

## Two new species of *Tetranchyroderma* (Gastrotricha, Macrotrichida) from a sandy beach in southeastern Australia

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**Abstract** Two new species of *Tetranchyroderma* are described from a sandy beach in southeastern Australia. Both species belong to the subgroup of the genus in which the dorsal surface of the body is covered with pentancretes and in which head sensorial organs (tentacles and/or pestle organs) are absent. *Tetranchyroderma pentaspersus* sp. nov. is small, 178–244  $\mu\text{m}$  long, and distinguished by possessing exceptionally large pentancretes, c. 30 rows, with the central tine c. 15  $\mu\text{m}$  long on the trunk, though shorter on the head; lateral tines c. 60% as long. The adhesive apparatus consists of four pairs of anterior adhesive tubules (TbA), nine pairs of ventro-lateral tubules (TbL), and eight posterior tubules (TbP), with two of the latter at the end of each caudal pedicle and four very small in between the caudal pedicles; dorsal and ventral tubules and “cirrata” are absent. *Tetranchyroderma australiense* sp. nov. is larger, 330–390  $\mu\text{m}$  long, possesses c. 70 rows of smaller pentancretes, with all five tines of equal length. The adhesive apparatus consists of four pairs of TbA, eleven pairs of TbL, one pair of dorsal adhesive tubules (TbD), and four TbP, the

latter equally distributed at the end of two caudal pedicles; ventral tubules and cirrata are absent. Possible synonymy between the new species and *Tetranchyroderma* sp. 7 of Valbonesi & Luporini (1988) from Somalia is discussed.

**Keywords** Australia; Gastrotricha; Macrotrichida; *Tetranchyroderma*; taxonomy; meiofauna; sandy beach; SEM

### INTRODUCTION

The Gastrotricha fauna of Australia, Southeastern Asia, New Zealand, and the adjacent Islands of the South Pacific is almost unknown. As an exception, Hochberg (2002a,b, 2003) described six new species from three families, collected from sandy beaches in Queensland, Australia. These comprised two species of Turbanellidae, two species of Thaumastodermatidae, and two species of Dactylopodidae (all belonging to the Macrotrichida). In addition, Nicholas & Todaro (2005) described *Halichaetonotus australis* (Chaetonotida) from a beach in New South Wales, Australia. They reported that at least five genera of Gastrotricha were common in the intertidal zone of two adjacent beaches. This paper describes two new species of *Tetranchyroderma* from South Broulee beach. Extensive use has been made of scanning electron microscopy (SEM) to describe the surface structures, which are sometimes very difficult to unambiguously describe with light microscopy because of their small size and superimposition of the complicated cuticular armature.

### MATERIALS AND METHODS

Sand was collected at low tide at the mid tide level from South Broulee beach in New South Wales, about 300 km south of Sydney (35°55'S, 150°53'E). Samples of c. 2 litres of wet sand were collected by forcing a plastic (PVC) tubular corer (20 cm long, 9 cm internal diam.) into the sand. The specimens

described in this paper were collected between 29 March and 1 April 2005. The methods used to recover gastrotrichs and to prepare them for examination have been described by Nicholas & Todaro (2005). Briefly, the specimens were recovered from samples of c. 2 litres of sand by repeated suspension in tap water (which paralyzes meiofauna), collection on a 60- $\mu\text{m}$  nylon sieve and back-washing into sea water (in which specimens recover normal movement). As fixative, 5% formalin in sea water was used; specimens were gradually transferred to anhydrous glycerol, in which they were mounted on glass slides under cover slips supported with small glass beads (Ballatini), and ringed with Gurr<sup>®</sup> glyceel (BDH Chemicals Ltd, Poole, England).

Some specimens were examined unstained, others were stained with rose bengal or cotton blue dissolved in glycerol, sufficient to very lightly colour the glycerol. Rose bengal improves the definition of the internal organs, cotton blue gives contrast for photomicroscopy, but unstained specimens were preferred for studying the cuticular armature and glandular tubules. Drawings were made using a camera lucida and measurements with a micrometer eye piece at a magnification of 65 $\times$ . The specimens tended to lie on their sides and were usually curved dorso-ventrally to varying degrees. The body length measured along the convex dorsal surface was greater than that along the concave ventral surface. Measurements along the median line accurately equal the mean of the two measurements. The position of body structures, expressed as percentages of body length, U, are distances along the median line from the anterior dorsal tip of the hood. In describing the new species, the terminology and abbreviations of Hummon et al. (1993) and Todaro (2002) are used.

