

A New Species of *Procephalothrix* (Anopla, Archinemertea) from North-Western Spain (Nemertea)

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With 10 Figures and 1 Table

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Abstract

A new species of archinemertean, *Procephalothrix oestrymnicus*, is fully described and illustrated from specimens collected intertidally in the Ensenada de Aguadulce, on the Galician coast of north-western Spain. The systematics of the genus and its contained species are discussed, and a key to the known species of *Procephalothrix* is provided.

More recent papers on the taxonomy of members of the family Cephalothricidae (e.g., GIBSON 1990; GIBSON et al. 1990) accept IWATA's (1960) proposal that this family should be removed from the Palaeonemertea and placed in a separate order, the Archinemertea. The only record of archinemerteans from Spanish waters is that of three specimens of *Cephalothrix rufifrons* (Johnston 1837) identified by ANADÓN (1980) from a beach of the Ría de Vigo, north-western Spain.

During a study of the nemertean fauna of the Spanish coast, nine specimens were collected which proved to belong to a previously unknown archinemertean species. Histological studies reveal that the nemerteans belong in the genus *Procephalothrix*, but that they possess features which exclude them from all the known members of this taxon. The nemerteans are accordingly named *Procephalothrix oestrymnicus* sp. nov.

Material and Methods

The nemerteans were anaesthetized in 7·5% MgCl₂ for external examination prior to being fixed in Bouin's fluid. Histological studies have been made on sections cut at 6 µm in 56°C m.p. paraffin wax, stained by the Mallory triple method described by PANTIN (1960).

Family Cephalothricidae McIntosh, 1873–1874

Genus *Procephalothrix* Wijnhoff, 1913

Diagnosis: The following diagnosis of the genus is amended from that given by GIBSON (1990) to include additional data tabulated by GIBSON et al. (1990).

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Archinemertean with two principal body wall muscle layers (outer circular, inner longitudinal), but with incompletely developed inner circular coat, mostly confined to foregut region but in some species extending more or less full body length; usually with longitudinal muscle plate situated between foregut wall and rhynchocoel; cerebral ganglia and lateral nerve cords situated in body wall longitudinal muscle layer; nervous system with neither neurochords nor neurochord cells; rhynchocoel typically less than half body length but in one species reaching posterior tip, housing proboscis containing outer circular and inner longitudinal muscle layers; blood vascular system normally consisting of paired lateral longitudinal vessels transversely connected only by cephalic and anal lacunae, without mid-dorsal vessel, but one species with elaborated blood supply; cerebral sensory organs missing; some species with two minute eyes, others eyeless; mouth located either far behind cerebral ganglia or not widely separated from them; cephalic region with four large nerves; sexes mostly separate, one species hermaphroditic.

Procephalothrix oestrymnicus sp. nov.

Type specimens: Series of transverse sections deposited in the Natural History Museum, London, Registration Numbers 1990.6.2 (holotype, female), 1990.6.3–4 (paratypes, both male).

Type locality: Galician coast of north-western Spain, Ensenada de Aguadulce, approximately 43°35'N, 7°14'W, under boulders in coarse sand, intertidal, nine specimens collected 10 January 1990 by Dr Juan Junoy and Mrs Cristina Rebollo Doncel.

Etymology: The species is named after the Oestrymnios, a tribe who supposedly first settled what is now Galicia during the Bronze Age.

Fig. 1. *Procephalothrix oestrymnicus* sp. nov. Part of the dorsal body wall in transverse section to show the appearance of the epidermis (EP), dermis, outer circular muscle layer (indicated by small arrowheads) and longitudinal musculature (LM). Large arrowheads point to the rhynchocoel circular muscle layer. PR, proboscis; RC, rhynchocoel. Mallory. Scale bar = 50 μ m.

Fig. 2. *Procephalothrix oestrymnicus* sp. nov. Part of the body wall in transverse section to show a lateral nerve cord (LN) and fibres of the delicate inner circular muscle layer (indicated by small arrowheads). The large arrowhead points to a lateral blood vessel. EP, epidermis; FE, foregut epithelium; LM, body wall longitudinal muscle layer. Mallory. Scale bar = 50 μ m.

Fig. 3. *Procephalothrix oestrymnicus* sp. nov. Transverse section through the foregut region to show the longitudinal muscle plate (indicated by arrowheads) running between the foregut epithelium (FE) and the rhynchocoel. FG, foregut lumen; PR, proboscis. Mallory. Scale bar = 50 μ m.

