their distance to the shore and in the degree of exposure to wave action. We evaluated if there were variations in nematode communities between the two algal substrates, also over time and if there were lower nematode richness and density in the wave impacted zone as a result of great physical disturbance. Moreover, we investigated the effect of sediment accumulation increase on nematode abundances, and whether epistrate feeders were dominant in both seaweeds, because diatoms and cyanobacteria may be the main food source on the seaweed surface. This information is important to understand local factors that may structure the nematode community.

In **Chapter 3**, we expanded our sampling area to look for geographical resemblance/difference of eight beaches along the Brazilian coast, distributed over two distinct main marine currents, providing us information on the diversity and patchiness

of the nematode communities present on algal and interstitial substrates. Moreover, we compare the nematodes from seaweed and sediment to infer whether they are composed by typical communities and reveal species specific preferences to one substrate over the other. This would provide us insight on possible latitudinal patterns for both substrates and whether nematodes from sediment can fully colonize and restore the biodiversity of phytal habitat in face of degradation.

Chapter 4 investigates the genetic structure of populations of the new species *Paracanthonchus gynodiporata* present in four beaches distributed over more than 1000 km along the northeastern coast of Brazil. This nematode was exclusively found associated with seaweeds, and thus making it suitable to study the dispersal capacity of nematodes associated with seaweeds. Because the beaches were under the influence of two opposite main marine currents, we tested whether those currents could have caused genetic breaks between those populations, by means of differences in haplotype frequencies. To investigate if possible haplotypes differences could be caused by a factor other than biogeographical barriers, the historical background was considered. Two beaches were in areas where historical seaweed harvesting took place while the other two did not. We searched for differences in genetic diversity between the two groups as a consequence of a genetic bottleneck. As the studied species is new to science, we have described the species in an integrative way, combining morphology and DNA data. In addition, we reconstructed for the first time a detailed 3D model of the head region for the genus *Paracanthonchus* and discussed the pitfalls of previous descriptions e.g. of the buccal armature. Finally, in view of the fact that cryptic speciation is common in marine nematodes, we confronted morphometric and molecular data to find whether phenotypic variation was reflected by the genotypes.

In **Chapter 5**, the **General Discussion** summarizes the main findings of the research and discusses general paradigms concerning the effect of the amount of sediment retained by the seaweed on the associated nematode community, the nematode diversity found on seaweeds and their level of distinctiveness from the community found in the sediment. We also call attention to the level of dispersal capacity and connectivity of marine nematodes associated with seaweed which could possibly affect the resilience of epiphytic communities to stressors. Furthermore, we discuss the relevance of studying seaweed beds as they are a productive ecosystem that is directly affected by human activities and the need to enrich our knowledge. We highlight the importance of carefully considering this ecosystem when conservation policies are formulated to preserve coastal areas.

Table 1. Sampling locations and corresponding environmental data. Abbreviations: Temp Air (air temperature); Temp Water (water temperature); High. Pluv. Period (Period of highest pluviometric values); High. Pluv. Month (month with peak pluviometric values); NE (Northeastern coast); SE-S (Southeastern-South Coast); P. Verde (Ponta Verde); S. Sebastião (São Sebastião). * indicates that data was not found.

Region	Location	Temp. Air (Mean)	Temp. Water (min-max)	High. Prec. Period	High. Prec. Month (mm - max; yearly mean)	Salinity (min - max)	Grain size range (dominant fraction)
NE	Flecheiras (CE)	26 - 28	27 - 32	Jan - June	April - 272; 1238	33 -37	Medium (medium sand)
	Pirambu (CE)	26 - 30	28	Jan - June	April - 384; 1500	35 - 36	Fine to course sand (course sand)
	Icapuí (CE)	20 - 32	27 - 28	Jan - Maio	June - * ; 949	33 - 35	Very fine to fine sand (fine sand)
	Muriú (RN)	25 - 27	33 -34	April - Junho	June - 260; 1562	33 - 40	Fine to medium sand (fine sand)
	Cupe (PE)	24 - 32	26 - 29	March - August	June - 415; 2050	32 - 38	Very fine sand to gravel (very course sand)
	P. Verde (AL)	22 - 29	26 - 29	March - September	June - 300; 2059	35 - 38	Medium to coarse sand (medium sand)
SE-S	S. Sebastião (SP)	20 - 25	16 - 31	December - January	January - 366; 1500	29 - 36	medium silt to medium sand (*)
	Ubatuba (SC)	16 - 20	17 - 26	January - Februry	February - 275; 1800	24 - 35	fine to medium sand (*)

Chapter 2: *Spatiotemporal variation and sediment retention effects on nematode communities associated with Halimeda opuntia (Linnaeus) Lamouroux (1816) and Sargassum polyceratium Montagne (1837) seaweeds in a tropical phytal ecosystem*

Modified from: De Oliveira DAS, Derycke S, Da Rocha CMC, Barbosa DF, Decraemer W, Dos Santos GAP. 2016. Spatiotemporal variation and sediment retention effects on nematode communities associated with *Halimeda opuntia* (Linnaeus) Lamouroux (1816) and *Sargassum polyceratium* Montagne (1837) seaweeds in a tropical phytal ecosystem. *Marine Biology* 163:102

Abstract

Knowledge on meiofauna associated with seaweed bed ecosystems, such as nematodes, is limited. Nematodes associated with *Sargassum polyceratium* and *Halimeda opuntia* were compared in two transects, 80 m apart and parallel to the beach line in Cupe Beach, Brazil. The temporal variation during the dry and rainy seasons and the effect of sediment retention by the seaweed on nematode density and composition were investigated. The differences in nematode assemblages between the two seasons were mainly caused by the increase in density of the most abundant genera in the rainy season. No significant difference was observed between the nematode assemblages of the two transects for *H. opuntia*. Moreover, the nematode assemblages of both seaweed species did not differ significantly in the same transect over time. The genus *Euchromadora* was dominant in both seaweed species. The amount of sediment retained by the seaweeds did not affect the overall nematode density. However, retained sediment was positively correlated with the density of *Draconema* and *Euchromadora* in both seaweeds, and both genera were exclusively found associated with seaweeds. This result opposes the idea that the more sediment retained by the seaweed, the higher the nematode overall density.

Key words: Cupe; Free-living; ecology; assemblage structure; Brazil.

2.1 Introduction

Seaweed beds and associated fauna form a highly productive ecosystem in shallow water coastal areas (Coull et al. 1983). Seaweeds harbor a variety of organisms belonging to almost all trophic levels of the food web, and also serve as a shelter, reproduction and/or grazing site for many organisms (Brewer et al. 1995, Kenyon et al. 1999, Nagelkerken et al. 2000, Ferreira et al. 2001, Da Rocha et al. 2006). They provide oxygen and are involved in many mineralization and chemical cycling processes (Vidotti and Rollemberg 2004).

Seaweed beds in tropical areas are frequently associated with geological formations such as sandstone or biological reefs, which provide protection by dissipating the wave energy (Ferreira Júnior 2005). The local hydrodynamics can strongly affect the macrophytal and epiphytal biomass, abundance and density, which in turn affect the distribution and activity of organisms that are grazing on the seaweeds (Schanz et al. 2002). Seaweed beds provide protection from currents and desiccation and can influence the spatial distribution of the associated organisms (Muralikrishnamurty 1983). Moreover, seaweed beds also play a role in decreasing the current velocity and increasing the sedimentation rate of sediment and other particles present in the water column (Fonseca and Cahalan 1992). It has been suggested that the accumulation of detritus by the seaweed correlates with the branching and structure of the macrophytes and increases microhabitat complexity, which would allow a higher density of small sized metazoans (Taylor 1967, Hicks 1980, Da Rocha et al. 2006). Seaweed beds are under the influence of tides and seasonality which also affect the associated organisms (Toyohara et al. 1999). However, for some small sized organisms examples are known where seasonality does not appear to be an important population driver, especially for

those species which reproduce throughout the year (Coull and Vernberg 1975, Song et al. 2010).

Small sized metazoans such as nematodes have a high capacity of colonizing seaweeds (Warwick 1977, Derycke et al. 2007c) and play a fundamental role in the maintenance of the benthic ecosystem (Riera and Hubas 2003). They are involved in processes such as biomineralization, bacterial population regulation, serve as food source for higher trophic levels and prey on the same and on lower trophic levels (Rysgaard et al. 2000, Schmid-Araya et al. 2002). With respect to seaweed nematofauna, differences in macroalgae morphology can cause differentiation between assemblages from different seaweed species (Warwick 1977, Gibbons 1991, Gee and Warwick 1994a, Gee and Warwick 1994b). Epistrate feeders are the most abundant nematode feeding type on seaweeds (Da Rocha et al. 2006) which may be related to the abundances of epiflora, and more specifically, of diatoms (Hagerman 1966, Tietjen and Lee 1973, Warwick 1977, Wetzel et al. 2002). Hence, nematodes may play an important role in controlling the densities of epiphytic organisms (e.g. diatom and cyanobacteria) that compete for light and nutrients with the macroalgae (Van Donk 1998, Ghobrial et al. 2007). Epiphytic nematodes also respond to seasonal variation (Jensen 1984). However, information on temporal and spatial variation of nematode assemblages associated with seaweeds is extremely limited. Such a knowledge would provide insights on the dynamics of small size organisms associated with macrophytal ecosystems, allowing for a better understanding of physical factors that are important for structuring the assemblages.

In this study, the nematode assemblages associated with a seaweed bed from the northeastern coast of Brazil were investigated. This area is characterized by a dry and rainy season with average temperatures of 32˚C and 24 ˚C, respectively (Chaves

1991). The seaweed species *Halimeda opuntia* (Linnaeus) Lamouroux (1816) and *Sargassum polyceratium* Montagne (1837) are abundantly present throughout the year. *H. opuntia* is a green calcareous seaweed sprawling along multiple axes, and forming mats over hard substrate. *S. polyceratium* is a brown seaweed with a more linear thallus and one main axis from where secondary branching originates, and can stand up perpendicularly to the rocky substrate (Fig. 1). *S. polyceratium* was distributed closer to the beach line compared to *H. opuntia. The latter* was also present near the reef barrier, which is directly exposed to wave action. Macrophytes decrease currents and increase sedimentation rate (Romano et al. 2003).

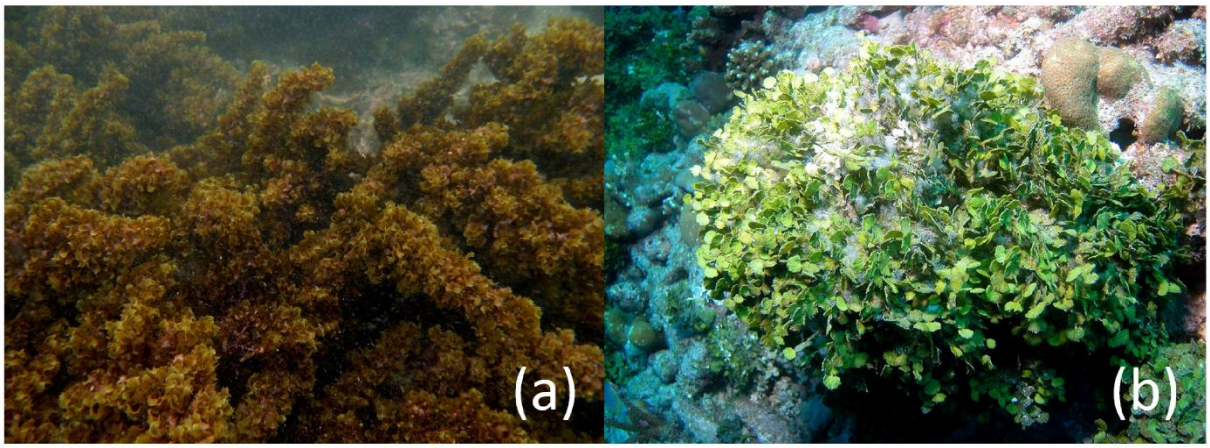

Figure 1. (a) *Sargassum polyceratium* and b *Halimeda opuntia* modified after Olga Camacho and Jimena Samper Villareal, (b) courtesy Denis-Ader).

The specific goals of this study were fourfold. **First**, the diversity, assemblage and feeding type structure of nematodes associated with *H. opuntia* and *S. polyceratium* were characterized and compared. Due to the different architectural structure of the two seaweed species it was expected to find seaweed species specific assemblages. Moreover, because of growth of diatoms and cyanobacteria on the seaweed surface (Stevenson and Stoermer 1982, Egan et al. 2000) a dominance of epistrate feeders

was expected to be found in the nematode assemblages of both seaweeds. **Second**, the temporal variability in nematode assemblages of *H. opuntia* and *S. polyceratium* was investigated by comparing the dry and rainy seasons and by comparing nematode assemblages over five months. Temporal fluctuations in abiotic parameters (e.g. the amount of rain, salinity, …) in Cupe beach may influence nematode abundances associated with *H. opuntia* and *S. polyceratium* and may cause shifts in the nematode assemblage. **Third**, spatial variation of nematode assemblages associated with *H. opuntia* in two transects parallel to the coast was investigated. These transects differed in their distance from the shore and in the degree of exposure to wave action. A higher variability in the nematode assemblages over time and lower nematode diversity and density were expected in the directly wave impacted zone because of the higher physical disturbance. **Finally**, the influence of sediment retention by the seaweeds *H. opuntia* and *S. polyceratium* on the nematode assemblages was assessed. The different architecture of *H. opuntia* and *S. polyceratium* may cause different sediment retention capacity resulting in a higher density and richness of nematodes in the seaweed with the highest sediment retention capacity because of an increase in habitat complexity and availability.

2.2. Material and Methods

2.2.1. ***Study area***

Cupe beach was chosen to test the impact of spatial and temporal variation and seaweed species on nematode assemblages. The beach is located in the northeast of the Brazilian coastline (coordinates 8º 45’ 48” - 8º 46’ 22” S and 34º 98’ 85” - 34º 97’ 99” W) and belongs to Ipojuca city, Pernambuco State. The beach is characterized by

arenite and stone reefs with natural swimming pools separating the beach from the open sea. Various seaweed species occur on the sandstone and its surrounding areas in the subtidal and intertidal zone. The water temperature ranges from 27.0 to 28.7 °C and the salinity varies between 28.88 and 37.16 according to the season. The sediment is composed mainly of quartz sand and is very rich in bioclast such as gastropods shells and pieces of calcareous algae (Dominguez et al. 1992).

2.2.2. ***Sample collection and processing***

Based on their high abundance throughout the year, two species of seaweed were selected: *Sargassum polyceratium* and *Halimeda opuntia*. *S. polyceratium* and *H. opuntia* have architectural differences. The first one is a brown seaweed which can stand up perpendicularly to the substrate, whereas *H. opuntia* is a green calcareous seaweed that tends to make mats over hard substrate. The sampling occurred during the dry season (December 2005, January 2006) and the rainy season (May, June, July 2006) at low tide in the subtidal zone. Two transects of about 160 m length and parallel to the beach were demarcated with a distance between each other of about 80 m. Transect 1 (T1) was further from the shore compared to transect 2 (T2) (Fig. 2). For all five time points and for each transect, three equidistant sampling points were chosen, and from each point three samples from each seaweed species were collected (Fig.2). The coordinates of each of the three sampling points are 8º 45' 78" S and 34º 98'19" W, 8º 45' 86" S and 34º 98' 23" W, 8º 45' 94" S and 34º 98' 29" W for T1 and 8º 45' 73" S and 34º 98' 30" W, 8º 45' 81" S and 34º 98' 34" W, 8º 45' 87" and 34º 98' 39" for T2.

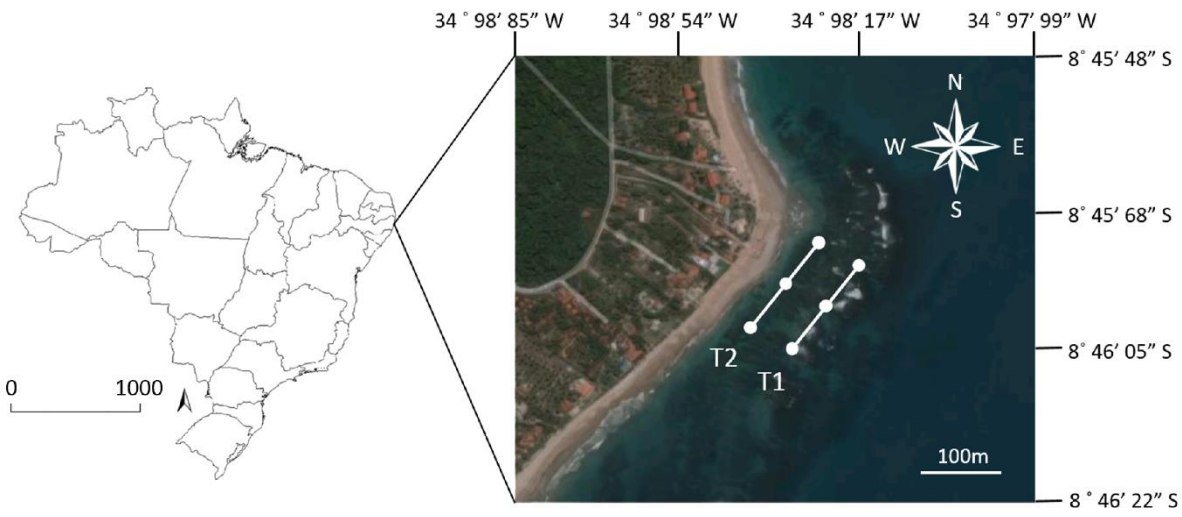

Figure 2. Location of transects in Cupe beach – Ipojuca – Pernambuco at the northeast of the Brazilian coast. T1 represents transect 1 which is more exposed to the waves, and T2 represents transect 2 which is closer to the beach and thus less exposed to the waves (modified from Da Rocha et al. 2006).

S. polyceratium only occurred in T2, while *H. opuntia* occurred in both transects. The seaweeds were collected by using a knife to detach the holdfast from the substrate. The whole seaweed was covered with a plastic bag before removal to prevent sediment loss, and fixed with 4% formalin. The seaweeds were washed under continuous water flow over a set of two sieves with mesh intervals for meiofauna of 500 and 44 micrometers (Elmgren 1973) and specimens retained on the latter were investigated. The volume of the seaweed was measured according to the methodology of Montouchet (1979) by measuring the difference between the initial and final water volume after the inclusion of seaweed in a graduated cylinder. To test the sediment retention capacity of *S. polyceratium* and *H. opuntia,* the sediment that was retained by the sieves for each seaweed sample was put in Petri dishes, dried in an oven and weighed (g). The nematodes were counted under a dissection microscope Olympus SZ51. When present, at least 100 nematodes were randomly and manually picked out

and mounted on slides for identification. In case less than 100 specimens were present in the sample, all were mounted on slides. Preparation and mounting of the nematode specimens occurred according to De Grisse (1969). The nematodes were identified under the light microscope Olympus CX31 to genus level by using the pictorial identification keys (Platt et al. 1985, Warwick et al. 1998) and dichotomous keys in Abebe et al. (2006). Due to time constrains, for the month of July, nematode assemblage of only five samples (three and two – *H. opuntia* and *S. polyceratium* respectively) were determined. Additionally, the nematode assemblage was classified according to the feeding types proposed by Wieser (1953) assigning four feeding guilds on the basis of their buccal cavity morphology: 1A – selective deposit feeders (narrow unarmed stoma, feed on bacteria and similarly sized particles), 1B – non-selective deposit feeders (wide(r) unarmed stoma, potentially feed on a broader range of particles, including microalgae and bacteria (Moens and Vincx 1997)), 2A – epistrate feeders (armed stoma with teeth and/or denticles, feed on microalgae and bacteria), and 2B – predators or omnivores (large armed stoma with teeth and/or mandibles, feed on other metazoans, but also on protists and perhaps even on bacteria) (Moens et al. 2004, Moens et al. 2014)

2.2.3. ***Data analyses***

The genus richness, densities and relative abundance of the nematode assemblage per seaweed sample were calculated. To compare the temporal (dry and rainy period, both seaweeds) and spatial variation (*H. opuntia* only) of the nematode assemblage associated with *S. polyceratium* and *H. opuntia*, the abundance of the nematode genera was converted to density (individuals/ml of seaweed), transformed to square roots and standardized by the total number of nematodes in the sample (relative

abundance) before the similarity analysis. All multivariate, Principal Coordinate (PCO), Permutation Dispersion (PERMDISP), Permutational Multivariate Analysis of Variance (PERMANOVA) and Similarity Percentages (SIMPER) analyses were performed based on Bray-Curtis similarity matrix using the software PRIMER v. 6.1.6 (Clarke and Gorley 2006). The fixed factors used in PERMANOVA were: seaweed species, season, and transect (*H. opuntia* only). The factors month (nested in season) and sampling point were treated as random variable when comparing transects. PERMANOVA was used to compare the nematode assemblage between **1**) *H. opuntia* and *S. polyceratium* occurring in the same transect over time (PERMANOVA: seaweed, season, month [season]), **2**) to compare the nematode assemblage in transects 1 and 2 over time for *H. opuntia* (PERMANOVA transect, season, month [season]). When significant differences were found, a SIMPER analyses was performed to determine the taxa that contributed to those differences.

The amount of sediment retained by the seaweeds was standardized to g/ml. The standardized amount of sediment retained by the seaweed, nematode densities and nematode richness were fourth root transformed to fulfill the assumptions for a parametric test (Kolmogorov-Smirnov normality test, Levene's homogeneity test, XY mean and standard variation plot). Analysis of Variance analysis (ANOVA) was performed to test whether there were: **1**) differences in nematode density and richness over time between the seaweeds in T2 (seaweed, month[season]), **2**) differences in nematode density and richness over time between transects for *H. opuntia (*transect, month[season]) , **3**) differences in sediment retention by *H. opuntia* over time between transects (transect, month[season]), and **4**) differences in sediment retention between the seaweeds over time in T2 (seaweed, month[season]). To test whether the amount of retained sediment correlated with the nematode density on the seaweeds, a

Spearman's correlation was done. The ANOVA and correlation analyses were performed using the statistical software STATISTICA v. 7 (Statsoft 2004).

2.3. Results

2.3.1. ***Nematode assemblages and feeding type structure of H. opuntia and S. polyceratium***

In total, 96 samples were analyzed: 35 for *S. polyceratium* (T2), and 61 for *H. opuntia* (T1 and T2). Identification of the nematode assemblages in these samples yielded 59 genera that were associated with both seaweeds (Table 1), from which 36 genera were found on *S. polyceratium* (T2: mean 6.74± 0.48) and 55 genera were associated with *H. opuntia* (T1: total= 49, mean 9.19± 0.61; T2: total= 41, mean 9.25 ± 0.75). The most abundant genera were *Euchromadora*, *Paracanthonchus* and *Halalaimus* for *H. opuntia* (35%; 10%; 8% respectively), and *Euchromadora*, *Paracanthonchus* and *Hypodontolaimus* for *S. polyceratium* (34%; 14%; 9% respectively). *Acanthonchus* and *Chromadora* reached two to threefold higher abundances in June compared to the other months, but only for *H. opuntia*.

Table 1: List of nematode generic relative abundance and feeding type associated with *H. opuntia* and *S. polyceratium* in Cupe Beach (Brazil) in 2005-2006. Feeding types: 1A Selective deposit feeders; 1B non-selective deposit feeders; 2A epistrate feeders; 2B predators or omnivores (Wieser 1953).

Genus	***H. opuntia T1***		***H. opuntia T2***		***S. polyceratium T2***		**Feeding type**
	Aver.	Std. Error	Aver.	Std. Error	Aver.	Std. Error	
Acantholaimus	-	-	-	-	0.04	0.04	2A
Acanthonchus	12.50	±2.08	4.09	±1.33	8.85	±2.53	2A
Acanthopharyngoides	0.03	±0.03	-	-	-	-	2A
Adoncholaimus	1.27	±0.49	0.23	±0.17	1.05	±0.35	2B

Camacolaimus	-	-	0.32	±0.25	-	-	2A
Chromadora	16.52	±2.78	3.42	±0.95	5.51	±1.15	2A
Chromadorina	0.23	±0.17	0.26	±0.26	0.09	±0.09	2A
Chromadorita	0.31	±0.14	0.43	±0.24	0.07	±0.07	2A
Chromaspirina	0.12	±0.12	-	-	0.52	±0.52	2B
Crenopharynx	-	-	0.37	±0.37	0.59	±0.59	1A
Cyatholaimus	2.65	±1.13	1.51	±0.57	1.75	±0.79	2A
Demonema	-	-	0.17	±0.17	-	-	2B
Desmodora	0.88	±0.57	-	-	-	-	2A
Desmolaimus	0.15	±0.15	-	-	-	-	1B
Desmolorenzenia	0.41	±0.37	-	-	-	-	1A
Desmoscolex	0.35	±0.17	1.41	±0.96	-	-	1A
Draconema	16.23	±2.85	5.02	±1.21	0.17	±0.12	1A
Enoplus	0.23	±0.23	0.09	±0.09	-	-	2B
Epsilonema	0.61	±0.28	1.67	±0.97	-	-	1A
Euchromadora	16.35	±2.09	35.09	±3.13	34.33	±3.07	2A
Eurystomina	6.34	±1.27	2.08	±0.58	7.44	±1.70	2B
Gammanema	0.07	±0.07	0.16	±0.16	-	-	2B
Gammarinema	-	-	-	-	0.09	±0.09	2A
Graphonema	0.04	±0.04	-	-	0.33	±0.20	2A
Halalaimus	5.63	±1.26	7.64	±1.36	0.98	±0.40	1A
Halichoanolaimus	0.55	±0.20	0.97	±0.54	1.04	±0.64	2B
Hypodontolaimus	0.20	±0.16	0.28	±0.17	14.49	±3.76	2A
Marylynnia	-	-	1.65	±1.01	0.10	±0.10	2B
Metachromadora	0.12	±0.12	-	-	0.10	±0.10	2A
Metepsilonema	0.18	±0.13	0.25	±0.19	-	-	1A
Meyersia	0.05	±0.05	0.10	±0.10	0.35	±0.20	2B
Micoletzkyia	0.04	±0.04	-	-	-	-	1A
Oncholaimus	0.45	±0.33	-	-	0.29	±0.15	2B
Oxystomina	0.03	±0.03	-	-	-	-	1A
Paracanthonchus	5.56	±1.34	9.55	±2.39	9.19	±2.24	2A
Paracyatholaimoides	0.04	±0.04	-	-	-	-	2A
Paracyatholaimus	0.15	±0.11	0.44	±0.33	0.09	±0.09	2A
Pareurystomina	-	-	-	-	0.04	±0.04	2B
Phanoderma	0.26	±0.18	1.84	±1.19	0.06	±0.06	2A

Polygastrophora	1.02	±0.64	7.17	±3.70	2.75	±0.88	2A
Praeacanthonchus	0.11	±0.08	-	-	0.67	±0.53	2A
Prochromadorella	-	-	0.05	±0.05	-	-	2A
Prooncholaimus	1.13	±0.38	3.39	±1.13	1.55	±0.64	2B
Pseudochromadora	0.80	±0.43	1.44	±0.56	-	-	2A
Quadricoma	-	-	-	-	0.36	±0.36	1A
Sabatieria	1.68	±1.03	0.28	±0.15	0.03	±0.03	1B
Setoplectus	0.17	±0.17	-	-	-	-	1B
Sigmophoranema	0.17	±0.13	-	-	-	-	2A
Spiliphera	0.08	±0.08	0.18	±0.12	-	-	2A
Spilophorella	1.06	±0.36	1.21	±0.93	0.08	±0.08	2A
Spirinia	0.79	±0.48	0.46	±0.22	0.10	±0.10	2A
Symplocostoma	0.42	±0.18	2.94	±1.14	4.44	±1.02	2B
Synonchiella	0.12	±0.12	0.63	±0.45	-	-	2B
Synonema	0.95	±0.86	0.05	±0.05	0.06	±0.06	2A
Thalassomonhystera	0.38	±0.26	0.41	±0.26	-	-	1B
Thoracostoma	-	-	0.12	±0.12	0.10	±0.10	2A
Tricoma	0.27	±0.13	1.00	±0.51	-	-	1A
Viscosia	2.05	±0.61	1.48	±0.60	2.33	±1.69	2B
Wieseria	0.25	±0.25	-	-	-	-	1A

The most frequent feeding type with more than 50% of the relative abundance in both seaweeds was epistrate feeders (2A) (53% and 56%), followed by predators (2B) (20% and 28%), selective deposit feeders (1A) (20% and 14%) and non-selective deposit feeders (1B) (7 and 3%) in *H. opuntia* and *S. polyceratium,* respectively.

2.3.2. ***Temporal variation of nematode assemblages associated with H. opuntia and S. polyceratium***

2.3.2.1. Nematode assemblage structure between seaweeds over time

The PERMDISP analysis showed no significant values for all the factors (seaweed, *P* = 0.843; season, *P* = 0.415; month, *P* = 0.255) indicating that significant PERMANOVA values are not due to dispersion of variances. Concerning nematode assemblage structure, no significant interaction between **seaweed and months[season]** (PERMANOVA, seaweed x months[season] Pseudo-*F*= 1.54, *P* = 0.071) were observed, indicating that variation in nematode assemblage structure between seaweed species over time was similar.

However, the factors individually (main effect seaweed and month[season]), showed significant differences. The overall significant difference between **seaweeds** (PERMANOVA, Pseudo-*F*= 4.73, *P* < 0.001), was mainly caused by the higher abundance of *Hypodontolaimus* in *Sargassum* (SIMPER: 8.95% contribution). Similarly, the genus that contributed the most to the differences between **seasons** (month[season] PERMANOVA, Pseudo-*F*= 2.62, *P*< 0.001) was *Hypodontolaimus* reaching higher abundances during the dry season (SIMPER, 9.57% contribution). In contrast, the most abundant genera *Euchromadora* and *Paracanthonchus* reached higher abundances during the rainy season (SIMPER, 9.50% and 7.63% contribution respectively). Although significant differences for the both factors were found, no clear pattern was observed in the PCO plot (Fig. 3).

