



Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev



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

M. Di Domenico^{a,b,c,*}, A. Martínez^a, P. Lana^b, K. Worsaae^{a,*}

^a Marine Biological Section, Department of Biology, University of Copenhagen, Strandpromenaden 5, 3000 Helsingør, Denmark

^b Laboratory of Benthic Ecology, Centre for Marine Studies, Federal University of Paraná, Brazil

^c 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 xxxx

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 (M. Di Domenico), lanau@ufpr.br (P. Lana), 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., in preparation). 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 MgCl₂ decantation technique and sieved through a 63- μ 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., in press).

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 μ 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 μ l, containing 2 μ l of template DNA, 1.5 μ M of each primer (10 μ M) and 18 μ l of ddH₂O. 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 (XXXX) | KF954476 | KF954434 | KF954455 | KF954495 |
| <i>Saccocirrus</i> sp. 3 (Bocas del Toro, Panama)* | <i>krusadensis</i> | Bocas del Toro, Panama | Central America – Caribbean Sea | ZMUC-Pol (XXXX) | KF954469 | KF954427 | KF954448 | KF954488 |
| <i>Saccocirrus</i> sp. 5 (Bocas del Toro, Panama)* | <i>krusadensis</i> | Bocas del Toro, Panama | Central America – Caribbean Sea | ZMUC-Pol (XXXX) | KF954475 | KF954433 | KF954454 | KF954494 |
| <i>Saccocirrus</i> sp. 9 (Belize) | <i>papillocercus</i> | Carrie Bow Cay, Belize | Central America – Caribbean Sea | ZMUC-Pol (XXXX) | KF954479 | KF954437 | KF954458 | KF954498 |
| <i>Saccocirrus sonomacus</i> | <i>krusadensis</i> | Bird Rock, CA, USA | North America – Pacific Ocean | ZMUC-Pol (XXXX) | KF954472 | KF954430 | KF954451 | KF954491 |
| <i>Saccocirrus sonomacus</i> | <i>krusadensis</i> | Chile | South America – Pacific Ocean | ZMUC-Pol (XXXX) | | KF954425 | KF954446 | |
| <i>Saccocirrus tridentiger</i> | <i>krusadensis</i> | North East Cay, Salomon Island | Indo-Pacific | ZMUC-Pol (XXXX) | KF954465 | KF954421 | KF954443 | KF954484 |
| <i>Saccocirrus tridentiger</i> | <i>krusadensis</i> | New South Wale, Australia | Indo-Pacific | ZMUC-Pol (XXXX) | KF954466 | KF954422 | KF954444 | KF954485 |
| <i>Saccocirrus jouinae</i> | <i>krusadensis</i> | Kennedy Island, Salomon Island | Indo-Pacific | ZMUC-Pol (XXXX) | KF954473 | KF954431 | KF954452 | KF954492 |
| <i>Saccocirrus krusadensis</i> | <i>krusadensis</i> | Ao Yon Beach, Phuket, Thailand | Indo-Pacific | ZMUC-Pol (XXXX) | KF954474 | KF954432 | KF954453 | KF954493 |
| <i>Saccocirrus</i> sp. 8 (Bermuda) | <i>papillocercus</i> | Windsor Beach, Bermuda | Central America – Caribbean Sea | ZMUC-Pol (XXXX) | KF954478 | KF954436 | KF954457 | KF954497 |
| <i>Saccocirrus</i> sp. 10 (Solomon Isl.) | <i>papillocercus</i> | Mono island, Salomon Island | Indo-Pacific | ZMUC-Pol (XXXX) | KF954480 | KF954438 | KF954459 | KF954499 |
| <i>Saccocirrus pussicus</i> | <i>papillocercus</i> | Barra Velha, Santa Catarina, Brazil | South America – Atlantic Ocean | ZMUC-Pol (XXXX) | KF954481 | KF954439 | KF954460 | KF954500 |
| <i>Saccocirrus</i> sp. 1 (Tenerife, Canary Isl.) | <i>papillocercus</i> | Playa Abades, Tenerife, Canary Island | Atlantic Island | ZMUC-Pol (XXXX) | KF954467 | KF954424 | KF954445 | KF954486 |
| <i>Saccocirrus</i> sp. 7 (Giglio, Italy) | <i>papillocercus</i> | Giglio Island, Italy | Mediterranean Sea | ZMUC-Pol (XXXX) | KF954477 | KF954435 | KF954456 | KF954496 |
| <i>Saccocirrus</i> sp. 4 (La Palma, Canary Isl.)* | <i>krusadensis</i> | La Palma, Canary Island | Atlantic Island | ZMUC-Pol (XXXX) | KF954471 | KF954429 | KF954450 | KF954490 |
| <i>Saccocirrus</i> sp. 2 (Lanzarote, Canary Isl.)* | <i>krusadensis</i> | Lanzarote, Canary Island | Atlantic Island | ZMUC-Pol (XXXX) | KF954468 | KF954426 | KF954447 | KF954487 |
| <i>Saccocirrus gabriellae</i> | <i>krusadensis</i> | Ponta do Balleiro, São Sebastião, SP, Brazil | South America – Atlantic Ocean | ZMUC-Pol (XXXX) | KF954470 | KF954428 | KF954449 | KF954489 |
| <i>Saccocirrus papillocercus</i> | <i>papillocercus</i> | Sevastopol Bay, Ukraine | Black Sea | ZMUC-Pol (XXXX) | KF954482 | | KF954461 | KF954501 |
| <i>Protodrilus purpureus</i> | outgroup | | | | EU418874 | AY527057 | AY340474 | DQ779760 |
| <i>Protodrilus ciliatus</i> | outgroup | Kallak, Faroe Island | Atlantic Island, North | | KF954464 | KF954420 | KF954442 | KF954505 |
| <i>Protodriloides symbioticus</i> | outgroup | Roscoff, France | North Sea, Atlantic | | KF954463 | KF954419 | KF954441 | KF954504 |
| <i>Protodriloides chaetifer</i> | outgroup | Ellekilde Hage, Denmark | North Sea, Atlantic | | KF954462 | KF954418 | KF954440 | KF954503 |
| <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 | GCGAAAGCATTTGCCAAGMA | 1 |
| G951 | 18s | 20 | R | GAGTCTCGTTCGTTATCGGA | 1 |
| G950 | 18s | 19 | F | GTTCGATCCGGAGAGGGA | 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 – Cunningham et al. (1994), 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 μm (=0); 200–400 μm (=1); thicker than 400 μm (=2)

