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**Research article**

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# Two new meiofaunal species of *Trilobodrilus* (Dinophilidae, Annelida) from California, USA

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[3]urn:lsid:zoobank.org:author:6BD1D9C0-2986-4A86-B3AF-33B117D092A0
[4]urn:lsid:zoobank.org:author:F4AAFAE4-85D9-44CA-8290-E0FC614E1983
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**Abstract.** We describe two new species of the annelid genus *Trilobodrilus* Remane, 1925 (Dinophilidae Verill, 1892) from an intertidal and a subtidal location in San Diego, California. These two species show morphological and molecular divergences between each other and the previously described, geographically distant species. Intertidal *T. windansea* sp. nov. differs from subtidal *T. ellenscrippsae* sp. nov. most remarkably in the number and pattern of ciliary tufts and bands on the prostomium and along the body length, besides showing ca. 15% difference in gene fragments of COI and CytB. *Trilobodrilus windansea* sp. nov., though nesting with *T. ellenscrippsae* sp. nov. in the molecular phylogenetic analyses, morphologically resembles the Japanese *T. itoi* Kajihara, Ikoma, Yamasaki & Hiruta, 2015 most closely, but still differs from this species in the higher number of apical ciliary tufts, an additional ciliary row posterior to the second ciliary band, and by lacking a forth ciliary band and segmentally arranged lateral ciliary tufts. *Trilobodrilus ellenscrippsae* sp. nov. is morphologically most similar to the Japanese *T. nipponicus* Uchida & Okuda, 1943, but is much shorter, has more apical ciliary tufts, and less regularly arranged lateral ciliary tufts along the body. All species differ significantly in all compared gene fragments, and no obvious correlation was found between habitat and the species morphology or relationships.

**Keywords.** Interstitial, intertidal zone, meiobenthos, morphology.

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## Introduction

The entirely meiofaunal annelid family Dinophilidae Verill, 1892 consists of 16 accepted species (Westheide 2008; WoRMS Editorial Board 2016) classified into two genera, *Dinophilus* O.Schmidt, 1848 (10 species) and *Trilobodrilus* Remane, 1925 (6 species). The monotypic genus *Apharyngtus* Westheide, 1971 has an uncertain phylogenetic relationship to Dinophilidae (Westheide 2008). All dinophilids are found in interstitial habitats of shallow marine waters in medium to coarse sand or shell gravel. Some species may also be found on biofilm of macroalgae in littoral marine regions (Harmer 1889; Jägersten 1944, 1951; Kerbl *et al.* 2016).

Dinophilidae are direct-developing microscopic annelids, 0.5–1.9 mm long, lacking appendages and chaetae, with a prostomium and ventral mouth, a trunk consisting of six inconspicuous segments with a midventral ciliary tract for gliding locomotion, and various degrees of transverse ciliation for swimming. The prostomium of the species of *Trilobodrilus* lacks eyes and appears swollen in the posterior part followed by a constriction at the border of the first segment, giving it a trilobed appearance compared to the rounded prostomium of *Dinophilus* species (Remane 1925; Westheide 1967, 2008). Compared to *Dinophilus*, *Trilobodrilus* lacks dorsal transverse ciliary bands except for one to two complete or incomplete bands on the first segment (Remane 1925; Uchida & Okuda 1943; Westheide 1967, 2008; Rao 1973; Kajihara *et al.* 2015). Similar to species of *Dinophilus*, species of *Trilobodrilus* are generally gonochoric (Remane 1925; Uchida & Okuda 1943; Westheide 1967; Ax 1968; Rao 1973; Kajihara *et al.* 2015), except for *T. hermaphroditus* Riser, 1999, which is hermaphroditic (Riser 1999). No species of *Trilobodrilus* exhibits sexual dimorphism as known from *D. gyrociliatus* O. Schmidt, 1848 and some other *Dinophilus* species (Schmidt 1858; Repiachoff 1886; Jones & Ferguson 1957; Fofanova *et al.* 2014; Kerbl *et al.* 2016). *Trilobodrilus axi* Westheide, 1967 and *T. heideri* Remane, 1925 have a life-span of approximately 12 months (Westheide 1967, 2008), including a reproductive period of 3–5 months (Remane 1925; Boaden 1963, 1966; Westheide 1967; Ax 1968).

The distribution of the six described species of *Trilobodrilus* shows regional overlap, but with differences in habitat: *Trilobodrilus heideri* Remane, 1925 is found in the German North Sea (Helgoland, Sylt) and in the Roscoff-area, where it co-occurs with *T. axi* Westheide, 1967, though the former is always found subtidally and the latter intertidally (Remane 1925; Westheide 1967, 2008; Ax 1968). *Trilobodrilus heideri* is also reported from the British coast at Anglesey, from Skagerak (Swedish West Coast, Gullmarfjord) and the Mediterranean Sea (Boaden 1963, 1966; Westheide 2008). *Trilobodrilus axi* is known from the coasts of Arcachon, France, and Massachusetts, USA, where this intertidal species co-occurs with the inter- and subtidal species *T. hermaphroditus* (Westheide 1967, 2008; Riser 1999). The intertidal species *T. itoi* Kajihara, Ikoma, Yamasaki & Hiruta, 2015 and *T. nipponicus* Uchida & Okuda, 1943 are both found in Hokkaido, Japan, but in different bays along the coast (Uchida & Okuda 1943; Kajihara *et al.* 2015). *Trilobodrilus nipponicus* was also possibly found in Seattle, USA, representing the single report of the genus from the west coast of the USA (Wieser 1957). *Trilobodrilus indicus* Rao, 1973 was described from the intertidal zone in the Bay of Bengal, India (Rao 1973).

Due to the limited number of obvious external characters, detailed morphological examinations of *Trilobodrilus* are necessary for species identification. Given that all Dinophilidae have six inconspicuous body segments and that all species of *Trilobodrilus* are of comparable size and shape, the number and continuity of ciliary bands, number and arrangement of ciliary tufts, and the shape and size of epidermal

inclusions have been shown to be the most taxonomically informative morphological characters (Westheide 1967, 2008; Kajihara *et al.* 2015).

The here presented study describes two species of *Trilobodrilus* from coastal southern California, based on light and electron microscopic examinations as well as on molecular sequencing and a phylogenetic analysis of the genus.

