



UNIVERSIDADE ESTADUAL DE CAMPINAS  
SISTEMA DE BIBLIOTECAS DA UNICAMP  
REPOSITÓRIO DA PRODUÇÃO CIENTÍFICA E INTELLECTUAL DA UNICAMP

**Versão do arquivo anexado / Version of attached file:**

Versão do Editor / Published Version

**Mais informações no site da editora / Further information on publisher's website:**

<https://www.sciencedirect.com/science/article/pii/S1055790314000542>

**DOI: 10.1016/j.ympcv.2014.02.003**

**Direitos autorais / Publisher's copyright statement:**

©2014 by Elsevier. All rights reserved.

DIRETORIA DE TRATAMENTO DA INFORMAÇÃO

Cidade Universitária Zeferino Vaz Barão Geraldo

CEP 13083-970 – Campinas SP

Fone: (19) 3521-6493

<http://www.repositorio.unicamp.br>



# Molecular and morphological phylogeny of Saccocirridae (Annelida) reveals two cosmopolitan clades with specific habitat preferences



M. Di Domenico<sup>a,b,c,\*</sup>, A. Martínez<sup>a</sup>, P. Lana<sup>b</sup>, K. Worsaae<sup>a,\*</sup>

<sup>a</sup> Marine Biological Section, Department of Biology, University of Copenhagen, Strandpromenaden 5, 3000 Helsingør, Denmark

<sup>b</sup> Laboratory of Benthic Ecology, Centre for Marine Studies, Federal University of Paraná, Brazil

<sup>c</sup> University of Campinas (UNICAMP), Biological Institute, Zoological Museum "Prof. Dr. Adão José Cardoso", Brazil

## ARTICLE INFO

### Article history:

Received 13 August 2013

Revised 7 February 2014

Accepted 10 February 2014

Available online 18 February 2014

### Keywords:

Protodrilida

Total evidence

Systematics

Interstitial habitats

Meiofauna

## ABSTRACT

Saccocirrids are tiny, slender annelids inhabiting the interstices among coarse sand sediments in shallow waters. The 22 nominal species can be grouped into two morphological groups "papillocercus" and "krusadensis", based on the absence/presence of a pharyngeal bulbus muscle, absence/presence of ventral ciliary patterns, bilateral/unilateral gonad arrangement and chaetal differences. We present herein the first phylogenetic analyses of Saccocirridae based on four molecular markers and 34 morphological characters, employing maximum likelihood and Bayesian methods. All analyses confirmed the monophyly of each morphological group with high nodal support. As a consequence and based on several apomorphic characters, a new genus, *Pharyngocirrus* gen. nov., is erected for the "krusadensis" clade. Remarkably, the habitat preferences and trophic guilds are also shown to differ between the two genera, yet show strong consistency within each group. Geographic distribution analyses underscore the family preference for subtropical areas, but reject the previously proposed restriction of *Pharyngocirrus* gen. nov. to the Indo-Pacific. The finding of two morphologically diverging, cosmopolitan sister clades with different habitat preferences suggest an early ecological diversification of the family, conserved during the later evolution, speciation processes and dispersal of the family.

© 2014 Elsevier Inc. All rights reserved.

## 1. Introduction

Saccocirridae is an annelid family with 22 nominal species, marine and interstitial, all of them belonging to the genus *Saccocirrus* Bobretzky, 1872. They are characterized by the presence of two long palps with internal canals provided with basal ampullae, as well as small cylindrical parapodia with simple chaetae and a bilobed adhesive pygidium. Seminal vesicles and penis are present in the males, and oviducts and spermathecae in females (Purschke and Jouin, 1988). Saccocirridae has morphologically been grouped with Protodriloididae and Protodrilidae in the clade Protodrilida (Purschke and Jouin, 1988; Westheide, 2008), which is left unresolved in the Canalpalpata clade in morphological phylogenies of Annelida (Rouse and Fauchald, 1997; Rouse and Pleijel, 2001). However, Protodrilida has never been recovered in molecular phylogenetic analyses, and Saccocirridae is either left unresolved

(Rousset et al., 2007), or as sister group of Polygordiidae (Struck et al., 2008; Golombek et al., 2013), sometimes within a clade including Protodrilidae, Protodriloididae and Nerilidae (Zrzavy et al., 2009).

Two morphological groups resembling either *Saccocirrus papillocercus* Bobretzky (1872) or *S. krusadensis* Alikunhi (1948) can be distinguished within the genus (Gray, 1969; Jouin, 1971; Martin, 1977; Brown, 1981; Sasaki, 1981; Sasaki and Brown, 1983; Jouin and Rao, 1987; Purschke and Jouin, 1988; Bailey-Brock et al., 2003; Jouin and Gambi, 2007; Westheide, 2008). The two groups have never received taxonomic value or been tested phylogenetically, but they seem well delineated by several morphological features (Brown, 1981; Purschke and Jouin, 1988; Westheide, 2008) and may contain species with different habitat preferences, trophic guilds and geographic distribution (Brown, 1981; Bailey-Brock et al., 2003; Jouin and Gambi, 2007; Westheide, 2008).

The "papillocercus" group consists of 10 nominal species distributed worldwide, all with bilateral gonads and no muscular pharyngeal organ or ventral ciliation. They possess three types of chaetae: long chaetae with short terminal prongs; medium chaetae with oar-shaped tips; and short chaetae with smooth or bifid shape tips (Jouin and Gambi, 2007). Conversely, the "krusadensis" group,

\* Corresponding authors. Address: Marine Biological Section, Department of Biology, University of Copenhagen, Strandpromenaden 5, 3000 Helsingør, Denmark (M. Di Domenico).

E-mail addresses: [maik2dd@gmail.com](mailto:maik2dd@gmail.com) (M. Di Domenico), [amartinez.ull@gmail.com](mailto:amartinez.ull@gmail.com) (A. Martínez), [iana@ufpr.br](mailto:iana@ufpr.br) (P. Lana), [kworsaae@bio.ku.dk](mailto:kworsaae@bio.ku.dk) (K. Worsaae).

with 12 described species, is mostly reported from the Indo-Pacific and characterized by the presence of unilateral gonads, pharyngeal muscular organ and anterior ventral ciliation (Brown, 1981; Jouin and Gambi, 2007). Species of this subgroup have long capillary lyrate chaetae (deeply bifid at tip), medium chaetae with a deep and denticulate terminal notch, and short bifid chaetae with a notched apex (see Brown, 1981; Sasaki, 1981; Jouin and Gambi, 2007;). The main difference in pharyngeal morphology and ciliation may reflect different trophic guilds. Gut contents indicate that species in the “papillocercus” group often are scavengers (Du Bois-Reymond Marcus, 1948; Westheide, 2008), whereas those in the “krusadensis” group seem more specialized in bacteria-diatom grazing (Du Bois-Reymond Marcus, 1946; Jouin and Gambi, 2007; Westheide, 2008). Species in each group also show different habitats preferences, with examples of species of the “krusadensis” group being common in sheltered sediments versus species of “papillocercus” preferring reflective beaches (Brown, 1981; Jouin and Gambi, 2007; Di Domenico et al., 2014). These overall differences in morphology, feeding and ecology may indicate two distinct evolutionary lineages within *Saccocirrus* (Brown, 1981; Westheide, 2008). However, this hypothesis remains to be tested with phylogenetic methods.

We present herein the first phylogenetic analyses of Saccocirridae based on four molecular markers and 34 morphological characters analyzed with maximum likelihood and Bayesian methods. We hereby aim to (i) address the monophyly of Saccocirridae, (ii) address the monophyly of the morphological groups “papillocercus” and “krusadensis”, (iii) trace the main morphological characters in order to test for apomorphies sustaining the family and the possible groups, as well as (iv) analyze the geographical distribution and habitat preferences.

## 2. Materials and methods

### 2.1. Taxon selection, sampling and fixations

This study included 19 species of *Saccocirrus* and 7 outgroups. Nine of the saccocirrids are described species and ten are undescribed taxa. Two Protodriloididae, two Protodrilidae, as well as one Sabellariidae, Ctenodrilidae, and Spionidae, were chosen as outgroups (Table 1). Since Saccocirridae have not been positioned with high support in any of the molecular phylogenies, the outgroup choices are instead based on the Protodrilida hypothesis (Purschke and Jouin, 1988; hereof Protodriloididae and Protodrilidae) as well as older morphological phylogenies placing them incertae sedis in Canalipalpata us hereof including representatives from each the three subclades (Rouse and Fauchald, 1997; Rouse and Pleijel, 2001). It is not an aim of this study to resolve the systematic position of Saccocirridae in Annelida and the small number of outgroups are just meant to reflect the morphological discussion.

Most of the specimens were extracted from sandy or gravelly sediments using the MgCl<sub>2</sub> decantation technique and sieved through a 63- $\mu$ m mesh (Higgins and Thiel, 1988). Sediments were collected by hand from intertidal zones of sandy beaches or sheltered bays, or by snorkeling or scuba diving from sublittoral bottoms. All specimens were sorted, identified and photographed alive in the field using dissecting and compound microscopes. Specimens for the molecular analyses were stored in 100% ethanol, whereas vouchers and specimens used for morphological studies were fixed in 2% glutaraldehyde in cacodylate buffer or 2% paraformaldehyde in PBS. All the investigated material is listed in Table 1.

Additionally, we examined type material of the following species: *Saccocirrus alanhongi* Bailey-Brock et al., 2003 (USNM POLY 1012494–1012497), *S. eroticus* Gray, 1969 (USNM POLY

36064–36066); *S. jouinae* Brown, 1981 (USNM POLY 62027–62033); *S. oahuensis* Bailey-Brock et al., 2003 (USNM POLY 1012490–1012491); *S. tridentiger* Brown, 1981 (USNM POLY 62034–62040); *S. waianaensis* Bailey-Brock et al., 2003 (USNM POLY 1012492–1012493); *S. sonomacus* Martin, 1977 (USNM POLY 53050–53052); as well as the neotypes of *Saccocirrus pussicus* Du Bois-Reymond Marcus, 1948 (ZUEC POL 14069) and *S. gabriellae* Du Bois-Reymond Marcus, 1946 (ZUEC POL 14053) (Di Domenico et al., 2014).

### 2.2. DNA extraction and amplification

DNA was extracted using Qiagen DNeasy Tissue and Blood kit (Düsseldorf, Germany), following protocols provided by the manufacturer. DNA elution was repeated twice with the same 80  $\mu$ l of buffer to maximize the amount of DNA yielded.

Three nuclear and one mitochondrial markers were consistently amplified for each of the species. Nuclear markers consisted of approximately 1800 base pairs of the small subunit ribosomal RNA (18S rRNA), 800 base pairs of the large subunit ribosomal RNA (28S rRNA, D1 region) and 350 base pairs of the protein-coding gene Histone 3 (H3). The mitochondrial marker consisted of 600 base pairs of the 16S ribosomal RNA (16S rRNA). Gene coverage among taxa was 96 % for the 18S rRNA, 92% for the 28S rRNA, 96% for the H3 and 100% for the 16S rRNA.

Polymerase chain reactions (PCR) were performed with Illustra PuReTaq Ready-To-Go PCR beads (Amersham Biosciences) following the protocol of the manufacturer. PCR reactions were performed in a volume of 25  $\mu$ l, containing 2  $\mu$ l of template DNA, 1.5  $\mu$ M of each primer (10  $\mu$ M) and 18  $\mu$ l of ddH<sub>2</sub>O. Details on the primers are summarized in Table 2. PCR reactions were carried out using a Bio-Rad S1000 Thermal Cycler with the following temperature profile: initial denaturation, 96°/2 min or 1 min – (denaturation, 94 °C/30 s or 60 s – annealing, 38–55 °C/30 s or 60 s – extension, 72 °C/60 s)\* 40–45 cycles – final extension, 72 °C/420 s. PCR products were checked in a E-Gel 2% SYBR safe agarose gels (Invitrogen, Life Technologies, CA, USA) and purified with E.Z.N.A. Cycle-Pure kit (Omega Bio-tek). Purified products were sequenced by MacroGen Europe Sequencing System on an ABI 3730XL DNA Analyzer (Applied Biosystems).

Chromatograms were read and assembled with Sequencer 4.10.1 (GeneCodes Corporation, Ann Arbor, MI, USA). Assembled sequences were blasted in GenBank and checked for possible contaminations. All sequences were deposited in GenBank (accession numbers are provided in Table 1).

### 2.3. Morphological data

A morphological matrix of 34 characters was compiled for 26 terminal taxa based on the original descriptions and new observations. Information on general features, as well as sexual and glandular characters were generally obtained from whole mounted specimens. Whole mounts were examined and photographed with an Olympus DP71 camera mounted on an Olympus BX50 microscope at the Marine Biological Section, University of Copenhagen (MBS).

Ciliary patterns and chaetae were preferably studied with electron scanning microscopy (SEM) on material fixed in 2% glutaraldehyde. Specimens were post-fixed in 1% osmium tetroxide for one hour, rinsed in demineralized water and dehydrated through a graded-ethanol series. Specimens in 100% ethanol were subsequently transferred to acetone, critical-point dried, mounted on stubs and sputter-coated with platinum. Coated specimens were examined with a JEOL JSM-6335F field emission scanning electron microscope at the Natural History Museum of Denmark, University of Copenhagen.

**Table 1**

Origin of sequenced terminals, morphological groups, localities, regions, vouchers, and GenBank accession numbers. New sequences are set in bold.

