

# A new species of *Prosorhochmus* (Hoploneurtea, Monostilifera) from the Chafarinas Islands (western Mediterranean)

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## Abstract

A viviparous hoploneurtean, *Prosorhochmus chafarinensis* sp. nov., is described from the Chafarinas Islands (western Mediterranean). The species lives in the low eulittoral under the encrusting alga *Lithophyllum lichenoides*. *Prosorhochmus chafarinensis* is compared and contrasted in detail with the other valid members of the genus, *P. claparedii* from European waters, *P. americanus* from South Carolina, and *P. adriatica* from Italy.

**Key words:** Nemertea, *Prosorhochmus chafarinensis*, new species, western Mediterranean

## INTRODUCTION

During an investigation of the benthic invertebrate fauna in the Chafarinas Islands (western Mediterranean) in 1991 and 1992, a large number of nemerteans was collected. Among these worms, several specimens of a new species were found. Morphological features of the specimens reveal that the nemerteans belong to the genus *Prosorhochmus* Keferstein, 1862. Gibson & Moore (1985) redescribed *Prosorhochmus claparedii* Keferstein, 1862 and at the same time redefined the genus, considering *P. delagei* Oxner, 1907 and *P. korotneffi* Bürger, 1895 as synonymous with *P. claparedii*, *P. viviparus* (Uljanin, 1870) and *P. albidus* (Coe, 1905) as inadequately described forms, and excluding *P. subterraneus* Friedrich, 1949 from this genus. Two additional *Prosorhochmus* species have since been described: *P. americanus* Gibson, Moore, Ruppert and Turbeville, 1986 by Gibson *et al.* (1986) from South Carolina and *P. adriatica* Senz, 1993 by Senz (1993) from Italy. The present form differs from these species in several respects, and is considered new for science. Here, we describe the new species *Prosorhochmus chafarinensis* and compare it with the other three valid members of the genus.

## MATERIAL AND METHODS

Specimens were collected by JJ on 17 September 1992 from the encrusting alga *Lithophyllum lichenoides* from

Isabel II Island (Chafarinas Islands, 35°11'N, 2°25' W). *L. lichenoides* is common in the low eulittoral exposed zones from the north of the archipelago, together with turf of *Rissoella verruculosa* and *Nemalion helmintoides*. Characteristic eulittoral fauna are the barnacle *Chthamalus stellatus*, the limpets *Patella aspera*, *P. rustica* and *P. ferruginea*, the topshells *Monodonta turbinata*, *M. articulata* and *Gibbula* sp. and the crabs *Pachygrapsus marmoratus* and *Eriphia verrucosa*. The nemerteans were examined alive both before and after anaesthetization in 7.5% MgCl<sub>2</sub>. Seven specimens were fixed in Bouin's fluid, embedded in 56°C m.p. paraffin wax, sectioned at 6 µm and stained with the Mallory triple method (Pantin, 1960).

## OBSERVATIONS

### Genus *Prosorhochmus* Keferstein, 1862

#### Diagnosis

Monostiliferous hoploneurteans with shallow lateral or ventrolateral cephalic furrows; four eyes, the anterior pair usually larger than the posterior pair; rhynchocoel reaching to posterior tip of body, with wall composed of two distinct muscle layers; proboscis large but not employed for locomotion; dermis distinct but thin; body wall musculature well developed; frontal organ a single exceptionally well-developed tubular ciliated canal opening from the tip of the head; cephalic glands extensive, posteriorly reaching far behind cerebral ganglia and containing three types of secretory cells; cerebral sensory organs small, anterior to brain; nervous

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system with neurochord cells, without accessory lateral nerve; blood vascular system with three longitudinal vessels, without capillary network, with distinct valves, mid-dorsal vessel with one vascular plug; excretory system restricted to foregut region, with mononucleate flame cells which lack cuticular support bars; intestinal caecum present, anteriorly reaching almost to brain; hermaphroditic, occasionally or invariably ovoviviparous (Gibson & Moore, 1985).