For SEM, formalin fixed specimens were washed in distilled water and freeze dried on a millepore membrane. The method is fully described by Nicholas & Todaro (2005). The freeze-dried specimens, mounted on the membrane, were coated with gold/palladium and photographed in a Cambridge 360 scanning electron microscope.

## TAXONOMY

Order Macrodaysida Remane, 1925 (Rao & Clausen, 1970)  
 Family Thaumastodermatidae Remane, 1926  
 Subfamily Thaumastodermatinae Ruppert, 1978  
 Genus *Tetranchyroderma* Remane, 1926

## *Tetranchyroderma pentaspersus* sp. nov.

Fig. 1 (drawings) and 2 (SEM photomicrographs); measurements Table 1.

### Diagnosis

Small, c. 178–244  $\mu\text{m}$  long; body almost cylindrical, slightly convex dorsally, almost flat ventrally; large thin-walled oral hood. Dorsal surface covered with very large pentancre, with the central tine substantially longer than four slightly incurved peripheral tines. Six ventro-anterior adhesive tubules (TbA) adjacent to the mouth rim, two more slightly posterior; nine pairs of ventro-lateral tubules (TbL), two pairs of very small posterior tubules (TbP) behind anus between pedicles. Caudal pedicles end in two adhesive tubules with fleshy portion covered with pentancre. Ventral field has transverse rows of long locomotor cilia. Cephalic tentacles, cirrata, pestle organs, and dorsal adhesive tubules (TbD) are absent. From mouth, the cylindrical pharynx leads into the intestine that terminates at posterior-ventral anus. About eight pairs of granular epidermal glands lie along each side of the trunk. Hermaphroditic; with single female and male gonads.

### Material examined

Twenty-one specimens were examined by light microscopy, but only six were considered suitable for type specimens, and two further specimens were examined by SEM. All specimens were collected by W. Nicholas from South Broulee beach.

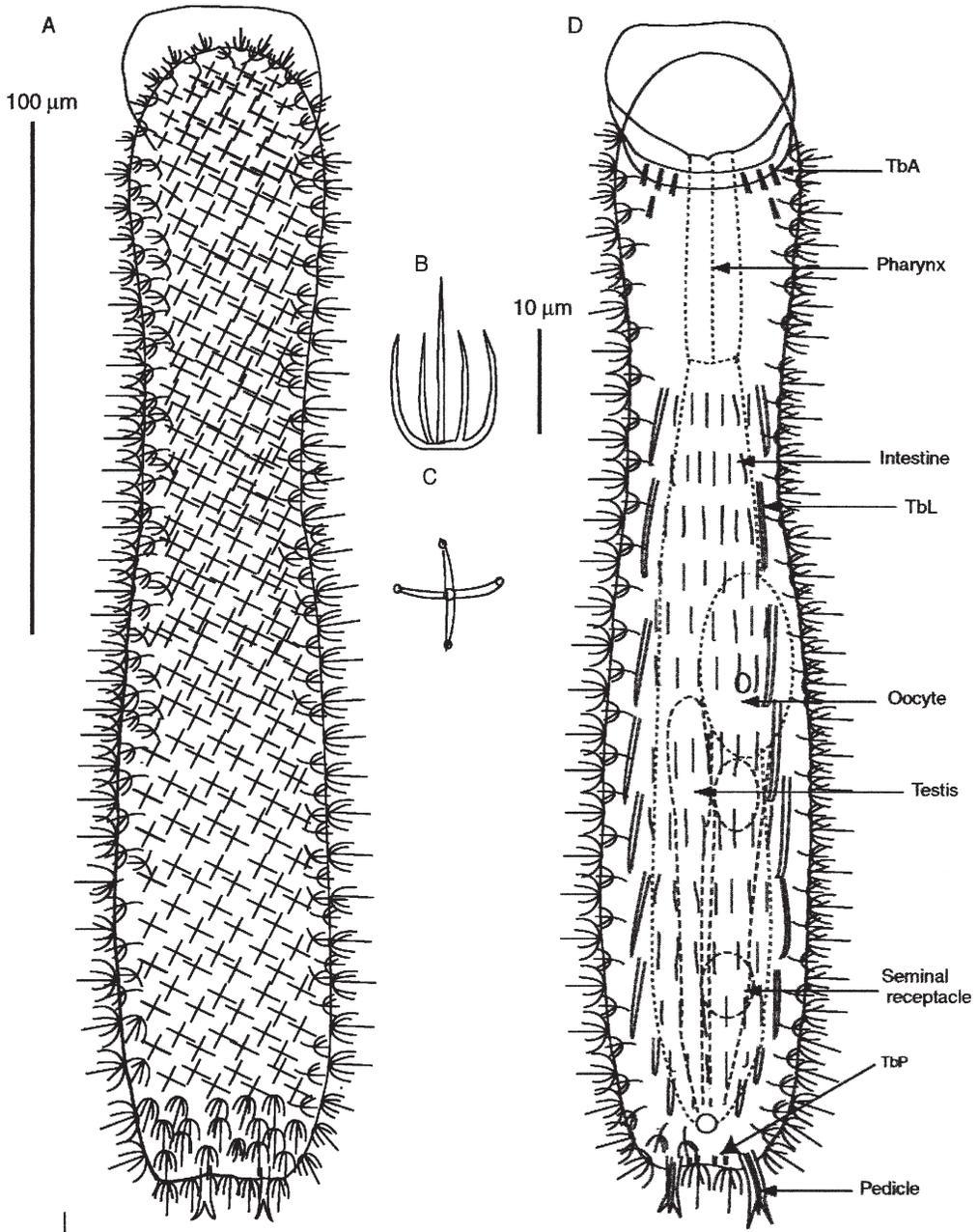
Holotype and five paratypes have been deposited in the Australian National Insect Collection, Nematode Collection (ANIC), CSIRO Entomology, GPO Box 1700, Canberra, ACT, Australia.

