New species of *Epacanthion* (Nematoda: Thoracostomopsidae) from Patagonian coastal areas, Río Negro and Chubut, Argentina

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Two new free-living marine nematodes belonging to the family Thoracostomopsidae, subfamily Enoplolaiminae and the genera *Epacanthion* are described from Patagonian coastal areas, Río Negro and Chubut provinces of Argentina. In *Epacanthion bicuspidatum* sp. nov. the main features are: two cusps on distal mandible end; presence of two rings of eight setae in the cervical region; and eight crown-shaped setae along the body, presence of one supplement distant two cloacal body diameter (cbd) from cloacae, gubernaculum and short spicule. *Epacanthion ampullatum* sp. nov. is characterized by having a tubular and long pre-cloacal supplement, short spicule, presence of gubernaculum, presence of three long setae on the tip of the tail, the presence of one supplement distant three cbd from cloacae and the presence of two small cusps on the distal end of the mandible.

**Keywords:** description, systematics, Patagonia

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**INTRODUCTION**

During an ecological and taxonomic study, focused on the meio-benthos of Patagonian bays (Río Negro and Chubut provinces, Argentina), new free-living marine nematodes were found. This is the fifth paper of the series. From these areas we describe two new species of the genus *Epacanthion* Wieser, 1953.

The genus *Epacanthion* belongs to the family Thoracostomopsidae Filipjev, 1927. This family is composed of three subfamilies: Thoracostomopsinae Filipjev, 1927, with only one genus (*Thoracostomopsis* Ditlevsen, 1918); Trileptiinae Gerlach & Riemann, 1974, that has only one genus too (*Trileptium* Cobb, 1933); and Enoplolaiminae De Coninck, 1965. The subfamily Enoplolaiminae is mainly marine species but has freshwater species. This subfamily has 18 genera and can be distinguished from the other subfamilies by the presence in the buccal cavity of three broad mandibles, formed of two longitudinal rods joined by a thin membrane between, and a cephalic capsule strongly sclerotized with three lips and the cephalic setae above them.

The most complete revision of the genus *Epacanthion* was made by Greenslade & Nicholas (1991). They established 21 species as belonging to it. Afterwards, Gagarin (2009) has described one new species (*E. macrolaimus*) from the Mediterranean Sea and Guilherme et al. (2009) have described another one (*E. agubernaculus*) from Brazil. In this last work, a new key for species identification was proposed.

We describe two new species of *Epacanthion*: *E. bicuspidatum* sp. nov. and *E. ampullatum* sp. nov.

**MATERIALS AND METHODS**

**Description of sites studied**

Samples were collected from the Patagonia littoral coastal region in seven separate locations at three different latitudes (Table 1; Figure 1).

The northernmost site (Banco Perdices) is in San Antonio Bay, Río Negro province. This bay is located in the north-west of San Matías Gulf. This bay is composed of a shallow channel system with small islands and sandbanks, which are completely covered at high tide. The tidal amplitude reaches 9.30 m, with low tides exposing a muddy area of 7 km width.

Four sampling sites are located in Nuevo Gulf, Chubut province. Three of these sites are in the coastal area of Puerto Madryn city (Puerto Madryn I, II and III), and the other is further south (Cerro Avanzado). Nuevo Gulf is situated in the southern part of Peninsula Valdés. This gulf is a semi-enclosed basin of 65 km in length and 46 km in width with 16.5 km in the mouth. Tidal current is the most important movement of water with tidal amplitude of 4 m.

The southernmost site (Rada Tilly) is located at the central coast of the San Jorge Gulf, Chubut province. This gulf is a semicircle with 145 km of radius and 220 km of width of mouth. The tidal amplitude varies between 4 and 6 m.

**Sample collection and treatment**

On each site, five sediment samples were taken with a cylindrical Plexiglas corer (10 cm high and 2.8 cm in diameter). The samples were preserved in 5% formaldehyde in filtered seawater, and then were sieved through both 500 μm and 50 μm mesh sieves. The nematodes present on the 50 μm sieve were separated by Ludox® TM. Nematodes were fixed following the...
method described by Ditlevsen (1911) and preserved in anhydrous glycerin, counted and mounted on slides sealed with Canada X resin. Then they were identified to species level.

**Specimen analysis**

Morphometrics data were obtained from camera lucida drawings using a Zeiss microscope with differential interference contrast (DIC). The measurements are in microns. Photographs were taken with Olympus BX51 microscopy equipment with DIC equipped with a Nikon D80 digital camera. Sediment analyses were carried out by dry sieving and classified according to the Wentworth scale. The literature has been obtained from NeMys (Deprez, 2006). Type specimens were deposited in the Museo Nacional de Ciencias Naturales 'Bernardino Rivadavia', and the paratypes were deposited at the Nematodes Patagonicos collection in the Centro Nacional Patagonico, Argentina. De Man’s ratios, a, b and c used in this paper were calculated as standard.

