Marine Ecology. ISSN 0173-9565

ORIGINAL ARTICLE

The ignored but common nemertine *Psammamphiporus elongatus* from the Galician beaches (Spain), affected by the Prestige oil spill

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Keywords
Beach; nemertea; prestige oil spill; *Psammamphiporus elongatus*.

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Conflict of interest
The authors declare no conflict of interests.

Abstract

Nemerteans have been recorded in several studies of the sandy beaches, but usually these specimens have been only regarded as members of the Phylum, with no attempt at identification at any taxonomic level. This lack of identification is partly because of the sampling procedures used to collect beach macroinfauna. Nemerteans are fragile and need special care. They must be isolated and observed *in vivo* before using histological procedures, which must be employed for the taxonomical studies. This study deals with a sandy beach hoplonemertean, *Psammamphiporus elongatus* (Stephenson 1911), only known for seven specimens from the Firth of Clyde (UK). This species is recorded for the first time from the Galician sandy beaches (NW Spain) where it reaches its maximum abundance. The species is described and illustrated and new data on its morphology and ecology are provided. The species was apparently affected by the 'Prestige' oil spill, and would be a good indicator of beach quality.

Problem

Although littoral and coastal nemerteans are quite well known from many marine habitats of the world (see Gibson 1995), the sandy beach forms have been almost entirely neglected. Several authors reported the occurrence of nemerteans from sandy beaches around the world and the Phylum is apparently quite common on this habitat, but species remain unidentified. Clearly, taxonomic studies on the sandy beaches nemerteans are long overdue.

Nemerteans have been recorded in several studies of the macroinfauna of the Galician sandy beaches (Viéitez & López Cotelo 1982; Viéitez & Baz 1988; Mazé *et al.* 1990; Pérez Edrosa & Junoy 1991; Junoy & Viéitez 1992; Lastra *et al.* 2006), but these specimens have only been regarded as members of the Phylum, with no attempt at identification at any taxonomic level. The lack of identification is partly because of the sample procedures. In those studies, all the sediment was sieved and the sedimentary debris and macroinfauna were stored with formalin. The correct identification of the specimens normally needs a live material and a good preservation for histological studies.

It is only with the sampling campaigns related to the study of the macroinfauna of the beaches affected by the 'Prestige' oil spill (Project VEM2004-08544, Spanish Ministerio de Educación y Ciencia) when living specimens were collected, and subsequently identified as *Psammamphiporus elongatus* (Stephenson 1911), a species that was previously known for seven specimens collected only at two localities in the Firth of Clyde (UK). For the last 75 years, the species *Amphiporus elongatus* Stephenson 1911 has only been known from a single specimen found in Fairlie sands, Ayshire (UK) (King 1911; Stephenson 1911; Gibson 1982). After that, Gibson (1989), studied six further specimens collected near the type locality (Kames Bay, Great Cumbrae Island, UK), reassessed the systematic position of the species, and established the genus *Psammamphiporus* for it.

In the present paper, the anatomy of this hoplonemertean is described and compared with that provided by Gibson (1989). The species is photographed for the first time: up to date, the external appearance of this species is only based on Stephenson's specimen drawings (Stephenson 1911: figures 12–14) and those redrawn by Gibson (1982: figures 30 A–C; 1994: figures 35 A–C). Notes on its ecology on the Galician beaches are provided; the species was apparently affected by the 'Prestige' oil spill.

Study Area

The 18 beaches studied, situated along the 1659 km of the Galician coast (NW Spain) were: América, La Lanzada, Corrubedo, Xuño, Louro, Carnota, Rostro, Area Longa, Traba, Seiruga, Baldaio, Barrañán, Doniños, Frouxeira, San Román, Esteiro, Llas, and Altar (Fig. 1, Table 1).