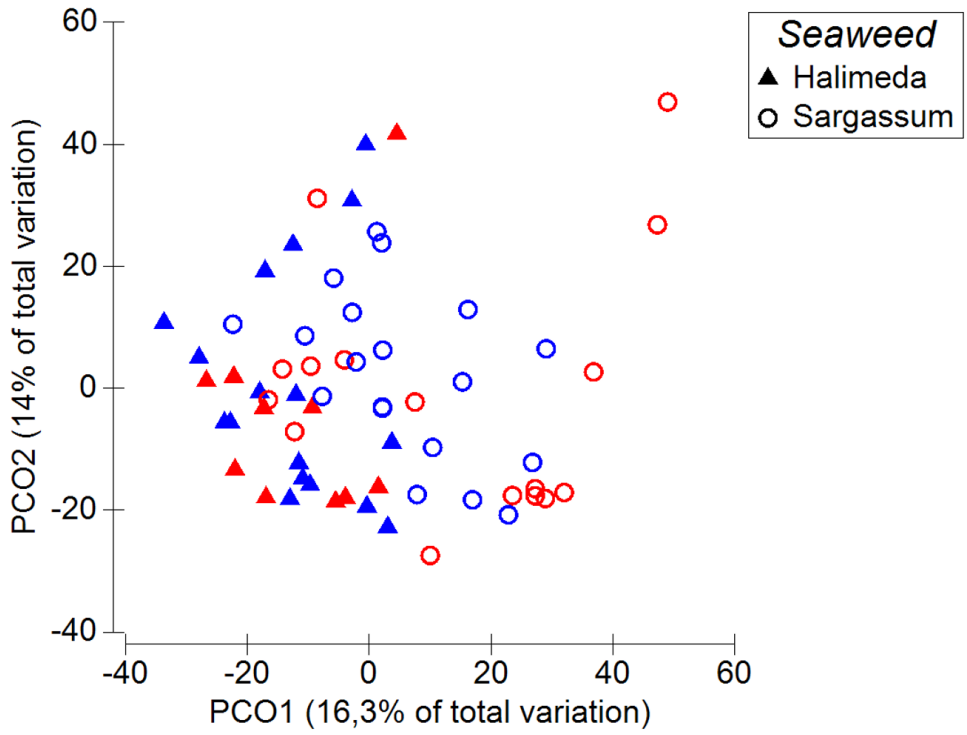

Figure 3. Principal Coordinate analisys (PCO) comparing the nematode assemblage structure between *H. opuntia* and S. polyceratium during the dry and rainy season in Cupe Beach (Brazil) in 2005-2006. The first two axes explained 30.3% of the variation.Red and blue colors represent the dry and rainy season respectively.

2.3.2.2. Nematode density and richness between seaweeds over time

A significant interaction between the factors **seaweeds and month[season]** was observed, indicating that differences between seaweeds were not consistent between season, with *H. opuntia* having higher nematode densities in the rainy season compared to *S. polyceratium*. (ANOVA, month[season] x seaweed, $F = 3.23$, $P = 0.029$; Table 2 and Fig. 4a),

No significant temporal variation in richness patterns between seaweeds (Fig. 4b) were observed (ANOVA, month x seaweed, $F = 0.39$, $P = 0.754$). However, an overall higher

nematode richness was observed in *H. opuntia* compared to *S. polyceratium* (ANOVA, seaweed, $F = 7.35$, $P = 0.009$).

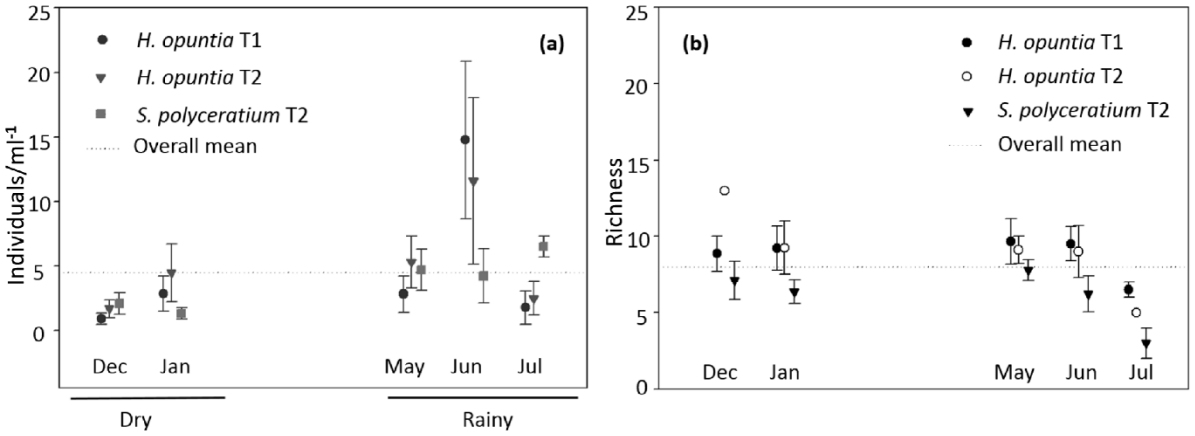

Figure 4. Temporal and spatial average (standard error bars) a densities and b richness of the nematode assemblage associated with *H. opuntia* (T1 and T2) and *S. polyceratium* (T2) in Cupe Beach (Brazil) in 2005-2006.

2.3.3. ***Spatial variation of the nematode assemblage of H. opuntia***

A total of 49 and 41 genera were found associated with *H. opuntia* in T1 and T2, respectively. The genera that presented the highest densities were *Euchromadora, Chromadora*, and *Acanthonchus* in T1 (1.01; 0.91; 0.88 individuals/ml, respectively) and *Euchromadora*, *Paracanthonchus* and *Halalaimus* in T2 (2.07; 0.80; 0.44 individuals/ml, respectively). The genera that reached the highest relative abundance in each transect were *Chromadora, Euchromadora* and *Draconema* in T1 (17%; 16%; 16%, respectively), and *Euchromadora*, *Paracanthonchus* and *Halalaimus* in T2 (35%; 10%; 8%, respectively; Fig. 5).

Table 2**:** ANOVA comparison of the nematode density and richness between *H. opuntia and S. polyceratium* in T2 over time; comparison of the nematode density and richness between the transects T1 and T2 for *H. opuntia* over time; and seaweed retention capacity between *H. opuntia and S. polyceratium* and between transects for *H. opuntia* over time in Cupe Beach Brazil in 2005-2006. The significant differences are marked in bold.

	SS	DF	MS	F	P
Nematodes					
H. opuntia x *S. polyceratium*					
Density					
Seaweed	0,13668	1	0,13668	1,7382	0,193263
Month [Season]	0,18663	3	0,06221	0,7911	0,504461
Seaweed x Month [Season]	0,76263	3	0,25421	3,2328	**0,029777**
Richness					
Seaweed	2,9653	1	2,9653	7,3527	**0,009104**
Month [Season]	2,7936	3	0,9312	2,3090	0,087412
Seaweed x Month [Season]	0,6374	3	0,2125	0,5268	0,665841
H. opuntia					
Density					
Transect	0,13773	1	0,13773	2,3836	0,129180
Point	0,20244	2	0,10122	1,7518	0,184371
Month [Season]	2,89517	3	0,96506	16,7017	**0,000000**
Transect x Month [Season]	0,25330	3	0,08443	1,4612	0,236894
Richness					
Transect	0,970	1	0,970	0,0665	0,797638
Point	5,427	2	2,713	0,1861	0,830826
Month [Season]	38,330	3	12,777	0,8761	0,460103
Transect x Month [Season]	26,976	3	8,992	0,6166	0,607648
Sediment accumulation					
H. opuntia x *S. polyceratium*					
Seaweed	2,12719	1	2,12719	8,20006	**0,006066**
Month [Season]	0,73917	3	0,24639	0,94980	0,423582
Seaweed x Month [Season]	0,52334	3	0,17445	0,67247	0,572880
H. opuntia					
Transect	0,01947	1	0,01947	0,1201	0,730421
Point	0,13078	2	0,06539	0,4034	0,670298
Month [Season]	0,55744	3	0,18581	1,1462	0,340039
Transect x Month [Season]	0,22969	3	0,07656	0,4723	0,703009

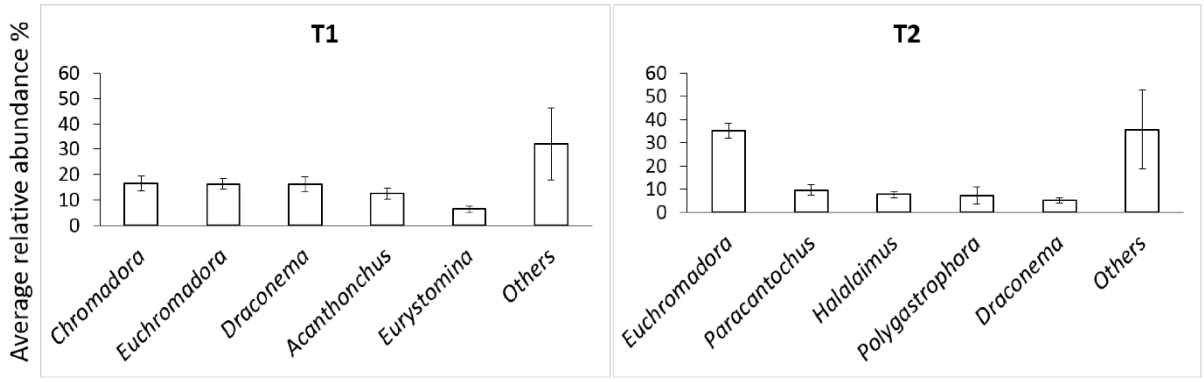

Figure 5. Average relative abundance of the five most abundant genera associated with H. opuntia in both transects in Cupe Beach (Brazil) in 2005-2006.

2.3.3.1. Nematode assemblage structure of H. opuntia *between transects over time*

No significant differences in nematode assemblage structure for any interaction between factors (PERMANOVA, transect x month[season], Pseudo-*F*= 0.82, *P* = 0.622; point x month Pseudo-*F*= 1.23, *P* = 0.131), suggesting that variation in nematode assemblage structure of *H. opuntia* did not vary according to time, the distance to the shore or to the level of exposure to wave action, although some genera preferences for one transect over the other was observed (e.g. *Euchromadora* in T2, Fig. 5).

2.3.3.2. Nematode density and richness of H. opuntia between transects over time

No significant interaction between the transects and time in nematode density (ANOVA, month[season] x transect, *F* = 1.21, *P* = 0.314) or in richness (ANOVA, month[season] x transect, *F* = 0.65, *P* = 0.584) were observed indicating that the observed pattern in density and richness was very similar over time in both transects. Also, no significant difference in richness (ANOVA, transect, *F* = 0.09, *P* = 0.769) between the T1 and T2 was found. However, for the factor **month[season]** regardless

transect, nematode density was significantly higher during the rainy season (ANOVA, season, F = 11.31, P = 0.001, Fig. 4a).

2.3.3. ***Comparison on sediment retention between seaweeds and for H. opuntia between transects***

In total 90 samples for *H. opuntia* (9 replicates per transect over 5 months) and 35 samples for *S. polyceratium* were analyzed. There were no differences in sediment retention over time between *H. opuntia* and *S. polyceratium* in T2 (ANOVA, seaweed x month[season], F = 0.67, P = 0.572). Yet, the difference in architecture of the two seaweeds yielded differences in overall sediment retention capacities in T2 (Table 2) where *H. opuntia* retained significantly more sediment than *S. polyceratium* (main effect ANOVA, seaweed, F= 8.20, P= 0.006). No significant differences between for the factor **months[season]** (main effect ANOVA, month, F= 0.94, P= 0.423) was observed.

For *H. opuntia,* no spatial pattern (Table 2) was observed in sediment retention between transects over time (ANOVA, transect x month[season], F = 1.46, P = 0.236) or between the transects (main effect ANOVA, F= 2.38, P= 0.129). Performing the Spearman's correlation, no correlation was found between the nematode density and the amount of sediment retained for *H. opuntia* or *S. polyceratium*. However, a positive correlation was observed between the amount of retained sediment and nematode richness for both seaweeds (*H. opuntia*: R = 0.32, P = 0.011 – *S. polyceratium*: R= 0.40, P = 0.014). Three of the most abundant genera showed a positive correlation between the amount of retained sediment and genus density in both seaweeds: *Draconema* (*H. opuntia*, R = 0.26, P = 0.03 - *S. polyceratium*, R = 0.34, P = 0.04),

Euchromadora (*H. opuntia*, $R = 0.41$, $p < 0.001$ - *S. polyceratium*, $R = 0.37$, $p = 0.02$) and *Paracanthonchus* only in *H. opuntia* ($R = 0.28$, $P = 0.026$). No correlation was found for *Acanthonchus*, *Chromadora*, *Eurystomina* or *Hypodontolaimus*.

2.4. Discussion

2.4.1. ***Co-occurring seaweed species harbor similar nematode communities and similar trophic composition but fluctuation of rare taxa may account for an overall difference between seaweeds***

No significant differences over time in nematode assemblage structure or richness. Similarity in nematode assemblages between seaweeds has been recorded in previous observations in a study involving four different macrophyte species (Da Rocha et al. 2006). However, *H. opuntia* showed higher overall nematode density during the rainy season (discussed in 2.4.2.) and higher overall richness compared to *S. polyceratium*. The most abundant genera were similar between seaweeds, e.g. *Euchromadora* and *Paracanthonchus* representing more than 44% of the total relative abundance for both macroalgae

Some nematodes appeared to prefer one seaweed species over the other as illustrated by *Hypodontolaimus* for *S. polyceratium*. In contrast, on *H. opuntia* a higher average relative abundance of the family Draconematidae was observed, also the occurrence of Epsilonematidae, which was not associated with *S. polyceratium*. Both families are typically found associated with corals and other hard substrate (Raes and Vanreusel 2006, Raes et al. 2008, Armenteros et al. 2012); their occurrence on *H. opuntia* is most likely related to the calcareous nature of *H. opuntia*. This kind of preference was already mentioned by other authors for seaweed and seagrass (Hopper and Meyers

1967, Hopper 1967, Warwick 1977). In epiphytic amphipods, no correlation has been found between seaweed morphology or complexity (ratio between surface area and biomass) and their abundance or species richness (Russo 1990). In contrast, ostracod species from California did show a strong correlation with complexity levels of the seaweed they were associated with (Frame et al. 2007). Therefore, it seems that different organisms have a different relationship with the macroalgal substrate. Regarding the feeding types in this study, the epistrate feeders (2A) were the most dominant in both seaweeds, as has been previously observed for seaweeds (Ólafsson et al. 1995, Da Rocha et al. 2006, Jaya et al. 2012b). However, this is in contrast with the nematode assemblage associated with the seagrass *Zostera* in which 1B was the most dominant feeding type (Alves et al. 2015) and with *Caulerpa taxifolia* which was dominated by the genus *Halichoanolaimus,* a predator/omnivore or 2B (Jaya et al. 2012b). Preferences for a type of food source is also regarded as an important factor shaping nematode assemblages (Rice and Lambshead 1994). Macroalgae cell wall structure play an important role on the epiphytic microbiota, providing a more or less suitable attachment site depending on the bacteria and diatom species (Egan et al. 2013). In our study, two types of seaweed cell wall are present, calcified and non-calcified (*H. opuntia* and *S. polyceratium*, respectively), and each may harbor different micro-epibionts, influencing nematode genus preference for a certain seaweed species.

2.4.2. ***Seasonal variation reveals higher nematode abundances during the rainy season but the assemblage composition was very similar***

Overall nematode density was significantly higher during the rainy season for both seaweeds. This could be a result of increase in food availability as the a higher

pluviosity increased allochthonous inputs of organic matter (Dell'Anno et al. 2002, Valentine and Duffy 2007). This hypothesis is corroborated by Machado et al. (2014) whom observed an increase in nutrients and phytoplankton productivity in the same region, as a result of a river plume during the rainy season. Salinity may also be affecting nematode densities since it appears to be the most variable physical factor for the region (28.8 to 37.1), e.g. compared to water temperature (27,0 a 28,7 ˚C) (Dominguez et al. 1992), but its role on shaping epiphytal nematode assemblages is unclear.

Although a similar trend was observed for both seaweeds (increase in average densities during the rainy season), the magnitude of this increase appeared to be seaweed species specific, as *H. opuntia* showed a significantly higher nematode density in the rainy season compared to *S. polyceratium* (interaction seaweed x month[season]) . Temporal variation in density of nematodes associated with seaweeds peaking in certain periods of the year has already been observed (Kito 1982). However, comparison between nematode assemblages from different seaweeds species over time is extremely limited. In current work, no variation in richness was observed between seasons and months for both seaweeds and for *H. opuntia* in both transects, showing a fairly stable composition throughout the year. In contrast, a significant difference in nematode assemblage structure has been found between the rainy and the dry seasons. Although the composition was very similar between the dry and rainy seasons, some abundant genera reached significantly higher relative abundances during the rainy season (e.g. *Euchromadora*). Temporal variation of the epifauna living on macrophytes can be related to seasonal morphological changes of the thallus (Travizi and Zavodnik 2004), and some nematode species may migrate to the sediment if thallus morphology is not suitable

(Jensen 1984a). Microarthropod species associated with the macrophyte *Ascophyllum nodosum* have also shown temporal variation (Jarvis and Seed 1996), with some species showing an increased density at a particular time point while the density decreased for other species. Meiofauna associated with the seagrass *Posidonia oceanica* showed higher temporal variability in density present on the leaf region than on the stem region, where the densities were higher with little variation throughout the year (Novak 1982). These differences were correlated with the seasonal development of the seagrass. Seaweeds, as *Sargassum muticum*, also show seasonal developmental variation, such as thallus size, which in turn may affect the associated fauna (Taylor 1997, Baer and Stengel 2010). In our study we used the species *Sargassum polyceratium* and *Halimeda opuntia* which were present throughout the year and without obvious thallus variation. Therefore, thallus seasonality is unlikely to be the most important factor explaining nematode seasonal variation. Moreover, it is important to emphasize that mentioned studies were performed in temperate higher latitudes (> 42°N or > 35°S) where there is a marked seasonal variation affecting the organisms life cycle. In contrast, the current work was performed in tropical low latitude (8°S) region with fairly stable temperatures with average of 26.5 °C during the rainy season and 27.9 °C during the dry season (Machado 2015).

2.4.3. ***No significant differences in nematode assemblages between transects were observed***

There were no significant differences in nematode assemblage structure, density or richness, for *H. opuntia* between transects. These results are in accordance with Arroyo et al. (2004) studying the meiofauna and nematode assemblage associated with the seaweed genus *Laminaria* in Spain. However, this result is unexpected

considering the distance between transects (about 80 m) and the differences in wave exposure. Moreover, according to the literature, the level of shelter from wave action appears to be a factor influencing nematode assemblages associated with the seaweed *Sargassum* in Brazil (Venekey et al. 2008) and on *Gelidium pristoides* in South Africa (Gibbons 1988). In present investigation, generally, the nematode assemblages associated with studied macrophytes reached a higher average density in more sheltered areas, although the data were not always statistically significant. One clear example was the genus *Euchromadora* which preferred areas closer to the beach thus more sheltered (T2) where it could reach twice the density of the area further away from the beach line (T1). Our results show no evidence for the effect of wave exposure on epiphytic nematodes but may suggest that the different level of exposure between the two transects was not high enough to observe significant changes in those nematode assemblages.

2.4.4. ***Sediment retention capacity differed between seaweeds, affecting the density of some specific genera but not the density of the whole assemblage***

There was no significant difference in sediment accumulation between the two transects over time. The sediment retention capacity related more to the seaweed species rather than to degree of exposure and appears related to the level of architectural complexity of the seaweed. Despite a significant difference in sediment retention capacity of the two seaweeds studied, it did not affect the overall nematode density. However, the retained sediment showed a positive correlation with the nematode richness for both seaweeds (*H. opuntia*: R = 0.32; p = 0.011 – *S. polyceratium*: R = 0.40; p=0.014). For some genera, a positive correlation was observed between the nematode density and seaweed species. For example in

Draconema and *Euchromadora*. This may suggest that the effect of the amount of retained sediment is species-specific, affecting the assemblage structure and richness, but not the overall nematode density.

Interestingly, *Draconema* and *Euchromadora* have not yet been recorded in the bottom sediment in the current studied location (de Oliveira et al. 2016), which could be either the result of morphological and locomotion adaptations of the first (Raes et al. 2008) or sampling underestimation. *Hypodontolaimus* occurred in the bottom sediment and on the seaweed but did not show any correlation with the retained sediment. This result contrasts one general assumptions found in a number of articles (Wieser 1951, Wieser 1952, Hopper and Meyers 1967, Hopper 1967, Moore 1971, Warwick 1977, Da Rocha et al. 2006), that the more sediment on the macrophyte the more nematodes can be found (density). None of the above mentioned authors measured the amount of retained sediment and tested its correlation with the nematode assemblage density or seaweed structure. Nematodes appear to choose actively the substrate on which they settle (Ullberg and Ólafsson 2003, Arroyo et al. 2006) rather than just passively be transported along with the sediment through the currents and retained by the seaweed. Experiments on colonization of macrophytes by nematodes have demonstrated that through time, the assemblage is dominated by species that are typically found associated with macrophytes (Arroyo et al. 2006, Derycke et al. 2007c). Our results show different sediment retention capacities between both seaweeds, but no significant differences in nematode densities. This result opposes the idea that the more sediment retained by the seaweed, the higher the nematode overall density.

2.5. Conclusion

Our results suggest that although there are similarities in nematode assemblages between seaweeds, there are preferences of some nematode genera to one seaweed over the other. Moreover, nematode densities significantly increase during the rainy season which might be a reflex of increase in nutrients and primary productivity during the period. Spatiotemporally, epiphytic nematode assemblages structure appears to be homogeneous and no major effect of wave exposure was observed. Contrary to what is generally expected in the literature, overall nematode densities did not increase with the increase of sediment load on the seaweeds. However, some genera appear to be positively correlated with sediment accumulation suggesting that this is a more genus specific rather than general relationship of epiphytic nematode assemblages.

Acknowledgements We would like to thank Prof. Dr. Carl Vangestel for the statistical advice, Prof. Dr. Verônica da Fonsêca-Genevois (*in memorian*) for the incentive and for sharing her experience. The Federal Rural University of Pernambuco (UFRPE) for the logistic support and the Flemish Interuniversity Council – University Development Cooperation (VLIR-UOS) for the grant and financing of the research.

Chapter 3: *Comparison of nematode assemblages from seaweed and surrounding sediment across a latitudinal gradient*

Abstract

Seaweed beds are important ecosystems around the globe and harbor a high biodiversity. Knowledge on the diversity of nematodes associated with seaweeds is still very limited and its relation with the surrounding sediment assemblages is unclear. We characterized and compared the nematode assemblages of the seaweed genera *Sargassum* and *Gracilaria* and sediment from eight locations at the Brazilian coast along 3540km coastline and tested whether biodiversity latitudinal patterns are present for both substrates. Our results showed that the nematode assemblages of seaweeds appear to be significantly distinct from those of adjacent sediment meaning that complete removal of seaweeds can cause a significant biodiversity loss in epiphytic nematode assemblages. Contrary to the sediment, we observed that few genera represented almost half of the total relative abundances in the seaweeds. No direct correlation of nematode biodiversity or density with the latitudinal gradient, for none of the substrates was observed. In general, within and between location similarity of nematode assemblages were higher for the ones in sediment. Our results indicate that the nematode diversity found on seaweeds may not be completely restored by the nematode assemblage found in the sediment. Instead, the recolonization source most likely comes from other seaweed beds.

3.1. Introduction

Abundances and diversity of benthic invertebrates are affected by local variation in sediment characteristics such as granulometry and organic matter content (Ward 1975, Heip et al. 1985, Urban-Malinga et al. 2005, Armenteros et al. 2010, Wang et al. 2011), but also by factors with regional to global influence such as temperature, which varies with latitude (Huston and Huston 1994, Hillebrand and Azovsky 2001), and water currents (Santos et al. 2006). Species richness of marine invertebrates generally increases towards lower latitudes. For meiofauna, latitudinal trends appear much less obvious (Finlay 1998, Hillebrand and Azovsky 2001, Allen et al. 2002, Mokievsky and Azovsky 2002), as they can either follow the general latitudinal pattern, strongly correlating with water temperature in sandy beaches along a stretch of the Pacific South American coast (Lee and Riveros 2012), or be more affected by local factors such as food availability rather than by latitudinal gradient as observed in deep-sea sediments (Lambshead et al. 2000; 2002). To our knowledge, most studies describing biodiversity patterns of marine invertebrates have focused on either the benthic or planktonic realm, whereas comparable studies on latitudinal patterns of invertebrates associated with phytal habitats are scant (Edgar 1982), and are even completely lacking in the case of meiofauna. Latitudinal patterns in biodiversity could allow us to understand if biodiversity distribution in small size metazoans lacking planktonic larvae, such as nematodes, follow the general global biodiversity trend, or if they are more stochastic. This is important because it gives us insights whether local factors can play a more important role in shaping those assemblages rather than a larger-scale trend.

Seaweed beds are affected locally and globally by anthropogenic pressures, either due to direct exploitation (Marinho-Soriano 2016) or to climate change (Jueterbock et al. 2013). Harvesting of seaweeds from the natural bed has shown to negatively affect the biomass, and abundances of the associated fauna such as mollusks *Perna perna* and *Fissurella mutobilis* (Lasiak and Field 1995), and lobsters *Panulirus argus, Panulirus laevicauda* (Costa et al. 2011). The increase of CO_2 uptake by the oceans has resulted in an increased growth rate of seaweeds (Gutow et al. 2014) and in a decrease of calcium carbonate fixation in coralline seaweeds (Brown 2012). Seaweed beds cause attenuation of the hydrodynamic energy and stabilize the sediment in coastal areas (Romano et al. 2003). They also serve as a food source and/or reproduction site for economically important organisms such as shrimps, lobsters (Miller et al. 1971) and fishes (Brüggemann 2012), but also for small metazoans such as nematodes (Warwick 1977), harpacticoid copepods and other meiobenthos (Coull et al. 1983). Many meiofauna on seaweeds presumably graze microalgae and other epigrowth organisms (copepods: Hicks 1977, Whatley and Wall 1975) and may thus contribute to controlling the densities of epiphytes, such as diatoms and cyanobacteria, which compete for light and nutrients with the seaweeds (Van Donk 1998, Ghobrial et al. 2007). Many nematodes living on macroalgal surfaces, for instance, belong to the so-called epistratum feeders (Da Rocha et al. 2006, De Oliveira et al. 2016) which feed by puncturing unicellular microalgae, and/or by scraping off epigrowth from the algal thalli (Moens and Vincx 1997).

True benthic or interstitial nematode assemblages are very diverse, reach high abundances (Lambshead 2004), and comprise species which are able to colonize seaweeds (Warwick 1977). Such colonization could be enhanced by the accumulation of sediment resulting from wave attenuation in macroalgal beds; this sediment

accumulation in turn increases the microhabitat complexity in algal beds (Gibbons 1988). Therefore, interstitial nematode assemblages could serve as a reservoir of biodiversity from which macroalgae in the process of recovery from human impacts such as culling can become recolonized. Rapid recolonization by associated organisms from a nearby reservoir may be important to the resilience of seaweed beds considering that seaweed exploitation can be very intensive (Marinho-Soriano 2016), can cause dramatic and frequently repeated habitat loss (Rocha 2013, de Paula et al. 2015), and that epiphytic invertebrates can be important in controlling microbial epigrowth on recovering algae (see above).

Unfortunately, only few studies have investigated nematode assemblages associated with seaweeds from at least genus level (e.g. Warwick 1977, Kito 1981, 1982, Coull et al. 1983, Gee and Warwick 1994, Pérez-García et al. 2015), and to our knowledge, only two studies have investigated nematode assemblages on seaweeds from the Brazilian coast (Da Rocha et al. 2006, Venekey 2008). Studies at the Brazilian coast, which corresponds to a large portion of the Atlantic South American continent could potentially provide relevant information on phytal habitat in tropical regions and improve our knowledge on nematode biodiversity in shallow-water ecosystems.

Therefore, in this work we aim to **1**) characterize and compare the nematode assemblages associated with two seaweed species and from adjacent beach sediments from eight locations along the southwestern Atlantic coast; **2)** assess whether interstitial nematode assemblages can be a reservoir from which recovering macroalgae can become recolonized after disturbance caused by algal exploitation; **3)** investigate and compare latitudinal patterns in the diversity of nematode assemblages

of sediments and macroalgae along 3450 km of southwestern Atlantic coast. We expect to observe differences in nematode assemblage structure between macroalgae and sediment because of differences in habitat (epiphytic and interstitial); that the sediment nematode assemblage may not fully restore epiphytic nematode diversity because a part of nematode diversity on seaweed would be absent in the sediment; and we expect higher diversity in lower latitudes because of latitudinal temperature gradient.

3.2. Material and methods

3.2.1. ***Nematode sampling***

Sampling occurred during the rainy seasons of 2012 and 2013 at eight locations along the east coast of Brazil (Table 1; Fig. 1). Six locations, Flecheiras (CE), Pirambú (CE), Icapuí (CE) (CE = Ceara state), Muriú (RN) (RN = Rio Grande do Norte state), Cupe (PE) (PE = Pernambuco state) and Ponta Verde (AL) (AL = Alagoas state), are located along the northeastern coast (northeast region) where arenitic reefs and coral reefs are present. They are under the influence of the warm Brazilian and North Brazilian currents, with average water temperatures >18˚C. The remaining two beaches, São Sebastião (SP) (SP = São Paulo state) and Ubatuba (SC) (SC = Santa Catarina state) are located at the southeastern and southern (southeast-south region) coast, and are under the influence of the Brazilian current and the rising of the colder South Atlantic Central Water which generates upwelling with average water temperature <18 ˚C (Coelho-Souza et al. 2012). The smallest and largest distances between all pairs of locations were 167 (Cupe and Ponta Verde) and 3546 km (Flecheiras and Ubatuba), respectively.

Figure 1. Sampling sites and main marine currents along the Brazilian coast. From North to South the sampling sites are Flecheiras-CE; Pirambú-CE; Icapuí-CE; Muriú-RN; Cupe-PE; Ponta Verde-AL; São Sebastião-SP; Ubatuba-SC.

