The smile of *Amphiporus nelsoni* Sanchez, 1973 (Nemertea: Hoplonemertea: Monostilifera: Amphiporidae) leads to a redescription and a change in family

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Abstract.—A common intertidal hoplonemertean species, *Amphiporus nelsoni* Sanchez, 1973, from Chile is re-described based on the investigation of material from the type locality and one other locality in Chile. The species is transferred to the genus *Prosorhochmus* Keferstein, 1862 (Prosorhochmidae) based on the presence of a dorsal epidermal fold, i.e., "prosorhochmid smile" on the bilobed head, truncated stylet basis, well-developed frontal organ with characteristic epithelial specialization, structure of the nephridial system and other characters of internal anatomy. Placement of this species into *Prosorhochmus* expands the geographical distribution of the genus, previously known only from the North Atlantic and Mediterranean, to the South Pacific. Interestingly, *Prosorhochmus nelsoni* (Sanchez, 1973) is characterized by separate sexes and oviparity, unlike all the other members of the genus, which combine hermaphroditism with ovoviviparity. We compare *P. nelsoni* to the other species of *Prosorhochmus* and reassess some of the morphological characters used in the systematics of the genus.

The genus *Prosorhochmus* currently comprises four marine intertidal species combining two relatively uncommon characteristics among nemerteans, hermaphroditism and viviparity. A total of nine species have been described in the genus, but two of these have been synonymized with *Prosorhochmus claparedii* Keferstein, 1862 and three others are considered species inquirendae. The four valid species are found in the North Atlantic or Mediterranean (Gibson & Moore 1985). The original description of *Amphiporus nelsoni* Sanchez, 1973 was mainly based on external appearance. In this paper we re-describe *A. nelsoni* on the basis of histological sections of material from the type locality and recently collected material from another locality, and we illustrate the description with a set of photomicrographs and diagrams. We demonstrate that morphological characteristics clearly place *A. nelsoni* in *Prosorhochmus,* and we compare it to the other valid species of the genus *Prosorhochmus.*

Materials and Methods

Three attempts over a 15-year period failed to locate the type material, consisting of a series of histological sections of a holotype and three unsectioned paratypes in 9% sea water formalin originally deposited with the Museo de Historia Natural, Santiago, Chile. The current redescription is based on investigation of the series of histological sections of three specimens (USNM 173162–173164) collected and identified by Malva Sanchez (who original-

Fig. 1. *Prosorhochmus nelsoni* (Sanchez, 1973). External appearance of relaxed individual. Scale bar = 3 mm.
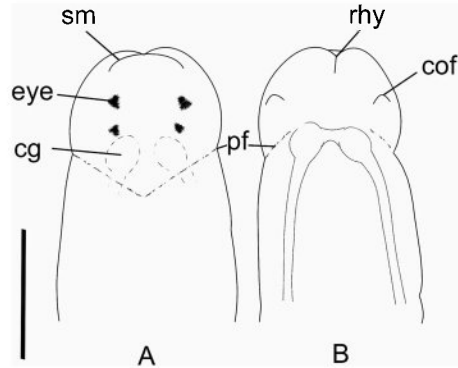

Fig. 2. Schematic of anterior end showing prosorhochmid "smile" and cephalic furrows of *Prosorhochmus nelsoni*. A. Dorsal view. B. Ventral view. Scale bar = 1 mm. Abbreviations: cg, cerebral ganglia; cof, cerebral organ furrow; pf, posterior cephalic furrow; rhy, rhynchopore; sm, prosorhochmid "smile."

ly described the species) from the type locality and five specimens (USNM 1019782, 1019784–86) collected from Coquimbo, Chile, by MT, of which three specimens were sectioned. Because the type material has been lost and with the purpose of clarifying the taxonomic status of *P. nelsoni* (Sanchez, 1973), a series of histological sections of a reproductive female (USNM 173164) collected from the type locality by the author of the species is hereby designated as the neotype and deposited in the National Museum of Natural History at the Smithsonian Institution, Washington D.C., U.S.A. along with the other catalogued specimens of this species mentioned above. Additional unsectioned specimens collected by one of us (MT) in La Pampilla Beach near Coquimbo, Chile, and preserved for histological studies have been deposited in the Museo de Historia Natural, Santiago, Chile (MNHNCL Nemer-No. 1009). Because nemertean species are generally difficult to differentiate and morphological characters are not interpreted consistently in the literature, we investigated the type material of three of the four valid *Prosorhochmus* species: *P. americanus* Gibson et al., 1986, *P. chafarinensis* Frutos et al., 1998, and *P. adriaticus* Senz, 1993. Type material of *P. claparedii* Keferstein, 1862 does not

exist; thus we utilized the material on which the species redescription was based (Gibson & Moore 1985), as well as additional material collected by us in reasonable proximity of the type locality (Roscoff, France; Bilbao, Spain). A complete list of examined material is presented in the Appendix.

Abbreviations.—USNM: National Museum of Natural History, Smithsonian Institution, Washington, D.C., U.S.A.; VMNH: Natural History Museum, Vienna, Austria; MNHM: Museo Nacional de Ciencias Naturales, Madrid, Spain; CMZ: Cambridge Museum of Zoology, Cambridge, UK.

Results and Discussion

Class Hoplonemertea
(sensu Thollesson & Norenburg, 2003)
Order Monostilifera
Family Prosorhochmidae
Prosorhochmus Keferstein, 1862
Prosorhochmus nelsoni (Sanchez, 1973),
new combination
Figs. 1–8, Tables 1, 2

Amphiporus nelsoni Sanchez, 1973:208–213, Figs. 10–13.

External appearance.—*Prosorhochmus nelsoni* is relatively small, with maximum

Table 1.—Measurements of stylet apparatus of immature specimens of *Prosorhochmus nelsoni* (Sanchez, 1973) from Coquimbo, Chile.

Specimen	Body length (anesthetized, mm)	Stylet length (S, μm)	Basis length (B, μm)	Basis diameter (μm)	S/B ratio
1	15	88.2	132.3	49	0.67
2	9.5	73.5	98	34.3	0.75
3	10	78.4	107.8	39.2	0.73
4	12	98	122.5	53.9	0.8
5	8	73.5	112.7	49	0.65
6	10	73.5	73.5	24.5	1
7	13	88.2	107.8	34.3	0.82
8	15	102.9	127.4	29.4	0.81
9	15	93.1	83.3	58.8	1.1
10	12	78.4	122.5	34.3	0.64
11	10	49	73.5	29.4	0.67
12	13	107.8	122.5	49	0.88
13	12	88.2	117.6	34.3	0.75
Average	11.88	84.05	107.80	39.95	0.79
SD	2.29	15.48	20.00	10.74	0.14
Min	8	49	73.5	24.5	0.64
Max	15	107.8	132.3	58.8	1.1

recorded length of reproductive specimens 45–50 mm and width 1.2–1.5 mm. The color in life is yellowish-orange dorsally, paler toward the ventral side. The body is slender and compact, dorso-ventrally flattened, wide at the anterior end, gradually tapering toward the posterior to end in a bluntly rounded tip (Fig. 1). The head is somewhat spatulate in shape and is wider than the remaining body, with a characteristic vertical anterior notch giving it a distinct bilobed appearance. An antero-dorsal horizontal epidermal fold anterior to the eyes separates two ventral apical lobes from a median dorsal lobe, creating the appearance of a "smile" (Fig. 2A), characteristic of the genera *Prosorhochmus* and *Pantinonemertes*. The four reddish-brown eyes are situated in front of the brain; the anterior pair is slightly larger than the posterior. The distance between the eyes of the anterior pair and the posterior pair is larger than between the two pairs. The rudimentary cerebral organ furrows, also referred to as the anterior cephalic grooves, appear as a pair of inconspicuous latero-ventral, whitish, semi-circular grooves approximately at the level of the anterior pair of eyes (Fig. 2B); these are not visible from the dorsal side. The shallow posterior cephalic furrow is indistinct and forms a dorsal, posteriorly directed "V" immediately behind the brain and a ventral, incomplete anteriorly directed "V" immediately anterior to the brain (Fig. 2A, B). The rhynchopore is subterminal.

