RAFFLES BULLETIN OF ZOOLOGY Supplement No. **34**: 8–12
Date of publication: 29 June 2016
http://zoobank.org/urn:lsid:zoobank.org:pub:52431AAA-09BD-4305-B9B7-EA78F989D8D7

First time surveying meiofauna in Singapore

Ricardo Cardoso Neves[1*], Jeremias Brand[2], Bin Qi Gan[3] & Heinrich Reichert[1]

Abstract. Meiofauna is the community of microscopically small, benthic animals living in and on soft aquatic substrates. Meiofaunal organisms, which have representatives of almost all invertebrate phyla, are smaller than macrofauna. The meiofauna of Singapore has never been explored. In the present study, we show results from the first exploration of the marine meiofauna of this geographical region. Several of the localities sampled yielded specimens of the meiofaunal groups Tardigrada, Kinorhyncha and/or meiofaunal Rhabditophora (Platyhelminthes). In the future, quantitative and qualitative studies would be required to assess the geographical distribution, as well as population density and taxon richness of the meiofaunal community in Singapore.

Key words. Metazoa, biodiversity, St. John's Island, Lazarus Island, Singapore Strait

INTRODUCTION

The marine environment constitutes the largest ecosystem on planet Earth. Despite the efforts made, the animal communities living in the marine realm are far less characterised than those inhabiting the terrestrial environment (Narayanaswam et al., 2013). This is particularly true in what concerns the study of marine meiofauna, which is a yet largely unknown community of microscopically small animals living in and on soft substrates – e.g., sand, shell gravel or mud – at all water depths (Mare, 1942; Giere, 2009). Meiofauna is defined operationally, i.e., meiofaunal organisms can pass through a sieve with a mesh width of 500 micrometers but is retained by a 63 (or 31) micrometer mesh sieve (Higgins & Thiel, 1988). Therefore, the members of meiofauna are mobile animals, which are smaller than macrofauna.

The meiofauna have representatives of almost all invertebrate phyla: the best represented are Arthropoda and Nematoda, which also include many macrofaunal organisms, while Gastrotricha (hairbacks), Tardigrada (water bears), Gnathostomulida (jaw worms), Loricifera (girdle wearers) and Kinorhyncha (mud dragons) are exclusively meiofaunal (Giere, 2009). Other phyla present are, e.g., Platyhelminthes, Acoela, Annelida, Mollusca and, only recently described, Hemichordata (Worsaae et al., 2012). But the importance of meiofauna is not only related to their taxonomic diversity, aberrant forms or dispersal ability (Swedmark, 1964; Curini-Galletti et al., 2012). These small benthic animals may occur in great abundance (e.g., ~10^6 ind m^{-2} in estuarine sediments worldwide). In general, meiofauna (i) facilitate biomineralisation of organic material and enhance nutrient regeneration; (ii) serve as food for a variety of higher trophic levels (e.g., crabs); and (iii) exhibit high sensitivity to anthropogenic inputs, making them excellent organisms for the study of estuarine pollution (Coull, 1999). Given the high socio-economical value of sandy beaches for the marine ecosystem – which is reflected in their importance for coastal fisheries and tourism – a better knowledge of the meiofauna is absolutely necessary to propose a sustainable management policy (Kotwicki et al., 2005a).

To date, information about the Singapore meiofauna is still lacking. Despite ongoing efforts to describe the marine fauna in general, this region is yet underexplored in what concerns groups solely present in the meiofaunal community, such as Tardigrada and Kinorhyncha. In order to have a comprehensive knowledge on the entire faunal biodiversity in Singapore, it is thus essential to have insights about the meiofauna that characterises this tropical region. Therefore, under the scope of teaching activities in the course "Unconventional Invertebrate Model Organisms" from the Biozentrum – University of Basel (Switzerland), we made a first survey on the marine meiofauna biodiversity of Singapore. Here we present preliminary findings on the presence and diversity of the meiofaunal groups Tardigrada, Kinorhyncha as well as meiofaunal Rhabditophora (Platyhelminthes). In addition, a detailed investigation of the kinorhynchs collected during this survey resulted in the recent publication of the first report of Kinorhyncha from Singapore (see Sørensen et al., 2016).