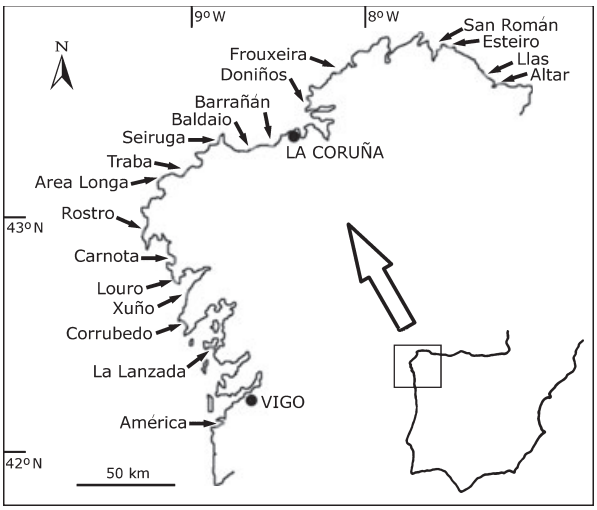

Fig. 1. Map showing location of sampled beaches.

Material and Methods

The 18 beaches were sampled once during May 2003 and May–June 2004 spring tides. A transect in the middle of the beach was extended from above the drift line to below the swash line, and sample stations marked to five intertidal levels. At each station, six 0.05 m^2 replicates were taken and sieved through 1 mm mesh and the residue preserved in 7% formalin (see Junoy *et al.* 2005 for details). For this study, additional specimens were collected alive in 2005 and 2006 and kept under laboratory conditions. These specimens were anaesthetised in 7.5% $MgCl_2$ and examined alive. Two of these specimens were fixed in cold Zenker's fluid, embedded in paraffin, sectioned (6 μm) and stained with the Mallory triple stain method.

Results

Systematics

Material examined

Twenty-nine specimens from the years 2003 and 2004 were preserved in 7% formalin: América beach (one specimen, 7 May 2004); La Lanzada beach (two specimens, 5 May 2004); Corrubedo beach (six specimens, 1 May 2004); Xuño beach (three specimens, 14 May 2003; two

Table 1. Summary table describing the locations and the principal characteristics of the beaches studied. W: wide in metres; L: long in metres; Md: range of median particle diameters (μm); OM: range of organic matter content (%).

beach	location		W	L	Md	Wentworth grades	OM
América	42°08′N	8°49′W	104	2300	240–370	fine–medium	1.2–2.5
Lanzada	42°28′N	8°51′W	127	2400	200–1570	fine–very coarse	1.3–2.2
Corrubedo	42°32′N	9°01′W	139	2900	210–460	fine–medium	0.8–2.1
Xuño	41°01′N	9°01′W	108	2700	460–1380	medium–very coarse	0.3–1.2
Louro	42°43′N	9°03′W	114	1470	380–560	medium–coarse	0.3–1.1
Carnota	42°41′N	9°07′W	133	7000	210–410	fine–medium	1.0–1.8
Rostro	42°90′N	9°12′W	125	2070	380–490	medium	0.4–1.3
Area Longa	43°10′N	9°11′W	185	310	450–770	medium–coarse	0.7–1.7
Traba	43°11′N	9°09′W	98	2630	350–810	medium–coarse	0.3–1.1
Seiruga	43°18′N	8°52′W	91	530	340–540	medium–coarse	1.7–2.6
Baldaio	43°41′N	8°41′W	163	3650	370–1290	medium–very coarse	0.8–1.4
Barrañán	43°18′N	8°32′W	140	1200	430–550	medium–coarse	0.8–1.4
Doniños	43°28′N	8°18′W	154	1700	480–620	medium–coarse	0.6–1.2
Frouxeira	43°35′N	8°10′W	215	3000	350–1070	medium–very coarse	0.4–1.2
San Román	43°44′N	7°39′W	153	910	330–390	medium	1.0–1.6
Esteiro	43°43′N	7°34′W	184	1160	360–430	medium	0.9–1.7
Llas	43°35′N	7°16′W	68	760	300–370	medium	0.5–1.4
Altar	43°34′N	7°14′W	220	950	260–350	medium	0.8–1.5

specimens, 2 May 2004); Louro beach (two specimens, 14 May 2003); Carnota beach (one specimen, 3 May 2004); Traba beach (one specimen, 15 May 2003; two specimens, 3 May 2004); Baldaio beach (one specimen, 1 June 2004); Frouxeira beach (one specimen, 2 June 2004), San Román beach (one specimen, 2 June 2004), Esteiro beach (two specimens, 17 May 2003; two specimens, 2 June 2004); and Altar beach (two specimens, 3 June 2004).

