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Neoentobdella gen. nov. for species of *Entobdella* Blainville in Lamarck, 1818 (Monogenea, Capsalidae, Entobdellinae) from stingray hosts, with descriptions of two new species

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Abstract

The knowledge that entobdelline (capsalid) monogeneans formerly in *Entobdella* fall into two natural groupings provides the background to this paper. It is proposed to retain *Entobdella* for *E. hippoglossi* (type species), *E. pugetensis* and *E. soleae* from teleost flatfishes and to erect *Neoentobdella* gen. nov. for those parasites formerly in *Entobdella* but infecting elasmobranch flatfishes (mostly dasyatid stingrays but one report from a rhinobatid host). *Neoentobdella diadema* comb. nov. is designated type species for the new genus, which also includes *N. apiocolpos* comb. nov., *N. australis* comb. nov., *N. bumpusii* comb. nov. and two new species, *N. natans* sp. nov. and *N. parvitesticulata* sp. nov. from the dasyatid stingrays *Pastinachus sephen* and *Himantura fai*, respectively, caught off the Great Barrier Reef, Queensland, Australia. A prominent feature of all *Neoentobdella* species is the possession of anterior adhesive pads with transverse rays, resembling a diadem, but the close relationship between *N. natans* and *N. parvitesticulata* is underlined by the presence in both species of a muscular pad armed with microsclerites inside the genital atrium and elaborate fleshy lips and folds on the dorsal surface near the common genital opening. Adults of both species are also able to swim. The validity of *Pseudoentobdella pacifica* is confirmed. Entobdellinae Bychowsky, 1957 is revised to accommodate the recently established *Listrocephalos*, as well as the proposal of *Neoentobdella*.

Key words

Platyhelminths, Monogenea, Capsalidae, Entobdellinae, *Entobdella*, *Neoentobdella* gen. nov., fishes, Dasyatidae

Introduction

During visits to the Heron Island Research Station of The University of Queensland, Australia, the opportunity arose to collect monogenean parasites from the stingrays *Pastinachus sephen* and *Himantura fai* (Dasyatidae). It became apparent after examination that capsalid (entobdelline) parasites from the skin of each of these hosts represented two closely related, but distinct, species. These two parasite species have remained undescribed since one of us (IDW) first discovered them in 1988, although brief reference has been made to them in several publications as follows. The species from *P. sephen* is the sub-adult *Entobdella* sp. of Kearn and Whittington (1991) and *Entobdella* sp. 2 of Whittington *et al.* (2004). The species from *H. fai* is the *Entobdella* sp. referred to by Whittington and Cribb (1998) and by Hamwood *et al.* (2002) and *Entobdella* sp. 1 of Whittington *et al.* (2004). Anatomical features not observed in any previously described species of *Entobdella* were detected in these specimens and prompted the current detailed anatomical study of the two species. Furthermore, in the context of some previous studies (e.g., Klassen *et al.* 1989, Whittington *et al.* 2004, Bullard *et al.* 2004), our investigation highlighted the need for a revision of *Entobdella* and an amendment of the subfamilial diagnosis.

Materials and methods

Heron Island is a small coral cay at the southern end of the Great Barrier Reef (23°27′S, 151°55′E), Queensland, Australia. During several visits between November 1988 and December 1995, many specimens of the dasyatid stingrays *Pastinachus sephen* (wingspans 53 to 86 cm) and *Himantura fai* (wingspans 53 to 84 cm) were caught either by handline or

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*Corresponding author: g.kearn@uea.ac.uk seine net in Shark Bay. Fish were identified from descriptions in Grant (1987), by consultation with Dr Peter Last (CSIRO Marine Research, Hobart, Tasmania) and by reference to Last and Stevens (1994). After capture, rays were transferred to aquaria at the Heron Island Research Station. Animals were kept in a large concrete pool (capacity: 7500 L) containing flow-through sea water for no longer than 5 days before examination for parasites. Rays were killed by pithing. Skin scrapings, taken gently from dorsal and ventral surfaces of rays, were placed in Petri dishes containing filtered sea water (FSW: filtered through two sheets of Whatman No. 1 filter paper) and searched using a stereomicroscope with transmitted illumination. Undamaged monogeneans that usually live on the skin of their host can attach themselves by the haptor (the posterior attachment organ) to glass surfaces and can move around taking leech-like steps. The behaviour of some of these living parasites was observed with a stereomicroscope and transmitted light. For anatomical studies, other living specimens were transferred to microscope slides, compressed slightly with coverslips and observed with a compound microscope fitted with bright field and phase contrast optics. Most specimens were then flattened and preserved beneath a coverslip either in 10% buffered neutral formalin or in Bouin's fluid. Some of these preserved parasites were stained with Ehrlich's haematoxylin or in acetocarmine, but most material was left unstained. All specimens were dehydrated in an ethanol series, cleared in methyl salicylate or cedar wood oil and mounted in Canada balsam. Some unflattened formalin-fixed specimens of the entobdelline from *P. sephen* were embedded in paraffin wax, sectioned serially at 4–5 µm, stained with haematoxylin and eosin, cleared in toluene and mounted in Canada balsam. Preserved juvenile and adult whole mounts and sections were examined with a compound microscope equipped with phase contrast optics and a drawing tube. Some adult parasites and a few eggs were preserved and processed for scanning electron microscopy (SEM) following Whittington *et al.* (1989) and examined using a Philips SEM 505 operating at 20 kV.

Measurements of adult and juvenile parasites were made using a digitising system similar to that described by Roff and Hopcroft (1986), but measurements of eggs were made using a calibrated ocular micrometer. All measurements are presented in micrometres as the mean followed by the range in parentheses and then the number of structures measured, unless stated otherwise. Where measurements are presented in paired sets separated by a multiplication sign, the first is length, the second width. Haptoral terminology for capsalids follows Whittington *et al.* (2001).

Sources of specimens and locations in which material has been deposited are abbreviated as follows: BMNH – Parasitic Worms, Department of Zoology, The Natural History Museum, London SW7 5BD, UK (contact: Dr David I. Gibson); GK – personal collection of Dr G.C. Kearn, School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, UK; LE – personal collection of Professor Louis Euzet, UMR CNRS 5555 Biologie des Populations d'Helminthes Parasites, Station Mediterranéenne de l'Environnement Littoral, 1 Quai de la Daurade, 34200 Sète, France; SAMA – The Australian Helminthological Collection, Parasitology Section, The South Australian Museum, North Terrace, Adelaide 5000, South Australia, Australia (contact: Dr Ian D. Whittington); USNPC – The United States National Parasite Collection, Beltsville, Maryland 20705, USA (contact: Dr Eric Hoberg).

The following material was examined for comparative purposes: *Entobdella diadema* ex *Dasyatis pastinaca* (Dasyatidae) (GK, 2 whole mounts, N109/6, N109/13); *Entobdella apiocolpos* ex *Taeniura grabata* (Dasyatidae) (LE, 6 mounted paratypes collected July 1951, Gorée, Senegal; 8 mounted specimens on 5 slides collected June 1996, Tunisia); *Entobdella bumpusii* ex *Pastinachus centrourus* (Dasyatidae) (USNPC No. 8148, 1 whole mount, voucher, collected July 1924, Woods Hole, Massachusetts, USA); *Entobdella squamula* ex *Taeniura melanospila* [= *T. meyeni* (Dasyatidae), see Froese and Pauly 2004] (USNPC No. 80216, 1 whole mount, voucher, collected July 1985, Okinawa, Japan); *Pseudoentobdella pacifica* (syn. *Epibdella pacifica*) ex *Myliobatis californica* (Myliobatidae) (USNPC No. 72834, 4 unmounted paratypes in ethanol, 3 of which were mounted in Canada balsam with permission from the USNPC; collected July 1927, Elkhorn Slough, Monterey Bay, California, USA).

Results

Capsalidae Baird, 1853

Entobdellinae Bychowsky, 1957

Amended subfamilial diagnosis: With features of the Capsalidae *sensu* Yamaguti (1963). Haptor aseptate, typically bearing three pairs of median sclerites, namely (in anterior to posterior succession): accessory sclerites, anterior hamuli, posterior hamuli. Median sclerites may be reduced (e.g., in *Listrocephalos* Bullard *et al.* 2004); one pair may be absent (in *Pseudoentobdella*; see below). Fourteen peripherally situated hooklets (pairs II to VIII), pair II between hamuli (numbering follows Llewellyn 1963). Haptor with or without ventral papillae; marginal valve conspicuous, reduced or absent. Pair of muscles in posterior region of body proper give rise to long tendons entering haptor; each tendon passes through proximal notch in accessory sclerite and typically attaches to anterior end of anterior hamulus (e.g., Kearn 1964 for *Entobdella soleae*). These body muscles and associated tendons may be reduced (e.g., in *Listrocephalos*, in the second new species of a new genus described below). Anterior attachment apparatus comprising pair of pads supplied with secretions from gland cells; each pad may be divided into three areas (e.g., *E. soleae*, see Kearn and Evans-Gowing 1998), into many transverse rays separated by narrow troughs, giving overall impression of a diadem (e.g., *Entobdella australis*, see Kearn 1978) or into numerous raised ovoid structures (e.g., *Listrocephalos*, see Bullard *et al.* 2004). Two pairs of eyes present (absent in *Pseudoentobdella*). Testes two, juxtaposed. One or two reservoirs for male accessory gland secretion. Glands of Goto present or absent. Reproductive ducts open on left side, at level of pharynx. Male duct and uterus sharing common opening, sometimes via genital atrium or opening separately (e.g., *Listrocephalos*). Vagina single, communicating proximally with vitelline reservoir or common vitelline duct (undetermined for *Listrocephalos*), running in anterolateral direction, opening on left side of body, ventrally or laterally. Proximal region of vagina typically serves as seminal receptacle, but additional seminal receptacles may be present, communicating with ovo-vitelline duct (e.g., *E. soleae*, see Kearn 1985). Vitelline reservoir single. Eggs tetrahedral or urn-shaped, with short or long appendage, often bearing adhesive material. Parasites of skin, gills or mouth cavity of marine elasmobranch and teleost flatfishes.

Type genus: *Entobdella* Blainville in Lamarck, 1818.

Other genera: *Listrocephalos* Bullard, Payne et Braswell, 2004; *Pseudoentobdella* Yamaguti, 1963; new genus described below.

Remarks: The diagnosis of Entobdellinae Bychowsky, 1957 is expanded to accommodate advancement in knowledge of morphology and proposals of *Listrocephalos* (see Bullard *et al.* 2004) and a new genus described below.

It is notable that species of a new genus described below and of *Listrocephalos* and *Pseudoentobdella* are skin parasites of stingrays. Species of *Benedeniella* and *Trimusculotrema* are capsalids also parasitising stingrays and the possibility that they are entobdellines requires investigation. These studies are in progress by IDW.

