

Marine Nematode Taxonomy in Africa: Promising Prospects Against Scarcity of Information

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Abstract: From the late 19th century, Africa has faced heavy exploitation of its natural resources with increasing land/water pollution, and several described species have already become extinct or close to extinction. This could also be the case for marine nematodes, which are the most abundant and diverse benthic group in marine sediments, and play major roles in ecosystem functioning. Compared to Europe and North America, only a handful of investigations on marine nematodes have been conducted to date in Africa. This is due to the scarcity of experienced taxonomists, absence of identification guides, as well as local appropriate infrastructures. A pivotal project has started recently between nematologists from Africa (Tunisia), India, and Europe (Italy) to promote taxonomic study and biodiversity estimation of marine nematodes in the African continent. To do this, as a first step, collection of permanent slides of marine nematodes (235 nominal species and 14 new to science but not yet described) was recently established at the Faculty of Sciences of Bizerte (Tunisia). Capacity building of next generation of African taxonomists have been carried out at level of both traditional and molecular taxonomy (DNA barcoding and next-generation sequencing [NGS]), but they need to be implemented. Indeed, the integration of these two approaches appears crucial to overcome lack of information on the taxonomy, ecology, and biodiversity of marine nematodes from African coastal waters.

Key words: Africa, barcoding, biodiversity, integrative taxonomy, molecular tools, morphology, next-generation sequencing.

Systematics or “the science of biodiversity” encompasses taxonomy, phylogeny, and nomenclature, and its goals are essentially recognition and naming of species (Blaxter, 2004). Interest in the discovery of new species has increased considerably in recent years, since the rate of species extinction appears to be far greater than that of new discoveries (Crisci, 2006). Knowledge of species is also essential for the reconstruction of evolutionary history, understanding their ecological importance, and the task of preserving variety of life on earth (Schander and Willassen, 2005).

It is important to protect biodiversity because today, just as always, humans are dependent on earth’s natural resources for their provision of goods and services (Balvanera et al., 2006). With the growing recognition of the importance of oceanic biodiversity on a planetary scale, scientists all over the world have become interested to understand and estimate species richness of marine prokaryotes and eukaryotes. The goals of this kind of research would be to gain an accurate baseline information on species biodiversity, determine if and how the ecosystem is changing, and develop ways to monitor marine ecosystems using marine organisms as proxy (Appeltans et al., 2012). Based on meta-analyses approach, the authors have estimated the presence of 0.7 to 1.0 million species in world’s oceans. Some studies have also shown that species diversity generally has a positive effect on ecosystem processes and functioning (e.g., Cardinale et al., 2006; Danovaro et al.,

2008). This let us to conclude that any loss in marine biodiversity could have serious negative effect on ongoing functional processes in marine environments (Loreau, 2008).

Nematodes are arguably the major component of benthic domain, which can range from shallow to deep-sea habitats (Lamshead and Boucher, 2003; Lamshead, 2004). The structural and functional organization of nematode assemblages can provide precious information on the “health” of ecosystem in which they live and are widely recognized as excellent bioindicators (Balsamo et al., 2010; Semprucci et al., 2015b).

The known number of marine nematode species based on morphological approach, after two centuries of research, is ~6,900 representing only the 19% of the total species estimated (Appeltans et al., 2012). Nematode identification is mainly carried out using morphological characters, but it may be time-consuming and sometimes problematic, mainly because of the high phenotypic plasticity among populations, few taxonomic diagnostic characters, and presence of cryptic species (Derycke et al., 2005; Bhadury et al., 2008; Rodrigues Da Silva et al., 2010).

The integration of morphological and molecular approaches may offer alternative ways to estimate biodiversity, in particular from unexplored geographical regions, and improve our understanding of cryptic species complexes and systematics of free-living nematodes. Furthermore, the addition of molecular techniques can be effectively used for large-scale assessment of marine biodiversity across various coastal ecosystems. In this respect, DNA barcoding, one of the promising sequence-based approaches, has gained increased importance in the field of nematology with a primary focus on elucidating biodiversity of nematodes from different habitats.