Species	Group	Locality	Region	Voucher	18S	28S	16S	Histone 3
<i>Saccocirrus</i> sp. 6 (CA, USA)*	<i>krusadensis</i>	Cove, La Jolla, CA, USA	North America – Pacific Ocean	ZMUC-Pol (2314)	<b>KF954476</b>	<b>KF954434</b>	<b>KF954455</b>	<b>KF954495</b>
<i>Saccocirrus</i> sp. 3 (Bocas del Toro, Panama)*	<i>krusadensis</i>	Bocas del Toro, Panama	Central America – Caribbean Sea	ZMUC-Pol (2311)	<b>KF954469</b>	<b>KF954427</b>	<b>KF954448</b>	<b>KF954488</b>
<i>Saccocirrus</i> sp. 5 (Bocas del Toro, Panama)*	<i>krusadensis</i>	Bocas del Toro, Panama	Central America – Caribbean Sea	ZMUC-Pol (2313)	<b>KF954475</b>	<b>KF954433</b>	<b>KF954454</b>	<b>KF954494</b>
<i>Saccocirrus</i> sp. 9 (Belize)	<i>papillocercus</i>	Carrie Bow Cay, Belize	Central America – Caribbean Sea	ZMUC-Pol (2317)	<b>KF954479</b>	<b>KF954437</b>	<b>KF954458</b>	<b>KF954498</b>
<i>Saccocirrus sonomacrus</i>	<i>krusadensis</i>	Bird Rock, CA, USA	North America – Pacific Ocean	ZMUC-Pol (2306)	<b>KF954472</b>	<b>KF954430</b>	<b>KF954451</b>	<b>KF954491</b>
<i>Saccocirrus sonomacrus</i>	<i>krusadensis</i>	Chile	South America – Pacific Ocean	ZMUC-Pol (2305)		<b>KF954425</b>	<b>KF954446</b>	
<i>Saccocirrus tridentiger</i>	<i>krusadensis</i>	North East Cay, Salomon Island	Indo-Pacific	ZMUC-Pol (2307)	<b>KF954465</b>	<b>KF954421</b>	<b>KF954443</b>	<b>KF954484</b>
<i>Saccocirrus tridentiger</i>	<i>krusadensis</i>	New South Wale, Australia	Indo-Pacific	ZMUC-Pol (2308)	<b>KF954466</b>	<b>KF954422</b>	<b>KF954444</b>	<b>KF954485</b>
<i>Saccocirrus jouinae</i>	<i>krusadensis</i>	Kennedy Island, Salomon Island	Indo-Pacific	ZMUC-Pol (2301)	<b>KF954473</b>	<b>KF954431</b>	<b>KF954452</b>	<b>KF954492</b>
<i>Saccocirrus krusadensis</i>	<i>krusadensis</i>	Ao Yon Beach, Phuket, Thailand	Indo-Pacific	ZMUC-Pol (2302)	<b>KF954474</b>	<b>KF954432</b>	<b>KF954453</b>	<b>KF954493</b>
<i>Saccocirrus</i> sp. 8 (Bermuda)	<i>papillocercus</i>	Windsor Beach, Bermuda	Central America – Caribbean Sea	ZMUC-Pol (2316)	<b>KF954478</b>	<b>KF954436</b>	<b>KF954457</b>	<b>KF954497</b>
<i>Saccocirrus</i> sp. 10 (Solomon Isl.)	<i>papillocercus</i>	Mono island, Salomon Island	Indo-Pacific	ZMUC-Pol (2318)	<b>KF954480</b>	<b>KF954438</b>	<b>KF954459</b>	<b>KF954499</b>
<i>Saccocirrus pussicus</i>	<i>papillocercus</i>	Barra Velha, Santa Catarina, Brazil	South America – Atlantic Ocean	ZMUC-Pol (2304)	<b>KF954481</b>	<b>KF954439</b>	<b>KF954460</b>	<b>KF954500</b>
<i>Saccocirrus</i> sp. 1 (Tenerife, Canary Isl.)	<i>papillocercus</i>	Playa Abades, Tenerife, Canary Island	Atlantic Island	ZMUC-Pol (2309)	<b>KF954467</b>	<b>KF954424</b>	<b>KF954445</b>	<b>KF954486</b>
<i>Saccocirrus</i> sp. 7 (Giglio, Italy)	<i>papillocercus</i>	Giglio Island, Italy	Mediterranean Sea	ZMUC-Pol (2315)	<b>KF954477</b>	<b>KF954435</b>	<b>KF954456</b>	<b>KF954496</b>
<i>Saccocirrus</i> sp. 4 (La Palma, Canary Isl.)*	<i>krusadensis</i>	La Palma, Canary Island	Atlantic Island	ZMUC-Pol (2312)	<b>KF954471</b>	<b>KF954429</b>	<b>KF954450</b>	<b>KF954490</b>
<i>Saccocirrus</i> sp. 2 (Lanzarote, Canary Isl.)*	<i>krusadensis</i>	Lanzarote, Canary Island	Atlantic Island	ZMUC-Pol (2310)	<b>KF954468</b>	<b>KF954426</b>	<b>KF954447</b>	<b>KF954487</b>
<i>Saccocirrus gabriellae</i>	<i>krusadensis</i>	Ponta do Balleiro, São Sebastião, SP, Brazil	South America – Atlantic Ocean	ZMUC-Pol (2300)	<b>KF954470</b>	<b>KF954428</b>	<b>KF954449</b>	<b>KF954489</b>
<i>Saccocirrus papillocercus</i>	<i>papillocercus</i>	Sevastopol Bay, Ukraine	Black Sea	ZMUC-Pol (2303)	<b>KF954482</b>		<b>KF954461</b>	<b>KF954501</b>
<i>Protodrilus purpureus</i>	outgroup				EU418874	AY527057	AY340474	DQ779760
<i>Protodrilus ciliatus</i>	outgroup	Kallak, Faroe Island	Atlantic Island, North		<b>KF954464</b>	<b>KF954420</b>	<b>KF954442</b>	<b>KF954505</b>
<i>Protodriloides symbioticus</i>	outgroup	Roscoff, France	North Sea, Atlantic		<b>KF954463</b>	<b>KF954419</b>	<b>KF954441</b>	<b>KF954504</b>
<i>Protodriloides chaetifer</i>	outgroup	Ellekilde Hage, Denmark	North Sea, Atlantic		<b>KF954462</b>	<b>KF954418</b>	<b>KF954440</b>	<b>KF954503</b>
<i>Ctenodrilus serratus</i>	outgroup				AY340426	AY364864	AY340452	DQ779727
<i>Polydora giardi</i>	outgroup				AY611455	AY611442	DQ779632	DQ779756
<i>Sabellaria alveolata</i>	outgroup				DQ140412	AY340416	AY340479	DQ779763

**Table 2**

PCR and sequencing primers to 18S do rDNA, 28S rDNA, 16S rDNA ribosomal and Histone 3.

Code	Gene	Bases	Direction	Sequence 5'–3'	Reference
G952	18s	20	F	GCGAAGCATTGCGCAAGMA	1
G951	18s	20	R	GAGTCTCGTTCGTTATCGGA	1
G950	18s	19	F	GTTCGATTCCGGAGAGGGA	1
G758	28s (D1)	19	F	ACC CSC TGA AYT TAA GCA T	2
G747	18s	20	R	CGG TAT CTG ATC GTC TTC GA	3
G51	18s	18	F	GGT TGA TCC TGC CAG TAG	3
G1275	28s	20	R	TCG GAA GGA ACC AGC TAC TA	4
G944	18S	24	R	TGA TCC TTC TGC AGG TTC ACC TAC	5
16Sar-L	16s	20	F	CGC CTG TTT ATC AAA AAC AT	6
16Sbr-H	16s	22	R	CCG GTC TGA ACT CAG ATC ACG T	6
H3f	H3	23	F	ATG GTC CGT ACC AAG CAG AC(ACG) GC	7
H3r	H3	23	R	ATA TCC TT(AG) GGC AT(AG) AT(AG) GTG AC	7

Fonte: 1 – Cohen et al. (2004), 2 – Brown et al. (1999), 3 – Hillis e Dixon (1991), 4 – Markmann (2000), 5 – Stoeckle (<http://www.coreocean.org>), 6 – Palumbi (1996), 7 – Colgan et al. (1998).

Independent morphological characters were scored as absence/presence or multistate characters. Linked characters were coded hierarchically following the principles of C-coding (Pleijel, 1995). First, absence/presence of features were coded. Subsequently, traits of each feature (e.g. length, shaped or position) were coded as independent multistate characters, hereby performing a combination

of binary and contingent coding. Absence of information was scored with question marks, and inapplicable states with a dash.

Scores of continuous characters were taken from the literature or measured from newly collected material. Unless otherwise indicated, a minimum of 10–15 whole mounted specimens were measured. All measures were taken from relaxed and fixed specimens.

All continuous characters were tested for correlations among them in order to avoid inclusion of redundant information. The characters maximum length, maximum width, maximum number of trunk segments and palps length were independent and subsequently coded into discrete states. Discrete states for each continuous character were defined after a k-means cluster analyses of the mean maximum values.

The morphological matrix (Table 3) was prepared using Mesquite software v2.75 (Maddison and Maddison, 2011). A summary of the morphological characters and states is present in Section 2.4.

#### 2.4. Description of the morphological characters

2.4.1. Total body length (1): shorter than 3 mm (=0); 3–5 mm (=1); 5–10 mm (=2); 10–20 mm (=3); longer than 20 mm (=4)

Total length was divided into five discrete states. *Polydora giardi* and *Sabellaria alveolata* are longer than 20 mm; *Protodriloides symbioticus*, *Protodriloides chaetifer* and *Protodrilus purpureus* are 10–20 mm (Jouin, 1966; Von Nordheim, 1989), *Protodrilus ciliatus* is 3–5 mm and *Ctenodrilus serratus* is shorter than 3 mm (Petersen and George, 1991). The different sizes of *Saccocirrus* species are summarized in Table 4.

2.4.2. Maximum width (2): thinner than 200  $\mu\text{m}$  (=0); 200–400  $\mu\text{m}$  (=1); thicker than 400  $\mu\text{m}$  (=2)

Maximum width was divided in three discrete states in our matrix. *Polydora giardi* and *Sabellaria alveolata* are thicker than 400  $\mu\text{m}$ ; *Protodriloides symbioticus*, *Protodriloides chaetifer*, *Protodrilus purpureus* are 200–400  $\mu\text{m}$ , *Protodrilus ciliatus* and *Ctenodrilus serratus* are thinner than 200  $\mu\text{m}$ . Variation within *Saccocirrus* is summarized in Table 4.

2.4.3. Maximum number of trunk segments (3): less than 50 segments (=0); 50–100 segments (=1); 100–150 segments (=2); more than 150 segments (=3)

All outgroups have less than 50 segments, except for *Polydora giardi* with 50–100 segments. In *Saccocirridae*, the number of segments varies from 50 to more than 150 segments. The maximum number of trunk segments is not covariant with the maximum body length.

2.4.4. Position of the palps (4): dorsal (=0); ventral (=1), terminal (=2)

Absence/presence of palps is uninformative with the selected taxa and the character is excluded from the analyses, since palps are only lacking in *Ctenodrilus serratus* in our matrix. Palp position however contains information and is defined by the place where palps insert on the prostomium. Palps insert ventrally in *Saccocirrus* and *Protodrilus*, terminally on the tip of the prostomium in *Protodriloides* (Purschke, 1993) and dorsally in *Polydora* (Blake, 1980; Worsaae, 2001; Orrhage and Müller, 2005). The palps are inserted dorsally anterior of the mouth in *Sabellariids* (Orrhage, 1978, 1980).

2.4.5. Maximum length of palps (5): shorter than 200  $\mu\text{m}$  (=0); 200–500  $\mu\text{m}$  (=1); 500–1000  $\mu\text{m}$  (=2); longer than 1000  $\mu\text{m}$  (=3)

Palp length varies among the examined species and it is not covariant with the total body length. Therefore, the maximum length of the palp may represent a source of phylogenetic information, tested in our analyses. Length of the palps ranges from 200 to 1500  $\mu\text{m}$  in *Saccocirrus*. Palps are longer than 1000  $\mu\text{m}$  in *Polydora* and *Protodrilus purpureus*, range between 200–500  $\mu\text{m}$  in *Protodriloides*, and 500–1000  $\mu\text{m}$  long in *Protodrilus ciliatus*.

2.4.6. Palp ciliation (6): sparse (=0); longitudinal bands (=1)

*Protodrilus ciliatus*, *P. purpureus*, *Polydora*, *Sabellaria* and a single species of *Saccocirrus* sp. 10 (Solomon Isl.) have the palp ciliation

arranged in longitudinal bands (Von Nordheim, 1989; Worsaae, 2001). In *Protodriloides*, the palps bear a ventral field of motile cilia extending from the tip to about two-thirds of their length (Purschke, 1993). Palps ciliation is organized as individual tufts uniformly scattered on the entire surface of the palp in the remaining *Saccocirrus* (Table 4).

2.4.7. Palp internal canal (7): absent (=0); present (=1)

Palps are supplied with one internal canal (coelomic cavities) in *Saccocirrus* and *Protodrilus*, and two (medial + lateral) internal canals in *Spionidae* (Orrhage, 1964; Purschke, 1993) and *Sabellaria* (Orrhage, 1978). The palp canals of *Protodrilus* and *Saccocirrus* may be similar to medial canal cavities of *Spionidae* (Purschke, 1993). The internal canal is absent in *Protodriloides* (Purschke and Jouin, 1988; Purschke, 1993), and the character is inapplicable for *Ctenodrilus* (Petersen and George, 1991; Orrhage and Müller, 2005).

2.4.8. Internal canal connected behind the brain (8): absent (=0); present (=1)

A connection of the internal canals behind the brain is only present in *Protodrilus* and *Saccocirrus* (Purschke, 1993) in our analyses. The internal palp canals of *Polydora* and *Sabellaria* are not connected (Orrhage, 1978; Purschke, 1993). The character is inapplicable for *Ctenodrilus* and *Protodriloides*.

2.4.9. Terminal ampulla in the palp internal canals (9): absent (=0); present (=1)

Palps in *Saccocirrus* are supplied with basal sac-like structures that extend longitudinally along the first segment. These sacs are connected by the internal canal. Sacs are also present in *Spionidae*, but they are relatively small, extend anteriorly into the prostomium and are not connected by the palp canals (Orrhage, 1964). Therefore, they are considered different than the ampulla in *Saccocirrus*, which is coded as absent for *Spionidae*. Sacs are absent in the palp canals of *Sabellaria* and *Protodrilus*. The character is inapplicable for *Ctenodrilus* and *Protodriloides* (Purschke, 1993).

2.4.10. Position of the nuchal organs (10): dorsal (=0), dorsolateral (=1)

Nuchal organs are probably homologous in Annelida (Purschke, 1997), but their external morphology and their position vary between groups. Nuchal organs are oval and elongated in *Saccocirridae*, but never extend laterally around the peristomium (Purschke, 1990; Westheide, 2008; pers. obs). Nuchal organ morphology greatly varies among *Protodrilidae* (see e.g. Martínez et al., 2013; Di Domenico et al., 2013). In *Protodrilus purpureus* and *Protodrilus ciliatus*, nuchal organs are oval and extend dorsolaterally around peristomium (Von Nordheim, 1989; Martínez et al., 2013). *Protodriloides*, *Ctenodrilus serratus*, *Polydora* and *Sabellaria* present dorsal nuchal organs (rounded or elongated) (Jouin, 1966; Rouse and Pleijel, 2001).

2.4.11. Epidermal eyes with microvilli in the shading pigment cells (11): absent (=0); present (=1)

Eyes are present in *Saccocirridae*, *Sabellaria* and all described *Protodrilus* larvae. However, *saccocirrid* eyes are bicellular (with a “shading pigment cell” and “light-sensitive cell”) and embedded in the epidermis. Both cells exhibit apical extensions with rhabdom-like microvilli and vestigial cilia (Eakin et al., 1977; Purschke, 1992). Pigmented eyes are only described in the adults of four species of *Protodrilidae* not included in our analyses, otherwise lacking the rhabdom-like microvilli extensions in the shading pigment cell. (Eakin et al., 1977). Eyes are absent in *Protodriloides*, *Ctenodrilus* and *Polydora giardi*.