**Abbreviations used in this paper**

bcl, buccal cavity length; bcw, buccal cavity width; cbd, cloacal body diameter; cs ant, anterior cervical setae; cs post, posterior cervical setae.

**Table 1.** List of sampling sites with sediment parameters (classification, mean grain size and fine fraction).

| Site              | Province     | Latitude    | Longitude   | Classification | MGS (mm) | FF (%) |
|-------------------|--------------|-------------|-------------|----------------|----------|--------|
| Banco Perdices    | Rio Negro    | 40° 47’S    | 64° 50’W    | Fine sand      | 0.134    | 14.26  |
| Puerto Madryn I   | Chubut       | 42° 45’S    | 65° 01’W    | Fine sand      | 0.180    | 2.47   |
| Puerto Madryn II  | Chubut       | 42° 46’S    | 65° 00’W    | Fine sand      | 0.182    | 1.64   |
| Puerto Madryn III | Chubut       | 42° 47’S    | 65° 00’W    | Fine sand      | 0.180    | 1.78   |
| Cerro Avanzado    | Chubut       | 42° 49’S    | 64° 52’W    | Fine sand      | 0.128    | 1.58   |
| Rada Tilly        | Chubut       | 45° 53’S    | 67° 37’W    | Fine sand      | 0.210    | 1.20   |

**Fig. 1.** Map showing the three sampling areas with the location of samples stations: (A) San Antonio Bay (Rio Negro); (B) Nuevo Gulf (Chubut); (C) San Jorge Gulf (Chubut).
cervical setae; daa, distance from anterior end to anus; daph, pharynx length; dav, distance from anterior end to vulva; mbd, maximum body diameter; hd, head diameter; lcs, length cephalic setae; llsi, length internal labial setae; llse, length external labial setae; Spic, spicule length in microns, along the arc; Spic%, spicule chord as proportion of anal body diameter; c’, tail in anal diameter; Gub, gubernaculum length; Gub%, gubernaculum length as proportion of anal body diameter; L, total body length; Lo ant, length antidromously reflected anterior ovary; Lo post, length antidromously reflected posterior ovary; V%, distance from the anterior end to the vulva opening in percentage of total length; T, tail length; PS, pre-cloacal supplement. Measurements are in μm.

RESULTS AND DISCUSSION

SYSTEMATICS
Order ENOPLIDA Filipjev, 1929
Suborder ENOPLINA Chitwood & Chitwood, 1937
Superfamily ENOPLOIDEA Dujardin, 1845
Family THORACOSTOMOPSIDAE Filipjev, 1927
Subfamily ENOPLOLAIMINAE De Coninck, 1965
Genus *Epacanthion* Wieser, 1953

*Epacanthion bicuspidatum* sp. nov.
(Figure 2; Plate 1; Table 2)

type material
Holotype: adult male. Registration number MACN-In 39287; type locality: Puerto Madryn I; coordinates: 42°45′S 65°01′W; low littoral. Collected by C.T. Pastor de Ward and V. Lo Russo, 1 March 2006.

Paratype: adult female. Registration number MACN-In 39288; type locality: Puerto Madryn I; coordinates: 42°45′S 65°01′W; low littoral. Collected by C.T. Pastor de Ward and V. Lo Russo, 1 March 2006.

other material
- Two males and one female. Registration numbers CNP NEM 1612–1614; type locality: Puerto Madryn II; coordinates: 42°46′S 65°00′W; low littoral. Collected by C. Harguinteguy, 15 June 2003.
- One male. Registration number CNP NEM 1615; type locality: Cerro Avanzado; coordinates: 42°49′S 64°52′W; low littoral. Collected by C. Harguinteguy, 15 June 2003.

Plate 1. *Epacanthion bicuspidatum* sp. nov.: (A) mandible and teeth of female paratype; (B) buccal cavity of female paratype; (C) view of teeth on proximal end of mandibles, and body of mandibles of male holotype (arrow); (D) entire male holotype; (E) view of mandibles cusps with tooth laterally of male holotype (arrows); (F) copulatory apparatus and pre-cloacal supplement of male paratype (arrow). Scale bars: A, B, C, E & F = 40 μm; D = 200 μm.
- One male and two females. Registration numbers CNP NEM 1616–1618; type locality: Puerto Madryn I; coordinates: 42°45'56"S 65°01'38"W; low littoral. Collected by C.T. Pastor de Ward and V. Lo Russo, 1 March 2006.
- One female. Registration number CNP NEM 1619; type locality: Puerto Madryn III; coordinates: 42°8'45"S 65°08'1"W; low littoral. Collected by C. Harguinteguy, 15 June 2003.
- Two juveniles. Registration numbers CNP NEM 1620 and 1621; type locality: Banco Perdices; coordinates: 40°8'47"S 64°58'1"W; high littoral. Collected by G. Villares and V. Lo Russo, 14 February 2009.

