Tools for identifying selected Australian aquatic oligochaetes (Clitellata: Annelida)

Adrian Pinder

TRIN Taxonomic Guide 2.

Taxonomy Research & Information Network
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Department of
Environment and Conservation
Our environment, our future

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INTRODUCTION

This document provides:

1) a guide to the characters required for oligochaete identification
2) a key to major annelid groups,
3) a key to families and some distinctive lower taxa
4) a key to the Naidinae.

Since the guide to identification of Australian oligochaetes was produced by Pinder and Brinkhurst (1994) there have been significant changes in oligochaete systematics and improved knowledge of Australian oligochaete diversity. Molecular work has confirmed that Oligochaeta is a paraphyletic group without inclusion of the leeches and branchiobdellidans. Clitellata is now commonly used to describe the larger group (e.g. Erseus and Kallersjo, 2004), though Oligochaeta will no doubt continue to be used as a convenient term for the 'non-leech, non-branchiobdellidan Clitellata', as it is here. Moreover, it appears to the oligochaetes may lie within the polychaete clade (e.g. Rouset et al. 2007).

The other major change is that the former Naididae sensu strictu was synonymised with the Tubificidae, with the name Naididae having precedence (Erseus et al. 2002; Erseus et al. 2005; Erseus and Gustavsson, 2002; IUZN, 2007). Thus the Naididae s.s is now a subfamily, Naidinae, within a larger Naididae that includes all the former tubificid subfamilies

(Tubificinae, Rhyacodrilinae, Phallodrilinae, Limnodriloidinae and Telmatodrilinae). This refining of clitellate systematics is continuing with indications that Naidinae itself is not a monophyly. In Australia, the former Telmatodrilinae have been placed within new genera of Rhyacodrilinae, so the subfamily is no longer represented in Australia (Pinder and Brinkhurst 2000). Finally, phallodriline naidids have been recorded in Australian limnic waters for the first time (Pinder et al. 2006).

On top of these systematic changes, the number of limnic species known from Australia has grown from 91 to almost 200, though a large proportion remains undescribed. The number of marine oligochaetes known from Australia also continues to grow.

Species richness within the aquatic families and subfamilies of Australia
(numbers in brackets are endemic).

Family	limnic described	limnic known undescribed	terrestrial	marine/ estuarine	total
Naididae	76 (23)	36 (36)	0	135	247
Phreodrilidae	32 (28)	32 (32)	0	1	65
Lumbriculidae	2 (0)	0	0	0	2
Capilloventridae	3 (3)	0	0	0	3
Enchytraeidae	4 (2)	probably many	6	33	43
Haplotaxidae	4 (1)	1 (1)	0	0	5
TOTAL	121	69	6	169	365

CLASSIFICATION

ANNELIDA

POLYCHAETA

Aphanoneura: Aeolosomatidae (have been allied with oligochaetes in the past)

CLITELLATA

Branchiobdelida (ectocommensal on fw crayfish - not known from Australia)

Euhirudinea (leeches and acanthobdellidans, phylogenetically within the former "Oligochaete" clade)

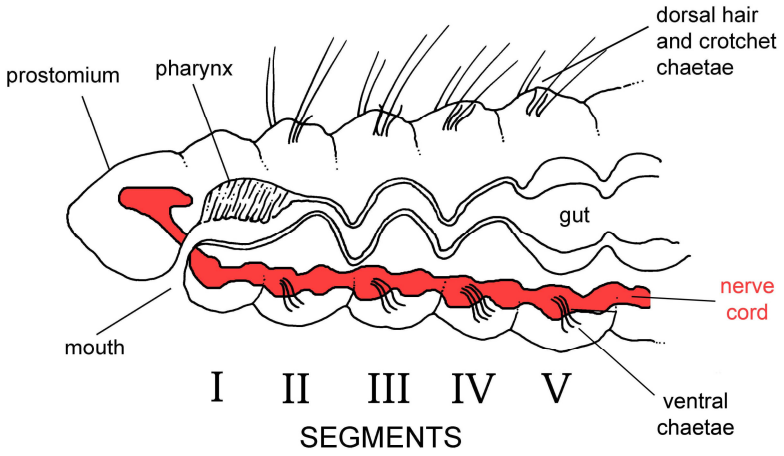
"**Oligochaeta**"

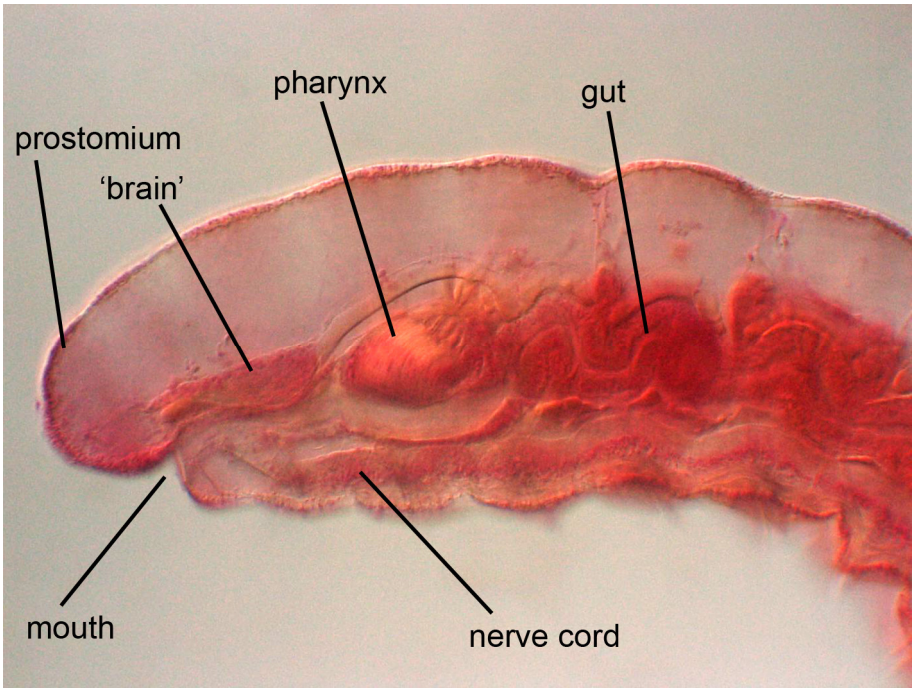
Several earthworm families	(some with aquatic or semi-aquatic representatives)
Haplotaxidae	(freshwater/interstitial/profundal, stygal)
Lumbriculidae	(2 introduced freshwater species)
Naididae	(freshwater, marine, stygal)
Tubificinae	(mostly freshwater, few marine)
Phallodrilinae	(mostly marine, few stygal)
Naidinae	(ex Naididae, mostly limnic, few stygal)
Rhyacodrilinae	(marine and freshwater)
Limnodriloidinae	(all marine)
Phreodrilidae	(Gondwanan, almost entirely freshwater)
Enchytraeidae	(marine, terrestrial and freshwater - poorly studied in Australia)
Propappidae	(not known from Australia)
Capilloventridae	(marine and freshwater)
Opistocystidae	(not known from Australia)
Narapidae	(not known from Australia)
Randidrilidae	(not known from Australia)

EXPLANATION OF CHARACTERS

Arrangement of the body. Roman numerals are used to denote segments while Arabic numerals are used to denote septa between segments (e.g. 1/2, 4/5 …). The head end is almost always broader than the anal end of a worm and has a ventral furrow (mouth) just behind the front of the worm. The most anterior part of the worm is the prostomium which is not counted as a segment. Segment I has the crescent-shaped ventral mouth but no chaetae. Chaetae generally start on segment II but dorsal chaetae may be absent on some anterior segments.

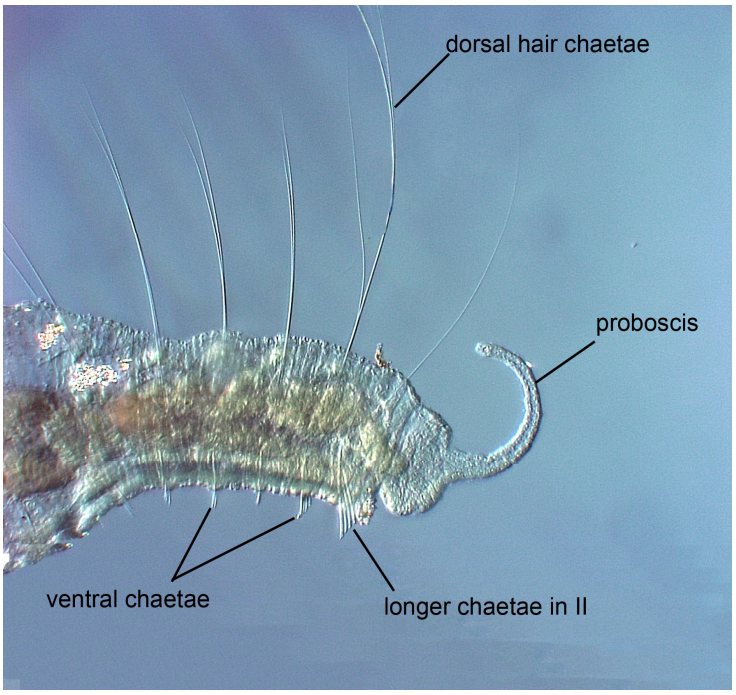
A useful orienting feature is the nerve cord which is normally easy to see and is always ventral. The nerve cord has a speckled appearance. The mouth is also ventral and normally long hairs (see below) are restricted to the dorsal side.



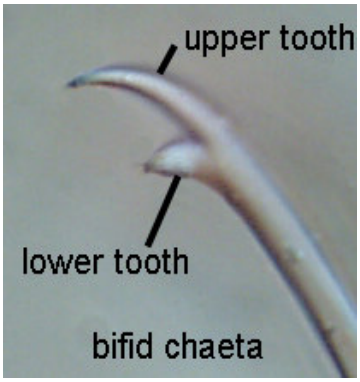


Chaetae (= setae of some authors): Chaetae occur in groups called **bundles** (although sometimes there will only be one chaeta/'bundle'). Most oligochaetes have 4 bundles per segment (2 ventro-lateral and 2 lateral to dorso-lateral). Chaetae are absent on the **prostomium** (head) and segment I but present on all or most segments thereafter, except for the anal segment (**pygidium**) and sometimes ventrally (rarely dorsally) on 1 or 2 segments containing the genitalia.

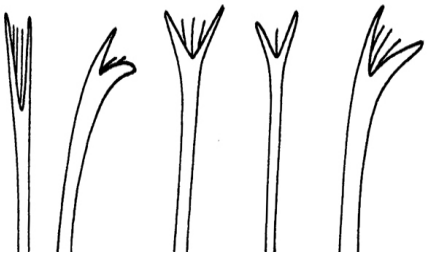
Hair chaetae (sometimes just called **hairs** or **capilliform** chaetae): long, very thin 'hair-like' chaetae, normally with a fine tapering tip. In most oligochaetes (except for the Capilloventridae, plus 2 naidids and 1 phreodrilid) these are restricted to dorsal bundles. Sometimes with annulations or serrations along the shaft or with secondary hairs giving a plumose appearance.