Fig. 4. *Procephalothrix oestrymnicus* sp. nov. Transverse section to show the appearance of the proboscis. Arrowheads point to two of the rhabditoid accumulations in the distal part of the proboscis epithelium. Mallory. Scale bar = 50 μ m.

Fig. 5. *Procephalothrix oestrymnicus* sp. nov. Transverse section through the mouth. Small arrowheads point to the lateral blood vessels, large arrowheads to the two foregut nerves. FE, foregut epithelium; PR, proboscis. Mallory. Scale bar = 150 μ m.

Fig. 6. *Procephalothrix oestrymnicus* sp. nov. Transverse section to show the general appearance of the foregut (FG). PR, proboscis. Mallory. Scale bar = 200 μ m.

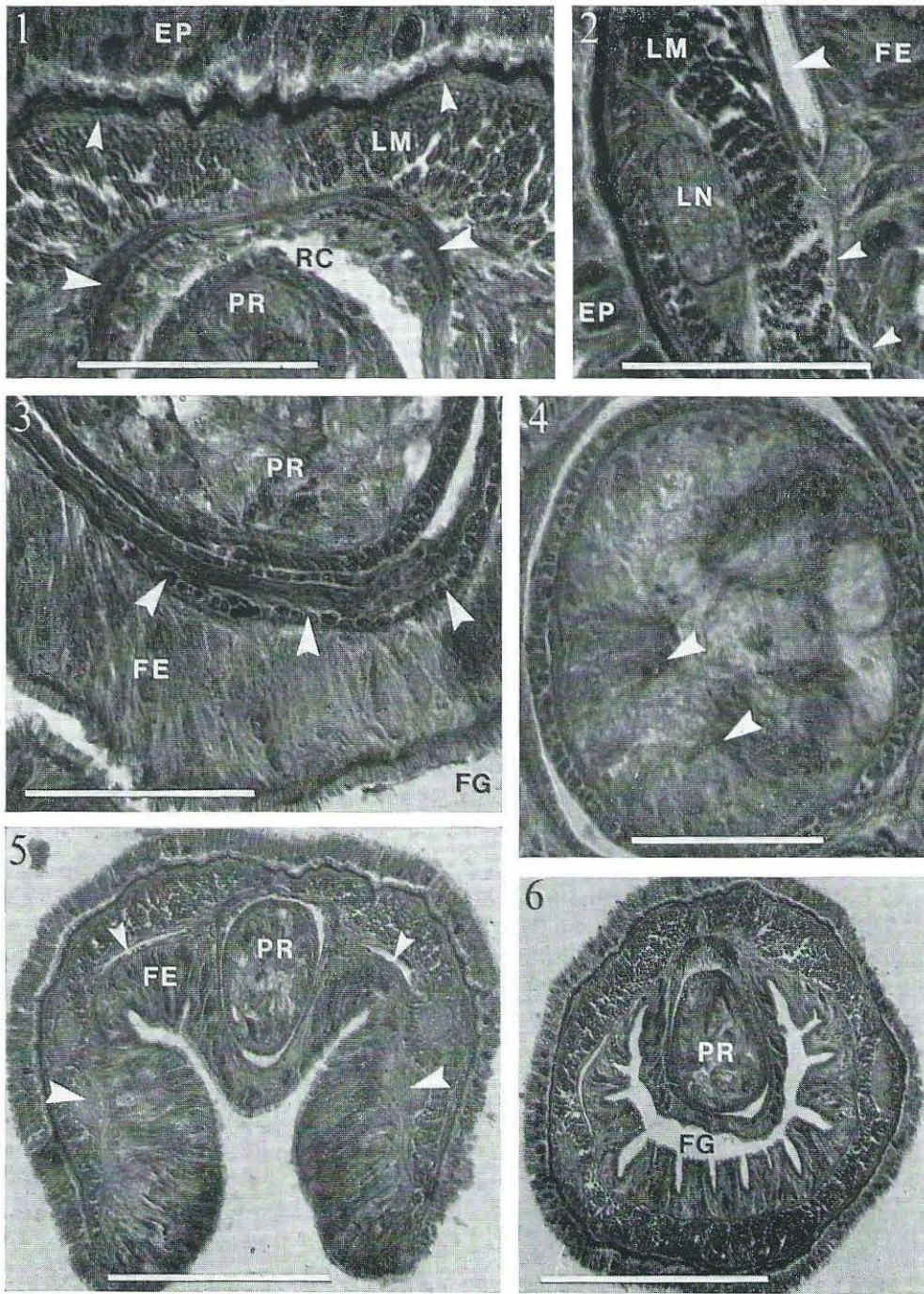


Fig. 1-6

External features: The only complete specimen obtained was, after anaesthetization, about 70 mm long but less than 0.5 mm in maximum diameter. The bluntly rounded head, which possesses neither eyes nor cephalic furrows, is slightly narrower than the succeeding body regions. The mouth is situated about 1–2 mm behind the anterior tip of the body, which gradually narrows caudally to end in a blunt rounded tail.

