

A new species of *Polygordius* (Polychaeta: Polygordiidae): from the inner continental shelf and in bays and harbours of the north-eastern United States

Patricia A. Ramey*[‡], Dieter Fiege[†] and Brian S. Leander[‡]

*Institute of Marine and Coastal Sciences, Rutgers the State University of New Jersey, 71 Dudley Road, New Brunswick, NJ, 08901, USA. [†]Forschungsinstitut Senckenberg, Sektion Marine Evertibraten II, Senckenberganlage 25, D-60325 Frankfurt/Main, Germany. [‡]Canadian Institute for Advanced Research, Program of Evolutionary Biology, Departments of Zoology and Botany, University of British Columbia, #3529-6270 University Boulevard, Vancouver, BC, V6T 1Z4, Canada. [‡]Corresponding author, e-mail: ramey@imcs.rutgers.edu

A new species of Polygordiidae, *Polygordius jouinae* sp. nov., along with its distribution, habitat, and reproduction is described. *Polygordius jouinae* sp. nov., the first North American *Polygordius*, is a dominant member of macrofaunal communities on the inner continental shelf, and in bays and harbours from Massachusetts to southern New Jersey. It is distinguished from most other *Polygordius* species by its non-inflated, heavily ciliated pygidium, absence of pygidial glands, and a conical (rather than rounded) prostomium. The 18S SSU rDNA from *P. jouinae* sp. nov. was sequenced and represents the first named *Polygordius* species with a DNA reference in GenBank. Spearman rank correlation of sediment grain size with density of *P. jouinae* sp. nov. at a New Jersey site showed that density was significantly ($P < 0.05$; $N = 92$) positively correlated with the proportion of medium to very coarse sand and negatively correlated with the fine sand fractions. Ecologically, *P. jouinae* sp. nov. is an important macrofaunal species given its widespread distribution and its fidelity for coarse sand habitats. Thus, its relative abundance may be useful as an indicator of changing sedimentary conditions.

INTRODUCTION

Members of the Polygordiidae Czerniavsky, 1881 are commonly found in intertidal and shallow subtidal sandy sediments, but have also been reported from depths of 152 m on the continental shelf (based on material from the Smithsonian Institution, Washington, DC; Maciolek & Grassle, 1987). They are small (length 10–100 mm; width <1 mm), have indistinct external segmentation, and lack certain defining polychaete characters such as parapodia and chaetae (except *Chaetogordius canaliculatus* Moore, 1904), but they do have well developed nuchal organs. Members of the Polygordiidae have been found in the Atlantic, Indian, Pacific, and Antarctic Oceans and most are described from European waters (Rouse & Pleijel, 2001). Fifteen species and two subspecies have so far been described in the genus *Polygordius* Schneider, 1868. According to Hartman (1959) other described genera synonymous with *Polygordius* include *Rhamphogordius* Rathke, 1843, *Linotrypane* McIntosh, 1875, and *Pseudogordius* Czerniavsky, 1881. The only other genus in the family Polygordiidae was established for *Chaetogordius canaliculatus* described by Moore (1904), which was based on fragmented material collected off Cape Cod, United States. This species has never been found again and is generally regarded as an invalid taxon (Hermans, 1969; Westheide, 1990; Rota & Carchini, 1999). The new species, *Polygordius jouinae* sp. nov., is the first *Polygordius* species described from North America. Although undescribed to this date, the

present study shows that it has been collected before (referred to as *Polygordius* sp. or *Polygordius* sp. A) and is a dominant member of sandy infaunal communities on the continental shelf and in bays and harbours of the north-eastern United States (e.g. Maciolek & Grassle, 1987; Snelgrove et al., 2001; Kropp et al., 2002; Battelle, 2003, 2004; Maciolek et al., 2004). On the continental shelf off New Jersey, *P. jouinae* sp. nov. at times makes up >50% of the macrofauna in sandy sediment communities with densities as high as 98,400 individuals m^{-2} (Snelgrove et al., 2001). Little is known about the ecology of *P. jouinae* sp. nov., or of the Polygordiidae in general (Rouse & Pleijel, 2001). A greater understanding of the natural history and reproductive biology of this species is needed if we are to understand observed spatial and temporal patterns. The present study describes this new species including its 18S SSU rDNA sequence, summarizes its distribution by looking at unidentified material (*Polygordius* sp. and *Polygordius* sp. A) collected along the east coast of the United States, and provides information on the species' reproductive biology, habitat preferences and ecological importance.

MATERIALS AND METHODS

Collection of Polygordius jouinae sp. nov.

LEO-15 research site

Specimens for the species description and examination of reproductive biology were collected from Station 9 at

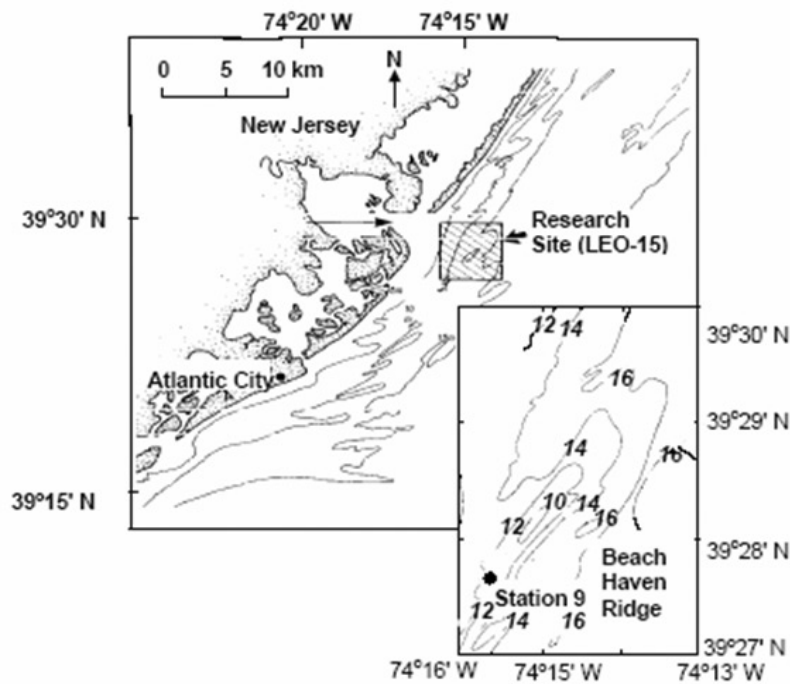


Figure 1. Map of research site LEO-15, continental shelf off New Jersey, United States. Smaller inset is of Beach Haven Ridge showing bathymetry (m) and type location, Station 9, of *Polygordius jouinae* sp. nov.

the LEO-15 research site (Von Alt & Grassle, 1992) on Beach Haven Ridge (39°27.69'N 74°15.81'W) (Figure 1). Beach Haven Ridge (5 km long by 1.5 km wide) is one of 71 shore oblique, sand ridges on the continental shelf off New Jersey (McBride & Moslow, 1991). Station 9 is on the shoreward side of the ridge at ~12 m depth, and is a coarse sand habitat (~2.0 mm) (Craghan, 1995). Samples were collected during spring and summer 2004/2005 by SCUBA divers using 7-cm-diameter corers (surface area 38.5 cm²) pushed ~10 cm into the sediment. Samples were sorted prior to fixation (live sorted) for *Polygordius jouinae* sp. nov. which were either frozen at -80°C for DNA analysis or fixed in 10% buffered formalin unless indicated otherwise, and transferred to 80% ethanol after 24 h. Fixed material was also obtained through loans from various benthic surveys that have collected *P. jouinae* sp. nov. from bays and harbours along the east coast of the United States including George's Bank, Massachusetts Bay, Cape Cod Bay, Rhode Island Sound, and Belmar (New Jersey).