Body wall, musculature and parenchyma.—Epidermis is of typical hoplonemertean structure (Figs. 3A, 6K). Dermis is represented by a thin layer of extracellular matrix. Body-wall musculature consists of an outer circular layer and an inner longitudinal layer. Diagonal (oblique) muscle fibers situated between the circular and longitudinal musculature of the body wall form a thin but distinct layer. This layer is best visualized on longitudinal sections (Fig. 3A) but also is visible on cross-sections (Fig. 3B). The precerebral septum is of split (Kirsteuer 1974) or mixed type (Chernyshev 2002) and corresponds to the description given by Gibson & Moore (1985:150, fig. 2). It is formed by individual muscle fibers emerging from the body wall longitudinal musculature at several levels. Behind the brain, separate bundles of oblique fibers diverge from the inner margins of the longi-
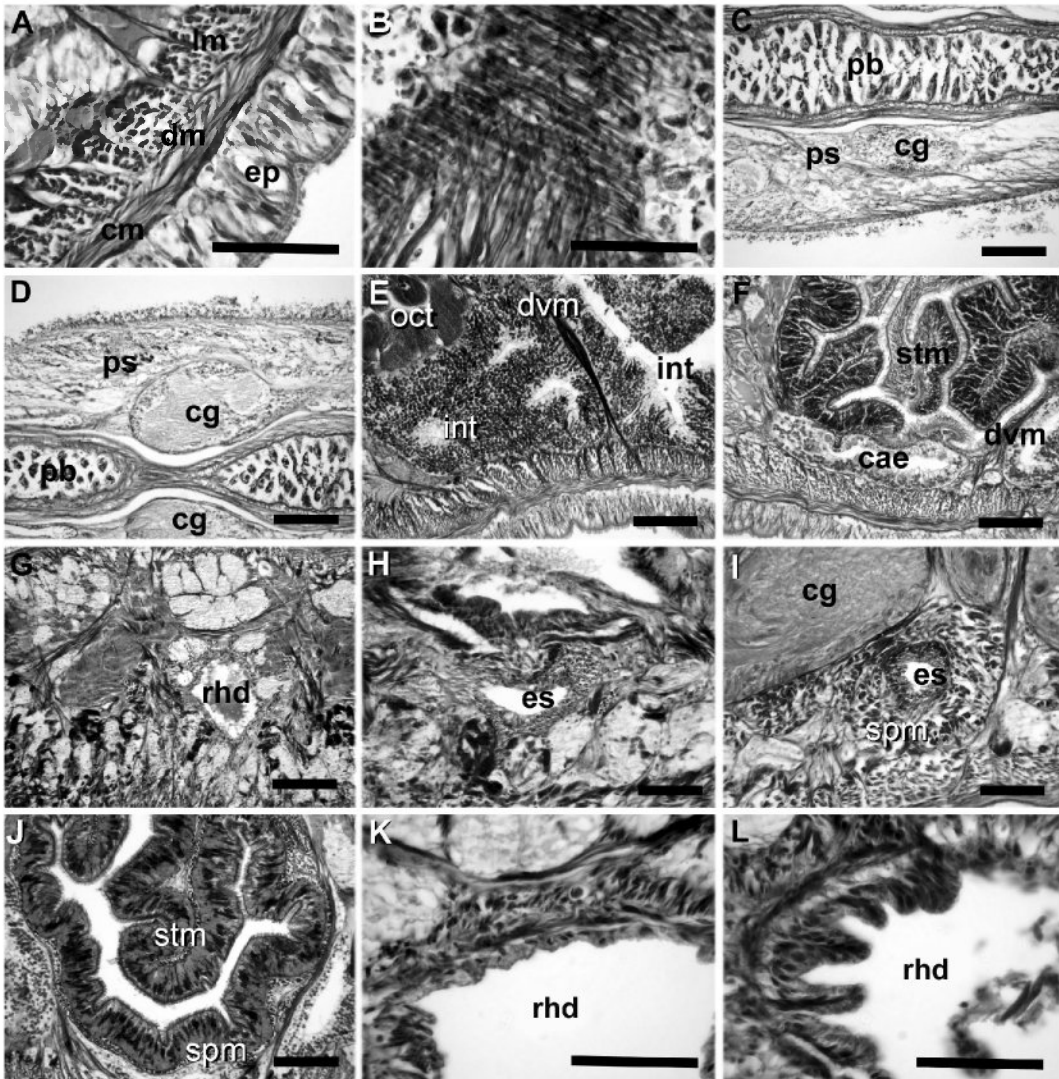
Fig. 3. *Prosorhochmus nelsoni*. A. Transverse section through body wall. B. Longitudinal section through body wall. C, D. Longitudinal frontal sections through cerebral region; anterior is to left. E. Transverse section showing well-developed dorso-ventral musculature in foregut region. F. Transverse sections through stomach and caecum. G. Transverse section through precerebral region showing numerous muscle fibers oriented dorso-ventrally, obliquely and horizontally. H, I. Transverse sections through esophageal region. J. Transverse section through stomach. K. Transverse section through rhynchodeum. L. Transverse section through rhynchodeum just anterior to proboscis insertion. A, B, H, I, K, L: scale bars = 50 μm; C–G, J: scale bars = 100 μm. Abbreviations: cae, caecum; cm, circular musculature; dm, diagonal musculature; dvm, dorso-ventral musculature; ep, epidermis; int, intestine; lm, longitudinal musculature; oct, oocytes; es, esophagus; pb, proboscis; ps, precerebral septum; rhc, rhynchocoel; rhd, rhynchodeum; spm, foregut or splanchnic musculature; stm, stomach.

tudinal muscle layer and lead forward toward the proboscis insertion. Here the oblique fibers are joined by additional (radial) fibers, which turn inward from the main layer (Fig. 3C, D). There is no clear demarcation between the radial and oblique fibers forming the septum. A few individual fibers from the inner portion of the longitudinal musculature continue into the head as cephalic retractors. Dorso-ventral mus-

cles are strongly developed between the gonads and intestinal diverticula (Figs. 3E, 8F). Anteriorly, thick bundles of dorso-ventral musculature are found between the lateral pouches of the caecum, between dorsal lobes of the mucus glands, and between the lateral nerve cords and lateral lobes of the mucus glands (Fig. 3F). Muscle fibers oriented dorso-ventrally, obliquely, and horizontally are abundant in the precerebral region (Fig. 3G). The musculature associated with foregut, in the literature often referred to as "splanchnic musculature," is very well developed and is continuous with the fibers surrounding the rhynchodeum. A layer of longitudinal muscles surrounds the esophagus from the point of its separation from the rhynchodeum to the beginning of the brain (Fig. 3H). The esophageal musculature becomes surrounded at the brain level by longitudinal fibers originating at the proboscis insertion and running parallel to the esophagus (Fig. 3I). These longitudinal muscles continue as a thin layer surrounding the stomach and are particularly obvious between the folds of the stomach wall (Fig. 3J). The amorphous extracellular matrix, the so-called "parenchyma," is developed somewhat better than in other species of *Prosorhochmus* (particularly in the foregut region) but is otherwise unremarkable.

