Marine Biodiversity

Saccocirridae (Annelida) from the Canary Islands with descriptions of a new species
--Manuscript Draft--

Manuscript Number:	MARB-D-19-00045
Full Title:	Saccocirridae (Annelida) from the Canary Islands with descriptions of a new species
Article Type:	S.I.: MeioLanza
Keywords:	taxonomy; interstitial; sandy beaches; waves; microscopy; DNA
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Abstract:	Two species of Saccocirrus, S. slateri sp. nov., and S. parvus Gerlach 1953, are recorded from samples collected in nine sandy beaches throughout the Canary Islands. Detailed descriptions combining live observations with light and electron scanning microscopical analyses are provided for each species; as well as an updated molecular phylogeny of the genus including all described European species. Saccocirrus slateri sp. nov. was found in sediments exposed to strong wave action in beaches and peers at two islands, Tenerife and La Palma; while S. parvus was found in three islands: Tenerife, La Palma, and Lanzarote, in more sheltered subtidal environments. Saccocirrus slateri sp. nov. resembles the European S. papillocercus, but it differs in possessing a longer trunk, more segments, and hooked chaetae. Saccocirrus parvus from Canary Island fits the description of S. parvus from the type locality, but it differs in the presence of unequal prongs in the longest chaeta, and minor details regarding the arrangement of the gonads. These differences are considered as intraspecific variation given that the available DNA sequences are identical. The description of S. slateri sp. nov. increases the number of species of Saccocirridae to 23. We discuss the habitat preferences of the species of Saccocirrus in the light of this newly available information.

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	Marco Curini-Galleti curini@uniss.it Very experienced zoologist, expert in Proseriats, with a long period of experience in interstitial environments.

Dear Lena Menzel,

Please find enclosed a manuscript prepared for publication in the Meiofauna Lanzarote special issue of Marine Biodiversity, entitled "**Saccocirridae (Annelida) from the Canary Islands with descriptions of a new species**".

The manuscript presents detailed morphological and molecular description of a new species, *Saccocirrus slateri* sp. nov., honoring after a famous surfer Kelly Slater. We tried to give audience for this group of animals since they live closed related to the sandy beaches environment, and many of the morphological adaptations, and behavior that distinguish this sandy-beach animals results from instability of the substratum coupled with wave action. Further, they have been reported ingesting microfibres (Gusmão et al., 2016; Environl Pol, 216:584-590). Since the interstitial organisms are key components of marine trophic web and their predation may cause a trophic transfer of microfibres and microplastics for higher trophic levels, we try to highlight the utility of this group as a marine sentinel. We hope it will give a broader audience for the saccocirrids. In case it is suitable for the Marine Biodiversity scope, and if accepted we will formally contact the social media for the divulgation.

We also include morphological and molecular data of *Saccocirrus parvus*, previously described from Italy, and reported at France and Tunisia. It is the first record of this species in the Atlantic Ocean. We discussed that the occurrence of several populations of *S. parvus* along the Mediterranean sea, and now in the Atlantic Ocean highlighting the habitat preferences of Saccocirridae

The submitted manuscript consists of a body text, 1 tables, and 6 figures. The figures are prepared as EPS, JPG (photo plates) or TIFF (line art), and vectored high-resolution files of each figure are available if needed.

If the manuscript can be considered for publication, I would suggest that the editor choses among following potential referees:

Hiroshi Kajihara, Hokkaido University, kajihara@eis.hokudai.ac.jp
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Very experienced zoologist, expert in Proseriats, with a long period of experience in interstitial environments.

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Sincerely,
Maikon Di Domenico

Saccocirridae (Annelida) from the Canary Islands with descriptions of a new species

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Abstract

Two species of *Saccocirrus*, *S. slateri* sp. nov., and *S. parvus* Gerlach 1953, are recorded from samples collected in nine sandy beaches throughout the Canary Islands. Detailed descriptions combining live observations with light and electron scanning microscopical analyses are provided for each species; as well as an updated molecular phylogeny of the genus including all described European species. *Saccocirrus slateri* sp. nov. was found in sediments exposed to strong wave action in beaches and peers at two islands, Tenerife and La Palma; while *S. parvus* was found in three islands: Tenerife, La Palma, and Lanzarote, in more sheltered subtidal environments. *Saccocirrus slateri* sp. nov. resembles the European *S. papillocercus*, but it differs in possessing a longer trunk, more segments, and hooked chaetae. *Saccocirrus parvus* from Canary Island fits the description of *S. parvus* from the type locality, but it differs in the presence of unequal prongs in the longest chaeta, and minor details regarding the arrangement of the gonads. These differences are considered as intraspecific variation given that the available DNA sequences are identical. The description of *S. slateri* sp. nov. increases the number of species of Saccocirridae to 23. We discuss the habitat preferences of the species of *Saccocirrus* in the light of this newly available information.

Keywords: Taxonomy, interstitial, sandy beaches, meiofauna, microscopy, DNA

Saccocirrus slateri sp. nov. is registered in Zoobank under: 95ACD363-37AD-4582-90D0-464AB3AA8B81

Introduction

Saccocirridae is a relatively small family of interstitial annelids common in shallow water marine sediment (Westheide 2008). The 22 species known from the family have been traditionally divided in two groups, "papillocercus" and "krusadensis", mainly based on the presence or absence of a ventral pharynx, as well as different ciliary patterns and chaetal types (Brown 1981). These groups have been largely recognized in the literature (Brown 1981; Jouin and Gambi 2007), but only recently phylogenetic analyses revealed that they actually constitute monophyletic clades (Di Domenico et al. 2014) leading to the formal definition of two respective genera, *Saccocirrus* Bobretzky 1872 and *Pharyngocirrus* Di Domenico, Martínez, Lana & Worsaae 2014. The position of Saccocirridae within Annelida has been controversial. The group was placed amongst, or closely related to, the archiannelids by early workers (see review by Hermans (1969)). This controversial phylogenetic position motivated detailed morphological investigations on the family (Eakin et al. 1977; Martin 1978; Sasaki and Brown 1983; Purschke and Jouin 1988; Purschke 1990; Purschke 1992), which in overall suggested a close relationship between Saccocirridae and the families Protodrilidae and Protodriloididae (Purschke 1988). This relationships was recovered by extensive cladistics studies (Rouse and Pleijel 2001), and more recently by phylogenomic analyses (Andrade et al. 2015; Struck et al. 2015; Helm et al. 2018).

Saccocirridae is known from all over the world. However, most of the European records have been attributed to *Saccocirrus papillocercus* Bobretzky, 1872, and until recently all the species of the "krusadensis" group were known solely from Brazil or the Indopacific (Purschke and Jouin 1988; Bailey-Brock et al. 2003; Jouin and Gambi 2007). However, soon after the description of the first *Pharyngocirrus* species in the Mediterranean (Jouin and Gambi 2007), more extensive sampling confirmed that both clades occur in all temperate and tropical oceans with the highest diversity around the equator (Di Domenico et al. 2014b). Despite their geographically overlap, the two genera rarely occur sympatrically, with species of *Pharyngocirrus* generally inhabiting sheltered sandy habitats, and members of *Saccocirrus* being more common in reflective sandy beaches (Di Domenico et al. 2014a, 2014b). However, these apparent differences in habitat preferences across each genus remain to be tested explicitly.

Until now, four species of saccocirrids are known from the Eastern Atlantic and the Mediterranean Sea (Westheide, 2008). *Saccocirrus papillocercus* is recorded from the Black Sea (Bobretzky 1868; Bobretzky 1870; Repiachoff 1881; Salensky 1907; Gusjewa 1929; Mastepanova, 2004; Surugiu, 2006), the Mediterranean Sea (Pierantoni 1906a; Pierantoni 1906b; Magagnini 1980; Villora-Moreno 1997; Jouin and Gambi, 2007), and the Atlantic Ocean (Langerhans 1880; Cabioch et al. 1968; Dauvin 1978; San Martin 1987; Núñez et al. 2005); whereas *Saccocirrus major* Pierantoni, 1907, *S. parvus* Gerlach, 1953 and *Pharyngocirrus goodrichi* Jouin-Toulmond & Gambi, 2007 are exclusively known from the Mediterranean Sea (Pierantoni 1907; Jouin and Gambi, 2007). Despite the family is commonly found in the Canary Islands (Gusmão et al. 2016; Riera et al. 2017), its diversity in the archipelago remains uninvestigated and so far only *Saccocirrus papillocerus* has been reported from the area (Núñez et al. 2005).