## Material and methods

### Sampling

Specimens were extracted from sediment samples collected at two different locations in San Diego County, California, USA, during April 2009; intertidally at Windansea Beach, La Jolla from holes dug 0.5 m and 1 m deep at the mid- and high-water marks in coarse sand, and subtidally using scuba diving, sampling the surface of silty shell gravel at 9 m depth off La Jolla Cove, La Jolla. All specimens were extracted by $MgCl_2$ anaesthetization with decantation and sorted using dissecting microscopes. Prior to fixation, all specimens were re-anaesthetized in a 1:1 solution of seawater and isotonic $MgCl_2$ and fixed either in paraformaldehyde (PFA, 3.7% in phosphate buffered saline (PBS)) or trialdehyde (3.7% paraformaldehyde/ 4% glutaraldehyde in 0.1 M sodium cacodylate buffer) overnight at 4°C. Subsequently, specimens fixed in paraformaldehyde were rinsed several times in PBS and stored with 0.05% $NaN_3$, while samples fixed in trialdehyde were transferred to 0.1 M sodium cacodylate buffer. Specimens for molecular analyses were stored directly in 99% ethanol.

### Light microscopy (LM)

Live animals were observed, photographed, and video recorded using a UI-1540 camera mounted on a Leica DMR compound microscope (with DIC). Eleven fixed specimens of *T. windansea* sp. nov. and four fixed specimens of *T. ellenscrippsae* sp. nov. were mounted individually in dorso-ventral orientation (whenever possible), and investigated using an Olympus IX 70 inverted compound microscope. Images were obtained using a mounted Olympus DP73 camera in combination with the CellSens Entry software package v. 1.6. Morphological measurements (Table 1) were conducted using either the previously mentioned software package or by using ImageJ v. 1.47 (Schneider *et al.* 2012). Two specimens were afterwards transferred to EtOH for preservation (*T. windansea* sp. nov. paratypes: SIO-BIC A8208, NHMD-210468), but the remaining specimens were unfortunately lost in the process and are not preserved.

### Scanning electron microscopy (SEM)

Three specimens of *T. windansea* sp. nov. and one specimen of *T. ellenscrippsae* sp. nov., which were fixed in trialdehyde and stored in 0.1 M sodium cacodylate buffer, were postfixed in an aqueous 2% $OsO_4$ solution for 1 h at room temperature (RT) and subsequently rinsed in demineralized water. Following dehydration via an ascending ethanol series and transferring to acetone, samples were critical-point dried (using an Autosamdri 815-machine at the Natural History Museum of Denmark, University of Copenhagen) and mounted on aluminum stubs. A high-resolution sputter coater (JFC-2300HR) applied an approximately 80–90 nm layer of platinum/palladium-mixture onto the samples prior to examination using a JEOL JSM-6335F field emission scanning electron microscope at the Natural History Museum of Denmark, University of Copenhagen.

Morphological measurements (Table 1) from SEM were scaled and measured using ImageJ v. 1.47 (Schneider *et al.* 2012).

**Table 1**. Measurements and morphological characters of holotype, as well as minimum, maximum and average of all measured *T. windansea* sp. nov. and *T. ellenscrippsae* sp. nov. The number of measured specimens is added to the table, all measurements are either given as µm (length or width), as distances (measured in µm from the anterior tip), or as relative measurements; "n/a" is used for measurements which are not applicable, since epidermal inclusions could not be detected in fixed material of *T. ellenscrippsae* sp. nov.

| **Species** | | ***Trilobodrilus windansea* sp. nov.** | | | | | ***Trilobodrilus ellenscrippsae* sp. nov.** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Type | | Holotype | Min. | Max. | Aver. | n | Holotype | Min. | Max. | Aver. | n |
| Length [µm] | | 715 | 499 | 1040 | 739 | 12 | 485 | 306 | 774 | 537 | 5 |
| Max. width [µm] | | 86 | 84 | 189 | 132 | 12 | 122 | 91 | 229 | 154 | 5 |
| Relative width/ length | | 0.12 | 0.12 | 0.31 | 0.18 | 12 | 0.25 | 0.23 | 0.39 | 0.29 | 5 |
| Position of neuropil [µm from anterior tip] | anterior | – | 26 | 41 | 32 | 9 | – | 18 | 24 | 20 | 4 |
| | middle | – | 36 | 55 | 44 | 9 | – | 28 | 36 | 30 | 4 |
| | posterior | – | 45 | 70 | 57 | 9 | – | 37 | 48 | 40 | 4 |
| Position of mouth [µm from anterior tip] | anterior | 102 | 64 | 109 | 90 | 9 | 59 | 59 | 88 | 70 | 5 |
| | middle | 110 | 77 | 133 | 105 | 9 | 64 | 64 | 92 | 81 | 5 |
| | posterior | 121 | 89 | 149 | 120 | 9 | 69 | 69 | 105 | 85 | 5 |
| Max. prostomial width [µm] | | 76 | 63 | 95 | 73 | 12 | 77 | 72 | 103 | 89 | 5 |
| Prostomial length [µm] | | 76 | 64 | 97 | 82 | 12 | 66 | 59 | 80 | 69 | 5 |
| Relative prostomial width/ body length | | 0.11 | 0.09 | 0.14 | 0.09 | 12 | 0.14 | 0.09 | 0.19 | 0.14 | 5 |
| Length of epidermal inclusion [µm] | | – | 6 | 12 | 9 | 12 | n/a | n/a | n/a | n/a | n/a |
| Width of epidermal inclusion [µm] | | – | 3 | 6 | 4 | 12 | n/a | n/a | n/a | n/a | n/a |

All holotypes and several paratype specimens are deposited at the Scripps Institution of Oceanography, San Diego, California, USA (SIO-BIC A8206–SIO-BIC A8209). One paratype of *T. windansea* sp. nov. (NHMD-210468) is deposited at the Natural History Museum in Copenhagen, Denmark.

**DNA extraction, amplification, and sequencing**

DNA extractions were performed using a Qiagen DNeasy Tissue and Blood kit (Qiagen, Düsseldorf, Germany) following the manufacturer's protocol. DNA elution was in 80–90 µL of buffer, and was repeated with the original buffer volume to maximize DNA yield. Genes used to reconstruct the phylogeny included 18S rRNA, 28S rRNA, cytochrome c oxidase subunit I (COI), and cytochrome B (CytB). Specific primer details, including references, are listed in Table 2. All PCR specifications, including visualization, purification, and sequencing follow that of Martínez *et al.* (2015) and Gonzalez *et al.* (2017).