**Holotype** Slide ANIC 508 no. 19472. Table 1, column 3.

**Paratypes** Slides ANIC 508 nos. 19473–19475; slide 509 no. 19476 and slide 510 no. 19477.

### Description of holotype

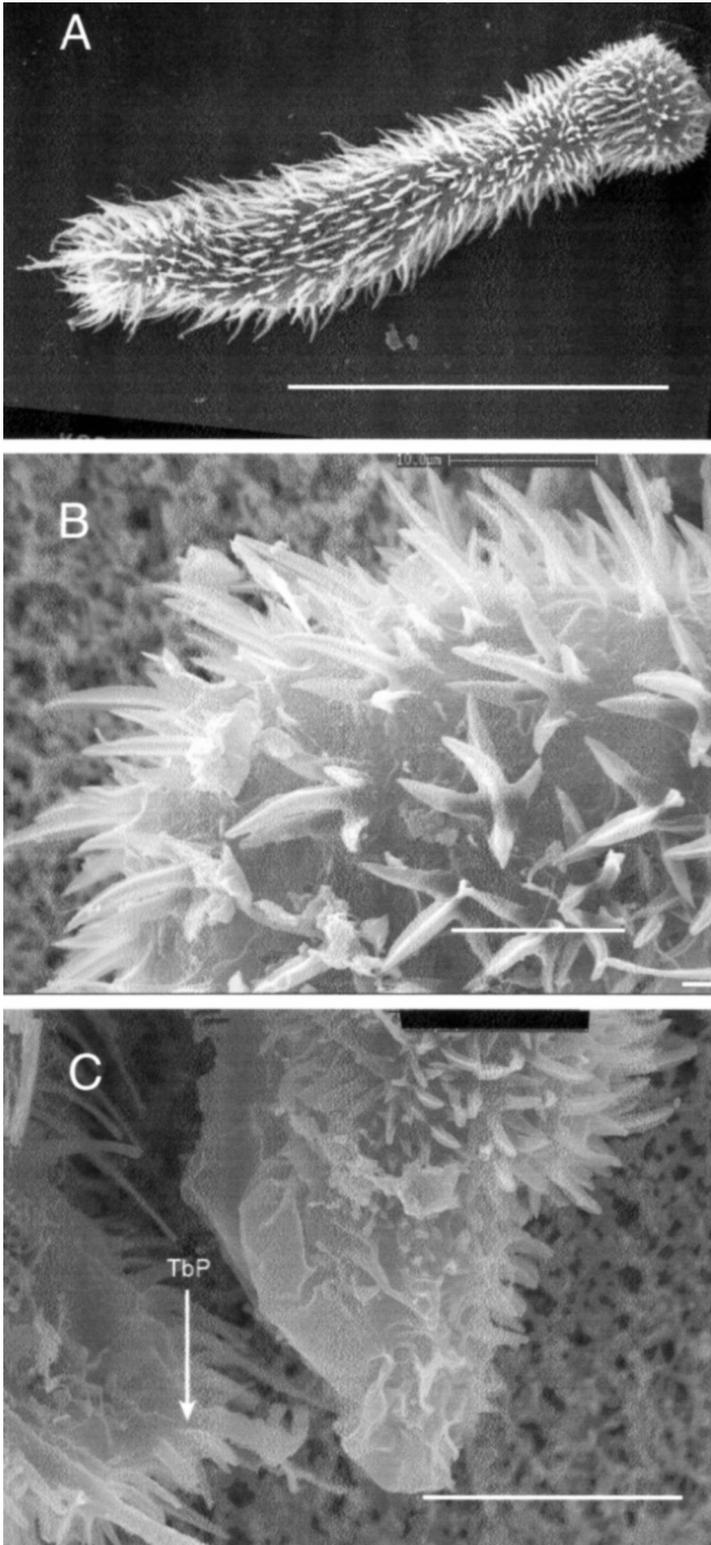
Small, 232  $\mu\text{m}$  long, lies on its side, partly curled up; anteriorly large oral hood projects 7  $\mu\text{m}$  in front of body. Dorsal surface covered by pentancre, each with slightly curved central and four shorter peripheral tines. On the trunk the pentancre are very large, with a central tine up to 15  $\mu\text{m}$  long and lateral tines c. 60% as long. Close to the head they become smaller, with a central tine 3.5  $\mu\text{m}$  long. Pentancre arranged in eight columns of c. 35 rows, but exact number is ambiguous because alignment in rows is not precise, with positions staggered