The goal of this study is to formally describe one species recovered from the Canary Islands during recent phylogenetic studies on the family (Di Domenico et al. 2014b), and include new information about

Saccocirrus parvus. We include new material collected during the I International Workshop to Marine and Anchialine Ecosystems, Lanzarote in November 2011. *Saccocirrus slateri* sp. nov. is described, according to the available material, combining light and scanning electron microscopy observations, as well as DNA sequences. Furthermore, we discuss the functional morphology of *Saccocirrus* and adaptations to occur in instable substratum coupled with wave action, highlighting their mobility and ability to deal with the surf and swash environment.

Material and methods

Sample collecting and processing

A total of nine sandy beach localities were sampled along the Canary Islands (Fig. 1), including Los Abades, Barranquilla, Punta del Hidalgo, and Punta Sama in Tenerife; Charco Verde in La Palma; Las Canteras in Gran Canaria; and Charco del Palo, Mala in Lanzarote. La Barranquilla, Punta Sama, Punta del Hidalgo, Charco Verde, Los Cancajos and El Golfo are exposed sandy beaches, with volcanic sand. Los Abades is a small semi-exposed beach serving as a fishermen harbor. Las Canteras beach is an organogen sandy beach, semi-exposed and protected by a fossil reef. Charco del Palo is an exposed rocky shore coast with sandy patches.

Sediment samples were collected manually at the intertidal or by SCUBA diving from subtidal environments. Animals were extracted using the $MgCl_2$ decantation technique through a 63-μm mesh (Higgins and Thiel 1988). Live animals were sorted and photographed in the field using a Nikon D300 (NIKON, Tokyo, Japan), mounted on an Olympus SZX16 stereomicroscope. After again relaxing the animals with isotonic $MgCl_2$, fixations were done in 2% glutaraldehyde in cacodylate buffer (24 h, subsequently transferred to 0.1 M cacodylate buffer) or 2% paraformaldehyde in PBS with 0.1 M sucrose (24h at 4ºC, subsequently rinsed in PBS and stored with 0.2 M sucrose and 0.01 NaN3).

Morphological examinations

Morphological examinations were done using light microscopy (LM) and scanning electron microscopy (SEM).

Light microscopy observations were performed on whole mounted specimens prepared in glycerin following a dilution series. Measurements and photographs were taken using an OLYMPUS DP73 camera mounted on an Olympus IX70 inverted compound microscope (OLYMPUS, Tokyo, Japan), equipped with CellSens Entry v.1.9 software.

Scanning electron microscopy examinations were preferably done on glutaraldehyde fixed specimens. After 60 min post-fixation in 1% osmium tetroxide (in 0.1 M cacodylate buffer), specimens were rinsed in demineralized water, dehydrated through a graded ethanol series, transferred to 100% acetone and critical-point dried. Dried specimens were mounted on aluminum stubs, sputter-coated with platinum and examined with a JEOL JSM-6335F field emission scanning electron microscope at the Natural History Museum of Denmark, University of Copenhagen.

DNA extraction and amplification

Specimens used for DNA extraction were preserved in 100% ethanol. DNA extractions were performed on single individuals using a Qiagen DNeasy Tissue and Blood kit following protocols provided by manufacturer. Approximately 600 base pairs of the 16S ribosomal RNA (16S rRNA), 650 base pairs of the cytochrome subunit I (COI) and 1750 of the 18S ribosomal RNA (18S rRNA) were amplified using universal primers (see Di Domenico et al. 2014). These markers were selected because 16S and COI are widely recognized as useful markers for species delimitation (Sekar et al. 2016; Silva et al. 2017), whereas 18S is the most widely use marker in high throughput sequencing studies, and therefore it is useful for OTUs identification (Fonseca et al. 2014). Polymerase chain reactions (PCR) were performed following the manufacturer's protocol for the Illustra PuReTaq Ready-To-Go PCR Beads, including 2 µl of template DNA and 1 µM of each primer. PCR reactions were carried out using a Bio-Rad S1000 Thermal Cycler. PCR protocol involved a 2-min initial denaturalization at 96ºC, followed by 40-45 cycles consisting of a denaturation step (94ºC, 30 s), annealing (38-55 ºC, 30 s), and extension (72ºC, 1 min), ending with a final extension at 72ºC for 7 min. PCR products were resolved on a E-Gel 2% SYBR Safe agarose gels (Invitrogen) and purified with E.Z.N.A. Cycle Pure kit. Purified products were sent to Macrogen Europe Lab for sequencing. Sequences were assembled with Sequencer 4.10.1 (GeneCodes Corporation, Ann Arbor, MI, USA) and submitted to GenBank (GenBank accession numbers included in each species description).

Alignment and phylogenetic analyses

We based our phylogeny on the dataset on Di Domenico et al. (2014), which we updated in the current study by including *S. minor* and *S. major.* Therefore, our phylogeny included 10 species of *Saccocirrus* with all Atlanto-Mediterranean species available for molecular comparison. The species *Pharyngocirrus sonomacus*, *Pharyngocirrus gabriellae*, *Pharyngocirrus jouinae* was selected as outgroup (see Di Domenico et al. 2014).

Sequences for each gene were visualized using Genious ® 7.1.9. and aligned using the MAFFT online platform (Katoh et al. 2010). Protein coding H3 was translated into amino acids and checked for indels and stop codons. We selected interactive refinement algorithm Q-INS-I (Katoh and Toh 2008) for alignments of 16S rRNA, 18S rRNA and 28S rRNA. The option 'nwildcard' was selected for in both cases as it does not designate missing data as gaps. Gene fragments H3 was constant in length and therefore trivial as sequences showed no variation in length, but we checked for directionality using the quick interactive refinement algorithm L-INS-I (Katoh et al. 2005). Individual gene datasets were concatenated using Genious ® 7.1.9.

Concatenated molecular datasets were analyzed using maximum likelihood (ML) and Bayesian inference (BI) methods. Maximum likelihood (ML) partitioned analyses were conducted using with RAxML version 7.2.8 (Stamatakis 2006). A default general time reversible, with corrections for a discrete gamma distribution (GTR + G) were specified for each partition. Nodal support was estimated via non-parametric bootstrap (Felsenstein 1985), with 1000 replicates and a GTR + G model. Bayesian analyses (BA) were performed using MrBayes version 3.2.5 (Ronquist and Huesenbeck 2003). Prior to the analyses, jModelTest (Posada 2008) was used to infer the optimal evolutionary model for each gene, which was selected using

the corrected Akaike information criterion (AICc) (Posada and Buckley 2004). A GTR+G and a proportion of invariable sites (GTR + I + G) was selected for 18S rRNA, 28S rRNA, 16S rRNA, and H3.

Additional material for comparison.

The types material from the following species examined as comparative material: *Saccocirrus alanhongi* Bailey-Brock, Dreyer and Brock, 2003 (USNM POLY 1012494 - 1012497); *S. oahuensis* Bailey-Brock, Dreyer and Brock, 2003 (USNM POLY 1012490 – 1012491); *S. waianaensis* Bailey-Brock, Dreyer and Brock, 2003 (USNM POLY 1012492 – 1012493); *S. pussicus* Du Bois-Reymond Marcus, 1948 (ZUEC-Pol 14069- 14099; ZMUC-Pol 2299); *Pharyngocirrus eroticus* Gray, 1969 (USNM POLY 36064 – 36066); *P. tridentiger* Brown, 1981 (USNM POLY 62034- 62040); *P. jouinae* Brown 1981 (USNM POLY 62027 – 62033)*P. sonomacus* Martin, 1977 (USNM POLY 53050- 53052); *P. gabriellae* Marcus, 1946 (ZUEC-Pol 14053 - 14063).