In this review, knowledge of free-living marine nematodes in the African continent is presented and

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summarized. Furthermore, the advantages of the molecular techniques as potential tools for taxonomic identification of nematodes are discussed.

WHY WE SHOULD STUDY MARINE NEMATODES FROM AFRICAN COASTAL ECOSYSTEMS?

Diversity of marine nematodes is of particular concern in tropical and subtropical environments (Semprucci and Balsamo, 2014), given the low level of knowledge on their systematics and biogeography (Liu, 2009). Published studies representing different habitats such as coral reefs (Ndaro and Ólafsson, 1999; Raes et al., 2007; Semprucci et al., 2010, 2013), seagrass beds (Danovaro and Gambi 2002; Danovaro et al., 2002), and mangrove sediments (Alongi, 1987; Ólafsson, 1995; Somerfield et al., 1998) indicate that tropical marine sediments are characterized by a well-diversified nematode fauna. However, the increase of diversity with the increasing latitude, which is well known for several terrestrial and marine animal taxa, has not been confirmed in case of marine nematodes. Marine meiofauna show only weak latitudinal gradients, mainly due to the presence of temporary meiofauna (Kotwicki et al., 2005). In contrast, free-living nematodes are not more diverse in tropical regions than in subtropical or temperate areas (Boucher and Lamshead, 1995; Gobin and Warwick, 2006). However, a huge amount of tropical areas remains unknown making these regions of crucial importance for marine biodiversity estimation. Furthermore, they are among the world regions that experience earliest emergences of unprecedented climate and resulting biodiversity changes (Solan et al., 2006).

Although the African continent shows a great heterogeneity of habitats, biogeographic sectors (e.g., Mediterranean Sea, Atlantic Ocean, Indian Ocean, and Red Sea), and climatic conditions (see north-south and east-west transects), current information on the distribution of marine nematodes is still limited and, in major cases, represented by ecological surveys in which only a genus level of identification was adopted. In detail, the highest amount of data is in the northern Africa coast, but exclusively focused in Tunisian coast (Aïssa, 1981, 1991; Beyrem and Aïssa, 2000; Mahmoudi et al., 2002, 2003a, 2003b; Boufahja et al., 2007, 2010, 2014). An overall high number of studies is also in the Eastern Africa coast. However, they have been carried out only in mangroves and shallow habitats along the coastal stretches of Kenya and were mainly conducted at genus level (Ólafsson, 1995; Muthumbi and Vincx, 1997; Muthumbi et al., 1997; Muthumbi and Vincx 1998a, 1998b; Ndaro and Ólafsson, 1999; Raes et al., 2007). Only dispersed studies have been undertaken along the coastline of South Africa (Inglis, 1961, 1963, 1966; Hendricks and Gibbons, 2010; Gyedu-Ababio, 2011) and Western Africa (Van Gaever et al., 2009).

Accordingly, the species list of marine nematodes from Tunisia appears to be most comprehensive compared to rest of the African continent (Boufahja et al., 2014).

Studies on Tunisian free-living marine nematodes started in early 70s (Aïssa, 1981) and were focused on estimating spatial trends of diversity (morphological taxonomy based) in eight Tunisian coastal environments (e.g., Bizerte bay, Bizerte lagoon, Bou Ghrara lagoon, Ghar El Melh lagoon, Old Harbor of Bizerte, Ichkeul lake, and northern and southern lakes of Tunisia) (Boufahja et al., 2014). About 249 species belonging to 133 genera and 31 families have been inventoried so far from Tunisia (Boufahja et al., 2014). Among them, Xyalidae (30 species), Cyatholaimidae (25), Chromadoridae, Linhomoeidae (21), and Comesomatidae (20) have been found to be the most diverse families (Boufahja et al., 2014). In this area, since 2005, studies focused on morphotaxonomy became increasingly fewer, and nematologists started to focus their research on the ecotoxicology of nematodes, which were exposed to toxic chemicals such as petroleum compounds (Mahmoudi et al., 2005; Hedfi et al., 2013), lubricants (Beyrem et al., 2010), heavy metals (Beyrem et al., 2007, 2011; Hedfi et al., 2007; Mahmoudi et al., 2007, 2008; Boufahja et al., 2011a), and pesticides (Boufahja et al., 2011c). However, many of these studies do not go into the details of nematode identification, rather focusing on the effects of toxic compounds on selected species.