**Table 3**  
Literature sources for geographic and habitats information on Saccocirridae.

Species	Group	Type locality	Distribution	References	Habitat description	Depth
<i>Saccocirrus papillocercus</i> Bobretzky, 1871	"papillocercus"	Sebastopol Bay, Russia (Baltic Sea, Europe)	English Channel (Plymouth, Isles of Scilly, North Wales). Irish Sea (Irish coast, North Wales). Mediterranean (Italy: Meloria; Spain: Valencia). Black Sea. Suez Channel. Madeira and Canary Islands	Abd-Elnaby (2009), Boaden (1963), Bobretzky (1872), Dauvin et al. (2003), Gusjewa (1929), Jouin and Gambi (2007), Cabioch et al. (1968), Langerhans (1880), Magagnini (1980), Mastepanova (2004), Núñez et al. (2005); Pierantoni (1906), Pierantoni (1907), Purschke (1990), von Repiachoff (1881), Smigielski e Souplet (1977), Villora-Moreno et al. (1991) and Wu and Yang (1962)	Swash zone, exposed beach	Intertidal
<i>Saccocirrus major</i> Pierantoni, 1907	"papillocercus"	Resina, Italy (Mediterranean)	Mediterranean (Italy. Spain: Chafarinas Isl.).	Jouin and Gambi (2007), Magagnini (1980), Pierantoni (1907), Uchida and Okuda (1953) and Villora-Moreno (1997)	Swash zone, exposed beach	Intertidal
<i>Saccocirrus orientalis</i> Alikunhi, 1946	"papillocercus"	Madras, India (Indian ocean)	Indian Ocean (Madras, South Andaman, Kavarathi and Laccadive, India; Durban, South Africa)	Alikunhi (1948), Jouin (1975) and Jouin and Rao (1987)	Swash zone, exposed beach	Intertidal
<i>Saccocirrus archiboldi</i> Kirsteuer, 1967	"papillocercus"	Middle Bay, Dominica (Caribbean)	Caribbean (Dominica)	Kirsteuer (1967)	Swash zone, exposed beach	Intertidal
<i>Saccocirrus minor</i> Aiyar and Alikunhi, 1944	"papillocercus"	Madras Coast (India)	Indian Ocean (Coast of India); New South Wales, Australia	Aiyar and Alikunhi (1944), Jouin and Rao (1987), Rao and Ganapati (1968)	Swash zone, exposed beach	Intertidal
<i>Saccocirrus pussicus</i> Du Bois-Reymond Marcus, 1948	"papillocercus"	São Sebastião, Santos (Brazil)	Brasil (São Sebastião, Santos)	Du Bois-Reymond Marcus (1948)	Swash zone, exposed beach	Intertidal
<i>Saccocirrus heterochaetus</i> Jouin, 1975	"papillocercus"	Amanzimtoti (Natal), South Africa	Eastern Africa (Amanzimtoti, Natal; Durban)	Jouin (1975)	Swash zone, exposed beach	Intertidal
<i>Saccocirrus parvus</i> Gerlach, 1953	"papillocercus"	Marina di Pisa, Italy	Mediterranean (France: Aigues-Mortes, Marseille; Tunes: Nabeul; Tyrrhenian Sea; Spain: Valencia Gulf, Chafarinas Islands)	Delamar-Deboutteville et al. (1954), Fize (1963), Gerlach (1953), Villora-Moreno et al. (1991), Villora-Moreno (1997) and Westheide (1972)	Swash zone, exposed beach	Intertidal
<i>Saccocirrus oahuensis</i> Baley-Brock et al., 2003	"papillocercus"	Hanauma Bay, O'ahu (Hawaii)	Hanauma Bay and Kamehameha Bay, O'ahu (Hawaii)	Baley-Brock et al. (2003)	Bay, reef, coarse sand	Subtidal
<i>Saccocirrus waianaensis</i> Baley-Brock et al., 2003	"papillocercus"	Barbers Point, O'ahu (Hawaii)	Barbers Point, O'ahu (Hawaii)	Baley-Brock et al. (2003)	Bay, reef, coarse sand	Subtidal
<i>Saccocirrus cirratus</i> Aiyar and Alikunhi, 1944	"papillocercus"	Madras Coast (India)	Indian Ocean (Coast of India)	Aiyar e Alikunhi (1944), Rao and Ganapati (1968)	Swash zone, exposed beach	Intertidal
<i>Saccocirrus krusadensis</i> Alikunhi, 1942	"krusadensis"	Krusadai Isl. (India)	India (Krusadai) , South Africa (Cape Town, Durban); French Polinesia (Moorea); New South Wales (Australia)	Alikunhi (1942), Jouin (1975), Jouin and Rao (1987), Purschke (1990) and Sasaki and Brown (1983)	Sheltered beach with organic matter, algae	Intertidal
<i>Saccocirrus gabriellae</i> Du Bois-Reymond Marcus, 1946	"krusadensis"	Guarujá Beach (Santos, Brazil)	Brasil (Guarujá, Santos); Mar do Japão (revisão)	Du Bois-Reymond Marcus (1946), Mastepanova (2004) (doubt) and Wu and Yang (1962) (S. major)	Tidal pool with organic matter, algae	Intertidal
<i>Saccocirrus eroticus</i> Gray, 1969	"krusadensis"	Orcas Isl. (Washington, USA)	Orcas Isl. (Washington, E. USA)	Gray (1969)	Sheltered beach with organic matter, algae	Intertidal
<i>Saccocirrus labilis</i> Yamanishi, 1973	"krusadensis"	Tanabe Bay, Wakayama (Japan)	Tanabe Bay, Wakayama (Japan)	Yamanishi (1973)	Sheltered beach with organic matter, algae	Intertidal
<i>Saccocirrus sonomacus</i> Martin, 1977	"krusadensis"	California (E. America)	California (E. America); Galapagos (Bahía Academy, Santa Cruz)	Brown (1981), Martin (1977); Salazar-Valejo and Lodonó-Mesa (2004) and Schmidt and Westheide (1977)	Exposed beach	Intertidal
<i>Saccocirrus jouinae</i> Brown, 1981	"krusadensis"	New South Wales, Australia	New South Wales, Australia		Among rocks, beach with organic matter, algae	Intertidal

Table 3 (continued)

Species	Group	Type locality	Distribution	References	Habitat description	Depth
<i>Saccocirrus tridentiger</i> Brown, 1981	"krusadensis"	New South Wales, Australia	New South Wales, Australia	Brown (1981)	Among rocks, beach with organic matter, algae	Intertidal
<i>Saccocirrus uchidai</i> Sasaki, 1981	"krusadensis"	Hokkaido (North Japan)	Hokkaido (North Japan)	Sasaki (1981) and Sasaki and Brown (1983)	Cliffed, sheltered beach	Intertidal
<i>Saccocirrus goodrichi</i> Jouin-Toulmond and Gambi, 2007	"krusadensis"	Gulf of Naples (Mediterranean)	Gulf of Naples (Mediterranean)	Jouin and Gambi (2007)	Amphioxus sand	Subtidal
<i>Saccocirrus burchelli</i> Silberbauer, 1969	"krusadensis"	Cape Agulhas, South Africa	Cape Agulhas, South Africa	Silberbauer (1969)	Among rocks, beach with organic matter, algae	Intertidal
<i>Saccocirrus atanhongi</i> Bailey-Brock et al., 2003	"krusadensis"	Wai'anae, O'ahu (Hawaii)	Wai'anae, O'ahu (Hawaii)	Bailey-Brock et al. (2003)	Reef flat	Subtidal

2.4.12. *Prostomial transverse ciliary band (12): absent (=0); present (=1)*

A transverse band of motile cilia extends latero-ventrally on the prostomium, from the palps to the delineation between prostomium and peristomium. The band is present in some *Saccocirrus* species belonging to the "krusadensis" group. The character is inapplicable in *Protodrilus*, *Protodriloides*, *Ctenodrilus*, *Sabellaria* and *Polydora*. This character is not present in the outgroups (Jouin, 1966; Von Nordheim, 1989; Petersen and George, 1991; Rouse and Pleijel, 2001).

2.4.13. *Ventral pharynx (13): absent (=0); present (=1)*

A ventral muscular pharynx is present in *Protodrilus*, *Protodriloides*, *Ctenodrilus*, *Polydora* and species from "krusadensis" group (Purschke, 1988; Purschke and Jouin, 1988). When the ventral pharyngeal apparatus is present, it consists of a ventral bulbus muscle with transverse muscular fibers and interstitial cells in all the terminals of our analyses (Purschke and Jouin, 1988; Purschke, 1988). The ventral pharynx is absent in the remaining species of the analyses.

2.4.14. *Ventral pharyngeal apparatus with tongue-like organ (14): absent (=0); present (=1)*

A tongue-like muscular organ is present in the "krusadensis" species of Saccocirridae, as well as Protodrilidae and Ctenodrilidae (Purschke and Tzvetlin, 1996). A tongue-like organ is absent in *Protodriloides* and *Polydora* species included in the current analyses and is inapplicable for species of the "papillocercus" group.

2.4.15. *Midventral ciliary band (15): absent (=0); present (=1)*

Midventral ciliary band, consisting of a longitudinal band of cilia extending along the trunk in a groove is present in adults of *Saccocirrus krusadensis*, *S. jouinae*, *S. tridentiger* ("krusadensis" group, Brown, 1981), as well as in *Protodrilus* and *Protodriloides*. Midventral ciliary band is absent in adults species in the "papillocercus" group, *Ctenodrilus*, *Sabellaria* and *Polydora* (Rouse and Pleijel, 2001; Westheide, 2008).

2.4.16. *Extension of the midventral ciliary band (16): from the mouth to the pygidium (=0); from the mouth to segment 10 (=1)*

Midventral ciliary band extends from the mouth to the pygidium in *Protodrilus* and *Protodriloides*. Midventral ciliary band extends maximum until segment 10 in *Saccocirrus krusadensis*, *S. jouinae* and *S. tridentiger* (Fig. 1B), although the presence and extension of band exhibit relatively high intraspecific variation the re-examined material of these species. The character is inapplicable for *Ctenodrilus*, *Sabellaria* and *Polydora* (Petersen and George, 1991; Rouse and Pleijel, 2001), and species of the "papillocercus" group.

2.4.17. *Mouth ciliary patches (17): absent (=0); present (=1)*

Ciliary patches are present around the mouth in saccocirrids from "krusadensis" group as well as in *Protodrilus* and *Protodriloides* (Fig. 1A). The character is absent in the remaining outgroups (Rouse and Pleijel, 2001; Westheide, 2008).

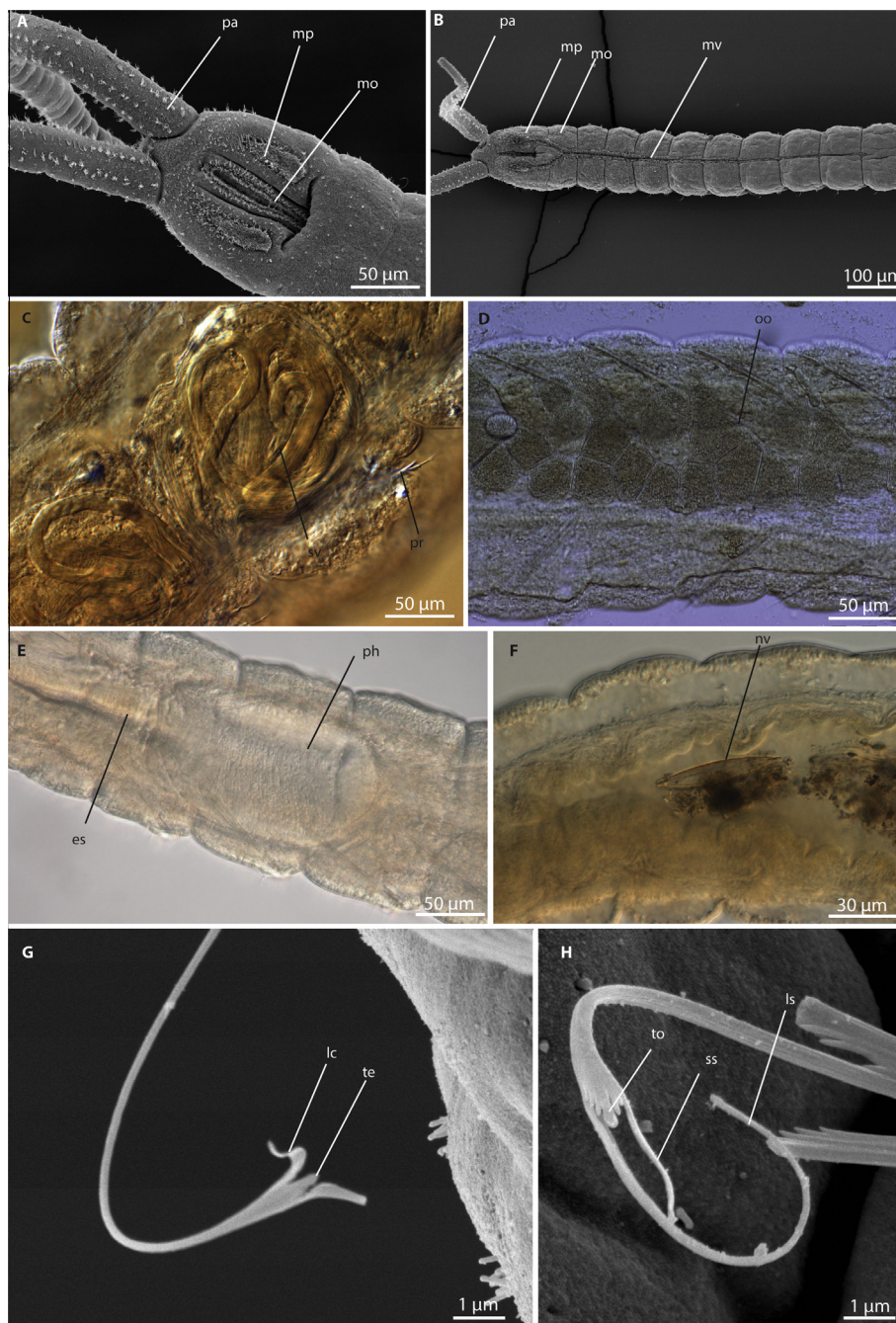
2.4.18. *Morphology of the mouth ciliary patches (18): arranged as a field around the mouth (=0); arranged as paired longitudinal bands, laterally to the mouths (=1)*

Mouth ciliary patches consist of two short longitudinal ciliary bands at each side of the mouth in species from "krusadensis" group, whereas they represent a triangular field extending posteriorly in *Protodrilus* and *Protodriloides* (Purschke and Jouin, 1988; Jouin and Gambi, 2007). The character is inapplicable in *Ctenodrilus*, *Sabellaria* and *Polydora* (Petersen and George, 1991; Rouse and Pleijel, 2001), and species in the "papillocercus" group.