Thirteen specimens alive: Corrubedo beach (one specimen, 20 September 2005; one male, 11 June 2006); Seiruga beach (one specimen, 27 April 2006); Frouxeira beach (two specimens, 19 September 2005; one specimen, 29 March 2006; one specimen, 28 April 2006; one specimen, 12 June 2006); San Román beach (four specimens, 28 April 2006); Altar beach (one female, 13 June 2006).

Diagnosis

Gibson (1989) established the genus *Psammamphiporus* with the following diagnosis: 'Monostiliferan enoplan nemerteans with rhynchocoel wall containing separate circular and longitudinal muscle layers; rhynchocoel extending almost to posterior tip of body, without diverticula; proboscis with three muscle layers (outer circular, middle longitudinal, inner circular) in wall of anterior chamber and single central stylet; pre-cerebral septum closed; mouth and proboscis pore opening via common anterior aperture, foregut without diverticula, intestinal caecum with anterior and lateral pouches, intestine with deeply branched lateral diverticula; body wall without diagonal muscle layer, longitudinal musculature not anteriorly divided and extending pre-cerebrally to tip of head; cerebral sensory organs well developed, situated close in front of pre-cerebral septum; excretory system well developed, extending from behind brain back to anterior intestinal region of body; four eyes; cephalic and submuscular glands present in the head; apical sensory organ present; blood vascular system with cephalic loop and three post-cerebral vessels, mid-dorsal vessel with single vascular plug, intestinal region without pseudometameric transverse connectives; sexes (probably) separate' (Gibson 1989: 357).

Specimens from the Galician beaches agree with this diagnosis and in general with de Gibson's redescription of *Amphiporus elongatus*. The species is true dioecious and mature specimens were collected in June 2006. The following description from the Galician nemertines is based on new observations.

Description

External features. Most of the nemerteans examined alive, before or after anaesthetization, were 20–60 mm long and less than 1 mm wide. They stretched the body out full length, but they contract very quickly when disturbed. Colour is light brown or orange to yellowish. There are four eyes grouped into two sets; the anterior pair is situated near the anterior tip of the body and they are some distance from the other pair, bigger, which is located over the large cerebral sensory organs in front of the cerebral ganglia and not far from them. The distance between the eyes of the anterior pair and the posterior pair is larger than the distance between both eyes in each pair. There are two pairs of posteriorly angled transverse cephalic grooves on the dorsal surface of the head; both pairs of furrows are almost parallel and extend ventrally and anteriorly towards the mid-line (Fig. 2A and C).

Body wall, musculature and parenchyma. The glandular epidermis, 15–25 μm thick, possesses a normal structure (Fig. 4A: EP). A connective tissue dermal layer up to 3 μm proximally borders the epidermis (Fig. 4A: DE). The body wall musculature comprises outer circular and inner longitudinal layers, respectively 6–10 and 30–90 μm thick (Fig. 4A: CM, LM). The deeper fibres of the longi-