[1]Biozentrum, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland; Email: ricardon.6@gmail.com (*corresponding author)

[2]Evolutionary Biology Zoological Institute, University of Basel, Vesalgasse 1, CH-4051 Basel, Switzerland.

[3]Tropical Marine Science Institute, National University of Singapore, S2S, 18 Kent Ridge Road, Singapore 119227.

ISSN 2345-7600 (electronic) | ISSN 0217-2445 (print)

Table 1. Summary of data on stations and taxa identified from the Singapore Strait in May 2014.

Date	Location	Position	Depth (m)	Substratum	Taxa
15 May	West of Jurong Island	1°14.139'N 103°39.706'E	18.5	Very compact mud	Most common meiofauna (MCM)*
15 May	West of Jurong Island	1°14.495'N 103°39.248'E	17	Mud	MCM, Kinorhyncha
15 May	West of P. Senang/ Raffles Lighthouse	1°09.337'N 103°44.362'E	26.5	Coral fragments	MCM
15 May	Near Sister's Island	1°12.494'N 103°48.501'E	26	Shell gravel with mud	MCM, Kinorhyncha
16 May	Between Bedok Jetty & Sungei Bedok	1°18.387'N 103°57.591'E	9	Sand with mud	MCM, Kinorhyncha
16 May	Near Tenah Merah	1°16.883'N 103°58.196'E	36.5	Shell gravel with mud	MCM
16 May	Eastern Fairway	1°15.589'N 103°56.680'E	52	Sand with mud	MCM, Kinorhyncha
17 May	Seringat Island	1°13.647'N 103°51.204'E	1.5	Black sand shore	MCM, Entoprocta
17 May	Lazarus Island	1°13.342'N 103°51.141'E	Intertidal	Rocky shore	MCM, Tardigrada
17 May	St. John's Island	1°13.225'N 103°50.987'E	Intertidal	Mangrove	MCM, Platyhelminthes, Acoela
18 May	St. John's Island	1°12.880'N 103°50.985'E	Intertidal	Rocky shore	MCM, Tardigrada
18 May	Seringat Island	1°13.531'N 103°51.299'E	Intertidal	Mud with seagrass	MCM, Platyhelminthes, Acoela
19 May	Seringat Island	1°13.647'N 103°51.204'E	Intertidal	Sandy beach	MCM, Tardigrada, Platyhelminthes, Acoela
19 May	Seringat Island	1°13.531'N 103°51.299'E	Intertidal	Seagrass beds	MCM, Kinorhyncha, Platyhelminthes, Acoela
19 May	St. John's Island	1°13.225'N 103°50.987'E	Intertidal	Mudflat	MCM, Platyhelminthes, Acoela
20 May	Seringat Island	1°13.531'N 103°51.299'E	Intertidal	Seagrass beds	MCM, Kinorhyncha, Platyhelminthes

*Crustaceans (e.g., copepods) and nematodes.

MATERIAL AND METHODS

The collections in Singapore waters were made in May 2014 in partnership with the Marine Biology and Ecology Laboratory of the Tropical Marine Science Institute, National University of Singapore (NUS). Specimens originated from seven subtidal sites in the Singapore Strait and nine intertidal sites at St. John's and Lazarus islands (see Table 1 for details on sampling locations). Sediments were collected by hand in the intertidal zone, while on board of the NUS vessel Galaxea for deeper sampling locations. In the latter case, we used rectangular and triangular dredges to collect the surface layer of different types of marine sediments, e.g., sand or shell gravel with fine particles, and mud. In order to cover the area of interest, several linear transects of approximately 100 m were defined. Afterwards, the sediments were transferred to the lab where they were processed to extract the meiofauna. Depending on the type of sediment, the methodology used was different: muddy sediments were processed by the 'Bubbling and Blot' method (see Higgins & Thiel, 1988; Sørensen & Pardos, 2008), while sandy sediments were freshwater-shocked and stirred vigorously. Alternatively, coarse or fine sand was processed with an isotonic solution of $MgCl_2$ and sea water (1:1) to narcotise the meiofauna and facilitate its detachment from the sand grains. Small plankton nets with mesh width of 45 micrometers were used to filter the water column after the settling of the sand. Afterwards, the concentrated sub-samples were inspected with the help of stereomicroscopes and the meiofauna of interest was sorted out. Specimens were fixed overnight at room temperature in 4% paraformaldehyde (PFA), and subsequently rinsed in 0.1 mol l^{-1} phosphate buffer solution (PBS). Afterwards,

