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A new species of *Ampharete* (Annelida: Ampharetidae) from the NW Iberian Peninsula, with a synoptic table comparing NE Atlantic species of the genus

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A new species of the genus *Ampharete* Malmgren, 1866 (Annelida; Ampharetidae) is described based on the specimens collected during several sampling campaigns in shallow coastal waters off Galicia, NW Iberian Peninsula. ***Ampharete santillani* sp. nov.** is morphologically similar to *Ampharete lindstroemi* Malmgren in Hessle, 1917 but differs by larger body size, the absence of eyes on the pygidium and the presence of short dorsal neuropodial cirri in posterior abdominal segments rather than minute rounded lobes. The external micro-morphology of the new species was studied using a scanning electron microscopy, and the gross internal anatomy was examined by means of micro-computed tomography. This new ampharetid is at least distributed from NW Spain to the Moroccan southern coast. A study of temporal variation in abundance showed that it may reach densities of up to 500–1000 ind./m^2. Molecular characterization is based on partial sequences of mitochondrial marker cytochrome *c* oxidase subunit 1, *COI* (DNA barcodes). Additional DNA barcodes were produced for five other species occurring in NE Atlantic waters. A molecular phylogenetic analysis, also including available *COI* sequences of *Ampharete* spp. from the BOLD database, is presented. A synoptic table summarizing diagnostic characters for NE Atlantic species of *Ampharete* is provided. The presence of peritrich ciliate epibionts is also reported.

ADDITIONAL KEYWORDS: DNA barcoding – ecology – epibiont ciliates – Iberian Peninsula – micro-CT – Morocco – Polychaeta – SEM.

INTRODUCTION

The genus *Ampharete* Malmgren, 1866 is by far the most species-rich genus of the family Ampharetidae Malmgren, 1866. The generic diagnosis of *Ampharete* has recently been emended to include several previously distinguished genera, for example *Asabellides* Annenkova, 1929 and *Sabellides* Milne Edwards in Lamarck, 1838 (Jirkov, 2011; Imajima, Reuscher & Fiege, 2012; Parapar *et al.*, 2012), and currently *c*. 45 species are referred to the genus *Ampharete*. At present, no apomorphies are known for *Ampharete*, and the diagnosis of the genus is based on a combination of several morphological characters, including presence of a prostomium with a distinct middle lobe, absence of prostomial glandular ridges, presence of papillose buccal tentacles, 11–12 thoracic uncingers, four (exceptionally three) pairs of branchiae arranged dorsally on fused segments II + III, presence of a single pair of nephridial papillae dorsally on segment IV, presence of two intermediate segments, absence of modified

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or elevated notopodia and absence of notopodial cirri (Imajima *et al.*, 2012).

The diversity of the genus *Ampharete* has been relatively well studied in the NE Atlantic Ocean (e.g. Holthe, 1986; Jirkov, 2001; Parapar *et al.*, 2012; Alvestad, Kongsrud & Kongshavn, 2014), and a total of 12 species are currently known between parallels 55°N and 80°N (Fig. 1): *A. acutifrons* (Grube, 1860), *A. baltica* Eliason, 1955, *A. borealis* (M. Sars, 1856), *A. falcata* Eliason, 1955, *A. finmarchica* (M. Sars, 1865), *A. goesi* Malmgren, 1866, *A. lindstroemi* Malmgren in Hessle, 1917, *A. octocirrata* (M. Sars, 1835), *A. petersenae* Jirkov, 1997, *A. undecima* Alvestad, Kongsrud & Kongshavn, 2014, *A. vega* (Wirén, 1883) and *A. villenai* Parapar, Helgason, Jirkov & Moreira, 2012. Two additional species are known from the high Arctic (see Jirkov, 2001): *A. crassiseta* Annenkova, 1929 and *A. sibirica* Wirén, 1883, and two species are known from more southern temperate latitudes: *A. minuta* Langerhans, 1881 (Madeira Islands) and *A. debrouweri* Jeldes & Lefevere, 1959 (off Angola) (Fig. 1).

Parapar, Besteiro & Urgorri (1993) from the study of several specimens collected in the Ría de Ferrol (Galicia), reported the presence of *A. finmarchica* in the Atlantic coast of the Iberian Peninsula. Later, Parapar *et al.* (2009) studied additional material of the same taxon and concluded that the specimens are morphologically more similar to *A. lindstroemi* based on the shape of the paleae and the number of abdominal uncingers, and consequently identified the specimens

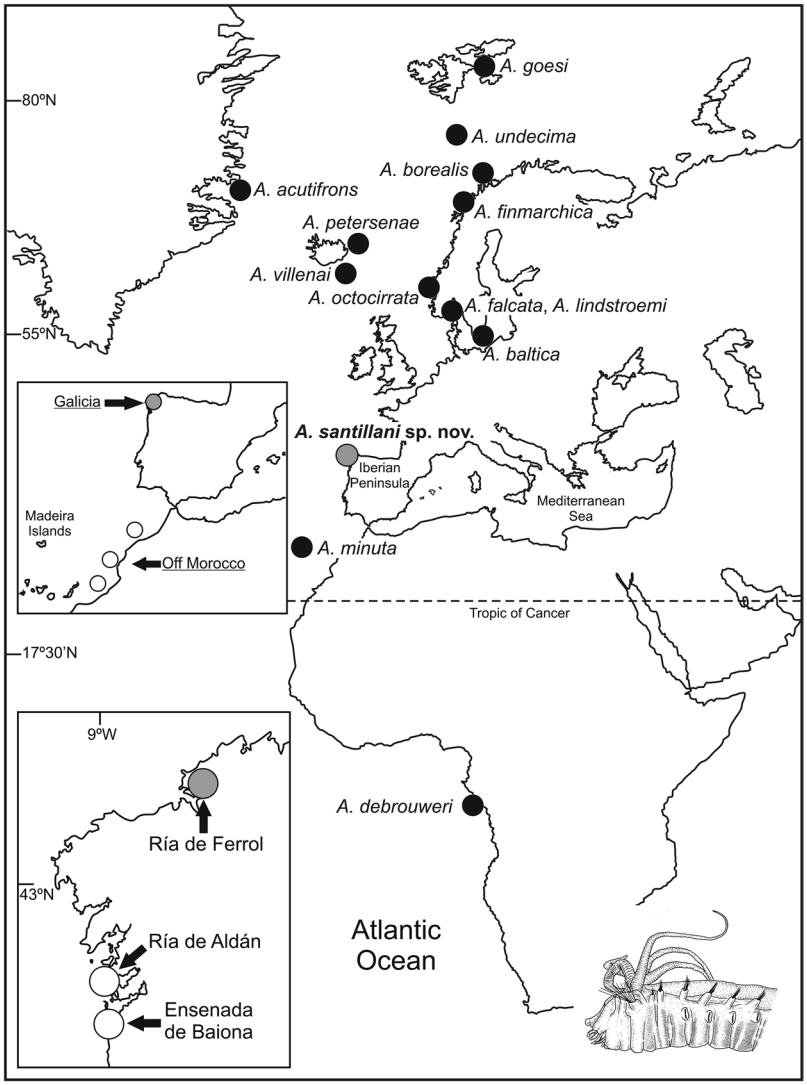

Figure 1. Map showing locations where specimens of ***Ampharete santillani* sp. nov.** were collected (grey circles: location of type material; white circles: additional non-type material). Type locality of other valid species of *Ampharete* occurring in the NE Atlantic is also indicated (black circles).

as *Ampharete* sp. On the basis of the combined morphological, molecular and ecological studies of these specimens, as well as additional material from the Galician coast and Morocco, a new species of the genus *Ampharete* is described herein: *Ampharete santillani* sp. nov.

The external micro-morphology of the new species has been examined with scanning electron microscopy (SEM), and the internal anatomy has been studied by histological sectioning and micro-computed tomography (micro-CT). Micro-CT is a non-invasive technique that allows exploration and 3D reconstruction of the internal anatomy of animals. It has been shown to be a powerful tool to uncover new potential characters for taxonomy, as well as improving the understanding of phylogenetic relationships (Dinley *et al.*, 2010; Faulwetter *et al.*, 2012; Sykes *et al.*, 2013; Paterson *et al.*, 2014).

Molecular characterization of the new species is based on the partial sequences of mitochondrial marker cytochrome *c* oxidase subunit I (*COI*) (DNA barcodes) of three specimens from Galicia and three specimens from off the Atlantic coast of Morocco. For comparison purposes, DNA barcodes were also produced for a number of specimens representing five other species of *Ampharete* occurring in European waters: *A. falcata*, *A. finmarchica*, *A. lindstroemi*, *A. octocirrata* and *A. undecima*. A molecular phylogenetic analysis based on the *COI* sequences is presented, also including additional sequences of *Ampharete* spp. from the BOLD database.

Polychaetes show a wide variety of ecological interrelationships with other marine organisms, which include different kinds of symbiotic associations such as mutualism, commensalism and parasitism (Martin & Britayev, 1998). In most cases, these symbionts are living inside (endobiont) or attached to the host body surface (epibiont). Polychaetes have been widely reported either as epibionts or as hosts (basibionts) of a large variety of marine animals (see Álvarez-Campos *et al.*, 2014 for details). The presence of ciliate protozoans as epibionts on polychaetes have been reported previously (Knox & Hicks, 1973; Magagnini & Verni, 1988; Arias, Anadón & Paxton, 2010; Álvarez-Campos *et al.*, 2014), but never in specimens of the genus *Ampharete*.

MATERIAL AND METHODS

This study is based on the material collected during several sampling campaigns along the coasts of Galicia (NW Spain) and Morocco (NW Africa). In total, 2919 specimens from the Ría de Ferrol, 1690 specimens from the Ría de Aldán and nine specimens from the Ensenada de Baiona (Galicia) were collected, mostly between 1988 and 2010. Twenty-six specimens were collected from five samples off the coast of Morocco during sampling cruise 2011410 of *R/V Dr. Fridtjof Nansen* in 2011 (Table 1). Holotypes and paratypes of the new species were deposited in the collections of the Museo Nacional de Ciencias Naturales de Madrid, Spain (MNCN 16.01/17841 to MNCN 16.01/17861) and the Department of Natural History, University Museum of Bergen, Norway (ZMBN 115540 to 115542, DNA vouchers). Non-type material from Spain is deposited in the personal collection of the first (J.P., Ría de Ferrol) and senior authors (J.M., Ría de Aldán and Ensenada de Baiona), and specimens from Morocco are deposited in the Department of Natural History, University Museum of Bergen (ZMBN 98157, 98164 and 98169).

For comparison purposes, type material of two non-European species of *Ampharete* now considered valid, but never reported after their original descriptions were attempted to be located: *Ampharete debrouweri* Jeldes & Lefevere, 1959 and *Ampharete minuta* Langerhans, 1881. The holotype of *A. debrouweri* was loaned for study by the Institut Royal des Sciences Naturelles de Belgique (Holotype, PY-987). Type specimens of *A. minuta* have been sought in several European Museums, including Museu Municipal de Funchal, Madeira, Portugal, Museum für Naturkunde, Berlin, Germany, Universität Hamburg, Germany, Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt, Germany and Naturhistorisches Museum Wien, Austria, but without success.

Specimens of *A. falcata*, *A. finmarchica*, *A. lindstroemi*, *A. octocirrata* and *A. undecima* from Norwegian and Icelandic waters were also studied.

STUDY AREA AND SAMPLE COLLECTION

Specimens of the new species were collected in three locations in NW Spain and off Morocco (Fig. 1, Table 1). Samples were taken by means of a Van Veen grab (Ensenada de Baiona, Ría de Aldán, Morocco) and a Naturalist dredge (Ría de Ferrol). Locations in NW Spain correspond to embayments of fully marine conditions, with salinity ranging from 30 to 36‰ (J.P., J.M., F.A., unpubl. data). Spatial distribution was studied in Ferrol and Baiona to map the presence of the new species in different types of sediments (gravel to mud). Temporal variation in abundance of the new species was studied in two muddy sites at the Ría de Aldán; five replicate samples were collected monthly in each site (total area per month and site: 0.28 m^2), between May 1998 and May 1999. In all cases, specimens were sorted from the sieved sediment; most specimens were fixed in 10% formalin and subsequently transferred to 75% alcohol for preservation; others were directly immersed in 96% ethanol.

Table 1. Geographical location and some abiotic data of sampling sites where specimens of ***Ampharete santillani* sp. nov.** were collected in the Galician and Moroccan coast

	Site	Date	Latitude N	Longitude W	Depth (m)	O. M.	Sediment
Ría de Ferrol (NW Spain)	6	6 July 1987	43°28′06″	08°19′04″	6	0.55	Muddy sand
	11	9 July 1987	43°27′30″	08°18′45″	20	0.41	Muddy sand
	12	9 July 1987	43°27′11″	08°18′56″	12	0.30	Muddy sand
	16	30 July 1987	43°27′44″	08°17′07″	16	1.45	Muddy sand
	18	24 June 1987	43°28′02″	08°16′37″	7	2.55	Muddy sand
	20	5 August 1987	43°27′37″	08°16′48″	12	0.71	Muddy sand
	21	8 August 1987	43°28′02″	08°16′22″	8	2.08	Muddy sand
	23	9 July 1987	43°27′25″	08°16′07″	1.5	0.76	Muddy sand
	24	12 September 1987	43°28′31″	08°15′34″	14	4.65	Mud
	25	12 September 1987	43°28′12″	08°15′44″	17	3.43	Muddy sand
	26	8 August 1987	43°27′52″	08°15′48″	15	0.42	Coarse sand
	27	5 August 1987	43°27′25″	08°15′57″	1.5	3.26	Sandy mud
	33	1 May 1987	43°27′56″	08°15′06″	8	1.72	Muddy sand
	36	6 September 1987	43°28′26″	08°14′47″	10	2.85	Muddy sand
	37	8 August 1987	43°28′10″	08°14′47″	15	4.60	Sandy mud
	38	18 July 1987	43°27′52″	08°14′47″	10	2.30	Muddy sand
	41	6 September 1987	43°28′10″	08°14′16″	13	2.38	Muddy sand
	42	11 August 1987	43°27′52″	08°14′16″	13	5.28	Muddy sand
	44	6 September 1987	43°28′10″	08°13′44″	15	3.09	Muddy sand
	45	11 August 1987	43°27′52″	08°13′44″	10	2.13	Mud
	47	22 July 1987	43°28′51″	08°13′02″	1.5	6.91	Mud
	48	25 August 1987	43°28′31″	08°13′14″	9	5.09	Mud
	49	11 August 1987	43°28′10″	08°13′14″	12	6.03	Sandy mud
	50	12 June 1987	43°27′52″	08°13′14″	9	3.09	Sandy mud
	51	18 July 1987	43°27′30″	08°13′18″	1.5	0.94	Muddy sand
	52	22 July 1987	43°28′51″	08°12′43″	2	4.88	Mud
	53	22 August 1987	43°28′31″	08°12′43″	8	5.92	Mud
	54	25 August 1987	43°28′10″	08°12′43″	8	5.98	Mud
	55	12 June 1987	43°27′52″	08°12′43″	4	2.05	Muddy sand
	57	25 July 1987	43°28′31″	08°12′14″	7	5.26	Sandy mud
	58	25 July 1987	43°28′10″	08°12′14″	6	5.45	Mud
	59	12 June 1987	43°27′52″	08°12′14″	2	2.45	Sandy mud
	62	31 March 1987	43°28′46″	08°11′45″	1.5	3.22	Sandy mud
	63	25 August 1987	43°28′31″	08°11′45″	5	5.70	Sandy mud
	66	30 August 1987	43°29′27″	08°11′13″	1	3.60	Sandy mud
	68	30 March 1987	43°28′51″	08°11′13″	5	1.14	Muddy sand
	69	3 October 1987	43°28′23″	08°11′13″	5	5.30	Mud
	71	30 March 1987	43°29′16″	08°10′44″	1.5	1.30	Muddy sand
	72	31 March 1987	43°28′51″	08°10′42″	1.5	1.5	Muddy sand
	MS	16 June 2010	43°27′43″	08°14′40″	1.69	5.13	Mud
	Z4R1	16 April 2015	43°27′45″	08°14′44″	2.15	N/A	Mud
	AN	22 April 2015	43°27′46″	08°14′46″	2.5	N/A	Mud
Ensenada de Baiona (NW Spain)	2	7 December 2015	42°08′50″	08°50′15″	7	1.91	Muddy sand
	4	6 December 2015	42°08′50″	08°49′44″	12	N/A	Gravel
	10	6 December 2015	42°08′10″	08°49′44″	8	2.20	Muddy sand
	17	7 December 2015	42°07′30″	08°50′15″	7	3.18	Muddy sand
	18	7 December 2015	42°07′30″	08°49′44″	8	2.48	Fine sand
	19	8 December 2015	42°07′19″	08°50′45″	2	8.45	Mud
	20	8 December 2015	42°07′10″	08°50′15″	3	7.28	Mud

Table 1. *Continued*

	Site	Date	Latitude N	Longitude W	Depth (m)	O. M.	Sediment
Ría de Aldán (NW Spain)	27	May 1999	42°17′45″	08°50′15″	18	3.19 ± 0.86	Muddy sand
	31	May 1999	42°17′15″	08°49′45″	17	12.72 ± 1.66	Mud
Morocco	GR37	8 December 2011	29°00′	11°13′	106	N/A	N/A
	GR44	10 December 2011	30°34′	09°48′	46	N/A	N/A
	GR45	11 December 2011	31°37′	09°45′	36	N/A	N/A
	GR50	13 December 2011	32°28′	09°16′	40	N/A	N/A
	GR56	15 December 2011	33°41′	07°37′	55	N/A	N/A

Site 47 now corresponds to harbour facilities. N/A, not available; O. M., organic matter content (%).

