

**SEM study of *Anisakis brevispiculata* Dollfus, 1966 and *Pseudoterranova ceticola* (Deardoff and Overstreet, 1981) (Nematoda: Anisakidae), parasites of the pygmy sperm whale *Kogia breviceps*\***

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**SUMMARY:** Scanning Electron Microscopy (SEM) was used to study different topo-morphological characteristics of the architecture (cuticle, excretory pore, lips and adjacent structures, number and distribution patterns of caudal papillae and papillae-like structures) of the anisakid nematodes *Anisakis brevispiculata* and *Pseudoterranova ceticola*, parasites in the stomach of the pygmy sperm whale *Kogia breviceps*. SEM micrographs herein help to accurately define the above surface topographical features by adding a more adequate 3-D picture to the original descriptions of both parasitic species. In *A. brevispiculata* the entire body cuticle structure, well-differentiated paraocloacal papillae and the wrinkle cuticle of the papillae are clear examples that enhance the above differentiation of structures as seen by LM or SEM. Similarly, in *P. ceticola* the cuticle striations, bulky cloacal lips, rectangular distribution pattern of distal papillae and the absence of a groove separating paraocloacal papillae which are obliquely arranged are all different to those features previously described.

*Key words:* *Anisakis brevispiculata*, *Pseudoterranova ceticola*, *Kogia breviceps*, SEM, anisakids, marine mammals.

**RESUMEN:** ESTUDIO A MEB DE *ANISAKIS BREVISPICULATA* DOLLFUS, 1966 Y *PSEUDOTERRANOVA CETICOLA* (DEARDOFF AND OVERSTREET, 1981) (NEMATODA: ANISAKIDAE), PARÁSITOS DEL CACHALOTE PIGMEO *KOGIA BREVICEPS*. – Se utilizó la técnica de Microscopía Electrónica de Barrido (MEB) para estudiar las diferentes características topo-morfológicas de la arquitectura (cutícula, poro excretor, labios y estructuras adyacentes, y el número y el patrón de distribución de las papilas caudales y de las estructuras papilares) de los nemátodos anisáquidos *Anisakis brevispiculata* y *Pseudoterranova ceticola*, parásitos en el estómago del cachalote pigmeo *Kogia breviceps*. Las microfotografías obtenidas al MEB permiten una mejor definición de las características topográficas de los nemátodos, ya que añaden una visión tridimensional a las descripciones originales de ambas especies parásitas. En *A. brevispiculata*, los caracteres de la estructura cuticular corporal, las papilas paraocloacales bien diferenciadas y la estriación cuticular de las papilas son claros ejemplos que muestran la diferenciación de las estructuras observadas a microscopía óptica de las observadas a microscopía electrónica. Igualmente, en *P. ceticola* las siguientes características (estriaciones cuticulares, labios cloacales abultados, patrón de distribución rectangular de las papilas distales, y ausencia de un surco que separe las papilas paraocloacales ordenadas oblicuamente), son todas ellas diferentes de las previamente descritas a microscopía óptica.

*Palabras clave:* *Anisakis brevispiculata*, *Pseudoterranova ceticola*, *Kogia breviceps*, MEB, anisáquidos, mamíferos marinos.

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## INTRODUCTION

Anisakid nematodes of biological, medical and economic importance in marine organisms, have received much attention notably concerning their biological characteristics, ecology, distribution and systematics. The adult stages of species of *Anisakis* Dujardin, 1845 and *Pseudoterranova* Mozgovoi, 1951 are found parasitizing the digestive tract of cetaceans and seals worldwide. The genus *Pseudoterranova* was erected as *Ascaris* L., 1758 by Krabbe (1878). Later, Baylis (1920) placed it in *Porrocaecum* Railliet & Henry, 1912, which he considered to be a senior synonym of *Terranova*. *Terranova* was resurrected at full generic level by Johnston & Mawson (1945). In 1959 Myers erected the genus *Phocanema*, which was subsequently suppressed by Gibson (1983) in favour of *Pseudoterranova*. Nowadays, the genus *Pseudoterranova* contains eight species: *P. kogiae* Johnston and Mawson, 1939, *P. ceticola* (previously named *Terranova ceticola*) (Deardorff and Overstreet, 1981), *P. cattani* (George-Nascimento, 2000) and the *P. decipiens* (Krabbe, 1878) complex comprising five biological species namely *P. decipiens* (s.s.), *P. krabbei*, *P. bulbosa*, *P. azarasi* and *P. decipiens* E (Bullini *et al.*, 1997; Paggi *et al.*, 1991, 2000; Mattiucci *et al.*, 1998). *P. ceticola* is characterized by measuring 15.5-25.5 mm long by 543-693 µm wide (greatest width). Furthermore, it possesses one pair of proximal papillae anterior to cloacal opening (absence of proximal papillae posterior to cloacal opening), one pair double paracloacal papillae and 4 pairs of distal papillae and spicules representing 1 to 2% of the body length. Cuticular plates at the posterior anal lip of males are lacking (Deardorff and Overstreet, 1981).