Chromatogram reading and contig assembly were performed in Sequencher 4.10.1 (GeneCodes Corporation, Ann Arbor, MI, USA). All chromatograms were blasted to check for contamination using

**Table 2**. List of primers used for amplification and sequencing, with original references.

| Gene | Primer | React. | Primer sequence (in 5'-3'-direction) | Dir. | Source |
|---|---|---|---|---|---|
| 18S rRNA | 18S-1F | A/S | ACCGWTTCGGCTTTTGTTGG | F | Giribet *et al.* (1996) |
| | 18S-5R | A/S | CTTGGCAAATGCTTTCGC | R | Giribet *et al.* (1996) |
| | G51 | | GGTTGATCCTGCCAGTAG | F | Hillis & Dixon (1991) |
| | G747 | | CGGTATCTGATCGTCTTCGA | R | Ibrahim *et al.* (2011) |
| | G952 | | GCGAAAGCATTTGCCAAGMA | F | Cohen *et al.* (2003) |
| | G944 | | TGATCCTTCTGCAGGTTCACCTAC | R | Lovejoy & Potvin (2011) |
| 28S rRNA (D1) | G758 | A/S | ACCCSCTGAAYTTAAGCAT | F | Brown *et al.* (1999) |
| | G1275 | A/S | TCGGAAGGAACCAGCTACTA | R | Markmann (2000) |
| CO1 | LCO1490 | A/S | GGTCAACAAATCATAAAGATATTGG | F | Folmer *et al.* (1994) |
| | HCO2198 | A/S | TAAACTTCAGGGTGACCAAAAAATCA | R | Folmer *et al.* (1994) |
| | dgLCO1490 | | GGTCAACAAATCATAAAGAYATYGG | F | Meyer (2003) |
| | dgHCO2198 | | TAAACTTCAGGGTGACCAAARAAYCA | R | Meyer (2003) |
| CytB | Cytb-424F | A/S | TGAGGYGCYACKGTTATTACTAA | F | Nickisch-Rosenegk *et al.* (2001) |
| | Cytb-876R | A/S | GCRTAWGCRAAWARRAARTAYCAYTCWGG | R | Nickisch-Rosenegk *et al.* (2001) |

**Table 3.** List of species and accession numbers included in the phylogenetic analysis. Sequences from *T. axi* Westheide, 1967 and *T. heideri* Remane, 1925, were taken from Struck *et al.* (2002), all sequences from *T. itoi* Kajihara, Ikoma, Yamasaki & Hiruta, 2015 and *T. nipponicus* Uchida & Okuda, 1943 were taken from Kajihara *et al.* (2015), and new sequences for *T. windansea* sp. nov. and *T. ellenscrippsae* sp. nov. were generated in this study.

| | Markers | | | | |
|---|---|---|---|---|---|
| **Species** | **18S** | **28S** | **COI** | **Cytb** | **Source** |
| *T. axi* | AF412806 | AY894292 | AY598742 | – | Struck *et al.* (2002) |
| *T. heideri* | AF412807 | AY732231 | AY598743 | – | Struck *et al.* (2002) |
| *T. itoi* | AB924372 | AB924373 | AB924371 | – | Kajihara *et al.* (2015) |
| *T. nipponicus* | LC009446 | LC009447 | LC009445 | – | Kajihara *et al.* (2015) |
| *T. windansea* sp. nov. | MG588089 | MG588091 | MG588093 | MG588095 | This study |
| *T. ellenscrippsae* sp. nov. | MG588090 | MG588092 | MG588094 | MG588096 | This study |
| *Dinophilus* sp. | FJ200245 | FJ200246 | – | – | Worsaae & Rouse (2008) |

NCBI BLAST. Sequences were visualized and trimmed with BioEdit (Hall 1999). All newly generated sequences were deposited in GenBank® with the accession numbers MG588089–MG588096 (Table 3).

Sequences of 18S rRNA, 28S rRNA, and COI were aligned using the MAFFT online platform (Katoh & Standley 2013), with sequences of 18S rRNA and 28S rRNA being aligned using the interactive refinement algorithm L-insi with the parameter 'nwildcard' selected (Katoh *et al.* 2005), while COI was aligned using the 'Auto' strategy. All genes were concatenated using Sequence Matrix (Vaidya

*et al.* 2011). Sequences of CytB (only available from the two here described *Trilobodrilus* species) were aligned with Clustal W in BioEdit.

### Phylogenetic analyses

Molecular datasets were analysed with maximum likelihood (ML) and Bayesian methods (BA). Phylogenetic analyses were run on the CIPRES Science Gateway (Miller *et al.* 2010), and specific phylogenetic parameter settings for ML and BA followed that of Gonzalez *et al.* (2017). jModelTest (Posada 2008) was used to infer optimal evolutionary models on alignments of individual genes estimated by the corrected Akaike information criterion (AICc) prior to BA. Both 18S rRNA and COI were analysed under a GTR model with gamma distribution and a proportion of invariable sites (GTR + I + Γ), while 28S rRNA was analysed under a GTR model with gamma distribution (GTR + Γ).

Pairwise similarities of individual genes were obtained using MEGA-7 (Kumar *et al.* 2016) and are listed in Tables 4–6.

## Results

Phylum Annelida Lamarck, 1809
Family Dinophilidae Verrill, 1892
Genus *Trilobodrilus* Remane, 1925

***Trilobodrilus windansea*** sp. nov.
urn:lsid:zoobank.org:act:A9DB66C3-C548-45C1-98F6-6EFBA019CF40
Fig. 1, Tables 1, 3–7

### Diagnosis

*Trilobodrilus* with two pairs of long prostomial compound cilia (1.5–2 times longer than locomotory cilia), encircled by five pairs of intermediate ciliary tufts anterior to the prostomial ciliary bands. First ciliary band with small dorsal gap. Dorsally incomplete second ciliary band with mid-dorsal ciliary tuft and additional posterior ciliary row. Dorsally incomplete third ciliary band posterior to nuchal organs, elongated epidermal inclusions, no spindle glands.

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**Fig. 1** (next page). *Trilobodrilus windansea* sp. nov. A, D, G, H. Light micrographs; B, D-F. SEM micrographs of several adult specimens. **A**. Adult live specimen in dorsoventral view. **B**. Adult specimen in lateral view. **C**. Adult specimen in ventral view. **D**. Detail of the prostomium of a live specimen in dorsoventral view. **E**. Detail of prostomium and anterior body region in lateral view, with the insert showing the additional ciliary row posterior to the second ciliary band. **F**. Detail of the prostomium in dorsal view. **G**. Detail of the posterior body region of an adult live female with two eggs. **H**. Detail of the dorsal epidermis of an adult live specimen with epidermal inclusions, one of them enlarged in insert. Abbreviations: acf = anterior ciliary field; acr, additional ciliary row; act = apical ciliary tuft; cb1–3 = ciliary bands 1–3; egg = eggs; ei = epidermal inclusion; ict = intermediate ciliary tuft; mdt = mid-dorsal ciliary tuft of the second ciliary band; mo = mouth opening; no = nuchal organ; np = neuropil; pcc = prostomial compound cilia; phb = pharyngeal bulb; pyg = pygidium; sto = stomach; vct = ventral ciliary tract. All images oriented with the anterior tip to the left. All scale bars = 50 µm, if not denoted otherwise.

