



Chiridota heheva—the cosmopolitan holothurian

Elin A. Thomas¹ · Ruoyu Liu^{2,3} · Diva Amon⁴ · Jon T. Copley⁵ · Adrian G. Glover⁴ · Sarah J. Helyar⁶ · Karine Olu⁷ · Helena Wiklund^{4,8} · Haibin Zhang² · Julia D. Sigwart¹

Received: 26 June 2020 / Revised: 6 October 2020 / Accepted: 15 October 2020
© The Author(s) 2020

Abstract

Chemosynthetic ecosystems have long been acknowledged as key areas of enrichment for deep-sea life, supporting hundreds of endemic species. Echinoderms are among the most common taxa inhabiting the periphery of chemosynthetic environments, and of these, chiridotid holothurians are often the most frequently observed. Yet, published records of chiridotids in these habitats are often noted only as supplemental information to larger ecological studies and several remain taxonomically unverified. This study therefore aimed to collate and review all known records attributed to *Chiridota* Eschscholtz, 1829, and to conduct the first phylogenetic analysis into the relationship of these chiridotid holothurians across global chemosynthetic habitats. We show that *Chiridota heheva* Pawson & Vance, 2004 is a globally widespread, cosmopolitan holothurian that occupies all three types of deep-sea chemosynthetic ecosystem—hydrothermal vents, cold seeps and organic falls—as an organic-enrichment opportunist. Furthermore, we hypothesise that *C. heheva* may be synonymous with another vent-endemic chiridotid, *Chiridota hydrothermica* Smirnov et al., 2000, owing to the strong morphological, ecological and biogeographical parallels between the two species, and predict that any chiridotid holothurians subsequently discovered at global reducing environments will belong to this novel species complex. This study highlights the importance of understudied, peripheral taxa, such as holothurians, to provide insights to biogeography, connectivity and speciation at insular deep-sea habitats.

Keywords Deep sea · Chemosynthetic environments · Opportunism · Widespread · Echinoderms

Communicated by S. Stöhr

✉ Elin A. Thomas
ethomas07@qub.ac.uk

¹ Queen's University Marine Laboratory, Queen's University Belfast, Portaferry, UK

² Institute of Deep-sea Science and Engineering, Chinese Academy of Sciences, Sanya, China

³ University of Chinese Academy of Sciences, Beijing, China

⁴ Department of Life Sciences, Natural History Museum, London, UK

⁵ Ocean and Earth Science, University of Southampton, Waterfront Campus, Southampton, UK

⁶ School of Biological Sciences, Queen's University Belfast, Belfast, UK

⁷ Deep-Sea Ecosystems Research Unit, IFREMER, EEP, F-29280 Plouzané, France

⁸ Department of Marine Sciences, University of Gothenburg, Box 463, 405 30 Gothenburg, Sweden

Introduction

Since the discovery of deep-sea chemosynthetic ecosystems just over 40 years ago, more than 600 species have been described from hydrothermal vent environments (Chapman et al. 2019) and at least 200 at cold seeps (Van Dover et al. 2002). Organic falls, such as whale falls and wood falls, have also been shown to be important deep-sea oases of organic enrichment, supporting a whole range of life including a chemosynthetic stage (Smith and Baco 2003). Echinoderms are among the least taxonomically represented invertebrates in these chemically reducing environments, suggesting that the colonisation of these habitats may be physiologically restricted for some taxa (Tunnicliffe 1992; Carney 2010).

The apodid holothurian *Chiridota heheva* Pawson & Vance, 2004 is one of the few echinoderms known exclusively from deep-sea reducing environments, having first been described from a wood fall off the east coast of Georgia, USA (type locality), and also recorded at cold seep and wood-fall habitats in the Gulf of Mexico, Caribbean Sea, and the Northwest Atlantic (Van Dover et al. 2003; Pawson

and Vance 2004). The genus itself is composed of 33 species, the majority of which are found in shallow-water habitats. Others are known from abyssal depths; however, *C. heheva* is distinguished from these species in tentacle and calcareous ring structure (Pawson and Vance 2004). The distinctive morphology of *C. heheva* also sets it apart from other apodid holothurians found at cold seeps and wood falls, such as *Rynkatorpa felderi* Pawson & Vance, 2005, from the lack of perforations to the calcareous ring to the six-spoked wheel ossicle structure (Pawson and Vance 2004). Another chiridotid species, *Chiridota hydrothermica* Smirnov et al., 2000, is the only holothurian described as endemic to hydrothermal-vent environments, having been recorded at vent fields in the West (type locality, Manus Basin) and Southeast Pacific (Smirnov et al. 2000). Though distinctive, there are strong morphological similarities between the congeners *C. heheva* and *C. hydrothermica*; the two species differ only in tentacle arrangement and digit structure (Pawson and Vance 2004).

These two species are apparently restricted to their respective reducing environments, but they generally inhabit the periphery of these deep-sea oases and, to date, neither species has been recorded to host chemosymbiotic bacteria (Smirnov et al. 2000; Pawson and Vance 2004). *Chiridota* sp. exhibited the highest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values among the species at the Longqi hydrothermal-vent field on the Southwest Indian Ridge (SWIR), indicating that the species is likely to be feeding on a mix of photosynthetically and chemosynthetically derived organic matter (Reid et al. 2012; Reid et al. 2013; Copley et al. 2016). Sulphur isotopic data for *C. hydrothermica* from the Manus Basin in the West Pacific also suggested that they are dependent on photosynthetically derived organic matter, supplemented by microbial chemoautotrophic production (Erickson et al. 2009). *Chiridota heheva* feeds on a mixture of sediment detritus, suspended material and wood fragments at wood-fall habitats (Pawson and Vance 2004; Carney 2010). Enriched $\delta^{15}\text{N}$ values of *Chiridota* aff. *heheva* from the REGAB Pockmark cold seep in the East Atlantic, as well as depleted $\delta^{13}\text{C}$ values, suggest some dependence on recycled material including chemosynthetic production in its diet (Olu et al. 2009). Both species share a peltato-digitate tentacle structure which would support the exploitation of various food sources by facilitating switching between deposit and suspension feeding (Smirnov et al. 2000; Carney 2010). These deep-sea chiridotids are therefore apparently reliant on organic enrichment rather than chemosynthetic primary production.