*Prosorhochmus chafarinensis* sp. nov.

*Type specimens*

Type material deposited with the Museo Nacional de Ciencias Naturales, Madrid, consists of complete series of stained sections; Registration Number is MNCN 5.01/1 (holotype and two paratypes).

*Type locality*

Isabel II Island, Chafarinas Islands, 35°11' N, 2°25' W.

*External appearance*

Anaesthetized animals 5–15 mm long, <1 mm wide, body tapering near tail to end in a blunt tip. Bilobed head, not demarcated from body, bearing anteriorly a single pair of shallow lateral cephalic furrows. Four eyes in front of the cerebral ganglia, anterior pair being larger than posterior pair (Fig. 1a).

The colour is typically orange, with black eyes; the cephalic and lateral body margins are often almost transparent. Some specimens have narrow bands and spots of dark brown pigment extending along the lateral body margins.

*Body wall and musculature*

The epidermis is mostly 20–50 µm thick and of a typical hoplonemertean construction; rhabditous glands, however, are limited to the anterior dorsal epidermis. The epidermis is internally bordered by a thin (2–4 µm) connective tissue dermis. Body wall muscles comprising circular and inner longitudinal layers, respectively, 4–10 µm and up to 30 µm thick (Fig. 2a). Dorsoventral muscles appear just behind the cerebral ganglia, in the stomach region, and extend across the body between gonads and/or lateral nerve cords and intestinal diverticula (Fig. 2a, c). Oblique and tangential muscle fibres pass between the pre- and post-cerebral lobes of the cephalic glands.

The oesophagus is enclosed by numerous longitudinal muscle fibres; these fibres also run adjacent to stomach epithelium, forming a splanchnic muscle layer (Fig. 2b, c).



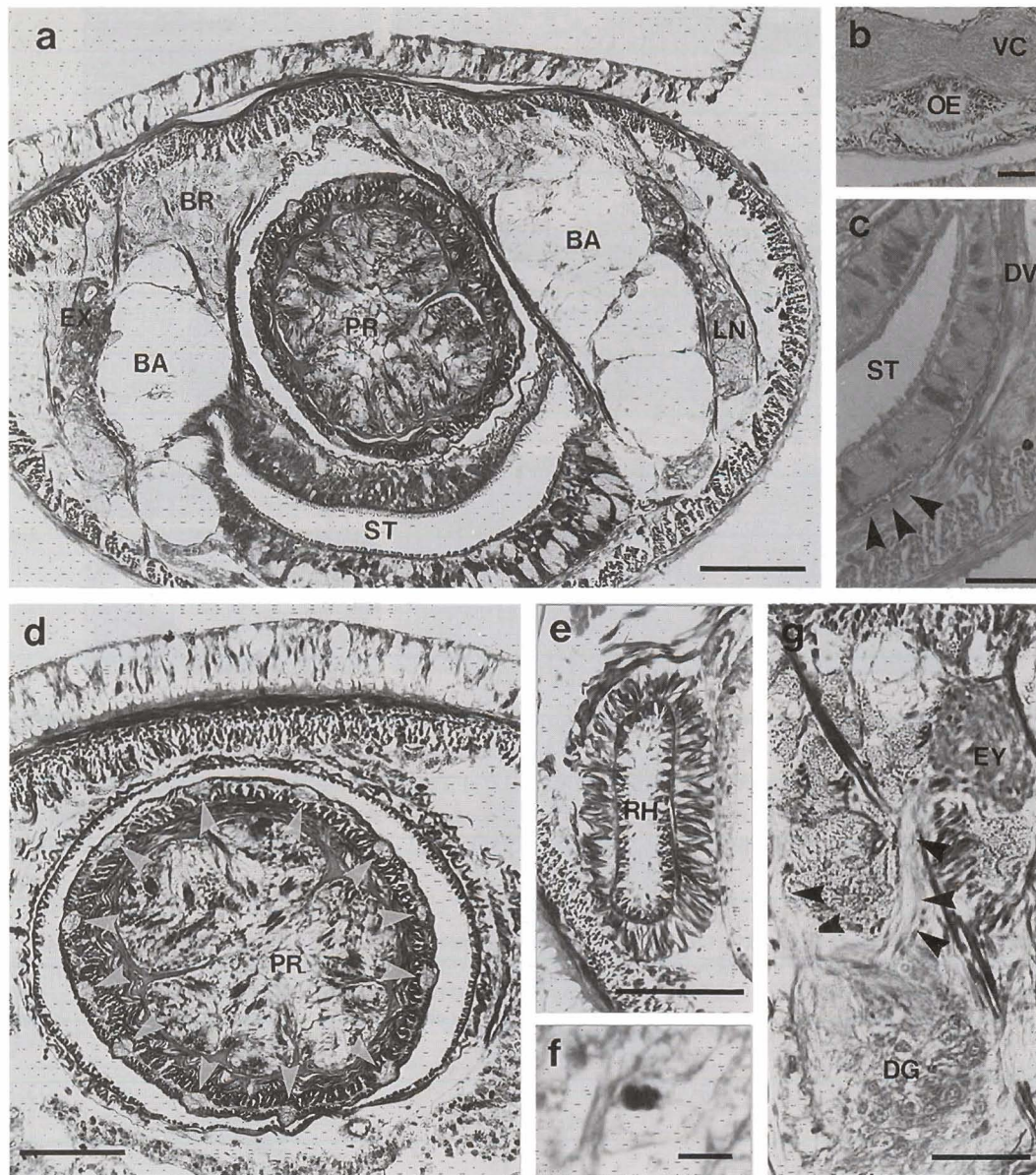
Fig. 1. *Prosorhochmus chafarinensis* sp. nov. (a) Drawing of anaesthetized animal to show the general external appearance in dorsal aspect. (b) Drawing of the central stylet and basis of a 5 mm length specimen. Scale bars: (a) = 1 mm; (b) = 50 µm.

