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## Research article

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# Hidden in plain sight, *Chaetopterus dewysee* sp. nov. (Chaetopteridae, Annelida) – A new species from Southern California

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<sup>1</sup>[urn:lsid:zoobank.org:author:89205DA4-C863-49CA-9C9B-4EFCF53B43B5](https://zoobank.org/author/89205DA4-C863-49CA-9C9B-4EFCF53B43B5)

<sup>2</sup>[urn:lsid:zoobank.org:author:F4AAFAE4-85D9-44CA-8290-E0FC614E1983](https://zoobank.org/author/F4AAFAE4-85D9-44CA-8290-E0FC614E1983)

**Abstract.** We describe a long-unnamed *Chaetopterus* Cuvier, 1830 species from southern California, using a combination of DNA barcoding and detailed morphological investigation employing high-resolution X-ray microtomography (micro-CT). *Chaetopterus dewysee* sp. nov. is not only one of the most dominant annelids in the benthic communities of the shallow end of the La Jolla submarine canyon, but also a well-established model for studying bioluminescence and has a published transcriptome. The description and naming of this southern Californian *Chaetopterus* is a step towards the much-needed revision of the group's taxonomy and towards resolving the confusion over the 'cosmopolitan' *Chaetopterus variopedatus* species complex. Micro-CT data showing details of both internal and external anatomy has been made freely available as the first annelid cybertype.

**Keywords.** Micro-CT, cybertype, COI, parchment worm, polychaete.

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## Introduction

Chaetopteridae Audouin & Milne Edwards, 1833 is a clade of mainly benthic, tubicolous annelids with an unusual body plan (Moore *et al.* 2017). The head is formed by a small prostomium and large peristomium, with two grooved palps. Following the head, chaetopterid bodies can be divided into three morphologically distinct regions: the anterior A region, middle B region and posterior C region (Fig. 1A). Each morphologically distinct body region undertakes a different function. In *Chaetopterus* Cuvier, 1830, region A, with its highly glandular ventral shield (plastron), is mainly involved in tube construction and mucus production, segments of region B are used for the elaborate mucous-feeding system (Fig. 1A). The notopodia of segments B3–5 form paddle like 'pistons' (Fig. 2A) that create suction and pump water through the worm's tube (Brown 1975, 1977). The cup-shaped cupule on segment B2 (Fig. 2A) forms and collects particles in a mucous net, which are then transported to the mouth opening along the ciliated groove (Fig. 2C). While grouped with spioniform annelids for the last

few decades (Rouse & Fauchald 1997; Rouse & Pleijel 2001), recent molecular phylogenies of annelids recover Chaetopteridae isolated from most spioniform taxa (Andrade *et al.* 2015; Weigert & Bleidorn 2016) forming a clade, Chaetopteriformia Fauchald, 1977, along with Apistobranchidae Mesnil & Caullery, 1898 and Psammodrilidae Swedmark, 1952 (Helm *et al.* 2018).

Chaetopterid taxonomy is not reliably resolved and a comprehensive revision is needed (Osborn *et al.* 2007; Moore *et al.* 2017). Especially within *Chaetopterus*, there is repeated confusion, mainly due to the synonymization of many species into a widely distributed and highly variable species *C. variopedatus* (Renier, 1804) by Fauvel (1927) and Hartman (1959), which has a type locality in the Mediterranean. In many other regions around the world, the name *C. variopedatus* was applied and no new species were named. Such is the case for US west coast, where Treadwell (1914) saw no reason not to use the European name for specimens he studied from southern California. This has been followed ever since for *Chaetopterus* from this region, with numerous records (see Hartman 1969) and studies (e.g., Brown 1975, 1977; Sumida 1983) referring to *C. variopedatus*, or more recently to *Chaetopterus* sp. (e.g., Deheyn *et al.* 2013; Weigand *et al.* 2017).

This taxonomic ‘lumping’ into a large, cosmopolitan species with an implied broad dispersal capacity was justified by the long planktonic stage of chaetopterid larvae in the water column (Scheltema 1974). However, it has been suggested repeatedly that *C. variopedatus* is a species complex of both morphologically (Petersen 1984a, 1984b; Petersen & Britayev 1997) and molecularly distinct species (Osborn *et al.* 2007; Martin *et al.* 2008). Herein, we describe a long-unnamed *Chaetopterus* species from southern California. *Chaetopterus dewysee* sp. nov. occurs in large densities and makes up a large portion of the benthic biomass in the shallow reaches of the La Jolla submarine canyon (Fig. 1D). It is one of the most abundant and visually conspicuous annelid species with a reported population density of on average 20.8 individuals per m<sup>2</sup> in Fisherman’s Cove, Santa Catalina (Chess & Hobson 1997). In addition to being one of the most dominant annelids in benthic communities, *Chaetopterus dewysee* sp. nov. is established as a model in the Scripps Institution of Oceanography and has been the focus of many studies investigating the biochemistry of light production and the bioluminescent properties of *Chaetopterus* mucus (Deheyn *et al.* 2013; Shah *et al.* 2014, 2015; Branchini *et al.* 2014; Rawat & Deheyn 2016; Weigand *et al.* 2017). *Chaetopterus dewysee* sp. nov. also has a sequenced transcriptome (Accession No: SRX755856) and was included (as *Chaetopterus* sp. nov.) in large scale phylogenomic studies of Annelida (Andrade *et al.* 2015; Lemer *et al.* 2015). Together with our description of *C. dewysee* sp. nov. we provide the first three-dimensional annelid ‘cybertype’. This micro-computed tomography dataset is freely available for future taxonomic and morphological investigations.

## Material and methods

### Morphology

Live specimens were studied and photographed with a Leica MZ9.5 stereo microscope mounted with a Canon EOS Rebel T5i digital camera. Chaetigers were dissected and placed on separate microscope slides, 50% bleach was used to dissolve the tissue. The tissue was slightly teased away from the chaetae. These were examined and photographed with a Leica DMR HC compound microscope. All animals were relaxed using MgCl<sub>2</sub>, fixed with 10% formaldehyde in seawater for a few days, rinsed in fresh water and transferred to 70% alcohol. Posterior parapodia were subsampled for DNA and fixed directly in 95% ethanol.