**ETYMOLOGY**
From Latin word *bicuspidatum* (adj.) = bicuspis, two cusps on the basis of the mandible.

**MATERIAL EXAMINED**
Measurements: see Table 2.

**DESCRIPTION**
Male (holotype): cylindrical body (L = 1450 μm). Smooth cuticle. Sclerotized cephalic capsule. Lips smooth and associated with three strongly sclerotized mandibles (10 μm long) composed each of one of two longitudinal solid bars united by a thin membrane. The mandibles are straight with a proximal end expanded laterally ending in two curved teeth on each side. Two cusps with two teeth on the basis of each mandible were observed. Each lip presents two labial setae (9–10 μm). There are six short (12 μm) and four long (19 μm) cephalic setae inserted at base of lip flats. Six long (17 μm) and six short (10 μm) cervical setae inserted below the position of the amphidial fovea. Eight setae arranged in two circles in the cervical region and below a third circle consisting of four setae. Somatic setae (6–8 μm) on the body follow a pattern standing in crowns, never found scattered.

Amphidial fovea slightly distinct, 4 μm width. Oesophagus cylindrical, long and muscular (250 μm long). Reproductive system dicrotic, tests located at the left of the intestine, extending to the anterior region of the body. Two short, equal spicules, 20 μm (1 cbd) in chord length. Gubernaculum present, triangular in shape. One tubular and sclerotized pre-cloacal supplement (5 μm long), 50 μm distant from the cloaca and 218 μm distant from the extreme of the tail. Eight setae (6 μm long) arranged in a circle below the pre-cloacal supplement and located 35 μm distant from the cloaca. Tail cylindrical–conical in shape (168 μm long). Caudal glands not observed.

Female (paratype): females are similar to males in general body shape. Reproductive system dicrotic, with two antidromously reflexed ovaries left to the intestine. Vulva 62% of body length, short vagina. Tail 180 μm long, cylindrical–conical in shape.

**DIAGNOSIS AND RELATIONSHIPS**
*Epacanthion bicuspidatum* sp. nov. is characterized by having two cusps on distal mandible end, the presence of two rings of...
eight setae in the cervical region and eight setae crown-shaped along the body, the presence of one supplement distant two cbd from cloacae, gubernaculum and short spicule.

Depending on the length of the spicules, the species of *Epacanthion* can be classified in two main groups. One with species with long spicules, more than 2 anal diameters, which includes: *E. agubernaculus* Guilherme *et al.*, 2009; *E. brevispiculosum* Mawson, 1958; *E. butschlii* (Southern, 1914); *E. durapelle* (Kreis, 1929); *E. microdentatus* Wieser, 1953; *E. nadjae* Sergeeva, 1974; *E. polystosum* (Jensen, 1986); *E. savelyeji* (Filipjev, 1927); and *E. stekhoveni* Greenslade & Nicholas, 1991. And the other with species with short spicules, less than 2 anal diameters, which includes: *E. brevispiculum* Mawson, 1956; *E. enoploidiforme* (Gerlach, 1953); *E. exploratoris* Greenslade & Nicholas, 1991; *E. galeatum* Boucher, 1977; *E. georgei* Inglis, 1971; *E. gorgonocepha-lum* Warwick, 1970; *E. macrolaimus* Gagarin, 2009; *E. mawsoni* Warwick, 1977; *E. multipapillatum* (Wieser, 1959); *E. oliffi* Inglis, 1966; *E. oweni* Keppner, 1986; and *E. pellucidum* (Saveljev, 1912). Into the second group there are

Fig. 3. *Epacanthion ampullatum* sp. nov.: (A) anterior end of male holotype; (B) anterior end of female paratype; (C) mandible of male holotype; (D) head and buccal cavity with mandible of male holotype; (E) detail of the precloacal supplement; (F) copulatory apparatus and pre-cloacal suplement; (G) head and buccal cavity with mandible of female paratype; (H) posterior end of female paratype. Scale bars: A–H = 20 μm.
species with several (*E. multipapillatum* and *E. oweni*) or no precloacal organs (*E. enoploidiforme*, *E. georgei*, *E. gorgonocephalum* and *E. oliffi*). The rest of the species with short spicules has one precloacal organ. Our species, *Epacanthion bicuspidatum* sp. nov., belongs to this group.

