

Organisms associated with the sandy-beach bivalve *Donax serra* Röding, with a description of *Cercaria serrae* sp. nov. (Trematoda)

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Examination of two collections of *Donax serra* from a South African west coast beach revealed the presence of Ciliophora, Trematoda, Nematoda and a parasitic pycnogonid. This is the first record of a pycnogonid from the genus *Donax* and the first published report of such a parasite from any southern African bivalve mollusc. One of the two trematode species found is described as new and its epidemiology is discussed briefly. No epibiota were found on the bivalve shell.

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The macrofauna of sandy beaches around South Africa is now well known, the dominant white sand mussel, *Donax serra*, being a species to which special attention has been accorded (de Villiers 1975a, b; McLachlan & Hanekom 1979; Donn 1986; Brown, Stenton-Dozey & Trueman 1989). However, nothing has been published on parasites or other organisms associated with this abundant bivalve, the only parasitological work on these beaches relating to the whelk *Bullia* (Webb 1991a; Brown & Webb 1994). The present communication reports on organisms associated with 200 *Donax serra* collected from the beach at Ou Skip, just north of Table Bay, on the west coast. It originates from an unpublished project (Tharme 1988), in which greater detail of these organisms and their epidemiology may be found.

Material and Methods

Two collections of *Donax serra* were made, each of 100 animals, on 19 February and 2 May 1988. A wide range of sizes was included in both collections and the shell length, height and breadth of each individual measured. The valves were then separated and the gills, mantle and viscera examined microscopically for evidence of parasites. Dissection of all internal organs was followed by progressive disruption in seawater, to flush out any associated organisms. The reproductive status of each bivalve was assessed and shell length employed to confirm identification of immature individuals (de Villiers 1975a). Trematode parasites were studied, measured and photographed using the methods described by Webb (1985, 1991a, b).

Results

Examination of the mussels revealed two species of digenean Trematoda, a parasitic species of pycnogonid, free and encysted ciliated Protista and nematode worms. Tumours and calcareous aberrations were also noted. However, the boring sponge *Clione* and the algal gardens associated with shells of the whelk *Bullia* (Harris, Da Silva, Bolton & Brown 1986; Brown & Webb 1994), inhabiting the same beaches, were not in evidence. In fact, no epibiota of any kind could be found associated with the shells. This may well be due to the much deeper burrowing of *Donax serra* as compared with *Bullia* (Brown & Trueman 1991).

Ciliophora

In many mussels, large numbers of a mobile, disc-shaped ciliate were found to be associated with the ctenidia. Average dimensions of the ciliate were $47,5 \times 49,0 \mu\text{m}$. It is a member of the family Trichodinidae and is tentatively assigned to the genus *Trichodina*. In one mussel, ciliophoran cysts were embedded in the gill tissue, distorting the filaments; morphological evidence indicated that these were probably the encysted form of the same species.

Trematoda

Two species of digenean trematode were apparent. One of these was represented by numerous daughter sporocysts in the digestive glands of three of the mussels, giving a prevalence of 1,5%. The sporocysts were not seen to move and no mother sporocysts were found. Mean sporocyst length was $66,0 \mu\text{m}$, mean width $52,4 \mu\text{m}$. Numerous cercariae, in initial stages of development, were visible within each daughter sporocyst. Germ balls were also noted.

A second species was represented by metacercariae, whose primary site of infection was the mantle, although in one mussel they were enveloped in the tissues of the labial palps. Metacercariae were either enclosed in proliferations of the mantle tissue or free and attached to the outer mantle surface, in the extrapallial space. Both free and enclosed forms were usually found at any infection site (Figure 1). Hypertrophy of mantle tissue surrounding each cluster of parasites was invariably evident. As this trematode did not conform to any description in the literature, it is described here as *Cercaria serrae* sp. nov. (Family Gymnophallidae).

Cercaria serrae sp. nov.