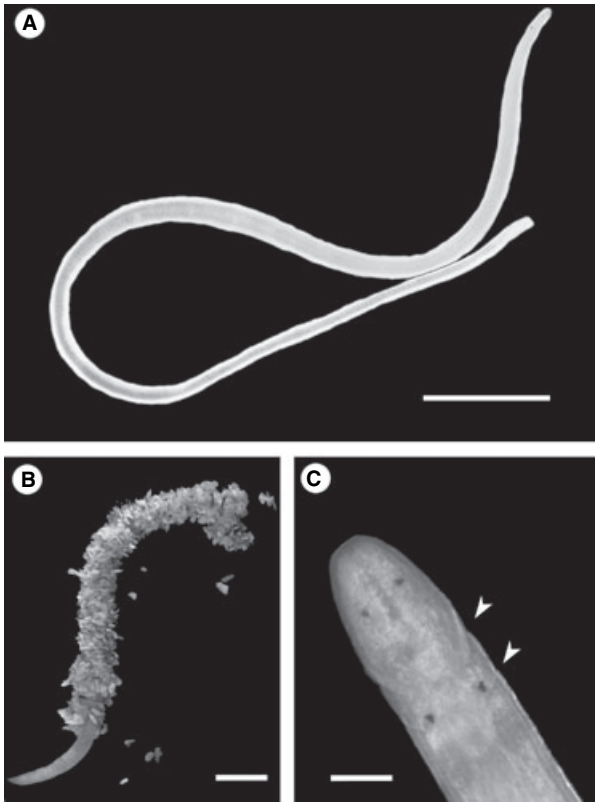

Fig. 2. *Psammamphiporus elongatus*: A: Photograph of complete specimen. B: The feeble tube constructed by the worm. C: Enlargement of the cephalic region in dorsal view to show the distribution of the cephalic furrows, both of them indicated by the arrowheads. Scale bars: A: 2.5 mm; B: 1 cm; C: 200 μm.

tudinal muscle layer lead radially inwards to the proboscis insertion and form the pre-cerebral septum (Fig. 4B).

Proboscis apparatus. The proboscis pore is situated antero-ventrally near the tip of the head; when everted it can be clearly seen that it is sparsely papillated (Fig. 3C). It opens into a thin-walled rhynchodaeum whose epithelium is neither ciliated nor glandular. The rhynchocoel almost reaches the posterior tip of the body. Its wall contains separate circular and longitudinal muscle layers. The proboscis is divisible into the three characteristic regions of the Monostiliferoidea (Figs 4C and 5A); nerve supply consists of a neural ring with 10 nerves (Fig. 5A). The proboscis armature consists of a single central stylet, 80–120 μm long, borne on a cylindrical basis. This basis, about 120–180 μm long and 70–80 μm wide, sometimes has a median waist, as noted by Stephenson (1911). These basis are longer and narrower than those described by Gibson (1989). The two accessory stylet pouches contain two to four reserve stylets (Table 2, Fig. 4D and G).

Alimentary canal. No differences with the Gibson description (Gibson 1989) are observed.

Blood system. The blood system follows the basic plan for enoplan nemerteans. The mid-dorsal blood vessel emerges as a branch of the right lateral vessel, and immediately enters the rhynchocoel as a thick-walled vascular plug, up to 85 μm in diameter (Fig. 4E: VP). For all the body length, the lateral vessels run below the lateral nerve cords (Fig. 4A: LV). Although the blood vessels possess the so-called valves noted by Gibson (1989), they could represent only artefacts of fixation (Maslakova *et al.* 2005).

Nervous system. The ventral cerebral lobes are much thicker than the dorsal ones. The dorsal cerebral commissure is long and slender (14–20 μm wide) and the ventral commissure is short and thick (33–40 μm wide) (Fig. 5C: DC, VC). The lateral nerve cords only contain fibres from the ventral brain lobes. There are some muscle fibres extending the length of the lateral nerves adjacent to the inner margin of the fibrous tissue core (Fig. 4A: arrowhead).

Frontal organ and cephalic glands. The frontal organ is a shallow epithelial depression on the tip of the snout (Fig. 4F). Cephalic gland lobules are poorly developed.

Sense organs. Cephalic cirri are observed at the tip of the head (Fig. 3D: arrowhead). There are four eyes grouped into two sets in each of the studied specimens. The eyes of the anterior pair are shorter in diameter than the posterior (15–17 and 22–23 μm respectively) (Fig. 2C). As noted by Gibson (1989), four eyes appear to be the typical number for the species; the Stephenson's (1911)