The data available for Africa as well as Asia are extremely negligible if they are compared with those from western Europe (especially British Isles and Belgian coasts) and North America (Semprucci and Balsamo, 2012). This is because pioneers' of nematode taxonomy and the majority of taxonomists are based in the latter areas. Furthermore, support and infrastructure required to motivate young scientists to pursue a career in taxonomy of marine nematodes is still at its dormancy in Africa. Upgrade of infrastructure along with capacity building of young African taxonomists in the field of DNA barcoding through application of highly innovative instrumentation (e.g., NGS platforms such as Illumina and 454) can significantly accelerate biodiversity studies with a focus on marine nematodes.

As of today, more than 800 marine nematode nucleotide sequences representing molecular markers such as nuclear small subunit ribosomal DNA (18S rDNA), large subunit ribosomal DNA (28S rDNA), and mitochondrial cytochrome c oxidase I (COI) are available in sequence databases such as GenBank, EMBL, DDBJ, and PDB. However, majority of these sequences represent marine ecosystems from western Europe and North America. Only 51 marine nematode nucleotide sequences in published databases currently represent the African continent (Fig. 1). Though tropical ecosystems can harbor novel species of marine nematodes as recently found in a highly productive tropical

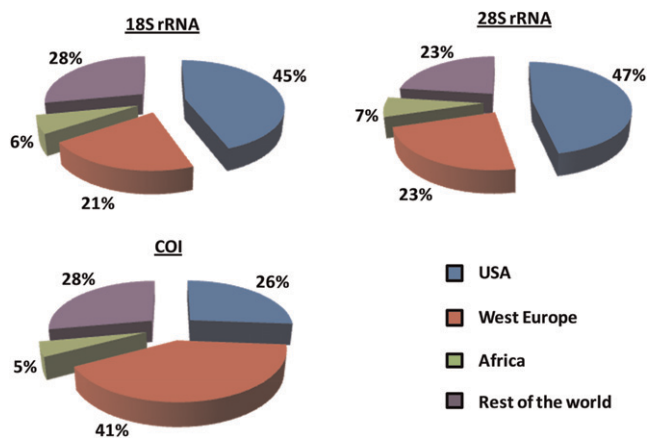


FIG. 1. Proportions of the available sequences in GenBank database for free-living marine nematodes. 18S rRNA = nuclear small ribosomal subunit; 28S rRNA = nuclear large ribosomal subunit; COI = mitochondrial cytochrome c oxidase I; USA = United States of America.

ecosystem of southeast Asia (Kumar et al., 2014), such a low representation of nematode sequences from Africa clearly reflects that biodiversity studies based on molecular tools are in their infancy here.

CONSTRAINTS IN THE TRADITIONAL TAXONOMY OF NEMATODES

Traditionally, although taxonomy of marine nematodes has relied on the use of morphological features for species identification (e.g., Platt and Warwick, 1983, 1988; Warwick et al., 1998), the morphological approach has its own limitations. Among the limits of traditional taxonomy there are the following issues: 1) as with other microscopic organisms, species identification requires specialist expertise, 2) accurate identification is mainly based on detailed examination of adult male specimens, 3) lack of museum collections of nematodes in tropical regions, and 4) relatively low amount of data that when present are predominantly focused on northwestern European coastal environments (Coomans, 2002; Semprucci and Balsamo, 2012). Furthermore, considerable emphasis is placed on the use of ratios of various body measurements, especially de Man's ratios. However, there are some limitations in the use of these tools because of the following reasons: nematodes may respond to various natural and anthropogenic stressors (e.g., salinity variation, and pollution) by speeding up some or all steps in their life cycle to reduce the exposure time (Boufahja et al., 2011b). The most apparent of such adaptive strategy is the differential variation in body dimensions, and thus biometric ratios as function of the sensitivity of each maturity stage. Moreover, these ratios may be affected during reproductive phases as adults may put more energy into reproduction and less into growth in comparison with juveniles (Boufahja et al., 2012). Thus, caution should be taken in the use of de Man's ratios. Nematoda and Mysida database, namely