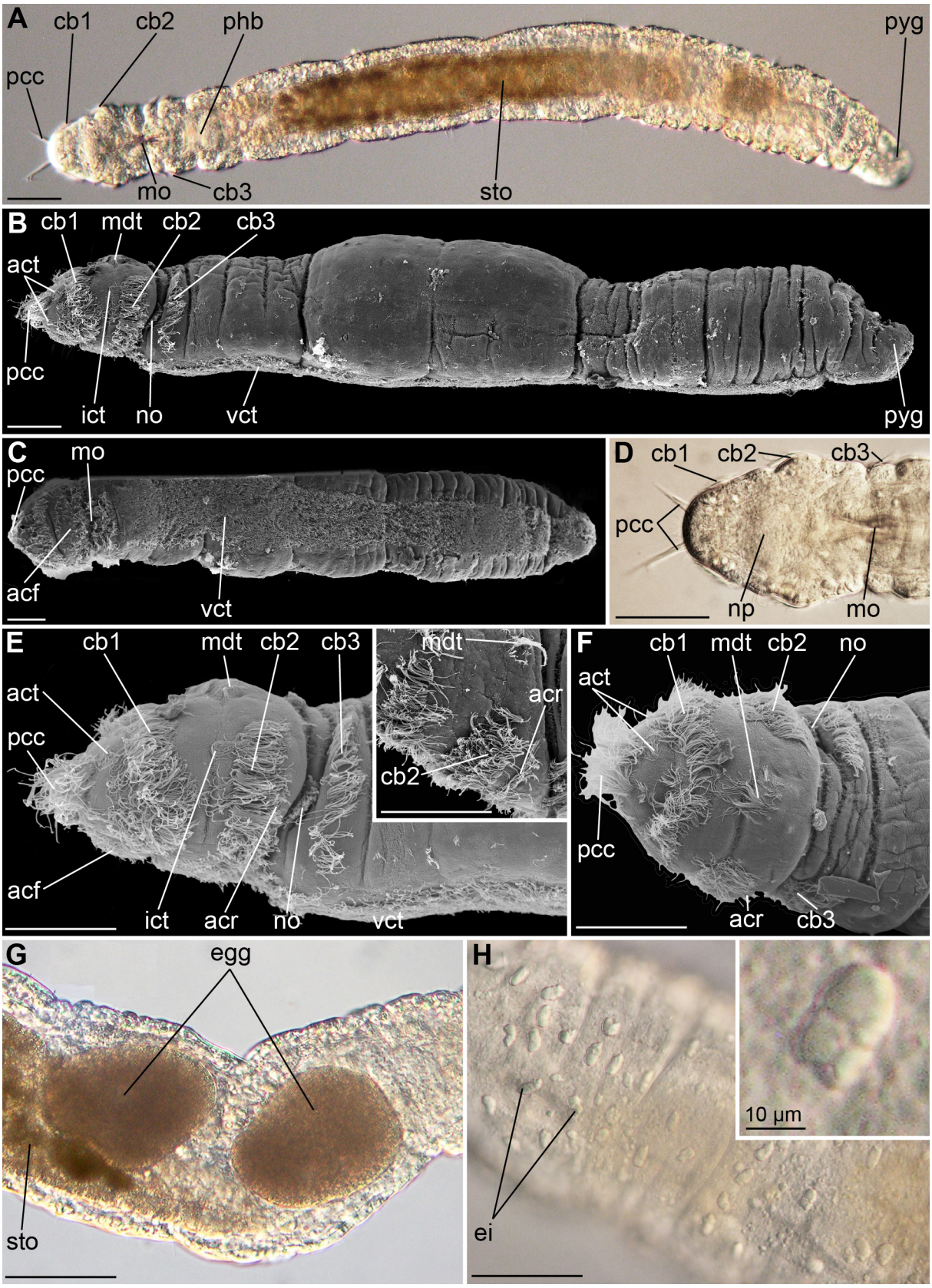
A
cb1
cb2
phb
pyg
pcc
mo
cb3
sto
B
cb1
mdt
cb2
cb3
act
pcc
ict
no
vct
pyg
C
mo
pcc
acf
vct
D
cb1
cb2
cb3
pcc
np
mo
E
cb1
mdt
cb2
cb3
act
pcc
acf
ict
acr
no
vct
mdt
acr
cb2
F
cb1
mdt
cb2
no
act
pcc
acr
cb3
G
egg
sto
H
ei
10 µm

**Table 4.** Comparison of similarities between 18S gene fragments in species of *Trilobodrilus* Remane, 1925. For accession numbers of the used sequences see Table 3.

| | *T. windansea* sp. nov. | *T. ellenscrippsae* sp. nov. | *T. itoi* | *T. nipponicus* | *T. axi* |
|---|---|---|---|---|---|
| *T. windansea* sp. nov. | | | | | |
| *T. ellenscrippsae* sp. nov. | 0.999 | | | | |
| *T. itoi* | 0.991 | 0.991 | | | |
| *T. nipponicus* | 0.990 | 0.991 | 0.993 | | |
| *T. axi* | 0.952 | 0.953 | 0.948 | 0.950 | |
| *T. heideri* | 0.955 | 0.955 | 0.951 | 0.953 | 0.982 |

**Table 5.** Comparison of similarities between 28S gene fragments in species of *Trilobodrilus* Remane, 1925. For accession numbers of the used sequences see Table 3.

| | *T. windansea* sp. nov. | *T. ellenscrippsae* sp. nov. | *T. itoi* | *T. nipponicus* | *T. axi* |
|---|---|---|---|---|---|
| *T. windansea* sp. nov. | | | | | |
| *T. ellenscrippsae* sp. nov. | 0.996 | | | | |
| *T. itoi* | 0.936 | 0.937 | | | |
| *T. nipponicus* | 0.944 | 0.943 | 0.969 | | |
| *T. axi* | 0.850 | 0.850 | 0.855 | 0.858 | |
| *T. heideri* | 0.859 | 0.860 | 0.862 | 0.863 | 0.901 |

**Table 6.** Comparison of similarities between COI gene fragments in species of *Trilobodrilus* Remane, 1925. For accession numbers of the used sequences see Table 3.

| | *T. windansea* sp. nov. | *T. ellenscrippsae* sp. nov. | *T. itoi* |
|---|---|---|---|
| *T. windansea* sp. nov. | | | |
| *T. ellenscrippsae* sp. nov. | 0.849 | | |
| *T. itoi* | 0.786 | 0.760 | |
| *T. nipponicus* | 0.769 | 0.760 | 0.776 |