*Epacanthion bicuspidatum* sp. nov. has a distance of 2.5 anal diameters between the precloacal organ and the cloaca, and a long tail of about 8 anal diameters. Hence, it can be distinguished from *E. brevispiculum*, *E. macrolaimus* and *E. pellucidum* because all of them have a shorter distance precloacal organ—cloaca (1.8, 2 and 2 anal diameter, respectively) and a shorter tail (5, 2.5 and 5.5 anal diameter). Our species has the somatic setae arranged in crows along the body and can be distinguished from *E. galeatum* that has long somatic setae scattered throughout the body. *Epacanthion exploratoris* has a gubernaculum with rounded posterior apophysis whereas *E. bicuspidatum* has not. The spicules are rather straight in the new species and arcuate in *E. mawsoni*.

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**Plate 2.** *Epacanthion ampullatum* sp. nov.: (A) amphidial fovea on head of female paratype (arrow); (B) bifid proximal end of the mandible with two lateral teeth (arrow) and view of two teeth on distal end of mandibles (arrows) of male holotype; (C) precloacal supplement, precloacal setae and gubernaculum of male holotype (arrows); (D) entire male holotype (M 1) and male paratype (M 2); (E) vulva (arrow); (F) posterior end and spicule of male holotype (arrow); (G) lip and body of mandibles of female paratype (arrows). Scale bars: A–C, E–G = 40 μm; D = 200 μm.
Genus *Epacanthion* Wieser, 1953

*Epacanthion ampullatum* sp. nov. (Figure 3; Plate 2; Table 3)

**Type Material**

Holotype: adult male. Registration number MACN-In 39289; type locality: Rada Tilly; coordinates: 45° 55′ S 67° 35′ W; mid-littoral. Collected by C.T. Pastor de Ward, 1 April 2006.

Paratype: adult female. Registration number MACN-In 39290; type locality: Rada Tilly; coordinates: 45° 55′ S 67° 35′ W; mid-littoral. Collected by C.T. Pastor de Ward, 1 April 2006.

**Other Material**

– Four males, four females and one juvenile. Registration numbers CNP NEM 1622–1630; type locality: Rada Tilly; coordinates: 45° 55′ S 67° 35′ W; mid-littoral. Collected by C.T. Pastor de Ward, 1 April 2006.

**Etymology**

From Latin word *ampullatum* (adj.) = blister, refers to presence of ampulla.

**Material Examined**

Measurements: see Table 3.

**Description**

Male (holotype): cylindrical body (*L* = 1450 μm). Smooth cuticle. Sclerotized cephalic capsule. Lips striated and composed of three sclerotized mandibles (18 μm long) of two longitudinal solid bars united by a thin membrane, mandibles expanded laterally in the proximal end with one tooth on each side. Two cusps with one small tooth on each were observed on their distal end.

Two labial (14 μm) setae were observed in each lip. Six short (20 μm) and six long (42 μm) cephalic setae inserted at base of lip flats. Somatic setae are present (8 μm) on the body, with no distinct pattern. Amphidial fovea very small, slightly distinct 2 μm width. Oesophagus cylindrical, long and muscular (360 μm long). Reproductive system dorchic, testes located at the left of the intestine, extending to the anterior region of the body. Two short, equal spicules, 18 μm (0.72 cbd) in chord length. Gubernaculum present, triangular in shape. One long tubular pre-cloacal supplement, sclerotized (15 μm long), 50 μm in front of the cloaca and 127 μm distant from the extreme of the tail. Tail 70 μm long, (2.8 cbd), approximately 1/2 cylindrical in shape. In the tip of the tail are seen two long setae (20 μm) and one short setae (9 μm). Caudal glands not observed.

Female (paratype): females are similar to males in general body shape. Reproductive system dorchic, with two antidromously reflexed ovaries left to the intestine. Vulva 65% of body length, short vagina. Tail 85 μm long, (3 cbd) approximately 1/2 cylindrical in shape. Two long setae (33 μm) and one short setae (8 μm) on the tip of the tail. Caudal glands not observed.