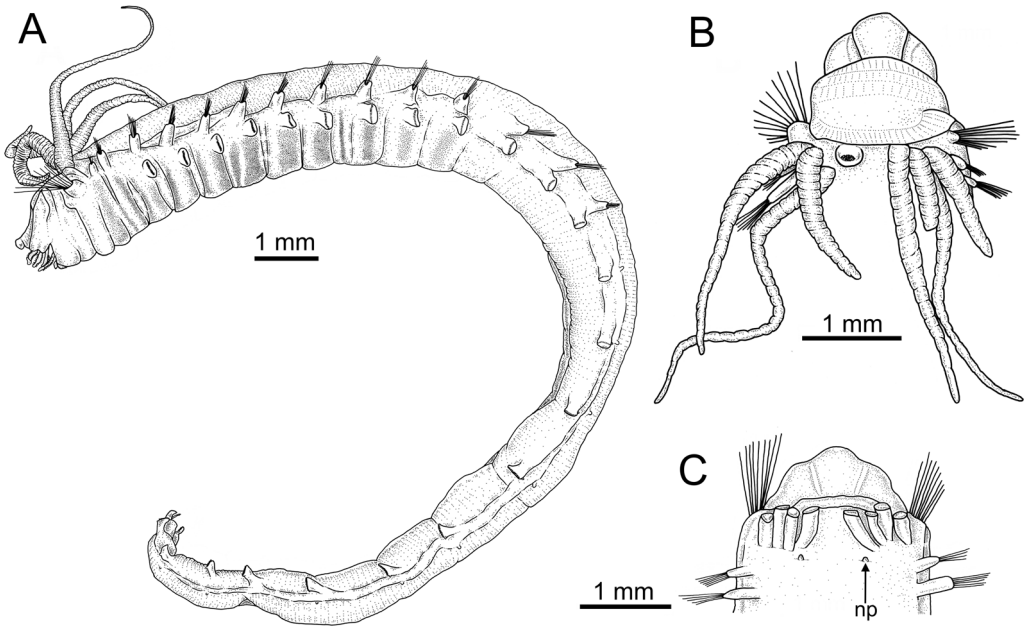

Figure 2. ***Ampharete santillani*** **sp. nov.** A, holotype MNCN 16.01/17841, left side in lateral view; B–C, paratypes MNCN 16.01/17849, anterior end in dorsal view of two specimens.

Optics and SEM

Specimens were studied using an Olympus SZX9 stereomicroscope and an Olympus BX40 compound microscope (Universidade da Coruña, UDC); micrographs of alive specimens were obtained with an Olympus SZX9 stereomicroscope (Universidade de Santiago de Compostela, USC); a Leica M205C stereomicroscope was used to obtain digital photos of specimens from Morocco (ZMBN). Line drawings of the holotype and paratypes were prepared using an OLYMPUS SZX9 stereomicroscope and an OLYMPUS BX40 compound microscope connected to a camera lucida (UDC). Specimens used for examination with SEM were prepared by critical point drying, coated with gold/palladium in a BAL-TEC SCD 004 evaporator, and examined and photographed under a JEOL JSM-6400 scanning electron microscope at the Servizos de Apoio á Investigación (SAI, UDC) and a ZEISS Supra 55VP SEM at the Laboratory for Electron Microscopy, University of Bergen. Some specimens were stained with methyl blue to aid in identification.

Anatomical study

The specimen used for histological sectioning was preserved in 70% ethanol, dehydrated through a series of graded ethanol baths and clearing agent, infiltrated with paraffin at 57 °C overnight and embedded in a paraffin block. The block was sectioned with a microtome in 8-µm sections, which were placed on microscope slides, hydrated and stained with haematoxylin–eosin, dehydrated and finally mounted on permanent slides with Canada balsam. The specimen used for micro-CT scan at the Estación de Bioloxía Mariña de A Graña (EBMG, USC) was originally preserved in 80% ethanol

and dehydrated in successive baths of ethanol 90 and 96%, then immersed 2 h in hexamethyldisilazane and allowed to air dry overnight (Alba-Tercedor & Sánchez-Tocino, 2011; Faulwetter *et al.*, 2013a, b). The specimen was not stained. Scanning was carried out with a Skyscan 1172 (Bruker, Belgium) microtomograph using the following parameters: 40 kV, 250 mA, unfiltered, image pixel size of 5 µm and no camera binning. Images were reconstructed with the NRecon software and cleaned with CT Analyzer software (both Skyscan software). To visualize the data, DataViewer and CTVox softwares (also from Skyscan) were used.

Molecular analysis

DNA barcodes (partial sequences of mitochondrial marker *COI*) were obtained for six specimens of *A. santillani* sp. nov.: three specimens from NW Spain and three specimens from Morocco. For comparison, DNA barcodes were also produced for a number of specimens of other related species occurring in the NE Atlantic: *A. falcata* (three specimens), *A. finmarchica* (two specimens) *A. lindstroemi* (three specimens), *A. octocirrata* (two specimens) and *A. undecima* (three specimens). DNA voucher specimens are deposited at ZMBN and at Senckenberg Museum Frankfurt, Germany (SMF). Available DNA barcodes of *Ampharete*, identified as *A. finmarchica*, *A. acutifrons*, *A. labrops* Hartman, 1961 and *A. manriquei* (Salazar-Vallejo, 1996), were downloaded from the BOLD database (http://boldsystems.org/). In total, 30 terminal taxa representing nine species of *Ampharete* were included in the analysis (Table 4). A *COI* sequence of *Anobothrus gracilis* (Malmgren, 1866) downloaded from GenBank was used to root the phylogenetic tree.

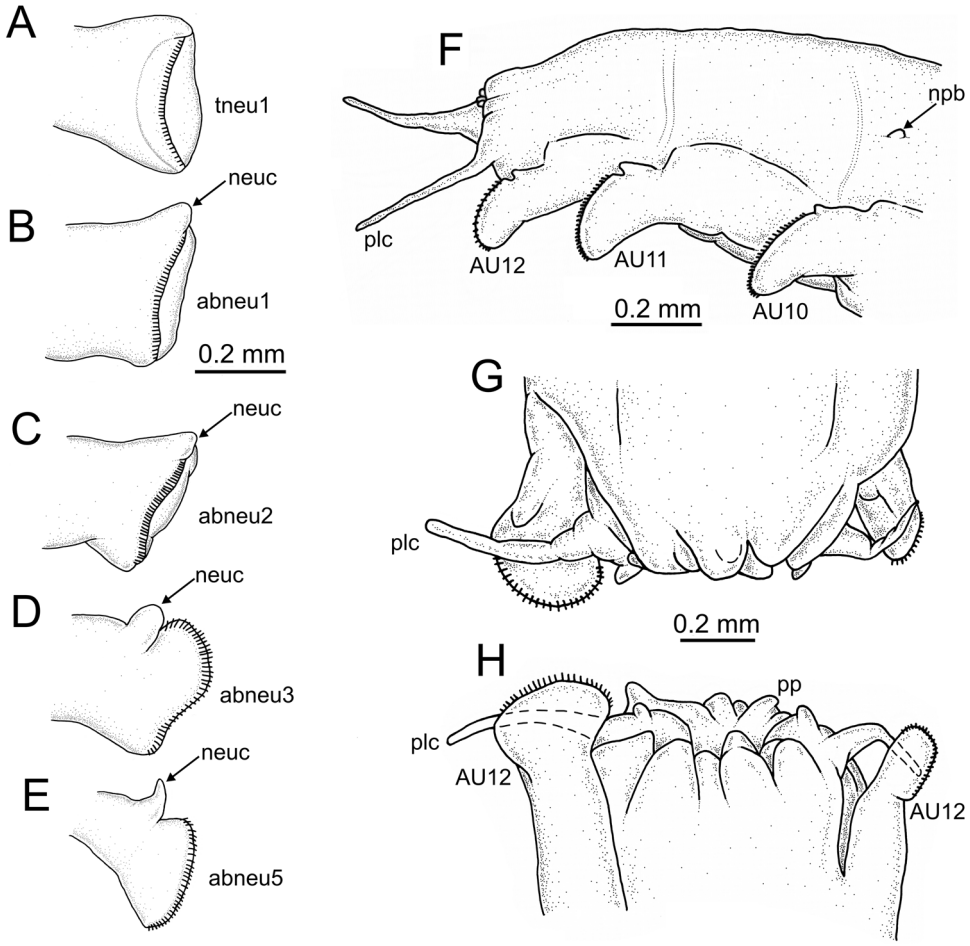

Figure 3. ***Ampharete santillani* sp. nov.**, A–E, holotype MNCN 16.01/17841. Lateral view of first thoracic neuropodium (tneu1), first two abdominal neuropodia of thoracic type (abneu1 and abneu2) and first and third 'normal' abdominal uncinigers (abneu3 and abneu5); F–H, paratypes MNCN 16.01/17849. F, right side of posterior end in lateral view, showing last three abdominal uncinigers (AU), notopodial button (npb) and pygidial lateral cirri (plc); G, pygidium in dorsal view; H, pygidium in ventral view, showing last abdominal unciniger (AU12), pygidial papillae (pp) and pygidial lateral cirri (plc).

The molecular work was performed at the University of Bergen and the Canadian Centre for DNA Barcoding (CCDB) in Guelph. DNA extraction and sequencing at the University of Bergen were carried out according to the methods described by Kongsrud *et al.* (2017), using standard primers for polychaetes (Carr *et al.*, 2011). For the molecular work at CCDB, tissue samples and metadata were submitted according to the routines in BOLD (http://www.boldsystems.org/). DNA extraction and sequencing at CCDB were carried out according to the protocols and procedures of the BOLD system (Ratnasingham & Hebert, 2007). All newly obtained sequences are available in BOLD and GenBank (see Table 4 for accession numbers).

COI sequences were aligned using MUSCLE (Edgar, 2004) in MEGA 6 (Tamura *et al.*, 2013). The best-fit evolutionary model for phylogenetic analysis was calculated in MEGA 6, yielding the GTR + G + I model as the best fit according to the Akaike information criterion. A molecular phylogenetic analysis, based on Maximum Likelihood method with 1000 bootstrap replications, and Kimura two-parameter (K2P) distances between and within species, were calculated in MEGA 6.

ABBREVIATIONS USED IN TEXT AND FIGURES

abneu, abdominal neuropodium; act, abdominal ciliated tuft; AU, abdominal unciniger; bc, buccal cavity; bd, basal disc; br, branchia; bt, buccal tentacle; cc, coelomic cavity; cil, cilia; dbs, dorsal blood sinus; dbv, dorsal blood vessel; dlm, dorsal longitudinal muscles; e, eye; epi, epidermis; gcs, gut circulatory sinus; hb, heart body; int, intestine; lml, longitudinal muscle layer; m, mouth; ma, macronucleus; neph, nephridium; neuc, neuropodial cirrus; np, nephridial papillae; npb, notopodial button; oes, oesophagus; oesv, oesophageal vessel; ooc, oocyte; pal, paleae; pha, pharynx; plc, pygidial lateral cirrus; pm, parapodial muscle; pp, pygidial papillae; pre, prostomial eye; prs, prostomium; pye, pygidial eye; S, segment; sbs, stomach blood sinus; sgo, segmental organ; sll, stomach lateral lobe; stl, stalk; stm, stomach; TC, thoracic chaetiger; tcha, thoracic chaetiger; tneu, thoracic neuropodium; tnot, thoracic notopodium; TU, thoracic unciniger; tut, thoracic uncinigerous torus; vbv, ventral blood vessel; vg, ventral gland; vlm, ventral longitudinal muscle; vm, ventral mesentery; vnc, ventral nerve cord; vpo, ventral pharyngeal organ; vs, ventral shield.

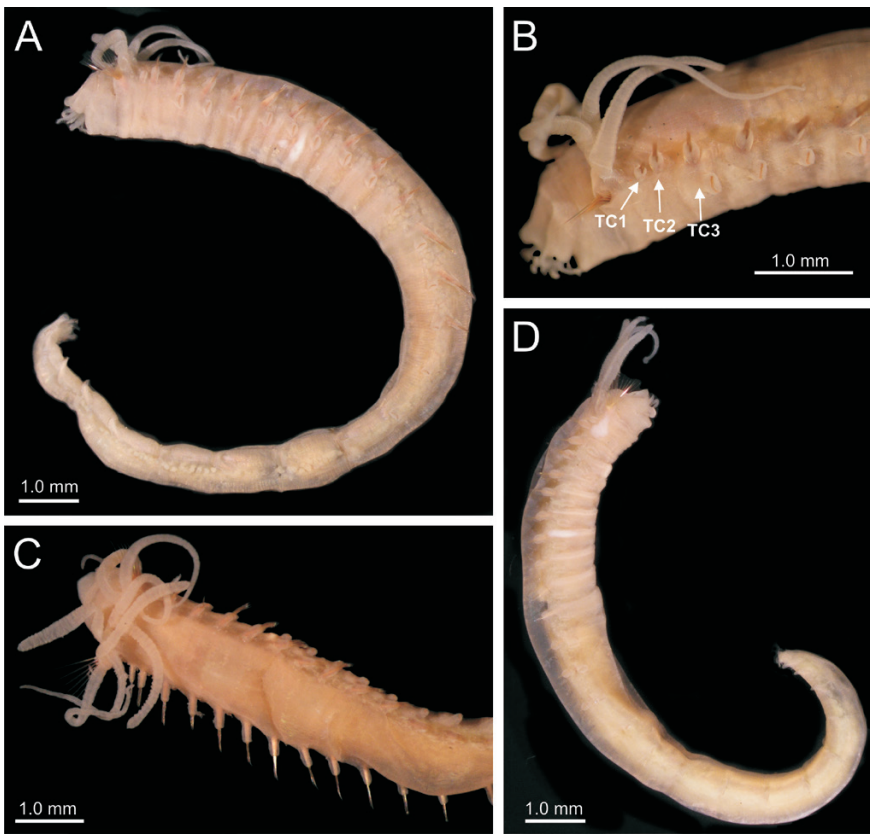

Figure 4. ***Ampharete santillani*** **sp. nov.**, stereomicroscope images. A–B, holotype MNCN 16.01/17841 in left lateral view [arrows pointing to first three thoracic chaetigers (TC)]; C–D, paratypes MNCN 16.01/17849 in dorsal and lateral views.