**Table 4**  
Morphology character matrix. “?” denotes missing data and “-” denotes inapplicable states.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
<i>Saccocirrus papilloercus</i>	4	1	3	1	3	0	1	1	1	0	1	0	0	0	0	-	0	-	1	1	1	1	1	0	0	-	0	0	1	0	1	1	1	1
<i>Saccocirrus</i> sp. 1 (Tenerife, Canary Isl)	4	2	2	1	3	0	1	1	1	0	1	0	0	0	0	-	0	-	1	1	1	1	1	0	0	-	0	0	1	0	1	1	1	3
<i>Saccocirrus</i> sp. 7 (Giglio, Italy)	2	1	1	1	3	0	1	1	1	0	1	0	0	0	0	-	0	-	1	1	1	1	1	1	0	-	0	0	1	0	1	1	1	3
<i>Saccocirrus</i> sp. 8 (Bermuda)	2	1	1	1	2	0	1	1	1	0	1	0	0	0	0	-	0	-	1	1	1	1	1	1	0	-	0	0	1	0	1	1	1	1
<i>Saccocirrus</i> sp. 9 (Belize)	2	1	1	1	2	0	1	1	1	0	1	0	0	0	0	-	0	-	1	1	1	1	1	1	0	-	0	0	1	0	1	1	1	1
<i>Saccocirrus pussicus</i>	3	1	1	1	2	0	1	1	1	0	1	0	0	0	0	-	0	-	1	1	1	1	1	0	0	-	0	0	1	0	1	1	1	0
<i>Saccocirrus</i> sp. 10 (Solomon Isl)	3	1	1	1	3	1	1	1	1	0	1	0	0	0	0	-	0	-	1	1	1	1	1	0	0	-	0	0	1	0	1	1	1	1
<i>Saccocirrus krusadensis</i>	4	1	2	1	2	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	-	1	1	1	2	1	1	1	1	1	1
<i>Saccocirrus jouinae</i>	3	2	2	1	2	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	-	1	1	1	2	1	1	1	1	1	2
<i>Saccocirrus tridentiger</i>	3	2	1	1	2	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	-	1	1	1	2	1	1	1	1	1	2
<i>Saccocirrus tridentiger</i>	3	2	1	1	2	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	-	1	1	1	2	1	1	1	1	1	2
<i>Saccocirrus gabriellae</i>	4	1	3	1	2	0	1	1	1	0	1	1	1	1	0	-	1	1	1	1	1	1	0	-	1	0	1	1	1	1	1	1	1	2
<i>Saccocirrus</i> sp. 5 (Panama 2)	2	0	2	1	2	0	1	1	1	0	1	1	1	1	0	-	1	1	1	1	1	1	0	-	1	0	1	1	1	1	1	1	1	1
<i>Saccocirrus</i> sp. 4 (La Palma, Canary Isl)	2	1	1	1	2	0	1	1	1	0	1	1	1	1	0	-	1	1	1	1	1	1	0	-	1	0	1	1	1	1	1	1	1	1
<i>Saccocirrus</i> sp. 2 (Lanzarote, Canary Isl)	3	1	1	1	2	0	1	1	1	0	1	1	1	1	0	-	1	1	1	1	1	1	0	-	1	0	1	1	1	2	1	1	1	2
<i>Saccocirrus</i> sp. 6 (CA, USA)	2	1	2	1	2	0	1	1	1	0	1	1	1	1	0	-	1	1	1	1	1	1	0	-	1	0	1	1	1	1	1	1	1	1
<i>Saccocirrus sonomacus</i>	4	1	2	1	2	0	1	1	1	0	1	1	1	1	0	-	1	1	1	1	1	1	0	-	1	0	1	1	1	2	1	1	1	2
<i>Saccocirrus sonomacus</i>	4	1	2	1	2	0	1	1	1	0	1	1	1	1	0	-	1	1	1	1	1	1	0	-	1	0	1	1	1	2	1	1	1	2
<i>Saccocirrus</i> sp. 3 (Panama 1)	2	1	1	1	2	0	1	1	1	0	1	1	1	1	0	-	1	1	1	1	1	1	0	-	1	0	1	1	1	1	1	1	1	1
<i>Protodrilus purpureus</i>	3	1	0	1	1	1	1	1	0	1	0	-	1	1	1	0	1	0	0	0	0	0	-	-	-	-	-	-	0	-	1	0	0	-
<i>Protodrilus ciliatus</i>	1	0	0	1	1	1	1	1	0	1	0	-	1	1	1	0	1	0	0	0	0	0	-	-	-	-	-	-	0	-	1	0	0	-
<i>Protodriloides symbioticus</i>	1	0	0	2	1	0	0	-	-	0	0	-	1	1	1	0	1	-	0	0	0	0	-	-	-	-	-	-	0	-	1	2	0	-
<i>Protodriloides chaetifer</i>	1	0	0	2	1	0	0	-	-	0	0	-	1	1	1	0	1	-	0	0	1	0	-	-	-	-	-	0	-	1	2	0	-	
<i>Ctenodrilus serratus</i>	1	0	0	-	-	-	-	-	0	0	0	-	1	1	0	-	-	-	0	0	1	0	-	-	-	-	-	0	-	0	?	0	-	
<i>Sabellaria alveolata</i>	4	2	0	0	0	0	1	0	0	0	0	-	0	-	0	-	-	-	1	0	1	0	-	-	-	-	-	0	-	0	?	0	-	
<i>Polydora giardi</i>	4	2	1	0	3	0	1	0	0	0	0	-	1	0	0	-	-	-	1	0	1	0	-	-	-	-	-	0	-	0	?	0	-	





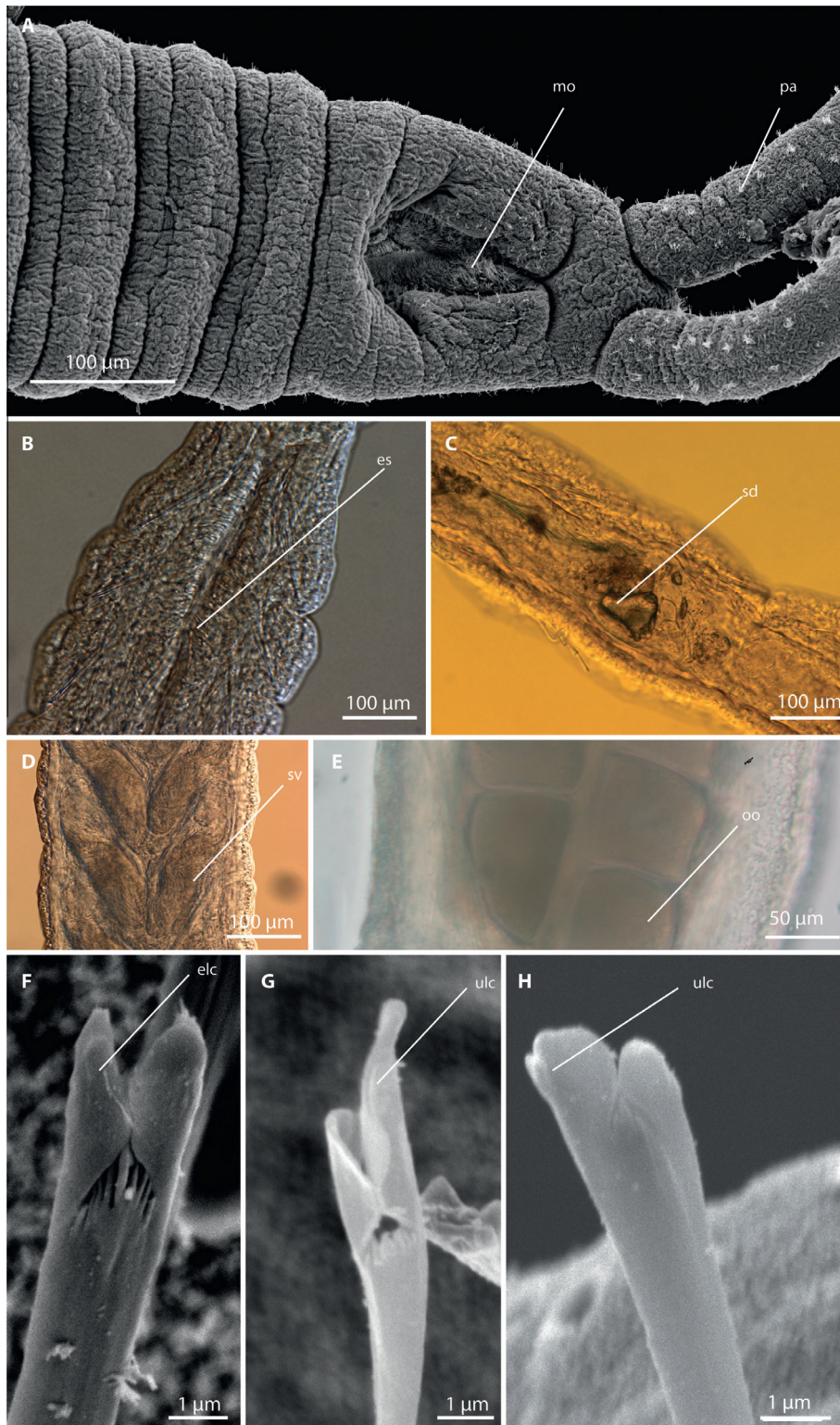
**Fig. 1.** Morphology of the “krusadensis” group (*Pharyngocirrus* gen. nov.), light and scanning electron micrographs. (A) *Saccocirrus sonomacus*, ventral view of the anterior end showing mouth ciliation. (B) *Saccocirrus jouinae*, ventral view of the anterior end showing the mouth ciliation and midventral ciliary band. (C) *Saccocirrus tridentiger*, mid body of a male showing the unilateral arrangement of seminal vesicles. (D) *Saccocirrus tridentiger*, midbody segments of a female showing the unilateral arrangement of oocytes. (E) *Saccocirrus gabriellae*, ventral pharyngeal bulbous on segments 2–4. (F) *Saccocirrus* sp. 4 (La Palma, Canary Island), showing a diatom inside the gut. (G) *Saccocirrus sonomacus*, equal lyrate longest chaeta; (H) *Saccocirrus tridentiger*, unequal lyrate longest chaeta. Abbreviations: lc, longest chaeta; ls, longest side of the longest chaeta; mo, mouth open; mp, Mouth ciliary patch; mv, midventral ciliary band; nv, Diatom; oo, oocytes; pa, palp; ph, pharynx; pr, parapodium; ss, shortest side of the longest chaeta; sv, seminal vesicle; te, teeth; to, tooth.

#### 2.4.19. Parapodia (19): absent (=0); present (=1)

Fleshy distinct parapodia are absent in *Protodrilus*, *Protodriloides* and *Ctenodrilus* (Jouin, 1978; Purschke and Jouin, 1988; Petersen and George, 1991; Rouse and Pleijel, 2001; Westheide, 2008), and present in the remaining species included in the analyses. In *Saccocirrus*, parapodia are uniramous, cylindrical, without lobes or cirri and bear 5–10 chaetae (Jouin and Gambi, 2007). In *Protodriloides chaetifer* and *Ctenodrilus serratus*, the hooked chaetae arise directly from the body wall.

#### 2.4.20. Parapodial ciliary tuft (20): absent (=0); present (=1)

Small ciliary tufts are present on the ventral side of the parapodia of all *Saccocirrus*. Each tuft consists of small groups of 5–10 cilia, up to 10 μm long. Similar parapodial ciliary tufts are absent in the adults of *Sabellaria* and *Polydora* (Rouse and Pleijel, 2001; Westheide, 2008). The character is inapplicable for *Protodrilus*, *Protodriloides* and *Ctenodrilus*.



**Fig. 2.** Morphology of the “papilocercus” group, light microscopic and scanning electron micrographs. (A) *Saccocirrus* sp. 7 from Giglio (Italy), ventral view of the anterior end showing the mouth. (B) *Saccocirrus pussicus*, segments 1–3 showing esophagus. (C) *Saccocirrus pussicus*; detail of the gut showing the presence of sand grains. (D) *Saccocirrus pussicus*, bilateral arrangement of seminal vesicles in a male. (E) *Saccocirrus pussicus*, bilateral arrangement of oocytes in a female. (F) *Saccocirrus* sp. 1 from Abades (Tenerife, Canary Island), equal forked longest chaeta. (G) *Saccocirrus* sp. 8 from Windsor Beach (Bermuda), unequal forked longest chaeta. (H) *Saccocirrus* sp. 7 from Giglio (Italy), unequal forked longest chaeta. Abbreviations: elc, equal longest chaeta; es, esophagus; mo, mouth; oo, oocytes; pa, palp; sd, sand grain; sv, seminal vesicle; ulc, unequal longest chaeta.

#### 2.4.21. Chaetae (21): absent (=0); present (=1)

Chaetae are present in all *Saccocirrus*, as well as *Protodriloides chaetifer*, *Polydora* and *Sabellaria*. Chaetae are absent in *Protodrilus* and *Protodriloides symbioticus* (Jouin, 1966; Westheide, 2008).

#### 2.4.22. Long chaetae with distinct apical morphology (22); absent (=0); present (=1)

All *Saccocirrus* presents three types of chaetae, except for *Saccocirrus* sp. 4 (La Palma, Canary Island), with five types. The three

types of chaetae common to all saccocirrids are usually identified as long, medium and short (Jouin and Gambi, 2007). The longest chaetae present a very characteristic morphology within each of the groups. Following the nomenclature proposed by Jouin and Gambi (2007), the longest chaetae in the species of the “papillocercus” group are coded as robust and forked (Section 2.4.25), whereas the delicate fan-shaped chaetae in the species of the “krusadensis” group are coded as lyrate (Section 2.4.26). This character is absent in all outgroups. *Sabellaria alveolata* and *Polydora giardi* present capillary chaetae, but they lack the distinct apical morphology present in saccocirrids (Petersen and George, 1991; Rouse and Pleijel, 2001; Surugiu, 2012). *Protodriloides chaetifer* and *Ctenodrilus serratus* only present a single type of short, hooked-like chaetae.

#### 2.4.23. Long forked chaetae (23): absent (=0); present (=1)

The robust and forked thick chaetae are present in the species of the “papillocercus” group (Section 2.4.25). The character is absent for species in the “krusadensis” group and inapplicable in the outgroups.

#### 2.4.24. Morphology of the long forked chaetae (24): with equal prongs (=0); with unequal prongs (=1)

The long forked chaetae of *Saccocirrus* sp. 7 (Giglio, Italy) (Fig. 2H), *Saccocirrus* sp. 8 (Bermuda) (Fig. 2G) and *Saccocirrus* sp. 9 (Belize) present unequal prongs; prongs are equal in the remaining species of “papillocercus” group included in the analyses (Fig. 2F). The character was inapplicable for species in the “krusadensis” group and outgroups.

#### 2.4.25. Long lyrate chaetae (25): absent (=0); present (=1)

Long, lyrate and delicate fan-shaped chaetae are present in the species of the “krusadensis” group (Section 2.4.27). The character was absent in the species of the “papillocercus” group and inapplicable for the outgroups.

#### 2.4.26. Morphology of the lyrate long chaetae (26): symmetrical (=0); asymmetrical (=1)

The lyrate chaeta was coded as asymmetrical in *Saccocirrus krusadensis*, *S. jouinae* and *S. tridentiger* (Fig. 1H), and symmetrical in the remaining species of the “krusadensis” group (Table 4). The character was inapplicable in the outgroups and species in the “papillocercus” group. Scanning electron microscopy examinations revealed the presence of three teeth (Fig. 1G and H) in all the lyrate chaetae examined in species of the “krusadensis” group, and not only in *S. tridentiger* as previously described (Brown, 1981). Therefore, the number of teeth was as a separate character is uninformative and not coded.