Table 1: Details of the sampled beaches referring to seaweed genera, and the year of sampling.

Beach	Coordinate	Seaweed	Year
Flecheiras (CE)	3°13'08"S 39°16'18"W	*Gracilaria*	2013
Pirambú (CE)	3°42'19"S 38°33'29"W	*Sargassum*	2012
Icapuí (CE)	4°41'02"S 37°22'01"W	*Grailaria*	2012
Muriú (RN)	5°33'43"S 35°14'21"W	*Gracilaria*	2013
Cupe (PE)	8°27'29"S 34°58'58"W	*Sargassum*	2012
P. Verde (AL)	9°39'55"S 35°41'54"W	*Sargassum*	2012
S. Sebastião (SP)	23°49'42"S 45°25'20"W	*Sargassum*	2012
Ubatuba-SC	26°11'48"S 48°31'35"W	*Sargassum*	2012

Sediment granulometry of all beaches ranged from very fine sand to gravel, with the dominance of fine sand in Icapuí (CE), and very course sand in Cupe (PE). An overview of relevant environmental data (e.g. air temperature, pluviosity and salinity) can be found in table 2. All samplings were performed during low tide in the subtidal zone. Three samples of each substrate (seaweed and adjacent sediment) per beach were collected yielding a total of six samples per beach. First, the top-5 cm of sediment was collected typically at a distance of no more than 50 cm from the collected seaweeds, by using a plastic cylinder with an inner diameter of 3.6 cm that was pushed vertically into the sediment. Second, the whole seaweed (holdfast and thalli) was collected manually by using a knife to detach the holdfast from the substrate, brought to the surface and put in a plastic recipient. Seaweeds used in this study belonged to the genera *Sargassum* C. Agardh (1820) and *Gracilaria* Greville (1830). All samples

were fixed *in situ* using DESS (Yoder et al. 2006) by submerging the whole algal and sediment sample in the DESS solution.

Table 2: Sampling locations and corresponding environmental data. Abbreviations: Temp Air (air temperature); Temp Water (water temperature); High. Prec. Period (Period of highest precipitation values); High. Prec. Month (month with highest total precipitation values); NE (Northeastern coast); SE-S (Southeastern-South Coast); P. Verde (Ponta Verde); S. Sebastião (São Sebastião). * indicates that data was not available. Environmental data was obtained from literature (Migotto et al. 1993, Carvalho et al. 1998, Barcellos and Furtado 1999, Horn Filho 2003, Dantas 2004, Araujo 2006, Vieira Hazin et al. 2008, Almeida 2010, Araujo and Rodrigues 2011, Bastos et al. 2011, Veras 2011, Zular 2011, Barros et al. 2012, Chaves 2012, Marino et al. 2013, Santos et al. 2014, Diniz and Pereira 2015).

Region	Location	Temp. Air (Mean)	Temp. Water (min-max)	High. Prec. Period	High. Prec. Month (mm - max; yearly mean)	Salinity (min - max)	Grain size range (dominant fraction)
NE	Flecheiras (CE)	26 - 28	27 - 32	Jan - June	April - 272; 1238	33 -37	Medium (medium sand)
	Pirambu (CE)	26 - 30	28	Jan - June	April - 384; 1500	35 - 36	Fine to course sand (course sand)
	Icapuí (CE)	20 - 32	27 - 28	Jan - May	June - * ; 949	33 - 35	Very fine to fine sand (fine sand)
	Muriú (RN)	25 - 27	33 -34	April - June	June - 260; 1562	33 - 40	Fine to medium sand (fine sand)
	Cupe (PE)	24 - 32	26 - 29	March - August	June - 415; 2050	32 - 38	Very fine sand to gravel (very course sand)
	P. Verde (AL)	22 - 29	26 - 29	March - September	June - 300; 2059	35 - 38	Medium to coarse sand (medium sand)
SE-S	S. Sebastião (SP)	20 - 25	16 - 31	December - January	January - 366; 1500	29 - 36	medium silt to medium sand (*)
	Ubatuba (SC)	16 - 20	17 - 26	January - February	February - 275; 1800	24 - 35	fine to medium sand (*)

3.2.2. ***Sample processing***

The seaweed samples were washed in the laboratory under a continuous flow of freshwater filtered with a Micro-Klean® (G78B2-1T, 5 µm) filter, over a pair of sieves with mesh sizes of 500 and 44 micrometers (Elmgren 1973, Gee and Warwick 1994b) and the retained fauna in the latter was analyzed. The algal volume was measured according to the methodology of Montouchet (1979) by immerging the seaweed in a beaker filled with a known volume of water and calculating the difference between the final and initial volume. For the sediment samples, nematodes were physically separated from the sediment by repeated (10 x) vigorous mixing of the sediment with freshwater followed by decantation over the same pair of sieves as mentioned above (Elmgren 1976). The elutriation procedure was repeated ten times per sediment sample. The nematodes were counted under a dissection microscope Olympus SZ51 (magnification up to 40 x). When present, at least 100 nematodes were randomly and manually picked out with a needle and mounted in slides for morphological identification. Preparation and mounting of the nematode specimens followed De Grisse (1969). The nematodes were identified to genus level under an Olympus CX31 light microscope by using specialized literature (Platt et al. 1985, Abebe et al. 2006).

Nematodes were also classified into feeding types according to (Wieser 1953), which essentially assigns nematodes to four feeding guilds on the basis of their buccal cavity morphology: 1A – selective deposit feeders (narrow unarmed stoma, feed on bacteria and similarly sized particles), 1B – non-selective deposit feeders (wide(r) unarmed stoma, potentially feed on a broader range of particles, including microalgae and bacteria (Moens and Vincx 1997)), 2A – epistrate feeders (armed stoma with teeth and/or denticles, feed on microalgae and bacteria), and 2B – predators or omnivores

(large armed stoma with teeth and/or mandibles, feed on other metazoans, but also on protists and perhaps even on bacteria) (Moens et al. 2004, Moens et al. 2014).

3.2.3. ***Data analyses***

3.2.3.1. Data treatment

The richness (the number of genera occurring in the sample, here expressed as S_{genera}) and Shannon's diversity index (H', a measure of species diversity in an assemblage) were calculated for each sample. To estimate the diversity loss from algal beds in case of complete removal of the seaweeds, we calculated the percentage of nematode genera that only occurred associated with seaweeds. A genus was only associated with a single type of substrate per location when not even a single specimen was found in the other substrate. Because nematode densities on algae were expressed as numbers per unit volume (individuals/ml), we have converted the densities of nematodes in the sediment from individuals/10cm² to individuals/ml by multiplying the core area by the sampling depth (5cm) and then standardizing per ml. In this manner we increased comparability between substrates.

Because two seaweed species were collected (*Gracilaria* sp*.* in Flecheiras-CE, Icapuí-CE and Muriú-RN; *Sargassum* sp. in Pirambu-CE, Cupe-PE, Ponta Verde-AL, São Sebastião-SP and Ubatuba-SC), a one-way Analysis of Variance (ANOVA) comparing the density and biodiversity, and a one-way Permutational Multivariate Analysis of Variance (PERMANOVA – Anderson et al. 2001) comparing the assemblage structure between the two seaweeds (factor: seaweed species) was performed. Should any significant difference be found for either analysis, the dataset for *Sargassum* sp. and *Gracilaria* sp. would be analyzed separately.

3.2.3.2. Comparison of nematode density and diversity between locations

We tested the hypothesis of **no significant differences in nematode total density, and S_{genera} and H'** by performing univariate analyses (one-way ANOVA, fixed factor: location) among the eight locations for sediment, three for *Gracilaria* and five for *Sargassum* along the Brazilian coast, separately (no interaction between substrate and location) because of the structural difference between habitats. We transformed the data to squared or fourth root to fulfill the assumptions when necessary (Kolmogorov-Smirnov normality test, Levene's homogeneity test, XY mean and standard variation plot) prior to the ANOVA analyses. When significant differences among locations were observed, Tukey's HSD test was performed to identify the differences between pairs of locations.

3.2.3.2. Comparison of nematode assemblage between substrate (seaweed, sediments) and locations

We tested the hypothesis of **no differences in nematode assemblage structure (taxa occurrence and proportions) between seaweed and sediment** in the studied locations. The data was transformed to Log (X+1) and standardized by the total (relative abundance) to decrease the effect of discrepancy in abundances between substrates, and then analysed using two-way PERMANOVA analyses with fixed factors location (such as in the univariate analyses) and substrate. A Permutation Dispersion analysis (PERMDISP) was performed to test whether significant results observed in PERMANOVA could be an effect of dispersion of the variances (heteroscedasticity). To visualize the similarity between samples we have generated a Principal Coordinates Analysis plot (PCO), and a Similarity Percentages analysis

(SIMPER) was performed to identify the taxa which contributed the most to the differences between seaweed and sediment nematode assemblages.

The multivariate analyses PERMANOVA, PERMDISP, PCO and SIMPER were based on Bray-Curtis similarity matrices and performed using the software PRIMER + v. 6.1.6 (Clarke and Gorley 2006) with PERMANOVA add-on.

3.2.3.3 Correlating nematode diversity and density with latitude

Gracilaria sp. only occurred in the northeast. Therefore, only *Sargassum* and sediment samples were used for the correlation analysis with latitude. To test the hypothesis of **no correlation between diversity and density and latitude**, a Spearman correlation analysis was performed between the S_{genera}, H', or density, with the latitudinal values (numbers) from Table 1.

The ANOVA, Tukey test and Spearman correlation analyses were performed using the software STATISTICA v. 7 (Statsoft 2004).

Table 3: Occurrence of the nematode genera per beach, substrate and corresponding feeding type. The Genera that only occurred on seaweeds, only in sediment and occurred in both substrates are marked as SW, SD, BO respectively.

Genus	**Beach**								**Feeding Type**
	Flecheiras(CE)	Pirambú(CE)	Icapuí(CE)	Muriú(RN)	Cupe(PE)	P. Verde(AL)	S. Sebastião(SP)	Ubatuba(SC)	
Acanthonchus	SW	SW	-	SD	-	-	SD	-	2A
Acanthopharingoides	-	-	-	-	SD	-	-	SW	2A
Actinonema	-	-	-	SW	-	-	-	BO	2A
Adoncholaimus	SW	-	-	-	-	-	-	-	2B
Amphimonhystera	-	-	-	SD	-	-	-	-	1B

Anoplostoma	-	-	-	-	SW	-	-	BO	1B
Anticoma	SW	-	-	-	-	-	SW	SW	1B
Anticomopsis	SW	-	-	-	-	-	-	-	1B
Apodontium	-	-	-	-	-	-	-	SD	1B
Araeolaimus	SW	-	-	-	-	-	-	SW	1A
Ascolaimus	SW	-	-	-	-	-	-	-	1B
Atrochromadora	-	-	-	-	-	-	-	SW	2A
Axonolaimus	-	-	-	-	-	-	-	SW	1B
Bathylaimus	-	-	SW	-	-	-	-	-	2B
Calyptronema	-	-	-	-	-	-	SD	-	2B
Camacolaimus	-	SW	-	BO	SD	SD	SD	SW	2A
Ceramonema	-	-	-	SD	-	-	-	-	1A
Chaetonema	BO	-	-	-	-	-	-	-	1B
Chromadora	SW	BO	SW	BO	BO	SW	-	SW	2A
Chromadorella	-	-	-	SD	SW	BO	SW	-	2A
Chromadorina	SW	SW	-	SW	SD	SW	SW	SW	2A
Chromadorita	-	-	SD	SD	SD	SW	SD	SW	2A
Comesoma	-	-	BO	-	-	BO	-	-	1B
Comesomoides	-	-	-	-	-	SW	-	-	1A
Crenopharix	-	-	-	-	-	-	-	-	1B
Cyatonema	-	-	-	SD	-	-	-	-	1B
Daptonema	SW	SD	BO	SD	-	BO	-	-	1B
Dasynemoides	-	-	-	SD	-	-	-	-	1A
Demonema	SW	-	-	-	SD	-	-	-	2B
Desmodora	-	-	-	-	SD	BO	-	SW	2A
Desmolaimus	-	-	-	-	-	SD	-	SW	1B
Desmolorenzenia	SD	-	-	BO	-	-	SW	-	1A
Desmoscolex	-	-	SD	SD	SD	-	BO	-	1A
Dolicholaimus	-	-	-	-	-	SW	-	-	2A
Dorylaimopsis	-	-	SD	-	SD	SD	-	-	2A
Draconema	-	-	-	-	SW	SW	SW	SW	1A
Endeolophos	BO	-	-	-	SW	-	-	SD	2A
Enoploides	-	-	-	-	-	-	-	SW	2B
Enoplus	-	-	-	-	-	-	-	SW	2B

Epachanthion	-	-	-	SD	SD	-	BO	-	2B
Epsilonema	-	-	-	-	BO	SD	-	SW	1A
Ethmolaimus	-	-	-	SW	-	-	-	-	2A
Euchromadora	SW	BO	-	SW	SW	SW	BO	SW	2A
Eurystomina	-	-	-	SW	SD	SD	-	-	2B
Glochionema	-	-	-	-	-	-	SD	-	1A
Gomphionema	-	-	-	-	-	BO	-	-	2B
Halalaimus	-	-	SD	-	SD	-	SW	SW	1A
Halichoanolaimus	SW	-	-	-	-	-	-	-	2B
Hopperia	-	-	SD	-	-	-	-	-	2A
Hypodontolaimus	BO	-	BO	SD	BO	-	-	-	2A
Latronema	SD	-	-	SD	SD	-	-	-	2B
Megadesmolaimus	-	-	-	-	-	SD	-	-	1B
Megeurystomina	-	-	-	-	-	-	SD	-	2B
Mesacanthion	-	-	-	-	SD	-	-	-	2B
Metachromadora	-	-	BO	SD	SD	SD	-	-	2A
Metadesmolaimus	-	-	-	-	-	-	-	-	1B
Metalinhonaeus	-	-	-	-	-	SD	-	-	1A
Metoncholaimus	SD	-	-	-	-	-	-	-	2B
Meyersia	BO	-	-	-	-	-	-	-	2B
Microlaimus	-	-	-	SD	SD	-	-	BO	2A
Molgolaimus	-	SD	SD	-	SD	-	-	-	2A
Monhystera	SW	-	-	-	BO	SW	-	-	1B
Nanolaimus	SD	-	-	-	-	-	-	-	1B
Nemanema	-	-	-	-	-	-	SW	-	1A
Nudora	-	-	-	-	SD	-	-	-	2A
Odontophora	-	-	-	SD	-	-	-	-	2B
Omicronema	SD	-	-	-	-	-	-	SD	1B
Onchium	SW	-	-	SW	SW	-	SD	-	2A
Oncholaimellus	-	-	SD	SD	SD	-	-	-	2B
Oncholaimus	BO	-	SW	-	BO	SW	-	BO	2B
Oxistomina	-	-	-	SW	-	-	-	-	1A
Paracanthonchus	SW	-	-	SW	SW	SW	-	-	2A
Paracomesoma	-	-	-	-	-	SD	-	-	1B

Paracyatholaimoides	-	-	-	-	-	-	SW	-	2A
Paracyatholaimus	-	-	-	-	-	SD	-	-	2A
Parallelocoilas	-	-	-	-	-	-	-	SW	1B
Paramonhystera	SD	-	BO	-	SD	-	SW	SW	2B
Paraondontophora	-	-	-	-	-	SD	-	-	2B
Pareurystomina	-	-	-	-	-	SW	-	-	2A
Parodontophora	-	-	-	-	-	BO	-	-	2B
Paroncholaimus	SD	-	-	-	-	-	-	-	2B
Perepsilonema	-	-	-	SD	SD	-	-	-	1A
Phanoderma	-	-	-	-	-	-	SW	SW	1B
Plectus	-	-	-	SD	SW	-	-	-	1A
Pomponema	-	-	-	SD	SD	SW	-	-	2B
pontonema	-	-	-	-	-	-	SW	-	2B
Praeacanthochus	-	-	-	BO	-	-	-	-	2A
Prochaetosoma	-	-	-	-	SD	-	-	-	2A
Prochromadora	-	-	-	-	BO	-	-	SW	2A
Prochromadorella	-	SD	-	SW	-	-	SW	-	2A
Prooncholaimus	SW	-	-	-	SW	-	SW	-	2B
Prorhynchonema	-	-	-	SD	-	SW	-	-	1B
Pselionema	-	-	-	-	-	-	SD	-	1A
Pseudochromadora	-	-	SW	SD	SD	SD	-	-	2A
Pseudosteineria	SD	SD	SD	-	SD	SW	-	-	1B
Rhips	SW	SW	-	-	-	SW	SW	BO	2A
Rhynchonema	-	SD	SD	SD	SD	-	-	SD	1B
Richtersia	SD	-	BO	-	-	-	-	-	1B
Sabatieria	-	-	SD	-	-	SD	-	SW	1B
Setosabatieria	-	-	SD	SD	-	SD	-	-	1B
Southerniella	-	-	-	-	-	-	SW	-	1A
Sphaerolaimus	-	-	SD	-	-	-	-	-	1B
Spilophorella	SW	SW	SW	SW	SW	SW	SW	SW	2A
Spirinia	-	SD	-	SD	-	SD	-	-	1A
Steineria	-	-	-	SW	-	SW	-	-	1B
Symplochostoma	SW	BO	-	SW	BO	-	-	SD	2B
Syringolaimus	SW	SW	-	-	SW	SD	-	SW	2B

Terschellingia	BO	-	SD	SD	-	SD	-	SW	1A
Theristus	SD	-	BO	-	BO	SW	SD	BO	1B
Thoracostoma	SW	-	-	-	-	-	-	SW	2A
Thoracostomopsis	-	-	-	-	-	SD	-	-	2A
Trichoteristus	SD	-	-	-	SD	-	-	BO	1B
Tricoma	-	SD	-	SD	SD	BO	-	-	1A
Trocamus	-	-	-	-	SD	-	SD	-	2A
Viscosia	BO	BO	SD	BO	BO	BO	SW	BO	2B
Xyala	-	-	-	-	SD	-	-	SD	1B

3.3. Results

3.3.1. ***Characterization and comparison of nematode assemblage structure between seaweed and sediment***

In total, 116 nematode genera were identified; 79 (average 9.43 ± 1.15 per sample) genera on seaweeds and 89 (average 11.6 ± 1.10 per sample) in sediments (Table 3, Fig. 2).

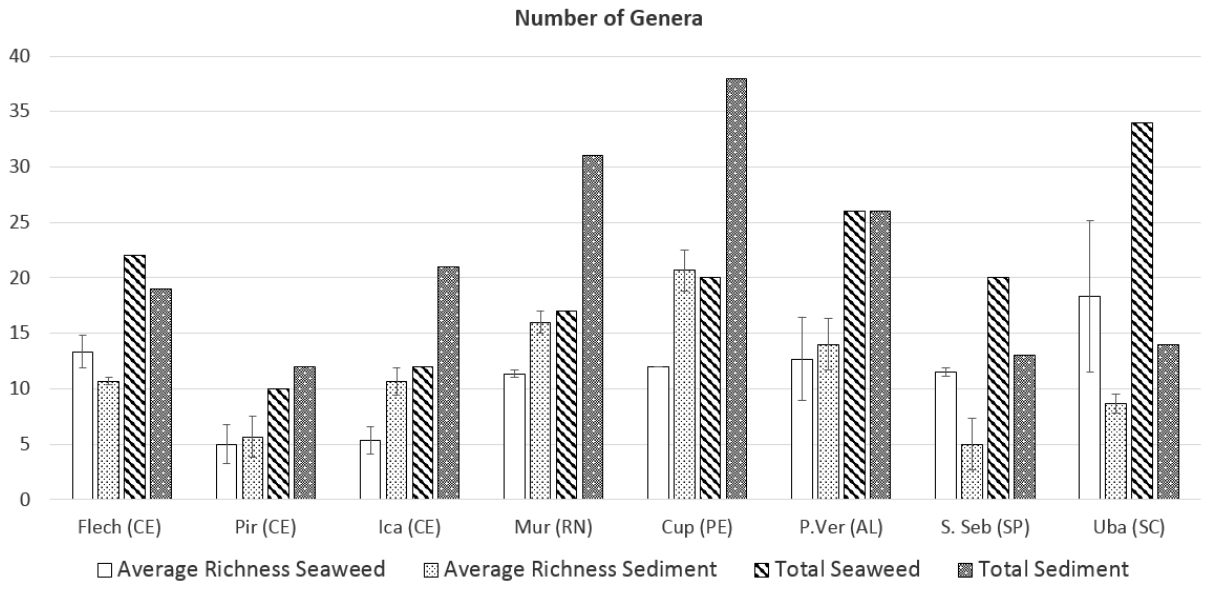

Figure 2. Total number of genera found per substrate per beach along the Brazilian coast. Abbreviations: Flech (CE) (Flecheiras (CE)); Pir CE (Pirambú (CE)); Ica (CE) (Icapuí (CE)); Mur-RN (Muriú (RN)); Cup (PE) (Cupe (PE)); P. Ver (AL) (Ponta Verde (AL)); S. Seb (São Sebastião (SP)); Uba (Ubatuba (SC)).

In total 26 of the nematode genera only occurred on seaweeds, 36 only in the sediment and 54 occurred in both substrates (Fig. 3). The five most abundant genera per location for *Gracilaria*, *Sargassum* and sediment are shown in Table 4. Feeding type 2A was represented by the highest number of genera in both substrates. However, in terms of relative abundance, 2A was dominant only for seaweeds while 1B was dominant in sediment (Fig. 4).

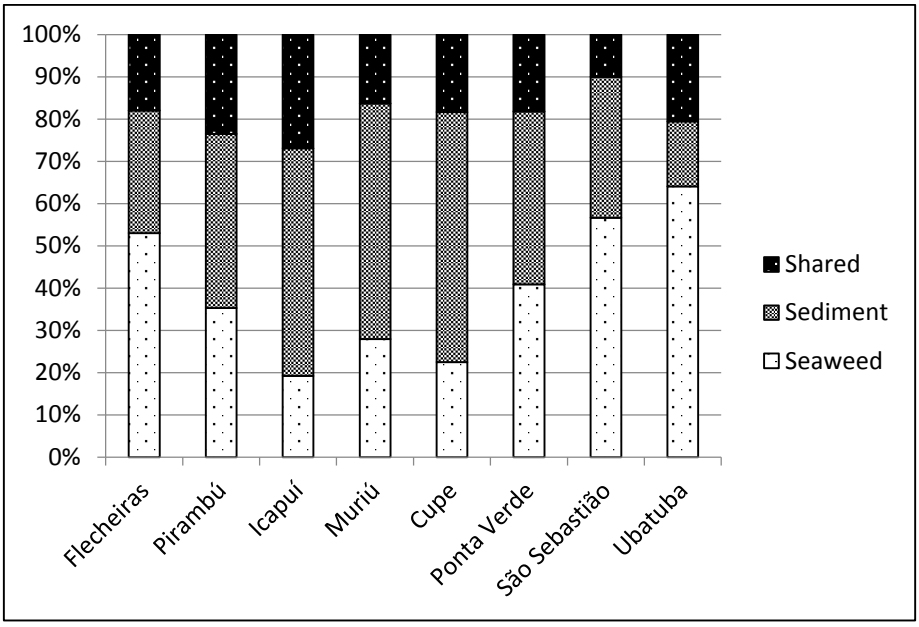

Figure 3. percentage of genera that only occurred on seaweeds, only in the sediment or in both substrates per beach.

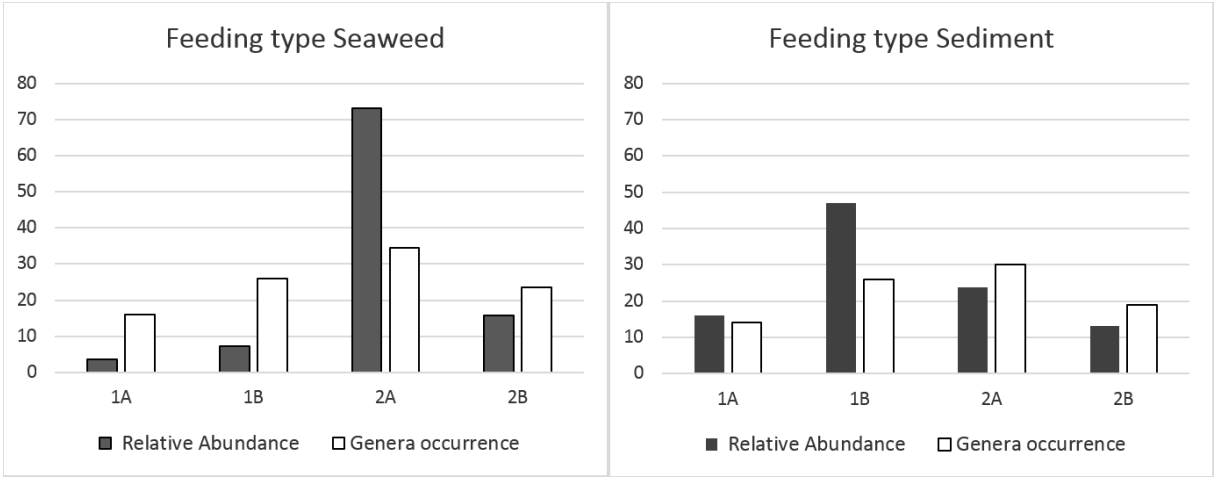

Figure 4. Relative abundance of the genera belonging to each feeding type for seaweeds (A) and sediment (B) along the Brazilian coast.

3.3.1.1. Density, S $_{genera}$, and H' between location and latitudinal correlation

The average densities fluctuated from 0.5 to 24.3 individuals/ml on seaweeds and from 0.4 to 29.5 in the sediment (Fig. 5). No significant differences in S $_{genera}$ were found between the two seaweed species (one-way ANOVA: F= 0.363; p= 0.552), but *Sargassum* showed a significantly higher nematode density compared to *Gracilaria* (one-way ANOVA: F= 5.450; p= 0.029), while *Gracilaria* showed significantly higher H' (one-way ANOVA: F= 4.711; p= 0.041) (Table 5). Hence, three locations for *Gracilaria,* five locations for *Sargassum* and eight locations for sediment were used separately for the latitudinal density and biodiversity analyses.

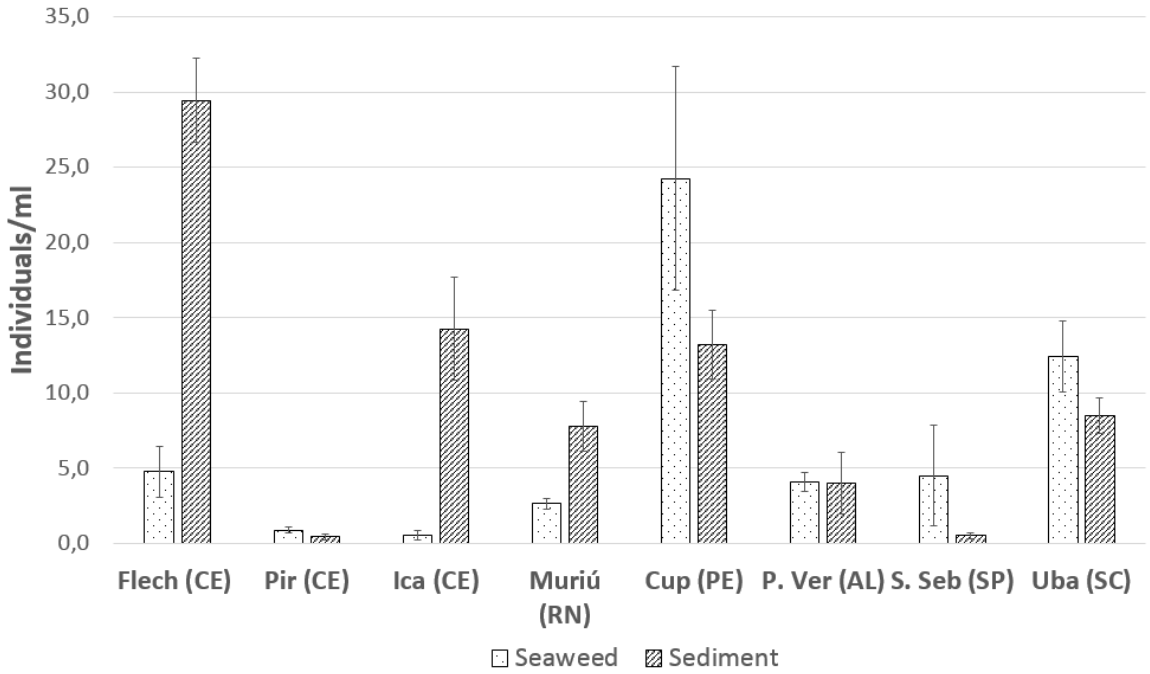

Figure 5. Average density and standard error bars of the nematode community associated with seaweeds and sediment along the Brazilian coast. Abbreviations: Flech (CE) (Flecheiras (CE)); Pir CE (Pirambú (CE)); Ica (CE) (Icapuí (CE)); Mur-RN (Muriú (RN)); Cup (PE) (Cupe (PE)); P. Ver (AL) (Ponta Verde (AL)); S. Seb (São Sebastião (SP)); Uba (Ubatuba (SC))

Table 4. The five most abundant nematode genera for each substrate in the eight studied locations along the Brazilian coast. Numbers represent the average relative abundance of the respective genus.

