New Zealand Journal of Marine and Freshwater Research, 2005, Vol. 39: 973–980
0028–8330/05/3904–0973

Observations on Gastrotricha from a sandy beach in southeastern Australia, with a description of *Halichaetonotus australis* sp. nov. (Gastrotricha, Chaetonotida)

WARWICK NICHOLAS
Division of Botany and Zoology
Australian National University
Canberra 0200
ACT, Australia
email: warwick@webone.com.au

M. ANTONIO TODARO
Dipartimento di Biologia Animale
Università di Moderna
via Università 4
I-41100 Moderna, Italy

Abstract Several species of Gastrotricha were found among samples of nematodes extracted from a sandy ocean beach, South Broulee beach, in southeastern Australia. One of these Gastrotricha turned out to be a species new to science which is described as *Halichaetonotus australis* sp. nov. in this study. Although the spines of the hydrofoil scales resemble those of the cosmopolitan *H. aculifer*, a distinctive feature of the new species is the presence of three prominent posterior dorsal spines. These are exaggerations of the keels found on the dorsal overlapping scales that cover the dorsal surfaces of the head, neck, and trunk. The spines are almost as long as the rami of the terminal furca. *H. australis* sp. nov. also resembles *H. marivagus*, but differs in lacking both a cephalion and hypostomion. Freeze drying is introduced as a preparation method for scanning electron microscopy of Gastrotricha.

Keywords Australia; Gastrotricha; Chaetonotida; *Halichaetonotus*; taxonomy; meiofauna; SEM method

M04153; Online publication date 17 June 2005
Received 15 November 2004; accepted 18 March 2005

INTRODUCTION

Apart from three papers by Hochberg (2002a,b, 2003), the marine gastrotrich fauna of Australia is unknown. These papers respectively described two species of Turbanellidae, two species of Thaumastodermatidae, and two species of Dactylopodolidae; all six from a sandy beach on and around Stradbroke Island, Queensland.

Gastrotrichs in the present study were found fortuitously when extracting nematodes from a sandy ocean beach in southeastern Australia in the course of a long-term study of marine nematodes (Nicholas 2001). The specific identity of most of the gastrotrichs, belonging to at least five genera, has yet to be determined. However, specimens belonging to the genus *Halichaetonotus* proved to be a previously undescribed species, and are described in this paper. We also present freeze-drying as a new method of preparing gastrotrichs for scanning electron microscopy (SEM), which facilitates the application of SEM for taxonomic studies of very small delicate meiofauna.

METHODS

All samples came from South Broulee beach, New South Wales, 300 km south of Sydney (35°55′S, 150°93′E). Samples of c. 2 litres of wet sand were dug up with a spade at mid-tide level at low tide. Samples were immediately transported to the laboratory for extraction of meiofauna. The sand was suspended in 5 litres of tap water (to paralyse the specimens). After several seconds to allow the sand to settle, the supernatant water was rinsed through a 60 µm mesh nylon sieve. Meiofauna retained on the sieve was back washed with sea water into a Petri dish allowing the meiofauna to recover normal movement. The procedure was repeated three times. Individual gastrotrichs were carefully extracted from the samples with a very fine pipette under a dissecting microscope, and fixed in 5% formalin in sea water.

For light microscopy, individual gastrotrichs were transferred to 5% aqueous glycerol in a solid watch glass. Water in the watch glass was evaporated in a drying oven at 40°C for 48 h and the specimens were mounted on glass slides in anhydrous glycerol. Cover slips were supported with glass beads (Ballatini) and ringed with glyceel (Gurr®). Measurements were made using a micrometer eyepiece at 65× or 100× magnification under oil immersion. The positions of different measurements were recorded as U, i.e., the distance along the body as a percentage of length from head end to caudum.

Specimens for SEM were washed in distilled water and freeze dried on 4.5 µm pore size Millipore filter membrane (10 µm diam.). The membrane was supported on filter paper in an open Petri dish, so that water could drain through the membrane into the filter paper. When the membrane was nearly dry, and contained several specimens near its middle, it was transferred to the flat top of a cold solid metal cylinder. The cylinder was pre-cooled by partial immersion in liquid nitrogen. The water on the filter froze instantly, and the filter was subsequently transferred to a pre-cooled metal cup in a vacuum freeze dryer. The specimens were freeze-dryed under vacuum c. 38 mPa, –25°C for c. 24 h. Following freeze drying the filter was affixed to a metal stub with double-sided adhesive tape. The specimens were coated with gold palladium and examined and photographed in a scanning electron microscope (Cambridge 360).

