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A new genus and species of Serpulidae (Annelida, Polychaeta, Sabellida) from the Caribbean Sea

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Abstract

A new genus and species of Serpulidae (Annelida, Polychaeta) from the Caribbean Sea. *Turbocavus secretus* (**gen. nov.** and **sp. nov.**) is described from shallow hard substrates (0.5–3 m) in wind-sheltered bays of St. John, U.S. Virgin Islands and Curaçao, as well as from diving depths (46−49 m) around Bonaire (Leeward Antilles), Caribbean Sea. The new taxon, which has from 7 to 19 thoracic chaetigers and up to 335 abdominal chaetigers, bears a unique type of thoracic chaeta which is multifolded at the base and continues with a groove tapering to the capillary tip. The new serpulid has unique 18S rRNA sequences and genetic analysis of the 18S rRNA gene situates the new genus at the basis of the serpulid cladogram, well separated from other genera, and close to *Filograna/Salmacina* and *Protula.*

Key words: U.S. Virgin Islands, Bonaire, Curaçao, grooved chaetae, multifolded chaetae

Introduction

The Serpulidae (Rafinesque, 1815) are a large family of annelid polychaete worms, which secrete and live within calcareous tubes affixed to hard surfaces. Common to all oceans, they exist from intertidal habitats to deep-sea zones (Rouse & Pleijel 2001; ten Hove & Kupriyanova 2009; Kupriyanova *et al.* 2014). The most recent review of the family, which does not include the spirorbin taxa (ten Hove & Kupriyanova 2009), lists 350 species within 46 genera. However, these authors acknowledge that much work needs to be done to examine the validity of 19 monophyletic genera, which may eventually be synonymized with other genera.

While the Serpulidae are easily recognized, their genera are considered as difficult to classify, since there are few taxonomic characters to clearly separate them. Frequently, genera and species are classified based on "negative traits", or the lack of specific characters, rather than for presenting combinations of unique characters (e.g. generic diagnoses in Fauchald 1977; ten Hove & Kupriyanova 2009). An analysis of thoracic blood vessel pattern was used to discern between the genera *Apomatus* and *Protula* (ten Hove & Pantus 1985), but blood vessel patterns can only be studied in fresh material. However the patterns of other genera are not known, and as a consequence the character has not been used in subsequent taxonomic literature. Taxonomists now routinely use scanning electron microscopy to identify unique characters, which cannot be detected with a light microscope.

Comparisons of DNA sequences also provide a powerful tool for taxon identification at genus and species level. The 18S rRNA nuclear gene has widely been employed for screening Serpulidae taxa and has been a valuable character for hypothesizing phylogenetic relationships within the family (Kupriyanova *et al.* 2006; Lehrke *et al.* 2007).

In this paper, a new serpulid genus, *Turbocavus*, is described on the basis of unique morphological characters and 18S rRNA gene sequence data.

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Material and methods

Sampling. Over 100 serpulid worms and associated tubes were hand collected while snorkeling in shallow water within the United States Virgin Islands Coral Reef National Monument (VICR) and Virgin Islands National Park (VIIS), St. John, U.S. Virgin Islands, between July 2010 and July 2013 (Fig. 1). St. John worms were collected as authorized by the U.S. National Park Service under the following collection permits: VICR-2010-SCI-0009, VICR-2011-SCI-0007, VICR-2012-SCI-0002, VICR-2012-SCI-0006, VIIS-2012-SCI-0024. Worm tubes were attached to the undersides of rocks (Fig. 2) from 0.25 to 3 meters depth in the wind-sheltered bays of Hurricane Hole: Princess Bay (18°21'10.7"N 64°41'37.1"W), Otter Creek (18°21'05.0"N, 64°41'33.1"W) and Water Creek (18°20'45.9"N 64°41'30.0"W), and in Little Lameshur Bay (18°19'06.5"N 64°43'31.6"W) and Great Lameshur Bay (west shore: 18°19'02.8"N 64°43'26.8"W and east shore: 18°18'56.9"N 64°43'17.4"W). A spatula was used to remove worm tube aggregations, which were affixed to the undersides of flat-bottomed basalt and plagiorhyolite rocks seated in fine sediment. Tubes were not visible unless the rocks were turned over, and most rocks within the habitat did not harbor worms. The worm tubes were never attached to dead coral boulders, common in the habitat. During collection, live worms were not observed extending outside of the tubes. Irregularly curving tubes extended from most tube coils to the rock edge (Fig. 2A). Younger tubes were thin, white and often fixed to older tubes, which were thicker and covered with what appeared to be an iron oxyhydroxide stain.

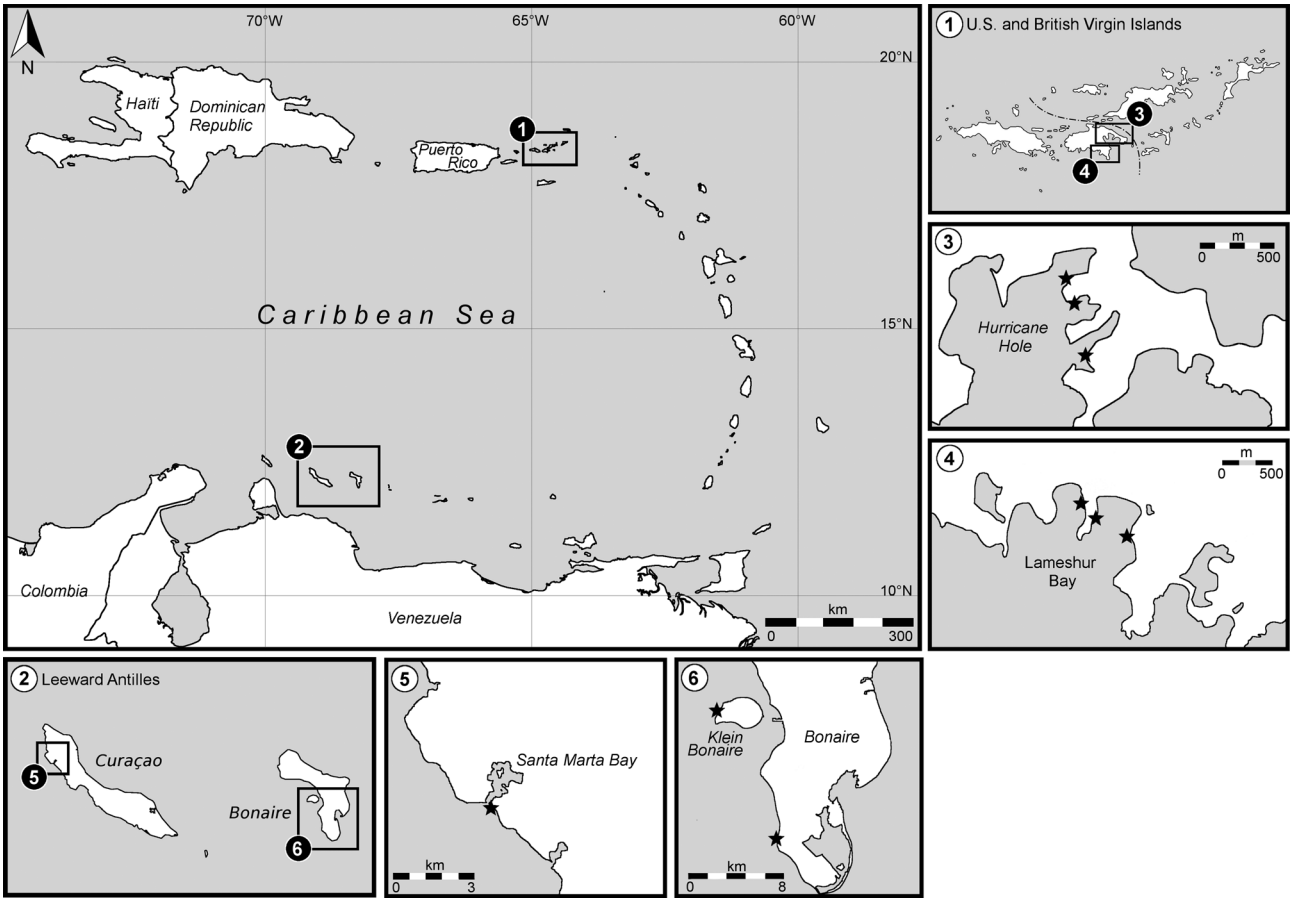

FIGURE 1. Distribution map of *Turbocavus secretus* **sp. nov.** Locations are marked with stars on maps 3−6.

During this study, one of the co-authors (HtH) provided several unidentified serpulid worms, worm fragments and associated tubes collected in the Leeward Antilles: in 1955 (by Wagenaar Hummelinck) and in 1970 (by ten Hove). These specimens were collected from coral rubble and sandy sediment wading at 0.25 m in a small lagoon in Curaçao, and diving in Bonaire and Little Bonaire between 46 and 49 m depth (Fig. 1). While these worms were incomplete (partially decomposed and lacking branchial crowns, probably due to imperfect sampling conditions), they provided morphological characters sufficient to confirm that they belonged to the same species as those collected in St. John.