Entobdella Blainville in Lamarck, 1818

Amended generic diagnosis: With features of the Capsalidae *sensu* Yamaguti (1963) and Entobdellinae (as amended above). Median haptoral sclerites relatively large. Marginal valve conspicuous. Haptor with ventral papillae (*E. hippoglossi*, *E. soleae*) or without (*E. pugetensis*). Tendons entering haptor from body musculature attach to proximal end of anterior hamuli. Anterior attachment apparatus comprising one pair of elongated anterolateral adhesive pads, sometimes with evidence of subdivision of each pad into three separate areas (e.g., *E. soleae*, see Kearn and Evans-Gowing 1998). Two reservoirs for male accessory gland secretion, one inside, other outside sac enclosing male copulatory organ. Male genital tract and short uterus share common opening. Parasites of skin or gills of teleost flatfishes.

Type species: *Entobdella hippoglossi* (Müller, 1776) Blainville, 1818 [syn. *Hirudo hippoglossi* Müller, 1776; *Phylline hippoglossi* (Müller, 1776) Oken, 1815; *Phyllonella hippoglossi* (Müller, 1776) Goto, 1899; *Entobdella* (*Entobdella*) *hippoglossi* (Müller, 1776) Johnston, 1929. Klassen *et al.* (1989) declared *E. brattstroemi*[1] Brinkmann, 1952, *E. curvunca* Ronald, 1957, *E. rosaceus* Crane, 1972, *E. squamula* (Heath, 1902) Johnston, 1929 and *E. steingroeveri*[2] (Cohn, 1916) Johnston, 1929 to be synonyms of *E. hippoglossi*].

Other species: *E. pugetensis* Robinson, 1961 [syn. *Pseudoentobdella pugetensis* (Robinson, 1961) Yamaguti, 1963]; *E. soleae* (van Beneden and Hesse, 1864) Johnston, 1929 (syn. *Phyllonella soleae* van Beneden and Hesse, 1864; *Epibdella producta* Linstow, 1903).

Remarks: Klassen *et al.* (1989) redescribed *E. hippoglossi* from specimens collected from Pacific halibut, *Hippoglossus stenolepis* and Petrale sole, *Eopsetta jordani* off the Pacific coast of Canada and declared the following species synonymous with *E. hippoglossi*: *E. brattstroemi*, *E. curvunca*, *E. rosaceus*, *E. squamula* and *E. steingroeveri*. It is our view that the synonymy proposed by Klassen *et al.* requires re-evaluation using fresh material prepared for histological and molecular study. This re-evaluation is underway and will be offered for publication separately.

Yamaguti (1963) transferred *Entobdella pugetensis* to *Pseudoentobdella*. This action seems entirely unjustified (the reasons for the transfer are not given by Yamaguti), since the main diagnostic features of *Pseudoentobdella*, namely the absence of one pair of median sclerites on the haptor, the presence of minute papillae on the ventral surface of the haptor and the presence of unique anterior adhesive organs described by Guberlet (1936) as 'sucking grooves' (see also below), are lacking in *E. pugetensis*. Klassen *et al.* (1989) referred to this species as *Entobdella pugetensis*, but gave no reasons for replacing the species in *Entobdella*. Egorova (1999) listed *Pseudoentobdella* as monotypic and *E. pugetensis* is presented as one of nine *Entobdella* species. We regard this as a logical and necessary move.

Dyer *et al.* (1989) collected a monogenean from the skin of a stingray, *Taeniura melanospila* (= *T. meyeni*, see Froese and Pauly 2004) (Dasyatidae) from the Okinawa Expo Memorial Park Aquarium in Japan. They identified this parasite as *E. squamula* (synonymised with *E. hippoglossi* by Klassen *et al.* 1989), but examination of a voucher specimen (USNPC No. 80216) revealed the presence of an anterior diadem, suggesting that the parasite belongs in a new genus described below. Other features of this single specimen indicate that it is distinct from the other species of this new genus and a description must await the availability of more specimens.

Amer (1990) described *E. aegyptiacus* from the gills of *Epinephelus gigas* (= *E. marginatus*, see Froese and Pauly 2004) (Serranidae) and *Morone labrax* (= *Dicentrarchus labrax*, see

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[1,2] Note that following Article 32.5.2.1 of the International Code of Zoological Nomenclature (1999), spellings for *brattströmi* and *steingröveri* are corrected to *brattstroemi* and *steingroeveri*, respectively.

Froese and Pauly 2004) (Moronidae) caught in the Mediterranean Sea near Port Said, Egypt. However, the account is insufficiently detailed to permit assignment with confidence to *Entobdella* and no reference is made to deposition of museum specimens. One of us (IDW) attempted to contact Dr Amer in the early 1990s with a view to examine any available material but no response was forthcoming. Consequently this monogenean must be regarded as a *species inquirenda*.

Listrocephalos Bullard, Payne et Braswell, 2004

Type species: *Listrocephalos corona* (Hargis, 1955) Bullard, Payne et Braswell, 2004 (syn. *Entobdella corona* Hargis, 1955).

Other species: *L. guberleti* (Caballero et Bravo-Hollis, 1962) Bullard, Payne et Braswell, 2004 (syn. *Entobdella guberleti* Caballero et Bravo-Hollis, 1962); *L. kearni* Bullard, Payne et Braswell, 2004; *L. whittingtoni* Bullard, Payne et Braswell, 2004.

Remarks: As well as describing two new species, *L. kearni* and *L. whittingtoni*, Bullard *et al.* (2004) transferred to *Listrocephalos* two species from elasmobranchs previously assigned to *Entobdella*, namely *E. corona* and *E. guberleti*. We consider these actions to be sound.

Pseudoentobdella Yamaguti, 1963

Type and only species: *Pseudoentobdella pacifica* (Guberlet, 1936) Yamaguti, 1963 [syn. *Epibdella pacifica* Guberlet, 1936; *Benedenia pacifica* (Guberlet, 1936) Price, 1939].

Remarks: The description of *P.* (as *Epibdella*) *pacifica* by Guberlet (1936) was based on two specimens, but no mention was made of any type material. In the USNPC we found four unmounted specimens collected by G.E. MacGinitie (20 July 1927) from the buccal cavity of *Myliobatis californica* (Myliobatidae) from Elkhorn Slough, a salt-water estuary of Monterey Bay, California, USA. Collector, collection date and locality are identical to those given by Guberlet (1936) and the specimens are designated as paratypes in the USNPC. This material is of poor quality and has been of limited use, but we provide the following clarifications and details to supplement Guberlet's description based on whole mounts of three of the four paratypes in spirit (USNPC No. 72834).

We verify that the haptor appears to be armed with only two pairs of median sclerites. The sclerites of one pair are stout crooked rods (Fig. 1A), measuring 330 to 360 µm in length and 16 to 31 µm in breadth, running from the centre of the haptor almost to the margin, where they end bluntly with no trace of a hook. The sclerites of the other pair are less easy to locate since they are much shorter, measuring between 12 and 22 µm in length. Each of the short rods has blunt ends and lies on the median side of the adjacent long rod near its anterior extremity (Fig. 1A). The positions of the sclerites on the haptor and their locations relative to each other are such that the short rods are likely to be modified accessory sclerites and the long rods anterior hamuli. No trace of structures that might represent posterior hamuli was found. Hooklets, not mentioned by Guberlet (1936), were found, but specimen quality precluded finding the expected total of fourteen. We also confirm that the ventral surface of the haptor bears numerous circular papillae. A marginal valve, illustrated by Guberlet (1936; see his fig. 1), is prominent.

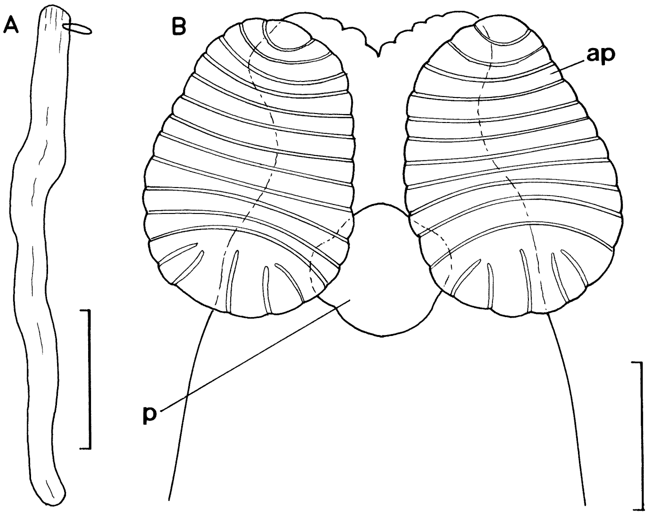


Fig. 1. *Pseudoentobdella pacifica*: **A** – long haptoral sclerite (anterior hamulus?) with tiny haptoral sclerite (accessory sclerite?) at proximal end (upper end in diagram); **B** – anterior region of the head in ventral view; ap – anterior adhesive pad, p – pharynx. Scale bars = 100 µm (A), 25 µm (B)

The newly mounted paratypes were of most use in clarifying the morphology of the anterior end of *P. pacifica*. Guberlet (1936) described 'a pair of sucking grooves', each 'marked on the inner surface by 10 or 12 minute longitudinal grooves'. In one of the paratypes mounted by us, these organs resembled those illustrated by Guberlet (1936; his fig. 1), but when another paratype was flattened between slide and coverslip we discovered that each so-called sucking groove is a roughly saucer-shaped ventral pad, slightly longer than broad (Fig. 1B), and that Guberlet's interpretation of their structure was based on a specimen in which the edges of the pad were folded inwards. We observed that 11–14 narrow bands, running mostly in a roughly transverse direction, cross the ventral surface of each pad. These bands seem likely to be grooves, but it is difficult to confirm this in the specimens available. Marginally, each ventral pad is slightly notched where the bands meet the edge of the pad.

In the anterior median region of the head between the adhesive organs, Guberlet (1936; his fig. 1) illustrated narrow longitudinal striations, similar to the 'longitudinal grooves' that he reported on the anterolateral adhesive organs. In the useful flattened specimen examined by us, these features were not apparent. However, in this specimen the anterior margin of the head was seen to be deeply notched medially, with marginal lobes on each side of the notch (Fig. 1B).

The quality of the material at our disposal was too poor to resolve any details of the reproductive system. Two of three specimens contained a tetrahedral egg in the ootype. Guberlet (1936) commented that the filament of the egg ended in a 'pad or adhesive disc', which may refer to a terminal adhesive droplet on the egg appendage. Guberlet noted the absence of eyes. We were unable to locate pigmented eyes in the three newly mounted paratypes that we studied. Reassessment of this species is needed, but must await the discovery of fresh material.

***Neoentobdella* gen. nov.**

Generic diagnosis: With features of the Capsalidae *sensu* Yamaguti (1963) and Entobdellinae (as amended above). Median haptoral sclerites relatively large. Ventral haptor surface apapillate. Marginal valve present, reduced and inconspicuous, or absent. Anterior attachment apparatus comprising one pair of elongate, anterolateral adhesive pads, each subdivided transversely to form many rays separated by narrow troughs, creating overall appearance of a diadem. Reservoir for male accessory gland secretion located inside sac enclosing male copulatory organ; reservoir single-chambered as in *N. apiocolpos* (see Euzet and Maillard 1967) or two-chambered as in *N. diadema* (see Llewellyn and Euzet 1964). Male copulatory organ a penis (as in the first of the two new species described below) or a cirrus (as in *N. apiocolpos* comb. nov., see Euzet and Maillard 1967, and below). Male genital tract and short uterus share common opening. Parasites of skin of elasmobranch flatfishes (dasyatids, myliobatids and rhinobatids).