Maximum width was divided in three discrete states in our matrix. *Polydora giardi* and *Sabellaria alveolata* are thicker than 400 μm; *Protodriloides symbioticus*, *Protodriloides chaetifer*, *Protodrilus purpureus* are 200–400 μm, *Protodrilus ciliatus* and *Ctenodrilus serratus* are thinner than 200 μm. 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 μm (=0); 200–500 μm (=1); 500–1000 μm (=2); longer than 1000 μm (=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 μm in *Saccocirrus*. Palps are longer than 1000 μm in *Polydora* and *Protodrilus purpureus*, range between 200–500 μm in *Protodriloides*, and 500–1000 μm 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., in preparation). *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 (1871), Dauvin et al. (2003), Gusjewa (1929), Jouin and Gambi (2007), Jouin in 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 (1966, 1967) | 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) | Delamare de Bouteville (1954), Fize (1963), Gerlach (1953, 1954), 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-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 (1966, (1967) | 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 | Intertidal |

(continued on next page)

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) | matter, algae Among rocks, beach with organic | Intertidal |
| <i>Saccocirrus uchidai</i> Sasaki, 1981 | "krusadensis" | Hokkaido (North Japan) | Hokkaido (North Japan) | Sasaki (1981) and Sasaki and Brown (1983) | matter, algae 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 | Intertidal |
| <i>Saccocirrus alanihongi</i> Baley-Brock et al., 2003 | "krusadensis" | Wai'anae, O'ahu (Hawaii) | Wai'anae, O'ahu (Hawaii) | Baley-Brock et al. (2003) | matter, algae Reef flat | Subtidal |

2.4.12. *Prostomial transverse ciliary band (12): absent (=0); present (=1)* 338
339
A transverse band of motile cilia extends latero-ventrally on the 340
prostomium, from the palps to the delineation between prosto- 341
mium and peristomium. The band is present in some *Saccocirrus* 342
species belonging to the "krusadensis" group. The character is 343
inapplicable in *Protodrilus*, *Protodriloides*, *Ctenodrilus*, *Sabellaria* 344
and *Polydora*. This character is not present in the outgroups (Jouin, 345
1966; Von Nordheim, 1989; Petersen and George, 1991; Rouse and 346
Plejel, 2001). 347

2.4.13. *Ventral pharynx (13): absent (=0); present (=1)* 348
349
A ventral muscular pharynx is present in *Protodrilus*, *Protodri-* 350
loides, *Ctenodrilus*, *Polydora* and species from "krusadensis" group 351
(Purschke, 1988; Purschke and Jouin, 1988). When the ventral pha- 352
ryngeal apparatus is present, it consists of a ventral bulbous muscle 353
with transverse muscular fibers and interstitial cells in all the ter- 354
minals of our analyses (Purschke and Jouin, 1988; Purschke, 1988). 355
The ventral pharynx is absent in the remaining species of the 356
analyses.

2.4.14. *Ventral pharyngeal apparatus with tongue-like organ (14):* 357
absent (=0); present (=1) 358
A tongue-like muscular organ is present in the "krusadensis" 359
species of Saccocirridae, as well as Protodrilidae and Ctenodrilidae 360
(Purschke and Tzetlin, 1996). A tongue-like organ is absent in *Pro-* 361
todriloides and *Polydora* species included in the current analyses 362
and is inapplicable for species of the "papilocercus" group. 363

2.4.15. *Midventral ciliary band (15): absent (=0); present (=1)* 364
365
Midventral ciliary band, consisting of a longitudinal band of cilia 366
extending along the trunk in a groove is present in adults of *Sac-* 367
cocirrus krusadensis, *S. jouinae*, *S. tridentiger* ("krusadensis" group, 368
Brown, 1981), as well as in *Protodrilus* and *Protodriloides*. Midven- 369
tral ciliary band is absent in adults species in the "papilocercus" 370
group, *Ctenodrilus*, *Sabellaria* and *Polydora* (Rouse and Plejel, 371
2001; Westheide, 2008).