**Fig. 1** *Tetranchyroderma pentaspersus* sp. nov. **A**, dorsal view; **B** and **C**, pentancretes; **D**, ventral view. (TbA, anterior adhesive tubules; TbL, lateral adhesive tubules; TbP, posterior adhesive tubules.)

in adjacent columns. Peripheral pentancretes lie on ventro-lateral surface of the trunk, but outside the ventral field, which is bordered by TbL. The ventral field possesses transverse rows of locomotor cilia.

There are three TbA on each side of the ventral mouth rim, with a fourth tubule located slightly further behind the rim. Nine pairs of TbL on each side of the trunk, starting at U30 and evenly spaced



**Fig. 2** *Tetranchyroderma pentaspersus* sp. nov. SEM photomicrographs. **A**, dorsal view of entire specimen; **B**, pentancre in lateral view; **C**, head and hind end, juxtaposed, in curled up specimen. (TbP, posterior adhesive tubules between pedicles.) Scale bars: A, 100  $\mu$ m; B, 20  $\mu$ m; C, 20  $\mu$ m.

up to U94, at the rounded posterior of the trunk. The tubules become longer in the middle and shorter towards the hind end of the trunk. The caudum is slightly indented between two 21- $\mu\text{m}$ -long caudal pedicles, each of which ends with two adhesive tubules backed by a dorsal spine, and with their proximal portions covered dorsally with pentancrees. Four very short tubules, TbP, lie between the pedicles. About eight granular epidermal glands are present on each side of the trunk.

Cylindrical pharynx, 41  $\mu\text{m}$  long, leads into the intestine; pharyngo-intestinal junction at U35; intestine terminates at the postero-ventral anus at U96, placed slightly anterior to and between the pedicles. Hermaphrodite with single female and male gonads dorso-laterally in the posterior half of the trunk. Sexual accessory organs, i.e., frontal and caudal organs, not observed.

### Description of paratypes

Light microscopic measurements of five specimens and SEM measurements of one specimen in Table 1.

Paratypes vary from 197 to 235  $\mu\text{m}$ , the two specimens examined by SEM measured 178  $\mu\text{m}$  and 244  $\mu\text{m}$  in length, respectively. The orientation of the second SEM specimen, with its dorsal surface uppermost did not provide a useful list of measurements. The four TbP, situated between the

pedicles, were very difficult to see because they were hidden by the pentancrees, except when viewed by SEM. An egg is visible in the trunk of three of the paratypes, 20  $\times$  16, 18  $\times$  15, and 18  $\times$  15  $\mu\text{m}$ , respectively.

### Type locality

Among sand grains at mid-tide level on high-energy ocean beaches in southeastern Australia. So far it has been found on South Broulee beach and adjacent Dolphin beach, but it has not yet been sought elsewhere.

### Etymology

The specific name alludes to the appearance of the pentancrees (Latin, sharp and rough).

### Remarks

Specimens of this species are often curved dorso-ventrally when fixed, but can be observed on a microscope slide unfixed in sea water among sand grains using a 65 $\times$  objective lens. Live specimens glide over large sand grains, fully extended, with the oral hood spread out over the substratum, apparently sweeping up microbial food. When disturbed, or usually when fixed, they tend to curl up in a ball. The hood then contracts around the pharyngeal opening, becoming pleated.