Origin of name: Species in the new genus have affinities with species in the older taxon *Entobdella*. *Neoentobdella* (gender: feminine) from *neos*, Greek for new referring to a new group of *Entobdella*-like species.

Type species: *Neoentobdella diadema* (Monticelli, 1902) comb. nov. [syn. *Epibdella diadema* Monticelli, 1902; *Entobdella* (*Entobdella*) *diadema* (Monticelli, 1902) Johnston, 1929; *Entobdella diadema* (Monticelli, 1902) Price, 1939].

Other species: *N. apiocolpos* (Euzet et Maillard, 1967) comb. nov. (syn. *Entobdella apiocolpos* Euzet et Maillard, 1967); *N. australis* (Kearn, 1978) comb. nov. (syn. *E. australis* Kearn, 1978); *N. bumpusii* (Linton, 1901) comb. nov. [syn. *Epibdella bumpusii* Linton, 1901; *Epibdella* (*Parepibdella*) *bumpusii* (Linton, 1901) Johnston, 1929; *Entobdella bumpusii* (Linton, 1901) Price, 1939]; two new species described below.

As indicated above, a further species of *Neoentobdella* from the stingray *Taeniura meyeni*, incorrectly identified as *Entobdella squamula* by Dyer *et al*. (1989), awaits description when more material becomes available.

Remarks: Linton's (1901) account (cited following Hargis and Thoney 1983) of *Epibdella bumpusii* predates that of Monticelli (1902) for *E. diadema*, but we propose *Neoentobdella diadema* (Monticelli, 1902) comb. nov. as the type species of *Neoentobdella* because the anatomy and biology of *N. diadema* is better known than those of *N. bumpusii*.

Euzet and Maillard (1967) described the male copulatory organ of *Entobdella apiocolpos* (= *Neoentobdella apiocolpos* comb. nov.) as a cirrus. This interpretation was confirmed in the present study by examination of paratypes and other whole mount preparations (LE).

Klassen *et al*. (1989) pointed out that *Entobdella* species segregate into two groups, one group comprising *E. hippoglossi*, *E. pugetensis* and *E. soleae* on teleost flatfishes and the other group comprising *E. diadema*, *E. apiocolpos*, *E. australis*, *E. bumpusii*, *E. corona* and *E. guberleti* on elasmobranch flatfishes. There are a few reports of *Entobdella* species from round-bodied teleosts, e.g., scorpaenids (Heath 1902, Egorova 2000), embiotocids (Crane 1972), serranids (Dyer *et al*. 1989, Amer 1990) and moronids (Amer 1990), but these are isolated or poorly described parasites and the host records require verification. While appreciating the implication that *Entobdella* species should be subdivided, Klassen *et al*. (1989) regarded it as inappropriate at that time. From molecular analyses, Whittington *et al*. (2004) demonstrated that *Entobdella* species from teleosts clustered separately from *Entobdella* species from elasmobranchs. This discovery provides further support for the separation of the entobdellines on teleost hosts from those on elasmobranch hosts. In addition, Bullard *et al*. (2004) have proposed *Listrocephalos* for two new species of entobdellines, namely *L. kearni* and *L. whittingtoni*, from the skin of dasyatid stingrays, and have transferred *E. corona* and *E. guberleti* from dasyatid and urolophid rays respectively, to this genus (see above). This transfer leaves a cohesive group of four parasite species from dasyatids and a rhinobatid, all of which possess anterior attachment pads subdivided to form many rays resembling a diadem. This feature was reflected in Monticelli's (1902) choice of specific name for *E. diadema*, and Llewellyn and Euzet (1964) used the term 'diadem' to describe this distinctive anterior attachment apparatus in the same species. The male accessory gland secretion in members of this group is stored inside the male copulatory sac, in single- or two-chambered reservoirs. The discovery on dasyatids of two additional species described in this paper, both of which possess a 'diadem' and a single-chambered accessory gland reservoir inside the male copulatory sac, reinforces this grouping and increases its members to six species.

This contrasts with the three species recorded from teleost flatfishes, namely *E. hippoglossi*, *E. pugetensis* and *E. soleae*, in which there is no diadem, each of the two anterior attachment pads being undivided, as illustrated by Klassen *et al*. (1989, fig. 1) for *E. hippoglossi*, or divided into three adhesive areas as in *E. soleae* (see Kearn and Evans-Gowing 1998) and *E. pugetensis* (see Robinson 1961, fig. 25). Moreover, the teleost parasite species have two male accessory gland reservoirs, only one of which is enclosed within the male copulatory sac. These characters provide strong support for subdivision of those species currently included in *Entobdella*, reserving the original genus for species parasitising teleosts and we propose a new genus, *Neoentobdella*, for the four previously described species from elasmobranch rays, together with the two new species described below, parasitising dasyatid elasmobranchs.

Species of *Neoentobdella*, *Pseudoentobdella* and *Listrocephalos* parasitise elasmobranch flatfishes (Dasyatidae, Rhinobatidae, Myliobatidae, Urolophidae), but the two last-named genera are readily distinguished from *Neoentobdella* species by reduction of the median haptoral sclerites and the absence of one pair in *Pseudoentobdella pacifica*. According to Guberlet (1936), *P.* (as *Epibdella*) *pacifica* is also distinguished by the absence of eyes, by the presence of minute papillae on the ventral haptor surface and by the presence of a pair of multinucleate bodies resembling glands of Goto located between the germarium and testes. Our studies of newly mounted paratypes of *P. pacifica* have revealed that each of the two anterolateral attachment organs is a saucer-shaped disc with numerous, narrow, roughly transverse bands (grooves?) crossing the ventral surface (Fig. 1). Reassessment of this species is needed but must await the availability of more specimens.

There are other differences between *Listrocephalos* and *Neoentobdella* in addition to the reduction in size of the median haptoral sclerites in species of the former genus. *Listrocephalos* species have papillae on the ventral haptor surface and each of the two anterior attachment areas is subdivided, forming numerous raised ovoid structures rather than elevated rays. The openings of the male reproductive system and the uterus are separate in *Listrocephalos* species.

***Neoentobdella natans* sp. nov.** (Figs 2–11)

Type host and locality: *Pastinachus sephen* (Dasyatidae) (cowtail stingray); Heron Island, Queensland, Australia (23°27´S, 151°55´E).

Other localities: Lizard Island, Queensland, Australia (14°40´S, 145°28´E) (see voucher specimen listed below).

Site on host: Ventral skin.

Prevalence and intensity: At Heron Island, prevalence 66.7% (based on 9 rays caught between November 1989 and July 1995; wingspan range 53–86 cm); intensity 1–36 (10.7) (based on 6 rays examined within 5 days of capture). At Lizard Island (June 1998), prevalence 100%, intensity 1 adult specimen [n = 1 ray (female); wingspan 57 cm].

Origin of name: The species name relates to the report by Kearn and Whittington (1991) of the swimming ability of juvenile specimens of this taxon. We have since determined that adult specimens of this species can also swim (see below). From Latin *natans*, present participle of the verb *nato*, to swim.

Holotype: SAMA AHC28664 (1 whole mount).

Paratypes: SAMA AHC28665-AHC28668 (4 whole mounts); SAMA AHC28669 (2 slides of sagittal sections); SAMA AHC28670 (6 slides of transverse sections); USNPC No. 95182 (4 whole mounts); BMNH No. 2004.10.5.1-4 (4 whole mounts).

Vouchers: Two mounted specimens from Heron Island lodged in SAMA AHC28430-1 (see *Entobdella* sp. 2 of Whit-

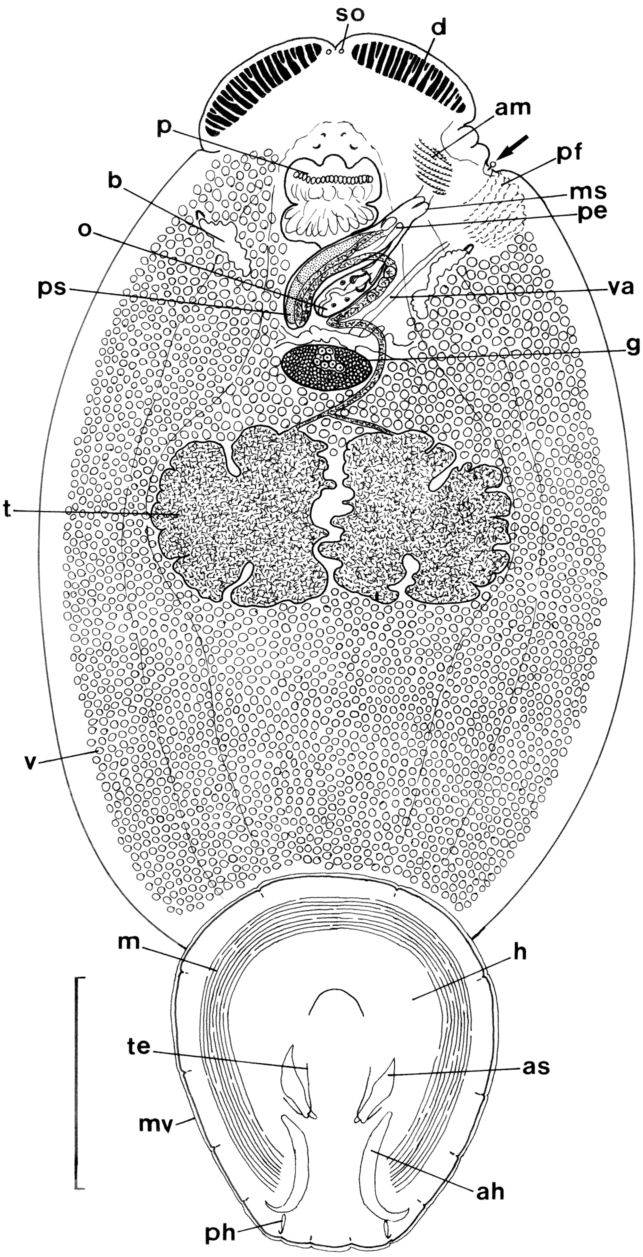


Fig. 2. *Neoentobdella natans* sp. nov. Whole animal in ventral view: ah – anterior hamulus; am – array of microsclerites (inside genital atrium); as – accessory sclerite; b – bladder; d – ‘diadem’; g – germarium; h – haptor; m – haptoral muscle (?) bands; ms – muscular structure (papilla?); mv – marginal valve; o – ootype; p – pharynx; pe – penis; pf – area of parallel folds (on dorsal surface; seen by transparency); ph – posterior hamulus; ps – penis sac; so – presumed sense organ; t – testis; te – tendon; v – vitellarium; va – vagina. Arrow indicates position of knob-like (sense?) organ. Scale bar = 1 mm

tington *et al.* 2004); SAMA AHC28675 (1 whole mount) ex ventral skin of *Pastinachus sephen* from Lizard Island (collected 19 June 1998).