NeMys (Steyaert et al., 2005), is an essential tool for nematode taxonomists and ecologists being the most comprehensive database of marine nematode species and a platform in which identification keys and literature are readily available. However, numerous original descriptions are not available even in NeMys, further complicating the identification process. As a result of the limits of the classic morphological approach, faunistic surveys have frequently been replaced with ecological studies that usually require a genus level of identification or, more rarely, putative species. These accepted practices have notably contributed to the current standstill in distribution knowledge of marine nematodes (Semprucci, 2013). However, some additional efforts involving ultrastructure-based taxonomic approaches such as scanning electron microscopy and transmission electron microscopy, 3D image-capturing technology, and confocal laser scanning microscopy have given a deeper resolution of some morphological details and can aid in resolving taxonomic issues (Abebe et al., 2011; Semprucci and Burattini, 2015a; Semprucci et al., 2015b).

DNA BARCODING

One of the ways to aid and accelerate nematode diversity estimation is to apply molecular biological approaches. One such approach is DNA barcoding that relies on the use of a standardized DNA region as a tag for rapid and accurate species characterization (Hebert et al., 2003; Hebert and Gregory, 2005). For animals, the locus of choice proposed for barcoding is a 658-bp region of the COI gene, involved in the respiratory chain of mitochondria (Valentini et al., 2008). This gene has an interesting level of variability, and the differences are low among individuals of the same species and high between individuals of different species. The goal of DNA barcoding primarily is to identify all eukaryotic species (Miller, 2007). It has been tested across several phyla and so far has shown promising results (e.g., Hebert et al., 2004; Radulovici et al., 2010).

In case of free-living marine nematodes, COI has proved to be relatively difficult to amplify because of rampant gene rearrangement, hypervariation among haplotypes, and frequent recombination in mitochondrial genome (Bhadury et al., 2006; Rodrigues Da Silva et al., 2010; Hyman et al., 2011). Therefore, other regions such as the nuclear 18S rDNA (Bhadury et al., 2006; Bhadury and Austen, 2010) and 28S rDNA (De Ley et al., 2005; Pereira et al., 2010) have been successfully evaluated as part of marine nematode barcoding initiatives. A list of commonly used primers targeting different molecular markers/gene for marine nematode barcoding has been detailed in Table 1.

However, in the last few years there have been developments with respect to the application of mitochondrial COI for barcoding of marine nematodes.

TABLE 1. List of commonly used primers for undertaking molecular studies of marine nematodes.

Molecular marker/gene of interest	Primer combinations	Size of amplicon	Reference
18S rDNA	G18S4 [5'-GCTTGCTCAAAGATTAAGCC-3']	1,600 bp approximately	Blaxter et al. (1998) Meldal et al. (2007) Bik et al. (2010b)
	26R [5'-CATTCTTGGCAAATGCTTTTCG-3']		
	22F [5'-TCCAAGGAAGGCAGCAGGC-3']		
	13R [5'-GGGCATCACAGACCTGTTA-3']	925 bp	Bhadury et al. (2006)
	24F1 [5'-AGAGGTGAAATCTTGGATC-3']		
	18P [5'-TGATCCWKCYGCAGGTTTCAC-3']		
	MN18F [5'-CGCGAATRGCTCATTACAACAGC-3']		
	NEM18S_R [5'-GGGCGGTATCTGATCGCC-3']		
	MN18F [5'-CGCGAATRGCTCATTACAACAGC-3']		
	22R [5'-GCCTGCTGCCTTCCTTGA-3']	345 bp	Bhadury et al. (2006)
	M18F [5'-AGRGGTCAAATYCGTGGAC-3']		
	M18R [5'-TCTCGCTCGTTATCGGAAT-3']	427 bp	Bhadury and Austen (2010)
	D2A [5'-ACAAGTACCGTGAGGGAAAGTTG-3']		
D3b [5'-TCGGAAGGAACCGCTACTA-3']			
28S rDNA	JB2F [5'-ATGTTTTGATTTTACCWGCWTTTYGGTGT-3']	396 bp	Derycke et al. (2007) Derycke et al. (2007)
	JB5GEDR [5'-AGCACCTAAACTTAAAACATARTGRAA RTG-3']		
COI	JB3F [5'-TTTTTTGGGCATCCTGAGGTTTAT-3']	418 bp	Derycke et al. (2005) Derycke et al. (2010a, 2010b)
	JB5R [5'-AGCACC TAAACTTAAAACATAATGAAAATG-3']		

