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### Revision of the smiling worms, genus **Prosorhochmus** Keferstein, 1862, and description of a new species, **Prosorhochmus belizeanus** sp. nov. (Prosorhochmidae, Hoplonemertea, Nemertea) from Florida and Belize

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**Revision of the smiling worms, genus *Prosorhochmus* Keferstein, 1862, and description of a new species, *Prosorhochmus belizeanus* sp. nov. (Prosorhochmidae, Hoplonemertea, Nemertea) from Florida and Belize**

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A new species of *Prosorhochmus* is described from Belize and Florida based on morphological, reproductive and sequence data. Similar to *Prosorhochmus nelsoni* (Sanchez, 1973), *Prosorhochmus belizeanus* sp. nov. is gonochoric and oviparous; all other species of *Prosorhochmus* are viviparous hermaphrodites. *Prosorhochmus belizeanus* sp. nov. differs from *P. nelsoni* by having significantly larger stylets and different arrangement of acidophilic cephalic glands. Sequence divergence between the two is 7.4% (16S) and 9.1% (COI), comparable to divergence from the viviparous hermaphroditic species. *Prosorhochmus* Keferstein, 1862 is revised based on re-evaluation of the type and voucher material as well as fresh specimens collected by us. We conclude that *Prosorhochmus adriaticus* Senz, 1993 is insufficiently described and cannot be distinguished from *Prosorhochmus claparedii* Keferstein, 1862. We re-establish *Prosorhochmus korotneffi* Bürger, 1895 from its previous synonymization with *P. claparedii* and designate it as type species of *Arhochmus* gen. nov.

**Keywords:** Nemertea; *Prosorhochmus*; *Arhochmus*; smiling worms; Cytochrome Oxidase I; 16S rDNA

## Introduction

The name “smiling worms” used by us for the hoplonemertean family Prosorhochmidae refers to the heart-warming crescent-like fold on the head of these worms – the prosorhochmid smile (see Figures 1B, 1H, 2A). The function of the smile is unknown, but its presence is correlated with the well-developed frontal organ and, associated with it, mucus cephalic glands. This, in turn, might have something to do with the unusual habitat of some of the prosorhochmids. Most nemerteans (phylum Nemertea) are marine oviparous worms with separate sexes. The family Prosorhochmidae continues to spark the interest of nemertologists because many of its members are terrestrial or semi-terrestrial. Even more intriguing, many of the species (from marine, semi-terrestrial or terrestrial habitats) acquired hermaphroditism in combination with viviparity.

This compact family includes 18 species in four genera: *Prosorhochmus* Keferstein, 1862, *Prosadenoporus* Bürger, 1890, *Pantionemertes* Moore and Gibson, 1981 and *Geonemertes* Semper, 1863. The first three genera are in need of revision. Maslakova in her Ph.D. thesis (2005), revised and redefined the family and its genera but disclaimed all the new taxon names and nomenclatural changes

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making them unavailable for nomenclatural purposes (ICZN Art. 8.3). Therefore, we make those changes available here for the first time.

Much has changed since the last revision of *Prosorhochmus* by Gibson and Moore (1985) that resulted in synonymization or invalidation of all previously described species but the type species *Prosorhochmus claparedii* Keferstein, 1862. Three new species have been described: *Prosorhochmus americanus* Gibson et al., 1986, the first New World representative, *Prosorhochmus adriaticus* Senz, 1993 from the Adriatic coast of Italy and *Prosorhochmus chafarinensis* Frutos et al., 1998 from the Chafarinas Islands (off the coast of Morocco, Western Mediterranean). One species, the Chilean *Amphiporus nelsoni* Sanchez, 1973, has been transferred to *Prosorhochmus* following its redescription by Maslakova et al. (2005). *Prosorhochmus nelsoni* (Sanchez, 1973) is the first reported species of *Prosorhochmus* with separate sexes and oviparity, all others being viviparous hermaphrodites.

Here we describe another oviparous *Prosorhochmus* with separate sexes from Belize and Florida – *Prosorhochmus belizeanus* sp. nov. To provide a proper comparison of the new species to other *Prosorhochmus* we took it upon ourselves to obtain and reinvestigate all the available type and voucher material from museums around the world and to attempt to collect fresh specimens for histology and sequencing as close as possible to the type localities of the previously described species.

## Material and methods

### *Specimen preparation*

Characters of external appearance were documented in living specimens, colour illustrations prepared by hand and photographs taken with a Nikon Coolpix 4500 digital camera mounted on the stereo- and compound microscopes of various brands. Stylets were viewed and photographed through the wall of dissected proboscis slightly compressed under the cover slip and the drawings subsequently made in Adobe Photoshop 5.0-6.0 as tracings. Measurements such as body length and width were taken on live specimens after relaxing them by gradually adding a 1:1 mixture of 7.5% MgCl<sub>2</sub> and local seawater to a container with animals in seawater, ultimately substituting it with 7.5% MgCl<sub>2</sub>. For histology, specimens were relaxed in MgCl<sub>2</sub> as described above, fixed for 24 h in 4% formaldehyde made in local seawater, briefly rinsed in tap water and post-fixed in Hollande's cupri-picri-formal-acetic fluid for 48–72 h. After post-fixation specimens were transferred to 70% ethanol for long-term storage. Specimens were dehydrated in a standard alcohol series, embedded in Tissueprep Paraffin compound (56°C melting point), serially sectioned at 8 µm and stained using Crandall's polychrome protocol – a combined variant of the Mallory, Gomori, Koneff and Gurr-McConail techniques (Frank Crandall, National Museum of Natural History, Washington, D.C., pers. comm.).

### *DNA extraction, amplification and sequencing*

Tissue for molecular work was obtained for *Prosorhochmus claparedii*, *Prosorhochmus americanus*, *Prosorhochmus cf. chafarinensis*, *Prosorhochmus nelsoni*, *Arhochmus korotneffi* comb. nov. (former *Prosorhochmus korotneffi*) and *Prosorhochmus belizeanus* sp. nov. (Table 1). Specimens were preserved in 95%

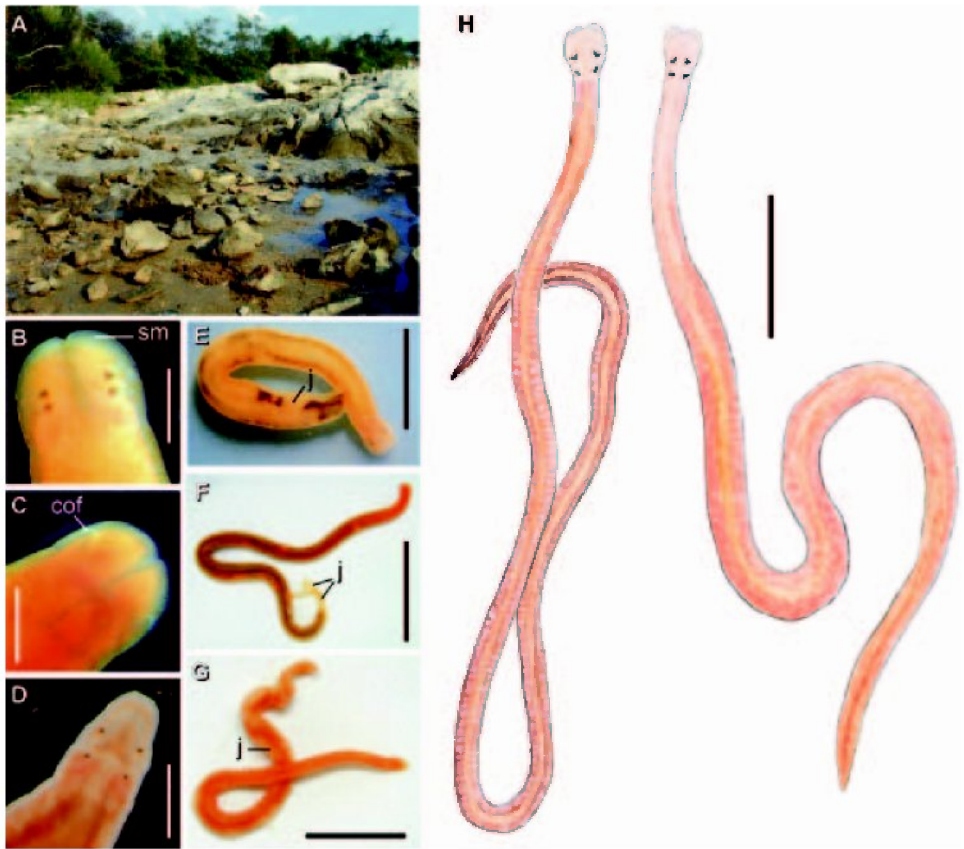


Figure 1. External appearance and habitat of *Prosorhochmus* species. (A) Typical intertidal habitat of *Prosorhochmus* spp. – wet coarse sand just above the low tide mark with partially embedded rocks. The pictured location is about halfway between Croatian towns Savudrija and Zabrata on the Adriatic coast; (B–C) *Prosorhochmus chafarinensis*, anterior end (B, dorsal view; C, ventral view); (D) *Arhochmus korotneffi* comb. nov., anterior end, dorsal view; (E) *Prosorhochmus claparedii*, external appearance of a viviparous hermaphroditic adult with several juveniles showing through the body wall between the gut diverticula; (F) *P. chafarinensis*, external appearance of viviparous hermaphroditic adult with two newly born juveniles; (G) *A. korotneffi*, external appearance of a viviparous hermaphroditic adult with several juveniles showing through the body wall; (H) *Prosorhochmus belizeanus* sp. nov. external appearance of sexually mature male (left) and female (right). Testes show through the body wall as whitish sacks regularly interspersed with gut diverticula on either side. Pinkish oocytes in ovaries are visible through the body wall of ripe females. Scales. (B–D): 0.5 mm; (E): 3 mm; (F, G): 6 mm; (H): 3.5 mm. Abbreviations: cof, cerebral organ furrow; j, juvenile; sm, prosorhochmid smile.

ethanol and stored at room temperature or  $-20^{\circ}\text{C}$  short-term and at  $-80^{\circ}\text{C}$  long-term. We used Qiagen DNAeasy miniprep kit (Qiagen Inc.) for DNA extractions. Partial sequences of Cytochrome Oxidase subunit I (COI), 658 bp long, and mitochondrial large subunit rDNA (16S), 458–467 bp long, were amplified using universal primers: 16sar-L [cgctgtttatcaaaaacat] and 16sbr-H [ccggtctgaactcagatcagct] from Palumbi et al. (1991) for 16S, and LCO1490 [gggtcaacaatcataaagatattgg]

Table 1. Accession numbers and specimen data for molecular analysis.

Species	Accession numbers	Collecting information and specimen numbers
<i>Prosorhochmus americanus</i>	EF157588, EF157576	Coll. J.M. Turbeville, Pawley's Island, SC, USA (#PISC. 7-30-97)
<i>Prosorhochmus belizeanus</i> sp. nov.	EF157591, EF157578	Coll. JLN, SAM, 2-11-2000, reef-crest berm of Carrie Bow Cay, Belize (#559-11-2-00-1,4)
<i>Prosorhochmus cf. chafarinensis</i>	EF157587, EF157575	Coll. SAM, 09-03-2002, Savudrija and Zabrata, Croatia, Adriatic Sea (#738-09-04-02-1,3,4.)
<i>Prosorhochmus cf. clapedii</i>	EF157589, EF157590, EF157577	Coll. SAM, 06-19-2000, Arminza, Bizkaia, Spain (#596-06-19-00-1,2). Coll. SAM, 08-14-2002, under rocks in front of the marine station, Roscoff, France (#722-08-14-02-3)
<i>Arhochmus (Prosorhochmus) korotneffi</i>	EF157592, EF157579	Coll. SAM, 09-03-2002, Savudrija and Zabrata, Croatia (#740-09-05-02-1-4).
<i>Prosorhochmus nelsoni</i>	EF157586, EF157574	Coll. M. Thiel, 5-20-2000, Coquimbo, Chile
<i>Tetrastemma albidum</i>	EF157598	Coll. JLN, SAM, 02-2002, La Jolla, CA, USA (sp. 644)

and HCO2198 [taaacctcagggtgaccaaataatca] from Folmer et al. (1994) for COI. In addition, we designed a pair of internal primers for each gene to amplify fragments where DNA degradation appeared to be a problem: P2619-Nem16S-intF [acaagaagaccctttgagct] and P2620-Nem16S-intR [taaagctcaaagggtcttct] for 16S, and P2638-COIF3-nemert [gtctagraatrttgcctatgctg] and P2683-COIR-nemert [ctyc-cagcatwgcaayatt] for COI. Each PCR reaction was performed with 1 µl of 1X and 0.1X dilution of unquantified template (genomic DNA) in a 25 µl volume, using pure TaqTMReady-To-GoTMPCR Beads (Amersham Biosciences) and 0.8 µM of each primer. Thermo-cycling was performed using an initial 2 min denaturation at 94°C, followed by five cycles of 30 s at 94°C, 15 s at 58°C and 45 s at 72°C, then an additional 30 cycles of 30 s at 94°C, 15 s at 55°C and 45 s at 72°C. The cycling ended with a 2 min sequence extension at 72°C. The PCR product was purified with QIAquick PCR Purification Kit (Qiagen). Where amplification produced multiple bands, they were separated by gel electrophoresis, excised and purified with QIAquick Gel Extraction kit (Qiagen Inc.). The DNA concentration was evaluated on the gel using a standard Low Mass ladder (Invitrogen) and the PCR products were used in cycle sequencing with dye-terminators using BigDye (BD3) chemistry (Perkin-Elmer) at a concentration of 0.04–0.14 ng/µl per 100bp of sequence, PCR primers at 0.16 µM and 25–30 cycles of 30 s at 94C, 15 sec at 55C, and 4 min at 60C. The products were sequenced on a 3100 ABI Capillary DNA Sequencer; both strands were sequenced at least once and proofread using Sequencher 4.1.4 (Gene Codes Corporation). Sequences are deposited with GenBank (see Table 1 for accession numbers).

**Measurements of stylet apparatus**

To determine whether interspecific differences in the stylet and basis length were statistically significant, we performed a one-way ANOVA correcting for multiple comparisons with Tukey-Kramer HSD method using statistical software package JMP version 6.0.3 (SAS Institute Inc.). Stylet measurements used in the analysis are presented in Table 2. Cases where individual measurements are not reported (marked with an asterisk in the Table 2) could not be used in the analysis. However, these measurements were used to adjust the range of variation in species diagnoses.

**Museum abbreviations**

USNM, US National Museum of Natural History, Smithsonian Institution, Washington, DC, USA; VMNH, Natural History Museum, Vienna, Austria; MNHM, Museo Nacional de Ciencias Naturales, Madrid, Spain; CMZ, University Museum of Zoology, Cambridge, England.