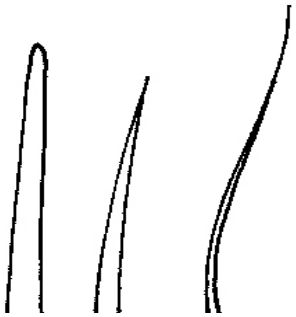
Crotchet chaetae: non-hair chaetae, often sigmoid in shape, usually with a swelling (**nodulus**) along the shaft, with tips either **single (=simple)** (a bluntly or sharply pointed tip), **bifid** (forked), **pectinate** (with comb-like teeth between the fork) or otherwise modified (e.g. paddle-shaped). In bifid and pectinate chaetae the **upper tooth** is on the convex side of the chaeta and the **lower tooth** is on the concave side. The relative length of these teeth is frequently used in keys.



Tip of bifid chaeta

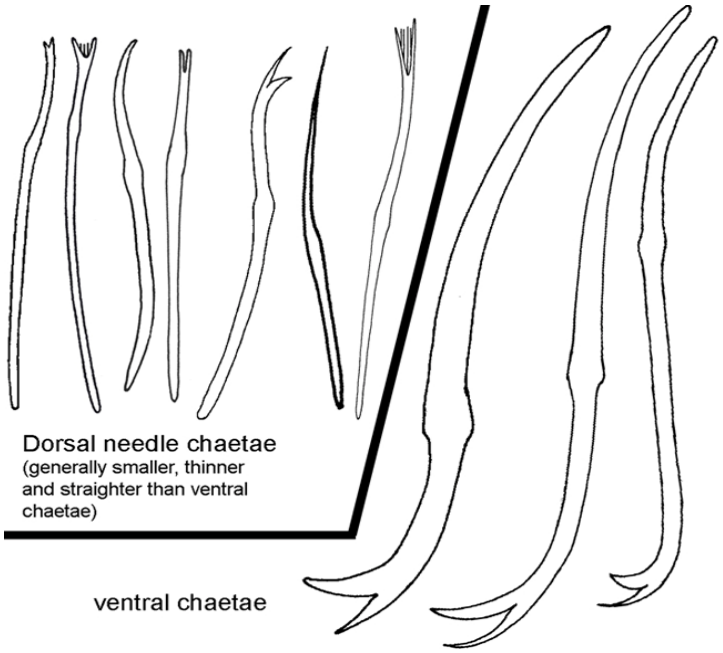


Tips of pectinate chaetae



Tips of single-pointed chaetae

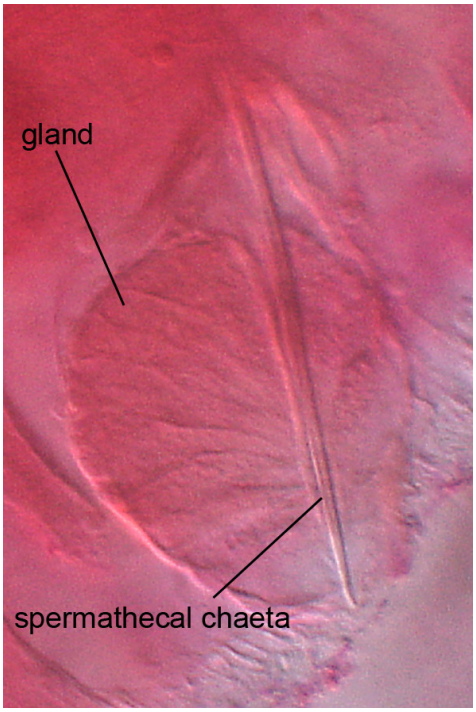
Needle chaetae: Most members of the subfamily Naidinae have short, fine, and usually fairly straight crotchet chaetae called **needles** (see previous page). These are generally much smaller than the bifid ventral chaetae and usually occur with hairs. High magnification is normally needed to see the form of the tips of needle chaetae.



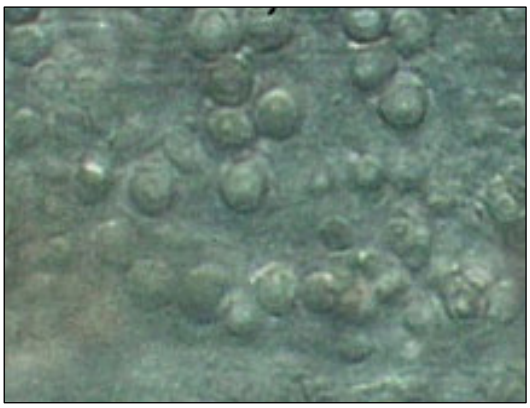
Naidine needle chaetae (left) compared to bifid ventral chaetae at same scale. Note nodulus (swelling part way along shaft).

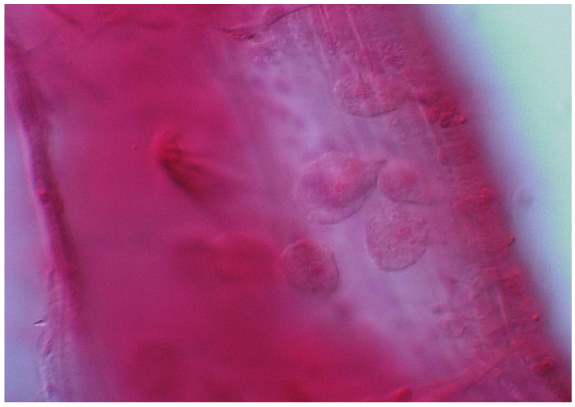
'Support' chaetae: Most Phreodrilidae have a pair of small fine chaetae 'support' surrounding the base each hair or crotchet chaeta. These look very much like especially fine needle chaetae (when they can be seen) but they do not emerge from the chaetal sac within the body wall and are only visible on slide mounted specimens by looking through the epidermis. Some Naidinae, especially some *Pristina*, have such fine needles that they appear much like support chaetae but do emerge from the body wall.

Genital chaetae: The ventral chaetae of mature specimens are usually absent or modified on the segments bearing the genital pores. These are mostly of two forms, 1) long straight single chaetae with hollow distal halves and sharp blade-like tips, associated with a large gland and normally associated with one much smaller chaeta or 2) one or more chaetae, often in parallel or with the distal ends bunched together, with bifid or bluntly simple tips. Both types are largely hidden within the body but can be seen in stained and cleared specimens. Genital chaetae of capilloventrids are long thickened hairs.



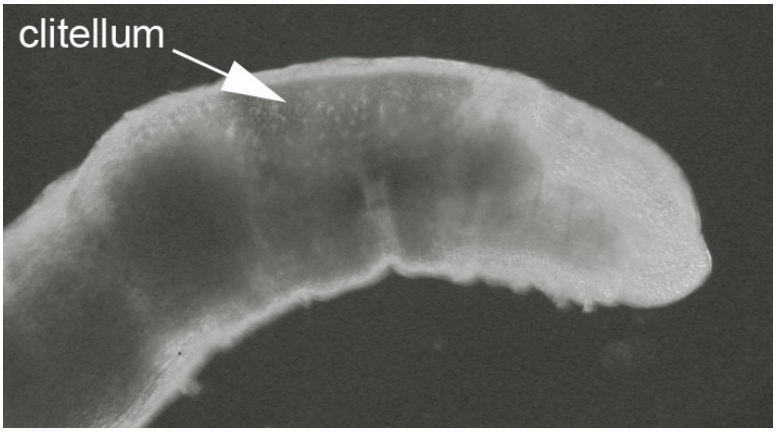
Coelomocytes: round to oval cells free floating in the coelomic cavity, in clusters or individually. Often very dense in naidine and rhyacodriline naidids. These can often be seen in glycerol mounted specimens.

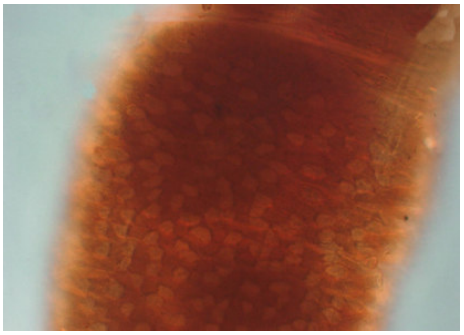


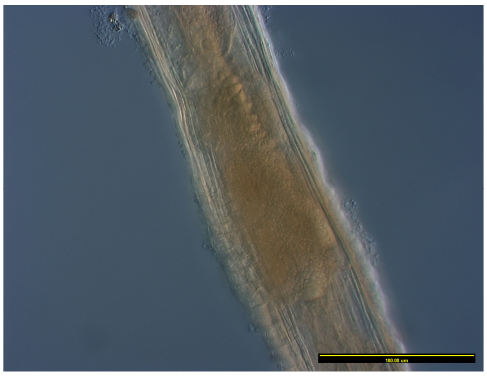


Genitalia: Although most specimens can be identified to family using external features, the most reliable way to identify a specimen to family is to use the genitalia. Knowing the segmental position of the spermatheca and male genital ducts is generally sufficient to arrive at a family, though this requires mature specimens which are not always present in a sample. Oligochaetes are hermaphrodites so have male and female genitalia. Examination of the genitalia is normally required for genus or species level identifications, with the notable exception of the Naidinae.

Clitellum: The easiest way to determine whether a worm is mature is to look for the clitellum. This is a thickened and more opaque region of the body with a different texture resulting from development of a glandular layer of cells around 2 or more segments in the region of the genitalia. This cell layer secretes the cocoon (below right) into which the sperm and eggs are deposited.







The clitellum is located as follows for the different families and subfamilies:

Naidinae	two consecutive segments between IV and VIII
Other Naididae	X and XI
Enchytraeidae	XI and XII
Phreodrilidae	½ XII and all of XIII
Lumbriculidae	several segments from VIII or IX
Capilloventridae	½ XII to segment XIV
Haplotaxidae	over many segments from about X or XI

Genital organs can often be seen in glycerol mounted specimens if the worms are small, but its generally best to stain and clear the specimens (see below), especially for long term storage as glycerol will eventually over clear soft tissues.

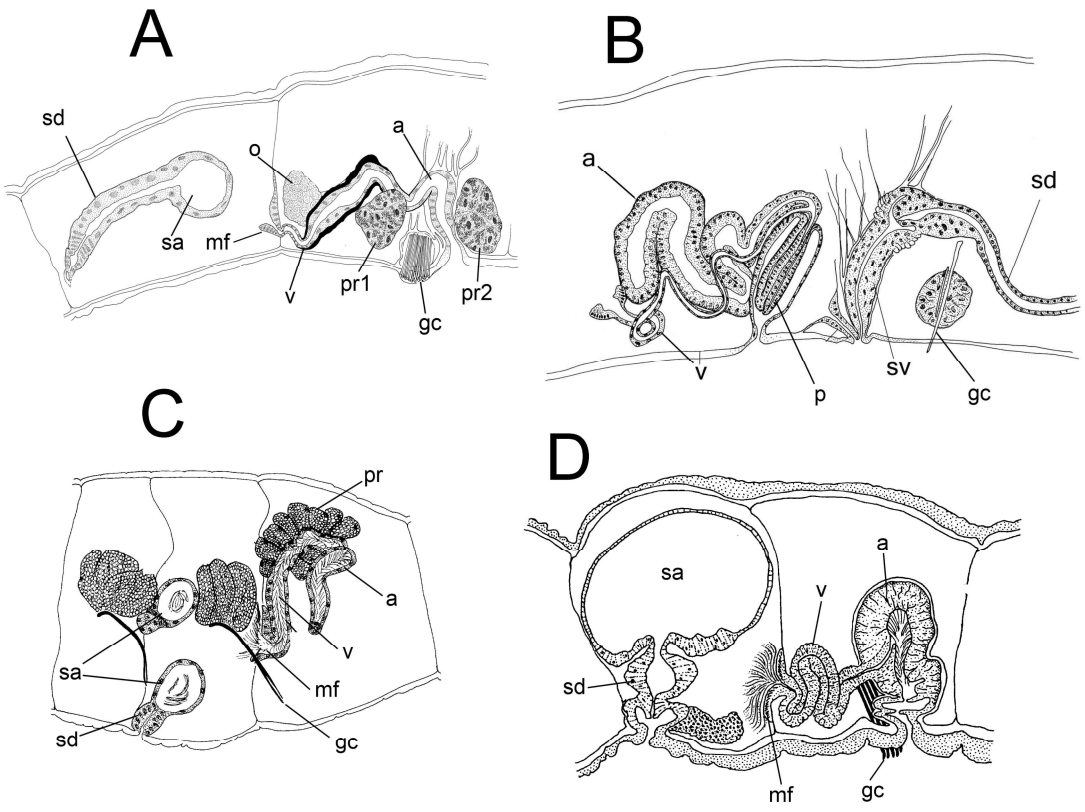
The **spermatheca** is an ovoid to elongate sac for storage of sperm after mating. There are normally two spermatheca present in one segment, usually with separate (and often indistinct) pores on the body wall of the same segment (normally ventro-lateral).