Both *C. hydrothermica* and *C. heheva* also exhibit widespread distributions, despite being restricted to specific insular deep-sea habitats (Smirnov et al. 2000; Pawson and Vance 2004). Studies have reported taxonomically unidentified chiridotid holothurians at deep-sea reducing habitats, as well as additional in situ records from remotely operated vehicle

(ROV) imagery attributed to *Chiridota* sp. These observations potentially expand the biogeographic range of *C. hydrothermica* in the West Pacific and to the Indian Ocean (Hashimoto et al. 2001; Podowski et al. 2010; Watanabe and Beedessee 2015; Copley et al. 2016; Zhou et al. 2018), and *C. heheva* in the West and to the East Atlantic (Pawson and Vance 2004; Olu-Le Roy et al. 2007; von Cosel and Olu 2009; Jones et al. 2014). Previous studies have already alluded to the widespread distribution of *Chiridota* Eschscholtz, 1829 across oceans (Olu et al. 2010); however, it remains unclear whether these records represent a chiridotid species with an unusually extensive distribution (Georgieva et al. 2015; Eilertsen et al. 2018) or constitute several phylogenetically distinct but unrecognised species within a species complex (Knowlton 1993).

Chiridotid holothurians are the most frequently observed echinoderms in deep-sea chemosynthetic ecosystems (Smirnov et al. 2000; Watanabe and Beedessee 2015), yet several records remain taxonomically unconfirmed, and the phylogenetic relationship of *Chiridota* spp. has never been tested, owing to the lack of molecular data for collected specimens. The evolutionary relationship between these species could provide insights to the colonisation of deep-sea reducing environments. The present study therefore aims to collate and review all known records attributed to or resembling *Chiridota* at deep-sea reducing environments, including both confirmed and taxonomically unverified observations, and conduct the first phylogenetic analysis using specimen material spanning their global distribution. Owing to the widespread distribution of chiridotid holothurians at these reducing environments, we hypothesised that these records capture a complex of multiple species belonging to the genus *Chiridota* (Knowlton 1993), including *C. heheva* and *C. hydrothermica*.

Material and methods

Material examined

We assembled specimens of chiridotid holothurians collected from deep-sea reducing environments, available globally in research collections (Table 1). Specimens preserved in ethanol were provided by the University of Southampton (one specimen), Natural History Museum, London (NHM, one specimen), and Ifremer Deep-Sea Ecosystems Research Unit (Ifremer, three specimens). Two additional specimens were flash frozen in liquid nitrogen and then stored in $-80\text{ }^{\circ}\text{C}$ by the Institute of Deep-sea Science and Engineering, Chinese Academy of Sciences (IDSSE, CAS). We further compiled all known records of chiridotid holothurians at deep-sea reducing environments from published literature, as well as

Table 1 Metadata for the *Chiridota* sp. holothurian specimens used in this study

| Habitat | Location | Coordinates | Depth (m) | Cruise details | Voucher number |
|-------------------|---|-------------------------------|-----------|----------------------------------|----------------|
| Hydrothermal vent | Longqi vent field, Southwest Indian Ridge, Indian Ocean | 37° 47.006' S, 49° 38.973' E | 2758 | RRS <i>James Cook</i> (JC67) | JC67-F-54/1b |
| Wood fall | Cayman Rise, Caribbean Sea | 18° 24.052' N, 81° 34.794' W | 3249 | E/V <i>Nautilus</i> (NA034) | NA034-013 |
| Cold seep | REGAB Pockmark, Gulf of Guinea, Atlantic Ocean | 5° 47.854' S, 9° 42.675' E | 3154 | RV <i>Pourquoi pas?</i> (WACS) | PL424/1 |
| Cold seep | REGAB Pockmark, Gulf of Guinea, Atlantic Ocean | 5° 47.854' S, 9° 42.675' E | 3154 | RV <i>Pourquoi pas?</i> (WACS) | PL424/2 |
| Cold seep | Worm Hole Pockmark, Gulf of Guinea, Atlantic Ocean | 4° 45.580' S, 9° 56.480' E | 3094 | RV <i>Pourquoi pas?</i> (WACS) | PL431 |
| Cold seep | Haima Seep, South China Sea | 16° 43.923' N, 110° 27.699' E | 1385 | HOV <i>Shenhaiyongshi</i> (TS07) | SQW58YC2 |
| Cold seep | Haima Seep, South China Sea | 16° 44.890' N, 110° 24.657' E | 1372 | HOV <i>Shenhaiyongshi</i> (TS07) | SY79YC1 |

additional in situ images of chiridotids at deep-sea reducing environments, to complement these records.