RESULTS

TAXONOMIC ACCOUNT

PHYLUM ANNELIDA LAMARCK, 1802

FAMILY AMPHARETIDAE MALMGREN, 1866

GENUS *AMPHARETE* MALMGREN, 1866

Type species: Amphicteis acutifrons Grube, 1860.

Diagnosis: Jirkov (2011: 86), Imajima *et al*. (2012: 79), Parapar *et al.* (2012: 333).

AMPHARETE SANTILLANI SP. NOV.

(FIGS 1–20, TABLES 1–4)

Ampharete finmarchica: Parapar *et al.* (1993), Moreira, Quintas & Troncoso (1998) (*non* M. Sars, 1865).

Ampharete sp.: Parapar *et al.* (2009).

Diagnosis: A medium-sized species of up to 21 mm in length and 2.5 mm in width. Branchiae arranged in two well-separated groups. Paleae thin and slender with filiform tips, 9–12 on each side. Twelve thoracic uncinigers and 12–13 abdominal uncinigers. Pygidium with two lateral cirri and several small rounded papillae. Single pair of prostomial eyes; no pygidial eyes.

Material examined

Type material: NW Iberian Peninsula, Atlantic coast of Spain. **Holotype**: Ría de Ferrol (Galicia, NW Spain), Museo Nacional de Ciencias Naturales, MNCN 16.01/17841 (site 62). **Paratypes**: Ría de Ferrol: Museo

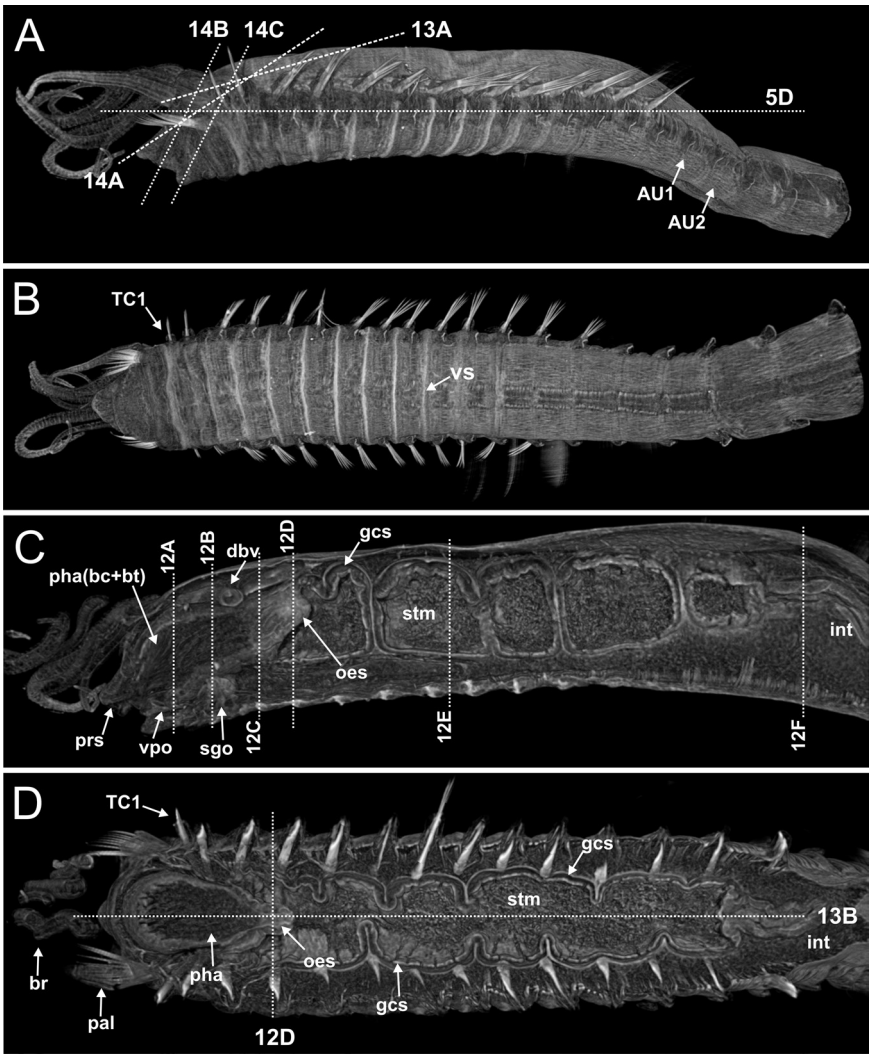

Figure 5. ***Ampharete santillani*** **sp. nov.** Micro-CT images of paratype MNCN 16.01/17852 with retracted buccal tentacles. A, left lateral view of body surface [broken lines showing oblique coronal views in Figs 13 and 14 and coronal view in (D)]; B, ventral view of body surface showing ventral shields (vs); C–D, sagittal and coronal sections showing digestive system (broken lines showing transversal view in Fig. 12).

Nacional de Ciencias Naturales, MNCN 16.01/17842 (site 25, 167 specimens); MNCN 16.01/17843 (site 38, 148 specs); MNCN 16.01/17844 (site 42, 99 specs); MNCN 16.01/17845 (site 45, 229 specs); MNCN 16.01/17846 (site 55, 2 specs); MNCN 16.01/17847 (site 57, 239 specs); MNCN 16.01/17848 (site 59, 3 specs); MNCN 16.01/17853 (site 59, 2 specs, in SEM stub); MNCN 16.01/17849 (site 62, 44 specs); MNCN 16.01/17854 (site 62, 3 specs, in SEM stub); MNCN 16.01/17850 (site 63, 635 specs); MNCN 16.01/17851 (site 71, 21 specs); MNCN 16.01/17852 (site MS, 1 spec. scanned for micro-CT); MNCN 16.01/17855–17861 (site 71, 1 spec. sectioned for histology); ZMBN 115540–115542 (DNA vouchers).

Non-type material: **Ría de Ferrol (Galicia)**: site 6, 11 specs; site 11, 1 spec.; site 12, 1 spec.; site 16, 2 specs; site 18, 49 specs; site 20, 10 specs; site 21, 82 specs; site 23, 1 spec.; site 24, 2 specs; site 26, 5 specs; site 27, 63 specs; site 33, 2 specs; site 36, 123 specs; site 37, 20 specs; site 41, 50 specs; site 44, 491 specs; site 47, 3 specs; site 48, 90 specs; site 49, 95 specs; site 50, 8 specs; site 51, 3 specs; site 52, 1 spec.; site 53, 8 specs; site 54, 30 specs; site 58, 25 specs; site 66, 1 spec.; site 68, 108 specs; site 69, 3 specs; site 72, 34 specs; site Z4R1, 1 spec.; site AN, 1 spec. **Ría de Aldán (Galicia)**: site 27, 1998: May, 23 specs; June, 2 specs; July, 69 specs; August, 72 specs; September, 48 specs; October, 85 specs; November, 7 specs; December, 49 specs; 1999: January, 75 specs; February, 52 specs; March, 14 specs; April, 14 specs; May, 36 specsite SITE 31, 1998: May, 25 specs; June, 65 specs; July, 148 specs; August, 66 specs; September, 27 specs; October, 79 specs; November, 120 specs; December, 33 specs; 1999: January, 75 specs; February, 149 specs; March, 92 specs; April, 42 specs; May, 295 specs. **Ensenada de Baiona (Galicia)**: site 2, 2 specs; site 4, 1 spec.; site 10, 1 spec.; site 17, 1 spec.; site 18, 2 specs; site 19, 1 spec.; site 20, 1 spec. **Morocco**: site GR37, 1 spec. (ZMBN 116931); site GR44, 17 specs (ZMBN 116932); site GR45, 2 specs (ZMBN 116933) and 1 spec. (DNA voucher, ZMBN 98169); site GR50, 4 specs (ZMBN 116934) and 1 spec. (DNA voucher, ZMBN 98164); site GR56, 3 specs (ZMBN 116935) and 1 spec. (DNA voucher, ZMBN 98157).

Additional studied material of other species: **Off Iceland**. *Ampharete finmarchica* (M. Sars, 1865). MNCN 16.01/15486 (BIOICE sample 2377, 1 spec.), MNCN 16.01/15487 (BIOICE sample 3252, 1 spec.), MNCN 16.01/15488 (BIOICE sample 2660, 3 specs), MNCN 16.01/15489 (BIOICE sample 2070, 4 specs). Specimens reported by Parapar *et al.* (2012). *Ampharete lindstroemi* Malmgren in Hessle, 1917. MNCN 16.01/15329 (BIOICE sample 2622, 2 specs), MNCN 16.01/15330 (BIOICE sample 3028, 1 spec.). Specimens reported by Parapar *et al.* (2012). **West Africa**. *Ampharete debrouweri* Jeldes & Lefevere, 1959. Holotype, PY-987, I.G. 20403, BS-551/12.09.1955. **Norway**. Specimens of *A. falcata*, *A. finmarchica*, *A. lindstroemi*, *A. octocirrata* and *A. undecima* used for DNA barcoding (Table 4).

External morphology (based on holotype): Complete specimen of 18 mm length and 1.5 mm width. Thorax thicker than abdomen and of same length (Figs 2A, 4A). Prostomium with median lobe delimited by deep lateral grooves; prostomial glandular ridges absent (Figs 2B, 4A, B, 6A, B, 10A). Two small black, circular eyespots located posteriorly on median prostomial lobe, next to lateral grooves (Fig. 11A). Nuchal organs not observed. Buccal tentacles with two rows of short papillae on one side (Fig. 6C–F). Peristomium and segment I only visible laterally and ventrally; peristomium forming well-developed lower lip (Fig. 6A). Four pairs of branchiae arranged in two groups with small median gap of about one to two times branchial width (Fig. 2B, C); branchiophores fused at base; branchiae thick at base and gradually tapering towards distal end, less than 1/6 of body length, reaching about TC6; with parallel ciliated rings from base to distal end (Figs 2A–C, 4B, C, 6A, B, D, 7A, 11A, C). Anterior three pairs of branchiae arranged in transverse row on segment II/III, fourth pair slightly posterior to anterior row, between second outermost and innermost branchiae. Branchiae of segment II in outermost position of anterior row, branchiae of segment III in mid-position of anterior row, branchiae of segment IV in innermost position of anterior row, branchiae of segment V in posterior position. Single pair of nephridial papillae located dorsally on segment IV (Fig. 2C). Dorsal part of thorax, behind branchial region, covered by patches of ciliated rounded tufts (only seen with SEM). Segment II with 12–15 long, flattened chaetae (paleae), with curved filiform tips, arranged in semicircle (Figs 6A, 7E, F, 10A). Thorax and abdomen of similar length; thorax slightly wider than abdomen; abdomen tapering posteriorly. Continuous ventral shields present to thoracic unciniger ten (Figs 5B, 11F). Fourteen thoracic segments with notopodia and capillary chaetae; last 12 also with neuropodial tori bearing single row of uncini. Notopodia as simple lobes, up to three times longer than wide; first notopodium somewhat reduced (Figs 2A, 5A, B, 6A, B, 8A, B). Notochaetae as simple spinulose capillaries (Fig. 8C), tapering into slender tips; notochaetae arranged in rows, capillaries from anterior row generally thinner and shorter than in posterior row. Anterior neuropodia oval, about three times higher than wide (Figs 2A, 3A); neuropodia gradually decreasing in size, becoming more rounded in posterior part of thorax (Fig. 2A). Thoracic uncini with about ten teeth in two vertical rows above rostrum and basal prow (Figs 8D, 10B). Twelve abdominal uncinigers (Fig. 2A). Anterior two abdominal segments with neuropodia of thoracic type (Figs 8E, 9A, B); remaining abdominal uncinigers

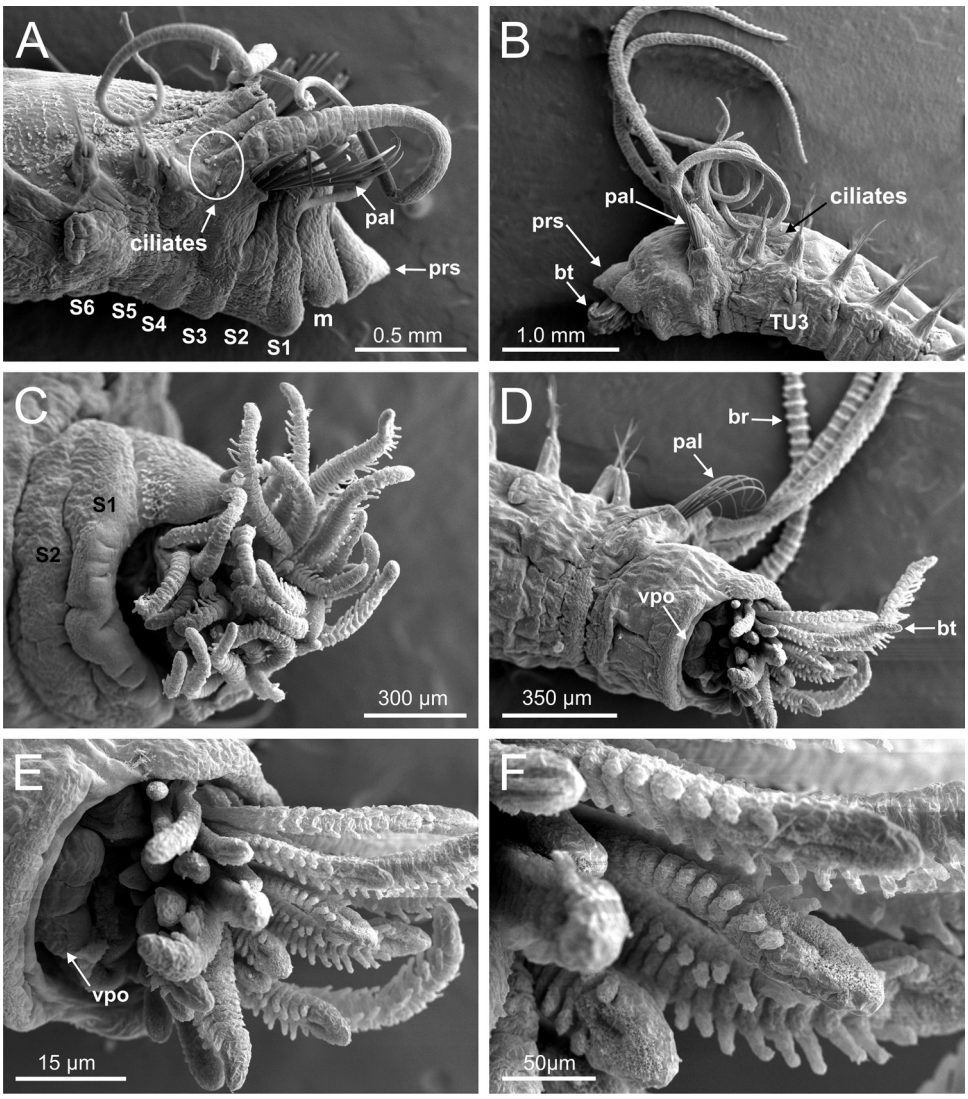

Figure 6. ***Ampharete santillani*** **sp. nov.** SEM micrographs of paratypes MNCN 16.01/17853 and MNCN 16.01/17854, showing position of epibiont ciliates and several body characters. A–B, anterior end in right and left lateral view; C–F, anterior end in ventral view, showing buccal tentacles (bt) and ventral pharyngeal organ (vpo).

with enlarged neuropodial pinnules, with short neuropodial cirrus (Figs 3B–E, 8E). Abdominal uncini with about five teeth in two vertical rows above rostrum and basal prow (Figs 9C, D, 10C, F). Ciliated tufts between parapodial rami in thorax on ventral bases of notopodia, and above neuropodia in abdomen (on parapodial ridge) (Figs 8E, 10E). Pygidium as short cylindrical tube, about as long as wide, with pair of lateral cirri of same length as width of last chaetiger and with about eight pygidial papillae (Figs 3F–H, 9E, F, 10D, E). Colour in life bright orange, epiderm transparent; thick dorsal blood vessel distinct in anterior dorsal thorax as deep red tube (Fig. 11A–C). Specimens in alcohol pale orange to yellow, with opaque epidermis (Fig. 4). Tube more or less quadrangular in cross section, incrusted with mud and relatively large pieces of shell fragments (Fig. 11E). Head and ventral shields deeply dyed in methyl blue (Fig. 11F).