#### 2.4.27. Morphology of the medium chaetae (27): spatulate (=0); bifid (=1)

The medium chaeta was coded as spatulated when it presents an oar-shaped or smooth apex, as in all the species of the “papillocercus” group (Jouin and Gambi, 2007). It was instead coded as bifid when it has two equally long prongs as in the species of the “krusadensis” group. The character was inapplicable in the outgroups.

#### 2.4.28. Morphology of the short chaetae (28): spatulated (=0); bifid (=1)

The shortest and thin chaeta present in all saccocirrids was coded as bifid when it bears a notched apex, as in all the species of the “krusadensis” group; or spatulated when it presents a smooth tip, as in the species of the “papillocercus” group (Jouin and Gambi, 2007). The character was inapplicable for the outgroups.

#### 2.4.29. Seminal vesicle (29): absent (=0); present (=1)

Seminal vesicles are present in all *Saccocirrus* and consist of a sperm-sac with short ciliated ejaculatory ducts. The ejaculatory ducts lead into a robust and long penis with a conical tip (Figs. 1C and 2D) (Purschke and Jouin, 1988). Different penis structures are known among other saccocirrid species (Purschke, 2006), but this information could not be coded with accuracy in our dataset.

#### 2.4.30. Arrangement of the seminal vesicle (30): bilateral (=0); right side (=1); left side (=2)

Seminal vesicles are arranged at both sides of the trunk in all the species of the “papillocercus” group (Fig. 2D), whereas they are arranged only in one of the sides in the “krusadensis” group. The character was inapplicable for the outgroups.

#### 2.4.31. Pygidial lobes (31): absent (=0); present (=1)

*Protodrilus*, *Protodriloides* and *Saccocirrus* bear paired pygidial lobes with adhesive glands (Jouin, 1966; Martin, 1978; Von Nordheim, 1989). Pygidial lobes are absent in *Polydora*, *Sabellaria* and *Ctenodrilus*. *Polydora giardi* has a cylindrical pygidium, dorsally incised (Fauvel, 1927).

#### 2.4.32. Shape of the pygidial lobes (32): paddle shaped (=0); cylindrical (=1); rounded (=2)

Pygidial lobes are cylindrical, with transverse adhesive ridges in all *Saccocirrus* (Martin, 1978). *Protodrilus* instead presents paddle-shaped pygidial lobes with terminal adhesive glands (Von Nordheim, 1989), whereas pygidial lobes are short and rounded in *Protodriloides* (Jouin, 1966). The character is inapplicable for *Polydora*, *Sabellaria* and *Ctenodrilus* (Rouse and Pleijel, 2001).

#### 2.4.33. Transverse pygidial adhesive papillae (33): absent (=0); present (=1)

In *Saccocirrus*, the two pygidial lobes are subdivided into a variable number of transverse papillae. Each pygidial ridge bears several adhesive duo-glands. Duo-glands are also described in *Protodriloides* and *Protodrilus* (Jouin, 1966; Martin, 1978), but they are not arranged in papillae but as terminal rows in the pygidium (Du Bois-Reymond Marcus, 1948). Transverse adhesive papillae are absent in *Polydora*, and inapplicable for *Sabellaria* and *Ctenodrilus* (Rouse and Pleijel, 2001).

#### 2.4.34. Number of pygidial adhesive papillae (34): 0–5 (=0); 6–10 (=1); 11–15 (=2); more than 16 (=3)

A species-specific range of transverse adhesive papillae is present on the pygidium of *Saccocirrus*. The character is inapplicable for *Protodrilus*, *Protodriloides*, *Ctenodrilus serratus*, *Polydora* or *Sabellaria* (Rouse and Pleijel, 2001; Westheide, 2008).

## 2.5. Alignments and data analyses

The molecular dataset was analyzed on static alignments inferred for each gene independently using MAFFT version 6 (Katoh et al., 2002; Katoh and Toh, 2010). L-ins-I (16s rRNA, H3 and 28s rRNA) and L-ins-E (18s rRNA) were the selected aligning strategies. Sequences for the protein-coding gene H3 were confirmed for reading frame before treatment with Gblocks. H3 sequences were constant in length; therefore, no gaps were permitted within blocks.

The initially aligned dataset consist of 1899 base pairs for 18S rRNA, 1163 bp for 28S rRNA, 689 bp for 16S rRNA and 331 bp for H3, reduced to 1713 bp (18S rRNA), 1022 bp (28S rRNA), 341 bp (16S rRNA) and 324 bp (H3) after treatment with Gblocks. The positions of the H3 gene fragment trimmed by Gblocks correspond to terminal positions missing for some terminals due to the

sequencing process. Individual gene alignments and the combined dataset were analyzed using maximum likelihood (ML) and Bayesian methods (BA).

ML analyses were performed using RaxML-VI-HPC (Stamatakis et al., 2008) at CIPRES Science Gateway implemented on the Trestles TeraGrid cluster (Miller et al., 2010; <http://www.phylo.org>) or RaxML GUI (Silvestro and Michalak, 2011). ML analyses of the combined data, partitioned genes, were run with a GTR +  $\Gamma$  model. Nodal support was calculated after 1000 pseudoreplicates of a non-parametric bootstrap, with a GTR +  $\Gamma$  model of evolution.

BA analyses were performed in MRBAYES v.3.1.2 (Ronquist and Huelsenbeck, 2003). JModelTest (Posada, 2008) was used to infer evolutionary models for each gene. Best-fit evolutionary model was selected after the Akaike information criterion. A K2 +  $\Gamma$  model was selected for H3, GTR +  $\Gamma$  for 16S rRNA and 28S rRNA, and GTR +  $\Gamma$  + I for 18S rRNA. The morphological data partition was analyzed with a MkV model and gamma correction. Four MCMCMC were run in two independent runs during 10,000,000 generations. Samples were set every 1000 generation and the first 25% of the samples were discarded as burn-in after assessing convergence using Tracer 1.4.1 (Rambaut and Drummond, 2007).

Morphological character evolution was reconstructed with the program Mesquite 2.75 (Maddison and Maddison, 2011) using parsimony on the fully resolved consensus tree from the Bayesian searches of the combined analysis (molecules and morphology). This tree was congruent with best tree from maximum likelihood analyses. Apomorphic states were plotted at the nodes corresponding to Saccocirridae as well as the “krusadensis” and “papillocercus” groups, in order to highlight the synapomorphies for each of the clades.

### 2.6. Analyses of geographic distribution patterns

Analyses of the geographical distribution patterns were performed on a matrix including all available geographical data for the 32 described and undescribed species of *Saccocirrus*. Both published and our own unpublished data were included for all described saccocirrids as well as the new species reported in this study. Geographical coordinates for our own records were directly taken in the field or estimated from maps (Table 1). Records from the literature were georeferenced with [www.getlatlon.com](http://www.getlatlon.com) site (Table 3). The distributions of the species in the “papillocercus” and “krusadensis” groups were plotted with the package ‘maps’ and ‘mapdata’ in R (Becker et al., 2013; Brownrigg, 2013).

The preferences for tropical, subtropical or polar zones were inferred using correlation between the numbers of species recorded at each 10 degrees of latitude. The “papillocercus” and “krusadensis” groups preferences for Indian Ocean, Pacific Ocean and Atlantic Ocean were tested using correlation between the numbers of species recorded in each subgroup every 40 degrees of longitude. Though surface water temperature is not the only factor to regulate the diversity of saccocirrids on a global scale, it may well limit the maximum attainable diversity for the family or subgroups. In the same way, Indo-Pacific preference of “krusadensis” species had never been tested. The maximum diversity of saccocirrids was used as an indication of the latitudinal and longitudinal preferences of the family. Geographical ranges with maximum diversity were estimated using quantile regression spline models (Koenker et al., 1994; Koenker, 2005; Anderson, 2008) built for the 95th percentile (i.e., the value below the diversity is expected to fall a 95%, the  $\tau = 0.95$  quantile), which is less sensitive to outliers (Anderson, 2008). Models were fitted using the functions `rq()` and `bs()`, part of the “quantreg” package for R (Hastie, 1993; Koenker, 2007; R Development Core Team, 2011). The function `bs()` provides a flexible way of constructing B-spline basis expansions to fit a piecewise polynomial of a specified degree. The degree of the

polynomial (resulting in a given number of parameters for the spline model) was optimized after the Akaike’s information criterion corrected for small-samples (Hurvich and Tsai, 1989; Burnham and Anderson, 2002). The model with the smallest AICc value from a set of models with a degree of polynomial of 2, 3, 4 or 5 was chosen. Peaks with optimal values were interpreted. Ninety-five percent bootstrap confidence intervals (Manly, 2006) were obtained for the estimated optimum using bias-corrected percentiles from the re-application of the model to each of the 10,000 bootstrapped sample pairs, using the polynomial degree that was chosen for the original data.

## 3. Results

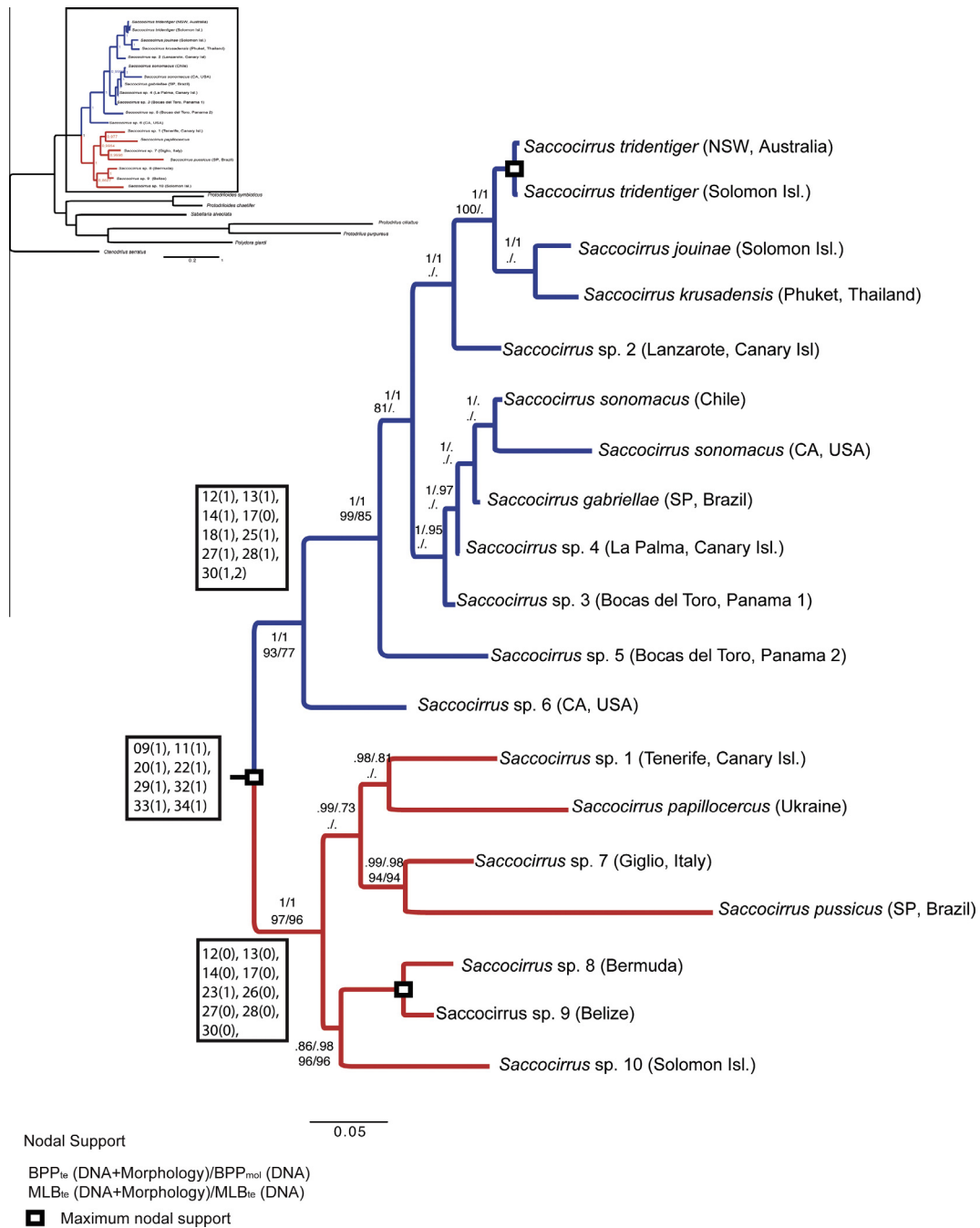
### 3.1. Phylogenetic analyses

Saccocirridae (Bayesian posterior probability,  $BPP_{te} = 1.00$ ,  $BPP_{mol} = 1.00$ ; maximum likelihood bootstrap,  $MLB_{te} = 100$ ,  $MLB_{mol} = 100$ ), as well as both “krusadensis” ( $BPP_{te} = 1.00$ ,  $BPP_{mol} = 1.00$ ;  $MLB_{te} = 88$ ,  $MLB_{mol} = 77$ ) and “papillocercus” groups ( $BPP_{te} = 1.00$ ,  $BPP_{mol} = 1.00$ ;  $MLB_{te} = 97$ ,  $MLB_{mol} = 96$ ) were monophyletic in all the analyses (Fig. 3). The Saccocirridae relationship with the outgroups were poorly supported, but the putative clade Protodrilida was never recovered.

The “papillocercus” group consisted of a monophyletic group with seven species in two sister clades. One of the clades included exclusively undescribed species ( $BPP_{te} = 0.86$ ,  $BPP_{mol} = 0.98$ ;  $MLB_{te} = 96$ ,  $MLB_{mol} = 96$ ) with *Saccocirrus* sp. 10 (Solomon Island) sister to *Saccocirrus* sp. 9 (Belize) and *Saccocirrus* sp. 8 (Bermuda) ( $BPP_{te} = 1.00$ ,  $BPP_{mol} = 1.00$ ;  $MLB_{te} = 100$ ,  $MLB_{mol} = 100$ ). The second clade, only recovered under BA ( $BPP_{te} = 0.99$ ,  $BPP_{mol} = 0.73$ ), included *S. pussicus* and *Saccocirrus* sp. 7 (Giglio, Italy) ( $BPP_{te} = 0.99$ ,  $BPP_{mol} = 0.98$ ;  $MLB_{te} = 94$ ,  $MLB_{mol} = 94$ ), sister to *S. papillocercus* and *Saccocirrus* sp. 1 (Tenerife, Canary Island) ( $BPP_{te} = 0.98$ ,  $BPP_{mol} = 0.81$ ).