Phylogenetic analyses

New sequences were obtained from seven deep-sea *Chiridota* specimens and sequences from eight additional taxa were downloaded from NCBI's GenBank based on the availability of cytochrome c oxidase I (COI) and 12S rRNA sequences, resulting in a total of 15 taxa used for phylogenetic analyses (Table 2). This covered a selection of taxa within the Suborder Synaptina (Smirnov 1998) and included the only chiridotid sequenced from a deep-sea reducing environment prior to this study, *Chiridota* sp. from the Longqi hydrothermal-vent field, SWIR (Zhou et al. 2018). The synaptid *Euapta tahitiensis* Cherbonnier, 1955 was chosen as the outgroup based on the findings of Miller et al. (2017).

Total genomic DNA was extracted and isolated from tissue samples using a Qiagen DNeasy Blood & Tissue Kit as per the manufacturer's protocol for animal tissues (Qiagen 2006). The two barcoding genes COI and 12S were targeted for analysis, to provide resolution in interspecies discrimination, amplified via polymerase chain reaction (PCR) with the following echinoderm-specific primer pairs: COI_{ef} (ATAATGATAGGAGGRTTTGG) and COI_{er} (GCTCGTGTRTCTACRTCCAT) (Arndt et al. 1996; Miller et al. 2017), 12SA (CTGGGATTAGATACCCCACTA) and 12SB (TGAGGAGGGTGACGGGCGGT) (Janies et al. 2011; Miller et al. 2017).

The PCR was conducted with GE Healthcare illustra™ PuReTaq Ready-To-Go™ PCR Beads in 25 µl volumes comprised of 1 µl forward primer, 1 µl reverse primer, and either 1 µl DNA template and 22 µl nuclease-free water or 5 µl DNA template and 18 µl nuclease-free water. The PCR was also performed with the Direct PCR kit B639289 (BBI) by a modified protocol: the 50-µl mixture contained 25 µl Direct

PCR mix, 1.25 µl sample homogenate (5 mg tissue crushed in 50 µl sterilised ddH₂O), 1.25 µl Hot Start DNA Polymerase, 2.5 µl forward primer, 2.5 µl reverse primer and 17.5 sterilised ddH₂O.

Thermocycling was undertaken using a VWR UNO96 gradient thermal cycler with the following protocols from Miller et al. (2017). For COI, initial denaturation at 95 °C for 3 min, followed by 40 cycles of (denaturation at 95 °C for 40 s, annealing at 45 °C for 40 s, extension at 72 °C for 50 s), ending with a final extension at 72 °C for 5 min. For 12S, initial denaturation at 95 °C for 3 min, followed by 35 cycles of (denaturation at 94 °C for 30 s, annealing

Table 2 GenBank accession numbers for cytochrome c oxidase I (COI) and 12S rRNA genes of the apodid taxa included in the phylogenetic analysis

| Taxon | Accession Numbers | |
|---------------------------------------|-------------------|----------|
| | COI | 12S |
| <i>Euapta tahitiensis</i> | KX874402 | KX856723 |
| <i>Euapta lappa</i> | KC626165 | - |
| <i>Sigmodota contorta</i> | - | KX856720 |
| <i>Chiridota rigida</i> | KX874401 | KX856721 |
| <i>Chiridota laevis</i> | KX874399 | KX856719 |
| <i>Chiridota albatrossi</i> complex 1 | KX874397 | - |
| <i>Chiridota albatrossi</i> complex 2 | KX874398 | - |
| <i>Chiridota</i> sp. | MH230104 | - |
| <i>Chiridota heheva</i> JC67-F-054/1b | MT707250 | MT724752 |
| <i>Chiridota heheva</i> NA034-013 | MT707251 | MT724753 |
| <i>Chiridota heheva</i> PL424/1 | MT707252 | MT724754 |
| <i>Chiridota heheva</i> PL424/2 | MT707253 | MT724755 |
| <i>Chiridota heheva</i> PL431 | MT707254 | MT724756 |
| <i>Chiridota heheva</i> SQW58YC2 | MT707255 | MT724757 |
| <i>Chiridota heheva</i> SY79YC1 | MT707256 | MT724758 |

at 55 °C for 60 s, extension at 72 °C for 90 s), ending with a final extension at 72 °C for 5 min. Amplification of the target genes was confirmed with 1% agarose gel electrophoresis, using either ethidium bromide or SYBR™ safe DNA gel stain, and the successful PCR products were sent for Sanger sequencing at Eurofins GATC or BGI.

Sequence contigs were trimmed for sequence quality and assembled using BioEdit (Version 7.0.5.3) (Hall 1999), and the resultant consensus sequences compared to published sequences stored in GenBank using the online Nucleotide Blast tool, to confirm chiridotid holothurian affinity. COI and 12S multiple alignments were conducted in Geneious (Version 11.0.5) using default settings. The COI and 12S alignments were manually inspected before being combined for analysis in Mesquite (Version 3.31). Monte Carlo Markov Chain (MCMC) Bayesian phylogenetic analyses were conducted in MrBayes (Version 3.2.6) using the GTR+G and HKY+G combined evolutionary models for COI and 12S respectively, as recommended by jmodeltest2 via the CIPRES Science Gateway (Miller et al. 2010). Analyses were run three times for 5 million generations, ensuring the standard deviation of split frequencies was below 0.01, with 25% of generations discarded as burn-in. MrBayes results were examined using Tracer (Version 1.7.1) to check that the analysis achieved convergence. The resultant phylogenetic tree was edited using FigTree (Version 1.4.3) and Adobe Acrobat Pro DC (Version 2020.006.20042).