Description: Based on 44 flattened whole mount preparations (23 adults; 21 juveniles), plus one adult specimen sectioned transversely and another sectioned in a sagittal plane. Sample of ten flattened mounted adult specimens measured. Body roughly oval in outline (Fig. 2). Total length and breadth: 6.35 (5.23–7.81) × 3.30 (2.67–4.51) mm. Haptor length and breadth: 2.05 (1.78–2.94) × 1.79 (1.52–2.51) mm. Length of median haptoral sclerites as follows: accessory sclerites 378 (320–499) (20) (Fig. 3A); anterior hamuli 568 (465–876) (20) (Fig. 3B); posterior hamuli 101 (86–108) (16) (Fig. 3C). Positions of hooklets on posterior and posterolateral borders of haptor often indicated by small marginal indentations (Fig. 2). Hooklets (measured in flattened juveniles) approximately 20 long (Fig. 3D). Anterior hamulus of juvenile found with sharp distal point broken off, but broken point close to original position relative to rest of hamulus (Fig. 3E), indicating that intact hamulus point strongly recurved and projecting towards centre of haptor. Points of anterior hamuli in adults reveal fracture planes, suggesting all may have lost recurved hook tips (Fig. 3B). Marginal valve present, narrow (confirmed in histological sections), inconspicuous in whole animal (Fig. 2). Tendons conspicuous; after passing through notches at proximal ends of accessory sclerites, travel obliquely in anterior direction alongside accessory sclerites; not attached to proximal end of anterior hamuli (Fig. 2). In some individuals, evidence that tendon divides after passing through notch and that slender, inconspicuous branch (?) may travel across to anterior hamulus (Fig. 4). Approximately nine narrow bands (muscle?) running parallel to each other and to haptor margin in anterior and lateral regions of haptor (Fig. 2).

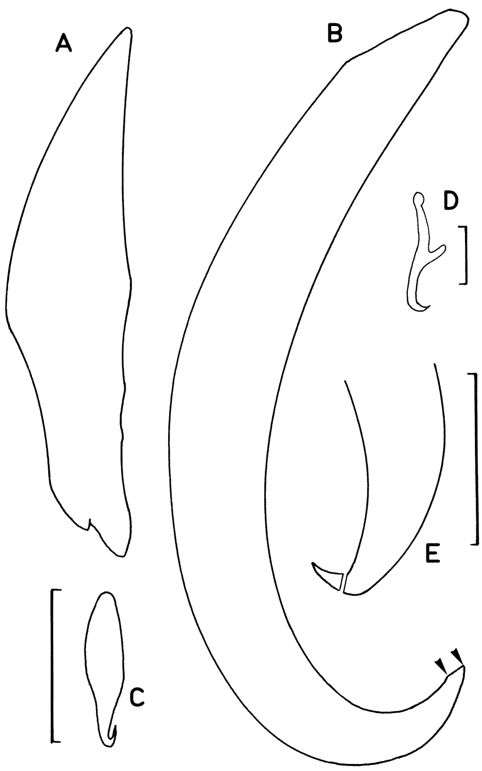


Fig. 3. *Neoentobdella natans* sp. nov. Haptoral sclerites of adult (except E): **A** – accessory sclerite; **B** – anterior hamulus; **C** – posterior hamulus; **D** – hooklet; **E** – posterior end of the anterior hamulus of a juvenile, showing fractured hook point. In B arrowheads indicate extent of fracture plane. Scale bars = 100 µm (A–C); 10 µm (D); 50 µm (E).

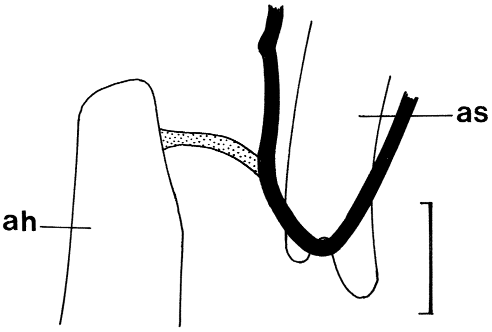


Fig. 4. *Neoentobdella natans* sp. nov. Proximal ends of anterior hamulus (ah) and accessory sclerite (as), showing path of tendon (black) and branch (structure uncertain) (stippled) linking tendon and anterior hamulus. Scale bar = 50 µm

Adhesive pads on anterolateral borders of head region subdivided to form 'diadem' (see Llewellyn and Euzet 1964); each pad comprising 17 (16–19) (20) flat, slightly elevated rays, separated by troughs from neighbouring rays and running across pad transversely from outer to inner borders (Figs 2 and 5). In addition to complete rays, 12 (6–27) (20) incomplete rays present, of varying lengths, occupying spaces between outer regions of complete rays. Number of rays in immature individuals (female reproductive system not yet functional) similar to adults. Pair of small circular structures (sense organs?) on ventral surface between two adhesive pads close to anterior border of head region (Figs 2 and 5). Bladders mostly conspicuous, orientated obliquely towards body margins in posterior-anterior direction, each bladder opening on urinary papilla (Figs 2 and 6). Pharynx 462 (347–583) × 501 (384–654). Intestinal caeca obscured by vitellarium.

General arrangement of reproductive organs and associated ducts as in other entobdellines (Figs 2 and 6). Testes relatively large: 929 (770–1070) (20) × 938 (760–1147) (20); numerous marginal indentations, two of which penetrate deeply into anterior and posterior margins of each testis. Glands of Goto not observed in adults or juveniles. Two short vasa efferentia unite posterior to germarium; after skirting left side of germarium, vas deferens develops thick wall containing prominent nuclei (Fig. 6). Vas deferens narrows, then expands and alongside ootype becomes convoluted with prominent nuclei in wall; enters penis sac and expands inside to form sperm-filled reservoir alongside reservoir for male accessory granular secretion. Contents of proximal and distal regions of male accessory secretion reservoir sometimes appear to differ in texture, creating sharp discontinuity; corresponding regional difference in staining of contents in histological sections stained with haematoxylin and eosin. Source of male accessory secretion not identified; no prominent ducts observed penetrating proximal end of penis sac. Sperm-filled reservoir and male accessory secretion reservoir each give rise distally to short duct; these fuse and common duct enters papilla-like penis and opens at distal tip. Penis housed in cavity receiving uterus laterally. Large muscular structure (papilla?), often hard to see, lying dorsal to penis and associated with opening of uterus into male reproductive tract. Common genital duct expands to form genital atrium bearing on inner surface muscular pad with 9–11 rows of closely spaced but separate microsclerites, each resembling a shark denticle, measuring 4–10 in height, 6–8 in width (Figs 6 and 7). Genital atrium opening on ventral surface close to body margin. Region of fleshy developments on dorsal surface adjacent to common genital opening, resolvable into two areas: anterior lip lying immediately dorsal to common genital opening; posterior to lip, a series of diagonal, roughly parallel folds (Figs 6–8). In adults and juveniles, single knob-like organ projecting from body margin just posterior to common genital opening (Figs 6 and 7).

Germarium with large centrally located fertilisation chamber; gives rise to short oviduct joining relatively long common vitelline duct to become ovo-vitelline duct (Fig. 6). Vitelline reservoir transversely elongated with prominent lobes. No seminal receptacles. Vagina originating proximally from common vitelline duct or from vitelline reservoir, following relatively straight path in anterolateral direction. In region of bladder, course of vagina no longer visible in whole mounts. In sections, vagina continues in anterolateral direction as narrow duct towards region of dorsal fleshy folds, but vaginal opening not detected. Many gland ducts entering ovo-vitelline duct at base of ootype; ducts appear to originate from cells between vitelline follicles adjacent to reproductive organs, but not posterior to testes.

Eggs roughly triangular in side view (Fig. 9), but urn-shaped when turned through 90°. Dimensions measured from freely deposited eggs: breadth in side view 161 (139–177) (8 eggs); total egg capsule length 234 (206–260) (8 eggs); opercular discontinuity to apex of egg 43 (36–52) (4 eggs); appendage length (measured along curves) 167 (114–234) (7 eggs); appendage bearing thick coating of granular adhesive material at tip (Fig. 9), capable of sticking eggs firmly to glass surfaces.

Differential diagnosis: The possession of a muscular pad armed with rows of microsclerites inside the genital atrium and elaborate fleshy developments on the dorsal surface in the region of the common genital opening, distinguishes *Neoentobdella natans* from all other species of the genus except the new species of *Neoentobdella* described below. The dorsal developments in *N. natans* are resolvable into an anterior lip adjacent to the common genital opening and, immediately posterior to the lip, an area of diagonally arranged, parallel, fleshy folds. The following features of *N. natans* distinguish it from the new species of *Neoentobdella* described below: the presence of a muscular structure (papilla?) in the reproductive tract distal to the penis; the microsclerites in the genital atrium are not fused to each other and in profile resemble shark denticles; the folds posterior to the dorsal lip lie roughly parallel; the presence of a knob-like sensory (?) structure on the body margin, near the common genital opening; the testes are relatively large; the eggs are relatively small.

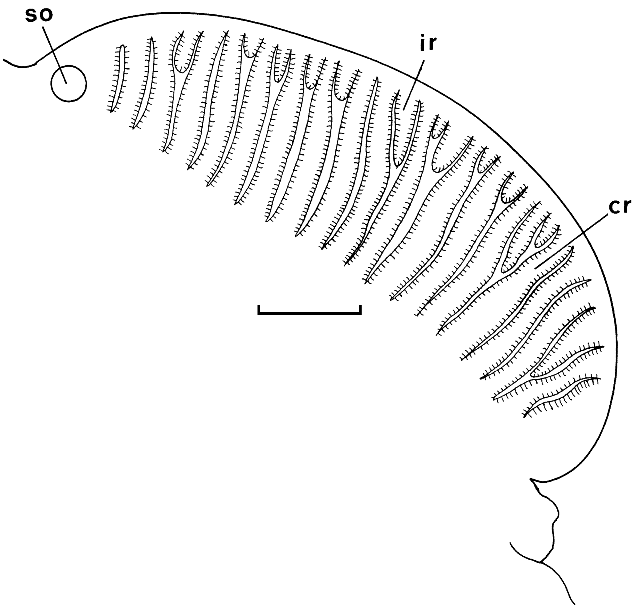


Fig. 5. *Neoentobdella natans* sp. nov. Left adhesive pad (half of 'diadem') in ventral view: cr – complete ray; ir – incomplete ray; so – presumed sense organ. Scale bar = 100 μm

Additional observations: In a previous report, Kearn and Whittington (1991) described swimming in juvenile parasites. This has been confirmed and swimming ability was also observed in adult specimens.