The **male ducts** transport sperm from the testes to the exterior. The normally paired male ducts start with **sperm funnels** on the posterior wall of the testes segment and in mature stained specimens this can be seen as a red mass looking like a dense tassel. The funnel feeds sperm into the vas deferens which leads to the male pores, usually via an **atrium** with associated glands (**prostate**) and a **penis** lying in an invagination of the body wall (**penis sac**), but there are many variations around this template. The pores are usually on the ventro-

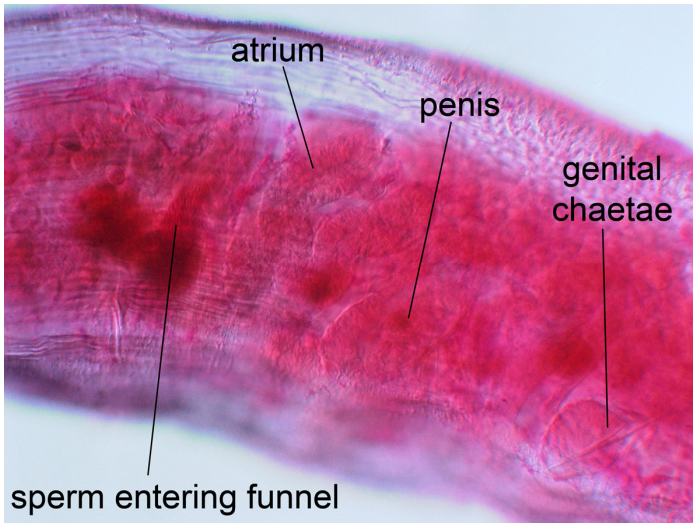
lateral body wall of the post-testes segment but in lumbriculids and earthworms pores can be 2 or more segments behind the testes.

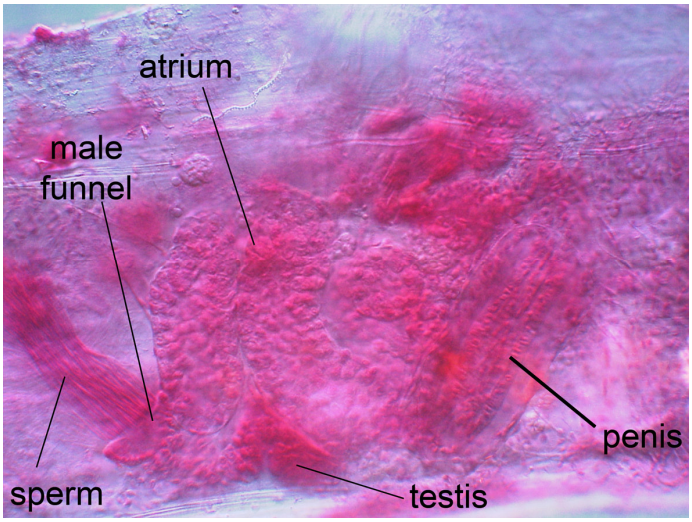
Below are cross sections of the segments containing the genitalia of four species from different oligochaete groups, showing variation in position and form of the genital organs. A: *Pectinodrilus ningaloo* (Naididae: Phallodrilinae); B: *Insulodrilus linae* (Phreodrilidae); C: *Pristina osborni* (Naididae: Naidinae) and D: *Ainudrilus nharna* (Naididae: Tubificinae). The *Pristina osborni* illustration is from Erseus and Grimm (1998).

sa = spermatheca, sd = spermathecal duct, sv = spermathecal vestibule, mf = male funnel (=sperm funnel), p = penis, o = ovary, v = vas deferens, a = atrium, pr = prostate, gc = genital chaetae.

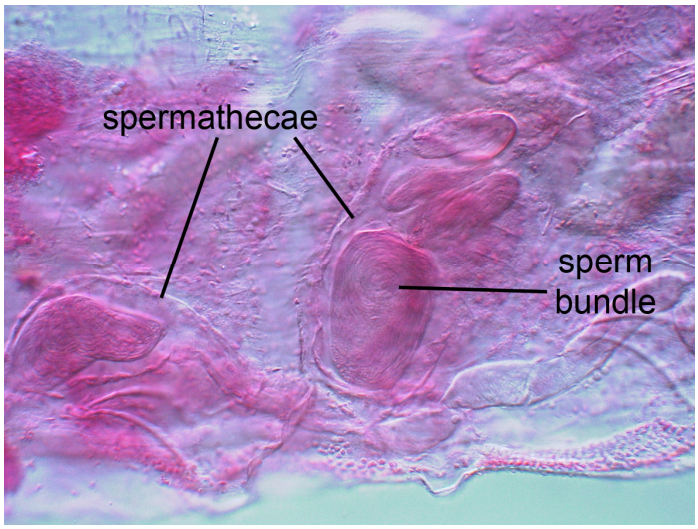


Images of genitalia on a stained and slide mounted specimen





close up of male genitalia



spermathecae with sperm bundles

METHODS FOR EXAMINING WORMS

Fixation and preservation

Worms may be killed and preserved in 5 to 10% formalin (2 to 4% formaldehyde) or in other histological fixatives such as Bouins, then stored in alcohol, although alcohol preserved material is normally adequate.

Examination of specimens

Few features can be seen with a dissecting microscope. The head (prostomium) is usually larger and more rounded than the tail and has a slit (mouth) along the ventral side. The prostomium may bear a proboscis and the tail may have knobby or elongate gills. The chaetae can often be seen but, apart from seeing whether hair chaetae are present and how long they are compared to the body width, little of their form can be seen.

Where more than one species are present, a preliminary separation of specimens into species (or groups of similar species), based on preserved colour, size, degree of coiling and contraction and presence and number of hair chaetae, can be made in the Petri dish. However, multiple specimens of each such separated group should still be made as some species can look identical at this gross level and examination of multiple specimens is often required to see all features.

Temporary slide mounts can be made in glycerol. For ease of mounting, worms are best temporarily transferred to water then into glycerol. Try to mount the worm laterally, so that both dorsal and ventral chaetae can be seen. When a worm is lateral the mouth can be seen as a ventral indentation between the prostomium and segment I. Conveniently, many worms naturally lie this way after preservation. For bulk identifications about 5 specimens are easily mounted under each of 2 coverslips on a slide. Specimens should not be left in glycerol for more than a few days as they will over clear.

The procedure for examining a worm mounted on a slide is as follows.

Start at the head end. Check the prostomium for a proboscis, segment I for eye spots (usually one purple coloured cell cluster on each side where present) and the body itself for gills or papillae. Next, examine the chaetae; determine which segment has the first dorsal chaetae and establish the number and form of the ventral and dorsal chaeta from a number of anterior and posterior segments. Don't forget that ventral chaetae start on segment II. The nerve cord is often usually visible and as it is always ventral (below the gut) is a useful indicator of the ventral side. Determine the relative lengths of the teeth of any bifid or pectinate chaetae. Examine at least three bundles in those segments examined to make sure that both dorsal and ventral chaetae have been seen. Care should be taken to examine several chaetae from an exactly lateral aspect because slight deviations in angle of view can produce apparent distortion of the relative lengths of the teeth. Some compression of the worm may be required (but not too much as the genitalia can be damaged if required later.

Check for presence of a clitellum which will indicate maturity.

The worm should then be searched for genital characters, if required. Carefully check the genital segments (X-XI in tubificids for example, see below) to see if the ventral chaetae of

mature specimens are modified. Check the segment with male ducts to see if there are penis sheaths (cuticular coverings of the penes); these may be thin and inconspicuous but often visible through the body wall. Other components of the genital system, such as penes, atria and sperm and eggs in sacs may also be visible through the body wall almost always requires the specimen to be stained and cleared (see below). If a specimen is mature and cannot be identified without examining the genitalia, then the following staining procedure should be used.

1. Soak the worm in water or alcohol to remove glycerol if required (a couple of minutes).
2. Place in about 0.5ml carmine stain (see recipe below).
3. After 10-30 minutes depending on size of specimen (10 minutes usually suffices for most worms), add a single drop of concentrated hydrochloric acid.
4. After a few minutes, remove specimen from stain and place in a solution of 3% HCl in 70% alcohol. This removes excess stain. The worm may need placing in fresh solution once or twice before it becomes a light pink colour.
5. Remove to 70% alcohol for a few minutes to remove acid.
6. Place in 100% isopropanol (not ethanol). Enchytraeids sometimes shrink at this stage and may need some intermediate concentrations.
7. Place in a solution of 50% Histoclear/50% isopropanol (few minutes).
8. Place in 100% Histoclear (few minutes).
9. Mount in a resinous mountant such as Permount, Canada Balsam or Ultramount, trying to get the worm to lie exactly on its side (rather than dorsally or ventrally) so that both ventral and dorsal chaetae can be seen and the genitalia can be viewed laterally. If done properly the indentation between the prostomium and segment I (the mouth) will be visible and the body will be straight (not twisted) thereafter (or at least not twisted for the first 15 or so segments). On animals that are coiled, these anterior segments need to be cut off before mounting so they can be mounted without overlapping the rest of the body.

Recipe for Grenacher's borax carmine

Carmine (C.I. 75470) 3.0g
Borax 4.0g
70% alcohol 100ml
Distilled water 100ml

Dissolve borax in water and then add carmine, boil or leave to stand until the carmine is dissolved. Add 70% alcohol and allow to stand for 1 to 2 days before filtering.