Variability: Complete specimens measure 11–21 mm in length and 0.5–2.5 mm in width. Several specimens from Galicia bear 13 abdominal chaetigers instead of 12. The pygidium showed variability regarding the number of papillae and length of lateral cirri; however, the actual variability of these characters is difficult to ascertain because the pygidium is often contracted in fixed specimens, sometimes inside the posterior end. Branchiae seem longer in live compared to fixed specimens (see Fig. 11A–C vs. Figs 2A, B, 4A–D, 6A, B), probably due to their intrinsic flexibility, and contraction

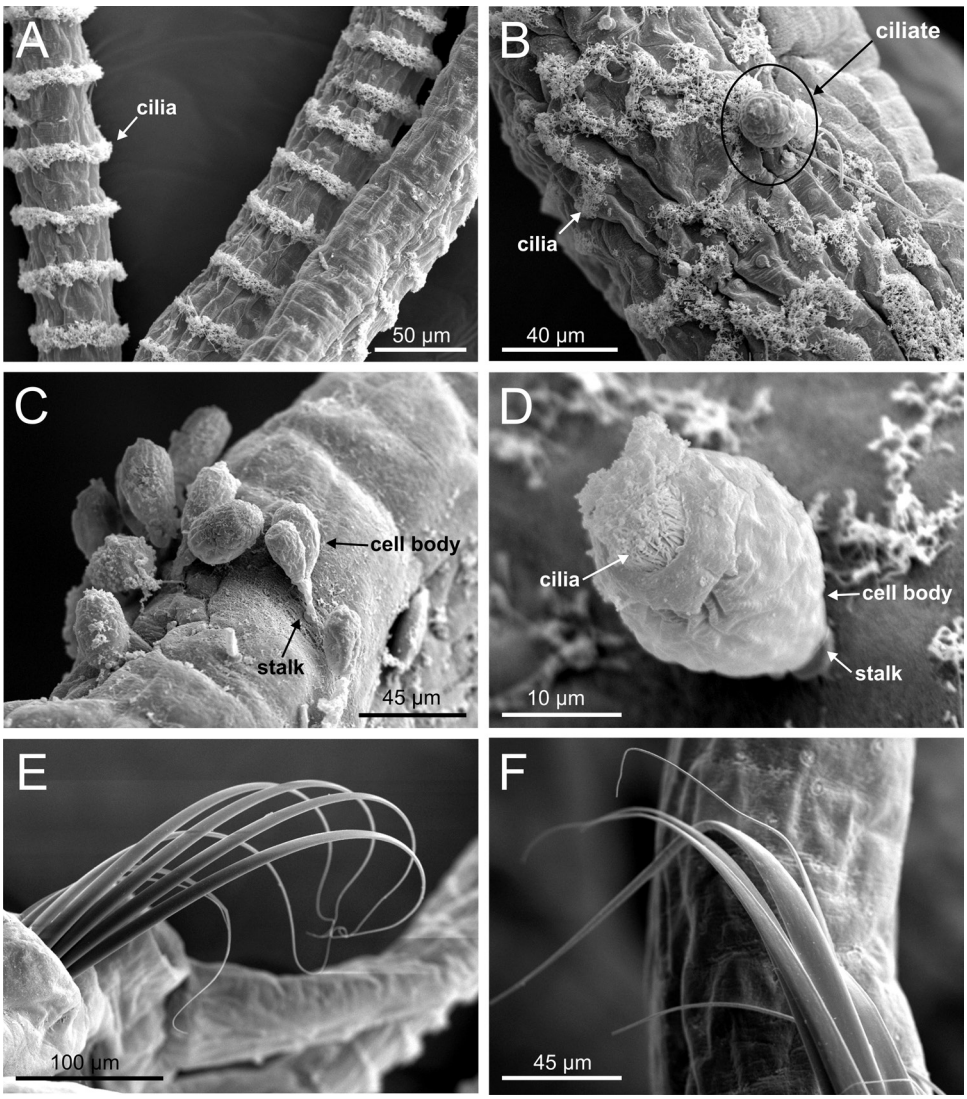

Figure 7. ***Ampharete santillani*** **sp. nov.** SEM micrographs of paratypes MNCN 16.01/17853 and MNCN 16.01/17854. A–B, general view and detail of ciliated rows in branchiae; C, clump of epibiont ciliates; D, detail of one epibiont ciliate; E–F, paleae.

caused by fixation. Oocytes were seen filling the coelomic cavity in live specimens (Fig. 11B).

*Anatomy (*Figs 5, 12–15*):* The micro-CT 3D reconstructions of internal longitudinal and transversal sections show the shape and position of musculature and organs in high quality, primarily those of muscular, digestive, circulatory, excretory and reproductive systems. The integument comprises the cuticle, epidermis and thin longitudinal musculature bands and is shown to be thicker ventrally (in thorax) due to the glandular epidermis (Fig. 12). The digestive system fills most of the coelomic cavity and has a wide anterior buccal cavity (pharynx or fore digestive) filled with retracted buccal tentacles in this specimen (Figs 5C, D, 12A–C), a short oesophagus connecting with a large stomach (median gut) supported by a muscular ventral mesentery (Fig. 12D, E), and a long, narrow and internally looped intestine (hind gut) (Figs 5C, D, 12F). The buccal cavity contains a ventral pharyngeal organ (Figs 5C, 13B) that is also discernible with SEM (Fig. 6D, E). The stomach shows an anterior pair of lobes surrounding the oesophagus (Figs 12C, D, 13A) followed by a continuous tube with irregularly distributed constrictions that may be related to the retraction of the buccal cavity (Fig. 5C, D). Transversal views do not show the circular musculature of the body wall; however, many thin bundles of longitudinal muscles can be seen dorsally and ventrally (Figs 13A, 14B, C). Thin bundles of oblique parapodial musculature connect the basal part of thoracic notopodia with the inner ventral body

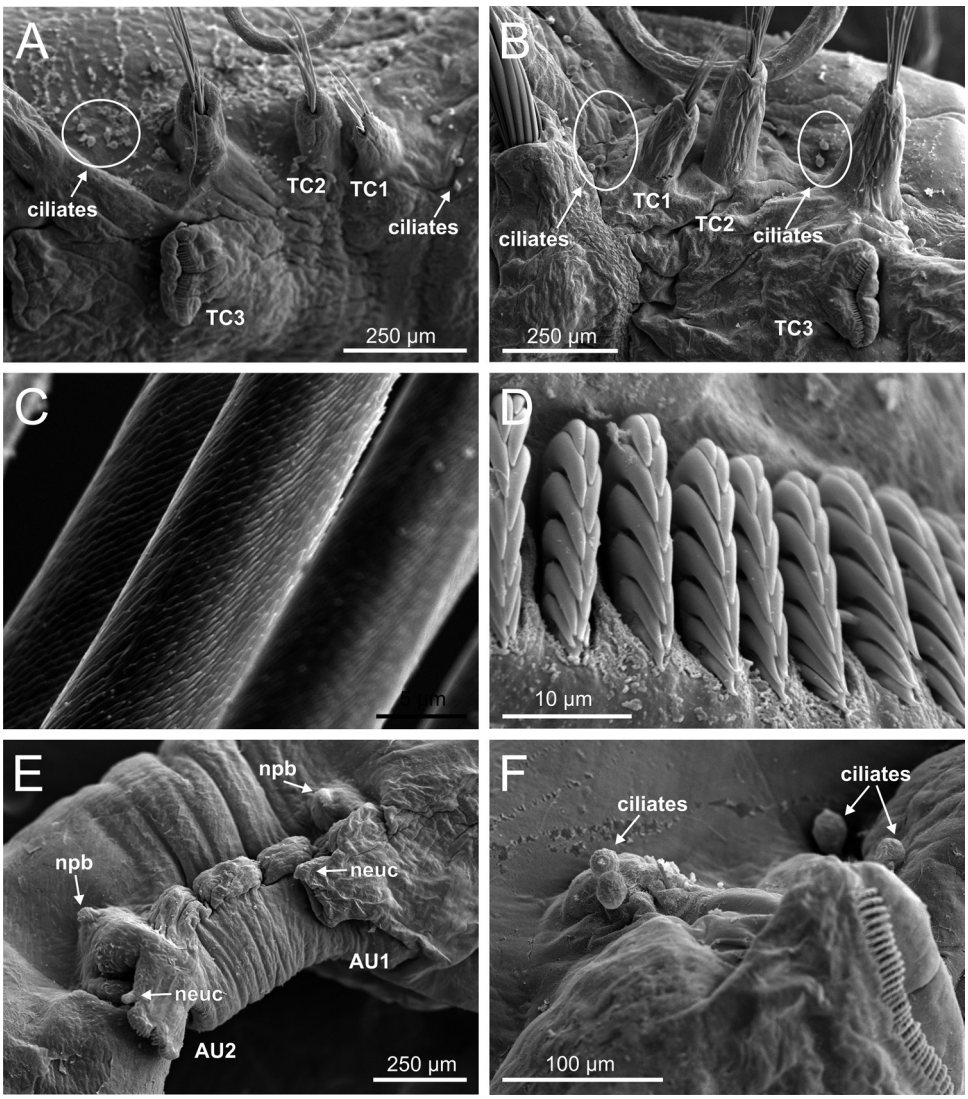

Figure 8. ***Ampharete santillani*** **sp. nov.** SEM micrographs of paratypes MNCN 16.01/17853 and MNCN 16.01/17854. A–B, first thoracic chaetigers (TC1–TC3) in left and right lateral view, showing location of epibiont ciliates; C, detail of spinulose notochaetae; D, thoracic uncini in fronto-lateral view; E, first two abdominal unciniger (AU1 and AU2) in right lateral view; F, detail of position of ciliates near to abdominal notopodial ciliated buttons.

surface (Fig. 12E, F). The circulatory system is represented by a well-developed dorsal blood vessel, which can be best seen anteriorly (Figs 5C, 12B–D, 14A, B), and an expanded blood sinus completely covering the stomach (Figs 12D, E, 13A, B). The only conspicuous part of the central nervous system is the nerve cord located ventrally, close to the integument (Fig. 12D). No brain or circumpharyngeal connectives were observed. Tissue masses located ventrally to the buccal cavity and stomach in the median thoracic region may be related to segmental organs (Figs 5C, 12B–E); their shape probably corresponds to different biological roles. Histological body sections of paratypes 16.01/17855 to 16.01/17860 taken from the anterior thoracic region (Fig. 15A) provide complementary information to that of micro-CT. Images show the presence of several anatomical structures inside a coelomic cavity partially filled with oocytes in different stages of maturation (Fig. 15B). Those structures are the stomach (Fig. 15B), the lateral branches of the segmental organs-nephridial ducts (Fig. 15C), the ventral nerve cord ganglion (Fig. 15D) and the stomach blood sinus (Fig. 15E).

*Molecular results (*Fig. 16, Table 2*):* All morpho-species were recovered as monophyletic (Fig. 16) with low genetic intraspecific K2P variation (< 0.5% in *COI*), except for *A. acutifrons* where the single specimen from Alaska

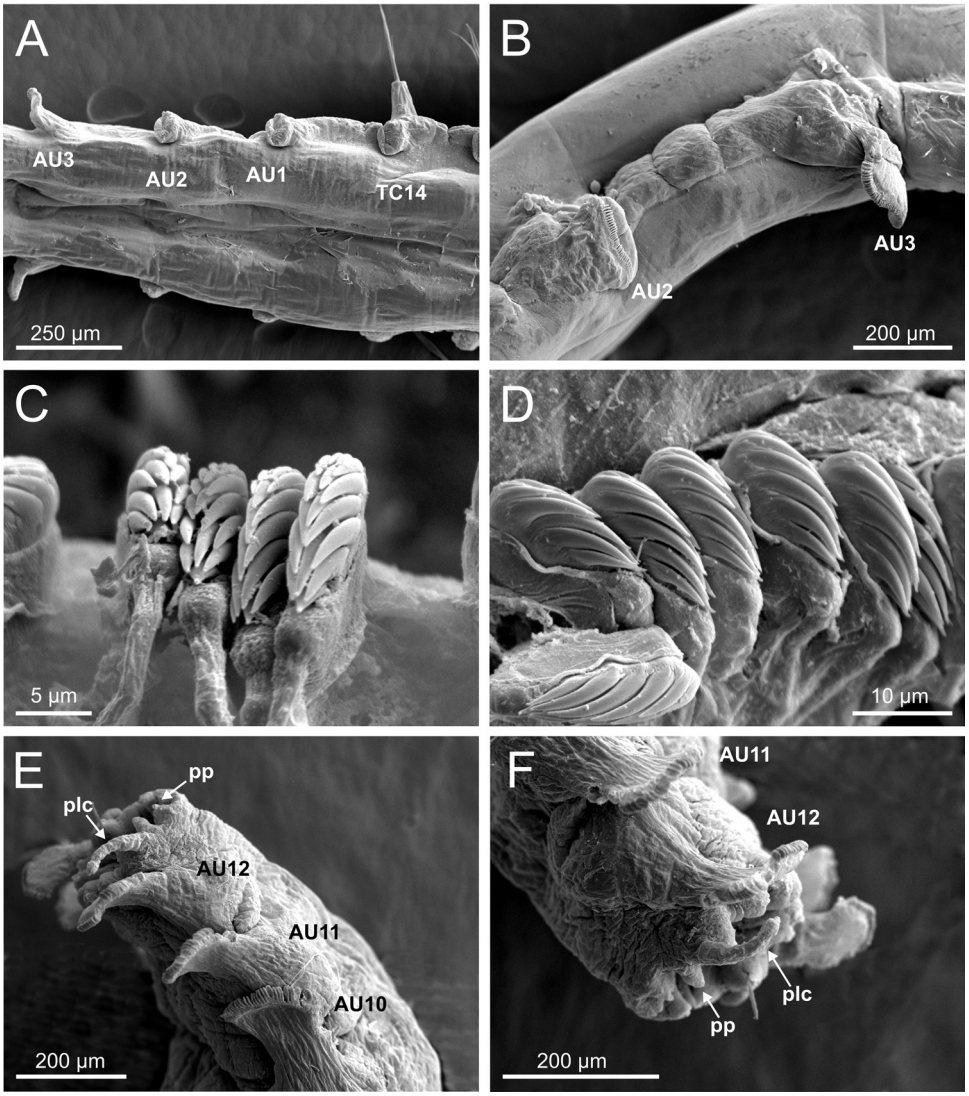

Figure 9. ***Ampharete santillani*** **sp. nov.** SEM micrographs of paratypes MNCN 16.01/17853 and MNCN 16.01/17854. A, last thoracic segment (TC14) and first three abdominal chaetigers (AU1–AU3) in ventral view; B, second (AU2) and third (AU3) abdominal uncinigers in left lateral view; C–D, abdominal uncini in frontal and lateral view; E, last three abdominal uncinigers (AU10–AU12) and pygidium in latero-ventral view showing pygidial papillae (pp) and pygidial lateral cirri (plc); F, detail of pygidium.

differed from the four specimens from California by 7.1% (Table 2). The mean interspecific K2P divergence between species was 17.4%, ranging from 3.3% between *A. manriquei* and *A. octocirrata* to 21.6% between *A. santillani* sp. nov. and *A. falcata* (Table 2). *Ampharete santillani* sp. nov. was recovered as sister species to *A. lindstroemi* with high bootstrap support, and the intraspecific distance between these two species was 15.1% (Table 2).