Belmar research site and habitat data

Infaunal samples from Belmar were collected by the New Jersey Division of Fish and Wildlife (NJDFW) in an independent study examining the impact of sand dredging on macrofauna (1996–2004, unpublished). *Polygordius jouinae* sp. nov. abundance and grain size data obtained by the NJDFW were examined in the present study. Belmar is located ~72 km north of LEO-15 and samples were collected from a reference site (~1.36×2.04 km), on the continental shelf (~40°11.09'N 73°57.08'W) at depths of 13–16 m. This site was divided into six sub-sites (~0.68×0.68 km), and within each of these, six infaunal, and one sediment core for grain size were collected seasonally when possible. Corers used in the NJDFW study were the same as those used in the LEO-15 sampling

and taken by the same divers. Sorting and identification of samples were completed by the Cove Corporation, Lusby, Maryland. Sediment samples were dried and sieved over nested sieves into eight grain size-categories. To determine if there was a relationship between grain size and density of *P. jouinae* sp. nov. Spearman rank correlations were calculated based on data provided by the NJDFW for the eight sediment grain size categories and density of *P. jouinae* sp. nov. (α 0.05 or 0.01; total=92). Per cent abundance of *P. jouinae* sp. nov. of total macrofauna was calculated for each sub-site/date (total=134).

Light and electron microscopy

Live and fixed specimens collected from LEO-15, New Jersey were studied using stereo and compound microscopes. Measurements were made on fixed material using a high resolution 1.4 megapixel, colour firewire camera with Image Pro Express manual measurement software. Total length was measured from the anterior margin of the prostomium (antennae not included) to the posterior margin of the pygidium containing the anus. Prostomium length was defined as the distance from the anterior margin of the prostomium to the head fold (Rota & Carchini, 1999). The width was taken at approximately the widest segment in the middle region of the body. Segmentation in preserved specimens is not visible externally making observations of whole, live specimens under a compound microscope essential for counting segments.

Scanning electron microscopy (SEM) was conducted to confirm certain observations such as the absence of pygidial glands. Worms were fixed using an OsO₄ vapour/OsO₄+glutaraldehyde protocol while in a Swinnex filter holder (Leander et al., 2003b). The SEM was also conducted on formalin-fixed material from Massachusetts

Bay, Rhode Island Sound, and Belmar. All SEM specimens were dehydrated using a graded ethanol series, critical-point dried using CO₂, mounted on aluminium stubs, and sputter coated with gold-palladium (Au-Pd). Stub-mounted specimens were examined with either a Hitachi S4700 SEM or a CamScan CS 24 SEM configured with Orion 5.08 software.

Transmission electron microscopy (TEM) was conducted on one of the many pores in the epithelium of the mid-trunk region. Worm fragments were collected in Eppendorf tubes and fixed in 2% glutaraldehyde/seawater at 4°C for 1 h. The fixed worm fragments were washed twice in seawater for 15 min and post-fixed with 1% OsO₄ at 4°C for 1 h. Worms were washed with distilled water, dehydrated with a graded series of ethanol, rinsed twice with acetone, infiltrated with an acetone-resin mixture, and flat embedded with Epon resin at 60°C. Worm fragments were excised with a razor blade and fixed to blank Epon blocks before being sectioned on a Leica UltracutT Ultramicrotome. Ultra-thin sections were post-stained with uranyl acetate and lead citrate and viewed under a Hitachi H7600 transmission electron microscope.

DNA extraction and PCR amplification

Ten individuals of *Polygordius jouinae* sp. nov. were washed three times in filtered seawater and placed in a 1.5 ml Eppendorf tube. DNA was extracted with a standard cetyltrimethylammonium bromide (CTAB) protocol (Zolan & Pukkila, 1986). Worms were suspended in 400 µl CTAB extraction buffer (1.12 g Tris, 8.18 g NaCl, 0.74 g EDTA, 2 g CTAB, 2 g polyvinylpyrrolidone, 0.2 ml 2-mercaptoethanol in 100 ml water), homogenized in a Knotes Duall 20 tissue grinder, incubated at 65°C for 30 min, and separated with chloroform:isoamyl alcohol (24:1). The aqueous phase was then precipitated in 70% ethanol. The 18S small subunit (SSU) rRNA gene from *P. jouinae* sp. nov. was amplified as a single fragment using universal eukaryotic primers (5'GAATTCAACCTGGTTGATCCTGCCAGT-3' and 5'CGGATCCTGATCC-TTCTGCAGGTTACCTAC-3') and a thermocycling protocol described previously (Leander et al., 2003a). A polymerase chain reaction (PCR) product corresponding to the expected size (1800 bp) was gel isolated and cloned into the PCR 2.1 vector using the TOPO TA cloning kit (Invitrogen, Frederick, Maryland, USA). Sixteen clones were screened for size using PCR, and two clones were sequenced with ABI big-dye reaction mix using the vector primers and four internal primers oriented in both directions. The SSU rDNA sequence from *P. jouinae* sp. nov. was detected by BLAST analysis and deposited in GenBank.

Authorship of *Polygordius triestinus* Hempelmann, 1906

The name *Polygordius triestinus* appears with varying authorship in the literature (e.g. Fauvel, 1927; Woltereck-Hempelmann, 1906 as well as Hempelmann, 1906; Remane, 1932; Hempelmann, 1906; Du Bois-Reymond Marcus, 1948; Woltereck, 1905; Hartman, 1959; Woltereck in Hempelmann, 1906; Jouin, 1970; Woltereck, Hempelmann, 1906; Rota & Carchini, 1999; Woltereck in Hempelmann,

1906) thus prompting us to carefully study the history of this name.

Woltereck introduced the name *Polygordius triestinus* nov. spec. (Woltereck, 1902: p. 7) as a *nomen nudum* based on specimens collected by Cori and intended to describe it (Cori, 1902: p. 362; Steuer, 1904: p. 229). Later he referred to the species as '... neuen Triestiner *Polygordius* Species...' (Woltereck, 1905: p. 182) when explicitly citing results from investigations by Friedrich Hempelmann. Finally, in 1906 the formal description of the new species was published by Hempelmann under the title '*Polygordius triestinus* Woltereck (nov. spec.)' as part of his PhD thesis (Hempelmann, 1906). This description is based on specimens given to him by Woltereck, the advisor on his thesis (Woltereck, 1905: p. 159; Hempelmann, 1906: p. 529). Although Hempelmann attributed the name *Polygordius triestinus* to his teacher Woltereck (Hempelmann, 1906: pp. 527, 530, 605), it was Hempelmann who produced the description (see also Hempelmann, 1908: p. 665) and there is no published evidence that Woltereck contributed to it in any form. Therefore, following the International Code of Zoological Nomenclature (1999) Hempelmann is to be cited as the single author of *Polygordius triestinus* Hempelmann, 1906.