2.4.16. *Extension of the midventral ciliary band (16): from the mouth* 372
to the pygidium (=0); from the mouth to segment 10 (=1) 373
374
Midventral ciliary band extends from the mouth to the pygidium 375
in *Protodrilus* and *Protodriloides*. Midventral ciliary band 376
extends maximum until segment 10 in *Saccocirrus krusadensis*, *S.* 377
jouinae and *S. tridentiger* (Fig. 1B), although the presence and 378
extension of band exhibit relatively high intraspecific variation 379
the re-examined material of these species. The character is inappli- 380
cable for *Ctenodrilus*, *Sabellaria* and *Polydora* (Petersen and George, 381
1991; Rouse and Plejel, 2001), and species of the "papilocercus" 382
group.

2.4.17. *Mouth ciliary patches (17): absent (=0); present (=1)* 383
384
Ciliary patches are present around the mouth in saccocirrids 385
from "krusadensis" group as well as in *Protodrilus* and *Protodri-* 386
loides (Fig. 1A). The character is absent in the remaining outgroups 387
(Rouse and Plejel, 2001; Westheide, 2008).

2.4.18. *Morphology of the mouth ciliary patches (18): arranged as a* 388
field around the mouth (=0); arranged as paired longitudinal bands, 389
laterally to the mouths (=1) 390
391
Mouth ciliary patches consist of two short longitudinal ciliary 392
bands at each side of the mouth in species from "krusadensis" 393
group, whereas they represent a triangular field extending posteri- 394
orly in *Protodrilus* and *Protodriloides* (Purschke and Jouin, 1988; 395
Jouin and Gambi, 2007). The character is inapplicable in *Ctenodri-* 396
lus, *Sabellaria* and *Polydora* (Petersen and George, 1991; Rouse 397
and Plejel, 2001), and species in the "papilocercus" 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 papillocercus</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 | - | 1 | 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 | - | 1 | 0 | 0 | - | - | - | - | 1 | 0 | 1 | 0 | - | - | - | - | - | 0 | - | 0 | ? | 0 | - | |

Please cite this article in press as: Di Domenico, M., et al. Molecular and morphological phylogeny of Saccocirridae (Annelida) reveals two cosmopolitan clades with specific habitat preferences. Mol. Phylogenet. Evol. (2014), <http://dx.doi.org/10.1016/j.ympev.2014.02.003>