In some whole mounts, granular material resembling the contents of the male accessory secretion reservoir in the penis sac was observed on the body surface close to the opening of the genital atrium and, in one adult specimen, attached to the fleshy folded area dorsal to the common genital opening. It is possible that this is discarded spermatophore material or perhaps spermatophores in the process of being offered to, or received from, another individual. A similar object (Fig. 10) found alongside a living adult parasite maintained in sea water in a glass dish may have been a discarded spermatophore.

In a single whole mount preparation (paratype: SAMA AHC28668) of a juvenile specimen (total length 5.55 mm; anterior hamulus length 470), the penis papilla was bent through almost 180° and inserted into the uterus (Fig. 11). The ootype of this individual contained granular material.

Neoentobdella parvitesticulata **sp. nov.** (Figs 12–17)

Type host and locality: *Himantura fai* (Dasyatidae) (pink whipray); Heron Island, Queensland, Australia (23°27′S, 151°55′E).

Site on host: Ventral skin.

Prevalence and intensity: Prevalence 35% (based on 41 rays caught between November 1988 and December 1995; wingspan range 53–84 cm); intensity 1–16 (6.47) (based on 15 fish examined within 5 days of capture).

Origin of name: The species name relates to the relatively small size of the testes. From: *parvus*, Latin for small; *testiculatus*, Latin for having testicles.

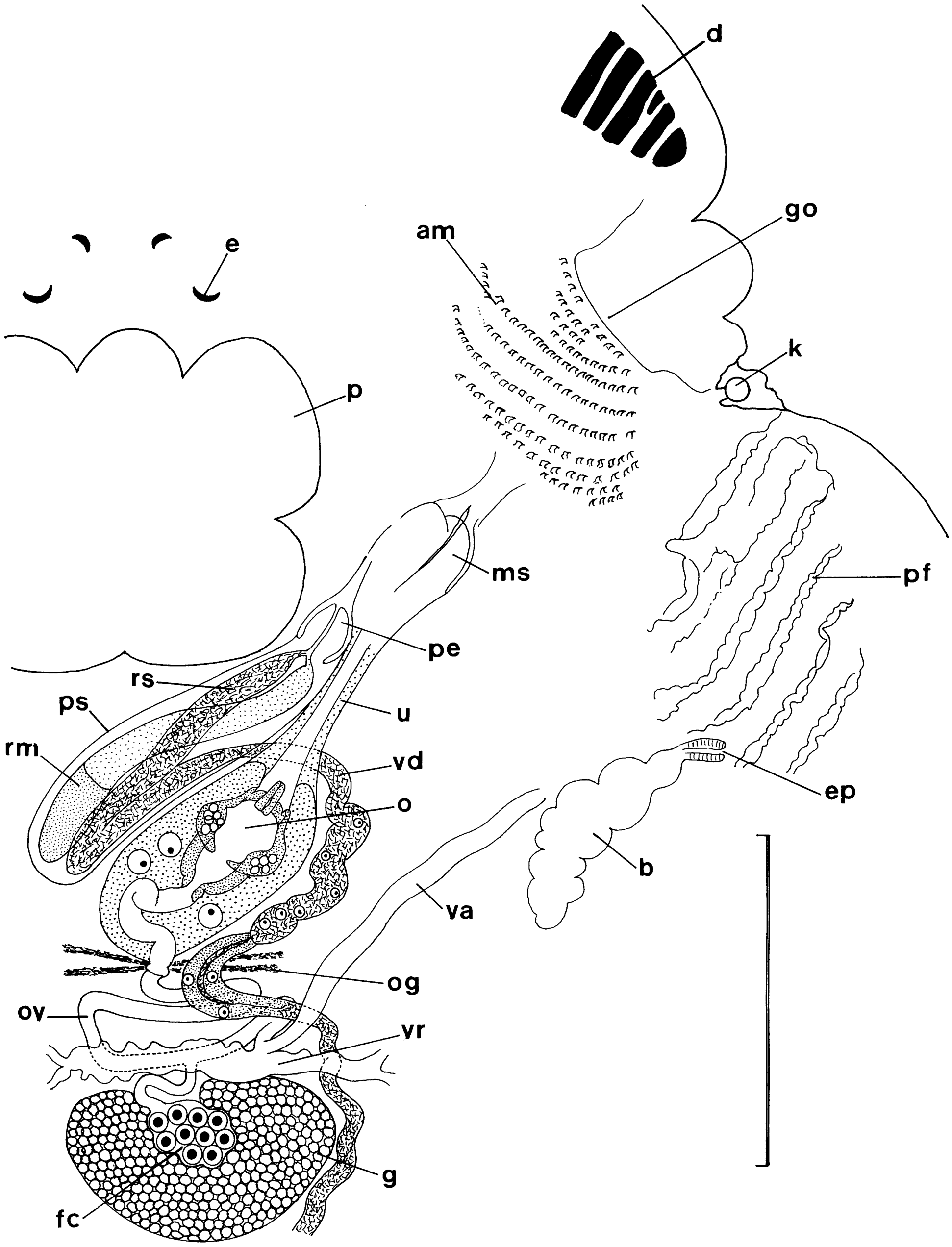
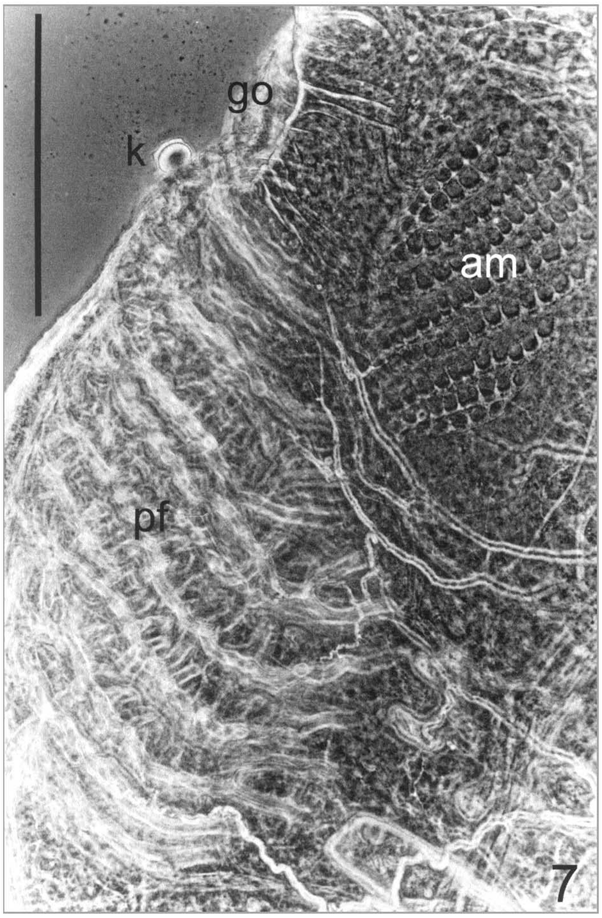


Fig. 6. *Neoentobdella natans* sp. nov. Anterolateral region of the body (left side) in ventral view, showing details of the reproductive system: e – eye, ep – excretory papilla, fc – fertilisation chamber, go – common genital opening, k – knob-like (sense?) organ, ms – muscular structure (papilla?), og – ootype glands, ov – ovo-vitelline duct, rm – reservoir containing male accessory secretion, rs – reservoir containing sperm, u – uterus, vd – vas deferens, vr – vitelline reservoir. Other lettering as in Figure 2. Note: the parallel folds (pf) posterolateral to the genital atrium are on the dorsal surface (see Figs 2 and 8) and are shown here as seen by transparency. Scale bar = 500 µm



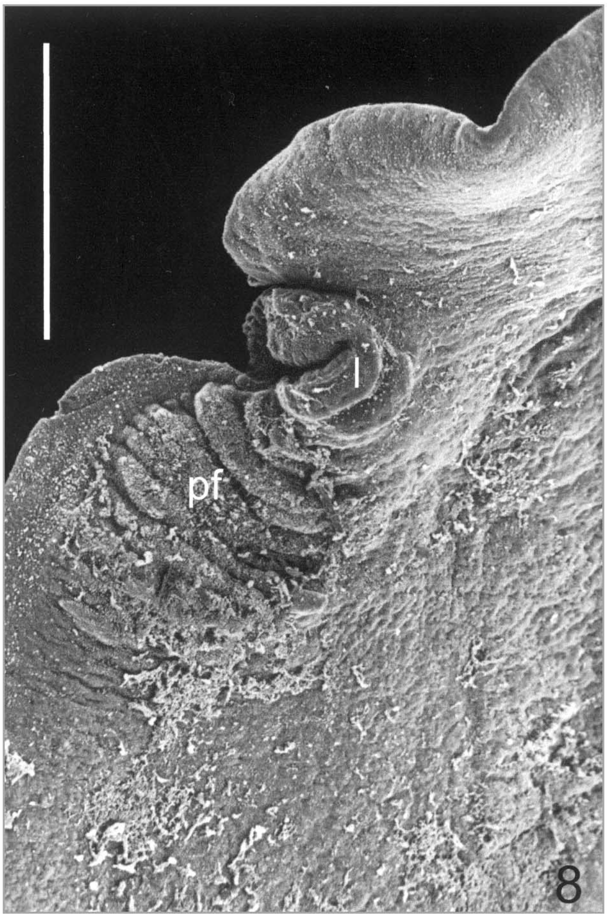
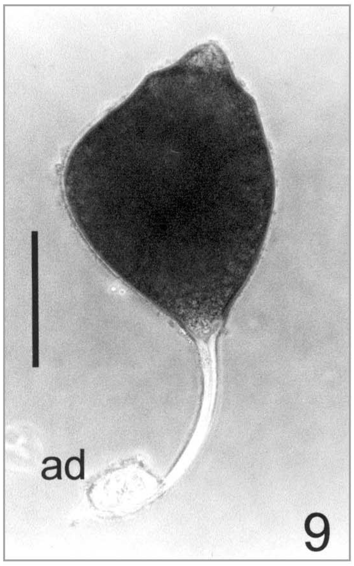


Fig. 7. *Neoentobdella natans* sp. nov. Phase contrast micrograph of the dorsal surface, left side, in the region of the opening of the genital atrium (go). The array of microsclerites (am) inside the genital atrium is sharply focussed; the parallel folds (pf) on the dorsal surface are out of focus; k – knob-like (sense?) organ. Scale bar = 250 µm. **Fig. 8.** Scanning electron micrograph of an area of the dorsal body surface adjacent to the common genital opening, similar to that shown in Figure 7. The fleshy development on the dorsal surface comprises anterior lip (l) and posterior parallel folds (pf). Scale bar = 500 µm