List of abbreviations

BMNH, The Natural History Museum, London; MCZ, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts; MNHN, Muséum National d'Histoire Naturelle, Paris; NBI, National Benthic Inventory, Charleston, South Carolina; NMW, National Museum and Gallery of Wales, Cardiff; USNH, National Museum of Natural History, Smithsonian Institution, Washington DC; SMF, Senckenberg Museum, Frankfurt/Main; SMNH, Swedish Museum of Natural History, Stockholm; YPM, Yale Peabody Museum of Natural History, Yale University, New Haven, Connecticut; ZMB, Museum für Naturkunde der Humboldt-Universität, Berlin; ZMH, Zoologisches Museum der Universität Hamburg, Hamburg.

SYSTEMATICS

Family POLYGORDIIDAE Czerniavsky, 1881

Genus *Polygordius* Schneider, 1868

Polygordius jouinae sp. nov.

(Figures 2–3)

Polygordius sp. A—Blake et al. (1983: 40–42); Maciolek & Grassle (1987: 303–309)

Polygordius sp.—Snelgrove (1999: 813–831; 2001: 1341–1347)

Polygordius sp. A—Kropp et al. (2002: 5–12 to 5–14); Maciolek et al. (2004: 5–11 to 5–15)

Polygordius sp. A – Battelle (2003: 7–9; 2004: 21–22)

Type material

Holotype: deposited at YPM (YPM 38050), a complete, sexually mature male, fixed in 80% ethanol, north-western Atlantic Ocean, United States, New Jersey, Tuckerton, Beach Haven Ridge, LEO-15 Station 9 (39°28'N 74°15'W), ~12 m depth in coarse sand (~2.0 mm), leg. P.A. Ramey, 19 May 2005.

Paratypes: six specimens, leg. P.A. Ramey in May 2004/2005, at same locality as holotype. Two paratypes deposited at the SMF (SMF 15970 SEM stub no. 679, leg. 27 May 2004; SMF 15971, male, fixed in 80% ethanol, leg. 19 May 2005), one each at YPM 38051 (SEM stub, leg. 27 May 2004), USNM 1086654 (male, fixed in 80% ethanol, leg. 19 May 2005), NBI V3709 (female, fixed in 80% ethanol, leg. 19 May 2005), and MCZ 68620 (male, 80% ethanol, leg. 19 May 2005).

Comparative material examined

Type material

Polygordius antarcticus Rota & Carchini, 1999: paratype (SMNH 5043), Antarctic Ocean, Ross Sea, Terra Nova Bay, sea between Tethys Bay, 74°40'S, and Adélie Cove, 74°46'S, between longitudes 164°01'E and 164°08'E, 50 m, leg. G. Carchini, 24 January 1988. *Polygordius eschaturus brevipapillosus* Jouin & Rao, 1987: holotype (MNHN 1392) and paratype (MNHN 1164), Indian Ocean, North Andaman Island, south island beach, 12°58'06"N 92°59'17"E, 5 cm below surface, near low water mark, sandy sediment (400–700 µm), leg. G.C. Rao, 28 April 1974. *Polygordius pacificus floreanensis* Schmidt & Westheide, 1977: holotype (ZMH P-13668), South Pacific Ocean, Galapagos Islands, Floreana, black beach, eulittoral, coarse black lava sand, leg. June 1972.

Non-type material

Polygordius appendiculatus Fraipont, 1887: 15 specimens (NMW 1989.104.1867), north-eastern Atlantic Ocean, Irish Sea, St George's Channel, 52°05.7'N 05°33.7'W, 112 m, sandy gravel, leg. 12 July 1989. *Polygordius jouinae* sp. nov., north-western Atlantic Ocean, United States: numerous specimens (USNH 1008249), George's Bank (United States shelf/slope break), 40°27'06"N 06°73'72'4"W, 152 m, leg. 10 May 1982, as *Polygordius* sp. A. (Blake et al., 1983; Maciolek & Grassle, 1987);—numerous specimens, Massachusetts Bay, leg. August 2004, as *Polygordius* sp. A. (Kropp et al., 2002; Maciolek et al., 2004), received from J. Blake, ENSR Marine and Coastal Center;—7 specimens on slides (MNHN MA22-28), leg. 1967–1969, and 3 specimens on slides (YPM: Gray 5339, 5340, 5338), leg. 1967–1969, Cape Cod Bay, all as *P. triestinus* Hempelmann, 1906 (det. C. Jouin-Toulmond);—numerous specimens, Rhode Island Sound, sandy sediments, leg. 29–30 July 2003, as *Polygordius* sp. A., det. Cove Corporation (Battelle, 2003, 2004);—numerous specimens, New Jersey, Belmar on the continental shelf, 40°11.09'N 73°57.08'W, leg. 2001, as *Polygordius* sp., det. Cove Corporation, New Jersey Division of Fish and Wildlife;—numerous specimens, New Jersey, Beach Haven Ridge, LEO-15, 39°27.69'N 74°15.81'W, leg. August to September 1994, as *Polygordius* sp., det. R. Petrecca (Snelgrove et al., 1999, 2001), received from F. Grassle, Institute of Marine and Coastal Sciences, Rutgers University. *Polygordius lacteus* Schneider, 1868: 1 specimen (USNH 53318), north-western Atlantic Ocean, United States, Maine, nearest place Crow Neck, North Tresscott, 0–1 m, leg. 20 February 1976, det. N. Riser;—1 specimen (SMF 9842), north-eastern Atlantic Ocean, France, Roscoff in Brittany, leg. in the 1970s. *Polygordius neapolitanus* Fraipont, 1887: numerous specimens (MNHN), Mediterranean Sea, Italy, Naples, leg. Dius, 10

May 1907;—3 parts (BMNH 1928.4.26.657/8), leg. Fauvel, 26 April 1928.

Diagnosis

Prostomium conical with two tapering antennae attached close together at their bases. Ratio of antenna length to prostomium length is ~1:1. Eyes absent. Pygidium densely ciliated, with terminal anus with seven small lobes directed interiorly. Pygidium not inflated and without glands or anal cirri. Species gonochoric.

Description (based on holotype)

Sexually mature male, clear to milky white in colour (live observation) due to presence of sperm. Opaque after fixation. Body elongated (length=19.4 mm) and cylindrical, tapering towards the head and terminal anus. A distinctive ventral groove present. All segments lacking parapodia and achaetous. Prostomium and peristomium separated by a ventral transverse groove or 'head fold' (Figure 2A). Prostomium conical (length=140 µm) with two tapering antennae (length=130 µm) attached very closely at their bases (Figure 2A). Antennae with sensory cilia. Length ratio of antenna to prostomium ~1:1 (Figure 2A; Table 1). One pair of oval, heavily ciliated nuchal organs present dorsolaterally, at the posterior margin of the prostomium (Figure 2A). Eyes absent. Mouth ventral with densely ciliated buccal lip (Figure 2A–D). Posterior end not inflated, and without pygidial glands or anal cirri (Figure 2E,F). Pygidium densely ciliated with a terminal anus with seven small lobes directed interiorly (Figure 2F). External segmentation not visible. Number of segments inconclusive (>65) due to fixation and subsequent opaqueness. Complete segment counts determined for other sexually mature individuals mounted live on slides (see Table 1). Internal segmentation best visible in live, sexually mature individuals with blood sinuses enlarged thus providing a reddish hue. Body widest in mid-region (width=0.23 mm). Body surface covered by thin cuticle with fine transverse striations (Figure 2G), perforated with many small irregularly distributed oval pores (length ~1 µm; width 0.5 µm) (Figure 2G–I). Variability in measurements in sexually mature specimens: antenna and prostomium length 0.11 to 0.15 mm, body length 13.0 to 43.1 mm, body width 0.23 to 0.38 mm, number of segments 82 to 93 (see also Table 1). No evidence of sexual dimorphism in this species (see Table 1 for female vs male measures). Information pertaining to distribution, habitat, and reproduction, addressed below based on additional paratype and non-type material.