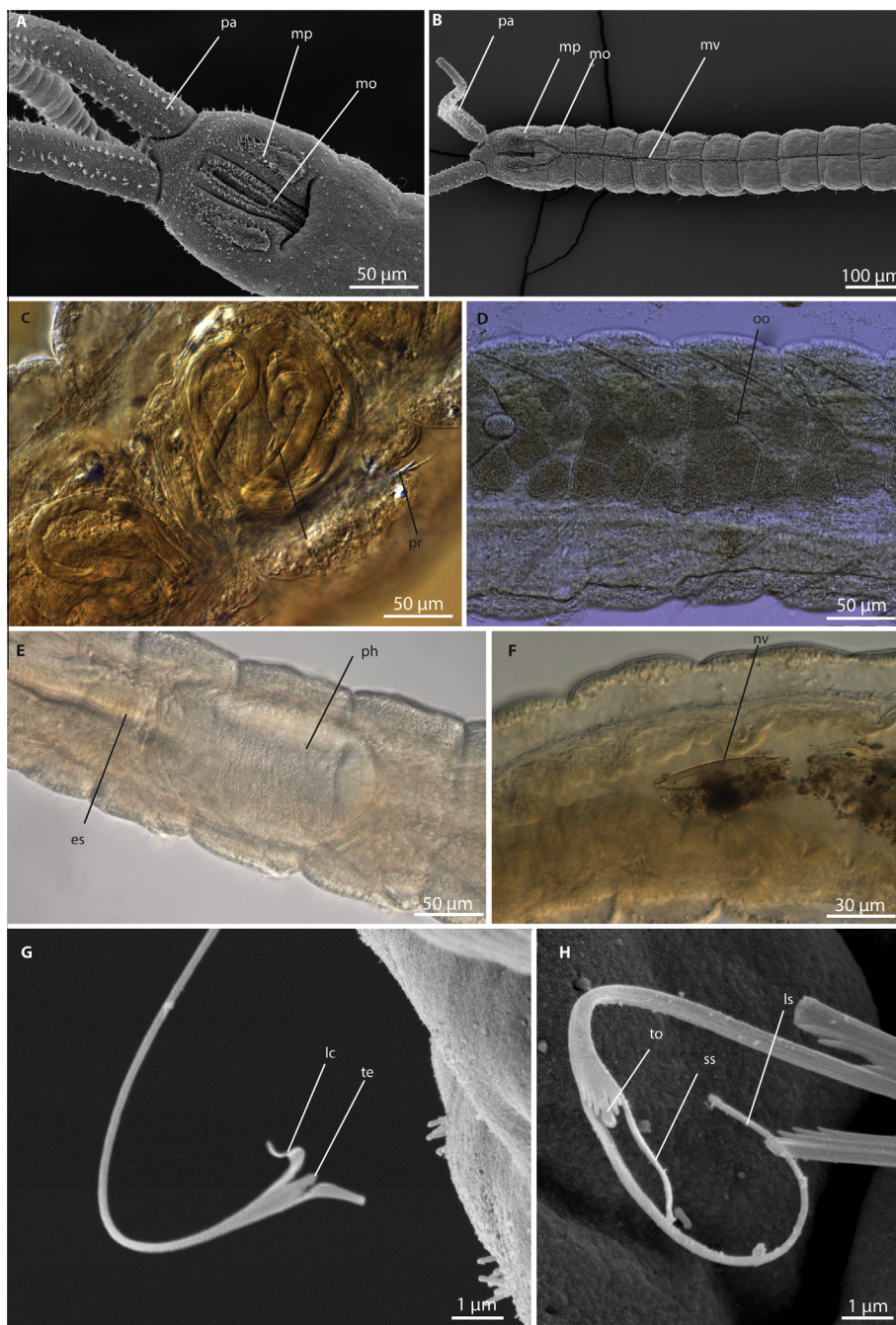


Fig. 1. Morphology of the "krusadensis" group (*Pharyngocirrus* gen. nov.), light and scanning electron micrographs. (A) *Saccocirrus sonomacus*, ventral view of the anterior end showing mouth ciliation. (B) *Saccocirrus jouinae*, ventral view of the anterior end showing the mouth ciliation and midventral ciliary band. (C) *Saccocirrus tridentiger*, mid body of a female 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*.

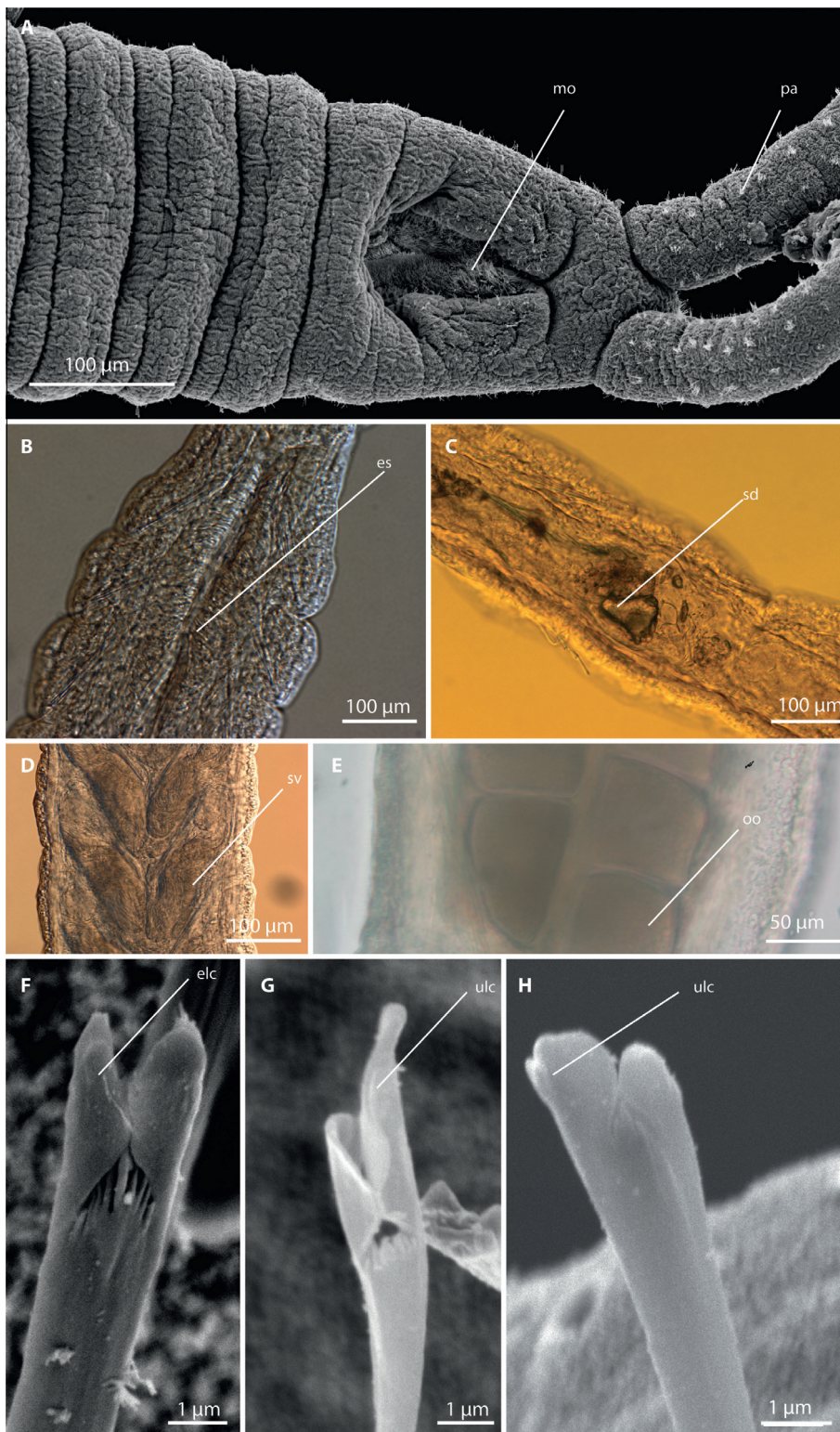


Fig. 2. Morphology of the “papilocercus” group, light microscopic and scanning electron micrographs. (A) *Saccocirrus* sp. 7 from Giglio (Italy), ventral view of the anterior end showing the mouth. (B) *Saccocirrus pussicus*, segments 1–3 showing esophagus. (C) *Saccocirrus pussicus*; detail of the gut 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.