### Micro-CT

The specimen fixed for micro-CT was preserved in 50% ethanol. In order to stain soft tissue and increase contrast, the specimen was transferred to a 0.3% phosphotungstic acid (PTA) solution in 70% ethanol (Metscher 2009). The specimen remained in this solution for 3 months and was scanned using a Skyscan

1272 (Bruker microCT, Kontich, Belgium) with the following scan parameters: 60 kV source voltage, 166  $\mu$ A source current, 741 ms exposure and a camera resolution of 1632  $\times$  1092 px. The voxel resolution was 8  $\mu$ m. An aligned image stack was generated with the software Nrecon (Bruker) and the surface renderings were generated with the software Drishti 2.6.5. (National University, Canberra, Australia). Micro-CT data together with a 3D surface rendering are deposited online in the morphological data repository MorphDBase (Grobe & Vogt 2009).

### DNA sequences

DNA was extracted from posterior parapodia of animals using the Zymo Research Quick-DNA™ Miniprep kit. DNA from the larva of *Chaetopterus dewysee* sp. nov. was extracted using the Quick-DNA™ Microprep kit. Up to 686bp of mitochondrial cytochrome c oxidase subunit I (COI) were amplified using the polyLCO/polyHCO primer set (Carr *et al.* 2011). Amplification was carried out using 8.5  $\mu$ l of ddH<sub>2</sub>O, 12.5  $\mu$ l of Apex™ 2.0x Taq RED DNA Polymerase Master Mix (Genesee Scientific), 1  $\mu$ l each of the forward and reverse primers (10  $\mu$ M), and 2  $\mu$ l of eluted DNA. The reactions were carried out in an Eppendorf thermal cycler. The COI reaction protocol was as follows: 94°C/60 s – (94°C/40 s – 45°C/40 s – 72°C/60 s) \* 5 cycles – (94°C/40 s – 51°C/40 s – 72°C/60 s) \* 35 cycles – 72°C/300 s. Successfully amplified products were purified using 2  $\mu$ l of ExoSAP-IT PCR product cleaning reagent. The cleaned products were then sequenced by Eurofins Genomics (Louisville, KY) and assembled with Geneious ver. 11.0.2 (<https://www.geneious.com>). The COI sequence was also pulled from the assembly of the published *Chaetopterus* transcriptome (SRX755856) using the direct sequencing results as a blast query. Other available *Chaetopterus* COI sequences were acquired from GenBank, mostly from the Moore *et al.* (2017) study, but also including one *C. dewysee* sp. nov. sequence as, “*Chaetopterus* sp. 1”, from Osborn *et al.* (2007) collected from Santa Barbara, California. Sequences of the sister group, *Mesochaetopterus* Potts, 1914, were used to root the phylogenetic analysis, following Moore *et al.* (2017).

All COI sequences included in the phylogenetic analysis (Table 1) were aligned using MAFFT (G-INS-i) (Kato & Standley 2013). The maximum likelihood analysis was executed with RaXML ver. 8.2.10 (Stamatakis 2014), with 1000 ‘thorough’ bootstrap pseudoreplicates to assess clade support. A haplotype network of the nine *Chaetopterus dewysee* sp. nov. COI sequences was created with PopART ver. 1.7 (Leigh & Bryant 2015) using the TCS algorithm.

### Repositories

FLMNH = Florida Museum of Natural History, Gainesville, USA  
 LACM = Natural History Museum of Los Angeles County, Los Angeles, USA  
 MNCN = Museo Nacional de Ciencias Naturales, Madrid, Spain  
 SAM = South Australian Museum, Adelaide, Australia  
 SIO-BIC = Scripps Institution of Oceanography Benthic Invertebrate Collection, La Jolla, USA

### Abbreviations

a = region A  
 b = region B  
 c = region C  
 cg = ciliated groove  
 ey = eyes  
 nt = notopodium  
 p = palps

## Results

Family Chaetopteridae Audouin & Milne Edwards, 1833

Genus *Chaetopterus* Cuvier, 1830

*Chaetopterus dewysee* sp. nov.

urn:lsid:zoobank.org:act:B4B305E7-9A28-4632-9E58-A5A9A8E0A6D3

Figs 1–4

*Chaetopterus variopedatus* – Treadwell 1914: 204. — Hartman 1959: 396; 1969: 209. — Brown 1972: 278.; 1975: 136, figs 1, 4–8.; 1977: 121. — Sumida & Case 1983: 249.

*Chaetopterus* sp. 1 – Osborn *et al.* 2007: 43. — Moore *et al.* 2017: 600.

*Chaetopterus* sp. – Deheyn *et al.* 2013: 702, fig. 1. — Branchini *et al.* 2014: 247, fig. 2. — Shah *et al.* 2014: 1, figs 1, 8.; 2015: 408, figs 1–6. — Lemer *et al.* 2015: 175. — Rawat & Deheyn 2016: 1, fig. 1.

*Chaetopterus* sp. nov. – Andrade *et al.* 2015: 2863.

*Chaetopterus* – Weigand *et al.* 2017: 1, fig. 1.; Weigand *et al.* 2018: 1.

## Diagnosis

*Chaetopterus dewysee* sp. nov. is characterized by having a long u-shaped tube partly buried in sediment, 10 region A chaetigers, 11–12 club-shaped a4 cutting chaetae with dark brown, coppery metallic coloration, a patch of notopodial uncini at the upper ventral margin of the modified b3–b5 notopodia.

## Etymology

Named for Mary ‘Dewy’ White, for her support of the Rouse lab and her passion for conservation and marine biological research. Based on her love of the sea we have incorporated the German word ‘See’ into the name.

## Material examined

### Holotype

UNITED STATES OF AMERICA • adult specimen with tube; California, San Diego, Scripps Submarine Canyon; 32°52′11.35″ N, 117°15′36.00″ W; depth 20 m; 2019; Phil Zerofski leg.; GenBank: MN991231; SIO-BIC A11476.