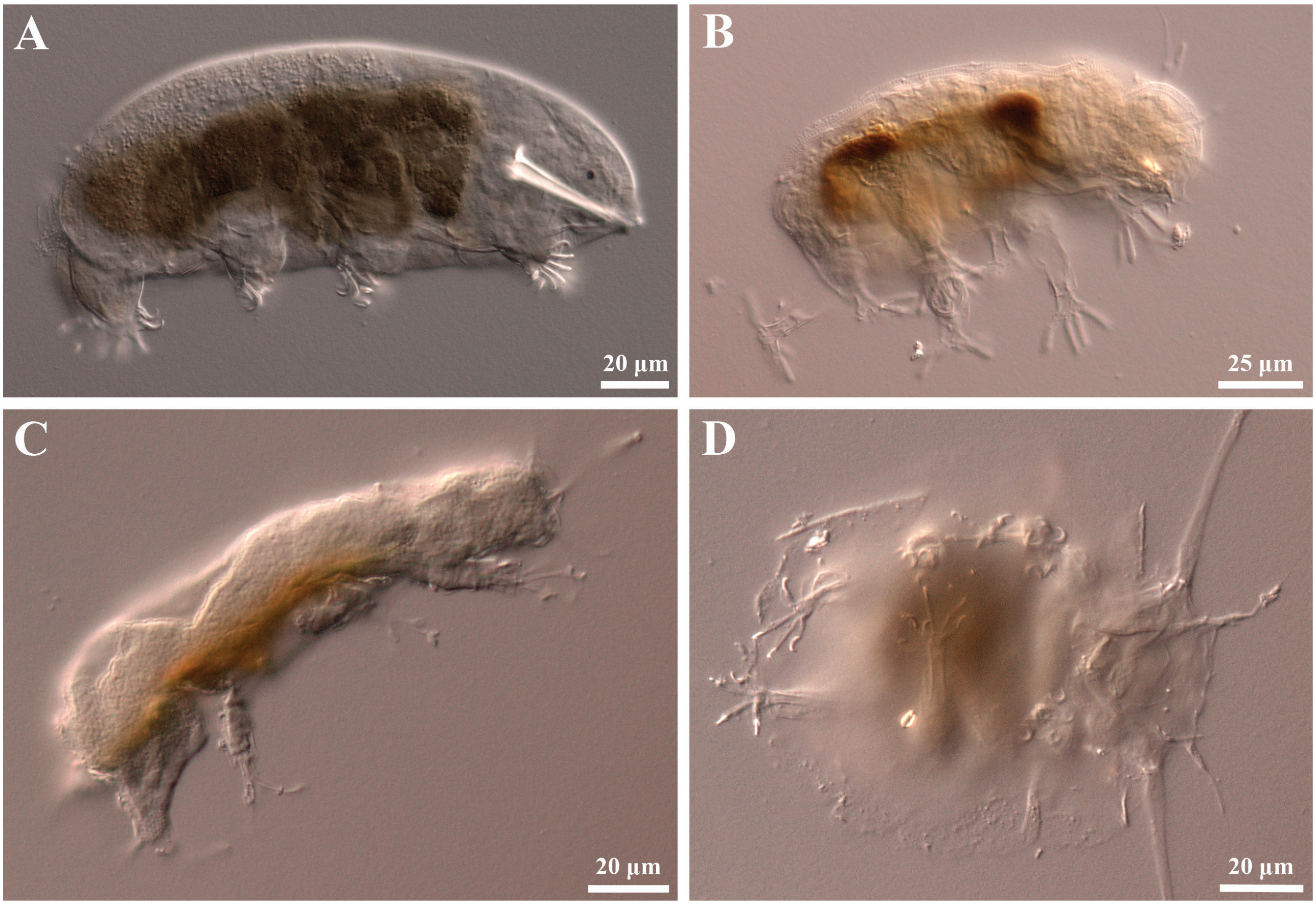

Fig. 1. Tardigrada specimens of distinct genera collected at St. John's Island; light micrographs. Anterior is facing right in all aspects. A, genus *Echiniscoides*; B, genus *Orzeliscus*; C, genus *Batillipes*; D, genus *Florarctus*.