Tubes containing living worms were placed in aerated seawater, to which a 5% solution of Epsom salts (magnesium sulfate) was gradually added to induce narcotization. Relaxed worms were photographed live and then were either fixed in 10% seawater-formalin and transferred to 70% ethanol, or fixed and preserved in 95% ethanol. Associated worm tubes were stored dry or kept in 70% ethanol.

Morphological analysis. The randomly collected worms commonly autotomized the branchial crown, thorax and abdomen, and readily decayed within the tubes. Thus, only 20 complete worms could be analyzed for all character measurements. Sixty worms with an intact thorax were assessed for the number of thoracic chaetigers. Summary statistics were obtained for the primary characters, which included maximum, minimum, range, average, standard deviation and the coefficient of variability of all measurement values. The following taxonomic characters were measured or counted: (a) lengths of: total body, crown, thorax, achaetigerous region and abdomen; (b) widths: at first and fifth thoracic chaetigers and first abdominal chaetiger; (c) numbers of thoracic chaetigers on right and left sides; (d) number of abdominal chaetigers; (e) number of posterior thoracic chaetigers with ventral glandular field; (f) extension of abdominal ventral shield (starting abdominal chaetiger to pygidium); (g) extension of thoracic membrane (start chaetiger and equivalent number of segments membrane extends over achaetigerous region); (h) thoracic chaetiger at which uncini first appear; (i) numbers of geniculate chaetae and of uncini at first and 100th abdominal chaetigers; (j) number of posterior abdominal chaetigers with long capillary chaetae.

Genetic methods. DNA was extracted from four samples of *Turbocavus secretus* **sp. nov.** Two DNA samples were processed at the Hellenic Centre for Marine Research (HCMR), where the DNA was extracted with the Quick Tissue/Culture Cells Genomic DNA extraction kit (Dongsheng Biotech, China). The other two DNA samples were processed at the University of Maine at Farmington (UMF), where the DNA was extracted with the DNeasy Blood and Tissue Kit (Qiagen). For all samples, an approximate 420 base pair fragment of the 18S rRNA gene was amplified with the PCR method, using the primer pair 18F509 CCCCGTAATTGGAATGAGTACA (Struck *et al*. 2002) - 18r GTCCCCTTCCGTCAATTYCTTTAAG (Hills & Dixon 1991). For the HCMR samples, each reaction was of 20 µl final volume and contained 1.5 µl of DNA template, 2 µl of 10x PCR buffer, 3.5 mM $MgCl_2$, 0.4 µl dNTPs (100 mM), 2 µl of each primer (10 mM), 0.1 µl KAPATaq DNA Polymerase (KAPA BIOSYSTEMS, Boston, USA). Reactions for the HCMR samples were carried out under the following thermal profile: 94ºC for 3 min; 35 cycles with 93ºC for 45 s, 52ºC for 60 s, 72ºC for 90 s; 72ºC for 10 min. The products were sequenced in an Applied Biosystems 3730 at the genetics laboratory of the Hellenic Centre for Marine Research in Crete, Greece. For the UMF samples, each reaction was of 25 µl final volume and contained 1.5 µl of DNA template, 2.5 µl of 10x PCR buffer, 0.5 µl of 3.5 mM $MgCl_2$, 0.5 µl dNTPs (100mM), 2.5 µl of each primer (10 mM), 0.1 µl Green *Taq* DNA Polymerase (GenScript). Reactions for the UMF samples were carried out under the following thermal profile: 94ºC for 5 min; 30 cycles with 94ºC for 30 s, 47ºC for 30 s, 72ºC for 40 sec; 72ºC for 7 min. The products were sequenced in an Applied Biosystems 3103xl at the Mount Desert Island Biological Laboratory in Salisbury Cove, Maine.

The 18S rRNA sequences for *Turbocavus secretus* **sp. nov.** are published on GenBank: KJ583233, KJ583234, KJ583235, KJ583236. Available 18S rRNA fragment sequences from known Serpulidae species were downloaded from GenBank (Table 1). All sequences were aligned in the MEGA v5 software with the Clustal W algorithm (Tamura *et al*. 2011). Maximum Likelihood analysis was run with the same software. For the Maximum Likelihood analysis the Tamura Nei substitution model was used (Tamura & Nei 1993) with gamma distribution and four categories. Sequences of *Sabella spallanzanii* (Gmelin, 1791), *Sabellaria alveolata* (Linnaeus, 1767) and *Amphicorina mobilis* (Rouse, 1990) were used as outgroups. Bayesian analysis was applied with MrBayes software (Huelsenbeck & Ronquist 2001). Analysis preferences were chosen after Lehrke *et al*. (2007). Sequences of *S. spallanzanii*, *Bispira melanostigma* (Schmarda, 1861), *Sabellaria alveolata* and *Gunnarea capensis* (Schmarda, 1861) were used as outgroups.

Scanning electron microscopy. Three individuals selected for obtaining Scanning Electron Microscope (SEM) images were first dehydrated, then critical point dried (Bal-Tec CPD 030), sputter-coated with gold (Bal-Tec SCD 050) and examined under a JEOL JSM-6390LV at the Department of Biology, University of Crete. Worm tubes were cut transversely and longitudinally using a fine jeweler saw and prepared for SEM imaging according to the methodology proposed by Vinn and Kupriyanova (2011). In compliance with their method, the tube fragments were first embedded in epoxy resin, polished at both longitudinal and cross-sectional direction, and etched with 1% acetic acid for 5−10 minutes prior to being sputter-coated and examined under the SEM.

TABLE 1. Taxa and GenBank accession numbers used for the phylogenetic analysis. Species names have been standardized against the World Register of Marine Species. * These two names are currently under review in Sun *et al.* (in preparation).

Taxon	Accession Numbers
Amphicorina mobilis (Rouse, 1990)	EF116206
Amplicaria spiculosa (Knight-Jones, 1973)	DQ242560
Apomatus voightae Kupriyanova & Nishi, 2010	GU441856
Bathyvermilia eliasoni (Zibrowius, 1970)	GU441857
Bispira melanostigma (Schmarda, 1861)	EF116211
Bushiella (*Bushiella*) *abnormis* (Bush, 1905) [as *B. abnormis*]	DQ242563
Chitinopoma serrula (Stimpson, 1854)	DQ317112
Chone sp.	EF116209
Circeis armoricana Saint-Joseph, 1894	DQ242545
Circeis spirillum (Linnaeus, 1758)	DQ242546
Crucigera inconstans Straughan, 1967	DQ317113
Crucigera tricornis Gravier, 1906	EU184056
Crucigera zygophora (Johnson, 1901)	DQ242543
Ditrupa arietina (O. F. Müller, 1776)	DQ140401; DQ317114
Eulaeospira cf. *orientalis*	DQ242553
Eulaeospira convexis (Wisely, 1962)	DQ242552
Ficopomatus enigmaticus (Fauvel, 1923)	AY577889; DQ317115
Ficopomatus macrodon Southern, 1921	EU167532
Ficopomatus miamiensis (Treadwell, 1934)	EU167531
Ficopomatus shezhensis Li, Wang & Deng, 2012	HQ433336
Filograna implexa Berkeley, 1835	DQ140402; DQ317116
Galeolaria caespitosa Lamarck, 1818	AB106257
Galeolaria hystrix Mörch, 1863	JX144801; JX144800
Gunnarea gaimardi (Quatrefages, 1848) [as *G. capensis* (Schmarda, 1861)]	DQ317111
Hyalopomatus biformis (Hartman, 1960)	GU441858
Hyalopomatus mironovi Kupriyanova, 1993	GU063862
*Hydroides brachyacanthus** Rioja, 1941	DQ317117
Hydroides ezoensis Okuda, 1934	EU184062
Hydroides minax (Grube, 1878)	EU184063
Hydroides norvegicus Gunnerus, 1768	AY611452
*Hydroides novaepommeraniae** Augener, 1925	EU184058
Hydroides pseudouncinatus Zibrowius, 1968	DQ140403
Hydroides sanctaecrucis Krøyer in Mörch, 1863	EU184061
Hydroides sp.	EU184060
Hydroides trivesiculosus Straughan, 1967	EU184060
Hydroides tuberculatus Imajima, 1976	EU184059
Janua pagenstecheri (Quatrefages, 1866)	DQ242548
Jugaria quadrangularis (Stimpson, 1854)	DQ242564
Laeospira corallinae (de Silva, Knight-Jones, 1962) [as *Spirorbis cor.*]	DQ242572
Laminatubus alvini ten Hove & Zibrowius, 1986	DQ317118

.....continued on the next page

TABLE 1. (Continued)