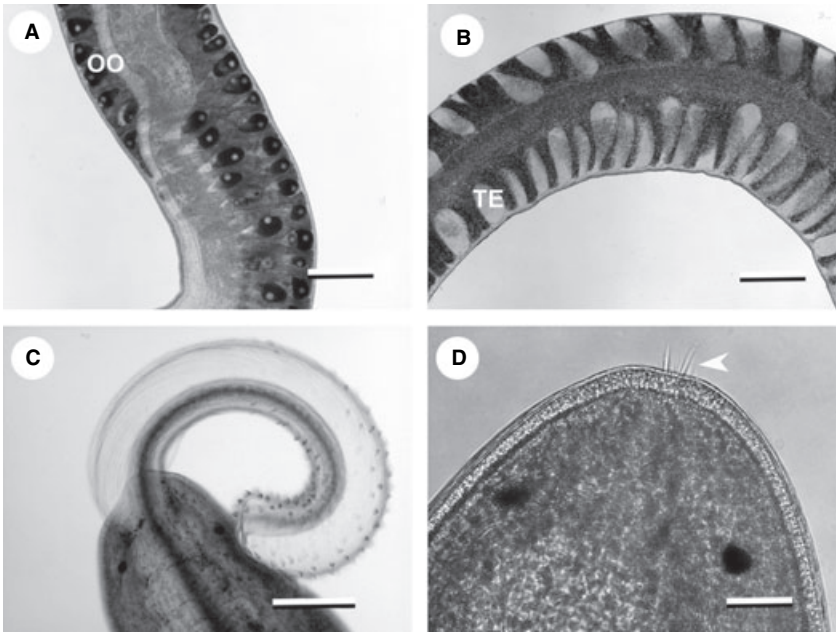

Fig. 3. *Psammamphiporus elongatus*: A: Female gonads showing an oocyte per gonad. B: Testes of a male specimen. C: Dorsal view of a specimen with everted proboscis showing papillae. D: Dorsal view of a live squeezed specimen showing the cephalic cirri (arrowhead). OO, oocyte; TE, teste. Scale bars: A, B: 500 μm; C: 250 μm; D: 50 μm.

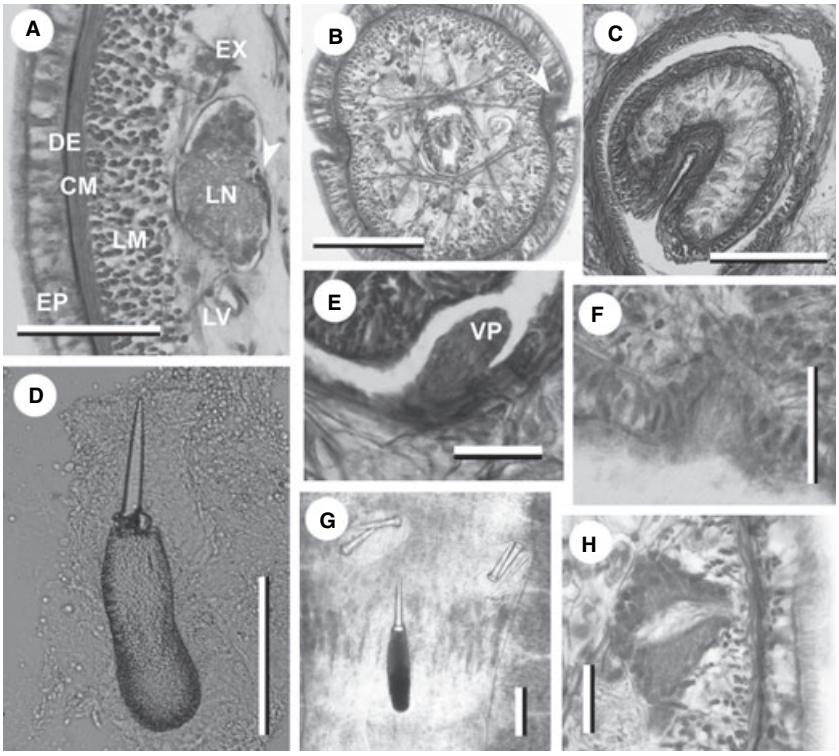

Fig. 4. *Psammamphiporus elongatus*: A: Transverse section to show the structure of the body wall, showing the relative positions of a nerve cord, lateral blood vessel and excretory tubule. The arrowhead indicates the position of the myofibrillae of the nerve cord. B: Transverse section to show the pre-cerebral septum, arrowhead indicates a cephalic furrow. C: Transverse section through the posterior portion of proboscis. D: Micro-photograph of a live squeezed specimen showing stylet with waist in its basis. E: Transverse section through the vascular plug. F: Transverse section to show the ciliated chamber of the apical sensory organ. G: Microphotograph of a live squeezed specimen showing both of the accessory stylet pouches and the central stylet without waist. H: Transverse section to show the ciliated canal of the cerebral sensory organ. CM, body wall circular muscle layer; DE, dermis; EP, epidermis; EX, excretory tubule; LM, body wall longitudinal muscle layer; LN, lateral nerve cord; LV, lateral blood vessel; VP, vascular plug. Scale bars: A: 50 μm; B, C, D, G: 100 μm; E, F, H: 25 μm.

