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ORIGINAL ARTICLE

Description of three new species of *Protodrilus* (Annelida, Protodrilidae) from Central America

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Abstract

Three new species of *Protodrilus* are described from the shallow Western Atlantic waters of Belize and Panama: *P. smithsoni* sp. nov., *P. draco* sp. nov. and *P. hochbergi* sp. nov. *Protodrilus smithsoni* sp. nov. resembles *P. jägersteni* and *P. submersus* from New Zealand, differing by (i) the presence of a dorsal ciliated area on segments 5–6 of males, (ii) lateral organs extending only to segment 15 (versus 16) and (iii) the smaller size of body and palps. *Protodrilus draco* sp. nov. is similar to the European *P. hypoleucus* and *P. helgolandicus*, but differs in (i) each pygidial lobe possessing a short cirrus and (ii) the posterior-most lateral organ extending dorsally. *Protodrilus hochbergi* sp. nov. resembles *P. purpureus* and *P. schneideri* from the Eastern Atlantic, but differs in (i) the extension of the salivary glands to segment 15 (versus 6) and (ii) the presence of paired lateral ciliary bands on the prostomium. This first exploration of *Protodrilus* along the Caribbean coast of Central America revealed five new species (three described here), but not *P. corderoi*, a species described from Brazil and recorded at Dominica. The findings indicate a putative high diversity of *Protodrilus* species in the Western Atlantic, comparable to the well-sampled Eastern Atlantic (18 reported species). The close resemblance to described species stresses the importance of detailed morphological studies, preferably including scanning electron microscopy, as well as DNA data, in order to describe and identify species of *Protodrilus*.

Key words: *Meiofauna, interstitial, scanning electron microscopy, barcoding, Caribbean*

Introduction

With 32 described species worldwide in shallow marine sediments, the Protodrilidae are, after the Nerillidae, the most species-rich family of meiofaunal annelids (Von Nordheim 1983, 1989; Worsaae & Kristensen 2005). In contrast to nerillids, all protodrilid species are restricted to the interstitial environment of sandy sediments, occupying the three-dimensional interstices among the sand grains (Westheide 2009). The family consists of the genera *Protodrilus* Hatschek, 1880 and *Parenterodrilus* Jouin, 1979, and is grouped with Protodriloididae and Saccocirridae in the clade Protodrilida (Purschke & Jouin 1988; Purschke 1990a, 1993). The interstitial success of the Protodrilidae is

attributed to their highly specialized and well-adapted body plan, sharing several synapomorphies: slender, elongated bodies, paired motile sensory palps, a specialized ventral muscular pharynx consisting of a bulbous muscle built up of muscle fibres and interstitial cells with small cell bodies and prominent tonofilaments, salivary glands lying in the oesophageal epithelium and opening in the buccal cavity, and two pygidial lobes provided with adhesive glands (Jouin 1970b; Purschke & Jouin 1988; Westheide 2009). Although the monophyly of Protodrilida is well supported by these morphological synapomorphies, its position within the Annelida remains unresolved. Morphological data support a derived position within

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Canalipalpata, as sister group to Spionida with a progenetic origin (Purschke 1993), while molecular data so far leave the family unresolved at the base of the Annelida (Bleidorn et al. 2003; Rousset et al. 2007; Struck et al., 2007, 2008; Sperling et al. 2009; Zrzavý et al. 2009).

The highest diversity of *Protodrilus* is found in the eastern Atlantic Ocean and Mediterranean Sea (18 species, 56% of the 32 described species). In contrast, only two species are reported from the Western Atlantic Ocean: *Protodrilus gelderi* Riser, 1997 from Massachusetts Bay (USA) and *Protodrilus corderoi* du Bois-Reymond, 1948 from São Sebastião Island, Brazil (du Bois-Reymond Marcus 1948) and possibly from Dominica, Caribbean Sea (Kirsteuer 1967). A few additional records from the western Atlantic Ocean are to genus level only (Ruebush 1939; Di Domenico et al. 2009). These data, however, may reflect a substantial regional sampling bias rather than the biogeographic history of this group, which therefore warrants further studies in Western Atlantic waters.

We describe here three species of *Protodrilus* from calcareous sand from shallow waters along the Caribbean coast of Central America, collected during two meiofauna surveys: one on the barrier reef of Belize and the other at Bocas del Toro, Panama. Two additional new species are recorded, but not described due to insufficient material.

Material and methods

Localities

Lighthouse Reef system is a sunken atoll situated approximately 75 km east of Belize City, 45 km in length and about 12.5 km wide and with four islands

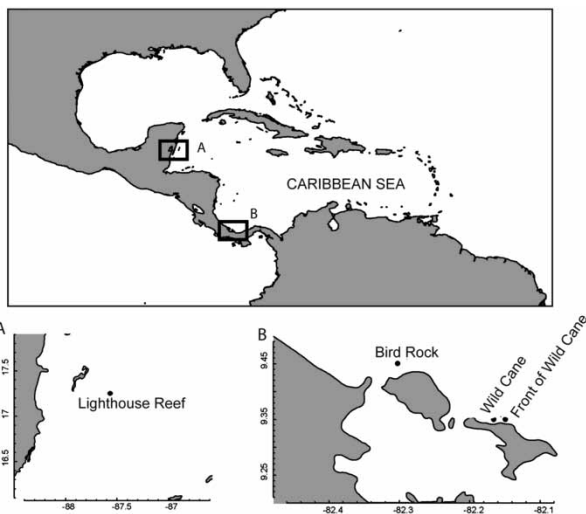


Figure 1. Map of the sampled localities. A, Lighthouse Reef, Belize. B, Bocas del Toro archipelago, Panama.

(Figure 1A): Half Moon Cay, Long Cay, Northern Cay and Sandbore Cay. Long Cay borders the southwestern side of the reef atoll, extending 3.5 km in length and 1.2 km at its widest point. Samples yielding *Protodrilus* were collected in coarse sand patches in the surf zone of the reef, among corals.

Bocas del Toro is an archipelago of islands, mainland bays, rivers and forested mountain slopes on the Caribbean side of the Panamanian isthmus (Figure 1B). Sampling was performed in different types of environments at Isla Colón, a 61-km long island. Samples yielding *Protodrilus* were from coarse sand patches in the surf zone of the reef and subtidal sandy sediments with medium-grained sand.

Sediment samples from oxygenated surface layers were collected manually by snorkel or SCUBA diving. SCUBA diving was performed by Katrine Worsaae and Peter R. Møller at Lighthouse Reef; and by Ashleigh Smythe, Daniel I. Gouge and Katrine Worsaae at Bocas del Toro. Snorkelling was performed at Bocas del Toro by all the participants at the Encyclopaedia of Life Course, Meiofauna Diversity and Taxonomy workshop.

Morphological studies

Animals were extracted using the $MgCl_2$ decantation technique through a 63- μm mesh (Higgins & Thiel 1988). Live animals were photographed and video recorded with a Zeiss Axio Scope A1 equipped with Sony HDR-XR500V HD camcorder. Several specimens were either fixed in Bouin's fixative (24 h and subsequently transferred to 70° ethanol through a gradient series) or 2% paraformaldehyde (24 h, followed by 6–7 rinses over 6 h, and then stored in PBS buffer with 0.3 M sucrose and 0.01% NaN_3). Subsequently, specimens were stained with mucicarmatein (10–15 s; Jägersten 1952) or Shirlastain (1 min) to increase the contrast of glandular structures in light microscopy (LM). Specimens were prepared as permanent whole mounts in glycerol and then examined, measured and photographed with an Olympus DP71 camera mounted on an Olympus BX50 microscope at the Marine Biological Section, University of Copenhagen (MBS, UC).

Scanning electron microscopy (SEM) studies were performed on material fixed in 2% glutaraldehyde (in 0.1 M cacodylate buffer with 0.3 M sucrose) for 24 h, and then transferred to 0.1 M cacodylate buffer with 0.3 M of sucrose. Material was post-fixed for 60 min in 1% osmium tetroxide (in 0.1 M cacodylate buffer), rinsed in distilled water, dehydrated through a graded ethanol series, transferred to 100% acetone and critical-point dried. Dried specimens were then mounted on aluminium stubs,

sputter-coated with platinum and examined with a JEOL JSM-6335F field emission scanning electron microscope at the Natural History Museum of Denmark, University of Copenhagen.

Schematic drawings summarizing the most important taxonomical features were prepared based on live specimens, and completed after examining fixed material with SEM and LM.

DNA barcoding

Specimens for DNA barcoding were identified alive with light microscopy and preserved in ethanol, voucher specimens being type material (see description of each species for details). DNA extractions were performed from entire single specimens using a Qiagen DNeasy Tissue and Blood kit, following protocols provided by manufacturer. DNA elution was repeated twice with the same 70 µl of buffer to maximize the amount of DNA yielded. Approximately 650 bp of the mitochondrial protein-coding gene cytochrome *c* oxidase subunit I (COI) and 1800 bp of the nuclear small subunit ribosomal RNA (18S rRNA) were amplified with universal primers (Hillis & Dixon 1991; Folmer et al. 1994; Meyer 2003; Cohen et al. 2004). Polymerase chain reactions (PCR) were performed following the manufacturer's protocol for the Illustra PuReTaq Ready-To-Go PCR Beads, including 2 µl of template DNA and 1 µM of each primer. PCR reactions were carried out using a Bio-Rad S1000 Thermal Cycler. PCR protocol involved a 2-min initial denaturalization at 94°C, followed by 40 cycles consisting of a denaturation step (94°C, 30 s), annealing (47°C for 18S rRNA and 48°C for COI, 30 s), and extension (72°C, 1 min), ending with a final extension at 72°C for 7 min. PCR products were resolved by E-Gel 2% SYBR Safe agarose gels (Invitrogen) and purified with E.Z.N.A. Cycle Pure kit. Purified products were sent to Macrogen Europe Lab for sequencing. Sequences were assembled with Sequencer 4.10.1 (GeneCodes Corporation, Ann Arbor, MI, USA). All COI barcodes were submitted to GenBank (see descriptions for GenBank accession numbers) as a reference for future studies.