Proboscis apparatus.—The proboscis pore opens terminally. It leads into a short, thin-walled rhynchodeum. Rhynchodeal epithelium comprises squamous cells with small elongated nuclei (Fig. 3K). Just anterior to the proboscis insertion, it is comprised of cells with acidophilic cytoplasm and large nuclei (Fig. 3L). It was not possible with light microscopy to determine whether rhynchodeal epithelial cells bear cilia or not. The rhynchodeal musculature is rather well developed and comprises both longitudinal and circular muscle fibers (Fig. 3K). There is no localized concentration of circular muscle fibers representing a distinct rhynchodeal sphincter. The rhynchocoel reaches almost to the posterior end of the

body. Its wall is of typical distromatonemertean (Thollesson & Norenburg 2003) structure and contains separate outer circular and inner longitudinal muscle layers (Fig. 4A). The thickness of the layers changes dramatically with the state of contraction of the animal. The proboscis is thin, longer than the body, somewhat translucent, and whitish or, occasionally, dull cream. The wall of the anterior chamber is composed of a tall glandular epithelium, arranged into conical papillae, a thin layer of extracellular matrix, an outer circular muscle layer, a longitudinal muscle layer, that is divided into concentric layers by the neural sheath, a delicate layer of inner circular muscles, and a thin endothelium (Fig. 4B). The neural sheath bears 10–12 proboscis nerves ($n = 3$; Fig. 4C).

The proboscis armature consists of a central stylet, mounted on a characteristically truncated basis, and two pouches each containing 1–3 accessory stylets (Fig. 4E). Measurements taken on 13 immature specimens (average length about 12 mm), collected during November 2003 at La Pampilla Beach near Coquimbo (Chile) are presented in Table 1. The average length of the central stylet (S) was 84 μm, average basis length was (B) −107.8 μm and average S/B ratio was 0.79. Sanchez (1973) reported the central stylet to be 80 μm long, but it is not known whether she measured the stylet in the mature or immature specimen. The wall of the posterior chamber of the proboscis consists of a glandular epithelium organized into papillae, outer longitudinal muscle layer, thin inner circular muscle layer, and a delicate endothelium (Fig. 4D). Similar to other described species of *Prosorhochmus,* but unlike *P. claparedii* from Anglesey [Gibson & Moore 1985:151–152, plate II(a)], there is no distinct nerve supply in the longitudinal muscle layer of posterior proboscis of *P. nelsoni.*

Alimentary canal.—The esophagus opens into the rhynchodeum in front of the precerebral septum. It is enclosed by longitudinal muscle fibers, which are confluent
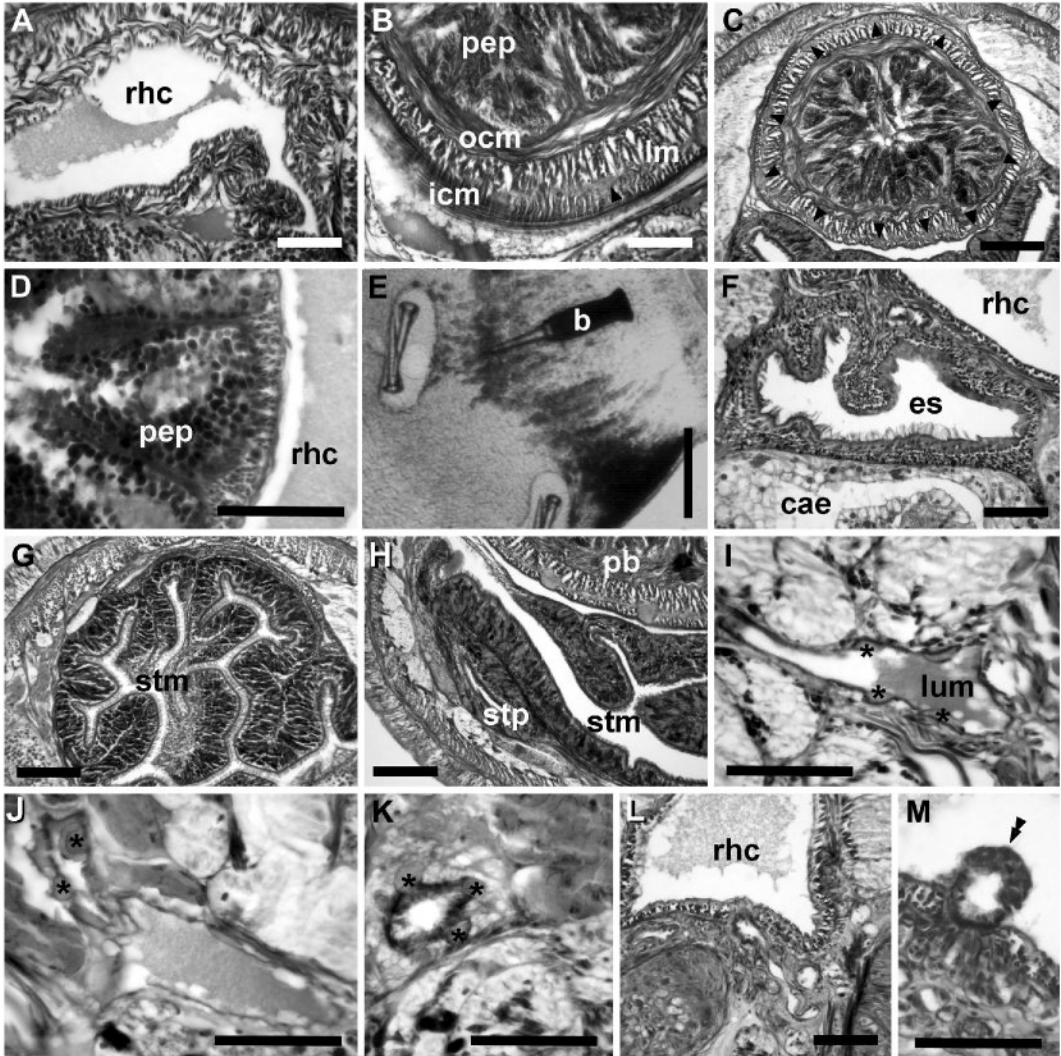
Fig. 4. *Prosorhochmus nelsoni.* A. Transverse section through rhynchocoel wall. B. Transverse (with tangential aspect) section through anterior proboscis wall; arrow indicates proboscis nerve. C. Transverse section through anterior proboscis; proboscis nerves indicated by arrow heads. D. Transverse section through posterior chamber of proboscis. E. Stylet apparatus. F. Transverse section through densely ciliated posterior esophagus. G. Transverse section through deeply folded glandular stomach. H. Transverse section through posterior region of stomach and its unpaired lateral diverticulum. I–K. Transverse sections through cephalic blood vessel in various states of contraction; thickened parts of wall (asterisks) in relaxed region of vessel (I), appear as valves or pouches in contracted regions (J, K). L. Transverse section through post-cerebral region. M. Transverse section through vascular plug: note modified rhynchocoelic epithelium (double arrow head). A, B, D, F, I–K, M: scale bars = 50 μm; C, E, G, H, L: scale bars = 100 μm. Abbreviations: ast, accessory stylet; b, basis; cae, caecum; cst, central stylet; icm, inner circular musculature; int, intestine; lm, longitudinal musculature; lum, blood vessel lumen; mbv, mid-dorsal blood vessel; ocm, outer circular musculature; os, esophagus; pb, proboscis; pep, proboscideal epithelium; rhc, rhynchocoel; spm, foregut or splanchnic musculature; stm, stomach; stp, stomach pouch.