**Table 1** Measurements of type specimens of *Tetranchyroderma pentaspersus* sp. nov. in  $\mu\text{m}$  or, alternatively, in U (percentage of body length). (TbA, anterior adhesive tubules; TbL, lateral adhesive tubules; nv = not visible.)

		Holo	Para 1	Para 2	Para 3	Para 4	Para 5	SEM	
Body length	$\mu\text{m}$	232	197	204	232	234	235	178	
Oral hood	U	3	6.0	3.0	9.2	4.5	3.2	6	
Widths									
Behind mouth rim U 13		16	18	17	16	15	10		
At end of pharynx U		13	20	16	14	14	12	nv	
Mid trunk, max.	U	13	29	18	15	16	17	14	
At level U30	U	10	23	12	10	10	9	11	
Pharynx length	U	18	21	20	20	21	19	nv	
Pharynx intestine junction U		35	39	26	31	31	31	nv	
Adhesive tubules									
TbA pairs	3+1	$\mu\text{m}$	6	5	6	nv	nv	6	4.5
TbL pairs	9	$\mu\text{m}$	11–16	15–22	14–30	11–24	11–25	11–21	7–16
anterior		at U	30	40	47	41	45	42	38
posterior		at U	94	nv	nv	97	96	nv	97
Pedicle length		$\mu\text{m}$	21	10	16	18	18	21	13
Oocyte		$\mu\text{m}$	–	20 $\times$ 16	–	18 $\times$ 15	18 $\times$ 15	–	nv

### *Tetranchyroderma australiense* sp. nov.

Fig. 3 (drawings) and 4 (SEM photomicrographs); measurements Table 2.

#### Diagnosis

Medium-sized *Tetranchyroderma*; almost cylindrical body that may narrow slightly in region of pharyngeal intestinal junction. Large oral hood encloses mouth, not crenulated. Cephalic tentacles, pestle organs, and cirrata lacking. Dorsal surface completely covered with pentancreas, with five tines of equal length. Pentancreas extend marginally onto ventral surface on each side of body; ventral field ciliated. Four TbA ventrally, on each side of mouth rim, eleven pairs of TbL, between about U40 and U94, the latter just in front of the anus, and one pair of dorsal TbD. Caudal pedicles terminating in two adhesive tubules; fleshy part covered dorsally with pentancreas.

#### Material examined

Fifty-five stained or unstained specimens were examined by light microscopy, of which six specimens were selected as suitable for type material and measured. In addition, two specimens were studied by SEM, but only one was orientated so as to give useful measurements. All specimens were collected by W. Nicholas from South Broulee beach.

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

**Holotype** Slide ANIC 505 no. 19466, Table 2, column 3.

**Paratypes** Slide ANIC 505 no. 19467; slide 506 nos. 19468 and 19469; slide 507 nos. 19470 and 19471.

#### Description of holotype

Length 390  $\mu\text{m}$ ; body almost cylindrical, 33–45  $\mu\text{m}$  wide (U8–11). Anteriorly, a thin-walled oral hood envelopes the mouth, projecting 6  $\mu\text{m}$  ahead of the main body. The dorsal surface of the trunk is covered by pentancreas, arranged in 12–14 staggered columns of c. 80 rows. On the trunk, pentancreas are 6  $\mu\text{m}$  across with four equal tines c. 5–6  $\mu\text{m}$  long. At the anterior end, the pentancreas are smaller, 2.25  $\mu\text{m}$  high and 2.25  $\mu\text{m}$  across. Each row of pentancreas may extend marginally onto the ventral surface on each side of the trunk. The oral hood bears no pentancreas.

Three pairs of TbA abut the ventral mouth rim, with another pair a little further posteriorly. Eleven pairs of TbL are present on the trunk from U42 to U94, bordering the ventral field, which carries transverse rows of locomotor cilia. The most posterior TbL lies near the anus. There are no adhesive tubules between the pedicles. A pair of TbD are situated at U57. Caudal pedicles, 18  $\mu\text{m}$  long, project beyond the rounded caudum and terminate in two adhesive tubules backed by a dorsal spine, with fleshy portions covered dorsally with pentancreas.

The mouth leads to a cylindrical muscular pharynx, 57  $\mu\text{m}$  long, which joins the intestine at U23. The intestine ends at the postero-ventral anus at U94, 13  $\mu\text{m}$  from the caudum. Hermaphrodite; with single ovary and testis lying side by side, occupying the posterior half of the trunk.