**Table 7** (next page). Morphological characters and their states in all described species of *Trilobodrilus* Remane, 1925. Measurements are given in mm for body length and width, and in µm for the size of epidermal inclusions; the latter are taken from Kajihara *et al.* (2015), and from the original sources (Rao 1973; Remane 1925; Riser 1999; Uchida & Okuda 1943; Westheide 1967, 2008). Abbreviations: G = gonochoristic; H = hermaphroditic.

| Species | *T. windansea* sp. nov. | *T. ellenscrippsae* sp. nov. | *T. heideri* Remane, 1925 | *T. axi* Westheide, 1967 | *T. hermaphroditus* Riser, 1999 | *T. indicus* Rao, 1973 | *T. nipponicus* Uchida & Okuda, 1943 | *T. itoi* Kajihara, Ikoma, Yamasaki & Hiruta, 2015 |
|---|---|---|---|---|---|---|---|---|
| Body length [mm] | 0.5–1.0 | 0.5–0.7 | 1.5–1.9 | 1 | 1.7 | 1.0–1.2 | 0.7–1.4 | 0.9–1.3 |
| Body width [mm] | 0.08–0.2 | 0.09–0.2 | 0.1–0.2 | 0.1 | 0.22 | 0.1–0.12 | 0.09–0.16 | 0.08–0.12 |
| Prostomial shape | Squared | Conical | Squared | Conical | Conical | Conical | Conical | Squared |
| Pairs of apical ciliary tufts | 5 | 4 | ? | ? | ? | ? | 2–3 | 3–4 |
| Mid-dorsal tufts on 2nd ciliary band (#) | 1 | 1 | 0 | 0 | 0 (but 2 stereocilia) | 0 | 1 | 1 |
| Interm. ciliary tufts (#) | present (1) | present (>2) | ? (absent) | ? (present) | ? | ? | present (1) | present (>2) |
| Add. ciliary row(s) posterior of 2nd ciliary band | present | absent | present | present | absent | ? | absent | absent |
| 3rd ciliary band dorsally continuous | absent | present | absent | absent | present (but not meeting ventral ciliary tract) | present | present | absent |
| 4th ciliary band | absent | present | absent | present | ? | ? | present | present |
| Segmentally arranged lateral ciliary tufts | absent | present (not as regular as in other species) | ? | ? | present | ? (absent) | present | present |
| Epidermal inclusions [µm × µm] | 4 × 9 | ? | ? | 10 × 15 | 6 × 9 | 3 × 12 | 5.5 × 16 | 7 × 9 |
| Spherules/ envelope (#) | 4–8 | ? | 3–10 | 5–19 | 3–7 | 7–11 | 9–13 | 3–22 |
| Length/ width of epidermal inclusions (mean) | 2.2 | ? | 1.41 | 1.81 | 1.42 | 2.42 | 2.91 | 1.29 |
| Spindle glands | absent | ? | present | present | present | present | absent | absent |
| Sex | G | G | G | G | H | G | G | G |
| Habitat | intertidal | subtidal | subtidal | intertidal | intertidal/ subtidal | intertidal | intertidal | intertidal |
| Type locality | Windansea Beach, La Jolla, California, USA | La Jolla Cove, La Jolla, California, USA 9 m depth | Helgo-land, Germany | Sylt, Germany | Nahant Bay, near middle of shor of Nahant, Massachusetts, USA 6 m depth | Palm Beach, Andhra Pradesh, India | Akkeshi Bay, Japan | Ishikari Beach, Japan |
| Source | This study | This study | Remane (1925) | Westheide (1967) | Riser (1999) | Rao (1973) | Uchida & Okuda (1943) | Kajihara *et al.* (2015) |

**Etymology**

This species is named after the beach where it was collected, Windansea Beach.

**Material examined**

**Holotype**

UNITED STATES OF AMERICA: complete adult, 805 µm long (platinum-palladinium-coated and mounted on stub for SEM), Windansea Beach, La Jolla, San Diego, California, 32°49′46″ N, 117°16′51″ W, coarse sand in holes at 0.5 m and 1 m at the mid- and high-water mark, respectively, 14 Apr. 2009, K Worsaae and G Rouse leg. (SIO-BIC A8206).

**Paratypes**

UNITED STATES OF AMERICA: 4 specimens (2 mounted on same stub as holotype for SEM, 2 in 70% EtOH), same locality and sampling site as the holotype, 14 and 17 April 2009 (SIO-BIC A8207, SIO-BIC A8208, NHMD-210468). Additional specimens mounted on slides were unfortunately lost after conducting the measurements.

**Description**

Measurements given from holotype, ranges given in parenthesis include paratypes and lost specimens.

Transparent body, light brown to dark brown tint (live and fixed specimens) (Fig. 1A). Body length 715 µm (499–1040 µm, n = 12), width 86 µm (84–189 µm, n = 12), body segments indistinct (Fig. 1A–C, Tables 1, 7).

Prostomial shape square (Fig. 1A–F, Table 7). Eyes lacking. Middle of mouth located 110 µm (77–133 µm, n = 9) posterior of the apical tip (mo, Fig. 1A, C–D, Table 1). Four compound cilia terminally on prostomium (pcc, Fig. 1B, E–F), each consisting of approximately 50–60 cilia (n = 3), spaced 32 µm apart (18–32 µm, n = 10). Compound cilia substantially (up to two times) longer than locomotory cilia in ciliary bands (19–43 µm relative to 8–20 µm in ciliary bands, n = 10, Fig. 1A, D, Table 1). Prostomial compound cilia surrounded by five pairs of semicircularly arranged apical ciliary tufts (act, Fig. 1B, E–F, Tables 1, 7). Two ciliary bands on prostomium and one ciliary band posterior to the nuchal organs (cb1–3, Fig. 1A–F, Table 7). First ciliary band (12–19 µm wide, n = 3) encircles prostomium with 7–8 µm wide dorsal gap (cb1, Fig. 1A–B, E–F, Table 7). One pair of intermediate ciliary tufts located laterally between first and second ciliary band (ict, Fig. 1B, E). Second ciliary band (13–19 µm wide) dorsally incomplete with 43–46 µm wide gap and one mid-dorsal ciliary tuft in center of gap (mdt, Fig. 1B, E–F, Table 7; consisting of 50–70 cilia, n = 3). One additional thin row of cilia (5–7 µm wide, n = 2) lines the second ciliary band postero-laterally, not extending dorsally for the entire length of the second ciliary band (acr, Fig. 1E–F, Table 7).

One lateral pair of nuchal organs located between the second and third ciliary band (no, Fig. 1B, E–F). Third ciliary band dorsally incomplete with wide gap (cb3, Fig. 1B, E–F, Table 7); width of third ciliary band 10–17 µm, width of gap 39–50 µm (n = 3). Individual cilia scattered across the body (Fig. 1B, E). The ventral ciliary tract extends from posterior prostomium to posterior pygidium (vct, Fig. 1B–C, E; width of tract relative to total body width approximately 0.6 (n = 3)). Anus opening dorso-anteriorly on pygidium.