Type material: South African Museum A 29426

Dorsoventrally flattened distome (Figure 2), with a pear-shaped body, slightly narrower anteriorly and tapering posteriorly. Body length variable, from $205 \mu\text{m}$ when contracted to $285 \mu\text{m}$ at full extension (Figure 3). Average widths at 25%, 50% and 75% of body length from anterior, were 145, 165 and $132 \mu\text{m}$ respectively. Spinous tegument, with recurved, simple triangular spines in horizontal rows across the body; spines small directly above oral sucker, well-developed along the sides of the body, becoming sparser in the final 25% of body length. Spines offset slightly one above the other in the

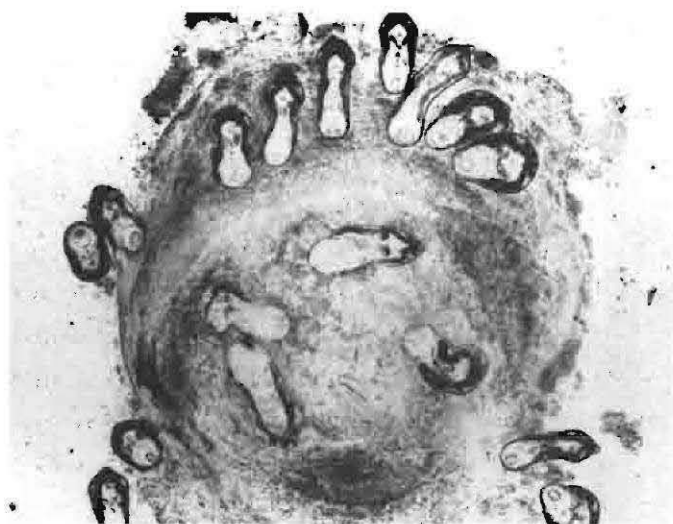


Figure 1 Infection focus of *Cercaria serrae* on *Donax serra* mantle, showing individuals enveloped in tissue and free.

rows. Average spine length 1,3 μm and pitch 1,25 μm . A series of three (but sometimes only two) rows of small, knob-like papillae are evident on the ventral surface of the tegument, midway between the acetabulum and the pharynx. Tegument uniformly thick and highly flexible.

Oral sucker large (mean length 117 μm , mean width 100 μm), opening slightly subterminally. Opening has a toothed appearance. A few scattered papillae commonly occur on the oral sucker, particularly around the opening. Two raised, spherical, pad-like areas occur, one on each side of the opening to the mouth, positioned towards the sides of the sucker;

average dimensions 12,5 \times 12,5 μm . Ventral sucker just less than one-third the size of the oral sucker and situated two-thirds of the body length from anterior; width 35–45 μm but length 27–40 μm , depending on degree of contraction. Pre-pharynx absent. Pharynx 32 μm long by 33 μm wide; walls muscular and capable of some outward expansion. A short (10 μm) thin-walled, highly flexible oesophagus leads from the pharynx; it is capable of great expansion and contraction. Two short (62 μm) digestive caeca bifurcate from the oesophagus, terminating above the ventral sucker. Numerous tear-shaped and irregularly globular gland cells encircle the midregion and base of the oral sucker, especially conspicuous as two major clusters on opposite sides of the base of the oral sucker. Gland cells frequently extend downwards in line with the base of the pharynx.

Two major excretory ducts extend on either side of the body, with blind endings positioned on either side of the oral sucker. Ducts extend posteriorly, fusing a short distance behind the ventral sucker. Two diverticula, immediately anterior to the junction, extend laterally towards the body midline until just behind the ventral sucker. A second pair of diverticula extend anterolaterally to each duct, to below the testes (Figure 2). The posterior, fused region of the ducts leads into an apparently thin-walled bladder, which in turn leads into a single, narrow excretory duct, opening terminally. The excretory system is stenostomate (La Rue 1957); the provisional flame-cell formula is 2(3+2+2) (Figure 4). The testes are oval, of approximately equal size, lying on either side of the ventral sucker (Figure 2); dimensions 25 to 37 μm wide, 27 to 60 μm long. Each testis contains block-like tissue, staining dark red with Neutral Red. The ovary is spherical and glandular, averaging 17 \times 13 μm , about 5 μm anterior to the acetab-

