





The freshwater ribbon-worm *Prostoma graecense* (Hoplonemertea: Monostilifera) in South America (Argentina)

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Abstract

The morphological species *Prostoma graecense* (Böhmig, 1892) is reported for the first time from Argentina and South America. Some diagnostic characters, such as the ciliated epithelium of the non-rhynchodaeal oesophagus and the backward extension of the cephalic glands up to the brain commisure, were confirmed for the Argentine material; the rhynchodaeal longitudinal muscles were present, but did not form a thick layer as expected from some previous descriptions. Adding South America to its distribution range supports the hypothesis that this morphotype *Prostoma graecense* actually has a cosmopolitan distribution.

Key words: Cosmopolitan distribution, Enopla, Nemertea, taxonomy, Tetrastemmidae

Introduction

Only two species of *Prostoma* have been recorded from South America, and only one, *Prostoma eilhardi* (Montgomery, 1894), was identified in Argentina and Brazil (Moretto & Durquet 1978; Corrêa 1986).

Prostoma graecense (Böhmig, 1892), a very widespread morphospecies, is deemed to be cosmopolitan (Corrêa 1986; Pennak 1989; Weidenbach 1995). However, while it was identified from North America, Europe, Asia, Africa, Australia, and New Zealand (Gibson & Moore 1976, 1978; Crandall et al. 2002; Gibson 2002), its presence in South America has not been factually established. Corrêa (1951) has mentioned it as inhabiting South America, but her statement was only based on the opinion that the nemerteans found in Venezuela by Cordero (1943) were Prostoma graecense, although that author had described and identified his material as P. asensoriatum (Montgomery, 1896). At present, the latter species is not considered a synonym of Prostoma graecense (Gibson & Moore 1976), and recent studies even show that P. asensoriatum may not be a member of the

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genus Prostoma (Gibson 1982).

This paper reports the finding of *Prostoma graecense* in a semi-arid region in southern Buenos Aires province, Argentina. It is the first documented record of *P. graecense* in South America, and provides additional evidence that this morphotype may actually be distributed over a cosmopolitan range.

Material and methods

The specimens were collected in the Napostá Grande stream, near Bahía Blanca city (38°40'S–62°11'W), under mesosaprobic conditions (Cazzaniga & Curino 1987; Pettigrosso & Cazzaniga 1987). The stream is permanent, its middle water flow being 0.425 m³ s⁻¹ (Paoloni et al. 1988).

Samples mainly consisted of bunches of *Cladophora* spp., one of the most abundant macroalgae in this environment (Parodi 1990). They were stored in 5–l containers without aeration to force the worms to crawl to the surface.

The specimens were relaxed before fixation; the best anaesthetic results were obtained by gradually adding an aqueous solution of MgCl₂ 2.5% and menthol crystals. Fixation was made in Bouin fluid for 24 hours. Serial sections 7 µm thick were obtained with a Minot rotary microtome (eight transverse and thirteen sagittal series) and stained with Carazzi haematoxylin and eosin, Masson Trichrome Stain, or Periodic Acid Schiff (PAS). Specimens that did not protrude the proboscis during fixation were preferred for microanatomical studies.

Voucher specimens (sets of serial sections) are deposited at the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (Buenos Aires, Argentina), under the numbers MACN—In 36374, 36375 and 36376.

Results

Family Tetrastemmidae

Genus Prostoma Dugès, 1828

Prostoma graecense (Böhmig, 1892) (Figures 1–2)

Description of the Argentine specimens

Worms of slightly flattened, round body section, about 0.5 to 2.5 cm long, with four to six turbellarian-like cup ocelli, five being a usual figure; all late embryos and newly hatched specimens bred in the laboratory showed four eyes. The smallest animals were

pale yellow whereas larger ones were orange-brown with small darker spots. A cupshaped frontal organ, dorsal to the rhynchopore, was easily noticeable by the active ciliar movement within it (Figure 1).