414 2.4.21. Chaetae (21): absent (=0); present (=1)

415 Chaetae are present in all *Saccocirrus*, as well as *Protodriloides*
416 *chaetifer*, *Polydora* and *Sabellaria*. Chaetae are absent in *Protodrilus*
417 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

418

419

420

421

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, 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, 2006) 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 + Γ model. Nodal support was calculated after 1000 pseudoreplicates of a non-parametric bootstrap, with a GTR + Γ 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 + Γ model was selected for H3, GTR + Γ for 16S rRNA and 28S rRNA, and GTR + Γ + I for 18S rRNA. The morphological data partition was analyzed with a Mk 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 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

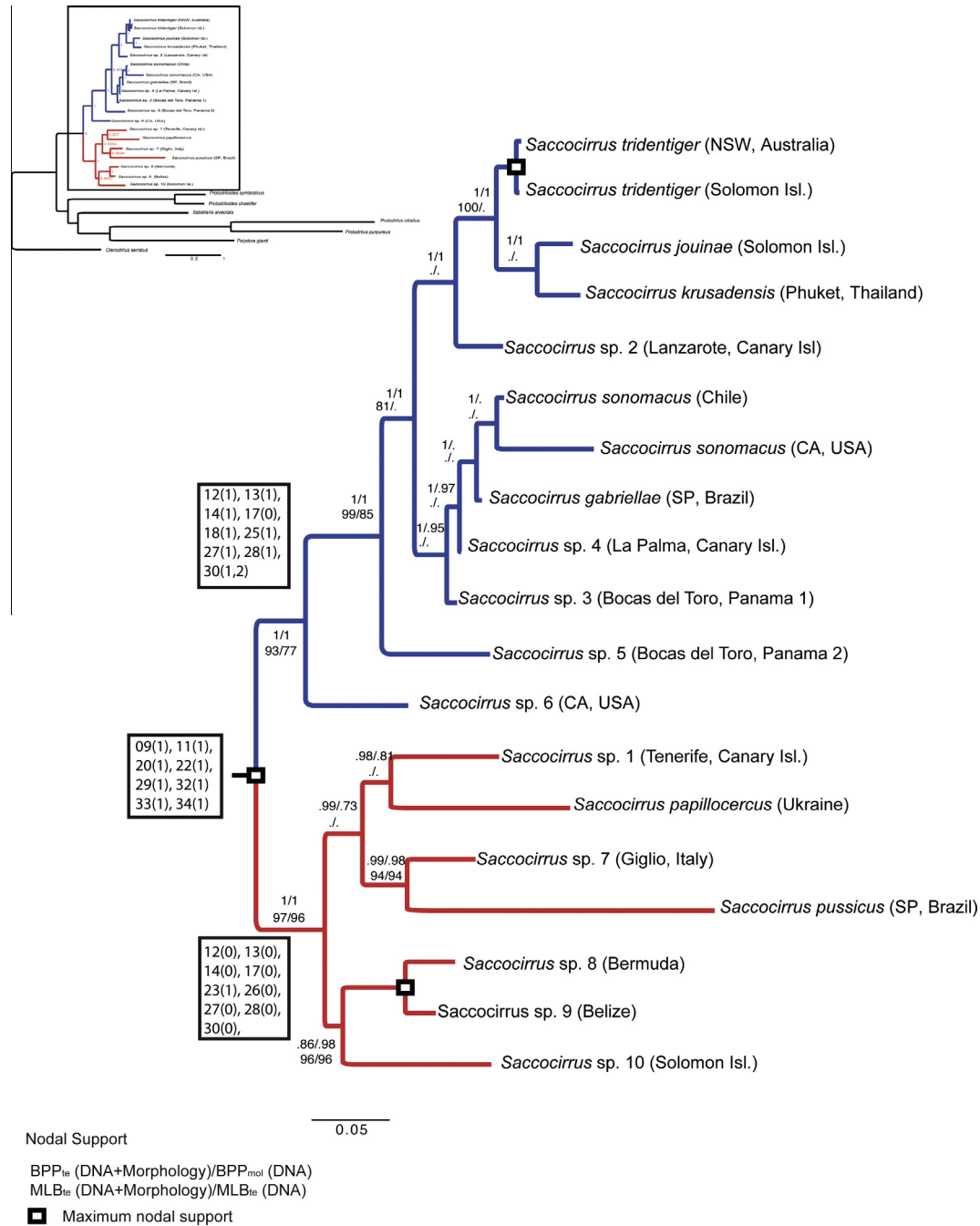


Fig. 3. Phylogenetic relationships of Saccocirridae. Tree from the Bayesian analysis, congruent with the maximum likelihood best tree. First line on the node represent Bayesian posterior probabilities (BPP) with molecular and morphological data and BPP only with molecular data; second line, maximum likelihood bootstrap (MLB) with molecular and morphological data and BPP only with molecular data. “Krusadensis” (*Pharyngocirrus* gen. nov.) clade is colored in blue and “papillocercus” 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 “papillocercus” 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 “papillocercus” 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

686 the clade lack prostomial transverse ciliary band (ch 12), pharyn-
687 geal bulbous muscle (ch 13 and 14) (Fig. 2B), ciliary patches around
688 the buccal area (ch 17) (Fig. 2A).