*Epibiosis (*Figs 6A, 7B–D, 8A, B, E, F, 10E, F, 17*):* A high infestation by ciliated protozoans on the external body surface was observed in specimens from both the Ría de Ferrol (Figs 6–8, 17) and Morocco (Fig. 10). Although they have been found in many body parts, such as attached to the abdominal uncini (Fig. 10E, F), their abundance seems to be higher in ciliated body parts, such as the branchial surface (Fig. 7B–D), the dorso-lateral area behind them (Figs 6A, 8A, B) and the ciliated buttons over the abdominal neuropodia (Fig. 8E, F).

Type locality: Ría de Ferrol (Galicia, NW Spain), 43°28′46″N, 08°11′45″W, sandy mud, 1.5 m depth, organic content in sediment = 3.22%.

*Ecology (*Figs 18–19*):* The new species was found in the Ría de Ferrol, Ría de Aldán and Ensenada de

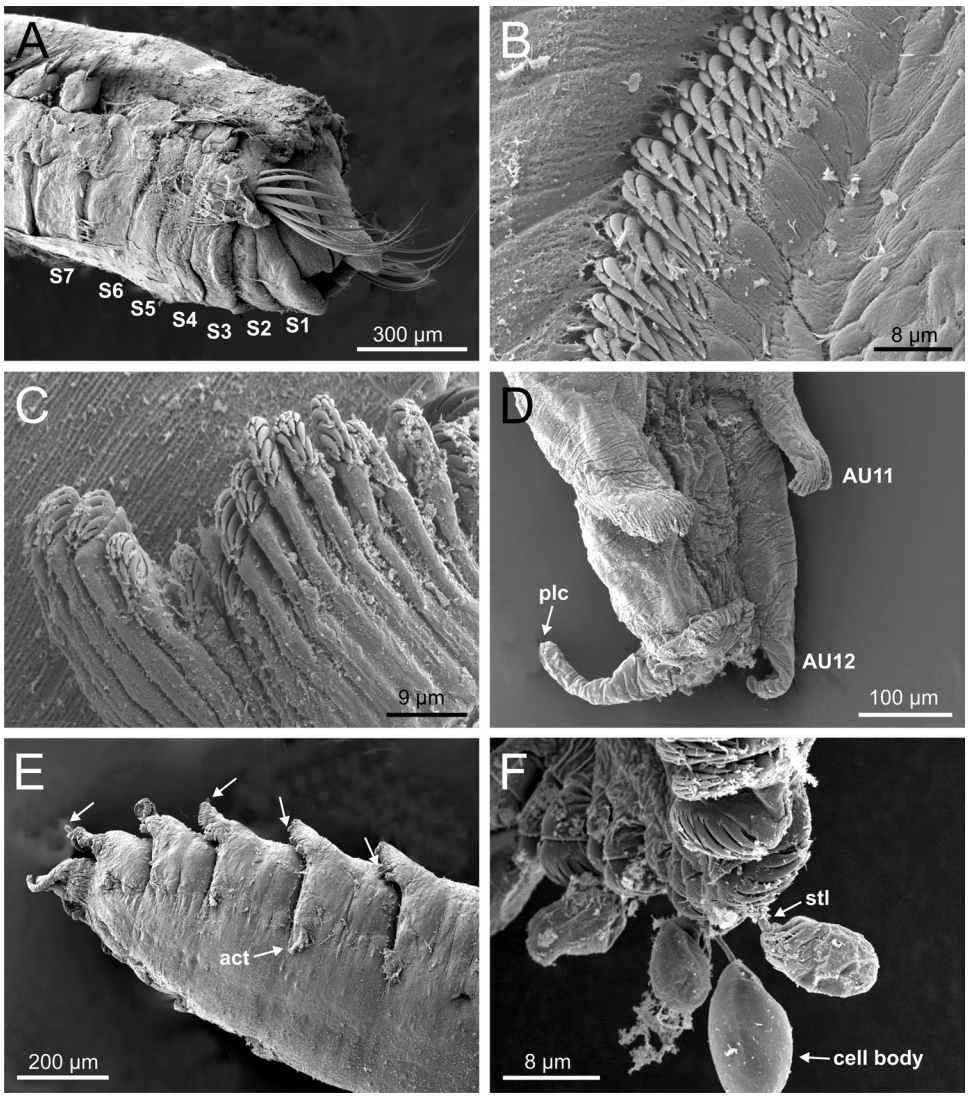

Figure 10. ***Ampharete santillani*** **sp. nov.** SEM micrographs of a specimen from Morocco (ZMBN 116934). A, anterior end in right lateral view, showing position of first seven segments (S1–S7); B, thoracic uncini in frontal view; C, abdominal uncini in frontal view; D, last two abdominal unciniger (AU11–AU12) and pygidium with lateral cirri (plc) in ventral view; E, posterior body end (arrows showing position of ciliates on abdominal neuropodia); F, detail of abdominal uncinigers with epibiont ciliates.

Baiona (NW Spain) mostly in fine sand and muddy sediments (sandy mud to mud) at inner areas of these rias at depths of between 1 and 20 m (Table 1); some specimens were occasionally found in gravel and coarse sand at the outer half of the Ría de Ferrol (Fig. 18). Specimens from Morocco were found in slightly deeper waters (36–106 m) than in NW Spain; however, no samples from shallower localities (< 30 m) were available from Morocco. Organic content of the sediment ranged from 0.3 to 15.1% and 0.3 to 8.5% in Ferrol and Baiona, respectively; in Aldán, the values in site 27 (muddy sand), ranged between 0.9 and 4.8%, while in site 31 (mud), they were much higher: 9.9–15.1%. There were, however, no significant correlations between organic matter and monthly abundance at any site (Spearman correlation coefficient, $P > 0.05$, d.f. = 11). Temporal variation in the Ría de Aldán showed that the new species may reach densities of up to 500–1000 ind./m^2 in site 31 and up to 300 ind./m^2 in site 27 (Fig. 19). In site 27, peaks of abundance were detected in summer/autumn and January, while in site 31, peaks (i.e. July, November, February, May 1999) were followed by conspicuous decreases in the following months.

Distribution: NW Iberian Peninsula, off southern coast of Morocco (Fig. 1). Previous reports of *A. acutifrons*/*A. grubei* in the Iberian Peninsula (e.g. Cantabrian coast,

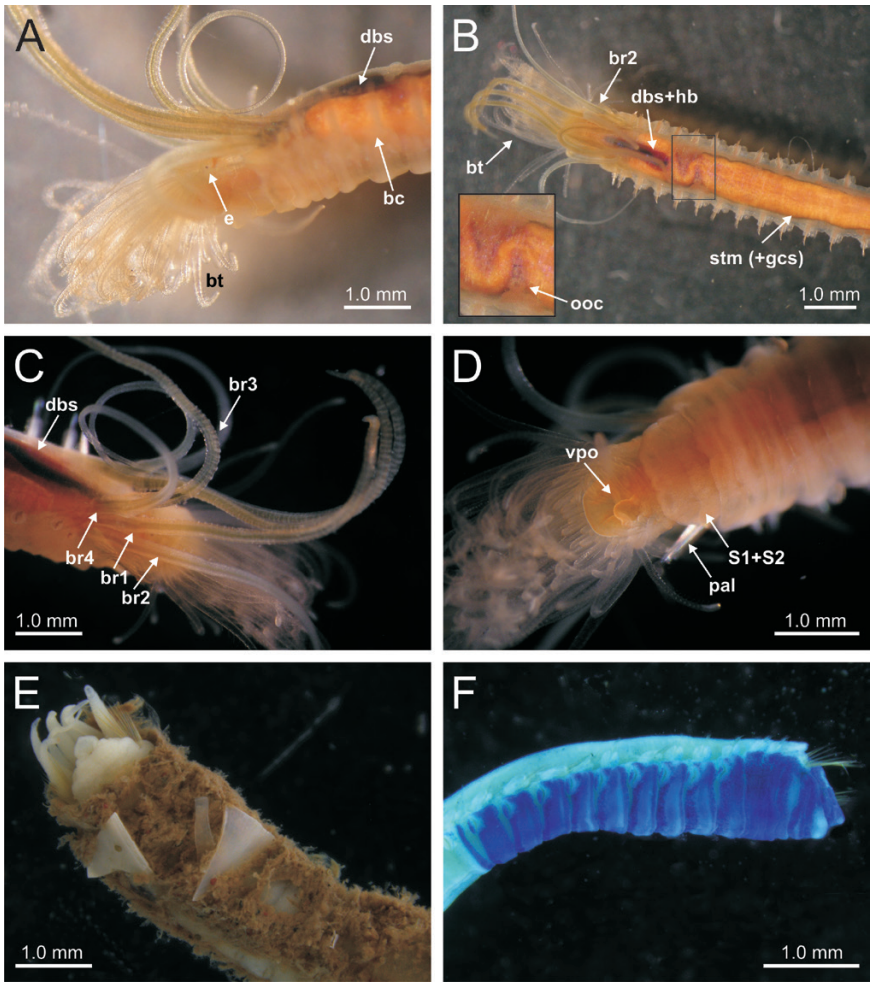

Figure 11. ***Ampharete santillani*** **sp. nov.** A–D, pictures of a live specimen with everted buccal tentacles (MNCN 16.01/17852) later used in micro-CT, showing several external and internal body characters; E, specimen from Morocco (ZMBN 116932) inside the tube in ventral view; F, same specimen stained with methyl blue showing coloration pattern.

Portuguese coast) may represent *A. santillani* sp. nov. (see Discussion).

Etymology: The species is named after the late Enrique Santillán (Quique), biologist, teacher at the Marista school of Cristo Rey (A Coruña, Spain) and friend of first author. Quique was an avid collector of marine sands around the world, and his kindness and love for nature has been an inspiration to many generations of young students.

Identification of NE Atlantic species of the genus *Ampharete*

A synopsis of diagnostic characters for all 13 valid species of *Ampharete* occurring in the NE Atlantic (including *A. santillani* sp. nov.) is given in Table 3. Traditionally, species of *Ampharete* have been identified based on the number of abdominal uncinigers, arrangement of the branchiae, shape of anal papillae, the shape of neuropodial dorsal cirri and their distribution along the body and the shape of paleae (Holthe, 1986; Imajima *et al.*, 2012; Parapar *et al.*, 2012). However, accepting *Sabellides* and *Asabellides* as junior synonyms of *Ampharete* (see Introduction), the presence/absence of paleae and number of thoracic uncinigers are also relevant characters to distinguish between species of *Ampharete* occurring in the NE Atlantic. Furthermore, we regard the presence and number (pairs) of prostomial and pygidial eyes as important characters to distinguish between certain species.

Ampharete minuta, originally described from the Madeira Islands and not reported after its original description, was not included in the table. The type

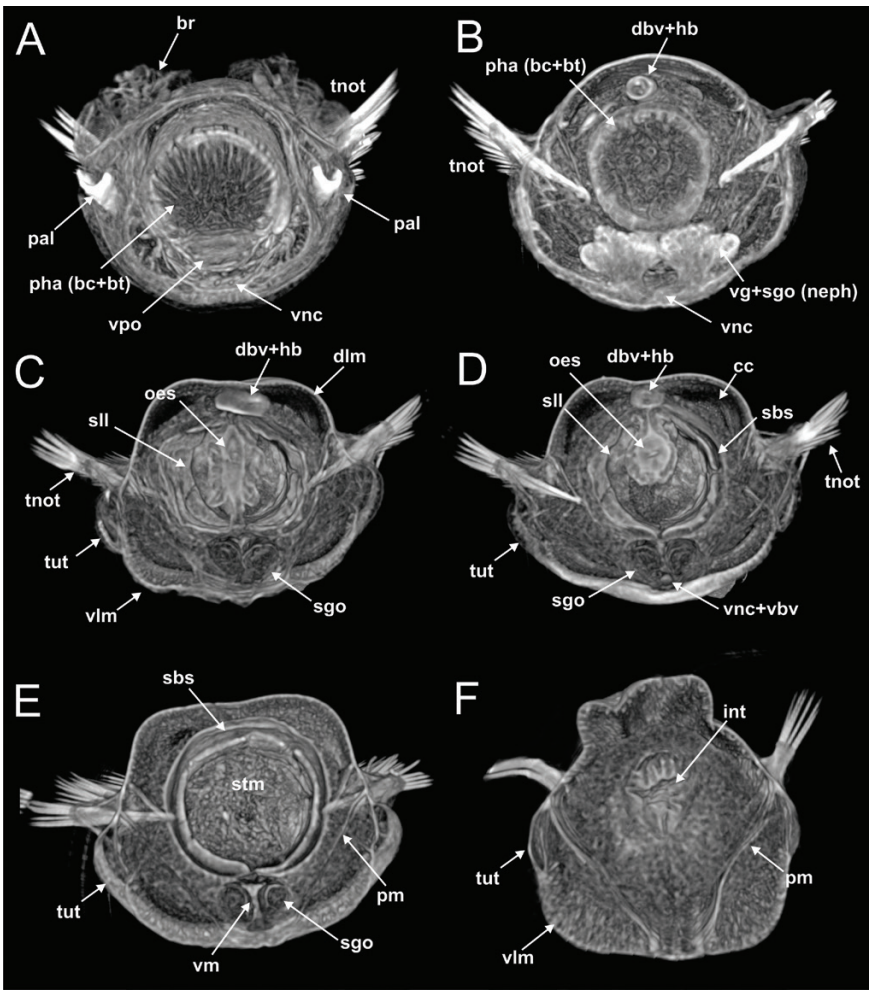

Figure 12. ***Ampharete santillani*** **sp. nov.** A–F, micro-CT images transversal sections at different body levels of paratype MNCN 16.01/17852 showing main body internal structures (see Fig. 5C for reference).

material could not be located, and the original description by Langerhans (1881) lacks detailed information about several taxonomical important characters (see Discussion). *Ampharete grubei* Malmgren, 1866, originally described from Greenland and N Europe, has long been considered as a synonym of *A. acutifrons*. However, Jirkov & Leontovich (2013) questioned this and suggested that this taxon should be reinstated. Pending a revision of the type material, we chose not to include it in the synoptic table presented here.