Pairwise distance analysis

Patterns of genetic distance within holothurian species were assessed following the method of Sigwart and Garbett (2018) by generating a pairwise-distance matrix for the trimmed COI gene in our focus specimens and other well-studied and commercially important holothurian species extracted from the BOLD database. The distance matrix used the dist.alignment function in SeqinR package (Charif and Lobry 2007) (R Version 1.2.5042) to determine the maximum and median pairwise distances as a measure of intraspecific genetic variability within each taxon.

Morphological analyses

Morphological analyses were only conducted for some of the specimens analysed using molecular techniques. These complementary morphological analyses were conducted alongside phylogenetic analyses for the specimens representing a new distribution range for *Chiridota* spp. to support species affinity, using specimen JC67-F-054/1b from the Longqi hydrothermal-vent field on the SWIR (Copley et al. 2016) and specimen SY79YC1 from the Haima cold seep in the South China Sea. External morphology was examined for

JC67-F-054/1b using a Leica EZ4 HD stereomicroscope. Tentacles were removed and rehydrated for further study, revealing the presence of the calcareous ring which was subsequently partially dissected for visualisation. Body wall and tentacle ossicles were extracted from JC67-F-054/1b and SY79YC1, and studied using a scanning electron microscope (SEM) at the NHM, London (Quanta FEG 650) and at the IDSSE, Sanya (Phenomenon ProX) following standard protocols (Smirnov et al. 2000, Purcell et al. 2012). Results were compared to the original species descriptions for *C. heheva* (Pawson and Vance 2004) and *C. hydrothermica* (Smirnov et al. 2000). Based on the broad anatomical similarities within the genus *Chiridota*, particularly between *C. hydrothermica* and *C. heheva* (Smirnov et al. 2000; Pawson and Vance 2004), only one specimen (JC67-F-054/1b) was fully dissected to study anatomical features included within the original species diagnoses, namely, the Polian vesicles, ciliated funnels and intestine (Smirnov et al. 2000).

Results

We examined 32 records attributed to *Chiridota* from global deep-sea reducing environments (Figs. 1 and 2, Table 3), and we obtained novel sequence data for seven specimens (Table 2).

Phylogenetics

Phylogenetic reconstruction based on COI and 12S mitochondrial gene fragments recovered a single clade encompassing all *Chiridota* sequences from global deep-sea reducing environments, with Bayesian inference posterior probability value (PP) > 95 (Fig. 3). Within this clade, there are some groupings that correlate with location and habitat. The two sequences from the Longqi hydrothermal-vent field on the SWIR form a basal grade to a clade of other exemplars that originate from non-vent reducing environments. However, support for the clade of sequences from other reducing environments is relatively low (PP = 89). Within that clade, sequences from the REGAB Pockmark and Worm Hole Pockmark cold seeps in the Gulf of Guinea, East Atlantic, are grouped with the Cayman Rise wood-fall specimen from the Caribbean (PP > 95). The two sequences from the Haima cold seep in the South China Sea are also grouped with strong support (PP > 95). The genus *Chiridota* is not recovered as monophyletic, with *Sigmodota contorta* resolved within the clade encompassing *Chiridota laevis* and *Chiridota albatrossi* (PP > 95).

The pairwise distances between the COI sequences of individual specimens attributed to *C. heheva* have a median pairwise distance of 0.028, and a maximum distance of 0.037 separating the Worm Hole and Haima cold seep

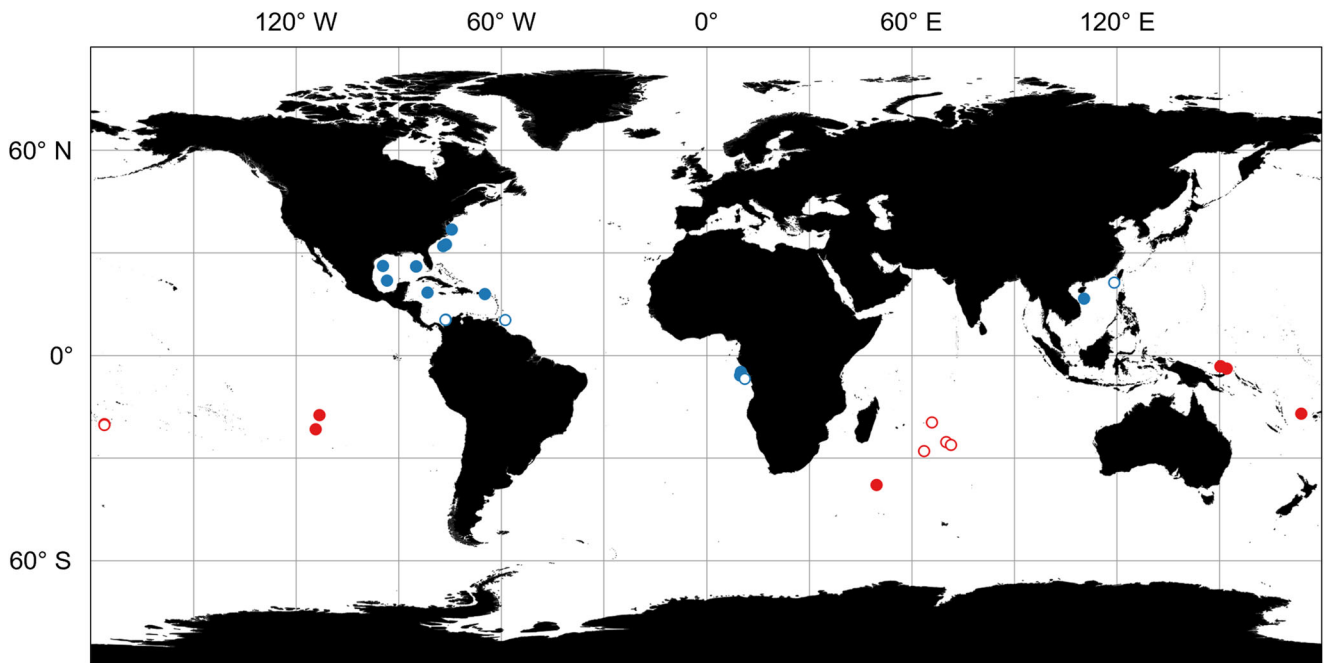


Fig. 1 Distribution map for *Chiridota* spp. at deep-sea reducing environments. Red circles denote hydrothermal-vent fields; blue circles denote other reducing environments (cold seeps and wood falls). Closed circles