The “krusadensis” group was recovered as a clade with 12 species. The new species *Saccocirrus* sp. 6 (CA, USA) and *Saccocirrus* sp. 5 (Bocas del Toro, Panama) branch off together near the root of the clade, sister to two derived clades. One clade ( $BPP_{te} = 1.00$ ,  $BPP_{mol} = 0.95$ ) contained the following taxa branching off subsequently from the root: *Saccocirrus* sp. 3 (Bocas del Toro, Panama) ( $BPP_{te} = 1.00$ ,  $BPP_{mol} = 0.97$ ), *Saccocirrus* sp. 4 (La Palma, Canary Isl) ( $BPP_{te} = 1$ ), *S. gabriellae* ( $BPP_{te} = 1$ ) and *S. sonomacus* (CA, USA) and *S. sonomacus* (Chile). The second clade ( $BPP_{te} = 1.00$ ,  $BPP_{mol} = 1.00$ ) included *Saccocirrus* sp. 2 (Lanzarote, Canary Island) branching off next to two sister clades ( $BPP_{te} = 1.00$ ,  $BPP_{mol} = 1.00$ ;  $MLB_{te} = 100$ ), with *S. krusadensis* and *S. jouinae* ( $BPP_{te} = 1.00$ ,  $BPP_{mol} = 1.00$ ) and *S. tridentiger* (NSW, Australia) and *S. tridentiger* (Solomon Island) ( $BPP_{te} = 1.00$ ,  $BPP_{mol} = 1.00$ ;  $MLB_{te} = 100$ ,  $MLB_{mol} = 100$ ), reciprocally monophyletic.

The monophyly of the family Saccocirridae was supported by unique morphological apomorphies: Palps with internal canal connected behind the brain (character, ch 08) and provided with basal ampullae (ch 09), epidermal eyes with microvilli in the shading pigment cell (ch 11), parapodial ciliary tuft (ch 20), presence of distinct long chaetae (ch 22), presence of seminal vesicle (ch 29), cylindrical pygidial lobes (ch 32) with transverse pygidial adhesive papilla (ch 33). The number of pygidial papillae presented two transformations within the family, being 6–10 in the ancestral state (ch 34), with transformations into 0–5 in *S. pussicus* and 16–20 in *Saccocirrus* sp. 1 (Tenerife, Canary Islands). This leaves the following species with the ancestral number of 6–10 transverse pygidial adhesive papillae: *Saccocirrus papillocercus*, *Saccocirrus* sp. 9 (Belize), *Saccocirrus* sp. 8 (Bermuda), *Saccocirrus* sp. 10 (Solomon Island), *Saccocirrus krusadensis*, *Saccocirrus* sp. 4 (La Palma, Canary



**Fig. 3.** Phylogenetic relationships of Saccocirridae. Tree from the Bayesian analysis, congruent with the maximum likelihood best tree. First line on the node represent Bayesian posterior probabilities (BPP) with molecular and morphological data and BPP only with molecular data; second line, maximum likelihood bootstrap (MLB) with molecular and morphological data and BPP only with molecular data. “Krusadensis” (*Pharyngocirrus* gen. nov.) clade is colored in blue and “papilloercus” clade in red. Box on the branches indicate unambiguous character transformations after character reconstructions, the character states are indicated between parentheses and explained in the Section 2.4. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Island), *Saccocirrus* sp. 2 (Lanzarote, Canary Island) and *Saccocirrus* sp. 5 (Bocas de Toro, Panama).

Additional morphological apomorphies delineated and distinguished the clades “papilloercus” and “krusadensis”. The “krusadensis” clade was characterized by the presence of prostomial transverse ciliary band (ch 12, unique apomorphy), presence of ventral pharyngeal apparatus with tongue-like organ (ch 13 and 14) (Fig. 1E), presence of mouth ciliation arranged as paired longitudinal bands (ch 17, unique apomorphy), unilateral arrangement of the seminal vesicle (ch 30) (Fig. 1C), long chaetae lyrate (ch 25, unique apomorphy) (Fig. 1G and H), bifid medium chaetae

and notched apex short chaetae (ch 27 and 28, unique apomorphy). The presence of asymmetrical lyrate chaetae (ch 26, unique apomorphy), presence of midventral ciliary band up to segment 10 (ch 17–18, see discussion) (Fig. 1B) were the synapomorphies supporting a clade with *Saccocirrus krusadensis*, *S. jouinae* and *S. tridentiger*.

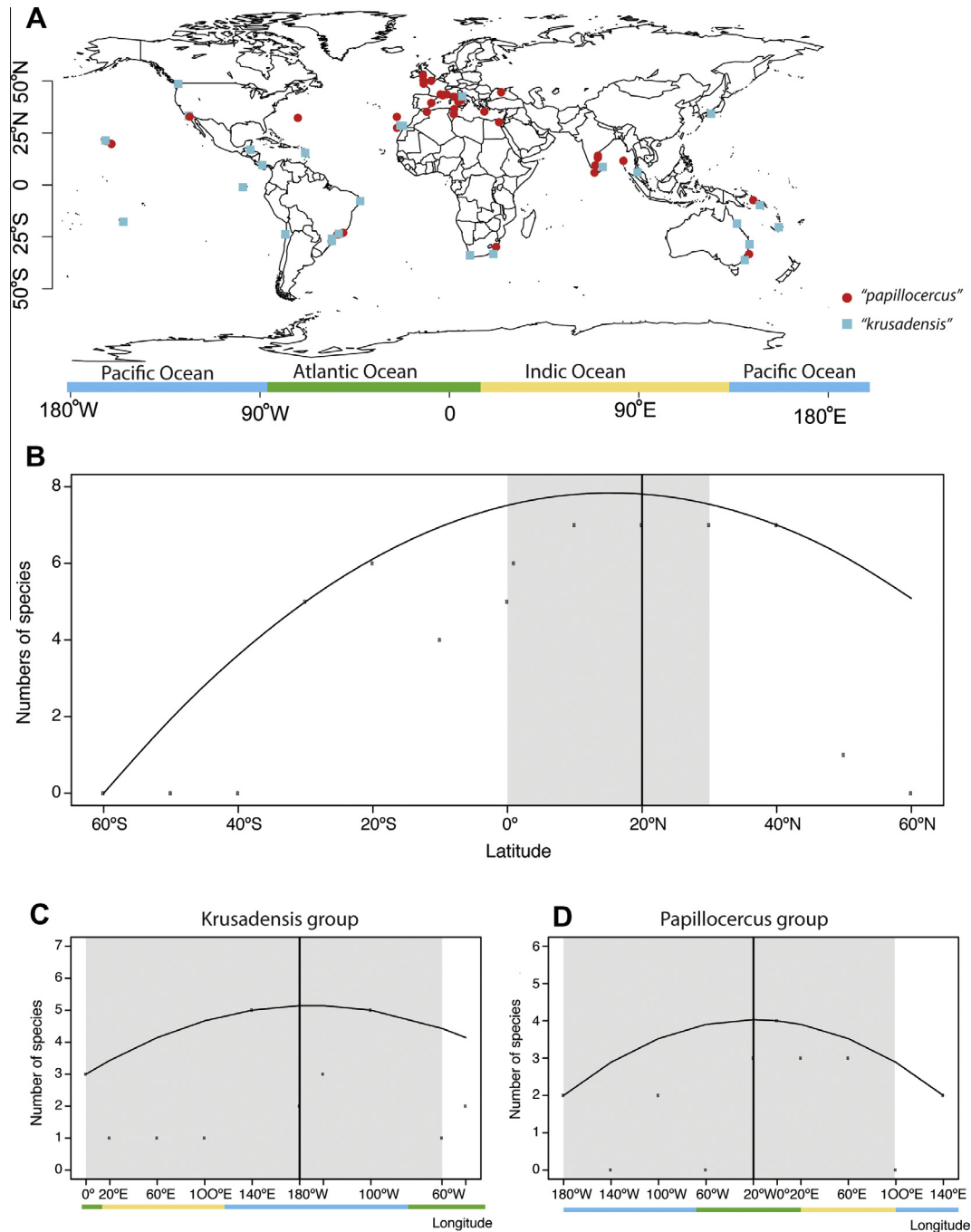
The “papilloercus” clade was delineated by the following synapomorphies: presence of bilateral seminal vesicle (ch 30, unique apomorphy) (Fig. 2D and E), robust forked chaetae (ch 23, unique apomorphy) (Fig. 2F–H), spatulated medium and short chaetae (ch 26–27, unique apomorphies). Additionally, all the species of

the clade lack prostomial transverse ciliary band (ch 12), pharyngeal bulbous muscle (ch 13 and 14) (Fig. 2B), ciliary patches around the buccal area (ch 17) (Fig. 2A).

### 3.2. Geographic distribution patterns

Geographic analyses yielded a well-supported diversity gradient of Saccocirridae, with a maximal diversity estimated at 20°N, with the latitudinal range between 0° and 30°N within the 95%. A steep decrease in diversity was registered from 0° to 30°N towards both poles (Fig. 4A and B).

The comparison of the longitudinal distribution patterns between “papillocercus” and “krusadensis” clades (Fig. 4C and D) shows the homogeneous diversity of both lineages throughout all longitudes, with no significant optimal values. The clade “krusadensis” showed a non-linear asymmetric unimodal relationship with longitudes. The longitude for the highest diversity was estimated in the middle of the Pacific Ocean at 180°W, but with wide ranges of longitudes (0–180°E; 180–60°W) within the 95% confidence interval. Species belonging to the “papillocercus” clade are distributed mainly in the Mediterranean and North Sea along the coast of Europe, with an optimal value estimated at 20°W, but with



**Fig. 4.** (A) Geographic distribution of the groups “papillocercus” and “krusadensis” (*Pharyngocirrus* gen. nov.) based on Tables 1 and 3. (B) Spline smoothing with polynomial regression ( $n = 14$ ) of latitude and number of *Saccocirrus* species showing the optimum value for diversity (line) and 95% confidence interval (gray). (C) Spline smoothing with polynomial regression ( $n = 10$ ) of longitudinal and number of *Saccocirrus* species of the krusadensis group showing the optimum value for diversity (line) and 95% confidence interval (gray). (D and C) Spline smoothing with polynomial regression ( $n = 10$ ) of longitudinal and number of *Saccocirrus* species of the papillocercus group showing the optimum value for diversity (line) and 95% confidence interval (gray).

wide ranges of longitudes 180°W to 100°E within the 95% confidence interval.

## 4. Discussion

### 4.1. Saccocirridae phylogeny

The monophyly of Saccocirridae was fully supported in all analyses, but as in other studies (Rousset et al., 2007; Struck et al., 2008; Zrzavy et al., 2009; Golombek et al., 2013), neither sister relationship between *Saccocirrus* and *Protodrilus* or a monophyletic Protodrilida were recovered. Yet, our study is not intended to address the position of Saccocirridae due to the limited number of outgroups and few sequenced genes.

Saccocirridae was sustained by several synapomorphies in our analyses, congruent with previous morphological studies (Purschke and Jouin, 1988). These synapomorphies included the presence of palp canals with basal ampullae, transverse pygidial adhesive ridges, parapodial ciliary tufts, penis and seminal vesicles, as well as the presence of specific type long chaetae. Several of these synapomorphies are clear adaptations to interstitial life. Transverse pygidial adhesive ridges provide attachment and stability among the sand grains and seminal vesicle and penis facilitate sperm transfer in these turbulent upper layers of the seafloor (Westheide, 1984). Other reproductive adaptations to these environments are the genital hooks in *S. heterochaetus* (Jouin, 1975) or the ovoviviparity in *S. burcheli* (Silberbauer, 1969) and *Saccocirrus* sp. 5 (Bocas del Toro, Panama). These features are not included in our analyses since they are apomorphies of terminals only.

Conversely, the adaptive value of other apomorphies of the family, e.g., presence of basal ampullae, parapodial tufts or presence of specific type of chaetae, is more difficult to assess. Basal ampullae aid to the stiffness control of the palps by varying the pressure within the canals (Purschke, 1993). Functionally similar ampullae are present in other groups, traditionally included in Canalipalpata, such as Spionidae or Magelonidae (Rouse and Fauchald, 1997). However, these sacs are smaller and normally included in the prostomium. Although homology of both structures has been proposed (Purschke, 1993), more recent molecular phylogenies indicate they alternatively may represent convergences, favoured by similar functional constraints related to independent evolution of long motile palps.

The monophyly of the clades “krusadensis” and “papillocercus” was fully supported in all analyses and well sustained morphologically, as expected from previous morphological studies (Brown, 1981; Purschke and Jouin, 1988; Purschke, 1992; Jouin and Gambi, 2007; Westheide, 2008). The most striking morphological difference between “papillocercus” and “krusadensis” clade is the lack of a ventral pharyngeal bulbus muscle in the “papillocercus” (Purschke and Jouin, 1988; Purschke and Tzvetlin, 1996). The lack of pharynx in the “papillocercus” has been previously interpreted as a loss under the Protodrilida hypothesis, since a similar pharynx is shared by Protodrilidae and Protodriloidae (Purschke and Jouin, 1988; Purschke, 1988; Westheide, 2008). However, Protodrilida was unrecovered in our analyses and the sister group of Saccocirridae remains unknown (Rousset et al., 2007; Struck et al., 2008; Zrzavy et al., 2009; Golombek et al., 2013), affecting the tracing of the pharynx on the root of Saccocirridae. On the other hand, ventral pharyngeal organs with bulbous muscle are common among polychaetes and might represent a plesiomorphy of annelids (Purschke and Tzvetlin, 1996; Tzvetlin and Purschke, 2005). This argument seems to favor the interpretation of absence of pharynx in “papillocercus” as a loss (Sasaki and Brown, 1983; Purschke and Jouin, 1988; Jouin and Gambi, 2007), until the position of Saccocirridae is fully resolved.

The presence of ventral mouth ciliation arranged as paired longitudinal bands is a synapomorphy for the “krusadensis” clade (Brown, 1981; Jouin and Gambi, 2007), whereas the presence of a midventral ciliary band only supports a derived subclade consisting of *Saccocirrus tridentiger*, *S. jouinae* and *S. krusadensis*. Mouth and midventral ciliation, as well as prostomial transverse ciliary band, may have evolved as retention of larval characters into the adult stage (underdevelopment, paedomorphosis). Midventral and mouth ciliation are at least present in the larva of *S. uchidai* (Sasaki and Brown, 1983), the only saccocirrid for which the complete larval development is known. During development of this species, the midventral ciliary band is progressively reduced and lost in most of the adults, and mouth ciliation is retained (Sasaki and Brown, 1983). Retention of larval features is common in other interstitial groups (Worsaae et al., 2012; Westheide, 1997; Worsaae and Kristensen, 2005). However, the retention of larval features in the clade “krusadensis” then seems restricted to the ciliary patterns. Other synapomorphies of the clade are difficult to interpret as retention of larval characters.