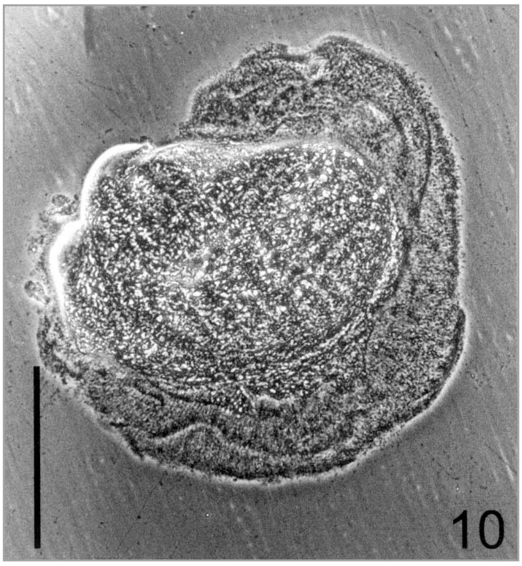


Fig. 9. *Neoentobdella natans* sp. nov. Light micrograph of egg: ad – adhesive material attached to free end of egg appendage. Scale bar = 100 µm. **Fig. 10.** Putative spermatophore. Scale bar = 250 µm

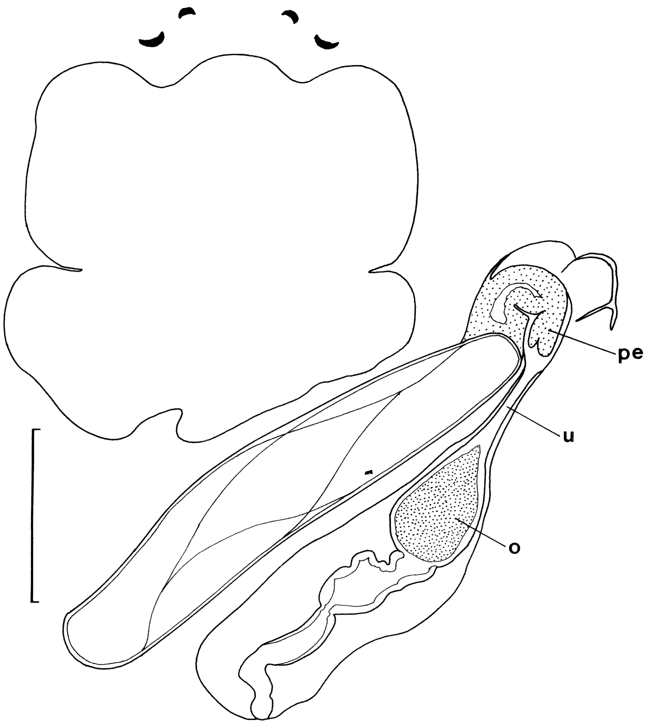


Fig. 11. *Neoentobdella natans* sp. nov. Juvenile (paratype: SAMA AHC28668) with penis (pe) deflected into the uterus (u). Note granular material in ootype (o). Scale bar = 200 µm

Holotype: SAMA AHC28671 (1 whole mount).

Paratypes: SAMA AHC28672-AHC28674 (3 whole mounts); USNPC No. 95183 (4 whole mounts); BMNH No. 2004.10.5.5-8 (4 whole mounts).

Vouchers: Two specimens lodged in SAMA AHC28428-9 (see *Entobdella* sp. 1 of Whittington *et al.* 2004).

Description of adult: Based on 60 flattened whole mount preparations (22 adults; 38 juveniles). Sample of ten flattened mounted adult specimens measured. Body outline tends towards oblong shape (Fig. 12). Total length and breadth: 6.4 (4.75–8.21) × 3.7 (2.25–4.51) mm. Haptor 2.01 (1.52–2.55) × 1.70 (1.44–2.13) mm. Lengths of median haptoral sclerites as follows: accessory sclerites 528 (337–575) (20) (Fig. 13A); anterior hamuli 547 (382–789) (20) (Fig. 13B-D); posterior hamuli 145 (118–168) (16) (Fig. 13E). Small indentations of haptor margin indicate positions of hooklets (Fig. 12). Hooklets (measured in flattened juveniles) approximately 25 long (Fig. 13F). Points of anterior hamuli strongly recurved, projecting towards centre of haptor (Fig. 12); points usually intact in juveniles; in most adults, points broken off and either still *in situ* or missing (Fig. 13C-D). Marginal valve absent (Figs 12 and 14). Tendons slender and inconspicuous; after passing through notch at proximal end of accessory sclerite, attach to proximal part of anterior hamulus (Fig. 12); evidence in some individuals of tendon branching after passage through notch, with second inconspicuous branch travelling close to accessory sclerite in anterolateral direction. Approximately nine narrow bands (muscle?) running parallel to each other and to haptor margin in anterior and lateral regions of haptor (Fig. 12).

Each ‘diadem’ pad with 15 (14–16) (10) complete rays and 4 (1–10) (10) incomplete rays (Fig. 12). Numbers of rays in immature individuals similar to adults. Pair of circular structures (sense organs?) on ventral surface between two adhesive pads, close to anterior border of head region (Fig. 12). Bladders conspicuous in all individuals, travelling obliquely towards body margins in posterior-anterior directions; bladder opening at apex of prominent urinary papilla, having

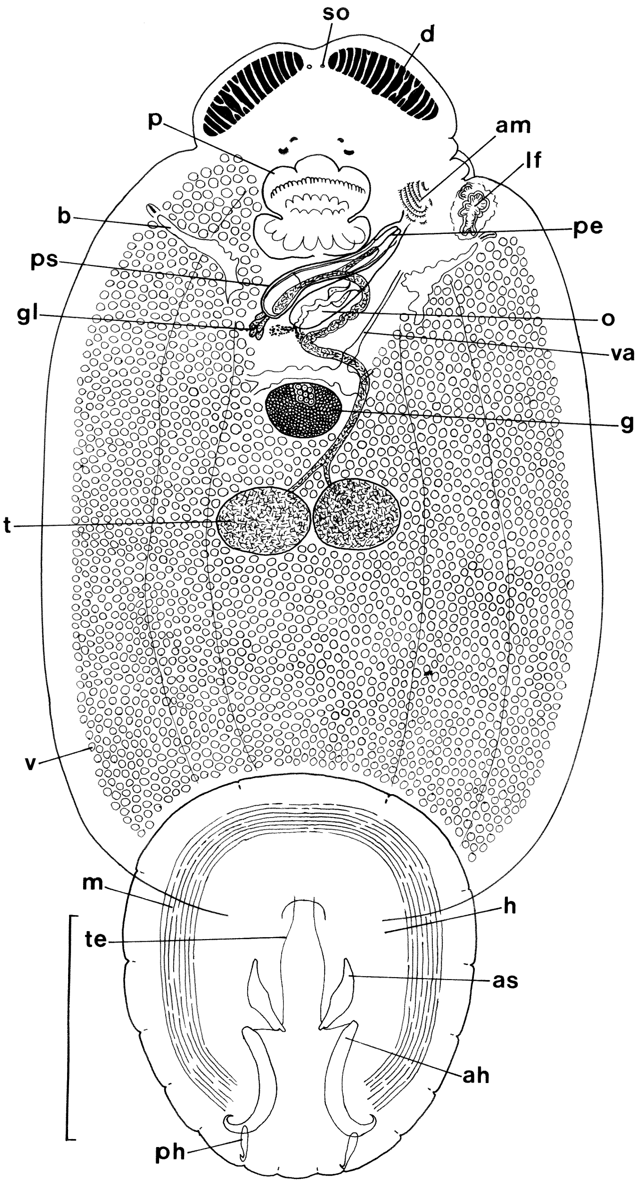


Fig. 12. *Neoentobdella parvitesticulata* sp. nov. Whole animal, in ventral view: gl – gland cells that may supply secretion to male accessory secretion reservoir; lf – labyrinthine folded area (on dorsal surface; seen by transparency). Other lettering as in Figure 2. Scale bar = 1 mm

affinity for acetocarmine. Pharynx 568 (401–846) × 622 (392–979). Intestinal caeca obscured by vitellarium.

Testes relatively small: 332 (235–433) (20) × 515 (302–858) (20); no prominent marginal indentations (Fig. 12). Glands of Goto not observed in adults or juveniles. Reproductive system similar to that of *N. natans*, but no muscular structure (papilla?) in reproductive tract between penis and genital atrium (Figs 12 and 15). Ridged muscular pad inside genital atrium, each ridge with sclerotised apical strip bearing closely spaced short projecting teeth, creating comb-like structure approximately 4 in height. Whole field comprising 4–6 roughly parallel, relatively long sclerotised combs, longest measuring about 138 in length, together with a few much shorter combs, shortest measuring about 16 long. Region of fleshy developments on dorsal surface adjacent to common genital opening, resolvable into two distinct areas: anterior lip lying immediately dorsal to common genital opening; labyrinthine, irregularly folded area lying immediately posterior to lip (Figs 12, 15 and 16). No knob-like organ observed on body margins of juveniles or adults near common genital opening. Vagina appears to originate from common vitelline duct or from vitelline reservoir; traced in whole mounts only as far as region of left bladder – vaginal pore not detected in preserved, mounted specimens, but observed opening ventrally in live specimens. Ducts containing granular secretion prominent among vitelline follicles adjacent to reproductive organs, but not seen posterior to testes. These ducts appear to converge on junction of ovo-vitelline duct and ootype (as in *N. natans*; see Fig. 6). Gland cells close to proximal end of penis sac (Fig. 12) may supply male accessory secretion to reservoir inside sac.

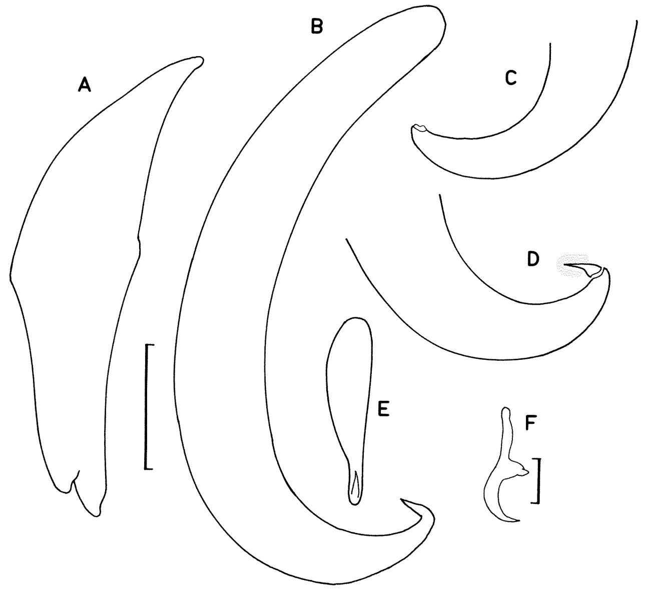


Fig. 13. *Neoentobdella parvitesticulata* sp. nov. Haptoral sclerites of adult: **A** – accessory sclerite; **B** – anterior hamulus with intact hook point; **C** and **D** – hooked distal regions of anterior hamuli, with hook point missing (C) and with hook point fractured but still *in situ* (D); **E** – posterior hamulus; **F** – hooklet. Scale bars = 100 µm (A-E), 10 µm (F)