SSU rDNA sequence from *P. jouinae* sp. nov. deposited in GenBank (Accession no. DQ153064) containing eight novel indels at positions 250, 711, 775, 851, 857, 884, 895, and 1732 (relative to the pairwise aligned sequence 'AF412809').

Remarks

Polygordius jouinae sp. nov. is distinguished from most other *Polygordius* species by its non-inflated, heavily ciliated pygidium, the absence of pygidial glands, and conical (rather than rounded) prostomium. Morphologically similar species include *Polygordius triestinus* Hempelmann, 1906 from the north Adriatic Sea, *Polygordius triestinus sensu* Jouin (1970) from New Caledonia, and *P. antarcticus*

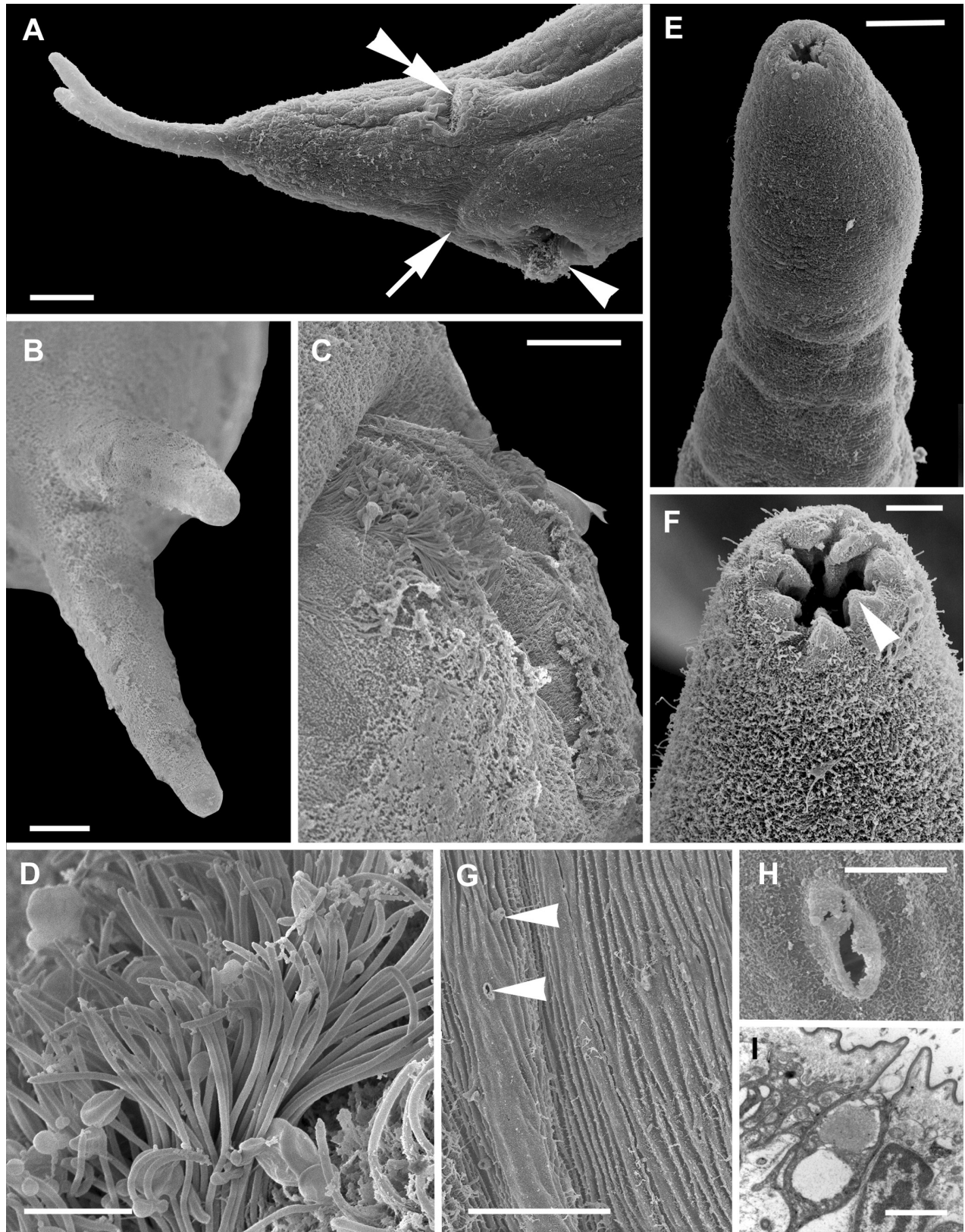


Figure 2. Morphology of *Polygordius jouinae* sp. nov. (A) Scanning electron microscopy (SEM) showing a lateral view of conical prostomium, ciliated nuchal organ (double arrowhead), head fold demarcating the prostomium from the peristomium (arrow), and ventral mouth (arrowhead); (B) SEM showing dorsal view of prostomium and paired antennae; (C) high magnification of ventral mouth and ciliated buccal lip; (D) higher magnification of cilia on buccal lip; (E) SEM showing lateral view of posterior end; (F) SEM showing lateral view of pygidium and seven anal lobes (arrowhead); (G) SEM showing cuticular striations and body pores (arrowheads); (H) high magnification SEM of body pore; and (I) transmission electron microscopy through body pore. Note: Figure 2A,E,F are from a paratype SEM stub located at the SMF (SMF 15970 SEM stub no. 679), and Figure 2B,C,D,G–I are from a paratype SEM stub located at the YPM (YPM 38051). Scale bars: A,E, 30 μm ; B, 12 μm ; C,F,G, 10 μm ; D, 2.5 μm ; H,I, 1 μm .

Table 1 Synoptic table of characters of *Polygordius jouinae* sp. nov. with morphologically most similar species of the genus (all based on fixed specimens). Morphological information for *P. triestinus* and *P. triestinus* sensu Jouin (1970) from original species descriptions because type material was either lost or never deposited. Information for *P. antarcticus* taken from description and confirmed on paratype since holotype was not available. Character information based on observations of sexually mature individuals with the exception of *P. antarcticus*, and population measurements for *P. jouinae* sp. nov., which included some immature individuals.