Additionally, we examined newly collected specimens of *Saccocirrus pussicus* Marcus, 1948 from Uruguay (Rodríguez et al. 2013) ; *P. gabriellae* from Colombia (Lagos *et al.* 2018), *P. sonomacus* Martin, 1977 from California, USA; *P. tridentiger* Brown, 1981 and *P. jouinae* Brown, 1981 from New South Wales, Australia; *P. krusadensis* Alikunhi, 1942, from New South Wales, Australia and Phuket, Thailand; and *S. papillocercus* Bobretzky, 1871 from Black Sea and Gulf of Napoli; as well as *S. major, S. parvus*, and *P. goodrichi* from Italy.

Results

Phylogenetic analyses

Bayesian consensus and maximum likelihood trees are congruent, except for the position of *Saccocirrus pussicus* (Fig. 2). All the species of *Saccocirrus,* recovered as a maximally supported monophylum (Maximum likelihood bootstrap, MLB=100; Bayesian posterior probability, BPP =1) branched off as two sister clades. Clade A (MLB =100, BPP = 0.99) included undescribed *Saccocirrus* sp. (France), *Saccocirrus* sp. 8 (Bermuda), *Saccocirrus* sp. 9 (Belize), as well as *Saccocirrus major*; while clade B (MLB = 100, BPP = 1) consisted of undescribed *Saccocirrus* sp. 10 from Mono Island sister to a well supported clade (MLB = 100, BPP = 1) with *S. slateri* sp. nov., *S. papillocercus*, *S. parvus*, and *S. pussicus. Saccocirrus slateri* sp. nov. was consistently recovered sister to *S. papillocercus* (MLB= 93, BPP=0.53); while all the sequences included for *Saccocirrus parvus* from the Canary Island and Italy were identical.

Systematic account

Genus *Saccocirrus* Bobretzky, 1872

Saccocirrus slateri sp. nov.

Figs. 3-4, Table 1

Saccocirrus sp. 1 (Di Domenico et al. 2014b; Gusmão et al. 2016)

Type material. Holotype (ZMUC-Pol 2309). 21 mm long female. Los Abades, Tenerife. Intertidal gravel at the ramp of the small fishermen harbor, 28°9'50.48"N, 16°25'54.14"W. Col: M. Di Domenico and A. Martínez. 22 December 2010. Paratypes: seven whole mounted specimens (MZUSP 3124 - 3130, same locality and date as the holotype; DNA information: 16S (GenBank Acc. KF954445), 18S (GenBank Acc. KF954467).

Additional material. Two whole mounted females (MZUSP 3131 - 3132), Charco Verde, La Palma. Exposed sandy beach, with volcanic sand, 0-1 m depth, 28°34'19.39"N, 17°53'58.63"W. 14 October 2011. and four specimens on SEM stubs (Worsaae's lab scientific collection), same locality and date as the holotype.

Etymology. Species named in honor of surfer Kelly Slater, who has been crowned World Surf League Champion 11 times. The genus *Saccocirrus* is often found at exposed sandy beaches, where they are able to cope with the turbulence produced by the waves by moving horizontally along the beach slope.

Diagnosis. Body greyish-brown with dark pigmentation along the gut. Trunk robust, with 100-155 segments, last 7 smaller and achaetous. Prostomium rounded with two pigmented eyes and long filiform palps. Palp ampullae expended posteriorly to segment 3. Ventral muscular pharynx absent. Parapodia with three types of chaetae: (1) 1-2 long chaetae, robust and forked with equal prongs; (2) 2-3 medium spatulated chaetae, with 5-6 denticles; and (3) 2-3 short simple chaetae, with notched apex. Paired pygidial lobes with 11-22 transverse adhesive ridges. Females with bilateral ovaries in segments 24-93 (along 65 segments), each with 10-14 large oocytes. Pyriform spermathecae present. Males with bilateral seminal vesicles in segments 22-72 (along 50 segments), with paired hooked penises in segments 46-52.

Description. (Measurements provided from holotype; ranges from adult paratypes in parentheses). Body long, very robust and dark greyish-brown (Fig. 3A, E), 21 mm long (18-25 mm, n=8) and 695 μm wide (460-730 μm, n=80), up to 117 segments (105-155, n=8). Last 7 segments (5-7 segments in adults, n=8) smaller and achaetous.

Prostomium rounded, 150 μm long (100-200 μm, n=8) and 200 μm wide (150-200 μm, n=3), with two dorsal pigmented eyes (Fig. 3B), 30 μm in diameter (20-35 μm, n=2). Paired annulated palps, 2740 μm long (1850-2980 μm, n=8), reaching to segment 12. Palp with internal channels connected to oval ampullae, 210 μm long (200-240 μm, n=6), extending posteriorly along peristomium until segment 3. Palp surface without motile cilia but with scattered non-motile ciliary tufts (Fig. 4A, D). Paired oval ciliated nuchal organs, extending dorsally between the prostomium and peristomium (no, Figs 3B, 4B), 30 μm long (25-35 μm, n=3).

Peristomium 200 μm long (200-300, n=3) and 250 μm wide (250-400, n=3), with a ventral slit-shaped mouth (mo, Figs 3C, 4D). Mouth cavity continues into glandular esophagus, extending along three trunk segments (Fig. 2F). Ventral muscular pharynx absent (Figs 3B, 4D).

Paired cylindrical retractile parapodia projecting laterally on each trunk segment, except the last 7. Each parapodium up to 10 μm long (10-15 μm, n=2) (pp, Figs 3I, 4C, G), uniramous, and with three types of chaetae. (1) 1-2 long forked chaetae (lc, Figs 3I, 4G, J), terminally bifurcated, 2 μm wide at the tip (n=3)

and twice times longer than the medium chaetae, with two short, equal, terminal prongs (pg, Fig. 4J), with a denticulated area between them (dt, Fig. 4J), (2) 2-3 spatulated chaetae (mc, Figs 3I, 4G-H), 5 μm wide terminally (n=2) with two thick lateral teeth (te, Fig. 4H) separated by a thinner area provided with 4-6 smaller denticles (dt, Fig. 4H). (3) 2-4 simple short chaetae (ss, Fig. 4G, I), thin (1μm wide, n=2) notched terminally (arrow head, Fig. 4I). Special penis structure called as uncini *sensu* Jouin (1975), 40 μm long (n=1), 10 μm wide (n=1) (hp, Fig 4J, L).

Epidermal ciliation absent except for few scattered ciliary tufts on peristomium (Fig. 4F), more abundant on the peristomium (Fig. 4B, D). A conspicuous ciliary band with abundant cuticular pores, presumably representing adhesive glands, present in each trunk segment (sg, Fig. 4K). Although these pores presumably connect to subepidermal glands, but these glands could not be observed with light microscopy.

Pygidium with two robust lobes (pyl, Figs 3M, 4K), 250 μm long (200-300 μm, n=6) and 200 μm wide (180-250 μm, n=3), each with 11-22 ventral adhesive ridges (ag, Figs 3M, 4K). Anus opening terminally between pygidial lobes, without distinct ciliation (an, Fig. 3M).

Females with ca. 70 fertile segments (max. 72, n=4) from segment 25 (24-26, n=4) to 97 (79-99, n=4). Each fertile segment with 10-14 mature oocytes (oo, Fig. 3D, G, K), 140-180 μm in diameter (110-195 μm, n=4), at both sides of the gut. Fertile segments with a pair of ventral gonoducts (gc, 4C, 4E). Spermathecae present from segment 60-67 to segment 100-104 (se, Fig. 3D, K), all piriform, ca. 200 μm long (150-200 μm, n=2). Males with 50 fertile segments (segments 22 to 72 (n=1)). Paired circular seminal vesicles (sv, Fig. 4J,), 300 μm in diameter, at each side of gut; with terminal hooked-shaped penis (hp, Fig. 4J, L).

