

Redescription of *Lineus acutifrons* Southern, 1913 (Nemertea: Pilidiophora) and comments on its phylogenetic position

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The nemertean *Lineus acutifrons* Southern (1913) is a species rarely encountered in sandy beaches of Europe and is only really known from its original and short description. This species has been found almost a century later in Galician sandy beaches (northwest Spain) and is re-described here. Data detailing its internal morphology and external anatomy, and digital photographs are included. In addition, sequences of the ribosomal gene 28S and the mitochondrial gene cytochrome oxidase I (*COI*) were obtained. These sequences were used in a phylogenetic analysis to test its phylogenetic position with respect to other sequences available for pilidiophorans.

Keywords: Pilidiophora; *Lineus acutifrons*; *COI* mtDNA; 28S rRNA; phylogeny; Galicia

Introduction

Lineus acutifrons was described by Southern in 1913 from western Ireland (Blacksod Bay, Mayo and Ballynakill Harbour, Connemara) from sand near the low tide line. Since then, this species has only been recorded twice: once by Gibson (1995), who noted it was also found on the Isle of Man (British Isles), and then by Müller (2004), who listed the species as present on the Belgian coast but has more recently said that it was only “probably present” (Müller personal communication 2009). Gibson mentions *L. acutifrons* in two other publications (Gibson 1982, 1994) and noted that no specific internal characters were adequately described.

Recently, specimens of a nemertean similar to the description by Southern (1913) of *L. acutifrons* were collected by sampling the beaches affected by the Prestige Oil spill (Project VEM2004-08544). These specimens were collected at four of 18 beaches surveyed on the Galician coast of northwest Spain. Additional living specimens were collected from Islas Atlánticas de Galicia National Park (Project 79/2003), also in northwest Spain.

The external morphology of the specimens fits well with the characteristics described by Southern (1913) for *L. acutifrons*: pink to bright red colouration, without differences between the dorsal and ventral surfaces, head acutely pointed in front, distinctly marked off from the body by a well-defined constriction, and without eyes. Details of the internal morphology, such as the size and relationships of the ganglia,

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cerebral organs and cephalic slits, and the size of rhynchocoel and body wall muscle layers also coincide with those described by Southern (Southern 1913: 8–9).

Here we re-describe the anatomy of *L. acutifrons* and report sequences for two molecular markers commonly used in phylogenetic studies, cytochrome oxidase I (COI) mitochondrial DNA, and 28S ribosomal RNA (28S rRNA). In addition, we evaluate its phylogenetic position and taxonomy based on the DNA sequence data.

Materials and methods

The 18 beaches sampled are located along 1659 km of the Galician coast of north-west Spain and include: América, La Lanzada, Corrubedo, Xuño, Louro, Carnota, Rostro, Area Longa, Traba, Seiruga, Baldaio, Barrañán, Doniños, Frouxeira, San Román, Esteiro, Llas, and Altar (Figure 1). All of these beaches were sampled once during spring tides from March to June between 2003 and 2006; three of the beaches (Corrubedo, Frouxeira and Altar) were sampled monthly between September 2006 and August 2007. A transect in the middle of the beach was extended from above the drift line to below the swash line, and sample stations were marked at five levels. At each station, six 0.05-m² replicates were taken and sieved through 1-mm mesh and

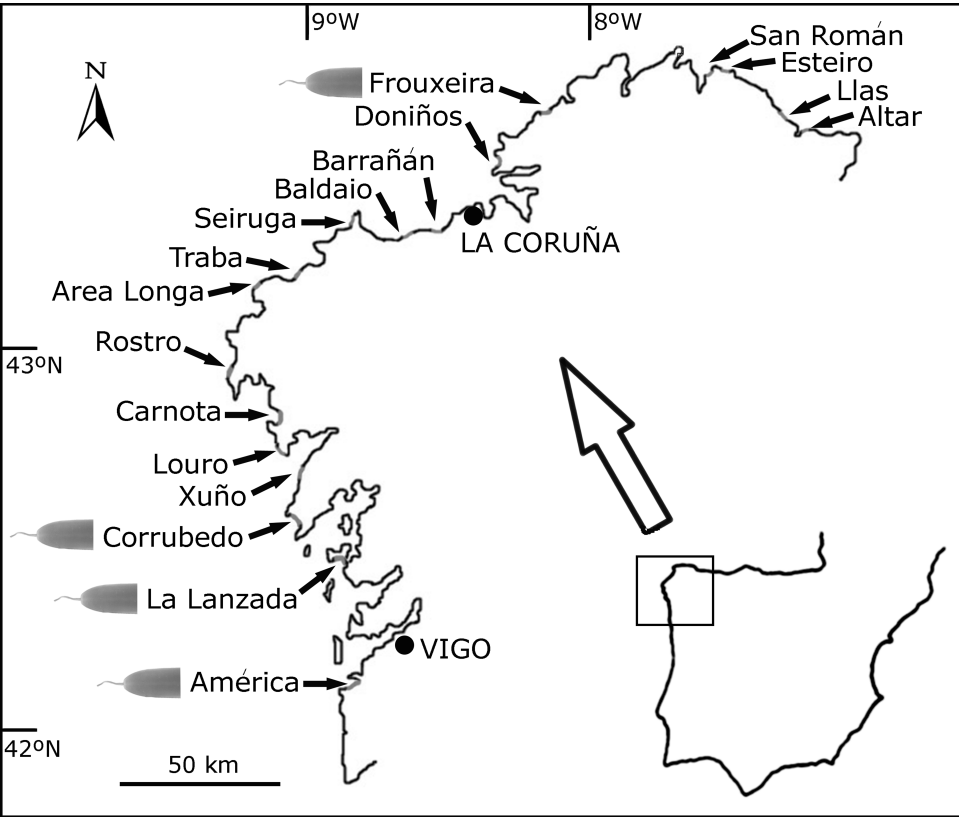


Figure 1. Map of beaches sampled in this study. Beaches where *Lineus acutifrons* was collected are marked with a caudal cirrus.