**Results**

Class **HOPLONEMERTEA** *sensu* Thollesson and Norenburg 2003  
(=Enopla Schultz 1851)

Order **MONOSTILIFERA** *sensu* Thollesson and Norenburg 2003  
(=**MONOSTILIFERA** Brinkman 1917+**BDELLONEMERTEA**)

Family **PROSORHOCHMIDAE** Bürger 1895

**Diagnosis**

Monostiliferous, marine, brackish-water, semi-terrestrial or terrestrial hoplonemerteans with rhynchocoel extending most or all the body length, body wall containing distinct outer circular and inner longitudinal muscle layers and a delicate layer of diagonal muscles between; longitudinal musculature may appear to be anteriorly divided; four simple eyes; proboscis may be small or large, and sometimes used in locomotion; stylet basis in most species characteristically truncated; head with characteristic antero-dorsal horizontal epithelial fold (the prosorhochmid smile); cerebral sensory organs anterior or anterolateral to brain, with unbranched canals opening ventro-laterally via reduced cerebral organ furrows; cephalic glands well-developed, with distinct granular proteinaceous components (orange-G and acidophilic gland cells) in addition to basophilic mucus lobules; frontal organ represented by an exceptionally well-developed tubular canal, in most species with laterally specialized epithelium; middorsal blood vessel with a single vascular plug; typically gonochoric and oviparous, occasionally hermaphroditic and viviparous.

**Composition**

The family currently contains four genera: *Prosorhochmus* Keferstein, 1862 (type genus), *Prosadenoporus* Bürger, 1890, *Pantinonemertes* Moore and Gibson, 1981 and *Geonemertes* Semper, 1863.

Table 2. Measurements of the stylet apparatus in *Prosorhochmus* species.

Species	Specimen	Stylet (S, $\mu\text{m}$ )	Basis (B, $\mu\text{m}$ )	S/B	Source
<i>P. cf. chafarinensis</i>	1	90	180	0.5	Coll. SAM, Croatia
<i>P. cf. chafarinensis</i>	2	90	200	0.45	Coll. SAM, Croatia
<i>P. cf. chafarinensis</i>	3	120	210	0.57	Coll. SAM, Croatia
<i>P. cf. chafarinensis</i>	4	130	260	0.5	Coll. SAM, Croatia
<i>P. cf. chafarinensis</i>	5	120	230	0.52	Coll. SAM, Croatia
<i>P. cf. chafarinensis</i>	6	130	200	0.65	Coll. SAM, Croatia
<i>P. chafarinensis</i>	7	120	240	0.5	Frutos et al. 1998
<i>P. chafarinensis</i>	8	96	176	0.55	Frutos et al. 1998
<i>P. chafarinensis</i>	9	96	256	0.38	Frutos et al. 1998
<i>P. chafarinensis</i>	10	104	200	0.52	Frutos et al. 1998
<i>P. chafarinensis</i>	11	80	136	0.59	Frutos et al. 1998
<i>P. nelsoni</i>	1	88.2	132.3	0.67	Maslakova et al. 2005
<i>P. nelsoni</i>	2	73.5	98	0.75	Maslakova et al. 2005
<i>P. nelsoni</i>	3	78.4	107.8	0.73	Maslakova et al. 2005
<i>P. nelsoni</i>	4	98	122.5	0.8	Maslakova et al. 2005
<i>P. nelsoni</i>	5	73.5	112.7	0.65	Maslakova et al. 2005
<i>P. nelsoni</i>	6	73.5	73.5	1	Maslakova et al. 2005
<i>P. nelsoni</i>	7	88.2	107.8	0.82	Maslakova et al. 2005
<i>P. nelsoni</i>	8	102.9	127.4	0.81	Maslakova et al. 2005
<i>P. nelsoni</i>	9	93.1	83.3	1.1	Maslakova et al. 2005
<i>P. nelsoni</i>	10	78.4	122.5	0.64	Maslakova et al. 2005
<i>P. nelsoni</i>	11	49	73.5	0.67	Maslakova et al. 2005
<i>P. nelsoni</i>	12	107.8	122.5	0.88	Maslakova et al. 2005
<i>P. nelsoni</i>	13	88.2	117.6	0.75	Maslakova et al. 2005
<i>P. claparedii</i> *	NA	30–45	90–165	1:3–1:4	Gibson and Moore 1985
<i>P. claparedii</i>	1	95	165	0.58	Coll. SAM, Roscoff, France
<i>P. claparedii</i>	2	85	145	0.59	Coll. SAM, Roscoff, France
<i>P. claparedii</i>	3	80	130	0.62	Coll. SAM, Roscoff, France
<i>P. americanus</i> *	NA	90	200	1:2–1:2.2	Gibson et al. 1986
<i>P. belizeanus</i> sp. nov.	1	185	225	0.82	Coll. SAM and JLN, Belize
<i>P. belizeanus</i> sp. nov.	2	150	250	0.6	Coll. SAM and JLN, Belize
<i>P. belizeanus</i> sp. nov.	3	250	375	0.67	Coll. SAM and JLN, Belize
<i>P. belizeanus</i> sp. nov.	4	250	300	0.83	Coll. SAM and JLN, Belize
<i>P. belizeanus</i> sp. nov.	5	185	270	0.69	Coll. SAM and JLN, Belize
<i>P. belizeanus</i> sp. nov.	6	235	350	0.67	Coll. SAM and JLN, Belize

Note: Cases where the individual measurements are not reported marked with an asterisk.

### Geographic distribution

Atlantic coast of the British Isles, France, Spain, USA (FL and SC) and Bermuda; Caribbean (Belize); Adriatic Sea (coast of Italy and Croatia); Mediterranean Sea (coast of Italy, France, Chafarinas Islands); Black Sea (Russian coast); Pacific coast of USA (Puget Sound, WA to CA, Hawaii) and Chile; Hong Kong, China (Fujian Province), northeastern coast of Australia (Queensland), Indopacific Islands (Noordwachter Is. off Sulawesi, Palau Bidan off Malay Peninsula, Papua New

Guinea, Japan, Seyshelles, Sri Lanka, Sulawesi, Pelew Is., Caroline Is., Samoan Is., Kei Is., Mauritius, Samarai, the Philippines), the West Indies (Dominica, Jamaica).

*Prosorhochmus* Keferstein, 1862

*Type species*

*Prosorhochmus claparedii* Keferstein, 1862, by monotypy.

*Etymology*

The name *Prosorhochmus* is a Greek compound formed from  $\pi\rho\sigma$  (pros)=in front, anterior+connective “ο”+ $\rho\omega\chi\mu\sigma$  (rhochmos)=cleft, runnel or gutter, which refers to the presence of the prosorhochmid smile in all species of this genus. The Greek has been Latinized by changing the terminal “os” to “us”. The name is masculine in gender.

*Diagnosis*

Modified from Gibson and Moore (1985): monostiliferous marine intertidal hoplonemertean with dorsal crescent-shaped horizontal cephalic epithelial fold (the prosorhochmid smile) (see Figures 1B, 1H, 2A). Cerebral organ furrows reduced to shallow ventro-lateral crescents (see Figures 1C, 2B). Four simple eyes, anterior pair may be slightly larger than posterior (see Figures 1B, 1H, 2A). Cephalic lobe spatulate in shape, equal or wider than adjacent body region in actively moving worms and with distinctly bifid anterior margin (see Figures 1E, 1F, 1H, 2). Rhynchocoel extends full body length, with wall composed of distinct outer circular and inner longitudinal muscle layers (see Figure 3A). Proboscis small with 9–13 proboscis nerves (see Figures 3B–C), a single pair of accessory stylet pouches, and characteristically truncated basis of central stylet. Body-wall musculature well-developed with a delicate layer of diagonal muscles between outer circular and inner longitudinal muscle layers (see Figure 4B). Body-wall longitudinal muscle layer is not anteriorly divided (see Figure 4C). Frontal organ well-developed and represented by a well-defined tubular canal with laterally differentiated epithelium opening into the prosorhochmid smile (Figures 4C–D, 5A–C, 5G–H). Cephalic glands extensive and contain at least three components: vacuolated basophilic mucus glands, finely granular acidophilic proteinaceous glands staining pink to dark red and coarsely granular proteinaceous glands staining yellow, golden brown or deep orange with Mallory trichrome or its modifications (orange-G glands) (see Figures 3K–L, 4C–D, 4G–H, 4K, 5B–C, 5E, 5H, 6A–D). Isolated gland cells occur as far back as the end of the rhynchocoel. Cerebral sensory organs small with unforked canal, anterior or antero-lateral to brain, opening ventro-laterally into reduced cerebral organ furrows (see Figures 1C, 2B, 5D–E, 6A–B). Neurochord cells and neurochords absent. Lateral nerve cords without accessory nerve. Oesophagus lacking acidophilic glands (Figures 4H–I). Caecum long, may be anteriorly bifid, with several lateral diverticula on each side (see Figures 4D, 4F, 6E, 6F). Anterior caecal diverticula reach posterior portion of brain. Blood system with three main longitudinal vessels, without transverse connectives. Mid-dorsal blood vessel with single vascular plug (Figure 3H); cephalic blood loop planar (not recurved). Extracellular matrix of the so-called parenchyma scarce. Excretory system restricted to foregut region, with mononucleate terminal nephridial cells without distinct support



bars, with thick-walled canals and a single pair of nephridiopores in pyloric region (Figures 3K–L, 6E). Dioecious oviparous species or viviparous hermaphrodites (Figures 1E–H, 4E, 5F, 6F).

#### *Composition*

The genus contains six species: *Prosorhochmus claparedii* Keferstein, 1862, *Prosorhochmus americanus* Gibson et al., 1986, *Prosorhochmus adriaticus* Senz, 1993 (insufficiently described), *Prosorhochmus chafarinensis* Frutos et al., 1998, *Prosorhochmus nelsoni* (Sanchez, 1973) and *Prosorhochmus belizeanus* sp. nov.

#### *Geographic distribution*

Atlantic coast of the British Isles, France, Spain, USA (FL and SC); Caribbean (Belize); Adriatic Sea (coast of Italy and Croatia); Mediterranean Sea (coast of Italy, France, Chafarinas Islands); Black Sea (Russian coast); Pacific coast of Chile.

#### ***Prosorhochmus adriaticus* Senz, 1993**

*Prosorhochmus adriatica* (Senz 1993; Gibson 1995; Frutos et al. 1998)

*Prosorhochmus adriaticus* (Senz 1999; Maslakova et al. 2005)

#### *Etymology*

Species is named after its place of discovery – Adriatic Sea.

#### *Type material*

*Prosorhochmus adriaticus* Senz, 1993. Holotype VMNH 3254. Coll. Wolfgang Senz, Venice, Italy.

#### *Material examined*

*Prosorhochmus adriaticus* Senz, 1993. Holotype VMNH 3254 plus two additional specimens VMNH 4292 and 4293. Coll. Wolfgang Senz, Venice, Italy.

#### *Diagnosis*

*Prosorhochmus adriaticus* does not have any unique characters. It differs from *Prosorhochmus nelsoni* and *Prosorhochmus belizeanus* sp. nov. by being viviparous and hermaphroditic. It differs from *Prosorhochmus americanus* by having but a single juvenile per ovary. *Prosorhochmus adriaticus* differs from *Prosorhochmus chafarinensis* by a smaller S/B ratio (0.25 compared to 0.38–0.65), however, the data at hand are insufficient to make a statistical comparison. The length of central stylet and basis is unknown. *Prosorhochmus adriaticus* appears to be morphologically indistinguishable from *P. claparedii*.

#### *Habitat and distribution*

The habitat of this species is not recorded. The only location from which it is reported is the Adriatic Sea (the coast of Venice, Italy) (Senz 1993).

### Remarks

The original description of *Prosorhochmus adriaticus* Senz, 1993, a species from Italy, lacks information about external appearance or stylet apparatus, asserting only that the species most resembles *Prosorhochmus claparedii* and S/B ratio is about 0.25. Senz (1993) emphasizes that *P. adriaticus* is different from *P. claparedii* and all other described nemerteans in its unique mode of embryonic nourishment via a direct connection between the ovary and specialized gut diverticula “cinched off” from the intestine, surrounded by a layer of extracellular matrix and filled with nutritional granules. We carefully studied the series of histological sections of the holotype and the other two specimens made available by the Natural History Museum in Vienna in attempt to confirm this particularly odd observation. However, we did not find anything unusual about the ovaries or the gut diverticula in these specimens and, certainly, there was no direct connection between the ovaries and the gut diverticula. We conclude that Senz (1993) misinterpreted the anatomy of this species. Moreover, in the follow up paper on the development of *P. adriaticus*, reporting their observations on sections of another specimen, Senz and Tröstl (1999) mention that the specialization of the gut diverticula is not nearly as prominent as in the holotype and that there is no direct connection between the lumen of gut diverticula and the gonads.

We could not determine with confidence whether *Prosorhochmus adriaticus* has purple cephalic glands or not, because of monochromatic and faded staining of the sections of the holotype and voucher specimens.

In the following paragraphs we debunk the other characters used to distinguish *Prosorhochmus adriaticus* from other *Prosorhochmus*. Take, for example, the presence of ciliation in the posterior oesophagus of *P. adriaticus* (Senz 1993). Although presence vs. absence of ciliation in oesophagus has been used to differentiate between the species of *Prosorhochmus* (Frutos et al. 1998), our observations on numerous nemertean species show that absence of ciliation can easily be an artifact of fixation, and sparse ciliation may be difficult or impossible to detect with standard light microscopy. We believe that this character cannot be used for diagnostic or identification purposes.

Without further explanation, Senz (1993) mentions that the frontal organ of *Prosorhochmus adriaticus* is different from the frontal organ of *Prosorhochmus claparedii*. We can only assume that he refers to the reported presence of the 90° bend in the frontal organ of *P. claparedii* (Gibson and Moore 1985, p.149, plate I, fig. e) and lack of it in *P. adriaticus*. After investigating serial sections of all relevant specimens we conclude that the 90° bend is a misinterpretation. All *Prosorhochmus* species have a frontal organ of a similar structure and complexity (see Maslakova et al. 2005 for discussion).

Other reported differences between *Prosorhochmus adriaticus* and *Prosorhochmus claparedii* (Senz 1993; Frutos et al. 1998) are the lack of dorsoventral muscles in the foregut region of *P. claparedii*, presence of the neural supply in the posterior chamber of the proboscis of *P. claparedii*, and the supposed lack of basophilic mucus glands in *P. adriaticus* (Frutos et al. 1998, p. 297, table 2). Our re-investigation of all the available type and voucher material showed that both species have well-developed dorso-ventral muscles in the foregut region, there is no unique neural supply in the posterior chamber of the proboscis of *P. claparedii* (or any other species in the genus) and basophilic mucus glands are present and well developed in both

species. The reported 0.25 S/B ratio in *P. adriaticus* is within the range of intra-specific variation of *P. claparedii* (Table 3).

In summary, *Prosorhochmus adriaticus* appears to be morphologically indistinguishable from *Prosorhochmus claparedii*. Material for molecular analysis of *P. adriaticus* is not available. We have not been able to get a hold of the species' author Wolfgang Senz for help with obtaining fresh material. Attempts by SAM to recollect *P. adriaticus* from the type locality (broadly defined as coast of Venice, Italy) or adjacent coastal areas of Italy (near Trieste) and Croatia (near Savudrija and Zabrata) in August 2002 failed, despite apparently finding the suitable prosorhochmid habitat and collecting several specimens of *Prosorhochmus* cf. *chafarinensis*.

***Prosorhochmus americanus* Gibson et al., 1986**

(Figures 7G, 8C–F, 9A; Tables 1–4)

*Prosorhochmus americanus* (Gibson et al. 1986; Senz 1993; Frutos et al. 1998; Maslakova et al. 2005).

*Etymology*

At the time of discovery this species was the only known New World representative of the genus and was accordingly named after the place of discovery – America.

