Museum Victoria Science Reports 13: 1–26 (2010)

ISSN 0 7311-7253 1 (Print) 0 7311-7260 4 (On-line)
https://doi.org/10.24199/j.mvsr.2010.13

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

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Abstract

Pinder, A. 2010. Tools for identifying selected Australian aquatic oligochaetes (Clitellata: Annelida). *Museum Victoria Science Reports 13: 1-26.*

Since Pinder and Brinkhurst (1994) produced a 'preliminary' guide to identifying Australian limnic oligochaetes there have been numerous new species described and substantial insights made into oligochaete phylogenetics, the latter reflected in recent changes to oligochaete classification. This report updates Pinder and Brinkhurst (1994) by 1) updating the taxonomy, 2) better describing and illustrating the characters and methods required to identify aquatic oligochaetes and 3) providing keys to the major groups of marine and freshwater aquatic oligochaetes (and some allied worms) and of all species of the subfamily Naidinae known from Australia. The impetus for this report was a workshop on aquatic invertebrate identification organised by La Trobe University and the Taxonomic Research and Information Network (TRIN).

Keywords Aquatic Oligochaeta, Clitellata, identification, Australia, Naididae, Naidinae

Introduction

The oligochaete fauna of Australian Inland waters was reviewed in the identification guide of Pinder and Brinkhurst (1994). Since that that publication there have been significant changes in oligochaete systematics and improved knowledge of Australian oligochaete diversity, and an update is timely. Molecular work has confirmed that leeches and branchiobdellidans form a monophyletic clade within Oligochaeta (Martin, 2001). Since these three groups constitute the entire Clitellata (annelids with a clitellum, Michaelsen, 1928), Oligochaeta and Clitellata are therefore synonymous. Nonetheless 'oligochaete' is still used as a convenient term to refer to clitellates other than leeches and branchiobdellidans, as here. Moreover, it appears that clitellates may lie within the polychaete clade (e.g. Rouset *et al.* 2007; Siddall et al. 2001).

The other major change is that the former family Naididae *sensu strictu* was synonymised with the Tubificidae, with the name Naididae having precedence (Erséus and Gustavsson, 2002; Erséus *et al.*, 2005; IUZN, 2007). Thus the former Naididae s.s. is now a subfamily, Naidinae, within a larger Naididae that also includes 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 (Envall et al., 2006). 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 (Table 1), although a large proportion remains undescribed and there are undoubtedly other species not yet collected. The number of marine oligochaetes known from Australia also continues to grow (e.g. De Wit *et al.*, 2009; Erséus, 2008).

Table 1. Numbers of species within each of the aquatic oligochaete families known from 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 OF AQUATIC ANNELIDS FROM NON-MARINE WATERS

POLYCHAETA

Twenty five families known from non-marine waters, including nine from Australia. See Glasby and Timms (2008) plus additional records in Halse *et al.* (2000) from Lake McLeod in north-western Australia.

Aeolosomatidae Beddard, 1895. Several species in freshwater.

Capitellidae Grube, 1862. Coastal/marine influenced wetlands.

Sabellidae Malmgren, 1867. Coastal/marine influenced wetlands and inland salt lakes (*Manayunkia* spp.)

Serpulidae Johnston, 1865. Coastal/marine influenced wetlands.

Histriobdellidae Vaillant, 1890. Coastal/marine influenced wetlands.

Nereididae Johnston, 1845. Coastal/marine influenced wetlands.

Orbiniidae Hartman, 1842. Coastal/marine influenced wetlands.

Polynoidae Malmgren, 1867. Coastal/marine influenced wetlands.

Spionidae Grube, 1850. Coastal/marine influenced wetlands.

CLITELLATA

Branchiobdellida Holt, 1965. Five families (Gelder, 1996). Ectocommensal on freshwater crayfish - not known from Australia.

Hirudinea Lamarck, 1818. Leeches. Of the fifteen aquatic families, the following four are known from Australia (Sket and Trontelj, 2008).

Hirudinidae Whitman, 1886 (including **Ornithobdellidae Govedich, 2001**).

Glossiphoniidae Vaillant, 1890.

Ozobranchidae Pinto, 1921.

Erpobdellidae Blanchard, 1884.

"Oligochaeta"

"Earthworms". Mostly terrestrial but eight families with non-marine aquatic representatives (Martin *et al.* 2008), some of which are comprised mostly or entirely of aquatic species. In Australia, earthworms are frequently encountered in inland waters but no obligate aquatic species these have been described.

Haplotaxidae (Michaelsen, 1900). Freshwater worms, generally interstitial, semi-terrestrial, profundal or stygal.

Lumbriculidae Vejdovský, 1884. Two introduced freshwater species in Australia.

Naididae Ehrenberg, 1828. Freshwater or marine.

Tubificinae Vejdovský, 1876. Mostly freshwater, few marine.

Phallodrilinae Brinkhurst, 1971. Mostly marine, few stygal.

Naidinae Ehrenberg, 1828. Former Naididae (sensu strictu), mostly freshwater.

Rhyacodrilinae Hrabe, 1963. Marine and freshwater.

Limnodriloidinae Erséus, 1982. All marine.

Phreodrilidae Beddard, 1891. Gondwanan, almost entirely freshwater.

Enchytraeidae Vejdovský, 1890. Marine, terrestrial and freshwater - poorly studied in Australia.

Propappidae Coates, 1986. Not known from Australia.

Capilloventridae Harman and Loden, 1984. Marine and freshwater, most endemic to Australia.

Opistocystidae Cernosvitov, 1936. Not known from Australia.

Narapidae Righi and Varela, 1983. Not known from Australia.

Explanation of characters

Arrangement of the body. Roman numerals are used to denote segments (Fig. 1) while Arabic numerals are used to denote septa between segments (1/2, 2/3 …). The head end is almost always broader than the anal end of a worm and the mouth is within a ventral furrow just behind the front of the worm. The most anterior part of the worm is the prostomium which is not counted as a segment (Figs 1 and 2). Segment I has the crescent-shaped ventral mouth but no chaetae. Chaetae generally start on segment II but dorsal (rarely ventral) 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 mid-ventral (Figs 1 and 2). The nerve cord is uneven in thickness and has a speckled appearance. The mouth is also ventral and normally long hairs (see below) are restricted to the dorsal side.