Table 1. Summary table describing the locations and principal characteristics of the beaches where nemerteans were found.

Beach	Location	W	L	Md	Wentworth grades
América	42°08' N, 8°49' W	104	2300	240–370	Fine–medium
La Lanzada	42°28' N, 8°51' W	127	2400	200–1570	Fine–very coarse
Corrubedo	42°32' N, 9°01' W	139	2900	210–460	Fine–medium
Frouxeira	43°35' N, 8°10' W	215	3000	350–1070	Medium–very coarse

Notes: W, width in metres; L, length in metres; Md, range of sediment median particle diameter (μm).

the residue was preserved in 7% formalin in seawater (Junoy et al. 2005). Twenty-one specimens of the red nemertean identified here as *L. acutifrons* were collected at four beaches (Table 1). Seven additional specimens were collected and examined alive at Corrubedo beach in February 2008. Four of these specimens were fixed in Bouin's fluid, embedded in paraffin, thick-sectioned (6 μm) and stained with the Mallory triple stain method.

Material examined

Twenty eight specimens were collected. América beach: two specimens, 13 May 2003, D3 and D5 stations; one specimen, 7 May 2004, B4 station; two specimens, 21 April 2005, C5 and D5 levels; two specimens, 10 June 2006, B5 and F5 stations. La Lanzada beach: two specimens, 15 May 2003, C4 and F4 stations; one specimen, 7 May 2004, B5 station. Corrubedo beach: two specimens, 22 April 2005, A3 and C4 stations; one specimen, 8 November 2006, D5 station; one specimen, 19 March 2007, B3 stations; three specimens, 17 April 2007, B4 station; three specimens, 15 May 2007, C5, E5 and F5 stations; 13 February 2009, low shore seven specimens: one in a set of 60 slides deposited at Museo Nacional de Ciencias Naturales, Madrid, Spain, MNCN 5.01/14; one specimen was fixed in 100% ethanol for molecular analyses. Frouxeira beach: one specimen, 18 March 2007, B5 station.

Molecular analysis

Total genomic DNA was extracted from the ethanol-fixed specimen using a DNeasy kit (Qiagen, Inc., Valencia, CA, USA), following the protocol described in the manual. A fragment of the 5' end (1071 base pairs) of 28S rRNA was amplified using the primer set 28rd1a/28rd5b (Edgecombe and Giribet 2006). A 590-base-pair fragment of the mitochondrial protein encoding gene *COI* was amplified using the primer pair LCO1490/HCO2198 (Folmer et al. 1994). Polymerase chain reactions were performed using AmpliTaq DNA polymerase (Perkin-Elmer, Waltham, MA, USA). Thermal cycling was initiated with 2 minutes of denaturation at 94°C followed by 35 cycles of 30 seconds at 94°C, 1 minute at 36–44°C and 1 minute at 72°C. After cycling, the reaction was completed with an extension phase at 72°C for 10 min and the reaction products were visualized in a 1% agarose gel and purified through enzymatic reaction with ExoSAP-IT (USB Corporation, Cleveland, OH, USA). For both 28S and

COI, sequencing reaction products were analysed using an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Alignment and phylogenetic analyses

The sequence alignment was performed using MUSCLE (Edgar 2004). Three phylogenetic analyses were performed using the sequences obtained for *L. acutifrons*, and sequences from GenBank (Table 2). Each gene was analysed independently and together. Four hoplonemertean, *Nipponnemertes punctatulus*, *Oerstedia zebra*, *Tetrastemma elegans* and *Tetrastemma wilsoni*, were chosen as outgroups in all analyses based on the results from Thollessen and Norenburg (2003).

MODELTEST 3.06 (Posada and Crandall 1998) module in HYPHY (Pond et al. 2005) was used to choose the best fit model of molecular evolution for our data set under the Akaike Information Criterion (Akaike 1974). The test indicated the general time reversible model (GTR) as best fitting the data for both nuclear and

Table 2. List of species included in the phylogenetic analysis and their respective accession numbers.

Taxa used	<i>COI</i> mtDNA	28S rRNA
<i>Cerebratulus lacteus</i> (Leidy, 1851)		AY145396
<i>Cerebratulus marginatus</i> Renier, 1804	AJ436931	AJ436876
<i>Hubrechtella dubia</i> Bergendal, 1902	EU489495	AJ436889
<i>Lineus alborostratus</i> Takakura, 1898	AJ436932	AJ436877
<i>Lineus acutifrons</i> Southern, 1913	GU590937	GU590936
<i>Lineus bilineatus</i> (Renier, 1804)		DQ279947
<i>Lineus longissimus</i> (Gunnerus, 1770)	DQ911374	AJ436880
<i>Lineus viridis</i> (Müller, 1774)	AJ436936	AJ436881
<i>Micrura alaskensis</i> (Coe, 1901)	AJ436937	AJ436882
<i>Micrura chlorapardalis</i> Schwartz and Norenburg, 2005		DQ022554
<i>Micrura ignea</i> Schwartz and Norenburg, 2005		DQ022553
<i>Micrura rubramaculosa</i> Schwartz and Norenburg, 2005		DQ022552
<i>Notospermus geniculatus</i> (Delle Chiaje, 1828)	AJ436934	AJ436879
<i>Parborlasia corrugatus</i> (McIntosh, 1876)	EU194806	AJ436884
<i>Parvicirrus dubius</i> (Verrill, 1879)	AJ436940	AJ436885
<i>Ramphogordius sanguineus</i> (Rathke, 1799)	AJ436938	AJ436883
<i>Riserius pugetensis</i> Norenburg, 1993	AJ436941	AJ436886
<i>Tenuilineus bicolor</i> (Verrill, 1892)	AJ436933	AJ436878
Outgroups		
<i>Nipponnemertes punctatulus</i> (Coe, 1905)	AJ436910	AJ436855
<i>Oerstedia zebra</i> (Chernyshev, 1993)	AJ436912	AJ436857
<i>Tetrastemma elegans</i> (Girard, 1852)	AJ436920	AJ436865
<i>Tetrastemma wilsoni</i> Coe, 1943	AJ436921	AJ436866

Notes: Accession numbers for the new sequences of *Lineus acutifrons* are in bold type.