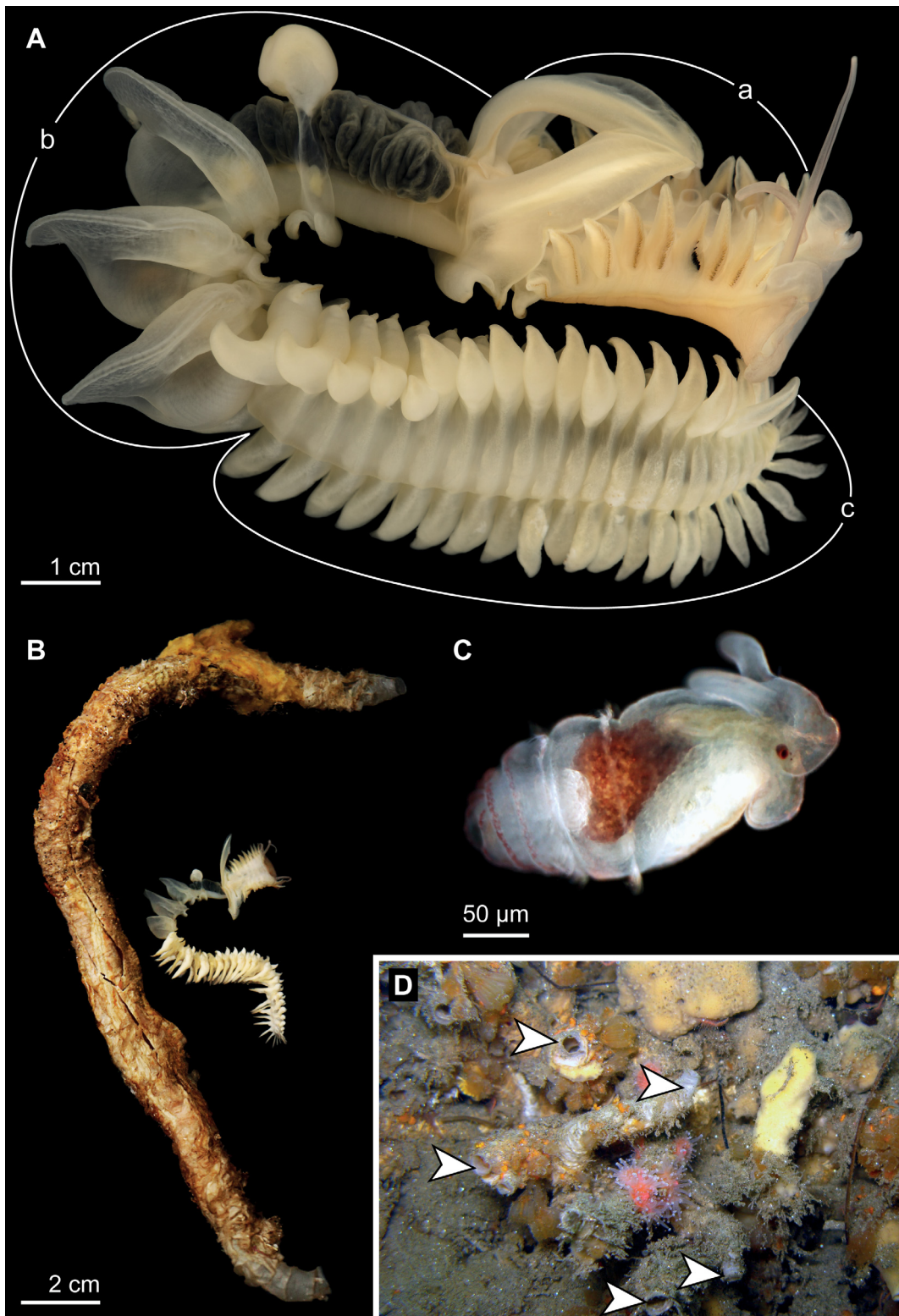
### Paratypes

UNITED STATES OF AMERICA • 1 adult specimen; same collection data as for holotype; GenBank: MN991233; SIO-BIC A10193 • 1 adult specimen; same collection data as for holotype; 18 Feb. 2010; Eddie Kisfaludy leg.; GenBank: MN991236; SIO-BIC A4193 • 1 adult specimen; California, San Diego, La Jolla Submarine Canyon; 24 Jul. 2008; Eddie Kisfaludy leg.; GenBank: MN991230; SIO-BIC A11649 • 1 adult specimen; same collection data as for holotype; 2017; Greg Rouse leg.; GenBank: MN991235; SIO-BIC A11652 • 1 adult specimen; California, San Diego, Scripps Institution of Oceanography pier; Nov. 2017; Avery Hatch and Ekin Tilic leg.; GenBank: MN991234; SIO-BIC A11653 • 1 adult specimen; same collection data as for preceding; 5 Jul. 2019; Ekin Tilic leg.; SIO-BIC A12034 (cybertype).

### Cybertype

Complete micro-CT scan of a paratype SIO-BIC A12034, designated as cybertype.

Direct link to online specimen: [www.morphdbase.de/?E\\_Tilic\\_20200122-S-5.1](http://www.morphdbase.de/?E_Tilic_20200122-S-5.1)



**Fig. 1.** *Chaetopterus dewysee* sp. nov. **A.** Live photograph of a paratype (SIO-BIC A11652), showing the three morphologically distinct regions of a chaetopterid annelid; a = region A, b = region B, c = region C. **B.** Holotype together with its tube (SIO-BIC A11476). **C.** Larva of *Chaetopterus dewysee* sp. nov. collected from a plankton sample off La Jolla and sequenced (GenBank: MN991232). **D.** Habitat of the new species, tubes are marked with arrow-heads.

Direct links to the micro-CT stack:

PART 1/3: [www.morphdbase.de/?E\\_Tilic\\_20200122-M-42.1](http://www.morphdbase.de/?E_Tilic_20200122-M-42.1)

PART 2/3: [www.morphdbase.de/?E\\_Tilic\\_20200122-M-41.1](http://www.morphdbase.de/?E_Tilic_20200122-M-41.1)

PART 3/3: [www.morphdbase.de/?E\\_Tilic\\_20200122-M-40.1](http://www.morphdbase.de/?E_Tilic_20200122-M-40.1)

Direct link to 3D surface rendering: [www.morphdbase.de/?E\\_Tilic\\_20200122-M-43.1](http://www.morphdbase.de/?E_Tilic_20200122-M-43.1)