In a revision of the genus *Anisakis*, Davey (1971) accepted only three valid species: *A. simplex* Rudolphi, 1809, *A. physeteris* Baylis, 1923 and *A. typica* Diesing, 1860 (with 14 synonyms between them). Later research, based on allozyme markers, showed the occurrence of three reproductively isolated biological species within the morphospecies *A. simplex*: *A. simplex* (s.s.), *A. pegreffii* Campana Rouget & Bioca, 1954 and *A. simplex* C (Nascetti *et al.*, 1986; Mattiucci *et al.*, 1997). In 1998, Paggi *et al.*, employing genetic markers, identified a new species of *Anisakis* (named *A. ziphidarum*) parasitizing the beaked whales. *A. brevispiculata* was originally described by Dollfus (1966) in the Atlantic Ocean from the pygmy sperm whale. Davey's (1971) re-

examination of the genus *Anisakis* considered *A. brevispiculata* to be a synonym of *A. physeteris*. Nevertheless, recent genetic studies showed that *A. brevispiculata* and *A. physeteris* are two distinct biological species (Mattiucci *et al.*, 2001). The main morphological diagnostic character distinguishing *A. brevispiculata* and *A. physeteris* appears to be the spicule length of male specimens which is significantly shorter in *A. brevispiculata*, as reported by Dollfus (1966) in his description of the species.

Anisakid nematodes are characterized by high stability in their structural traits and very few morphological characters of taxonomic significance are so far, available (i.e. morphology of excretory system, number and distribution of caudal papillae), and are often applicable only to adults (Fagerholm, 1991). Scanning electron microscopy (SEM) provides a means to define the surface topographical features of species more accurately than possible using light microscopy (LM) (Fagerholm and Gibson, 1987; Fagerholm *et al.*, 1996). The high magnification and three-dimensional pictures obtained with the SEM has enabled us to understand the spatial relationships of surface structures and to evaluate them as taxonomic characters (Gibbons, 1986).

This paper provides a better understanding of morphological characters of taxonomic significance by complementing the previous LM morphological description of *A. brevispiculata* and *P. ceticola*.

## MATERIALS AND METHODS

The nematode material studied was collected from the stomachs of two adult females of the pygmy sperm whale, *K. breviceps*, stranded on Galician coasts (NW Spain: 42°05'-45°15'N 07°00'-09°20'W).

The nematodes were washed and freed from debris by shaking them in 0.9% NaCl solution and preserved in 70% ethanol. For diagnostic morphological studies, the nematodes were cleared in lactophenol and examined by light microscopy (LM), following standard procedures (Gibson 1984; Fagerholm and Lovdahl 1984). The identities of *A. brevispiculata* and *P. ceticola* were verified by referring to the LM observations of Dollfus (1966) and Deardorff and Overstreet (1981). They were identified by examining the following morphological characters: anatomy of the oesophago-intestinal region, lip morphology, opening of the excretory pore, tail morphology and shape and length of spicules. Moreover,