COI mtDNA, mitochondrial DNA of cytochrome oxidase I gene; 28S rRNA, ribosomal RNA of 28S gene.

mitochondrial genes with gamma shape parameter alpha (G) to model rate heterogeneity (GTR + G). Maximum likelihood analysis was performed with RAXML v7.0.4 (Stamatakis 2006; Stamatakis et al. 2008). For each analysis of concatenate genes, a search that combined four separate maximum likelihood estimates with 1000 fast bootstrap replicates was conducted to evaluate the support for each node. Bootstrap values $\geq 70\%$ were considered to indicate strong support, given that bootstrap values appear to be biased but conservative measures of phylogenetic accuracy (Felsenstein 2004).

Results

Lineus acutifrons Southern, 1913

Lineus acutifrons Southern 1913: 7–9, pl. I, fig. 1A–D; Gibson 1982: 84, fig. 20A; Gibson and Knight-Jones 1990: 153, fig. 5.3G; Gibson 1994: 88, fig. 21A.

Description

External features. Live specimens measured up to 250 mm long and 5 mm maximum diameter before and after being anaesthetized. Anterior body region rounded in cross-section, flattened towards mid to posterior parts (Figure 2A); this body regionalization more apparent in larger worms. Smaller worms cylindrical throughout body length. Head ovate and acutely pointed in front, marked off from body by well-defined rounded constriction in front of mouth (Figure 2B). Long, wide, deep pair of horizontal cephalic slits present, posteriorly expanded to form deep bays (Figure 2C). Eyes absent. Pale caudal cirrus is present (Figure 2D). Body dark or brick red, fades to pink in anterior region, until head, which is pink to white. No differences in colouration between dorsal and ventral surfaces.

Body wall and musculature. Epidermis ciliated and glandular 20–25 μm thick, possesses typical heteronemertean arrangement. Thin subepidermal muscle layer, four or five fibres, 6 μm thick. Dermal gland cells form no distinctive layer, basophilic gland cells interspersed between outer longitudinal muscle fibres (36–40 μm thick). Body wall muscles well-developed throughout the post-cerebral regions consist of outer longitudinal muscle, circular muscle and inner longitudinal muscle layers, each respectively 180–200, 45–50 and 10–12 μm thick; thin connective tissue layer separates outer longitudinal muscle from circular muscle. The former is composed of large muscle fibre bundles, penetrated regularly by radial connective tissue and muscle fibre strands. These radially oriented fibres penetrate the ganglionic zone of lateral nerve cords (Figure 3A). Dermal gland cells distributed in homogeneous layer in the intestinal region. Thickness of three muscle layers, outer longitudinal muscle, circular muscle and inner longitudinal muscle, is 100–120, 30–35, and 40–45 μm , respectively (Figure 3B).

Rhynchodaeum and cephalic lacuna surrounded by a coat of longitudinal and circular muscle in the pre-cerebral region. Radial and diagonal fibres run through the head, intermingled with some basophilic glandular areas and several anteriorly directed nerves. Typical heteronemertean body wall morphology becomes apparent at the cerebral ganglia, the three body wall muscle layers distinguishable at the opening of the mouth about midway.