Body wall with two muscular layers, outer circular and inner longitudinal, as typical for monostiliferous Hoplonemertini; no diagonal fibres observed.

Nervous system consisting of a pair of dorsal and a pair of ventral cerebral ganglia connected by commissures forming the neural ring around the rhynchodaeum. The cerebral organ connected with two external, oblique cephalic grooves. A pair of lateral nerve cords arose from the ventral ganglion, with no accessory nerve. The highly basophilous cephalic glands connecting to the frontal organ extend backwards up to, at least, the cerebral dorsal commissure in all the specimens sectioned (Figure 2).

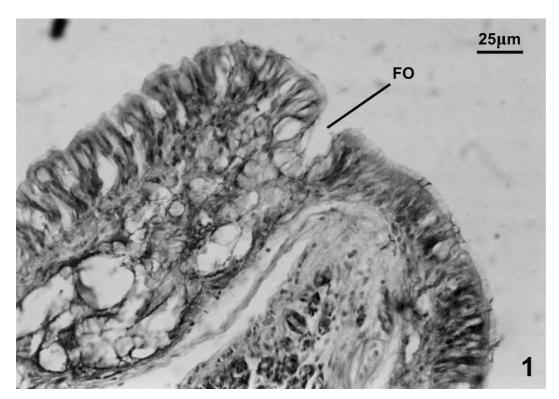


FIGURE 1. Prostoma graecense (Böhmig 1892) from Argentina. Sagittal section through the cephalic region showing the cup-shaped frontal organ (FO).

Rhynchocoel about two thirds the body length, with two muscular layers (outer circular, inner longitudinal). Rhynchodaeum with a thin layer of longitudinal fibres, noticed only in a few specimens. Proboscis differentiated into three regions (anterior, middle bulb, and posterior), with three muscular layers (outer longitudinal, middle circular, inner longitudinal). The principal stylet—nearly as long as its base—laid in the basis of the middle bulb region; the two lateral pouches containing accessory stylets were

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surrounded by some unicellular glands. Late embryos and newly hatched worms already possess the main stylet.

Oesophagus non-rhynchodaeal, short, ciliated (Figure 2); its cilia were not always visible due to lumen collapse. Stomach with a strongly ciliated epithelium.

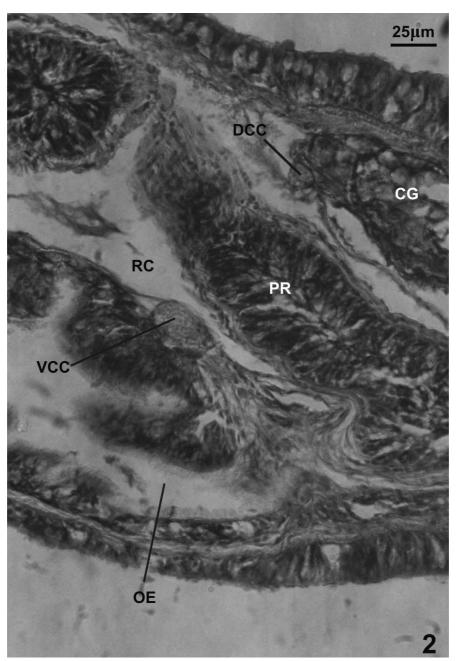


FIGURE 2. *Prostoma graecense* (Böhmig 1892) from Argentina. Sagittal section through the fore region of the body, showing the ciliated epithelium of the oesophagus (OE) and the extension of the cephalic glands (CG): dorsal cerebral commisure (DCC); PR, proboscis; RC, rhynchocoel; VCC, ventral cerebral commisure.

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Ovotestes located between the intestinal diverticula. A large ovum and a few remaining spermatic cells formed each ovotestis in mature specimens. One of the smallest specimens had only testes. Spherical eggs laid in the laboratory were 0.032 mm in diameter.