denote taxonomically confirmed records of *Chiridota* spp. at the site; open circles denote in situ observations of *Chiridota* spp. at reducing environments that have not been confirmed by specimen collection

specimens (Table 4). The genetic variability of the COI gene among individuals for other well-studied holothurian species shows comparable levels of intraspecific variation, with *Cucumaria frondosa* exhibiting a median pairwise distance

of 0.007 and a maximum distance of 0.023 ($n = 366$), *Holothuria atra*: median distance of 0.018 and maximum distance of 0.112 ($n = 109$) and *Leptosynapta clarki*: median distance of 0.000 and maximum distance of 0.026 ($n = 12$).

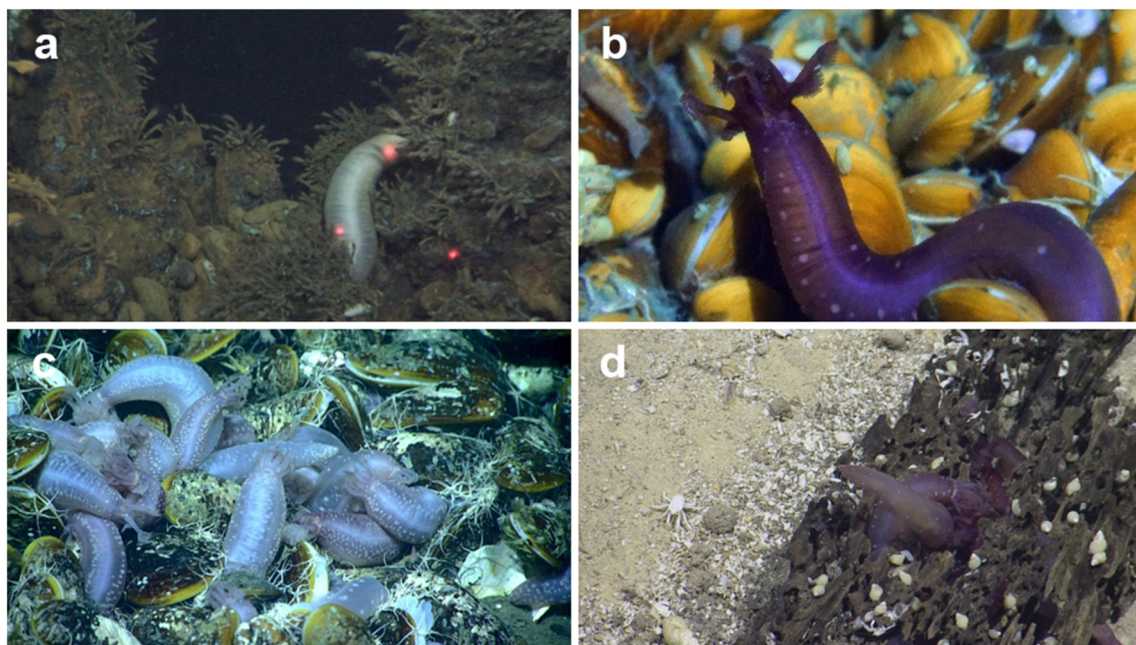


Fig. 2 In situ images of live holothurians attributed to *Chiridota* in different reducing habitats. **a** *Chiridota heheva* at the Longqi hydrothermal-vent field, Southwest Indian Ridge, with 0.1-m scale from lasers (RRS *James Cook*); **b** *Chiridota heheva* at the Haima cold seep, South China Sea (HOV *Shenhaiyongshi*); **c** *Chiridota* sp. at a cold seep in

the Gulf of Mexico (NOAA Ship *Okeanos Explorer*, Copyright NOAA Office of Ocean Exploration and Research); **d** *Chiridota heheva* at a wood fall on the Cayman Rise (E/V *Nautilus*, Copyright Ocean Exploration Trust)

Table 3 Complete list of published records attributed to *Chiridota* sp. at deep-sea reducing environments in global locations. Reference page numbers are included for records with in situ or specimen photographs