	Flecheiras (CE)		Pirrambú (CE)		Icapuí (CE)		Muriú (RN)		Cupe (PE)		Ponta Verde (AL)		S.Sebastião (SP)		Ubatuba (SC)	
Sediment	*Hypodontolaimus*	24	*Viscosia*	29	*Paramonhystera*	26	*Spirinia*	30	*Chromadorita*	16	*Terschellingia*	33	*Epachanthion*	37	*Trichotheristus*	50
	Pseudosteineria	21	*Pseudosteineria*	26	*Richtersia*	25	*Hypodontolaimus*	8	*Monhystera*	14	*Spirinia*	12	*Theristus*	25	*Theristus*	18
	Chaetonema	18	*Rhynchonema*	18	*Oncholaimellus*	19	*Rhips*	8	*Hypodontolaimus*	12	*Megadesmolaimus*	7	*Trocamus*	15	*Omicronema*	16
	Paramonhystera	11	*Euchromadora*	13	*Daptonema*	8	*Prorhynchonema*	7	*Rhynchonema*	6	*Paracomesoma*	7	*Acanthonchus*	4	*Microlaimus*	6
	Paroncholaimus	6	*Prochromadorella*	3	*Hypodontolaimus*	3	*Rhynchonema*	5	*Oncholaimus*	6	*Sabatieria*	6	*Calyptronema*	3	*Rhynchonema*	3
Gracilaria	*Chromadora*	26			*Chromadora*	37	*Chromadora*	36								
	Paracanthonchus	16			*Hypodontolaimus*	18	*Paracanthonchus*	27								
	Oncholaimus	10			*Metachromadora*	11	*Euchromadora*	16								
	Anticoma	6			*Paramonhystera*	7	*Praeacanthochus*	7								
	Endeolophos	5			*Bathylaimus*	4	*Spilophorella*	4								
Sargassum			*Chromadora*	40					*Paracanthonchus*	38	*Chromadora*	41	*Pontonema*	74	*Chromadora*	41
			Spilophorella	28					*Hypodontolaimus*	21	*Paracanthonchus*	23	*Viscosia*	4	*Chromadorina*	10
			Euchromadora	11					*Euchromadora*	14	*Euchromadora*	16	*Halalaimus*	3	*Epsilonema*	7
			Acanthonchus	6					*Chromadora*	7	*Viscosia*	6	*Epachanthion*	3	*Araeolaimus*	6
			Camacolaimus	4					*Chromadorella*	7	*Monhystera*	3	*Southerniella*	3	*Paramonhystera*	4

Table 5: ANOVA results of (a) the nematode density, S_{genera} and H' comparison of the nematode community between the two seaweeds *Sargassum* and *Gracilaria*; (b) the nematode density, S_{genera} (S) and biodiversity (H') of *Sargassum* between beaches; (c) the nematode density, S_{genera} and H' comparison of the nematode community of *Gracilaria* between beaches; (d) the nematode density, S_{genera} and H' between beaches along the Brazilian coast. Significant results are marked in bold.

Dependent variable	Seaweed	location
Effect (F/R)	Fixed	Fixed
(a) *Sargassum* and *Gracilaria*		
Density	df=1; *F*=5.454; *p*= **0.029**	na
S_{genera}	df=1; *F*= 0.363; *p*= 0.552	na
H'	df=1; *F*= 4.711; *p*= **0.041**	na
(b) *Sargassum*		
Density	na	df=3; *F*= 10.545; *p*= **0.001**
S_{genera}	na	df=3; *F*= 2.222; *p*= 0.147
H'	na	df=3; *F*= 1.172; *p*= 0.385
(c) *Gracilaria*		
Density	na	df=2; *F*= 7.835; *p*= **0.021**
S_{genera}	na	df=2; *F*= 13.164; *p*= **0.006**
H'	na	df=2; *F*= 8.057; *p*= **0.019**
(d) Sediment		
S_{genera}	na	df=6; *F*= 26.410; *p*<**0.001**
H'	na	df=6; *F*= 11.630; *p*<**0.001**
S_{genera}	na	df=6; *F*= 18.497; *p*= **0.001**

For ***Sargassum*****,** an overall significant difference in density was observed between locations (one-way ANOVA: *F*= 10.545; *p*= 0.001). Pirambú-CE showed significantly lower densities compared to Cupe-PE (*p*= 0.001) and Ubatuba (SC) (*p*= 0.020), and Cupe-PE showed significantly higher nematode density compared to P. Verde-AL (*p*= 0.011). No significant differences in S_{genera} (ANOVA: *F*= 2.222; *p*= 0.147) or H' (one-way ANOVA: *F*= 1.172; *p*= 0.385; Fig. 2, 6; Table 5) among locations were observed.

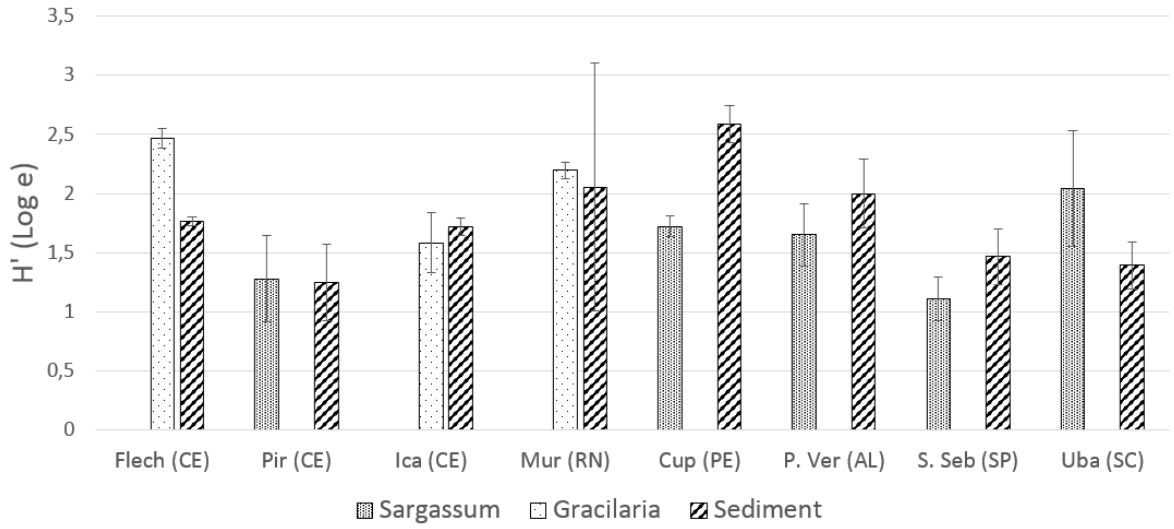

Figure 6. H' values of the nematodes associated with the seaweed genera *Sargassum* and *Gracilaria* and those found in sediments along the Brazilian coast. Abbreviations: Flech (CE) (Flecheiras (CE)); Pir CE (Pirambú (CE)); Ica (CE) (Icapuí (CE)); Mur-RN (Muriú (RN)); Cup (PE) (Cupe (PE)); P. Ver (AL) (Ponta Verde (AL)); S. Seb (São Sebastião (SP)); Uba (Ubatuba (SC)).

For ***Gracilaria***, overall significant differences in density (one-way ANOVA: F= 7.835; p= 0.021), S_{genera} (one-way ANOVA: F= 13.164; p= 0.006) and H' (one-way ANOVA: F= 8.057; p= 0.019) were observed. Nematode densities (p= 0.018) and H' (p= 0.018) were significantly lower in Icapuí (CE) than in Flecheiras (CE), and S_{genera} was significantly lower in Icapuí (CE) compared to Flecheiras (CE) (p= 0.006) and Muriú (RN) (p= 0.019). Icapuí (CE) is a location with known historical seaweed exploitation, and when it was excluded from the analysis, no significant differences in density and biodiversity (S_{genera} or H') between the remaining beaches (Flecheiras (CE) and Muriú (RN)) were observed. Additionally, by not including Icapuí into the analyses comparing the two seaweeds (*Gracilaria* vs *Sargassum*), the only significant difference was in H' (one-way ANOVA: F= 9.495; p= 0.006).

For **sediment**, significant differences between locations in the three variables were observed (one-way ANOVA: nematode density, F= 26.410; p< 0.001- S_{genera} F= 11.630; p< 0.001 – H', F= 18.497; p< 0.001; Table 5). Nematode density in the sediment varied considerably. The location Flecheiras (CE) had the highest nematode densities except compared to Icapuí (CE) and Cupe (PE). In contrast, Pirambú (CE) had significantly the lowest densities followed by S. Sebastião (SP).

The S_{genera} was significantly higher in the location Cupe (PE) followed by Muriú (RN) and P. Verde (AL). Again, Pirambú-CE showed significantly lower S_{genera} except when compared to S. Sebastião (SP) (Fig. 2; Table 7). Similarly, H' was significantly higher in Cupe (PE) followed by Muriú-RN and P. Verde-AL. However, for H', Ubatuba (SC) showed the lowest value instead of Pirambú (CE), compared to the other two analyses (density and S_{genera}). Generally, the location of Pirambú (CE) had the lowest values for the three variables. Detailed pairwise comparisons are provided in table 7.

3.3.1.2. Assemblage structure between substrates and locations

No significant differences in nematode assemblage structure between the seaweeds were found (one-way PERMANOVA: Pseudo-F= 1.538; p= 0.125)(Fig. 7). PERMDISP analysis also showed that this result was not affected by dispersion of the variance between seaweed species (factor seaweed species: p(perm)= 0.146). The resemblance in assemblage structure was due to high abundances of the same genera structuring the assemblages of both seaweed (SIMPER: *Chromadora* and *Paracanthonchus* cumulative contribution of 78.71% and 60.01% in *Gracilaria* and *Sargassum* respectively), accounting for about 50% of the nematodes on both seaweeds. In the absence of significant differences between the nematode

assemblages of the two algal species, the data of both seaweeds from all locations were pooled for the analysis of assemblage structure between substrates (seaweed and sediment).

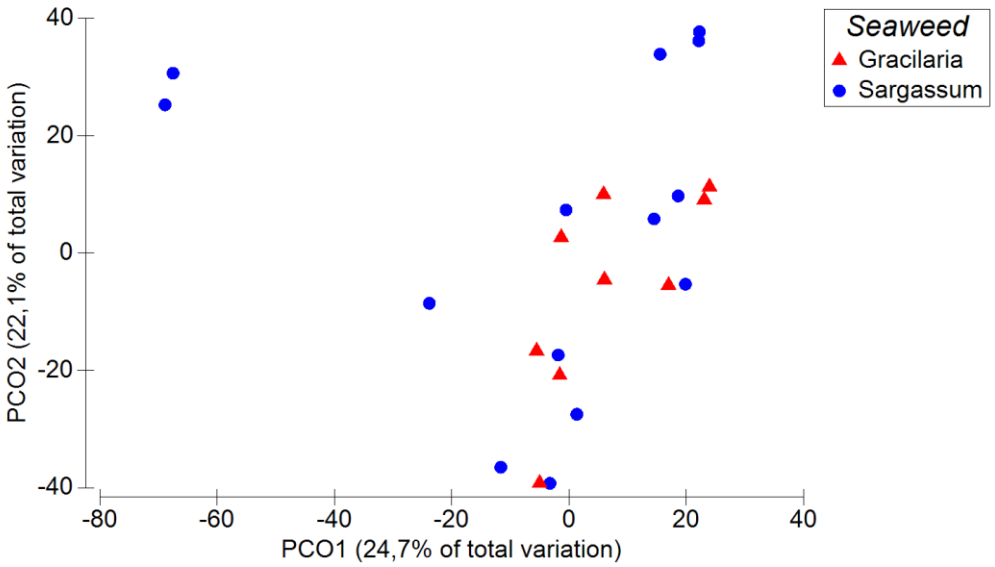

Figure 7. PCO showing the variability in nematode assemblages between *Gracilaria* and *Sargassum*. The first two axes explaining 30% of the variability.

A significant effect of the interaction of the factors substrate and location (two-way PERMANOVA: Pseudo-*F*= 4.1369; *p*< 0.001) on nematode assemblage structure was observed, showing that differences between substrates were not consistent across locations. All nematode assemblages associated with seaweeds were significantly different from those in sediments (Pairwise PERMANOVA, *p*<0.05), except in S. Sebastião-SP (*p*= 0.130). The genera that reached the highest average relative abundances on **seaweeds** were *Chromadora* (30% ± 4.3%), *Paracanthonchus* (11% ± 2.6%) and *Euchromadora* (10% ± 2.3%), with the second one occurring exclusively and the first and the third one predominantly on seaweeds. Those genera contributed 26.41% of the difference in nematode assemblage composition between substrates

(SIMPER). For **sediment**, *Trichotheristus* (7% ± 3.5%), *Spirinia* (6% ± 2.8%) and *Hypodontolaimus* (6% ± 2.0%) reached the highest relative abundances, with the second one occurring exclusively and the first and the third ones predominantly in sediment (table 3). The observed differences between seaweed and sediment have to be interpreted with caution, because PERMDISP demonstrated a significant (p=0.0001) dispersion effect. However, the PCO (Fig. 8) confirms a clear separation between the two substrates, indicating that they do differ substantially.

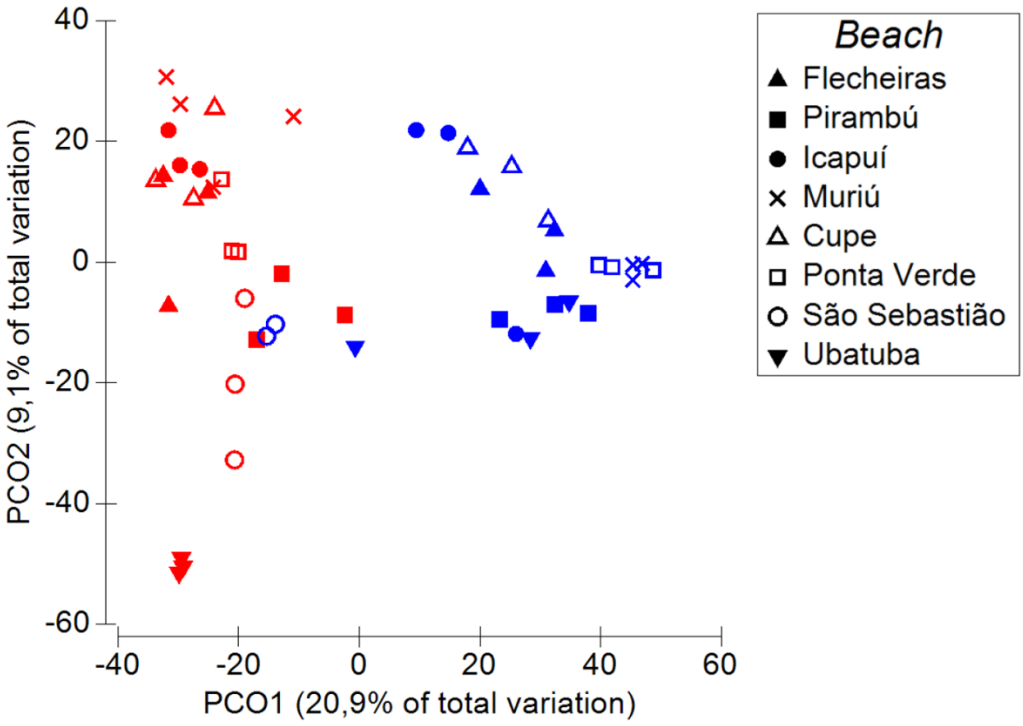

Figure 8. PCO showing the variability in nematode assemblages from eight beaches along 3450 km of Brazilian coast line. The first two axes explaining 30% of the variability. Blue and red symbols correspond to seaweed and sediment assemblages, respectively.

Table 6: Pairwise within and between similarities for seaweed (a) and sediment (b) nematode assemblages of the eight studied locations at the Brazilian coast. The * marks within-site similarities. Bold values represent significant differences between sampling sites.

	(a)	Flecheiras	Pirambú	Icapuí	Muriú	Cupe	P. Verde	S. Sebastião	Ubatuba
	Flecheiras	54.245*							
	Pirambu	**26.146**	46.272*						
	Icapuí	**28.647**	31.224	39.516*					
	Muriú	**41.765**	40.149	**32.091**	74.648*				
Seaweed	Cupe	**34.375**	**19.603**	**19.243**	**49.508**	62.288*			
	P. Verde	**40.211**	39.139	**32.393**	69.647	**46.712**	67.502*		
	S. Sebastião	**5.681**	**3.3835**	**0.49203**	**3.5445**	**2.8227**	**5.4554**	61.761*	
	Ubatuba	**30.177**	30.446	30.71	**33.088**	**16.986**	**35.868**	**5.9413**	48.977*
	(b)								
	Flecheiras	42.641*							
	Pirambu	10.931	24.86*						
	Icapuí	***15.312***	**3.3572**	49.69*					
Sediment	Muriú	**9.3028**	***7.8986***	**7.4734**	37.259*				
	Cupe	**16.276**	**7.6458**	***12.696***	**22.899**	46.832*			
	P. Verde	**1.2743**	**5.3805**	**5.467**	***14.774***	**3.8163**	33.271*		
	S. Sebastião	2.8312	0.34097	1.0322	4.9555	*4.8836*	0.85756	8.8988*	
	Ubatuba	**13.184**	**4.8426**	**1.5517**	**3.895**	**9.8494**	***0.94195***	**11.101**	72.518*

Significant differences between locations for both substrates (seaweed and sediment) significantly fluctuated, and S. Sebastião appears to be an outlier compared to all the other locations as observed in the ordination and in the pairwise similarity (table 5; Fig. 6). When not taking S. Sebastiao into account, six non-significant results (p>0.05) between locations were observed for seaweed whereas only one comparison was not

significant for sediment (Flecheiras (CE) vs Pirambu (CE), *p*= 0.055). Replicate sediment samples of S. Sebastiao showed very low similarities (8.89%) and all pairwise comparisons involving this location were not significant (Table 5). The most abundant genera in all locations varied considerably for sediment, while for seaweeds *Chromadora* was dominant in almost all locations it occurred representing between 26% to 41% of the relative abundances (Table 4). When comparing between substrates, epiphytic nematode assemblages showed higher average similarities compared to the interstitial ones (SIMPER, group average similarity: seaweed= 56.46 – sediment= 39.25; Fig. 6; Table 5).

3.3.2. ***density, S $_{genera}$ and H' of seaweed and sediment along the latitudinal gradient***

No significant correlation (Spearman) between density, S $_{genera}$, or H' and the latitudinal values were observed for none of the substrates (*Sargassum*, *Gracilaria* and sediment).

Table 7: ANOVA pairwise comparison of the nematode community in the sediment of the eight studied locations along the Brazilian coastline. Table A shows the p-values of nematode density (bottom left) and S *genera* (S; top right), and B the p-values of the Shannon–Wiener index (H'). Significant values are highlighted in bold.

A

	Flecheiras (CE)	**Pirambu (CE)**	**Icapuí (CE)**	**Muriú (RN)**	**Cupe (PE)**	**P. Verde (AL)**	**S. Sebastião (SP)**	**Ubatuba (SC)**
Flecheiras		0.289746	1.000000	0.169888	**0.003419**	0.731845	0.746322	0.972123
Pirambu	**0.000175**		0.289746	**0.001360**	**0.000199**	**0.016151**	0.998713	0.816805
Icapuí	0.159298	**0.000179**		0.169888	**0.003419**	0.731845	0.746322	0.972123
Muriú	**0.002523**	**0.000289**	0.496456		0.292050	0.960473	**0.014003**	**0.025636**
Cupe	0.113830	**0.000182**	0.999999	0.619961		0.074873	**0.000559**	**0.000650**
P. Verde	**0.000242**	**0.017189**	**0.016719**	0.354416	**0.024399**		0.106632	0.226628
S. Sebastião	**0.000175**	0.999984	**0.000235**	**0.001106**	**0.000258**	0.067668		0.994783
Ubatuba	**0.008503**	**0.000313**	0.758394	0.999960	0.854704	0.277035	**0.001098**	

B

	Flecheiras (CE)	**Pirambu (CE)**	**Icapuí (CE)**	**Muriú (RN)**	**Cupe (PE)**	**P. Verde (AL)**	**S. Sebastião (SP)**	**Ubatuba (SC)**
Flecheiras								
Pirambu	0.538217							
Icapuí	1.000000	0.634341						
Muriú	0.875188	0.054594	0.796191					
Cupe	0.100228	**0.002511**	0.074885	0.530181				
P. Verde	0.984190	0.154760	0.960608	0.999904	0.398020			
S. Sebastião	0.966727	0.994105	0.985762	0.383746	**0.028322**	0.633891		
Ubatuba	0.844471	0.999061	0.908246	0.156082	**0.007076**	0.360857	0.999996	

3.4. Discussion

3.4.1. ***Nematode assemblages from seaweeds appear to be distinct from those of the surrounding sediment***

Our data demonstrate that nematode assemblages in the sediment differ significantly from those on seaweeds. The genera *Chromadora* and *Euchromadora*, both epistrate feeders, reached higher relative abundances on seaweeds when compared to sediment (Table 4), in agreement with previous papers (Kito 1982, Da Rocha et al. 2006, De Oliveira et al. 2016). They are also among the more abundant genera on hard substrata in coastal habitats (Fonseca-Genevois et al. 2006, Corrêa et al. 2014). The genus *Chromadora* was present in all locations except S. Sebastião (SP), and was the dominant genus in six locations, constituting up to more than 40% of the epiphytic nematode assemblages. A new species of the genus *Paracanthonchus* (see chapter 4), which was also considered an epistrate feeder by Wieser (1953), was also abundant on macroalgae but was not recorded so far in the sediment. Not surprisingly, then, epistrate feeders were the most abundant feeding type on seaweeds (often more abundant than the sum of the three remaining feeding types), whereas selective deposit feeders were the least abundant. The dominance of epistrate-feeders on seaweeds agrees with previous studies (Ólafsson et al. 1995, Da Rocha et al. 2006, Jaya et al. 2012a). This dominance is possibly caused by the growth of diatoms and cyanobacteria biofilms on the surface of the seaweeds. Nematodes specialized for grazing this biofilm tend to reach high abundances as a consequence of food availability (Da Rocha et al. 2006). Those diatoms and cyanobacteria compete with the seaweed for light and nutrients (Van Donk 1998, Ghobrial et al. 2007), and it has been observed that 'micrograzers' can have a positive, neutral or negative effect on the seaweed productivity depending on the species (Brawley and Adey 1981, Norton and

Benson 1983, D'Antonio 1985, Duffy 1990). However, currently, it is unclear whether grazing by epiphytic nematodes can have any significant effect on seaweed primary productivity. In our study, the most abundant epistrate-feeders on seaweeds were chromadorids, which have been recorded to actively emerge from sediment into the water column and swim towards submerged macrophyts (Jensen 1981). Such behaviour has been atributed to phyto-chemical signaling which atracted the nematode *Chromadorita tenuis* (typically epiphytic) towards the macroalgae *Cladophora glomerata*. Well developed caudal glands allow nematodes (not necessarely epistrate-feeders) to better attach to surfaces such as artificial hard substrate (da Fonsêca-Genevois et al. 2006), as observed for the genus *Oncholaimus*, also found in sediment.

Sediment nematode assemblages varied considerably and did not show a dominance of a specific genus across locations. Overall, Xyalidae was the most abundant family as expected for sandy beaches (Gheskiere et al. 2005). In southern locations (S. Sebastião (SP) and Ubatuba (SC)) *Theristus, Omicronema* and *Trichotheristus* reached high densities, and are typically found in subtropical and temperate areas (Wieser and Hopper 1967, Nicholas and Hodda 1999, Gheskiere et al. 2005, Lee and Riveros 2012), whereas in northern locations (tropical), *Theristus* was one of the least abundant and *Omicronema* and *Trichotheristus* were absent. The heterogeneity observed in the nematode assemblages in sediment may reflect differences in granulometry. *Oncholaimellus* was one of the three most abundant nematode genera in Icapuí (CE), which was granulometrically characterized by very fine to fine sand. This result agrees with (Maria et al. 2012) who studied a fine-sandy beach at the Belgian coast. Fine to medium sands are usually dominated by nematodes from the family Xyalidae (Gourbault and Warwick 1994, Nicholas and Hodda 1999, Gheskiere et al. 2004, Hourston et al. 2005, Moreno et al. 2006, Mundo-Ocampo et al. 2007).

Fine to medium sands were present in three beaches in our study, Flecheiras (CE), Muriú (RN) and Ubatuba (SC). The family Xyalidae was dominant in two of those beaches, represented by the genera *Pseudosteineria* and *Paramonhystera* in Flecheiras (CE), *Trichotheristus*, *Theristus* and *Omicronema* in Ubatuba (SC), but not for Muriú (RN), dominated by the genus *Spirinia* (Desmodoridae).

Heterogeneity in sediment nematode assemblages could be further explained by a more diverse food source availability than compared to the food source on seaweeds, particularly due to the accumulation of detrital matter from the seaweeds (Cebrian 1999) and from allochthonous inputs of organic matter (Dell'Anno et al. 2002, Valentine and Duffy 2007). This may explain the more scattered distribution of the sediment samples in the PCO plot compared to the seaweed samples (Fig. 4). As a logical consequence of the prominence of Xyalidae, the feeding type 1B was the most abundant in sediments, followed by 2A (see also de Jesús-Navarrete and Herrera-Gómez (2002).

Differences between substrates were further substantiated by the number of genera that exclusively occurred on seaweeds, which varied between 19% (Icapuí (CE)) to 64% (Ubatuba (SC)) depending on the location. This suggests that epiphytic nematode assemblages are not simply a subset of sediment assemblages, implying that a complete recovery of seaweed assemblages, with their epiphytic invertebrates, after disturbance would require the presence of nearby ‘unaffected’ macroalgal habitats.

In such a scenario, other seaweed beds would be the most plausible ‘reservoir’ of nematodes for the recolonization of other, disturbed seaweed habitats. Rafting on drifting algae is a commonly known dispersal mechanism in epiphytic organisms (Thiel and Haye 2006) and has been suggested for nematodes too (Derycke et al. 2008, 2013). Alternatively, re-colonization could happen from nematodes which are passively

transported in the water column. However, nematode taxa found in the water column more commonly reflect the sediment assemblages (Bell and Sherman 1980, Commito and Tita 2002). We found that some genera that were exclusive for a certain substrate in one location, were present in both substrates in another location; this was, for instance, the case for *Chromadora* and *Acanthonchus*. We could be either dealing with different species of the same genus, or if they do belong to the same species, it could reflect temporary migration of nematodes to the sediment as a strategy to avoid adverse conditions in the algal habitat (Jensen 1984a). In total we found that 26 out of 116 genera were exclusively found on seaweeds. However, a considerably more extensive sampling of different sediments, and identification of nematodes to species level, are required to confirm or discard the idea that a substantial portion of seaweed-associated nematodes are confined to seaweed habitats. Nevertheless, our data showed that some genera appear to prefer one substrate over the other and some may only occur in a particular substrate (e.g. *Paracanthonchus gynodiporata* sp. n. chapter 4).

Differences in seaweed morphology may also provide more favorable conditions for certain nematodes to colonize and dominate (Warwick 1977). In our work, despite the differences in seaweed morphology between *Sargassum* (brown seaweed, usually one longer main straight thallus, branched, with clear leaf-like structure; Agardh, 1820) and *Gracilaria* (red seaweed, thalli cylindrical to flattened, holdfast giving rise to one to many erect axes; Iyer et al., 2004), we observed no significant difference in nematode assemblage structure at genus level, in line with similar observations for other seaweed species (Da Rocha et al. 2006, Pérez-García et al. 2015).

3.4.2. ***Nematode assemblages from seaweeds are more similar than nematode assemblages from sediment***

Our results indicated an overall higher average similarity between seaweed samples (56%) compared to sediment (39%) as observed in the PCO plot (Fig.5). We have found higher within-site variability for sediment nematode assemblages in 6 out of 8 locations, which exhibited a higher patchiness compared to the epiphytic ones. This result suggests that ecological pressure acting on nematode assemblages from the two habitats is different. Alternatively, sediments may provide a more heterogeneous habitat, e.g. higher food patchiness (Lee et al. 1977), higher interstitial space variation due to different grain sizes and shapes (Conrad 1976) and higher variation in oxygen levels (Jogensen and Revsbech 1985), compared to the epiphytic habitat, leading to a more homogeneous nematode assemblages in the latter. The between-site variability was also higher in sediment nematode assemblages, showing a higher beta-diversity in the interstitial habitat compared to the epiphytic. The higher similarity between epiphytic nematode assemblages compared to the ones in sediment, becomes more evident when looking at differences in nematode density, S_{genera}, and H' between locations. For instance 44% of all pairwise comparisons between locations (the proportion of significant results in relation to the total number of comparisons), excluding assemblage structure, were significantly different for sediment, while only 17% of all pairwise comparisons were significantly different for seaweeds (sum of the results for *Sargassum* and *Gracilaria* combined). Moreover, excluding the location of S. Sebastião, which appears to be an outlier, there were more significant differences between locations in sediment than for epiphytic nematode assemblage structure ($p<0.05$ 97% and 79% of the pairwise comparison for sediment and seaweed respectively).

Gracilaria is an economically important seaweed, and the three locations where the genus occurred have undergone historical seaweed harvesting. However the level of this activity was different between locations. For instance, in the 1980's, while in Flecheiras (CE) up to 17 tons of seaweed per month were harvested (Rocha 2013), as much as 45 tons were culled per week in Icapuí (CE) (de Paula et al. 2015). Indeed, even at present, Icapuí (CE) is known for an extremely high level of seaweed bed degradation due to historical seaweed harvesting (Costa et al. 2011). Interestingly, Icapuí (CE) had significantly lower density, S_{genera} and H' compared to the other two locations with *Gracilaria.* No significant differences in density, S_{genera} or H' between the two other locations (Flecheiras-CE and Muriú-RN) were observed. In fact, even when comparing nematode assemblages from locations irrespective of the identity of the seaweed species (*Gracilaria* vs *Sargassum*), hardly any significant differences (with the exception of H') between locations were found when Icapuí(CE) was left out of the analysis. This suggests that the intensity of historical seaweed harvesting had a strong effect on nematode assemblage structure and composition. There is no evidence that seaweed exploitation in this particular location had any effect on nematode assemblages in sediment, as density and biodiversity (S_{genera} and H') showed no clear pattern.

Interestingly, nematodes colonizing artificial hard substrate resemble those found on seaweeds (Fonseca-Genevois et al. 2006), and a number of genera present on aluminum plates were absent in the sediment (12 genera out of 19). This was unexpected considering that the study premise was that upwelling currents, would suspend and transport sediment nematodes (passive dispersal, Palmer 1988), which in turn, would colonize the aluminum plates. Although sediment nematodes were present in the water column, the nematode assemblages on *Sargassum furcatum*, a

common seaweed in the studied region, clearly were more efficient in colonizing the aluminum plates. It suggests a colonization capacity of epiphytic nematodes compared to the interstitial ones.