with the rhynchodeal musculature and continue posteriorly as the musculature of the stomach. The posterior part of the esophagus is ciliated, but lacks acidophilic or basophilic glands present in the wall of the stomach (Fig. 4F). The stomach is of typical hoplonemertean structure with densely ciliated, deeply folded epithelium, containing numerous basophilic and acidophilic glands (Fig. 4G). Presence or absence of the posteriorly directed pouches in the stomach had been used to differentiate between the species of the genus (Frutos et al. 1998). Although it appears quite plausible that these pouches are merely an artifact of fixation, a more thorough study of the intra- and interspecific variation of this character would be necessary to reject this character altogether. In *P. nelsoni* there appears to be intraspecific variation in this character: four specimens studied from series of cross-sections lack a ventral posterior pouch in the stomach, while one additional specimen (USNM 1019785, anterior c.s. slide 5) possesses two consecutive postero-lateral diverticula on the left side of the stomach, each 80–100 μm long (Fig. 4H). The intestinal caecum is well developed, anteriorly divided, up to 5.3 mm long (measured in the largest sectioned specimen), and bears numerous lateral diverticula throughout its length. The anterior lobes or pouches reach the posterior portion of the dorsal cerebral ganglia. Intestinal diverticula are lobed.

Blood system.—The blood system is of a common hoplonemertean type. A cephalic suprarhynchodeal loop crosses just behind the posterior chamber of the frontal organ and continues posteriorly as the body's paired lateral vessels. The middorsal blood vessel originates near the ventral cerebral commissure from the left cephalic vessel (observed in one specimen) (Fig. 4L) and immediately penetrates the rhynchocoel floor to form a single vascular plug. The wall of the vascular plug consists of endothelium of the blood vessel, a thin layer of extracellular matrix, and a modified rhynchocoel endothelium (Fig. 4M). Similar to other described *Prosorhochmus* species, but unlike *P. claparedii* from Anglesey (Gibson & Moore 1985), *P. nelsoni* lacks transverse connectives linking mid-dorsal and lateral blood vessels in the intestinal region. The blood vessels are thin-walled with a well-defined extensive lumen and irregular thickenings of the wall (Fig. 4I). During contraction of the blood vessel wall, depending on whether the muscle fibers of the wall lie toward the inner or outer side of the thickening, the latter appear as "pouches" or "valves" (Fig. 4J, K). This is confirmed by the fact that neither distinct valves nor pouches are visible in relaxed regions of blood vessels, while both appear in the contracted regions (Fig. 4I–K). In addition, many kinds of intermediate states can be observed between the valves and the pouches. Perhaps these regions of thickened extracellular matrix serve to add rigidity to the otherwise elastic walls of the blood vessels.