COI = cytochrome c oxidase I; rDNA = ribosomal DNA.

Derycke et al. (2010b) evaluated and successfully amplified COI region from 41 species of marine nematodes from European coasts. They found that variability pattern observed in the COI could be used to aid in identification of nematode species provided a good reference database is equally available. However, paucity of reference sequences from different geographical regions in published sequence databases currently restricts to some extent the applicability of DNA barcoding for marine nematodes on a global scale. One of the ways to overcome such constraints would be to start a global initiative for marine nematode barcoding on the lines of Census of Marine Life programs.

NGS APPROACHES TO ASSESSMENT OF MARINE NEMATODE DIVERSITY

In the last five years, some studies have applied newer sequencing technologies such as NGS toward assessment of biodiversity including marine environments (e.g., Lecroq et al., 2011; Fonseca et al., 2010, 2014; Bik et al., 2012). NGS platforms such as Roche 454, Illumina (HiSeq and MiSeq), and ABI SOLiD with

capacities to process millions of sequences in parallel, have revolutionized biological research in recent years. Specification of some of the NGS platforms have been detailed in Table 2. Individual read lengths in majority of next-generation platforms are usually limited, but the depth of coverage per base pair and advanced sequence assembly software allow sequencing of giga base pairs in a single run. Concurrently, there has been significant development in bioinformatic pipelines, which can process these large set of raw data generating out of NGS platforms (Stoltzfus et al., 2013; Darling et al., 2014).

NGS has been tested toward assessment of marine nematode diversity from coastal areas of United Kingdom. Creer et al. (2010) analyzed sediment samples collected from intertidal zone at Littlehampton on the south coast of England, United Kingdom, as well as soil, litter, and understory habitats sampled at La Selva Biological Station, Costa Rica. Community polymerase chain reaction and 454 sequencing based on 18S rDNA approaches yielded a total of 29,756 high-quality sequences over 200 bases from the marine samples whereas these approaches for the tropical rain forest samples yielded a total of 40,334 high-quality sequences of at least

TABLE 2. Specification of some of the NGS platforms.

Sequencing platform	454	Illumina	ABI SOLID	Ion Torrent
Year of availability	2005	2006	2006	2010
Length of sequenced fragment	200–700 bp	150 bp approximately	35–50 bp	200 bp approximately
Run time	23 hr	27 hr to 11 d	7–8 d	2 hr
Technology used for sequencing	emPCR, pyrosequencing	Polonies, cleavable dye terminators	emPCR, ligation with cleavable dye terminators	emPCR, H ⁺ detection

NGS = next-generation sequencing.

200 bases. In the terrestrial data set, the soil habitat had fewer nematode operational cluster taxonomic units (OCTU) (35) than either the marine littoral habitat (149) or canopy (97). Plant-parasitic nematodes were more diverse and abundant in soil environment, with bacterial- and fungal-feeding nematodes predominating in the litter and canopy. This study evaluated the effectiveness of 454 sequencing in nematode diversity studies from varied environments including marine realms.