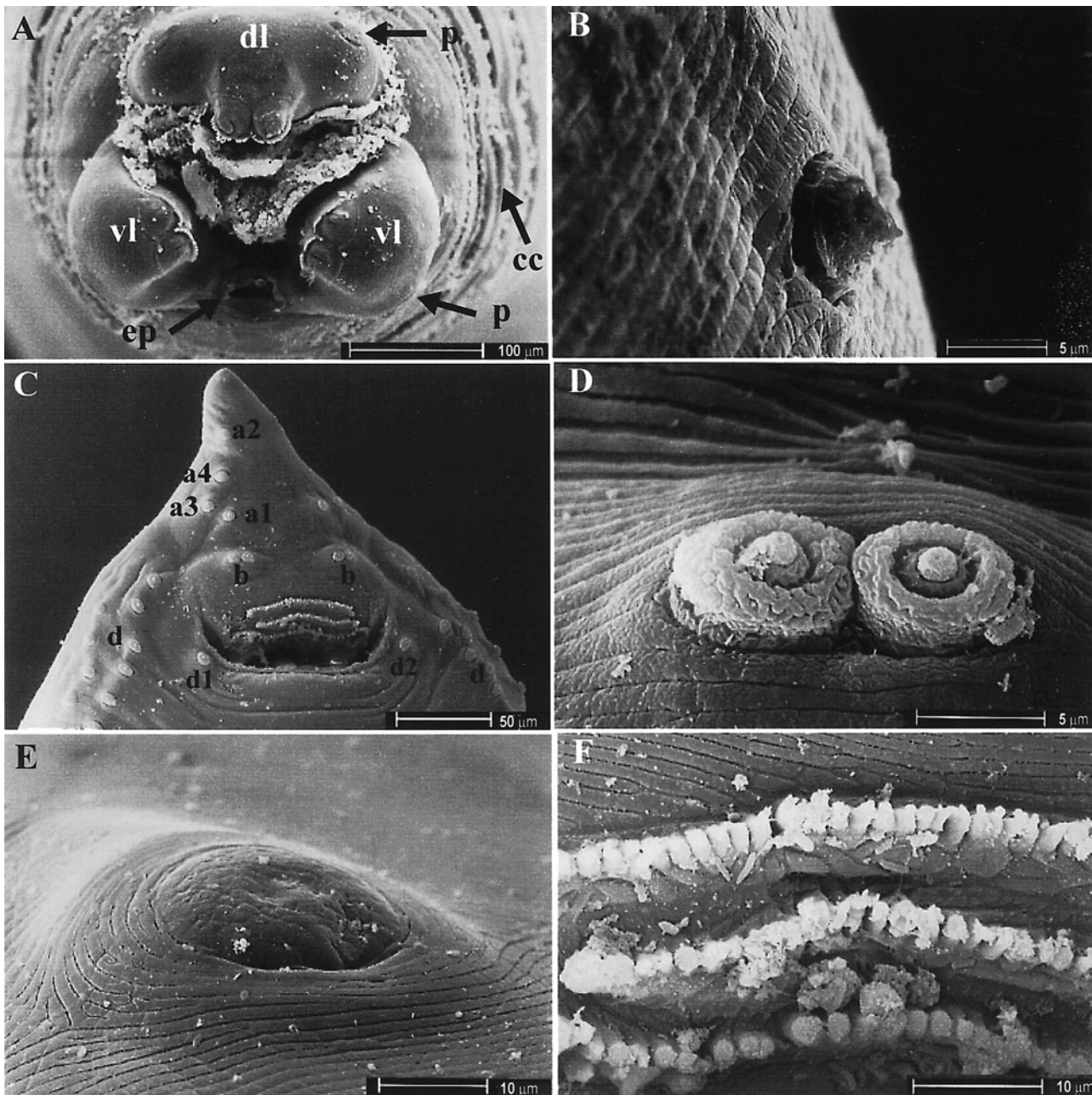


FIG. 1. – SEM micrographs of adult *Anisakis brevispiculata*. **A**, Apical view of the anterior end showing the cuticular collar (cc), excretory pore (ep), two ventrolateral lips (vl), one dorsolateral lip (dl), lips' papillae (p) and dentigerous ridges (dr). **B**, deirids. **C**, ventral view of the posterior end of a male, showing the number and distribution pattern of caudal papillae: distal papillae (a), paracloacal papillae (b), single precloacal median papillae (c) and proximal papillae (d). **D**, a pair of paracloacal papillae. **E**, phasmid. **F**, plectanes.

previous research by Mattiucci *et al.* (2001) using allozyme markers has validated the specific status of *A. brevispiculata* collected from *K. breviceps* stranded on the Galician coast.