*Proboscis apparatus*

The proboscis pore is situated anteroventrally, near the tip of the head, just below the medioventral chamber of frontal organ. The rhynchodaeum is lined by a thin (5 µm thick) epithelium lacking both cilia and glands. Some longitudinal muscle fibres surround the rhynchodeum.

Rhynchocoel reaching posterior tip of body; wall with separate circular and longitudinal muscle layers, together about 10 µm thick. Some muscular fibres from body wall add to these muscle layers in pyloric region; these muscle fibres with a zigzag arrangement are at first dorsal and lateral, but posteriorly form a continuous layer (up to 30 µm thick) in the intestinal region (Fig. 2e). Some specimens carry gregarine sporozoans in rhynchocoel fluid (Fig. 3f).

Proboscis diameter up to 350 µm. Anterior chamber wall composed of a glandular epithelium 70–130 µm across, an outer circular muscle layer (5 µm thick) surrounded on both sides by connective tissue, a longitudinal muscle coat 20 µm wide with 10 to 12 proboscis nerves, a thin inner circular muscle layer and a slender endothelium (Fig. 2d). Proboscis armature a single central stylet borne on a cylindrical basis (Fig. 1b), with two accessory stylet pouches each containing two or three reserve stylets. Measurements of the stylet

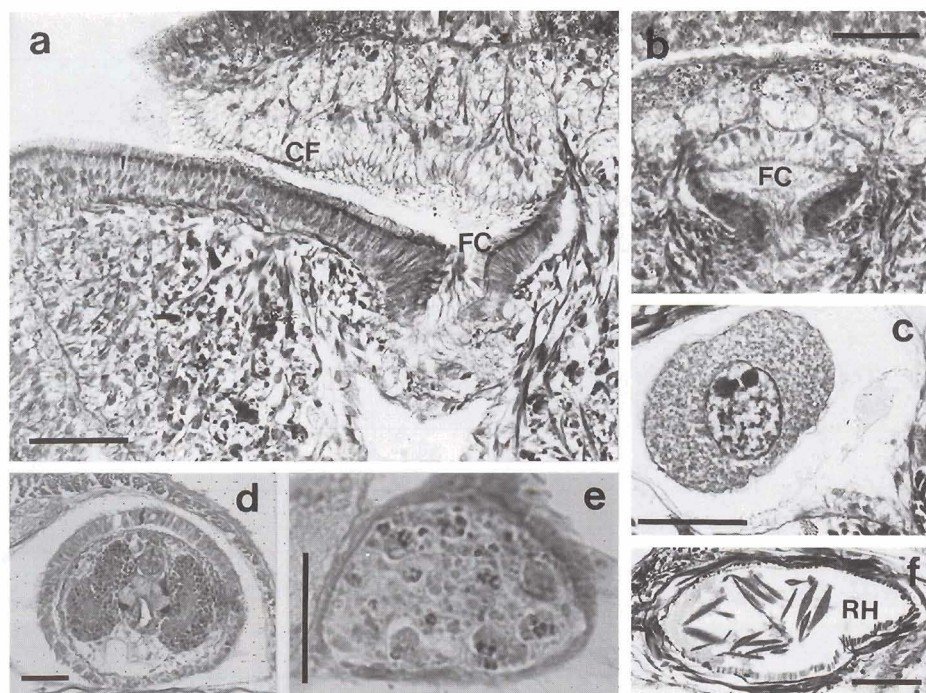


**Fig. 2.** *Prosorhochmus chafarinensis* sp. nov. (a) Transverse section through the stomach region to show the post-cerebral extension of the cephalic glands and the general organization of this body region. The epidermis has separated from the underlying dermal layer. (b) Transverse section through the cerebral ganglia at the level of the ventral cerebral commissure to show the longitudinal muscle fibres adjacent to the oesophagus. (c) Part of the stomach in transverse section to show the longitudinal splanchnic muscles (arrowed) and the dorsoventral muscle fibres. (d) Transverse section to show the organization of the anterior portion of the proboscis. Arrowheads indicate the proboscis nerves. (e) Transverse section through the rhynchocoel wall in the intestinal region. (f) Transverse section through the lateral nerve cord to show the myofibrillae. (g) Transverse section through the dorsal cerebral ganglia to show two cephalic nerves (arrowed). Abbreviations: BA, basophilic cephalic glands; BR, brown cephalic glands; DG, dorsal ganglionic lobe; DV, dorsoventral muscle fibres; EX, excretory tubules; EY, eye; LN, lateral nerve cord; OE, oesophagus; PR, proboscis; RH, rhynchocoel; ST, stomach; VC, ventral cerebral commissure. Scale bars: (a, d) = 20 µm; (b, c, e, g) = 10 µm; (f) = 1 µm.

apparatus recorded for five specimens are shown in Table 1. Wall of posterior proboscis chamber composed of a glandular epithelium, delicate outer longitudinal and inner circular muscle layers and a thin endothelium; no proboscis nerves can be distinguished.

#### *Alimentary canal*

Oesophagus, with ciliated epithelium in posterior portion, emerging from rhynchodaeum just in front of pre-cerebral septum, opening into the stomach. Folded



**Fig. 3.** *Prosorhochmus chafarinensis* sp. nov. (a) Vertical longitudinal section through the tip of the head to show the structure of the frontal organ. (b) Transverse section through the frontal organ chamber. (c) Transverse section through a gonad containing an egg. (d) Transverse section through an embryo within a gonad. (e) Transverse section through the intestinal region to show the accumulation of pigment granules in a regression gonad. (f) Transverse section through the rhynchocoel in the intestinal region to show gregarine sporozoans. Abbreviations: CF, horizontal canal of frontal organ; FC, frontal organ chamber; RH, rhynchocoel. Scale bar (a–f): 10  $\mu$ m.