Fonseca et al. (2010) applied a metagenetic approach using NGS of the 18S nuclear small subunit ribosomal RNA (rRNA) marker to assess simultaneously the relative levels of richness and patterns of diversity of multiple metazoan phyla from eight benthic samples collected from low-tide zone of an estuarine beach near Prestwick on the west coast of Scotland and from one sample from a beach in Littlehampton in the south of England. The amplicons were processed for sequencing on a Roche 454 FLX platform generating a total of 353,896 sequences, which were subsequently filtered to 305,702 for downstream analysis. Based on the annotation, 374 OCTU were assigned to phylum level. Of the metazoan OCTU, 182 were from Nematoda, at least three times more than from any other individual meiofaunal taxon. Platyhelminthes (61 OCTU) was the second richest phylum, followed by Arthropoda (29 OCTU including Copepoda, Ostracoda, and Malacostraca), Mollusca (22 OCTU), Gastrotricha (7 OCTU), Annelida (6 OCTU), and five less-rich phyla (e.g., Bryozoa, Echinodermata, Cercozoa, Rotifera, and Alveolata with between 1 and 3 OCTU each). On the basis of the comparisons of the OCTU sequences with the NCBI databases, authors found that majority (95%) of Nematoda OCTU have never been sequenced before. For other phyla, only a small fraction of the OCTU showed 100% identity with previously sequenced specimens. Only 15 of the OCTU with <90% identity (300 sequences) were robustly placed within identified phyla (mainly Nematoda). For Prestwick samples, the authors detected 182 Nematoda OCTU, compared with 450 species of free-living marine nematodes that have been described from around the entire British Isles. Geographically, these datasets represent the discovery of 40% of the previously known phylum richness from transect that represents 0.004% of the length of the British coastline (~17,820 km, Ordnance Survey). In all, 70% of Nematoda OCTU were unique to Prestwick and 58% to 100% of the OCTU for the other phyla were only present in Prestwick. Based on 454 sequencing approach, a study was undertaken to assess microbial eukaryotic communities across depth (shallow water to abyssal) and ocean basins (deep-sea Pacific and Atlantic) (Bik et al., 2012). Within the 12 sites examined, the authors found that some taxa can maintain eurybathic ranges and cosmopolitan deep-sea distributions, but majority of species appear to be regionally restricted. Contrary to previous observation of nematodes being the most abundant

members in sediment, the authors found equal or more dominant role for other taxonomic groups in some of the deep-sea sites (e.g., unicellular eukaryotes in the Pacific) based on 454 sequencing. In addition, low genetic divergence between geographically disparate deep-sea sites suggested either a shorter coalescence time between deep-sea regions or slower rates of evolution across this vast oceanic ecosystem (Bik et al., 2012). In another study involving deep-sea sites in the southern Ocean, the authors investigated association between deep-sea nematodes and marine fungi and potential implications including food preference of nematodes based on capillary and 454 sequencing approaches (Bhadury et al., 2011).

Several studies including the ones mentioned above have shown that despite the application of high-throughput sequencing, rarefaction curves of the operational taxonomic units (OTU) do not reach saturation in many of the studied areas, indicating that biodiversity is possibly much higher than expected in marine realms (see review by Carugati et al., 2015). Tang et al. (2012) have stated that lack of saturation of rarefaction curves could be caused by low resolution of 18S rDNA in discriminating meiofaunal groups such as marine nematode at the species level.

NGS approaches can provide information of marine nematode diversity from less explored sites or regions; however, at the same time these studies also reflect that a significant proportion of the sequences generated show limited sequence identity with sequences that are available in published databases such as GenBank/EMBL/DBJ. This is due to the restricted number of deposited sequences for valid marine nematode species and also many biogeographic regions such as those located in tropical and subtropical realms (e.g., Africa) are yet to be fully explored using morphotaxonomy and molecular approaches. In addition, some technological impediments associated with NGS techniques such as amplification and sequencing errors, lack of availability of broad set of primers, arbitrary OTUs, and unreliable inferences on species richness at times can lead to under- or overestimation of nematode biodiversity (Carugati et al., 2015).