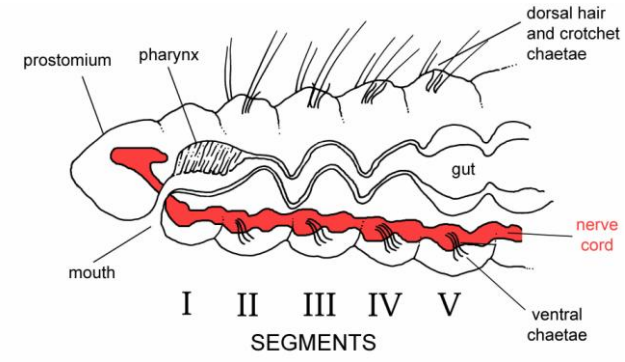


Figure 1. Major features of the anterior end of an oligochaete.

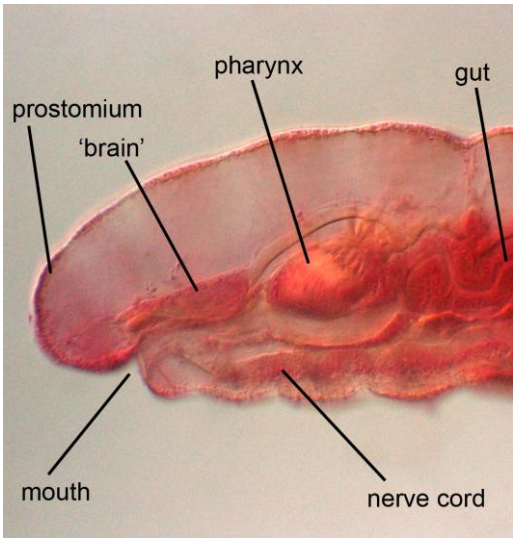


Figure 2. Photograph of a stained and slide mounted oligochaete showing major features.

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. There are several main kinds of chaetae as follows.

Hair chaetae (sometimes just called **hairs** or **capilliform** chaetae): long, thin 'hair-like' chaetae, normally with a fine tapering tip (Fig. 3). In most oligochaetes (except for the Capilloventridae, plus 2 naidids and 1 phreodrilid) these are restricted to dorsal bundles. Hairs sometimes have annulations or serrations along the shaft or have secondary hairs giving a frayed or plumose appearance.

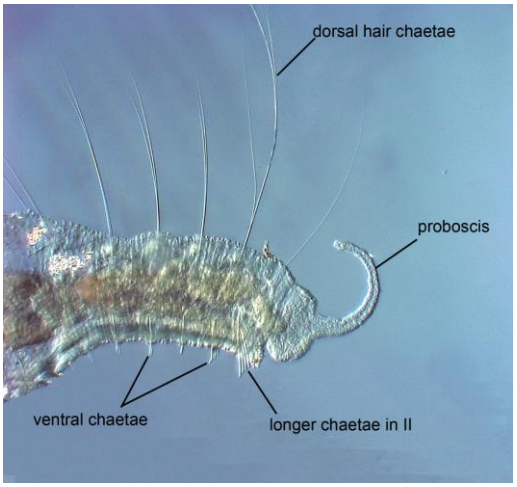


Figure 3. Pristina longiseta showing position of dorsal hair chaetae and ventral crotchet chaetae.

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, Fig. 4), **bifid** (forked, Fig. 5), **pectinate** (with comb-like teeth between the fork, Fig. 6) 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, though sometimes curvature is difficult to detect. The relative length of these teeth is frequently used in keys.

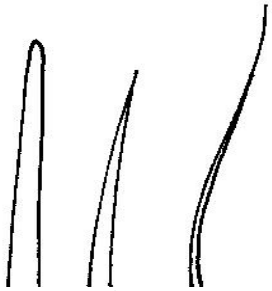


Figure 4. Tips of single-pointed chaetae

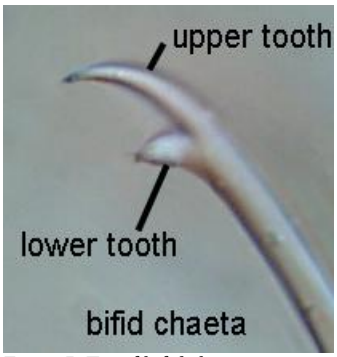


Figure 5. Tip of bifid chaeta

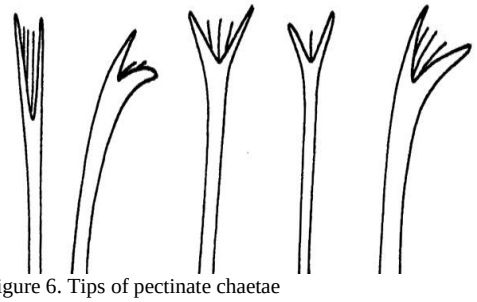


Figure 6. Tips of pectinate chaetae

Needle chaetae: Most members of the subfamily Naidinae have short, fine, and usually fairly straight crotchet chaetae called **needles**. These are generally much smaller than the bifid ventral chaetae (Fig. 7) and usually occur with hairs. High magnification is normally needed to see the form of the tips of needle chaetae.

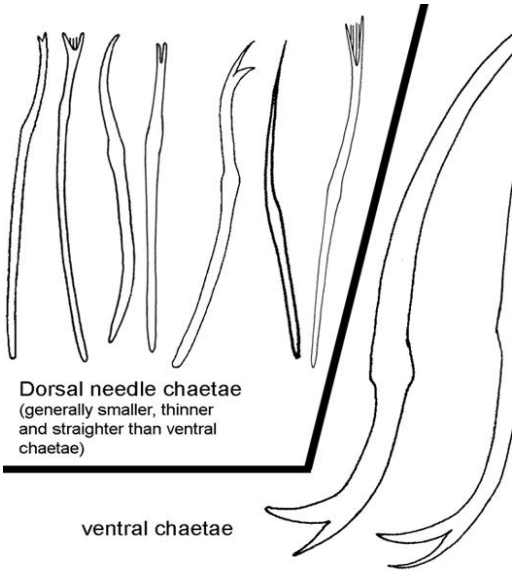


Figure 7. Naidine needle chaetae (left) compared to bifid ventral chaetae at same scale. Note nodulus (swelling part way along shaft).

'Support' chaetae: Most Phreodrilidae have a pair of small fine chaetae 'support' surrounding the base each hair or crotchet chaeta (Fig. 8). 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.

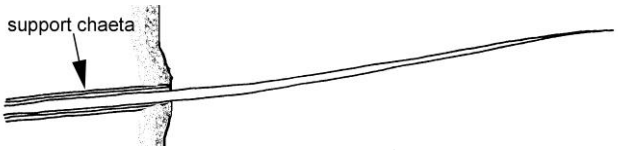


Figure 8. Phreodrilids hair chaeta with a pair of basal support chaetae.