TAXONOMY

Order Chaetonotida Remane, 1925 [Rao & Clausen, 1970]
Suborder Paucitubulatina d'Hondt, 1971
Family Chaetonotidae Zelinka, 1889
Genus *Halichaetonotus* (Remane, 1936) Schrom, 1972
Type species *Halichaetonotus pleuracanthus* (Remane, 1926)

Halichaetonotus australis sp. nov.

Fig. 1–2 (drawings) and 3 (scanning electron micrographs), measurements Table 1.

Diagnosis

Medium-sized *Chaetonotida* head, neck, and trunk well defined; head rounded, lacking cephalion and hypostomion; a medium-long furca projects from the posterior of the trunk. Body enveloped by 13 columns (7 dorsal, 2 + 2 ventrolateral hydrofoil, 1+1 ventral small) each with 17–18 alternating oval scales. Keels on head scales extend beyond the edge of the scales as short spiny processes; keels on dorsal trunk scale do not extend beyond edge of scales except on three posterior scales, one median and two lateral, forming long robust spines extending beyond end of trunk; keels on dorsal scales aligned along anterior-posterior axis, two small keeled scales on dorsal base of each furca branch.

Ventrolaterally two columns of hydrofoil scales with lamellaeform spines of varying length, medial to hydrofoil scales; a further column of smaller scales bearing spines and two perianal ovoid keeled scales; ventrally two bands of locomotory cilia, interciliary ventral field naked.

Almost circular mouth opens into muscular pharynx, then sack-like intestine, short rectum, and terminal anus. All specimens observed parthenogenetic, sometimes with single large egg dorsal to mid intestine.

Material examined

Six specimens were examined by light microscopy and four specimens by SEM. All specimens were collected by W. Nicholas from South Broulee beach (35°55′S, 150°93′E). between December 2003 and April 2004.

All measurements were made with an ocular eyepiece using either 65× or 100× oil immersion objectives.

Type specimens

Holotype and five paratypes were deposited in Australian National Insect Collection, Nematode Collection (ANIC), CSIRO Entomology, GPO Box 1700, Canberra, ACT, Australia.

Holotype Slide 502 number 19461 (Table 1, columns 2 and 3).

Paratypes Slide 502 number 198462; Slide 503 numbers 19463, and 19464; Slide 504 number19465 (Table 1, columns 4–8).

Holotype

Measurements in Table 1 column 2 from dorsal aspect, column 3 the same holotype specimen from lateral aspect (after the specimen had rotated to lie on its side, the stable condition). The head, trunk, neck, and posterior end are proportionally wider in dorsal view compared with lateral view. Fig. 1A shows the holotype from dorsal aspect.

The ventral almost circular, slightly protuberant mouth (Fig. 3B,C) carries a single row of tiny knobs along rim; a tuft of short sensory cilia lie anterior to