Comparative material

Several species of *Protodrilus* were examined for comparison, including type material from *P. jägersteni* von Nordheim, 1989 (holotype from Museum of the University of Hamburg, MUH P-18978), *P. submersus* von Nordheim, 1989 (holotype, MUH, P-18984) and *P. helgolandicus* von Nordheim, 1983 (holotype, MUH, P-17407), as well as newly collected material of *P. cf. jägersteni* von Nordheim,

1989 and *P. cf. submersus* von Nordheim, 1989 from Lord Howe and Kennedy Island (Australia) and Mono Island and Nusa Anghana (Solomon Islands); *P. hypoleucus* Armenante, 1903 and *P. hypoleucus tenuis* Jouin, 1970b from Roscoff (France); *P. helgolandicus* von Nordheim, 1983 from Tjärno (Sweden); *P. purpureus* Schneider, 1868 from Sardinia (Italy) and Tjärno (Sweden); and *P. schneideri* Langerhans, 1880 from Lanzarote (Canary Islands).

Taxonomy

Family Protodrilidae (Czerniavsky 1881)

Genus *Protodrilus* Hatschek, 1880

Diagnosis

See von Nordheim (1989).

Protodrilus smithsoni sp. nov.

(Figures 2–4; Table I)

Holotype

ZMUC-POL-2161: Male, 3.0 mm long, whole-mounted. Bird Rock, Isla Colón, Bocas del Toro, Panama. 9°27.5'N, 82°18.0'W, exposed sandy patches, coarse well-sorted sand, 1–3 m depth. 07 Jun. 2010. Coll.: K. Worsaae and D.I. Gouge.

Paratypes

Three SEM specimens on a stub (ZMUC-POL-2170–ZMUC-POL-2172), nine specimens as whole mounts (seven adults and two juveniles; ZMUC-POL-2162–2169 and ZMUC-POL-2207) and a vial containing several specimens preserved in ethanol (ZMUC-POL-2208), from same locality and date as holotype; DNA barcoding (GenBank Acc. JX402097 and JX270810). One specimen as whole mount (ZMUC-POL-2173), Wild Cane Reef, Isla Colón, Bocas del Toro, Panama. 9°21.0'N, 82°10.4'W, sandy bottom, medium coarse sand with ripples, 15 m. 11 Jun. 2010. Coll.: K. Worsaae and D.I. Gouge.

Diagnosis

Hyaline body, with 31–39 segments. All segments with well-developed septa. Salivary glands in segments 1 to 10–12. Prostomium with two long palps, no eyes, but large rounded unpigmented ciliary receptors dorso-anteriorly; two conspicuous straight dorso-lateral nuchal organs. Pygidium bilobed with square adhesive lobes. External ciliation lacking

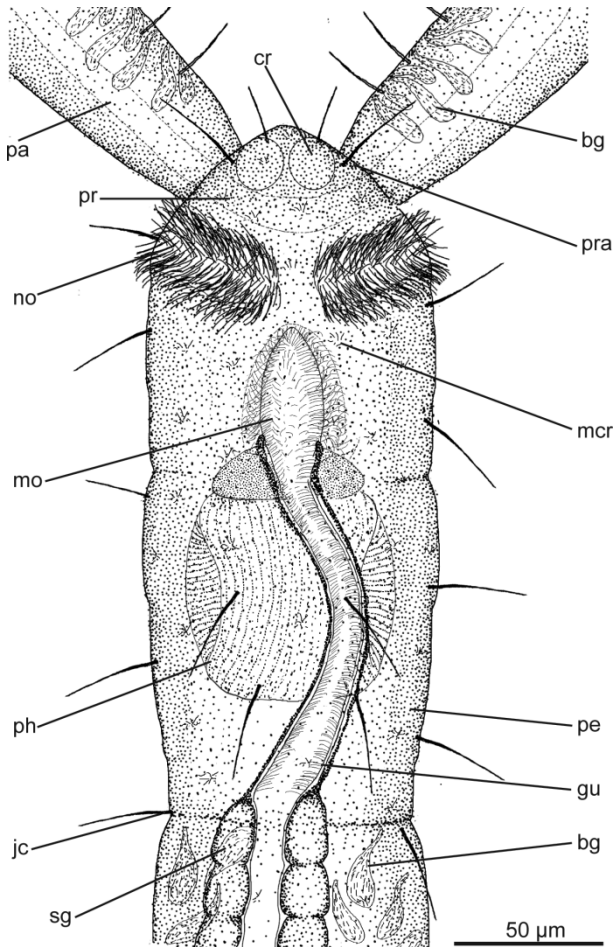


Figure 2. *Protodrilus smithsoni* sp. nov. Schematic drawing of the anterior end in dorsal view from live holotype (completed after SEM and LM observations from fixed specimens). bg, bacillary glands; cr, unpigmented ciliary receptor; gu, gut; jc, compound cilia (multiciliary sensory cell 'type 1a' in the text); mcr, multiciliary receptor (multiciliary sensory cell 'type 2' in the text); mo, mouth opening; no, nuchal organ; pa, palp; pe, peristomium; ph, pharynx; pr, prostomium; pra, prostomium apical cilia; sg, salivary glands.

ciliary rings but consisting of abundant multiciliated cells and bacillary glands on trunk, head and palps. Continuous lateral organs on segments 7–15 of males; first separated and rounded. Sperm in body cavity from segment 10 to pygidium. Four pairs of spermi ducts with gonopores on segments 12–15. Dorsal ciliated area on segments 4–6, consisting of transversely oriented bandlets of short cilia. Females with large oocytes from segment 15 to 17.

Description

(Measurements provided from holotype; ranges of all adult types in parentheses; measurements given in Table I.) Body slender and hyaline, 3.0 mm long (2.4–3.6 mm, $n=6$) and maximum 130 μm wide

(110–150 μm , $n=7$, LM) with 38 segments (31–39, $n=6$), all with well-developed septa (Figures 2, 3A, arrowheads). Prostomium rounded, 50 μm long (25–50 μm , $n=7$, LM) and 60 μm wide (45–70 μm , $n=7$) (pr, Figure 2, Figure 3A,F,G), with two filiform motile palps, 440 μm long (420–520 μm , $n=5$) bearing compound cilia (pac, Figure 3C). Conspicuous unpigmented ciliary receptors (*sensu* Purschke 1990b) dorso-anteriorly in the prostomium (up to 15 μm diameter, $n=7$) (cr, Figures 2, 3F,G). Nuchal organs 40 μm wide, densely ciliated, oval and extending dorso-laterally in a furrow between prostomium and peristomium (no, Figure 4A,D). Nuchal organ cilia beat metachronally, producing water currents perpendicular to their longitudinal axis. Salivary glands in segments 1–11 (segments 1 to 10–12 in paratypes) (sg, Figure 3A), with rounded/squared cells, each 15–25 μm wide, surrounding the entire gut (sg, Figure 3D).

Bacillary glands on trunk and palps (bg, Figure 3B,C), most evident in live specimens. Glands open as small pores slightly raised above the cuticle (bg, Figure 4G). Pygidium bilobed with square lobes, sometimes arranged in a funnel, 50 μm long (length: 50–60 μm , $n=5$; width: 40–60 μm , not measurable in holotype, $n=3$, LM) (pyl, Figures 3E, 4B), with about 50 distal adhesive gland openings (ag, Figure 4B).

Prostomium with four anterior bundles of 5–10 compound cilia dorsally (length of cilia: 50–70 μm , $n=5$, LM) (pra, Figures 2, 3G, 4D) and ventrally with anterior tufts of cilia and a transverse ciliary band near the border of the peristomium (prv, Figure 4C). Palp with a continuous ventral ciliary band (length of cilia: 10–15 μm , $n=2$, SEM) (pav, Figure 4C), beating in diaplectic metachrony; and an abfrontal band of compound cilia beating in unison as occasional flicks (pac, Figure 4D) (length of cilia: 15–20 μm , $n=2$, SEM). No ciliary rings on the head or trunk. Midventral ciliary band from peristomium to pygidium as two bands of multiciliated cells (mv, Figure 4A,B), with double ring around mouth (length of cilia: about 5 μm , $n=2$, SEM) (mv, Figure 4C). Transverse rows of cilia in midventral ciliary band beat in antiplectic metachrony. Mouth ciliary area extends posteriorly from mouth lateral folds and tongue, consisting of scattered ciliary tufts of short cilia (mc, Figure 4C) (length of cilia: about 5 μm , $n=2$, SEM) and two posterior groups of longer compound cilia (mcl, Figure 4C) (length of cilia: about 10 μm , $n=1$, SEM), beating in unison as occasional flicks towards the mouth.