The higher patchiness of interstitial nematode assemblages may indicate, but not only (e.g. granulometry. see next section), that nematodes typically found in sediment may be more isolated and have a more limited dispersal capacity compared to nematodes associated with seaweeds. Although benthic nematodes can become suspended and disperse in the water column (Palmer 1988, Boeckner et al. 2009, Thomas and Lana 2011), nematodes are generally considered bad swimmers and are unlikely to remain suspended for much longer than 2 hours which limits the distance over which benthic nematodes can disperse (Ullberg and Ólafsson 2003). Moreover, although even very weak currents can suspend the nematodes in the water column (Boeckner et al. 2009), many sediment nematode species try to avoid being suspended by migrating downwards in the sediment (Steyaert et al. 2001, Sedlacek and Thistle 2006). Nevertheless, several studies have found nematode species composition in water samples to be very similar to that of the bottom sediment (Bell and Sherman 1980, Commito and Tita 2002, Boeckner et al. 2009).

3.4.3. ***No clear latitudinal patterns for the nematode assemblages from seaweeds or sediments***

Latitudinal patterns of nematode assemblages in intertidal zone along the Pacific coast of South America have shown to be strongly correlated with water temperature (Lee and Riveros 2012). However, no direct correlation between the latitude and nematode densities and biodiversity (S_{genera} and H') were observed in current study for both

substrates in shallow water subtidal zone. For marine shallow-water ecosystems, studies have not found clear latitudinal meiofauna patterns for sedimentary nematodes (Kotwicki et al. 2005, Gobin and Warwick 2006) which is in agreement with our results for both substrates (seaweed and sediment). However, our result might not be representative for the whole Brazilian coast considering the gap between northern and southern sampling locations and the full extension of the coastline (more than 7000 km). Additionally, looking at geographical distances may also provide relevant information than latitudinal gradients only.

It is argued that latitudinal patterns in abundance appear to vary depending on the type of environment, where nematodes tend to reach high abundances at low latitude in coastal areas (Kotwicki et al. 2005) and higher abundances at higher latitudes in the deep sea. This appears to be correlated with the productivity (Lambshead et al. 2000). Because of the upwelling phenomenon in the two southernmost beaches (S. Sebastião (SP) and Ubatuba (SC) – Pereira et al. 2009, Coelho-Souza et al. 2012), we expected nematode assemblages to show significantly higher abundances there than compared to the northeastern ones. However, we did not observe higher abundances in the SE-S for either substrate.

One local factor that could explain biodiversity of interstitial nematode in current study is grain size. Granulometry profiles varied across locations. As grain size is one important factor shaping nematode assemblages in sediment (Steyaert et al. 2003), this variation may explain the observed differences. However, we could not test it since we did not obtain granulometric data ourselves. Generally, we found higher biodiversity in beaches with coarse sand and gravel as observed in P. Verde (AL) and especially for Cupe (PE) where the sediment has high content of bioclasts such as fragments of the seaweed *Halimeda* (Dominguez et al. 1990). A positive correlation with grain size

has been already reported in coastal areas (Steyaert et al. 1999, Maria et al. 2013). The only exception to this was Pirambú (CE) (fine to coarse sand), which exhibited significantly lower biodiversity compared to most other locations. This was the only beach located in an urban area within a large capital in the Brazilian NE (Fortaleza). There, other factors, such as domestic sewage (Pereira et al. 2009b), may play a more important role than granulometry.

3.5. Conclusion

Our results show that nematode assemblages of seaweeds and sediment are distinct, with few genera representing almost half of the relative abundances in the seaweed. Moreover it suggests that because epiphytic nematodes are not simply a subset of the interstitial assemblages, the latter may not completely restore the nematode diversity of the macroalgal habitat. At a large scale (thousands of km), nematode sediment assemblages were more heterogeneous compared to those on seaweeds. There was no clear latitudinal pattern of density or diversity. This observation corroborates with the idea that local factors may play a more relevant role on shaping the assemblages of small-size metazoans, such as nematodes, that lack planktonic larvae. Finally, further studies in the same and other locations along the Brazilian coast are necessary to increase coastal area coverage, and improve our knowledge in this macro-puzzle that is nematode biodiversity in shallow water habitats.

Chapter 4: *Low genetic but high morphological variation over more than 1000 km coastline refutes omnipresence of cryptic diversity in marine nematodes*

Modified from: De Oliveira DAS, Decraemer W, Moens T, Dos Santos GAP, Derycke S. 2016. Low genetic but high morphological variation over more than 1000 km coastline refutes omnipresence of cryptic diversity in marine nematodes. *BMC Evolutionary Biology* (under peer-review phase)

Abstract

Seaweed beds form a dynamic shallow water ecosystem influenced by climate change and human exploitation. The resilience of ecosystems to negative impacts is generally higher when high gene flow, species diversity and genetic diversity are present. We studied the population genetic structure of the new nematode species *Paracanthonchus gynodiporata* associated with seaweeds in northeastern Brazil. Nematodes are generally believed to have a limited dispersal capacity because of the lack of planktonic larvae. Yet, they can drift on seaweeds, and water currents might be a natural barrier for their dispersal. Populations of *P. gynodiporata* were sampled over more than 1000 km coastline in regions across major oceanic currents with and without historical exploitation of seaweed. *P. gynodiporata* is described in an integrative way using mitochondrial and nuclear sequences and morphological data. The 3D model of the head region shows for the first time a detailed view of the ventrosublateral teeth, a character often overlooked in older taxonomic studies of the genus. A total of 17 mitochondrial COI haplotypes were found with one haplotype representing 63% to 83% of the frequencies in each population. AMOVA showed overall little population genetic structure (F_{ST}= 0.05204), and no genetic subdivision between the populations under the influence of the two different water currents were found. Effects of historical seaweed exploitation on population genetic diversity were not detected. In contrast, significant differences between populations were found in morphometric characters. This discrepancy in genetic and morphological differentiation between populations across 1000 km of coastline is surprising in view of the frequently observed presence of several cryptic species at small geographical scale in other macroalgal associated nematodes.

Our results show that cryptic species are not omnipresent in marine nematode species, suggesting that nematodes associated with seaweeds have been able to disperse over large distances across well-known biogeographic barriers.

Keywords: COI - connectivity - morphometry - population genetics

4.1. Introduction

Morphologically similar but genetically distinct species, i.e. cryptic species, are prevalent in many taxonomic groups (Pfenninger and Schwenk 2007) and have been reported from marine environments since decades (Knowlton 1993). Cryptic species are invoked when genetic variation within species exceeds that typically found between morphologically well-known species. Genetic differentiation between populations within a species depends on selection, genetic drift and gene flow (Hartl 1988). Selection favors individuals that are better adapted to the environmental conditions at play and can increase or decrease genetic differentiation between populations through disruptive or balancing selection, respectively. Genetic drift is the random loss of alleles (Knowlton 1993), and leads to an increase in genetic differentiation between populations. Finally, gene flow homogenizes allele frequencies between populations through dispersal of individuals and reduces differentiation between populations.

Many marine organisms were initially thought to have high dispersal capacity because of the passive dispersal potential via currents and the perceived 'homogeneity' of marine habitats over extensive spatial scales (Kinlan and Gaines 2003). However, population genetic structuring among marine populations can be surprisingly high (Hohenlohe 2004, Jones et al. 2005), even in organisms with a planktonic larval stage (Cowen et al. 2000). Organisms which lack planktonic larvae, such as free-living marine nematodes, have a population structuring which strongly varies depending on the species, distance and the environmental conditions (Derycke et al. 2013). Dispersal can be substantial on fairly small geographical scales (≤ 100 km), leading to rapid colonization and moderate to little population-genetic structuring (Derycke et al. 2005, Derycke et al. 2007c). Nematodes that occur on seaweeds can use the seaweed

drifting mechanism for their dispersal (Thiel and Gutow 2005), and this may even occur over oceanic scales (Derycke et al. 2008b). Yet, such long-distance dispersal in marine nematodes is thought to be rare, and substantial cryptic diversity has been observed in marine nematodes associated with macroalgae (Derycke et al. 2005, Derycke et al. 2007a, Derycke et al. 2010a).

Dispersal of marine organisms can be hampered by biogeographic barriers which may result in genetic breaks within species (Hohenlohe 2004). Well known examples are the Gulf of Mexico and the Atlantic coast in Florida (for instance for the black sea bass (Roy et al. 2012)), the Indo-Pacific barrier (for instance for populations of the fish *Lutjanus fulvus* (Gaither et al. 2010)), and Point Conception in California (for instance for shark (Chabot et al. 2015)). Along the northern Brazilian coast, known biogeographical barriers are the Amazon-Orinoco Plume and the North Brazilian current which prevent some Caribbean species from dispersing to Brazil (Luiz et al. 2013, da Silva et al. 2015). In addition, the split of the South Equatorial current (SEC) in the northeastern coast of Brazil (Santos et al. 2006) forms two different currents: the above mentioned north Brazil current towards the north and the Brazil Current towards the south. The importance of the latter current as a barrier for dispersal between populations of marine species associated with seaweeds has yet to be clarified.

Seaweed beds can cover areas of thousands of square kilometers (Takao et al. 2015) but are often discontinuous along the coastline (Metri 2006, Rocha 2013). Although seaweed beds may be hundreds of kilometers apart, such distances may not represent a strong barrier to dispersal of seaweeds (Tom Dieck and De Oliveira 1993) and of associated fauna which can drift/raft along with seaweeds (Thiel and Gutow 2005, Arroyo et al. 2006). In addition, harvesting of seaweed beds creates a highly dynamic

environment (Rocha 2013) where recolonization, founder effects and genetic bottlenecks can affect allele frequencies of the associated fauna (Derycke et al. 2007c), which may lead to reduced genetic diversity when compared to areas where no exploitation took place.

One of the most abundant and widespread nematode genera on seaweeds along the NE Brazilian coast is *Paracanthonchus* Mikoletzky (1924; Paracanthonchinae, Cyatholaimidae) (Venekey et al. 2008, De Oliveira et al. 2016). The validity of a number of species within this genus has been debated because of the poor representation of structures in the buccal cavity, among others (Miljutina and Miljutin 2015). Here, we describe the new species *Paracanthonchus gynodiporata* sp. n. which has hitherto only been found associated with seaweeds, in an integrative way based on a large number of specimens (38) from four different populations spanning a wide geographical distribution (> 1000 km). Mitochondrial (COI) and nuclear (18S and the D2/D3 fragment of the 28S rDNA) sequences were obtained and morphometric variation across populations was addressed to capture morphological variation. In addition, a 3D reconstruction of the mouth structure, one of the most important diagnostic characters within the genus, was made. Second, genetic structure and diversity of this new species were investigated using mitochondrial COI sequences of nematodes occurring on seaweed beds separated by the north Brazil and Brazil current (Santos et al. 2006). In view of the large genetic structure observed in coastal nematodes from the Atlantic at distances >100 km (Derycke et al. 2013), we expected to find distinct genetic breaks among the Brazilian beaches and across the northeastern split of the south equatorial current. Third, we also investigated the effect of seaweed harvesting on population genetic diversity by comparing samples from two locations with and without historical

seaweed harvesting. We expected to find lower genetic diversity in the algal beds that had been disturbed by harvesting because the latter will lead to population bottlenecks.

4.2. Material and Methods

4.2.1. ***Field sampling and collection of nematodes***

Five seaweed samples of the genera *Sargassum* C. Agardh, 1820 and *Gracilaria* Greville, 1830 and five sediment samples were collected from natural seaweed beds on each of four beaches along the northeastern coast of Brazil, spread over a distance of about 1040km (Fig. 1). All sampling sites were within the Northeastern Brazil ecoregion and sampling took place during the rainy season between April and July. The nematode community associated with both seaweeds is very similar in the northeastern coast of Brazil (Da Rocha et al. 2006, De Oliveira et al. 2016). The two northernmost beaches are located in Flecheiras (CE) and Muriú (RN), both sampled in 2013, and are under the influence of the north Brazil Current. In those locations, seaweeds from the natural bed, mostly *Gracilaria*, were continuously harvested for about 30 years (historical harvesting), followed by a period of 11 years with no harvesting. Currently, in both locations the natural seaweed beds are no longer harvested. However, in Flecheiras (CE), seaweed cultivation outside the natural seaweed bed started in 2003 in an area smaller than the natural bed, and is still ongoing. The cultivation technique consists of floating ropes of about 25 m long to which the seaweeds are attached. The two southern beaches, Cupe (PE) (sampled in 2011 and 2012) and Ponta Verde (AL) (sampled in 2012), are under the influence of the Brazil current and algal beds are dominated by *Sargassum*. No historical or contemporal harvesting or seaweed cultivation has taken place in these southern

locations. Distance between sampled seaweed beds ranged from about 167 to 1045 km (Fig 1), and each seaweed bed had a total area ranging from ca 0.3 to 4.54 km² (Table 1). Sampling was performed during low tide in the subtidal zone. Only seaweeds attached to the substrate were collected by cutting the base of the holdfast with a knife, put in plastic recipients and fixed with DESS (Yoder et al. 2006). Five samples containing an entire seaweed plant were collected ca 50 m apart from each other per beach (*Gracilaria*: Flecheiras and Muriú - *Sargassum*: Cupe and P. Verde). Five samples of the top 5 cm of the adjacent bottom sediment were collected using a plastic cylinder with inner diameter of 3.6 cm that was vertically pushed into the sediment. Three seaweed samples and three sediment samples were used for characterization of the nematode community (Apolônio Silva De Oliveira 2016), and two were used to collect nematode specimens for the population genetic study. The seaweed samples were washed under a continuous stream of filtered freshwater over a pair of sieves with mesh sizes of 500 and 44 micrometers.

Table1: Coordinates of the studied locations with the approximate total area of the seaweed bed in km² between brackets, historical background concerning exploitation of the natural seaweed bed and the average relative abundance of *P. gynodiporata* sp. n. in the respective locations. Haplotype occurrence per beach and h (haplotype diversity), π (nucleotide diversity), Tajima's D and Fu's Fs neutrality test values with the corresponding p-values between brackets of the studied populations at the northeastern Brazilian coast. Abbreviation: Rel. Abund. (relative abundance).

Beach	**Coordinate**	**Seaweed**	**Historical background**	***P. gynodiporata sp. n. Rel. Abund.***	**nº haplotype**	**Year**	***n***	***h***	***π***	***D***	***Fs***
Flecheiras(CE) (1.49km²)	3°13'08''S 39°16'18''W	*Gracilaria*	Exploited	15.56 ±6.39	5 (F1, F2, C7, C8, PV2)	2013	27	0.5783 ±0.0961	0.0034 ± 0.0024	-0.3561 (0.3920)	0.0766 (0.5110)
Muriú(RN) (1.86km²)	5°33'43''S 35°14'21''W	*Gracilaria*	Exploited	26.73 ±8.31	4 (M1, M2, M3, C8)	2013	24	0.3080 ±0.1180	0.0008 ± 0.0009	-1.4943 (0.0430)	-2.3829 (0.0030)
Cupe(PE) (0.30km²)	8°27'29''S 34°58'58''W	*Sargassum*	Not exploited	37.73 ±7.16	6 (M2, C2, C5, C7, C8, C9) - (C3, C4, C6, C7, C8, C10)	2011-2012	33 - 25	0.3667 ±0.122 - 0.3807 ±0.1058	0.0012 ± 0.0012- 0.0011 ± 0.0011	-2.0875 (0.0040) -1.6480 (0.0160)	-4.3714 (0.0000) -4.0239 (0.0000)
P. Verde(AL) (4.54km²)	9°39'55''S 35°41'54''W	*Sargassum*	Not exploited	22.95 ±2.47	5 (PV1, PV2, PV3, C4, C8)	2012	20	0.3684 ± 0.1351	0.0019 ± 0.0016	-1.9723 (0.0130)	-1.7287 (0.0590)

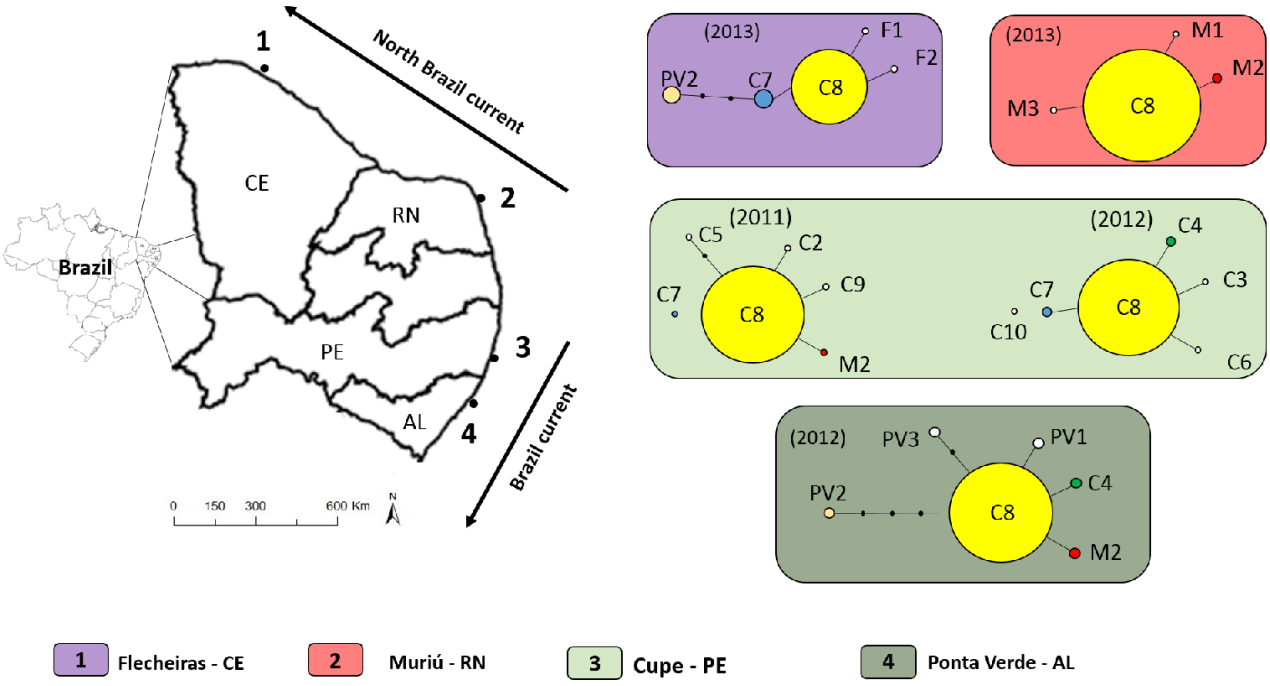

Figure 1. Location of the four sampling sites (indicated by numbers) in the northeastern region of Brazil. The direction of the two main water currents (North Brazil current and the Brazil current) is indicated with arrows. The haplotype networks of *P. gynodiporata* sp. n. in each of the four studied beaches are in boxes with colors corresponding to the sampling sites indicated by the numbers in the map: 1. Flecheiras - Ceará State (CE); 2. Muriú - Rio Grande do Norte State (RN); 3. Cupe - Pernambuco State (PE); 4. Ponta Verde - Alagoas State (AL). Haplotypes are indicated by letters (corresponding to the first letter(s) of the name of the sampling site) followed by numbers (corresponding to the order in which haplotypes were detected in this study) and the size of the circles correspond to the haplotype frequency.

4.2.2. ***Species selection***

The new species *Paracanthonchus gynodiporata* sp. n. was one of the most abundant species on the seaweed samples from the four locations. This new species was

systematically absent from all the sediment samples from all four locations in the current study and also in four other locations (Pirambu-CE; Icapuí-CE; São Sebastião-SP; Ubatuba-SC) along the Brazilian coast (Apolônio Silva De Oliveira 2016), and was selected for population genetic and phenotypic analyses.

4.2.3. ***DNA extraction, PCR and sequencing of COI, 18S and D2D3 sequences***

Because of the very high number of juveniles at different developmental stages and the paucity of adults in the four populations, we were unable to use the same individuals for morphometry and molecular analyses. Instead, individuals of a mix of adults (males and females) and juveniles of *P. gynodiporata* sp. n. from two out of five seaweed samples per beach were taken for molecular processing. No other *Paracanthonchus* species was recorded in our samples. Each individual was stored in 0.5 mL centrifuge tubes with 25 µL Worm Lysis Buffer (50 mM KCl, 10 mM Tris–HCl pH 8.3, 2.5 mM $MgCl_2$, 0.45% NP40, 0.45% Tween 20) and stored at -20 °C until DNA extraction. The samples were digested for 1 h at 65 °C and for 10 min at 95 °C with 1 µL of Proteinase K (10 mg mL^{-1}). Tubes were centrifuged at maximum speed (21 000 *g*) for 1 min and stored at -20 °C. DNA was subjected to PCR to amplify a 396 bp fragment of the cytochrome oxidase c subunit I (COI) gene with the primers JB3 (5'-TTTTTTGGGCATCCTGAGGTTTAT-3') and JB5 (5'-GCACCTAAACTTAAAACATAATGAAAATG-3') (Derycke et al. 2005). PCR was performed in 25 µL reaction mixtures and contained: 0.125 µL TOPTAQ polymerase (Qiagen), 2.5 µL of 10 X PCR buffer with 15 mM $MgCl_2$, 2.5 µL PCR coral load concentrate, 2 µL $MgCl_2$ 25 mM, 0.5 µL deoxynucleotide triphosphate (10 mM), 0.125 µL of each primer (25 µM), 1 µL DNA and 16.125 µL sterile distilled water. For COI, the thermocycling conditions were: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 50 °C

for 30 s and 72 °C for 30 s, plus a final extension step at 72 °C for 10 min. The D2D3 region of the large subunit of the nuclear ribosomal DNA was amplified with the primers D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and D3B (5′-TCCTCGGAAGGAACCAGCTACTA-3′) (Derycke et al. 2010a) with amplification starting with a denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 60 s, and a final extension period of 10 min at 72 °C. The large ribosomal subunit region was amplified using the primers G18S4 (5'-GCTTGTCTCAAAGATTAAGCC-3') and 4R (5'-GCTTGTCTCAAAGATTAAGCC-3') with thermocycling conditions of (Derycke et al. 2010a). The sequencing reaction was performed with BigDye Terminator v. 3.1 Mix (PE Applied Biosystems) and under the following conditions: an initial denaturation of 2 min at 98 °C was followed by 40 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 5 s, and extension at 60 °C for 60 s. The bidirectional sequences can be found under GenBank accession numbers KX352221 - KX352239. Haplotypes were named after the place where they occurred using the first letter(s) followed by a number which corresponds to the order in which haplotypes were recorded (e.g. Flecheiras (CE) first found haplotype = F1; Ponta Verde-CE second found haplotype = PV2).

4.2.4. ***Species description, morphometry and 3D reconstruction of the head region***

Digital pictures of eight males and twelve females of *P. gynodiporata* sp. n. from the type location Cupe (PE) mounted in permanent slides were taken at different magnifications using a light microscope (Leica DAS microscope type R) with differential interference contrast (DIC), and equipped with a Leica DFC420 camera. The entire habitus and selected body regions (head, mid-body, and tail) with important

taxonomic structures were photographed and measured using Leica Application Suite v. 3.4.1. Slides were deposited in the Royal Belgian Institute of Natural Sciences under the numbers RIT848 (holotype), RIT849 and RIT850 (Paratypes) and in the Zoology Museum at the Faculty of Sciences, Ghent University, under the numbers UGMD 104316. In addition to the type specimens, four, six and four males from Flecheiras (CE), Muriú (RN) and P. Verde (AL), respectively, were measured and 15 somatic and 6 sexual characters were used to investigate morphometric variability. Within the genus *Paracanthonchus*, there is substantial variation in stoma structure (armature) according to previous descriptions, varying from a single hollow dorsal tooth to one hollow dorsal tooth and two pairs of small ventrosublateral teeth combined or not with three cuticular ridges (Miljutina and Miljutin 2015). The stoma structure with feeding apparatus is an important character to differentiate congeneric species, though not always easy to interpret. To this end, a head section of one male individual of *P. gynodiporata* sp. n. from the type location Cupe (PE) from 2012 was mounted in a glycerol-gelatin mixture (60 g distilled water, 10 g gelatin, 70 g glycerol, and 1.4 g phenol) and observed under a light microscope. In total, 52 pictures at different optical sections of the head were taken. The pictures were used for constructing the 3D model in the software AMIRA 3.1.1. (TGS Software, San Diego, California, USA).

4.2.5. ***Data analyses***

Population genetic structure: The sequence chromatograms from the three markers COI, D2D3, and 18S were investigated with DNASTAR LASERGENE SeqMan v. 7.1.0. Sequences from the three markers were separately aligned by ClustalW. COI sequences were translated to amino acid sequences before the alignment to ensure that no stop codons would be present. P-distances and the number of variable sites

were calculated using the software MEGA 6 (Tamura et al. 2013). Genetic diversity within sampling sites was investigated by calculating nucleotide diversity (π) and haplotype diversity (h) according to (Nei 1979, Nei 1987, Excoffier and Lischer 2010). Lower genetic diversity was expected in sampling sites where seaweed was harvested because smaller population sizes lead to increased genetic drift. Population genetic structure was assessed by Analysis of MOlecular VAriance (AMOVA), using the frequencies of the COI haplotypes to calculate overall and pairwise F_{ST} (Tamura and Nei 1993). The level of population genetic structure followed Wright's division (Wright 1978): little (0.0-0.05), moderate (0.05-0.15), large (0.15-0.25) and very large (above 0.25) genetic differentiation. To investigate whether sequence evolution followed a neutral model, Tajima's D and Fu's Fs neutrality tests were performed. When both tests were significantly different from zero, a mismatch analysis was performed by comparing the frequency distribution of the pairwise sequence differences with the expected distribution based on the sudden expansion model to investigate whether the populations experienced an expansion (Rogers and Harpending 1992). This is particularly relevant to investigate effects of bottlenecks and population growth in the sampling sites that have been harvested in the past. To investigate whether the North Brazil and Brazil sea currents create a biogeographical barrier for *P. gynodiporata* sp. n., a hierarchical AMOVA was conducted by grouping Flecheiras (CE) and Muriú (RN) in a northern group under the North Brazil current and Cupe (PE) and P. Verde (AL) in a southern group under the Brazil current. The pairwise F_{ST} p-values were corrected based on the sequential Bonferroni method (Rice 1989). Additionally, we compared haplotype frequencies from 2011 and 2012 in Cupe (PE) to investigate temporal variation in population genetic structuring. All population genetic analyses were performed using the Arlequin 3.5.1.2 software (Excoffier and Lischer 2010). To

investigate evolutionary relationships and mutational differences between haplotypes, as well as the geographical distribution of haplotypes, a haplotype network was built based on the median joining algorithm implemented in NETWORK 4.6.1.4 (Bandelt et al. 1999) and edited with Microsoft PowerPoint software.

Phenotypic variability: All morphometric data analyses were performed with the software PRIMER v. 6.1.6 (Clarke and Gorley 2006). In all four locations, a high dominance of juveniles and variable but low numbers of females and males were present. Variability in morphometric characters was assessed based on males present in the samples (Flecheiras: 4; Muriú: 6; Cupe: 8; P. Verde: 4). Only from one beach (Cupe) there was a suitable number of both sexes to analyze possible sexual dimorphism (12 females and 8 males). The characters used in the analysis were chosen based on what is used in the literature to differentiate congeneric species of the genus *Paracanthonchus* (Miljutina and Miljutin 2015) (Table 2). The somatic and sexual characters (copulatory apparatus and precloacal supplements) were analyzed separately (15 somatic and 6 sexual characters described in table 2). The dataset was normalized and a dissimilarity matrix based on Euclidean distance was constructed. No transformations were performed. Nonmetric multidimensional scaling (nMDS) and one-way PERMANOVA with fixed factor location were performed. In addition, a similarity of percentages (SIMPER) analysis was performed to detect which characters contributed most to the observed differences, if any, between the different populations. If significant differences were found, the highest ranked characters were compared among populations by performing a one-way ANOVA after verification of the assumptions (Kolmogorov-Smirnov normality test, Levene's homogeneity test, XY mean and standard variation plot) to test whether the character alone is able to differentiate the populations or whether the differences were a result of a combination

of characters. Correlation between morphometric characters was investigated using STATISTICA v. 7 (Statsoft 2004).

Table 2: Somatic and sexual characters used for the morphometric analysis of *Paracanthonchus gynodiporata* sp. n. from the four studied populations along the northeastern coast of Brazil. Morphometry of the holotype (holo) and paratypes of *Paracanthonchus gynodiporata* sp. n. from Cupe-PE in the northeastern coast of Brazil. Abbreviations: L (body length); Ventr. pore dist. ant. end. (ventral pore distance from the anterior end); abd (body diameter at anus level); Amphid dist. ant. end (Amphid distance from the anterior end); cbd base pharynx (corresponding body diameter at the base of the pharynx); a (body length / body width); b (body length / pharynx length); c (body length / tail length).