Examination of the genitalia

The location of the genital organs is fixed depending on the family or subfamily as follows:

Enchytraeidae: spermathecae in V, testes in XI, male ducts in XII.
Naidinae (except Naidinae): spermathecae and testes in X, ovaries and male ducts in XI
Naididae (Naidinae): spermathecae and testes in V, ovaries and male ducts in VI.
Phreodrilidae: testes in XI, ovaries and male ducts in XII, spermathecae in XIII

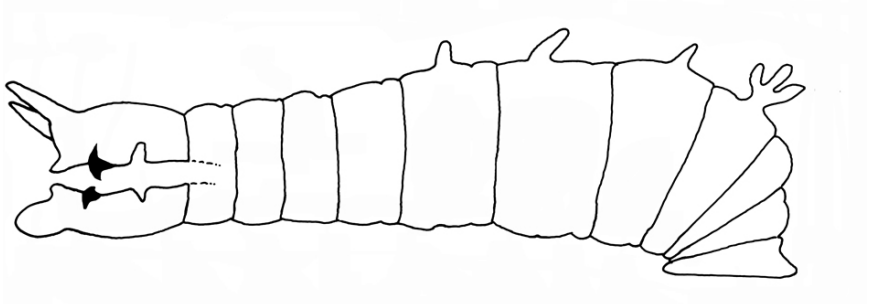
The easiest to find genital structure is generally the sperm (or male) funnel (located at the rear of the testes segment) as it will have a mass of darkly stained sperm crowding into the funnel. From here the thin and usually transparent vas deferens can usually be followed to the atrium in the next segment. The atrium is usually the next most visible organ as it is often large, though may be obscured if large prostate glands are present. The spermathecae are also easy to find, especially in mated specimens as they will be filled with darkly stained sperm. It is necessary to focus up and down a lot to follow the often convoluted 3D paths of these ducts.

KEY TO ANNELID GROUPS

1 Chaetae absent; anterior and (usually) posterior attachment organs ('suckers') present 2

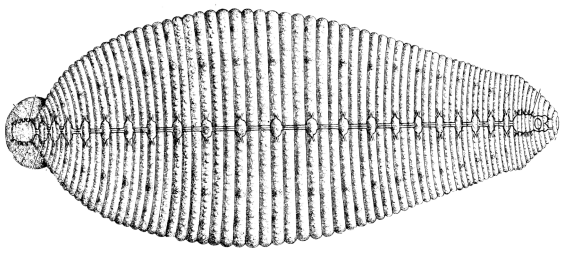
- Chaetae present (may be hard to see in some earthworms); attachment organs absent 3

2 Sometimes with short tentacles around the mouth and/or digitiform or multibranched dorsal appendages on some segments; protrusible proboscis absent; body consisting of head plus 11 body segments; ectosymbiotic on astacid crayfish and a few other crustacean **Clitellata**: **Branchiobdellida** (not known from Australia but present in Asia)



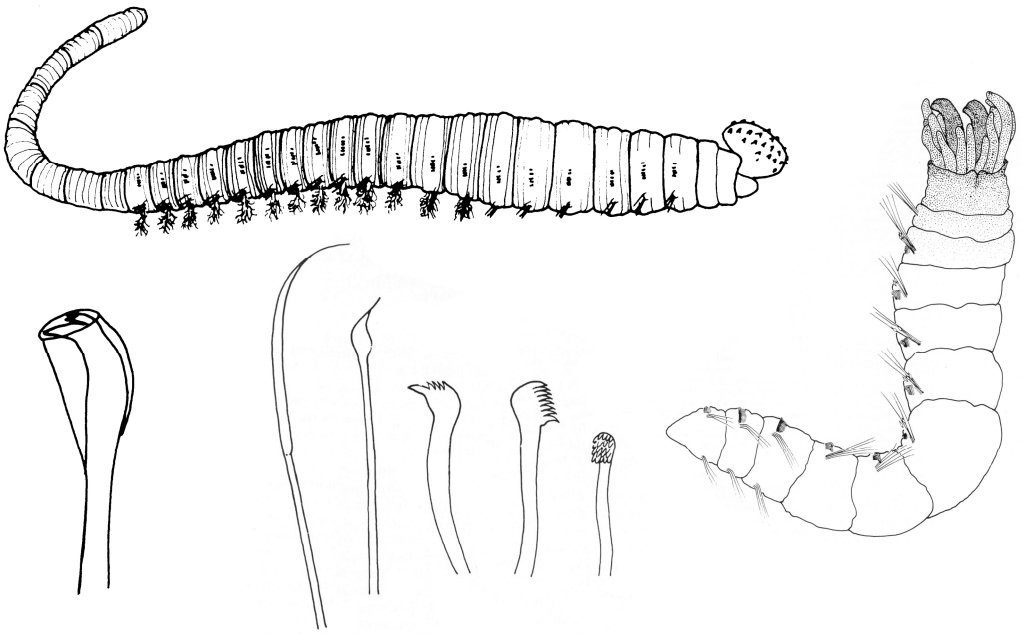
General body plan of a branchiobdellid

- Without tentacles and usually without dorsal appendages (lateral gills or fins may be present). Pharynx may be modified as a protrusible proboscis; body consisting of 34 segments (though externally sometimes appearing as more or less); blood-feeding forms temporarily parasitic on a range of hosts, otherwise mostly free-living .... **Clitellata**: **Euhirudinea (leeches)**



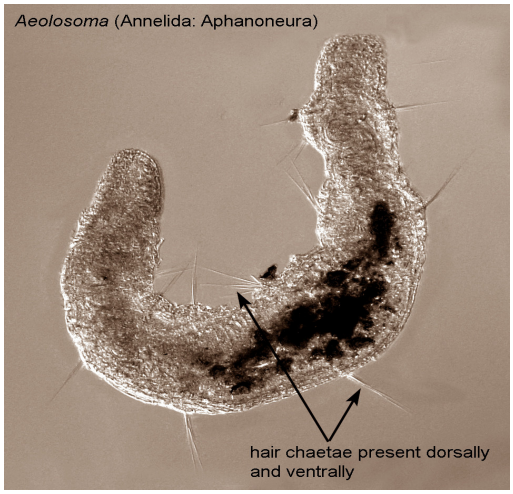
from Sawyer 1986

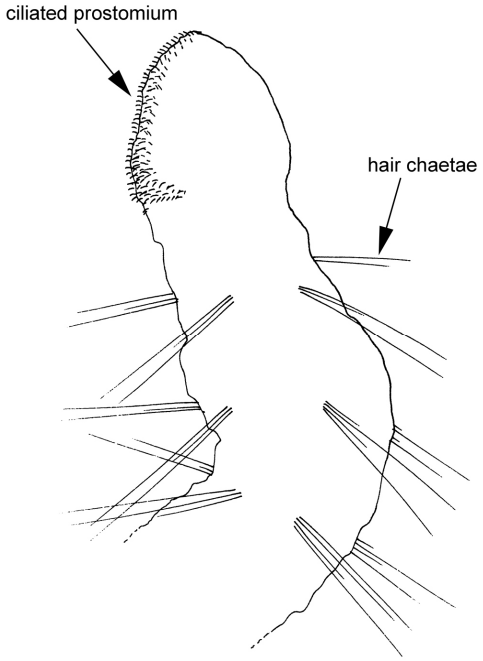
3. Head may bear multiple appendages; jaws may be present; body usually divided into distinct regions with different widths and types of chaetae; often separate male and females; chaetae often complex or hooded and usually borne on a pair of lateral outgrowths (parapodia); mostly marine but a few freshwater/athalassic saline species... ..... **Polychaeta**



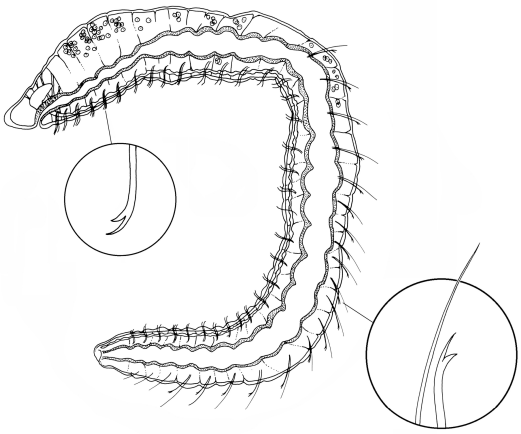
- Head usually without appendages other than a simple proboscis in a few species; jaws absent; body generally more or less uniform in width (tapering slightly posteriorly, expanding slight at head) with chaetae generally changing only gradually in form along body; hermaphroditic; usually with hairs and/or relatively simple chaetae with single-pointed or forked tips (sometimes with intermediate teeth), occurring in four 'bundles' per segment (usually dorso and ventro-lateral), not on lateral outgrowths; marine, terrestrial or freshwater **4**

4. Minute worms (1-2 mm long or chains of zooids up to 10 mm); hair chaetae in dorsal and ventral bundles; prostomium covered with cilia used for feeding and to propel worm in a gliding motion; intersegmental furrows usually inconspicuous or absent; head (pre-mouth segment) often broader than rest of body when alive; body wall with refractile epidermal oil glands which are usually coloured in live specimens; clitellum absent ..... **Aphanoneura**

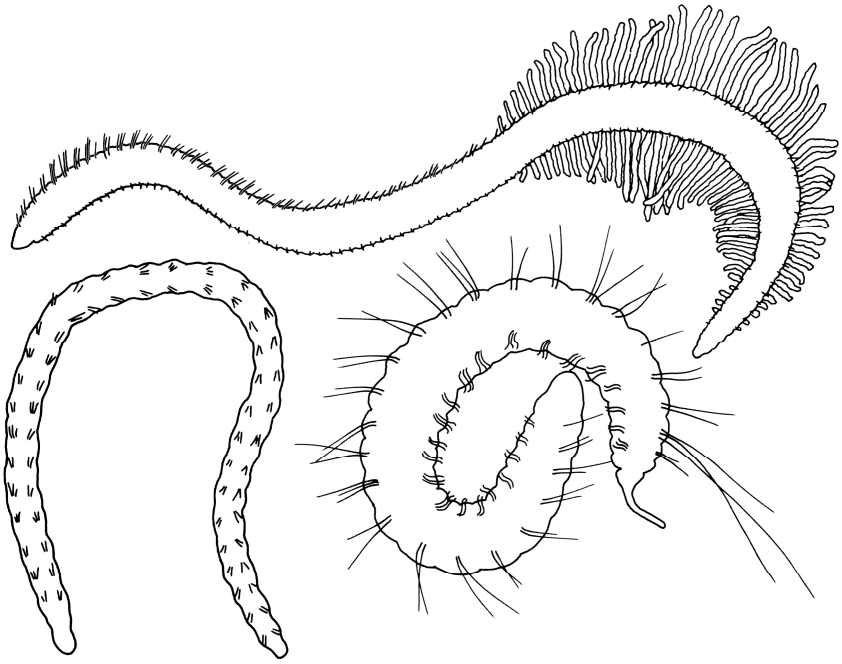




- Generally larger; hair chaetae normally restricted to dorsal bundles (except for Capilloventridae and two tubificids) or absent; prostomium not ciliated, locomotion by use of body wall musculature (peristalsis or undulating swimming); generally with obvious intersegmental furrows; head not larger than rest of body; epidermal oil glands absent; clitellum present in mature specimens ..... **Clitellata: Oligochaeta**



Nais barbata (Naididae) showing hair chaetae in dorsal bundles only



A variety of oligochaete body forms

KEY TO THE FAMILIES, SUBFAMILIES AND SOME ABERRANT SPECIES OF AQUATIC OLIGOCHAETES

(mature specimens required for some groups)

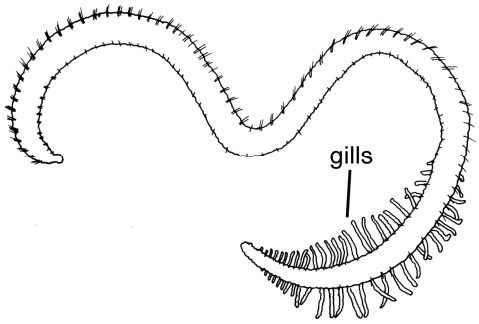
1. Hair chaetae present in ventral bundles 2

- Hair chaetae absent in ventral bundles 5

2. Hair chaetae present in anterior-most chaetal bundles; chaetae present from II, III or IV 3

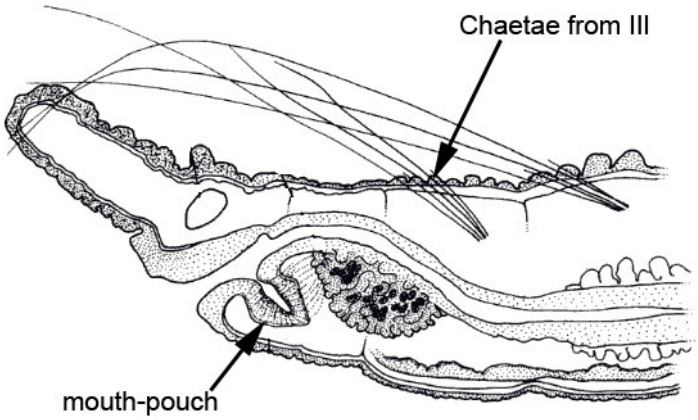
- Hair chaetae absent in anterior-most chaetal bundles; ventral chaetae present from II .... **Naididae: Rhyacodrilinae:** ***Breviatria papillatus*** (Tas only), see Pinder and Brinkhurst (2000).