*Type material*

Holotype USNM 98550, paratypes USNM 98551–98552, coll. J.M. Turbeville, Winyah Bay, Georgetown, South Carolina, USA.

*Material examined*

*Prosorhochmus americanus* Gibson et al. 1986. Holotype USNM 98550, paratypes USNM 98551–98552. Additional material: USNM 1020649–1020651, coll. JLN, Sebastian Inlet, Florida, USA; USNM 1107441–1107443, coll. JLN, Virginia Key, Florida, USA.

*Diagnosis*

*Prosorhochmus americanus* differs from all other species of the genus in having well-developed purple cephalic glands (Figure 9A, Table 3). Additionally, it differs from *Prosorhochmus nelsoni* and *Prosorhochmus belizeanus* sp. nov. in being viviparous and hermaphroditic and from *P. belizeanus* sp. nov. in having acidophilic and purple cephalic glands intermixed with the basophilic mucus cephalic glands in the pre-cerebral and cerebral region (compare Figures 6B–C and 9A). It differs from *P. claparedii*, *P. adriaticus* and *P. chafarinensis* in having up to three juveniles per ovary (compared to one). Central stylet (S) 90 µm long, basis (B) truncated, 200 µm long; S/B ratio 0.45–0.5 (Gibson et al. 1986); data at hand are insufficient to make statistical comparisons with other species.

*Habitat and distribution*

Intertidal, under valves of the oyster *Crassostrea virginica* attached to the large irregular granite blocks of the North Jetty at Winyah Bay entrance near Georgetown (33°12'10" N, 79°09'00" W), North Jetty at Murrell's Inlet near Garden City

Table 3. Morphological comparison between the *Prosorhochmus* species.

Characters	<i>P. clarearii</i>	<i>P. adriaticus</i>	<i>P. americanus</i>	<i>P. chafarinensis</i>	<i>P. nelsoni</i>	<i>P. belizeanus</i> sp. nov.
Stylet length (S, $\mu\text{m}$ )	30–90	N/A	90	80–130 (109.6)	49–107.8 (84)	185–250 (209.1)
Basis length (B, $\mu\text{m}$ )	90–160	N/A	200	136–260 (208)	73.5–132.3 (108)	225–375 (295)
Stylet to basis length (S/B) ratio	0.25–0.62	0.25	0.45–0.5	0.38–0.65 (0.52)	0.64–1.1 (0.79)	0.60–0.83 (0.71)
Purple cephalic glands	Absent or poorly developed	?	Well-developed	Absent or poorly developed	Absent or poorly developed	Absent or poorly developed
Acidophilic cephalic glands	Intermix with basophilic glands	Intermix with basophilic glands	Intermix with basophilic glands	Intermix with basophilic glands	Intermix with basophilic glands	Form a dense cluster
Sex/life history	Viviparous hermaphrodite	Viviparous hermaphrodite	Viviparous hermaphrodite	Viviparous hermaphrodite	Oviparous, gonochoric	Oviparous, gonochoric
Juveniles per ovary	1	1	1–3	Several embryos may start cleaving, but only one per ovary develops into a juvenile	N/A	N/A

Note: Where available, the average for stylet length, basis length and S/B ratio is provided in parenthesis.

(33°31'48" N, 79°01'48" W), North Jetty at the Charleston Harbor entrance near Charleston (33°43'42" N, 79°49'00" W) and on isolated boulders embedded in a sandy oceanic beach at Pawley's Island (33°24'42" N, 79°07'45" W) in South Carolina, USA. Gregarious with several (10 or more) individuals occasionally found below a single oyster valve. The heteronemertean *Lineus socialis* and the hoplonemertean *Nemertopsis bivittata* occur on the same granite blocks just below the *Crassostrea*–*Prosorhochmus* zone.

Additional specimens were collected by JLN in 1983 in Florida underneath and among coral rubble from mid- to high-tide region, on tidal flat in back of north-side breakwater at Sebastian Inlet; among littoral rubble on flats in back of the breakwater and from north-side jetty near Fort Pierce Inlet; and from freestanding concrete piling along the Intercoastal Waterway (Indian River Lagoon) near Lake Worth Inlet, Florida. In the latter locality, *Prosorhochmus americanus* co-occurs with *Nemertopsis bivittata* and vermetid gastropods. In 2006 JLN collected three more specimens from the littoral portion of fouling communities on cement pilings of the boat pier at Rosenstiel School of Marine and Atmospheric Science, Virginia Key, Florida.

#### Remarks

Surprisingly, sequences of both 16S and COI from *Prosorhochmus americanus* (specimens collected by J. "Clint" Turbeville from Pawley's Island, South Carolina, USA) turned out to be identical to sequences of *Prosorhochmus claparedii* collected by SAM in vicinity of Station Biologique de Roscoff in Roscoff, France. DNA extraction, amplification reactions and sequencing were repeated three times on each of these samples to exclude the possibility of cross-contamination. If these are the same species it means that there is intra-specific variation in the number of juveniles per ovary and presence and degree of development of purple cephalic glands. Alternatively, this might reflect hybridization accompanied by introgression, in which case the mitochondrial genome of one species could have taken over. The latter hypothesis is supported by comparing partial sequences of the nuclear gene 28S rDNA, which display a small difference (Maslakova, unpublished). This, combined with their subtle morphological difference provides a justification for keeping them as separate species.

Table 4. Sequence divergence between species of *Prosorhochmus* (%).

	<i>P. nelsoni</i>	<i>P. belizeanus</i> sp. nov.	<i>P. claparedii</i>	<i>P. americanus</i>	<i>P. cf. chafarinensis</i> (from Adriatic Sea)
<i>P. nelsoni</i>	–	–	–	–	–
<i>P. belizeanus</i> sp. nov.	7.4/9.1	–	–	–	–
<i>P. claparedii</i>	7.6/9.7	7.8/10.2	–	–	–
<i>P. americanus</i>	7.6/9.7	7.8/10.2	0.0	–	–
<i>P. cf. chafarinensis</i> (from Adriatic Sea)	7.4/10.2	8.6/10.6	2.2/0.6	2.2/0.6	–
<i>Arhochmus korotneffi</i>	15.6/13.5	14.9/14.0	14.6/13.7	14.6/13.7	14.8/13.8

Note: The first number corresponds to 16S, the second – to COI sequences.

*Prosorhochmus belizeanus*, sp. nov.

(Figures 1H, 2–6, 7I; Tables 1–4)

*Etymology*

The species is named after the country of its type locality – Belize.

*Type material*

Serial histological sections of the holotype (mature female, USNM 1020503) and six paratypes (USNM 1020501, 1020502, 1020504–07) are deposited in the collection of US National Museum of Natural History, Smithsonian Institution. Specimens USNM 1020502–07 were collected by JLN and SAM from the type locality at Carrie Bow Cay, Belize in February 2000. Specimen USNM 1020501 was collected by JLN from Lake Worth Inlet near West Palm Beach, FL, USA in February 1998.

*Material examined*

*Prosorhochmus belizeanus* sp. nov. USNM 1020501–1020507. Additional material: *Prosorhochmus* sp. 137 USNM 1020648 (coll. JLN, Peanut Island, near West Palm Beach, FL, USA).

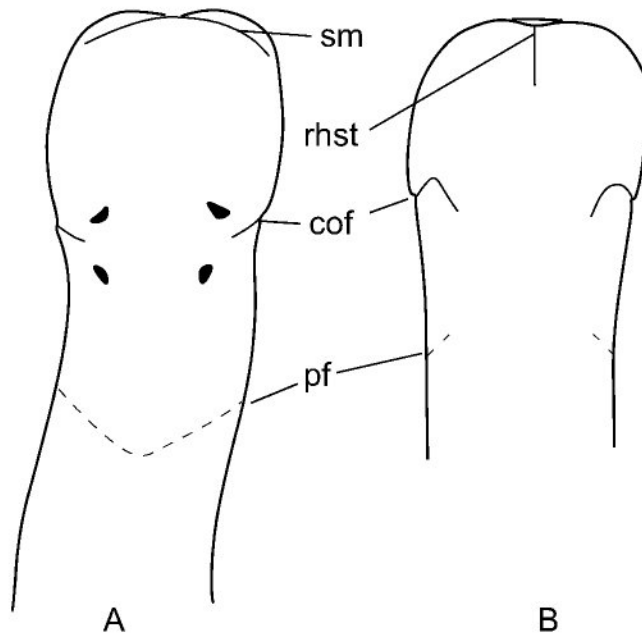


Figure 2. *Prosorhochmus belizeanus* sp. nov., diagram of anterior end. (A) Dorsal view; (B) ventral view. Abbreviations: cof, cerebral organ furrow; pf, posterior cephalic furrow; rhst, rhynchostome; sm, prosorhochmid smile.

*Diagnosis*

*Prosorhochmus belizeanus* sp. nov. possesses a unique apomorphy of acidophilic cephalic glands forming a compact cluster in the precerebral region (Figures 4C–D, G–H, K). Additionally, it differs from *P. claparedii*, *P. adriaticus*, *P. americanus* and *P. chafarinensis* in being gonochoric and oviparous (Figures 1H, 4E, 5F, 6F) and from *P. americanus* in lacking well-developed purple cephalic glands (compare Figures 4G and 9A). Central stylet (S) 185–250 µm long, average 209.1 µm, significantly different (longer) from that of *Prosorhochmus chafarinensis*, *Prosorhochmus nelsoni* and *Prosorhochmus claparedii* ( $p=0.05$ ); basis (B) truncated, 225–375 µm long, average 295 µm, significantly different from that of *P. chafarinensis*, *P. nelsoni* and *P. claparedii* ( $p=0.05$ ); central stylet to basis (S/B) ratio is 0.60–0.83, average 0.72, significantly different from that of *P. chafarinensis*, but not from that of *P. claparedii* or *P. nelsoni* ( $p=0.05$ ) (see Table 2).

*Habitat, type locality and distribution*

The type locality is Carrie Bow Cay, site of the Smithsonian Institution's Caribbean Coral Reef Ecosystems Station, located 18 km offshore on the barrier reef in Belize (16°48' N, 88°05' W). The specimens were obtained by breaking coral rubble exposed during low tide on the reef flat on the SE side of the island. A single specimen was collected from a bivalve-vermetid community encrusting a concrete piling at Lake Worth Inlet near West Palm Beach, Florida.

*Description*

*External appearance.* *Prosorhochmus belizeanus* sp. nov. is relatively small, with maximum recorded length of reproductive specimens 35 mm and width 1.0 mm. The colour in life is yellowish-rosy, orange-yellow or salmon, head and ventral side slightly paler than the rest of the body. In some specimens narrow bands or specks of dark brown pigment extend posteriorly along the edges of lighter rhynchocoel, fading out toward the anterior end (Figure 1H). The body is slender and compact, dorso-ventrally flattened, wider at the anterior end, gradually tapering toward the posterior to end in a bluntly rounded tip. The head is spatulate in shape and wider than the adjacent body region, with a characteristic vertical anterior notch giving it a distinct bifid appearance. A dorsal horizontal epidermal fold anterior to the eyes separates two ventral apical lobes of the head from a median dorsal lobe, creating the appearance of a "smile" (Figures 1H, 2A–B), characteristic of the genus. 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, reaching slightly over onto the dorsal side (Figures 2A–B). 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 (Figures 2A–B). The rhynchopore is subterminal.

*Body wall, musculature and parenchyma.* Epidermis is of typical hoplonemertean structure (Figure 4A). 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 fibres situated between the circular and longitudinal musculature of the body wall form a thin but distinct layer. This layer is best visualized in longitudinal sections (Figure 4B). The precerebral septum is of split (Kirsteuer 1974) or mixed type (Chernyshev 2002). It is formed by individual muscle fibres emerging from the body-wall longitudinal musculature at several levels. Behind the brain, separate bundles of oblique fibres diverge from the inner margins of the longitudinal muscle layer and lead forward toward the proboscis insertion. Here the oblique fibres are joined by additional (radial) fibres, which turn inward from the main layer (Figure 4C, D). A few individual fibres from the inner portion of the longitudinal musculature continue into the head as cephalic retractors. Dorso-ventral muscles are strongly developed between the gonads and intestinal diverticula (Figure 4E). Anteriorly, thick dorso-ventral muscles are found between the lateral pouches of the caecum, lobes of the mucus cephalic glands and the lateral nerve cords (Figures 4F, 6D, E). Muscle fibres oriented dorso-ventrally, obliquely and horizontally are abundant in the precerebral region (Figure 4G). The musculature associated with the foregut, in the literature often referred to as “splanchnic musculature”, is very well developed and is continuous with the fibres surrounding the rhynchodeum. A longitudinal muscle layer surrounds the oesophagus from the point of its separation from the rhynchodeum to the brain region (Figure 4H). At this point, oesophageal muscles become surrounded by additional longitudinal fibres originating at the proboscis insertion (Figure 4I). These muscles continue as a thin layer surrounding the stomach and are particularly apparent between its folds (Figure 4J). The amorphous extracellular matrix, of the so-called “parenchyma”, is scarce and 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 (Figure 4K). Just anterior to the proboscis insertion, it is comprised of cells with acidophilic cytoplasm and large nuclei (Figure 4L). It was not possible to determine with light microscopy whether rhynchodeal epithelial cells bear cilia or not. The rhynchodeal musculature is rather well developed and comprises both longitudinal and circular muscle fibres. There is no localized concentration of circular muscle fibres representing a distinct rhynchodeal sphincter. The rhynchocoel reaches almost to the posterior end of the body. Its wall is of typical distromatonemertean (Thollesson and Norenburg 2003) structure i.e. contains separate outer circular and inner longitudinal muscle layers (Figure 3A). 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 to dull cream. Immediately after proboscis insertion its wall consists of a thin non-glandular epithelium, a thin layer of extracellular matrix, an outer circular muscle layer, a longitudinal muscle layer divided into two concentric layers by the neural sheath with distinct proboscis nerves, a delicate layer of inner circular muscles, and a thin endothelium (Figure 3B). Further posterior, the proboscis wall comprises all the same layers except for the proboscideal epithelium, which is thick, glandular and arranged into conical papillae (Figures 3C, D). The neural sheath