	Flecheiras-CE				**Muriú-RN**				**Cupe-PE**				**P. Verde-AL**			
	Min	*Max*	*Average*	*Std*	*Min*	*Max*	*Average*	*Std*	*Min*	*Max*	*Average*	*Std*	*Min*	*Max*	*Average*	*Std*
Somatic																
L	860	952	905	42	779	1090	961	114	800	1119	1059	110	903	1248	1048	160
Pharynx length	123	138	129	7	113	144	126	10	123	152	143	9	127	156	139	13
Ventr. pore dist. ant. end	20.9	29.8	24.3	3.9	21.9	24.0	23.0	0.8	21.0	31.1	27.8	3.1	23.8	28.3	26.5	2.0
Tail length	103	128	114	11	106	132	124	10	114	133	124	6	111	131	120	9
Abd	42.6	52.0	46.5	4.0	35.4	51.0	43.6	5.1	32.2	45.5	41.9	4.2	35.7	44.7	39.3	4.0
Head diameter	21.5	22.6	22.0	0.5	21.4	33.1	27.1	4.0	21.8	25.8	24.0	1.4	21.3	23.0	22.1	0.8
Cephalic Sensilla Length	3.1	3.8	3.5	0.3	3.0	3.3	3.2	0.1	2.8	4.4	3.9	0.5	3.6	5.0	4.2	0.6
Buccal width	7.9	10.4	9.7	1.2	7.1	7.9	7.6	0.3	7.5	10.9	9.2	1.2	7.8	8.7	8.4	0.5
Buccal length	6.8	8.5	7.9	0.8	8.6	11.5	9.4	1.1	7.7	9.7	8.5	0.7	6.1	9.2	7.8	1.3
Amphid. fovea length	8.6	10.2	9.5	0.7	8.2	10.5	9.8	0.9	8.8	10.6	9.5	0.6	9.4	10.1	9.7	0.3
Amphid. fovea width	9.5	11.1	10.5	0.8	9.1	11.8	10.4	0.9	10.0	12.7	11.2	0.8	9.4	11.0	10.2	0.6

Amphid. dist. ant. end	8.6	11.3	10.4	1.2	6.5	8.7	7.9	0.8	8.8	14.2	12.0	1.7	7.8	12.4	9.7	2.1
Pharynx width base	19.6	26.3	24.0	3.1	20.5	26.7	23.2	2.6	18.3	24.6	20.8	1.9	18.3	21.1	19.7	1.3
cbd base pharynx	41.6	47.5	45.3	2.6	40.0	51.5	47.0	4.4	36.0	46.6	42.2	3.0	35.7	41.8	38.9	2.6
Body width	43.5	55.2	50.6	5.0	46.6	60.2	55.5	4.8	40.4	54.2	48.5	4.0	41.4	52.5	45.4	5.1
a	16.5	21.9	18.1	2.6	15.3	19.7	17.3	1.7	19.8	23.4	21.8	1.2	21.8	24.3	23.0	1.2
b	6.2	7.6	7.0	0.6	6.9	8.3	7.6	0.5	6.5	7.8	7.4	0.4	6.7	8.4	7.5	0.8
c	7.5	8.5	8.0	0.4	7.0	8.3	7.7	0.6	7.0	9.5	8.6	0.8	8.1	9.5	8.7	0.7
Sexual																
Spicule length	33.7	34.4	34.0	0.3	35.3	42.8	37.8	3.6	35.6	45.6	40.6	3.5	37.9	41.7	39.3	1.6
Gubernac. length	31.9	33.8	33.1	0.9	32.6	37.0	34.7	1.5	35.0	37.3	36.4	0.6	34.2	38.6	36.3	1.8
Supplement length 4	20.2	23.1	21.2	1.3	18.5	25.7	23.2	2.6	21.7	30.8	27.2	3.0	22.5	27.6	24.4	2.3
Supplement length 3	20.1	22.2	20.8	1.0	17.8	25.2	23.1	2.9	23.2	28.5	26.6	1.6	22.6	28.3	24.7	2.6
Supplement length 2	20.1	20.9	20.5	0.3	14.3	25.0	21.7	3.9	20.2	29.1	25.9	2.7	22.4	26.2	24.3	1.7
Supplement length 1	18.3	20.4	19.5	1.0	14.5	22.8	20.0	2.9	16.8	24.8	23.0	2.6	21.3	24.9	23.1	2.0

4.3. Results

4.3.1. ***Population genetic structure***

In total, 27 (Flecheiras (CE)), 24 (Muriú (RN)), 25 (Cupe (PE 2011)), 33 (Cupe (PE 2012)) and 20 (P. Verde (AL)) individuals from *P. gynodiporata* n. sp. yielded good COI sequences (396 bp sequences and alignment with 19 variable sites). The best blastn identity for all COI sequences was *Paracanthonchus* sp. (FN998914.1, identity 87% - 88%, query cover 59%). In total, 17 COI haplotypes were found, with haplotype C8 being the most abundant in all four locations (average 77% ± 8). Only four other haplotypes were shared between at least two beaches, while the remaining 12 haplotypes were restricted to a single location and occurred at low frequencies (Fig. 1 and 2). The p-distances ranged from 0.003 to 0.015 and number of differences ranged from 1 to 6 base pairs. The haplotype network further revealed a low number of mutations between haplotypes and a star-shaped pattern with no geographical clustering of haplotypes (Fig. 2). The AMOVA analysis revealed a little but statistically significant genetic structuring (F_{ST}= 0.05204; p= 0.00391). The pairwise analysis showed moderate separation between the populations of Flecheiras (CE) and Muriú (RN), and between Flecheiras (CE) and Cupe (PE) 2012 (Table 3). Within Cupe (PE), no significant temporal variation in haplotype composition was observed between 2011 and 2012 (F_{ST}= 0.00091; p= 0.38739).

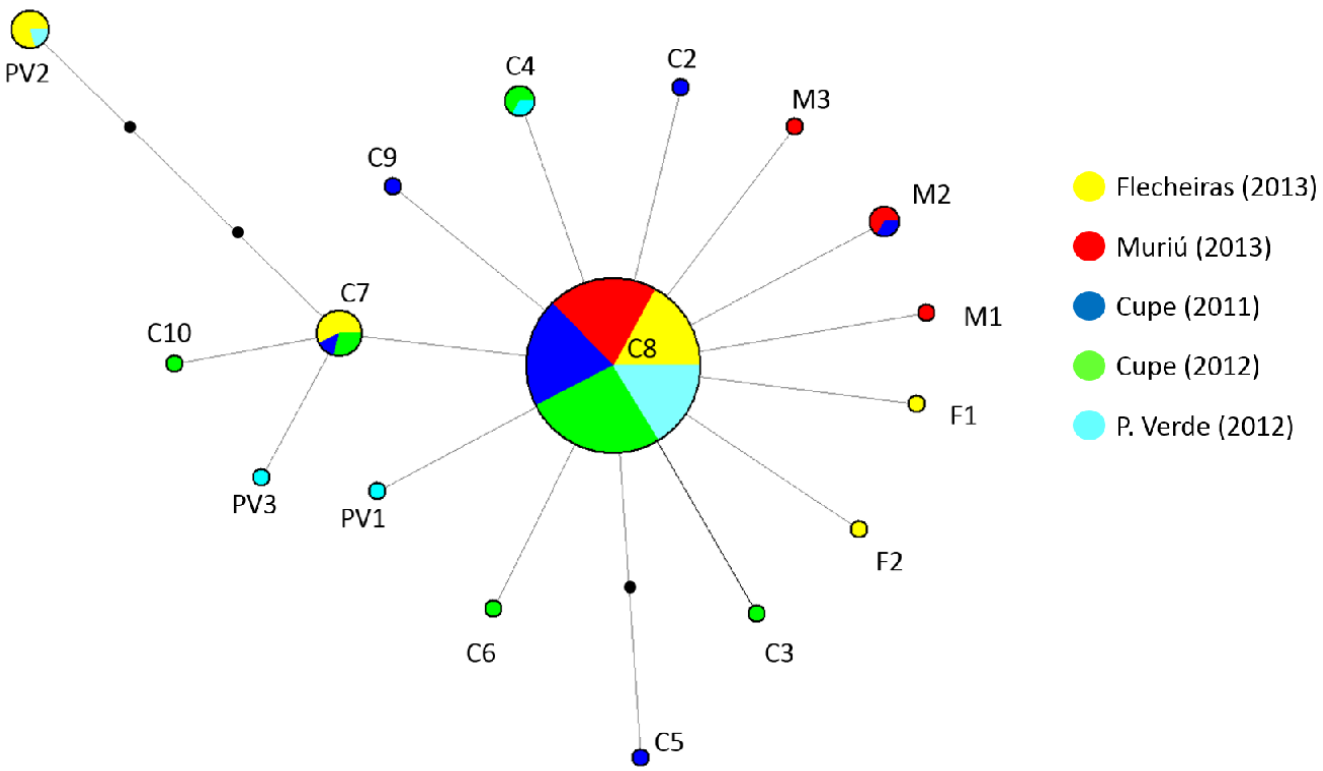

Figure 2. Overall haplotype network of *P. gynodiporata* n. sp from the four studied beaches. The size of the circles correspond to the haplotype frequency in the total dataset. Beaches are represented by different colors.

Haplotype networks for each location showed the same pattern as the overall network, with one dominant haplotype and a low number of rare haplotypes with few mutations between them. Genetic diversity appeared to be higher in Flecheiras (CE) Beach (*h*= 0.5783 ± 0.0961), where the seaweed bed was considered to be most impacted because of historical and ongoing seaweed exploitation; however, the standard deviation overlapped with those of the diversity estimates observed in the other locations except Muriú (RN) (Table 1). Tajima's *D* and Fu's *Fs* neutrality test statistics were negative and significantly different from zero for the beaches Muriú (RN) and Cupe (PE - 2011 and 2012) (Table 1), and point to recent expansion or purifying

selection. The mismatch distribution analyses were unimodal and fitted the sudden expansion model, indicating that those populations experienced a recent sudden expansion (Fig. 3 Muriú – RN: *SSD* = 0.00801, *p*= 0.409, Raggedness = 0.23591, *p*= 0.641; Cupe – PE, 2011 and 2012: *SSD* = 0.00007, *p*= 0.872, Raggedness = 0.17240, *p*= 0.745 and *SSD* = 0.00003, *p*= 0.95730, Raggedness = 0.15770, *p*= 0.563). A significant but little genetic structure was observed (F_{ST}= 0.05204; *p*= 0.00391 ±0.00185) between the northern group under the influence of the north Brazil current (Flecheiras (CE)) and Muriú (RN)) and the southern group under the influence of the Brazil current (Cupe (PE - 2011, 2012) and P. Verde (AL)), indicating that the split of the South Equatorial Current along the Brazilian coast imposes only a weak biogeographical barrier for the *P. gynodiporata* sp. n. populations. The D2D3 (747 bp; sequences: 4 in Cupe (PE) and 17 in P. Verde (AL)) and 18S (914 bp; sequences: 8 in Cupe (PE) and 19 in P. Verde (AL)) sequences were identical. The best blastn identity for D2D3 sequences was *Paracanthonchus* sp. (KX270432.1, identity 82%, query cover 100%) while for 18S it was *Paracyatholaimus intermedius* (AJ966495.1, identity 94%, query cover 98%).

Table 3: Pairwise F_{ST} values between the four populations of *P. gynodiporata* sp. n. in Flecheiras (CE), Muriú (RN), Cupe (PE) and P. Verde (AL). Signifcant F_{ST} values after Bonferonni correction are indicated in bold. Negative values were converted to zero.

	Flecheiras	Muriú	Cupe 11	Cupe 12	P. Verde
Flecheiras					
Muriú	**0.13125**				
Cupe 11	0.10175	0			
Cupe 12	**0.09177**	0.02486	0.00091		
P. Verde	0.02502	0.020	0	0	

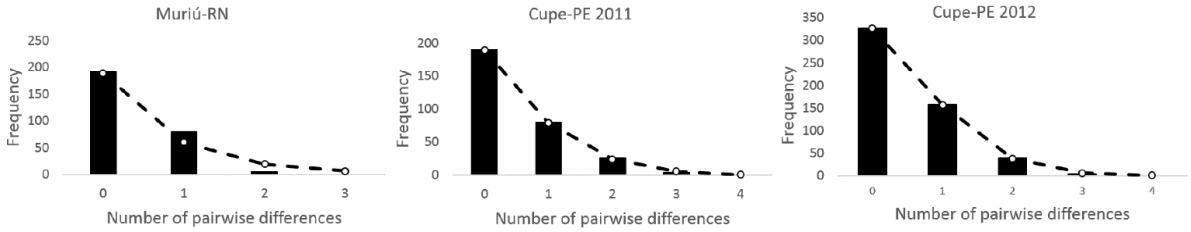

Figure 3. Mismatch distribution of the pairwise differences of the haplotypes occurring in Muriú (RN) 2013, Cupe (PE) 2011 and Cupe - PE 2012. Muriú - RN (SSD = 0.00801, p= 0.409; Raggedness = 0.23591, p= 0.641) and Cupe - PE, 2011 and 2012 (SSD = 0.00007, p= 0.872; Raggedness = 0.17240, p= 0.745 - SSD = 0.00003, p= 0.95730; Raggedness = 0.15770, p= 0.563).

4.3.2. ***Phenotypic variability***

Morphometric variation among males from different populations was significant (PERMANOVA: Pseudo-*F*= 4.7107; *p*< 0.001; Fig. 4). However, significant non-overlapping measurements were restricted to three characters. Buccal cavity width

(one-way ANOVA: *F*= 5.657; *p*= 0.006), distance of the amphidial fovea from anterior end (one-way ANOVA: *F*= 8,5366; *p*< 0.001), and cephalic setae length (one-way ANOVA: *F*= 7,048; *p*= 0.002) were significantly different and non-overlapping between Flecheiras (CE) and Muriú (RN); Muriú (RN) and Cupe (PE); and Muriú (RN) and P. Verde (SIMPER and Tukey pairwise comparison). In contrast, specimens between Flecheiras (CE) and P. Verde (AL) and between Cupe (PE) and P. Verde (AL) did not differ significantly. With respect to sexual characters, substantial variability was observed in the precloacal supplement length (PERMANOVA: Pseudo-*F*= 4,4825; *p*= 0.002). The anteriormost precloacal supplement (SP4) was the top ranked character that contributed to the differences between populations: SP4 of individuals from Cupe was longer than that of individuals from Flecheiras (SIMPER: Cupe (PE) x Flecheiras (CE) = 25.65% contribution). In addition, the posterior most precloacal supplement (SP1) of individuals from P. Verde (AL) was longer than the one of individuals from Flecheiras (CE) (SIMPER: P. Verde (AL) x Flecheiras (CE) = 23,03% contribution), and SP3 of the individuals from Cupe (PE) was longer than the one of specimens from Muriú (SIMPER: Cupe (PE) x Muriú (RN)= 30% contribution). Spicule and gubernaculum did not differ significantly among populations. Because of considerable overlap, no single sexual character by itself could distinguish individuals of one location from those of other locations. The character measurements are presented in table 2.

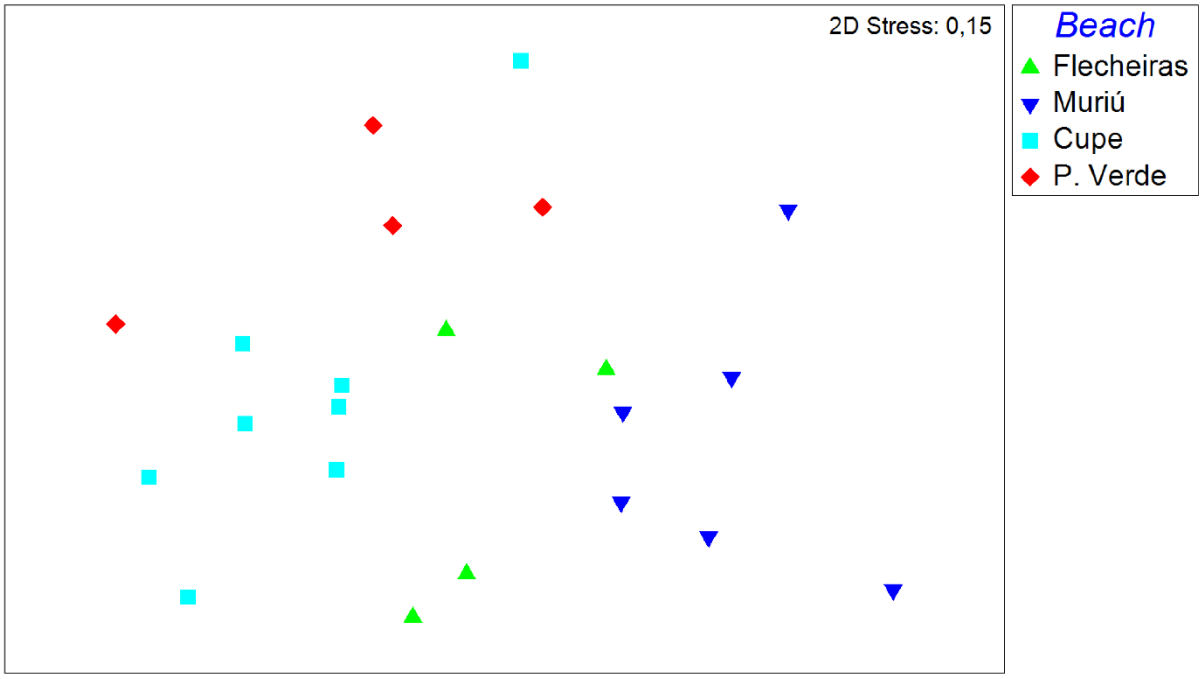

Figure 4. Non-metric multidimensional scaling of the somatic characters among the individuals of *P. gynodiporata* sp n. populations of Flecheiras - Ceará State (CE), Muriú - Rio Grande do Norte State (RN), Cupe - Pernambuco State (PE) and Ponta Verde - Alagoas State (AL) along the Brazilian coast. Used characters: body length, pharynx length, distance of ventral pore to anterior end, tail length, anal body diameter, head diameter, cephalic sensilla length, buccal width, buccal length, amphidial fovea, length, amphidial fovea width, distance of amphidial fovea to anterior end, width at the base of the pharynx, corresponding body diameter at the base of the pharynx, body width, body length divided by the width, body length divided by pharynx length, body length divided by tail length, spicule length, gubernaculum length, length of the 4th, 3rd, 2nd and 1st precloacal supplements.

4.3.3. ***Species description***

Genus *Paracanthonchus* Bastian, 1865

Paracanthonchus gynodiporata sp. n. (Fig. 5 – 8, Table 4)

Holotype. Male (Fig. 5).

Paratypes. 5 males, 5 females, 3 juveniles at fourth stage (two molting specimens, one to male, one to female).

Type locality. Brazil, Pernambuco State, Cupe Beach (8°27'29''S 34°58'58''W), subtidal zone, associated with brown seaweed *Sargassum polyceratium*

Other Localities. Praia de Flecheiras, Trairí – Ceará – Brazil (3°13'08''S 39°16'18''W); Praia de Muriú, Ceará-Mirim – Rio Grande do Norte – Brazil (5°33'43''S 35°14'21''W), Praia de Ponta Verde, Maceió – Alagoas – Brazil (9°39'55''S 35°41'54''W)

Sequences from type location:

Type Haplotypes: COI – KX352225 (C2), KX352226 (C3), KX352227 (C4), KX352228 (C5), KX352229 (C6), KX352230 (C7), KX352231 (C8), KX352232 (C9), KX352233 (C10), KX352235 (M2).

Type Genotypes: 18S - KX352221; D2D3 - KX352222

Life Science Identifier (LSID): urn:lsid:zoobank.org:pub:B6FBE6B8-B482-4206-A9D3-842A7014A505

Etymology: The species name refers to the pre- and post-advulvar body pores in the female (Fig. 6).

The number of sequences available for the genus *Paracanthonchus* is very restricted in GenBank.

COI (396 bp): 317 conserved and 76 variable sites compared to *Paracanthonchus* sp. (FN998914.1)

18S (914 bp): 848 conserved and 61 variable sites compared to *P. caecus* (AF047888.1)

D2D3 (747 bp): 532 conserved and 127 variable sites compared to *Paracanthonchus* sp. (KJ638031.1)

4.3.3.1. Diagnosis and relationships

Body medium-sized (779.3 – 1058.5 µm), largely cylindrical with rounded truncated head with conical tail; body cuticle with transverse rows of fine dots, slightly larger at level of lateral field, more visible posterior to the neck region; amphidial fovea ventrally spiral, smaller in females with 3.5 turns and 4 turns in males. Buccal cavity with a small dorsal tooth and two pairs of minute ventrosublateral teeth. Spicules paired and slightly ventrally bent, 34 – 46 µm long, gubernaculum with double apophyses and complex crura ridge dorsally with large thorn and lateral protuberances; four large well sclerotized tubiform precloacal supplements; and two short weekly developed tubiform supplements with similar structure between SP1 and cloacal opening. Females with vagina flanked by a pre- and post-vulvar body pore. *Paracanthonchus gynodiporata* sp. n. appears morphologically similar to *P. perspicuus* Kito, 1981 by the presence of a small dorsal tooth, overall spicule shape and gubernaculum structure with a crura ridge with dorsal thorn. However, *P. gynodiporata* sp. n. can be distinguished from *P. perspicuus* by the smaller body length (779 – 1120 µm vs 1269 – 1287 µm), presence

of ventrosublateral teeth vs absence in the latter, the presence of only one instead of two crura dorsal thorns, and the presence of two weakly developed precloacal supplements instead of one weakly developed precloacal supplement near the cloacal opening in *P. perspicuus*. Finally, the presence of the pre- and post-advulvar body pores observed in *P. gynodiporata* sp. n. has not been reported in any other species in the literature.

4.3.3.2. Description

Male (*holotype*)

Body largely cylindrical, slightly narrowing in anterior neck region but more pronounced in conical tail. Punctated cuticular ornamentation, consisting of transverse rows of dots, forming the tip of inner cuticular struts; at the level of the lateral field punctation slightly larger though hardly differentiated in the neck region. Eight longitudinal rows of body pores, at mid body the largest pores bordering the lateral field. Somatic setae arranged in four sublateral longitudinal rows; setae longest (6 µm) and most numerous in neck region. Head region anteriorly rounded and truncated; lip region with six separate lips. Anterior sensilla arranged in two crowns: an anterior crown of six inner labial papillae and an outer crown of six external labial setae (4.5 µm) and four slightly longer cephalic setae (3.7 µm); both types of setae bipartite with open tip. Amphidial fovea spiral (4 turns), ventrally wound and surrounded by punctation. No ocelli present. Buccal cavity with cheilostome reinforced by 12 cheilorhabdia and wide cup-shaped; pharyngostome short funnel-shaped with a small well developed dorsal tooth and two pairs of minute ventrosublateral teeth. Pharynx largely cylindrical, just posterior mid-way surrounded by the nerve ring. Outlet of a pair of posterior ventrosublateral pharyngeal glands at

the level of the nerve ring; outlet of dorsal gland far anteriorly. Cardia surrounded by intestinal cells and apparently with associated glands; intestine usually with diatoms visible in its lumen. Secretory-excretory pore at short distance from anterior end (26 µm); short outlet sclerotized, swollen anterior ampulla; ventral gland at level of anterior intestine. Tail conical with three well developed caudal glands and nucleus, anteriorly extending along rectum. Spinneret well developed.

Male reproductive system diorchic, anterior testis outstretched on the right side of the intestine, posterior one reflexed and lying on the left side of the intestine; sperm cells small globular (1.5 µm); vas deferens surrounded by muscular sheath and showing differentiation in granulation; spicules paired, slightly ventrally bent, strongly sclerotized with capitulum narrower than blade; blade about equally wide but tapered distally. Gubernaculum, strongly sclerotized and complex structure, composed proximally of a pair of (slightly twisted) apophyses with narrower tip, and crura (wider distal part) embracing retracted spicules, dorsal wall of the crura provided with dentate ridge with one larger thorn and laterally pointed protuberance visible in ventral view. Four oblique anteriorly orientated large, well sclerotized mid-ventral tubiform precloacal supplements; the posteriormost one (SP1) at about 30 µm from cloacal opening; each supplement with a central sensillar canal surrounded by a cuticular wall. In between SP1 and cloacal opening, two short, weakly developed tubiform supplements with similar structure (Fig. 7).

Females

General appearance (body shape, cuticular ornamentation), digestive and secretory-excretory system as in male. Head region similar but smaller (but slightly narrower i.e. about 24% of corresponding body diameter in females and 36% in males), fovea with 3.5 turns. Reproductive system didelphic-amphidelphic with antidromously reflexed

ovaries, anterior ovary right of the intestine, posterior on the opposite side; uteri with up to three developed oocytes observed in both uteri together; well-developed muscles at level of ovejector. Vagina, rather short, surrounded by vaginal constrictor muscles; vulva at mid-body and flanked by a pre- and post-advulvar body pore. No sperm observed.

4.3.3.3. Remarks

The most important characters to distinguish species in the genus *Paracanthonchus* are the number of teeth in the buccal cavity (Fig. 8) and the number of precloacal supplements [53]. The presence of ventrosublateral teeth is quite variable and was not mentioned in the species descriptions before the 1950's; it is not clear if teeth have been overlooked or not. The number of precloacal supplements is an easier character to observe and supposedly more reliable. However, the presence and number of minute precloacal supplements near the cloacal opening is an object of discussion.

Table 4: Morphometry of the holotype (holo) and paratypes of *Paracanthonchus gynodiporata* sp. n. from Cupe-PE in the northeastern coast of Brazil. Abbreviations: L (body length); Ventr. pore dist. ant. end. (ventral pore distance from the anterior end); abd (body diameter at anus level); Amphid dist. ant. end (Amphid distance from the anterior end); cbd base pharynx (corresponding body diameter at the base of the pharynx); a (body length / body width); b (body length / pharynx length); c (body length / tail length). The codes RIT848, RIT849, RIT850 and UGMD 104316 correspond to one slide each.

	RIT848				**RIT849**			**RIT850**			**UGMD 104316**		
	Male (Holo)	*Female (NG)*	*J4 stage*	*Male*	*Female (NG)*	*J3 stage*	*Male*	*Male*	*Female (G)*	*Female (G)*	*Male*	*Female (G)*	*J3 stage*
L	1099	1238	796	1072	1001	819	1116	1146	1184	1145	1060	1075	500.83
Distance vulva anterior end	n/a	500	373	n/a	466	n/a	n/a	n/a	521	552	n/a	488	n/a
Pharynx length	151	156	132	144	154	131	147	162	172	172	151	160	97
Ventr. pore. Dist. ant. end	26.0	24.3	n/a	26.6	33.1	27.9	30.3	30.6	29.5	32.7	27	26	28.3
Tail length	130	126	107	120	120	115	126	130	132	132	121	121	76
abd	40	39.1	34.3	38.6	36.2	37.8	41.1	43.4	39	39.9	40.6	37.7	24.9
Head diameter	22.4	22.6	24.9	21.4	22.1	22.9	21.5	22.9	24.5	24.2	20.5	25.9	15.3
Sensila Length	4.5	3.5	3	3.7	4.7	2.5	3.7	4.6	4.9	4.4	2.9	4.2	2.3
Buccal width	9.7	10.8	n/a	10.4	11.7	n/a	9.6	8.9	10.6	11.1	9.8	10.8	6.8
Buccal length	8.5	8.7	n/a	8.9	10	n/a	8.5	9.6	9.9	10.8	9.5	10.3	6.6
Amphid. fovea length	10	7.3	n/a	9.8	6.7	n/a	9.5	9.2	6.9	7.1	9.3	7.4	4.
Amphid. fovea width	10	7.4	6.2	10.5	8.1	n/a	10.7	9.6	8.3	8.6	9.2	7.8	4.8
Amphid. dist. Ant. end	11.5	11.5	n/a	11.2	12	n/a	11.3	11.4	11.5	1188	12.9	12.3	9.1
Pharynx width base	21	24.5	21.9	19.9	25.5	18.6	19.5	23.4	28.7	27.5	18.9	26.9	15.9
cbd base pharynx	40	46.1	40.6	42.0	47.4	38.8	39.9	47.1	52.80	48.6	40.9	46.6	29.5

Pre-vulvar body pore	n/a	16.5	14.8	n/a	16.2	n/a	n/a	n/a	16.5	17.2	n/a	15.6	n/a
Post-vulvar body pore	n/a	15.5	12.6	n/a	15.6	n/a	n/a	n/a	16.8	16.3	n/a	16.1	n/a
Body width	40	55.9	46	46.8	58.5	41.4	48	n/a	65.90	63.6	50.4	58.2	30.8
Spicule length	40	n/a	n/a	38.9	n/a	n/a	39.2	39.5	n/a	n/a	42.1	n/a	n/a
Gubernac. length	34.6	n/a	n/a	36.1	n/a	n/a	35.9	40.8	n/a	n/a	34.5	n/a	n/a
Supplement length 4	21.5	n/a	n/a	21.7	n/a	n/a	23.2	22.5	n/a	n/a	23.7	n/a	n/a
Supplement length 3	25.4	n/a	n/a	24.9	n/a	n/a	25.2	23.2	n/a	n/a	24.3	n/a	n/a
Supplement length 2	24.6	n/a	n/a	25.4	n/a	n/a	25.1	24.4	n/a	n/a	24.8	n/a	n/a
Supplement length 1	26	n/a	n/a	25.8	n/a	n/a	25.7	25.5	n/a	n/a	25.8	n/a	n/a

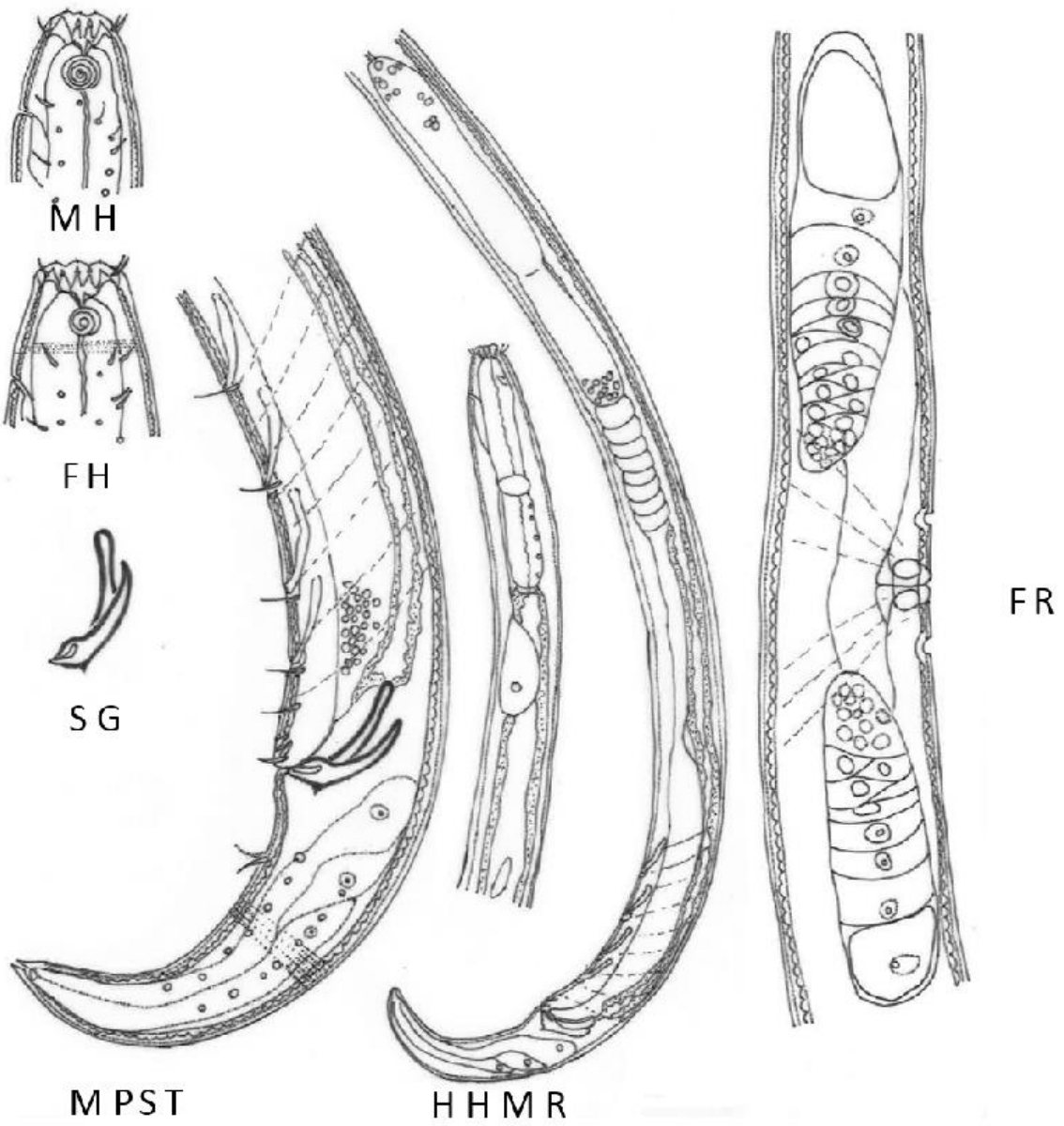

Figure 5. *Paracanthonchus gynodiporata* sp. n. Abbreviations: MH - Head region male holotype; FH - head region female paratype showing sexual dimorphism in amphidial fovea; SG - detail of holotype spicule and gubernaculum; MPST - male holotype posterior region with precloacal supplements and tail; HHMR - holotype habitus with male reproductive system; FR - female paratype reproductive system.