MOLECULAR APPROACH IN THE TIME OF "TAXONOMIC CRISIS"

In recent years, approaches to nematode taxonomy have become more holistic, and molecular tools are also applied toward identification and description of new species. Over the last decade, some studies have used molecular phylogeny (based on 18S rDNA and mitochondrial COI) as a tool for describing new taxa (e.g., Armenteros et al., 2014; Rho et al., 2011; Cunha et al., 2013; Tchesunov et al., 2015). However, rate of description of new species has not exponentially increased, as initially expected. Indeed, the advantages of molecular taxonomy often remain incomplete lacking

the final step of formal species description that requires use of traditional morphological approach and involvement of experienced nematologists. In the last decade, molecular tools have also revealed the existence of cryptic species that based only on morphology were identified as conspecific (e.g., Derycke et al., 2005, 2006; Bhadury et al., 2008; Bik et al., 2010a). Thus, integrative taxonomy, a combination of molecular and morphological approaches, is fundamental also to describe valid new species from cryptic complexes. An attempt that has been carried out until now by only a handful of studies (e.g., Derycke et al. 2010a).

WAY FORWARD FOR AFRICAN MARINE NEMATODE STUDIES

To accelerate studies on nematode diversity in Africa, there is a need to integrate morphotaxonomy based on the most recent microscopic techniques with molecular tools. One of the advantages of molecular tools lies in the fact that it can be applied to any life stages (juvenile or adult) and, thus, can help in taxonomical identification in conjunction with morpho-approach.

The first step in this direction has been the creation of the first collection on permanent slides of marine nematodes from Africa. The collection was recently established at the Faculty of Sciences of Bizerte (Tunisia) (Boufahja et al., 2014). In detail, 235 nominal species and 14 new to science but not yet described belonging to 133 genera and 31 families have been deposited, becoming a reference point for all taxonomists.

Second, there was an urgent need to train the next generation of African taxonomists in the field of marine nematology, and this action is possible through several international opportunities that are currently available including the Royal Society Department for International Development Africa Capacity Building Initiative, Chandrasekhara Venkata Raman International Fellowships for African Researchers, and European Master of Science in Nematology (Erasmus Mundus). In addition to capacity building at the level of classical taxonomy, there was an action to train existing and future nematologists from Africa in DNA barcoding and NGS for an improved assessment of marine nematode biodiversity. The infrastructure facilities required to undertake such studies at African institutions have been augmented in the last years, but they have to be implemented.

As stated earlier there is an increasing need to undertake global initiative for DNA barcoding of marine nematodes with emphasis on biodiversity rich ecosystems such as in the African continent. Such an initiative within a stipulated time frame through involvement of multi-institutions including those from Africa can pave the way for accelerated identification of species based on integrative taxonomy. Among the most significant advantages of a such initiative will be not only an estimation of the marine biodiversity of rich ecosystems across different continents, but also it will give new

insights on the biogeography of the phylum and aid in the process toward discovery of new species. Additionally, the global initiative on marine nematode barcoding, can also help toward populating reference sequence databases with a much broader coverage that may ultimately improve inferences drawn from methods such as NGS.

No one to date knows how many species of animals in particular metazoans, exist on earth. Africa, a largely unexplored continent, has experienced pressures (exploitation of natural resources and introduction of industrial technologies) from the late 19th century until the middle of 20th century, and many of the emerging developing countries in this continent in this century are undergoing rapid industrialization and development. Despite increased efforts in conservation, losses in biodiversity continue to date (Groombridge and Jenkins, 2002). For free-living marine nematodes, the rapid loss of “healthy” environments in Africa (Groombridge and Jenkins, 2002), even before the discovery and morphological descriptions of present species, means that we are currently losing valuable knowledge. For this reason, in Africa, the implementation of the molecular methods in conjunction with morphotaxonomy toward improved identification of marine nematodes could offer three-in-one solution, in view of 1) the rareness of experienced taxonomists, 2) the huge lack of data, and 3) the massive extinctions of species due to human activity. At present, a pilot project has been initiated between nematologists from Tunisia, India, and Italy keeping the above points in mind. Indeed, a large amount of information accrued on various aspects of nematode biology and ecology is currently linked with morphology (Abebe et al., 2011) and an estimation of the richness or diversity, even if fast, does not give information on the life cycles or define ecological niches of the species, or it cannot give indications of their role within an assemblage or ecosystem (Boero, 2010).

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