**Table 1.** Measurements recorded for the stylet apparatus of five specimens of *Prosorhochmus chafarinensis* sp. nov.

Length of specimen (mm)	Length of central stylet ( $\mu$ m)	Length of basis ( $\mu$ m)	Maximum width of basis ( $\mu$ m)	Ratio of basis length to central stylet length	Ratio of basis length to basis width
15	120	240	72	2.00	3.33
10	96	176	49	1.83	3.59
9	96	256	88	2.66	2.90
8	104	200	72	1.92	2.77
5	80	136	40	1.70	3.40

stomach with a densely ciliated epithelium 80  $\mu$ m or more thick; at its rear the stomach merges directly into the pyloric portion of the foregut without forming a posterior ventral pouch as described in *P. claparedii* by Gibson & Moore (1985) and *P. adriatica* by Senz (1993). Pylorus an unfolded canal; gland cell density decreases posteriorly. Intestine with lobed lateral diverticula throughout its length. Intestinal caecum also with lateral diverticula, ending near rear of oesophagus; most anterior pair of pouches continuing forward dorsolaterally, terminating closely behind dorsal cerebral lobes.

#### Blood system

Blood system consists of paired lateral vessels which are transversely joined by a cephalic loop just behind rear of frontal organ. Branches off to form a mid-dorsal blood

vessel, immediately entering rhynchocoel floor and forming a single vascular plug. Blood vessels are thin-walled throughout body, with so-called valves similar to those described by Gibson & Moore (1985) in *P. claparedii*.

#### Nervous system

Well-developed brain; dorsal lobes more widely separated than ventral. Brain enclosed by thin outer neurilemma, but no inner neurilemma could be distinguished. Ventral cerebral commissure thicker (45–50  $\mu$ m) than dorsal (13–20  $\mu$ m). Several cephalic nerves leading forward from anterior borders of brain lobes; one thick pair of nerves from the dorsal ganglia supplies the eyes whereas ventral ganglia provide the neural supply to the cerebral sensory organ and proboscis (Fig. 2g).

**Table 2.** Comparison between certain anatomical features recorded for species of *Prosorhochmus*. Data compiled from Gibson & Moore (1985), Gibson *et al.* (1986) and Senz (1993)

Species	1	2	3	4	5	6	7	8	9	10	11
<i>P. claparedii</i>											
Keferstein, 1862		+			+	9-13	1,2,3	1	+	30-45	1
<i>P. americanus</i>											
Gibson, Moore, Ruppert & Turbeville, 1986	+	-	-	-	-	10-11	1,2,3,4	1-3		90	1
<i>P. adriatica</i> Senz, 1993	+	+	+	+	?	11	2,3	1?	-	?	1
<i>P. chafarinensis</i> sp. nov.	+	+	+	-	-	10-12	1,2,3,4	1	+	80-120	3-8

1: Dorsoventral musculature in foregut region present (+) or absent (-).

2: Oesophageal musculature present (+) or absent (-).

3: Oesophageal epithelium ciliated (+) or unciliated (-) in the posterior portion.

4: Posterior ventral pouch of the stomach present (+) or absent (-).

5: Neural supply in posterior proboscis chamber present (+) or absent (-).

6: Number of nerves in the anterior proboscis chamber.

7: Type of cells in the cephalic glands: basophilic (1), acidophilic (2), brown (3), purple (4).

8: No of eggs/young within each gonad.

9: Frontal organ: with 90° bend between the canal and the chamber (+); without bend (-).

10: Length of central stylet (µm).

11: Number of muscular fibres in the lateral nerve cords.

Lateral nerve cords with a single fibre core, accessory lateral nerve missing; occasional neurochord cells have been observed. Three to eight myofibrils running along inner dorsal margin of fibrous core (Fig. 2f).

### Eyes

Four eyes of pigment cup ocellus type; anterior pair 50-60 µm in diameter, situated 30-40 µm beneath epidermal basement layer; posterior pair diameter 20 µm, 15-20 µm beneath epidermal basement layer.