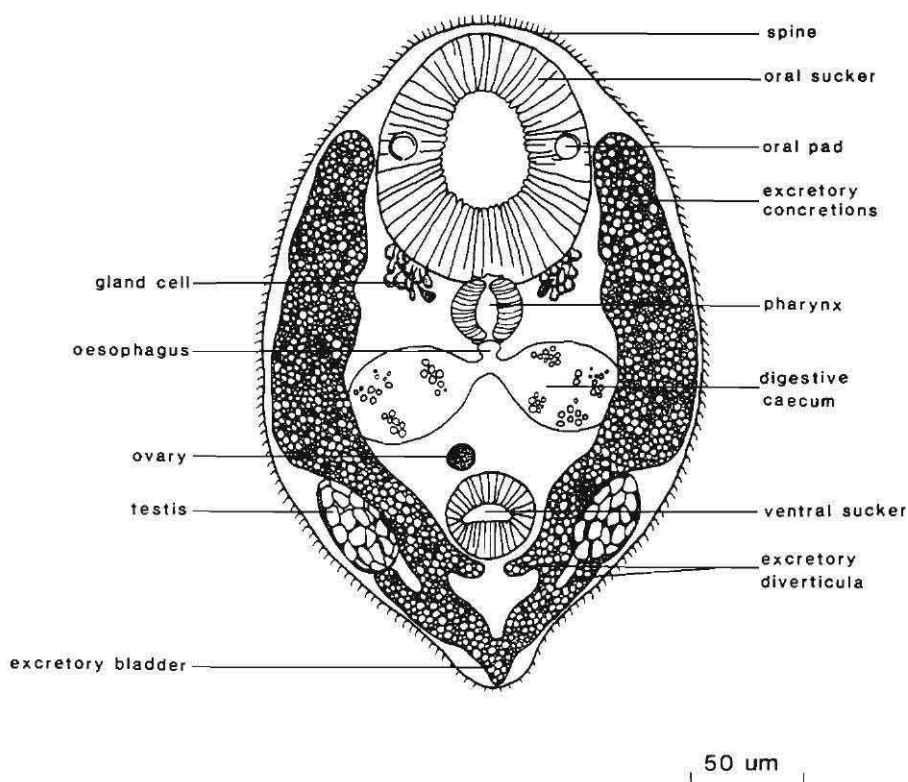


Figure 2 Dorsal view of *Cercaria serrae*.