Discussion

Criteria for the morphological recognition of *Prostoma* species were discussed by Stiasny-Wijnhoff (1938), Corrêa (1951, 1964) and Gibson and Moore (1976). There is no general agreement on the extent of intraspecific variability, and some characters are still controversial because they are seldom observable, naturally variable, or are very sensitive to alterations induced by methods employed in preparation.

The presence of a definite frontal organ in the Argentine material excludes *Prostoma* asensoriatum from the discussion. This species, tentatively cited in South America (Cordero 1943), is actually a nomen dubium (Gibson and Moore 1976).

Three crucial characters that discriminate *Prostoma graecense* from its close relatives are the ciliation of the oesophagus epithelium; the presence of a distinct layer of longitudinal muscles in the rhynchodaeum of the former; and the backward extension of the cephalic glands, which reach the brain commissure or extend beyond them.

The ciliated oesophagus was used to separate *P. graecense* from its allies: *P. eilhardi* (Stiasny-Wijnhoff 1938; Corrêa 1951, 1986), *P. jenningsi* Gibson and Young, 1971, (Gibson & Moore 1976), and *P. canadiensis* Gibson and Moore, 1978. Gibson and Moore (1976) retained this character as reliable even though epithelial cilia were not visible in the oesophagus of three of their ten specimens analysed from different localities. Those Argentine specimens where the oesophagus lumen did not collapse during fixation showed strong ciliation in the oesophagus.

The layer of longitudinal muscles in the rhynchodaeum of *P. graecense* may be either thick, as described by Corrêa (1951) for South African specimens, or not strongly developed, as mentioned by Gibson and Moore (1976) for specimens from Israel, Australia, New Zealand and part of the North American material. The Argentine worms had a relatively weak, thin muscular layer, which was hardly visible in some entirely relaxed specimens.

The backward extension of the cephalic glands up to the cerebral commissure is generally accepted as a consistent specific character of *P. graecense* (Iwata 1954; Müller 1966; Gibson & Moore 1971, 1976; Moretto & Durquet 1978; Gamo 1986; Cook & Herrmann 1997). However, Corrêa (1951) argued that this character is not endorsed by the original description, and that she also never found any systematic difference in this respect between the samples she identified as *P. graecense* and *P. eilhardi*. All the Argentine specimens showed extensive cephalic glands that extended at least to the brain, and sometimes beyond it.



Previous records of *Prostoma* in South America can be summarized as follows:

- —*Prostoma asensoriatum* (Montgomery, 1896) in Caracas (Venezuela) (Cordero 1943).
- —*Prostoma eilhardi* (Montgomery, 1894) in the states of São Paulo (Marcus 1942), Paraná (Marcus 1943), Pará (Corrêa 1951) (Brazil), and in the La Plata river (Argentina) (Moretto & Durquet 1978).
- —*Prostoma* sp. in Montevideo (Uruguay), Buenos Aires and Misiones (Argentina) (Cordero 1943). The material was not identified to species level and no histological data were given.

Corrêa (1951: 257) stated that the presence of *Prostoma graecense* was verified "in Mexico (Rioja 1941) and Venezuela (Cordero 1941, p. 195; 1943, p. 143)", but her opinion was based on inconclusive evidence. In fact, the former reference (Cordero, 1941) refers to species of *Hydra* and does not include any specific name for his "fairly frequent nemertean" from Caracas. Instead, Cordero (1943, p. 129) describes and identifies it as *Prostoma asensoriatum*; later in his paper (p. 132) he mentions that Rioja (1941) had given the name *Stichostemma rubra* (Leidy, 1850) to the Mexican specimens of the same species, and concludes that only *P. asensoriatum* and *P. eilhardi* are present in the Americas to the south of Mexico. This opinion is still valid, except for the doubt over the validity of *P. asensoriatum* (Gibson & Moore 1976). At the time, Corrêa (1951) seemed to assume the synonymy of *Prostoma rubrum* and *P. asensoriatum* with *P. graecense*, but this statement is no longer acceptable. While Corrêa (1951) identified *Prostoma eilhardi* and *P. graecense* in her material from South Africa, all her specimens from South America were *P. eilhardi*, the only *Prostoma* species now recognised in Brazil (Corrêa 1986). Therefore, *P. graecense* has not until now actually been identified from South America.