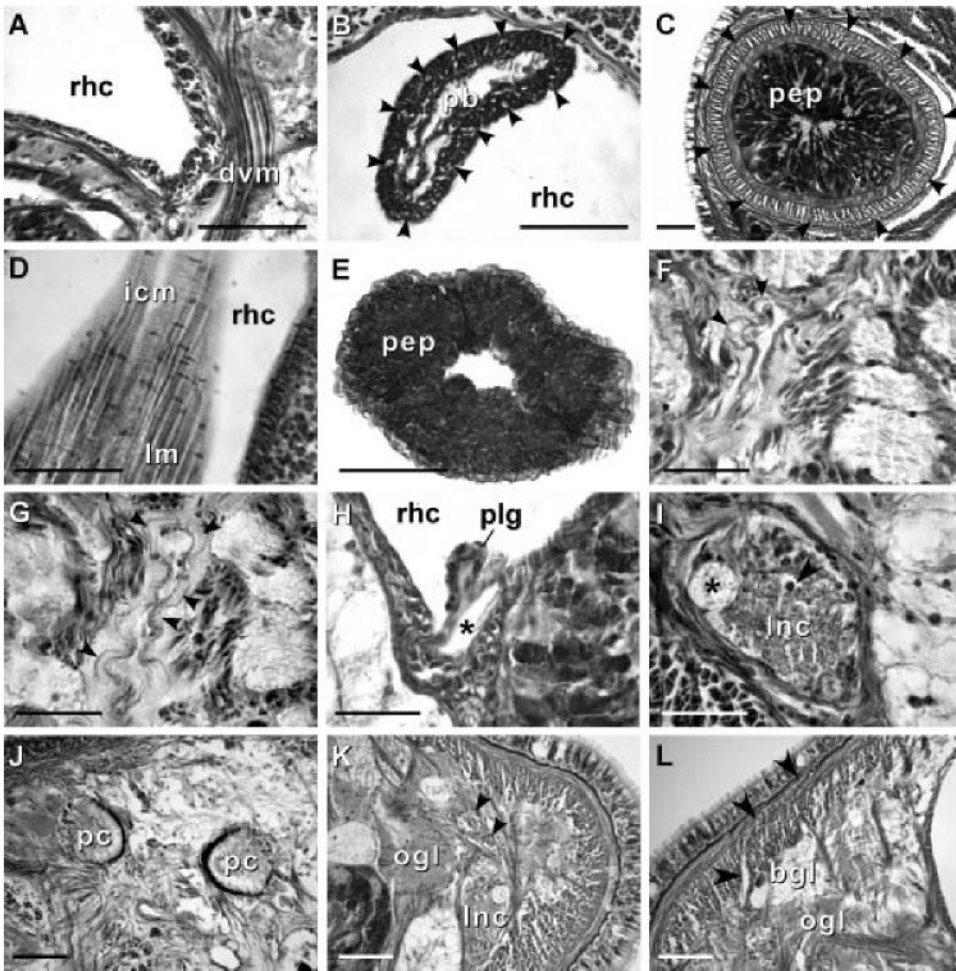


Figure 3. *Prosorhochmus belizeanus* sp. nov. (A) Transverse section through the rhynchocoel wall; (B) transverse section through the anterior-most portion of the anterior proboscis. Note the non-glandular epithelium and proboscis nerves (indicated by the arrowheads). (C) Transverse section through the middle region of anterior proboscis; proboscis nerves (arrowheads); (D) longitudinal section through the wall of anterior proboscis showing the delicate layer or inner circular muscles overlaying longitudinal muscles; (E) transverse section through the posterior chamber of the proboscis; (F, G) transverse sections through the cephalic blood vessels showing “pouches” and “valves” (arrowheads); (H) transverse section through the vascular plug; lumen of the mid-dorsal blood vessel marked by an asterisk; (I) transverse section through the lateral nerve cord showing the upper nerve (asterisk) and nerve cord muscles (arrowhead); (J) longitudinal section through the ocelli; anterior to the right; (K) transverse section through the foregut region showing nephridial tubules in cross-section (arrowheads); (L) transverse section at the level of nephridioduct (arrowheads). Scales. (A, B, D, E and J–L): 50  $\mu$ m; (C): 100  $\mu$ m; (F–I): 25  $\mu$ m. Abbreviations: bgl, basophilic cephalic glands; dvm, dorso-ventral muscles; icm, inner circular muscles; lm, longitudinal muscles; lnc, lateral nerve cord; ogl, orange-G cephalic glands; pb, proboscis; pc, pigment cup of ocellus; pep, proboscis epithelium; plg, vascular plug; rhc, rhynchocoel.

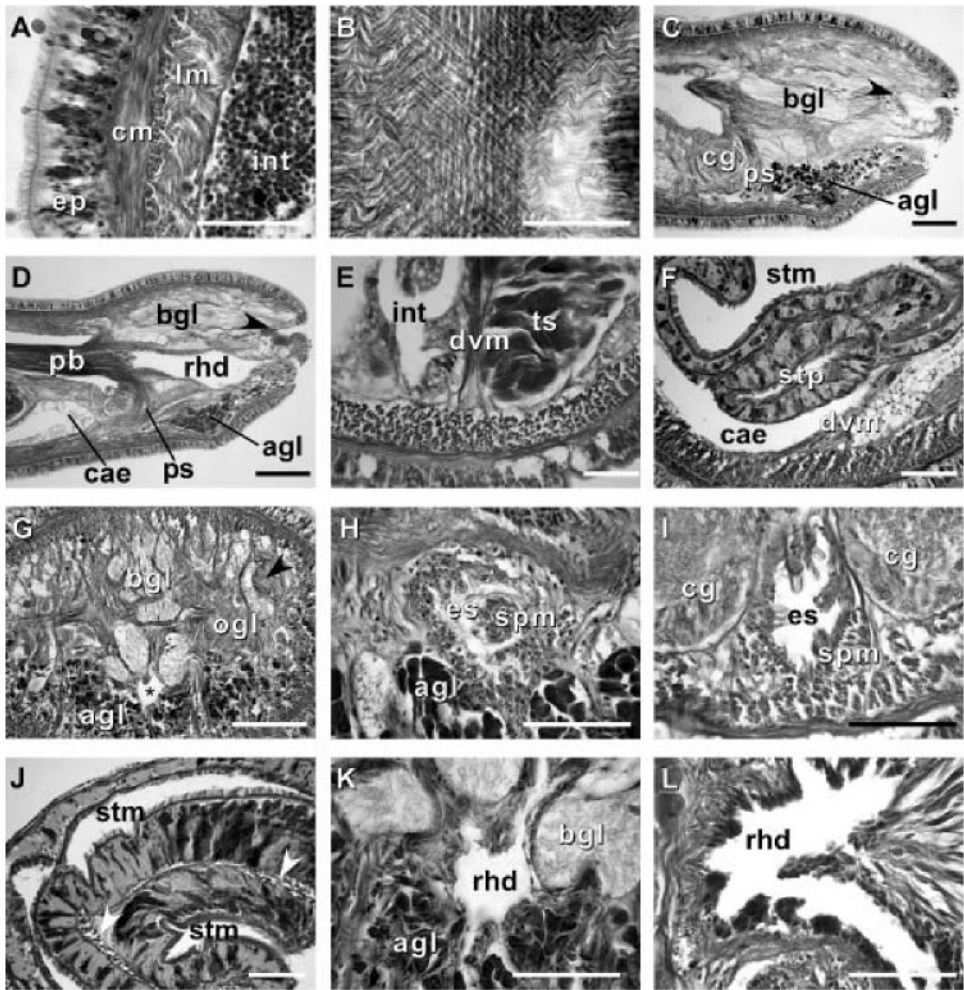


Figure 4. *Prosorhochmus belizeanus* sp. nov. (A) Transverse section through the body wall; (B) longitudinal section through the body wall showing diagonal muscles; (C, D) longitudinal sagittal sections through the precerebral and cerebral region showing frontal organ (arrowhead), precerebral septum and acidophilic cephalic glands; anterior is to the right; (E) transverse section showing well-developed dorso-ventral muscles in the midgut region; (F) transverse sections through the stomach showing the ventral stomach pouch and well-developed dorso-ventral muscles; (G) transverse section through precerebral region showing numerous muscle fibres oriented in all possible directions, rhynchodeum (asterisk) and an ocellus (arrowhead); (H) transverse section through the anterior oesophageal region showing foregut or "splanchnic" muscles; (I) transverse section through the sparsely ciliated posterior oesophagus; (J) transverse section through the deeply folded stomach showing "splanchnic" muscles (arrows); (K) transverse section through the anterior rhynchodeum; (L) transverse section through the rhynchodeum just in front of the proboscis insertion. Scales. (A, B, E, F, H-L): 50  $\mu$ m; (C, D, G): 100  $\mu$ m. Abbreviations: agl, acidophilic cephalic glands; bgl, basophilic cephalic glands; cae, caecum; cg, cerebral ganglia; cm, circular muscles; dvm, dorso-ventral muscles; ep, epidermis; es, oesophagus; int, intestine; lm, longitudinal muscles; ogl, orange-G cephalic glands; pb, proboscis; ps, precerebral septum; rhd, rhynchodeum; spm, foregut or "splanchnic" muscles; stm, stomach; stp, stomach pouch; ts, testis.

bears 11–14 proboscis nerves (Figure 3C). The proboscis armature consists of a central stylet, mounted on a characteristically truncated basis (Figure 7I) and two pouches each containing 1–3 accessory stylets. The length of the central stylet (S) ranges from 185 to 250  $\mu\text{m}$  (average 209.1  $\mu\text{m}$ ), basis length (B) ranges from 225 to 375  $\mu\text{m}$ , (average 295  $\mu\text{m}$ ) and S/B ratio ranges from 0.60 to 0.83 (0.71 on average), see Tables 2–3. The wall of the posterior chamber of the proboscis consists of glandular epithelium, outer longitudinal muscle layer, inner circular muscle layer and a delicate endothelium (Figure 3E). We did not observe distinct nerve supply in the longitudinal muscle layer of the posterior proboscis of *P. belizeanus* sp. nov.

*Alimentary canal.* The oesophagus opens into the rhynchodeum in front of the precerebral septum. It is enclosed by longitudinal muscle fibres (Figures 4H–I), which are confluent with the rhynchodeal musculature and continue posteriorly as the musculature of the stomach. The posterior part of the oesophagus is ciliated and lacks acidophilic or basophilic glands (Figure 4I). The stomach is of typical hoplonemertean structure with densely ciliated, deeply folded epithelium, containing numerous basophilic and acidophilic glands (Figure 4J). Specimens from Belize lack a ventral posterior stomach “pouch”, while the only specimen from Florida (USNM 1020501) possesses a single “pouch” about 80  $\mu\text{m}$  long (Figure 4F). We do not attribute any taxonomic significance to the presence or absence of such “pouches”, as they are likely a result of folding of the voluminous stomach. The intestinal caecum is well developed and may be anteriorly bifid: divided portion up to 100  $\mu\text{m}$  long, reaching the posterior portion of the dorsal cerebral ganglia. The caecum bears numerous lateral diverticula throughout its length. These and intestinal diverticula are lobed.

*Blood system.* The blood system is of usual 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 and immediately penetrates the rhynchocoel floor to form a single vascular plug. The wall of the vascular plug consists of thickened endothelium of the blood vessel, a thin layer of extracellular matrix and a modified rhynchocoel endothelium (Figure 3H). We did not observe any transverse connectives linking mid-dorsal and lateral blood vessels in the intestinal region. The blood vessels are thin-walled with a well-defined lumen and irregular thickenings of the wall. Apparently, during contraction of the blood vessels the latter may appear as “pouches” or “valves” (Figures 3F–G).

*Nervous system.* As is typical for nemerteans, the brain consists of two ventral and two dorsal ganglia, joined by ventral (subrhynchocoelic) and dorsal (suprarhynchocoelic) commissures, respectively. The smaller 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 and no neurochords in the lateral nerve cords. The lateral nerve cords contain a single fibrous core throughout their length. The so-called “upper” nerve is present – a

bundle of nerve fibres in the dorsal part of the fibrous core of the lateral nerve cord, distinguished by their lighter colour, which we observed in all other species of *Prosorhochmus* (Figures 3I, K). The difference between this upper nerve and a real accessory nerve is that the upper nerve is never separated from the main fibrous core by cell bodies, and it is derived from the ventral cerebral ganglion, as opposed to the dorsal cerebral ganglion. As observed in most monostiliferans studied in the last three decades, each lateral nerve cord contains a single delicate muscle bundle (several fibres thick) running within or adjacent to the fibrous core (Figure 3I). In addition, there are several less conspicuous muscle fibres running along the inner lateral side of the fibrous core. Muscle fibres associated with the lateral nerve cords can usually be traced to their extracerebral origin near the proboscis insertion. Cephalic nerves lead anteriorly from the brain ganglia to supply various structures of the head. Two stout nerves originating from the ventral ganglia innervate paired cerebral sensory organs.

*Eyes.* The four eyes are well-developed pigment cups. The eyes of the anterior pair are slightly larger than the posterior. The pigment cups of the anterior pair are facing antero-laterally, while those of the posterior pair are directed posterolaterally (Figure 3J).

*Frontal organ.* The frontal organ consists of a ciliated canal 50 – 85  $\mu\text{m}$  long, lined by a regionally differentiated epithelium (Figures 4C–D, 5A–C, G, H). The anterior portion of the canal is often triangular in cross-section, becoming rounded or oval toward the posterior end. Anteriorly, the ventral wall of the frontal organ comprises strongly acidophilic epithelium, clad in densely-arranged short cilia, which soon divides to run on lateral borders of the canal. The portions of the canal, through which the basophilic mucus cephalic glands discharge, have vacuolated appearance and bear much longer, sparsely distributed cilia. There do not appear to be 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.

*Cephalic glands.* Cephalic glands are extremely well developed. As in other members of the genus, they include three distinct types: strongly vacuolated basophilic lobules (mucus glands), coarsely granular proteinaceous gland cells staining golden-yellow to brown with Mallory trichrome or orange with Crandall's method (orange-G glands), and finely granular proteinaceous acidophilic cells, staining pink to red with Mallory or Crandall's technique (acidophilic or red glands).

Basophilic (mucus) glands are well developed and open through the dorsal, ventral and posterior epithelium of the frontal organ (Figures 4C–D, 5B–C). Dorsal lobes reach their maximum development in the cerebral region and reach as far back as the anterior pyloric region, while the two ventro-lateral lobes running parallel to the oesophagus reach the anterior end of stomach.

Red acidophilic glands are well developed but restricted to the precerebral region. At the level of the frontal organ they are most abundant dorsally, in some individuals forming almost a continuous layer between the dorsal basophilic lobules and the longitudinal musculature of the body wall.