The nemerteans are a general pinkish-white colour with a paler head.

Body wall, musculature and parenchyma: The richly glandular epidermis (Fig. 1) is 15–36 μm thick in the anterior half of the body but is reduced in thickness posteriorly. Below it the distinct connective tissue dermis is mostly 2–8 μm deep.

The body wall muscles principally comprise an extremely thin outer circular layer, about 1–3 μm across, and an inner longitudinal layer approximately 70–80 μm in maximum thickness. Both muscle layers extend to the tip of the head, although the longitudinal fibres in the cerebral and pre-cerebral regions tend to be displaced by the nervous system. Post-cerebrally the circular layer does not, as in some *Procephalothrix* species, divide to enclose the lateral nerve cords. In the anterior body region an incomplete inner circular muscle layer (Fig. 2), rarely more than about 3 μm thick, is confined to the ventral half of the body and does not enclose the lateral blood vessels. This muscle layer extends for most of the foregut length but does not reach its posterior part.

Above the foregut and intestine is a plate of longitudinal muscles, often only a single fibre thick (Fig. 3). Anteriorly the plate runs between the rhynchocoel and dorsal gut wall.

Parenchymatous connective tissues are sparsely developed in all parts of the body.

Proboscis apparatus: The subterminal ventral proboscis pore leads into a tubular rhynchodaeum, enclosed by a delicate layer of longitudinal muscle fibres, whose epithelium possesses neither cilia nor gland cells. Just in front of the proboscis insertion a sphincter-like band of circular muscles surrounds the rear of the rhynchodaeum.

The rhynchocoel extends for about one-third of the body length. Its wall contains separate circular and longitudinal muscle layers which are together about 5 μm thick.

The proboscis (Fig. 4) is up to about 150–160 μm in overall diameter and possesses a more or less uniform and simple construction throughout its length. Its glandular epithelium contains large numbers of acidophilic rhabditoids, each of which is slightly curved, 5–6 μm long and 1 μm or less wide. Below the epithelium delicate outer circular and inner longitudinal muscle layers, together about 6–10 μm across, are internally bordered by a delicate endothelium.

Alimentary canal: The large mouth (Fig. 5) is situated approximately 1–2 mm back from the tip of the head, 0.7–1.2 mm behind the ventral cerebral commissure. The epithelium on either side of the mouth forms thick ventrolateral lips 80 μm or more in height. The mouth leads into a foregut whose richly glandular epithelium, mostly 25–90 μm tall, is distinctly folded (Fig. 6).

As in other *Procephalothrix* species, the junction between foregut and intestine is marked by an abrupt change in epithelial construction, the gastrodermis being distinguishable by its much more sparsely distributed cilia and lack of basophilic gland cells. The intestine (Fig. 7) bears a few shallow folds but no distinct lateral diverticula. The anus opens terminally.

Blood system: The blood system belongs to the simple grade typical of the Cephalothricidae (OUDEMANS 1885) and is similar to that described in other species of *Procephalothrix* (GIBSON 1990; GIBSON et al. 1990). It consists of a pair of lateral longitudinal vessels (Figs. 5, 6) which are joined only by cephalic and anal lacunae.