Taxon	Accession Numbers
Marifugia cavatica Absolon & Hrabe, 1930	EU167530
Metalaeospira tenuis Knight-Jones, 1973	DQ242554
Metavermilia acanthophora (Augener, 1914)	DQ317119
Neodexiospira alveolata (Zachs, 1933) [as *N. nipponica* (Okuda, 1934)]	DQ242549
Neodexiospira brasiliensis (Grube, 1872)	DQ242550
Neodexiospira steueri (Sterzinger, 1909)	DQ242551
Paradexiospira (*Spirorbides*) *vitrea* (Fabricius, 1780) [as *Pa. vitrea*]	DQ242547
Paralaeospira sp.	DQ242555
Pileolaria marginata Knight-Jones, 1978	DQ242565
Pileolaria militaris Claparède, 1870	DQ242567
Pileolaria sp.	DQ242568; DQ242562
Protis hydrothermica ten Hove & Zibrowius, 1986	DQ317122
Protis sp.	GU063863
Protolaeospira (*Protolaeospira*) *capensis* (Day, 1961)	DQ242558
Protolaeospira (*Protolaeospira*) *eximia* (Bush, 1905)	DQ242556
Protolaeospira (*Protolaeospira*) *tricostalis* (Lamarck, 1818)	DQ242557
Protula atypha Bush, 1905	DQ318595
Protula bispiralis (Savigny, 1822)	JX144827; JX144826
Protula palliata (Willey, 1905)	DQ317124
Protula sp.	DQ140406; AY611453; U67142
Protula tubularia (Montagu, 1803)	DQ317123
Pseudochitinopoma occidentalis (Bush, 1905)	DQ242542
Romanchella quadricostalis Knight-Jones, 1973	DQ242559
Sabella spallanzanii (Gmelin, 1791)	DQ318594
Sabellaria alveolata (Linnaeus, 1767)	DQ140412
Salmacina sp.	DQ140407; DQ317126; DQ317125; JX144829; JX144828; DQ242544
Serpula columbiana Johnson, 1901	DQ317127
Serpula concharum Langerhans, 1880	DQ140408
Serpula jukesi Baird, 1865	DQ317129
Serpula uschakovi Kupriyanova, 1999	EU184065
Serpula vermicularis Linnaeus, 1767	AY732224; AY395721; DQ317128; DQ140409
Serpula vittata Augener, 1914	EU184064
Serpula watsoni Willey, 1905	EU184057
Simplaria potswaldi (Knight-Jones, 1978)	DQ242566
Spirobranchus cariniferus (Gray, 1843)	JX144819
Spirobranchus corniculatus (Grube, 1862)	DQ140410
Spirobranchus lamarckii (Quatrefages, 1866) [as *Pomatoceros ditto*]	DQ140404
Spirobranchus latiscapus (Marenzeller, 1885)	JX144825; JX144824; JX144823; JX144822; JX144821; JX144820
Spirobranchus lima (Grube, 1862)	DQ317130
Spirobranchus taeniatus (Lamarck, 1818) [as *Pomatoceros ditto*]	DQ317120

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TABLE 1. (Continued)

Taxon	Accession Numbers
Spirobranchus triqueter (Linnaeus, 1758) [as *Pomatoceros ditto*]	DQ140405; DQ317121
Spirorbinae sp.	JX144830
Spirorbis (*Spirorbis*) *bifurcatus* Knight-Jones, 1978 [as *Sp. bifurcatus*]	DQ242569
Spirorbis (*Sp.*) *marioni* Caullery & Mesnil, 1897 [as *Sp. bushi* Rioja, 1942]	DQ242570
Spirorbis (*Spirorbis*) *rupestris* Gee, Knight-Jones, 1962	DQ242571
Spirorbis (*Spirorbis*) *spirorbis* (Linnaeus, 1758)	AY577887; AY527060
Spirorbis (*Spirorbis*) *tridentatus* Levinsen, 1883	DQ242573
Vermiliopsis infundibulum (Philippi, 1844)	DQ140411
Vermiliopsis labiata (O. G. Costa, 1861)	DQ317131
Vermiliopsis pygidialis (Willey, 1905)	DQ317132
Vermiliopsis striaticeps (Grube, 1862)	DQ317133
Vinearia koehleri (Caullery & Mesnil, 1897)	DQ242561

Micro-CT scanning. One individual within a tube and an empty tube were imaged through micro-computed tomography. Prior to scanning, the individual in the tube was stained for 48 h with 0.3% phosphotungstic acid in 70% ethanol following the protocol of Metscher (2009). Images were obtained with a SkyScan 1172 at the Hellenic Centre for Marine Research, Crete, Greece. Specimens were scanned at 76 kV and 100 µA with a 0.5 mm aluminum filter. Images were obtained at a resolution of 13.75 µm/pixel (worm in tube) and 4.88 µm/pixel (tube only). The resulting projection images were reconstructed into cross sections using the software NRecon (Bruker, Kontich, Belgium) and rendered with the software packages Dristhti (http://anusf.anu.edu.au/Vizlab/drishti/) and CTVox (Bruker, Kontich, Belgium).

Additional online material. Additional material (images, videos, specimen info, etc.) not included in this publication on the new species, has been made available through the Polychaetes Scratchpads (http://polychaetes.lifewatchgreece.eu).

Results

Family Serpulidae Rafinesque, 1815

Turbocavus gen. nov.

(Figs 2–4)

Diagnosis. *Turbocavus* is diagnosed from all other Serpulidae genera by its thoracic chaetae, each one of which has a plicate or multifolded base and a grooved shaft that extends to a capillary tip (Fig. 2B–F). This chaetal structure has not been reported for any polychaete species. In addition, there is a variable number of thoracic chaetigers (7 to 19) (Fig. 2G, H). While other genera (e.g. *Filograna* and *Spiraserpula*) also have a variable number of thoracic chaetigers, none have been reported to have more than 14 in total. *Turbocavus* also has an unusually high number of abdominal chaetigers (up to 335) in comparison to other genera, including *Spirobranchus* (reported to sometimes having over 200 abdominal chaetigers). Furthermore, *Turbocavus* lacks an operculum, special collar chaetae or *Apomatus* chaetae. The 18S rRNA genetic sequences obtained for the *Turbocavus* genus are distinguished from the closest neighbor of other sequenced genera (Table 1) by the intergeneric genetic distance of 10.5–12.9%.

Etymology. This genus is named for Hurricane Hole, "Hurricane (Latin: *Turbo-)* Hole (Latin: *cavus*)", the region of the Virgin Islands Coral Reef National Monument, St. John, U.S. Virgin Islands, where it abundantly occurs. Also, the tube forms concentric, ever-widening circles, like a hurricane. Masculine noun.

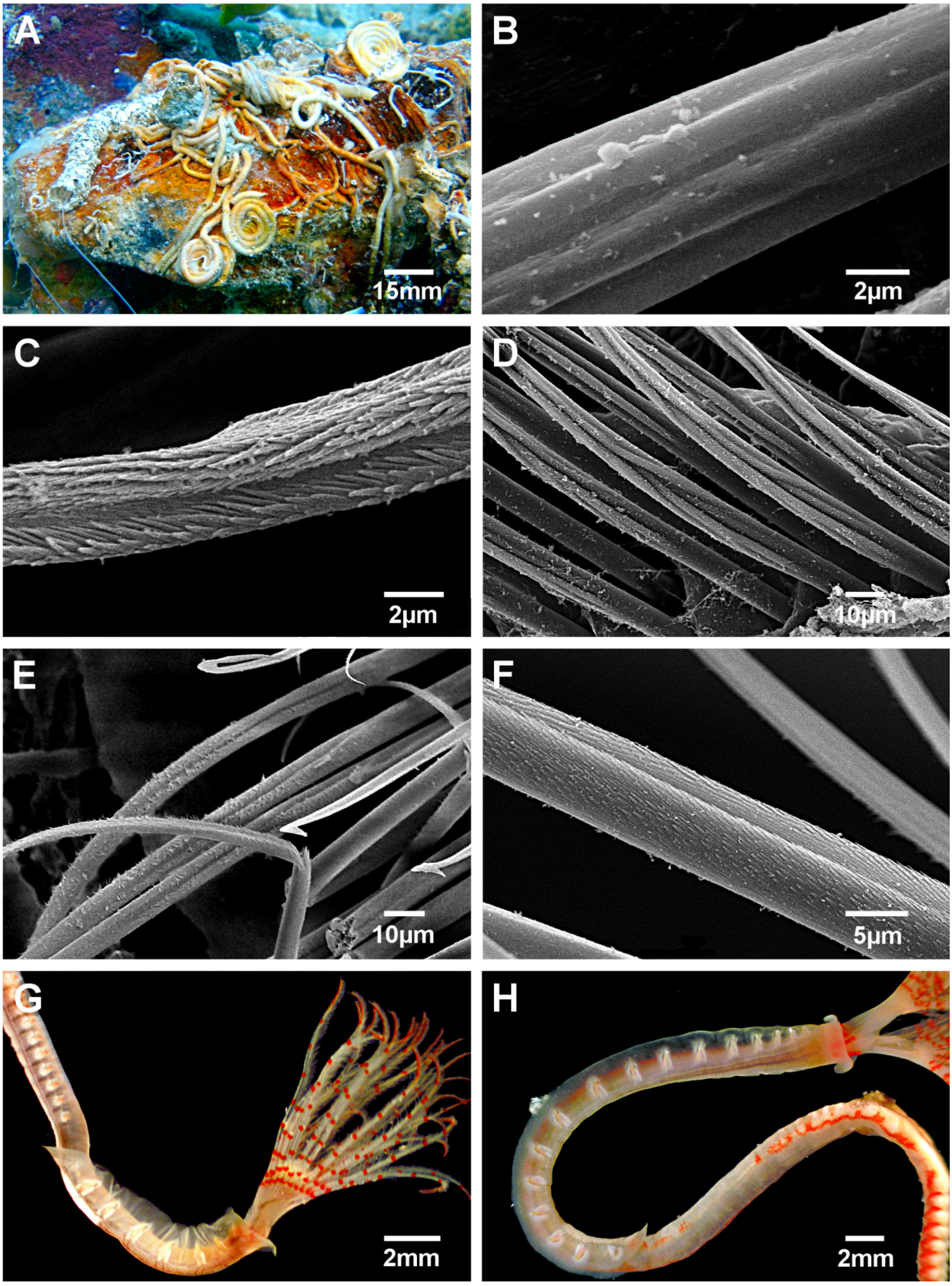

FIGURE 2. A–H. *Turbocavus secretus* **sp. nov.** A. Aggregation of coiled tubes, *in situ*, on the underside of rock. B. SEM image of plicate base of collar chaeta. C. SEM image of grooved limba of collar chaeta. D. SEM image of multifolded bases of thoracic capillary chaetae at chaetiger 7. E–F. SEM images of grooved limbate parts of thoracic notochaetae from chaetiger 7. G. Live worm with 7 thoracic chaetigers. H. Live worm with 17 thoracic chaetigers.