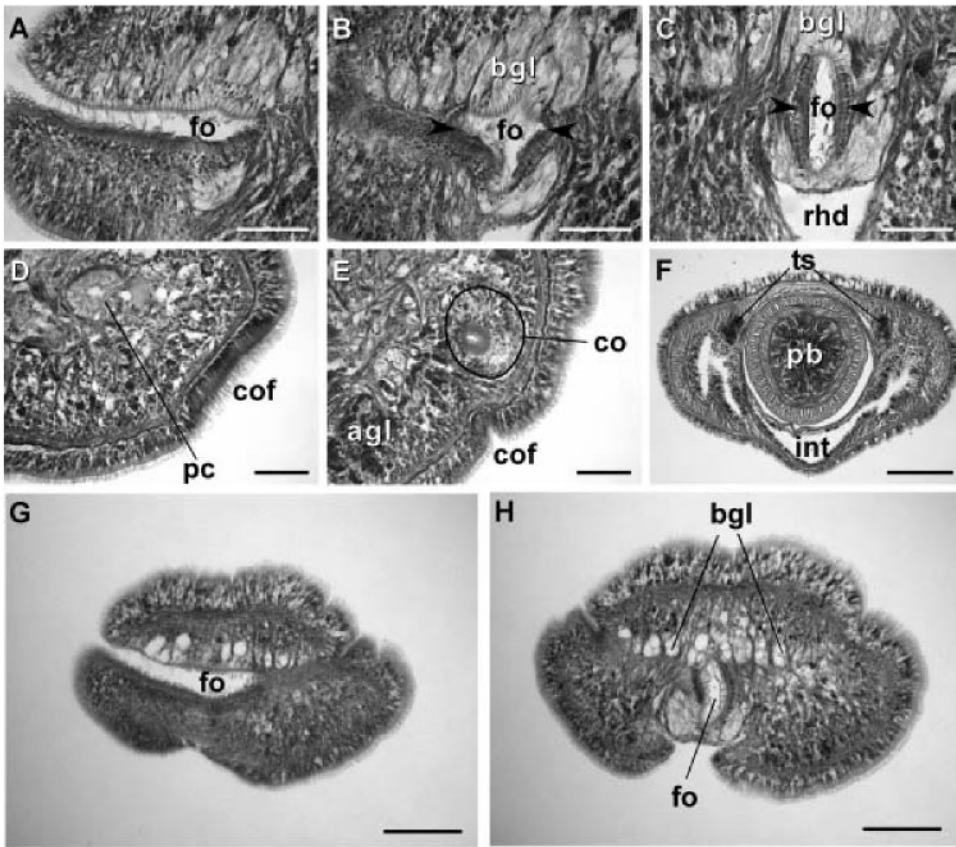


Figure 5. *Prosorhochmus belizeanus* sp. nov. (A–C) A series of transverse sections through the frontal organ from anterior to posterior; lateral acidophilic regions marked by arrowheads. (D) Transverse section through the cerebral organ furrow and posterior ocellus. (E) Transverse section through the cerebral organ (outlined). (F) Transverse section through the intestinal region of a mature male. (G, H) Slightly oblique transverse sections through the tip of the head: prosorhochmid smile (G) and frontal organ (H). Scales on (A–E) - 50  $\mu\text{m}$ ; on (G, H) - 100  $\mu\text{m}$ ; on (F) - 200  $\mu\text{m}$ . Abbreviations: agl - acidophilic cephalic glands, bgl - basophilic cephalic glands, co - cerebral organ, cof - cerebral organ furrow, fo - frontal organ, int - intestine, pb - proboscis, pc - pigment cup of ocellus, rhd - rhynchodeum, ts - testis.

Numerous red gland cells are also scattered ventrally and laterally on both sides of the rhynchodeum. Necks of the red glands reach to the epidermis and open via numerous pores dorsally, ventrally and laterally. Further back, at the level of the cerebral organs, dorsal red glands decrease in numbers, while the ventral red glands become much more abundant, and form a dense cluster below the oesophagus, reaching their maximum abundance in front of the precerebral septum (Figures 4C–D, G, H, K, 6A–B). A few isolated red gland cells persist after the septum, mostly in the dorsal region, interspersed with the orange-G and mucus glands.

Orange-G glands are very strongly developed, particularly in the cerebral and foregut regions (Figures 3K–L, 4G, 6A–D). They open via improvised ducts in

the dorsal epithelium and can be found as far back as the end of the rhynchocoel. On series of transverse sections, orange-G gland cell bodies first appear near the anterior pair of eyes, on both sides of the rhynchodeum, although their glandular necks can be traced all the way into the anterior-most tip of the head, where they lie interspersed with the cell bodies of the red glands and open via numerous pores just above the frontal organ. Orange-G glands gradually increase in number further back and reach their maximum abundance immediately behind the brain, where they form dense dorso-lateral clusters on each side of the rhynchocoel adjacent to the nerve cords and nephridial tubules (Figure 3K). Near the end of the pylorus, orange-G glands become restricted to the two narrow dorso-lateral strips – one on each side of the rhynchocoel between the diverticula of the gut and the longitudinal body wall musculature (Figure 6F).

*Cerebral organs.* Small paired cerebral organs are situated almost entirely in front of the brain between the anterior and posterior pairs of eyes. The posterior portion of the cerebral organs slightly overlaps with the anterior portion of the brain. Each organ opens at the level of the anterior pair of eyes into a reduced ventro-lateral cerebral organ furrow, which is nothing more than a shallow ventro-lateral crescent-shaped groove (Figures 1C, 2B) lined with a strongly acidophilic epithelium (Figures 5D–E). The cerebral organ canals are not branched. The posterior portion of the cerebral organ is a glandular lobe with finely granular acidophilic secretion (Figure 5E).

*Excretory system.* The protonephridial system extends from the dorsal brain ganglia to the anterior pyloric region, most of it immediately dorsal to the lateral nerve cords and the lateral blood vessels. Ciliated nephridial tubules are thick-walled and not regionally specialized. Paired nephridia open dorso-laterally immediately posterior to the brain via two nephridiopores - one on each side (Figures 3L, 6E). Small and hardly noticeable mononucleate flame cells are found embedded in the extracellular matrix in the vicinity of the lateral blood vessels.

*Reproductive system and life history:* *Prosorhochmus belizeanus* sp. nov. is gonochoric. Reproductive males and females were observed in February in Belize and Florida. As in most other nemerteans, gonads alternate with the lobes of intestinal diverticula (Figures 1H, 4E, 5F, 6F). Similar to *Prosorhochmus nelsoni*, up to 20–30 mature oocytes can be observed within the same ovary (Figure 6F), indicative of oviparity. Pinkish oocytes can be readily observed through the body wall of mature females (Figure 1H).

#### *Remarks*

Characters, such as bifid anterior cephalic margin with the “prosorhochmid smile”, truncated stylet basis, well-developed frontal organ with laterally differentiated epithelium, well-developed cephalic glands, combining mucus and at least two kinds of proteinaceous components (staining pinkish-red and golden-orange with Mallory trichrome) and protonephridial system with

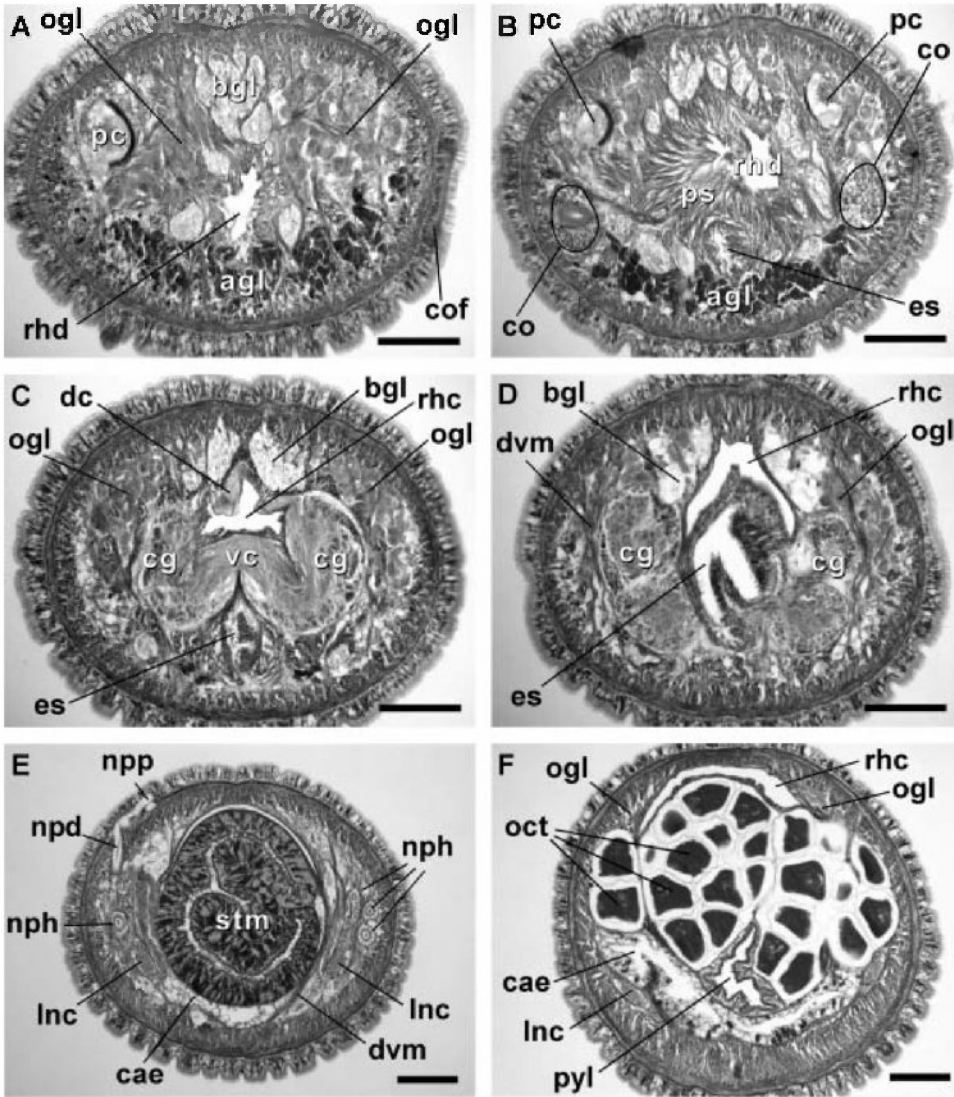


Figure 6. A series of slightly oblique transverse sections of *Prosorhochmus belizeanus* sp. nov. from precerebral to pyloric region (proboscis is missing). (A) Section through one of the cerebral organ furrows and the anterior ocellus on the opposite side. (B) Posterior ocelli, precerebral septum and cerebral organs (outlined). (C) Cerebral ganglia and brain commissures. (D) Posterior portion of brain. (E) Section through the stomach and nephridia. (F) Pyloric region and ovaries. Scales 100  $\mu$ m. Abbreviations: agl - acidophilic cephalic glands, bgl - basophilic cephalic glands, cae - caecum, cg - cerebral ganglia, co - cerebral organ, cof - cerebral organ furrow, dc - dorsal brain commissure, dvm - dorso-ventral muscles, es - oesophagus, lnc - lateral nerve cord, npd - nephridioduct, npn - nephridial tubule, npp - nephridiopore, oct - oocyte, ogl - orange-G cephalic glands, pb - proboscis, pc - pigment cup of ocellus, ps - precerebral septum, pyl - pylorus, rhc - rhynchocoel, rhd - rhynchodeum, vc - ventral brain commissure.

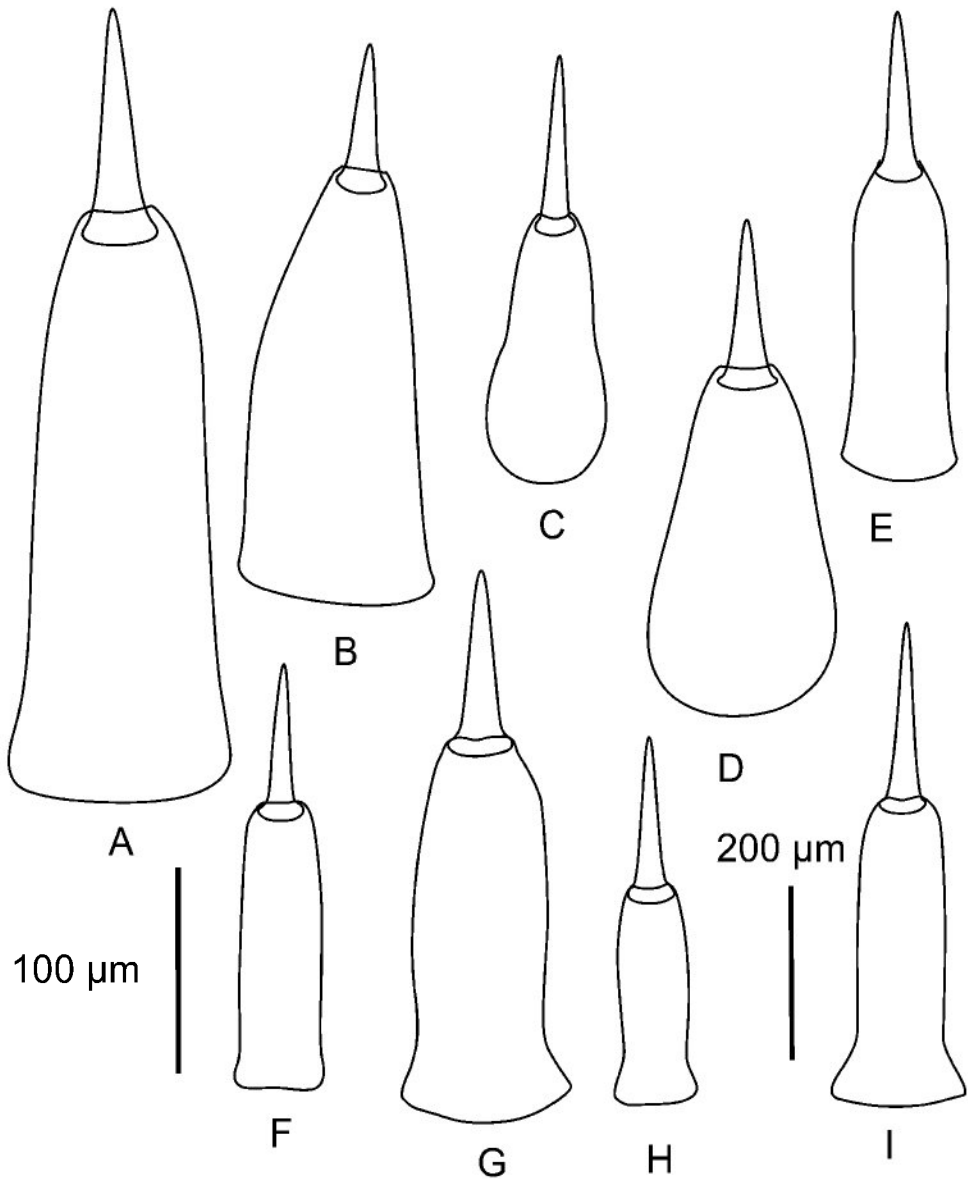


Figure 7. Central stylets. The 100µm scale bar applicable to all, except (I). (A, B) *Prosorhochmus chafarinensis* (specimens from Croatia); (C, D) *Arhochmus korotneffi* comb. nov. (specimens from Croatia); (E) *Prosorhochmus claparedii* (specimen from Roscoff, France); (F) *P. chafarinensis* (after Frutos et al., p. 294, fig. 1b); (G) *Prosorhochmus americanus* (after Gibson et al. 1986, p. 330, plate I, fig. e); (H) *Prosorhochmus nelsoni* (specimen from Coquimbo, Chile); (I) *Prosorhochmus belizeanus* sp. nov. (200µm scale bar).

mononucleate flame cells without distinct support bars, thick-walled excretory tubules and a single pair of nephridiopores place this species in the genus *Prosorhochmus*. Sequence divergence between *Prosorhochmus belizeanus* sp. nov. and its closest congener *Prosorhochmus nelsoni* is 7.7% for 16S rDNA and 9.1%



for COI, which is comparable to the sequence divergence from the European (viviparous and hermaphroditic) species of *Prosorhochmus* (Table IV). One additional *Prosorhochmus* specimen, a female with numerous mature oocytes in each ovary, histologically indistinguishable from the *P. belizeanus* sp. nov., but much larger than all other known specimens of this species (50 mm long and 1–1.5 mm wide), was collected by JLN in March, 1983 from a split *Coquina* rock at the low tide near Peanut Island, Fort Worth Inlet, Florida (*Prosorhochmus* sp. 137). Unfortunately, the specimen was collected without its proboscis. Thus, stylet characteristics could not be evaluated. Material for molecular analysis is not available from this specimen to determine with certainty whether it belongs to *P. belizeanus* sp. nov. Serial histological sections of this specimen are stored at the Smithsonian Institution's National Museum of Natural History in Washington D.C. (USNM 1020648). Repeated efforts to recollect similar specimens from Florida in subsequent years were unsuccessful.