Genital chaetae: The ventral chaetae of mature specimens are usually absent or modified on the segments bearing the genital pores. Modified chaetae are mostly of two forms. 1) long straight single-pointed chaetae with grooved distal halves and sharp blade-like tips and usually associated with a large gland, often occurring with one much smaller chaeta and most frequently associated with the spermathecal segment(Fig. 9). 2) one or more chaetae, often in parallel or with the distal ends bunched together, with bifid or bluntly simple tips and generally associated with the segment with the male pores (Fig. 10). 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.

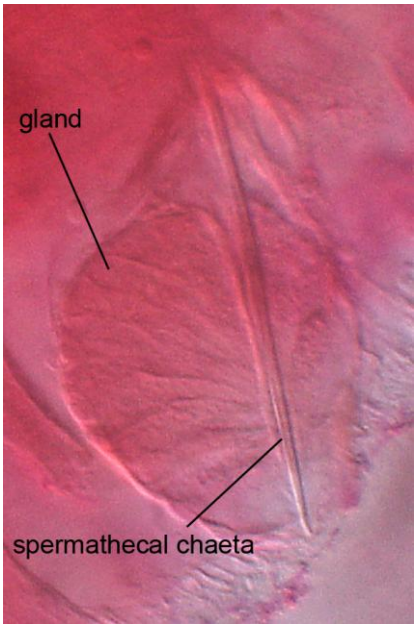


Figure 9. Grooved genital chaeta and gland

Figure 10. Parallel genital chaetae.

Coelomocytes: round to oval cells free-floating in the coelomic cavity, in clusters or individually (Fig. 11). Often very dense in naidine and rhyacodriline naidids. These can often be seen in glycerol mounted specimens.

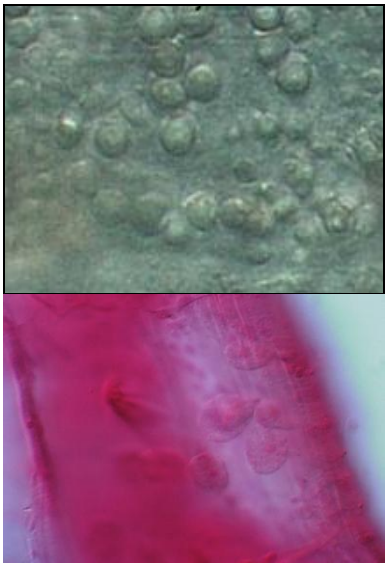


Figure 11. Coelomocytes in body cavity.

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 (Figs 12 to 15). This cell layer secretes the cocoon (Fig. 16) into which the sperm and eggs are deposited and the embryo develops.

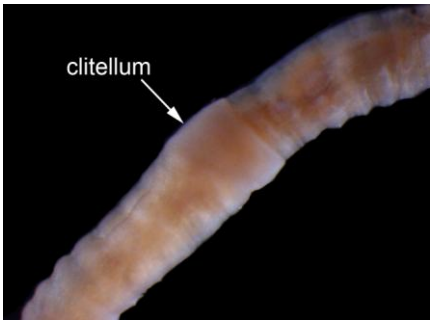


Figure 12. Clitellum appearing as an opaque pale area over two segments.

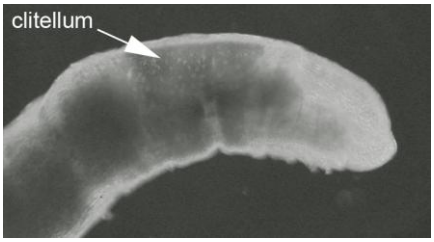


Figure 13. textured clitellum on a naidine oligochaete.

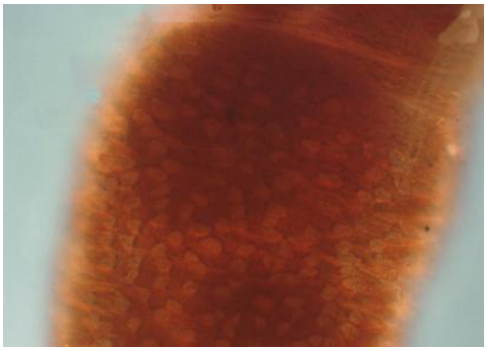


Figure 14. Glandular clitellum.

Figure 15. Clitellum of an enchytraeid worm.

Figure 16. Oligochaete cocoon with two embryos.

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

Naididae: 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 several segments from about X or XI

Genital organs (Figs 17 and 18) 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 (Fig. 17D). 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.