689 3.2. Geographic distribution patterns

690 Geographic analyses yielded a well-supported diversity
691 gradient of Saccocirridae, with a maximal diversity estimated at
692 20°N, with the latitudinal range between 0° and 30°N within the
693 95%. A steep decrease in diversity was registered from 0° to 30°N
694 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 “krusad-
ensis” showed a non-linear asymmetric unimodal relationship
with longitudes. The longitude for the highest diversity was esti-
mated 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% confi-
dence 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

695
696
697
698
699
700
701
702
703
704
705

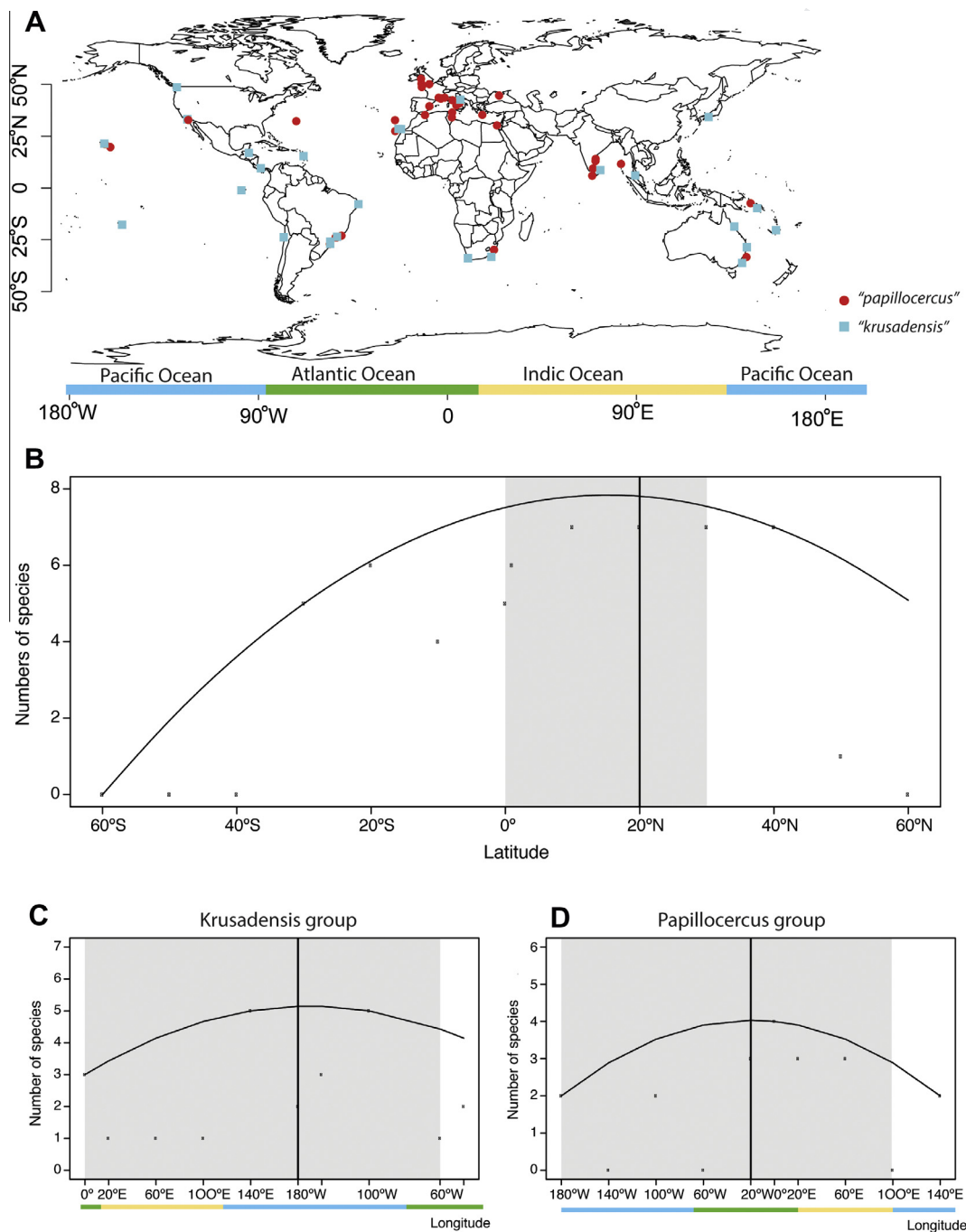


Fig. 4. (A) Geographic distribution of the groups “papillocercus” and “krusadensis” (*Pharyngocirrus* gen. nov.) based on Tables 1 and 3. (B) Spline smoothing with polynomial regression ($n = 14$) of latitude and number of *Saccocirrus* species showing the optimum value for diversity (line) and 95% confidence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

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’s 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 Indo-pacific, 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, 1980; Jouin and Gambi, 2007; Di Domenico et al., submitted for publication). 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. alanhongi* (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_{mol} = 1.00; MLB_{mol} = 77), as well as by total evidence analyses including morphological data (BPP_{te} = 1.00; MLB_{te} = 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, 1871, emended.

Type species: *Saccocirrus papillocercus* Brobretzky, 1871.