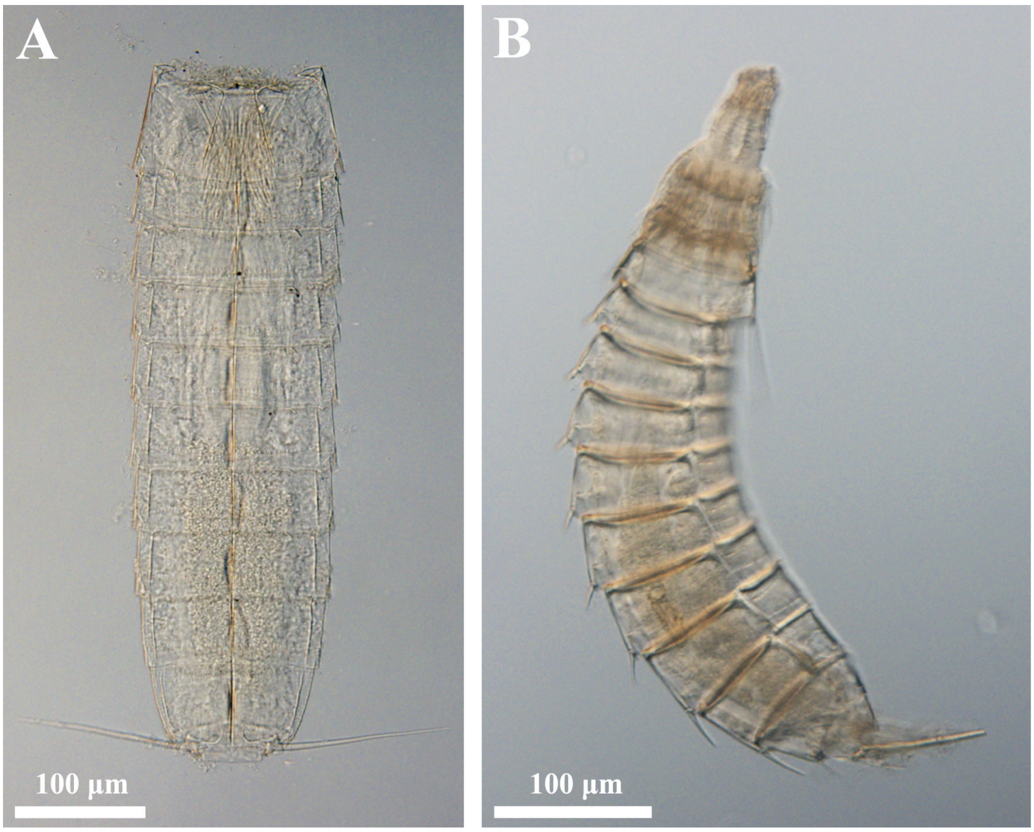

Fig. 2. Kinorhyncha specimens of different orders collected at Singapore; light micrographs. Anterior is facing up in all aspects. A, genus *Pycnophyes*; B, genus *Centroderes*.