Three types of multiciliated cells scattered all over the body, most likely sensory in function. Multiciliated cells types Ia and Ib (presumed to correspond to multiciliary sensory cells type I, after Purschke 1993) are observable as compound cilia

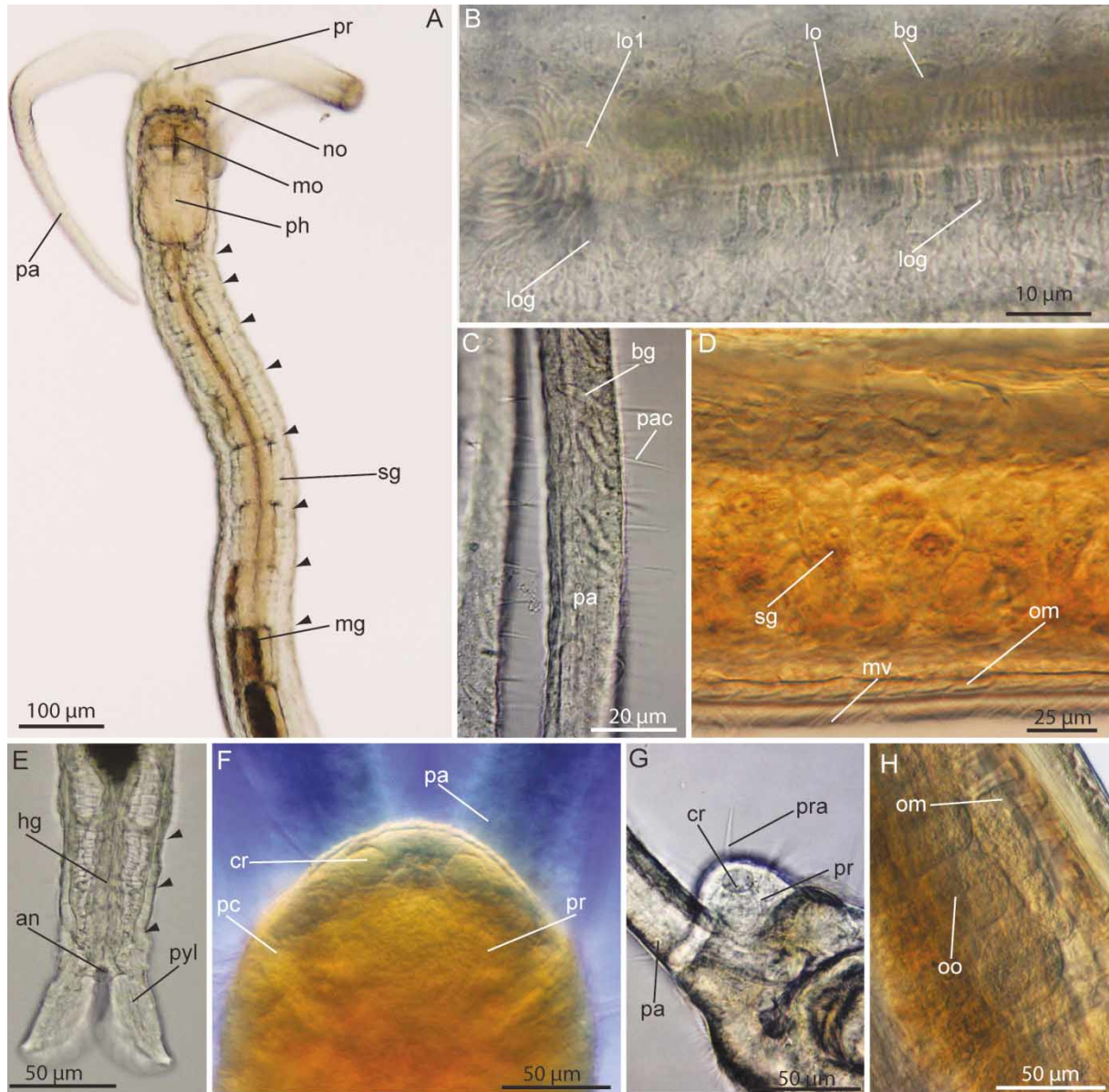


Figure 3. *Protodrilus smithsoni* sp. nov. Light micrographs. A, Anterior end of live holotype, dorsal view (arrowheads pointing at septa). B, Lateral organs on segments 7–8 of a live paratype, lateral view. C, Palp ciliation of a live paratype, lateral view. D, Salivary glands in segment 4, of whole-mounted holotype stained with Shirlastain, lateral view. E, Pygidium of the live holotype, dorsal view, arrowhead pointing to the septa. F, Prostomium of the fixed holotype, showing the unpigmented ciliary receptors, dorsal view. G, Prostomium of a live paratype showing the unpigmented ciliary receptors, lateral view. H, Oocytes of a fixed female, stained with Shirlastain, lateral view. an, anus; bg, bacillary gland; cr, unpigmented ciliary receptor; hg, hindgut; lo, lateral organ; lo1, first lateral organ; log, glands associated with lateral organ; mg, midgut; mo, mouth; mv, midventral ciliary band; no, nuchal organ; om, oblique muscle; oo, oocytes; pa, palp; pac, palp abfrontal ciliary band; pc, palp canal; ph, pharynx; pr, prostomium; pra, prostomium apical ciliation; pyl, pygidium; sg, salivary glands.

in live animals; type Ia with about 5 very long cilia per group (length of cilia: 20–40 µm, $n=3$, SEM) (ms1a, Figure 4G,H) corresponding to long compound cilia in live specimen; type Ib with about 20 long cilia per group (length of cilia: 5–10 µm, $n=3$, SEM) (ms1b, Figure 4D,H,I), corresponding to shorter compound cilia in live specimens. Multiciliated cells type II (corresponding to multiciliary sensory cells type II: Purschke 1993), are only visible

with scanning electron microscope, as about 7–10 short cilia arranged in a circle projected in different directions (length of cilia: about 3 µm, $n=3$, SEM) (ms2, Figure 4G,H). The posterior segments carry rings of about 20 ‘type Ia’ multiciliated cells, spaced about 10 µm apart (length of cilia: 20 µm, $n=2$, SEM) (ms1a, Figure 4G). A unique ciliated area is present on the dorsal surface of segments 5–6 of males (dca, Figure 4A,E), with