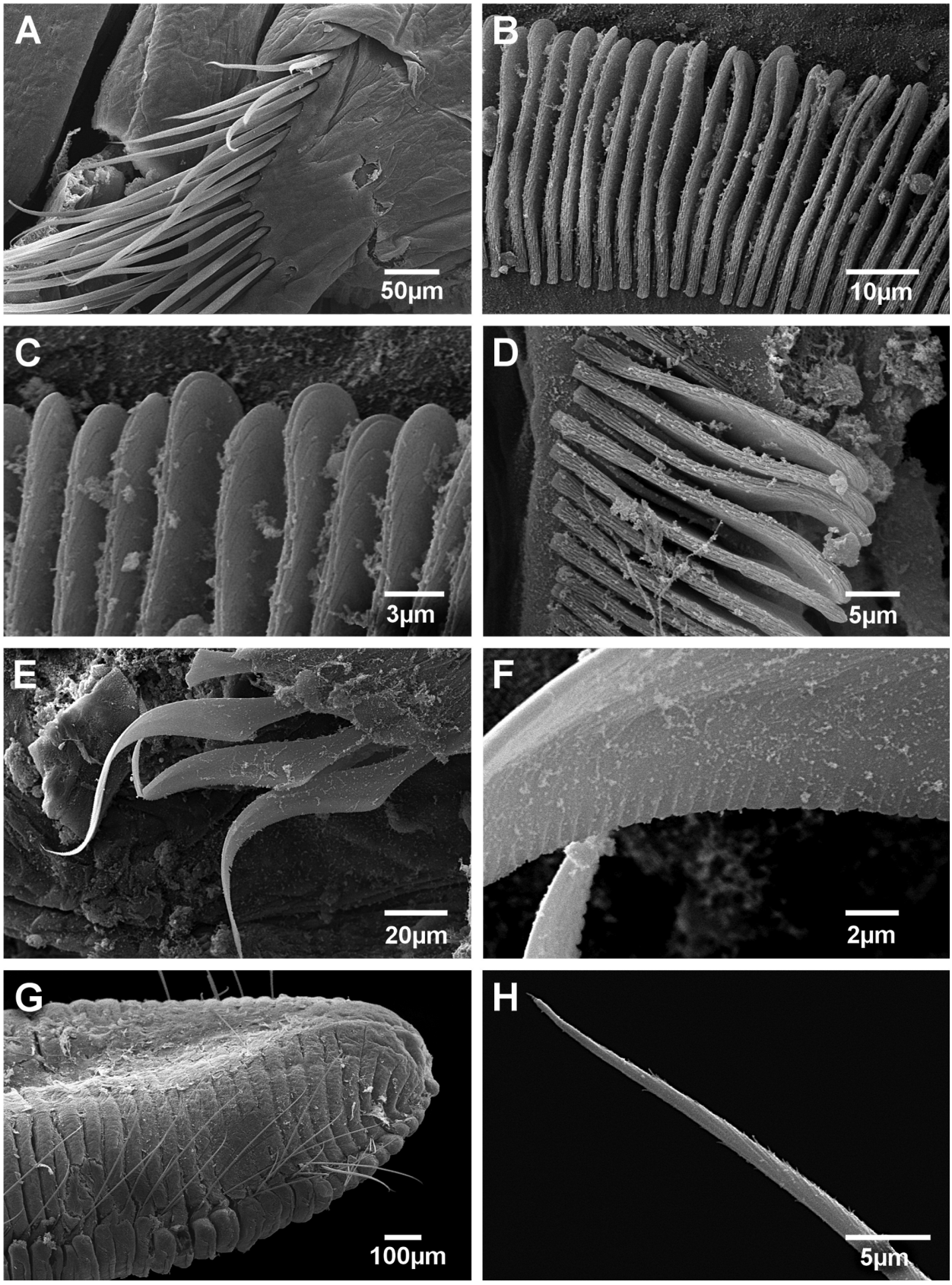

FIGURE 3. A−H. *Turbocavus secretus* **sp. nov.** A. SEM image of chaetal bundle at thoracic chaetiger 7. B−C. SEM images of thoracic uncini at chaetiger 7. D. SEM image of uncini in anterior abdomen. E−F. SEM images of flat narrow geniculate neurochaetae in anterior abdomen. G. Pygidium and posterior abdomen with fine capillary neurochaetae. H. Fine capillary neurochaeta from the posterior abdomen.

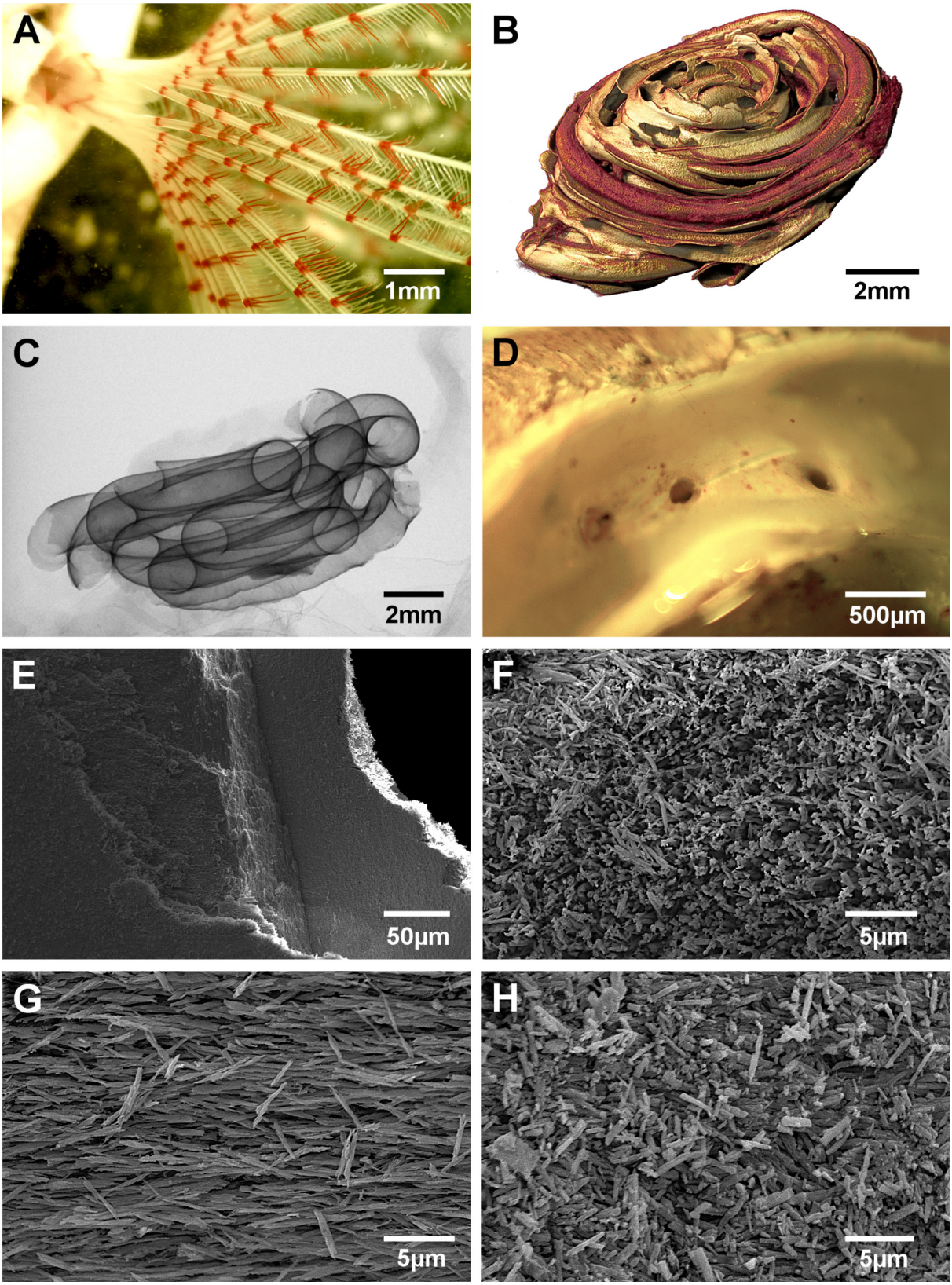

FIGURE 4. A–H. *Turbocavus secretus* **sp. nov.** A. Branchial crown (with ocellar clusters) of live worm. B. Three-dimensional, false colour volume rendering of micro-CT scan: partially opened tube, exposing preserved individual. C. Shadow (x-ray micro-CT) image of empty tube, side view. D. Interior of tube with perforations. E. SEM image of tube showing three ultrastructurally different layers. Transverse cut, layers from front to back of image: inner, middle, outer. F. SEM image of outer tube layer (longitudinal cut) showing irregularly oriented prismatic (IOP) structure. G. SEM image of middle tube layer (longitudinal cut) showing spherulitic prismatic structure. H. SEM image of inside of the tube with IOP structure.