Nervous system.—The brain is of typical nemertean construction and consists of two ventral and two dorsal ganglia, joined by ventral (subrhynchodeal) and dorsal (suprarhynchodeal) commissures, respectively. The dorsal ganglia are more widely separated than the ventral. A thin but distinct outer neurilemma encloses the brain as a whole, but there is no inner neurilemma dividing the fibrous and ganglionic tissues. There are no neurochord cells in the brain ganglia (as defined by Bürger 1895:355) and no neurochords in the lateral nerve cords. The redescription by Gibson & Moore (1985) of *Prosorhochmus claparedii,* which served as a basis for the generic diagnosis, appears to misidentify type III neurons (as defined by Bürger 1895:320) as neurochord cells. As in other species of *Prosorhochmus,* the lateral nerve cords contain a single fibrous core throughout their length. However, in one specimen an unpaired accessory nerve, derived from the dorsal cerebral ganglion, was found in the right lateral nerve cord in the region adjacent to the brain (Fig. 5A). This unpaired
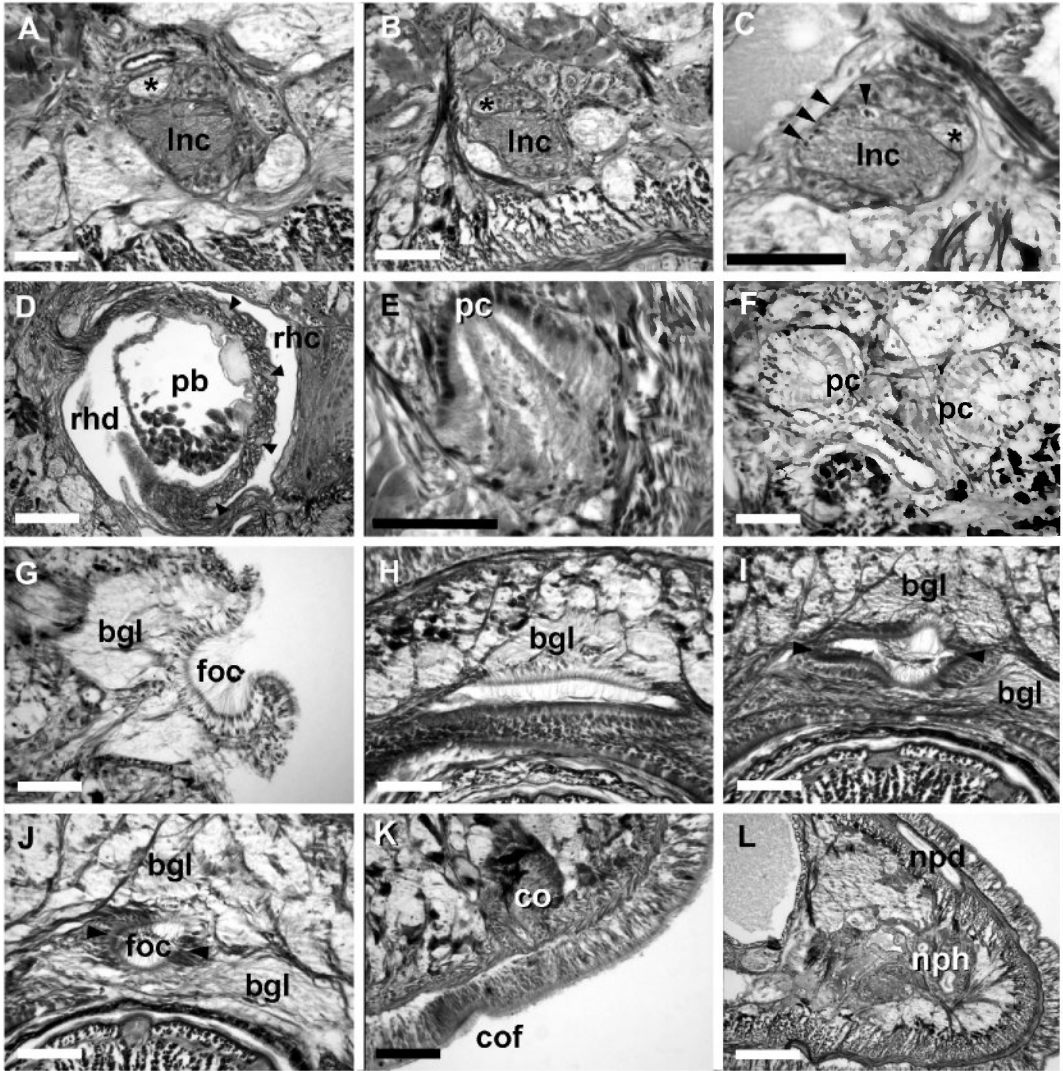
Fig. 5. *Prosorhochmus nelsoni.* A, B. Consecutive transverse sections through right lateral nerve cord in region adjacent to brain; note change in position of unpaired accessory nerve (asterisk). C. Transverse section through left lateral nerve cord; "upper nerve" (asterisk) and muscle fibers (arrows). D. Transverse section through proboscis insertion, proboscis nerves (arrows). E. Section through eye. F. Longitudinal sagittal section through both anterior and posterior eyes. G. Longitudinal section through middle part of the frontal organ. H. Transverse section at anterior (slit-like) part of frontal organ. I. Transverse section through frontal organ of same individual as (H), acidophilic epithelium (arrows). J. Transverse section through posterior part of frontal organ chamber of same individual. K. Transverse section through cerebral organ. L. Transverse section through post-cerebral region. A–C, E–K: scale bars = 50 μm; D, L: scale bars = 100 μm. Abbreviations: bgl, basophilic (mucus) cephalic glands; co, cerebral organ; cof, cerebral organ furrow; foc, frontal organ chamber; lnc, lateral nerve cord; npd, nephridioduct; nph, nephridial tubule; pb, proboscis; pc, eye pigment cup; rhc, rhynchocoel; rhd, rhynchodeum.
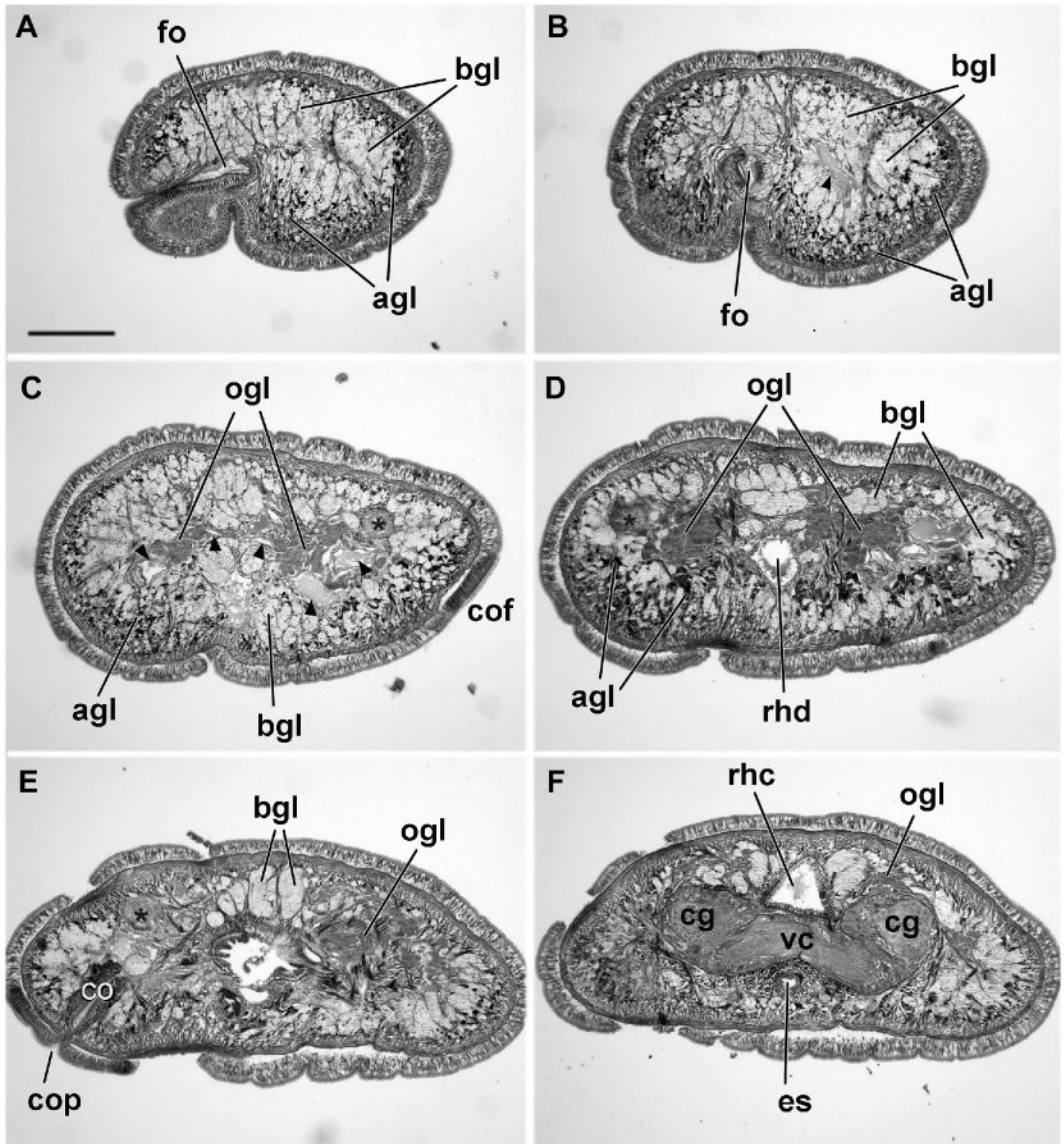
Fig. 6. Slightly oblique transverse sections of *Prosorhochmus nelsoni* from tip of head to brain region: proboscis is absent. A. Opening of frontal organ. B. Frontal organ. C. Section at the level of anterior eye (indicated by asterisk) and anterior portion of ventro-lateral semi-circular cephalic groove, corresponding to anterior cephalic groove. D. Precerebral region. E. Section at level of the right cerebral organ and hind part of right anterior cephalic groove. F. Ventral cerebral commissure. Scale bar = 200 μm. Abbreviations: agl, acidophilic (proteinaceous) cephalic glands; bgl, basophilic (mucus) cephalic glands; cg, cerebral ganglia; co, cerebral organ; cof, cerebral organ furrow; cop, cerebral organ opening; fo, frontal organ; es, esophagus; ogl, orange-G stained (proteinaceous) cephalic glands; rhc, rhynchocoel; rhd, rhynchodeum; vc, ventral brain commissure.

accessory nerve cord later came to lie close to the dorsal part of the main fibrous core, forming the so-called "upper nerve," a bundle of nerve fibers in the dorsal part of the fibrous core of the lateral nerve cord, distinguished by their lighter color, which we observed in all species of *Prosorhochmus* (Fig. 5B). The difference between this

Table 2.—Comparison between stylet measurement of *Prosorhochmus nelsoni* and other described species of *Prosorhochmus.*

Species	Stylet length (μm)	Central stylet length to basis length (S/B) ratio
P. claparedii Keferstein, 1862 (after Gibson & Moore 1985)	30–45	0.25–0.33
P. americanus Gibson et al., 1986	90	0.45–0.5
P. adriaticus Senz, 1993	?	0.25
P. chafarinensis Frutos et al., 1998	99	0.5
P. nelsoni (Sanchez, 1973)	84	0.8

upper nerve and a real accessory nerve is that the upper nerve is never separated from the main fibrous core by cell bodies, as is the accessory nerve, and it is derived from the ventral cerebral ganglion. As observed in most monostiliferans studied in the last three decades, each lateral nerve cord contains a single delicate muscle bundle, consisting of 3–7 fibers and running within or adjacent to the fibrous core, near its dorsal border. In addition, there are several less conspicuous muscle fibers running along the inner lateral side of the fibrous core (Fig. 5C). Muscle fibers associated with the lateral nerve cords can be traced to their extracerebral origin near the proboscis insertion. Numerous cephalic nerves lead anteriorly from the brain lobes to supply various structures of the head. A stout nerve joins each ventral lobe with the appropriate cerebral sensory organ. A single proboscis nerve trunk originates from the ventral ganglion on each side near the ventral cerebral commissure and branches before entering the proboscis (Fig. 5D).