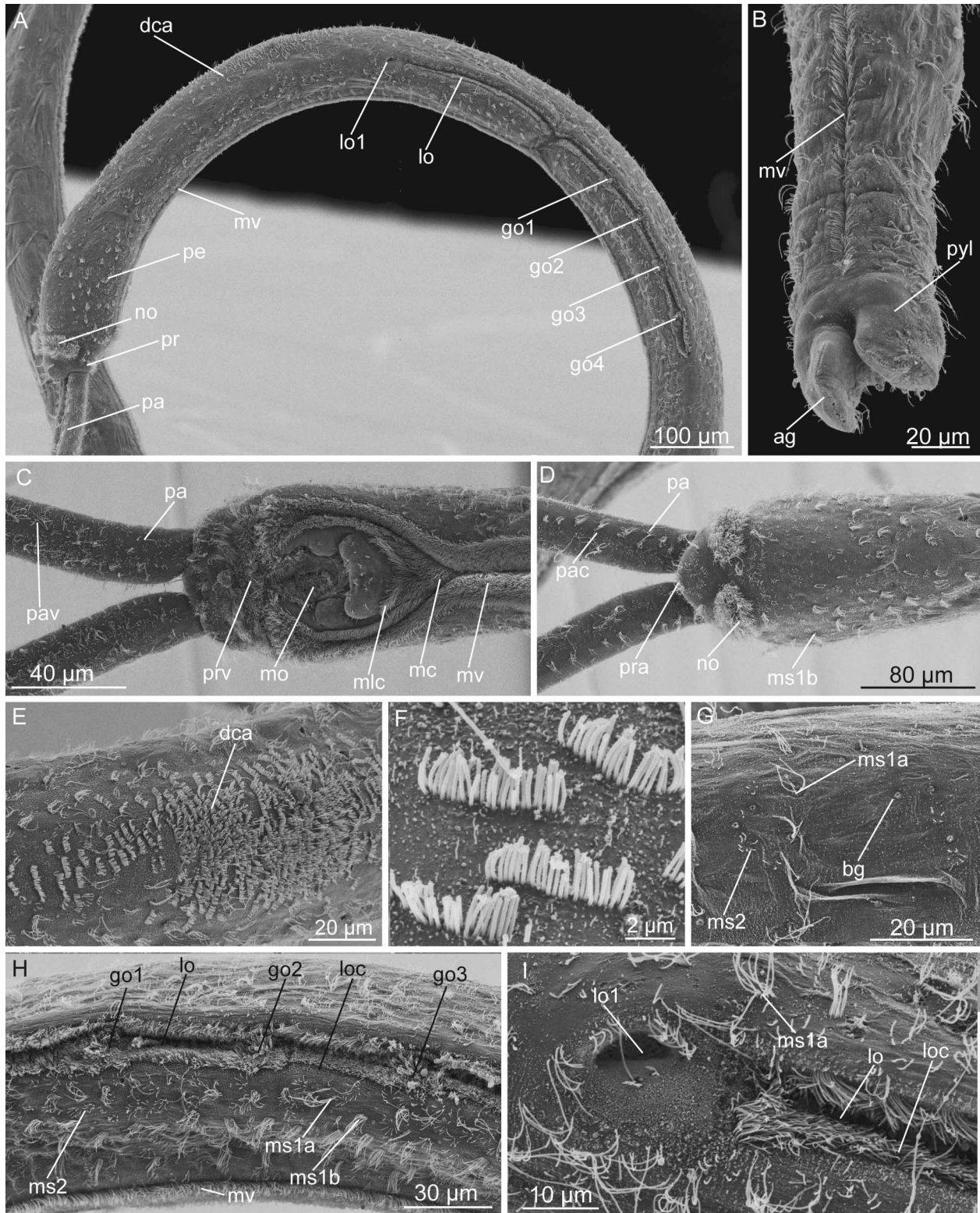


Figure 4. *Protodrilus smithsoni* sp. nov. Scanning electron micrographs. A, Anterior end of a mature male, showing the arrangement of the lateral organs, lateral view. B, Pygidium, ventral view. C, Prostomium and peristomium, showing the mouth ciliation, ventral view. D, Prostomium and peristomium, showing arrangement of nuchal organs, dorsal view. E, Ciliary area on segments 4–5 of a male, dorsal view. F, Bandlet of cilia at the dorsal ciliary area. G, Posterior segments of a male, showing the arrangement of the compound cilia, lateral view. H, Lateral organs on segments 12–14, showing the position of the gonopores. I, First lateral organ on segment 7. ag, adhesive gland openings; bg, bacillary gland pore; dca, dorsal ciliary area; go1–4, gonopores 1–4; lo, lateral organs; lo1, first lateral organ; loc, lateral organ ciliation; mc, mouth ciliation; mlc, mouth lateral cilia; mo, mouth; ms1a, multiciliated sensory cell ‘type Ia’; ms1b, multiciliated sensory cell ‘type Ib’; ms2, multiciliated sensory cell ‘type II’; mv, midventral ciliary band; no, nuchal organ; pa, palp; pac, palp abfrontal ciliation; pav, palp ventral ciliation; pe, peristomium; pr, prostomium; pra, prostomial apical ciliation; prv, prostomial ventral ciliation; pyl, pygidial lobe.

Table I. Meristic and morphometric characters of holotype and whole-mounted type material of *Protodrilus smithsoni* sp. nov.; numbers in parentheses correspond to juvenile specimens. Measures in mm. Width is always measured at maximum width. *Reduced to only one decimal place as measured under a dissecting scope. Abbreviations: L, length; N, number; W, width.

	Holotype	Mean	Min.	Max.	N
Total L*	3.0	3.1 (1.2)	2.4 (1.1)	3.6 (1.4)	6 (2)
Max. W	0.130	0.135 (0.105)	0.110 (0.095)	0.150 (0.115)	7(2)
L, prostomium	0.050	0.040 (0.035)	0.025 (0.035)	0.050 (0.035)	7(2)
W, prostomium	0.060	0.060 (0.060)	0.045 (0.045)	0.070 (0.070)	7 (2)
L, peristomium	0.190	0.180 (0.140)	0.130 (0.135)	0.230 (0.145)	7 (2)
W, peristomium	0.110	0.140 (0.120)	0.110 (0.110)	0.160 (0.135)	7 (2)
L, palp	0.440	0.475 (0.330)	0.420 (0.330)	0.520 (0.330)	5 (1)
W, palp	0.040	0.030 (0.030)	0.030 (0.030)	0.040 (0.030)	5 (1)
L, pygidium	0.050	0.055 (0.055)	0.050 (0.050)	0.060 (0.065)	5 (2)
W, pygidium	–	0.045	0.040	0.060	3 (0)
N segments	38	37 (17)	31 (15)	39 (18)	6 (2)

numerous transversely oriented bandlets of short cilia, each with about 20 short non-motile cilia (length: about 2 μm , $n=2$, SEM) (Figure 4F).

Males with paired lateral organs from segment 7 to 15 (lo, Figures 3B, 4A); first consisting of a rounded ciliated pit (lo1, Figure 3B, 4I). Subsequent lateral organs following as continuous ciliated bands (length of cilia: 2–3 μm , $n=2$, SEM) (lo, loc, Figures 3B, 4A,H,I) surrounded by glands (log, Figure 3B). Four pairs of spermi ducts in segments 11–14, with gonopores opening in the anterior part of the lateral organs in the following segments 12–15 (go1-4, Figure 4A). Sperm present from segment 10, occupying the whole body cavity. Mature females with 6–7 oocytes (30–50 μm diameter, $n=2$) and 10–15 smaller oocytes (10–15 μm diameter) per segment (oo, Figure 3H), from segment 15 to 17, occupying the whole body cavity. Oviducts not observed.

Motility

Gliding propelled by antiplectic metachronal beating of cilia of the midventral ciliary band. Palps waving continuously around the head.

Molecular data

1740 base pairs of the 18S rRNA (GenBank Acc JX402097) with a guanine–cytosine content of 49.5%, and 628 base pairs of the cytochrome *c* oxidase subunit I (GenBank Acc JX270810), coding for 209 amino acids with a guanine–cytosine content of 42.6%.

Etymology

Named after the Smithsonian Institution, which hosted and financially supported the surveys at Bocas del Toro, Panama.

Remarks

Protodrilus smithsoni sp. nov. resembles *P. jägersteni* and *P. submersus*, differing by the presence of a dorsal ciliated area in males on segments 4–5; the position of lateral organs, which extend only to segment 15 in *P. smithsoni* sp. nov. (up to 16 in *P. jägersteni* and *P. submersus*); the less numerous segments, shorter body and palps as well as occurrence of multiciliated cells arranged in rings on posterior-most body segments.

Protodrilus draco sp. nov.

(Figures 5, 6; Table II)

Holotype

ZMUC-POL-2174, male, 2.7 mm long, as whole mount; Wild Cane Cay, Isla Colón, Bocas del Toro, Panama. 9°21.102'N 82°09.720'W, 1.3 m wide channels with calcareous sand between coral heads, 6 m depth. 13 Jun. 2010. Coll.: K. Worsaae and D.I. Gouge.

Paratypes

Three SEM specimens (ZMUC-POL-2175–002177) and ten whole mounts (ZMUC-POL-2178–2184, ZMUC-POL-2209–2211) and a vial containing several specimens preserved in ethanol (ZMUC-POL-2212), same locality and date as for holotype; DNA barcoding (GenBank Acc JX402098 and JX270807).

Diagnosis

Hyaline body with 31–40 segments, all with well-developed septa. Salivary glands in segments 1 to 14–16. Prostomium with two palps, no eyes, but rounded unpigmented ciliary receptors at the base of the palps; two circular dorsal nuchal organs.

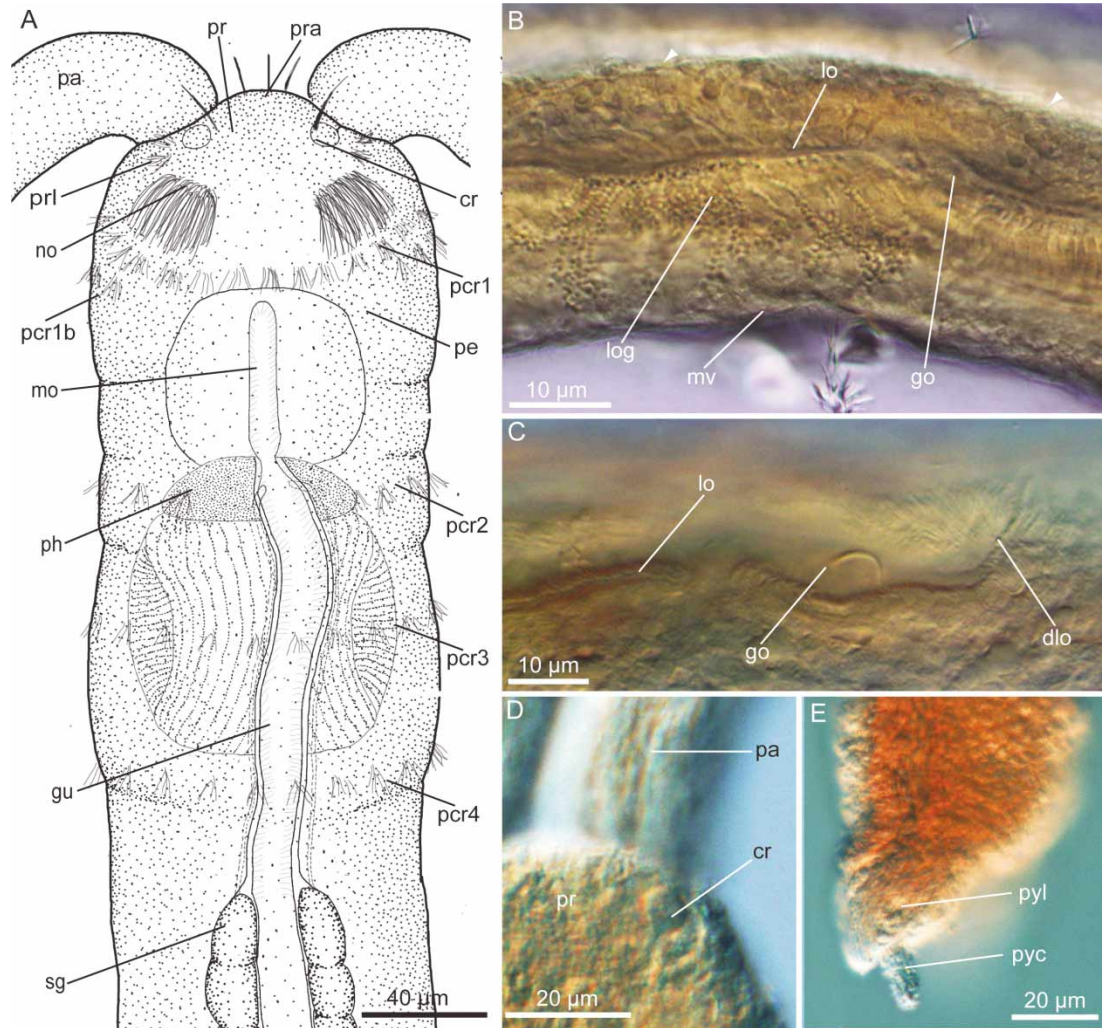


Figure 5. *Protodrilus draco* sp. nov. Schematic drawing and light micrographs. A, Schematic drawing of the anterior end of the species made after a scaled micrograph of a live specimen and completed with SEM and LM observations on fixed paratypes. B, Lateral organs on segments 6–8, showing lateral organs glands. C, Lateral organs on segments 6–8, showing lateral organ on segment 8 pointing dorsally. D, Prostomium of a fixed specimen, showing the unpigmented ciliary receptor. E, Pygidium and pygidial cirri of a fixed specimen, lateral view. cr, unpigmented ciliary receptor; dlo, dorsal extension of lateral organ; go, gonopore; gu, gut; lo, lateral organ; log, glands associated with the lateral organ; mo, mouth; mv, midventral ciliary band; no, nuchal organ; pa, palp; pcr1–4, peristomial ciliary ring 1–4; pcr1b, peristomial incomplete ciliary band; pe, peristomium; ph, pharynx; pr, prostomium; pra, prostomium apical ciliation; prl, prostomium lateral ciliary band; pyc, pygidial cirrus; pyl, pygidial lobe; sg, salivary glands.