**Paratypes** Five paratypes vary from 330 to 382  $\mu\text{m}$  in length; measurements in columns 4–8 in Table 2, measurements of one SEM specimen in column 9. There is considerable variation in some of the measurements, despite some compensation for differences in size by expressing body proportions as percentages of body length (U). These gastrotrichs are flexible, non-rigid and curved. Probably the most generally useful measurements are those taken from the SEM photomicrographs. On other specimens, various parts of the reproductive system were found. A single female gonad, sometimes with an egg, a single testis and what is probably a seminal receptacle, posterior to the ovary, is sometimes visible.

#### Type locality

Among sand grains at mid-tide level on a high-energy ocean beach in southeastern Australia. So far it has been found on only one beach, but it has not yet been sought elsewhere.

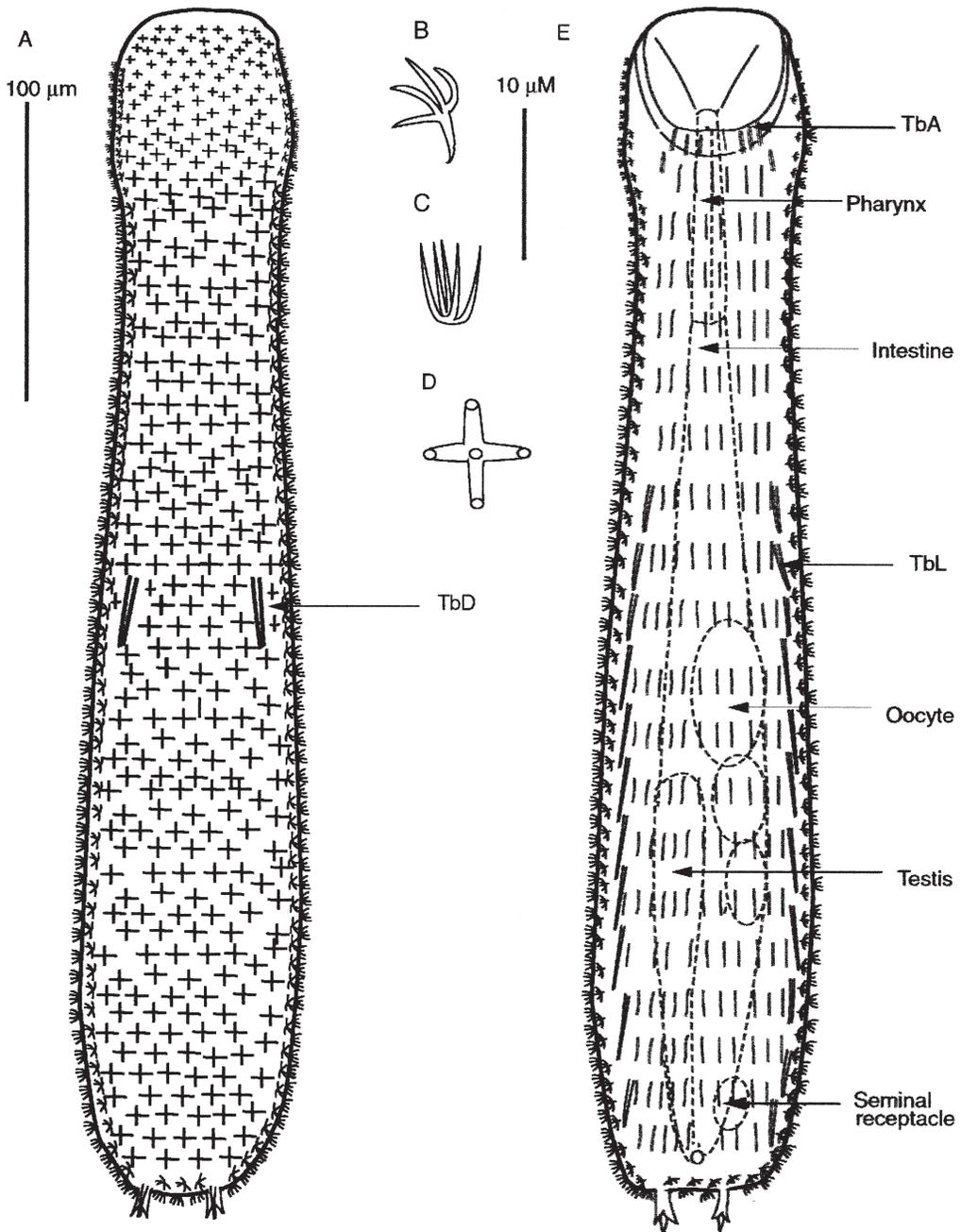
#### Etymology

Named after the geographical region where the new species was found.