The status of *P. graecense* as a cosmopolitan morphospecies appears to be legitimate since it is common in Europe, Asia, and North America (Gibson & Moore 1976; Crandall et al. 2002); it is also present in Africa (Corrêa 1951; Gibson & Young 1974); in Australia and New Zealand (Gibson & Moore 1978; Gibson 2002); and is now confirmed from South America. The scarcity of records in the Southern Hemisphere is possibly but a consequence of the limited interest of local zoologists.

Traditional taxonomy of the Tetrastemmidae based on morphological characters only, does not necessarily reflect actual phylogenetic relationships (Strand & Sundberg, 2005a). Nemertean species that have direct development, with no free-swimming stages of larvae, face obvious restrictions on dispersal and gene flow (Strand & Sundberg, 2005b). So, under the present day evidence on related groups, such an ubiquitous species as *Prostoma graecense* is subject to the suspicion that it might represent a species-complex or of having been introduced into at least some areas of its world-wide distribution. There is still no molecular evidence to show if the South American *P. graecense* are genetically similar to the morphologically identical specimens collected from other continents.

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Literature cited

- Cazzaniga, N.J. & Curino, A.C. (1987) On *Dugesia anceps* (Kenk, 1930) (Turbellaria Tricladida) from Argentina. *Bolletino di Zoologia*, 54,141–146.
- Cook, J.L. & Herrmann, S.J. (1997) New record of *Prostoma graecense* (Nemertea: Hoplonemertea) from southern Colorado, with observations on its feeding behaviour and habitat. *Southwestern Naturalist*, 42, 62–169.
- Cordero, E.H. (1941) Observaciones sobre algunas especies sudamericanas del género *Hydra*. *Anais da Academia Brasileira de Ciências*, 13, 195–201.
- Cordero, E.H. (1943) Hallazgos en diversos países de Sud América de Nemertinos de agua dulce del género *Prostoma. Anais da Academia Brasileira de Ciências*, 15, 125–134, 1 pl.
- Corrêa, D.D. (1951) Freshwater nemertines from the Amazon region and from South Africa. Boletim da Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo, Zoologia, 16, 257–269.
- Corrêa, D.D. (1964) Nemerteans from California and Oregon. *Proceedings of the California Academy of Sciences*, 31, 515–538.
- Corrêa, D.D. (1986) Nemertini. *In:* R. Schaden (ed.) *Manual de identificação de invertebrados lím-nicos do Brasil* 9, 1–20. Brasilia: CNPq Assessoria Editorial.
- Crandall, F.B., Norenburg, J.L., Chernyshev, A.V., Maslakova, S.A., Schwartz, M.L. & Kajihara, H. (2002) Checklist of the nemertean fauna of Japan and northeastern Asia. http://nemertes.si.edu/PDFs/epub2917.pdf. pp. i–ii, 1–43.
- Gamo, J. (1986) Presencia del nemertino de agua dulce *Prostoma eilhardi* (Montgomery, 1894) (Hoplonemertea) en la Península Ibérica. *Miscelania Zoologica*, 10, 373–375.
- Gibson, R. (1982) Nemertea. *In Parker SP. Synopsis and classification of living organisms*, vol. 1. New York: McGraw-Hill, pp. 823–846.
- Gibson, R. (2002) The invertebrate fauna of New Zealand: Nemertea (Ribbon Worms). NIWA *Biodiversity Memoir*, 118, 1–87.
- Gibson, R. & Moore, J. (1971) Occurrence of the freshwater hoplonemertean *Prostoma graecense* (Böhmig) in Tasmania. *Freshwater Biology*, 1, 193–195.
- Gibson, R. & Moore, J. (1976) Freshwater nemerteans. *Zoological Journal of the Linnean Society*, 58, 177–218, 2 pl.
- Gibson, R. & Moore, J. (1978) Freshwater Nemerteans: New records of *Prostoma* and a description of *Prostoma canadiensis* sp. nov. *Zoologischer Anzeiger*, 201, 77–85
- Gibson, R. & Young, J.O. (1974) First records of *Prostoma* (Hoplonemertea) from East Africa. *Zoologischer Anzeiger*, 193, 103–109.
- Iwata, F. (1954) The fauna of Akkeshi Bay. XX. Nemertini in Hokkaido (revised report). *Journal of the Faculty of Science, Hokkaido University*, Series 6, 12, 1–39.
- Marcus, E. (1942) Sôbre um nemertino de agua doce do Brasil. Anais da Academia Brasileira de