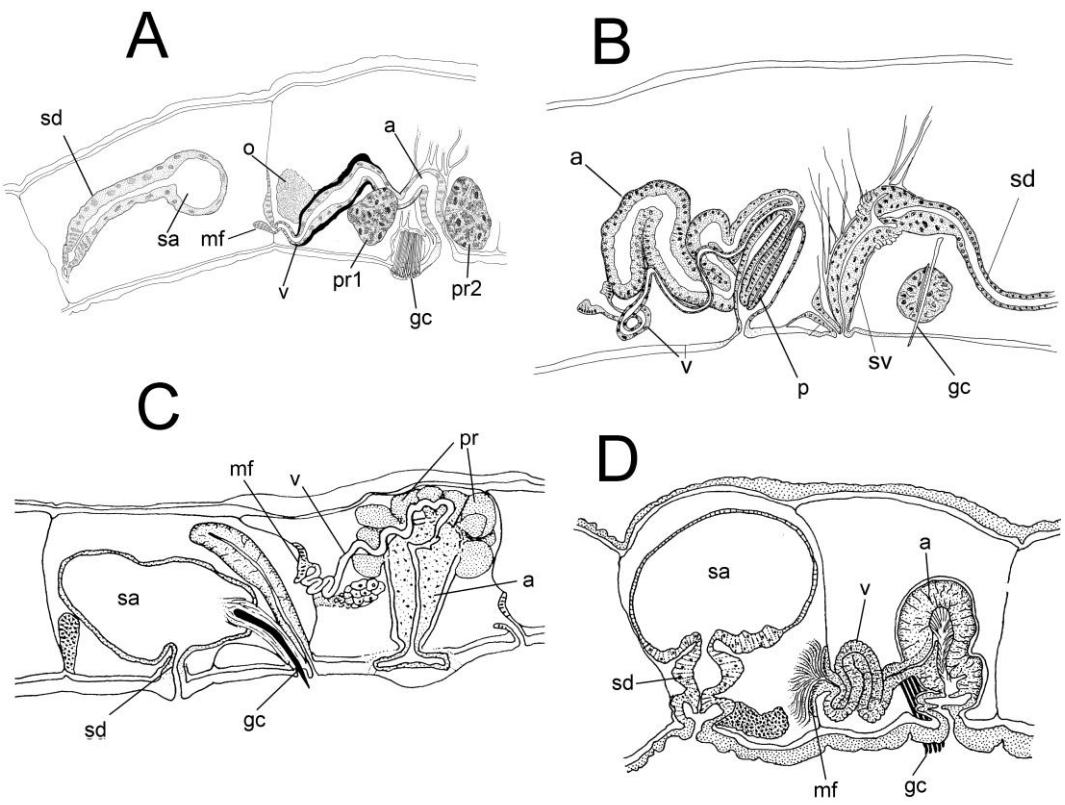


Figure 17. Cross sections of the segments containing the genitalia of four species from different oligochaete groups, showing variation in position and form of the genital organs. A: *Pectinodrilus ningaloo* (Naididae: Phallodrilinae); B: *Insulodrilus linae* (Phreodrilidae); C: *Breviatria multiprostatus* (Naididae: Rhyacodrilinae); D: *Ainudrilus nharna* (Naididae: Tubificinae). a = atrium, gc = genital chaetae, mf = male funnel, o= ovary, p = penis, pr = prostate gland, sa = spermathecal ampullae, sd = spermathecal duct, sv = spermathecal vestibule, v = vas deferens.

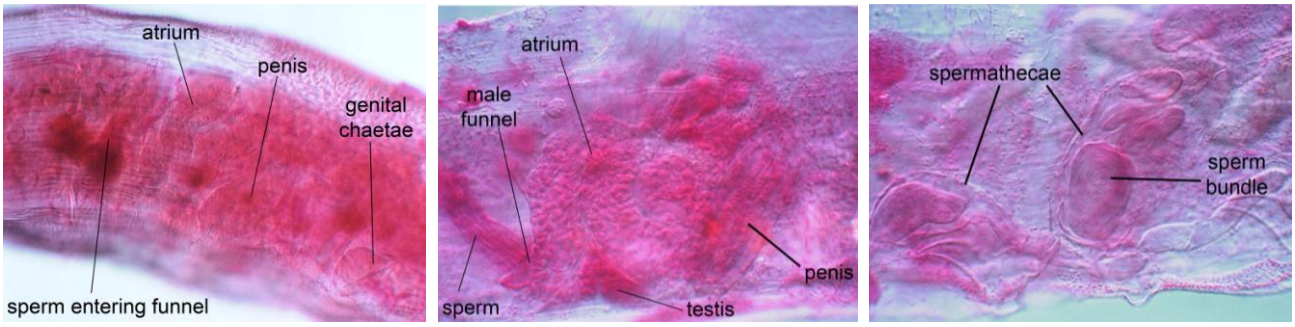


Figure 18. Images of genitalia from a stained and slide mounted specimens

METHODS FOR EXAMINING WORMS

Fixation and preservation

Worms may be killed and fixed in 5 to 10% formalin (2 to 4% formaldehyde) or in other histological fixatives such as Bouins, then stored in alcohol, although material preserved in high strength alcohol is normally adequate, especially if bulk samples are elutriated to remove sediment before preservation.

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 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 examined as some species can look identical at this gross level.

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 square 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 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. Gentle compression of the worm may be required, but not too much as the tissues can become compressed hindering examination of the genitalia, 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, but usually the specimen needs to be stained and cleared to see these structures (see below). If a specimen is mature and cannot be identified without examining the genitalia, then the following staining procedure should be used. It is often useful to mount several specimens as usually not all aspects of the genitalia can be seen on one specimen.

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 to 15 minutes usually suffices for most worms), add a single drop of 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. It is important not to under or overstain.
5. Remove to 70% alcohol for a few minutes to remove acid.
6. Place in 100% isopropanol (<u>not</u> ethanol). Enchytraeids sometimes shrink at this stage and may need some intermediate concentrations.
7. Place in a solution of 50% isopropanol /50% Histoclear (or other clearing agent (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 or more posteriad.

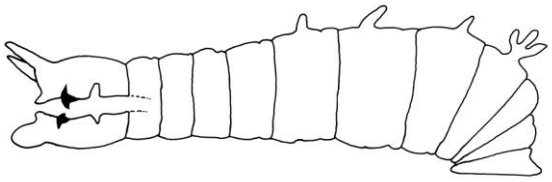
The easiest to find genital structure is generally the sperm (or male) funnel (located at the rear of the testes segment) as it will have a mass of darkly stained sperm crowding into the funnel. From here the thin and usually transparent vas deferens can usually be followed to the atrium in the next 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

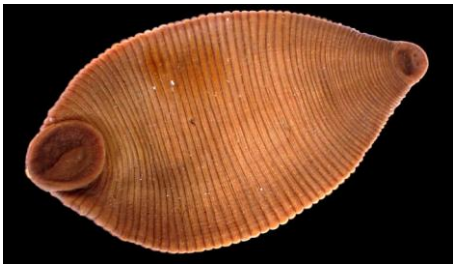
- Chaetae present (may be hard to see in some earthworms); attachment organs absent 3

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 crustaceans ..... **Clitellata**: **Branchiobdellida** (not known from Australia, but present in Asia)



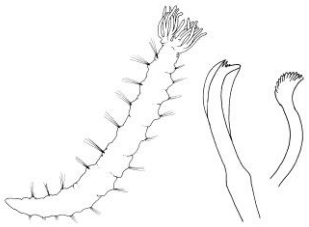
General body plan of a branchiobdellid

- 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)



Helobdella papillornata leech (Glossiphoniidae)

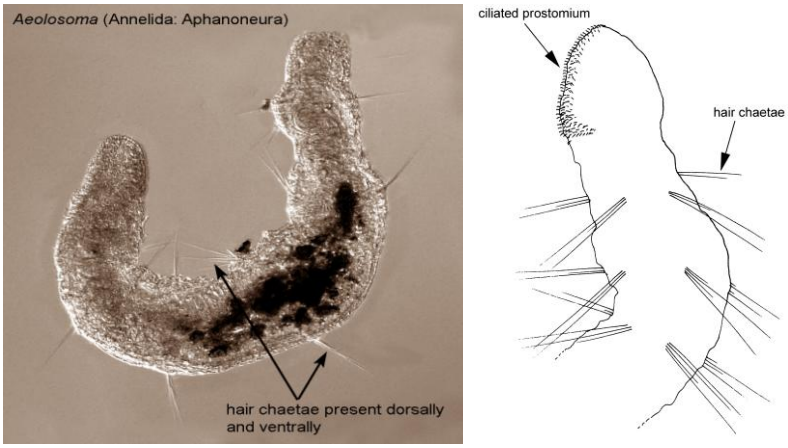
3 Head may bear multiple appendages, including tentacles; jaws may be present; body usually divided into distinct regions with different widths and types of chaetae; often separate males and females; chaetae often ornamented or hooded and usually borne on a pair of lateral outgrowths (parapodia); mostly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