| Location | Record(s) | Habitat | | |
|------------------------|---|--------------------|------------|------------|
| | | Hydrothermal vents | Cold seeps | Wood falls |
| Manus Basin | <i>Chiridota</i> n. sp. (Galkin 1997) <i>Chiridota hydrothermica</i> (Smirnov et al. 2000*; Desbruyères et al. 2006: 480; Erickson et al. 2009) | x | | |
| North Fiji Basin | <i>Chiridota</i> n. sp. (Desbruyères et al. 1994: 234) <i>Chiridota hydrothermica</i> (Smirnov et al. 2000*; Desbruyères et al. 2006: 480) | x | | |
| Southeast Pacific Rise | <i>Chiridota hydrothermica</i> (Smirnov et al. 2000: 326*; Desbruyères et al. 2006: 480) | x | | |
| Lau Basin | <i>Chiridota hydrothermica</i> (Desbruyères et al. 2006: 480; Podowski et al. 2010, Sen et al. 2016)† | x | | |
| Central Indian Ridge | Synaptidae (Hashimoto et al. 2001)† Apodacean (Nakamura et al. 2012; Watanabe and Beedessee 2015)† | x | | |
| Southeast Indian Ridge | <i>Chiridota</i> sp. (Gerdes et al. 2019)† | x | | |
| Southwest Indian Ridge | <i>Chiridota</i> sp. (Copley et al. 2016: 10; Zhou et al. 2018: 7; Sun et al. 2020: 6, 9†) <i>Chiridota heheva</i> (this study) | x | | |
| Central East Atlantic | Synaptidae holothurians (Olu-Le Roy et al. 2007)† Synaptid holothurians (von Cosel & Olu 2009: 2378)† <i>Chiridota</i> sp. (Olu et al. 2009: 2384, 2389) <i>Chiridota</i> aff. <i>heheva</i> (Olu et al. 2010: 8) Holothurian species (Jones et al. 2014: 134)† <i>Chiridota heheva</i> (this study) | | x | |
| Gulf of Mexico | <i>Chiridota</i> sp. (Hecker 1985; Van Dover et al. 2003; D. Amon, pers. comm. 2019, Fig. 2 this study†) <i>Chiridota heheva</i> (Pawson and Vance 2004: 5*; Carney 2010; Olu et al. 2010; MacDonald et al. 2020†; Wegener et al. 2020: 117) | | x | |
| Caribbean Sea | <i>Chiridota heheva</i> (Pawson and Vance 2004*; Borrero-Pérez et al. 2020†; this study) | | x | x |
| Northwest Atlantic | <i>Chiridota</i> (Herdendorf 1995: 57) <i>Chiridota</i> sp. (Van Dover et al. 2003: 288) <i>Chiridota heheva</i> (Pawson and Vance 2004: 6*; Olu et al. 2010; Turner et al. 2020: 9†) | | x | x |
| South China Sea | <i>Chiridota</i> sp. (C. Chen, pers. comm. 2019)† <i>Chiridota heheva</i> (this study) | | x | x |

*Taxonomic description

†Taxonomically unconfirmed record(s)

Morphological observations

Morphological examination of new *Chiridota* specimen material was found to be consistent with Pawson and Vance's (2004) description of *Chiridota heheva*. External morphology of JC67-F-054/1b is typical of order Apodida: vermiform in shape, with peltato-digitate tentacles and lacking tube feet. The appearance of specimens differs in colour and size owing to different collection and preservation techniques. The papillae are inconspicuous in preservation, but in situ images (Fig. 2) and photographs of specimens immediately post-collection (Fig. 4) confirm the presence of papillae in the interradia, as described by Pawson and Vance (2004). The calcareous ring consists of 10 pieces, five radial and five interradial, and contrary to most *Chiridota* species, lacks perforations for the passage of the radial nerve. Study of the internal anatomy confirmed the presence of more than 20 slender Polian vesicles, with ciliated funnels absent or rare and intestine looped.

All specimens possess 12 conspicuous peltato-digitate tentacles, each with approximately 20 digits arranged along the margin of a flattened disc, which occupies the upper side of the tentacle in a 'palm' shape (Fig. 4). Digit structure appears to be discrete as finger-like processes (Pawson and Vance 2004). No ventral gap was observed in the arrangement of the tentacles examined in the present study, as described by Pawson and Vance (2004).

The body wall ossicles of JC67-F-054/1b and SY79YC1 are mainly six-spoked wheels, 66 to 207- μ m diameter and typical of *Chiridota*, which bear small denticles uniformly spaced around the inner edge of the uppermost rim (Fig. 4). The wheels have complex central hubs which protrude above the wheel rim as cap-like bulbs and an inner surface which is characterised by a star structure, as described by Smirnov (1998). Wheel ossicles are generally clustered in interradial papillae but