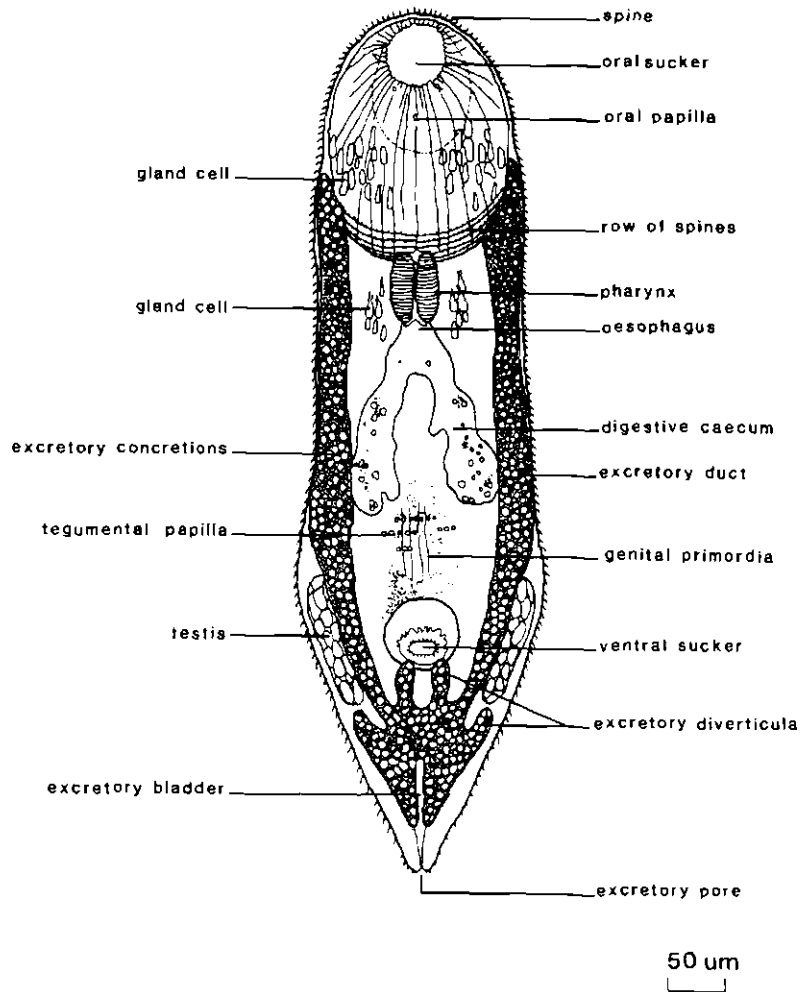


Figure 3 Ventral view of *Cercaria serrae*, extended.

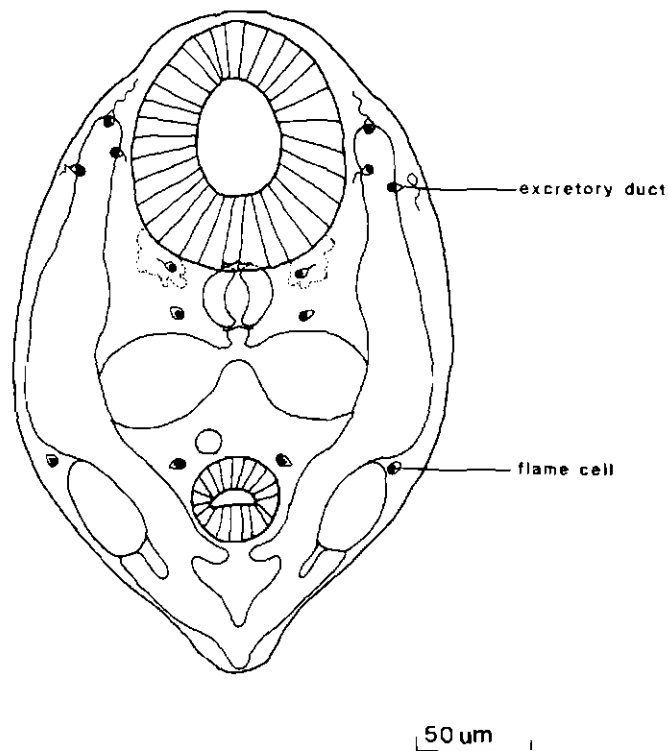


Figure 4 Location of flame cells in *Cercaria serrae*.

ulum and slightly to the left of the midline (Figure 2).

While we have no doubt that *C. serrae* belongs in the family Gymnophallidae, assigning it to a genus is problematic at this stage, one reason being its flame-cell formula of $2(3+2)+(2) = 14$. There are distinct similarities with *Lacunovermis macomae* (Pekkarinen 1986) but the latter has a smaller oral sucker and more extensive digestive caecae; moreover, *L. macomae* lacks the conspicuous oral papillae of *Cercaria serrae*. *C. serrae* and *C. granosa* (Holliman 1961) have similar lateral papillae on the oral sucker but the latter species has a flame-cell formula of $2(2+2)+(2) = 12$ and its caecae are much more extensive. Also the excretory vesicle of *C. granosa* has a short stem, which is absent in *C. serrae*. The flame-cell formula in the genus *Gymnophallus* is $2(2+2+2)+(2+2+2) = 24$ (James 1964) and the oesophagus and the stem of the Y-shaped excretory vesicle may be longer than in *C. serrae*. *C. serrae* has some similarities with the genus *Gymnophalloides* but here the flame-cell formula is $2(2+2)+(2+2) = 16$; also the Y-shaped excretory vesicle of *Gymnophalloides* differentiates them (see James 1964). *Meiogygnophallus* has a flame-cell formula of either $2(2+2)+(2+2) = 16$ or $2(2+2+2)+(2+2+2) = 24$ (Bowers & James 1967). Members of this genus also have a proportionately smaller oral sucker and the ovary may be more lateral; the oesophagus is longer and the caecae extend more to the posterior.