Included species: *Saccocirrus papillocercus* Brobretzky, 1871; *S. major* Pierantoni, 1907; *Saccocirrus orientalis* Alikunhi, 1946; *Saccocirrus minor* Aiyar e Alikunhi, 1944; *Saccocirrus pussicus* Du Bois-Reymond Marcus, 1948; *Saccocirrus heterochaetus* Jouin,

1975; *Saccocirrus parvus* Gerlach, 1953; *Saccocirrus oahuensis* Baley-Brock et al., 2003; *Saccocirrus waianaensis* Baley-Baley-Brock et al., 2003; *Saccocirrus 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_{mol} = 1.00; MLB_{mol} = 97), as well as by total evidence analyses including morphological data (BPP_{te} = 100; MLB_{te} = 96).

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

5. Uncited references

Altschul et al. (1997), Barnes (2002), Dordel et al. (2010), Gelder and Palmer (1976), Giere (2009), Hausen (2005), Ingólfsson (1995), Katoh and Toh (2008), Orrhage (1974), Rosenzweig (1995), Stamatakis et al. (2008), Struck (2011, 2007), Struck et al. (2011), Tamura et al. (2007), Todaro et al. (1996), Westheide and Haß-Cordes (2001) and Westheide and Rieger (1987).

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 Anchioline 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 Pardelas 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-Elhaby, 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.
- Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.
- 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.
- Barnes, D.K.A., 2002. Invasions by marine life on plastic debris. Nature 416, 808–809.
- 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 Boccara 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' novogo semeistra annelid. Sravnitel'no-Anatomichskii obchek. Mem. Kiev. Univ. Odsch. Estest. Zapisky 2, 211–259.
- 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).
- 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.
- 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., 1–16.
- Di Domenico, M., Martínez, A., Amaral, C., Lana, P.C., Worsaae, K., in press. Saccocirrus (Saccocirridae, Annelida) from the southern and southeastern Brazilian coasts. Mar. Biodivers.
- Dordel, J., Fisse, F., Purschke, G., Struck, T.H., 2010. Phylogenetic position of Sipuncula derived from multi-gene and phylogenomic data and its implication for the evolution of segmentation. J. Zool. Syst. Evol. Res. 48, 197–207.
- 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.
- Gelder, S.R., Palmer, R., 1976. The nervous system of the marine polychaete *Ctenodrilus serratus* and its importance in the taxonomic position of the Ctenodrilidae. Trans. Am. Microsc. Soc. 95 (2), 156–164.
- 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.
- Giere, O., 2009. Meiobenthology, The Microscopic Fauna in Aquatic Sediments, second ed. Springer-Verlag, Berlin.
- 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.
- Hausen, H., 2005. Chaetae and chaetogenesis in polychaetes (Annelida). Hydrobiologia 535 (536), 37–52.
- Hillis, D.M., Dixon, M.J., 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Quart. Rev. Biol. 66, 411–453.
- Huston, M.A., 1994. Biological Diversity – The Coexistence of Species on Changing Landscapes. Cambridge University Press, New York.
- Ingólfsson, A., 1995. Floating clumps of seaweed around Iceland: natural microcosms and a means of dispersal for shore fauna. Mar. Biol. 122, 13–21.
- 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. papillocercus* 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., Toh, H., 2008. Recent developments in the MAFFT multiple sequence alignment program. Brief. Bioinform. 9 (4), 286–298.
- 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., Ng, P., Portnoy, S., 1994. Quantile smoothing splines. Biometrika 81, 673–680.
- Langerhans, P., 1880. Die Wurffaua 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>>.
- Manly, B.F.J., 2006. Randomization, Bootstrap and Monte Carlo Methods in Biology. Chapman and Hall, London.
- Martin, G.G., 1977. *Saccocirrus sonomacus* 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 monocoel. Zoomorphology 91, 63–75.
- Martínez, A., Di Domenico, M., Jörger, 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).
- 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 und Poecilochaetidae. Zool. Bidr. Uppsala 36, 335–405.
- Orrhage, L., 1974. Über die Anatomie, Histologie und Verwandtschaft der Apistobranchidae (Polychaeta Sedentaria) nebst Bemerkungen tiber die Systematische Stellung der Archianneliden. Z. Morph. Ökol. Tiere 79, 1–45.
- 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.
- Pierantoni, U., 1907. Il genere *Saccocirrus* Bobretzky e le sue specie. Ann. R. Mus. Zool. R. Univ. Napoli 2, 1–11.
- Pleijel, F., 1995. On character encoding for phylogeny reconstruction. Cladistics 11, 309–315.
- 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., Tzetlin, A.B., 1996. Dorsolateral ciliary folds in the polychaete foregut: structure, prevalence and phylogenetic significance. Acta Zool. 77, 33–49.
- 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 papillocercus* 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.
- Rosenzweig, M.L., 1995. Species Diversity in Space and Time. Cambridge University Press, Cambridge.
- 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.
- 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.
- Silberbauer, B.L., 1969. Archiannelids of the genus *Saccocirrus* from southern Africa. Trans. Roy. Soc. S. Afr. 38 (2), 165–182.