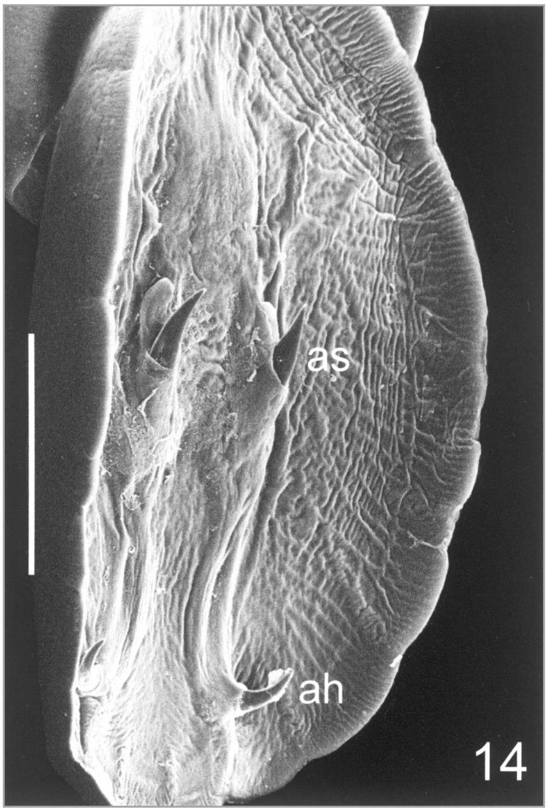


Fig. 14. *Neoentobdella parvitesticulata* sp. nov. Scanning electron micrograph of haptor in oblique view: ah – anterior hamulus, as – accessory sclerite. Note absence of marginal valve. Scale bar = 500 µm

Eggs roughly triangular in side view, but urn-shaped when turned through 90° (Fig. 17). Measurements of freely deposited eggs: breadth in side view 207 (201–212) (5 eggs); total egg capsule length 325 (310–341) (6 eggs); opercular discontinuity to apex of egg 60 (52–72) (3 eggs); appendage length (measured along curves) 164 (114–200) (11 eggs); appendage with thick coating of granular adhesive material at tip. Eggs laid singly, or retained with appendage lodged in genital atrium until up to eleven have accumulated, then released as group attached by adhesive material at ends of appendages (Fig. 17).

Differential diagnosis: The possession of a muscular pad armed with rows of microsclerites inside the genital atrium and elaborate fleshy developments on the dorsal surface in the region of the common genital opening, distinguishes *Neoentobdella parvitesticulata* from all other species of the genus except *N. natans* (see above). In *N. parvitesticulata*, the dorsal developments are resolvable into an anterior lip adjacent to the common genital opening and, immediately posterior to the lip, a labyrinthine area of irregularly arranged fleshy folds.

The following features of *N. parvitesticulata* distinguish it from *N. natans*: there is no muscular structure (papilla?) in the reproductive tract distal to the penis; the microsclerites in the genital atrium are fused to sclerotised ridges creating comb-like structures; the folds posterior to the dorsal lip are arranged irregularly (labyrinthine), not parallel; there is no knob-like sensory (?) structure on the body margin near the common genital opening; the testes are relatively small; the eggs are relatively large.

Additional observations: Adult and juvenile specimens attach readily to glass surfaces by the anterior attachment regions and by the haptor. When undergoing leech-like locomotion, adult specimens of *N. parvitesticulata* can extend their bodies over 2 cm in length. Stationary parasites attached by the haptor undulate their body continually in the manner described for *E. soleae* by Kearn (1962). Adult specimens laid eggs readily when maintained in glass dishes containing filtered sea water at 23–24°C. Strong peristaltic contractions of the ootype were evident during egg manufacture. To expel a fully formed egg from the ootype, parasites displayed a characteristic series of contractions of the body whereby the anterior third of the parasite contracted strongly and, simultaneously, the edges of the body curled dorsally. During observation, one specimen laid an egg every 4–5 min over a period of 2.5 h. Egg expulsion is rapid and some eggs are released singly, but others are retained temporarily and released in groups of up to 11 eggs (see above) (e.g., Fig. 17). On a few occasions during the anterior body contractions described above, groups of eggs were seen to be relocated from the common genital pore to the region of dorsal, irregular folds where eggs remained attached to the dorsal surface of the parasite for several minutes. Once free of the parasite, eggs, whether single or in groups, attach tenaciously to glass surfaces by their adhesive droplets. Strong jets of water from a Pasteur pipette fail to dislodge attached eggs and they require prising off glass surfaces using a needle.

A description of the oncomiracidium of *N. parvitesticulata* will be submitted for publication separately.

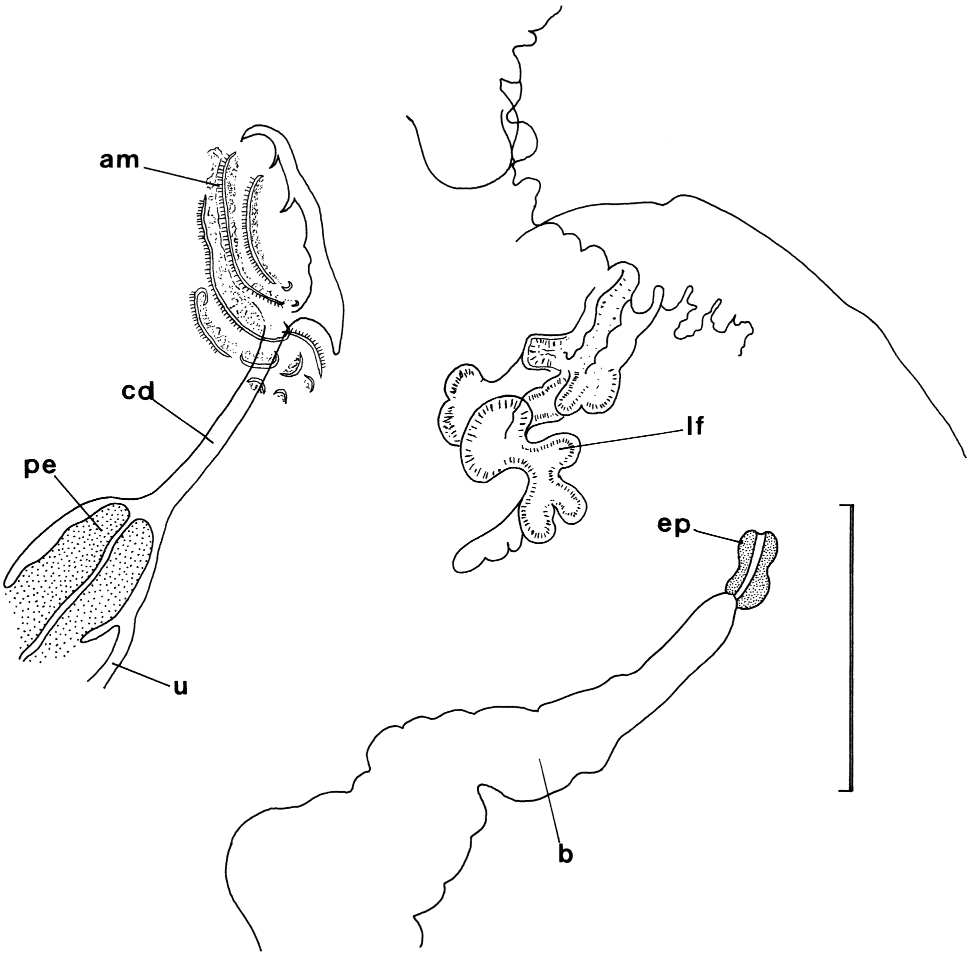
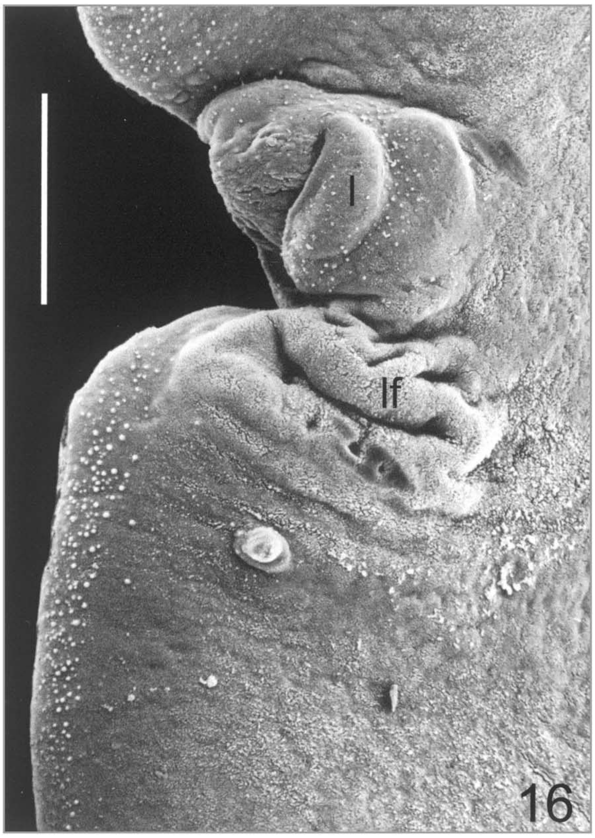


Fig. 15. *Neoentobdella parvitesticulata* sp. nov. Anterolateral region of the body (left side) in ventral view, showing termination of common genital duct (cd) and associated structures: am – array of microsclerites (inside genital atrium), b – bladder, ep – excretory papilla, lf – labyrinthine folded area (on dorsal surface; seen by transparency), pe – penis, u – uterus. Scale bar = 100 µm



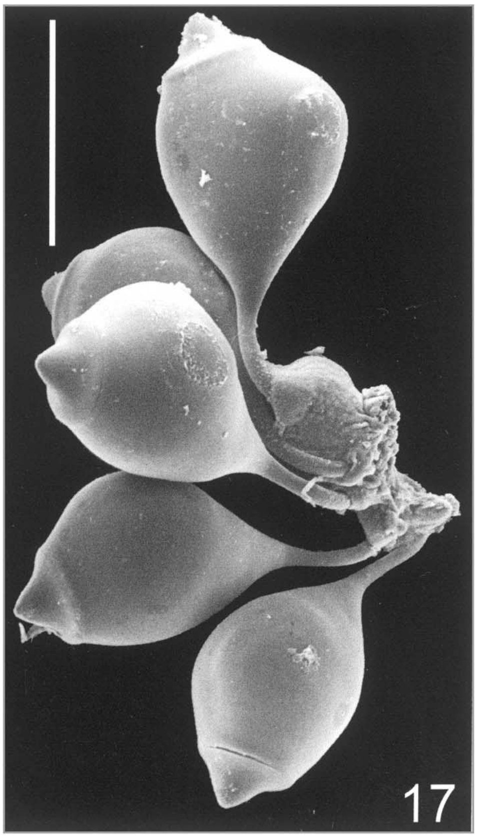


Fig. 16. *Neoentobdella parvitesticulata* sp. nov. Scanning electron micrograph of an area of the dorsal surface adjacent to the common genital opening. The fleshy development on the dorsal surface comprises anterior lip (l) and a labyrinthine system of irregular folds (lf). Scale bar = 200 µm. **Fig. 17.** Scanning electron micrograph of a group of five eggs attached to each other by adhesive secretion at the free ends of the appendages. Scale bar = 200 µm

During manipulation of parasites, one sub-adult and two adult specimens of *N. parvitesticulata* were seen to swim, like their close relative *N. natans*.