Cercaria serrae appears to have closest affinities with the genus *Parvatrema*, although flame-cell formulae in this genus are either $2(2+2)+(2) = 12$ or $2(2+2)+(2+2) = 16$ (Bowers & James 1967). The excretory vesicle is V-shaped, as in *Cercaria serrae*. *Parvatrema borinquenae* has lateral projections on its oral sucker (James 1964), as does *P. affine*; however the ventral sucker in *P. borinquenae* occurs at mid-body. *P. affine* is quite similar to *Cercaria serrae* (see Lauckner 1983) but the oral sucker is smaller in proportion to the ventral sucker. In *Parvatrema homoeotectum* the ovary is more anteriorolateral and the excretory vesicle not so extensive anteriorly (Bowers & James 1967).

Satisfactory location of the new species must thus await description of the adult reproductive system.

The overall infection prevalence of *Cercaria serrae* was 48%, making it by far the most common parasite of *Donax serra* in the study area. However, infection prevalence for the 100 mussels collected in February was only 17%, compared with 80% in May of the same year. Analysis of overall infection prevalence in relation to host sex gave results of 51,5% for males, 40% for females and 60% for immature individuals. The lower size limit for infection was a shell length of about 20 mm, above which infection frequency increased with increasing shell length until, at 30 to 35 mm, there were more infected than non-infected mussels. A marked increase in frequency at 35 to 40 mm immediately preceded or was coincidental with the onset of sexual maturity. For males, infection reached 100% at a shell length of 47 mm and again at 63 to 64 mm; a decline followed with increasing size. In females, the prevalence peak coincided with that of the older males but never reached 100%, the maximum being 70,5%. The largest males and females were invariably free of infection.

The mean intensity of infection was 24 metacercariae per

infected mussel. In males there was an inverse relationship between infection intensity and infection prevalence, although this was less apparent in females. Young females appeared to be less susceptible to infection than were the males, intensity being only one or two metacercariae per mussel. The onset of sexual maturity coincided with a marked drop in intensity.

There was an apparent correlation between infection and the formation of calcareous, non-nacreous concretions in the tissues or on the inner surfaces of the valves. Shallow pits in the valves, owing to erosion of calcareous material, were also in evidence. In most cases, these shell aberrations occurred directly opposite sites of infection. However, the frequency of aberrations also increased with increasing shell length so that, despite an extensive literature linking calcareous deposition and erosion with the presence of metacercariae (Lauckner 1983), the relationship needs to be confirmed for *Donax serra*.

Nematoda

Two mussels were found to harbour nematode worms. In one case, three motile worms were discovered, which appeared to be free living. In a second mussel, ten worms emerged, under coverslip pressure, from a discrete body of tissue embedded in the mantle at the base of the foot. These ostensibly parasitic nematodes averaged a body length of 300 μm , with a maximum width of 13,75 μm .

Pycnogonida

Eight pycnogonids, apparently belonging to a single species were discovered, each in a different mussel. All came from the February collection. Six were young adults, while two were in the protonymphon stage. Seven of them were firmly attached to the gill filaments, by means of their chelae, while the eighth, a protonymphon, was attached to a labial palp. Local tissue destruction was strongly in evidence in all cases. There are no other published records of pycnogonids parasitic in bivalve molluscs from southern Africa and the present species appears to be undescribed. An interesting point is that it does not seem to belong to the family Nymphonellidae as described by Ohshima (1937), these being the common pycnogonid parasites of bivalves in other parts of the world.