**Diagnosis and Relationships**

*Epacanthion ampullatum* sp. nov. is characterized by having a tubular and long pre-cloacal supplement distant three cbd from cloacae, short spicule, presence of gubernaculum,

Table 3. Measurements (μm) of *Epacanthion bicuspidatum* sp. nov. (range, mean value in parentheses).

| Holotype | Paratype | Males | Females | Juvenile |
|----------|----------|-------|---------|----------|
| male     | female   | N = 4 | N = 4   | N = 2    |
| L        | 1450     | 1718  | 2200    | 1550     |
| a        | 45       | 69    | 59      | 47       |
| b        | 40       | 4     | 5.7     | 3.4      |
| c        | 20.7     | 19    | 25      | 15       |
| mbd      | 32       | 26    | 39      | 29.5     |
| bcl      | 14       | 19    | 17      | 16.5     |
| bcw      | 4        | 7     | 7       | 7        |
| Hd       | 21       | 20    | 25      | 21       |
| Lcs (4)  | 42       | 37    | 47      | 32       |
| Lcs (6)  | 20       | 15    | 16      | 15       |
| lli      | 14       | 12    | 10      | 10.5     |
| lls      | 14       | 12    | 10      | 10.5     |
| daph     | 360      | 413   | 407     | 430      |
| cbd      | 25       | 17    | 25      | 23       |
| Spic     | 18       | 15    | –       | –        |
| Spic%    | 0.72     | 1     | –       | –        |
| Gub      | 15       | 6     | –       | –        |
| Gub%     | 0.6      | 0.4   | –       | –        |
| c'       | 2.8      | 5     | 3.5     | 4.1      |
| T        | 70       | 92    | 88      | 90       |
| Ps       | 1        | 1     | –       | –        |
| Ps length| 18       | 11    | –       | –        |
| Ps-tail  | 127      | 141   | –       | –        |
| Ps-cloaca| 50       | 52    | –       | –        |
| daa      | 1380     | 1626  | 2112    | –        |
| dva      | –        | 1360  | –       | –        |
| Lo ant   | –        | 161.7 | –       | –        |
| Lo post  | –        | 156.7 | –       | –        |
| V%       | –        | 65    | –       | –        |
presence of three long setae on the tip of the tail, the presence of one supplement and the presence of two small cusps on the distal end of the mandible.

*Epacanthion ampullatum* as *Epacanthion bicuspidatum*, also belongs to the group characterized by short spicules and one precloacal supplement, but differs from all of them by having the largest distance between the precloacal supplement and the cloaca (3 anal diameters in the new species versus 2 to 2.5 in the others). *Epacanthion galeatum*, *E. mawsoni* and *E. pellucidum* have a long tail (more than 5 anal diameters) and no setae on its tip whereas *E. ampullatum* has a short tail (less than 3 anal diameters) and three long setae on the tip of the tail. From *E. macrolaimus* and *E. brevispiculum* our species can be distinguished by the length of the spicules that is about 1.5 anal diameters in our species can be distinguished by the long setae on the tip of the tail. From *E. macrolaimus* and *E. brevispiculum* our species can be distinguished by the length of the spicules that is about 1.5 anal diameters in them instead of less than 1 anal diameter in *E. ampullatum.*

As *E. ampullatum*, *E. galeatum* has long terminal setae, but differs in the number of them (3 versus 2). The other difference between these two species is the shape of the gubernaculum that has apophysis in *E. galeatum* but not in *E. ampullatum.*

### ADDITIONAL CONSIDERATIONS

As *Epacanthion murmanicum* Saveljev, 1912 was described without figures and appears to be similar to *E. georgei*, it was considered as *species inquirendae* by Greenslade & Nicholas (1991). For that reason it was not considered in the previous comparisons. *Epacanthion flagellicauda* Gerlach, 1956 also was not considered because it is only known from one juvenile.

The last key for *Epacanthion* is from Guilherme et al. (2009), that is a modified version of that present in Greenslade & Nicholas (1991). We modified here a part of that key to add our two new species and *E. macrolaimus* Gagarin, 2009.

10. Spicules 2.5 anal diameters, gubernaculum present, lips not striated. ................. *E. microdentatus*  
   – Spicules less than 2.5 anal diameters, lip flaps striated .................................. 10’

10’. Spicules about 2 anal diameters .......... *E. macrolaimus*  
   – Spicules about 1 anal diameter .......... 11

11. Small species (1.5—2.0 mm long), posterior rim of cephalic capsule crenelated .......... 11’  
   – Large species (2.2—2.9 mm long), posterior rim of cephalic capsule not crenelated ...... *E. exploratoris*

11’. Tail with caudal setae .......... *E. ampullatum* sp. nov.  
   – Tail without caudal setae .............. 11”

11”. Somatic setae following a pattern standing in crowns .............. *E. bicuspidatum* sp. nov.  
   – Somatic setae sparse without a pattern .... *E. galeatum.*

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