The morphologically diverging clades “papillocercus” and “krusadensis” are corroborated by the phylogenetic analyses and are shown to represent systematic significant entities and good candidates for well sustained taxonomic units (see Section 4.3). This divergence is correlated with different trophic guilds and habitat preferences in each clade. Members of both groups are sometimes categorized as deposit-feeders, but species of “krusadensis” are more specialized bacteria-diatom grazers (e.g. diatoms, Fig. 1F), typically occurring in sheltered intertidal and subtidal reefs and rocky shores (see Table 3, Brown, 1981; Du Bois-Reymond Marcus, 1946; Jouin and Gambi, 2007). In these species, a muscular ventral pharyngeal bulbous and ventral ciliation around the mouth may facilitate grazing on biofilms and ingestion of deposited organic matter. In contrast, members of the clade “papillocercus” are scavengers or microphagous (e.g. gut with sand grains, Fig. 2C), more common in the intertidal zones of sandy beaches with strong hydrodynamics (Du Bois-Reymond Marcus, 1948; Di Domenico et al., 2009; Jouin and Gambi, 2007). The high turbulence in these habitats prevents the deposition of organic matter, so palps (rather than pharynx) possibly play a more decisive role in feeding in these species (Di Domenico et al., 2014).

### 4.2. Geographic distribution

The highest species diversity of saccocirrids is estimated between 0° and 30°N, which correspond to the equatorial and north subtropical region. Similar diversity patterns are reported in several other marine groups (Huston, 1994). The species diversity decreases towards the poles, although the decrease is steeper in the southern hemisphere than in the northern hemisphere. This asymmetrical decrease in the diversity is most likely related to a higher sampling and taxonomical effort in the Mediterranean Sea and the North Atlantic (Table 3), however, it may also be influenced by the longer coastline of the northern hemisphere containing more km of suitable coarse sandy beaches.

Previous studies on Saccocirridae suggested vicariant distribution patterns for the “krusadensis” and “papillocercus” clades. Species in the clade “krusadensis” were mostly reported in the Indopacific, while “papillocercus” clade was more abundant at the Atlantic (Jouin and Gambi, 2007). However, our more extensive sampling shows that both the “krusadensis” and “papillocercus” clades are geographically widespread.

The wide geographic distribution exhibited by Saccocirridae suggests an ancient origin, as for other annelid families. Though saccocirrids disperse by free-living larvae, their planktotrophic stage has a short-time duration. This, as well as the lack of consistent geographical distribution patterns, makes it unlikely that the

current wide distribution of the family is the result of recent dispersal events. In contrast to this geographical ubiquity, species in each clade seemingly exhibit distinct habitat preferences, with members of the “krusadensis” group inhabiting sheltered sandy habitats, and members of the “papillocercus” group specialized to exposed sandy beaches.

The worldwide distribution patterns of each clade, the short-time planktotrophic larval stages (Sasaki and Brown, 1983), and the seeming habitat preferences of the species in each clade indicate an early ecological diversification of Saccocirridae into two diverging interstitial habitats (Gerlach, 1977; Sterrer, 1973). Further characterization of the habitats of a variety of Saccocirridae is warranted in order to test this hypothesis.

#### 4.3. Taxonomic implications

The phylogenetic analyses and reconstructed morphological apomorphies of the “krusadensis” and “papillocercus” clades are here addressed for taxonomic consequences. Several works proposed these two groups of Saccocirridae as monophyletic (Westheide, 2008) or at least as morpho-functionals (Brown, 1981; Jouin and Gambi, 2007; Di Domenico et al., 2014). We hereof propose a new generic name for the “krusadensis” clade and provide an emended diagnosis for *Saccocirrus*. Both genera include the species from the analyses herein as well as other described species that fit their diagnoses.

*Pharyngocirrus*, gen. nov. Di Domenico, Martínez, Lana and Worsaae.

Type species: *Pharyngocirrus gabriellae* (Du Bois-Reymond Marcus, 1946).

Included species: *Pharyngocirrus gabriellae* (Du Bois-Reymond Marcus, 1946); *P. krusadensis* (Alikunhi, 1948); *P. archiboldi* (Kirsteuer, 1967); *P. eroticus* (Gray, 1969); *P. burchelli* (Silberbauer, 1969); *P. labilis* (Yamanishi, 1973); *P. sonomacus* (Martin, 1977); *P. jouinae* (Brown, 1981); *P. tridentiger* (Brown, 1981); *P. uchidai* (Sasaki, 1981); *P. goodrichi* (Jouin-Toulmond and Gambi, 2007); *P. alanhongii* (Bailey-Brock et al., 2003).

Diagnosis: Brown body. Prostomium with two pigmented eyes and long filiform palps. Presence of prostomial transverse ciliary band. Mouth surrounded by ciliary patches consisting of paired longitudinal bands. Mid-ventral ciliary band can be present. Ventral muscular pharynx present. Uniramous parapodia with three types of chaetae: (1) long capillary chaetae lyrate (equal or unequal sides) with a small median tooth; (2) medium bifid chaetae with equal lateral prongs and (3) short chaetae with notched apex. Females with unilateral ovaries at the right or left side of the gut. Males with unilateral seminal vesicles at the right or left side of the gut.

Molecular evidence: The monophyly of *Pharyngocirrus* gen. nov. is well supported by molecular data including both nuclear and mitochondrial genes (BPP<sub>mol</sub> = 1.00; MLB<sub>mol</sub> = 77), as well as by total evidence analyses including morphological data (BPP<sub>te</sub> = 1.00; MLB<sub>te</sub> = 93).

Habitat and distribution: Species of *Pharyngocirrus* gen. nov. occur intertidally at sheltered beaches, bays or coves, between rocks in tidal pools or subtidally. Generally they occur in coarse sand with a well-defined redox layer.

Etymology: From the Latin, “*Pharyngo*” (pharynx), as a reference to the characteristic ventral pharynx; “*cirrus*”, from latin (curl, tuft) with reference to the sister taxon *Saccocirrus*.

*Saccocirrus* Brobretzky, 1872, emended.

Type species: *Saccocirrus papillocercus* Brobretzky, 1872.

Included species: *Saccocirrus papillocercus* Brobretzky, 1872; *S. major* Pierantoni, 1907; *S. orientalis* Alikunhi, 1946; *S. minor* Aiyar e Alikunhi, 1944; *S. pussicus* Du Bois-Reymond Marcus, 1948; *S. heterochaetus* Jouin, 1975; *S. parvus* Gerlach, 1953; *S. oahuensis*

Bailey-Brock et al., 2003; *S. waianaensis* Bailey-Brock et al., 2003; *S. cirratus* Aiyar and Alikunhi, 1944.

Emended diagnosis: Brown body. Prostomium with two pigmented eyes and long filiform palps. Uniramous parapodia with three types of chaetae: (1) 1–2 long chaetae, robust and forked with equal or unequal prongs; (2) 2–3 medium spatuled chaetae; and (3) 2–3 short spatuled chaetae, with notched apex. Females with bilateral ovaries. Males with bilateral seminal vesicles.

Molecular evidence: The monophyly of *Saccocirrus* is well supported by molecular data including both nuclear and mitochondrial genes (BPP<sub>mol</sub> = 1.00; MLB<sub>mol</sub> = 97), as well as by total evidence analyses including morphological data (BPP<sub>te</sub> = 100; MLB<sub>te</sub> = 96).

Habitat and distribution: Species of *Saccocirrus* are found intertidally in well-oxygenated coarse sand of exposed beaches.

#### Acknowledgments

We thank our colleagues, technicians and staffs of the Marine Biological Section – University of Copenhagen, especially Aleksandra Tofteby for her help with DNA extraction and amplification. Field efforts were in part supported by a grant from Encyclopedia of Life Biodiversity Synthesis Group to J.L. Norenburg and R. Collin. We are greatly indebted to R. Collin and the rest of the staff at the Bocas Marine Laboratory of the Smithsonian Tropical Research Institute. We thank all the students and professors who participated at the surveys at Bocas del Toro, and who helped sorting out valuable material. We thank K. Jörger for the material collected in Italy and Thailand, Elena Mastepanova for the material collected in Black Sea, and P.R. Møller and D.I. Gouge for assisting with collecting (often by SCUBA diving) at Belize and Bocas del Toro. Special thanks to G. Rouse, SCRIPPS Institute of Marine Science, UCSD for the material from Australia, and his assistance in finding the Californian material as well as for his great hospitality. The project was further supported by the participants of the I International Workshop on Marine and Anchialine Meiofauna in Lanzarote, especially our students K. Kvindebjerg and A. Partavian. The staff at the Aula de la Naturaleza and Carlos Dizzi and his family from Las Paredas Park kindly hosted us during our field trips. We are grateful to Marcio Pie for the comments to the first draft of the manuscript. We thank two anonymous reviewers for their constructive comments. This research is a result of the Freja grant of K.W. as well as research grants to K.W. from the Danish Independent Research Council (Grant # 272-06-0260) and Carlsberg Foundation (Grant # 2010\_01\_0802), which funded the laboratory work and salaries. This study was also supported by the Brazilian National Council for Technological and Scientific Development (CNPq – Process 140611/2008-8), which provided the PhD fellowship of MDD, and São Paulo Research Foundation (FAPESP – Process 2012/08581-0, 2013/04358-7) which provided postdoctoral fellowships and grants for MDD. The collections at the Canary Islands were mainly funded by Reserva de la Biosfera (Government of Lanzarote).

#### References

- Abd-Elnaby, F.A., 2009. New records from the south part of Suez Canal, Egypt. *World J. Fish Mar. Sci.* 1 (1), 07–19.
- Aiyar, R.G., Alikunhi, K.H., 1944. On some archiannelids of the Madras Coast. *Proc. Natl. Inst. Sci. Ind.* 10 (1), 113–140.
- Alikunhi, K.H., 1946. On a new species of *Saccocirrus* from the Madras Beach. *Curr. Sci. (Bangalore)* 15, 149.
- Alikunhi, K.H., 1948. On some archiannelids of Krusadai Island. *Proceedings of the National Institute of Sciences of India* 14, 373–383.
- Anderson, M.J., 2008. Animal-sediment relationships re-visited: characterising species' distributions along an environmental gradient using canonical analysis and quantile regression splines. *J. Exp. Mar. Biol. Ecol.* 366, 16–27.
- Bailey-Brock, J.H., Dreyer, J., Brock, R.E., 2003. Three new species of *Saccocirrus* (Polychaeta: Saccocirridae) from Hawai'i. *Pac. Sci.* 57, 463–478.