Pygidium bilobed with rounded lobes bearing a short terminal cylindrical cirrus. External ciliation consisting of four ciliary rings on peristomium, no ciliary rings on trunk and abundant compound cilia on trunk and palps. Continuous lateral organs on segments 5–8 of males, extending dorsally on segment 8. Sperm from segments 4 to 5. Three pairs of spermi ducts with gonopores at lateral organs on segments 6–8. Females with large oocytes from segment 15.

Description

(Measurements provided from holotype; ranges of all adult types in parentheses; principle measurements given in Table II.) Body slender and hyaline, 2.7 mm

long (2.2–4.0 mm, $n = 5$, LM) and maximum 70 μm wide (50–100 μm , $n = 10$, LM), with 37 segments (31–40, $n = 5$, LM) all with well-developed septa. Prostomium rounded, 40 μm long (25–50 μm , $n = 10$, LM) and 50 μm wide (30–85 μm , $n = 10$, LM) (pr, Figures 5A, 6A,F), with two filiform palps, 315 μm long (260–425 μm , $n = 8$, LM), bearing sensory cilia (pa, paj, Figure 6F). Unpigmented ciliary receptors (*sensu* Purschke 1990b) in the base of the palps (up to 15 μm in diameter), inconspicuous in fixed material (cr, Figures 5A,D). Nuchal organs 10–15 μm in diameter, densely ciliated (length of cilia: 15 μm , $n = 3$, SEM), rounded and dorsal (no, Figures 5A, 6E,F). Cilia of nuchal organs beating in diaplectic metachrony producing water currents

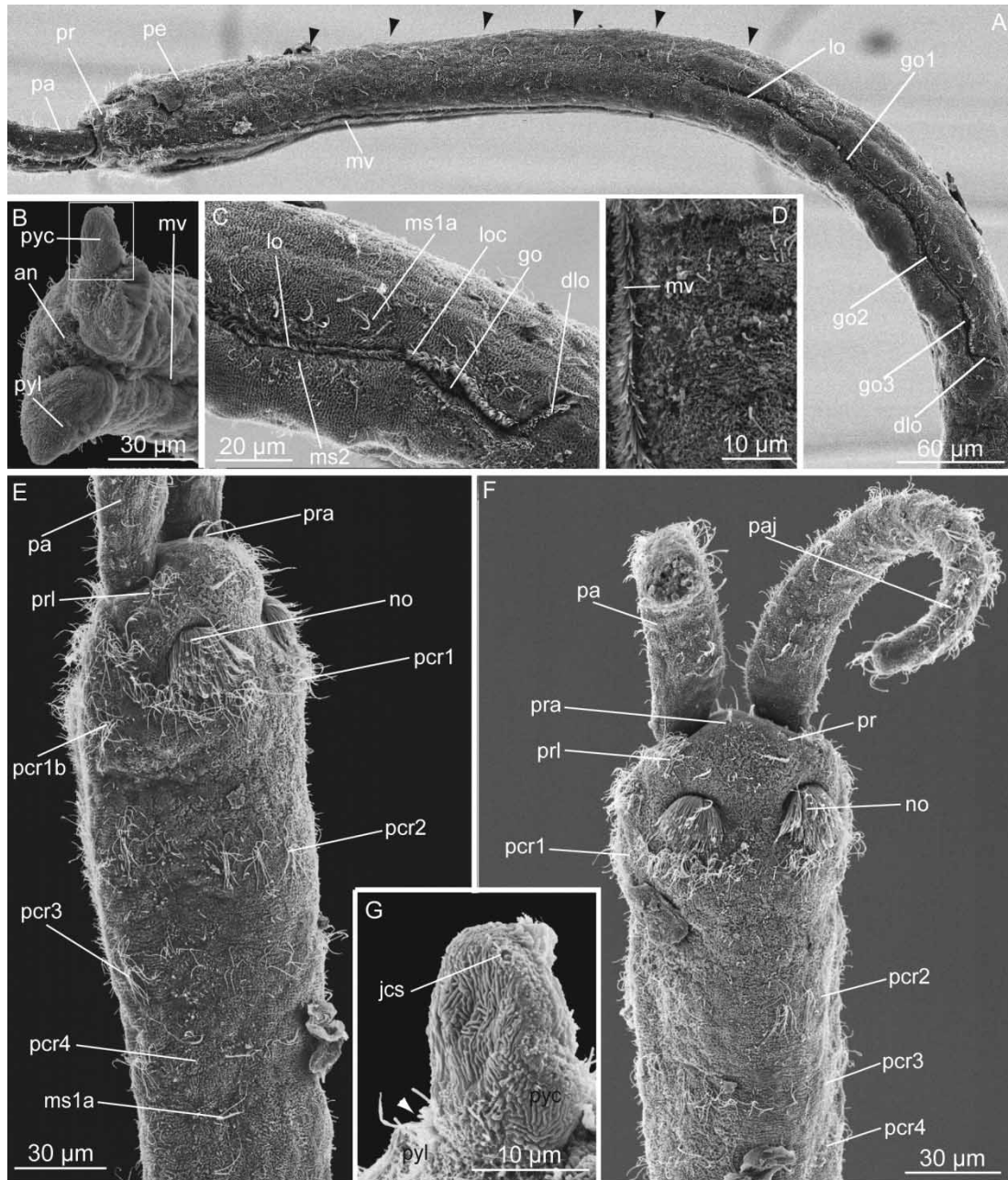


Figure 6. *Protodrilus draco* sp. nov. Scanning electron micrographs. A, Anterior end of a mature male, lateral view (arrowheads pointing to the septa). B, Pygidium, showing a pygidial cirrus, frontal view (squared area shown in G). C, Segments 7–8 of a mature male, showing the dorsal extension of the lateral organ. D, Cuticle ornamentation, ventral view, midbody segment. E, Prostomium and peristomium, showing the ciliary rings and nuchal organs, dorsolateral view. F, Prostomium and peristomium, dorsal view. G, Pygidial cirrus, lateral view, corresponding to the square in B (arrowhead pointing to the joint between the cirrus and the pygidial lobe). an, anus; dlo, dorsal prolongation of lateral organ; go1–3, gonopores 1–3; jcs, compound cilium scar; lo, lateral organ; loc, lateral organ ciliation; ms1a, multiciliated sensory cell ‘type Ib’; ms2, multiciliated sensory cell ‘type II’; mv, midventral ciliary band; no, nuchal organ; pa, palp; paj, palp compound cilia; pcr1–4, peristomial ciliary rings 1–4; pcr1b, peristomial incomplete ciliary ring; pe, peristomium; pr, prostomium; pra, prostomial apical ciliation; prl, prostomial lateral ciliary band; pyc, pygidial cirrus; pyl, pygidial lobe.

parallel to the longitudinal axis of the body. Salivary glands reaching to segment 15 (14–16), more distinct ventrally.

Pygidium with a pair of rounded lobes, 35 μm long (30–45 μm , $n=4$, LM) and 30 μm wide (30–50 μm , $n=4$, LM) (pyl, Figures 5E, 6B). Each with about 50

distal adhesive gland openings and a cylindrical cirrus (pyc, Figures 5E, 6B,G), 15 μm long (15–30, $n=3$, LM) with terminal compound cilia.

Ciliation on prostomium consisting of apical compound cilia (length of cilia: 10–12 μm , $n=2$, LM and SEM) (pra, Figure 6F) and two short

Table II. Meristic and morphometric characters of holotype and whole-mounted type material of *Protodrilus draco* sp. nov. Measures in mm. Width is always measured at maximum width. *Reduced to only one decimal place as measured under a dissecting microscope. Abbreviations: L, length; N, number; W, width.