Nervous system: The brain and lateral nerve cords are situated in the body wall longitudinal muscle layer. The cerebral ganglia commence about 220–290 μm behind the tip of the head. They are joined transversely by dorsal and ventral commissures, respectively some 15 μm and 30 μm thick. Within the brain an inner neurilemma, separating the fibrous and neuroganglionic components, can be distinguished, but there is

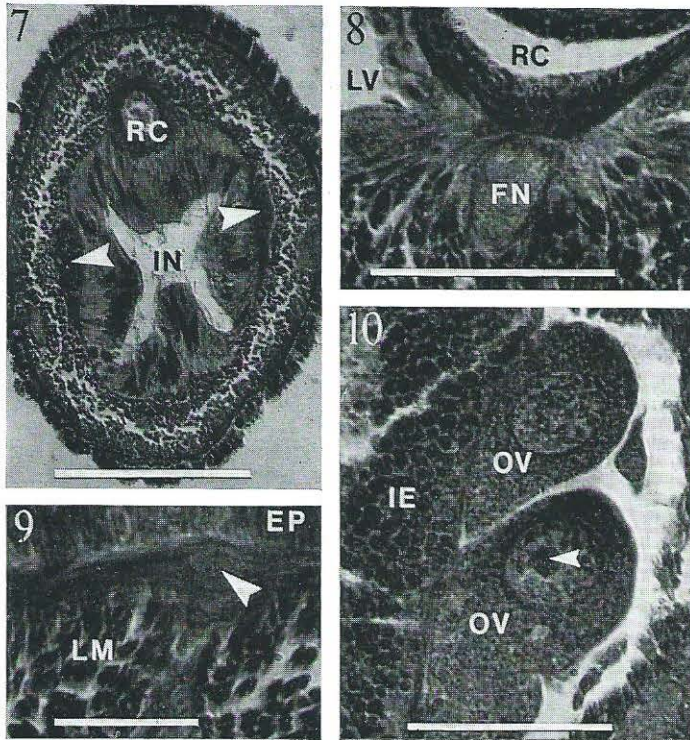


Fig. 7. *Procephalothrix oestrymnicus* sp. nov. Transverse section in the vicinity of the posterior part of the rhynchocoel (RC) to show the appearance of the intestine (IN) flanked by two testes (indicated by arrowheads). Mallory. Scale bar = 200 μm .

Fig. 8. *Procephalothrix oestrymnicus* sp. nov. Transverse section close behind the brain to show the unpaired foregut nerve root (FN) running below the rhynchocoel (RC). LV, lateral blood vessel. Mallory. Scale bar = 50 μm .

Fig. 9. *Procephalothrix oestrymnicus* sp. nov. Part of the body wall in transverse section to show the mid-dorsal nerve, indicated by the arrowhead, running between the dermis and outer circular muscle layer. EP, epidermis; LM, body wall longitudinal muscle layer. Mallory. Scale bar = 25 μm .

Fig. 10. *Procephalothrix oestrymnicus* sp. nov. Transverse section through an ovary to show two developing ova (OV) and their large nuclei. The arrowhead points to the distinct nucleolus of one nucleus. IE, intestinal epithelium. Mallory. Scale bar = 50 μm .

no outer equivalent. The lateral nerve cords extend directly back from the rear of the ventral cerebral lobes. Neither the brain nor nerve cords contain neurochord cells or neurochords.

The peripheral nervous system is well developed. Delicate neural plexi can be distinguished running in the rhynchocoel wall, immediately below the foregut epithelium, and between the dermis and underlying body wall muscles; occasional nerve fibrils leading from the lateral nerve cords appear to supply the latter plexus.

A single stout foregut nerve root, 15–20 μm or more in diameter, emerges from the rear of the ventral cerebral commissure and runs back below the rhynchocoel (Fig. 8). Close in front of the mouth this nerve divides into two, the nerves passing back on either side of the mouth (Fig. 5) to meet below the foregut by a sub-alimentary commissure. Occasional fibrils connect the foregut nerves with the neural plexus running adjacent to the foregut epithelium.

A distinct mid-dorsal nerve (Fig. 9), up to about 15 μm in diameter, leads from the rear of the dorsal cerebral commissure to extend posteriorly between the dermis and body wall outer circular muscle layer. This nerve does not, as in some species of *Procephalothrix*, branch into upper and lower components.

A large cephalic nerve, enclosed by bean-shaped accumulations of neuroganglionic cells and up to 100 μm in diameter, leads forwards from the front of each cerebral lobe. The four cephalic nerves together occupy much of the space in the head.

Frontal organ and cephalic glands: Neither a frontal organ nor typical basophilic cephalic gland lobules could be distinguished in the present nemerteans, and there are no acidophilic submuscular glands as, for example, occur in *P. hermaphroditicus* (GIBSON et al. 1990).

Sense organs: The present nemerteans do not possess eyes and, as in all other members of the genus, also lack cerebral sensory organs. No evidence of other sensory structures could be found.