- Becker, R.A., Wilks, A.R., Brownrigg, R., Minka, T.P., 2013. Maps: Draw Geographical Maps. R Package Version 2.3-2. <<http://cran.r-project.org/package=maps>> (accessed 13.03.13).
- Blake, J.A., 1980. *Polydora* and *Boccaria* species (Polychaeta: Spionidae) from western Mexico, chiefly from calcareous habitats. *Proc. Biol. Soc. Wash.* 93 (4), 947–962.
- Boaden, P.J.S., 1963. The interstitial fauna of some New Wales beaches. *J. Mar. Biol. Assoc. UK* 43, 79–96.
- Bobretzky, N.V., 1872. *Saccocirrus papillocerus*, n. gen., n. sp. Tip' novago semeistra annelid. *Sravmitel'no-Anatomichskii obcherk. Mem. Kiev. Univ. Odsch. Estest. Zapisky* 2, 211–259.
- Brown, S., Rouse, G., Hutchings, P., Colgan, D., 1999. Assessing the usefulness of histone H3, U2 snRNA and 28S rDNA in analysis of polychaete relationships. *Australian Journal of Zoology* 47, 499–516.
- Brown, R., 1981. Saccocirridae (Annelida: Archiannelida) from the central coast of New South Wales. *Aust. J. Mar. Freshwater Res.* 32, 439–456.
- Brownrigg, M.R., 2013. Mapdata: Extra Map Databases. R Package 'mapdata' 2.2-2. <<http://cran.r-project.org/package=mapdata>> (accessed 01.03.13).
- Burnham, K.P., Anderson, D.R., 2002. Model selection and multi-model inference: a practical information-theoretic approach (2nd edition), Springer, New York.
- Cabioch, L., L'Hardy, J.P., Rullier, F., 1968. Inventaire de la faune marine de Roscoff. *Annélides. Trav Stn Biol Roscoff* 17, 1–95.
- Cohen, B.L., Nadia Améziane, N., Eleaume, M., Forges, B.R., 2004. Crinoid phylogeny: a preliminary analysis (Echinodermata: Crinoidea). *Marine Biology* 144, 605–617.
- Colgan, D.J., McLauchlan, A., Wilson, G.D.F., Livingston, S.P., Edgecombe, G.D., Macaranas, J., Cassis, G., Gray, M.R., 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Aust. J. Zool.* 46, 419–437.
- Dauvin, J.C., Dewarumez, J.M., Gentil, F., 2003. Liste actualisée des espèces d'Annélides Polychètes présentes en Manche. *Cahiers de Biologie Marine* 44, 67–95.
- Delamare-Deboutteville, C., Gerlach, S., Siewing, R., 1954. Recherches sur la faune des eaux souterraines littorales du Golfe de Gascogne. *Vie Milieu* 5, 373–407.
- Di Domenico, M., Lana, P.C., Garraffoni, A.R.S., 2009. Distribution patterns of interstitial polychaetes in sandy beaches of southern Brazil. *Mar. Ecol.* 30, 47–62.
- Di Domenico, M., Martínez, A., Lana, P.C., Worsaae, K., 2013. *Protodrilus* (Annelida, Protodrilidae) from the southern and southeastern Brazilian coasts. *Helgoland Mar. Res.* 67, 733–748.
- Di Domenico, M., Martínez, A., Amaral, C., Lana, P.C., Worsaae, K., 2014. Saccocirridae (Annelida) from the southern and southeastern Brazilian coasts. *Mar. Biodivers.* <http://dx.doi.org/10.1007/s12526-014-0208-5>.
- Du Bois-Reymond Marcus, E., 1946. On a New Archeannelid, *Saccocirrus gabriellae*, from Brazil. *Commun. Zool. Mus. Hist. Nat. Montev.* 37 (2), 1–11.
- Du Bois-Reymond Marcus, E., 1948. Further archiannelids from Brazil. *Commun. Zool. Mus. Hist. Nat. Montev.* 2, 1–22.
- Eakin, R.M., Martin, G.G., Reed, C.T., 1977. Evolutionary significance of fine structure of archiannelid eyes. *Zoomorphologie* 88, 1–18.
- Fauvel P., Faune de France 16. Polychètes sédentaires. P. Lechevallier, Paris, 1927, 494 pp.
- Fize, A., 1963. Contribution à l'étude de la microfaune des sables littoraux du Golfe d'Aigues-Mortes. *Vie et Milieu* 14, 669–774.
- Gerlach, S.A., 1953. Zur Kenntnis der Archianneliden des Mittelmeeres. *Kiel. Meeresforsch.* 9, 248–251.
- Gerlach, S.A., 1977. Means of meiofauna dispersal. In: Sterrer, W., Ax, P. (Eds.), *The Meiofauna Species in Time and Space*, vol. 61. Mikrofauna Meeresbod, pp. 89–103.
- Golombek, A., Tobergte, S., Nesnidal, M.P., Purschke, G., Struck, T.H., 2013. Mitochondrial genomes to the rescue – Diuridrilidae in the myzostomid trap. *Mol. Phylogenet. Evol.* 68 (2), 312–326.
- Gray, J.S.A., 1969. New species of *Saccocirrus* (Archiannelida) from the West Coast of North America. *Pac. Sci.* 23, 238–251.
- Gusjewa, S., 1929. Zur Kenntnis von *Saccocirrus*. *Zool. Anz.* 84, 151–157.
- Hastie, T.J., 1993. Generalized additive models in J.M. Chambers, T.J. Hastie (Eds.), *Statistical Models*, S. Chapman and Hall, New York, pp. 249–307.
- Higgins, R.P., Thiel, H., 1988. Introduction to study of meiofauna, first ed., Smithsonian Institution Press, Washington D.C.
- Hillis, D.M., Dixon, M.J., 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. *Quart. Rev. Biol.* 66, 411–453.
- Hurvich, C.M., Tsai, C.L., 1989. Regression and time-series model selection in small samples. *Biometrika* 76, 297–307.
- Huston, M.A., 1994. Biological Diversity – The Coexistence of Species on Changing Landscapes. Cambridge University Press, New York.
- Jouin, C., 1966. Morphologie et anatomie comparée de *Protodrilus chaetifer* Remane et *Protodrilus symbioticus* Giard, création du nouveau genre *Protodriloides* (Archiannelides). *Cah. Biol. Mar.* 7, 139–155.
- Jouin, C., 1971. Status of the knowledge of the systematics and ecology of Archiannelida. *Smithson. Contrib. Zool.* 76, 47–56.
- Jouin, C., 1975. Étude de quelques archiannelides des côtes d'Afrique du Sud: description de *Saccocirrus heterochaetus* n. sp. (Archiannelide, Saccocirridae). *Cah. Biol. Mar.* 16, 97–110.
- Jouin, C., 1978. Anatomical and ultrastructural study of the pharyngeal bulb in *Protodrilus* (Polychaeta, Archiannelida). I. Muscles and myo-epithelial junctions. *Tissue Cell* 10, 269–287.
- Jouin, C., Gambi, C., 2007. Description of *Saccocirrus goodrichi* sp. nov. (Annelida: Polychaeta: Saccocirridae), a new Mediterranean species and new data on the chaetae of *S. papillocerus* and *S. major*. *Cah. Biol. Mar.* 48, 381–390.
- Jouin, C., Rao, G.C., 1987. Morphological studies on some Polygordiidae and Saccocirridae (Polychaeta) from the Indian Ocean. *Cah. Biol. Mar.* 28, 389–402.
- Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* 30, 3059–3066.
- Katoh, K., Toh, H., 2010. Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics* 26, 1899–1900.
- Kirsteuer, E., 1967. Bredin–Archbold–Smithsonian biological survey of Dominica. 3. Marine archiannelids from Dominica. *Proc. U.S. Natl. Mus.* 123 (3610), 1–6.
- Koenker, R., 2005. *Quantile Regression*. Cambridge University Press, New York.
- Koenker, R., 2007. *quantreg: Quantile Regression*, R package version 4.10. <http://www.r-project.org>.
- Koenker, R., Ng, P., Portnoy, S., 1994. Quantile smoothing splines. *Biometrika* 81, 673–680.
- Langerhans, P., 1880. Die Wurffauna von Madeira. *Z. wiss. Zool. Bd.* 34, 88–143.
- Maddison, W.P., Maddison, D.R., 2011. Mesquite: A Modular System for Evolutionary Analysis. Version 2.75. <<http://mesquiteproject.org>>.
- Magagnoli, G., 1980. Archiannelidi della Mleoria (Livorno). *Atti Soc. Tosc. Sci. Nat. Mem. Ser. B* 87, 299–308.
- Manly, B.F.J., 2006. *Randomization, Bootstrap and Monte Carlo Methods in Biology*. Chapman and Hall, London.
- Markmann, M., 2000. Entwicklung und Anwendung einer 28S rDNA-Sequenzdatenbank zur Aufschlüsselung der Artenvielfalt limnischer Meioentofosfauna im Hinblick auf den Einsatz moderner Chiptechnologie (PhD thesis).
- Martin, G.G., 1977. *Saccocirrus sonomacis* sp. nov., a new archiannelid from California. *Trans. Am. Microsc. Soc.* 96, 97–103.
- Martin, G.G., 1978. The Duo-Gland adhesive system of the archiannelids *Protodrilus* and *Saccocirrus* and the turbellarian moncelis. *Zoomorphology* 91, 63–75.
- Martínez, A., Di Domenico, M., Jörgen, K., Norenburg, J., Worsaae, K., 2013. Description of three new species of *Protodrilus* (Annelida, Protodrilidae) from Central America. *Mar. Biol. Res.* 9 (7), 676–691.
- Mastepanova, E.A., 2004. Interstitial polychaetes of the seas of Russia. *Invertebr. Zool.* 1 (1), 59–64 (in Russian).
- Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov., New Orleans, LA 1–8.
- Núñez, J., Brito, M.C., Docoito, J.R., 2005. Anélidos poliquetos de Canarias: Catálogo de especies, distribución y hábitats. *Vieraea* 33, 297–321.
- Orrhage, L., 1964. Anatomische und morphologische Studien fiber die Polychaetenfamilie Spionidae, Disomidae and Poecilochaetidae. *Zool. Bidr. Uppsala* 36, 335–405.
- Orrhage, L., 1978. On the structure and evolution of the anterior end of the Sabellariidae (Polychaeta Sedentaria). With some remarks on the general organization of the polychaete brain. *Zool. Jb. Abt. Anat.* 100, 343–374.
- Orrhage, L., 1980. On the structure and homologues of the anterior end of the polychaete families Sabellidae and Serpulidae. *Zoomorphology* 96, 113–168.
- Orrhage, L., Müller, M.C.M., 2005. Morphology of the nervous system of Polychaeta (Annelida). *Hydrobiologia* 535 (536), 79–111.
- Palumbi S.R., 1996. Nucleic acids II: The polymerase chain reaction. In: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.), *Molecular Systematics*, second edition, Massachusetts: Sinauer, Inc, pp. 205–247.
- Petersen M.E. and George J.D., A new species of *Raricirrus* from northern Europe, with notes on its biology and a discussion of the affinities of the genus (Polychaeta: Ctenodrilidae). *Ophelia*, 5, 1991, 185–208 in M. E. Petersen & J. B. Kirkegaard, eds., *Systematics, biology and morphology of world Polychaeta*. Proceedings of the 2nd International Polychaete Conference, Copenhagen 1986. *Ophelia* 5.
- Pierantoni, U., 1907. Il genere *Saccocirrus* Bobretzky e le sue specie. *Ann. R. Mus. Zool. R. Univ. Napoli* 2, 1–11.
- Pierantoni, U., 1996. Osservazioni sullo sviluppo embrionale e larvale del *Saccocirrus papillocerus* Bobr. *Mitteilungen aus der Zoologischen Station zu Neapel Berlin* 18, 46–72.
- Pleijel, F., 1995. On character encoding for phylogeny reconstruction. *Cladistics* 11, 309–315.
- Posada, D., 2008. jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25, 1253.
- Purschke, G., 1988. Pharynx. In: Westheide, W., Hermans, C.O. (Eds.), *The Ultrastructure of Polychaeta. Microfauna Marina*, vol. 4. Gustav Fischer, Stuttgart, pp. 177–197.
- Purschke, G., 1990. Comparative electron microscopic investigation of the nuchal organs in *Protodriloides*, *Protodrilus* and *Saccocirrus* (Annelida, Polychaeta). *Can. J. Zool.* 68, 325–338.
- Purschke, G., 1992. Ultrastructural investigations of presumed photoreceptive organs in two *Saccocirrus* species (Polychaeta, Saccocirridae). *J. Morphol.* 211, 7–21.
- Purschke, G., 1993. Structure of the prostomial appendages and the central nervous system in the *Protodrilida* (Polychaeta). *Zoomorphology* 113, 1–20.
- Purschke, G., 1997. Ultrastructure of nuchal organs in Polychaetes (Annelida). *Acta Zool.* 78 (2), 123–143.

- Purschke, G., 2006. Problematic annelid groups. In: Rouse, W., Pleijel, F. (Eds.), *Reproductive Biology and Phylogeny of Annelida. Reproductive Biology and Phylogeny*, vol. 4. Science Publishers, Enfield, pp. 639–667.
- Purschke, G., Jouin, C., 1988. Anatomy and ultrastructure of the ventral pharyngeal organs of *Saccocirrus* (Saccocirridae) and *Protodriloides* (Protodriloidae fam. n.) with remarks on the phylogenetic relationships within the Protodrilida (Annelida: Polychaeta). *J. Zool.* 215, 405–432.
- Purschke, G., Tzvetlin, A.B., 1996. Dorsolateral ciliary folds in the polychaete foregut: structure, prevalence and phylogenetic significance. *Acta Zool.* 77, 33–49.
- Rao, G.C., Ganapati, P.N., 1968. The interstitial fauna inhabiting the beach sands of Waltair coast. *Proc. Natn. Inst. Sci. India B* 34, 82–125.
- R Development Core Team, 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <<http://www.R-project.org>> ISBN 3-900051-07-0.
- Rambaut, A., Drummond, A.J., 2007. Tracer v1.4. <<http://tree.bio.ed.ac.uk/software/tracer/>>.
- von Rapiachoff, W., 1881. Zur Entwicklungsgeschichte des *Polygordius flavocapitatus* Uljan. und *Saccocirrus papillocerus* Bobr. *Zool. Anz.* 73–100, 518–520.
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.
- Rouse, G.W., Fauchald, K., 1997. Cladistics and polychaetes. *Zool. Scr.* 26 (2), 139–204.
- Rouse, G.W., Pleijel, F., 2001. *Polychaetes*. Oxford University Press, London.
- Rousset, V., Pleijel, F., Rouse, G.W., Erséus, C., Siddall, M.E., 2007. A molecular phylogeny of annelids. *Cladistics* 23, 41–63.
- Salazar-Vallejo, S.I., Londoño-Mesa, M.H., 2004. Lista de especies y bibliografía de poliquetos (Polychaeta) del Pacífico Oriental Tropical. *Anales del Instituto de Biología de la Universidad Nacional Autónoma de México, Zoología* 75 (1), 9–97.
- Sasaki, S., 1981. A new species of the genus (Archiannelida) from Hokkaido, northern Japan. *Annot. Zool. Jpn.* 54 (4), 259–266.
- Sasaki, S., Brown, R., 1983. Larval development of *Saccocirrus uchidai* from Hokkaido, Japan and *Saccocirrus krusadensis* from New South Wales, Australia (Archiannelida, Saccocirridae). *Annot. Zool. Jpn.* 56, 299–314.
- Schmidt, P., Westheide, W., 1977. Interstitielle fauna von Galapagos. XVII. Polygordiidae, Saccocirridae, Protodrilidae, Nerillidae, Dinophilidae (Polychaeta). *Mikrofauna des Meeresbodens* 62, 1–38.
- Silberbauer, B.L., 1969. Archiannelids of the genus *Saccocirrus* from southern Africa. *Trans. Roy. Soc. S. Afr.* 38 (2), 165–182.
- Silvestro, D., Michalak, I., 2011. RaxmlGUI: a graphical front-end for RAXML. *Org. Divers. Evol.* <http://dx.doi.org/10.1007/s13127-011-0056-0>.
- Smigielski, F., Souplet, A., 1977. Présence dans le Pas-de-Calais de trois espèces d'annélides nouvelles pour la région. *Cah. Biol. Mar.* 18 (4), 475–481.
- Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAXML web servers. *Syst. Biol.* 57, 758–771.
- Sterrer, W., 1973. Plate tectonics as mechanism for dispersal and speciation in interstitial sand fauna. *Syst. Zool.* 21, 151–173.
- Struck, T.H., Nesnidal, M.P., Purschke, G., Halanych, K.M., 2008. Detecting possibly saturated positions in 18S and 28S sequences and their influence on phylogenetic reconstruction of Annelida (Lophotrochozoa). *Mol. Phylog. Evol.* 48, 628–645.
- Surugi, V., 2012. Systematic and ecology of species of the *Polydora*-complex (Polychaeta: Spionidae) of the Black Sea. *Zootaxa* 3518, 45–65.
- Tzvetlin, A.B., Purschke, G., 2005. Pharynx and intestine. *Hydrobiologia* 535 (536), 199–225.
- Uchida, T., Okuda, S., 1953. Notes on an archiannelid, *Saccocirrus major*. *J. Fac. Sci. Hokkaido Univ. Ser. Vi Zool.* 11 (2), 175–181.
- Villora-Moreno, S., 1997. Environmental heterogeneity and the biodiversity of interstitial polychaeta. *Bull. Mar. Sci.* 60, 494–501.
- Villora-Moreno, S., Capaccioni-Azzati, R., Garcia-Carrascosa, A.M., 1991. Meiobenthos of sandy beaches from the Gulf of Valencia (Western Mediterranean): ecology of interstitial polychaetes. *Bull. Mar. Sci.* 48, 376–385.
- Von Nordheim, H., 1989. Six new species of *Protodrilus* (Annelida, Polychaeta) from Europe and New Zealand, with a concise presentation of the genus. *Zool. Scripta* 18, 245–268.
- Westheide, W., 1972. Nouvelles recoltes d'annelides interstitielles dans les plages sableuses du bassin d'Arcachon. *Vie et Milieu* 23, 365–370.
- Westheide, W., 1984. The concept of reproduction in polychaetes with small body size: adaptations in interstitial species. *Fortschr. Zool.* 29, 265–287.
- Westheide, W., 1997. The direction of evolution within the Polychaeta. *J. Nat. Hist.* 31, 1–15.
- Westheide, W., 2008. *Polychaetes: Interstitial Families*, second ed. London, Publ. The Linnean Society of London and The Estuarine and Coastal Science Association.
- Worsaae, K., 2001. The systematic significance of palp morphology in the *Polydora* complex (Polychaeta: Spionidae). *Zool. Anz.* 240, 47–59.
- Worsaae, K., Kristensen, R.M., 2005. Evolution of interstitial Polychaeta (Annelida). *Hydrobiologia* 535 (536), 319–340.
- Worsaae, K., Sterrer, W., Kaul-Strehlow, S., Hay-Schmidt, A., Giribet, G., 2012. An anatomical description of a miniaturized acorn worm (Hemichordata, Enteropneusta) with asexual reproduction by paratomy. *PlosOne* 7 (11), e48529.
- Wu, B.L., Yang, D.J., 1962. The taxonomy and distribution of the genus *Saccocirrus* Bobretzky (Archiannelida). (Russian abstract). *Oceanol. Limnol. Sin.* 4, 169–179.
- Yamanishi, R., 1973. A new species of *Saccocirrus* (Archiannelida) in Japan. *Publ. Seto Mar. Biol. Lab.* 21 (2), 73–76.
- Zrzavy, J., Riha, P., Pialek, L., Janouskovec, J., 2009. Phylogeny of Annelida (Lophotrochozoa): total-evidence analysis of morphology and six genes. *BMC Evol. Biol.* 9, 189.