	Holotype	Mean	Min.	Max.	N
Total L*	2.7	3.0	2.2	4.0	5
Max. W	0.070	0.085	0.050	0.100	10
L, prostomium	0.040	0.040	0.025	0.050	10
W, prostomium	0.050	0.060	0.030	0.085	10
L, peristomium	0.135	0.145	0.105	0.190	10
W, peristomium	0.080	0.090	0.075	0.120	10
L, palp	0.315	0.335	0.260	0.425	8
W, palp	0.030	0.030	0.020	0.030	8
L, pygidium	0.035	0.035	0.030	0.045	4
W, pygidium	0.030	0.040	0.030	0.050	4
L, pygidial cirri	0.015	0.020	0.015	0.032	3
N segments	37	35	31	40	5

transverse bands (length of cilia: about 10 μm , $n=2$, SEM) between the palps and nuchal organs (prl, Figures 5A, 6E,F). Palps with scattered compound cilia (paj, Figure 6F), performing occasional flicks. Peristomium with four transverse rings of motile cilia (pcr1–pcr4, Figures 5A, 6E,F). First ring posterior of nuchal organs with dense ciliation, 40–50 μm from anterior margin of prostomium ($n=2$, SEM) (length of cilia: 10–15 μm , $n=2$, SEM) (pcr1, Figures 5A, 6E,F). An additional dorsally incomplete band consisting of few tufts only present posterior to the first band (pcr1b, Figure 6E). Following three ciliary rings (pcr2–4, Figures 5A, 6E,F) less dense, consisting of 12–16 tufts of cilia, 5–10 μm apart, each with 10–15 cilia ($n=2$) and distances of 100–105 μm , 130–135 μm and 150–160 μm from the anterior end ($n=2$, SEM) (length of cilia: 10–15 μm , $n=2$, SEM). Midventral ciliary band from peristomium to pygidium (mv, Figure 6A,B), as two longitudinal bands of multiciliated cells extending into a double ring around mouth (length of cilia: about 5 μm , $n=1$, SEM). Transverse rows of cilia in midventral ciliary band beat in antiplectic metachrony.

Compound cilia found on entire body: some scattered on all surfaces (ms1a, Figure 6E), others arranged in longitudinal bands (ms1a, Figure 6C), extending laterally along the trunk (length of cilia: about 10 μm , $n=2$, SEM). Multiciliary sensory cells type II, with about 7–10 short cilia arranged in a circle (ms2, Figure 6C), spread along body surface (length of cilia: about 2 μm , $n=2$, SEM). Cuticle with hairy ornamentation in transverse rows, visible in SEM ($n=3$) (Figure 6C,D).

Males with paired lateral organs from segment 5 to 8 (lo, Figure 6A); as a continuous ciliated band (loc, Figure 6C) extending dorsally on segment 8 (length

of cilia: about 2 μm , $n=3$, SEM) (dlo, Figures 5C, 6A,C) and surrounded by glands (log, Figure 5B). Three pairs of spermi ducts, with gonopores at lateral organs of segments 6–8 (go1–3, Figure 6A). Sperm from segment 5, occupying the entire body cavity. Mature females with 10–15 oocytes (about 10 μm in diameter) per segment from segment 15. Oviducts not observed.

Motility

Gliding propelled by coordinated beating of cilia of midventral ciliary band, in antiplectic metachrony. The relatively short palps are passive during gliding.

Molecular data

1721 base pairs of the 18S rRNA (GenBank Acc JX402098), with a guanine–cytosine content of 47.5%; and 657 base pairs of the cytochrome *c* oxidase subunit I (GenBank Acc JX270807), coding for 218 amino acids, with a guanine–cytosine content of 43.8%.

Etymology

Named for Isla del Drago, the first name given to the Bocas del Toro region upon its discovery in 1502.

Remarks

Protodrilus draco sp. nov. resembles *P. hypoleucus* and *P. helgolandicus*, but differs in the presence of a short cylindrical cirrus on each pygidial lobe as well as shorter body, palps and head. In addition, *P. draco* sp. nov. has fewer body segments and it has lateral organs extending dorsally to segment 8 (versus segment 9 in *P. hypoleucus*, and segment 8 but not extending dorsally in *P. helgolandicus*).

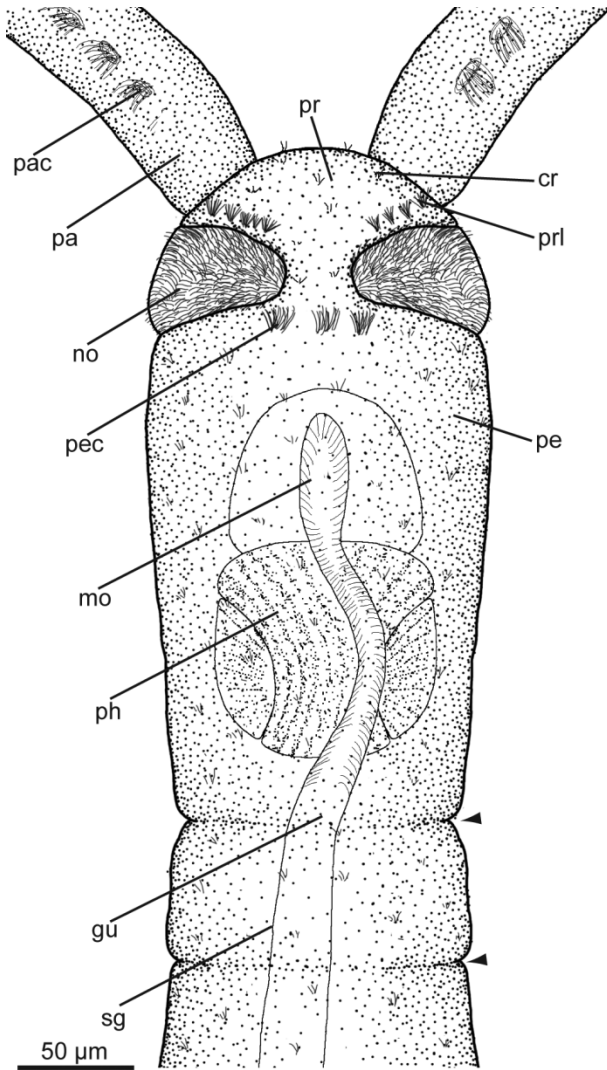


Figure 7. *Protodrilus hochbergi* sp. nov. Schematic drawing of the anterior end of the species made after a scaled micrograph of a live specimen and completed with SEM and LM observations on fixed paratypes (arrowheads pointing to the septa). cr, unpigmented ciliary receptor; gu, gut; mo, mouth; no, nuchal organ; pa, palp; pac, palp abfrontal ciliation; pe, peristomium; pec, peristomium ciliation; ph, pharynx; pr, prostomium; prl, prostomial lateral ciliation; sg, salivary glands.

Protodrilus hochbergi sp. nov.

(Figures 7–9; Table III)

Holotype

ZMUC-POL-2185, female, 7.1 mm long, whole mounted. Wild Cane Rock, Isla Colón, Bocas del Toro, Panama. 9°21.04'N, 82°10.35'W, very coarse well-sorted sand among rocks and coral pebbles, 3 m depth. 11 Jun. 2010. Coll.: A. Martínez and S. Pytaeva.

Paratypes

Six SEM specimens (ZMUC-POL-2196–002201), 12 whole mounts (ZMUC-POL-2186–2195 and

ZMUC-POL-2213–2214) and a vial containing several specimens preserved in ethanol (ZMUC-POL-2215), same locality and date as holotype; DNA barcoding (GenBank Acc JX402096 and JX270808). Four whole mounts (ZMUC-POL-2202–2205), one SEM specimen (ZMUC-POL-2206) and a vial containing several specimens preserved in ethanol (ZMUC-POL-2216), Lighthouse Reef, Long Cay Island, Belize. 17°13'42"N, 87°35'35"W, coarse well-sorted sand, 1–3 m depth, 27 Dec. 2009, coll.: K. Worsaae and P.R. Møller; DNA barcoding (GenBank Acc JX402099 and JX270809).

Diagnosis

Yellowish body with reddish pharynx, up to 49 segments. Salivary glands in segments 1 to 15–16. Prostomium with two long palps having ventral and abfrontal longitudinal ciliary bands; no eyes and small round unpigmented ciliary receptors dorso-anteriorly. Two conspicuous nuchal organs in dorso-lateral furrows. Pygidium with three lobes. Ciliary rings absent on peristomium or trunk, but abundant ciliary tufts scattered on the whole body. Separated lateral organs on segments 6–11, first is a rounded ciliated pit. Sperm from segment 8. Four pairs of spermiducts with gonopores at lateral organs on segments 8–11. Females with large, abundant oocytes from segment 15, filling entire body cavity.

Description

(Measurements provided from holotype; ranges of all adult types in parentheses; principle measurements given in Table III.) Body cylindrical and yellowish, 7.1 mm long (6.8–8.3 mm, the four paratypes are incomplete, yet mature and longer than 30 segments, LM) and maximum 190 μm wide (150–280 μm, $n=15$, LM), with 49 segments (30–49, $n=4$). Prostomium rounded, 70 μm long (50–115 μm, $n=15$, LM) and maximum 75 μm wide (75–220 μm, $n=15$, LM) (pr, Figures 7, 8A, 9A,B), with paired filiform ciliated palps (lost in the holotype) (570–1040 μm length, $n=4$, LM) (pa, Figures 8A, 9F). Unpigmented ciliary receptors (*sensu* Purschke 1990b) dorso-anterior in the prostomium (up to 10–15 μm in diameter, $n=8$, LM), indistinct in fixed material (cr, Figures 7, 8G). Nuchal organs densely ciliated, extending dorso-laterally in a furrow between prostomium and peristomium (length: 280 μm, width: 80 μm, $n=1$, SEM) (no, Figures 9A,B,E). Cilia of the nuchal organ beat in diaplectic metachrony. Salivary glands in segments 1–15 (12–16, $n=15$, LM) more distinct ventrally (sg, Figure 8I) and consisting of cells with