Eyes.—The four eyes are of typical pigment cup structure and well developed (Fig. 5E). The eyes of the anterior pair are slightly larger in diameter than the posterior (90 μm and 75 μm, respectively). The pigment cups of the anterior pair are directed anterolaterally, while those of the posterior pair are directed posterolaterally (Fig. 5F).

Frontal organ.—The frontal organ, opening at the tip of the head, is of typical structure for the genus *Prosorhochmus.* Its structure, although described slightly differently for different species of *Prosorhochmus* (Gibson & Moore 1985, Gibson et al. 1986, Frutos et al. 1998), appears to be remarkably uniform within the genus. The frontal organ of this species consists of a ciliated canal about 80–100 μm long, lined by a regionally differentiated epithelium. Initially narrow and somewhat dorsoventrally flattened (Figs. 6H, 7A), the canal quickly expands to form a rounded chamber (Figs. 6G, 6I–J, 7B). The ventral wall of the canal comprises a strongly acidophilic epithelium, clad in densely-arranged short cilia, whereas the dorsal wall of the canal and ventral, dorsal, and posterior walls of the chamber, through which the basophilic mucus cephalic glands discharge, has a vacuolated appearance and bears much longer, sparsely distributed cilia. The acidophilic epithelium divides just in front of the chamber to run on its lateral borders. The lateral part of the lumen of the chamber is dorsoventrally compressed (Fig. 5I). There are no subepidermal acidophilic glands associated with the acidophilic epidermis of the frontal organ. It seems that the acidophilic appearance comes from the densely arranged elongated nuclei of the ciliated cells. The apparent higher degree of development of the frontal organ (comprising the supposed 90° bend) in *Prosorhochmus claparedii* seems to be a misinterpretation by Gibson & Moore (1985), carried over to the description of *Prosorhochmus chafarinensis* (Frutos et al., 1998). They misinterpreted a slightly oblique cross-section as a vertical longitudinal (i.e., sagittal) section of the frontal end (Gibson & Moore 1985:149, plate I, fig. e; Frutos et al. 1998:296, fig. 3a). As a result, they believed that the canal of the frontal organ first runs horizontally and then turns ventrally through 90° to end in a flask-shaped chamber. Our re-investigation of the original slides of *P. claparedii* and *P. chafarinensis* shows that what they
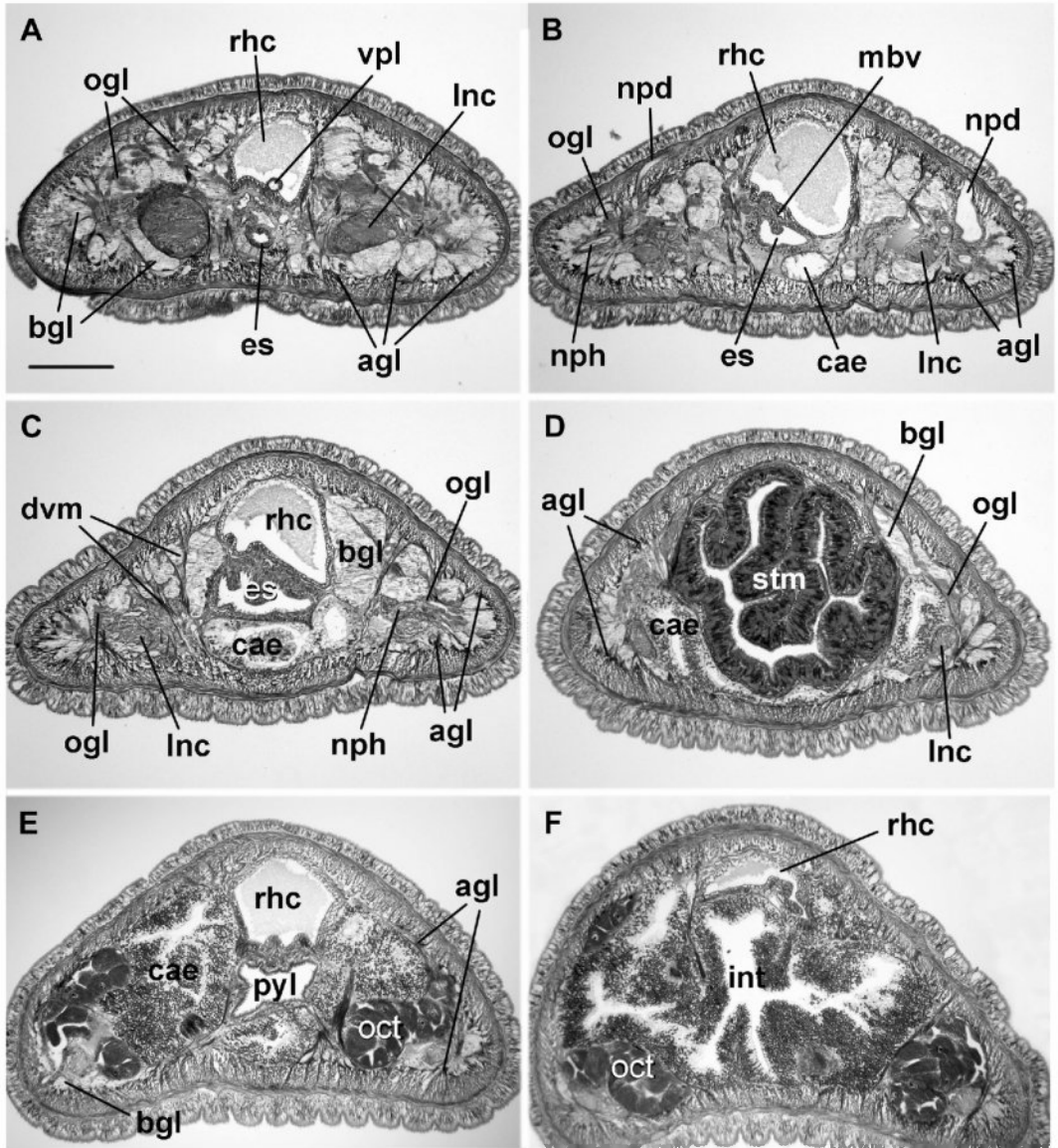
Fig. 7. Slightly oblique transverse sections of *Prosorhochmus nelsoni* from posterior brain region to intestinal region. A. Section through posterior region of cerebral ganglia. B. Openings of nephridial ducts. C. Posterior, densely ciliated portion of esophagus and anterior caecal diverticula. D. Stomach. E. End of pylorus. F. Midbody region. Scale bar = 200 μm. Abbreviations: agl, acidophilic (proteinaceous) cephalic glands; bgl, basophilic (mucus) cephalic glands; cae, caecum; dvm, dorso-ventral muscles; int, intestine; lnc, lateral nerve cords; mbv, mid-dorsal blood vessel; npd, nephridioduct; nph, nephridial tubule; oct, oocytes; es, esophagus; ogl, orange-G (proteinaceous) cephalic glands; pyl, pylorus; rhc, rhynchocoel; vpl, vascular plug.

believed to be a vertical portion of the canal is actually a ventral "groove" of the canal.