Excretory system: The excretory system is located in the foregut and anterior intestinal regions of the body. It closely conforms to the metanephridial arrangement, originally described by COE (1930a), and consists of an undetermined number of separate nephridia intimately associated with the lateral blood vessels. Each nephridium comprises a mushroom-shaped 'nephrostome', about 20 μm in diameter, protruding into the blood vessel lumen. Efferent ducts could not be found.

Reproductive system: The sexes are separate, the gonads being distributed on either side of the intestine. Each ovary contains 3–6 developing eggs, up to 55 μm or more in diameter, with a nucleus 15–20 μm across in which the single nucleolus, about 5 μm wide, is usually very distinct (Fig. 10). The testes appear as ovoid sacs with a maximum dimension of about 80 μm (Fig. 7).

Systematic Discussion

Major anatomical features of the Spanish nemerteans include two principal body wall muscle layers (outer circular, inner longitudinal), cerebral ganglia and lateral nerve cords situated in the longitudinal muscle layer, no cerebral sensory organs, and a simple blood system with no mid-dorsal vessel. This combination of characters identifies the nemerteans as members of the sole archinemertean family Cephalothricidae, comprising the genera *Cephalothrix*, *Cephalotrichella* and *Procephalothrix*. In her key to these taxa

WIJNHOF (1913: 294) separated *Procephalothrix* from the other two by an "Innere Ringmuskelschicht vorhanden"; the present worms have an incomplete inner circular muscle layer and are accordingly placed in the genus *Procephalothrix*.

Twelve species of *Procephalothrix* are at present recognised (Table 1) and, as noted by GIBSON et al. (1990: 285), "many of these ... resemble ... each other ... and a reliable

Table 1. A summary of some of the anatomical features recorded for species of *Procephalothrix* compared with those of the Spanish nemerteans. Data compiled from PUNNETT (1903), WIJNHOF (1913), COE (1930b), FRIEDRICH (1935), IWATA (1952, 1954), MORETTO (1974), FERRARIS (1978), KOROTKEVITSCH (1982), GIBSON (1990) and GIBSON et al. (1990).

Species	1	2	3	4	5	6	7
<i>P. aliena</i> (Punnett 1903)	0? ^a	+	0	+ ^b	0	0?	0?
<i>P. arenarius</i> Gibson 1990	0	+	+	0	+	0	0
<i>P. fasciculus</i> Iwata 1952	+	++	++	0?	0	0	0?
<i>P. filiformis</i> (Johnston 1828—1829)	0	++	++	+	0	0	0
<i>P. hermaphroditicus</i> Gibson, Sánchez and Méndez 1990	0	+	0	0	++	0	+
<i>P. kiliensis</i> Friedrich 1935	0	+?	?	+	0	0	?
<i>P. major</i> (Coe 1930)	0	++	+++	+	++	0	0
<i>P. mokievskii</i> Korotkevitch 1982	0	+	+	+	+?	0	0
<i>P. orientalis</i> Gibson 1990	0	+	+	0	0	+	0?
<i>P. quequenensis</i> Moretto 1974	0?	+	++	+ ^c	?	0	?
<i>P. simulus</i> Iwata 1952	0	++	++	+	0	0	?
<i>P. spiralis</i> (Coe 1930)	0	++	++	0?	+	+	0
<i>P. oestrymnicus</i> sp. nov.	0	+	0	0	0	0	0

^a PUNNETT (1903) commented that the posterior portion of the body was missing from his specimen.

^b Whether or not the inner circular muscle fibres enclose the lateral blood vessels in *P. aliena* is not certain: PUNNETT (1903: 103) said that the muscles "continued laterally to enclose the two lateral blood vessels", but his diagram of the foregut region (pl. IV, Fig. 16) suggests that the fibres pass only on the inner margins of the vessels.

^c It is not certain whether the inner circular muscle fibres pass on one or both sides of the blood vessels in *P. quequenensis*; MORETTO (1974: 12) reported that the muscles "abrazando a los vasos sanguíneos laterales" but he did not illustrate this feature and the Spanish "abrazando" can be translated to mean either condition.

1: Rhynchocoel at most about half body length (0) or extending to posterior tip (+).

2: Mouth close to or immediately behind brain (+) or far behind rear of brain (++).

3: Cephalic glands absent (0), sparse (+), well developed (++) or voluminous (+++).

4: Inner circular muscle layer divides to enclose lateral blood vessels (+) or does not divide to surround them (0).

5: Inner circular muscle layer restricted entirely to foregut region of body (0), extending into anterior intestinal region (+) or reaching for more or less full length of body (++).