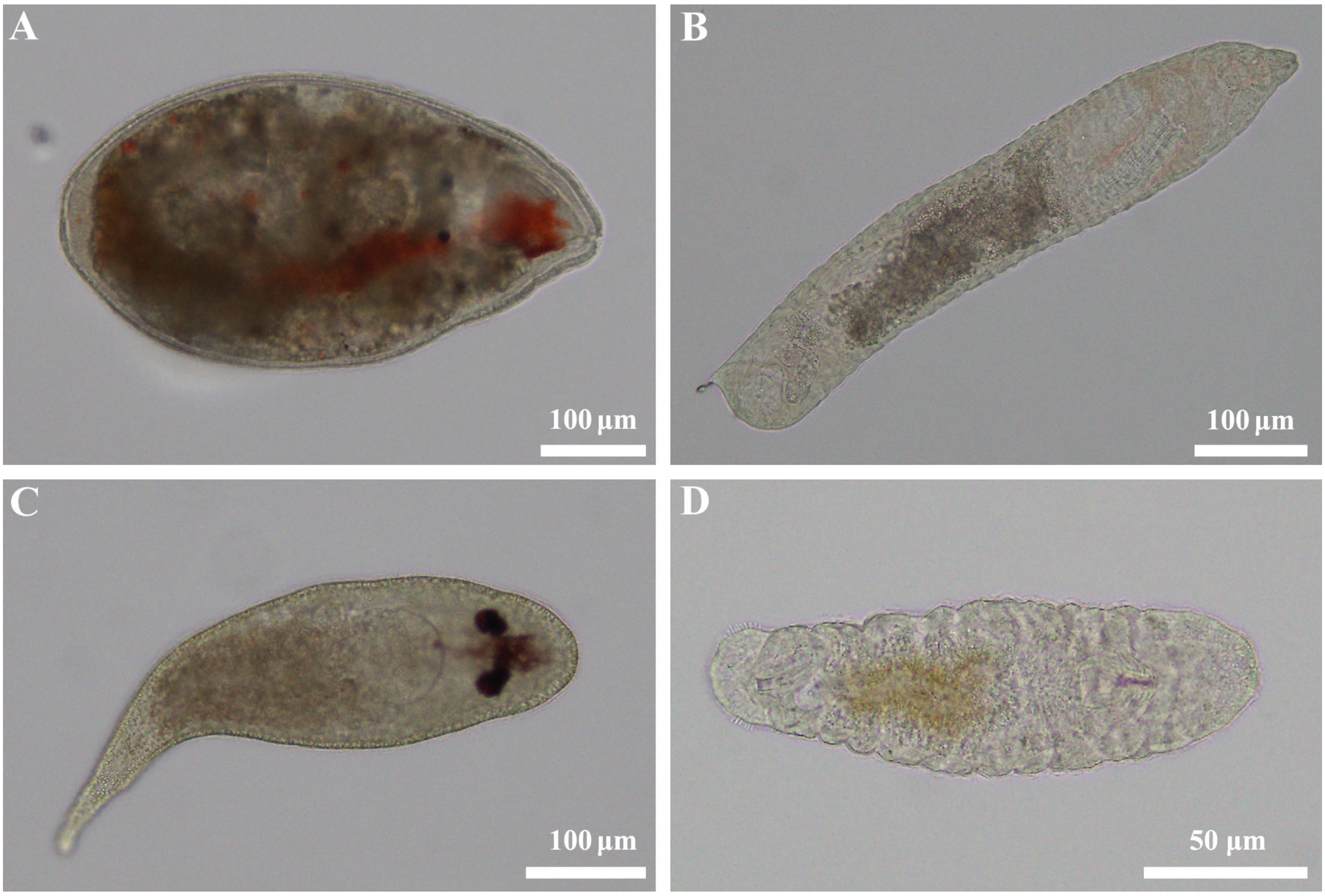

Fig. 3. Free-living Platyhelminthes specimens of various taxa collected at Singapore; light micrographs. Anterior is facing right in all aspects. A, infraorder Eukalyptorhynchia; B, infraorder Schizorhynchia; C, suborder Dalyellioida; D, genus *Macrostomum*.

specimens were mounted in Fluoromount G (Southern Biotech) on glass slides and studied with an Olympus BX43 microscope fitted with differential interference contrast (DIC) optics, and photographs were taken with a Olympus DP21 digital camera. Alternatively specimens were studied with (i) a Leica DM6000 B light microscope with phase contrast and DIC optics and color camera; or (ii) with an Olympus BX43 microscope fitted with DIC optics and photographed with a Olympus DP20 camera.