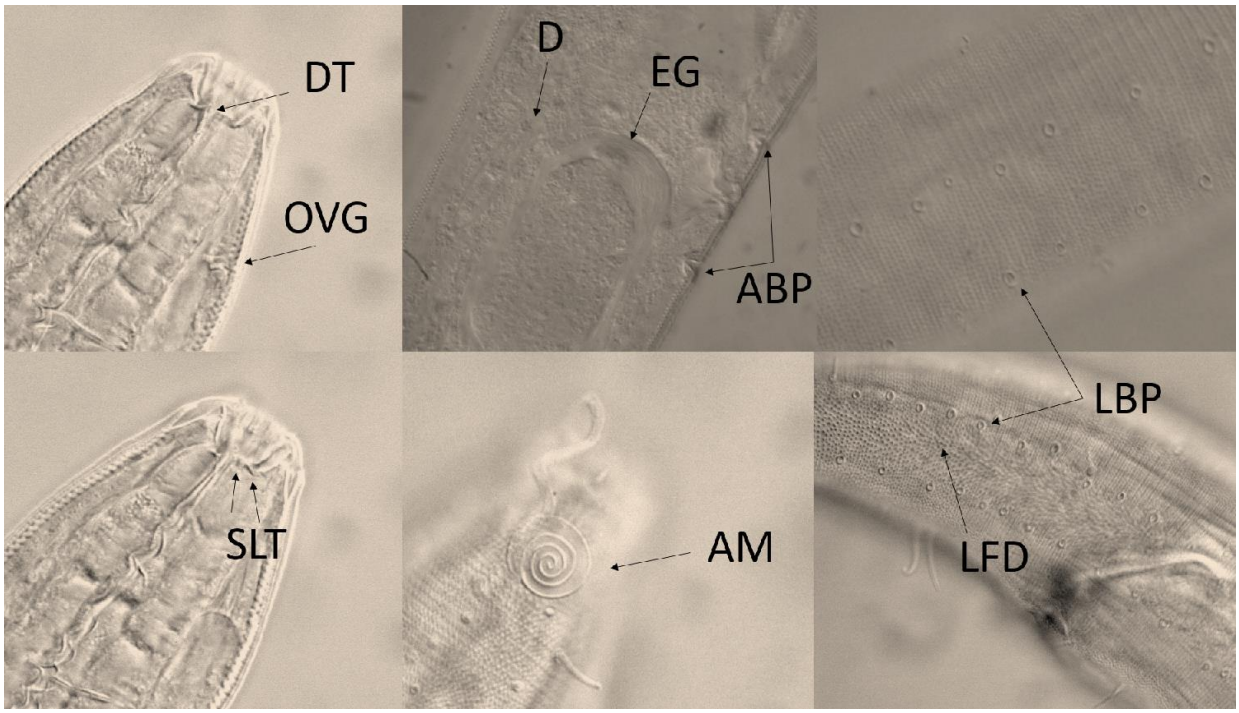

Figure 6. *Paracanthonchus gynodiporata* sp. n. Abbreviations: DT - dorsal hollow tooth; OVG - sclerotized outlet of the ventral gland; SLT - ventrosublateral teeth; D - detail of diatom in the intestine; EG - egg in the uterus; ABP - pre and post-advulval body pores; AM - male amphidial fovea; LBP - longitudinal rows of large body pores bordering the lateral field; LFD - lateral field and differentiation.

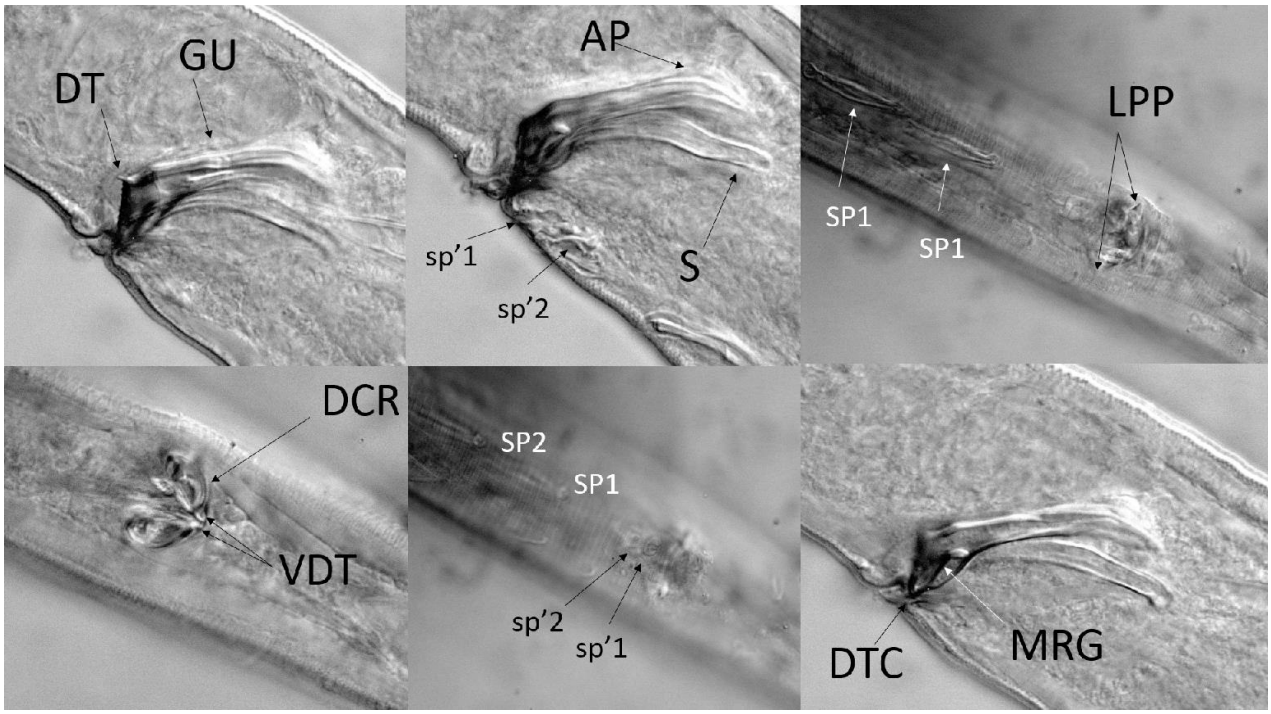

Figure 7. *Paracanthonchus gynodiporata* sp. n. Abbreviations: DT - Gubernaculum with dorsal thorn of crura; GU - gubernaculum; DCR - dorsal crura ridge; VDT - ventral view of the dorsal thorns; AP - gubernaculum apophysis; S - spicule; LPP - lateral pointed protuberance; DTC - gubernaculum showing distal thorn of crura; MRG - mid-rib gubernaculum; SP - precloacal supplements; sp' minute precloacal supplements.

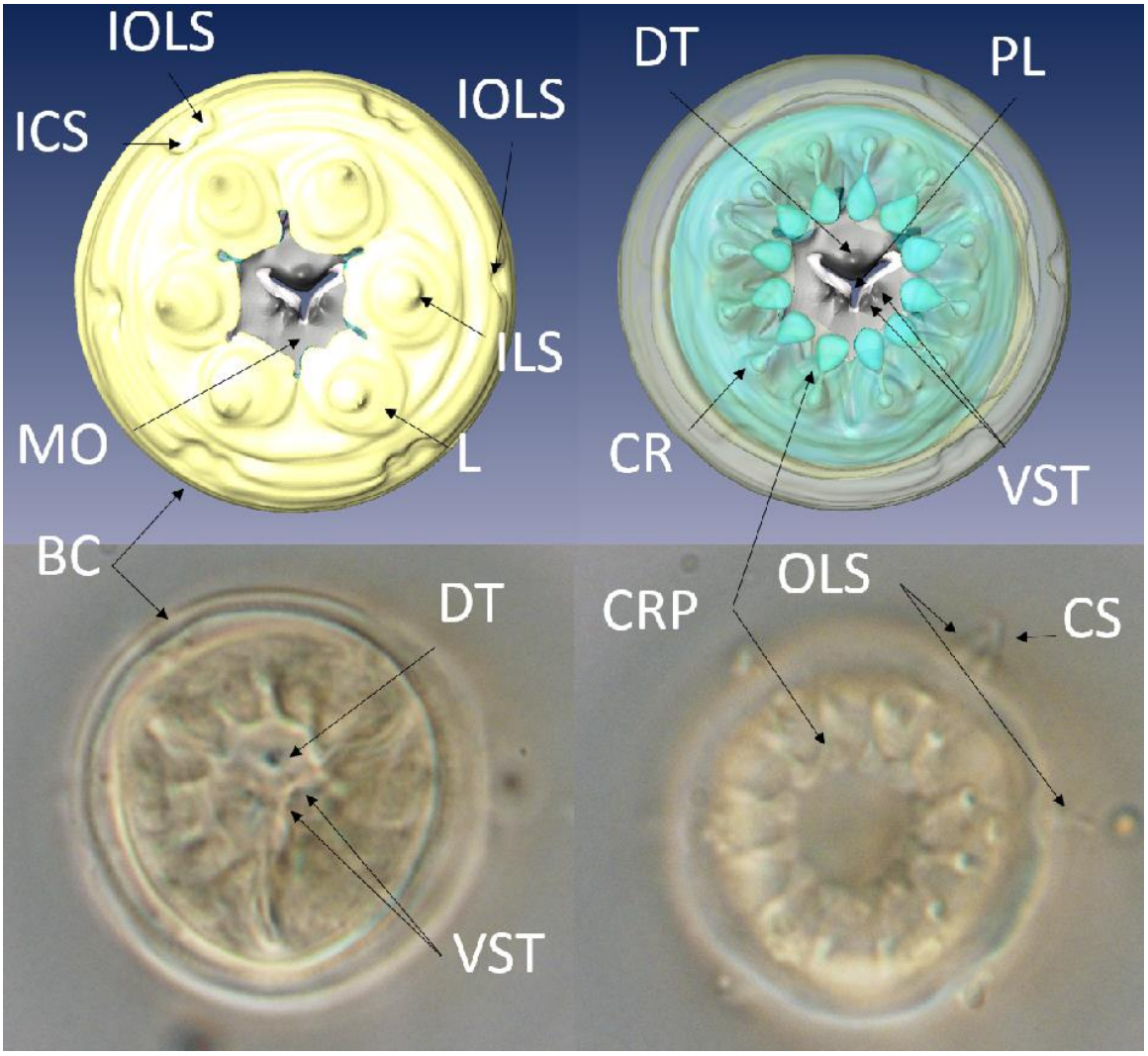

Figure 8. *Paracanthonchus gynodiporata* sp. n. Detailed reconstruction of the head region showing the mouth armature indicating the number and position of teeth, especially the ventrosublateral, which are variable and an important feature in the literature. The numbers between brackets correspond to the number of the referred structure when different from 1. Abbreviations: ICS - Insertion of the cephalic setae (4); IOLS - insertion of the outer labial setae (6) which is at the same level of the outer labial setae composing a single circle with 10 sensilla (4+6); ILS - inner labial sensilla (6); MO - mouth opening; L - labium (6); DT - dorsal hollow tooth; VST - ventrosublateral teeth; PL - pharynx lumen; BC - body cuticle; CR - cheilorhabdia (12); CRP - prolongation of the cheilorhabdia beneath labial cuticle (12); OLS - outer labial setae; CS - cephalic setae.

4.4. Discussion

4.4.1. ***Paracanthonchus gynodiporata sp. n. associated with seaweeds shows little population genetic structuring across large geographical distances.***

Our F_{ST} values show little overall population genetic structure (F_{ST}= 0.05388). This result was remarkable considering that nematodes in general lack planktonic larvae and dispersal is likely to be limited (Derycke et al. 2007a). Previous population genetic studies showed that nematodes associated with seaweeds have moderate to very large genetic structuring as observed in a species of *Halomonhystera disjunta* species complex in the region of the Westerschelde estuary (GD3, Φ_{ST} = 0.11 – 0.13, p< 0.001) (Derycke et al. 2013), in species of *Litoditis marina* species complex in the North Sea along the Belgian and Dutch coast (Pm I, Φ_{ST} = 0.22, p< 0.001), from the Bay of Biscay to the Baltic Sea (Pm II, Φ_{ST} =0.37, p< 0.001), and at transatlantic distances (Pm III, Φ_{ST} = 0.19, p< 0.001) (Derycke et al. 2008b). Large genetic structuring is expected for those two species complexes, because they have very short generation times and high reproductive output (Derycke et al. 2005, Derycke et al. 2008b). However, very large genetic structuring has also been observed for other seaweed-nematode species, with presumably long generation times and low reproductive output such as for species of the *Thoracostoma trachygaster* species complex (Clade II, Φ_{ST} = 0.28, p< 0.001) (Derycke et al. 2010a).

The northern most population, Flecheiras, was differentiated from Muriú (RN) and Cupe. The latter two populations had significant negative neutrality tests and fitted the recent expansion model, suggesting that the differentiation with Flecheiras may be caused by a rapid expansion of the latter two populations. In addition, Flecheiras was the closest population to the border between the northeastern Brazil province and the Amazon province (Spalding et al. 2007, Boehm et al. 2013). The proximity of this

location to the Amazon river may affect the genetic diversity of the northernmost population, but this remains very speculative. All four locations were dominated by the same nematode haplotype (C8) and showed very similar haplotype networks, with a small number of rare haplotypes and only very few mutations between them. This suggests that all four populations are evolutionary quite young. No geographical structuring was present in the overall network, and together with the weak genetic structuring observed in AMOVA, this suggests that these populations have been well connected. Alternatively, balancing selection may be responsible for the high dominance of the C8 haplotype and the generally low diversity in these populations. Generally, mitochondrial DNA is considered a neutral marker, but it has already been demonstrated that it may be under selection (Ballard et al. 2007). This would require that selection for the C8 haplotype happened independently in each of the four populations and that not enough time has passed to accumulate new mutations. A second alternative may be the lack of mutation-drift equilibrium, which can also lead to low overall F_{st} values despite a lack of ongoing gene flow (Hartl 1988, Hellberg 2009). Especially for low-dispersal species with high effective population sizes, such as marine nematodes, time to reach a mutation-drift equilibrium may take thousands of generations, which may not be achieved in habitats with strong colonization-extinction dynamics such as macro-algal beds.

Although data on the age of seaweed beds in the northeast of Brazil is extremely limited, seaweed beds have a close relationship with coral reefs because many seaweed species need hard substrate to attach and develop (Biber 2007). Reef ecosystems in the northeastern coast of Brazil are estimated to have originated around 7 Myr ago, i.e. between the late Miocene and early Pliocene (Leãoa et al. 2003), and have been under the influence of sea-level fluctuations during the Pleistocene (Barreto

et al. 2002). However, to what extent those sea-level fluctuations affected the genetic connectivity among populations of marine small metazoans along the northeastern coast of Brazil remains unclear. About 2.7 Myr ago, the Central America Seaway was still open, allowing the Pacific Upper Ocean water to flow towards the Atlantic causing the North Brazil current to flow SE, which is the opposite direction observed today (Heinrich and Zonneveld 2013). In this way, the sea current along the northeastern coast of Brazil was continuous and allowed passive dispersal from north to south via rafting on algae. This could explain the presence of similar haplotypes in all populations observed today. The populations of Muriú (RN) beach showed the highest F_{ST} value in the pairwise comparison with Flecheiras (CE) (F_{ST}= 0.13125; $p < 0.001$). However, the F_{ST} values appear to decrease with distance and become insignificant. Such chaotic patterns are not uncommon in marine environments, and adding local environmental data might shed light to understand this apparent chaos (Selkoe et al. 2010).

The two main currents at the Brazilian northeastern coast did not appear to constitute a strong physical barrier for *P. gynodiporata* sp. n. as observed by the weak genetic structure between those two regions (F_{ST}= 0.05204). Our sampling area covered only one biogeographical province, the Northeastern Brazil province. Provinces are classified upon a hierarchical system based on taxonomic configurations, influenced by evolutionary history, patterns of dispersal, and isolation (Spalding et al. 2007). The lack of large population genetic structure among the *P. gynodiporata* sp. n. agrees well with the above mentioned biogeographical province. In all, our data point to a very low genetic differentiation across a large geographic area suggesting that *P. gynodiporata* sp. n. has performed long-distance dispersal during some time along its evolutionary history. Since the studied species has not been found in the sediment so far (Apolônio

Silva De Oliveira 2016), drifting seaweeds are known to be used as a dispersal mechanism for diverse marine organisms (Ingólfsson 1995, Arroyo et al. 2006) including nematodes (Thiel and Gutow 2005). However this kind of dispersal is limited by the direction of the carrying current and does not fully explain the lack of large genetic structure between locations under the influence of opposite currents in our study. The diverging force of the water currents in this area has to be overcome if mutation-drift equilibrium is present and ongoing gene flow is the major homogenizing force. Nematodes are able to colonize hard artificial substrata (Atilla et al. 2003, Fonseca et al. 2008), for example turtle shells (Corrêa et al. 2014), and might thus hitchhike on sea turtles when they forage between seaweed beds (Bjorndal 1985). *Paracanthonchus* is a frequent genus found associated with turtles (Corrêa 2012) and could possibly feed on epibiont diatoms growing on the turtle shell (Majewska et al. 2015). Personal recent observations in an *Acanthonchus* nematode species associated with the sea turtle *Eretmochelys imbricata* have shown the presence of an identical mitochondrial DNA haplotype in two beaches more than 900 km apart. Whether the amount of nematodes using this particular dispersal mechanism would be sufficient to establish a population in the new patch remains unclear, as priority effects may hamper the establishment of newly arriving individuals (Derycke et al. 2007c).

4.4.2. ***Historical exploitation of the natural seaweed bed does not affect the haplotype frequencies of associated nematode populations***

Colonization dynamics can strongly impact the mitochondrial haplotype diversity over time (Derycke et al. 2006, Derycke et al. 2007c). Yet, no variation was observed in genetic composition in Cupe (PE) between years. Moreover, the beach with the highest genetic diversity was the one where historical exploitation of the natural seaweed bed

was prominent (Flecheiras (CE)). Commercial seaweed exploitation has gradually disappeared since the 1970s in Brazil and has been replaced from 2002 onwards by seaweed cultivation. It has been argued that seaweed cultivation may increase biological diversity, attracting marine life by creating a harbour where marine species can find shelter and food (Bergman et al. 2001). In Flecheiras, the fishermen stopped the seaweed extraction from the natural bed and started seaweed cultivation in 2003 which persists until today (Rocha 2013). Yet, all four populations presented a very similar haplotype diversity and no evidence of founder effects, bottlenecks or genetic drift in *P. gynodiporata* sp. n. was found. Considering that seaweeds were harvested monthly during the peak production period in the 1980's in the northeastern coast of Brazil (Rocha 2013), the seaweed beds in those regions could also be considered as an ephemeral substrate with a dynamic recolonization rate. It seems the effect of seaweed harvesting on the nematode population is limited: if the population was affected at all, it was able to fully regain its genetic diversity in the 11 years after the harvesting stopped. The source population to re-establish genetic diversity probably came from other seaweed beds since *P. gynodiporata* sp. n. has not been observed in the sediment in the studied locations nor in four other locations along the Brazilian coast (Pirambu (CE), Icapuí (CE), São Sebastião (SP) and Ubatuba (SC)) (Apolônio Silva De Oliveira 2016). Also, historical seaweed exploitation did not lead to genetic changes in nematode haplotype frequencies. This may be caused by the presence of large population sizes, or by substantial gene flow to prevent population genetic structuring even over very large distances (≈1080 km).

4.4.3. ***Nematodes can show considerable phenotypic variation among populations potentially biasing species description***

There are no synapomorphies at subfamily and genus level within the family Cyatholaimidae (Lorenzen 1994). The subfamily Paracanthonchinae shows variation in e.g. lateral differentiation (present or absent) and precloacal supplements (rarely absent). Likewise, within the subfamily, the genus *Paracanthonchus* shares the presence of tubular precloacal supplements with *Acanthonchus,* differing from the latter by the presence of lateral differentiation in cuticular punctation, but no synapomorphy is observed (Miljutina and Miljutin 2015). At species level within *Paracanthonchus*, the difficulty in interpretation of the stomatal armature, especially with respect to the presence and number of ventrosublateral teeth, the number of precloacal supplements, the structure of the gubernaculum as well as the interspecific overlap of morphometric features such as body length and spicule length, complicate species differentiation based on light microscopic observations. This also hampers comparison with older descriptions, in which some features were overlooked or misinterpreted (Decraemer and Backeljau 2015). In Brazil, there are three described species, *P. batidus* (Gerlach 1957), *P. digitatus* (Gerlach 1957) and *P. cochlearis* (Gerlach 1957), which can be distinguished from the new species by the number of precloacal supplements (5 in *P. batidus*; 4 in *P. digitatus*; 4 + 2 *P. gynodiporata* n. sp.) and the number of turns of the amphidial fovea (6 *P. cochlearis*; *P. gynodiporata* n. sp. 4). Substantial overlap exists for other characters (e.g. body length). One possible example of phenotype misinterpretation concerning diagnostic characters is the poorly developed precloacal supplement near the cloaca. In *P. perspicuus*, which is very similar to *P. gynodiporata* sp. n., Kito (Kito 1981) claimed that the poorly developed supplement is a single structure with a single opening, while two tubular-like structures

are illustrated. However, in the new species we observed the presence of two poorly developed tubular precloacal supplements, each with its own opening. It is unclear, however, whether the presence or absence and number of those very small precloacal supplements in other *Paracanthonchus* species are a result of misinterpretation or not. The combination of the following characters could be used to distinguish between species from the genus *Paracanthonchus*: 1) number of precloacal supplements including the poorly developed tubular supplement near the cloaca, 2) the mouth armature including the number of ventrosublateral teeth, 3) the ornamentation of the gubernaculum (e.g. ridges, thorn like protuberances) and 4) the number of loops in the amphidial fovea. Body length, body width and pharynx length should be given less weight.

Interestingly, the observed phenotype variability is not accompanied by genotypic variability in *P. gynodiporata* sp. n. A threshold between intra and interspecific genetic distances for the COI gene in marine nematodes has been set at 4.8% p-distance (Ferri et al. 2009, Derycke et al. 2010b). In our work, the highest difference between haplotypes was 1.5%. A combination of morphological characters differentiated the four studied *P. gynodiporata* sp. n. populations and non-overlapping morphometric characters such as buccal cavity width, distance of amphidial fovea from anterior end, and cephalic setae length were able to differentiate populations. It has been demonstrated that body and tail length can substantially vary within a single species progeny (Fonderie et al. 2013) and even the presence or absence of teeth in a single species (Kiontke and Fitch 2010) can be affected by environmental variables (e.g. food source), but it has only rarely been documented from field collected specimens. In contrast to the substantial phenotypic variability observed, a maximum of 6 haplotypes per location per year was observed, with 17 haplotypes in total over more than 1000

km. Surprisingly, the nuclear sequences were identical for all individuals of the two studied locations Cupe (PE) and P. Verde (AL). The opposite pattern (high genetic variation and no morphological variation) is well documented in a wide range of species (Bickford et al. 2006), including marine nematodes (Derycke et al. 2006, 2007a, Derycke et al. 2008a, Fonseca et al. 2008, Apolônio Silva De Oliveira et al. 2012). Due to the limited number, small size and the high risk of losing individuals of *P. gynodiporata* n. sp. during voucher procedure, we have not used the same individuals for DNA sequencing and morphometry. However, because of the low haplotype richness (maximum of 6 haplotypes per location) and the high dominance of a single haplotype, at times representing more than 80% (Muriú (RN)) of the haplotype frequencies, it is very likely that the individuals used for the morphometry are from the same or similar haplotypes. Because of initial differences in number of adults among the four populations, we have added a number of juveniles to increase the balance of our design for the molecular analysis. However, because 1) only one species of the genus *Paracanthonchus* occurred associated with seaweeds in our samples in the four studied locations, and 2) very few overall mutations were present among their sequences (maximum of 6 mutations out of 396 bp for COI, and identical nuclear D3D3 and 18S sequences between two beaches), it is highly unlikely that the added juveniles belonged to a different species.

Many morphological traits are encoded by multiple genes, and it is possible that other regions of the genome could show more variation than the three genes we have studied. Moreover, environmental factors can play a role in nematode phenotypic variation without similar levels of genetic differentiation as a result of epigenetic mechanisms (Bossdorf et al. 2008). Gene expression usually can be reduced by methylation of CpG sites (cytosine followed by a guanine with one phosphate in

between). Reduced activity of the Hsp90 (a heat shock protein) caused morphological variations in an isogenic *Drosophila melanogaster* strain (Sollars et al. 2003), showing that epigenetic modification can be expressed in the organism's morphology. Such changes can be heritable and may imply that in natural populations, mutation is not the only source of heritable variation (Bossdorf et al. 2008), and epigenetic changes are also an important mechanism underlying microevolutionary processes.

Clearly, an integrative approach using independent data sources can lead to scientifically valid species delineation. Some morphological characters of nematodes are difficult to observe. Similarly, the genus *Paracanthonchus* presents a wide variation in mouth structure (Miljutina and Miljutin 2015). The pairs of ventrosublateral teeth can be strongly reduced and barely visible in lateral view, and consequently they could have easily been overlooked using light microscopy in previous publications (Gerlach 1957, Inglis 1962). Ideally, morphometric data from different populations, as provided in this study, should be included to give an idea of the morphological variability of a species. However, it is comprehensible that this is not always possible if sampling requires intensive logistic effort (e.g. deep sea). Our data provides another clear-cut example of the need to combine multiple approaches (morphology and DNA sequences) to describe and determine species boundaries.

4.5. Conclusion

Nematodes associated with seaweeds can show low genetic structuring over large distances (>1000 km), suggesting dispersal capacity of nematodes can be high throughout the evolutionary history of the species. There is no evidence that historical seaweed exploitation has affected genetic diversity or haplotype frequencies of epiphytic marine nematodes. Morphometric variation in natural populations can be substantial, showing interspecific overlap, and one should combine at least molecular

and morphological data in an integrative way to establish species boundaries and describe diversity.

Acknowledgement

We would like to thank Ghent University and the Federal Rural University of Pernambuco (UFRPE) for the logistic support.

Chapter 5: *General discussion*

5. **General discussion**

In tropical areas, especially in South America, substantial study effort is still needed to properly document marine biodiversity. Brazil's 7000 km coastline accounts for 50% of the entire South American continent's coast line. Current study on genetic diversity, connectivity and structure of marine nematodes along the Brazilian coast contributes to decrease the knowledge gap on the free-living marine nematofauna of South America. It represents the first study comparing the nematode assemblages associated with seaweeds with those from the sediments in different beaches over a large scale (>3000 km) in tropical (mostly) and subtropical regions. Additionally, current study also made an attempt to explore whether historical human activities such as seaweed exploitation in shallow water ecosystems could affect nematode diversity based on population genetic information from a single species present in several sampling sites along the Brazilian coast. For the first time, it is shown that nematodes associated with seaweeds may be able to disperse over distances much longer than 100 km, and one of the few studies showing that phenotypes between populations can be significantly variable while genotypes are conserved. The direct testing of the assumption that the more sediment accumulated on the seaweeds, the higher the nematode abundances is, as far as I know, also new to science. The latter assessment combined with the assemblage analysis between sediment and seaweed, is very important to understand whether the sediment is a source of diversity and recolonization for the seaweed beds upon disturbance and degradation. In the next section we will discuss the fundamental questions of this thesis into detail.

5.1. Do nematode communities associated with macroalgae differ from those inhabiting the nearby sediment because of ecological differences between macroalgal and sediment as habitats?