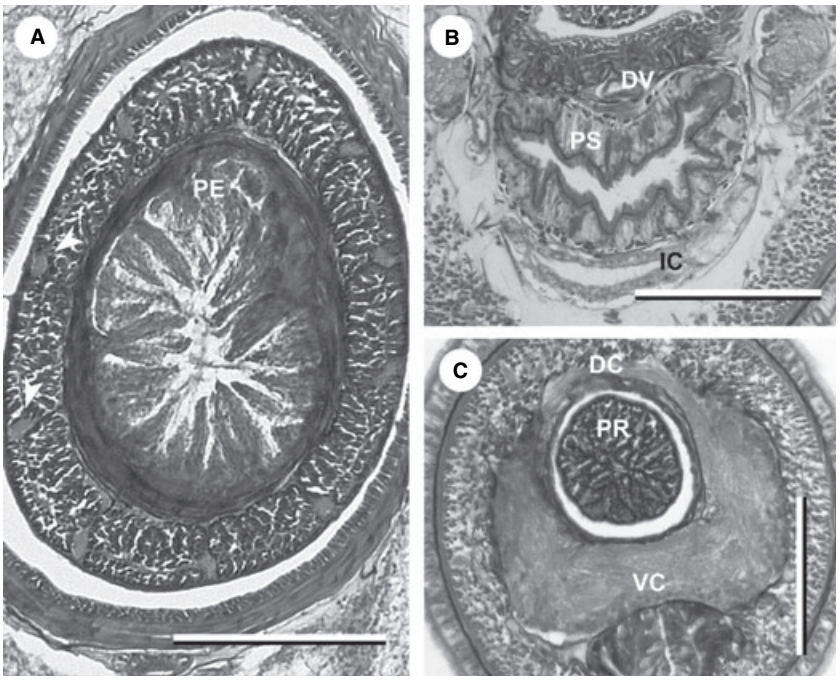

Fig. 5. *Psammamphiporus elongatus*: A: Transverse section showing the organisation of the anterior portion of the proboscis. The arrowheads indicate two of the 10 proboscis nerves. B: Transverse section through the posterior stomach region showing also the intestinal caecum. C: Transverse section through the cerebral region showing the ventral and the dorsal commisure. DC, dorsal commisure of the cerebral region; DV, mid-dorsal blood vessel; IC, intestinal caecum; PE, proboscis epithelium; PR, proboscis; PS, epithelium of posterior stomach region; VC, ventral commisure of the cerebral region. Scale bars: A–C: 100 μm.

specimen possessed five eyes. The cerebral sensory organs are large and located in front of the brain. Each one opens laterally by a ciliated canal leading inwards from the anterior cephalic furrow on either side of the head (Fig. 4H). The canals turn posteriorly when they have passed through the body wall layers and they reach the neural tissues. A well-developed cap of acidophilic glands extends a short distance along the upper and lower mar-

Table 2. Measurements recorded for the stylet apparatus of six specimens of *Psammamphiporus elongatus*. Length and width in μm.

	range	average
length of central stylet	80–130	99
length of basis	119–230	154.50
maximum width of basis	35–53	44.66
ratio of basis length to central stylet length	1.36–1.76	1.54
ratio of basis length to basis width	2.60–4.33	3.45
number of accessory stylet pouches	2	2
number of reserve stylets per pouch	2–4	3

gins of the organs. The cerebral organs are 30–45 μm in height, 30–40 μm wide and 60 μm long (including their ciliated canal).

Excretory system. It extends from close behind the back of the brain to the anterior intestinal regions. It consists of one to several thick-walled collecting tubules, 15–20 μm wide, running above or below the lateral nerve cords on both sides of the body (Fig. 4A: EX).