Discussion

Larval digenean trematodes have been reported from many marine bivalves, including *Donax* from many regions, and are regarded as the most important group of parasites in these molluscs (Lauckner 1983). The presence of two trematode species in *Donax serra* from Ou Skip beach is thus not remarkable, nor is the extremely high prevalence of one of them surprising. More noteworthy is the marked difference in the prevalence of *Cercaria serrae* between February and May of the same year, possibly indicating seasonal variation. Also of interest is the fact that the largest mussels of both sexes were all non-infected. The apparent association of calcareous aberrations with infection sites requires confirmation.

The presence of parasitic Pycnogonida in a South African bivalve is reported for the first time, although in fact they are known to occur in rocky-shore mussels in the region (S.C. Webb, G.M. Branch, pers. comm.) and nymphonellid Pycno-

gonida have been dredged off the South African coast (Stock 1958). It is also the first record of pycnogonids parasitic in a member of the genus *Donax*, although their occurrence in sandy-beach bivalves other than *Donax* is well documented (Ohshima 1927, 1937; Kikuchi 1976).

There are several records of ciliated Protista infesting bivalve molluscs, including *Donax* (Chatton & Lwoff 1923, 1929). In some cases, such infestations have been held responsible for the mass mortality of host populations (Michaelis 1977; Van Banning 1979). However, it seems likely that most ciliate/bivalve associations are transitional along a continuum from commensalism to parasitism, a shift towards parasitism taking place when the physiological state of the bivalve deteriorates and the numbers of the ciliate become unusually high (Noble & Noble 1964; Lauckner 1983). However that may be, the encysted form of the ciliate reported here certainly appears to cause some malfunctioning of the gill cilia, thus inevitably stressing the mussel.

A final point to consider is that *Donax serra* appears to have considerable potential as an indicator of pollution (Cook 1985; Stenton-Dozey 1989). Researchers of pollution-related stress are usually careful to ensure that other types of stress, as might arise from overcrowding, inappropriate temperatures or salinities, or inadequate nutrition, are minimal. However, parasite load has never been taken into account as an additional stress factor. It is high time it was.