Character/species	<i>P. jouinae</i> sp. nov.	<i>P. triestinus</i> Hempelmann, 1906	<i>P. triestinus</i> sensu Jouin (1970)	<i>P. antarcticus</i> Rota & Carchini, 1999
Prostomium shape	conical	conical	conical	conical
Antenna length (mm)	holotype (0.13) range (0.11–0.15; N=43)	n.d.	n.d.	0.075
Prostomium length (mm)	holotype (0.14) range (0.11–0.15; N=43)	n.d.	n.d.	~0.15
Ratio (antenna: prostomium)	1:1	n.d.	n.d.	0.5:1
Antennae (close or spaced)	close	close	n.d.	close
Eyes	absent	absent	absent	absent
Pygidium shape	not inflated	not inflated	not inflated	inflated
Pygidial glands	absent	absent	present	present 28–30
Pygidial gland shape	N/A	N/A	n.d.	round
Pygidial cirri	absent	absent	absent	absent
Anal lobe (no.)	7	n.d.	5 (F)	6–7
Body length (mm)	holotype (19.4) females (13.0–42.7; N=20) males (13.8–43.1; N=23) population (3.0–23.5; N=318)	maximum 30	10–20	20 (subadult)
Body width (mm)	holotype (0.23) female (0.24–0.38; N=20) males (0.23–0.34; N=23) population (0.06–0.32; N=318)	n.d.	n.d.	0.22–0.32 (subadult)
Segment (no.)	holotype (>65) females (82–91; N=10) males (86–93; N=10)	n.d.	~60	82–98 (subadult)
Reproduction	gonochoristic	hermaphrodite	only males found	n.d.
Type locality	north-western Atlantic Ocean, USA, New Jersey, Tuckerton, Beach Haven Ridge	Northern Adriatic Sea, Italy, near Trieste	South Pacific Ocean, Bay of Saint-Vincent, New Caledonia	Antarctic Ocean, Ross Sea, Terra Nova Bay
Sediment type	medium to very coarse sand	muddy, oxygen poor sediments in relatively quiescent environments	medium sand with some fine material susceptible to variable sali- nities and strong currents	medium to coarse grained, well- oxygenated sands
Depth range (m)	5–152	n.d.	6–7	31–61

N/A, not applicable; n.d., no data available, N, number of specimens measured; F, observations taken from a figure rather than being explicitly stated in the text.

Rota & Carchini, 1999 from the Ross Sea, Antarctic Ocean. However, *Polygordius jouinae* sp. nov. can be clearly distinguished from the three species mentioned above in the following characters (see also Table 1). *Polygordius jouinae* sp. nov. is gonochoristic, whereas Hempelmann's *P. triestinus* is a hermaphrodite, described as having oocytes and sperm in close proximity to each other in the coelom (Hempelmann, 1906). Currently *P. triestinus* is the only known hermaphroditic species in the Polygordiidae, however, hermaphroditism cannot be ruled out for *P. triestinus* sensu Jouin, as only sexually mature males were found. *Polygordius jouinae* sp. nov. lacks pygidial glands, whereas they are present in *P. triestinus* sensu Jouin and *P. antarcticus*. *Polygordius jouinae* sp. nov. also lacks an

inflated pygidium present in *P. antarcticus*. Additionally the length ratio of antenna to prostomium is 1:1 for *P. jouinae* sp. nov. in contrast to 0.5:1 in *P. antarcticus*.

Unfortunately, direct comparison was impossible since specimens of *P. triestinus* sensu Jouin (1970) were not deposited in Paris (MNHN) and are not available in the personal collection of C. Jouin-Toulmond (C. Jouin-Toulmond, personal communication). We were unsuccessful, as were Rota & Carchini (1999), in locating type material for *P. triestinus*. The latter also reported that specimens were not present in the Laboratory of Marine Biology, Trieste. Since Hempelmann and Woltereck, who provided Hempelmann with the original specimen(s) for the description of *P. triestinus* and was

Table 2 Spearman's rank correlation between grain size and density of *Polygordius jouinae* sp. nov. (number m^{-2}) at each subsite, Belmar, New Jersey over all sampling dates.

Grain size	mm	Spearman's correlation	P-value
Pebble	>4	0.123	0.243
Granule	2–4	0.174	0.098
Very coarse	1–2	0.297**	0.004**
Coarse sand	0.5–1	0.314**	0.002**
Medium sand	0.25–0.5	0.265*	0.011*
Fine sand	0.125–0.25	–0.249*	0.019*
Very fine sand	0.062–0.125	–0.353**	0.001**
Mud	<0.062	–0.166	0.113

Total N=92; *, $P < 0.05$; **, $P < 0.01$.

therefore subsequently often erroneously quoted as author of this taxon (see Materials & Methods), both worked at the University of Leipzig, Germany we also requested type specimens from other major German collections, i.e. in Berlin (ZMB) and Hamburg (ZMH) besides checking the collections of Senckenberg (SMF). However, our requests were unsuccessful. Furthermore, *P. triestinus* has not been reported in benthic samples from the Gulf of Trieste from 1966 to 2003. To the best of our knowledge it has not been collected again since the original species description (V. Solis-Weiss, personal communication). Unfortunately, our loan request to the Museo Civico di Zoologia di Roma, Rome, for the holotype of *P. antarcticus* remained unanswered.

In addition to the distinction based on morphological characters, especially in a polychaete taxon lacking