DISCUSSION

External morphology and taxonomy

Ampharete santillani sp. nov. belongs to the group of species traditionally referred to as *Ampharete* (see e.g. Holthe, 1986), characterized by the presence of paleae and 12 thoracic uncinigers. The species is similar to *A. lindstroemi* Malmgren in Hessle, 1917 in the presence of 12 abdominal uncinigers, number and shape of the paleae and the presence of a pygidium with two long lateral cirri and a number of small, rounded papillae. However, *A. santillani* sp. nov. differs from *A. lindstroemi* by larger body size, the absence of eyes on the pygidium and the presence of short dorsal neuropodial cirri in posterior abdominal segments rather than minute rounded lobes.

Ampharete lindstroemi has been recorded from widespread localities in the NE Atlantic, and the possible presence of a species complex confounded under this species name was indicated by Jirkov (2001) and Parapar *et al.* (2012). Type material of *A. lindstroemi* is presumably lost (Holthe, 1986), but the DNA voucher specimens of *A. lindstroemi* examined here were collected from the south coast of Norway, which is relatively close to the type locality, Bohuslän, Sweden. These specimens fit very well with the description of *A.*

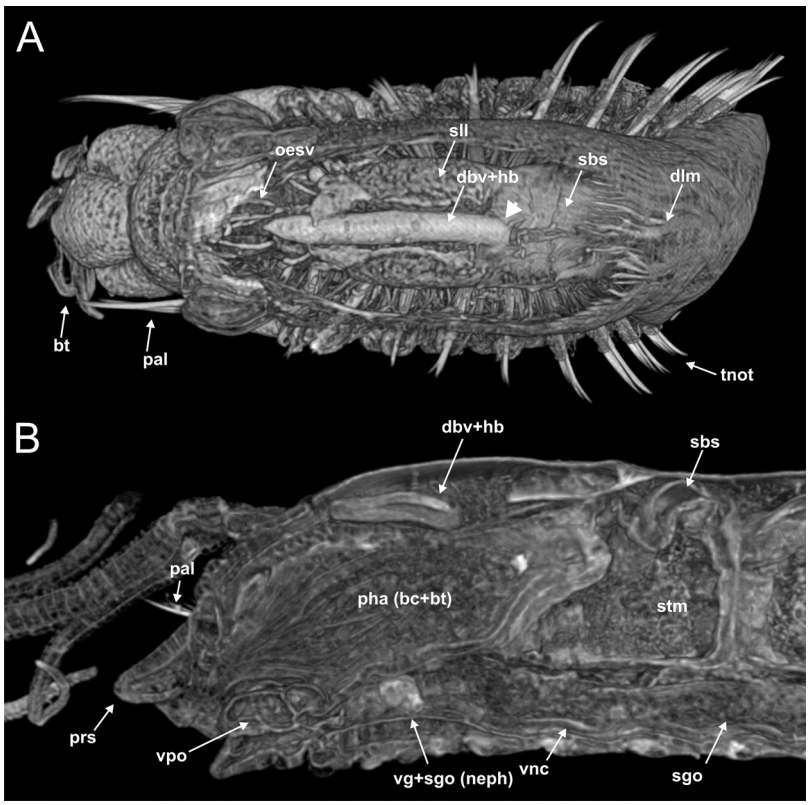

Figure 13. ***Ampharete santillani*** **sp. nov.** Micro-CT images of paratype MNCN 16.01/17852. A–B, coronal and sagittal views of anterior end, showing dorsal blood vessel (dbv) and stomach lateral lobes (sll) area, and buccal cavity (bc) area with buccal tentacles (bt), respectively. Arrowhead points to the area connecting the dorsal blood vessel (dbv) with the stomach blood sinus (sbs).

lindstroemi given by Hessle (1917) and Holthe (1986), and the presence of pygidial eyes is distinct. The specimens reported as *A. lindstroemi* from Icelandic waters by Parapar *et al.* (2012) were re-examined in this study. These specimens differ from the DNA voucher specimens from Norway in the presence of conspicuously long paleae (see Parapar *et al.*, 2012: fig. 4A) and the absence of pygidial eyes. We consider these specimens from Iceland to represent an undescribed species, closely related to *A. lindstroemi* and *A. santillani* sp. nov.

Records of *A. acutifrons* from the Cantabrian Sea (Aguirrezabalaga, 1984), Galician rias (e.g. López-Jamar, 1978, 1979, 1981, 1982; Rodríguez-Castelo & Mora, 1984) and the Atlantic shelf off Spain (Amoureux, 1972, 1974; Tenore *et al.*, 1984; López-Jamar & González, 1987), and also from the Portuguese coast (Marques, 1944; Bellan, 1960) and the Mediterranean (Rioja, 1920, 1931), merit a revision. This will be done elsewhere and may reveal an expanded distribution of *A. santillani* sp. nov. or, alternatively, (1) the existence of new undescribed taxa or (2) the reinstatement of species synonymized with other *Ampharete* species, for example *A. grubei* and *A. intermedia* Marion, 1876 (see Fauvel, 1927).

The holotype specimen of *A. debrouweri* was re-examined for comparative purposes; two microscope slides with thoracic and abdominal uncini are presumably lost (Y. Samyn, pers. comm.). The holotype is incomplete and broken in two pieces; the two branchiae reported attached to the body in the original description (Jeldes & Lefevere, 1959) are now lost. However, the original description and the study of the holotype reveal that it differs from *A. santillani* sp. nov. in several features: (1) the paleae are bayonet shaped in *A. debrouweri* (Fig. 20I), while in *A. santillani* sp. nov., they are evenly tapered; (2) the paleae are distinctly longer in *A. debrouweri* (see Fig. 20G, H); (3) thoracic uncini bear more teeth in *A. debrouweri* (15) than in *A. santillani* sp. nov. (11); and (4) *A. debrouweri* lacks prostomial eyes. Moreover, Jeldes & Lefevere (1959) describe a change in the shape of thoracic neuropodia from the sixth chaetiger, that is from low pinnules to elevated tori (Fig. 20G, arrow). The latter character was observed in the holotype, but whether this is relevant to characterize the

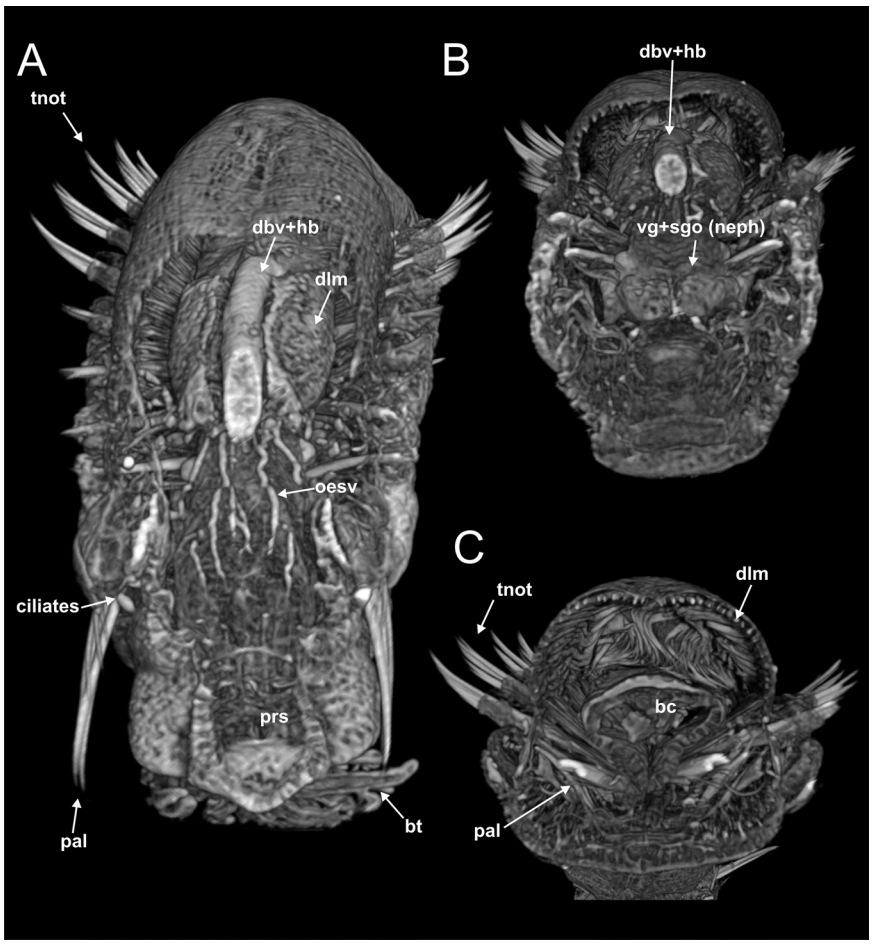

Figure 14. ***Ampharete santillani*** **sp. nov.** A–C, micro-CT images of paratype MNCN 16.01/17852, showing three oblique sections at different levels (see Fig. 5A for reference).

species cannot be evaluated, as only one specimen is available.

The type material of *A. minuta* could not be located, and the species has not been reported in the literature since the original description. The description and drawings by Langerhans (1881) refer to the presence of a pair of large eyes in the prostomial lateral lobes (and a third one in the posterior part of central lobe reported by the author in some specimens; Fig. 20L) and a long dorsal cirrus in abdominal neuropodia (Fig. 20N) like that of *A. acutifrons*; these two features clearly separate this taxon from *A. santillani* sp. nov.

Molecular study

In this study, DNA barcodes were produced for six species occurring in the North Atlantic: *A. falcata*, *A. finmarchica*, *A. lindstroemi*, *A. octocirrata*, *A. santillani* sp. nov. and *A. undecima*. For these species, DNA barcodes are now available from the type locality or from relatively close to their type locality. For *A. acutifrons*, originally described from Greenland, barcodes are only available from Pacific specimens, representing two distinct clades. At present, it is not possible to decide if any of these clades represents the true *A. acutifrons*. DNA barcodes are still lacking for five species known from the North Atlantic, *A. baltica*, *A. borealis*, *A. goesi*, *A. petersenae* and *A. villenai*. This contribution is thus only a first step to establish a reference library of barcodes for the genus *Ampharete* in the NE Atlantic.

DNA barcoding (*COI* sequences) has proven to be very useful in delineating species boundaries and to examine gene flow among widespread populations (see e.g. Carr *et al.*, 2011; Havermans *et al.*, 2011). In this study, a close relationship between morpho-species and DNA barcode data were identified, and the new species described herein, *A. santillani* sp. nov., was found to be > 15% different (K2P distance) from the morphologically most similar species, *A. lindstroemi*. Furthermore, DNA barcoding data document the distribution of the new species from the NW coast of Spain to southern Morocco.

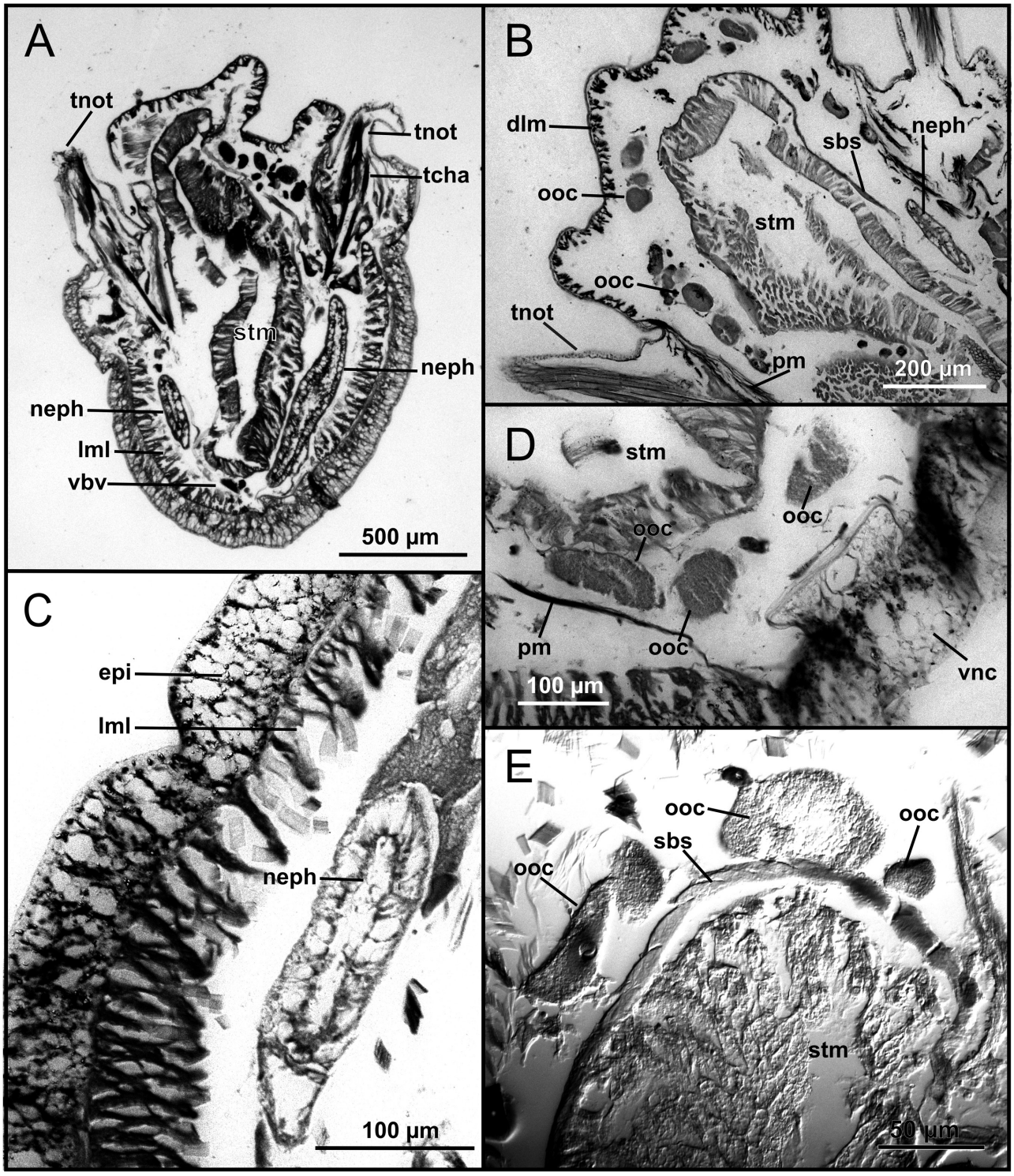

Figure 15. ***Ampharete santillani*** **sp. nov.** Histological sections of paratypes MNCN 16.01/17855 to 16.01/17860 showing main internal organs. A, complete body transversal section at thoracic level; B, detail of dorsal part between thoracic notochaetal bundles (tnot); C, detail of right nephridial duct; D, detail of one thoracic ventral nerve cord (vnc) ganglion; E, dorsal half of stomach (stm) with surrounding blood sinus (sbs) and oocytes (ooc) in coelomic cavity.