Reproductive system. The specimens collected in June 2006 have mature gonads; the male is white in colour and female light orange. Gonads are numerous and extend around the intestine and between the diverticula from the dorsal to the ventrolateral margins. Every ovary bears only one ovum 130–250 μm in diameter, with a 40–45 μm nucleus (Fig. 3A). The mature male worms contain ripe testes closely packed within the parenchymal tissue along the body (Fig. 3B). This observation confirms the dioecious sexuality of *Psammamphiporus elongatus.*

Remarks

Gibson & Crandall (1989) in the revision of the genus *Amphiporus* concluded that the designated type species for the genus is a *nomen dubium* and thus, the status of the genus *Amphiporus* is uncertain. They asked themselves what to do with the species included in this genus, and Gibson (1989) resolves the question with *Amphiporus elongatus*. He redescribed the species, established the genus *Psammamphiporus* for it, and designed the neotype. This would be possible because six new specimens were collected in the year 1987 close to the type locality, whereas only Stephenson's one specimen was known up to that date. Present data indicate that *Psammamphiporus elongatus* is a common species of the Galician beaches, where 42 specimens were collected during the years 2003–2006. The species is also listed in the North East Atlantic Taxa checklist from Germany (Hansson 1998), but the author did not mention any details about this record. Thus, the species is well known only from two very distant places, Scotland (UK) and Galicia (Spain). Although apparently the species is more abundant in Spain, this fact is probably the result of the much higher sampling intensity in the Galician beaches. This species should be expected to be present in other sandy beaches of the European Atlantic.

The external appearance of the species is now well known and illustrated. Gibson (1989: 357–358) mentioned that '[…] external features of the present specimens closely conformed with Stephenson's (1911) original description, although all were smaller and rather more orange than yellow in colour'. The Galician nemerteans are shorter than Stephenson's specimen (40–60 mm *versus* 75 mm) and their colour is light orange in females, whitish in males and light brown to yellowish in immature specimens. As noted by Gibson (1989), all specimens possess four eyes. New observations on their gonads and proboscis armature contribute to a better knowledge of the species. The morphology of Spanish specimens is similar to that described by Gibson (1989); minor differences can be due to the fixation.

Ecology

Habitat

Characteristics of the sampled beaches are given in Table 1. The median diameter of the sand grains varies between 200 and 1570 μm. The organic matter content of the sediments was relatively low throughout the beaches and ranged from 0.3 to 2.6%. The worm lives in a feeble tube constructed by aggregating sand grains (Fig. 2).

Macroinfaunal community

The macroinfaunal community of these beaches consists of typical psammophilous species dominated by crustaceans and polychaetes. *Psammamphiporus elongatus* was collected at the medium to low intertidal levels of these sandy beaches. No specimens of the nemertean were collected in the supralitoral and drift line levels. Air breathers like talitrid amphipods (*Talitrus saltator* Montagu, 1808, *Talorchestia brito* Stebbing, 1891 and *Talorchestia deshayesii* (Audouin, 1826)), the isopod *Tylos europaeus* Archangeli, 1938, and insects inhabit these upper levels.

Psammamphiporus elongatus inhabited the three lower levels sampled in the beaches, at densities ranging from 1.1 to 6.6 indiv m^{-2}. Table 3 shows the frequency and abundance of the accompanying macroinfaunal taxa in the samples with *P. elongatus.* The most frequent and abundant species were the amphipod *Pontocrates arenarius* (Bate, 1858), the mysid *Gastrosaccus roscoffensis* Bacescu, 1970, and the spionid polychaetes *Scolelepis mesnili* (Bellan & Lagardère, 1971) and *Scolelepis squamata* Müller, 1806.