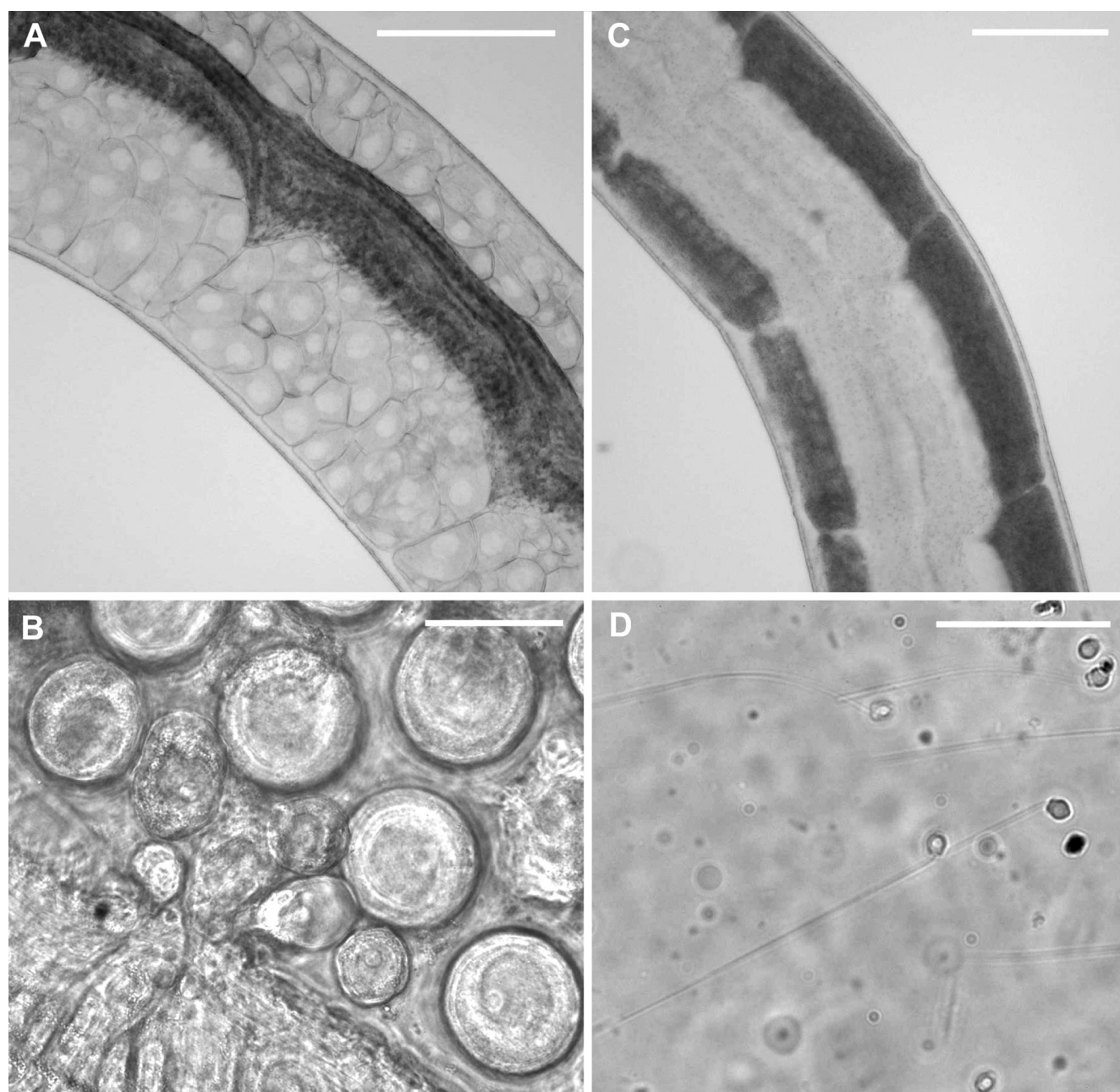


Figure 3. Light micrographs of live mounted *Polygordius jouinae* sp. nov. (A) Female with oocytes tightly packed within coelom; (B) oocytes bursting through body wall to surrounding medium; (C) male with sperm packed within coelom; and (D) morphology of spermatozoa. Scale bars: A, 190 μm ; B, 45 μm ; C, 135 μm ; D, 25 μm .

'typical' characters as chaetae and parapodia, we analysed the 18S ribosomal RNA sequence from *P. jouinae* sp. nov. and deposited it in GenBank as a reference for further systematic studies of this group. Furthermore, we compared the 18S ribosomal RNA sequence from *P. jouinae* sp. nov. (Accession no. DQ153064) to the sequence from an unnamed *Polygordius* species collected from Anse Forbans and Mahé, Seychelles, India (Accession no. AF412809; Struck et al., 2002) that is also deposited in GenBank. The sequence from *P. jouinae* sp. nov. was 98.2% identical to the sequence from the *Polygordius* sp. from India (32 differences over 1814 compared sites), however, it contained eight novel indels at positions 250, 711, 775, 851, 857, 884, 895 and 1732 (relative to sequence AF412809), thus adding to the distinction of *P. jouinae* sp. nov.

Etymology

The species is named after Dr Claude Jouin-Toulmond who studied specimens from Cape Cod Bay, Massachusetts in the 1970s and noted that this species was gonochoristic.

Distribution and habitat

Sandy habitats on the inner continental shelf, and in bays and harbours from Massachusetts to New Jersey (42°N and 39°N) to maximum depth of 152 m on George's Bank.

Specimens referred to as *Polygordius* sp. or *Polygordius* sp. A in several studies are here referred to *P. jouinae* sp. nov. (i.e. Blake et al., 1983; Maciolek & Grassle, 1987; Snelgrove et al., 1999, 2001; Kropp et al., 2002; Battelle, 2003, 2004; Maciolek et al., 2004). Spearman rank correlations of sediment grain size with density (individuals m⁻²) of *P. jouinae* sp. nov. collected at Belmar showed that density was significantly ($P < 0.05$; $N = 92$) positively correlated with the proportion of medium to very coarse sand (0.25–1.00 mm) and negatively correlated with the fine sand (0.125–0.25 mm) fractions (Table 2). *Polygordius jouinae* sp. nov. was present at all sub-site/dates from 1996–2003, (with the exception of three sampling dates) and comprised up to 78% (mean = 27%) of the total macrofaunal abundance (individuals m⁻²).

The habitat where *P. jouinae* sp. nov. was found is most similar to the habitat of *Polygordius triestinus sensu* Jouin, which was reported from a depth of 6–7 m in an area with strong currents. Bottom sediments were described as medium sand with some fine material, however, this area was also susceptible to variable salinities (Jouin, 1970). *Polygordius triestinus* is the only *Polygordius* species reported from muddy, oxygen poor sediments in relatively quiescent environments (Hempelmann, 1906). *Polygordius antarcticus* was reported from medium to coarse grained, well-oxygenated sands at depths ranging from 31–61 m (Rota & Carchini, 1999).

Reproduction

Polygordius jouinae sp. nov. collected on 19 May 2004 and 27 May 2005 from the LEO-15 research site comprised only sexually mature individuals. The proportion of males to females was ~1:1. Both sexes were notably swollen with gametes and had enlarged blood sinuses giving them a reddish hue when viewed with the naked eye. Oocytes were tightly packed in the coelom and appeared clear

and irregular in shape (Figure 3A). They were present from the first 19–27 segments until the last 10–16 and moved freely throughout the coelom. It is likely that oocytes and sperm are set free by rupture of the body wall (Figure 3B). Released from the body cavity, oocytes were rounder and varied in size (diameter 22–62 µm). Males were milky white in appearance and sperm were present from the first 15–25 segments until the last 10–20. Spermatozoa had round heads (3.30–3.56 µm), tiny pointed acrosomes, short mid-pieces, and long tails (50–60 µm), (Figure 3C,D).

Gut parasites

Stereomicroscopic observations of live *Polygordius jouinae* sp. nov. from LEO-15 revealed that the intestines were heavily infected with single-celled, ovoid parasites within the gut. This parasite is gregarine-like and may be closely related to the intestinal parasites reported for *P. antarcticus* and for another species, *P. neapolitanus* Fraipont, 1887. A separate study has been conducted on the intestinal parasites (Leander & Ramey, 2006).

DISCUSSION

Rota & Carchini (1999) summarized the characters previously used to identify species of *Polygordius* such as, e.g. body size, segment number, colour, shape of pygidium, ciliation on the body surface, pattern of the circulatory system, etc. (Rota & Carchini, 1999: table 1). During the present study, observations on living and fixed material of *Polygordius jouinae* sp. nov. showed that colour differs depending on whether specimens are live or fixed, or sexually mature males or females. In sexually mature individuals the oocytes and sperm packed into the coelom greatly influence body width leaving external segmentation invisible thus making it difficult to count segments in preserved material. Identifications based on differences in the arrangement of the circulatory system have been controversial (Rota & Carchini, 1999) and we consider this character of limited diagnostic value. In our study of specimens of *P. jouinae* sp. nov. features of the circulatory system, specifically the vascular loops connecting dorsal and ventral vessels in the anterior part of the body, proved very similar to those observed for *P. triestinus sensu* Jouin (1970) (based on personal notes kindly provided by Dr C. Jouin-Toulmond) and those reported for *P. antarcticus* by Rota & Carchini (1999).

Specimens collected from Cape Cod Bay, Massachusetts in 1968–1969 had been identified as *Polygordius triestinus* by Dr C. Jouin-Toulmond based primarily on details of the blood vascular system as described by Hempelmann (1906) for the respective species. Our careful re-examination of these Cape Cod specimens, which had meanwhile been deposited in the collections of the Yale Peabody Museum (YPM) as well as at the Muséum National d'Histoire Naturelle (MNHN), considering reproductive mode (gonochorism/hermaphroditism), additional features of the pygidium such as overall shape (inflated/non inflated) and the presence/absence of pygidial glands, revealed that these specimens belong to *P. jouinae* sp. nov. Moreover, species for which the circulatory system has been discussed include different levels of detail, are fraught with inconsistent terminology, and re-evaluation

in specimens that have been preserved for a long time proved very difficult.