Manayunkia polychaete (Sabellidae) (left), chaeta of Capitellidae (middle) and chaeta of *Manayunkia* (right)

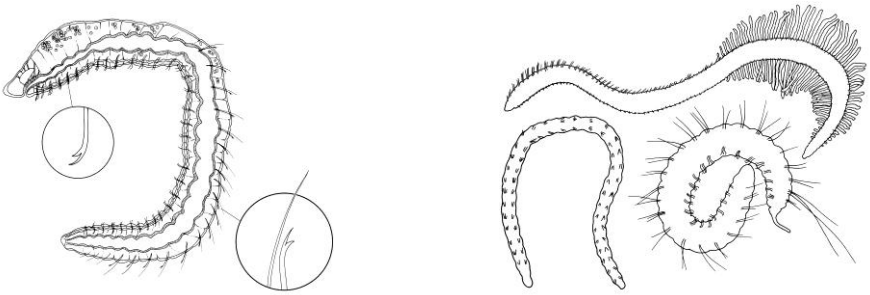
- Head usually without appendages other than a simple proboscis in a few species (one genus has lateral gills on some anterior segments and others have gills on posterior segments); jaws absent; body generally more or less uniform in width (tapering slightly posteriorly, expanding slightly towards head) with body and 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 ..... **Aeolosomatidae (Polychaeta)**



Photograph of *Aeolosoma* sp.(left), drawing of anterior end of *Aeolosoma* sp. showing hairs in all bundles and ciliated prostomium (right)

- 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 (left), a variety of oligochaete body forms(right)

Key to the families, subfamilies and some aberrant species of aquatic oligochaetes

(note that mature specimens required for some groups)

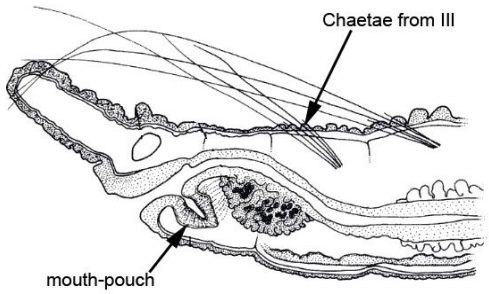
1 Hair chaetae present in some ventral bundles 2

- Hair chaetae absent in ventral bundles 4

2 Chaetae present from II, III or IV; hair chaetae present in anterior-most bundles 3

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

3 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***



Anterior segments of *Capilloventer longicapitus*.

- Chaetae present from II; ventral mouth-pouch absent; mature specimens with ventral chaetae of XI modified as hollow-tipped straight chaetae (as in Fig. 9) .... **Naididae: Tubificinae**: ***Antipodrilus* n. sp. GABS4** (known only from Great Artesian Basin mound springs)

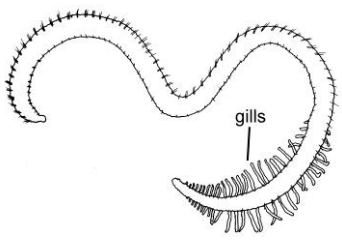
4 Filamentous gills present either on posterior segments only or along much of body but longest anteriorly 5

- Filamentous gills absent (or if present then only on terminal segment) 6

5 Dorso-lateral filamentous gills present on most segments, becoming shorter posteriorly .... **Naididae: Naidinae: *Branchiodrilus hortensis***

- Lateral gills present on posterior segments only ..... **Phreodrilidae: *Phreodrilus branchiatus***

- Dorsal and ventral gills present on posterior segments only ..... **Naididae: Rhyacodrilinae: *Branchiura sowerbyi***



Naididae: Naidinae, *Branchiodrilus hortensis* with gills longest anteriorly (left); **Naididae: Rhyacodrilinae,** *Branchiura sowerbyi* showing dorso-ventral gills on posterior segments (right)

6 All chaetae simple-pointed crotchets (Fig. 4) 15

- At least some chaetae with teeth (albeit sometimes rudimentary) and/or some bundles with hair chaetae 7

7 Dorsal chaetae absent in II 8

- Dorsal chaetae present in II (i.e. same segment as first ventral chaetae) 9

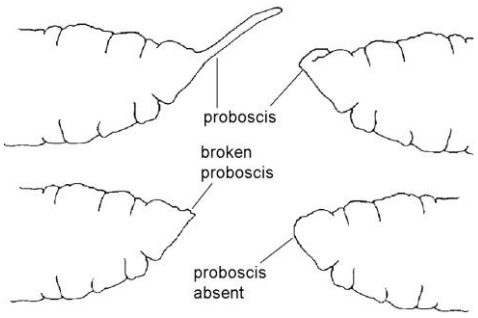
8 Ventral chaetae paired; dorsal chaetae usually with fine 'support' chaetae which do not emerge from the body wall (Fig. 8); spermathecae in XIII; male ducts in XII; clitellum usually covering 1/2 of XII and all of XIII ..... **Phreodrilidae**

- 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)

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

- At least anterior segments with >2 chaetae/bundle 10

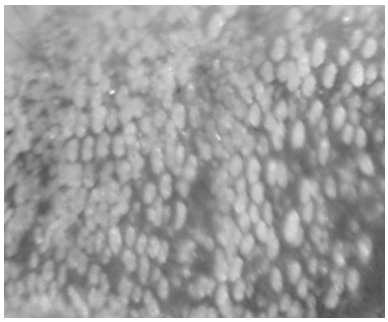
10 Proboscis may be present; dorsal chaetae (other than hairs) are crotchet chaetae that are much finer and smaller (= needles) than the ventral chaetae (see Fig. 7) 11



Prostomia with or without proboscis

- Proboscis absent; dorsal chaetae (other than hairs) of similar size to ventral chaetae ..... **Naididae** (in part) 14

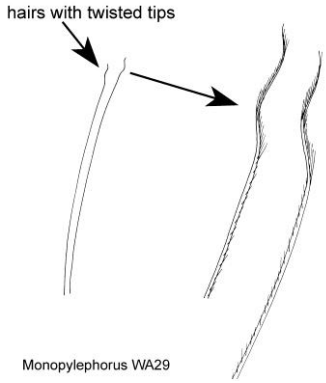
11 Proboscis absent; body densely covered by papillae with adhering foreign matter .... **Naididae: Tubificinae: *Embolocephalus yamaguchii*** (known only from Japan and South Australia) or ***Spirosperma* sp**. (undescribed species from South-eastern Australia)



Body wall papillae on *Embolocephalus yamaguchii*.