***Turbocavus secretus* sp. nov.**
(Figs 2–4).

Holotype. U.S. Virgin Islands, St. John, Hurricane Hole, Otter Creek (18°21'05.0"N, 64°41'33.1"W), north shore of marine bay, affixed to underside of flat rock embedded in soft sediment at 1 m depth, May 27, 2013, Prentiss (hand collecting while snorkeling), fixed and preserved in 95% ethanol, Smithsonian National Museum of Natural History (NMNH) (USNM-1251873).

Paratypes. Same data as for holotype; also in Princess Creek and Water Creek, and in Great Lameshur Bay and Little Lameshur Bay. All paratypes (including worms and worm tubes) deposited in NMNH (USNM 1251849-1251872; 1251874-1251905). Specific information on collection dates, locations, coordinates and fixatives and preservatives is presented in Table 2.

TABLE 2. *Turbocavus secretus* **sp. nov.** collection information. Most specimens fixed and preserved in 95% ethanol (etoh). Other specimens fixed in 10% formalin (form) and preserved in 70% ethanol (etoh). Virgin Islands specimens were obtained from two areas: Coral Reef National Monument [VICR] Hurricane Hole area: Princess Bay, Otter Creek and Water Creek, and Virgin Islands National Park [VIIS] Lameshur Bay area: Great Lameshur Bay (two sites) and Little Lameshur Bay; Paratypes include both worms and tubes. Included are catalogue numbers for the Smithsonian Museum of Natural History (USNM) and National Park Service (NPS). Leeward Antilles specimens obtained from Klein Bonaire, Bonaire and Curaçao are deposited in the Naturalis Biodiversity Center, Leiden, the Netherlands.

Date	Location	NPS Code	USNM Code	Depth (m)	Fix/Preserve	Designation
	Hurricane Hole Area					
12-Jan-11	18°21'05.0"N, 64°41'33.1"W	VICR-00001-1	1251849	1	form/etoh	PARATYPE
17-Jul-11	18°21'05.0"N, 64°41'33.1"W	VICR-00001-2	1251850	0.5	form/etoh	PARATYPE
17-Jul-11	18°21'05.0"N, 64°41'33.1"W	VICR-00001-3	1251851	0.5	95% etoh	PARATYPE; DNA
31-Mar-12	18°21'05.0"N, 64°41'33.1"W	VICR-00001-4	1251852	0.25	95% etoh	PARATYPE
26-May-12	18°21'05.0"N, 64°41'33.1"W	VICR-00001-5	1251853	1	95% etoh	PARATYPE
26-May-12	18°21'05.0"N, 64°41'33.1"W	VICR-00001-6	1251854	1	95% etoh	PARATYPE; DNA
26-May-12	18°21'05.0"N, 64°41'33.1"W	VICR-00001-7	1251855	1	95% etoh	PARATYPE; DNA
26-May-12	18°21'05.0"N, 64°41'33.1"W	VICR-00001-8	1251856	1	95% etoh	PARATYPE
26-May-12	18°21'05.0"N, 64°41'33.1"W	VICR-00001-9	1251857	1	95% etoh	PARATYPE; DNA
26-May-12	18°21'05.0"N, 64°41'33.1"W	VICR-00001-10	1251858	1	95% etoh	PARATYPE; DNA; Micro-CT
26-May-12	18°21'05.0"N, 64°41'33.1"W	VICR-00001-11	1251859	1	95% etoh	PARATYPE
26-May-12	18°21'05.0"N, 64°41'33.1"W	VICR-00001-12	1251860	1	95% etoh	PARATYPE
4-Jan-13	18°21'05.0"N, 64°41'33.1"W	VICR-00001-13	1251861	1	95% etoh	PARATYPE
4-Jan-13	18°21'05.0"N, 64°41'33.1"W	VICR-00001-14	1251862	1	95% etoh	PARATYPE

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TABLE 2. (Continued)

Date	Location	NPS Code	USNM Code	Depth (m)	Fix/Preserve	Designation
4-Jan-13	18°21'05.0"N, 64°41'33.1"W	VICR-00001-15	1251863	1	95% etoh	PARATYPE; DNA
7-Jan-13	18°21'10.7"N 64°41'37.1"W	VICR-00001-16	1251864	1	95% etoh	PARATYPE
7-Jan-13	18°21'10.7"N 64°41'37.1"W	VICR-00001-17	1251865	1	95% etoh	PARATYPE
7-Jan-13	18°21'10.7"N 64°41'37.1"W	VICR-00001-18	1251866	1	95% etoh	PARATYPE
7-Jan-13	18°21'10.7"N 64°41'37.1"W	VICR-00001-19	1251867	1	95% etoh	PARATYPE
27-May-13	18°21'05.0"N, 64°41'33.1"W	VICR-00001-20	1251868	1	95% etoh	PARATYPE
27-May-13	18°21'05.0"N, 64°41'33.1"W	VICR-00001-21	1251869	1	95% etoh	PARATYPE
27-May-13	18°21'05.0"N, 64°41'33.1"W	VICR-00001-22	1251870	1	95% etoh	PARATYPE
27-May-13	18°21'05.0"N, 64°41'33.1"W	VICR-00001-23	1251871	1	95% etoh	PARATYPE
27-May-13	18°21'05.0"N, 64°41'33.1"W	VICR-00001-24	1251872	1	95% etoh	PARATYPE
27-May-13	18°21'05.0"N, 64°41'33.1"W	VICR-00001-25	1251873	1	95% etoh	HOLOTYPE
27-May-13	18°21'05.0"N, 64°41'33.1"W	VICR-00001-26	1251874	1	95% etoh	PARATYPE
27-May-13	18°21'05.0"N, 64°41'33.1"W	VICR-00001-27	1251875	1	95% etoh	PARATYPE
27-May-13	18°20'45.9"N 64°41'30.0"W	VICR-00001-28	1251876	1	95% etoh	PARATYPE
30-Jul-10	18°21'05.0"N, 64°41'33.1"W	VICR-00001-29	1251877	1	70% etoh	PARATYPE
13-Jan-11	18°21'05.0"N, 64°41'33.1"W	VICR-00001-30	1251878	1	70% etoh	PARATYPE
17-Jul-11	18°21'05.0"N, 64°41'33.1"W	VICR-00001-31	1251879	0.5	70% etoh	PARATYPE
17-Jul-11	18°21'05.0"N, 64°41'33.1"W	VICR-00001-32	1251880	0.5	70% etoh	PARATYPE
26-May-12	18°21'05.0"N, 64°41'33.1"W	VICR-00001-33	1251881	1	70% etoh	PARATYPE
27-May-13	18°21'05.0"N, 64°41'33.1"W	VICR-00001-34	1251882	1	70% etoh	PARATYPE
27-May-13	18°21'05.0"N, 64°41'33.1"W	VICR-00001-35	1251883	1	70% etoh	PARATYPE
27-May-13	18°21'05.0"N, 64°41'33.1"W	VICR-00001-36	1251884	1	70% etoh	PARATYPE
27-May-13	18°21'05.0"N, 64°41'33.1"W	VICR-00001-37	1251885	1	70% etoh	PARATYPE
27-May-13	18°21'05.0"N, 64°41'33.1"W	VICR-00001-38	1251886	1	Dry	PARATYPE 9 empty tubes

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TABLE 2. (Continued)

