

KINORHYNCHS FROM SEA-GRASS BEDS IN SOUTH EASTERN AUSTRALIA- WITH NOTES ON A NEW COLLECTING METHOD

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ABSTRACT

Kinorhynchs are usually collected from soft bottom and sandy sediments, whereas their occurrence in sea-grass beds rarely has been explored. In the present study we developed two new collecting methods that can recover specimens from amongst sub-tidal sea-grass beds and mangrove mud. Several species of kinorhynchs were found and one could be identified as *Echinoderes teretis* Brown, 1985.

The species was originally found in silty mud and described from localities in Sydney Harbour and Broken Bay. We recorded the species in the Clyde River Estuary and Candlagan Creek, some 250 km further south, and in sub-tidal mud and tidal mud, also in Lake Wellington in the Gippsland Lakes, Victoria. The presence of *E. teretis* and several unidentifiable kinorhynch species in sea-grass beds and mangrove vegetation indicates that the provided collecting methods can reveal new information about kinorhynch diversity in such habitats.

INTRODUCTION

The Kinorhyncha is an often overlooked phylum of marine invertebrates, no doubt, because of their small size, less than 1 mm long, and because they are inhabitants of marine sediments from which it may be difficult to isolate them. The largest genus, with a worldwide distribution

and currently 65 described species, is *Echinoderes* Claparède, 1862. Up to present days, only 5 species of Kinorhyncha have been recorded from Australia. Brown and Higgins (1983) collected and described *Kinorhynchus phyllotropis* Brown and Higgins, 1983 from sandy mud in estuaries running into Hunter Bay, Sydney Harbour.

Two years later, in a PhD thesis submitted to Macquarie University, Sydney, Brown (1985) described *Pycnophyes faveolus* Brown, 1985 from a locality nearby, and *Echinoderes teretis* Brown, 1985 from both Broken Bay and the Sydney Harbour estuaries. More recently, *Echinoderes cavernus* Sørensen *et al.*, 2000 and *Pycnophyes australensis* Lemburg, 2002 were described. *Echinoderes cavernus* was collected in a submarine cave north of Sydney (Sørensen *et al.*, 2000), whereas *P. australensis* inhabits a sandy beach on Magnetic Island (Lemburg, 2002).

Kinorhynch sampling from silty mud or sand sediments is often done with a meiobenthic dredge that can be pulled behind a boat or, in shallower water, by hand. It travels on two broad runners, between which an angled steel bar scrapes off several centimetres of sediment that is collected in a following canvas bag. The sampling depth into the sediment can be adjusted so that only the top layer with the highest kinorhynch densities is collected (see

Sørensen and Pardos (2008) for further details on various kinds of dredges). Less efficient, but still useable, alternatives to the Higgins' dredge are the anchor dredge, triangular dredge, or even a HAPS or McIntyre grab. However, whereas these collecting devices work well in soft or sandy sediments, they are not suitable at all in seaweed aggregations because they cannot penetrate the tight network of roots. Perhaps for this reason, the kinorhynch diversity in such substrates has rarely been explored. In the present contribution we demonstrate a method that enables collecting of samples from sediments under or within vegetation that cannot be sampled by a meiobenthic dredge. The collected samples yielded specimens of *Echinoderes teretis*, hence we can extend its known distributional range further south, along the East Coast of Australia.

METHODS

Collections of Kinorhyncha were made on the south eastern coast of Australia at Candlagan Creek, 247 km south of Sydney, close to the point where this small creek empties into the sea at Broulee. Collections were also made in the much larger Clyde River estuary, close to Batemans Bay, 235 km south of Sydney. Although a number of kinorhynch species were found only *E. teretis* was identified.

Several methods of collection were used. In the first, used for sea-grass sub-tidal beds in Candlagan Creek, a small scoop was lowered from a canoe, and dragged across the weed bed bringing up a collection of mud and sea-grass roots.

The cup-shaped metal scoop was 6.5cm in diameter at its opening, 4cm deep with an internal volume of 40.6cm³ (Fig. 1). The metal cup was suspended from a cylindrical metal weight, and this in turn was attached to a rope so that the cup and weight



Figure 1. The metal scoop used for sampling from sub-tidal sea-grass beds. Opposed to more commonly used meiofauna sampling devices, such as dredges, the robust metal scoop was able to penetrate the dense root web in the sea-grass beds, making it possible to sample this otherwise less accessible habitat

could be lowered from a canoe into the sub-tidal sea-grass beds in Candlagen Creek. The mud was washed through a 100 µm mesh brass sieve and then re-suspended in seawater. After a few minutes, the suspension was passed through a 60 µm mesh nylon sieve. When the sieve became clogged with fine detritus, the detritus was allowed to briefly dry in air, then inverted and back washed in to a 20cm diameter Petri dish.

The surface of the water in the Petri dish was searched under a dissecting microscope. Because of their hydrophobic cuticle, kinorhynchs, copepods and many nematodes become trapped in the surface film. If the re-suspension is done in seawater, the kinorhynchs repeatedly invert and evert their heads making it easier to find them in the surface film.

Kinorhynchs are picked up on an eyebrow hair (glued to a wooden stick) or an Irwin loop and transferred to 5% aqueous glycerol in a watch glass. The watch glasses were left for 48 h in a 40°C oven to evaporate water, and the specimens were mounted in anhydrous glycerol on glass microscope slides. Cover slips were supported with small glass beads (ballatini). The cover slips were held in place by ringing the cover slips with Glyceel (Gurr, Poole, England).