RESULTS

All sediment samples collected during this survey rendered a diverse array of meiofaunal specimens (see Table 1). The commonly found meiofauna, such as Copepoda (Crustacea, Arthropoda) and Nematoda, was found in all sites investigated. However, we focused our activities on other typical meiofaunal groups such as Platyhelminthes, Kinorhyncha and Tardigrada, which were collected only from specific locations. The latter group was found in the intertidal zone of rocky or sandy beaches. For instance, specimens of the genus *Echiniscoides* (Fig. 1A) were found associated with barnacles settled on a large rock located in a rocky shore at St. John's Island. From a sandy beach at Seringat Island, we found two genera of interstitial Tardigrada, namely *Orzeliscus* (Fig. 1B) and *Batillipes* (Fig. 1C). Specimens from these two genera were collected from the intertidal zone, in sand layers located at a depth of 0.3–0.5 m. A fourth genus of Tardigrada, *Florarctus* (Fig. 1D), was found in the surface layers of sand collected from a rocky shore located at Lazarus Island.

Kinorhyncha were found exclusively in samples of mud or in those containing a high quantity of fine sediments. Surveys made on the subtidal and the intertidal both rendered kinorhynchs of the two orders, i.e., Homalorhagida and Cyclorhagida (see Table 1). We collected a single species of *Pycnophyes* (Order Homalorhagida; Fig. 2A) from subtidal locations with mud, e.g., located at west of Jurong Island, as well as from a seagrass (*Zostera* sp.) area at the intertidal zone of the Seringat Island. Specimens of *Centroderes* (Fig. 2B), *Condyloderes* – which is a genus described from the Indian Ocean (Higgins, 1969) – and *Echinoderes* (Order Cyclorhagida) were found in sand with mud from a location between Bedok Jetty and Sungei Bedok. Based on these data, three new species of kinorhynchs were recently described by Sørensen and colleagues (2016). This study also provides notes on two species already known to science (for a complete list of species found in Singapore see Sørensen et al., 2016).

In samples from the intertidal zone we found numerous taxa of Platyhelminthes. Among the Kalyptorhynchia we found representatives of both subtaxa Eukalyptorhynchia (Fig. 3A) and Schizorhynchia (Fig. 3B). Moreover, we also found members of the Dalyellioida (Fig. 3C), Proseriata and several species of *Macrostomum* (Fig. 3D). Other meiofaunal groups found during this survey include, e.g., Entoprocta and Acoela.

DISCUSSION

The meiofauna present at Singapore appears thus to be diverse and abundant. However, quantitative and qualitative studies are desirable to perform in order to assess the complete richness of taxa, geographical distribution, and population density of this community. The putative outcome from this investigation should necessarily be interpreted in the light of previous studies on biodiversity patterns of meiofauna, especially in the tropics (e.g., Dexter, 1996; Kotwicki et al., 2005b; see also Sørensen et al., 2016). Furthermore, it would be interesting to investigate and compare the meiofaunal assemblages that characterise places affected by human activities with that from regions where the impact is less obvious.

We also suggest the rigorous identification of the species present in this region, as well as the description of new species accompanied by DNA extraction whenever possible. Indeed, new insights on the morphological and molecular characterisation of, e.g., the *Echiniscoides* species (Tardigrada) present in Singapore could shed light on their yet unreliable phylogeny and phylogeographical history in the Indian Ocean (Faurby et al., 2011, 2012).