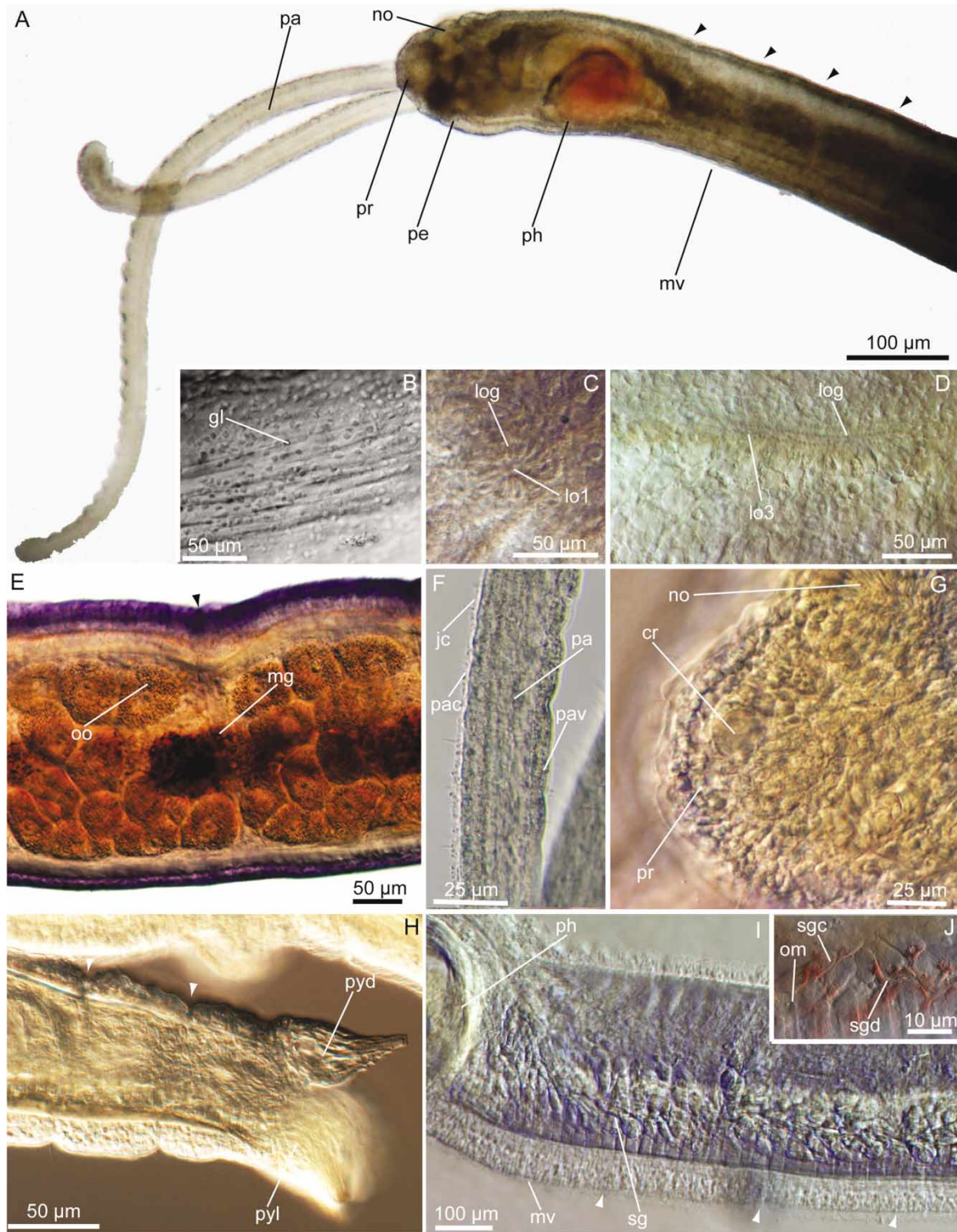


Figure 8. *Protodrilus hochbergi* sp. nov. Light micrographs. A, Anterior end of a live specimen, lateral view (arrowheads pointing to septa). B, Epidermal glands on a midbody segment of a fixed specimen, lateral view. C, First lateral organ on segment 6 of a fixed mature male. D, Lateral organ on segment 8 of a fixed mature male. E, Segments 20–21 of the holotype, showing the oocytes, dorsal view. F, Palp of live a specimen, ventro-lateral view. G, Prostomium of a fixed specimen, showing the position of the unpigmented ciliary receptor, lateral view. H, Pygidium of a fixed specimen, lateral view (arrowheads pointing to the septa). I, Segments 1–3 of fixed specimen stained with Shirlastain, lateral view (arrowheads pointing to the septa). J, Salivary glands at segment 12 of the fixed holotype stained with Shirlastain. cr, unpigmented ciliary receptor; gl, epidermal gland; jc, compound cilia; lo1, first lateral organ; lo3, third lateral organ; log, glands associated with lateral organs; mg, midgut; mv, midventral ciliary band; no, nuchal organ; om, oblique muscles; oo, oocytes; pa, palp; pac, palp abfrontal ciliation; pav, palp ventral ciliation; pe, peristomium; ph, pharynx; pr, prostomium; pyd, pygidial dorsal lobe; pyl, pygidial lateral lobe; sg, salivary glands; sgc, salivary gland cell; sgd, salivary gland duct.

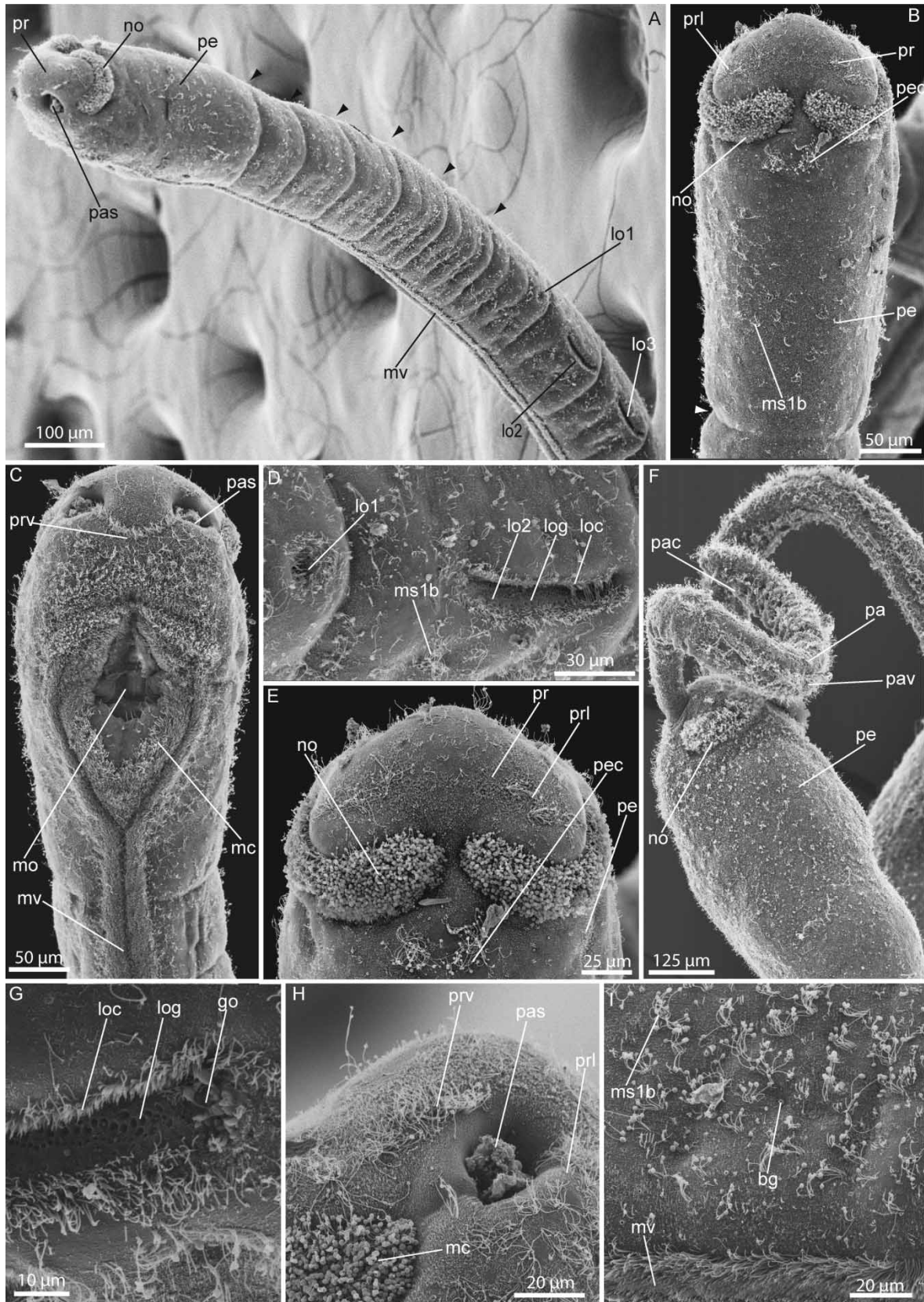


Figure 9. *Protodrilus hochbergi* sp. nov. Scanning electron micrographs. A, Anterior end, lateral view; palps are lost (arrowheads pointing to the septa). B, Prostomium and peristomium, dorsal view. C, Prostomium and peristomium, showing the mouth ciliation, ventral view. D, Segments 6–7, showing lateral organs 1–2, lateral view. E, Prostomium, showing ciliary patterns and nuchal organs, dorsal view. F, Prostomium and palps, lateral view. G, Lateral organ on segment 10, showing gonopore. H, Palp insertion showing prostomial lateral ciliation. I, Epidermis on midbody segments, showing the ciliary patterns and opening of bacillary glands. bg, bacillary glands; go, gonopore; lo1–3, lateral organs 1–3; loc, lateral organ ciliation; log, glands associated with lateral organ; mc, mouth ciliation; mo, mouth; ms1b, multiciliated sensory cell ‘type Ib’; mv, midventral ciliary band; no, nuchal organ; pa, palp; pac, palp abfrontal ciliation; pas, palp insertion; pav, palp ventral ciliation; pe, peristomium; pec, peristomium ciliation; pr, prostomium; prl, prostomium lateral ciliation; prv, prostomium ventral ciliation.