Samples from the tidal zone of Candlagen Creek, were taken from among the pneumatophores of *Avicennia marina*, by forcing a corer made from the barrel of a 20 ml plastic syringe into the mud. Extracting kinorhynchs from the mud followed the same procedure as that just described.

A quite different method of extracting kinorhynchs from mud uses centrifugal flotation in colloidal silicate. It has the advantage that mud samples do not need to be processed immediately. It can be used on mud cores fixed in 5% formalin in seawater and returned to a laboratory for later processing.

The colloidal silicate, Ludox Tm, is manufactured by Du Pont de Nemours, Delaware, USA, but they generally do not sell or ship it in small quantities. It is very widely used internationally for studies of meiofauna from muddy samples. One must therefore ask somebody in a large marine biological institution to donate some of the Ludox solution. Ours was obtained from M. Hodda, CSIRO Black Mountain Laboratories in Canberra, ACT.

A muddy core, containing much organic detritus, must be washed through a coarse sieve, a brass sieve. A mesh width of 100 µm is suitable. The filtrate is distributed to 15 ml glass centrifuge tubes and spun at 5000 rpm in a bench centrifuge for 5 minutes. The supernatant is discarded and the pellet is re-suspended and thoroughly dispersed in about 12 ml of diluted Ludox. The Ludox must be diluted to a specific gravity of 1.15 with distilled water. The specific gravity is critical and should be measured with a hydrometer. The tubes are spun again at 5000 rpm. This time the supernatant is retained and passed through a 60 µm mesh nylon sieve. The very small amount of material retained by the sieve is washed into a 12cm diameter Petri dish. Kinorhynchs trapped in the surface film are searched for under a dissecting microscope. Specimens found are then collected and mounted as described above.



Fig. 2. SEM *T. teretis* showing , head and first segment (zonite) and terminal setae

RESULTS

The collections show that *E. teretis* can occur in surprisingly large numbers in sea-grass beds of *Posidonia australis* (Table 1). They could not have been collected by a meiobenthic dredge or any other of the commonly used devices for kinorhynch collection, as these would have difficulties penetrating the dense beds of sea-grass. Several other species of kinorhynchs were collected from the mangrove mud, but these could not be identified. Also mangrove, *Avicennia maritima* produces dense fields of pneumatophores which would impede the dredge.

Kinorhynchs are usually considered inhabitants of mostly muddy, and to some extent sandy habitats, and a vast majority of the known diversity are reported from such habitats. However, a few studies have demonstrated their occurrence in different environments as well, including the coralline algae *Lithothamnion* or kelp holdfasts (Moore 1973; Higgins and

Kristensen 1988). Furthermore, Kirsteuer (1964) collected a then new species, *Echinoderes caribiensis* Kirsteuer, 1964 from mangroves in Venezuela. Our observations indicate that sea-grass beds represent another kinorhynch-inhabited habitat, and stress the importance of exploration of such atypical kinorhynch habitats. Further exploration of such habitats will most likely lead to an improved insight in kinorhynch biodiversity and distribution, and the collecting methods provided in the present contribution will make sea-grass bed inhabiting kinorhynch accessible for more future research.



Fig. 3. SEM showing *T.teretis* with head and first segment everted by fresh water.

Table 1. Details of *Echinoderes teretis* collections. Furthermore one poorly preserved specimen was found in sub-tidal mud from Lake Wellington in the Gippsland Lakes, while searching for nematodes.

Locality	Clyde River	Candlagan Creek	Candlagan Creek	Candlagan Creek	Candlagan Creek	Candlagan Creek
Collecting date	5.02.1985	28.12.2005	11.01.2007	14.01.2006	30.08.2006	10.02.2007
Habitat	Mangrove mud	Subtidal sea-grass roots	Subtidal sea-grass roots	Mangrove mud	Tidal sea-grass roots	Tidal sea-grass roots
Sample processing	Ludox flotation	Sieving	Sieving	Ludox flotation	Sieving	Sieving
No. of specimens	10 specimens	53 specimens	3 specimens	35 specimens	3 specimens	6 specimens

REFERENCES

- Brown, R. 1985. Developmental and taxonomic studies of Sydney Harbour Kinorhyncha. Ph.D. thesis, School of Biological Sciences, Macquarie University, Sydney, (193pp).
- Brown, R. and Higgins, R.P. 1983. A new species of Kinorhynchus (Homalorhagida, Pycnophyidae) from Australia with redescription and range extension of other Kinorhyncha from the South Pacific. *Zoologica Scripta*, **12**:161-169.
- Higgins, R.P. and Kristensen, R.M. 1988. Kinorhyncha from Disko Island, West Greenland. *Smithsonian Contributions to Zoology* **458**:1-56.
- Kirsteuer, E. 1964. Zur Kenntnis der Kinorhynchen Venezuelas. *Zoologischer Anzeiger* **173**:388-393.
- Lemburg, C. 2002. A new kinorhynch *Pycnophyes australensis* sp. n. (Kinorhyncha: Homalorhagida: Pycnophyidae) from Magnetic Island, Australia. *Zoologischer Anzeiger* **241**:173-189.
- Moore, P.G. 1973. *Campyloderes macquariae* Johnston, 1938 (Kinorhyncha: Cyclorhagida) from the northern hemisphere. *Journal of Natural History* **7**:341-354.
- Sørensen, M.V., Jørgensen, A. and Boesgaard, T.M. 2000. A new *Echinoderes* (Kinorhyncha: Cyclorhagida) from a submarine cave in New South Wales, Australia. *Cahiers de Biologie Marine* **41**:167-179.
- Sørensen, M.V. and 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.