Cephalic glands.—Cephalic glands are extremely well developed. As in other members of the genus, they include three distinct types of cells: basophilic lobules with vacuolated appearance (mucus glands), coarsely granular proteinaceous gland cells staining golden-yellow to brown with Mallory trichrome or orange-G with
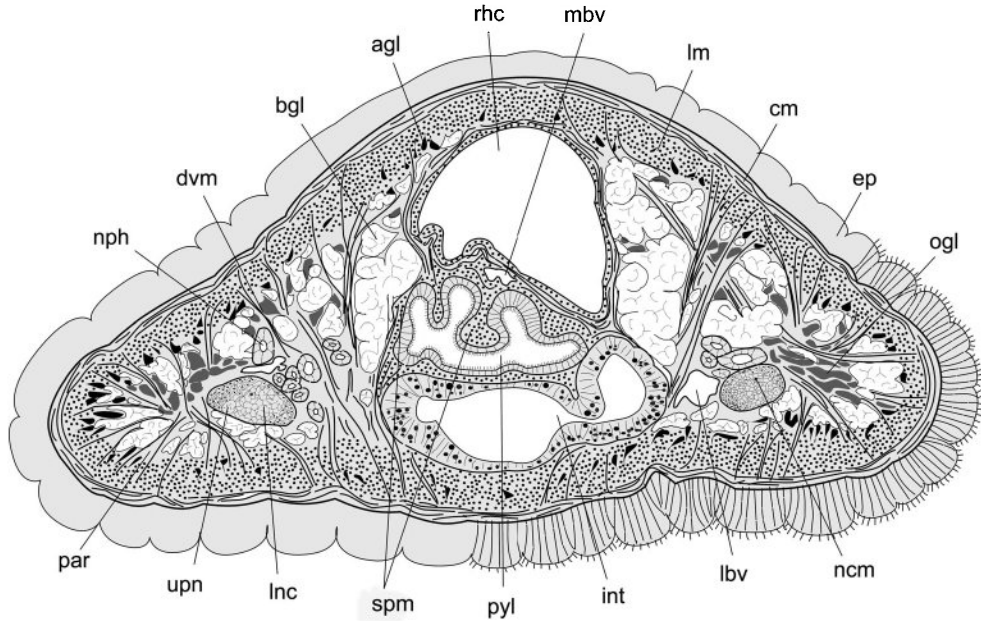
Fig. 8. Diagrammatic cross-section of *Prosorhochmus nelsoni* in pyloric region. Abbreviations: agl, acidophilic cephalic glands; bgl, basophilic cephalic glands; cm, circular musculature; dvm, dorso-ventral musculature; ep, epidermis; int, intestine; lbv, lateral blood vessel; lm, longitudinal musculature; lnc, lateral nerve cord; mbv, mid-dorsal blood vessel; ncm, nerve cord muscles; nph, nephridial tubule; ogl, orange-G cephalic glands; pyl, pylorus; rhc, rhynchocoel; spm, foregut or splanchnic musculature; upn, upper nerve.

Crandall's method (orange-G glands), and finely granular proteinaceous acidophilic cells, staining red with Mallory or Crandall's technique (acidophilic or red glands). However, the degree of development and distribution of the different types of cephalic glands is somewhat different from that described for other species of the genus. We refer to these cells as "glands" for the sake of briefness and also because they are traditionally referred to as "glands" in nemertean literature. These cells are clearly of secretory nature, but as opposed to real glands they do not form discrete collectives, functioning as units. Moreover, gland cells of different types may intermix with each other.

Basophilic (mucus) glands are well developed and, as in other species of the genus, they open through the dorsal and posterior epithelium of the frontal organ. The mucus glands are restricted to the dorsal hemisphere precerebrally in some other prosorhochmids. In this species, the mucus glands are situated centrally, filling most of the precerebral region, whereas the acidophilic glands are situated on the periphery, both in dorsal and ventral hemispheres (Fig. 6A, B). As acidophilic glands become increasingly more abundant in the ventral hemisphere toward the cerebral region, mucus glands become interspersed with acidophilic glands ventrally and with orange-G glands dorsally and laterally (Fig. 6D–F). Mucus glands in the cerebral region are distributed on the perimeter—dorso-lateral of the rhynchocoel, lateral of and under the brain lobes (Figs. 6F, 7A). Mucus glands are also well developed postcerebrally and retain essentially the same arrangement as in the cerebral region (Fig. 7B, C). Lateral lobes of the mucus glands together with the orange-G glands partially surround excretory ducts of the nephridia (Figs. 6L, 7B, C). Dorsal and ventral lobes gradually disappear in the stomach-pylorus region (Figs.

7D, E, 8), while lateral lobes gradually diminish in numbers but reach into the intestinal region (Fig. 7D–F).

Orange-G glands are well developed. Overall, they demonstrate typical prosorhochmid distribution in two dorso-lateral tracts starting around the frontal organ canal and reaching as far back as the end of the rhynchocoel. Precerebrally, orange-G glands are restricted to the dorso-lateral regions above the cephalic blood vessels and below the mucus gland lobes (Fig. 6C–E). In the cerebral region they mostly lay dorso-laterally above the brain lobes, either forming dense clusters or interspersing with the mucus gland lobes. They rarely surround mucus gland lobes and mostly lie below the mucus glands (Figs. 7F, 8A). Postcerebrally, orange-G glands become less abundant than in the pre-cerebral or cerebral region. They are interspersed with the lobes of mucus glands and occupy dorsolateral regions lying above the lateral nerve cords and nephridia.

Acidophilic glands are well developed and most abundant just anterior to the brain (at the level of the cerebral organs). Anteriorly (at the level of frontal organ), they are located marginally, inside the perimeter of the body wall, surrounding the mucus gland lobes (Fig. 6A, B). Behind the frontal organ they gradually become more abundant in the ventral hemisphere (Fig. 6C, D). At the level of the cerebral organs acidophilic glands are richly distributed and form clusters among the lobes of mucus glands in the ventral hemisphere (Fig. 6D, E). They also are present as a thin layer dorsally and laterally just under the body-wall musculature. Acidophilic glands become far less numerous behind the proboscis insertion, remaining as a thin layer underneath the body wall musculature around the perimeter of the body, more prominent ventrally and ventro-laterally (Fig. 6F). The distribution evens out in the postcerebral region (Figs. 7A–C, 8). Dorsal and ventral components of the acidophilic glands gradually disappear in the stomach and anterior pyloric region, while the lateral components persist (although also diminished) beyond the end of the pylorus (Fig. 7D–F). There are no purple cephalic glands, associated with the acidophilic glands in the precerebral region described for *P. americanus* (Gibson et al., 1986). Although the purple glands cells had been described in *P. chafarinensis* (Frutos et al., 1998), our re-investigation of the type material showed that the authors apparently mistook acidophilic gland cells for the purple cephalic glands.