## Description

Total body length of holotype 55 mm (paratypes: A12034 = 35 mm, A11652 = 180 mm, A11653 = 107 mm). Region A with 10 chaetigers. Prostomium small, with anterior border rounded, entire. Peristomium extended, completely covering prostomium; wide horseshoe-shaped in anterior view (Figs 2A, 4C). Two grooved palps extending beyond peristomium, length 7.5 mm (Fig. 2A). Paired eyes present, located at the base of palps (Fig. 2B). Middorsal ciliated groove (cg) extending from region B through region A, ending near the mouth opening with a conical ridge (Fig. 2C–B). Ventral surface of region A with a smooth plastron (ventral shield) (Figs 1A, 4B): length 9 mm, width 3.9 mm. First 9 chaetigers uniramous. Tenth chaetiger (a10) biramous, with long notopodium and a neuropodial lobe (Fig. 3A). Each notopodium with 2–3 rows of light yellow lanceolate chaetae. Neuropodial uncini of chaetiger a10 with 5–6 teeth, short rostral and long adrostral process (Fig. 3B). Chaetiger a4 with 11–12 modified cutting chaetae (Fig. 2C–E). Modified chaetae dark brown, with coppery metallic shimmer, club-shaped, tip asymmetrical, truncated with sharp apical point (Fig. 2E). Larger portion of cutting chaetae embedded inside the chaetiger (Figs 2D, 4E). Region B with five chaetigers, with biramous parapodia. Digestive gland blackish green in living specimens; coloration lost in ethanol-preserved specimens. Chaetiger b1 with distally tapering, aliform notopodia extending to the middle of region A (Figs 2C, 3D): length 9.2 mm (paratypes: A12034 = 11 mm, A11652 = 28 mm, A11653 = 26.4 mm). Chaetiger b2 modified with large cupule (Figs 2A, 4A–B). Notopodia of b3–b5 fused middorsally, forming enlarged fans (Figs 2A, 4A). Small pocket with patch of uncini at the upper ventral margin of the enlarged fan (Fig. 3C, E). Notopodial uncini of chaetigers b3–b5 with 9–10 teeth, shorter adrostral process and a long rostral process (Fig. 3F). Neuropodium of chaetiger b1 with upper and lower row of uncini (Fig. 3D), b3–5 only with one lower row of uncini (Fig. 3C). Region C with 16 chaetigers (paratypes: A12034 = 10, A11652 = 19, A11653 = 17). Parapodia biramous. Notopodia long, with distally tapering tip and internal acicular chaetae (Fig. 3G). Neuropodia bilobed; lateral lobe with no cirrus on lateral side; ventral neuropodial lobe without cirrus (Fig. 3G). Lateral lobe uncini of region C arranged in 2–3 rows (Fig. 3H), chaetiger c1 uncini similar to those of chaetiger a10, with 5–6 teeth (Fig. 3I).

## Tube

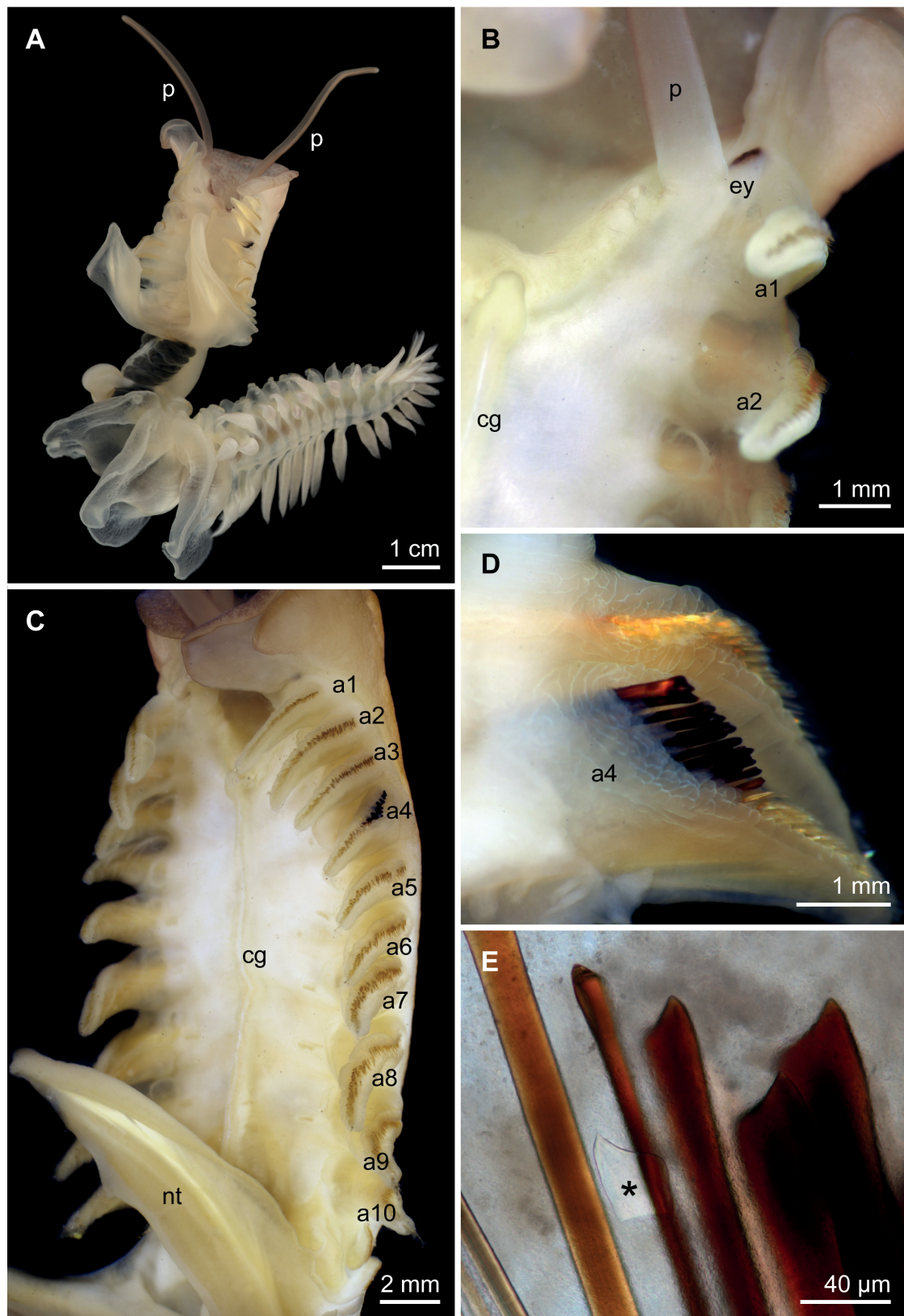
Parchment like, U-shaped tube, sometimes with sand debris and shell fragments on the outer surface (Fig. 1B). Both tube openings almost half in diameter (8 mm) compared to the middle section of the tube (16 mm). Total tube length 293 mm.