- 1127 Silvestro, D., Michalak, I., 2011. RaxMLGUI: a graphical front-end for RAxML. Org.
1128 Divers. Evol. <http://dx.doi.org/10.1007/s13127-011-0056-0>.
- 1129 Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the
1130 RAxML web servers. Syst. Biol. 57, 758–771.
- 1131 Sterrer, W., 1973. Plate tectonics as mechanism for dispersal and speciation in
1132 interstitial sand fauna. Syst. Zool. 21, 151–173.
- 1133 Struck, T.H., 2011. Direction of evolution within Annelida and the definition of
1134 Pleistoannelida. J. Zool. Syst. Evol. Res. 48, 197–207.
- 1135 Struck, T.H., Schult, N., Kusen, T., Hickman, E., Bleidorn, C., McHugh, D., Halanych,
1136 K.M., 2007. Annelida phylogeny and the status of Sipuncula and Echiura. BMC
1137 Evol. Biol. 7, 57.
- 1138 Struck, T.H., Nesnidal, M.P., Purschke, G., Halanych, K.M., 2008. Detecting possibly
1139 saturated positions in 18S and 28S sequences and their influence on
1140 phylogenetic reconstruction of Annelida (Lophotrochozoa). Mol. Phylogen.
1141 Evol. 48, 628–645.
- 1142 Struck, T.H., Paul, C., Hill, N., Hartmann, S., Hösel, C., Kube, M., Lieb, B., Meyer, A.,
1143 Tiedemann, R., Purschke, G., Bleidorn, C., 2011. Phylogenomic analyses unravel
1144 annelid evolution. Nature 471, 95–98.
- 1145 Surugiu, V., 2012. Systematic and ecology of species of the *Polydora*-complex
1146 (Polychaeta: Spionidae) of the Black Sea. Zootaxa 3518, 45–65.
- 1147 Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular Evolutionary
1148 Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.
- 1149 Todaro, M.A., Fleeger, J.W., Hu, Y.P., Hrincevich, A.W., Foltz, D.W., 1996. Are
1150 meiofaunal species cosmopolitan? Morphological and molecular analysis of
1151 *Xenotrichula intermedia* (Gastrotricha: Chaetonotida). Mar. Biol. 125, 735–742.
- 1152 Tzvetlin, A.B., Purschke, G., 2005. Pharynx and intestine. Hydrobiologia 535 (536),
1153 199–225.
- 1154 Uchida, T., Okuda, S., 1953. Notes on an archiannelid, *Saccocirrus major*. J. Fac. Sci.
1155 Hokkaido Univ. Ser. Vi Zool. 11 (2), 175–181.
- 1156 Villora-Moreno, S., 1997. Environmental heterogeneity and the biodiversity of
1157 interstitial polychaeta. Bull. Mar. Sci. 60, 494–501.
- 1158 Villora-Moreno, S., Capaccioni-Azzati, R., Garcia-Carrascosa, A.M., 1991.
1159 Meiobenthos of sandy beaches from the Gulf of Valencia (Western
1160 Mediterranean): ecology of interstitial polychaetes. Bull. Mar. Sci. 48, 376–385.
- Von Nordheim, H., 1989. Six new species of *Protodrilus* (Annelida, Polychaeta) from
1161 Europe and New Zeland, with a concise presentation of the genus. Zool. Scripta
1162 18, 245–268.
- Westheide, W., 1984. The concept of reproduction in polychaetes with small body
1163 size: adaptations in interstitial species. Fortschr. Zool. 29, 265–287.
- Westheide, W., 1997. The direction of evolution within the Polychaeta. J. Nat. Hist.
1164 31, 1–15.
- Westheide, W., 2008. Polychaetes: Interstitial Families, second ed. London, Publ.
1165 The Linnean Society of London and The Estuarine and Coastal Science
1166 Association.
- Westheide, W., Haß-Cordes, E., 2001. Molecular taxonomy: description of a cryptic
1167 *Petitita* species (Polychaeta: Syllidae) from the island of Maheé (Seychelles,
1168 Indian Ocean) using RAPD markers and ITS2 sequences. J. Zool. Syst. Evol. Res.
1169 39, 103–111.
- Westheide, W., Rieger, R.M., 1987. Systematics of the *Microphthalmus listensis*
1170 species-group (Polychaeta: Hesionidae): facts and concepts for reconstruction
1171 of phylogeny and speciation. Z. Zool. Syst. Evolutionsforsch. 25, 12–39.
- Worsaae, K., 2001. The systematic significance of palp morphology in the *Polydora*
1172 complex (Polychaeta: Spionidae). Zool. Anz. 240, 47–59.
- Worsaae, K., Kristensen, R.M., 2005. Evolution of interstitial Polychaeta (Annelida).
1173 Hydrobiologia 535 (536), 319–340.
- Worsaae, K., Sterrer, W., Kaul-Strehlow, S., Hay-Schmidt, A., Giribet, G., 2012. An
1174 anatomical description of a miniaturized acorn worm (Hemichordata,
1175 Enteropneusta) with asexual reproduction by paratomy. PlosOne 7 (11),
1176 e48529.
- Wu, B.L., Yang, D.J., 1962. The taxonomy and distribution of the genus *Saccocirrus*
1177 Bobretzky (Archiannelida). (Russian abstract). Oceanol. Limnol. Sin. 4, 169–179.
- Yamanishi, R., 1973. A new species of *Saccocirrus* (Archiannelida) in Japan. Publ.
1178 Seto Mar. Biol. Lab. 21 (2), 73–76.
- Zrzavy, J., Riha, P., Pialek, L., Janouskovec, J., 2009. Phylogeny of Annelida
1179 (Lophotrochozoa): total-evidence analysis of morphology and six genes. BMC
1180 Evol. Biol. 9, 189.