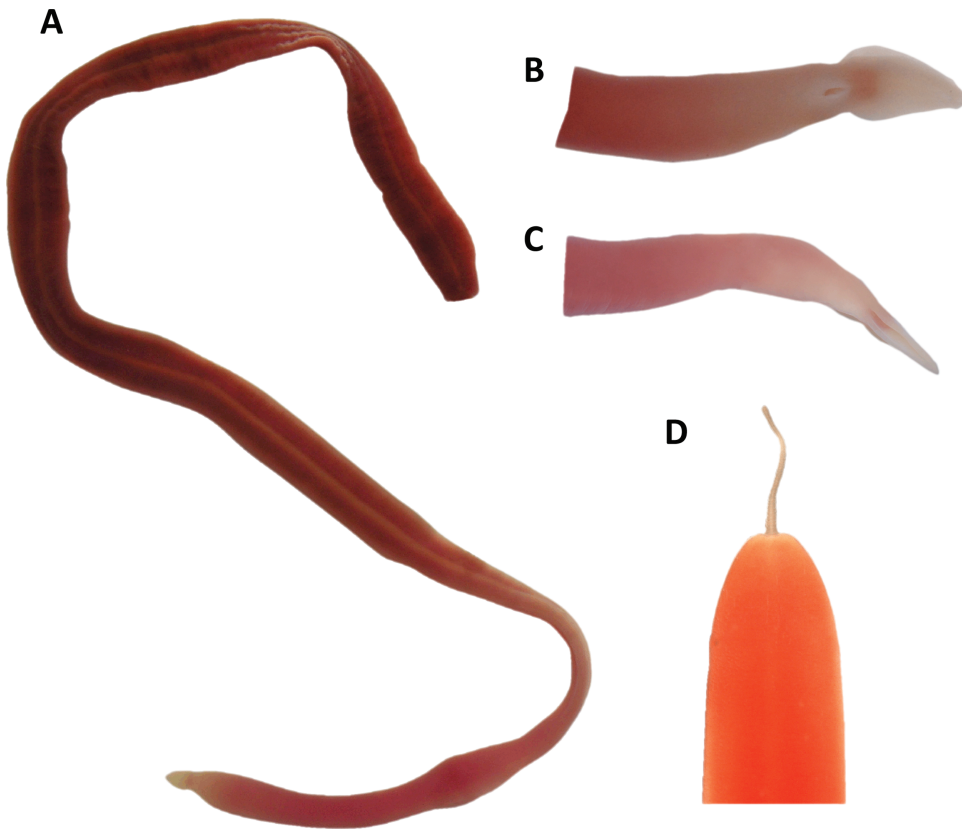


Figure 2. External appearance of *Lineus acutifrons*. (A) Specimen showing the difference between the cylindrical anterior part and the flattened posterior part; (B) enlargement of cephalic region in ventral view to show head shape and mouth; (C) enlargement of cephalic region in lateral view to show cephalic slits; (D) enlargement of posterior region to show caudal cirrus. (A–C) are from living specimens; (D) is an image of an anesthetized specimen.

Well-developed horizontal muscle plate between rhynchocoel and mouth epithelium, 10–20 μm thickness (Figure 3C). At the intestinal region, dorsoventral fibres from the body wall run between intestinal diverticula (Figure 3B).

Proboscis apparatus. Proboscis pore ventral, close to head, tip continuous with the rhynchodaeum. Rhynchocoel extends from the proboscis insertion to the posterior end of the body. Its wall consists of two muscle layers; longitudinal layer one or two fibres thick and thicker circular muscle layer. Rhynchocoel circular muscle layer is thicker in the intestinal region than foregut region. At the villus, rhynchocoel circular musculature interweaves with foregut musculature ventral and laterally (Figure 3D).

Proboscis relatively slender, approximately one-fifth to one-sixth of the body's diameter. Thick glandular acidophilic epithelium, underlain by connective tissue. Below this connective tissue, some longitudinal muscle fibres irregularly interspersed but without forming a distinct layer. A pair of proboscis nerves distinguishable at the anterior part and are the origin of posterior neural plexus. This palaeotype proboscis