## Habitat

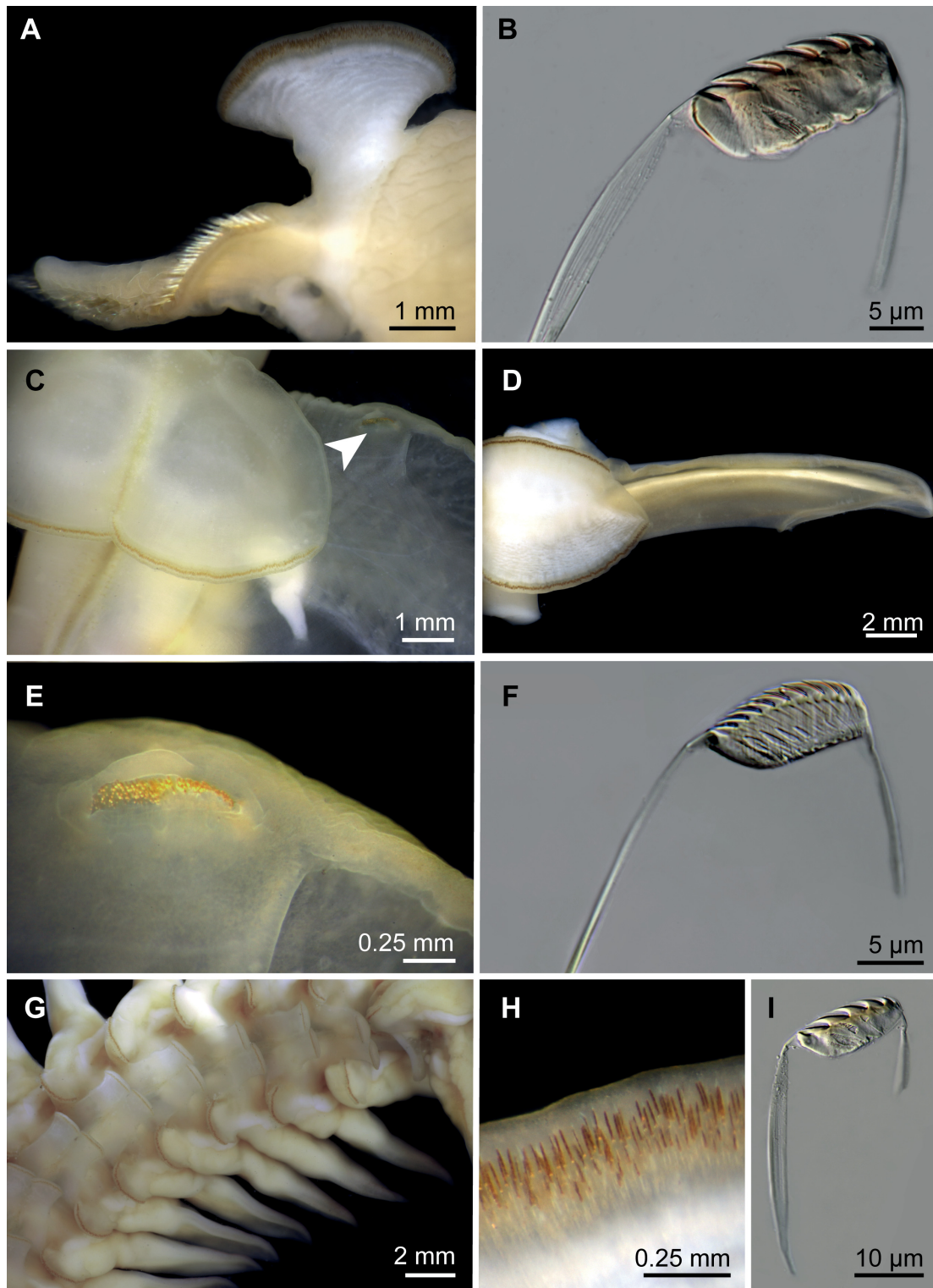
Commonly found partially buried along canyon walls in large assemblages of solitary, intermingled tubes and sediment with other fauna, such as sponges and tunicates.

## Distribution

Southern California. COI sequences confirm the identity of *Chaetopterus* sp. 1 from Santa Barbara (Osborn *et al.* 2007; Moore *et al.* 2017) as *C. dewysee* sp. nov. (Fig. 5)