Distribution and ecology. The new species was collected in volcanic coarse sand at exposed reflective beaches and gravelly cinders at the slope of a little fishermen harbor. Accompanying fauna included the protodrilid annelids *Lindrilus* sp. and *Claudrilus* cf. *hypoleucus* (in Martínez et al. 2015), as well as tricladid flatworm *Procerodes* sp., several species of proseriate flatworms, nemertean *Ototyphlonemertes* sp. and the amphipod *Ingolfiella* cf. *canariensis* Vonk & Sánchez, 1991 (Vonk and Sánchez 1991). *S. slateri* sp. nov. is omnivorous non-selective feeder, as unicellular algae, pieces of animals, organic debris, as well as microfibers (probably microplastic) has been found in the stomach of several individuals (Gusmão et al. 2016)

Taxonomical remarks. *Saccocirrus slateri* sp. nov. and *S. heterochaetus* are the only species of *Saccocirrus* described with hooked-shaped penis. However, they differ in the presence of longer pygidial lobe with up to 22 pygidial papillae, absent in *S. heterochaetus*, and the longest chaeta with equal prongs length, unequal in *S. heterochaetus*. *Saccocirrus slateri* sp. nov. morphologically resembles *S. papillocercus, S. minor, S. major* and *S. orientalis* in the presence of forked chaetae with equal prongs. It differs from *S. papillocercus* in the presence of fewer fertile segments, more pygidial papillae, and the presence of hooked-shape penis; *Saccocirrus slateri* sp. nov. differs from *S. major* by its smaller size, presence of fewer fertile segments, less oocytes per segment, and presence of teeth in the median chaetae, which are absent in *S. major*. It differs from *S. minor* and *S. orientalis* in the larger size, and shape of the pygidium. The differences with the remaining species of the family are summarized in Table 1.

Saccocirrus parvus **Gerlach, 1953**

Delamare-Deboutteville et al. (1954), Fize (1963), Gerlach (1953), Villora-Moreno et al. (1991), Villora-Moreno (1997) and Westheide (1972)

Figures 5-6, Table 1.

Type material. whole mounted, female,13 mm long (MZUSP 3133). Punta Sama, Tenerife. Patches of coarse pumitic sand amongst boulders, 2-3 m depth, 28°7'22.21"N, 16°27'29.64"W, 12 December 2007. Col: A. Martínez and G. González. *Paratypes:* Two whole mounted specimens, immature (MZUSP 3134 - 3135), same locality, date and collectors as the female. DNA information: 18S (GenBank Acc. MK604223).

Additional material. One whole mount (MZUSP 3136) Playa de las Canteras, Las Palmas de Gran Canaria. Coarse sand in a sheltered bay, 3-4 m depth, 28°8'38.56"N, 15°26'13.61"W. 8 May 2008. Col: A. Martínez and K. Worsaae. One whole mount (MZUSP 3137). 01 specimen mounted on SEM stubs (Worsaae's lab scientific collection). Charco del Palo, Mala, Lanzarote. 4-6 m depth. Coarse sand amongst rocks. 29°4'59.75"N, 13°26'58.32"W. 13 October 2011. Col: M. Curini-Galetti.

Diagnosis. Body brownish, 54-70 segments; last 5-10 smaller and achaetous. Prostomium rounded with two pigmented eyes, transverse ciliary band extends between palps and nuchal organs, long filiform palps. Ventral muscular pharynx absent. Parapodia with three types of chaetae: (1) 1-2 long chaetae, robust and forked with unequal prongs; (2) 2-3 medium long spatulated chaetae with 8-10 denticles; and (3) 2-3 short simple chaetae with notched apex. Paired pygidial lobes with 3-5 transverse adhesive ridges. Females with bilateral ovaries from segments 31 to 53 (along 22 segments), each with 5 to 6 large oocytes. Spermathecae absent.

Description. Body brownish (Fig. 5A), 12 mm long (5-13 mm, n=5) and 280 μm wide (210-270 μm, n=5), up to 70 segments (54-70, n=5). Last 7 segments (5-7 segments, n=5) smaller and achaetous.

Prostomium rounded, 100 μm long (90-110, n=5) and 150 μm wide (120-170, n=5) with two dorsal pigmented eyes (oc, Fig. 5A), 8 μm in diameter (4-10 μm, n=3). Short ciliary band extending transversally between palp insertion and nuchal organ (plc, Fig. 6H). Paired palps, annulated and hollow, 1300 μm long (720-1300 μm, n=5), extending to segment 7 (6-9, n=5) (pa, Fig. 5A). Palp ampullae oval, 20 μm long (n=1). Palp without motile cilia, but with scattered non-motile ciliary tufts on the palp surface (Fig. 6B, G). Paired nuchal organ dorsal, oval and ciliated (no, Fig. 6A), 20 μm long (n=1).

Peristomium 350 μm long (300-350 μm, n=2) and 150 μm wide (100-150 μm, n=2), with a ventral slit-shaped mouth. Ventral muscular pharynx absent (Fig. 5A).

Parapodia cylindrical and retractile, up to 30 μm long (n=1), projecting transversally from each trunk segment, absent on last 7 segments (5-7, n=5) (pp, Fig. 6C, E). All parapodia uniramous with three types of chaetae. (1) 1-2 long forked chaetae (lc, Fig. 6C-D), thin and bifurcated, 2 μm wide at the tip (n=2) and ca. twice longer than the medium chaetae; bearing two short, unequal, terminal prongs (pg, Fig. 6D) with a denticulated area between them (dt, Fig. 6D). (2) 2-3 spatulated medium chaetae (mc, Fig. 6C, E), 4 μm wide terminally (n=2) with 8-10 denticles (dt, Fig. 6C, E). (3) 2-3 simple short chaetae (ss, Fig. 6C, E), thin (2 μm wide, n=2) and terminally notched (arrowhead, Fig. 6E).

Epidermal ciliation absent, except for prostomial ciliary bands and few, small ciliary tufts, more abundant on the peristomium (Fig. 6B, H). A transverse band with abundant cuticular pores, presumably representing adhesive glands, present in each trunk segment (sg, Fig. 6B).

Pygidium with two short lobes (pyl, Fig. 6F), 100 μm long (60-100 μm, n=2) and 100 μm wide (100-120 μm, n=3), each with five (3-5, n=5) ventral adhesive ridges (ag, Fig. 6F). Anus opening terminally between pygidial lobes, without distinct ciliation (an, Figs 5C, 6F).

Females with ca. 22 fertile segments (n=1), from segment 31 (n=1) to 53 (n=1). Each fertile segment with 5-6 mature oocytes, 140-190 μm in diameter (n=1), at both sides of the gut. Spermathecae absent, pairs of gonoducts present in each fertile segment (gc, Fig. 6C). Males were not found.

Distribution and ecology. The *Saccocirrus parvus* from Canary Island has been collected in shallow water (depth 2-5 m) in semi-exposed well-sorted coarse sandy sediments. Fauna accompanying the new species included several species of annelids such as the nerillids *Trochonerilla* sp. *Mesonerilla* cf. luederitzi and *Nerillidium* sp., the psammodrilid *Psammodrilus* sp. (in Worsaae et al. 2018)*, and the protodrilids *Meiodrilus* sp. nov. 3 and *Claudrilus* cf. *hypoleucus* (in Martínez et al. 2015). Further details of the accompanying fauna in Lanzarote are provided in Martínez et al. (this issue). *Saccocirrus parvus* has also been found at several beaches in the Mediterranean, at France, and Tunisia (Gerlach 1953, Delamare-Deboutteville et al. 1954, Fize 1963, Westheide 1972, Villora-Moreno et al. 1991, Villora-Moreno 1997).

Taxonomical remarks. *Saccocirrus parvus* from Canary Island is genetically identical to *S. parvus* from Italy. Our specimens also fit well the original description of the species, although the showed some differences in the presence of more pygidial ridges (one in *S. parvus* from Italy, versus 3-5 in *S. parvus* from the Canary Islands), and unequal prongs in the longest chaeta (described as equal in *S. parvus*). Compared to other species of *Saccocirrus, S. parvus* from the Canary Island resembles *S. pussicus, S. heterochaetus,* and *S. minor* in size, number of segments and pygidial length size. However, *S. parvus* from Canary Island possesses 3-5 transverse adhesive ridges in the pygidial lobes (versus one in *S. pussicus*, *S. heterochaetus*, and *S. minor),* unequal prongs in the longest chaeta (equal in *S. pussicus*, and notched apex in *S. minor*). *Saccocirrus parvus* differs from *S. slateri* sp. nov. by the absence of spermathecae.