Eggs 126–143 µm long, located 534–464 µm posterior to the prostomial tip and 209–194 µm (n = 2) anterior to pygidial tip (Fig. 1G).

Epidermal inclusions in the epidermis (Fig. 1H, Table 7); average measurement of envelope 4 × 9 µm, 4–8 spherules per envelope (n = 12); no spindle glands.

**Molecular information**

The following sequences were determined by standard sequencing from a single, non-type specimen collected on 14 Apr 2009, for which no morphological voucher remains: 18S rDNA, MG588089 (1857 nucleotides (nt), Table 4); 28S rDNA, MG588091 (1126 nt, Table 5); COI, MG588093 (644 nt, Table 6); CytB, MG588095 (421 nt). In the following, the sequences of *T. windansea* sp. nov. are first compared to the most similar sequences found in *T. ellenscrippsae* sp. nov., and subsequently the range of similarities to the respective species is listed.

*Trilobodrilus windansea* sp. nov. 18S rDNA fragment is 99.9% similar to the 18S rDNA of *T. ellenscrippsae* sp. nov., and 99.1% (*T. nipponicus*) – 99.5% (*T. axi*) similar to the 18S rDNA fragments of the other sequenced species (Table 4). Its 28S rDNA fragment is 99.9% similar to the 28S rDNA of *T. ellenscrippsae* sp. nov., and 98.6% (*T. axi*) – 99.4% (*T. nipponicus*) similar to the 28S rDNA fragments of the other sequenced species (Table 5). COI is 84.9% similar to the COI of *T. ellenscrippsae* sp. nov., and 76.9% (*T. itoi*) – 78.6% (*T. nipponicus*) similar to the sequences of the other species (Table 6). Cytochrome B is 85.6% similar to *T. ellenscrippsae* sp. nov.

In both the maximum-likelihood as well as the Bayesian analyses, *T. windansea* sp. nov. was found to be the sister species of *T. ellenscrippsae* sp. nov. with full support, the two of them forming the sister group to the *T. itoi* – *T. nipponicus* clade (Fig. 2). These Pacific species were shown to be the sister clade

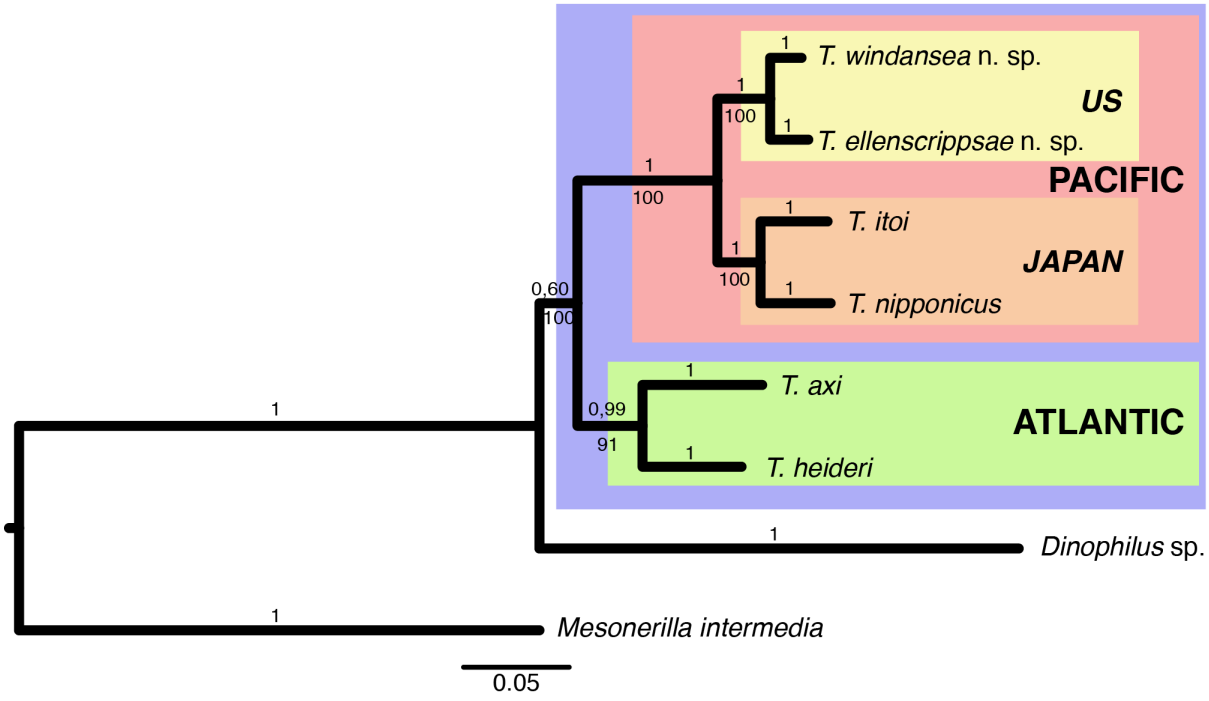


**Fig. 2.** Phylogenetic tree resulting from Bayesian analysis based on combined COI, 18S, and 28S rRNA gene sequences of already known and sequenced species and the newly described species of *Trilobodrilus* Remane, 1925. Numbers above nodes indicate maximum-likelihood bootstrap support values in percent ( > 60%), and numbers below nodes indicate posterior probabilities from Bayesian inference ( > 0.90), respectively. The yellow box labels the two species from the West Coast of the USA, the orange box the species from Japan, which are collectively labelled by the red box as Pacific species. The green box labels species recorded from the Atlantic, and all these species form the clade *Trilobodrilus* (violet box).

to the group formed by the Atlantic species *T. axi* and *T. heideri* (Fig. 2). All *Trilobodrilus*-species form a sister-clade to *Dinophilus* sp., a representative of the second dinophilid genus (Fig. 2).

### Habitat

Intertidal zone of a clean, coarse, well-sorted sandy beach, 0.5–1 m below the mid- and high water mark.

### Distribution

*Trilobodrilus windansea* sp. nov. is only known from Windansea Beach, La Jolla, San Diego, California.

### Remarks

*Trilobodrilus windansea* sp. nov. most closely resembles *T. itoi* in morphology (but *T. ellenscrippsae* sp. nov. in its molecular profile), but still differs by having more apical ciliary tufts, by having an additional row of cilia posterior to the second ciliary band, and by lacking a fourth ciliary band as well as segmentally arranged ciliary tufts along the body (Fig. 1, Table 7). Furthermore, the epidermal inclusions are more elongated and include fewer spherules than in *T. itoi* (Table 7). *Trilobodrilus windansea* sp. nov. resembles the other Californian species, *T. ellenscrippsae* sp. nov., in having a dorsally incomplete second ciliary band with mid-dorsal tuft, but differs in having longer prostomial compound cilia, one more pair of apical ciliary tufts, a small dorsal gap in the first ciliary band, an additional row of cilia posterior to the second ciliary band, a dorsally incomplete third ciliary band, and by lacking a fourth ciliary band (Fig. 1, Table 7).