#### Remarks

Table 2 lists taxonomically significant measurements of organs and body proportions. Some degree of compensation for differences in body length, when comparing the new species with other described species was made by expressing proportions as percentages of body length (as U), but considerable variation in the proportions is evident in Table 2.

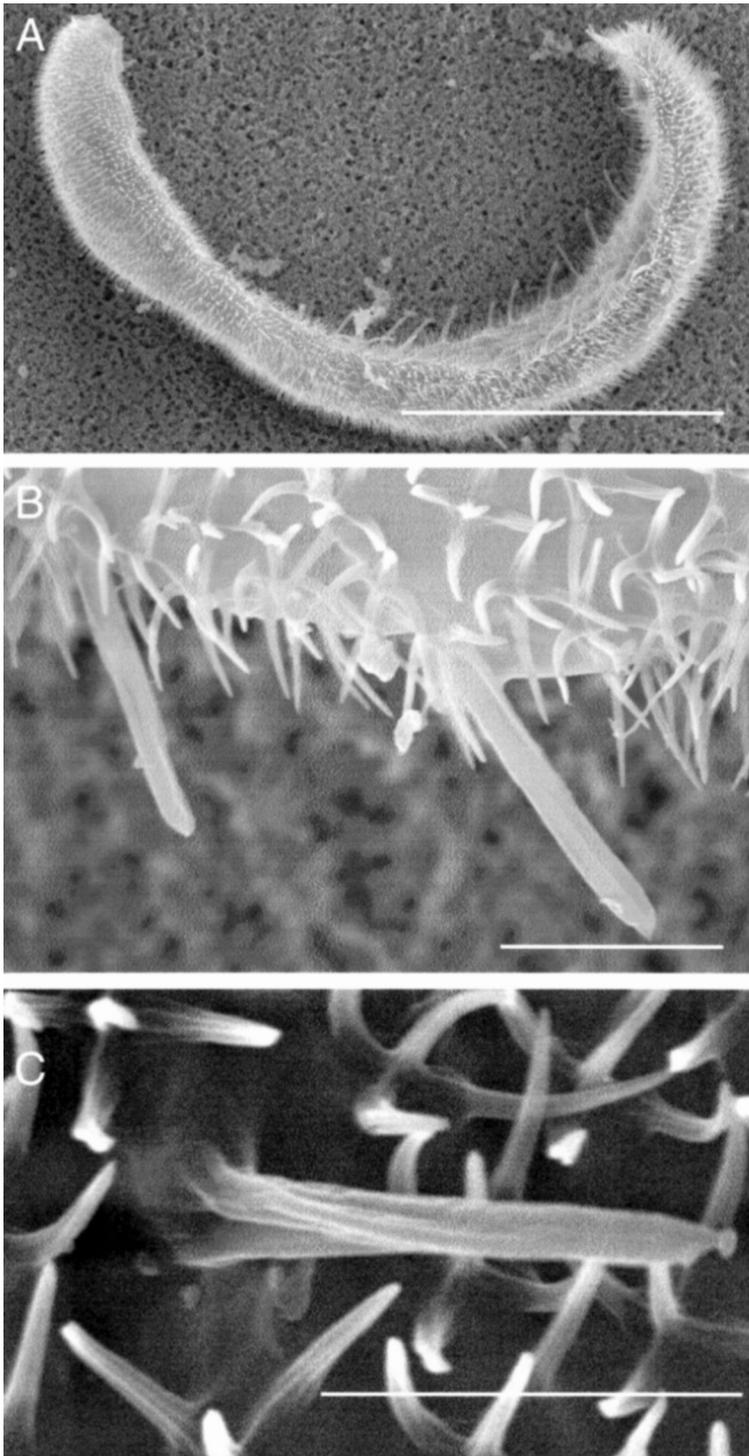
The body of specimens was flexible and always curved to some degree when mounted. Measurements



**Fig. 3** *Tetranychoderma australiense* sp. nov. **A**, dorsal view of specimen; **B**, **C**, and **D**, different views of pentancre; **E**, ventral view of specimen. (TbA, anterior adhesive tubules; TbL, lateral adhesive tubules; TbD, dorsal adhesive tubules.)

along the convex dorsal surface are greater than along the ventral concave surface, depending on the degree of curvature. Taking the median line along the lateral surface takes the mean of these

measurements. It is impossible to make a precise count of the number of pentancre in rows because the pentancre are not strictly aligned. There are more pentancre along the dorsal surface than the



**Fig. 4** *Tetranchyroderma australiense* sp. nov. SEM photomicrographs. **A**, lateral view of entire specimen; **B**, lateral adhesive tubules and pentaneres; **C**, dorsal adhesive tubule. Scale bars: A, 100  $\mu$ m; B and C, 10  $\mu$ m.

ventro-lateral surface. We estimated that there are c. 80 rows along the dorsal surface. The number of columns is also imprecise because placements in adjacent columns are staggered so that we had to arbitrarily decide whether to count alternating pentancreas as lying in one or two rows. Taking the alternating pentancreas as one row we estimated there are c. 13 to the row.