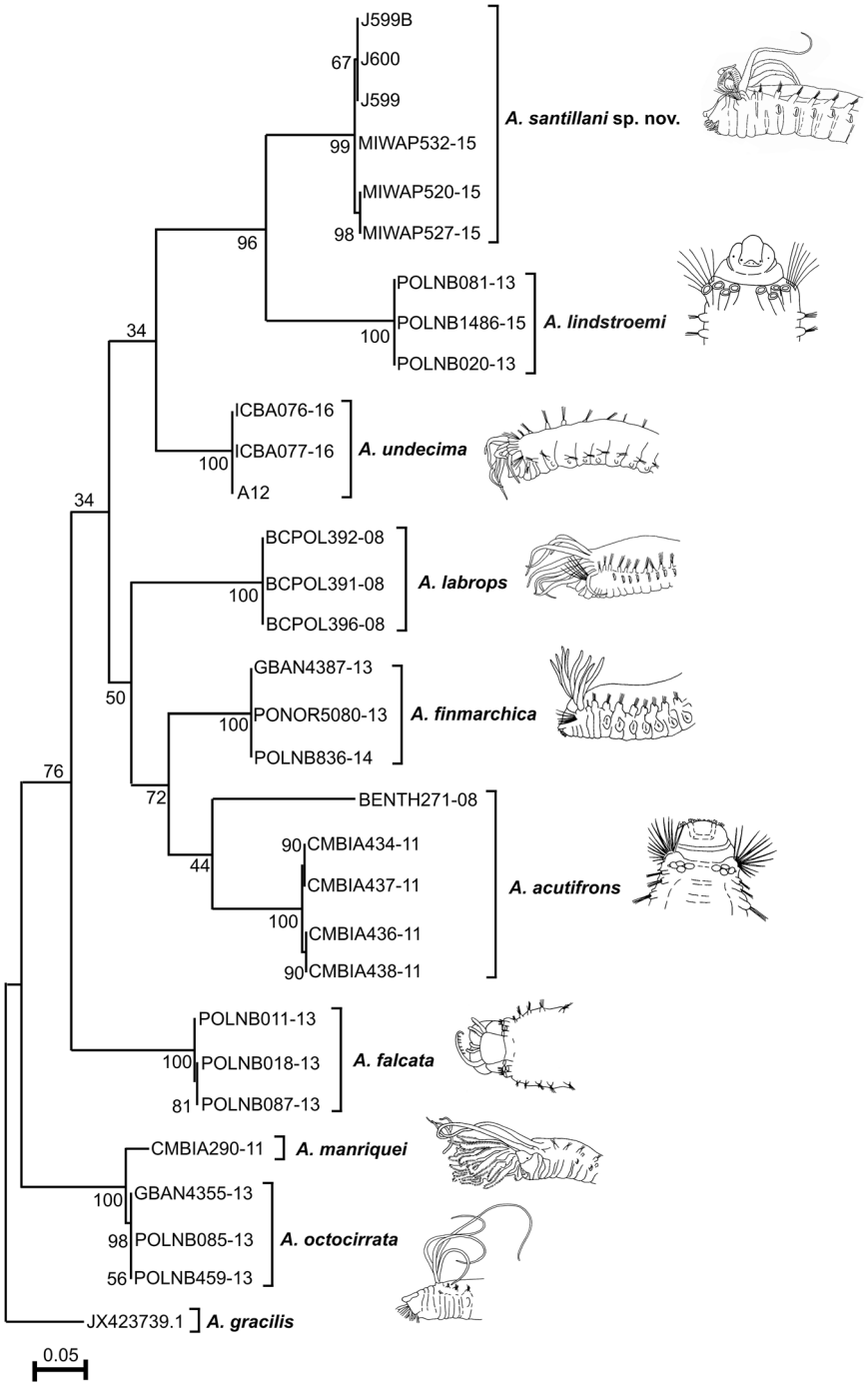

Figure 16. Maximum likelihood tree based on partial *COI* sequences, with bootstrap supports (1000 replications) shown on branches. Specimen data are summarized in Table 4. Figures redrawn from Eliason (1955) (*A. falcata*), Holthe (1986) (*A. acutifrons*, *A. finmarchica*, *A. lindstroemi*, *A. octocirrata*), Hilbig (2000) (*A. labrops*), Alvestad *et al.* (2014) (*A. undecima*), Salazar-Vallejo (1996) (*A. manriquei*) and this study (***Ampharete santillani* sp. nov.**).

INTERNAL ANATOMY

Several detailed anatomical studies on Ampharetidae were made in the late 19th century (e.g. Fauvel, 1897) and early 20th century (e.g. Hessle, 1917). This was later summarized by Day (1964), who points out that the gut in *Ampharete* is a relatively narrow tube

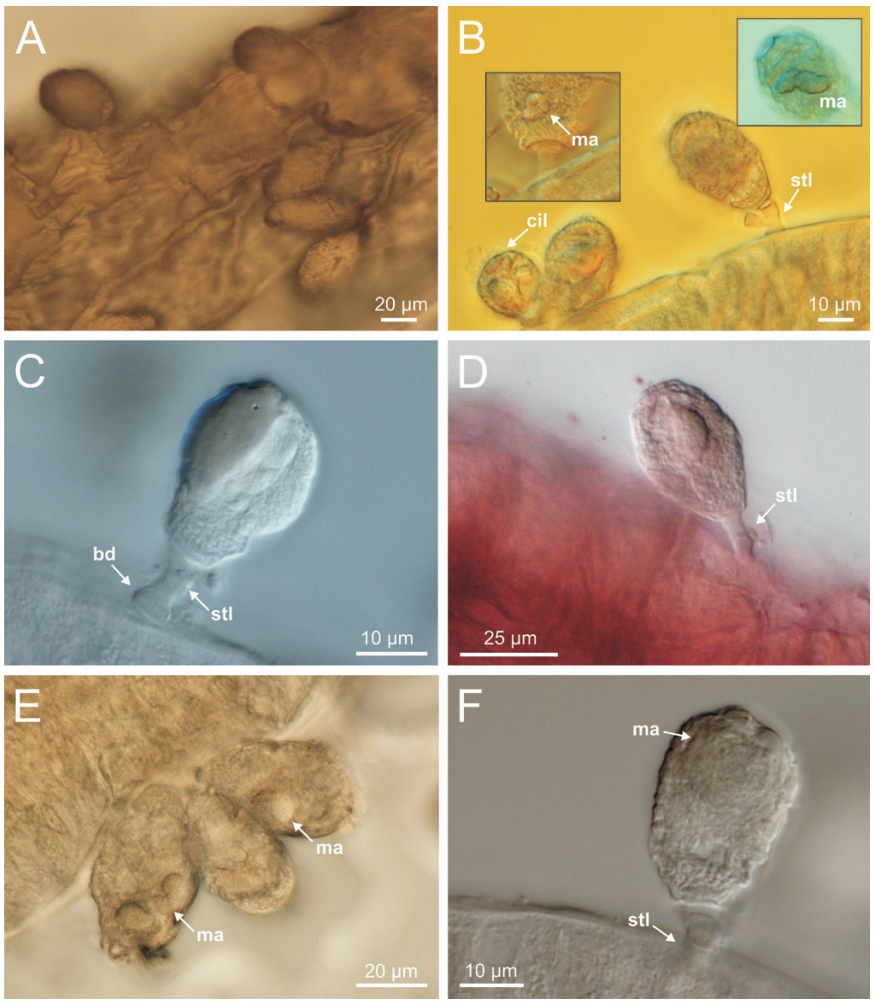

Figure 17. ***Ampharete santillani*** **sp. nov.** A–F, microscope images of peritrich ciliates attached to body surface of paratype MNCN 16.01/17849.

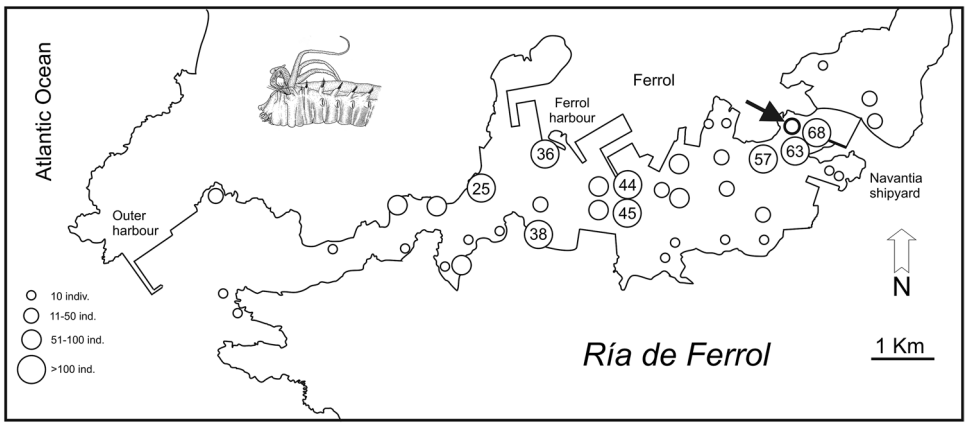

Figure 18. Map of the Ría de Ferrol showing the presence and range of abundance per sampling site of ***Ampharete santillani*** **sp. nov.** Number of sampling sites with higher abundance data are also indicated. Arrow points to location of site 62 where holotype was collected.

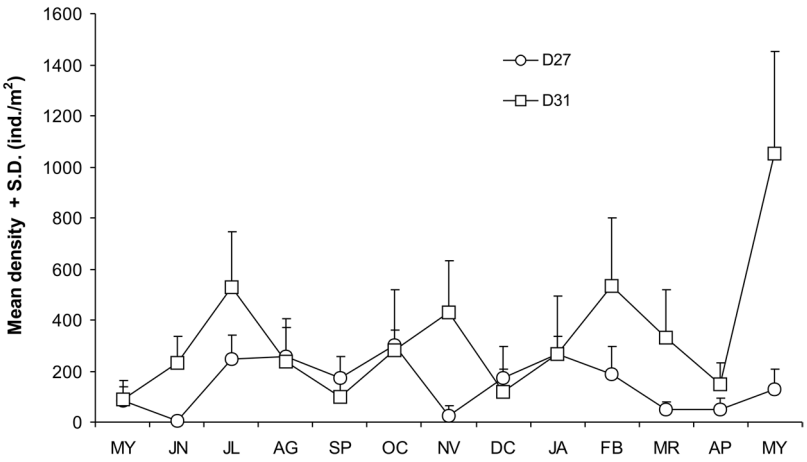

Figure 19. Temporal variation in abundance (number of individuals per square metre; mean + SD) of ***Ampharete santillani* sp. nov.** at the Ría de Aldán (May 1998–May 1999) in sites 27 (muddy sand) and 31 (mud).

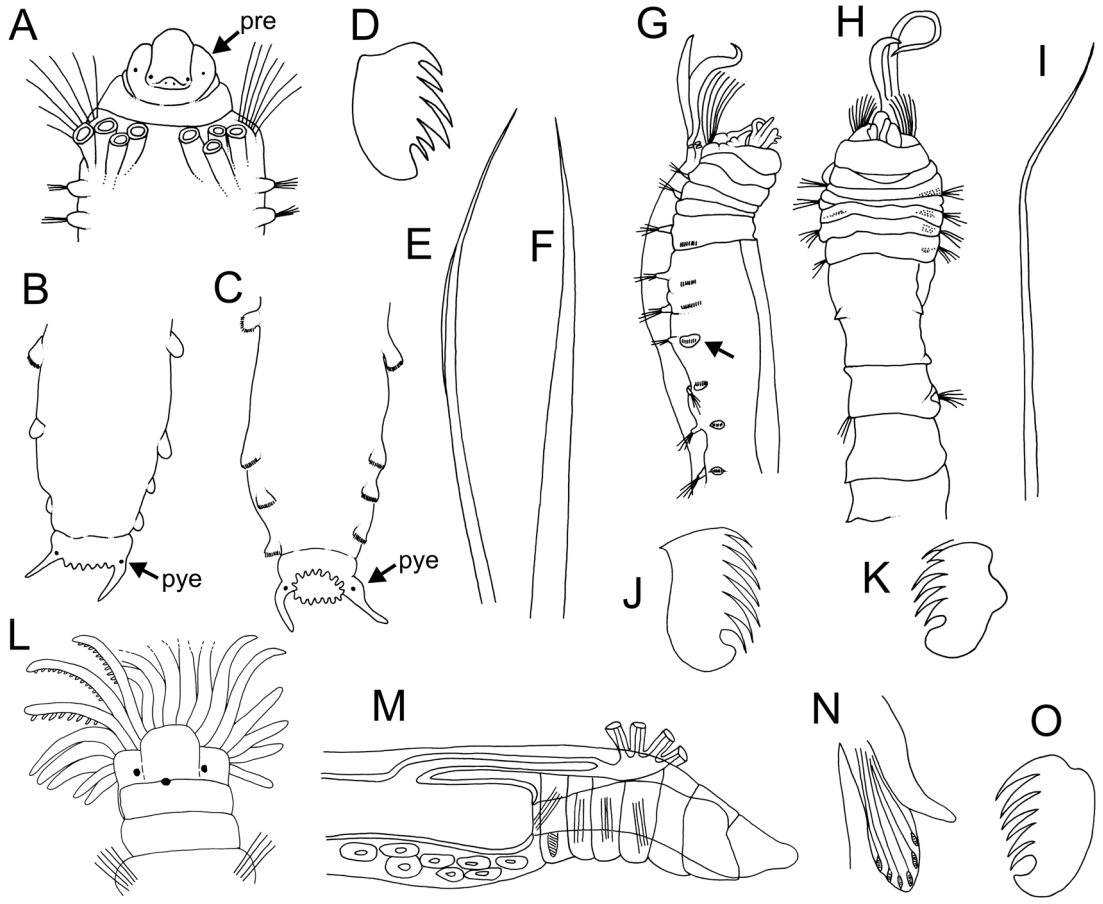

Figure 20. A–F, *Ampharete lindstroemi* Malmgren in Hessle, 1917. A, anterior end in dorsal view; B–C, posterior end in dorsal and ventral view; D, thoracic/abdominal uncinus; E, dorsal chaeta; F, palea. G–K, *Ampharete debrouweri* Jeldes & Lefevere, 1959. G–H, anterior end in lateral and ventral view (arrow marking fourth thoracic unciniger); I, bayonet palea; J, thoracic uncinus; K, abdominal uncinus. L–O, *Ampharete minuta* Langerhans, 1881. L–M, anterior end in dorsal and lateral view (anterior gut and oocytes seen through body wall); N, abdominal neuropodium; O, thoracic uncinus. A–C redrawn from Holthe (1986); D–F from Kirkegaard (1996); G–K from Jeldes & Lefevere (1959); L–O from Langerhans (1881). All redrawn from original.

divided into pharynx, oesophagus, stomach and intestine. Dales (1963) identified a buccal organ located ventrally in the buccal cavity and considered this organ as a lip and a food sorter. Hutchings (2000), following Fauvel (1897), reports a pair of lateral pouches at the junction of the oesophagus and the stomach in some Ampharetid genera, while in others, an internal diverticulum is present in the ventral wall of the stomach. Images obtained from transversal sections using micro-CT confirm the absence of dorso-lateral ciliary folds in the anterior part of the gut that Purschke & Tzetlin (1996) previously documented for several Ampharetid species (Figs 12A, B, 13B). Rouse (2001) refers to a gular membrane that is present between segments IV and V; this was not seen in the examined specimens.