3. Dorsal and ventral digitate gills present on posterior segments ....**Naidinae: Rhyacodrilinae**: ***Branchiura sowerbyi***



- Digitate lateral gills absent, though may be present as small processes within a shallow chamber on the anal segment only or may be present on anterior segments 4

4. Chaetae absent in segment II and sometimes III; ventral mouth-pouch present; mature specimens with ventral chaetae of XII modified as thickened hairs .... **Capilloventridae:** *Capilloventer*



- Chaetae present from II; ventral mouth-pouch absent; mature specimens with ventral chaetae of XI modified as hollow-tipped straight chaetae .....
  Naididae: Tubificinae: *Antipodrilus* **n. sp. GABS4** (known only from Great Artesian Basin mound springs)

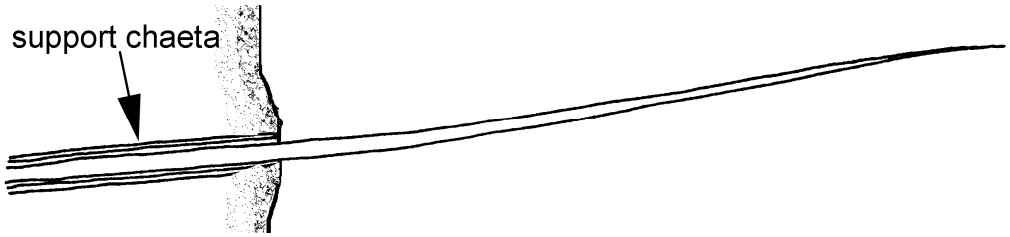
5. All chaetae simple-pointed crotchets 14

- At least some chaetae with teeth (albeit sometimes rudimentary) and/or some bundles with hair chaetae 6

6. Dorsal chaetae absent in II 7

- Dorsal chaetae present in II (i.e. same segment as first ventral chaetae) 8

7. Ventral chaetae paired; dorsal chaetae usually with fine 'support' chaetae which do not emerge from the body wall; spermathecae in XIII; male ducts in XII; clitellum usually covering 1/2 of XII and all of XIII ..... **Phreodrilidae**



- More than two chaetae per ventral bundle (at least in anterior segments); support chaetae absent (some naidid needle chaetae look similar but protrude from the body wall); genitalia and clitellum located between segments IV to VIII .....
  ..... **Naididae; Naidinae** (in part, see key below)

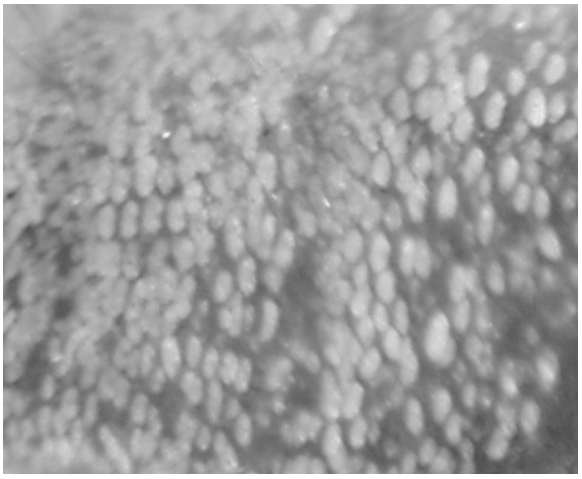
8. All chaetae paired and bifid with rudimentary upper tooth .... **Lumbriculidae** (2 introduced species)

- At least anterior segments with >2 chaetae/bundle 9

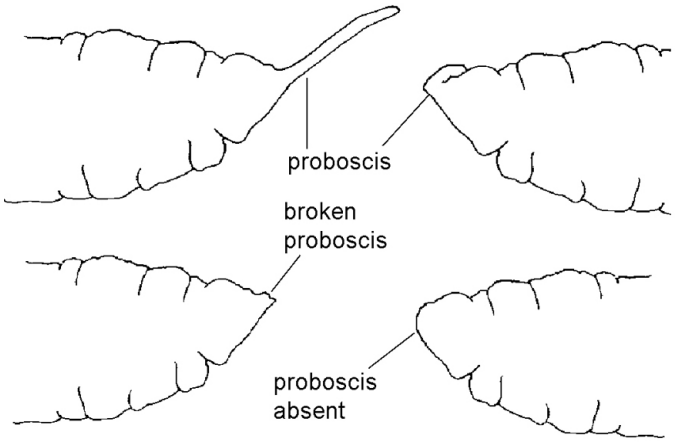
9. Proboscis may be present; dorsal chaetae (other than hairs) consisting of crotchet chaetae that are much finer and smaller (= needles) than the ventral chaetae (see illustrations above) 10

- Proboscis absent; dorsal chaetae (other than hairs) of similar size to ventral chaetae .... **Naididae** (in part) 13

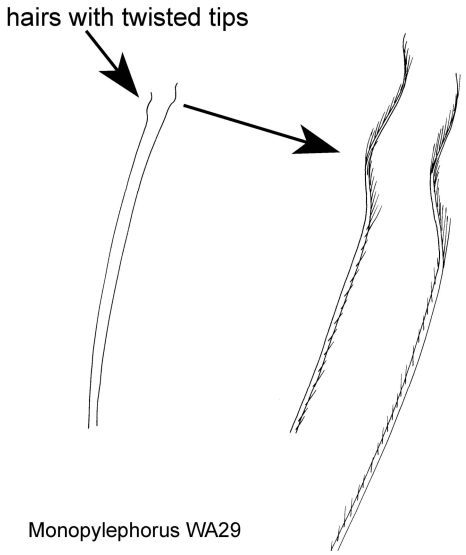
10. Proboscis absent; body densely covered by papillae with adhering foreign matter (below. ..... **Naididae: Tubificinae: *Embolocephalus yamaguchii*** (known only from Japan and South Australia)



- Proboscis present or absent; body without papillae 11



11. Hair chaetae with twisted plumose tips; proboscis absent .....
Naididae: Rhyacodrilinae; *Monopylephorus* n. sp. WA29 (known only from Pilbara groundwater)



Monopylephorus WA29

- Hair chaetae without twisted tips; proboscis may be present 12

12. Proboscis absent; dorsal chaetae deeply pectinate, outer teeth about 7 µm long .....
.....?***Monopylephorus* sp. WA2** (known only from groundwater in the Kimberley region)

- Proboscis may be present; chaetae single-pointed or bifid with short teeth .....
..... **Naididae: Naidinae** (see key below)

13. Each male duct with a single discrete prostate gland; hair chaetae may be present in dorsal bundles; coelomocytes absent or sparse; mostly inland waters ....**Naididae: Tubificinae**

- Each male duct with a pair of prostate glands attached asymmetrically, usually one ectal and one ental; hair chaetae absent; coelomocytes generally absent; marine/estuarine/brackish/rarely freshwater ..... **Naididae: Phallodrilinae**

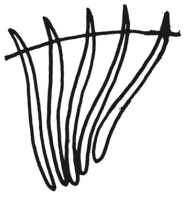
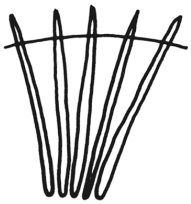
- Male duct with no prostate tissue or a pair of prostate glands arranged symmetrically on the atrium or with numerous small glands on the atrium or with a diffuse covering of prostatic cells arranged over the atrium; hair chaetae may be present in dorsal bundles; coelomocytes usually present and frequently abundant; mostly inland waters .... **Naididae: Rhyacodrilinae**

14. Up to 8 chaetae per segment, usually in 4 pairs (or if more than 8 per segment, then chaetae arranged evenly around the segment - perichaetine), rarely with all chaetae absent 16

- More than 8 chaetae per segment (at least anteriorly), always grouped into distinct bundles..... 15

15. Up to 8 chaetae per bundle anteriorly with tips broadly recurved; clitellum on X and XI; spermatheca in X; ventral chaetae of IX or X enlarged and blade-like; known only from Tasmania (records from New Zealand are probably enchytraeids) IDENTITY CAN ONLY BE CONFIRMED FROM EXAMINATION OF THE GENITALIA – SEE PINDER AND BRINKHURST 2000 - IMMATURES LIKELY TO BE CONFUSED WITH ENCHYTRAEIDS ..... . **Naididae: Rhyacodrilinae; *Breviatria multiprostatus***

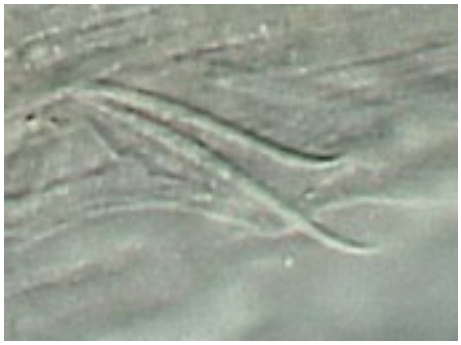
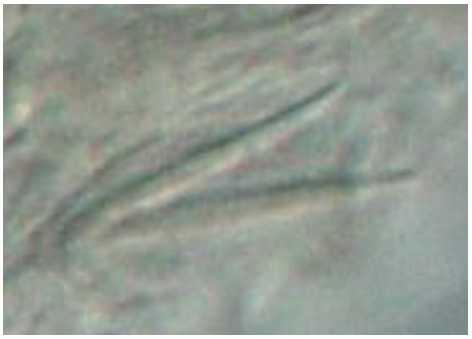
- Usually fewer than 6 chaetae per bundle; clitellum on XI and XII; spermathecae in V; ventral chaetae of IX or X not modified; widespread ..... **Enchytraeidae (in part)**