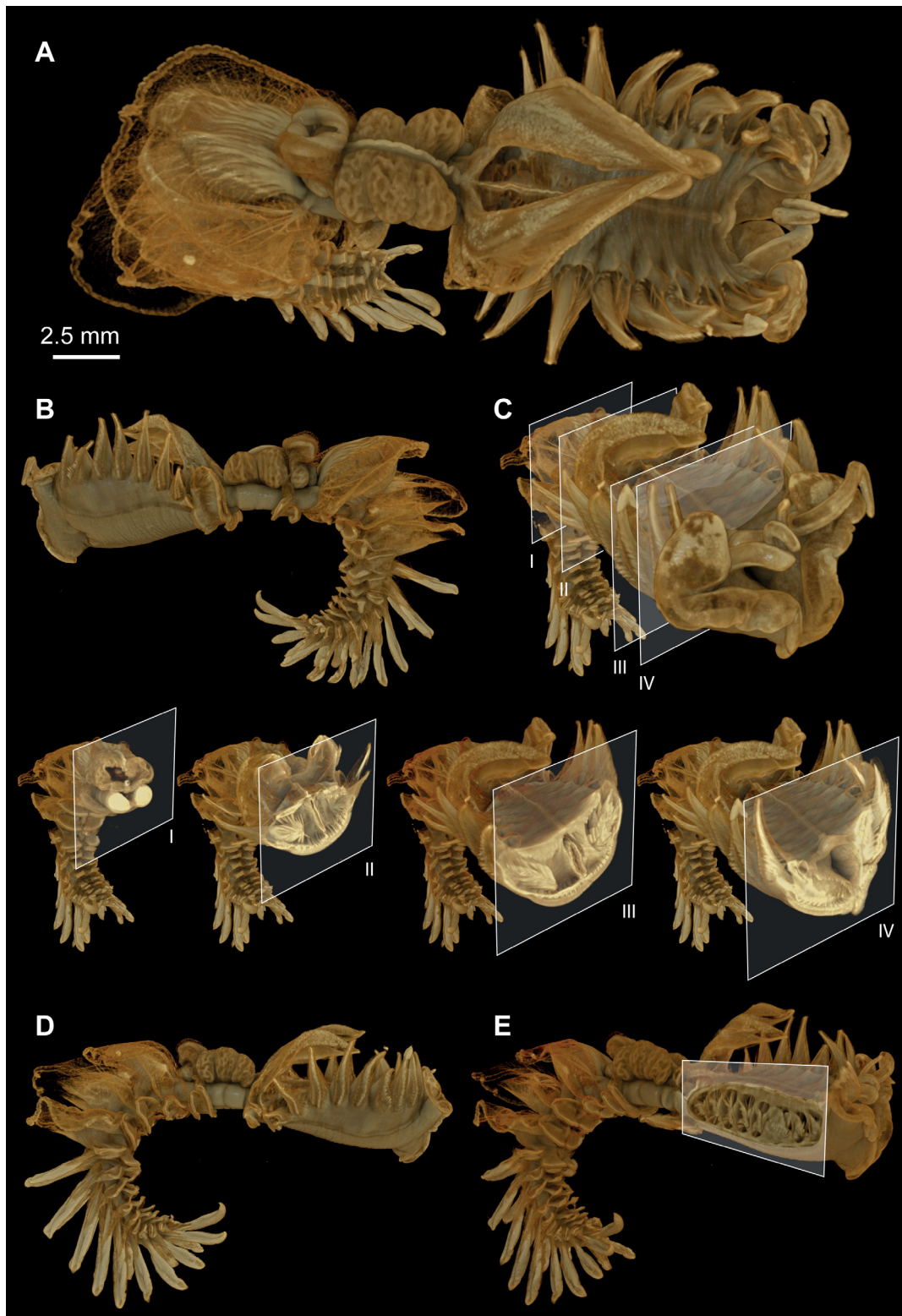


**Fig. 2.** *Chaetopterus dewysee* sp. nov. **A.** Habitus of a paratype (SIO-BIC A11653). **B–C.** Anterior region A, chaetigers are numbered a1–10. **D–E.** Details of chaetiger a4 cutting chaetae; \* marks a developing cutting chaeta. p = palps, ey = eyes, cg = ciliated groove, nt = notopodium.

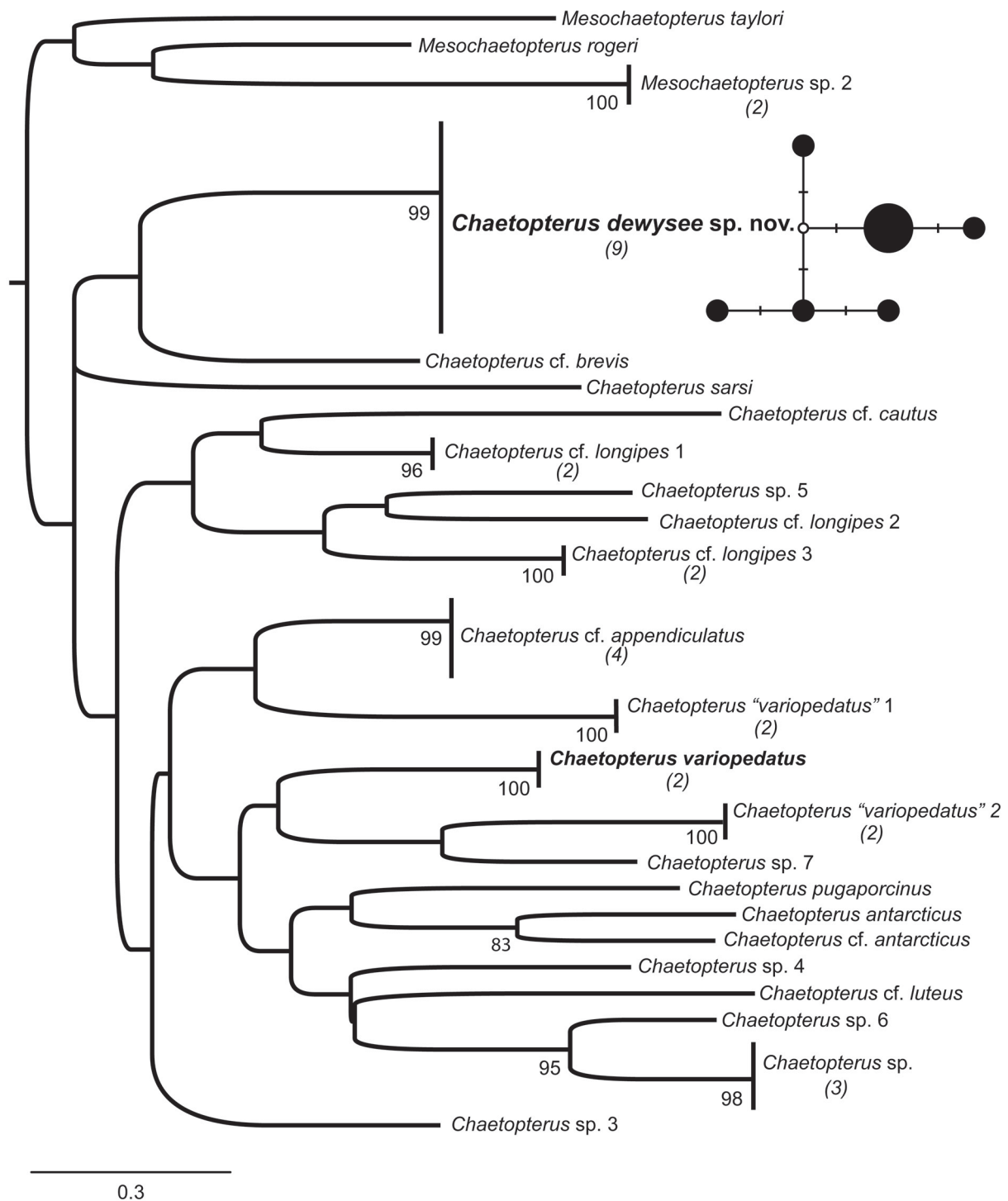


**Fig. 3.** *Chaetopterus dewysee* sp. nov. **A.** Biramous chaetiger a10 parapodium with a long notopodium and a neuropodial lobe. **B.** Neuropodial uncinus of chaetiger a10. **C.** Chaetiger b3 neuropodium with one row of uncini, arrow-head marks the patch of uncini (**E**) at the upper ventral margin of enlarged fan. **D.** Aliform notopodium of chaetiger b1. **F.** Notopodial uncinus of chaetiger b3. **G.** Biramous region C parapodia. **H.** Uncini of region C lateral lobe arranged in 2–3 rows. **I.** Chaetiger c1 uncinus of lateral lobe.