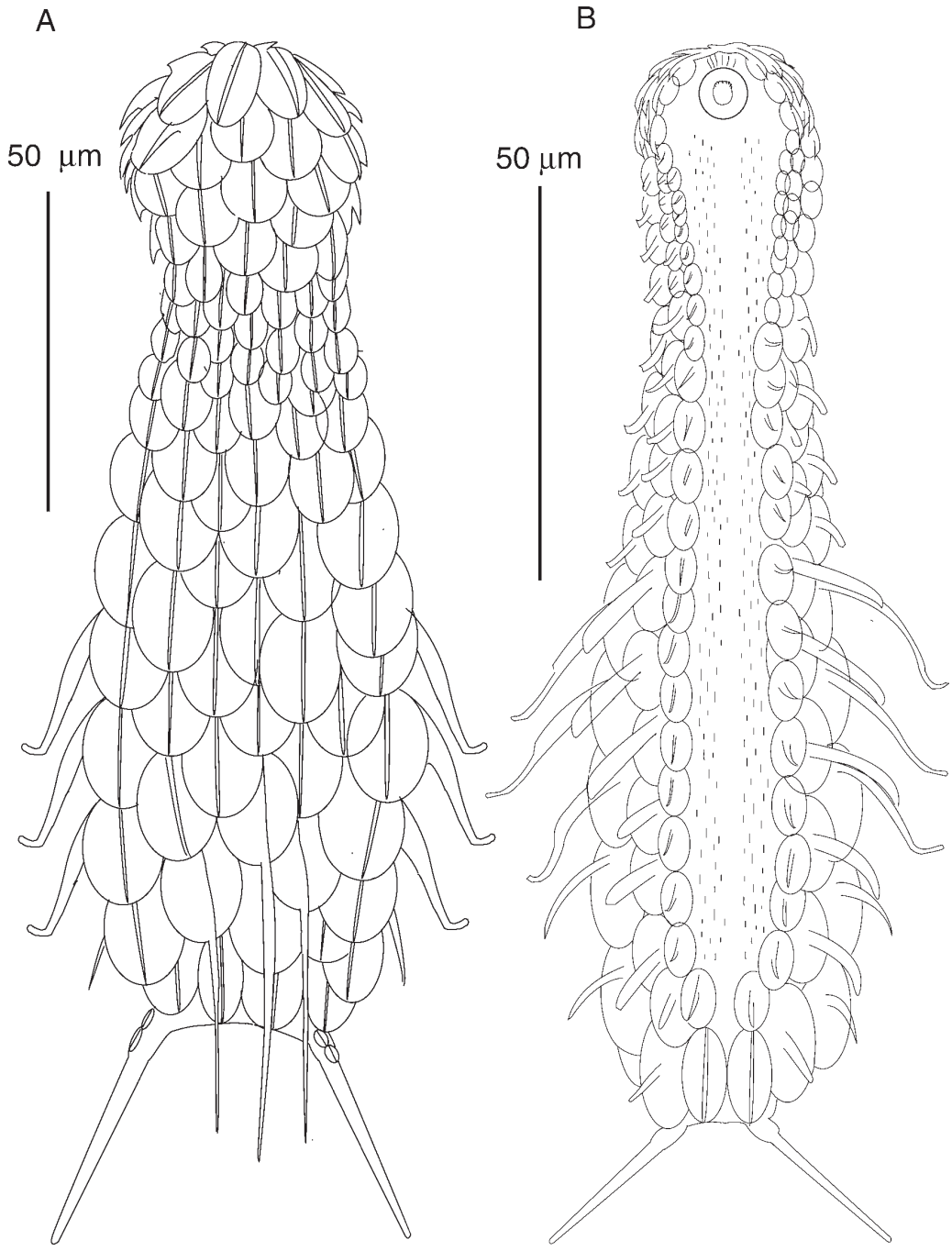

Fig. 1 *Halichaetonotus australis*, sp. nov. **A**, dorsal view; **B**, ventral view. The furcal rami in B appear shorter than their true length in this camera lucida drawing: they are foreshortened by the angle between the rami and the axis of the trunk.