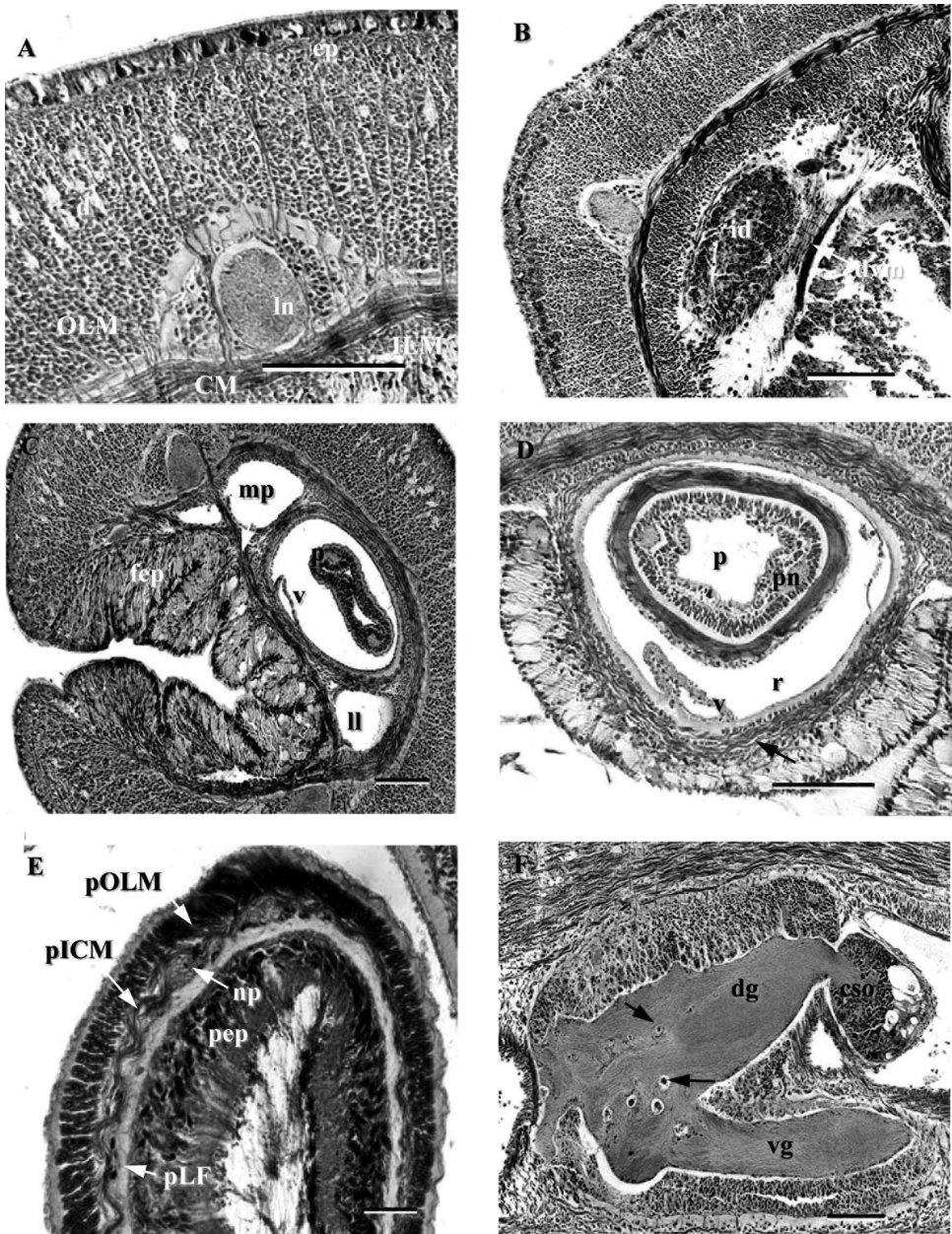


Figure 3. *Lineus acutifrons*. (A) Transverse section showing the body wall; (B) transverse section at intestinal region; (C) transverse section at foregut; (D) transverse section at foregut showing the anterior portion of the proboscis; (E) transverse section at main portion of the proboscis; (F) longitudinal section through the brain showing gregarine parasites (arrows). Scale bars A–F: 100 μ m. Abbreviations: CM, circular muscle layer; cso, cerebral sensory organ; d, dermis; dg, dorsal ganglia; dvm, dorsoventral musculature; ep, epidermis; fep, foregut epithelium; id, intestinal diverticula; ILM, inner longitudinal muscle layer; ll, lateral lacunae; ln, lateral nerve cord; mp, muscle plate; np, proboscis neural plexus; OLM, outer longitudinal muscle layer; p, proboscis; pep, proboscis epithelium; pICM, proboscis inner circular muscle layer; pLF, proboscis longitudinal fibres; pn, proboscis nerves; pOLM proboscis outer longitudinal muscle layer; r, rhynchocoel; v, villus; vg, ventral ganglia.

formed also by very thick circular muscle layer (15–20 μm), outer longitudinal muscle stratum, connective tissue and outer epithelium. No indication of muscle crosses has been observed (Figure 3D, E).

Alimentary canal. Mouth opens ventrally near the brain, just behind cephalic constriction (Figure 2B). Beneath the thick (80–160 μm) ciliated glandular folded epithelium, a vascular plexus and two subepithelial nerves can be distinguished (Figure 3C). These nerves are not visible at posterior foregut. Intestine well developed bears long lateral diverticula (Figure 3B).

Blood system. Single large median blood lacuna at the tip of head, divided into two spacious lateral cephalic lacunae by a dorsoventral extension of rhynchodaeum (Figure 4A). At cerebral ganglia, these two lacunae extend ventrally and meet below rhynchocoel to form “U”-shaped ventral lacuna (Figure 4B); this lacuna is divided by connective tissue strands into medial ventral lacunae (Figure 4C). Posteriorly, the latter lacuna is again divided in two; at this level, two lateral lacunae and two mid-ventral lacunae are present (Figure 4D). An upper mid-ventral lacuna penetrates rhynchocoel forming a villus, which runs posterior to the end of foregut, and is the origin of a medial blood vessel (Figure 3D). At the mouth, lateral lacunae spread through lateral regions forming foregut vascular plexus as described for many pilidiophorans. Three longitudinal blood vessels run along most of body length with no transverse connective between them.

Nervous system. Well-developed brain, both inner and outer neurilemma evident, outer layer thicker (Figure 4B–D). Dorsal and ventral lobes similar in size. Dorsal commissure long and slender (20–30 μm wide), ventral commissure comparatively short and thick (40 μm wide). As in other pilidiophorans, dorsal ganglia bifurcates posteriorly into the cerebral sensory organ (Figure 3F). Two thick nerves from the ventral ganglia innervate the mouth. Ganglia lacks neurochord cells. Putative gregarine parasites observed in the cerebral ganglion tissue (Figure 3F). Lateral nerve cords emerge from ventral brain lobes, and inner and outer persist. No neurochord cells were observed within the lateral nerve cords.

Sense organs. Head bears a pair of long, wide and deep horizontal cephalic slits as in Lineidae (*sensu* Friedrich 1960; Gibson 1985); in living specimens, upper and lower margins of the slit do not meet medially, slit is open (Figure 2C). Ciliated cerebral canals emerge from posterior dorsal wall of cephalic bays, which is more glandular and has longer cilia (Figure 4E). Cerebral sensory organs situated behind dorsal brain lobes, inside lateral blood lacunae (Figure 3F). Single apical sense organ observed as small invagination at very tip of head in longitudinal sections (Figure 4F). No pigment cup ocelli observed.

Excretory system. No traces of nephridial structures observed.

Reproductive system. No mature specimens found.