16. Earthworms, rarely aquatic; body wall thick, sometimes squarish in cross-secion; all chaetae fairly straight and generally not protruding far from the body wall; usually 8 chaetae per segment in pairs (if more then chaetae spread evenly around the segment, perichaetine); male pores on or after XIII, located two or more segments posterior to the last or only pair of testes; clitellum obvious, more than one cell thick, usually located behind the male pores .....**earthworms (several possible families)**

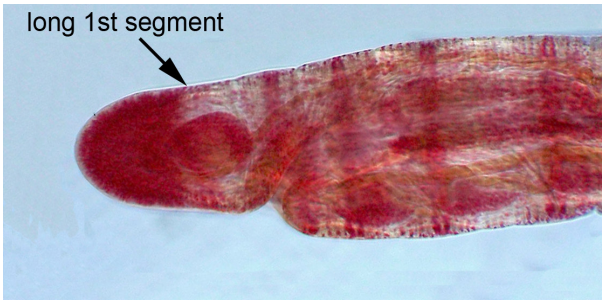
- Aquatic or semi-aquatic worms; body wall usually thin; chaetae paired, ≤ 8 per segment, usually protruding well out of the body wall and either straight or sigmoid; male pores always anterior to XIII; clitellum often inconspicuous, one-cell thick, covering the segment(s) with male pores 17

17. Thin, small (usually < 15mm) worms, usually cream to white when alive, body wall often noticeably cuticular (stiff and refractile); chaetae sigmoid or straight, usually without a distinct nodulus, often splayed; dorsal chaetae more lateral, sometimes absent, rarely with all chaetae absent; Male ducts in XII; spermathecae in V .... **Enchytraeidae (in part)**

- Usually larger (> 10 mm), usually pink to brown when alive, body wall not as above; chaetae with a nodulus; some chaetae always present, but may be absent dorsally; genitalia other than as above, often with more than one pair of genital organs 18

18. Worms very elongate in relation to their width (usually 2-10 cm long) and with a long 1st segment (peristomium), creating a particularly elongate region in front of the mouth; some characters modified for a predatory habit, including: 1 or 2 large sickle-shaped chaetae per ventral bundle (below left); dorsal chaetae usually smaller and reduced in number or absent; pharynx modified as a non-eversible muscular gizzard .... **Haplotaxidae, in part (genus *Haplotaxis*)**



- Not as above ..... **Other Haplotaxidae**

KEYS TO THE AUSTRALIAN NAIDINAE

Characters and states used in the interactive key to Naidinae

First segment with dorsal chaetae
- segment II (= first segment with ventral chaetae)
- segment III
- segment IV
- segment V
- segment VI
- segment VII or more posterior

Form of single-pointed needles (no teeth present at tip)
- evenly tapering to a sharp point
- tapering but with a minutely swollen tip
- tapering to very fine whip-like tips
- with a blunt tip

Gross form of needle chaetae
- with a bifid tip (forked, teeth sometimes minute)
- with a flared or spatulate tip
- with a pectinate tip (forked with fine intermediate teeth)
- with a single-pointed tip (sometimes with a swelling at the very tip)

Hair chaetae present or absent
- absent
- present

Needle chaetae fan-shaped or spatulate
- Needle chaetae with a fan-shaped tip
- Needle chaetae with a spatulate tip

Relative length of hair chaetae in some anterior segments
- Hairs absent in III or not much longer than those of other segments
- Some hairs of segmwent III much longer (up to 3x) than those of other segments (but these often missing)
- Some hairs of VI much longer than those of other segments

Relative length of teeth on bifid and pectinate needles
- with teeth approximately equal in length and width
- with teeth unequal in length and width
- with teeth unequal in length but not greatly different in width

type of non-hair dorsal chaetae
- needle chaetae, not of same general form as ventral chaetae, usually much smaller
- non-needle chaetae, of same general form as ventral chaetae

Length of needle chaetae in anterior segments
- Not yet included in key

Number of hair chaetae in anterior dorsal bundles
- Not yet included in key

Uniformity of ventral chaetae
- Anterior-most chaetae (usually II to V or VI) clearly longer and sometimes a little thicker, but not greatly enlarged
- some chaetae in one or more segments from IV to X greatly enlarged
- Ventral chaetae all of about the same size
- Ventral chaetae of II to IV slightly shorter than the rest

Presence and position of nodulus on sharply single-pointed needles
- Nodulus absent, needle shaft smoothly tapering
- Nodulus median
- Nodulus distal

Ventral chaetae in segment II
- 4-8 per bundle, less than 110 microns long, teeth curved
- 5-20 per bundle, teeth long and strongly recurved
- 6-13 per bundle, more than 140 microns long, teeth curved

U:L ratio of ventral chaetae of posterior chaetae
- Not yet included in key

U:L ratio of ventral chaetae of II-IV
- Not yet included in key

Divergence of teeth on bifid and pectinate needles
- Teeth almost parallel
- Teeth strongly divergent
- Teeth moderately divergent

Dorsal chaetae present/absent
- absent
- present

Serrations on hair chaetae
- present
- absent - hairs smooth

Length of longest tooth on bifid and pectinate needles
- Not yet included in key

Body wall papillae
- present, with adhered foreign matter
- absent

Symbiosis
- endosymbiotic in ureters of frogs
- not symbiotic
- ectocommensal on snails (may be found free living)

Palps on posterior edge of gill chamber
- present
- absent

Gills
- absent
- knob or finger-like within a chamber (fossa) on anal segment only
- long dorso-lateral gills on at least anterior segments, enclosing many dorsal chaetae

Number of pairs of gills on anal segment
- 1
- 2
- 3
- 4
- 5

Eyes on peristomium (segment I)
- present

absent

Proboscis on prostomium
present
absent

Raised ridge on body wall of each segment
absent
present

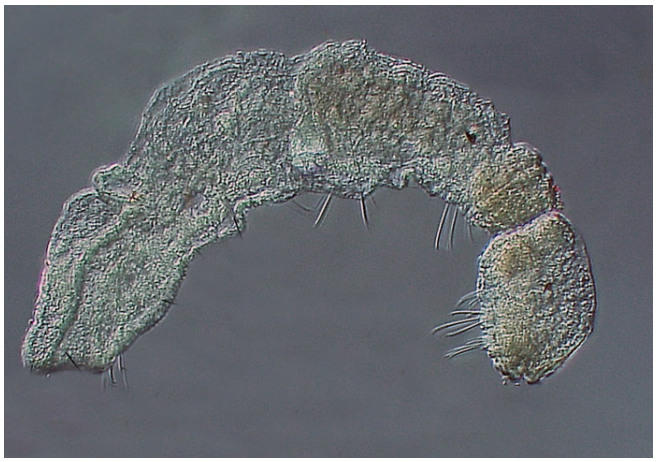
Dichotomous key to the Australian Naididae

1. Dorsal chaetae absent; body elongate or short and broad .... ***Dero*** (in part) or ***Chaetogaster*** 2

- Dorsal chaetae present on at least some segments; body long and narrow 5

2. Body elongate; ventral chaetae present on all anterior segments from II; stygal, known only from Pilbara groundwater ..... ***Dero*** **WA1**

- Body body short and broad, ventral chaetae of III-V missing; epigean .... ***Chaetogaster 3***



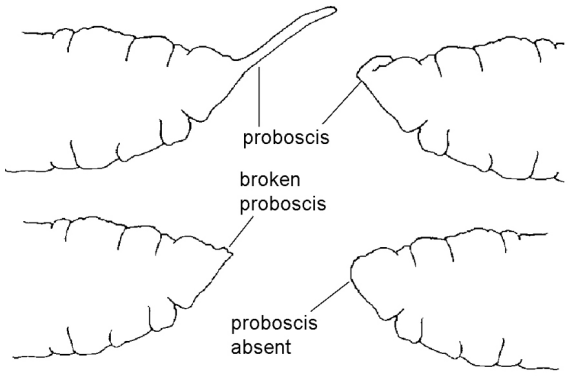
3. Ventral chaetae with strongly recurved teeth, subequal in length; commensal on gastropods ..... ***Chaetogaster limnaei***

- Ventral chaetae of II with teeth more gently curved, generally with upper longer than lower 4

4. Ventral chaetae of II 140 to 350 um long ..... ***Chaetogaster diaphanus***

- Ventral chaetae of II 70 to 110 um long ..... ***Chaetogaster diastrophus***

5. Proboscis on prostomium (may be partly broken off) ... ***Stylaria* and *Pristina* (in part)** 6

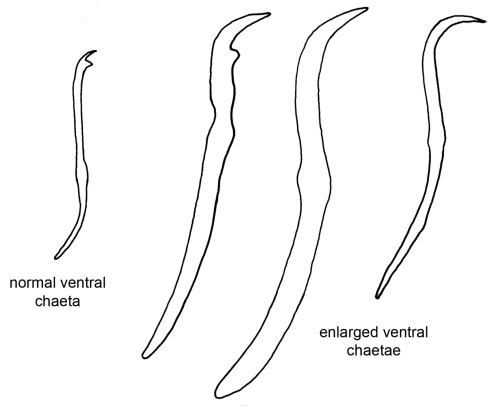


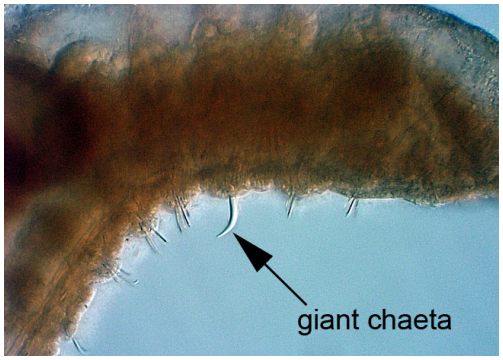
- Without proboscis 10

6. Dorsal chaetae from VI; ventral chaetae with two kinks at the basal end .... ***Stylaria lacustris***

- Dorsal chaetae from II; ventral chaetae smoothly sigmoid ..... ***Pristina*** (in part) 7

7. Needle chaetae distinct, with distinct teeth easily visible at 400x; hairs of III not much longer than rest; ventral chaetae of IV, V or VI enlarged with lower tooth absent or less than half as long as upper ..... ***Pristina aequiseta***



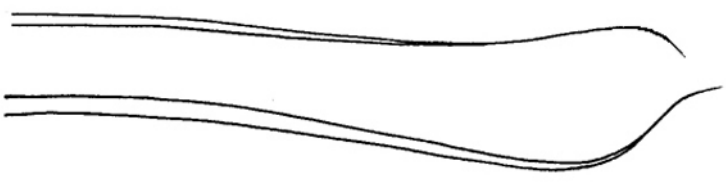


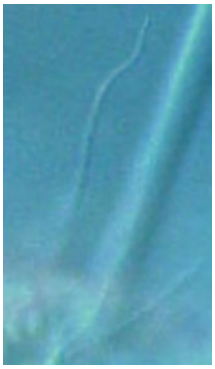
- Needle chaetae fine, difficult to detect at the base of the hairs, and either very finely tapering to a point or with minute teeth (hardly visible at 400x); hairs of III may be much longer than the rest; ventral chaetae of II and III may be longer and/or thicker than the rest, but those of IV, V and VI of normal size 8

8. Needle chaetae minutely bifid (usually need 1000x magnification to confirm, teeth about 1 µm long) ..... ***Pristina leidyi***
   (has been considered a subspecies of *Pristina longiseta* by some and many Australian specimens identified as *longiseta* in the past)