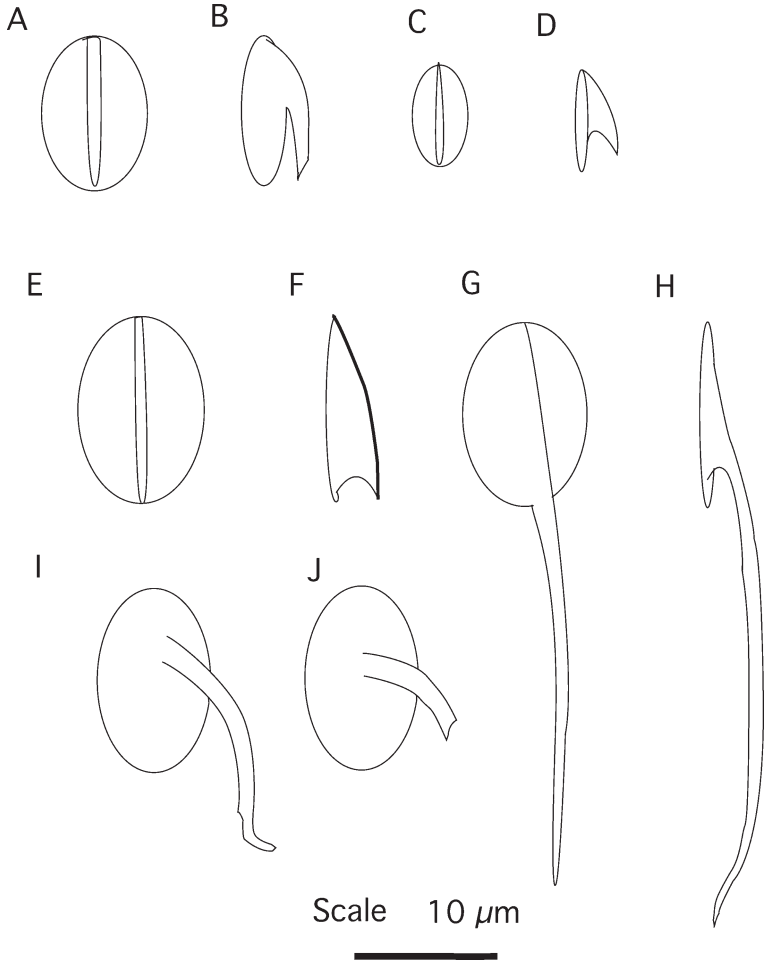

Fig. 2 Scales from different parts of the body of *Halichaetonotus australis*, sp. nov. **A** and **B**, head scales; **C** and **D**, ventral scales; **E** and **F**, dorsal trunk scales; **G** and **H**, median posterior dorsal scale with long spine; **I** and **J**, hydrofoil scales.

mouth. Head oval, slightly elongated along anterior/posterior axis, without pleural lobes and visible hypostomion; neck narrower, trunk sac-like, terminating in a furcate caudum.

Body widths at the head/neck/trunk/caudum are 38, 29, 54, and 27 μm, at U10, 25, 73, and 100, respectively. Caudum of medium length, paired laterally divergent furcal branches with slightly swollen bases, surmounted by two keeled scales.

Cuticular armature Head, neck, and trunk covered dorsally and ventrolaterally by alternating columns (7 dorsal, 2 + 2 ventrolateral hydrofoil, 1+1 ventral) of 17–18 overlapping, oval scales. Head scales are 10 × 7.5 μm, neck scales 7 × 5 μm, and dorsal trunk scales 13 × 9 μm. Keels on dorsal scales extend the full anterior-posterior axis of scale, but (with the following exceptions) not beyond the edge of the scale. The keels on each column are aligned along the anterior-posterior axis giving the appearance of raised ridges (Fig. 3A). On head scales, keels protrude beyond posterior margin as a short spine (Fig. 3D). On one median and two lateral posterior trunk scales, at U82 and U86, respectively, the keels form robust, very long spines projecting 30 μm beyond the scales. Ventrolateral hydrofoil scales bear flattened lamellae of increasing length from anterior to posterior, 7.5 μm to 25 μm; with each scale possessing a narrow, crescent-shaped lamella over its proximal 4/5. On either side of the posterior trunk region, the three longest hydrofoil lamellae extend beyond the lateral body margins (Fig. 3A). Two very small keeled scales, 3–4 μm