*Trilobodrilus windansea* sp. nov. further differs from *T. ellenscrippsae* sp. nov., *T. itoi*, *T. axi* and *T. heideri* in comparison of gene fragments, see Molecular information above or Tables 4–6.

## ***Trilobodrilus ellenscrippsae*** sp. nov.

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Fig. 3, Tables 1, 3–7

### Diagnosis

Short *Trilobodrilus* with two pairs of prostomial compound cilia encircled by four pairs of intermediate ciliary tufts anterior to first ciliary band. Additional ciliary tufts between first and second ciliary band. Dorsally continuous third ciliary band. Incomplete fourth ciliary band, posterior of third ciliary band with broad dorsal gap.

### Etymology

This species is named (as a noun in the genitive case) to honour Ellen Browning Scripps (1836–1932), a founding benefactor of the Scripps Institution of Oceanography (McClain 2017) in La Jolla, California.

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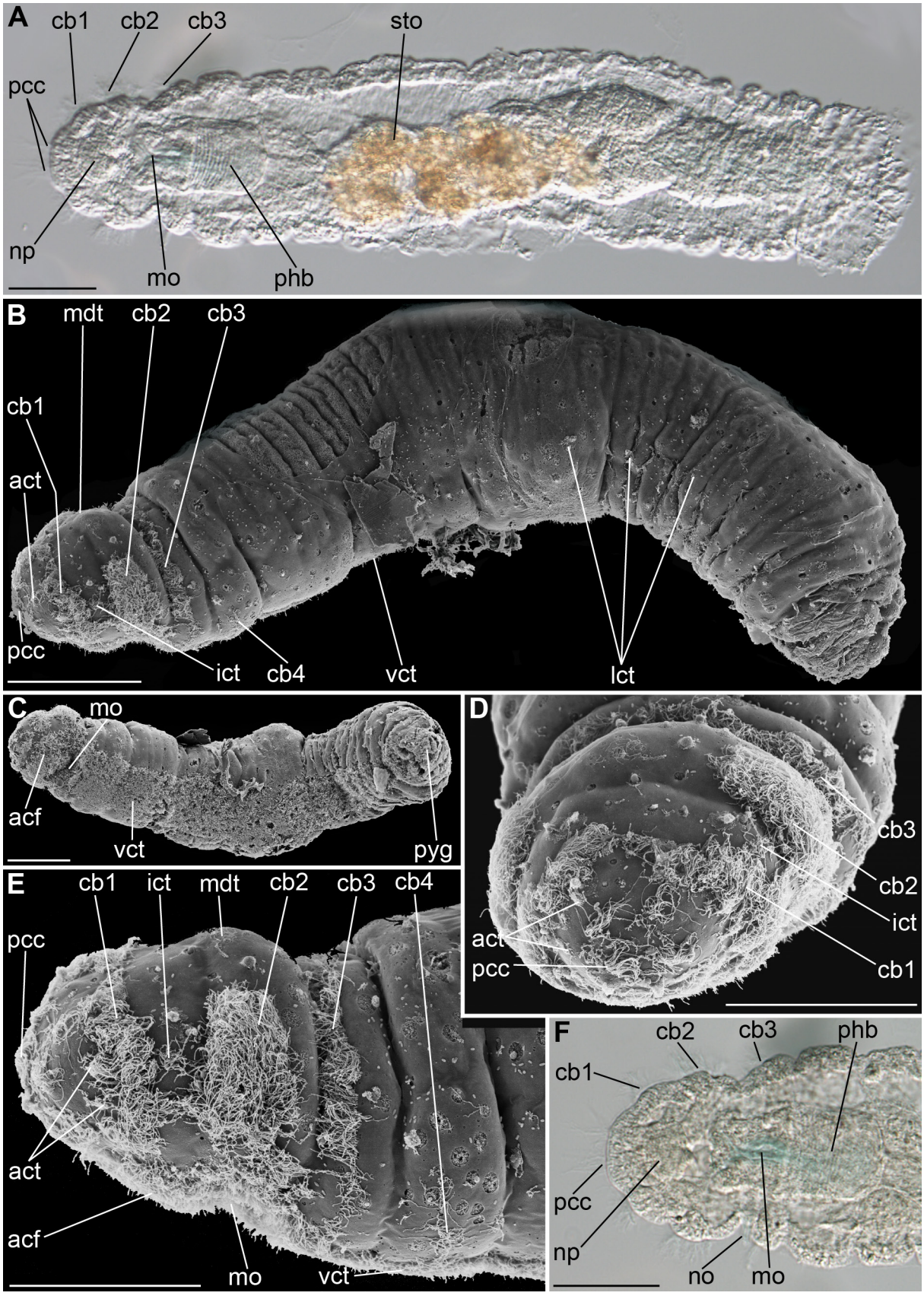
**Fig. 3** (next page). *Trilobodrilus ellenscrippsae* sp. nov. A, F. Light micrographs; B-E. SEM micrographs of several adult specimens. **A**. Adult fixed specimen in dorsoventral view, pygidium missing. **B**. Adult specimen in lateral view. **C**. Adult specimen in ventral view. **D**. Apical view of the prostomium of an adult specimen. **E**. Detail of the prostomium and anterior body region of and adult specimen in lateral view. **F**. Detail of the prostomium of a fixed adult specimen in dorsoventral view. Abbreviations: acf = anterior ciliary field; act = apical ciliary tuft; cb1–4 = ciliary bands 1–4; ict = intermediate ciliary tuft; lct = lateral ciliary tuft; mdt = mid-dorsal ciliary tuft of the second ciliary band; mo = mouth opening; no = nuchal organ; np = neuropil; pcc = prostomial compound cilia; phb = pharyngeal bulb; pyg = pygidium; sto = stomach; vct = ventral ciliary tract. All images oriented with the anterior tip to the left. All scale bars = 50 µm, if not denoted otherwise.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No species have been named for her to date, yet Ellen Browning Scripps' impact on science has been very important (see McClain 2017).

**Material examined**

**Holotype**

UNITED STATES OF AMERICA: complete adult, 485 µm long (platinum-palladinium-coated and mounted on stub for SEM), La Jolla Cove, La Jolla, California, 32°51′03″ N 117°16′20″ W, 9 m depth, 19 Apr. 2009, K. Worsaae and G. Rouse leg. (SIO-BIC A8209).