***Prosorhochmus chafarinensis* Frutos et al., 1998**

(Figures 1B–C, 1F, 7A–B, 7F, 9C–F; Tables 1–4)

*Prosorhochmus chafarinensis* (Frutos et al. 1998; Maslakova et al. 2005)

*Etymology*

The species is named after the place of discovery, the Spanish Chafarinas Islands off the coast of Morocco (western Mediterranean).

*Type material*

Holotype and two paratypes MNHM 5.01/1. Isabel II Island, Chafarinas Islands, Spain.

*Material examined*

*Prosorhochmus chafarinensis* Frutos, 1998. Holotype and two paratypes MNHM 5.01/1. Additional specimens collected by SAM in Savudrija and Zabrata, Croatia, Adriatic Sea and identified as *P. cf. chafarinensis*: USNM 1020514 a series of transverse sections of anterior end, a series of longitudinal frontal sections of midbody and a series of longitudinal saggital sections of the posterior; USNM 1020515 and 1020517 two series of longitudinal frontal sections of anterior and posterior; USNM 1020516 a series of longitudinal saggital sections of anterior and posterior; USNM 1020518 and 1020519 two complete series of transverse sections. These and several unsectioned specimens coll. by SAM from Croatia deposited at the Smithsonian Institution's National Museum of Natural History in Washington D.C., USA.

*Diagnosis*

*Prosorhochmus chafarinensis* has no known morphological apomorphies. It differs from *Prosorhochmus nelsoni* and *Prosorhochmus belizeanus* sp. nov. in being viviparous and hermaphroditic (Figure 1F) and from *P. belizeanus* sp. nov. in

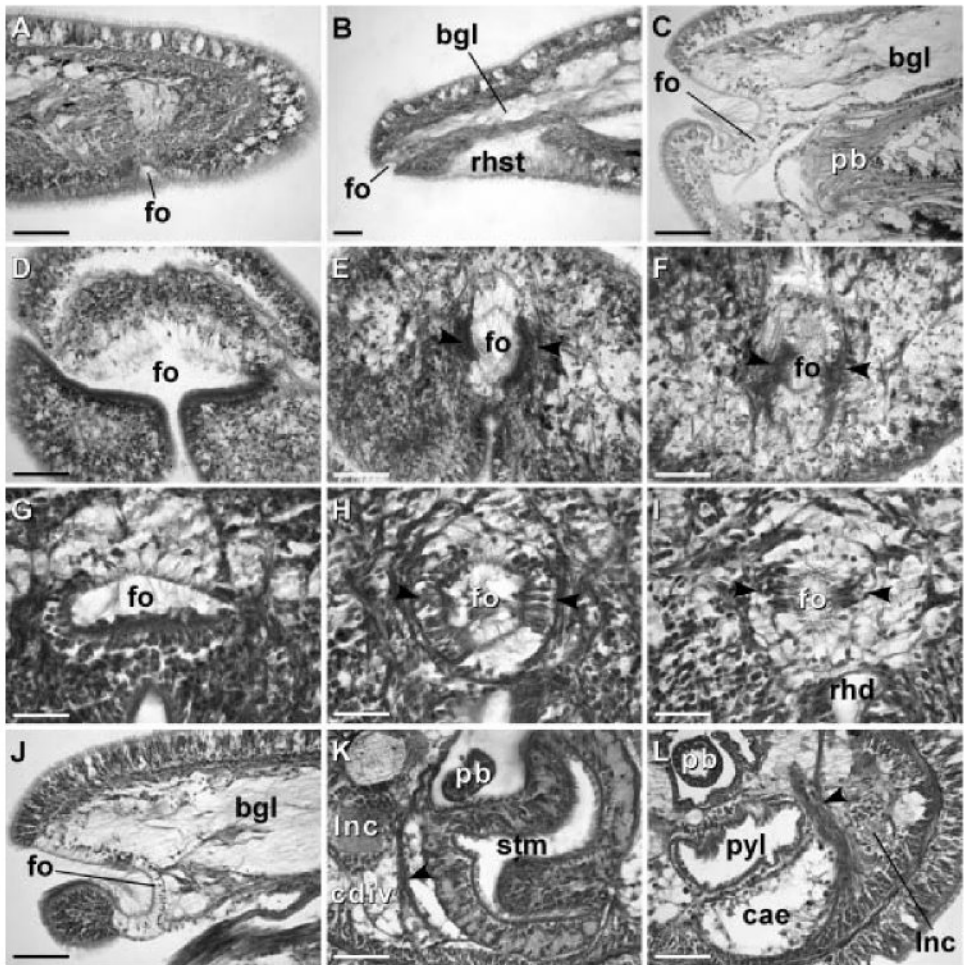


Figure 8. (A) Slightly oblique transverse section through frontal organ of *Arhochmus korotneffi* comb. nov.; (B) longitudinal sagittal section through frontal organ of *A. korotneffi*; anterior to the left; (C) longitudinal sagittal section through frontal organ of *Prosorhochmus americanus*; anterior to the left; (D–F) a series of transverse sections through the frontal organ of *P. americanus* from anterior to posterior showing lateral acidophilic regions of frontal organ (arrowheads); (G–I) a series of transverse sections through the frontal organ of *Prosorhochmus clapedii* showing lateral acidophilic regions of frontal organ (arrowheads); (J) longitudinal sagittal section through frontal organ of *P. clapedii*; anterior to the left; (K–L) transverse sections through stomach (K) and pylorus (L) of *P. clapedii* showing well-developed dorso-ventral muscles (arrowheads). Scales (A–F, J–L): 50  $\mu$ m; (G–I): 25  $\mu$ m. Abbreviations: bgl, basophilic cephalic glands; cae, caecum; cdv, caecal diverticulum; fo, frontal organ; lnc, lateral nerve cord; pb, proboscis; pyl, pylorus; rhst, rhynchostome; stm, stomach.

having acidophilic cephalic glands intermixed with the basophilic mucus cephalic glands in the pre-cerebral region (compare Figures 9C and 6A). It differs from *Prosorhochmus americanus* in lacking well-developed purple cephalic glands (compare Figures 9C and 9A) and in having but a single juvenile per ovary. It

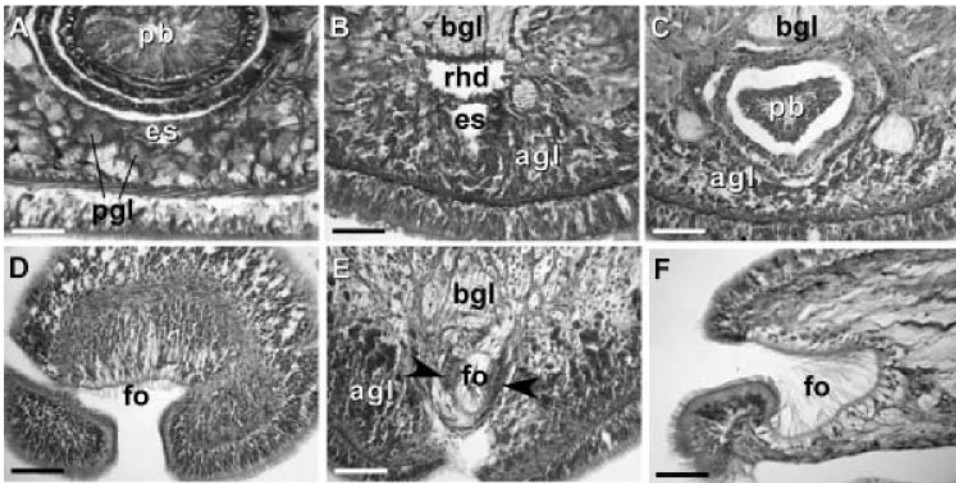


Figure 9. (A–C) Transverse sections through the precerebral region at the level of cerebral organs to show acidophilic, purple and basophilic cephalic glands: *Prosorhochmus americanus* (A), *Prosorhochmus claparedii* (B) and *Prosorhochmus chafarinensis* (C); (D–E) two slightly oblique transverse sections through the frontal organ of *P. chafarinensis*; (D) anterior opening into prosorhochmid smile; (E) middle portion of the canal; lateral acidophilic regions marked with arrowheads; (F) longitudinal sagittal section through frontal organ of *P. chafarinensis*. Scales: 50  $\mu\text{m}$ . Abbreviations: agl, acidophilic cephalic glands; bgl, basophilic cephalic glands; es, oesophagus; fo, frontal organ; pb, proboscis; pgl, purple cephalic glands; rhd, rhynchodeum.

differs from *P. adriaticus* in having a larger S/B ratio (0.52 on average compared to 0.25), but the data at hand are not sufficient to determine statistical significance. Central stylet (S) 80–130  $\mu\text{m}$  long (109.6  $\mu\text{m}$  average), significantly different from that of *P. belizeanus* sp. nov. but not *P. nelsoni* or *Prosorhochmus claparedii* ( $p=0.05$ ), basis (B) truncated, 136–260  $\mu\text{m}$  long (208  $\mu\text{m}$  average), significantly different from that of *P. nelsoni* and *P. belizeanus* but not *P. claparedii*; S/B ratio 0.38 to 0.65 (0.52 average), significantly different from that of *P. nelsoni* and *P. belizeanus* but not *P. claparedii* (Tables 2, 3). *Prosorhochmus chafarinensis* most closely resembles *P. claparedii* but differs from it by longer stylet and basis (see Tables 2, 3), although the data at hand are not sufficient to demonstrate statistical significance.

#### *Habitat and distribution*

On the encrusting alga *Lithothamnion lichenoides* on Isabel II Island (Chafarinas Island, 35°11' N, 2°25' W) (Frutos et al. 1998). Additional specimens collected by SAM from the coast of Adriatic Sea near Croatian coastal towns Savudrija and Zabratiya in the northwestern part of the Istrian Peninsula. In this location several individuals were found together on the moist sand under stones or on the lower surface of stones just above the low water mark at low tide (Figure 1A). The worms seemed to prefer medium size stones resting on the fairly coarse, somewhat muddy sand. Specimens in Croatia were associated with another viviparous hoplonemertean – *Arhochmus korotneffi* (Bürger, 1985) comb. nov.

*Remarks*

Specimens collected by SAM from the Adriatic coast of Croatia were slightly longer than *Prosorhochmus chafarinensis* from Chafarinas Islands but otherwise conformed to the species description. Stylet characteristics, measured on six specimens from Croatia were not statistically significant from the originally described for *P. chafarinensis* by Frutos et al. (1998) ( $p=0.05$ ). We pooled the measurements and adjusted the ranges and means accordingly in the species diagnosis.

As reported by Frutos et al. (1998), *Prosorhochmus chafarinensis* is different from other species of the genus in having 3–8 muscle fibres (instead of a single fiber) in the lateral nerve cords. Our observations over the years show that hoplonemertean typically have several muscle fibres associated with the lateral nerve cords. Often these fibres stick together giving the appearance of a single fiber, most likely due to fixation and subsequent dehydration of the tissues. We believe that this character has no taxonomic value. Similar to *Prosorhochmus claparedii*, *P. chafarinensis* is described as having a more complex frontal organ ending in a 90° bend compared to *Prosorhochmus americanus* (Gibson and Moore 1985, p.149, plate I, fig. e; Frutos et al. 1998). As we mentioned before, this is a misinterpretation. All *Prosorhochmus* species examined by us have frontal organ of a similar structure and complexity (see Figures 4C–D, 5A–C, 5G–H, 8A–J, 9D–F).

The differences between *Prosorhochmus chafarinensis* and *Prosorhochmus claparedii* are summarized by Frutos et al. (1998, p.297, Table 2). These include lack of dorso-ventral musculature in the foregut region of *P. claparedii*, and presence of the neural supply in the posterior chamber of proboscis of *P. claparedii*. Our reinvestigation challenges both assertions. We did not find distinct nerves on cross-sections of the posterior proboscis in *P. claparedii*. On the contrary, we found well-developed dorso-ventral muscles in the stomach and pylorus region in this species (see Figures 8K, L). Other suggested differences include absence of ciliation in the oesophageal epithelium of *P. claparedii*. We argue that this cannot be determined with confidence using standard light microscopy. Additionally, *P. chafarinensis* is supposed to differ from *P. claparedii* in lacking the postero-ventral stomach pouch. We observed that this character is intraspecifically variable in *Prosorhochmus belizeanus* sp. nov. and argue that it should not be used to distinguish between the species of *Prosorhochmus*.

To summarize, we found that *Prosorhochmus chafarinensis* is morphologically indistinguishable from *Prosorhochmus claparedii*, except for the difference in stylet (S) and basis (B) length and, possibly, S/B ratio. The respective stylet measurements of specimens from England used by Gibson and Moore (1985) in redescription of *P. claparedii* are nearly half the size of those of *P. chafarinensis* (see Tables II, III). The difference is somewhat obscured when our three specimens of *P. claparedii* from Roscoff, France are added to comparison. And since only the latter can be used for statistical comparisons, the measurements of the two species do not come out as significantly different. However, we believe that if more specimens of *P. claparedii* were available the difference would turn out to be significant. Furthermore, *P. claparedii* and *P. chafarinensis* can be distinguished molecularly. Sequence divergence between *P. cf. chafarinensis* (specimens from Croatia), and *P. claparedii* (material from the Atlantic coast of Spain and France) is 2.2% and 0.6% for 16S rDNA and COI, respectively (Table 4).

***Prosorhochmus claparedii*** Keferstein, 1862

(Figures 1E, 7E, 8G–L, 9B; Tables 1–4)

*Planaria flava* (Montagu 1808)

*Polia fumosa* (Quatrefages 1846; 1849)

*Nemertes fumosa* (Diesing 1850)

*Tetrastemma fumosum* (Diesing 1863)

*Prosorhochmus claparedii* (Keferstein 1862; Diesing 1863; McIntosh 1869; Bürger 1904; Campbell 1982)

*Prosorochmus claparedii* (Claparède 1863)

*Prosorhochmus claparedii* (McIntosh 1873–1874; Dewoletzky 1880, 1887; Chapius 1886; Sheldon 1896; Bürger 1897–1907; Gontcharoff 1955; Pantin 1969; Gibson 1972, 1982a, 1982b; Friedrich 1979; Anadón 1980; Gibson and Moore 1985; Gibson et al. 1986; Senz 1993; Frutos et al. 1998; Maslakova et al. 2005)

*Prosorochmus claparedii* (Czerniavsky 1880; Joubin 1890; Oxner 1907b)

*Phrosorochmus claparedii* (Joubin 1889), [*Lapsus calami*]

*Prosorochmus claparedii*

*Prosorochmus claparedei* (Joubin 1894), [*Lapsus calami*]

*Prosorhochmus claparedi* (Bürger 1895, 1897–1907; Friedrich 1936; Campbell 1976), [*Lapsus calami*]

*Prosorhochmus claparedi* (Friedrich 1955; Marine Biological Association 1957; Bruce et al. 1963), [*Lapsus calami*]

*Prosorhochmus claparedei* (Pantin 1961), [*Lapsus calami*]

*Etymology*

The species is named after Professor Jean Louis René Antoine Édouard Claparède, a Belgian zoologist, who originally drew attention to the species and provided illustrations of live specimens.

*Type material*

Type material was never designated and the original specimens are almost certainly lost.