### Frontal sensory organ

Frontal organ (Fig. 3a, b) structure similar to that described in *P. claparedii* by Gibson & Moore (1985), with a ciliated canal that runs back for about 40 µm, then turns ventrally through 90° to end in a medioventral chamber 100 µm wide. Ventral wall of canal and lateral walls of medioventral chamber are lined by an epithelium clad in densely arranged cilia 4 µm long with acidophilic gland cells. Both dorsal border of canal and ventral wall of chamber have a vacuolated epithelium; with cilia, 10 µm long in the canal and up to 30 µm long in the chamber.

### Cephalic glands

Four types of cephalic glands exist. Typical basophilic lobules are the most abundant, occupying much of the cephalic space; behind the brain they are increasingly restricted to the dorsal half of the body, extending back well into the pyloric region (Fig. 2a). The second type of glands corresponds to the 'brown glands' described by Gibson & Moore (1985: 154); these glands, confined to

the dorsal region throughout the body length, appear dorsally just in front of the cerebral septum, being at their maximum in the stomach region (Fig. 2a, g). Third, ventrally, pre-cerebral and cerebral regions have acidophilic glands. Fourth, 'purple glands' described by Gibson *et al.* (1986: 334) in *P. americanus*, are present in all sectioned specimens in a various arrangement; mainly located in the pre-cerebral region.

### Cerebral sensory organs

Cerebral sensory organs, in front of the brain, about 80 µm in diameter. Each organ opens laterally from the pair of cephalic furrows, via ciliated canals 70 µm long and 20 µm in diameter; canals ending in a neuroglandular complex with finely granular acidophilic glands in the central part and peripheral coarsely granular brown glands.

### Excretory system

The excretory system consists of two pairs of ciliated coiled tubules, about 10 µm in diameter, situated on each side of the body near lateral nerve cords in the foregut region (Fig. 2a). No evidence of the characteristic flame cells described by Pantin (1969) could be found.

### Reproductive system

Hermaphroditism in combination with viviparity (see Bierre, 1983); gonads distributed irregularly between lobes of intestinal diverticula. Immature gonads with 10 gonadal cells in which the nucleus is 10-12 µm across. Mature gonad typically contains a single oocyte,

80–90 µm in diameter, in which the nucleus is 40 µm across (Fig. 3c). The holotype with gonads contains both spermatozoa and gonadal cells, gonads with mature oocytes and six gonads containing, each, one young worm (Fig. 3d).

Dense accumulations of coarse granules staining dark brown with Mallory, surrounded by connective tissue distributed between lobes of intestinal diverticula may be regression gonads (Fig. 3e)

### Systematic discussion

The general morphology of the Chafarinas specimens agree with the diagnosis of *Prosorhochmus* provided by Gibson & Moore (1985), and there is not doubt that they belong to this genus. From all the characters mentioned in this diagnosis, only ovoviviparity is uncertain. Whilst species of *Prosorhochmus* give birth to live young, differences of opinion exists in the literature as to whether they are viviparous (Bierne, 1983; Senz, 1993) or ovoviviparous (Gibson & Moore, 1985; Gibson *et al.* 1986; Gibson, 1994).

Three valid species of *Prosorhochmus* (see Gibson, 1995) (Table 2) are known at present. In their external features, these species resemble both each other and the Chafarinas form, and a reliable distinction between them cannot be achieved without recourse to histological studies of their internal morphology.

The various internal features which can be used to distinguish between the different species are summarized in Table 2. *P. claparedii* is the only species of the genus that lacks dorsoventral musculature in the foregut region and has distinct nerves in the posterior chamber of the proboscis (Gibson & Moore, 1985). This species also has a posterior stomach chamber, as recorded in *P. adriatica*, but not present in *P. americanus* nor in *P. chafarinensis*. The presence of four types of cells in the cephalic glands and the measurements of the stylet apparatus are similar in *P. americanus* and *P. chafarinensis*, but these two species differ in the degree of development of the frontal organ, the oesophageal

ciliation and musculature, and the number of eggs/young within each gonad. The combination of these characters justify the separation of the Chafarinas nemertean into the new species, *P. chafarinensis*. A further distinguishing character is the number of muscular fibres in the lateral nerve cords.

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