Additional specimens mounted on slides were unfortunately lost after conducting the measurements.

**Description**

Measurements given first in the text from holotype, ranges in in parenthesis include measurements taken from the lost specimens.

Compact body, light brown tint (fixed specimens, Fig. 3A). Body length 485 µm (306–774 µm, n = 4, Tables 1, 7), width 122 µm (91–229 µm, n = 4, Tables 1, 7), body segments indistinct (Fig. 3A–C).

Prostomial shape conical (Fig. 3, Table 7). No eyes. Middle of the mouth located 69–88 µm (n = 4) posterior of terminal prostomium (mo, Fig. 3A, C, F, Table 1). Four compound cilia terminally on prostomium (pcc, Fig. 3, Tables 1, 7), each consisting of approximately 30 cilia (n = 3); dorsal compound cilia spaced 19 µm apart (16–29 µm, n = 3), length similar to the cilia in the prostomial ciliary bands (10–18 µm long, n = 3). Prostomial compound cilia surrounded by four pairs of semicircular arranged apical ciliary tufts (act, Fig. 3B, D–E, Table 7). Two prostomial ciliary bands on prostomium and one ciliary band posterior to the nuchal organs, as well as one additional incomplete fourth ciliary band posterior to these bands (cb1–4, Fig. 3B, E, Table 7). First dorsally continuous ciliary band (13 µm wide, n = 1) encircles prostomium (Fig. 3A, D–E, Table 7). Several intermediate ciliary tufts located laterally between first and second ciliary band (ict, Fig. 3B, D–E, Table 7). Second ciliary band (cb2, Fig. 3A–B, D–F; 24 µm wide, n = 1) dorsally incomplete with 48 µm wide gap (n = 1) and one mid-dorsal ciliary tuft in center of gap (mdt, Fig. 3B, E, Table 7, 40–60 cilia, n = 1).

One lateral pair of nuchal organs located between second and third ciliary band (no, Fig. 3F). Third ciliary band (10 µm wide, n = 1) dorsally continuous (cb3, Fig. 3B–E, Table 7) posterior to nuchal organs. Fourth ciliary band extends laterally approx. 24 µm (n = 1) from the ventral ciliary tract (cb4, Fig. 3B, E, Table 7). Ciliary tufts arranged laterally along the body (lct, Fig. 3B, Table 7). Ventral ciliary tract extends from posterior prostomium to posterior pygidium (vct, Fig. 3B–C, E; width of tract relative to total body width approximately 0.44 (n = 1)). Anus opening dorso-anteriorly on pygidium.

No eggs present in the investigated specimens.

Epidermal inclusions and spindle glands in the epidermis could not be described and measured due to insufficient preservation in the investigated specimens.

**Molecular information**

The following sequences were determined by Sanger sequencing from a single, non-type specimen collected on 19 April 2009, for which no morphological voucher remains: 18S rDNA, MG588090 (1857 nucleotides (nt), Table 4); 28S rDNA, MG588092 (1126 nt, Table 5); COI, MG588094 (644 nt, Table 6); CytB, MG588096 (426 nt). In the following, the sequences of *T. ellenscrippsae* sp. nov. are first compared to the most similar sequences found in *T. windansea* sp. nov., and the range of similarities with the addition of the respective species are subsequently listed.

*Trilobodrilus ellenscrippsae* sp. nov. 18S rDNA is 99.9% similar to the 18S rDNA of *T. windansea* sp. nov., and 99.5% (*T. axi*) – 99.9% (*T. nipponicus*) similar to the other sequenced species (Table 4). Its 28S rDNA is 99.9% similar to the 28S rDNA of *T. windansea* sp. nov. and 98.5% (*T. axi*) – 99.4% (*T. nipponicus*) similar to the other species (Table 5). COI is 84.9% similar to *T. windansea* sp. nov., and 76% similar to the *T. itoi* and *T. nipponicus* (Table 6). Cytochrome B resembles the sequence of *T. windansea* sp. nov. to 85.6%.

**Habitat**

Subtidal in coarse to silty shell gravel at the surface layer at 9 m depth (subtidal).

**Distribution**

*Trilobodrilus ellenscrippsae* sp. nov. is known from La Jolla Cove, La Jolla, San Diego, California only.

**Remarks**

*Trilobodrilus ellenscrippsae* sp. nov. shows closest morphological resemblance to *T. nipponicus* (but nests with *T. windansea* sp. nov. in the molecular tree), but is much shorter, has a higher number of apical ciliary tufts on the prostomium, and its ciliary tufts along the body are not arranged in an as distinctive a pattern as in *T. nipponicus* (Fig. 3, Table 7). *Trilobodrilus ellenscrippsae* sp. nov. resembles *T. windansea* sp. nov. by having apical and intermediate ciliary tufts on the prostomium, and a dorsally incomplete second ciliary band with a mid-dorsal tuft (Fig. 3B, D–E, Table 7). However, *T. ellenscrippsae* sp. nov. differs from *T. windansea* sp. nov. in the shape of the prostomium, the additional ciliary row posterior to the second ciliary band, and by having a dorsally continuous third ciliary band as well as an incomplete fourth ciliary band (Fig. 3, Tables 1, 7).

*Trilobodrilus ellenscrippsae* sp. nov. further differs in its genetic sequences from all other species, see Molecular information above or Tables 4–6.

## Discussion

*Trilobodrilus* has been reported from the west coast of the USA only once prior to this study by Wieser (1957). Although the author identified the single specimen found on a beach in Seattle as *T. nipponicus*, he also noted discrepancies, and this record may instead have been the here described *T. ellenscrippsae*. However, the description of Wieser (1957) does not allow a clear re-identification.

Several of the previously described species are found in close proximity to each other, though either at different beaches such as the Japanese species from Hokkaido (Kajihara *et al.* 2015) or at different depths such as the European species *T. axi* and *T. heideri* (Remane 1925; Westheide 1967, 2008). Interestingly, the two new species from California show a similar niche separation as seen for the European species, with both species found within the same coastal area, but with *T. windansea* sp. nov. living intertidally (similar to *T. axi*) and *T. ellenscrippsae* sp. nov. subtidally (similar to *T. heideri*) (Fig. 2, Table 7). Although the here described two Californian species show obvious differences in the configuration of their ciliary patterns (number of ciliary tufts and continuity of bands) and the length of the prostomial compound cilia between them (Figs. 1, 3), there were no clear matches of these characteristics to other species with similar habitats or any obvious relation to, e.g., different grain size or turbulence.

The presented study shows the usefulness of external morphological characters such as ciliary bands and tufts to identify superficially similar microscopic annelids, and furthermore emphasizes the importance of combined approaches (LM, SEM, molecular data) in taxonomic studies of meiofauna specimens.

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