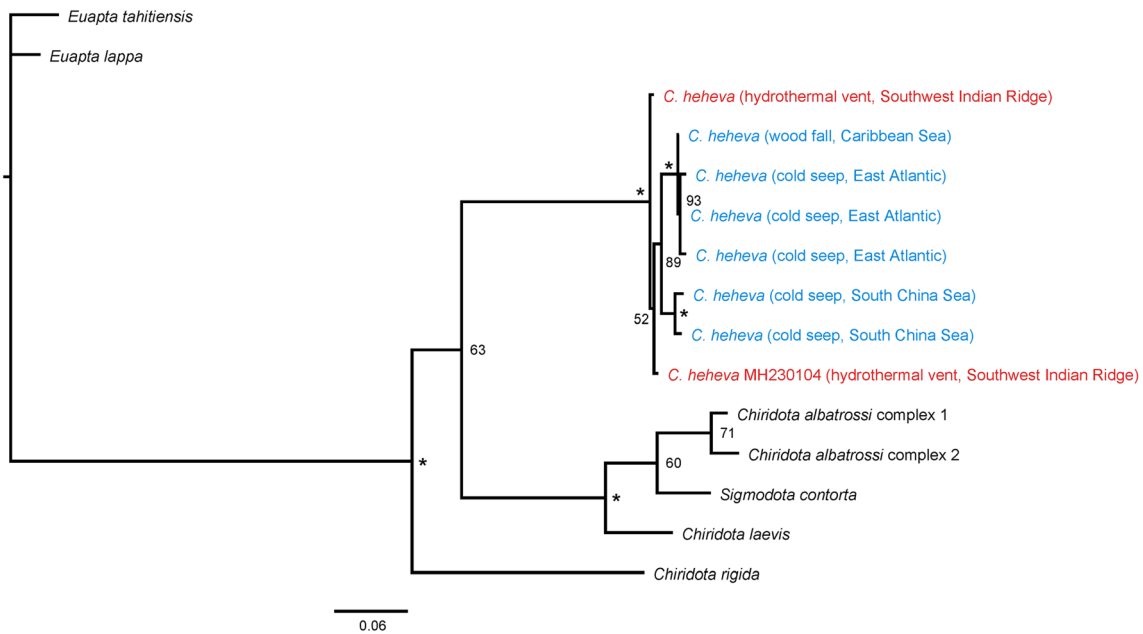


Fig. 3 Phylogenetic tree obtained from combined Bayesian analysis of the cytochrome c oxidase I (COI) and 12S rRNA genes from 15 apodid taxa, showing the phylogenetic relationship between holothurian material attributed to *Chiridota heheva* and published holothurian sequences from NCBI GenBank. Numbers indicate the Bayesian Inference posterior probability value as a percentage, with significant values of 95 and above

marked by asterisks (*). Scale bar represents the quantity of genetic change, with 0.06 nucleotide substitutions per site. *Chiridota heheva* specimens collected from hydrothermal-vent environments are highlighted in red. *Chiridota heheva* specimens collected from other deep-sea reducing environments (cold seeps and wood falls) are highlighted in blue

may also occur individually, scattered throughout the body wall in specimens with indiscernible papillae. Rare five-spoked wheels are reported in some specimens (Smirnov et al. 2000). Tentacle ossicles are rod-shaped, 100 to 280 µm in length, with highly variable distal branching within and between specimens (Fig. 4). Width also varies from ossicle to ossicle, especially in the central region of the rods. The rod exteriors are irregular with protuberances across the surface.

Discussion

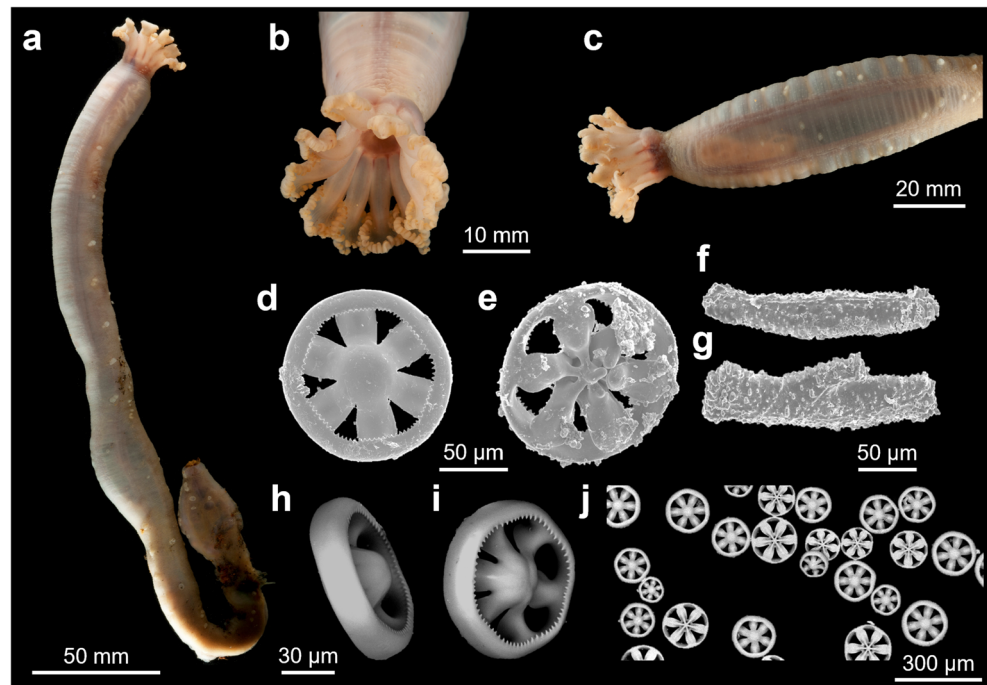
Biogeography and habitat of *Chiridota heheva*

Based on the results of this study, we expand the geographic range and report a new habitat record for the deep-sea holothurian, *Chiridota heheva*. *Chiridota heheva* has a global distribution, spanning the Caribbean Sea, Gulf of Mexico, Atlantic Ocean, Indian Ocean and South China Sea, with 20

Table 4 Pairwise distances demonstrating intraspecific genetic variability of the cytochrome c oxidase I (COI) gene between specimens of *Chiridota heheva* from this study and published sequence MH230104 (Zhou et al. 2018). Median pairwise distance = 0.028, maximum pairwise distance = 0.037