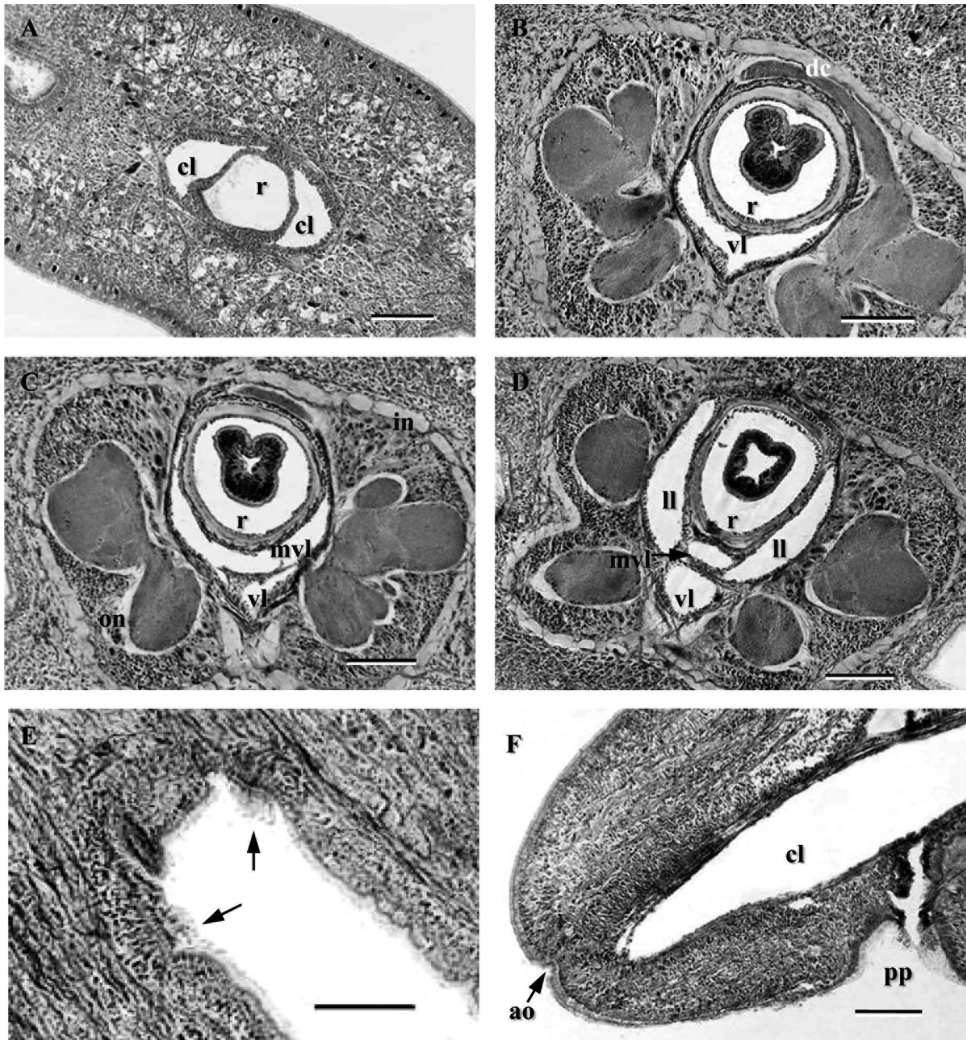


Figure 4. *Lineus acutifrons*. (A) Transverse section showing the two lateral cephalic lacunae; (B) transverse section at brain level showing ventral lacuna; (C) transverse section at brain level showing two ventral lacunae; (D) transverse section at brain level showing four lacunae; (E) longitudinal section of the cephalic slit showing the aperture of the ciliated canal; glandular area with long cilia are marked with arrows; (F) longitudinal section at the tip of the head. Scale bars A–F: 100 μ m. Abbreviations: ao, apical organ; cl, cephalic lacunae; dc, dorsal commissure; ll, lateral lacunae; mvl, mid-ventral lacuna; pp, proboscis pore; r, rhynchocoel; vl, ventral lacuna.

Habitat and behaviour

Lineus acutifrons lives in sandy beaches, in the lower part of the intertidal slope, buried in the sediment. From the 18 beaches surveyed, the species was only collected from four that have a flat dissipative area, with sediments from fine to medium sands (Junoy

et al. 2005). Density in these beaches was very low, with a maximum of one specimen per 30 m². The macroinfaunal community of these beaches consisted of typical psammophilous species dominated by crustaceans and polychaetes. The most frequent and abundant species were the amphipod *Pontocrates arenarius* (Bate, 1858), the mysid *Gastrosaccus roscoffensis* Bacescu, 1970 and the spionid polychaetes *Scolecopsis mesnili* (Bellan and Lagardère, 1971) and *Scolecopsis squamata* Müller, 1806. Other accompanying species were the isopods *Eurydice naylori* Jones and Pierpoint, 1997 and *Eurydice affinis* Hansen, 1905, the amphipod *Haustorius arenarius* (Slabber, 1769) and the polychaete *Nephtys cirrosa* Ehlers 1868. These species were offered to *L. acutifrons* in individual containers, but no proboscis eversion was observed and no prey were consumed. This nemertean is the largest macrofaunal species of the beaches and presumably eats larger polychaetes present in the habitat, such as *S. squamata* and *N. cirrosa*.

Specimens maintained in the laboratory were surrounded by a mucus sheath with sand grains; however, this cover was not visible at collecting times. A singular behaviour was observed in Petri dishes whereby the posterior end waved around while the anterior part was still, which is the opposite of that seen in other Pilidiophora.

Phylogenetic analysis

The phylogenetic hypothesis obtained using exclusively 28S data supports a relationship between *Tenuilineus bicolor*, *Lineus alborostratus* and *Lineus bilineatus* with bootstrap support of 77% (Figure 5). The individual phylogenies from *COI* and

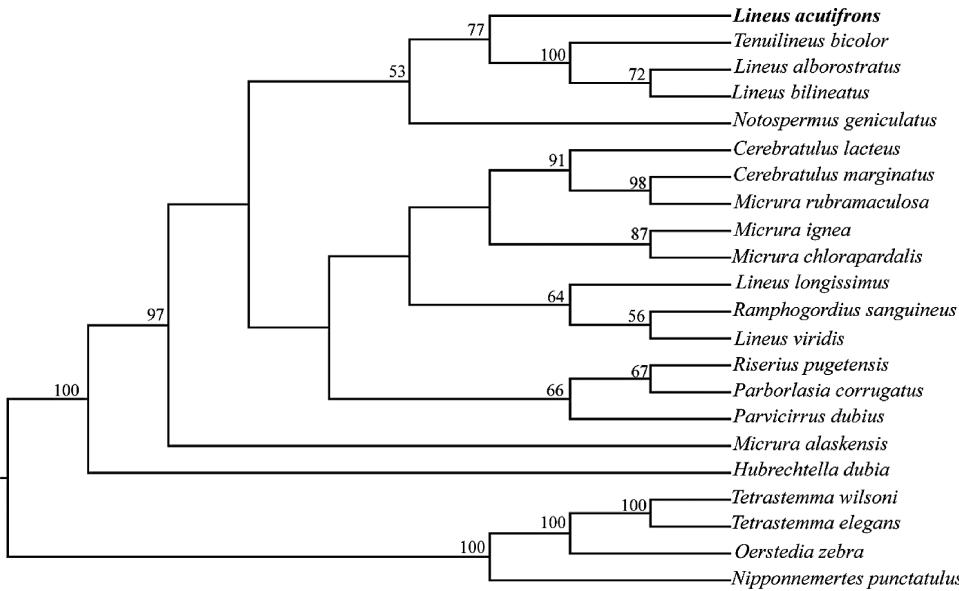


Figure 5. Phylogeny resulting from a maximum likelihood analysis of 28S ribosomal RNA (likelihood = 8692.363) with *L. acutifrons* in bold. Numbers adjacent to nodes indicate bootstrap $\geq 50\%$. Outgroup species are *Nipponnemertes punctatulus*, *Oerstedtia zebra*, *Tetrastemma elegans* and *Tetrastemma wilsoni*.