*Material examined*

*Prosorhochmus claparedii* Keferstein, 1862. CMZ #A6, #A7, #CH1, “Ray J”, “Ray”. Coll. R. Gibson, Anglesey, England. Additional material: USNM 1020508, 1020509. Coll. SAM, Bilbao, Spain; USNM 1020510–1020513. Coll. SAM. Roscoff, France.

*Diagnosis*

*Prosorhochmus claparedii* does not possess any known morphological apomorphies. It differs from *Prosorhochmus nelsoni* (Sanchez, 1973) and *Prosorhochmus belizeanus* sp. nov. in being viviparous and hermaphroditic (Figure 1E) and additionally from *P. belizeanus* sp. nov. in having acidophilic cephalic glands intermixed with the basophilic mucus cephalic glands in the pre-cerebral and cerebral region (Figure 9B). It differs from *Prosorhochmus americanus* in lacking well-developed purple cephalic glands and in having but a single juvenile per ovary. *Prosorhochmus claparedii* closely resembles *P. chafarinensis*, however, they can be differentiated by stylets (although

the data at hand do not show them to be statistically significantly different) (see Tables 2, 3) and sequence data (Table 4). Central stylet (S) 30–95  $\mu\text{m}$  long, statistically different from that of *P. belizeanus* sp. nov. but not *P. chafarinensis* or *P. nelsoni* ( $p=0.05$ ), basis (B) truncated, 90–165  $\mu\text{m}$  long, statistically different from that of *P. belizeanus* sp. nov. but not *P. chafarinensis* or *P. nelsoni* ( $p=0.05$ ); S/B ratio 0.25–0.62, statistically different from that of *P. nelsoni* but not *P. belizeanus* sp. nov. or *P. chafarinensis* ( $p=0.05$ ), (see Tables 2, 3). At present *P. claparedii* cannot be distinguished from the insufficiently described *Prosorhochmus adriaticus* Senz, 1993.

#### *Habitat and distribution*

Typically found beneath stones and boulders on coarse sand or in rock fissures in the mid- to upper-shore zones, but can also be obtained by sublittoral dredging. In the Plymouth district of England it extends to crevices at the top of the *Pelvetia* belt, where it co-occurs with collembolans, myriapods, chernetids and gastropods transitional to land forms (Pantin 1969). Gregarious, with several specimens often found together under the same rock. Wembury and the Yealm, Plymouth; Trwyn Du Point, Anglesey (Gibson and Moore 1985); Port Sr. Mary in the Isle of Man (Bruce et al. 1963), Port Trefadog, Anglesey (Eason 1973); the southern shores of England and St. Peter Port, Guernsey (McIntosh 1873–1874) around the British Isles; from north of Tisaoson (ty-Zaoson) near Roscoff, Finistère (Chapius 1886; Gontcharoff 1955), Roscoff (Joubin 1890; SAM), Île Bréhat, Côtes-du-Nord (Quatrefages 1846), St. Vaast-la-Hougue, Manche (Quatrefages 1846; Keferstein 1862) and Le Portel, Pas-de-Calais (Joubin 1894) on the northern coast of France; San Esteban de Pravia, Asturias, (Anadón 1980) and in the vicinity of Bilbao, northern Spain (SAM). Near Trieste in the Adriatic Sea (Dewoletzky 1880), Naples in the Mediterranean (Bürger 1895); possibly Black Sea (Bürger 1895, Bürger 1904).

#### *Remarks*

We compared sections of the material on which Gibson and Moore (1985) based their redescription of the species and those of specimens collected by SAM from Bilbao, Spain and Roscoff, France and found them to be morphologically indistinguishable. However, we noted that the higher degree of development of the frontal organ reported by Gibson and Moore (1985, p. 153) for *P. claparedii* is apparently a result of misinterpreting a slightly oblique cross-section as a vertical longitudinal (i.e. sagittal) section of the frontal end (Gibson and Moore 1985, p. 149, plate I, fig. e). Our reinvestigation shows that the frontal organ of this species is similar in structure and shape to that of the other species of *Prosorhochmus* (see Figures 4C–D, 5A–C, 5G–H, 8A–J, 9D–F). Furthermore, after re-investigating all of the available material, we cannot confirm the reported presence of distinct nerves in the posterior proboscis chamber of this species (Gibson and Moore 1985, p. 151), transverse blood vessel connectives (Gibson and Moore 1985, p. 153) and the presence of the neurochord cells (Gibson and Moore 1985, p. 153). Originally defined by Bürger (1895, p. 355), neurochord cells represent a single pair of giant

neurons located in the inner portion of the ventral cerebral ganglia in the vicinity of the ventral commissure. Other large cells in the cerebral ganglia – most likely the type III neurons as defined by Bürger (1895, p. 320), have apparently been mistaken for neurochord cells by Gibson and Moore (1985). Stylet measurements of three specimens collected by SAM in Roscoff, France (close to the type locality) are as follows: central stylet (S) 80–95 µm long, basis (B) 130–165 µm long; S/B ratio ranging from 0.58 to 0.62 (0.60 on average). These are larger than the measurements reported by Gibson and Moore (1985) (see Table 2). However, because only ranges of variation are available and the number of specimens and average values are not known, it is not possible to determine whether this difference is statistically significant. Because of the geographical proximity of our collecting sites to the type locality and England (where Gibson and Moore's specimens came from) and the fact that our specimens are otherwise indistinguishable from the English *P. claparedii*, we assume that our specimens belong to the same species and that if more specimens were available the difference in stylet measurements would turn out to be insignificant. This is why we pool the measurements to adjust the ranges of variation in species diagnosis.

***Prosorhochmus nelsoni* (Sanchez, 1973)**

(Figure 7H, Tables 1–4)

*Amphiporus nelsoni* (Sanchez 1973)

*Prosorhochmus nelsoni* (Maslakova et al. 2005)

*Etymology*

Specific epithet is derived from the masculine name Nelson.

*Type material*

Neotype USNM 173164, coll. Malva Sanchez from the type locality; designated by Maslakova et al. (2005).

*Material examined*

*Prosorhochmus nelsoni* (Sanchez, 1973). USNM 173162–173164. Coll. M. Sanchez, Quintero, Chile; USNM 1019782, 1019784–6. Coll. M. Thiel, Coquimbo, Chile.

*Diagnosis*

*Prosorhochmus nelsoni* possesses no known morphological apomorphies. It differs from *Prosorhochmus claparedii*, *Prosorhochmus adriaticus*, *Prosorhochmus americanus* and *Prosorhochmus chafarinensis* by being gonochoric and oviparous (Sanchez 1973; Maslakova et al. 2005) and, additionally, from *P. americanus* in lacking well-developed purple cephalic glands (Maslakova et al. 2005, p. 495). Central stylet (S) 49–108 µm long (84 µm average) significantly different from that of *P. belizeanus* sp. nov. but not *P. chafarinensis* or *P. claparedii* ( $p=0.05$ ), basis (B) truncated, 74–132 µm long (108 µm average) significantly different from that of *P. belizeanus* sp. nov. and *P. chafarinensis*, but not *P. claparedii* ( $p=0.05$ ); S/B ratio 0.64–1.1 (average 0.79), significantly different from that of *P. chafarinensis* and *P. claparedii*, but not

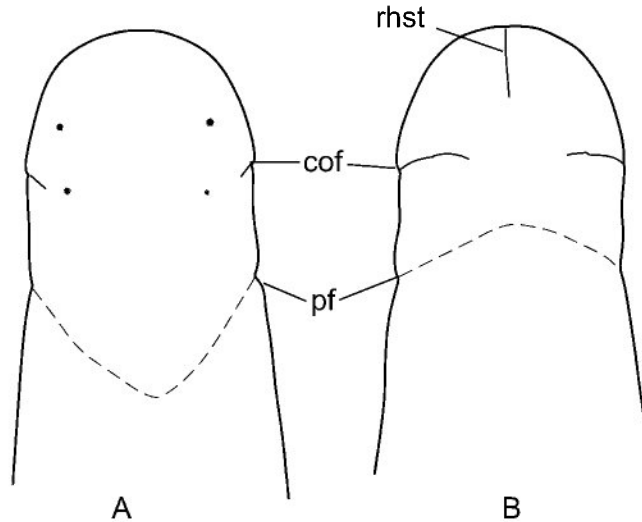


Figure 10. *Arhochmus korotneffi* comb. nov., diagram of anterior end. (A) Dorsal view; (B) ventral view. Abbreviations: cof, cerebral organ furrow; pf, posterior cephalic furrow; rhst, rhynchostome.

*P. belizeanus* sp. nov. ( $p=0.05$ ). *Prosorhochmus nelsoni* most resembles gonochoric and oviparous *P. belizeanus* sp. nov. but differs from it by having acidophilic cephalic glands intermixed with the basophilic mucus cephalic glands in the pre-cerebral and cerebral region, so that they do not form a compact cluster (Maslakova et al. 2005, Figures 3G–H, 6A–F).

#### *Habitat, type locality and distribution*

*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 (polychaetes, bivalves, and crustaceans, representing the most abundant groups; Thiel et al. 2001). *Prosorhochmus nelsoni* is reportedly the most common nemertean species in the rocky intertidal of the Chilean coast (Thiel, pers. comm.). It is commonly found during morning low tides, particularly under overcast conditions. 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 (18°29' S, 70°20' W) and Concepción (36°50' S, 73°03' W), Chile (Maslakova et al. 2005).

#### *Arhochmus* gen. nov.

##### *Type species*

*Prosorhochmus korotneffi* Bürger, 1895.



### *Etymology*

The name *Arhochmus* is formed by adding a negating “a” to a Greek *ρῶχος* (rhochos)=cleft, runnel or gutter. It refers to lack of the prosorhochmid smile in this genus, in contrast to *Prosorhochmus*. The Greek has been Latinized by changing the terminal “os” to “us”. The name is masculine in gender.

### *Diagnosis*

Monostiliferous marine intertidal hoplonemerteans with rhynchocoel extending full body length, with wall composed of distinct outer circular and inner longitudinal muscle layers. Four simple eyes. Cephalic lobe is somewhat narrower than adjacent body region in actively moving worms. Proboscis small with a single pair of accessory stylet pouches and rounded basis of central stylet. Body-wall musculature well-developed with a delicate layer of diagonal muscles between outer circular and inner longitudinal muscle layers. Body-wall longitudinal muscle layer is not anteriorly divided. Frontal organ represented by a small epithelial pit. Cephalic glands extensive and contain at least three components: vacuolated basophilic mucus glands, finely granular acidophilic proteinaceous glands staining pink to dark red and coarsely granular proteinaceous glands staining yellow, golden brown or deep orange with Mallory trichrome or its modifications (orange-G glands). Cerebral sensory organs small with unforked canal, anterior or antero-lateral to brain, opening ventro-laterally into simple oblique cerebral organ furrows. Neurochord cells and neurochords absent. Lateral nerve cords without accessory nerve. Oesophagus lacking acidophilic glands. Caecum long, may be anteriorly bifid, with several lateral diverticula on each side. Anterior caecal diverticula reach posterior portion of brain. Blood system with three main longitudinal vessels, without transverse connectives. Mid-dorsal blood vessel with single vascular plug; cephalic blood loop planar (not recurved). Extracellular matrix of the so-called parenchyma scarce. Excretory system restricted to foregut region, with mononucleate terminal nephridial cells without distinct support bars, with thick-walled canals and up to six pairs of nephridiopores. Viviparous hermaphrodites.

### *Composition*

Currently contains but a single species *Arhochmus korotneffi* (Bürger, 1895).

### *Geographic distribution*

Currently only known from the Mediterranean coast of France (near Villefranche-sur-Mer) and the Adriatic coast of Croatia (near Savudrija and Zabratajka).

*Arhochmus korotneffi* (Bürger, 1895) comb. nov.

(Figures 1A, 1D, 1G, 7C–D, 8A–B, 10; Tables 1, 4)

*Prosorhochmus korotneffi* (Bürger 1895, 1897–1907, 1904; Oxner 1907a, 1907b; Friedrich 1955; Gibson 1982b, 1995)

*Prosorhochmus claparedii* (Gibson and Moore 1985)

### *Etymology*

The species is named after Professor Korotneff, who first drew attention to the species and provided live and preserved specimens for description.

### *Type material*

The type material was never designated and original specimens have almost certainly been lost. None of Bürger's material, except two or three specimens, could be found, after exhaustive search efforts in likely European museums. In lieu of an available original type and with the purpose to clarify the taxonomic position of this species, we hereby designate a series of cross-sections of a gravid individual collected by SAM on Adriatic coast near Croatian coastal town Zabratiya as a neotype. The neotype is deposited in the Smithsonian Institution's National Museum of Natural History in Washington D.C., USA (USNM 1020575) together with two additional sectioned (USNM 1020573, 1020574) and four unsectioned (USNM 1025341) specimens collected from the same locality in Croatia.

### *Material examined*

*Arhochmus korotneffi* (Bürger, 1895). USNM 1020573-1020575, 1025341. Coll. SAM, Zabratiya, Croatia.

### *Habitat and distribution*

Intertidal to shallow sublittoral near Villefranche-sur-Mer, Provence, on the Mediterranean coast of France (Bürger 1895). Additional specimens were collected by SAM from the coast of the Adriatic Sea near the Croatian coastal town Zabratiya, in the north-western part of the Istrian Peninsula. Several individuals were found together crawling on the wet sand under the stones or on the lower surface of the stones just above the low water mark at low tide. The worms seem to prefer medium-size stones resting on the fairly coarse, somewhat muddy sand (Figure 1A). Found in the same habitat as another viviparous hoplonemertean – *Prosorhochmus chafarinensis*.

### *Remarks*

*Arhochmus korotneffi* (Bürger, 1895) is a viviparous, hermaphroditic intertidal species from the European coast of the Mediterranean Sea. Adults reach 60–70 mm in length and 1–1.5 mm in width. Body dorso-ventrally flattened, slender, tapering toward both ends. Colour in life is pinkish-orange, lateral margins of the body somewhat translucent, rhynchocoel appearing as a mid-dorsal stripe of deeper orange (Figure 1G). Only four eyes have been observed in Croatian specimens (Figure 1D), although Oxner (1907a) reported that there are occasionally five to seven eyes. Brooded young show through the body wall of the mature individual as large opaque, lighter-coloured areas between the intestinal diverticula (Figure 1G). Proboscis with 12 proboscis nerves. Nephridia open via multiple distinct nephridiopores (up to six pairs). Central stylet (S) 90–110 µm long, basis (B) pear-shaped, rounded, 115–170 µm long; S/B ratio 0.65–0.83 (average 0.76) (Figures 7C–D).