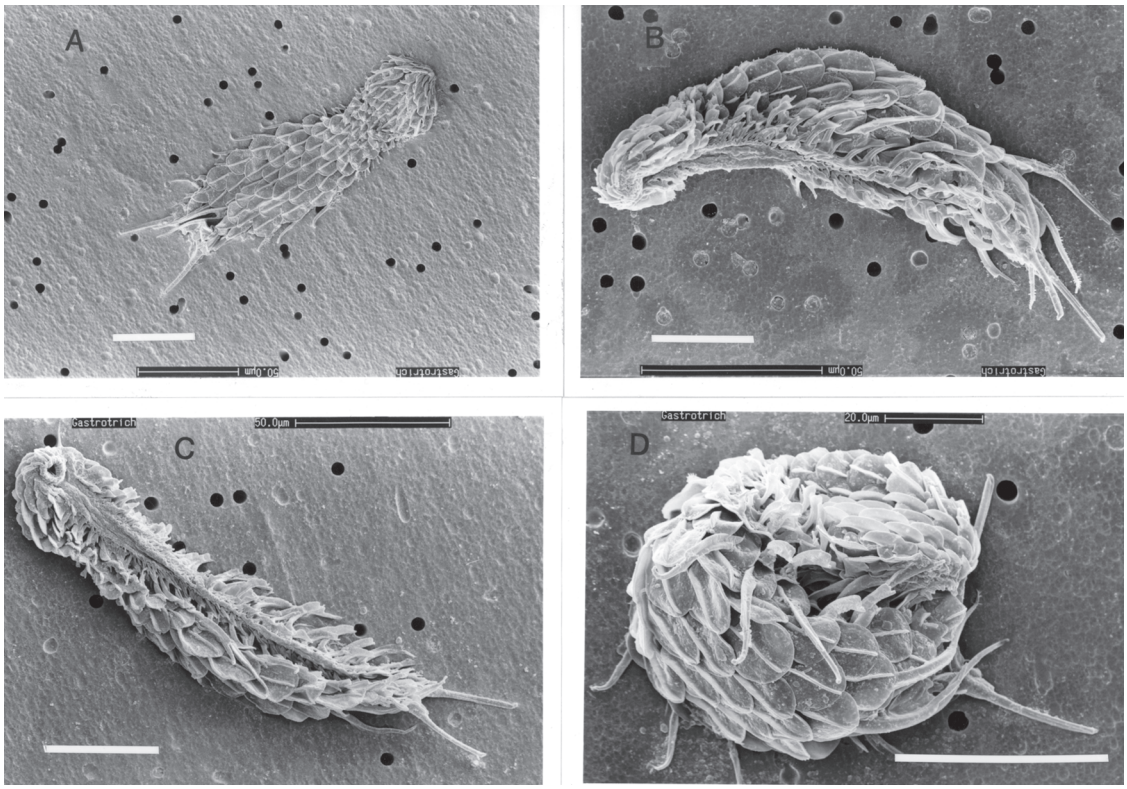

Fig. 3 Scanning electron micrographs of *Halichaetonotus australis* sp. nov. **A**, dorsal view; **B**, lateral view; **C**, lateral view; **D**, strongly curved specimen to show dorsal, cephalic, and hydrofoil scales. Scale bars: A–C 40 µm; D 50 µm.

Table 1 Measurements of holotype, and 5 paratypes of *Halichaetonotus australis* sp. nov. Measurements in µm.

Type:	Holo		Para	Para	Para	Para	Para
Aspect	Dorsal	Lateral	Dorsal	Ventral	Lateral	Lateral	Lateral
Length	153	151	128*	138	150	157	141
Head to neck	40	41	48	34	42	48	33
Pharynx	30	33	33	39	32	39	41
Longest dorsal spine	46	39	29	43	33	39	39
Longest hydrofoil lamella	18	22	–	23	24	24	20
Furca	38	33	–	27	32	37	30
Head width	38	35	30	24	24	24	35
Neck width	29	33	21	18	18	17	33
Trunk max.	54	36	39	37	38	33	36
Width at anus	22	15	30	15	18	18	15
Egg, length × width	67 × 25	–	none	none	none	48 × 23	45 × 23
Neck/length%	26	27	38	24	23	28	23
Pharynx/length%	20	22	25	28	21	25	29
Furca/length%	25	22	–	22	21	21	21
Head width/length	30	26	23	31	22	11	28
Trunk width/length%	35	24	31	27	25	21	26

*Measurements influenced by curvature leading to foreshortening.

long, lie on the enlarged soft base of the furcal rami. Interciliary field naked except for a couple of oval keeled scales (12 × 5.5 µm) in the perianal region.

Paired longitudinal rows of locomotory cilia extend along the ventral surface, poorly resolved by SEM, and difficult to observe by light microscopy except in living specimens. The anterior and posterior extent of the bands have not been clearly determined.

Digestive tract The mouth of medium size, c. 10 µm in diameter, projects very slightly, and is surrounded by a ring of tiny spherical knobs (Fig. 3B,C); leads successively into a cylindrical muscular pharynx, showing a slight bulb anteriorly; pharynx connected to a sack-like intestine at pharyngo-intestinal junction at U18–22; intestine occupying most of trunk, except where compressed by a very large egg, short cylindrical rectum, terminal anus.