Date	Location	NPS Code	USNM Code	Depth (m)	Fix/Preserve	Designation
17-Jul-11	18°21'05.0"N, 64°41'33.1"W	VICR-00001-39	1251887	1	Dry	PARATYPE used for SEM
17-Jul-11	18°21'05.0"N, 64°41'33.1"W	VICR-00001-40	1251888	1	Dry	PARATYPE used for SEM
	Lameshur Bays Area					
10-Jul-13	18°19'02.8"N 64°43'26.8"W	VIIS-00314-49155	1251889	1	95% etoh	PARATYPE
10-Jul-13	18°19'02.8"N 64°43'26.8"W	VIIS-00314-49156	1251890	1	95% etoh	PARATYPE
15-Jul-13	18°18'56.9"N 64°43'17.4"W	VIIS-00314-49157	1251891	1	95% etoh	PARATYPE
15-Jul-13	18°18'56.9"N 64°43'17.4"W	VIIS-00314-49158	1251892	1	95% etoh	PARATYPE
15-Jul-13	18°18'56.9"N 64°43'17.4"W	VIIS-00314-49159	1251893	1	95% etoh	PARATYPE
15-Jul-13	18°18'56.9"N 64°43'17.4"W	VIIS-00314-49160	1251894	1	95% etoh	PARATYPE
22-Jul-13	18°19'02.8"N 64°43'26.8"W	VIIS-00314-49161	1251895	2	95% etoh	PARATYPE
22-Jul-13	18°19'02.8"N 64°43'26.8"W	VIIS-00314-49162	1251896	2	95% etoh	PARATYPE
22-Jul-13	18°19'02.8"N 64°43'26.8"W	VIIS-00314-49163	1251897	2	95% etoh	PARATYPE
22-Jul-13	18°19'02.8"N 64°43'26.8"W	VIIS-00314-49164	1251898	2	95% etoh	PARATYPE
22-Jul-13	18°19'02.8"N 64°43'26.8"W	VIIS-00314-49165	1251899	2	95% etoh	PARATYPE
22-Jul-13	18°19'02.8"N 64°43'26.8"W	VIIS-00314-49166	1251900	2	95% etoh	PARATYPE
22-Jul-13	18°19'02.8"N 64°43'26.8"W	VIIS-00314-49167	1251901	2	95% etoh	PARATYPE
9-Jul-13	18°19'06.5"N 64°43'31.6"W	VIIS-00314-49168	1251902	1	95% etoh	PARATYPE
22-Jul-13	18°19'02.8"N 64°43'26.8"W	VIIS-00314-49169	1251903	1	70% etoh	PARATYPE
22-Jul-13	18°19'02.8"N 64°43'26.8"W	VIIS-00314-49170	1251904	1	70% etoh	PARATYPE
22-Jul-13	18°19'02.8"N 64°43'26.8"W	VIIS-00314-49171	1251905	1	70% etoh	PARATYPE

Date	Location	Naturalis Biodiversity Center Code	Depth (m)	Fix/Preserve	Designation
01-Jul-70	12°09'28.58"N 68°19'34.13"W	RMNH.VER. 19907	49	form/etoh	not included in typeseries
03-Jul-70	12°03'41.27"N 68°16'55.39"W	RMNH.VER. 19908	46	form/etoh	not included in typeseries
03-Mar-55	12°16'03.35"N 69°07'39.43"W	RMNH.VER. 19909	0.5	form/etoh	not included in typeseries

Other material. Leeward Antilles, Klein Bonaire, N, 0.8 km E of Westpunt, July 1, 1970. Sand-flat below reef; 49 m. From coral debris in sand, legit H.A. ten Hove, stn. 2105C. (Naturalis Biodiversity Center (NBC), RMNH.VER. 19907; several tubes); Bonaire, 250 m N of Witte Pan, July 3, 1970. Sand-flat below reef; 46 m. Mainly from sides of boulders in sand partly above sand, legit H.A. ten Hove, stn. 2117B (NBC, RMNH.VER. 19908; several tubes); Curaçao, Santa Marta Baai, second lagoon, March 3, 1955, 0.5 m, legit P. Wagenaar Hummelinck, stn. 1322 (NBC, RMNH.VER. 19909; seven tubes). All Leeward Antilles material fixed in 10% formalin and preserved in 70% ethanol.

Etymology. Named for its hidden (Latin: *secretus)* nature, in that so far, it has only been observed, *in situ,* attached to the undersides of rocks, firmly embedded in fine sediment. Adjective reflects the masculine noun.

Diagnosis. As for genus by monotypy.

Description. Morphometric data are presented in Table 3. Largest specimens up to 84 mm long (holotype is 86 mm long) and 1.7 mm wide at 5th thoracic chaetiger (holotype 5th thoracic chaetiger is 1.7 mm wide); thorax with 7−19 chaetigers (holotype has 19L/17R thoracic chaetigers); thoracic membrane forms well developed ventral apron extending beyond last thoracic segment over the abdomen for equivalent of two segments (Figs 2G−H); abdominal achaetigerous zone maximally as long as thoracic region (Fig. 2H); abdomen up to 335 chaetigers (holotype has 335 abdominal chaetigers). Branchial crown with up to 19 pairs of radioles arranged pectinately (Fig. 2G); crown without operculum, pseudoperculum or modified radioles; radiole tips of different lengths but end at same level (longest radioles are dorsal due to the horseshoe shape of branchial lobe); paired pinnules (up to 100 per radiole) extend to radiole tips; some individuals with short apinnulate tips. Stylodes absent. Without interradiolar membrane, lips or mouth palps. Branchial eyes present (Figs. 2G, 4A). Peristomium with tri-lobed collar: one median ventral and two dorsal flaps, continuous with thoracic membranes, forming apron across anterior abdominal achaetigerous zone. All thoracic chaetae essentially limbate. *Apomatus* chaetae absent. Collar chaetae plicate at base and grooved tapering to a capillary tip; plicate collar chaetae appear to have "pleats" at the base, with each "pleat" visible (Figs 2B, C). Collar chaetae equal in size to the smallest anterior-most notochaetae of the following thoracic chaetigers. All remaining thoracic chaetae multifolded at base and grooved tapering to capillary tip. Multifolded bases of thoracic chaetae have "folds" which appear to fold into, over and under adjacent folds (Figs 2D−F). Well-developed thoracic tori; torus bears two rows of notochaetae, with up to 53 chaetae per torus and nearly equal numbers of shorter and longer chaetae; notochaetae emerging from anterior side of the torus about half the length of those emerging from posterior side (Fig. 3A); Thoracic uncini first appear in posterior thorax (average = 9th thoracic chaetiger). Thoracic uncini rasp-shaped with approximately 40 teeth in profile, and 1-2 rows of teeth apically to 5-6 rows immediately above and continuing onto elongated (rounded to) rectangular peg, not unlike those figured by ten Hove & Kupriyanova (2009, Fig. 39C) for *Protula* (dental formula P:5:5:4:4... ...2:2:2:1; Figs 3B, C). Achaetous anterior abdominal zone present. Ventral glandular field extends along posterior-most thorax for up to four segments, and extends up to two segments across achaetigerous zone. Width at 1st abdominal chaetiger up to 2.5 mm (holotype 1st abdominal chaetiger is 1.6 mm wide). Abdominal uncini rasp-shaped with round/rectangular peg and up to 30 transverse rows of seven teeth, decreasing to four teeth per row distally (dental formula P:6:6:6:5... ...4:4:3:2; Fig. 3D). Anterior abdominal neurochaetae flat narrow geniculate (Figs 3E, F). Posterior abdominal neurochaetae are long capillaries (Figs 3G, H), with 1−2 pairs emerging from each segment (average = 16% of posterior of abdomen). Posterior abdominal segments compressed; ventral glandular pad triangular. All specimens of undetermined sex; gametes were never observed in any intact or dissected individuals.

Colour. Live specimens white, strongly accented with vermilion-red pigment (Figs 2G, H; 4A). Radioles white, with evenly-spaced red ocelli (simple eyespots) or ocellar clusters (Fig. 4A) (terminology on ocelli follows ten Hove & Kupriyanova 2009). Ocellar cluster length extends over 2−3 pairs of pinnules, occurring at regular intervals (between every six to 10 sets of white pinnules) along radioles for up to 10 clusters per radiole. Most pinnules associated with ocellar clusters pigmented; small individuals with red pigmented radiole tips. Streaks of pigment extend longitudinally from ventral base of crown to ventral collar lobe and transversally to form irregular band on dorsum just posterior to collar lobes. Thorax dorsum strongly red-pigmented from collar to last thoracic chaetiger. Abdominal achaetigerous region lacks pigment. Red pigment patches occur at each chaetal bundle along the entire length of abdomen. All pigmentation lost from worms during fixation in formalin or ethanol; ocelli not visible in preserved material.

TABLE 3. Morphometric data: summary statistics for primary taxonomic characters.

CHARACTER	MIN	MAX	RANGE	MEAN (X)	SD	CV = SD/X	n	HOLO-TYPE
body length (mm)	2.3	86.0	83.7	43.5	24.3	0.56	16	86.0
crown length (mm)	0.3	13.0	12.7	5.5	3.3	0.60	16	13.0
thorax length (mm)	0.6	31.1	30.5	9.0	6.6	0.74	20	14.0
achaetigerous zone (mm)	0.2	10.5	10.3	2.8	2.5	0.9	17	4.0
abdomen length (mm)	1.1	58.1	57.0	30.9	18.9	0.61	19	55.0
number of thoracic chaetigers	7	19	12	11.4	3.8	0.34	60	19L/17R
1st thoracic chaetiger width (mm)	0.3	1.9	1.5	1.4	0.5	0.4	20	1.7
5th thoracic chaetiger width (mm)	0.3	1.8	1.5	1.3	0.5	0.4	20	1.7
thoracic chaetiger where uncini start	2	17	15	9.6	4.7	0.49	16	17
number of chaetigers ventral glandular field extends over posterior thorax (field extends the equivalent of 1−2 chaetigers across achaetigerous zone)	1	4	3	1.5	1	0.67	13	1
number of equivalent chaetigers membrane extends across achaetigerous region	1	3	2	2.0	0.5	0.25	15	1
number of abdominal chaetigers	31	335	304	217.2	85.6	0.39	14	335
1st abdominal chaetiger width (mm)	0.2	2.5	2.3	1.3	0.5	0.38	20	1.6
number of flat narrow geniculate chaetae at 1st abdominal chaetiger	1	6	5	3.6	1.5	0.42	16	4
number of uncini at 1st abdominal chaetiger	9	250	241	112.7	62.7	0.56	16	180
number of flat narrow geniculate chaetae at 100th abdominal chaetiger	3	6	3	5.1	0.9	0.17	10	4
number of uncini at 100th abdominal chaetiger	42	250	208	183.2	59.8	0.33	13	230
abdominal chaetiger at which ventral glandular pad starts	146	305	159	228.2	42.3	0.19	11	305
number of posterior segments with long capillary chaetae	0	45	45	27.2	14.7	0.54	11	5

Min, minimum; Max, maximum; Mean, arithmetic mean; SD, standard deviation; CV, coefficient of variation; *n*, sample size.