We agree with Rota & Carchini (1999) that it is likely that many more species of *Polygordius* remain to be described and that the pygidium and its associated structures will be important defining characters. Especially the shape and number of pygidial glands can be useful (P. Ramey, unpublished data), although SEM is needed for accurate observation and counting.

Macrofaunal samples collected seasonally from six sub-sites at Belmar over eight years showed *P. jouinae* sp. nov. to be the most abundant macrofaunal species in sandy sediment communities. This dominance in sandy sediments is not restricted to Belmar. In Boston Harbor, *P. jouinae* sp. nov. made up 98% of the macrofaunal (300 µm sieve) abundance at a single sandy site with a mean density of 7483 individuals m⁻² (Kropp et al., 2002), and the same trends were found in Rhode Island Sound (area E) where it was the most abundant species in sediments composed of 85–98% sand (Battelle, 2003). In the same location, sediments containing >20% mud (<0.063 mm) were dominated by a deposit feeding bivalve, *Nucula annulata* Hampson, 1971 (Battelle, 2003). On George's Bank *P. jouinae* sp. nov. was one of the ten most abundant species present seasonally at 14 out of 17 regional stations (~80–152 m deep). At shallower locations (stations 1, 4, 10; all 60 m deep) with coarser sediments it was the dominant macrofaunal species (Maciolek & Grassle, 1987).

The widespread distribution of relatively high densities of *Polygordius jouinae* sp. nov. in coarse sandy sediments may be related in part to its mode of reproduction. The coelomic spaces of mature *P. jouinae* sp. nov. from LEO-15 were packed with large numbers of small oocytes (N=several 1000; diameter <63 µm) or sperm. Sperm structure indicated that gametes are most likely freely spawned into the water column (Rouse & Jamieson, 1987) and small eggs usually give rise to planktotrophic larvae (e.g. Strathmann & Strathmann, 1982). Moreover, planktotrophic larvae occur in the four species of *Polygordius* where development mode is known (Rota & Carchini, 1999). In contrast, brooding with direct development of a limited number of eggs is prevalent in interstitial polychaetes (e.g. Spioninae, Fabriciinae, Dorvilleidae, Syllidae) (reviewed by Giangrande, 1997). Greater dispersal ability is associated with planktonic larvae and although larval survival may be low during the pelagic phase, the greater numbers of offspring produced by *P. jouinae* sp. nov. compared to other interstitial polychaetes may help explain its relatively high abundance in sandy sediment communities. In support of this the next most abundant species often found to co-occur with *P. jouinae* sp. nov. is the free spawning bivalve *Spisula solidissima* (Dillwyn, 1817). Other relatively less abundant co-occurring species include representatives of brooding interstitial polychaetes such as Dorvilleidae, Syllidae, and some members of the Hesionidae and Protodrilidae.

Changes in sediment composition and concomitant increases in organic loading affect the abundance and distribution of *Polygordius jouinae* sp. nov. thus making it a possible indicator of changing environmental conditions. A good example comes from the Rhode Island Region, long-term, dredged material disposal site (Battelle, 2003). Prior to disposal of dredged material (primarily made up

of muddy estuarine sediments), *P. jouinae* sp. nov. was the most abundant macrofaunal species at sites in the Rhode Island Sound Region (autumn 2001). Following mud disposal (July 2003), *P. jouinae* sp. nov. no longer numbered among the top ten species being replaced by the deposit feeding bivalve *Nucula annulata*. During this same time period the proportion of mud (<0.063 mm) in sediments increased from 7% to 30% (Battelle, 2004).

We thank Claude Jouin-Toulmond for kindly providing notes and information on deposition of specimens. Much appreciation goes to the New Jersey Division of Fish and Wildlife who provided us with abundance data for *Polygordius jouinae* sp. nov. collected as part of a study examining the impact of sand dredging on benthic communities off Belmar, New Jersey (1996–2004). Mike Celestino was especially helpful and provided us with specimens from the unpublished Belmar study. Special thanks are also extended to the many other people who provided us with material for identification (listed in Materials and Methods). Rosemarie Petrecca and Institute of Marine and Coastal Sciences (IMCS) staff collected the infaunal cores at LEO-15 research site. Special thanks to Fred and Judy Grassle for their support and encouragement. Judy Grassle, Pedro Quijón, Jim Blake and two anonymous referees provided helpful comments on the manuscript. P.A. Ramey was supported by an IMCS graduate assistantship and graduate student research funds from the Rutgers University Marine Field Station. This is contribution no. 2005-12 from the IMCS. Thanks also to the Director of the Senckenberg Museum for providing laboratory space to P.A. Ramey. B.S. Leander is a Canadian Institute for Advanced Research scholar and was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC 283091-04).

REFERENCES

- Battelle, 2003. Benthic infauna data for Area E and Area W, July 2003. Rhode Island Region. Long-term dredged material disposal site evaluation project. Prepared under contract No. DACW33-01-D-0004, Delivery order no. 02., 17 pp.
- Battelle, 2004. Appendix C. Final site management and monitoring plan (SMMP) for the Rhode Island Region long-term dredged material disposal site evaluation project, 46 pp. http://www.epa.gov/boston/eco/ridredge/pdfs/eis_final/Final%20Appendix%20C%20RIR%20EIS.pdf
- Blake, J.A., Grassle J.F., Maciolek-Blake N., Neff J.M. & Sanders H.L., 1983. The Georges Bank Benthic Infauna Monitoring Program. Battelle New England Marine Research Laboratory, Duxbury MA and Woods Hole Oceanographic Institution, Woods Hole MA. Contract no. 14-12-0001-29192, 153 pp.
- Cori, C.I., 1902. Mitteilungen aus der k.k. zoologischen Station in Triest. Nr. 3. Über das Vorkommen des *Polygordius* und *Balanoglossus* (Ptychodera) im Triester Golfe. *Zoologischer Anzeiger*, **25**, 361–365.
- Craghan, M., 1995. *Topographic changes and sediment characteristics at a shoreface sand ridge—Beach Haven Ridge, New Jersey*. MS thesis, Rutgers University, New Brunswick, USA.
- Czerniavsky, V., 1881. Material ad zoographiam Ponticam. Fasc. III Vermes. *Bulletin de la Société Impériale des Naturalistes de Moscou*, **55**, 211–363.
- Dillwyn, L.W., 1817. *A descriptive catalogue of recent shells arranged according to the Linnean method; with particular attention to the synonymy*, vol. I. London: J. & A. Arch.
- Du Bois-Reymond Marcus, E., 1948. Further archiannelids from Brazil. *Comunicaciones Zoológicas del Museo de Historia Natural de Montevideo*, **48**, 1–17.
- Fauvel, P., 1927. Polychètes sédentaires. *Faune de France*, vol. 16. Paris: Lechevalier.