Paratypes

Five paratypes are listed, measurements in Table 1 columns 4–8, one from dorsal aspect, one from ventral (see Fig. 1B), and three from lateral aspect. Four other specimens in Fig. 3A–D are scanning electron micrographs.

The paratypes are similar to holotype, but vary in length from 128 to 153 µm. Cuticular armature the same as holotype. Dorsal spines slightly curved making exact measurements difficult, 33–39 µm long at U78–89. Three posterior ventrolateral hydrofoil lamellae consistently project beyond lateral margins of the trunk.

Reproductive tract Most of the specimens were in parthenogenetic phase with single ovary of few cells, holotype and two paratypes with large eggs filling much of trunk.

Differential diagnosis Very varied cuticular armature distinguishes the 25 species of *Halichaetonotus* described so far. The new species most closely resembles *H. marivagus*, Balsamo, Todaro & Tongiorgi, 1992, in that both species are distinguished by possessing three dorsal spines close to the posterior of the trunk. However, these are much less developed in *H. marivagus* than in *H. australis*. *H. marivagus* also differs by possessing both a cephalion and hypostomion, absent in *H. australis*.

The new species also resembles *H. aculifer* (Gerlach, 1953) based on size, and most importantly the shape of the hydrofoil scales. However, the presence of three long spines on the posterior trunk and the absence of ventral interciliary field scales in *H. australis* are features that can easily differentiate this species from *H. aculifer* (see Gerlach, 1953). There are additional differences between *H. australis* sp. nov. and *H. aculifer*, as redescribed by Kisielewski (1988), notably the presence in the latter species of a ventral polygonal hypostomal plate, the anterior margin of which adheres to the mouth ring.

Type locality

Among sand grains on a high-energy ocean beach at mid-tide level on South Broulee beach, New South Wales, Australia (35°55′S, 150°93′E).

Etymology

The specific name alludes to the only geographic region from where the new species has been found to date.

DISCUSSION

It is surprising that species from five genera of gastrotrichs were found in samples designed to extract nematodes from a single location on one beach. Two of the genera belong to the Macrodasyida, i.e., *Turbanella* and *Tetranchyroderma*, and three to the Chaetonotida, i.e., *Aspidiophorus*, *Heteroxenotrichula*, and *Halichaetonotus australis*. Two species of *Tetranchyroderma* were commonly observed among extracted nematodes from South Broulee beach and several nearby beaches (Nicholas 2001). When several monthly samples from South Broulee beach were carefully examined the other genera were of regular occurrence, but in very small numbers, probably because the methods used were not designed to collect fauna as small as the representatives of these genera. In particular, the use of sieves during the extraction process may have contributed to the loss of gastrotrichs from the samples (Todaro et al. 1995). Moreover, osmotic shock could have destroyed soft bodied taxa like most non-thaumastodermatid Macrodasyida.

Only one of these genera, *Turbanella*, possibly the same species, was included in the two genera described by Hochberg (2002a) from more northerly beaches in Queensland, Australia.

Many species known from the Northern Hemisphere have very wide geographical distributions, often on both sides of the North Atlantic and Mediterranean Sea. *H. decipiens*, for example, has been found in The North Sea, English Channel, Baltic Sea, Atlantic coasts of France and the United States, and the Black Sea (cf. Balsamo et al. 1992). It is perhaps significant that an abundant species like

H. australis sp. nov. has not been found in numerous investigations in the Northern Hemisphere, nor along the Indian coasts (Naidu & Rao 2005). However, the wide distribution of the known species makes it likely that the new species also has a wide distribution, but, apart from Hochberg's (2002a,b, 2003) three papers on Australian gastrotrichs, nothing is known of the marine gastrotrich fauna of the South Pacific and Indian Oceans. The greatest impediment to studying the gastrotrichs of these ocean coasts is, perhaps, the difficulty of obtaining the early European taxonomic literature outside European libraries.

Since the pioneering work by Luporini & Tongiorgi (1972), SEM has been widely applied to gastrotrich research. In most studies, critical point drying with carbon dioxide has been used for dehydration before SEM (e.g., Ruppert 1978; Balsamo 1981; Balsamo & Todaro 1987; Todaro 1992, 1998, 2002; Clausen 2000; Hochberg 2001; Lee & Chang 2003). As an alternative, chemical dehydration by means of hexamethyldisilazane has been used (Fregni 1998; Hochberg & Litvaitis 2000). In both methods, the minute dried specimens are individually attached to a metal stub. We can now add the method of freeze drying, as described by us, to the former methods. As our method involves a membrane to which several specimens are attached, it minimises the risk of specimen loss.