**Fig. 4.** *Chaetopterus dewysee* sp. nov. micro-CT surface renderings of the cybertype (SIO-BIC A12034). **A.** Dorsal view of the whole specimen. **B.** Lateral view of the whole specimen. **C.** Frontal view with virtual dissection planes (I–III) showing cross-sections along the body. **D–E.** Lateral view with a virtual dissection window showing the parapodial musculature of region A. Direct link to cybertype: [www.morphdbase.de/?E\\_Tilic\\_20200122-S-5.1](http://www.morphdbase.de/?E_Tilic_20200122-S-5.1)



**Fig. 5.** Maximum likelihood tree of *Chaetopterus* Cuvier, 1830 spp. COI sequences with the *Mesochaetopterus* Potts, 1914 clade as outgroup, based on Moore *et al.* (2017). Only bootstrap supports > 80 are shown. The new species *Chaetopterus dewysee* sp. nov. and *C. variopedatus* (Renier, 1804) from the type locality are in bold. Number of sequences included for each terminal is in brackets. Details on sequences that were analyzed can be found in Table 1. Haplotype network for the nine *Chaetopterus dewysee* sp. nov. sequences is shown next to the tree.

**Table 1.** List of sequenced *Chaetopterus dewysee* sp. nov. specimens and analyzed COI sequences with GenBank repository, voucher and locality information. Species names are listed as they are shown in Fig. 5. New sequences are in bold. Voucher holding institution abbreviations are: SIO-BIC = Scripps Institution of Oceanography Benthic Invertebrate Collection; FLMNH = Florida Museum of Natural History; SAM = South Australian Museum; LACM = Natural History Museum of Los Angeles County; MNCN = Museo Nacional de Ciencias Naturales.

Terminal	GenBank No.	Voucher ID	Locality
<i>Mesochaetopterus taylori</i>	DQ209251	SAM E3570	Friday Harbor, WA
<i>Mesochaetopterus rogeri</i>	AM503098	MNCN 6.01/10145	Blanes, Spain
<i>Mesochaetopterus</i> sp. 2	KX896518	FLMNH UF 1971	Moorea, FP
<i>Mesochaetopterus</i> sp. 2	KC706790	—	Moorea, FP
<i>Chaetopterus dewysee</i> sp. nov.	DQ209252	SAM E3511	Santa Barbara, CA
<b><i>Chaetopterus dewysee</i> sp. nov.</b>	<b>MN991233</b>	<b>SIO-BIC A10193</b>	<b>San Diego, CA</b>
<b><i>Chaetopterus dewysee</i> sp. nov.</b>	<b>MN991236</b>	<b>SIO-BIC A4193</b>	<b>San Diego, CA</b>
<b><i>Chaetopterus dewysee</i> sp. nov.</b>	<b>MN991231</b>	<b>SIO-BIC A11476</b> <i>holotype</i>	<b>San Diego, CA</b>
<b><i>Chaetopterus dewysee</i> sp. nov.</b>	<b>MN991230</b>	<b>SIO-BIC A11649</b>	<b>San Diego, CA</b>
<b><i>Chaetopterus dewysee</i> sp. nov.</b>	<b>MN991235</b>	<b>SIO-BIC A11652</b>	<b>San Diego, CA</b>
<b><i>Chaetopterus dewysee</i> sp. nov.</b>	<b>MN991234</b>	<b>SIO-BIC A11653</b>	<b>San Diego, CA</b>
<b><i>Chaetopterus dewysee</i> sp. nov.</b>	<b>MN991232</b>	— * <i>larva not vouchered</i>	<b>San Diego, CA</b>
<i>Chaetopterus</i> cf. <i>brevis</i>	DQ209255	—	Banyuls, France
<i>Chaetopterus sarsi</i>	DQ209254	SAM E3557	Trondheimsfjord, NO
<i>Chaetopterus</i> cf. <i>cautus</i>	KX896511	FLMNH UF 4676	Kavieng, PNG
<i>Chaetopterus</i> cf. <i>longipes</i> 1	KX896491	SIO-BIC A2831	Moorea, FP
<i>Chaetopterus</i> cf. <i>longipes</i> 1	KX896515	FLMNH UF 1697	Guam
<i>Chaetopterus</i> sp. 5	KX896510	FLMNH UF 4675	Kavieng, PNG
<i>Chaetopterus</i> cf. <i>longipes</i> 2	KX896502	SIO-BIC A3625	Lizard Island, AUS
<i>Chaetopterus</i> cf. <i>longipes</i> 3	KX896513	FLMNH UF 551	Moorea, FP
<i>Chaetopterus</i> cf. <i>longipes</i> 3	KX896519	FLMNH UF 2652	St. Martin
<i>Chaetopterus</i> cf. <i>appendiculatus</i>	KY124465	—	Vietnam
<i>Chaetopterus</i> cf. <i>appendiculatus</i>	KY124466	—	Vietnam
<i>Chaetopterus</i> cf. <i>appendiculatus</i>	KY124467	—	Vietnam
<i>Chaetopterus</i> cf. <i>appendiculatus</i>	KY124468	—	Vietnam
<i>Chaetopterus</i> “ <i>variopedatus</i> ” 1	MF120993	—	Alaska, USA
<i>Chaetopterus</i> “ <i>variopedatus</i> ” 1	MF121353	—	Alaska, USA
<i>Chaetopterus</i> “ <i>variopedatus</i> ” 2	AM503096	—	Norwich, Norfolk, UK
<i>Chaetopterus</i> “ <i>variopedatus</i> ” 2	DQ087501	—	—
<i>Chaetopterus variopedatus</i>	AM503094	—	Naples, Italy
<i>Chaetopterus variopedatus</i>	KX896523	FLMNH UF 4257	Adriatic Sea
<i>Chaetopterus</i> sp. 7	KX896506	—	Baja California
<i>Chaetopterus pugaporcinus</i>	DQ209257	LACM POLY 2173-2175	Monterey Bay, CA
<i>Chaetopterus antarcticus</i>	KX896494	SIO-BIC A2929	Burdwood Bank
<i>Chaetopterus</i> cf. <i>antarcticus</i>	KX896493	SIO-BIC A2978	Scotia Arc
<i>Chaetopterus</i> sp. 4	KX896509	FLMNH UF 4674	Kavieng, PNG
<i>Chaetopterus</i> cf. <i>luteus</i>	DQ209253	SAM E3510	South Australia
<i>Chaetopterus</i> sp. 6	KX896505	FLMNH UF 4678	Red Sea
<i>Chaetopterus</i> sp.	KY124469	—	Vietnam
<i>Chaetopterus</i> sp.	KY124471	—	Vietnam
<i>Chaetopterus</i> sp.	KY124470	—	Vietnam
<i>Chaetopterus</i> sp. 3	KX896508	FLMNH UF 4673	Kavieng, PNG