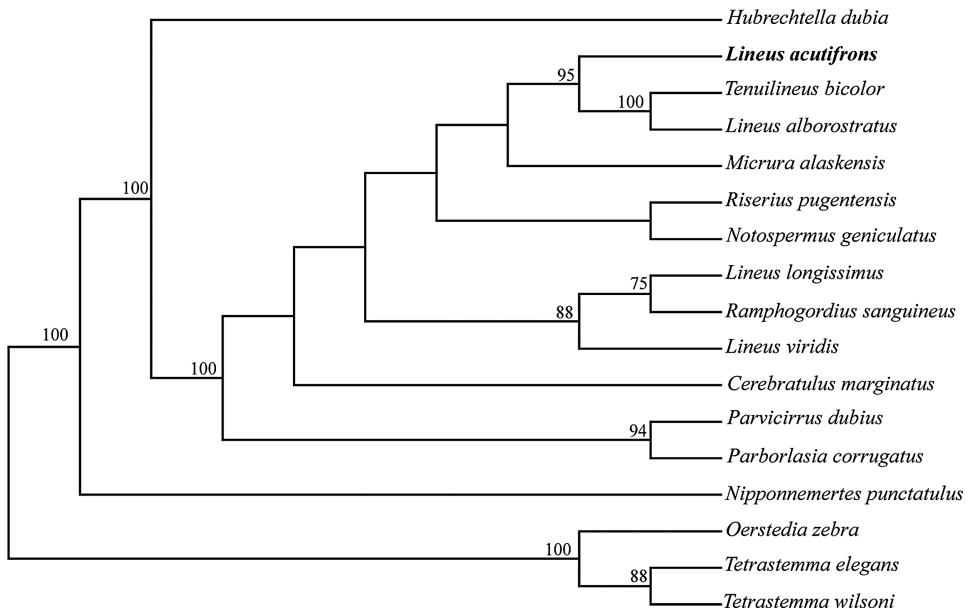


Figure 6. Phylogeny resulting from a maximum likelihood analysis of combined DNA datasets [cytochrome oxidase I (*COI*) mitochondrial DNA and 28S ribosomal RNA; likelihood – 8692.363] with *Lineus acutifrons* in bold. Numbers adjacent to nodes indicate bootstrap $\geq 50\%$. Outgroup species are *Nipponnemertes punctatulus*, *Oerstedia zebra*, *Tetrastemma elegans* and *Tetrastemma wilsoni*.

28S, and the combined phylogeny of both genes demonstrate similar results: *Lineus acutifrons* is in a clade with other members of the Lineidae family, which was supported by high bootstrap values. In both the *COI* and combined phylogenies, *L. acutifrons* is a sister species to *L. alborostratus* and *T. bicolor* with 95% bootstrap support (Figure 6).

Discussion

Scientific investigations of sandy beaches did not begin until 50 years after the first intensive studies of rocky shores (Fairweather 1990; McLachlan and Brown 2006). Consequently, they are not as extensively investigated. On the other hand, in benthic studies, nemerteans are usually listed only as members of the Phylum Nemertea so it is not surprising that littoral nemertean species have been anonymous for a long time in these habitats (Herrera-Bachiller et al. 2008). The only available data for large red heteronemerteans found in European sandy beaches are records from Southern (1913), identified as *L. acutifrons*. Other red European nemerteans, such as *Lineus ruber* or *Ramphogordius sanguineus*, can live in sand but not in the sandy beaches exposed to ocean waves, the typical habitat of the present species, and these species are clearly not similar to the one described herein.

Southern (1913) noted that *L. acutifrons* differed markedly from other British species of *Lineus*, but related it to two red Mediterranean species of *Lineus* described by Bürger (1892), *Lineus rufocaudatus* and *Lineus coccinus*. The specimens collected at

the Galician beaches agree well with Southern's description, except for minor details: (1) Southern described the body as cylindrical and round in cross-section, but this is only true for the anterior part of the Spanish specimens which are flat in the intestinal region of larger worms; and (2) the rhynchocoel circular muscle, which Southern described as thin, is wider at the intestinal region in our specimens. Additionally, Southern's interpretation of the rhynchocoel circular muscle "attached to the dorsal body wall by a sheet of radiating vertical muscles" is in fact the proboscis insertion.

Our specimens are similar in external appearance and some morphological details to another sandy beach species, *Fragilonemertes rosea* Riser, 1998. However, the present species differs from *F. rosea* which has proboscidial muscle crosses and neurochord cells. Both species show extensive development of the preoral diagonal muscle which appears to be an adaptation to the burrowing way of life (Riser 1998).

None of the specimens collected by Southern were complete (Southern 1913: 55.7) so he never observed the caudal cirrus. The caudal cirrus, however, is a primary character differentiating *Lineus* Sowerby, 1806 from the two other large Pilidiophora genera, *Cerebratulus* Renier, 1804 and *Micrura* Ehrenberg, 1831. These two genera were traditionally distinguished respectively by the presence or absence of neurochord cells (Gibson 1985; but see Schwartz 2009). According to the states of these two characters, caudal cirrus and neurochord cells, many species have been historically attributed to one or another of these three genera, whereas pilidiophoran species without horizontal lateral cephalic slits were attributed to *Baseodiscus*. Taken together, these four genera comprise 70% of the pilidiophoran species (Table 3).