Tubes. Concentrically-coiled worm tubes (Figs 2A, 4B) occur in tight, overlapping aggregations loosely affixed to undersides of smooth rocks; tubes easily removed from substrate with spatula; younger tubes overlie surfaces of older tubes; occupied tubes with straight or curving extension leading to rock edge (Fig. 2A). Individual tubes in less densely-packed aggregates exhibit loose coiling or loosely-curved extensions along rock surface. Tube diameter up to 2 mm, concentrically coiled (diameter to 25 mm); individual coil diameter up to 25 mm (Fig. 2A). Tabulae have not been observed. Tubes opaque and circular in cross-section without internal or external keels or structures (Fig. 4C). External tube surface smooth to fine granular or with transverse growth striations. Internal tube surface smooth and glossy. Younger tubes off-white color, older tubes thicker and with an iron oxyhydroxide stain. Internally, some tubes with a pattern of three perforations through the tube wall, with one hole offset from the other two (Fig. 4D). SEM analysis of tube shows three ultrastructurally different layers: outer layer with irregularly oriented prismatic (IOP) structure; middle layer with spherulitic prismatic structure; inner layer with IOP structure (Figs 4E−H).

Remarks. Some individuals show evidence of crown regeneration, where one half (side) of the branchial crown is fully formed, while the other half has newly forming radioles emerging from the peristomium. The variation in length of the abdominal achaetigerous zone may result from varying degrees of shrinkage during fixation as this zone appears not to have the same structural integrity of chaetigerous regions. Long capillary

neurochaetae of posterior abdomen easily break off during transfer and are not present on some individuals. No evidence of intratubular brooding. Tube perforations, from the lumen of one whorl to that of another whorl (Fig. 4D), have been regularly observed and may function to allow circulation of water, with the worm body acting like a piston. Serpulid tubes vacated by serpulid worms can be occupied by polychaete species of other families including Amphinomidae, Aphroditidae, Dorvilleidae, Eunicidae, Oenonidae and Terebellidae. Live worms were observed *in situ* in shallow (0.5−3 m) water, however material collected in the Leeward Antilles indicated that the species here occurred at depths to at least 49 m. It is expected that the species is widely distributed throughout the Greater Caribbean region.

Genetic analysis. Phylogenetic trees presented in the paper are downsized. The complete trees are available through the Polychaetes Scratchpads website (http://polychaetes.lifewatchgreece.eu). The phylogenetic tree inferred by the Bayesian analysis (Fig. 5) showed the existence of three major, strongly supported clades: (a) All *Turbocavus* sequences were clustered alone in Clade 1 (Posterior Probability, PP=100%); (b) Clade 2 was formed by *Filograna*, *Salmacina* and *Protis* species (PP=100%); (c) Clade 3 included the remaining sequences of Serpulidae (PP=99%). Within Clade 3, sequences of *Protula* (PP=97%) and *Chitinopoma* (PP=100%) formed separate groups. *Vermiliopsis* sequences were arranged in a distinct group together with the *Apomatus* and *Metavermilia* sequences (PP=84%). *Serpula*, *Crucigera* and *Hydroides* species (PP=100%) were a sister-group to the clade, which was formed by the *Spirobranchus, Galeolaria, Ficopomatus, Ditrupa* species (PP=100%). All Spirorbinae species were included in one well-supported group (PP=100%).

The phylogenetic tree produced by the Maximum Likelihood analysis (Fig. 6) clustered Serpulidae into eight groups: (a) Sequences of *Serpula*, *Crucigera* and *Hydroides* species formed one group (Clade 4), though not strongly supported (Bootstrap value=77); (b) *Spirobranchus, Galeolaria* and *Ficopomatus* species formed Clade 3 (Bootstrap value=94); (c) The remaining species were clustered into two well supported clades: Clades 1 and 2; Bootstrap value=96. Clade 2 represented the Spirorbinae species. The analysis resulted in four different groups within Clade 1 of the remaining serpulid species: (d) *Turbocavus* sequences formed a well-supported group (Bootstrap value=100), whereas (e) *Protula* sequences were grouped in a poorly supported sub-clade (Sub-clade 2c; Bootstrap value=69). Two more sub-clades were included: (f) sub-clade 2b with *Chitinopoma* and *Vermiliopsis* species (Bootstrap value=95) and (g) sub-clade 2a with the *Salmacina, Filograna* and *Protis* species (Bootstrap value=100).

Discussion

Traditional overviews of Serpulidae employed the character "operculum" as one of the key ones for the classification of the taxa within the family, non-operculate genera (and a few taxa with a simple opercular bulb on a non-modified radiole) were classified in the subfamily of "Filograninae" and thought to be primitive, plesiomorphic (e.g. Fauvel 1927; Hartman 1959; Fauchald 1977). However, later studies (ten Hove 1984; Kupriyanova *et al.* 2006, 2008; Lehrke *et al.* 2007) concluded that the—essentially non-operculate—"Filograninae" group as well as the—essentially operculate—“Serpulinae” were paraphyletic.

Turbocavus secretus **sp. nov.** lacks an operculum, which in the traditional morphology based systematics would have suggested close affinity with the genera traditionally placed within the "Filograninae". Taxonomic keys (e.g. Bastida-Zavala 2008; ten Hove & Kupriyanova 2009), which often mistakenly are attributed a phylogenetic basis, use the presence or absence of an operculum as a taxonomic character convenient for grouping species into genera (but see below). Using these keys and the associated descriptions of the genera, *T. secretus* **sp. nov.** is most closely aligned with *Protis* (in part), *Protula* (in part) or *Filogranella*. However, the only choice offered in the key by ten Hove and Kupriyanova (2009) is to determine whether the worm has 7, 9 or 11−14 thoracic chaetigers, leading to *Filogranella*. Worms described in the present paper as *T. secretus* **sp. nov.** have between 7 and 19 thoracic chaetigers, and thus at best, would end up as *Filogranella*, by extending the range of this character in this genus. This finding underpins the limits of identification keys, as implicated by ten Hove and Kupriyanova (2009) in their formulation “Key to serpulid genera (described before 2008)”. Such keys are dependent on a heuristic choice of arguments (characters), moreover changing with increasing knowledge. This fact is well known to taxonomists, but not always to frequent users of keys such as ecologists.