Discussion

Seven of the nine species of *Entobdella* regarded by Klassen *et al*. (1989) as valid remained after the transfer of *E. corona* and *E. guberleti* to *Listrocephalos* by Bullard *et al*. (2004). These seven species fall into two distinct subgroups. *Entobdella hippoglossi*, *E. pugetensis* and *E. soleae* parasitising the skin or gills of teleost flatfishes lack a 'diadem' (subdivision of the adhesive pads into numerous narrow rays) and have two reservoirs for storage of male accessory gland secretion, one lying inside the male copulatory sac and the other outside. *Entobdella diadema*, *E. bumpusii*, *E. apiocolpos*, *E. australis* and two new species from Queensland, Australia, described herein, all parasitise the skin of elasmobranch flatfishes (predominantly dasyatid stingrays; *E. apiocolpos* is also reported from a rhinobatid, *Zanobatus schoenleinii*, see Euzet and Maillard 1967), possess a 'diadem' and have a single- or two-chambered male accessory gland reservoir located inside the male copulatory sac (single-chambered in *E. apiocolpos* and *E. australis* according to Euzet and Maillard 1967, and Kearn 1978, respectively; two-chambered in *E. diadema*, according to Llewellyn and Euzet 1964). These different characters provide strong support for the proposal to retain *Entobdella* for the three teleost parasite species (including *E. hippoglossi*, the original type species of the genus) and to propose a new genus, *Neoentobdella*, for the six species that parasitise elasmobranchs, with *N. diadema* comb. nov. as type species. These actions are also consistent with a preliminary phylogeny for the Capsalidae based on large subunit ribosomal DNA sequence data proposed by Whittington *et al*. (2004) in which *Entobdella* species from teleosts fell in a separate clade from '*Entobdella*' species (including *N. parvitesticulata* and *N. natans* as *Entobdella* sp. 1 and 2, respectively) from elasmobranchs.

Detailed anatomical descriptions are provided for *N. natans* from the skin of *Pastinachus sephen* and *N. parvitesticulata* from the skin of *Himantura fai*, both dasyatid host species from the Great Barrier Reef, Queensland, Australia. These two monogenean species are closely related, similar in size, with similar male copulatory organs (a penis) and a similar vaginal morphology. Inside the genital atrium, both species possess a muscular pad carrying arrays of microsclerites and have elaborate fleshy developments on the dorsal surface in the region of the common genital opening. These embellishments of the genital atrium and dorsolateral body surface serve to distinguish the two new species from all species previously ascribed to *Entobdella*. The two new species are also readily distinguished from each other by differences between these features. In *N. natans*, the microsclerite array in the genital atrium consists of rows of tiny individual microsclerites, each resembling a shark denticle in profile, while in *N. parvitesticulata* each row resembles a comb, the tiny tooth-like microsclerites being fused to a narrow sclerotised strip. The dorsolateral fleshy developments are resolvable in both species into two distinct areas: anterior lips and fleshy folds immediately posterior to the lips. The lips are similar in both species, but the posterior folds in *N. natans* lie roughly parallel to each other, while in *N. parvitesticulata* the folds have an irregular (labyrinthine) arrangement. These differences are reinforced by the presence in *N. natans* of a knob-like structure on the body margin near the genital atrium. Another distinguishing feature concerns the testes, which are significantly larger in *N. natans* and have an irregular outline with two deep and conspicuous indentations in each organ, one penetrating the anterior margin of each testis and the other penetrating its posterior margin. A muscular structure (papilla?) in the male reproductive tract and distal to the penis occurs in *N. natans* but not in *N. parvitesticulata*. This structure is less reliable as a distinguishing feature since it sometimes lies dorsal to the penis and is hard to see. The eggs of *N. natans* are significantly smaller than those of *N. parvitesticulata*.

The rays of each anterior adhesive area ('diadem') also differ in number although there is overlap between them. *Neoentobdella natans* has an average of 17 (16–19) complete rays, plus 12 (6–27) incomplete rays, while *N. parvitesticulata* has 15 (14–16) complete rays and 4 (1–10) incomplete rays. Juvenile parasites have approximately the same number of rays as adults suggesting that the adhesive pads increase in size by growth of existing rays rather than by addition of new rays. This also indicates that incomplete rays are not in the process of developing into complete rays but serve to increase the overall adhesive area of a pad by filling the gaps between complete rays, especially where these rays diverge near the external borders of the pads. A pair of circular features on the ventral surface between the median ends of the pads of both species is more likely to be sensory than adhesive in function.

Neoentobdella natans has a roughly oval body shape but the body of *N. parvitesticulata* has a more oblong profile. Living specimens of *N. natans* are able to swim as reported by Kearn and Whittington (1991). Subsequent observations on live specimens of *N. parvitesticulata* by one of us (IDW) demonstrated that this species is also capable of swimming. Further study of live specimens of other *Neoentobdella* species is required to determine whether this ability is widespread throughout the genus.

The terminal regions of the common genital duct and vagina and the areas of the body surface adjacent to these duct terminations are particularly elaborate in *Neoentobdella* species. The genital opening of *N. diadema* is equipped with a pair of lips (Llewellyn and Euzet 1964) and muscular structures (lips or papillae) occur inside the common reproductive canals distal to the penis in *N. australis* (see Kearn 1978) and in *N. natans*. Kearn (1978) reported the presence of a muscular sucker in the genital atrium of *N. australis*, and the genital atria of *N. natans* and *N. parvitesticulata* have pads bearing rows of microsclerites. In *N. diadema*, *N. apiocolpos* and *N. bumpusii*, the terminal regions of the vaginae are expanded and elaborated to form muscular/glandular sacs, being particularly spacious in the last-named species (see Llewellyn and Euzet 1964, Euzet and Maillard 1967 and Linton 1901 respectively). In *N. australis* (see Kearn 1978), *N. natans* and *N. parvitesticulata*, the vagina is not dilated distally and in the two last-named species is hard to trace in whole mounts. An additional unique feature in *N. natans* is the presence of a knob-like structure on the body margin near the genital atrium.

The knob-like structure on the anterolateral body margin of *N. natans* is likely to be sensory and may have a role in mating, but it is difficult to assign functions to the other sexual embellishments of *N. natans* and *N. parvitesticulata* until more is known about their reproductive behaviour. However, the microsclerite-bearing pads in the genital atrium of these two species may have a role in controlling the release of eggs, since, as eggs are assembled by *N. parvitesticulata* and leave the uterus, the genital atrium may fail to release the terminal regions of their egg appendages until as many as eleven eggs have accumulated. Temporary relocation of newly released bunches of eggs to the dorsal region of irregular fleshy folds has also been observed in living *N. parvitesticulata*.

Some entobdellines are known to assemble and transfer spermatophores. In *E. soleae* mutual exchange of soft, jelly-like spermatophores occurs, while in *N. diadema* spermatophores with a thin outer casing have been reported (see Llewellyn and Euzet 1964). Our observations on *N. apiocolpos* (LE specimens, ex *Taeniura grabata*, 1996, Tunisia) revealed that the spermatophores reported by Euzet and Maillard (1967) also have a thin outer casing. The male accessory gland reservoirs of *N. natans* and *N. parvitesticulata* contain material that, by analogy with *E. soleae*, *N. diadema* and *N. apiocolpos*, seems likely to contribute towards the matrix or outer casing of spermatophores, but encased spermatophores have not been identified in *N. natans* and *N. parvitesticulata*. However, masses of material resembling the jelly-like spermatophores of *E. soleae* have been observed attached to the body surface near the common genital opening in slide-mounted specimens of *N. natans*, alongside a living parasite in a dish of sea water and attached to the folded dorsal area.

The source of the male accessory gland secretion, stored inside the penis sac of *N. natans* and *N. parvitesticulata* is not clear. In *N. apiocolpos*, Euzet and Maillard (1967) failed to find ducts entering the penis sac and this was also our experience with *N. natans*. A few gland cells were found near the proximal end of the sac in *N. parvitesticulata* but whether these contribute significantly to the contents of the penis sac is uncertain. In contrast, Llewellyn and Euzet (1964) found prominent ducts originating from an extensive follicular gland dispersed among the inner band of vitelline follicles and converging on and penetrating the male copulatory sac. In *N. parvitesticulata*, there is a conspicuous follicular gland interspersed with vitelline follicles anterior and lateral to the testes, but the ducts from this gland appear to converge on the proximal end of the ootype and may supply the adhesive secretion observed on the egg appendage. Similar glands and ducts occur in *N. australis*, in which each egg appendage is furnished with a large drop of sticky secretion (Kearn 1978).

The discovery of a single juvenile specimen of *N. natans* in which the penis was deflected into the uterus (Fig. 11) raises the possibility that self-insemination may occur in this species. The penis is so short and so near the opening into the uterus that little manoeuvrability is needed to achieve this, and before egg production commences it would be theoretically possible for sperm to reach the oviduct and the fertilisation chamber of the germarium via the ootype. There is evidence that self-insemination via the uterus takes place in *Benedeniella macrocolpa* before egg assembly begins (Kearn and Whittington 1992), but experimentally isolated individuals of *E. soleae* did not self-inseminate (Kearn *et al.* 1993).

It was observed that the points of the anterior hamuli of most juvenile specimens of *N. parvitesticulata* are angled abruptly inwards and point towards the centre of the haptor. In a few mounted adults these delicate points persist, but in most preparations they are broken off and are either still *in situ* or missing. In *N. natans* the impression was gained at first that recurved hook tips were absent until a juvenile was found with the sharp recurved point broken off, but lying close to its original position relative to the rest of the hook. Closer examination of the hook points of adults revealed that they possessed fracture planes at their free terminations compatible with the earlier loss of a delicate recurved hook tip. Thus it seems likely that the anterior hamuli of *N. natans*, like those of *N. parvitesticulata*, acquire recurved hook tips during larval development. It is possible that the recurved hook tips break off as a consequence of the stresses and strains of removal from the host by scraping, but it is also possible that loss of hook tips is a natural feature. The recurved points may be sufficiently strong to provide anchorage for relatively small juveniles without breaking, but as the parasite grows and stresses increase, the points may break off. In spite of the loss of the delicate hook tip, the hamulus point remains sharp. The loss of the tip also changes the direction in which the hook tip points and this may reflect a change in the role of the anterior hamulus in attachment of larger individuals.

In adult *N. natans*, the tendons associated with the accessory sclerites are conspicuous, but appear to have lost their connection with the anterior hamuli, except for a possible inconspicuous branch in some individuals (Fig. 4). In adult *N. parvitesticulata*, the tendons retain their terminal connection to the anterior hamuli, but the tendons themselves are reduced to slender threads and probably make little or no contribution to haptor function. In *E. soleae*, the intrinsic musculature of the haptor is capable of generating suction (Kearn 1988), and it seems likely that corresponding haptor muscles in adult *N. natans* and *N. parvitesticulata* compensate for reductions in the effectiveness of the extrinsic muscle/tendon/sclerite system.

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