Discussion

Geographic distribution of Saccocirridae

Saccocirridae is a relatively species-poor family with 23 described species (Di Domenico et al. 2014a, b). The family was traditionally divided in two groups, so called "krusadensis" and "papillocercus", which were believed to be restricted to the Indopacific and Atlantic oceans respectively. However, recent phylogenetic analyses erected these groups as two separated genera, and showed that these patterns reflected previously sampling bias rather than real biogeographical patterns (Di Domenico 2014b). Today, both *Saccocirrus* and *Pharyngocirrus* are considered widespread in tropical and temperate seas (Di Domenico, 2014b)

In contrast to other interstitial annelids, species of Saccocirridae exhibit comparatively large distribution areas, probably facilitated to the presence of larvae (Curini-Galletti et al. 2012). Therefore, the discovery of *S. parvus* in our samples is not surprising, despite it is the first record of the species outside the Mediterranean. Large distribution areas have also been found in ongoing molecular studies on the genus *Pharyngocirrus.* Sequences of 16S rRNA gene fragments indicate that *P. gabriellae,* originally described from Brazil (MDD, unpublished data), might occur along the entire Atlantic and Pacific coasts of South America, as well as the Canary Islands (see Martínez, this issue; or Teodoro et al. in preparation). However, whether these high genetic similarities reflect the presence of gene flow among distant populations of saccocirrids, or are an artifact derived from the election of very conservative markers demands more exhaustive studies.

In contrast to these high molecular similarities, morphological traits seem to vary much more than previously reported (Brown 1981; Gambi & Jouin 2007). Species of saccocirrids are mainly diagnosed using the chaetal morphology, the arrangement of the gonads, and the shape and number of pygidial adhesive ridges (Brown 1981; Jouin-Toulmond and Gambi 2007). However, our results here, as well as the examination of type and newly collected material, suggested that these traits might vary more than previously expected. These variation could be linked to different developmental stages, which might affect the arrangement of the gonads or the size of the pygidium. If this is the case, this variation could potentially be understood by sampling the same localities at different times of the year to get complete developmental series (Sasaki & Brown 1983; Westheide 2008). However, variation in chaetal morphology is more difficult to explain in this way, since the shape of chaetae is determined by the temporal and spatial modification of the microvilli pattern of the chaetoblast during the chaetogenesis (Hausen 2005), and just seems more plastic than previously reported.

While these observations are preliminary, they collectively highlight the importance of an integrative approach in the future studies of the family, preferably including large number of specimens collected at different periods of the year, and combining morphological observations with several independent molecular markers. This approach has been already followed in several studies done in other groups of interstitial annelids, such as Protodrilidae (Di Domenico et al. 2013; Martínez et al. 2013), Nerillidae (Worsaae et al. this issue a,b), Psammodrilidae (Worsaae et al. 2018), and Parergodrilidae (Struck et al. 2017), which collectively suggests that the evolutionary histories of each lineage of these ancient lineages is unique, and more complex than any simple single general model can predict (Martínez et al 2015; Gonzalez et al. 2017; Ramey-Balcı et al. 2018; Worsaae et al. in review). This is not surprising, as this has also been showed to be the case in many other interstitial lineages of metazoans (Fontaneto 2011; Curini-Galletti et al. 2012; Jörger et al. 2014; Sánchez et al. 2016)

Habitat preferences of Saccocirridae

While both cosmopolitan, it has been proposed that each genus of Saccocirridae exhibit distinct habitat preferences with members of the *Pharyngocirrus* preferring sheltered sandy habitats, in contrast to the specialization of exposed sandy beaches showed by members of *Saccocirrus*. Although this hypothesis remains to be explicitly tested, it was congruent with the results obtained in the last phylogenetic analysis of the family (Di Domenico et al. 2014b).

These differences in habitat preferences seem to be related to the presence of different trophic guilds and morphological traits in each clade. Species of *Pharyngocirrus* are specialized to selectively graze on biofilms of bacteria-diatom using their muscular ventral pharyngeal bulbous and ventral ciliation around the mouth. In contrast, members of *Saccocirrus* are non-selective microphagous, as indicated by the presence of sand grains and even microfibers in the gut content of many specimens (d.B-R. Marcus, 1948; Di Domenico et al., 2009; Jouin and Gambi, 2007; Gusmão 2016). Since most of the species of *Saccocirrus* has been found in exposed beaches, where the high turbulence prevents the deposition of organic matter, it has been proposed that palps, rather than pharynx, play a more decisive role in feeding in these species (Di Domenico et al. 2014b, a). Since food is more difficult to locate and catch under turbulent conditions, palps might be essential for locating and reaching food particles in flow. High rates of ingestion, efficient digestion and assimilation, and subsequent energy conservation are common adaptation found in other animals dwelling in sandy beaches (McLachlan & Brown, 2017).

However, our findings provide new information on habitat preferences of species of Saccocirridae, suggesting that different species of *Saccocirrus* might be adapted to specific grain sizes and turbulence. These adaptations might involve changes in the number and position of adhesive glands, as well as body and palps size (Martin 1978; Domenico et al. 2014b). *Saccocirrus slateri* sp. nov. has been exclusively found in very exposed, coarse sediments. It is a large species with well-developed palps, two features found in other annelids adapted to this energetic environment. For example, the protodrilid *Protodrilus albicans* Jouin 1970 (Jouin 1970; Martínez et al. 2018) is one of the largest species in the genus and undulates its long body to swim back into the sediments after being suspended in the water column by turbulence; once in the bottom, it uses its ciliated palps to gather suspended particles of organic matter. *Saccocirrus slateri* sp. nov. does not swim, but instead curls around heavy sand grains, adhering to them using both pygidial and segmentally arranged adhesive glands (Di Domenico et al. 2014a). The large body of the species might therefore provide a better grip to the large particles integrating the coarse sediments that the species inhabits. In contrast, *Saccocirrus parvus* has been found in subtidal semi-exposed environment, a habitat resembling those where species of *Pharyngocirrus* are typically found. *Saccocirrus parvus* is also one of the smallest species in the genus, possessing comparatively short palps. Its small body more likely represent an evolutionary secondary reduction in size, given the position of *Saccocirrus parvus* in our phylogenies, consistently recovered nested among larger species of *Saccocirrus*. The available information suggests that this reduction might facilitate the exploration of smaller interstitial spaces found in the semi-exposed environments where the species was found, favoring the access to interstitially deposited organic matter (Westheide 1987). However, this remains speculative until more detailed behavioral studies and ecological data allow an explicit test for this hypothesis,

Extant sandy beach environments may be considered recent sedimentary deposits, dating only from the Holocene. Meanwhile, fossil records of organisms of the Annelida group date back to the beginning of the Cambrian Period (~ 535 m) (Perry et al., 2014). Considering the ancient evolutionary history of the interstitial annelids (Andrade et al., 2015; Struck at el. 2015), we hypothesize that sediments in the beach environments may have been conquered by *Saccocirrus* for a relatively short time (Di Domenico et al 2014a, b). Many of the morphological adaptations and behaviors that distinguish this sandy-beach animals may be driven by the instability of the substratum coupled with wave action (Di Domenico et al. 2013;

2014c; McLachlan & Defeo, 2017). Thus, burrowing behavior or adhesive glands to attach to the sand grains of high-energy sandy beaches may be essential to not be swept away by incoming waves and swash (Martin 1978; Di Domenico et al. 2014c).

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Sampling and field studies

All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements, if applicable.

Data availability

All data generated or analyzed during this study are included in this published article.

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Fig. 1. Map of study area showing sampled localities.

Fig. 2. Phylogenetic relationships of *Saccocirrus* based on four molecular markers. Tree topology is based on Bayesian analyses, whereas nodal values correspond to maximum likelihood bootstrap (MLB) followed by Bayesian posterior probabilities (BPP). *i* indicate incongruence between ML and BI trees topologies.