Despite the differences in head shape and stylet armature Gibson and Moore (1985) synonymized *Prosorhochmus korotneffi* Bürger, 1895 with *Prosorhochmus claparedii* Keferstein, 1862, arguing that these characters may be intraspecifically variable. We have never observed these characters to vary intraspecifically in *Prosorhochmus* or other nemerteans. Moreover, our specimens from Croatia fit Bürger's (1895) description of *Prosorhochmus korotneffi* exactly. Our observations of live specimens and serial histological sections suggest that *P. korotneffi* is so morphologically different from *P. claparedii* and other *Prosorhochmus* that its inclusion in the genus is unwarranted. Unlike the true *Prosorhochmus*, *P. korotneffi* has a narrow head (vs. broad, bifid) without a prosorhochmid smile (Figures 1D, 1G, 10A–B), poorly developed frontal organ without lateral specialization of the epithelium of the frontal organ canal (Figures 8A–B), several pairs of nephridiopores (vs. single pair) and, finally, a pear-shaped (vs. the typical truncated) central stylet basis of other *Prosorhochmus* (Figures 7C–D). Juveniles of *P. korotneffi* are whitish in contrast to the yellowish-orange juveniles in *P. claparedii* and *Prosorhochmus chafarinensis*. The average sequence divergence between *P. korotneffi* and other *Prosorhochmus* species is about twice as much as between the other species of *Prosorhochmus*: 15.2% and 13.7% for 16S rDNA and COI, respectively (Table 4). All of the above suggests that *P. korotneffi* is not only distinct from *P. claparedii* but that it is not a true *Prosorhochmus*, despite its viviparity. Urged by our colleagues, we create a new monotypic genus *Arhochmus* gen. nov. for *Prosorhochmus korotneffi*.

***Tetrastemma albidum* Coe, 1905**

*Tetrastemma albidum* (Coe 1905a, 1905b)

*Prosorhochmus albidus* (Coe 1940, 1944; Friedrich 1955; Corrêa 1964; Gibson 1982b, 1995; Gibson and Moore 1985)

*Etymology*

The specific epithet refers to the milky-white colour of live specimens.

*Type material*

Type material had not been designated. One specimen found in Coe's collection and identified as *Prosorhochmus albidus* by Coe was sectioned by Frank Crandall (Washington, D.C.) and is currently deposited with the Smithsonian Institution's National Museum of Natural History, Washington, D.C., USA (USNM 126765).

*Material examined*

"*Prosorhochmus*" *albidus* – series of transverse sections, USNM 126765; additional material: numerous live specimens coll. by SAM and JLN at Bird Rock, near La Jolla, CA, USA (*Tetrastemma* sp. 644, deposited at the National Museum of Natural History in Washington D.C., USA).

*Habitat and distribution*

Roots of sea grass, kelp holdfasts and coralline algae in the intertidal zone between Monterey Bay, California, USA and Ensenada, Mexico.

*Remarks*

*Tetrastemma albidum*, currently referred to as *Prosorhochmus albidus* (Coe, 1905), is a small milky-white or cream-coloured four-eyed hoplonemertean reaching maximum length of 25 mm, found in the intertidal zone between Monterey Bay, California and Ensenada, Mexico. The species was originally described as *Tetrastemma albidum* (Coe 1905a) and subsequently transferred to *Prosorhochmus* because it appeared to be occasionally viviparous, i.e. while most specimens were oviparous, some retained fertilized eggs within the ovaries until juveniles were formed (Coe 1940). Gibson and Moore (1985) commented that *P. albidus* was insufficiently described to determine its taxonomic status, however, Gibson (1995) included *P. albidus* as a valid species. Several characters described and illustrated by Coe (1905a, p. 294, pl. 17, figs 104–105, pl. 22, figs. 145–149; 1940, p. 294, pl 25, fig. 20) make prosorhochmid affinity of this species doubtful: the head is narrow, not anteriorly bifid and lacks a prosorhochmid smile, basis of the central stylet is rounded (compared to truncated bases of all other *Prosorhochmus*) and, finally, cerebral organs are large and extend ventro-laterally to the posterior portion of brain (compared to small cerebral organs, located anteriorly to brain in true *Prosorhochmus*).

We collected numerous specimens of small four-eyed cream-coloured hoplonemerteans from the roots of sea grass, kelp holdfasts and coralline algae in January 2002 at Bird Rock, La Jolla, CA, which appear to fit Coe's description (1905a). Specimens collected by us were 10–15 mm long, whitish or cream-coloured, with a typical tetrastemma-like narrow head without anterior notch or a prosorhochmid smile; and base of the central stylet was rounded. These specimens also resembled Coe's description (1905a, 1940) by having a colour pattern on the head formed by diffuse brownish pigment spots. This pattern was quite variable: two pigment spots covering the anterior eyes, sometimes connected; four pigment spots – one over each eye, anterior spots connected, while posterior not; posterior spots connected while anterior not; anterior pair of eyes entirely covered with pigment in addition to diffuse pigment lines connecting anterior and posterior eyes of each side. Our specimens fixed for histology are deposited at the Smithsonian Institution's National Museum of Natural History (*Tetrastemma sp.* 644). If it is Coe's *Prosorhochmus albidus*, it definitely does not belong to *Prosorhochmus*, based on the characters of external appearance and stylet apparatus.

Another line of evidence supporting non-prosorhochmid affinity of *Prosorhochmus albidus* comes from investigation of material that was found in Coe's collection and identified as *P. albidus* by Coe. This material was sectioned by Frank Crandall and is currently deposited at the Smithsonian Institution's National Museum of Natural History (USNM 126765). The lack of well-developed frontal organ, lack of lateral differentiation of frontal organ epithelium and lack of prosorhochmid smile; poorly developed basophilic mucus cephalic glands; lack of orange-G glands; and, finally, large cerebral organs situated postero-lateral to brain

indicate that this species does not belong to *Prosorhochmus* or the family Prosorhochmidae.

We are urged by colleagues not to return *P. albidus* to its original home, the potpourri that is *Tetrastemma*. However, until there is an acceptable revision and diagnosis of *Tetrastemma*, it cannot be determined that *T. albidum* would not properly be associated with the designated type species of the genus. Until such time, there is no increased functional benefit to erecting what would be a monotypic genus of convenience – we know as little about systematic affiliation either way. We believe it is best to re-establish the original combination *Tetrastemma albidum* for *P. albidus*.

### Discussion and conclusions

*Prosorhochmus* is the only fully marine genus of the family. Currently, it includes five species: the three European viviparous hermaphrodites *Prosorhochmus claparedii* Keferstein, 1862, *Prosorhochmus adriaticus* Senz, 1993 and *Prosorhochmus chafarinensis* Frutos et al., 1998, the North American viviparous hermaphrodite *Prosorhochmus americanus* Gibson et al., 1986 and the oviparous gonochoric *Prosorhochmus nelsoni* (Sanchez, 1973) from the coast of Chile (Maslakova et al. 2005).

We describe another oviparous species with separate sexes from Belize and Florida – *Prosorhochmus belizeanus* sp. nov., which appears to be as distantly related to its Pacific counterpart *Prosorhochmus nelsoni* as to the viviparous hermaphroditic *Prosorhochmus* according to the sequence data from 16S and COI mitochondrial genes. The average sequence divergence between *P. belizeanus* sp. nov. and other *Prosorhochmus* is 7.98% (16S) and 10.03% (COI). The differences from its nearest sequenced congener *P. nelsoni* are 7.4% and 9.1%, respectively. The two gonochoric oviparous species are widely separated geographically. The most apparent morphological difference between them is their stylet armature. *P. belizeanus* sp. nov. has a significantly longer central stylet and basis compared to *P. nelsoni*. There is another, more subtle, anatomical difference between the new species and all the other *Prosorhochmus* – the acidophilic proteinaceous cephalic glands form a very compact cluster in the ventral precerebral region in *P. belizeanus* sp. nov. In other species these glands tend to intermix with the basophilic mucus lobules. This, obviously, is a less objective and harder to quantify character, which requires preparation of histological sections and direct comparison of sectioned material from other species. Reproductive characters and stylet armature can be observed on living specimens and only require a standard light microscope with an ocular micrometer.

To provide a proper comparison of *Prosorhochmus belizeanus* sp. nov. to the previously described species we obtained all the available type and voucher material of the previously described species. More often than not, the voucher specimens are not in ideal condition; for example, the worms may not have been relaxed prior to fixation, sections may be missing or the staining is faded. To ensure the best results, we recollected fresh specimens as close to the type locality as possible, prepared serial histological sections and obtained partial sequences of mitochondrial genes 16S and COI of all previously described species, except *Prosorhochmus adriaticus*.

One of the main conclusions we reached upon investigating all of the available material is that the species of *Prosorhochmus* are not nearly as different from each other as one used to believe based on the species descriptions. This is probably because descriptions of new species are often prompted by discovery of what generally looks like a species of the genus from a new geographical location and the morphological differences are added as an afterthought. The phylum Nemertea is a classical morphology-poor taxon. Nemerteans are soft bodied worms and have very few or no parts that can be easily measured, and few external features. The difference between closely related species can be very subtle (e.g. the way the worm moves, shape of its body or colour) and although apparent to specialists intimately familiar with the appearance of the species in question, it can be very difficult to describe in definitive terms. To find enough characters to differentiate between closely related species nemertologists traditionally rely on characters of internal anatomy reconstructed from serial histological sections. This requires a considerable amount of expertise both in preparation of histological sections and, more important, in interpreting the results. Because it is a time consuming business, few complete series are usually studied and the extent of intra-specific variation of the characters in question is not known. Moreover, to save time, many nemertologists often rely on published descriptions instead of making direct comparisons with type and voucher material of other species, thus propagating the mistakes in the literature. Subtle differences and artifacts are often overinterpreted to emphasize the species distinctiveness. In this light, molecular data, such as 16S and COI mitochondrial gene sequences, become invaluable for differentiating between closely related and morphologically indistinct species.

While comparing the type and voucher material of all species of *Prosorhochmus* it became apparent to us that most of the species can only be reliably distinguished based on reproductive characters and sequence data. The only morphological character that can be evaluated objectively and requires little time and expertise is the proboscis armature, i.e. the length of the central stylet (S), length of basis of central stylet (B) and the S/B ratio. The problem remains that for the most species there are not enough measurements to evaluate the degree of intra-specific variation and make statistical comparisons to other species. In a few cases where the data are available it appears that variation in S, B and S/B can be statistically significant between species of *Prosorhochmus*.

After debunking the majority of the characters of internal anatomy previously used in prosorhochmid systematics, we are left with the following impressions. There are two groups of species within *Prosorhochmus* easily distinguishable by reproductive strategy. The first includes gonochoric and oviparous *Prosorhochmus nelsoni* (Sanchez, 1973) and *Prosorhochmus belizeanus* sp. nov. The second includes the four viviparous hermaphroditic species *Prosorhochmus claparedii* Keferstein, 1862, *Prosorhochmus americanus* Gibson et al., 1986, *Prosorhochmus adriaticus* Senz, 1993 and *Prosorhochmus chafarinensis* Frutos et al., 1998. While the species within the first group appear well separated geographically, morphologically and molecularly, species within the second group are all very similar to one another morphologically and sequence-wise.

Among the viviparous hermaphroditic *Prosorhochmus*, the only species widely separated geographically is the New World *Prosorhochmus americanus*. Apparently, this is also the only species that can bear more than a single developing juvenile per

ovary, although it is not clear whether this character exhibits intra-specific variation. In contrast to all the other congeners *Prosorhochmus americanus* has an additional type of cephalic glands – the so-called purple cephalic glands. However, these are only discernible on sections stained with polychromatic techniques. Although we accept this character as potentially useful for distinguishing between species of *Prosorhochmus* it is rather a subtle one and can be easily misinterpreted, because staining procedures for sectioned material are not standardized, colour perception varies between people and it is still difficult and expensive to publish illustrations in colour.

Interestingly, partial sequences of 16S and COI mitochondrial genes of *P. americanus* turned out identical to those of *Prosorhochmus claparedii*, a northern European species. There may be two reasons why this is so. Either these two species are really the same (and the number of juveniles per ovary and presence of purple cephalic glands are intra-specifically variable) or the two species somehow managed to cross-hybridize across the Atlantic Ocean and the mitochondrial genome of one had taken over. Future studies might be able to solve this problem by determining the variability of morphological characters in question and obtaining sequences of nuclear genes for both species.

The three European viviparous hermaphroditic species *Prosorhochmus claparedii*, *Prosorhochmus adriaticus* and *Prosorhochmus chafarinensis* present another challenge for a taxonomist. The morphological difference between the three is very small if any. Our study shows that *P. adriaticus* is insufficiently described and at present cannot be distinguished from *P. claparedii*. Our attempts to recollect *P. adriaticus* from its type locality (Venice, Italy) or nearby coastal areas (Adriatic coast of Italy and Croatia) yielded two viviparous hermaphroditic hoplonemertean species.

One was identified by us as *Prosorhochmus korotneffi* Bürger, 1895, previously synonymized with *P. claparedii* by Gibson and Moore (1985). The outright non-prosorhochmid appearance of *Prosorhochmus korotneffi* (e.g. no smile!), shape of stylet armature and structure of frontal organ indicate that synonymization with *Prosorhochmus claparedii* is unjustified. We have performed due diligence in studying *P. korotneffi* and its potential systematic relationship to other *Prosorhochmus* species. We conclude that a diagnosis of *Prosorhochmus* that would permit inclusion of *P. korotneffi*, under either ICZN or PhyloCode principles, becomes relatively uninformative and dilutes understanding of the special relationship shared by remaining species of the genus. We are urged by colleagues whom we respect to move *P. korotneffi* to a new or different genus. At present we have no morphological or molecular justification to move *P. korotneffi* into any other hoplonemertean genus. Hence, we bow to convention and erect a new genus and combination, *Arhochmus korotneffi*, for *P. korotneffi* to avoid confusion about membership of *Prosorhochmus*. DNA sequence data suggest that *A. korotneffi* comb. nov. is twice as genetically distant from *Prosorhochmus* as the species of *Prosorhochmus* are from each other, and suggest a possible sister relationship between *Arhochmus* and *Prosorhochmus* (Maslakova 2005). It also is about 15% different for 16S rDNA sequence (unpublished obs.) from *Cyanophthalma obscura* (Schultze, 1851), another viviparous distromatonemertean, reported from the Baltic Sea, the northwest Atlantic coast, and the Black Sea (Norenburg 1986). However, *A. korotneffi* much more closely resembles *Prosorhochmus* species in post-cerebral distribution and types of cephalic glandular cells.

The other viviparous species SAM collected from the Adriatic coast of Croatia is a true *Prosorhochmus*. Internal anatomy of these specimens corresponds to the description of *Prosorhochmus chafarinensis* Frutos et al., 1998 and the stylets are not significantly different from those of *P. chafarinensis* from Chafarinas Islands. It is particularly the S/B ratio that led us to conclude our specimens do not belong to *Prosorhochmus adriaticus* as the average S/B ratio of our specimens is 0.53 (n=6), more than twice 0.25, the S/B ratio reported for *P. adriaticus* (Senz 1993). *Prosorhochmus chafarinensis* has larger stylet, basis and S/B ratio compared to that reported for *P. claparedii* by Gibson and Moore (1985), but not our *P. claparedii* from Roscoff, France. Sequence data revealed that our Adriatic *P. chafarinensis* are different, albeit only slightly, (2.2% and 0.6% for 16S and COI, respectively), from the specimens collected by us in northern Europe (Roscoff, France and near Bilbao, Spain) and identified as *P. claparedii*.

This leaves the three species rather in a tangle and it is not clear whether all three European *Prosorhochmus* represent distinct species. However, we cannot exclude the possibility that there are three distinct populations or species of European *Prosorhochmus*. To solve this riddle it would really help to obtain sequence data from the *Prosorhochmus chafarinensis* from Chafarinas Islands, more sequences and stylet measurements from *Prosorhochmus claparedii* (particularly from England) as well as fresh material of *Prosorhochmus adriaticus* from Venice to properly measure proboscis armature, make new serial sections and obtain sequence data.

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