- Proboscis present or absent; body without papillae 12

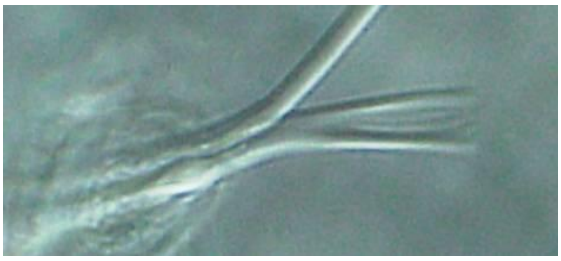
12 Hair chaetae with twisted plumose tips; proboscis absent .... **Naididae: Rhyacodrilinae; *Monopylephorus* n. sp. WA29** (known only from Pilbara groundwater)



Monopylephorus n. sp. WA29 hair chaetae

- Hair chaetae without twisted tips; proboscis may be present 13

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



Pectinate dorsal chaeta of *Monopylephorus* sp. WA2

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

14 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 (Fig. 17C); hair chaetae absent; coelomocytes generally absent; marine/Coastal/brackish/rarely freshwater ..... **Naididae: Phallodrilinae**

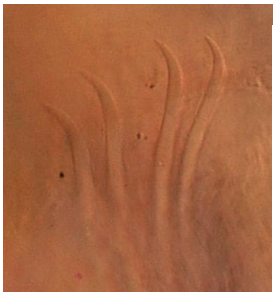
- Male duct with no prostate tissue or a pair of prostate glands arranged symmetrically on the atrium or with numerous small glands on the atrium or with a diffuse covering of prostatic cells arranged over the atrium; hair chaetae may be present in dorsal bundles; coelomocytes usually present and frequently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

15 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 17

- More than 8 chaetae per segment (at least anteriorly), always grouped into distinct bundles 16

16 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 .... **Naididae: Rhyacodrilinae; *Breviatria multiprostatus*** (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)

- 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)



Naididae: Rhyacodrilinae, Chaetae of *Breviatria multiprostatus* (left photo); **Enchytraeidae,** Chaetae of various enchytraeid species (illustrations and right photo)

17 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)**

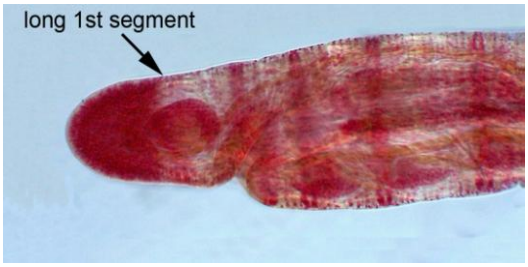
- Aquatic or semi-aquatic worms; body wall usually thin; chaetae paired, ≤ 8 per segment, usually protruding well out of the body wall and either straight or sigmoid; male pores always anterior to XIII; clitellum often inconspicuous, one-cell thick, covering the segment(s) with male pores 18

18 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)**

Paired enchytraeid chaetae without distinct nodulus

- 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 19

19 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 scythe-shaped chaetae per ventral bundle; dorsal chaetae usually smaller and reduced in number or absent; pharynx modified as a non-eversible muscular gizzard ..... **Haplotaxidae, in part (genus *Haplotaxis*)**



Long peristomium of *Haplotaxis* (left), *Haplotaxis* ventral chaeta (right)

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

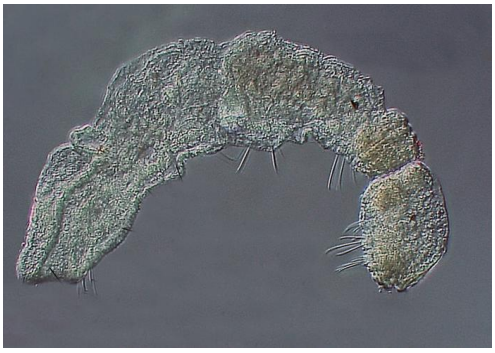
Key to the Australian Naidinae

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 elongate 5

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

- Body short and broad; prostomium greatly reduced so mouth is directed anteriorly; ventral chaetae of III-V missing; epigean .... ***Chaetogaster*** 3



Chaetogaster sp.

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

Chaetogaster limnaei chaeta

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

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

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

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

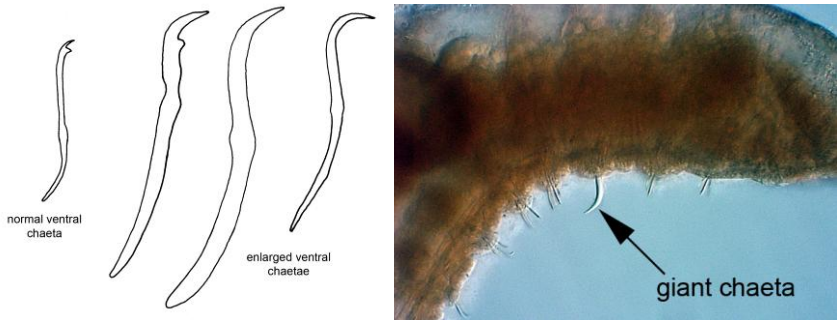
- Without proboscis 10

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

Stylaria lacustris with proboscis (left), *Stylaria lacustris* ventral chaeta with kinked shaft (right)

- 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 usually enlarged with lower tooth absent or less than half as long as upper ..... ***Pristina aequiseta***



Pristina aequiseta ventral chaetae (left), *Pristina aequiseta* giant chaeta (right)

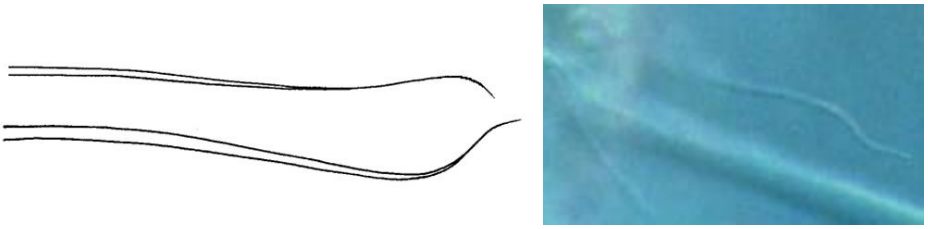
- Needle chaetae very fine, difficult to detect at the base of the hairs, and either very finely tapering to a point or with minute teeth (hardly visible at 400x); hairs of III may be much longer than the rest; ventral chaetae of II and III may be longer and/or thicker than the rest, but greatly enlarged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