Fig. 3. *Saccocirrus slateri* sp. nov. light micrographs of male holotype **a** whole animal dorsal view; **b** anterior end in dorsal view; **c** details of the mouth; and paratypes showing **d** bilateral oocytes and spermatheca from a female; **e** glandular esophagus; **f** transition between esophagus and gut; **g** bilateral oocytes; **h** female ventral gonopore; **i** parapodium showing a bundle of chaeta; **j** male circular seminal vesicle and hooked-penis; **k** female oocytes and spermatheca; **l** male hooked-penis shape; **m** pygidium in dorsal view. ag, adhesive glands; bp, black pigmentation; oe, oesophagus; go, glandular oesophagus; gu, gut; hp, hoocked-penis; lc, longest chaeta; mc, medium chaeta; mo, mouth; no, nuchal organ; oc, oceli; oo, oocytes; pa, palp; pp, parapodium; pr, prostomium; py, pygidium; pyl, pygidium lobe; s, setae; sc, short chaeta; se, spermatheca; sv, seminal vesicle; tr, transition.

Fig. 4. *Saccocirrus slateri* sp. nov. scanning electron micrographs of **a** anterior end in lateral view; **b** prostomium and peristomium in dorsal view; **c** mid-body segments in lateroventral view; **d** mouth and prostomium in ventral view; **e** gonoducts in ventral view; **f** ciliary tufts on the epidermal ciliary; **g** parapodium showing a bundle of chaeta; **h** medium chaeta; **i** short chaeta; **j** long chaeta; **k** pygidium in dorsal view. ag, adhesive glands; dt, denticle; go, gonad opening; lc, longest chaeta; mc, medium chaeta; mo, mouth; no, nuchal organ; pa, palp; pg, prongs; pp, parapodium; pr, prostomium; py, pygidium; pyl, pygidium lobe; sc, short chaeta; sp, spatulated chaeta.

Fig. 5. *Saccocirrus parvus* light micrographs of fixed paratype **a** anterior end animal view; **b** chaetae; **c** pygidium in lateral view. lc, longest chaeta; pa, palp; oc, oceli; py, pygidium.

Fig. 6. *Saccocirrus parvus* scanning electron micrographs **a** anterior end in dorsal view; **b** mouth and prostomium, ventral view; **c** mid-body segments in ventral view showing the gonoducts; **d** parapodium showing a bundle of chaeta in lateral view; **e** olongest chaeta; **f** medium chaeta; **g** pygidium in ventral view; **h** palp ciliation; **i** patch of lateral ciliation on the prostomium. ag, adhesive glands; an, anus; dt, denticle; fo, forked chaeta; go, gonad opening; lc, longest chaeta; mc, medium chaeta; mo, mouth; no, nuchal organ; pa, palp; pg, prongs; plc, palp lateral ciliation; pp, parapodium; pr, prostomium; py, pygidium; pyl, pygidium lobe; s, setae; sc, short chaeta; sp, spatulated chaeta.
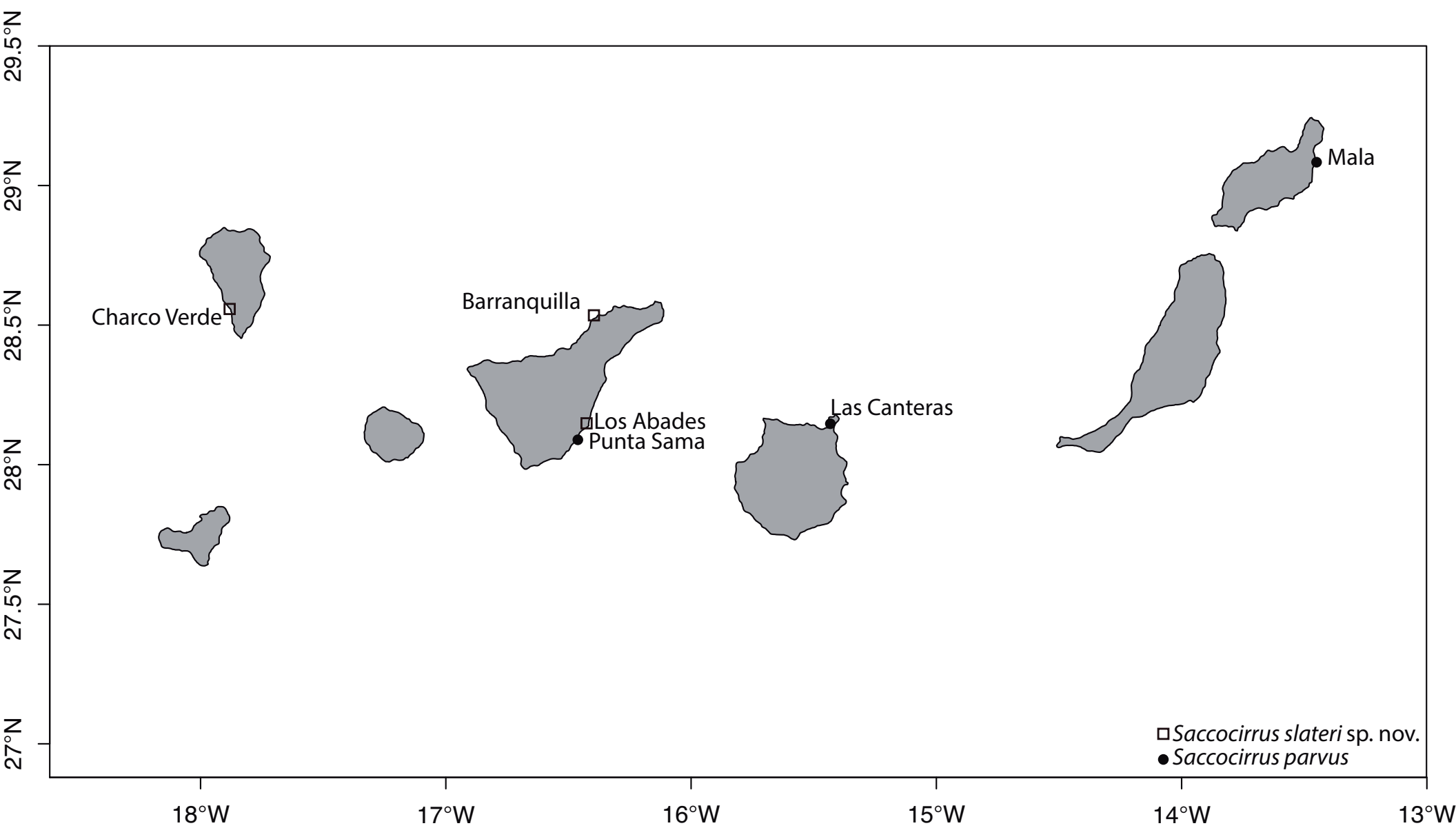
Figure 1

Figure 2

Pharyngocirrus sonomacus

Pharyngocirrus jouinae

Pharyngocirrus gabriellae

Saccocirrus sp. 10 (Mono Island)

Saccocirrus slateri sp. nov.

Saccocirrus papillocercus

Saccocirrus pussicus

Saccocirrus parvus (Italy)

Saccocirrus parvus (Canary Island)

Saccocirrus major

Saccocirrus sp. 9 (Belize)

Saccocirrus sp. (France)

Saccocirrus sp. 8 (Bermuda)