5.1.1. ***Main differences between seaweeds and sediment as habitat for meiofauna***

M**acroalgal substrate**. Seaweeds are a fundamental part of the autotrophic food web, providing oxygen, feeding site and shelter. Physical factors such as wave exposure and sediment accumulation appear one of the most important physical factors in the literature (see also chapters 1 and 2). However, direct interactions between the algal substrate and its associated organisms are also important. Morphological differences in algal cell wall structure and presence/absence of secondary metabolites are responsible for shaping epiphytic communities, e.g. the growth of microphytobenthos (Paul and Fenical 1986, Egan et al. 2000, Wikström and Pavia 2004).

Some epiphytic meiofauna organisms possess specific adaptations, such as claw-like structures (in copepods) which allow the attachment of the organism onto seaweed thallus to withstand currents (Hicks 1985). Secondary metabolites exuded by the macroalgae form another type of adaptation. Compounds as diterpene alcohols, pachydictyol—A and dictyol—E, are produced by some brown seaweeds for example *Dictyota dichotoma*. Those compounds appear to decrease seaweed palatability for the herbivore fish *Diplodus holbrooki* and the sea urchin *Arbacia punctul*ata (Hay et al. 1987). In contrast to fishes, herbivore amphipods feed on *D. dichotoma* and are abundant, indicating that they are tolerant to the seaweed secondary metabolites.

In our study, we have compared nematode assemblages of the seaweeds *Sargassum polyceratium* (brown seaweed) and *Halimeda opuntia* (green calcified seaweed). The

most abundant genera were similar between seaweeds (e.g. *Euchormadora* and *Paracanthonchus*), but an overall difference in nematode assemblage was observed; the latter could be a result of fluctuations of rare taxa. Some nematode genera apparently showed a preference for one seaweed species over the other (e.g. *Hypodontolaimus* for *S. polyceratium* and *Draconema* for *H. opuntia*). Apart from the presence of caudal glands, most epiphytal nematodes do not possess obvious morphological adaptations. However, draconematids (found in current study) and epsilonematids form an exception since they possess in addition transformed glandular setae (ambulatory setae) as well as an S- or epsilon-shaped appearance. Nematodes have a well-developed chemosensory system in the head region as well as gustatory sensory elements in the pharynx (Knowlton 2000, Goetze and Kiørboe 2008) which may contribute to seaweed preferences. Biochemical interactions such as seaweed metabolite tolerance and sensitivity to other seaweed exudate (chemical signalling, Jensen 1981) could perhaps be one non-visible nematode adaptation to the phytal habitat.

Differences in seaweed morphology have been reported as important structuring factors for epiphytic meiofauna including nematode assemblages (Warwick 1977, Gibbons 1991, Gee and Warwick 1994a, Gee and Warwick 1994b). In our study (chapter 2), the most obvious effect of different thallus architecture between *S. polyceratium* and *H. opuntia*, was reflected in the amount of retained sediment, which was higher in the latter. Although no significant differences in nematode overall density between both seaweed species or correlation between nematode density and the amount of retained sediment were found, the amount of sediment positively correlated with the density of some particular nematode genera. So the amount of retained sediment present seems to be important for specific genera/species rather than for the

whole nematode assemblage. Counterintuitively, the positive correlations were with nematode genera that were typically found on seaweeds (*Paracanthonchus*, *Euchromadora* and *Draconema*), but not for one frequently found in sediment in our work (*Hypodontholaimus*). However, the data on nematode assemblages in the sediment was obtained in a different year and based on relatively few samples (chapter 3). Because this result seems contradictory with the idea of higher loads of sediment leads to higher abundances of nematodes typically found in sediment on seaweeds, more data would be necessary to clarify whether there is such a straightforward relationship.

In the **bottom sediment** however, factors such as oxygen percolation, organic matter content and grain size have been demonstrated as important factors structuring meiofauna (Steyaert et al. 2003, Udalov et al. 2005, Soetaert et al. 2009). For instance, while macroalgae are important oxygen producers for the associated meiofauna, oxygen can be more limited in the sediment as high levels of organic matter content increase metabolic activity of bacteria, consequently increasing the rate in which oxygen is depleted (Jogensen and Revsbech 1985, de Beer et al. 2005). Bioturbation plays an important role on the microdistribution of food sources (Dauwe et al. 1998), affecting the distribution of the meiofauna such as nematodes. Grain size and shape have also important factors because they influence porosity and organic matter retention (e.g. lower organic matter content in medium sand compared to fine sand (Williams 1972, Franco et al. 2007). Moreover, high abundances of certain nematode families have been attributed to granulometric profiles (Gheskiere et al. 2005, Maria et al. 2012).

In our study (chapter 3), we did not generate granulometric data and our comparisons are based on what is found in the literature for the same location. The granulometry of

the bottom sediment ranged from medium silt to gravel with medium sand being the most common grain size. We have found very similar families which correlated with certain grain size ranges in the literature. For instance, the dominance of the family Xyalidae in fine to medium sand (Gourbault and Warwick 1994, Nicholas and Hodda 1999, Gheskiere et al. 2004, Hourston et al. 2005, Moreno et al. 2006, Mundo-Ocampo et al. 2007).

5.1.2. ***Nematode assemblages from seaweeds appear to be distinct from those in sediment***

Taking into account the above mentioned differences in habitat conditions of the macroalgal and sediment substrates, the **first** main question of this study was: are nematode communities from seaweeds and adjacent sediment significantly different? The answer is important if one wants to understand the sources of recolonization and possible restoration capacity of the seaweed beds. Considering 1) the significant difference in nematode assemblages between seaweed and sediment in seven out of eight studied locations (despite a significant effect in dispersal of the variances) and 2) the absence of some macroalgal nematode species in the sediment, such as the *Paracanthonchus gynodiporata* n.sp. and the nearly absence (one or two individuals in the sediment in 3 out of 7 locations) of the most abundant species on the algae *Chromadora macrolaimoides*, epiphytic nematodes may not simply be a subset of those found in the surrounding sediment, clearly showing substrate preferences. Our findings contrast with the idea that the more sediment, the higher the general nematode density (Wieser 1951, Wieser 1952, Hopper and Meyers 1967, Hopper 1967, Moore 1971, Warwick 1977, Da Rocha et al. 2006).

Nematodes that are typically found in the sediment possibly do not stay on the seaweed when currents carry them along with the sediment, but apparently move back to the bottom sediment illustrating that nematodes can actively choose their habitat (Ullberg and Ólafsson 2003, Arroyo et al. 2006). Conversely, nematodes typically found on seaweeds may not colonize the sediment as observed in experiment with drifting seaweeds, in which epiphytic nematodes arriving in a new location did not migrate to the sediment and remained on the seaweed instead (Arroyo et al. 2006). Although the investigation by Arroyo et al. (2006) was at genus level, this finding indicates that some nematodes only or mainly colonize seaweeds.

From a functional point of view, 2A feeding type nematodes (= epistratum feeders which scrape off particles from surfaces by small buccal teeth) was dominant in seaweeds which is typical for phytal habitats (Hopper and Meyers 1967, Lewis and Hollingworth 1982, Da Rocha et al. 2006, De Oliveira et al. 2016) while 1B feeding type nematodes (= ciliate or non-selective deposit feeders with a non-armed buccal cavity) were dominant in the sediment and typically found in detritivore marine food webs (de Jesús-Navarrete and Herrera-Gómez 2002). It indicates that type of food availability probably differs between substrates and food preference is likely playing a role on the distribution and structuring of the nematode communities.

Hereby, we have demonstrated that differences in nematode assemblages are likely to be caused by differences in substrate characteristics. Our hypothesis that nematode communities differ between different substrates, is confirmed.

5.2. Do nematode assemblages from non-harvested regions differ from harvested regions because of the higher temporal turnover in the latter?

5.2.1. **Exploitation of natural seaweed bed and cultivation**

The effect of seaweed exploitation has been observed on seaweed bed communities, for example in the decrease in biomass of both the exploited seaweed *Gelidium* (the main harvested seaweed) and macrofauna species such as a holoturian (*Pentacta dolioculum*), a bivalve (*Perna perna*), a gastropod (*Fissurella mutobilis*) and an amphipod (*Elasmopus japonicus*) (Lasiak and Field 1995). Seaweed cultivation in general as well as in Brazil has been proposed as a way to exploit economically important seaweeds, mitigating the impact on the natural bed communities (Rocha 2013). However, the effects of seaweed cultivation are still controversial as some studies claimed either a negative or a positive effect, which appear to be related to the taxon and/or habitat. For instance interstitial macrofauna appear to be negatively affected by seaweed cultivation as farms are easily accessible on foot and the bottom substrate is disturbed by trampling (de la Torre-Castro and Rönnbäck 2004, Eklöf et al. 2005). In contrast, seaweed farming areas have shown to harbour a more abundant and diverse fish community (Bergman et al. 2001).

5.2.2. **Seaweed exploitation and nematode assemblages**

The **second** main question of our study focused on possible changes in nematode assemblage between locations with and without historical seaweed exploitation, and latitudinal patterns for both epiphytic and sediment nematodes. Except for one beach (Icapuí-CE), we observed in general no significant difference between nematode communities with and without historical exploitation as parameter, showing that if those

nematode communities were disturbed by harvesting in the past, nowadays the impact can no longer be observed. However, the number of samples used in our study was limited, and we will avoid strong conclusion to this respect.

Jensen (1984) assumed that in the Baltic Sea epiphytic nematodes were able to migrate to the bottom sediment during adverse conditions such as seasonal variation which causes changes on seaweed thallus. Therefore, nematodes associated with seaweeds in exploited areas might be able to mitigate the effect of habitat loss by migrating to the bottom sediment, although it can be very limited (Arroyo et al. 2006). In our study, especially in the northeastern coast, climatic conditions are fairly stable (Dominguez et al. 1992), no variation on seaweed thallus were observed and it is uncertain whether tropical species exhibit this type of avoidance behaviour.

5.2.3. ***Differences in nematode assemblages between locations within seaweeds and sediment***

Overall, there was significant variation in nematode assemblage between locations for both substrates (seaweeds and sediment) and no correlation between diversity or abundance with latitudinal gradient. However one particular pattern was observed. Nematode communities associated with seaweeds are more similar to each other over more than 3000 km coast line in all measured variables, density, diversity and assemblage structure compared to the nematode communities found in the adjacent sediment. We found the same macroalgae-exclusive species *P. gynodiporata* in four locations over more than 1000 km coast line, showing little genetic structuring as well as a widespread epiphytic species, *Chromadora macrolaimoides* which occurred in all locations except in one, and when recorded from the sediment this was only by one or

two individuals. Both species, when co-occurring, accounted for more than 40% of the relative abundances except one location in the southeast (São Sebastião), where both species were absent. In contrast, for the sediment, we found no dominance of a particular nematode genus and hardly the same co-occurring genera across locations.

This pattern might be related to the dispersal capacity of some typical epiphytic nematodes e.g. on drifting seaweeds which may float for long periods over long distances (Sudhaus 1974, Thiel and Gutow 2005, Derycke et al. 2008b). In contrast, nematodes from the sediment can be suspended in the water column (Palmer 1988, Boeckner et al. 2009, Thomas and Lana 2011) and carried by sea currents for a much more limited time (Ullberg and Ólafsson 2003).

We observed that the nematodes which only occurred in seaweeds accounted for 20% to more than 60% of the relative abundance depending on the location. Although the percentages are likely to change by increasing sampling effort, differences in nematode assemblages at genus level between seaweed and sediment were consistent, i.e. seven out of eight beaches showed significant differences between substrates. Consequently, it seems unlikely that nematode diversity on seaweeds upon disturbance can be fully restored from sediment nematode assemblages. It suggests that there might be a local loss of epiphytic nematode diversity as a result of extensive uncontrolled exploitation/degradation of seaweeds beds

Here, we found no evidence of seaweed exploitation and no latitudinal pattern. Therefore, **2**) we reject the hypothesis that nematode communities between the locations with and without historical harvest would be different as a result of temporal turnover and patterns in nematode assemblage would be found along the latitudinal gradient

5.3. Will intraspecific genetic differences be higher among populations in non-harvested vs. harvested regions due to the higher colonization dynamics in the latter?

*5.3.1. **Environmental pressure and genetic diversity***

Habitat loss (e.g. deforestation, fragmentation, etc.) is known as a strong factor affecting genetic diversity (Lowe et al. 2005, Rauch and Bar-Yam 2005), decreasing population size, and increasing inbreeding rates, culminating in a lower population fitness (inbreeding depression – Dolgin et al. 2007, Indrioko and Ratnaningrum 2015). It directly affects the capacity of populations to withstand environmental pressure (Ehlers et al. 2008, Wilkinson et al. 2010), which can endanger species. Responses on genetic diversity in meiofauna resulting from environmental stress have been recorded in many studies (Street and Montagna 1996, Street et al. 1998, Schizas et al. 2001, Gardeström et al. 2006, Gardeström et al. 2008). However, response to adverse environmental conditions has shown both to decrease genetic diversity by the same mechanism mention above (inbreeding) or increase genetic diversity by increasing mutation rates and the selection favouring heterozygotes (Depledge 1996, DiBattista 2008). In nematodes, changes in genetic diversity caused by adverse environmental conditions are still unclear (Derycke et al. 2007b). However, previous meiofauna studies dealing with genetic diversity variation were focused on **pollution** rather than on direct physical decrease of habitat.

5.3.2. ***The third main question dealt with the influence of seaweed harvesting at molecular level: Is intraspecific genetic diversity in non-harvested areas higher compared with areas which were historically harvested?***

After many attempts to find a good candidate nematode species and testing different primer sets for the mitochondrial COI for a variety of species with a wide enough distribution, we succeeded with one new species, *Paracanthonchus gynodiporata* sp. n., of the family Cyatholaimidae.

Similarly as for nematode assemblages (chapter 3), no significant genetic diversity difference was observed between locations where historical harvesting of natural seaweed beds took place compared with those without any harvesting record. We observed however, higher genetic diversity in one beach with historical harvesting. This could have been caused by a selective force favouring heterozygosity for example, as mentioned in the literature (Depledge 1996, DiBattista 2008). This result was also surprising as bottleneck and founder effect could have happened as a result of habitat loss. This would decrease the genetic diversity and affect allele frequencies as a result of recurrent recolonization, also observed in shorter terms experiment with nematode recolonization (Derycke et al. 2007c).

Meiofauna in shallow water ecosystems has shown to quickly recover from disturbance, such as trampling, in few days in a mudflat, or after months in a phytal habitat (Johnson et al. 2007, Sarmento et al. 2013). Possibly, in our study area enough time has passed to allow the restoration of the local genetic diversity, considering that the exploitation of the natural seaweed beds has stopped more than 10 years ago. However, the time needed to restore the genetic diversity is still unclear as life history between nematode species varies considerably (Derycke et al. 2013). Alternatively,

seaweed harvesting may have little, if any, effect on nematode assemblages associated to the selected macrophytes.

Our hypothesis related to the main question **3**: “nematodes from non-exploited areas exhibit higher genetic variability” could be rejected for *P. gynodiporata* sp. n.

5.4. Will genetic differentiation be pronounced along the Brazilian coast as a result of historical long term isolation?

Populations that are for a long time isolated, for instance by a geographical barrier, are likely to exhibit genetic structuring because of random **variation in allele frequencies** over time (genetic drift - Masel 2011) and lack of gene flow. This is especially true for populations that have undergone strong decrease in population size, such as caused by habitat loss, which increases genetic drift (Ellstrand and Elam 1993). Moreover, differences between populations can also be caused by the emergence of new alleles resulting from **mutation** (Coyne and Orr 2004).

Mitochondrial genes are fast evolving, up to nine times the nuclear ones (DeSalle et al. 1987, Moriyama and Powell 1997, Monteiro and Pierce 2001, Lin and Danforth 2004), making them ideal for studying microevolutionary processes. Fossils are used to calibrate **molecular clocks**, which in turn, take into consideration mutation rates to estimate divergence time between lineages (Thomas et al. 2006). Studies estimating mitochondrial mutation rate in different vertebrate and invertebrate phyla have shown no values that could be applied to calibrate molecular clocks across all metazoans (Thomas et al. 2006). For vertebrates, faster molecular evolution has been correlated with smaller body size and higher metabolic rates (Martin and Palumbi 1993). The third codon position of the mitochondrial gene cytochrome *b* has revealed to be renewed

every 1-2 Myr year(s) in fast evolving mammals (Nabholz et al. 2008). However, the study of Nabholz et al. (2008) did not include any species of the Phylum Nematoda. Although there is no evidence for the correlation between body size and higher evolution rate in invertebrates (Thomas et al. 2006), and estimation of molecular clocks in nematodes can be very challenging because of the lack of a fossil register (Dorris et al. 1999), nematodes are known as **fast evolving** compared to other invertebrate phyla (Aguinaldo et al. 1997, Coghlan 2005).

Nematodes are species diverse and can have contrasting life histories, from few days generation time in the *Litoditis marina* species complex to annual/semi-annual in *Thoracostoma trachygaster* (Derycke et al. 2010a). Information on overall mutation rate is available for two model organisms *Pristionchus pacificus* and *Caenorhabditis elegans*, both hermaphrodites with a life cycle of about 4 days at 20 °C. A study scanning the mitochondrial genome of *P. pacificus* estimated an overall mutation rate of 7.6 X 10^{-8} per site per generation, or based on a 4 days generation time, about 7.6 mutations per site per Myr (Molnar et al. 2011) while *C. elegans* has a slightly higher mitochondrial mutation rate of 9.7 X 10-8 mutations per site per generation, or about 8.9 per site per Myr (Denver et al. 2000). Therefore, nematode populations that are isolated (no gene flow) in a time scale of 1 Myr are expected to exhibit a number different haplotypes that are not shared as a result of mutation.

5.4.1. ***The fourth main question to answer: Will the distances and the opposite sea currents function as a biogeographic barrier and cause genetic breaks over large distances?***

Because of the high mutation rate of mitochondrial DNA, the observation of large population genetic structuring in nematodes for distances inferior to 100 km (Derycke et al. 2007a) and the presence of a known geographical barrier, we expected to find in our study a substantial genetic structuring along more than 1000 km coastline. However, we found little genetic structure in the new epiphytic species *Paracanthonchus gynodiporata* we investigated This result was unexpected as gene flow at those distances are supposedly limited as nematodes lack planktonic larvae that could be passively dispersed by water currents (Derycke et al. 2013). Moreover, speciation rate might be higher in tropical areas (Allen et al. 2006) which could have been another factor contributing to differences between populations. The four locations studied were grouped per two according to the two major sea currents they were influenced by but apparently the latter did not represent a biogeographical barrier preventing genetic flow.

Nematodes can passively disperse over at least a 100 km distance using drifting seaweeds (Derycke et al. 2013). However, considering that in the current contribution, the distances between seaweed beds ranged from 167 to 1045 km and little genetic structure was found, this might indicate that nematodes associated with seaweed can exceed a distance of more than 1000 km in tropical areas. Although this kind of dispersal could be true for sites under the same major sea current, it does not explain the dominance of a single haplotype (Hellberg et al. 2002) (C8) and how a number of shared haplotypes (e.g. C7, PV2) occurred between extreme sites under opposite sea currents.

Nematodes have been recorded associated with sea turtle shells for example the genus *Paracanthonchus* (Corrêa 2012, Corrêa et al. 2014). Sea turtles occurred in the studied locations and are known to be able to overcome long distances. When the sea turtle forages between seaweed beds, nematodes could become associated and transported from one location to another (Bjorndal 1985). Alternatively, the dominant haplotype C8 (average 77% ± 8 abundance) present in the four studied populations in the northeastern coast of Brazil, could have been selected in the studied locations. However, it is unlikely that the same haplotype would have been independently selected four times (four studied locations).

Although 17 haplotypes were found, the number of haplotypes per location did not exceed 6, showing very few mutations between them (1 to 6 base pairs), resulting in a star-like haplotype network without any biogeographical subdivision. This suggests that the four *P. gynodiporata* n. sp. populations are young and did not accumulate enough mutations to differentiate between populations. Sea currents change over geological time inducing changes in biogeographical barriers. The Central America Seaway was gradually narrowing and ending around 2.7 Myr ago. Before the closure of this way, the North Brazil Current to flowed SE which is the opposite direction observed nowadays (Heinrich and Zonneveld 2013). It is possible that during the period of the Central America Seaway those communities were connected and dispersal occurred passively via seaweed drift.

The D2D3 and 18S sequences of all individuals of *P. gynodiporata* populations were identical between the locations of Cupe-PE and P. Verde-AL which are more than 165 km apart. It shows an overall conserved genotype over large distances. Moreover, it suggests that nematode dispersal capacity might be much higher than previously

expected and that provided enough time, the genetic diversity of nematode associated with seaweeds can be restored.

Our hypothesis related to the main question **4**) “genetic differentiation as a result of long term isolation among nematode populations would be found”, could be rejected.

5.4.2. ***A plea for integrative taxonomy in marine nematodes***

Although genotypes in *P. gynodiporata* sp. n. populations were conserved, the phenotypes between the populations varied considerably, which contrasts with what is generally expected for marine nematodes in view of the substantial presence of cryptic speciation (Derycke et al. 2005, Derycke et al. 2007a, Apolônio Silva De Oliveira et al. 2012). It has already been observed in bioassays that the quality and the amount of the food can affect the morphometrics of specimens within a single species (Fonderie et al. 2013) and even the phenotype such as the absence or presence of teeth in the same nematode species (Kiontke and Fitch 2010), but this has rarely been observed directly in nature. It can make species description solely based on morphology e.g. based on structures such as stoma armature, at times, misleading. Within the genus *Paracanthonchus*, there is substantial variation in morphological characters, and important characters are not mentioned in some species descriptions (Miljutina and Miljutin 2015). That is the case for the ventrosublateral teeth which vary in number and presence in the genus. The genus *Paracanthonchus* exhibits also a variety of gubernaculum shapes and structures (e.g. presence/absence of denticles, crura ridge), and a variable number of precloacal supplements, which in some cases, are difficult to interpret (e.g. reduced precloacal supplement(s) closest to the cloacal opening).

Accurate interpretation and the combination of characters, could provide a more reliable way to differentiate *Paracanthonchus* species when molecular data are not available. In our work we have reconstructed a detailed 3D view of the head region of *P. gynodiporata* sp. n. showing the presence and disposition of minute ventrosublateral teeth that are less visible in lateral view, and could have been ignored in previous species descriptions within the genus. The 3D reconstruction of taxonomically important characters has been used before to discriminate between cryptic species (Apolônio Silva De Oliveira et al. 2012), which by conventional lateral view with light microscopy, would not have been possible. It shows that at different angles the same or perhaps other anatomical structures not considered diagnostic features in the literature, may provide extra and relevant morphological information to be integrated in species description. The same integrative approach (combining morphology with diverse molecular data), used in the current study for free-living marine nematodes, could also be applied to other species across the phylum. Finally, integrative taxonomy can potentially help researchers to select a set of characters to support species delimitation, which is an issue in the grey zone of independently evolving lineages (De Queiroz 1998, 1999, 2005).

5.5. General conclusion

In sum, we demonstrated that 1) nematode assemblages associated with seaweeds are very diverse. In tropical regions nematode differences, if any, between algal substrates may be barely perceptible, and nematode assemblages may vary little throughout the year. It is the first time that the amount of sediment retained by the seaweed and its effect on nematode assemblage was tested directly. We showed that

the accumulation of sediment affected specific genera but no overall effect in nematode density was observed.

2) Nematodes may exhibit preferences for a substrate, in some cases, occurring either in seaweeds or sediment. In the eight locations along 3000 km coastline, similarity between epiphytic nematode assemblages was higher compared to the ones in sediment. Although more data is necessary, epiphytic nematode assemblages may not be simply a subset of those found in the sediment. It suggests that nematode assemblages found in the sediment may not completely restore the diversity found on seaweeds upon disturbance, and part of the epiphytic diversity may come from other seaweed beds.

3) Nematodes appear to be capable of overcoming known biogeographical barriers over more than 1000 km, and no evidence for effect of seaweed harvesting was observed on genetic diversity. Provided that the habitat loss caused by intense seaweed harvesting affected epiphytic nematode population structure, in a long term (≈10 years), marine epiphytic nematode populations may stabilize over time and disturbance may no longer be detectable, as observed for *P. gynodiporata* sp. n. This is one of the few studies evaluating historical human mediated impact on marine nematode populations at genetic level. Moreover, this is one of the very few demonstrating that in natural populations, genotypes can be very conserved while phenotypes can substantially vary. It shows that cryptic species are not omnipresent in free-living marine nematodes.

4) Despite the difficulty of interpreting some morphological characters in the literature, we could differentiate our new species by the presence of pre- and post advulvar pores flanking the vulva in females, and by the gubernaculum with a crura ridge with adorsal thorn and lateral protuberance in males. Additionally, the use of 3D reconstruction

provided us a comprehensive detailed representation of the head morphology using light microscopy. This is a relatively cheap and accessible technique and can be used to support morphological differences between species.

5.6. Future perspectives

We have provided some relevant information on phytal nematode assemblages, population genetics of one species, and discussed the possible impact of historical harvesting. However, more information is necessary to draw stronger conclusions about the effect of seaweed exploitation on nematode assemblages. Our study was performed about 10 years after seaweed harvesting on the natural seaweed bed has stopped. Ideally, samples before and after seaweed harvesting would provide direct information on how diversity and haplotype frequencies respond to this specific human activity. Because nematodes may exhibit high colonization turnover at ecological time scales, short term experiments would be necessary to address how nematode assemblages from seaweeds and sediment behave in terms of macroalgae recolonization. Moreover, to clarify whether nematodes typically found in sediment or on seaweeds differ in dispersal capacity, more sequences from different nematode species from both substrates are necessary to estimate population genetic structuring.

There are two main points that are considered harmful to organisms present on macrophytal beds in literature (including seagrasses): 1) seaweed farms that shade the natural bed decrease the biomass and abundances of the natural flora and fauna (Eklöf et al. 2005); and 2) if farming areas are accessible on foot, the local infauna may suffer from trampling (Lyimo et al. 2008). Therefore, we would recommend to avoid such farming conditions. None of the obtained samples in our study were under those

farming conditions. The technique used consisted of floating ropes where seaweeds were attached to. They were far from the natural bed and only accessible by boat. In the future, it would be worthwhile to look at different farm methods and their effect on epiphytic and infaunal assemblages. In this way, we could answer the question whether different farming methods have negative, neutral or even positive effects on local nematode assemblages or meiofauna communities.

Because of the geographical gap between the two main regions (Northeast and South-Southeast) samples between those two regions, including environmental data such as granulometry, would improve our knowledge on nematode biodiversity in a latitudinal profile. It would be important to design specific primers to obtain sequences from the most widespread nematode species in our study, *Chromadora macrolaimoides*, which would provide a broader view of population genetic structuring covering an area three times larger than *Paracanthonchus gynodiporata* sp. n. Finally, the development of sequence libraries (databases) would be useful to estimate biodiversity and the effects of human activities in shallow water ecosystems.

List of publications and contributions

Publications in SCI-indexed journals

Apolônio Silva de Oliveira D, Decraemer W, Holovachov O, Burr J, De Ley IT, De Ley P, Moens T, Derycke S. 2012. An integrative approach to characterize cryptic species in the *Thoracostoma trachygaster* Hope, 1967 complex (Nematoda: Leptosomatidae). Zoological Journal of the Linnean Society: 164, 18–35.

Apolônio Silva de Oliveira D, Derycke S, Da Rocha CMC, Barbosa DF, Decraemer W, Dos Santos GAP. 2016. Spatiotemporal variation and sediment retention effects on nematode communities associated with Halimeda opuntia (Linnaeus) Lamouroux (1816) and Sargassum polyceratium Montagne (1837) seaweeds in a tropical phytal ecosystem. Marine Biology 163:102.

Apolônio Silva de Oliveira D, Decraemer W, Moens T, Dos Santos GAP, Derycke S. Low genetic but high morphological variation over more than 1000 km coastline refutes omnipresence of cryptic diversity in marine nematodes. BMC evolutionary Biology (under review)

Dos Santos GAP, Corrêa GV, Apolônio Silva de Oliveira D, G Fonsêca-Genevois VG, Vazquez YV, Siva AC, Pontes LP, Dolan E, Ingels J. Eretmochelys imbricata shells present a dynamic substrate for a facilitative epibiont relationship between macrofauna richness and nematode diversity, structure and function. Experimental Marine Biology and Ecology (under review)

Other publications

Apolônio Silva de Oliveira D, Dos Santos GAP, Derycke S, Moens T, Decraemer W. 2014. Biodiversity and connectivity of marine nematodes associated with algae from two tropical beaches. Journal of nematology 46:152-152.

Active contributions

Third European Conference for the Barcode of Life (ECBOL3) 17-21 September 2012, Brussels - Belgium. Oral presentation. “An integrative approach to characterize cryptic species in the *Thoracostoma trachygaster* Hope, 1967 complex (Nematoda: Leptosomatidae). Apolônio Silva de Oliveira D, Decraemer W, Holovachov O, Burr J, De Ley IT, De Ley P, Moens T, Derycke S.”

6th International Congress of Nematology, 4 to 9 May 2014, in Cape Town – South Africa. Oral presentation. Section: Nematode Biodiversity. “Biodiversity and connectivity of marine nematodes associated with algae from two tropical beaches Apolônio Silva de Oliveira D, Dos Santos GAP, S. Derycke S, Moens T, Decraemer W”

5º Brazilian Congress of Marine Biology (5º CBBM) 17 to 21 de maio de 2015,– Brazil. Poster. “Efeito letal de bário sobre as populações de nematóides de vida livre Litoditis marina em estarvação. Luna CA, Apolônio Silva de Oliveira D, Souza PO, Dos Santos GAP”

Poster. “Nematóides de vida livre e suas interações com a macrofauna em cascos de Eretmochelys imbricata. Dos Santos GAP, Corrêa G, Silva D, Spagnolo G, Apolônio Silva de Oliveira D”

32nd Symposium of the European Society of Nematologists (ESN) 2016 in Braga, Protugal from, 28 August to 1 September, Oral presentation. “A marine epiphytic nematode (Cyatholaimidae) revels low genetic structure but significant morphological variation over more than 1000 km coast line”

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