8 Needle chaetae minutely bifid (usually need 1000x magnification to confirm, teeth about 1 µm long); hair chaetae of III usually much longer than the rest (unless missing)..... ***Pristina leidyi*** (has been considered a subspecies of Pristina longiseta by some and author has frequently identified Australian specimens as longiseta in the past)

Needle chaeta of *Pristina leidyi*

- Needle chaetae tapering to a fine point; hair chaetae of III may be much longer than the rest 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***



Drawing of *Pristina longiseta* needle chaetae (left), Photograph of *Pristina longiseta* needle chaetae at base of hair (right)

- Needle chaetae with fine straight 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 .... ***Pristina proboscidea*** (presence of this species in Australia is uncertain)

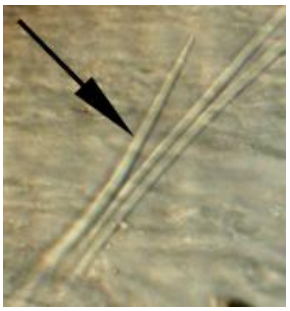
Pristina proboscidea chaeta

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***



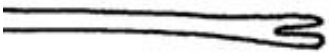
Simple-pointed needle of *Bratislavia unidentata*

- 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

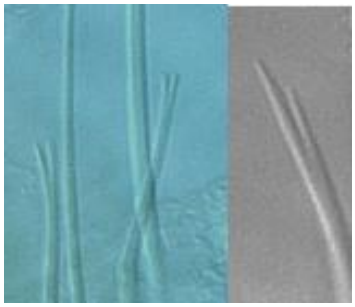
13 Needle chaetae teeth short (<2 um), parallel and of same length ..... ***Pristina bilobata***



Bifid tip of *Pristina bilobata* needle

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

(A species tentatively named *Monopylephorus* WA2 [Rhyacodrilinae], known only from the Kimberley region to date, 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 long but equal in length)



Bifid needle chaetae of *Pristina jenkinae*

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

(Rodriguez (2002) provides a good comparison of this species with *P. osborni*)

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

Pectinate needle chaeta of ***Pristina sima*** (left), tips of needle chaetae of ***Pristina osborni*** (right)

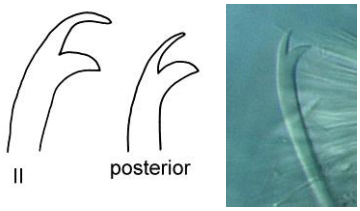
15 Needles with sharp simple tip (see photo at couplet 11)..... ***Bratislavia unidentata***

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

16 Hair chaetae absent; usually in brackish to saline waters ..... ***Paranais*** 17

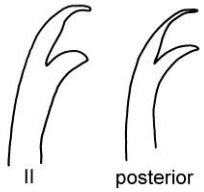
- Hair chaetae present; fresh to brackish water 18

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***



Chaetae of *Paranais litoralis* (left: ventral chaetae of segment II; middle and right: posterior ventral and dorsal chaetae.

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



Chaetae of *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***

Branchiodrilus hortensis

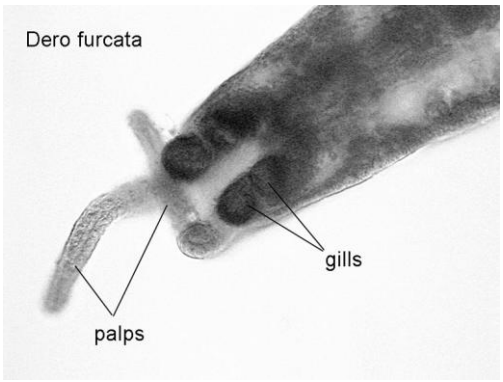
- Paired dorso lateral gills absent 19

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

Gill chambers of *Dero* spp.

- 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



Dero (Aulophorus) furcata with palps on posterior edge of gill chamber

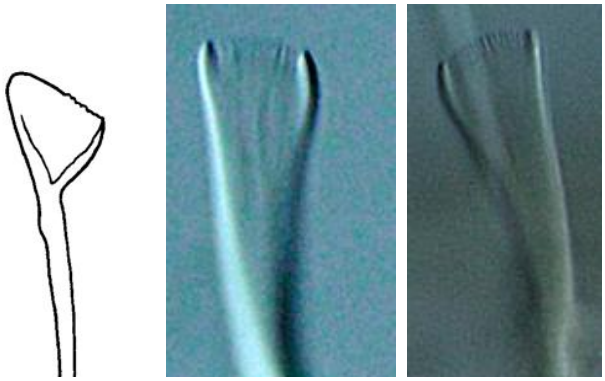
- Without caudal palps 24

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

- Needle chaetae with bifid tips 23

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

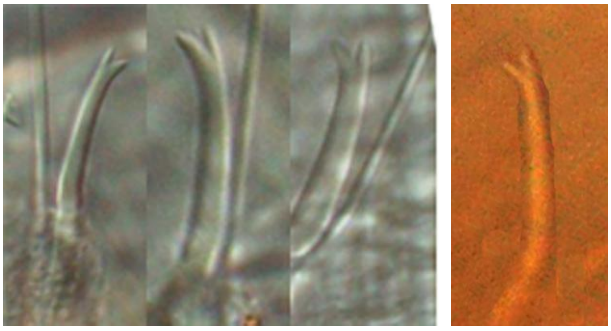
- Needle chaetae more moderately and symmetrically expanded ..... ***Dero (Aulophorus) vagus***



Dero flabelliger needle chaeta (left), ***Dero vagus*** needle chaetae (centre and right)

23 With 3 or 4 pairs of elongate 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**

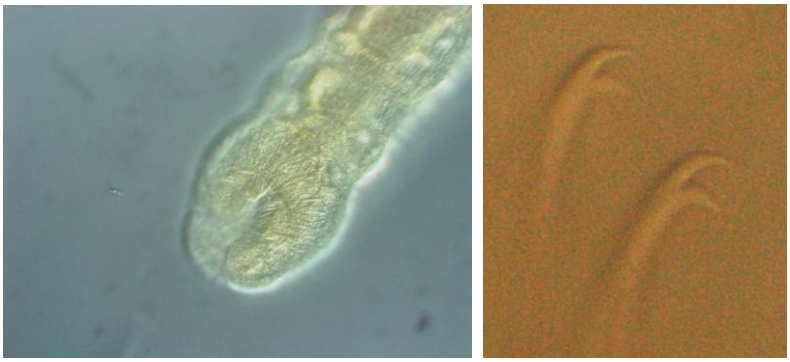


Dero furcata needle chaetae (left), ***Dero* WA4** needle chaeta (right)