- Needle chaetae tapering to a fine point 9

9. Needle chaetae with small very fine whip-like tip at bases of hairs; hairs of III much longer than the rest (unless missing); ventral chaetae of II longer and slightly thinner than the rest and those of III longer and slightly thicker; posterior chaetae with teeth subequal in length.....***Pristina longiseta***





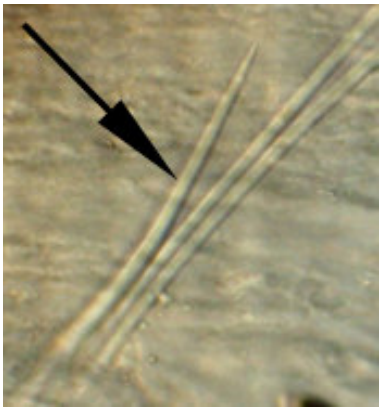
- Needle chaetae with straight fine tapering tip; hairs of III not longer than rest; ventral chaetae of II longer and thinner than rest, all ventral chaetae with upper teeth longer than the lower PRESENCE OF THIS SPECIES IN AUSTRALIA IS UNCERTAIN..... ..... ***Pristina proboscidea***

10. Dorsal chaetae from II ..... ***Bratislavia unidentata* (in part), *Pristina* (in part)** 11

- Dorsal chaetae from III ..... ***Bratislava*** 15

- Dorsal chaetae from IV or more posterior 16

11. Needles with single sharp point ..... ***Bratislavia unidentata***

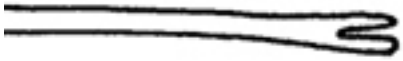


- Needles bifid or pectinate..... ***Pristina*** (in part) 12

12. Needle chaetae bifid, teeth parallel or only slightly diverging (see below)..... 13

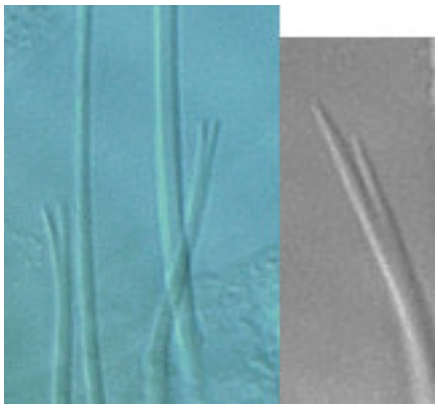
- Needle chaetae pectinate (sometimes bifid), outer teeth strongly diverging 14

13. Needle chaetae teeth short (<2 um), parallel and of same length ..... ***Pristina bilobata***



- Needle teeth long (up to 6 µm) and either parallel or slightly diverging and may be unequal in length ..... ***Pristina jenkinae***

(?*Monopylephorus* WA2 [Rhyacodrilinae] will key out here if assumed to be a Naidinae because of the needle-like dorsal crotchet chaetae). This species has teeth on needles about 7 µm but equal in length)



14. Upper tooth of needles substantially shorter and slightly thinner than the lower, lower tooth about 3 um long (2.8 to 4.7 in lit.), upper 1.5-2 µm long (1.8 to 3.5 in lit.)..... ..... ***Pristina sima***

(Rodriguez (2002) Graelliana 58(2): 3-19, provide a good comparison of this species with *P. osborni*)

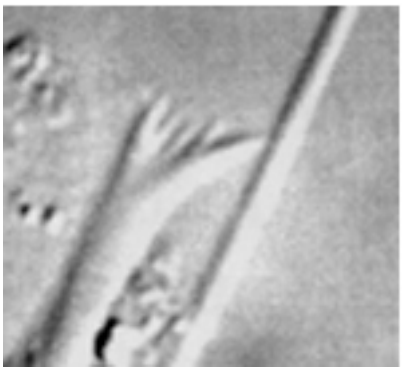


Photo Rodriguez (2002)

- Needle teeth subequal in length, 1.5 to 2 µm (1 to 3.3 in lit.) and width ***Pristina osborni***

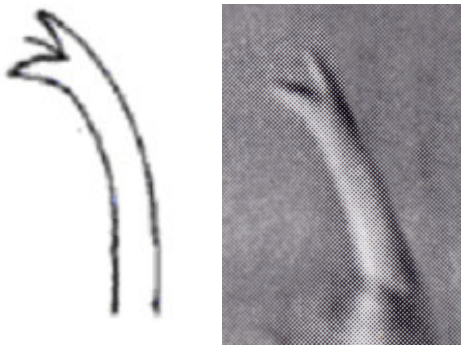


Photo Rodriguez (2002)

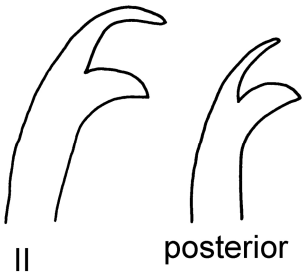
15. Needles with sharp simple tip ..... ***Bratislavia unidentata***

- Needles bifid ..... ***Bratislavia* WA1**

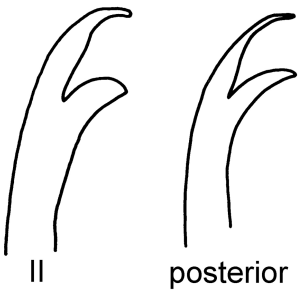
16. Hair chaetae absent ..... ***Paranais*** 17

- Hair chaetae present 18

17. Ventral chaetae of II with upper teeth longer than lower (below left), ventral chaetae of some other anterior chaetae with upper teeth slightly longer than lower, all other dorsal and ventral chaetae with teeth subequal (below middle and right)..... ***Paranais litoralis***



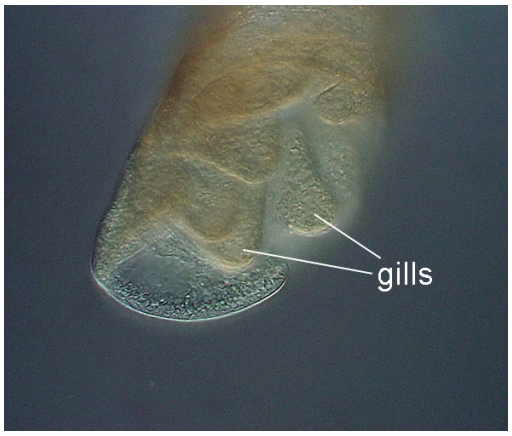
- All ventral chaetae of with upper teeth longer than lower ..... ***Paranais frici***



18. Gills present as paired dorso-lateral processes on each segment from between IV to VII onwards, enclosing some of the hair chaetae .....***Branchiodrilus hortensis***

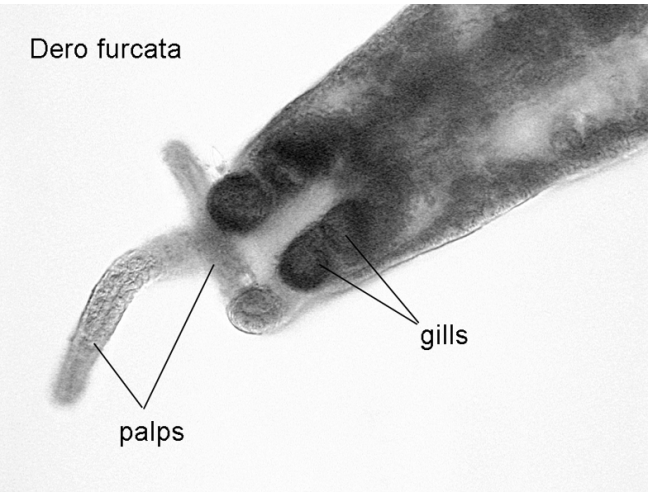
- Paired dorso lateral gills absent 19

19. Gills present as knobby or finger like processes arising from a shallow dorsal chamber on the last segment (may be inconspicuous), some species also with a pair of long caudal palps arising from the posterior edge of this chamber ..... ***Dero*** 20



- Without gills or palps on last segment 30

20. With a pair of caudal palps on the posterior edge of the gill chamber .... ***Dero*** (***Aulophorus***) 21



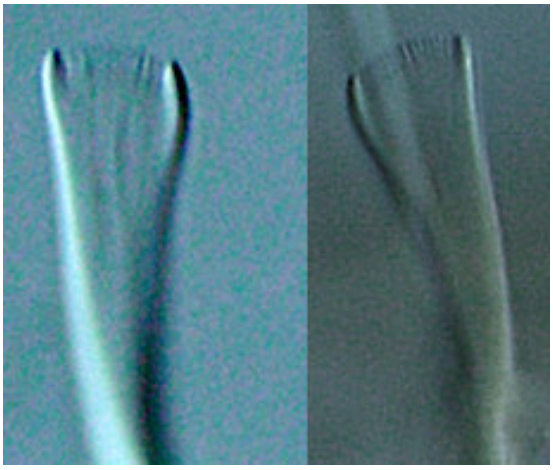
- Without caudal palps 24

21. Needle chaetae with flared and flattened tips (see figures in next couplet)..... 22

- Needle chaetae with bifid tips 23

22. Needle chaetae assymmetrically expanded into a fan-shaped tip .....
..... ***Dero (Aulophorus) flabelliger***

- Needle chaetae more moderately and symetrically expanded ***Dero (Aulophorus) vagus***

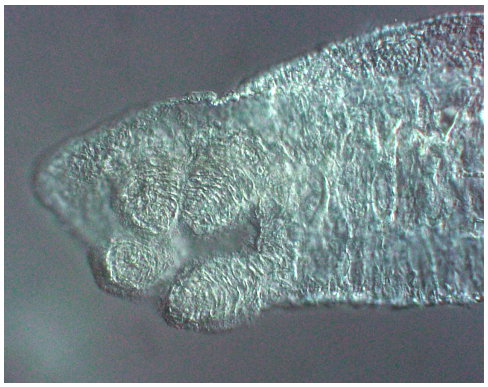


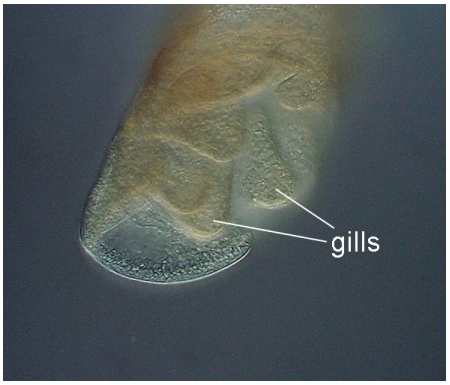
23. With 3 or 4 pairs of long thin gills; dorsal chaetae from V; needles with lower teeth slightly thicker, more curved and longer than upper ..... ***Dero*** (***Aulophorus***) ***furcata***

- With 3 pairs of short gills; dorsal chaetae from VI; needles with teeth subequal .... ***Dero*** (***Aulophorus***) **WA4**