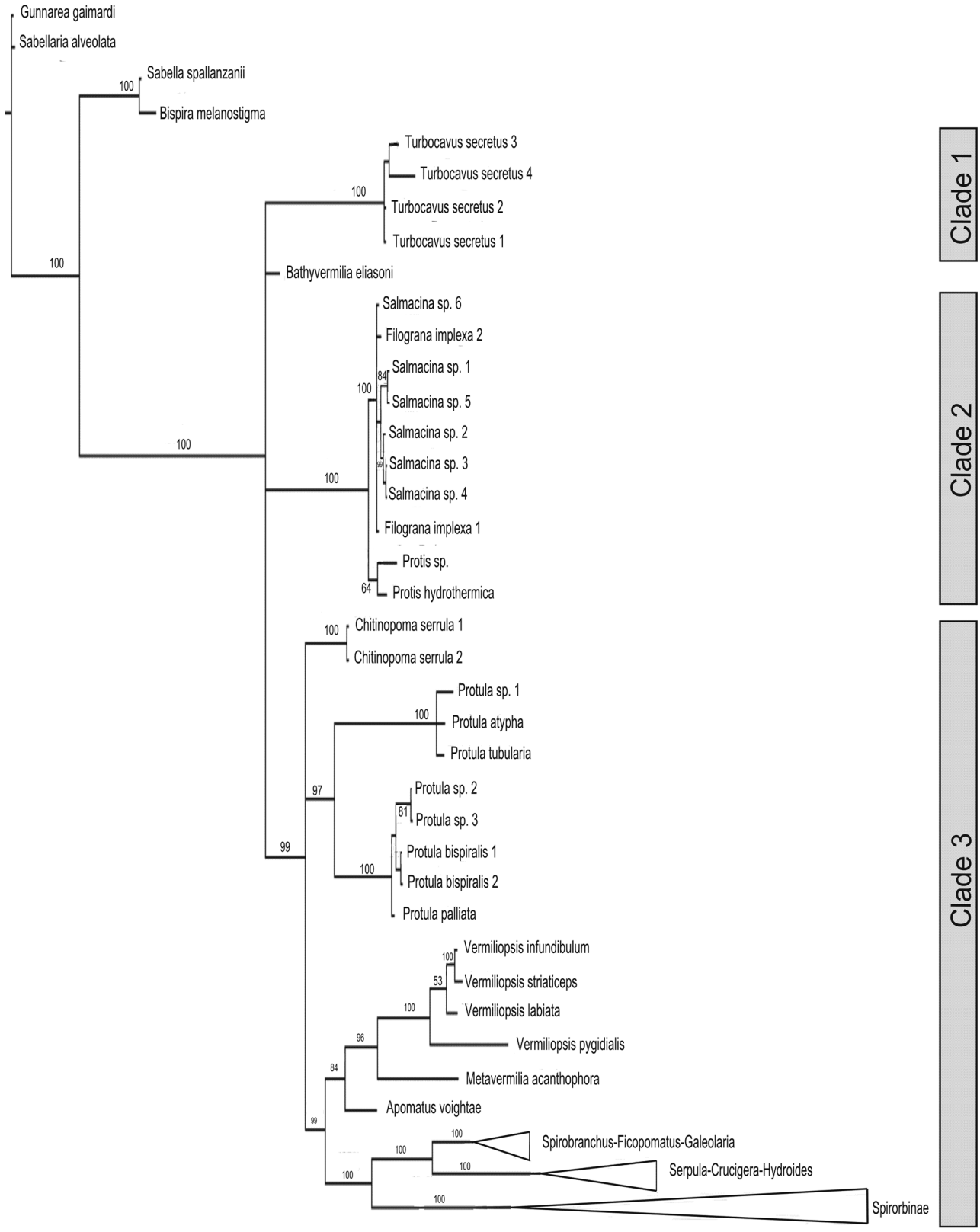

FIGURE 5. Phylogenetic tree produced by Bayesian analysis. Numbers on the nodes are posterior probabilities. The tree is downsized. The complete trees are available through the Polychaetes Scratchpads (http://polychaetes.lifewatchgreece.eu).

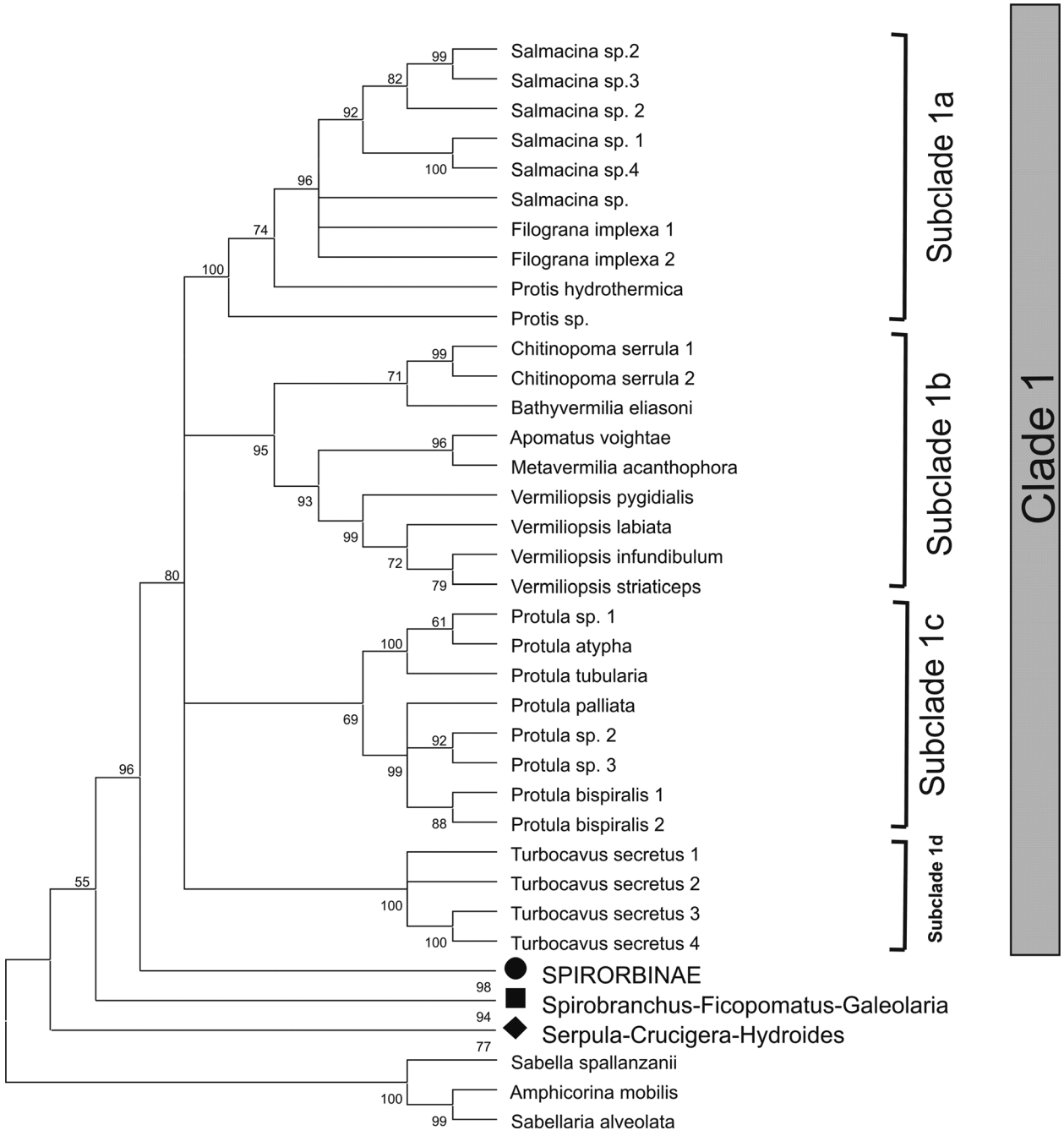

FIGURE 6. Phylogenetic tree inferred from Maximum Likelihood analysis. Numbers on the nodes are bootstrap values. The tree is downsized. The complete trees are available through the Polychaetes Scratchpads (http://polychaetes.lifewatchgreece.eu).

Functionally, the operculum is used for protection against possible invaders and to prevent water loss (Day 1967; Rouse & Pleijel 2001). However, many authors report the existence of both operculate and non-operculate taxa in genera, sometimes even within the same colony (Day 1967; ten Hove & Jansen-Jacobs 1984; Lehrke *et al*. 2007). Lack of an operculum might be due to ecological selection. *Hydroides spongicola* Benedict, 1887 frequently does not have (75-95%, ten Hove 1984) a functional operculum. Occurring symbiotically in the chemically aggressive do-not-touch-me sponge *Neofibularia nolitangere* (Duchassin & Michelotti, 1864), as mentioned by ten Hove (1989), it would have less need for opercula against possible invaders. The latter might apply too for *Spirobranchus nigranucha* (Fischli, 1903), the only non-operculate member of its genus, occurring well sheltered deep between the branches of *Acropora* spp. (ten Hove 1989). Knight-Jones *et al.* (1997) discuss a number of other possible examples of functional absence of opercula. Among these that of *Hyalopomatus cancerum* Knight-Jones,

Knight-Jones, Oliver & Mackie, 1997, which might have adapted to low oxygen conditions of the deep sea by losing its operculum, which would hinder respiration. However, reading Kupriyanova *et al.* (2014), operculate taxa appear to be more common than non-operculate ones in the deep sea. Ecological selection (fewer predators and/or lower oxygen levels) could perhaps account for the absence of an operculum in *Turbocavus secretus* **sp. nov.** Individuals were found subtidally under rocks, where the colonies would not only be safeguarded from water loss, but also be relatively protected from predators. *Turbocavus secretus* **sp. nov.** seems to inhabit an oxic-anoxic interface. Their tubes are underneath rocks firmly embedded in fine sediment and appear to be stained orange by iron oxyhydroxides.

The importance of chaetal characters for the classification within the Serpulidae was stressed by ten Hove (1984). This might, in part, be reflected in the molecular results of the present study: *Turbocavus secretus* **sp. nov.** forms a distinct clade, a result which concurs with the unique thoracic chaetae observed.

It is not the intention of the present paper to discuss the phylogeny of serpulids in depth, only to illustrate the genetic basis, if any, for our morphology initiated decision to attribute generic rank to the new taxon. Although different genes and analyses have been used by Kupriyanova *et al*. (2006, Fig. 5B; 2009, Fig. 5; 2010, Fig. 4), Lehrke *et al*. (2007, Fig. 2) and Kupriyanova & Nishi (2010, Fig. 7), and the used number of taxa varies between these papers, some general trends can be discerned. For example, all show clades with *Spirobranchus*-*Ficopomatus*-*Galeolaria*, *Serpula*-*Crucigera*-*Hydroides* on the one hand, *Salmacina*/*Filograna*, *Protis*, as well as a *Protula* clade on the other. Relationships between the clades, however, are unclear, with recurrent polytomies. Differences in topology of the cladograms, for instance the position of the Spirorbinae and of the *Vermiliopsis*-like genera, indicate that a relatively stable phylogeny reconstruction on the basis of a single gene is not to be expected. Nevertheless, in both our maximum-likelihood and Bayesian analysis, *Turbocavus* clearly stands out as a well-supported separate clade with a jackknife value respectively posterior probability of 100, substantiating a generic status for the taxon.

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