6: Foregut neural supply originating from unpaired (0) or paired (+) nerve roots.

7: Sexes separate (0) or hermaphroditic (+).

distinction between them cannot be achieved without recourse to histological studies of their internal morphology."

Significant features of the Spanish nemerteans include a rhynchocoel about one-third of the body length, a mouth situated far behind the brain, no cephalic glands, a weakly developed inner circular muscle layer restricted to the foregut region of the body and not dividing to enclose the lateral blood vessels, a foregut nerve supply originating from an unpaired nerve, and separate sexes. This combination of features is not found in any of the existing *Procephalothrix* species (Table 1) and, since the Spanish worms cannot thus be identified with any of the known procephalothricids, the new species *Procephalothrix oestrymnicus* is established for them.

Procephalothrix oestrymnicus sp. nov. can also be distinguished from individual species in other ways: *P. mokievskii* has a much bulkier body form and a more elaborate blood system than any other member of the genus known (KOROTKEVITSCII 1982); *P. kiliensis* possesses a short mid-dorsal blood vessel (FRIEDRICH 1935); *P. major*, the largest member of the genus described, has an extremely long slender snout extending far in front of a minute mouth (COE 1930b); in *P. aliena* the epidermis is characterized by a double row of nuclei (PUNNETT 1903); *P. arenarius* and *P. orientalis* both possess a frontal organ, the latter taxon also being for most of its body length marked by broad median dorsal and ventral pale brown stripes (GIBSON 1990); *P. fasciculus* is anteriorly greenish-ochre in colour, and ochre with translucent lateral margins in its intestinal region (IWATA 1952); and *P. quequenensis* possesses a constriction between its cephalic and trunk regions which is marked by a colourless band, whilst the remainder of its body is dorsally reddish-yellow (MORETTO 1974).

Key to the *Procephalothrix* species of the world

Because of the high degree of similarity in the external appearance shown by many species of *Procephalothrix*, the following key is based entirely upon features distinguishable in histological sections.

1. Rhynchocoel at most about half length of body 2
 Rhynchocoel extending to posterior tip of body. *P. fasciculus* Iwata 1952
2. Blood system elaborated, with one or two vessels running adjacent to rhynchocoel wall in addition to lateral longitudinal vessels 3
 Blood system simple, without additional vessels running adjacent to rhynchocoel wall 4
3. With single short mid-dorsal blood vessel *P. kiliensis* Friedrich 1935
 With two lateroventral blood vessels running adjacent to rhynchocoel wall
 *P. mokievskii* Korotkevitsch 1982
4. With horizontal longitudinal muscle plate between foregut and rhynchocoel 5
 Without horizontal longitudinal muscle plate between foregut and rhynchocoel (there is some confusion over this point; IWATA 1952: 132, when he originally established this species, stated that "The longitudinal muscle plate is entirely wanting", but subsequently [IWATA 1954: 6] noted that "The longitudinal muscle plate runs posteriorly between the proboscis sheath and the oesophagus") *P. simulus* Iwata 1952
5. Foregut neural supply arising from single nerve root which divides into two in front of mouth 6
 Foregut neural supply arising from two separate nerve roots 8
6. Cephalic glands absent 7
 Cephalic glands present 9

7. Inner circular muscle layer restricted to foregut region of body *P. aliena* (Punnett 1903)
 Inner circular muscle layer extending more or less full length of body
 *P. hermaphroditicus* Gibson, Sánchez and Méndez 1990
8. Inner circular muscle layer extending into anterior intestinal region of body
 *P. spiralis* (Coe 1930)
 Inner circular muscle layer restricted to foregut region of body *P. orientalis* Gibson 1990
9. Mouth close to or immediately behind brain 10
 Mouth very far behind rear of brain 12
10. Inner circular muscle layer divides to enclose lateral blood vessels (see footnote 'c' in Table 1)
 *P. quequenensis* Moretto 1974
 Inner circular muscle layer does not divide to enclose lateral blood vessels 11
11. Inner circular muscle layer extends into anterior intestinal region of body
 *P. arenarius* Gibson 1990
 Inner circular muscle layer restricted to foregut region of body *P. oestrymnicus* sp. nov.
12. Inner circular muscle layer restricted to foregut region of body
 *P. filiformis* (Johnston 1828–1829)
 Inner circular muscle layer extends more or less full length of body *P. major* (Coe 1930)

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