ACKNOWLEDGMENT

We are indebted to the Electron Microscope Unit of the Australian National University for the use of their facilities.

REFERENCES

Balsamo M 1981. Gastrotrichi della Toscana: il lago di Sibolla. Bollettino del Museo Civico di Storia Naturale di Verona 7: 547–571.

Balsamo M, Todaro MA 1987. *Aspidiophorus polystictos*, a new marine species (Gastrotricha, Chaetonotida) and its life cycle. Bollettino di Zoologia 54: 147–153.

Balsamo M, Todaro MA, Tongiorgi P 1992. Marine gastrotrichs from the Tuscan Archipelago (Tyrrhenian Sea): II. Chaetonotida, with description of three new species. Bollettino di Zoologi 59: 487–498.

Clausen C 2000. Gastrotricha Macrodasyida from the Tromso region, northern Norway. Sarsia 85: 357–384.

Fregni E 1998. The spermatozoa of macrodasyid gastrotrichs: observations by scanning electron microscopy. Invertebrate Reproduction and Development 34: 1–11.

Gerlach S 1953. Gastrotrichen aus dem Küstengrundwasser des Mittelmeeres. Zoologischer Anzeiger 150: 203–211.

Hochberg R 2001. A special form of sensory cilia in *Lepidodermella squamata* (Gastrotricha : Chaetonotida). Ophelia 55: 137–139.

Hochberg R 2002a. Two new species of Turbanellidae (Gastrotricha: Macrodasyida) from a high-energy beach on North Stradbroke Island, Australia. New Zealand Journal of Marine and Freshwater Research 34: 311–319.

Hochberg R 2002b. Two new species of *Pseudostomella* (Gastrotricha: Macrodasyida) with scaled triancres from the east coast of Australia. New Zealand Journal of Marine and Freshwater Research 36: 571–579.

Hochberg R 2003. Two new species of *Dactylopodola* (Gastrotricha, Macrodasyida) from islands off the Queensland coast, Australia. Meiofauna Marina 12: 37–46.

Hochberg R, Litvaitis MK 2000. Hexamethyldisilazane for scanning electron microscopy of Gastrotricha. Biotechnology and Histochemistry 75: 41–44.

Kiesielewski J 1988. New records of marine Gastrotricha from the French coasts of Manche and Atlantic. II Chaetonotida, with description of four new species. Cahiers de Biologie Marine 29: 187–213.

Lee JM, Chang CY 2003. Two new marine gastrotrichs of the genus *Ptychostomella* (Macrodasyida, Thaumastodermatidae) from South Korea. Zoological Science 20: 481–489.

Luporini P, Tongiorgi P 1972. Observation au microscope a balayage de quelques gastrotriches chaetonotoides. Cahiers de Biologie Marine 13: 299–303.

Naidu KV, Rao GC 2005. Fauna of India: Gastrotricha. Zoological Survey of India.

Nicholas WL 2001. Seasonal variations in nematode assemblages on an Australian temperate beach; the effect of heavy seas and unusually high tides. Hydrobiologia 464: 17–26.

Ruppert EE 1978. The reproductive system of gastrotrichs. II. Insemination in Macrodasys: a unique mode of sperm transfer in Metazoa. Zoomorphologie 89: 207–228.

Todaro MA 1992. Contribution to the study of the Mediterranean meiofauna: Gastrotricha from the island of Ponza, Italy. Bollettino di Zoologia 59: 321–333.

Todaro MA 1998. Meiofauna: Il popolo della sabbia. Otello De Batte ed. Livorno. 79 p.

Todaro MA 2002. An interesting new gastrotrich from littoral meiobenthos (Long Beach Island, USA), with a key to species of *Tetranchyroderma* (Gastrotricha : Macrodasyida). Journal of the Marine Biological Association of the United Kingdom 82: 555–563.

Todaro MA, Fleeger JW, Hummon WD 1995. Marine gastrotrichs from sand beaches of the northern Gulf of Mexico: species list and distribution. Hydrobiologia 310: 1207–117.