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

Dero (Dero) anterior ventral chaeta (left), *Dero (Dero*) posterior ventral chaeta (centre), *Dero (Dero)* gill chambers (right, two photos)

- 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

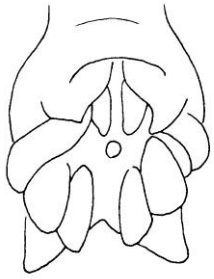


Dero (Allodero) gill chamber (left), *Dero (Allodero)* chaetae (right)

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***



Dero dorsalis gills

- 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***

Dero cf. *sawayai* chaeta

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

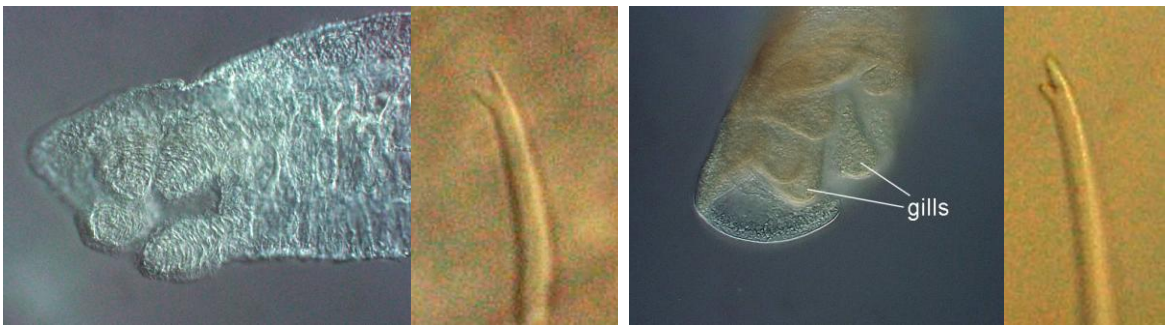
28 Hair chaetae plumose, needle chaetae pectinate ..... *Dero (Dero) pectinata*

Dero pectinata dorsal needle chaeta (left) and plumose hair chaeta (right)

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

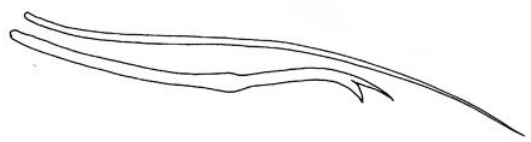
29 Needle chaetae with short teeth of about same length and width (or lower slightly thicker), two pairs of gills in a chamber that is not much wider than rest of body but sometimes with posterior extension ..... ***Dero (Dero) nivea***

- 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***



Dero nivea gill chamber and needle chaeta (left); ***Dero digitata*** gill chamber and needle chaeta (right)

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***



Haemonais waldvogeli dorsal chaetae

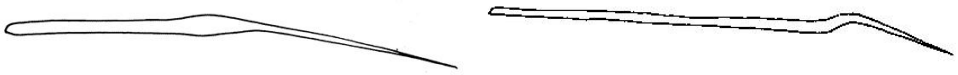
- Dorsal chaetae much finer than the ventral chaetae (dorsal chaetae are 'needle' chaetae) and present from IV to VI onwards 31

31 Needle chaetae sharply single-pointed 32

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

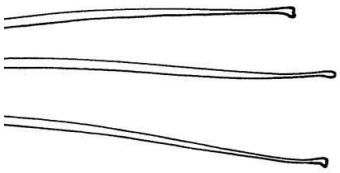
32 Needle chaetae with nodulus near mid-way along the needle shaft; posterior ventral chaetae with upper teeth shorter than lower ..... ***Nais barbata***

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



Nais barbata needle chaeta (left); ***Nais pseudobtusa*** needle chaeta (right)

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

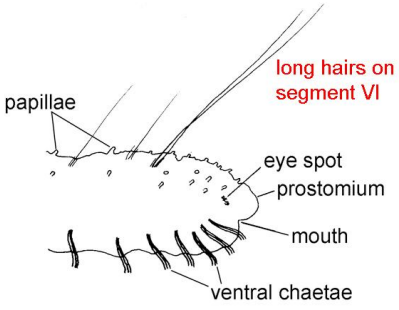


Slavina appendiculate needle chaetae

- 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***

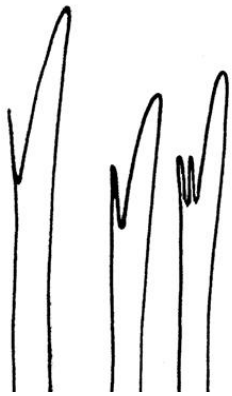


Slavina appendiculata anterior segments

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***



Tips of *Allonais paraguayensis* needle chaetae

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

Tips of *Allonais inequalis* needle chaetae

37 Needle chaetae pectinate 38

- Needle chaetae bifid 39

38 Needle chaetae with short (about 3 μm long) and strongly divergent teeth ..... ***Allonais pectinata***

- Needle chaetae with longer (about 5 μm long) and less strongly divergent teeth ..... ***Allonais ranauana***

Tip of ***Allonais pectinata*** needle chaeta (left); tip of ***Allonais ranauana*** needle chaeta (right)

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; needle teeth large (5 to 7.5 µm long, about 1 µm wide at base) .... ***Nais elinguis***

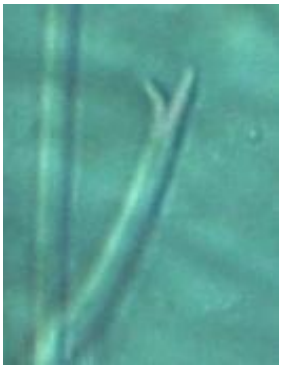
Nais elinguis ventral chaeta (left) and needle chaeta (right)

- Ventral chaetae with upper teeth < 1.5 times as long as the lower (frequently subequal or even with upper slightly shorter than lower, at least posteriorly); needles teeth short (<2 µm long and <0.5 µm wide) 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*** (Note: this species and *Nais communis* are notoriously difficult to separate, but these characters seem consistent)

Nais variabilis needle chaeta (left), anterior ventral chaetae (middle) and posterior ventral chaetae (right)

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



Tip of *Nais communis* needle chaeta

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

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

? = presence in Australia not confirmed

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