For microtopographical diagnosis under the scanning electron microscopy (SEM), the head and tail portions of each adult nematode were processed by passing the material through a series of increasing concentrations of ethanol prior to drying in a critical point apparatus (Polaron E3000). Before

coating with gold, in a Polaron SC500 according to standard procedures, any debris, which often entirely covered the nematode cuticle, was removed by sonication. The observations were made using a Philips XC-30 microscope with an accelerating voltage of 15-20 kV.

The numbers and distribution patterns of caudal papillae and papillae-like structures in the adult males were analysed according to Fagerholm (1991).

## RESULTS

### *Anisakis brevispiculata* Dollfus, 1966 (Fig. 1)

*Description:* Transverse cuticular striae present on entire body posterior to lips, with branching and incomplete transverse striations. Presence of a cuticular collar posterior to the lips. Three lips, two ventro-lateral and one dorsal (Fig. 1A). Each lip has a bilobed anterior projection which bears the dentigerous ridge on the inner surface. Dorsal lip with a broader basal portion than ventrolateral lips. The base of the dorsal lip has two double papillae, one at each corner, and each ventro-lateral lip has one such papilla. Interlabia absent. Excretory pore located between the ventro-lateral lips. Prominent deirids situated in first-third of the body (Fig. 1B).

*Male* (10 specimens). Tail conical with rounded extremity (Fig. 1C). Cuticular striations more prominent on posterior than on anterior end. Four pairs of subventral distal papillae (a1-a4) situated posterior to the cloacal aperture were not at the same level. Two pairs of very closed subventral paracloacal papillae (b) situated between the distal papillae and the cloaca (Fig. 1D). Median papilla (sensory plaque) present on the anterior cloacal border. Numerous proximal caudal papillae forming single subventral rows. In the region of the cloaca the papillae are clustered and 8 pairs of them were found posterior to the cloaca. Paracloacal proximal and distal papillae similar in size. The papillae all have the same structure, are circular in shape, surrounded by wrinkled cuticle and provided with a small central knob. A single pair of phasmids situated laterally on the tail, circular and conical in shape (Fig. 1E). Three transverse rows of cuticular plates (plectanes) at the posterior cloacal lip (Fig. 1F).

### *Pseudoterranova ceticola* (Deardorff and Overstreet, 1981) (Fig. 2)

*Description:* Cuticle with fine transverse striations. Absence of cuticular collar posterior to the lips. Lips approximately equal in size, widest at base (Fig. 2A). Dorsal lip with a broader basal portion than ventro-lateral lips. Dentigerous ridges at the outer margin of the internal surface of each of the large lips and separated from the outer surface by a

groove. Dorsal lip with two lateral double papillae, one at each corner, and ventro-lateral lips each with a single lateral papilla (amphid) and mediolateral double papilla. Interlabia absent. Excretory pore opening between bases of ventrolateral lips.

*Female* (10 specimens). Tail conical with rather pointed tip. Pairs of phasmids situated sublaterally on tail (Fig. 2B).

*Male* (15 specimens). Tail relatively long, conical and curved ventrally. Four pairs of distal papillae (a1-a4), with 2 pairs located subventrally (a1 and a2) and 2 pairs oriented laterally (a3 and a4) on the posterior half of the tail, with a rectangular distribution (Fig. 2C). Papillae a2-a4 and a1-a3 at about the same level. A single pair of phasmids situated more dorsally to and closely associated with distal papillae a4 (Fig. 2D). Two pairs of subventral paracloacal papillae (b) fused to form double papillae (Fig. 2E), larger than distal (a) and proximal papillae (d). Distal papillae a1 closer to paracloacal papillae (b) than to distal papillae a2 or a4. Median papillae or plaque reduced. Cloaca prominent with two bulky lips. Numerous proximal caudal papillae (44-48 pairs) forming single subventral rows. In the region of the cloaca the proximal papillae are clustered and 6 pairs of them are posterior to the cloaca. A pair of these caudal papillae (d1 and d2) were always found on precloacal lip (Fig. 2F). Absence of plectanes at the posterior cloacal lip.