98/1

100/1

100/1

97/1

100/1

60i/0.6

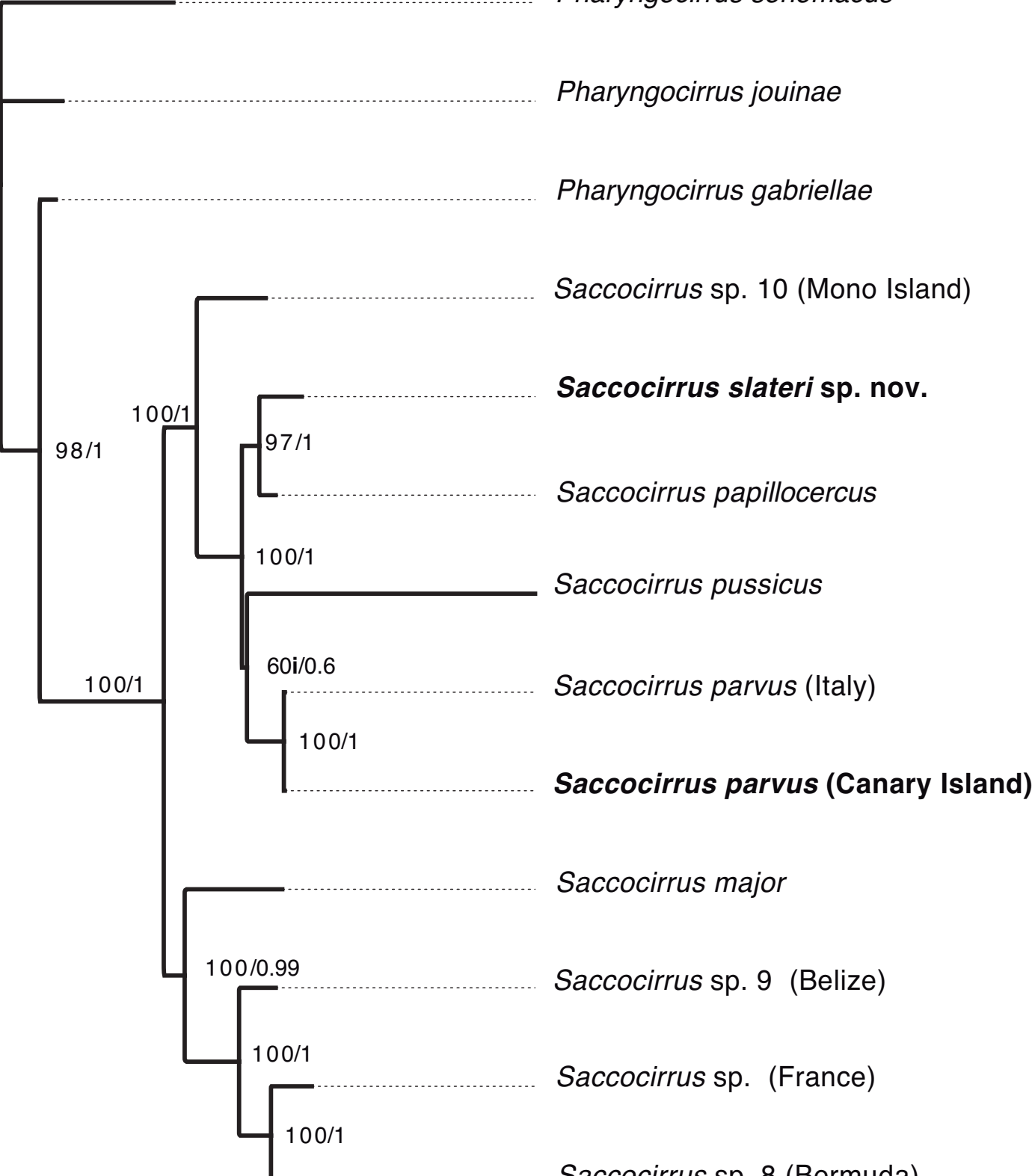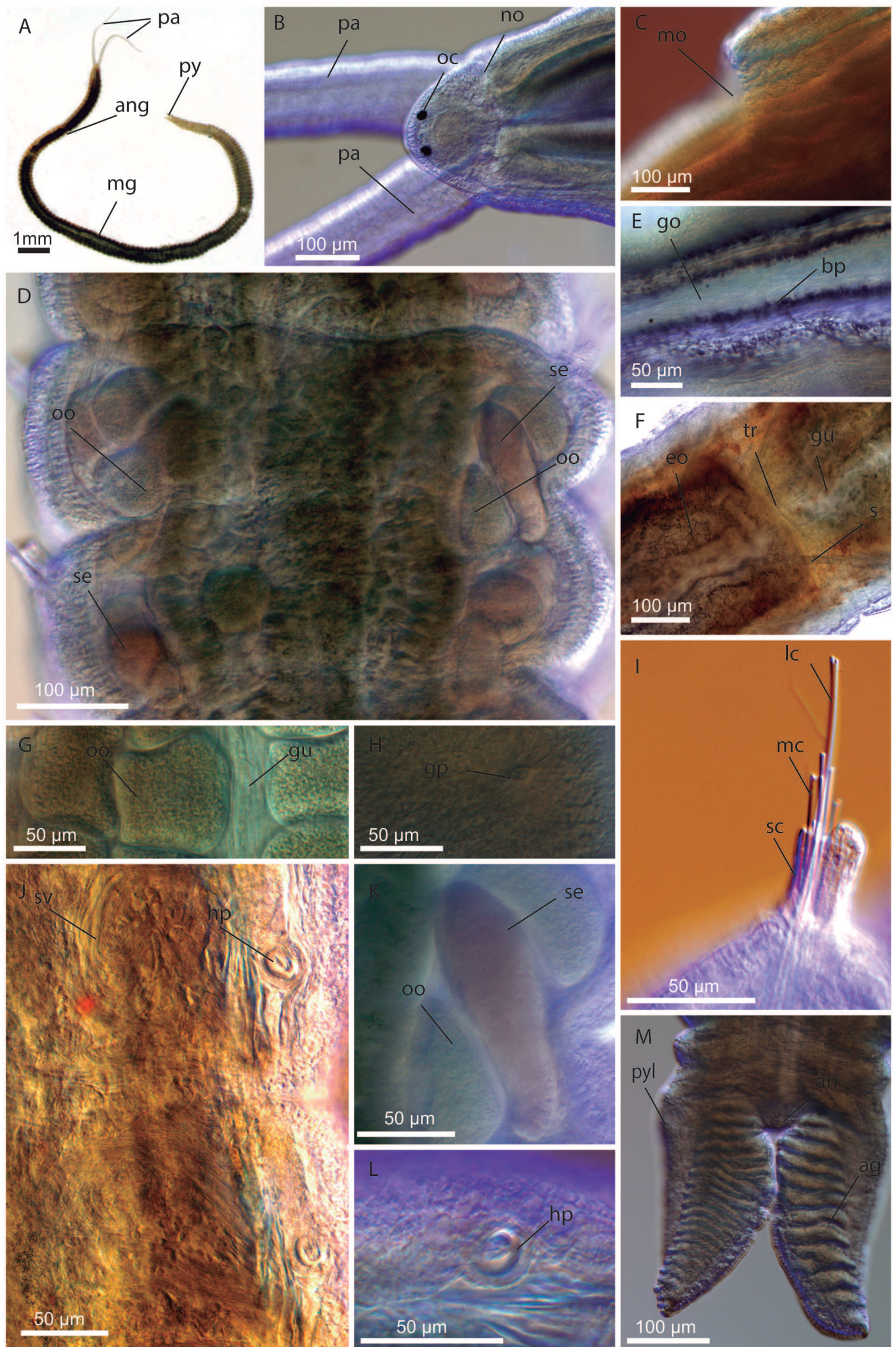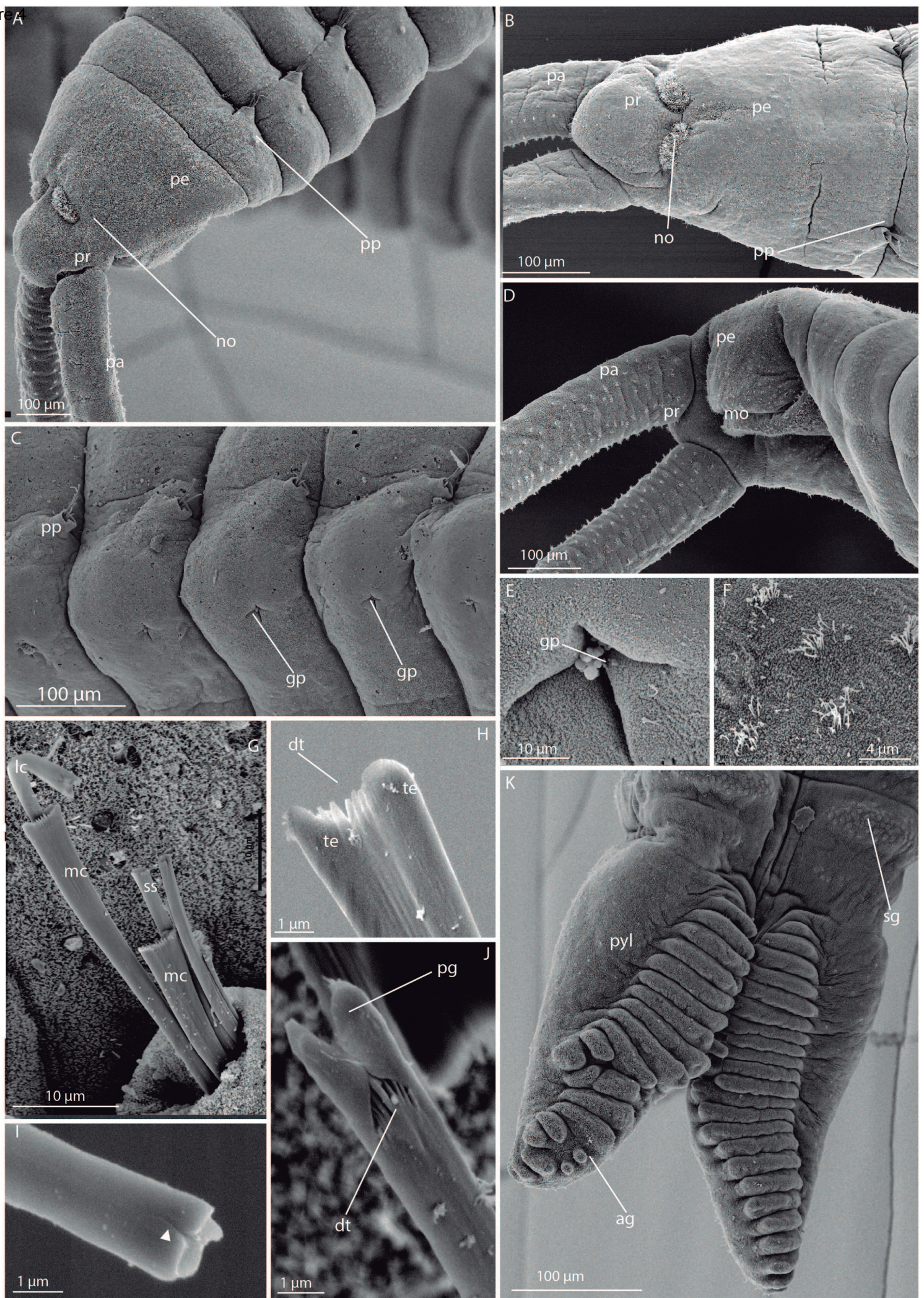100/1