## DISCUSSION

Todaro (2002) provided a comprehensive dichotomous key to species of *Tetranchyoderma* including 57 taxa. To these, four more species should be added to the list; two described before 2002 (i.e., *T. arcticum* and *T. symphorocheatum*, see Hummon et al. 1998; Clausen 2000), and two described afterwards (*T. canariense* and *T. feroense*, see Todaro et al. 2003; Clausen 2004). Consequently, up to now the genus consists of 61 known species, including six species that are described and figured but not named. Todaro (2005) reports a complete list of known species whereas Schrom (1972) and Valbonesi & Luporini (1984) account for unnamed species.

Of the 61 known species, 29 species possess pentancreas, 29 species tetrancreas, and 3 species possess triancreas or a mixture of ancreas. *Tetranchyoderma pentaspersus* sp. nov. and *T. australiense* sp. nov. should be added to the list of 29 species with

pentancreas. Because *T. pentaspersus* sp. nov. bears a complete dorsal covering of pentancreas, the central tines of which are distinctly longer than the outer four, and lacks cephalic sensorial organs and dorsal and ventral adhesive tubules, it resembles most closely *T. polyacanthus* and *T. tanymesatherum*. However, the tines of the ancreous covering are much stouter in the Australian species than in the other two taxa. Moreover, in the new species the difference in length between the central and outer tines is not as great as the 4–5-fold difference reported in *T. polyacanthus*, the number of TbA is less than *T. tanymesatherum* (8 versus 12), and there are fewer tubules at the end of the pedicles (2 versus 3). It should be emphasised that the single pair of ventral adhesive tubules reported in the original description of *T. tanymesatherum* (Hummon et al. 1996) is not always present in this species (M.A. Todaro pers. obs.).

*Tetranchyoderma australiense* sp. nov., by virtue of its complete dorsal covering of pentancreas possessing tines of equal length, and the absence of both cephalic sensorial organs and ventral adhesive tubules, most closely resembles *Tetranchyoderma* sp. 7 of Valbonesi & Luporini (1984), from which it can be differentiated by the possession of fewer adhesive tubules at the end of each caudal pedicle (2 versus 3), more numerous TbL (11 versus 9), the absence of adhesive tubules between the caudal pedicles. However, owing to the weakness of these

**Table 2** Measurements of type specimens of *Tetranchyoderma australiense* sp. nov. in  $\mu\text{m}$  or, alternatively, in U (percentage of body length). (TbA, anterior adhesive tubules; TbL, lateral adhesive tubules; TbD, dorsal adhesive tubules; nv = not visible.)

		Holo	Para1	Para2	Para3	Para4	Para5	SEM	
Body length	$\mu\text{m}$	390	330	374	342	382	354	367	
Oral hood	U	1.5	?	3.0	4.5	4.5	1.5	1.8	
Widths									
Behind mouth rim	U	8	8	8	15	9	13	9	
At end of pharynx	U	10	6	8	11	6	6	nv	
Mid trunk, max.	U	11	7	9	19	10	14	9	
At U94	U	9	5	8	7	9	7	7	
Pharynx length	U	15	15	14	14	13	15	nv	
Pharynx intestine junction	U	23	16	18	20	22	26	nv	
Adhesive tubules									
TbA pairs	3+1	$\mu\text{m}$	11	nv	6	4.5	nv	nv	5.7
TbL pairs	11	$\mu\text{m}$	11–21	15–18	11–21	11–18	14–24	16–18	11–17
anterior		at U	42	52	33	40	35	42	38
posterior		at U	94	94	89	97	96	92	94
Length TbD		$\mu\text{m}$	18	18	18	18	14	nv	13
TbD No. pairs 1		at U	57	47	40	56	63	nv	58
Pedicles		$\mu\text{m}$	18	16	22	15	18	18	10

diagnostic characters, only future research on Somalian specimens can ascertain whether the two populations belong to the same species, at least from a morphological perspective.

#### ACKNOWLEDGMENTS

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