## DISCUSSION

Although the main features of the morphology of *A. brevispiculata* and *P. ceticola* are well established (Dollfus, 1966; Deardorff and Overstreet, 1981), there is a need for a fine analysis of some surface features which could be of use in taxonomic studies. It could be argued that such information is of restricted value, as genetic markers are confirmed as a sound approach to validate the taxonomic status of biological species (Mattiucci *et al.*, 1997, 2001; Paggi *et al.*, 1991, 1998). However, it is possible to pose pertinent questions in future work regarding taxonomy, zoogeography and phylogeny only if detailed basic morphological studies are available (Fagerholm, 1989). On the other hand, although the morphology can be readily studied by LM, a better perception of the surface structure can be obtained with SEM observations.

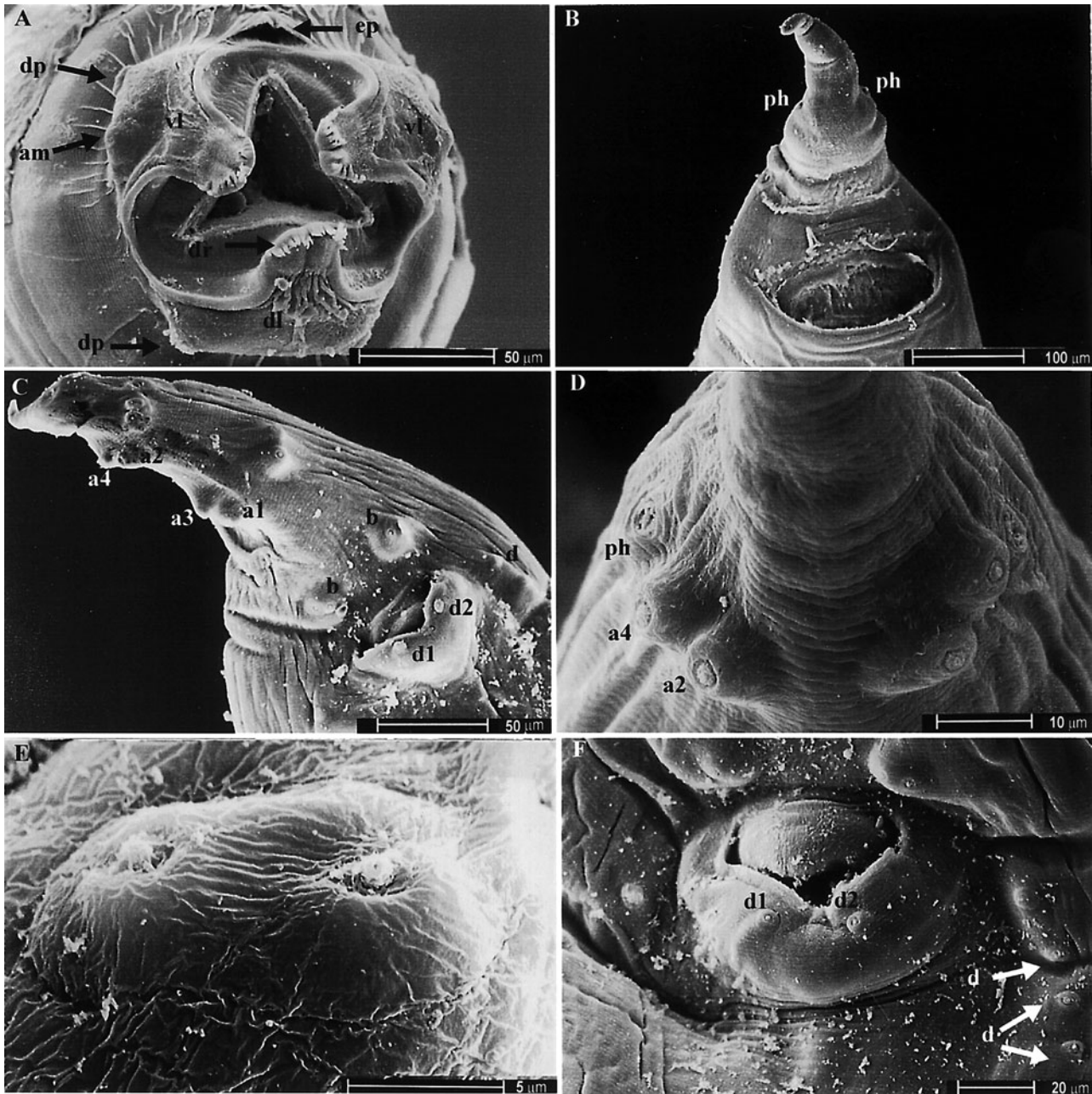


FIG. 2. – SEM micrographs of adult *Pseudoterranova ceticola*. **A**, apical view of the anterior end showing the excretory pore (ep), two ventrolateral lips (vl), one dorsolateral lip (dl), amphids (am), double papillae (dp), and dentigerous ridges (dr). **B**, ventrolateral view of the posterior end of a female, showing the phasmids (ph). **C**, ventrolateral view of the posterior end of a male showing the number and distribution pattern of caudal papillae: distal papillae (a), united paracloacal papillae (b) and proximal papillae (d). **D**, distal papillae (a2 and 4) and phasmids (ph). **E**, united paracloacal papillae pair forming double papillae. **F**, proximal caudal papillae (d) showing a pair of papillae (d1 and d2) on precloacal lip (white arrows).

Results suggest that *A. brevispiculata* is morphologically very similar to *A. physeteris*. Moreover, the distribution pattern and numbers of caudal papillae and papillae-like structures in males of different species of the genus is also very similar. Nevertheless, paracloacal papillae in *A. brevispiculata* are not united to form double papillae and they are not located immediately posterior to the cloaca as in other species of the genus, such as

*Anisakis simplex* sensu lato (Abollo *et al.*, 1998; Fagerholm, 1991). Moreover these papillae have a characteristic wrinkled cuticle, while in *A. simplex* s.l. the cuticle is smooth (Abollo *et al.*, 1998; Fagerholm, 1991).

The posterior extremity of *A. brevispiculata* herein reported was much longer and tapered than that of the *A. simplex* described by Abollo *et al.* (1998). In the latter species, papillae a2-a4 and a1-