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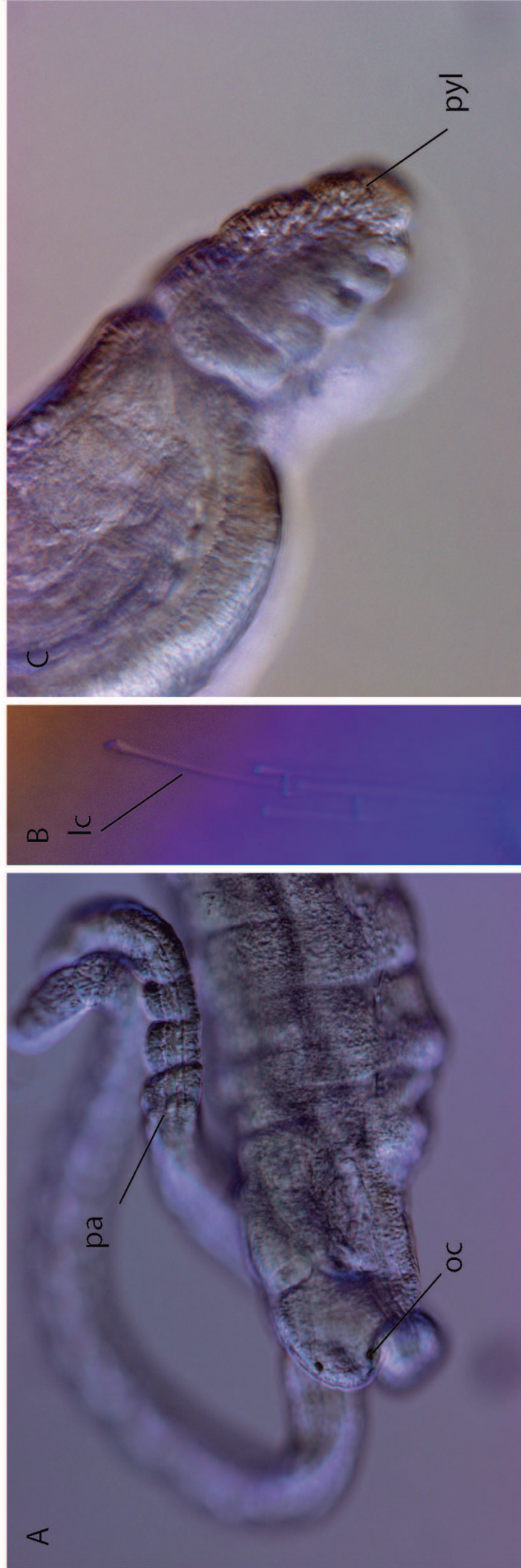Figure 3

Figure A

Figure 5

Figure 6

Table 1

Table 1. Meristic and morphometric characters of the species of *Saccocirrus*. Abbreviations: L, lenght; W, width; Max, maximum; N, number; Fem, Female; unk, unkonow; ca. circa (about), segm, segment

	Max. L. (mm)	Max. W. (µm)	Max. N. segments	Max. palps L. (µm)	Pharyngeal bulb	Pygidial lobes shaped	Pygidial lobes length (µm)	Max N. papillae pygidium	Pygidial anal cirri	Gonads Fem.	N. fertile segments	Size mature oocytes (µm)	N. oocytes/ segm.	Gonads Males	Ciliary groove	Ciliary patches mouth	Longest chatae	Prongs length	Medium chaetae	Median chaetae, number of denticles in each prong	Short chaetae	Ampullae length
Genus Saccocirrus	25	730	155	727	absent	long		22	absent		72	195	10 to 14	bilateral	absent	absent	forked					
S. slateri sp. nov.							250			bilateral								equal	spatulated	5 to 6	spatulated	until segm. 3
S. papilocercus	30	400	150	1500	absent	long	?	8	absent	bilateral	120	90	10	bilateral	absent	Absent	forked	equal	spatulated	ca. 7	spatulated	until segm. 1
S. major	70	1000	200	?	absent	long	?	14	absent	bilateral	175	65	40	bilateral	absent	Absent	forked	equal	spatulated	absent	spatulated	until segm. 1
S. minor	15	200	100	900	absent	rounded	?	absent	1 ventral adhesive papilla	bilateral	40	45	2	bilateral	absent	Absent	forked	equal	spatulated	?	spatulated	until segm. 2
S. orientalis	12	?	170	?	absent	rounded	?	4	2 ventral adhesive papillae	bilateral	60	60	?	bilateral	absent	Absent	forked	equal	spatulated	?	spatulated	until segm. 1
S. parvus Canary Island	13	280	70	280	absent	long	100	3 to 4	absent	bilateral	22	190	5 to 6	?	absent	absent	forked	unequal	spatulated	8 to 10	spatulated	until segm. 1
S. pussicus	30	400	120	1600	absent	long	?	12	absent	bilateral	36	80	5	bilateral	absent	Present	forked	unequal	spatulated	?	spatulated	until segm. 2
S. heterochaetus	9	300	74	800	absent	rounded	?	absent	absent	bilateral	20	80	4	bilateral	absent	Absent	forked	unequal	spatulated	?	spatulated	until segm. 2
S. parvus	3	180	48	550	absent	rounded	?	absent	absent	bilateral	?	?	?	bilateral	absent	Absent	forked	unequal	spatulated	?	spatulated	?
S. oahuensis	10,5	400	119	1400	absent	long	200	6	short	bilateral	?	?	?	bilateral	absent	Absent	forked	unequal	spatulated	?	spatulated	?
S. waianaensis	10	450	210	1310	absent	long	250	absent	absent	bilateral	?	?	?	bilateral	absent	Absent	forked	unequal	spatulated	?	spatulated	?
S. cirratus	45	?	200	1500	present	rounded	?	absent	long	bilateral	115	?	?	bilateral	absent	Present	lyrid	unequal	spatulated	?	spatulated	until segm. 1