Table III. Meristic and morphometric characters of holotype and whole mounted type material of *Protodrilus hochbergi* sp. nov. Measures in mm. Width is always measured at maximum width. *Reduced to only one decimal place as measured under a dissecting microscope. Abbreviations: L, length; Lat, lateral; N, number; Pyg, pygidium; W, width.

	Holotype	Mean	Min.	Max.	N
Total L*	7.1	7.6	6.8	8.3	4
Max. W	0.190	0.220	0.150	0.280	15
L, prostomium	0.070	0.080	0.050	0.115	15
W, prostomium	0.075	0.130	0.75	0.220	15
L, peristomium	0.250	0.340	0.250	0.415	14
W, peristomium	0.200	0.235	0.165	0.300	14
L, palp	–	0.885	0.570	0.1040	4
W, palp	0.050	0.065	0.050	0.080	2
L, Pyg. Lat. lobe	0.060	0.050	0.035	0.060	2
L, Pyg. dorsal lobe	0.055	0.045	0.035	0.055	2
N segments	49	41	30	49	4

variable shape, connected to small ducts (sgc, sgd, Figure 8J), progressively fusing into two main canals that open dorsally of the mouth cavity.

Small rounded epidermal glands on entire body and palps (gl, Figure 8B). Pygidium with three lobes: two triangular ventral lobes, 60 μm long (35–60 μm , $n=2$, LM) and 35 μm wide (30–50 μm , $n=2$, LM), bearing openings of numerous distal adhesive glands (pyl, Figure 8H) and one dorsal lobe, leaf-shaped, 65 μm long (length: 65–75 μm , $n=2$, LM) (pyd, Figure 8H).

Prostomium with paired lateral (prl, Figure 9B,E) and ventral (prv, Figure 9C,H) motile ciliary bands; lateral band extending between nuchal organs and insertion of palps, consisting of about 4–5 ciliary tufts spaced 10–15 μm apart (length of cilia: 10–15 μm , $n=2$, SEM) (prl, Figure 9B,E); ventral band extending between mouth ciliated area and palp insertion (length of cilia: 10–15 μm , $n=2$, SEM) (prv, Figure 9C,H). Palps with abfrontal and ventral bands of motile cilia (pac, pav, Figures 8F, 9F). Abfrontal band consisting of ciliary tufts with 15–20 cilia, spaced 15 μm apart, beating in diaplectic metachrony (length of cilia: 10–15 μm , $n=1$, SEM), and intercalated longer compound cilia (jc, Figure 8F), beating in unison as occasional flicks (length of cilia: 20–25 μm , $n=2$, LM). Ventral band of palps similar to abfrontal band, but with ciliary tufts very close together, as a continuous row, beating in diaplectic metachrony. No ciliary rings on peristomium or trunk, but a densely ciliated area around mouth (length of cilia: about 10 μm , $n=2$, SEM) (mc, Figure 9C) and two groups of 3–4 ciliary tufts, dorsally between nuchal organs (length of cilia: 10–15 μm , $n=2$, SEM) (pec, Figure 9E). Midventral ciliary band from peristomium to pygidium as two rows of multiciliated cells (mv, Figure 9A), with double ring around mouth opening (mv, Figure 9C) (length of cilia: 5–10 μm , $n=3$, SEM). Transverse rows of cilia in midventral ciliary band beat in antiplectic metachrony. Groups of small cilia scat-

tered over the entire body surface (length of cilia: about 10 μm , $n=3$, SEM) (ms1b, Figure 9B).

Males with paired separated lateral organs from segment 6 to 11 (lo, Figure 9A) (maximum length: 55–60 μm on segment 11, $n=4$, LM), ciliated (length of cilia: about 2 μm , $n=2$, SEM) (loc, Figure 9D,G) and surrounded by glands (log, Figure 8D). First lateral organ consisting of a rounded ciliated pit (about 15 μm diameter, $n=4$, LM and SEM) (lo1, Figure 8C, 9A,D). Four pairs of spermiducts with gonopores at lateral organs on segments 8–11 (go, Figure 9G). Sperm from segment 10 to 11 ($n=3$) occupying the whole body cavity. Females with 10–15 (up to 25) oocytes per segment (60–75 μm in diameter) occupying the entire body cavity from segment 15–17 (oo, Figure 8E) ($n=4$). Oviducts not observed.

Motility

Gliding propelled by antiplectic metachronal beating of cilia of midventral ciliary band. Palps very motile around head and towards the mouth, generating water currents perpendicular to the longitudinal axis of the palp by ciliary activity (see above). Mostly under stress conditions, animals can swim by undulatory muscular activity for short periods and with limited control of swimming direction.

Molecular data

18S rRNA and cytochrome *c* oxidase subunit I gene fragments were amplified from single specimens from Bocas del Toro (Panama) and Lighthouse Reef (Belize). 18s rRNA fragments consist of 1740 base pairs with a guanine–cytosine content of 48.6%, both for Boca del Toro (GenBank Acc JX402096) and Lighthouse Reef (GenBank Acc JX402099). COI consist of 658 base pairs (218 amino acid positions), with a guanine–cytosine content of 40.7% (Bocas del Toro, GenBank Acc

JX270808 and Lighthouse Reef, GenBank Acc JX270809).

Etymology

Named in honour of the great meiobenthologist Dr Rick Hochberg, who also helped with collecting, sophisticated processing and photography during our joint field trips to Carrie Bow Cay and Bocas del Toro. Although trying to resist, he could not help himself consistently finding these worms, for which we are very grateful.

Remarks

Protodrilus hochbergi sp. nov. resembles *P. schneideri* Langerhans, 1880 and *P. purpureus* Schneider, 1868. However, its salivary glands extend to segment 15 (versus segment 6) and it has four pairs of spermi ducts (versus 3).

Discussion

This is the first record and focused collecting of the genus *Protodrilus* of the Caribbean coast of Central America, although there is a record of *P. corderoi* from Dominica, in the Lesser Antilles (Kirsteuer 1967). The discovery of five new species (only three here described) from this limited region may indicate a diversity in the Caribbean that is comparable to the most studied areas of Europe.

Of the five species recorded, only *Protodrilus hochbergi* was found both in Belize and Panama, in similar coarse well-sorted sediments in the surf zone of the reefs. Further studies are required to determine whether the restricted distribution of the four species is an artifact of limited sampling or reflects low dispersal capabilities, which would, in turn, suggest potential high diversity of the genus in the Caribbean area.

Although some morphological differences exist, at least two of the species recorded in this study strongly resemble European species. This underlines the importance of detailed morphological studies, including scanning electron microscopy, to unravel the actual global diversity of the group. It also indicates that wide distributions recorded for some species of *Protodrilus* should be taken with caution (Jouin 1970a; Bailey-Brock et al. 2010). This leads us to be cautious about the identity of the Dominica specimens assigned to *P. corderoi* (see Kirsteuer 1967), which was originally described from southeastern Brazil (du Bois-Reymond Marcus 1948). *P. corderoi* differs significantly from all the three newly described species herein by the presence of continuous lateral organs on segments 7–12, with two pairs of spermi ducts with

gonopores on segments 9–12 and salivary glands in segments 1–16 (di Domenico, Martínez & Worsaae, personal observations).

Interestingly, many of the morphological similarities among European and American species seem to be adaptations to specific interstitial habitats. The presence of short palps and slender body in *P. draco* can be regarded as an adaptation to the subtidal medium-fine sand environments where the species was collected. A slender body facilitates exploration of tight interstices, where long palps would be a disadvantage, while short palps with abundant sensory cilia facilitate food detection (Giere 2009). In contrast, the long motile palps with bands of motile cilia and bacillary glands of *P. hochbergi* seem to have a major role in feeding, correlated to the larger interstices of coarse gravel environments in exposed surf areas, where the species was collected. Similar palp morphology, as well as undulatory swimming behaviour, has been observed in the big European *P. schneideri* and *P. purpureus*, both common in coarse sand environments affected by wave action (Von Nordheim 1989; Martínez & Worsaae, unpublished data).

The morphological diversity of *Protodrilus* species is shown here to be higher among different habitats in the same geographical region (*P. draco* in fine sand versus *P. hochbergi* in coarse sand in Panama) than among similar habitats in different geographical areas (*P. draco* from Panama versus *P. hypoleucus* or *P. helgolandicus* in fine sand from Europe; or *P. hochbergi* versus *P. purpureus* or *P. schneideri* from Europe and Macaronesia). The results also indicate that colonization is highly restricted by the specific demands of the environment. Future studies of the phylogeny of *Protodrilus* will test whether the shared traits of geographically distant species are homologous or convergent adaptations to the environment.

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and Bocas del Toro. We also thank Prof. Angelika Brandt, curator of the NT II collection at the Zoologisches Museum of Hamburg, as well as Kathrin Philipps-Bussau and Petra Wagner, for facilitating us access to the type material of three species of *Protodrilus*. The travelling costs of AM and MDD were partially financed by an Encyclopedia of Life fellowship. The travel costs of KW and laboratory work was financed by grants for K. Worsaae from the Carlsberg Foundation and a Freja fellowship (University of Copenhagen). K. Jörger received funding by a PhD scholarship from the Volkswagen Foundation.

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