References

- BOWERS, E.A. & JAMES, B.L. 1967. Studies on the morphology, ecology and life-cycle of *Meiogymnophallus minutus* (Cobbold, 1859) comb. nov. (Trematoda: Gymnophallidae). *Parasitol.* 57: 281–300.
- BROWN, A.C. & TRUEMAN, E.R. 1991. Burrowing of sandy-beach molluscs in relation to penetrability of the substratum. *J. moll. Stud.* 57: 134–136.
- BROWN, A.C. & WEBB, S.C. 1994. Organisms associated with burrowing whelks of the genus *Bullia*. *S. Afr. J. Zool.* 29: 144–151.
- BROWN, A.C., STENTON-DOZEY, J.M.E. & TRUEMAN, E.R. 1989. Sandy-beach bivalves and gastropods: a comparison between *Donax serra* and *Bullia digitalis*. *Adv. mar. Biol.* 25: 179–247.
- CHATTON, E. & LWOFF, A. 1923. Un cas remarquable d'adaptation: *Ellobiophrya donacis*, n.g., n.sp., péritriche inquilin des branchies de *Donax vittatus* (Lamellibranche). *C. v. Seanc. Soc. Biol.* 88: 749–752.
- CHATTON, E. & LWOFF, A. 1929. Contribution à l'étude de l'adaptation. *Ellobiophrya donacis* Ch. et Lw., péritriche vivant sur les branchies de l'acéphale *Donax vittatus* da Costa. *Bull. biol. Fr. Belg.* 63: 321–348.
- COOK, P.A. 1985. Koeberg Nuclear Power Station: marine environmental monitoring programme. Unpublished report, University of Cape Town.
- DE VILLIERS, G. 1975a. Reproduction of the white sand mussel *Donax serra* Röding. *Sea Fish. Brnch Invest. Rep.* 102: 1–33.
- DE VILLIERS, G. 1975b. Growth, population dynamics, a mass mortality and arrangement of white sand mussels, *Donax serra* Röding, on beaches in the south-western Cape Province. *Sea Fish. Brnch Invest. Rep.* 109: 1–31.
- DONN, T.E. (ed.) 1986. Biology of the genus *Donax* in southern Africa. Institute for Coastal Research, University of Port Elizabeth.
- HARRIS, S.A., DA SILVA, F.M., BOLTON, J.J. & BROWN, A.C. 1986. Algal gardens and herbivory in a scavenging sandy-beach nassariid whelk. *Malacologia* 27: 299–305.
- HOLLIMAN, R.B. 1961. Larval trematodes from the Apalachee Bay area, Florida, with a checklist of known marine cercariae arranged in a key to their superfamilies. *Tulane Stud. Zool.* 9: 1–74.
- JAMES, B.L. 1964. The life cycle of *Parvatrema homoeotecnum* sp. nov. (Trematoda: Digenea) and a review of the family Gymnophallidae Morozov, 1955. *Parasitol.* 54: 1–41.
- KIKUCHI, T. 1976. Rediscovery of the bivalve-infesting Pycnogonida *Nymphonella tapetis* Ohshima 1927, in Amakusa. *Publ. Amakusa mar. biol. Lab., Kyushu Univ.* 4: 51–56.
- LA RUE, G. 1957. The classification of digenetic Trematoda: a review and a new system. *Exp. Parasitol.* 6: 306–344.
- LAUCKNER, G. 1983. Diseases of Mollusca: Bivalvia. In: Diseases of marine animals, Vol. 2: Bivalvia to Scaphopoda (ed. O Kinné): 477–961. Biologische Anstalt Helgoland, Hamburg.
- MCLACHLAN, A. & HANEKOM, N. 1979. Aspects of the biology, ecology and seasonal fluctuations in biochemical composition of *Donax serra* in the east Cape. *S. Afr. J. Zool.* 14: 183–193.
- MICHAELIS, H. 1977. Untersuchungen über das sterben wirbelloser Wattentiere seit dem Sommer 1976. Forschungsstelle für Insel und Küstenschutz, Nordency (informal report).
- NOBLE, E.R. & NOBLE, G.A. 1964. Parasitology: the biology of animal parasites. Lea & Febiger, Philadelphia.
- OHSHIMA, H. 1927. *Nymphonella tapetis* n. gen., n. sp., a pycnogon parasite in a bivalve. *Annot. Zool. Japon.* 11: 257–263.
- OHSHIMA, H. 1937. Nymphonellidae, a new family of Pantopoda. *Annot. Zool. Japon.* 17: 229–233.
- PEKKARINEN, M. 1986. Development of the cercaria of *Lacunovermis macomae* (Trematoda: Gymnophallidae) to the metacercaria in brackish-water *Macoma balthica* (Bivalvia). *Ann. Zool. Fennici* 23: 237–250.
- STENTON-DOZEY, J.M.E. 1989. The physiology and energetics of the sandy-beach bivalve *Donax serra* Röding with special reference to chlorine and temperature tolerance. Ph.D. thesis, University of Cape Town.
- STOCK, J.H. 1958. On some South African Pycnogonida of the University of Cape Town ecological survey. *Trans. R. Soc. S. Afr.* 35: 549–567.
- THARME, R.E. 1988. A survey of digenetic trematodes in the white sand mussel *Donax serra* Röding on the western Cape coast. Unpubl. project in the Niven Library, University of Cape Town.
- VAN BANNING, P. 1979. Protistan parasites observed in the European flat oyster (*Ostrea edulis*) and the cockle (*Cerastoderma edule*) from some coastal areas of the Netherlands. *Haliotis* 8: 33–37.
- WEBB, S.C. 1985. A survey of helminth parasites in surfing whelks on the western Cape coast of South Africa. M.Sc. thesis, University of Cape Town.
- WEBB, S.C. 1991a. *Cercaria hastata* sp. nov. (Digenea: Trematoda) in *Bullia digitalis*, a surfing whelk from the western Cape coast of South Africa: epidemiology and sex linked phenomena. *J. nat. Hist.* 25: 543–558.
- WEBB, S.C. 1991b. Use of acetic orcein as a fast method to relax, fix, clear and stain cercariae for light microscopy. *J. Helminthol.* 65: 234–238.