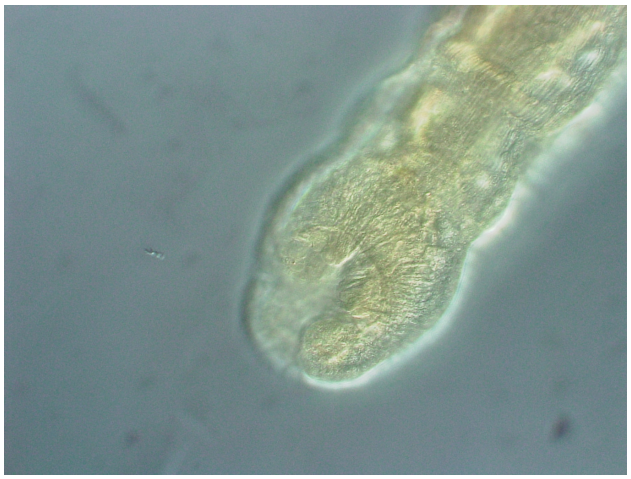
24. Ventral chaetae of first few segments (usually II to VI) longer and thinner than the rest and with teeth long and curved, the upper longer than the lower (below top left); posterior

ventral chaetae with teeth subequal or the upper shorter (below top right); dorsal chaetae starting on segments IV to VI; gills and gill chamber conspicuous (below, bottom) .... ***Dero*** (***Dero***) 26





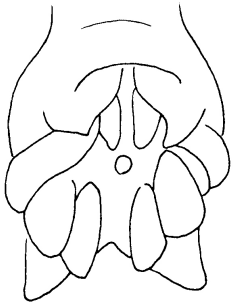
- Ventral chaetae of first few segments not much different to rest, all with teeth subequal; dorsal chaetae starting on segment IV; gills and gill chamber often inconspicuous .... ***Dero*** (***Allodero***) 25



25. Commensal within the urogenital tract of frogs, though possibly will be found free living; epidermis forming a raised ridge around each segment, most noticeable dorsally..... ..... ***Dero*** (***Allodero***) ***litoria***

- Not commensal; collected only from groundwater in the Pilbara; epidermis without raised ridges ..... ***Dero*** (***Allodero***) **sp. WA2**

26. Dorsal chaetae from IV; gill chamber with 5 pairs of gills and with the chamber with postero-lateral extensions ..... ***Dero (Dero) dorsalis***



- Dorsal chaetae from V or VI; gill chamber with 2 to 4 pairs of gills 27

27. Dorsal chaetae from V, with upper teeth shorter than lower (NOTE: easily confused with *D. nivea*) ..... ***Dero*** (***Dero***) cf. ***sawayai***

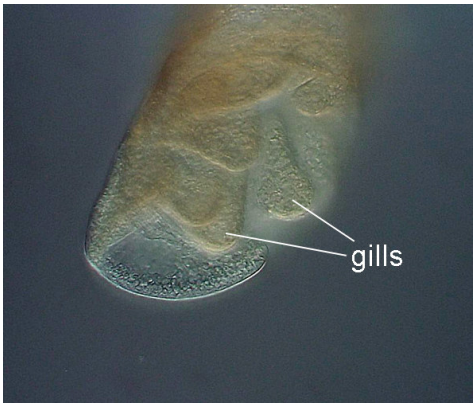
- Dorsal chaetae from VI, with teeth equal or the upper tooth longer 28

28. Hair chaetae plumose, needle chaetae pectinate ..... ***Dero*** (***Dero***) ***pectinata***

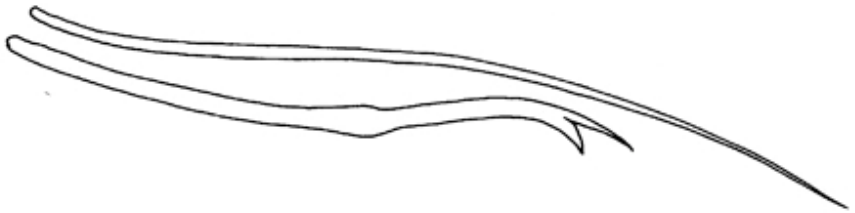
- Hair chaetae not plumose, needle chaetae at most with one small intermediate tooth but generally bifid 29

29. Needle chaetae with short teeth of about same length and width (or lower slightly thicker), two pairs of gills in a chamber that is not much wider than rest of body but sometimes with posterior extension..... ***Dero* (*Dero*) *nivea***

- Needle chaetae with upper tooth somewhat longer and thicker than the lower; 4 pairs of gills in a chamber normally expanded to be broader than rest of body .... ***Dero* (*Dero*) *digitata***



30. Dorsal bifid chaetae similar in size to ventral chaetae, with upper tooth twice as long as lower; dorsal chaetae starting on segments XVI to XXI ..... ***Haemonais waldvogeli***



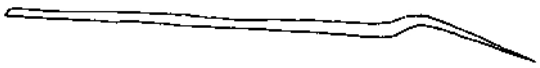
- Dorsal chaetae much finer than the ventral chaetae (dorsal chaetae are 'needle' chaetae) and present from IV to VI 31

31. Needle chaetae sharply single-pointed 32

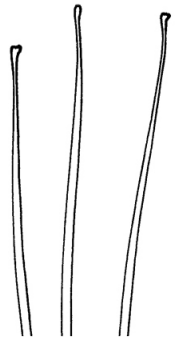
- Needle chaetae bifid or pectinate or having an apparently single-pointed tip with a blunt or slightly swollen tip or with the upper tooth rudimentary and hardly visible..... 33

32. Needle chaetae with nodulus near mid-way along the needle shaft; posterior ventral chaetae with upper teeth shorter than lower ..... ***Nais barbata***

- Needle with nodulus near the distal end of the shaft; posterior ventral chaetae with upper teeth longer than lower ..... ***Nais pseudobtusa***



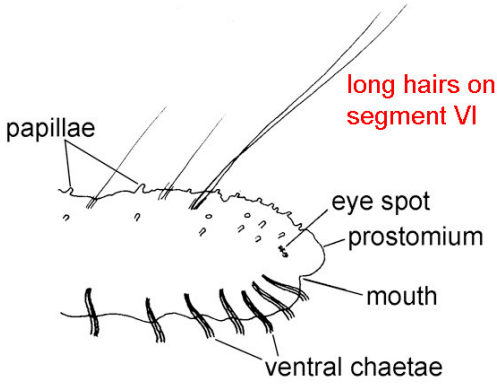
33. Needle chaetae very fine with a slightly swollen or minutely bifid tip; body wall with rings of papillae and frequently with a closely adhering layer of organic matter; hairs of VI may be much longer than the rest ..... ***Slavina*** 34



- Needle chaetae not so fine and with distinctly bifid or pectinate tips or with a single large tooth and one or two very fine reduced teeth; without an adhered layer of organic matter; hairs of VI about same size as rest 35

34. Anterior ventral chaetae with upper tooth 2.5 to 3 times longer than lower; hairs of VI not longer than on other segments; needle chaetae minutely bifid; eyes absent .... ***Slavina proceriseta***

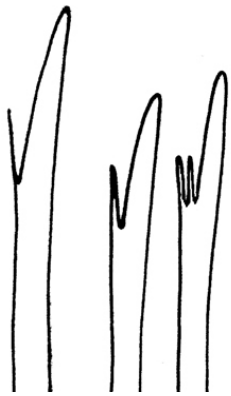
- Anterior ventral chaetae with upper teeth up to 1.5 times longer than the lower; hairs of VI much longer than on other segments; needle chaetae minutely swollen or seemingly notched; pigmented eye spots present ..... ***Slavina appendiculata***



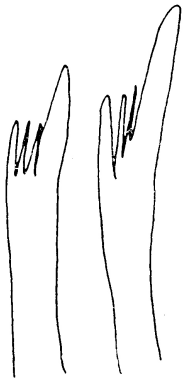
35. Needle chaetae either pectinate with upper tooth smaller than lower or bifid with an upper tooth much smaller than the lower and sometimes duplicated 36

- Needle chaetae bifid or pectinate with outer teeth equal (or subequal) in size 37

36. Needles bifid with upper tooth very fine, much smaller than the lower (and sometimes replicated)..... ***Allonais paraguayensis***



- Needles pectinate with pectinations finer than outer teeth ..... ***Allonais inequalis***



37. Needle chaetae pectinate..... 38

- Needle chaetae bifid 39

38. Needle chaetae with short strongly divergent teeth ..... ***Allonais pectinata***

- Needle chaetae with longer less strongly divergent teeth ..... ***Allonais ranauana***

39. Some anterior ventral chaetae of segments VI onwards greatly enlarged with upper teeth 2 to 3 times longer than the lower ..... ***Nais bretscheri***

- All ventral chaetae of normal size 40

40. Ventral chaetae all with upper teeth twice as long as lower ..... ***Nais elinguis***

- Posterior ventral chaetae with teeth subequal 41

41. Needle chaetae with parallel teeth; ventral chaetae of II-V distinctly longer and thinner than the rest and with upper teeth much longer than the lower (below centre); posterior ventrals with upper teeth shorter than lower (below right) ..... ***Nais variabilis***

- Needle chaetae with divergent teeth (like *Dero nivea*, see above); ventral chaetae of II-V only slightly longer than the rest ..... ***Nais communis***

KNOWN OCCURRENCE OF NAIDINE SPECIES IN AUSTRALIA

Australian Capitol Territory included with NSW and WA split into north (nWA) and south (sWA).

	Species	sWA	NSW	VIC	TAS	SA	nWA	NT	QLD
Allonais	*inaequalis* (Steph.)							+	+
	paraguayensis (Mich.)						+	+	+
	pectinata (Stephenson)						+		+
	ranauana (Mich. et Boldt)					+	+	+	
Branchiodrilus	*hortensis* (Stephenson)		+			+	+	+	+
?Bratislavia	*unidentata* (ex sp. NT1)			+			+	+	
	sp. WA1	+							
Chaetogaster	*diaphanus* (Gruithuisen)	+		+		+	+		
	diastrophus (Gruithuisen)	+	+	+		+	+		
	limnaei von Baer	+	+	+		+			
Dero	*digitata* (Müll.)	+	+	+	+	+	+	+	+
	dorsalis Ferronniere								?
	litoria Pinder, Hill et Green								+
	nivea Aiyer	+	+	+	+	+	+	+	+
	? nr. *cooperi* Steph. (*)							?	
	nr. sawayai Marcus						+		
	pectinata Aiyer						+		+
	flabelliger (Steph.)		+	+		+	+	+	+
	furcata (Müll)	+	+	+		+	+	+	
	vagus (Leidy)						+	+	
	sp. WA1						+		
	sp. WA2						+		
	sp. WA4	+							
Nais	*bretscheri* Mich.	+		+		+			
	communis Piguet	+	+	+	+	+	+		+
	variabilis Piguet	+	+	+	+	+			+
	elinguis Mull.			+		+			+
	pseudobtusa Piguet								+
	barbata Muller		+						
Paranais	*frici* Hrabe			+					
	litoralis (Mull.)	+		+	+	+			
Pristina	*aequiseta* Bourne	+	+	+		+	+	+	+
	longiseta Ehrenberg	+	+	+		+	+	+	+
	leidyi Smith	+					+		
	sima (Marcus)	+					+		
	bilobata (Bretscher)			?					
	proboscidea Beddard	?		?		?	?	?	?
	jenkinae (Steph.)	+		+		+	+		
	osborni (Walton)	+		+		+		+	
Haemonais	*waldvogeli* Bretscher		+				+		
Slavina	*appendiculata* d'Udekem		+	+		+			
	proceriseta Pinder et Brink.			+		+	+		
Stylaria	*lacustris* (Linnaeus)		+	+		+	+		

** = tentatively id from North Qld by Brinkhurst (1971)*

? = presence in Australia not confirmed

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