Cerebral organs.—Relatively small and compact paired cerebral organs reach a maximum diameter of about 145 μm in the adults and are situated in front of the brain, between the anterior and posterior pairs of eyes. Each organ opens anteriorly near the anterior pair of eyes into reduced cephalic furrows. The latter are shallow semi-circular grooves lined by a modified ciliated epithelium, which appears strongly acidophilic in sections stained with Crandall's method (Figs. 6K, 7C–E). The cerebral organ canals are unbranched. Two types of glandular cells [described by Gibson & Moore (1985) in the Anglesey specimens] can be distinguished in the cerebral organs. The coarsely granular cells, staining dark reddish-brown with Crandall's method form two clusters, on the dorso- and ventro-anterior margins of the ciliated canal (Fig. 5K). The second type of cell is finely granular, stains less intensively red to brownish purple and forms a single cluster on the inner-lateral and posterior side of each organ. Both types of cells open into the cerebral organ canal (Figs. 5K, 6E).

Excretory system.—The excretory system extends from a short distance behind the dorsal brain lobes back to the anterior pyloric region, most of it lying dorsal and close to the lateral nerve cords and the lateral blood vessels, which at this point lie dorsal to the lateral nerve cords. It consists of thick-walled ciliated collecting tubules, up to 60–65 μm in diameter in the distal part. Collecting tubes are not regionally specialized, although they are smaller in the

proximal part (10–15 μm in diameter). Nephridial ducts are surrounded by the mucus gland lobes and orange-G glands (Figs. 6L, 8A–C, 9). They open dorso-laterally, about halfway along the length of the system, via a single nephridiopore on each side (Figs. 5L, 7B). Mononucleate flame cells can be observed embedded in the extracellular matrix in the vicinity of the lateral blood vessels. They possess indistinct and irregular transverse support bars, which can be easily missed.

Reproductive system and life history.— Unlike all other described species of *Prosorhochmus, P. nelsoni* is gonochoric and oviparous. Reproductive males and females were observed in July–August near Coquimbo. Gonads are distributed irregularly between the lobes of the intestinal diverticula. Up to 20–30 mature oocytes can be observed within the same ovary (Fig. 7F). Females maintained in the laboratory laid eggs in cocoons. Eggs are approximately 100–150 μm in diameter. Females feed again following spawning, possibly indicating iteroparity. The embryonic development from fertilization until the formation of the planuliform swimming larvae takes about 20 days at room temperature.

Habitat and ecology.—*Prosorhochmus nelsoni* is fully marine and occurs in the mid-intertidal zone on the rocky shore, among the mytilid *Perumytilus purpuratus* and barnacles *Jehlius cirratus,* according to Sanchez (1973), and on rocks and boulders covered by algal turf, inhabited by a wide variety of marine invertebrates (polychaets, bivalves, and crustaceans representing the most abundant groups; Thiel et al. 2001). One of us (MT personal observations) considers *P. nelsoni* to be the most common nemertean species in the rocky intertidal of the Chilean coast. It is commonly found during morning low tides, particularly under overcast conditions.

Geographic distribution.—The Pacific coast of South America. Only known from Chile. Type locality: Quintero (32°46′S, 71°31′W) (Sanchez 1973); other known localities: La Pampilla Beach near Coquimbo (29°57′S, 71°21′W) (Thiel et al. 2001), Arica (Arica: 18°29′S, 70°20′W) and Concepción (36°50′S, 73°03′W) (MT, personal observations).

Conclusions

Characters such as bilobed head with a dorsal epidermal fold (the "smile"), truncated stylet basis, well-developed tubular frontal organ with laterally differentiated epithelium, well-developed cephalic glands, combining mucus and proteinaceous components and nephridial system with thick excretory tubules, and a single pair of nephridioducts place this species into the genus *Prosorhochmus.* This species differs from all other described species of *Prosorhochmus* in being gonochoric and oviparous (vs. hermaphroditic and ovoviviparous). Additionally, *P. nelsoni* differs from other described species of *Prosorhochmus* in having a greater S/B ratio (Table 2). However, the possibility exists that this is because we measured stylets in sexually immature individuals and that the S/B ratio is smaller in mature specimens. Our unpublished sequence data from the two mitochondrial genes 16S rDNA and Cytochrome Oxidase Subunit I show that the average sequence divergence between *P. nelsoni* and other species of *Prosorhochmus* (*P. americanus, P. claparedii, P. cf. adriaticus* and an undescribed species from Belize) is comparable to that between the other species: 6.82% for 16S and 8.82% for COI. Lastly, *P. nelsoni* is found only on the Pacific side of South America (coast of Chile), whereas the other described species are known only from the Atlantic and Mediterranean.

A critical observation for prosorhochmids is that presence vs. absence of a 90° bend in the frontal organ is a misinterpretation and cannot be used to distinguish members of the genus. Reinvestigation of the material of all described species of *Prosorhochmus* also revealed that no member

of this genus possesses neurochord cells, in contrast to the generic diagnosis (Gibson & Moore 1985:146). Finally, we suggest that the so-called valves and pouches in the blood vessels represent artifacts of fixation and their presence or absence has no diagnostic significance. Morphological characters previously used in *Prosorhochmus* systematics, such as presence/absence of dorso-ventral musculature in the foregut region, esophageal musculature, ciliation in the posterior esophageal region, neural supply in the posterior proboscis chamber, posterior stomach pouch, and number of muscle fibers in the lateral nerve cords must be used with caution and need to be thoroughly re-evaluated, as they might be inconsistent with species boundaries. That is outside the scope of this article but is work in progress.

Acknowledgments

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Appendix
Examined histological material

P. nelsoni (Sanchez, 1973). USNM (173164): series of cross-sections of anterior part and foregut region. Coll. Sanchez, Quintero, Chile. Reproductive female; USNM (173163): series of frontal sections of anterior part and cross-sections of posterior part. Coll. Sanchez, Quintero, Chile. Immature individual; USNM (173162): series of sagittal sections of anterior part, cross-sections of posterior part. Coll. Sanchez, Quintero, Chile. Reproductive female; USNM (1019782): series of cross-sections of anterior third of body. Coll. MT, Coquimbo, Chile. Reproductive female; USNM

(1019784): series of cross-sections of anterior fourth of body. Coll. MT, Coquimbo, Chile. Reproductive female; USNM (1019785): series of cross-sections of anterior part and adjacent midbody region. Coll. MT, Coquimbo, Chile. Reproductive female; USNM (1019786): two unsectioned specimens in alcohol. Coll. MT, Coquimbo, Chile.

P. claparedii Keferstein, 1862. CMZ (A6): series of cross-sections of single specimen. Coll. Gibson, Anglesey, UK; series of transverse and longitudinal sections of four specimens from personal collection of Ray Gibson. Coll. Gibson. Anglesey, England; USNM (1020508, 1020509): series of cross-sections of anterior parts of two immature individuals. Coll. SAM.

Bilbao, Spain; USNM (1020510–1020513): series of cross-sections of anterior ends of two specimens, series of cross-sections of one whole specimen and series of frontal sections of anterior end of one specimen. Coll. SAM. Roscoff, France.

P. americanus Gibson et al., 1986. Series of cross-sections of holotype (USNM 98550), two paratypes (USNM 98551, 98552) and three additional specimens from Florida, USA collected by JLN.

P. chafarinensis Frutos et al., 1998. Series of cross-sections of holotype (MNHM AA1) and two paratypes (MNHM AA2, AA5).

P. adriaticus Senz, 1993. Series of cross-sections of holotype (VMNH 3254) and two other specimens from type locality collected by Senz. (VMNH 4292, 4293).