### Molecular information

All type specimens of *Chaetopterus dewysee* sp. nov., except for the cybertype, were subsampled and sequenced for COI (Table 1). The specimen chosen for the micro-CT scan was kept intact as a cybertype. All COI sequences for specimens identified as *Chaetopterus dewysee* sp. nov. were >98.8% similar. These included the COI sequence pulled from the available *Chaetopterus* transcriptome, the sequence from Santa Barbara, published by Osborn *et al.* (2007) and the COI sequence from the larva (Fig. 1C). The haplotype network for the nine *C. dewysee* sp. nov. sequences (Fig. 5) shows minor variation amongst the specimens. The most frequent haplotype is shared by four individuals (the sequenced larva, the holotype A11476, A10193 and A11653). In addition to this, there are 5 low-frequency haplotypes, each represented by a single specimen. The haplotypes are separated by one to three mutational steps. On the maximum likelihood tree sequences that were more than 97% similar were given the same terminal name and the branches were collapsed (Fig. 5). Average identity between the COI sequences of different *Chaetopterus* spp. was 78.7% (min. 72.1%, max 85.2%).

### Remarks

*Chaetopterus dewysee* sp. nov. most resembles the two European species, *C. brevis* Lespés, 1872 and *C. variopedatus*. The main difference between the 3 species is in the morphology of a4 cutting chaetae. *Chaetopterus variopedatus* has a4 cutting chaetae with teeth, whereas the cutting chaetae of *C. brevis* are symmetrical and distally inflated. In *C. dewysee* sp. nov. the cutting chaetae have a smooth, asymmetrical tip with a sharp apical point. Furthermore, region C notopodia of *C. dewysee* sp. nov. have no lateral cirrus, which is present in both *C. brevis* and *C. variopedatus*. *Chaetopterus brevis* also differs from the other two species in having a gregarious habit, that can be occupied by multiple individuals. Relationships were not supported across most of the phylogeny generated here using COI (Fig. 5). *Chaetopterus dewysee* sp. nov. is sister group to *Chaetopterus* cf. *brevis* from France, but with low support. In Moore *et al.* (2017), which also used nuclear 18S and 28S data, this relationship was also recovered, but with very strong support.

Other *Chaetopterus* species described from the eastern Pacific (Galapagos Islands) are *C. galapagensis* Nishi, Hickman & Bailey-Brock, 2009, *C. adunctus* Nishi, Hickman & Bailey-Brock, 2009 and *C. charlesdarwinii* Nishi, Hickman & Bailey-Brock, 2009. *Chaetopterus adunctus* has a J-shaped and *C. darwinii* a U-shaped tube, both attached to rocks (Nishi *et al.* 2009), whereas the new species *C. dewysee* sp. nov. has tubes partly buried in sediment. The obvious main difference among the species is the number of region A chaetigers: *C. charlesdarwinii* only has 9, *C. galapagensis* 13–15 and *C. dewysee* sp. nov. has 10. The three species also differ in the number of teeth on the chaetiger c1 lateral lobe uncini; *C. dewysee* sp. nov. only has 5–6 teeth whereas *C. adunctus* has 10–11, *C. charlesdarwinii* has 9–11 and *C. galapagensis* 7–8 teeth (Nishi *et al.* 2009).

### Discussion

The concept of ‘cybertypes’ was introduced by Faulwetter *et al.* (2013) and in the same paper the potential of micro computed tomography as a taxonomic resource was illustrated using examples from different annelid species. Micro-CT scanning has been used for species descriptions in arthropods and there are several cybertypes available for myriapods (Stoev *et al.* 2013; Akkari *et al.* 2015) and ants (Hita Garcia *et al.* 2017). According to the definition of Faulwetter *et al.* (2013), a cybertype needs to: (a) provide morphological and anatomical information of at least the same accuracy as a physical type, that is not linked to a specific research question; (b) a cybertype should be associated with an original type; (c) a cybertype has to be made freely accessible.

Virtual dissections together with contrast enhancing stains significantly improve the resolution of anatomical details of otherwise inconspicuous soft-tissue (Fig. 4C, E) and micro-CT is becoming more

common as a tool to study the internal anatomy of annelids (Paterson *et al.* 2014). The advantages of a non-invasive technique like micro-CT are clear as this technique allows a detailed imaging of historical and valuable museum specimens. Furthermore, these methods are significantly less labor-intensive than traditional histology and allow a more automated workflow that can generate large amounts of morphological data in a shorter period of time. Another great advantage is how the volume data generated shows anatomical structures in their original arrangement and thus enables automated image processing for anatomical 3D reconstructions. Micro-CT might not be able to provide the high cell-level resolution of serial histology, but certain internal details of the myoanatomy (Fig. 4C, E), digestive system (Parapar *et al.* 2017), nervous system (Beckers *et al.* 2019) and hard structures, like jaws, in annelids (Watson & Faulwetter 2017) can be visualized in great detail.

The micro-CT dataset and 3D renderings of *Chaetopterus dewysee* sp. nov. (SIO-BIC A12034) we provide here constitute the first annelid cybertype which is freely available for future research on this species.

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