Table 3. Accompanying macroinfaunal taxa in the samples with *Psammamphiporus elongatus* in 2003 and 2004.

species	frequency	indiv m^{-2}
Pontocrates arenarius	72	107.20
Scolelepis mesnili	40	18.40
Gastrosaccus roscoffensis	40	14.40
Scolelepis squamata	24	56.00
Lekanesphaera sp.	16	5.60
Oligochaeta	12	3.20
Talitrus saltator	12	3.20
Protodriloides chaetifer	8	0.80
Pisione remota	8	3.20
Saccocirrus cf. *papillocereus*	8	11.20
Donax trunculus	8	1.60
Haustorius arenarius	8	3.20
Eurydice affinis	8	1.60
Eurydice pulchra	4	8.80
Cumopsis fagei	4	20.00

Other accompanying species were the isopods *Eurydice naylori* Jones & Pierpoint, 1997, *Eurydice affinis* Hansen, 1905, the amphipod *Haustorius arenarius* (Slabber, 1769) and the polychaete *Saccocirrus* cf. *papillocercus* Bobretzky, 1872.

Prestige's oil spill impact

Psammamphiporus elongatus was only identified in the 2003 and 2004 sampling campaigns, 6 and 18 months after the spill, respectively. In 2003, the species was only collected at four beaches: Xuño, Louro, Traba and Esteiro beaches. Except for Louro beach, in 2004, *P. elongatus* was also present in seven more beaches: América, La Lanzada, Corrubedo, Baldaio, Frouxeira, San Román and Altar beaches. No specimens were collected in both sampling occasions at five beaches with heavy pollution (Rostro, Area Longa, Seiruga, Barrañán and Doniños beaches) and one beach with light pollution (Llas beach).

Geographical distribution

Scotland, UK and Galicia, NW Spain. This species is apparently also present in Germany (Hansson 1998; M. Thiel, personal communication).

Discussion

As noted by Junoy *et al.* (2005) the total macroinfaunal abundance was significantly reduced after the spill in the Galician beaches. Nemerteans in general were more abundant before the spill, from a density of 6.33 indiv 1.5 m^{-2} to a density of 2.50 indiv 1.5 m^{-2} after the spill (Junoy *et al.* 2005). These authors indicated that the twelve most affected beaches, classified as heavily polluted, were those situated in the arc between Corrubedo and Frouxeira beaches whereas the remaining six were relatively unimpacted by the oil, and considered as lightly polluted (Junoy *et al.* 2005).

There are no data about the presence of *Psammamphiporus elongatus* before the Prestige oil spill; the nemertean is now identified for the first time from the Galician beaches in the samples taken after the spill. The species was probably eliminated from many beaches by the oil, and their populations are now recovering. Six months after the spill, it was present in only 22% of the beaches, whereas 18 months after it was present in 61% of the sampled beaches. No specimens were collected in either sampling occasion at five beaches with heavy pollution. These data do not clearly indicate that the spill was affecting the presence of *Psammamphiporus elongatus*, but it raises founded suspicions.

Like other carnivorous, its abundance in the beaches is low in comparison with the other sandy beach macroinfauna. Rare species, with very low density in the beaches, were eliminated by the spill (Junoy *et al.* 2005), and this was the case for *P. elongatus*.

Nemerteans prey primarily on polychaetes and crustaceans (McDermott & Roe 1985; Thiel & Kruse 2001) and it could be that the reduction of preys by the spill affected the populations of *P. elongatus*. This could be true if the nemertean preyed only on the polychaete *Scolelepis* and the isopod *Eurydice*, whose populations were reduced after the spill (Junoy *et al.* 2005). But another potential prey is the amphipod *Pontocrates arenarius*, the most abundant species in the samples with *P. elongatus*, and whose populations increased after the spill (Junoy *et al.* 2005). Thus, the alternative hypothesis, i.e. the oil directly affected the nemertine, is more probable.

Acknowledgements

The authors wish to thank Carolina Castellanos, Dr Viéitez and the Benthos Team from Universidad de Alcalá ('The Cribians') for assistance in the field. In addition, they are very grateful to Dr Eduardo de Miguel for his valuable suggestions to improve this manuscript.

This research was in part supported by the Project CGL2004-00709, Spanish Ministerio de Educación y Ciencia and the Project 79/2003, Spanish Organismo Autónomo de Parques Nacionales. The Project VEM2004-08544, Spanish Ministerio de Educación y Ciencia, provided funds for the sampling campaigns on the beaches.

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