- Fraipont, J., 1887. Le genre *Polygordius*. *Fauna und Flora des Golfes von Neapel*, **14**, 1–125.
- Giangrande, A., 1997. Polychaete reproductive patterns, life cycles and life histories: an overview. *Oceanography and Marine Biology. Annual Review*, **35**, 323–386.
- Hampson, G.R., 1971. A species pair of the genus *Nucula* (Bivalvia) from the eastern coast of the United States. *Proceedings of the Malacological Society of London*, **39**, 333–342.
- Hartman, O., 1959. *Catalogue of the polychaetous annelids of the world*, part II. Los Angeles, California: University of Southern California Press.
- Hempelmann, F., 1906. Zur Morphologie von *Polygordius lacteus* Schn. und *Polygordius triestinus* Woltereck, nov. spec. *Zeitschrift für Wissenschaftliche Zoologie*, **84**, 527–618.
- Hempelmann, F., 1908. Neuere Arbeiten über *Polygordius* und einige an diesen Anneliden anknüpfende theoretische Erörterungen aus der Literatur der letzten Jahre. *Zoologisches Zentralblatt*, **15**, 649–672.
- Hermans, C.O., 1969. The systematic position of the Archiannelida. *Systematic Zoology*, **18**, 85–102.
- International Commission on Zoological Nomenclature, 1999. *International code of zoological nomenclature*, 4th edn. London: International Trust for Zoological Nomenclature.
- Jouin, C., 1970. Archiannelides interstitielles de Nouvelle Calédonie. In *Expédition française sur les récifs coralliens de la Nouvelle Calédonie, 1960–1963. Editions de la Fondation Singer-Polignac, Paris*, **4**, 147–167.
- Jouin, C. & Rao, G.C., 1987. Morphological studies on some Polygordiidae and Saccocirridae (Polychaeta) from the Indian Ocean. *Cahiers de Biologie Marine*, **28**, 389–402.
- Kropp, R.K., Diaz, R.J., Dahlen, D.T., Boyle, J.D. & Hunt C.D., 2002. *2000 Harbor Benthic Monitoring Report*. Boston: Massachusetts Water Resources Authority. Report ENQUAD 2002-02.
- Leander, B.S., Clopton, R.E. & Keeling, P.J., 2003a. Phylogeny of gregarines (Apicomplexa) as inferred from SSU rDNA and beta-tubulin. *International Journal of Systematic and Evolutionary Microbiology*, **53**, 345–354.
- Leander, B.S., Harper, J.T. & Keeling, P.J., 2003b. Molecular phylogeny and surface morphology of marine aseptate gregarines (Apicomplexa): *Selenidium* and *Lecudina*. *Journal of Parasitology*, **89**, 1191–1205.
- Leander, B.S. & Ramey, P.A., 2006. Cellular identity of a novel small subunit rDNA sequence clade of apicomplexans: description of a marine parasite *Rhytidocystis* sp. nov. (Host: *Polygordius* sp., Polychaeta). *Journal of Eukaryotic Microbiology*, in press.
- Maciolek, N.J. et al., 2004. *2002 Outfall Benthic Monitoring Report*. Boston: Massachusetts Water Resources Authority. Report ENQUAD 2003-13.
- Maciolek, N.J. & Grassle, J.F., 1987. Variability of the benthic fauna, II: seasonal variation, 1981–1982. In *Georges Bank* (ed. R.H. Backus and D.W. Bourne), pp. 303–309. Cambridge, Massachusetts: MIT Press.
- McBride, R.A. & Moslow, T.F., 1991. Origin, evolution, and distribution of shoreface sand ridges, Atlantic inner shelf, USA. *Marine Geology*, **97**, 57–85.
- McIntosh, W.C., 1875. On a new example of Opheliidae (*Linotrypane apogon*) from Shetland. *Proceedings of the Royal Society of Edinburgh*, **8**, 386–390.
- Moore, P., 1904. A new generic type of Polygordiidae (*Chaetogordius*). *American Naturalist*, **38**, 519–520.
- Rathke, H., 1843. Beiträge zur Fauna Norwegens. *Verhandlungen Kaiserlichen Leopoldinisch—Carolinischen Akademie der Naturforscher, Breslau*, **20**, 1–264.
- Remane, A., 1932. Archiannelida. Die Tierwelt der Nord- und Ostsee. Lieferung XXI, Teil VIa. Akademische Verlagsgesellschaft, Leipzig.
- Rota, E. & Carchini, G., 1999. A new *Polygordius* (Annelida: Polychaeta) from Terra Nova Bay, Ross Sea, Antarctica. *Polar Biology*, **21**, 201–213.
- Rouse, G.W. & Jamieson, B.G.M., 1987. An ultrastructural study of spermatozoa of the polychaetes *Eurythoe complanata* (Amphinomidae), *Clymenella* sp. and *Micromaldane* sp. (Maldanidae), with definition of sperm types in relation to reproductive biology. *Journal of Submicroscopic Cytology*, **19**, 573–583.
- Rouse, G.W. & Pleijel, P., 2001. *Polychaetes*. London: Oxford University Press.
- Schmidt, P. & Westheide, W., 1977. Interstitielle Fauna von Galapagos. 17. Polygordiidae, Saccocirridae, Protodrilidae, Nerillidae, Dinophilidae (Polychaeta). *Mikrofauna des Meeresbodens*, **62**, 1–38.
- Schneider, A., 1868. Über Bau und Entwicklung von *Polygordius*. *Archiv für Anatomie Physiologie und Wissenschaftliche Medizin, Leipzig*, **10**, 51–60.
- Snelgrove, P.V.R., Grassle, J.F., Grassle, J.P., Petrecca, R.F. & Stocks, K.I., 2001. The role of colonization in establishing patterns of community composition and diversity in shallow-water sedimentary communities. *Journal of Marine Research*, **59**, 813–831.
- Snelgrove, P.V.R., Grassle, J.P., Grassle, J.F., Petrecca, R.F. & Ma, H., 1999. *In situ* habitat selection by settling larvae of marine soft-sediment invertebrates. *Limnology and Oceanography*, **44**, 1341–1347.
- Steuer, A., 1904. Mitteilungen aus der k.k. zoologischen Station in Triest. Nr. 9. Über zwei interessante Larvenformen aus dem Plankton des Triester Golfes. *Zoologischer Anzeiger*, **28**, 228–230.
- Strathmann, R.R. & Strathmann, M.F., 1982. The relationship between adult size and brooding in marine invertebrates. *American Naturalist*, **119**, 91–101.
- Struck, T.H., Westheide, W. & Purschke, G., 2002. Progenesis in Eunicida ('Polychaeta,' Annelida)—separate evolutionary events? Evidence from molecular data. *Molecular Phylogenetics and Evolution*, **25**, 190–199.
- Von Alt, C.J. & Grassle, J.F., 1992. LEO-15, an unmanned long-term environmental observatory. *Proceedings of Oceans '92*, **2**, 849–854.
- Westheide, W., 1990. Polychaetes: interstitial families. *Synopsis of the British Fauna*, **44**, 1–152.
- Woltereck, R., 1902. Trochophora-Studien I. Ueber die Histologie der Larve und die Entstehung des Annelids bei den *Polygordius*-Arten der Nordsee. *Zoologica*, **34**, 1–71.
- Woltereck, R., 1905. Zur Kopffrage der Anneliden. *Verhandlungen der Deutschen Zoologischen Gesellschaft*, **15**, 154–186.
- Zolan, M.E. & Pukkila, P.J., 1986. Inheritance of DNA methylation in *Coprinus cinereus*. *Molecular Cell Biology*, **6**, 195–200.

Submitted 21 October 2005. Accepted 24 May 2006.