ACKNOWLEDGEMENTS

The Singapore Strait marine biodiversity workshop was held on St. John's Island, Singapore from 20 May to 7 June 2013, and was organised by the National Parks Board and National University of Singapore. The workshop, as part of the Comprehensive Marine Biodiversity Survey (CMBS) benefited greatly from generous contributions provided by Asia Pacific Breweries Singapore, Care-for-Nature Trust Fund, Keppel Care Foundation, Shell Companies in Singapore and The Air Liquide Group. We are grateful to Tan Koh Siang for receiving us at the Tropical Marine Science Institute. Although this work was carried out one year after the Singapore Strait biodiversity workshop, we greatly appreciate the technical support provided by both staff and students at the Marine Biology and Ecology Laboratory: Chim Chee Kong, Helen Wong Pei San, Teresa Stephanie Tay, Rebecca Loh Ker and Mohammad Razali bin Duriat. In addition, Martin Vinther Sørensen and Reinhardt Kristensen (both Natural History Museum of Denmark) are acknowledged for helping with the identification of Kinorhyncha and Tardigrada, respectively. We also thank Lukas Schärer (Zoological Institute, University of Basel) for his help with the identification of Platyhelminthes.

LITERATURE CITED

Coull BC (1999) Role of meiofauna in estuarine soft-bottom habitats. Australian Journal of Ecology, 24: 327–343.

Curini-Galletti M, Artois T, Delogu V, De Smet WH, Fontaneto D, Jondelius U, Leasi F, Martínez A, Meyer-Wachsmuth I, Nilsson KS, Tongiorgi P, Worsaae K & Todaro MA (2012) Patterns of diversity in soft-bodied meiofauna: dispersal ability and body size matter. PLoS One, 7(3): e33801.

Dexter DM (1996) Tropical sandy beach communities of Phuket Island, Thailand. Phuket Marine Biological Center Research Bulletin, 61: 1–28.

Faurby S, Jørgensen A, Kristensen RM & Funch P (2011) Phylogeography of North Atlantic intertidal tardigrades: refugia, cryptic speciation and the history of the Mid-Atlantic Islands. Journal of Biogeography, 38: 1613–1624.

Faurby S, Jørgensen A, Kristensen RM & Funch P (2012) Distribution and speciation in marine intertidal tardigrades: testing the roles of climatic and geographical isolation. Journal of Biogeography, 39: 1596–1607.

Giere O (2009) Meiobenthology: the Microscopic Motile Fauna of Aquatic Sediments. Second Edition. Springer Verlag, Berlin, 538 pp.

Higgins RP (1969) Indian Ocean Kinorhyncha: 1, Condyloderes and Sphenoderes, new cyclorhagid genera. Smithsonian Contributions to Zoology, 14: 1–13.

Higgins RP & Thiel H (eds) (1988) Introduction to the Study of Meiofauna. Smithsonian Institute Press, Washington, 488 pp.

Kotwicki L, Szymelfenig M, De Troch M, Urban-Malinga B & Węslawski J-M (2005a) Latitudinal biodiversity patterns of meiofauna from sandy littoral beaches. Biodiversity and Conservation, 14: 461–474.

Kotwicki L, Marleen T, Urban-Malinga B, Gheskiere T & Wesawski JM (2005b) Horizontal and vertical distribution of meiofauna on sandy beaches of the North Sea (The Netherlands, Belgium, France). Helgoland Marine Research, 59(4): 255–264.

Mare MF (1942) A study of a marine benthic community. Journal of Marine Biological Association, 25: 517–554.

Narayanaswamy BE, Coll M, Danovaro R, Davidson K, Ojaveer H & Renaud P (2013) Synthesis of knowledge on marine biodiversity in European seas: From census to sustainable management. PLoS ONE 8(3): e58909.

Sørensen MV, Gąsiorowski L, Randsø PV, Sánchez N & Neves RC (2016) First report of kinorhynchs from Singapore, with the description of three new species. Raffles Bulletin of Zoology, 64: 3–27.

Sørensen MV & Pardos F (2008) Kinorhynch systematics and biology an introduction to the study of kinorhynchs, inclusive identification keys to the genera. Meiofauna Marina, 16: 21–73.

Swedmark B (1964) The interstitial fauna of marine sand. Biological Reviews, 39(1): 1–42.

Worsaae K, Sterrer W, Kaul-Strehlow S, Hay-Schmidt A & Giribet G (2012) An anatomical description of a miniaturized acorn worm (Hemichordata, Enteropneusta) with asexual reproduction by paratomy. PLoS One, 7(11): e48529.