Cladistic analyses based only on morphological characters have failed to provide resolution of the relationships among the heteronemertean genera, showing extensive homoplasy (Schwartz and Norenburg 2001; Schwartz 2009). Inadequate description of species is the unresolved problem within nemertean systematics and further confounds the problem of generic placement. Molecular (Sundberg and Saur 1998; Tholleson and Norenburg 2003; Strand et al. 2005) and combined molecular and morphological studies (Schwartz 2009) have basically supported the monophyly of the nemertean orders, and the genus *Baseodiscus*, but have also demonstrated that poorly defined genera, *Lineus*, *Cerebratulus* and *Micrura*, are not monophyletic.

Many authors have preferred to use tables of combined morphological characters to describe new genera when describing a new nemertean species (e.g. Gibson 1990; Rogers et al. 1993; Wern 1998; Gibson and Sundberg 2002) or justify taxonomic changes on this basis (e.g. Gibson 1989; Envall and Norenburg 2001). Differences in character combinations established up to 70 monotypic genera (Table 3), but without any phylogenetic significance (Sundberg 1993; Sundberg and Saur 1998). These character combinations are used widely among pilidiophorans to delineate genera, yet their value as a taxonomic tool is questionable. For example, the cirrus has been used as a diagnostic character for about 30 genera, but Schwartz (2009) demonstrates that presence or absence of a caudal cirrus "is clearly not informative for generic placement as it has been historically used". The caudal cirrus appears to be a plesiomorphic character retained by burrowing species (Riser 1998).

Pilidiophoran systematics is still obscure: *Lineus longissimus*, the type species for the genus *Lineus*, is more closely related to *Riseriellus occultus* and *Ramphogordius sanguineus* (Sundberg and Saur 1998; Strand et al. 2005; Schwartz 2009) or *Lineus viridis* (this paper), than with other *Lineus* species. Our data suggest that *L. acutifrons* is not a member of the genus *Lineus*.

Table 3. Number of species of Pilidiophorans within the genera *Baseodiscus*, *Cerebratulus*, *Lineus*, *Micrura*, genera with more than one species and monotypic genera (updated from Gibson 1995).

Taxa	No. of species	% species
<i>Cerebratulus</i>	126	26.3
<i>Lineus</i>	102	21.3
<i>Micrura</i>	55	11.5
<i>Baseodiscus</i>	41	8.5
Other 14 genera	81	16.9
Monotypic genera	73	15.2

How should one deal with *L. acutifrons*? The alternatives are (1) transfer the species to the indefinable genus *Micrura*; (2) create a new monotypic genus for the species; or (3) retain use of the name *Lineus acutifrons*. The first option simply moves the problem to another place. *Micrura* is represented by up to four of the seven clades shown by Schwartz (2009) in her heteronemertean analyses, making it impossible to diagnose the genus. Friedrich (1960) listed the absence of a connective tissue sheath around the ganglia layer of the brain as a distinctive character of the genus *Micrura*; this neurilemma is present in Spanish specimens. The transfer from the genus *Lineus* to another genus when a caudal cirrus was observed was made for *Micrura wilsoni* by Coe (1940); Coe chose the first alternative but cladistic and Bayesian analyses have not really supported the change (Schwartz 2009). As for a new monotypic genus, Riser (1990) pointed out that the proliferation of monotypic genera as a result of more complete descriptions will facilitate rather than handicap phylogenetic analyses, and before molecular analyses, we chose this solution.

However, given our phylogenetic analyses, we have retained the name until more data clarify the taxonomic status. Molecular analyses demonstrate that *L. acutifrons* is related to *Tenuilineus bicolor* and *Lineus alborostratus* (Figures 5 and 6). According to Schwartz (2009) the depth of penetration of the dermal glands through the body wall is phylogenetically informative and can be used as a diagnostic feature for Clade III of her analysis, which includes *Tenuilineus bicolor*, *Lineus bilineatus* and *L. rubescens*. However, characters used by Riser (1993) in the diagnosis of the genus *Tenuilineus*, such as the single dorsal/dorsolateral cephalic blood lacuna or the lack of caudal cirrus, do not apply to *L. acutifrons*, and consequently the species is not transferred to this genus. Schwartz (2009) also mentioned that the organization of the ocelli at the corners of the anterior tip of the head supports her Clade III. However, *L. bilineatus*, in common with *L. acutifrons*, lacks eyes.

Lineus acutifrons is also related to *L. alborostratus* Takakura, 1898, a species from Japan re-described by Yamaoka (1940). Both nemerteans have a body rounded in cross-section in the anterior part, whereas the posterior part is dorsoventrally flattened, but differ in internal morphology: *L. acutifrons* has an inner longitudinal muscle layer, but proboscoidal muscle crosses and nephridial structures were not observed.

Except for the single specimen from Frouxeira beach, the species was collected only from the beaches that are more influenced by the seasonal upwelling located in the northwest coast of Iberian Peninsula (Bode et al. 1996); these beaches have the highest macrofaunal density of the Atlantic coast of Spain (Lastra et al. 2006; J. Junoy

personal data). The nemertean *L. acutifrons* could be a useful bioindicator species of beach quality because it is only present in beaches where macrofauna density is high. As opposed to *Psammamphiporus elongatus*, the other nemertean species found in the same beaches (Herrera-Bachiller et al. 2008), *L. acutifrons* is present in such low densities that it is not possible to assess whether it has been affected by the Prestige oil spill.

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