a3 are at about the same level. A clearly defined distinct medial papilla was also observed in *A. simplex* unlike that of *A. brevispiculata* which resembled a small elevation. The number of proximal papillae anterior to the cloaca was also different in both *Anisakis* species: 8 in the case of *A. brevispiculata* and 2 in *A. simplex*. The paraoccal papillae are fused to form double papillae, but each papilla is separated by a groove, as occurs in other species of the genus. In *P. ceticola* the absence of the groove separating each papilla is noteworthy. In our material the presence of well-developed bulbous anal lips and prominent distal papillae in *P. ceticola*, which have not been recorded in other species within the genus or family, is worthy of notice. Similarly, plectanes are also absent in *P. ceticola* but present in other species within the genus (Paggi *et al.*, 1991, 2000) or family (Abollo, *et al.*, 1998; Fagerholm, 1991). In addition, paraoccal papillae were obliquely arranged rather than horizontally arranged as in the original description (Deardorff and Overstreet, 1981) and distinct also from those in *Pseudoterranova* and *Anisakis* species which are vertically and horizontally arranged, respectively. In our material, papillae a1-a3 of *P. ceticola* were at about the same level unlike the original LM description in which the papillae a1-a3 were clearly much more separated.

According to Willmott (1974) and Gibbons (1986) annulations are defined as deep transverse grooves occurring at regular intervals giving the body a segmented appearance. Fine transverse grooves occurring at regular intervals were seen in our *P. ceticola* material. According to the above authors these fine transverse markings in the cuticle should be considered striations and not inconspicuous annulations as was previously reported in the original description of the species by Deardorff and Overstreet (1981).

The species *A. brevispiculata* was recorded from the stomach of *K. breviceps* stranded at Charente-maritime (France) by Dollfus (1966), and on the Galician coast (Spain) and South African coast by Mattiucci *et al.* (2001). *P. ceticola* was recorded from the stomach of *K. breviceps* stranded at Charente-maritime (France) by Dollfus (1966) and from the stomach of a dwarf sperm whale (*K. simus*) stranded on the Mississippi Coast by Gunter and Overstreet (1974) and Deardorff and Overstreet (1981). This paper therefore widens the geographical distribution of *P. ceticola* which has not been previously reported from Galician waters.

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