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- Ciências, 14, 371-383.
- Marcus, E. (1943) Novos achados de nemertinos limnicos. *Anais da Academia Brasileira de Ciências*, 15, 11–17.
- Moretto, H.J.A. & Durquet, J. (1978) El hoplonemertino *Prostoma eilhardi* (Montgomery, 1894) en el Río de la Plata. *Ciencia e Investigación*, Buenos Aires, 34, 36–38.
- Müller, G.J. (1966) Beitrag zur Kenntniss der Nemertinen-Fauna Ungarns. *Acta Zoologica Academiae Scientiarum Hungaricae* 12, 129–131.
- Paoloni, J.D., Vázquez, R. & Fiorentino, E.C. (1988) La topografía y variación de las precipitaciones y escurrimientos en el Sistema de Ventania. *Actas 2^{as} Jornadas Geológicas Bonaerenses*, Bahía Blanca, Argentina, 651–661.
- Parodi, E.R. (1990) Biologia, cariología, ultraestructura y taxonomía de Cladophoraceae del sur de la Provincia de Buenos Aires. *Doctoral Thesis*, Universidad Nacional del Sur, 229 pp.
- Pennak, R.W. (1989) Fresh-water invertebrates of the United States, 3 ed. John Wiley and Sons, New York, 628 pp.
- Pettigrosso, R.E. & Cazzaniga, N.J. (1987) Registro de tres especies de *Aspidisca* (Ciliophora: Hypotrichida) en la Argentina. *Anales del Museo de Historia Natural de Valparaíso*, 18, 5–12.
- Rioja, E. (1941) Hallazgo en Xochimilco de *Stichostemma rubrum* (Leidy), nemerte de agua dulce. *Anales del Instituto de Biología, Universidad Nacional Autónoma de México*, 12, 663–668.
- Stiasny-Wijnhoff, G. (1938) Das Genus *Prostoma* Dugès, eine Gattung von Süsswasser-Nemertinen. *Archives Néerlandaises de Zoologie* 3, Suppl., 219–230.
- Strand, M. & Sundberg, P. (2005a) Delimiting species in the hoplonemertean genus *Tetrastemma* (phylum Nemertea): morphology is not concordant with phylogeny as evidenced from mtDNA sequences. *Biological Journal of the Linnean Society*, 86, 201–212.
- Strand, M. & Sundberg, P. (2005b) Genus *Tetrastemma* Ehrenberg, 1831 (Phylum Nemertea)—a natural group? Phylogenetic relationships inferred from partial 18S rDNA sequences. *Molecular Phylogenetics and Evolution*, 37, 144–152.
- Weidenbach, R.P. (1995) Freshwater pond life, a guide to the animals and plants of Hawaii's freshwater aquaculture ponds. Aquaculture Development Program, DLNR, State of Hawaii, 84 pp.