The segmental organs of Ampharetidae were studied in detail by Hessle (1917) and summarized by Day (1964), who reported several pairs of nephridia (two to five) in anterior segments, which may correspond to the mixonephridia type *sensu* Goodrich (1945). Goodrich (1945) affirms that in the genus *Ampharete* the anteriormost pair (supposedly having an excretory function) has funnels in segment III or IV (in front of the gular membrane) and opens to the exterior in segment IV. The following pairs serve for gamete release as well. The micro-CT images show a pair of well-delimited organs in the ventral part of an anterior thoracic segment (Figs 12B, 13B) that may correspond to a nephridial organ, which is probably what Fauvel (1897) named 'ventral glandules' plus nephridia, and paired masses of tissue at both sides of the ventral mesentery, which could, in turn, be developing gonads.

The circulatory system in Ampharetidae was studied by Wirén (1885), Meyer (1887) and Fauvel (1897); it is complete and closed, with a major dorsal longitudinal vessel connected by transverse vessels and lacunae. A heart body associated with the dorsal blood vessel has been documented by Kennedy & Dales (1958); this is barely seen in the transversal 3D reconstructions, but the plug of tissue almost occluding the lumen of the vessel at supra-oesophageal level can be seen more clearly in the oblique images presented in Figure 14A–B. Langerhans (1881) and later Fauvel (1897) described the close relationship of the digestive and circulatory systems for, respectively, *A. minuta* and *A. grubei*. Langerhans (1881) described the anterior pharyngeal cavity (i.e. buccal cavity) and its connection with the midgut at about TC5 (Fig. 20M) and a wide circulatory, red-coloured sinus surrounding this part of the gut up to the end of the thorax, which also envelops a digestive branch arising mid-dorsally from this region, and then connecting with the basal part of branchiae as blood vessels. This was both observed in live specimens of *A. santillani* sp. nov. (Fig. 11A–C) and in micro-CT images (Figs 5C, D, 12D, E, 13A, 14A, B).

A pair of nephridial ducts located ventrally in anterior thoracic segments (see fig. 11 in plate XVI and figs 100–102 in plate XXII) were identified by Fauvel (1897). Curiously, this conspicuous segmental organ was not well observed in the specimens studied under the micro-CT, but it was clearly observed in the specimen studied with traditional histology techniques, documenting their internal organization in two separate spaces (Fig. 15C).

Epibiosis

Few studies have reported the the presence of peritrich ciliates in polychaetes although they are ubiquitous symbionts with other invertebrates

Table 2. Kimura two-parameter (K2P) distances between and within selected species of *Ampharete*. N/A, not applicable

	Between species									Within species
		1	2	3	4	5	6	7	8	9	
1	*A. acutifrons*										0.071
2	*A. falcata*	0.189									0.001
3	*A. finmarchica*	0.173	0.178								0.005
4	*A. labrops*	0.191	0.188	0.170							0.005
5	*A. lindstroemi*	0.214	0.209	0.219	0.205						0
6	*A. manriquei*	0.197	0.187	0.200	0.196	0.204					N/A
7	*A. octocirrata*	0.200	0.181	0.194	0.193	0.202	0.033				0.003
8	***A. santillani* sp. nov.**	0.200	0.216	0.198	0.209	0.153	0.178	0.179			0.005
9	*A. undecima*	0.195	0.169	0.176	0.173	0.189	0.169	0.164	0.178		0
10	*Anobothrus gracilis*	0.214	0.187	0.198	0.192	0.227	0.148	0.152	0.210	0.184	N/A

Table 3. Type locality and morphological characteristics for species of *Ampharete* occurring in NE Atlantic waters

Species	Reference	Type locality, region	Length, maximum (mm)	Number of TU	Number of AU	Prostomial eyes	Pygidial eyes	Branchial gap
A. acutifrons (Grube, 1860)	Holthe (1986), T.A. and J.A.K. pers. observ.	East Greenland	80	12	12	1 pair	–	Yes (1–2)
A. baltica Eliason, 1955	Holthe (1986), T.A. and J.A.K. pers. observ.	The Baltic Sea	18	12	12	1 pair	–	Yes (1)
A. borealis (M. Sars, 1856)	Holthe (1986), T.A. and J.A.K. pers. observ.	Northern Norway	50	11	12	1 pair	–	Yes (4)
A. falcata Eliason, 1955	Holthe (1986), T.A. and J.A.K. pers. observ.	Bohuslän, Sweden	18	12	12	1 pair	–	Yes (6)
A. finmarchica (M. Sars, 1865)	Holthe (1986), T.A. and J.A.K. pers. observ.	Troms, Norway	50	12	13	1 pair	–	No
A. goesi (Malmgren, 1866)	Holthe (1986), T.A. and J.A.K. pers. observ.	Spitsbergen, Svalbard	50	12	17	1 pair	–	Yes (1)
A. lindstroemi Malmgren in Hessle, 1917	Holthe (1986), T.A. and J.A.K. pers. observ.	Bohuslän, Sweden	12	12	12	1–3 pairs	1–2 pairs	Yes (1–2)
A. octocirrata (Sars, 1835)	Holthe (1986), T.A. and J.A.K. pers. observ.	W Norway	13	11	15–18	1 pair	–	Yes (4)
A. petersenae Jirkov, 1997	Parapar *et al.* (2012)	E Iceland, shelf	11	12	16	–	–	No
***A. santillani* sp. nov.**	This study	Galicia, NW Spain	21	12	12–13	1 pair	–	Yes (1–2)
A. undecima Alvestad *et al.*, 2014	Alvestad *et al.* (2014), T.A. and J.A.K. pers. observ.	Norwegian Sea	5	12	11	–	–	No
A. vega (Wirén, 1883)	Holthe (1986)	Bering Sea	50	12	26	1 pair	–	No
A. villenai Parapar *et al.*, 2012	Parapar *et al.* (2012)	S Iceland, slope	12	12	12	–	–	No

Table 3. *Continued*

Species	Paleae, number on each side	Paleae, shape	Neuropodial dorsal cirrus, distribution and shape	Pygidial lateral cirri	Pygidial papillae	Other characteristics
A. acutifrons (Grube, 1860)	10–30	Long, thin, gradually tapering to long filiform tip	Last 2 TU and all AU with long cirrus	Long	Long cirriform	
A. baltica Eliason, 1955	6–10	Long, thin, gradually tapering to long filiform tip	Posterior AU with minute rounded lobes	Long	Long cirriform	
A. borealis (M. Sars, 1856)	< 10	Short, thin, gradually tapering to filiform tip	First 2 AU with short and remaining with long cirrus	Long	Small rounded	
A. falcata Eliason, 1955	6–8	Short, thin, gradually tapering to filiform tip	Anterior 3–4 AU with large rounded lobe	Long	Small rounded	
A. finmarchica (M. Sars, 1865)	12–16	Long, stout, gradually tapering to short curved tip	Posterior AU with minute rounded lobe	Long	Small rounded	
A. goesi (Malmgren, 1866)	10–23	Long, stout, gradually tapering to short curved tip	AU 3 and onwards with small cirrus	Long	Small rounded	
A. lindstroemi Malmgren in Hessle, 1917	7–10?	Long, thin, gradually tapering to long filiform tip	AU 3 and onwards with minute, rounded lobes	Long	Small rounded	
A. octocirrata (M. Sars, 1835)	< 10	Short, thin, gradually tapering to filiform tip	AU 3 and onwards with long cirrus	Long	Small rounded	Anterior end of tube thinner and curls shut when the worm retracts
A. petersenae Jirkov, 1997	0	–	AU 3 and onwards with long cirrus	Long	Short cirriform	
***A. santillani* sp. nov.**	9–12	Long, thin, gradually tapering to long filiform tip	AU 3 and onwards with very small cirrus	Long	Small rounded	Tube more or less quadrangular in cross section
A. undecima Alvestad *et al.*, 2014	9–12	Long, thin, gradually tapering to long filiform tip	Absent	Short	Small rounded	Branchiophores fused, forming high ridge
A. vega (Wirén, 1883)	Unknown	Long (details not known)	?	?	?	
A. villenai Parapar *et al.*, 2012	13–19	Long, stout, abruptly tapering to short curved tip	Absent	Absent	Absent	

Branchial gap: width as number of branchial base. AU, abdominal uncinigers; TU, thoracic uncinigers.

Table 4. Specimens included in the molecular analysis, with museum voucher number, BOLD/GenBank accession numbers, sampling location and reference to published sequences

Specimens	Voucher	BOLD/GenBank accession number	Location	Reference
A. acutifrons (Grube, 1860)	SCCWRP	CMBIA434-11	California, San Francisco Bay, 37.868°N, 122.641°W, 21 m	Not published
A. acutifrons (Grube, 1860)	SCCWRP	CMBIA437-11	California, San Francisco Bay, 37.868°N, 122.641°W, 21 m	Not published
A. acutifrons (Grube, 1860)	SCCWRP	CMBIA436-11	California, San Francisco Bay, 37.868°N, 122.641°W, 21 m	Not published
A. acutifrons (Grube, 1860)	SCCWRP	CMBIA438-11	California, San Francisco Bay, 37.868°N, 122.641°W, 21 m	Not published
A. acutifrons (Grube, 1860)	–	BENTH271-08/ HM473718	Alaska, N Bering Sea, 64.492°N, 170.441°W, 47 m	Carr *et al.* (2011)
A. falcata Eliason, 1955	ZMBN 89835	POLYNB011-13	Norway, Bergen area, 60.26910°N, 5.11570°E, 98 m	This study
A. falcata Eliason, 1955	ZMBN 89842	POLYNB018-13	Norway, Skagerrak, 58.3466°N, 8.9283°E, 250 m	This study
A. falcata Eliason, 1955	ZMBN 89911	POLYNB087-13	Norway, W coast, 62.27842°N, 5.45413°E, 170 m	This study
A. finmarchica (M. Sars, 1865)	SIO-BIC A1100	GB4387-13/JX423738	Norway, Svalbard, Hornsunddjupet, 76.77°N, 14.97°E, 291 m	Stiller *et al.* (2013)
A. finmarchica (M. Sars, 1865)	ZMBN 94828	POLYNB836-14	Norway, Barents Sea, 72.30983°N, 32.34133°E, 312 m	This study
A. finmarchica (M. Sars, 1865)	NTNU-VM 68245	PONOR080-13	Norway, Porsangerfjorden, 70.476°N, 25.397°E, 107 m	This study
A. labrops Hartman, 1961	–	BCPOL396-08/ HM473292	Canada, British Columbia, 48.831°N, 125.129°W, intertidal	Carr *et al.* (2011)
A. labrops Hartman, 1961	–	BCPOL391-08/ HM473290	Canada, British Columbia, 48.831°N, 125.129°W, intertidal	Carr *et al.* (2011)
A. labrops Hartman, 1961	–	BCPOL392-08/ HM473291	Canada, British Columbia, 48.831°N, 125.129°W, intertidal	Carr *et al.* (2011)
A. lindstroemi Malmgren in Hessle, 1917	ZMBN 89844	POLYNB020-13	Norway, Bergen area, 60.26910°N, 5.11570°E, 98 m	This study
A. lindstroemi Malmgren in Hessle, 1917	ZMBN 95899	POLYNB1486-15	Norway, SW coast, 59.02985°N, 5.44881°E, 58–60 m	This study
A. lindstroemi Malmgren in Hessle, 1917	ZMBN 89905	POLYNB081-13	Norway, Skagerrak, 57.9282°N, 9.2809°E, 247–290 m	This study
A. manriquei (Salazar-Vallejo, 1996)	–	CMBIA290-11	California, Huntington Beach, 33.573°N, 117.985°W, 57 m	Not published
A. octocirrata (M. Sars, 1835)	SIO-BIC A1109	GB4355-13/JX423770	Norway, Trondhjemsfjord, 63.52°N, 10.42°E, 90 m	Stiller *et al.* (2013)
A. octocirrata (M. Sars, 1835)	ZMBN 89909	POLYNB085-13	Norway, Skagerrak, 57.9282°N, 9.2809°E, 247–290 m	This study
A. octocirrata (M. Sars, 1835)	ZMBN 91772	POLYNB459-13	Norway, W coast, 62.36917°N, 4,46317°E, 193 m	This study
***A. santillani* sp. nov.**	ZMBN 98169	MIWAP532-15	Morocco, Atlantic, 31.6147°N, 9,7558°W, 36 m	This study
***A. santillani* sp. nov.**	ZMBN 98157	MIWAP520-15	Morocco, Atlantic, 33.6879°N, 7.6144°W, 55 m	This study

Table 4. *Continued*

Specimens	Voucher	BOLD/GenBank accession number	Location	Reference
***A. santillani* sp. nov.**	ZMBN 98164	MIWAP527-15	Morocco, Atlantic, 32.4725°N, 9,2744°W, 40 m	This study
***A. santillani* sp. nov.**	ZMBN 115540	MG230531 (J599B)	Spain, Ría de Ferrol, 43.47°N, 8.23°W, shallow subtidal	This study
***A. santillani* sp. nov.**	ZMBN 115541	MG230530 (J599)	Spain, Ría de Ferrol, 43.47°N, 8.23°W, shallow subtidal	This study
***A. santillani* sp. nov.**	ZMBN 115542	MG230532 (J600)	Spain, Ría de Ferrol, 43.47°N, 8.23°W, shallow subtidal	This study
A. undecima Alvestad *et al.*, 2014	ZMBN 104774	POLNB1781-15 (A12)	Norwegian Sea, 63.0372°N, 4,6890°E, 760–766 m	This study
A. undecima Alvestad *et al.*, 2014	SMF	ICBA076-16	Norwegian Sea, 62.27°N, 0.02°W, 846 m	This study
A. undecima Alvestad *et al.*, 2014	SMF	ICBA077-16	Iceland, Denmark Strait, 67.868°N, 23.696°W, 1281 m	This study
Anobothrus gracilis (Malmgren, 1866)	SIO-BIC A1106	JX423739	Norway, Trondhjemsfjord, 63.48°N, 10.37°E, 271–334 m	Stiller *et al.* (2013)

SCCWRP, Southern California Coastal Water Research Project; SIO-BIC, Scripps Institution of Oceanography, Benthic Invertebrate Collection; NTNU-VM, Department of Natural History, NTNU University Museum, Trondheim, Norway; ZMBN, Department of Natural History, University Museum of Bergen, Norway; SMF, Senckenberg Museum Frankfurt, Germany.

(Álvarez-Campos *et al.*, 2014). Knox & Hicks (1973), and Arias *et al.* (2010) found *Vorticella* sp. and *Epistylis* sp. on the onuphid polychaetes *Brevibrachium maculatum* (Estcourt, 1966) and *Diopatra marocensis* Paxton, Fadlaoui & Lechapt, 1995, respectively; Magagnini & Verni (1988) reported *Scyphidia* Dujardin, 1841 on the archiannelid *Nerilla antennata* Schmidt, 1848, and Álvarez-Campos *et al.* (2014) described three species of *Cothurnia* Ehrenberg, 1831 and *Rhabdostyla* Kent, 1881 on several species of Syllidae. However, epibionts have not previously been reported on any Ampharetid species, although a large number of gregarines have been reported in the coelom of several species of the genus *Melinna* Malmgren, 1866 (Hutchings, 2000).

These epibionts mainly occur close to ciliated areas on the new species, usually near the anterior end (branchiae and dorsal postbranchial area) that is partially out of the tube. This suggests that the symbiont may take advantage of the water currents created by the polychaete ciliature for feeding. No damage was observed on the polychaete body surface, and therefore, this association is regarded as ectocommensalism, as Magagnini & Verni (1988) observed in the case of *Scyphidia–N. antennata* (see above).

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