| | Hydrothermal vent, Southwest Indian Ridge JC67-F-054/1b | Wood fall, Caribbean Sea NA034-013 | Cold seep, East Atlantic PL424/1 | Cold seep, East Atlantic PL424/2 | Cold seep, East Atlantic PL431 | Cold seep, South China Sea SQW58YC2 | Cold seep, South China Sea SY79YC1 | Hydrothermal vent, Southwest Indian Ridge MH230104 |
|-----------------|--|---------------------------------------|-------------------------------------|-------------------------------------|-----------------------------------|--|---------------------------------------|---|
| JC67-F-054/1b \ | | 0.028 | 0.029 | 0.028 | 0.031 | 0.024 | 0.022 | 0.007 |
| NA034-013 0.028 | \ | | 0.006 | 0.000 | 0.007 | 0.029 | 0.028 | 0.029 |
| PL424/1 0.029 | 0.006 | \ | | 0.006 | 0.013 | 0.031 | 0.029 | 0.031 |
| PL424/2 0.028 | 0.000 | 0.006 | \ | | 0.007 | 0.029 | 0.028 | 0.029 |
| PL431 0.031 | 0.007 | 0.013 | 0.007 | \ | | 0.037 | 0.035 | 0.033 |
| SQW58YC2 0.024 | 0.029 | 0.031 | 0.029 | 0.029 | 0.037 | \ | 0.002 | 0.024 |
| SY79YC1 0.022 | 0.028 | 0.029 | 0.028 | 0.028 | 0.035 | 0.002 | \ | 0.022 |
| MH230104 0.007 | 0.029 | 0.031 | 0.029 | 0.029 | 0.033 | 0.024 | 0.022 | \ |

Fig. 4 Morphology of *Chiridota heheva*. **a–c** full-length and tentacle view of specimen JC67-F-054/1a collected from the Longqi hydrothermal-vent field, Southwest Indian Ridge (RRS *James Cook*, JC67); **d–g** SEM images of wheel-shaped body wall ossicles and rod-shaped tentacle ossicles from specimen JC67-F-054/1b; **h–j** SEM images of wheel-shaped body wall ossicles from specimen SY79YC2 from Haima cold seep, South China Sea (HOV *Shenhaiyongshi*, TS07)



taxonomically confirmed records of the chiridotid species at deep-sea reducing environments to date (Fig. 1). Furthermore, *C. heheva* is now established as a deep-sea species endemic to hydrothermal vents, cold seeps and organic falls. This overlap in niche, as well as morphological similarities, could indicate that *C. heheva* and *C. hydrothermica* are conspecific, but we have been unable to obtain sequence data for any confirmed *C. hydrothermica* specimens.

Chiridota heheva from diverse habitats are resolved in a single monophyletic clade, including individuals sequenced from a wood fall in the Caribbean, cold seeps in the East Atlantic and South China Sea, and a hydrothermal-vent field on the SWIR. Prior records of morphologically similar specimens from the vent periphery have all been attributed to *C. hydrothermica* (Smirnov et al. 2000; Desbruyères et al. 2006; Erickson et al. 2009; Podowski et al. 2010; Sen et al. 2016). Pairwise distances within the species provide evidence that *C. heheva* has a similar level of intraspecific variability in the COI marker as other well-studied holothurian species, in spite of this geographical and ecological breadth. The pairwise distances among individuals of different populations for shallow-water taxa, such as *Holothuria*, are comparable to those of *C. heheva*, with *H. atra* exhibiting a median distance of 0.018, compared to a median distance of 0.028 in *C. heheva*. Our morphological analyses of specimens from the Longqi vent field and Haima cold seep confirm the phylogenetic result, with specimens positively identified as *C. heheva* based on the radial symmetry of the tentacles and their conspicuous finger-like digits, as described by Pawson and Vance (2004).

In most studies of deep-sea diversity, detailed investigations and molecular barcoding commonly reveal cryptic species (Knowlton 1993; Vrijenhoek 2009). Vesicomid clams of the subfamily Pliocardiinae, for example, are one of the most diverse clades endemic to deep-sea reducing environments (Vrijenhoek et al. 1994; Kojima et al. 1995; Audzijonyte et al. 2012). Owing to their morphologically cryptic shells, DNA barcoding studies found the known species richness of vesicomid clams to be nearly double the estimate based on conchological morphospecies designations (Vrijenhoek et al. 1994; Vrijenhoek 2009; Audzijonyte et al. 2012). A similar pattern was found in lepetodrilid limpets, with minor variances in morphological features that supported new phylogenetic species delimitations only discovered after molecular evidence prompted additional examination of the microscopic anatomy (Johnson et al. 2008; Vrijenhoek 2009).

Our data suggest a contrary, cosmopolitan distribution for *C. heheva*, which is all the more surprising given that global hydrothermal-vent systems have different species assemblages with many regional endemics (Rogers et al. 2012). This is not the only such case of confirmed geographic and ecological cosmopolitanism (Georgieva et al. 2015; Eilertsen et al. 2018). Conspecific populations of the annelids *Sclerolinum contortum* and *Nicomache lokii* occur pole to pole, across hydrothermal vents in the Norwegian Arctic, mud volcanoes in the Barbados Prism, cold seeps in the Gulf of Mexico and hydrothermal vents along the East Scotia Ridge in the Antarctic (Georgieva et al. 2015; Eilertsen et al. 2018), demonstrating a range comparable to that of *C. heheva*. The two annelids also demonstrate differing

levels of regional genetic structuring (Eilertsen et al. 2018), indicating that variable dispersal and connectivity may produce similarly broad distributions.

Reproduction and life history in *Chiridota heheva*

Efficient larval recruitment strategies are central to facilitating broad dispersal in any benthic animals with limited mobility at ecologically relevant timescales. The larva of *C. heheva* has never been directly observed, but Pawson and Vance (2004) reported an egg diameter ranging from 97 to 125 µm for female *C. heheva* specimens, which is small compared with eggs ranging up to 15 mm in other deep-sea holothurians (Pawson et al. 2003). Indeed, these may be among the smallest eggs recorded for any deep-sea holothurian (Galley et al. 2008). Such a small egg size suggests that the larvae of *C. heheva* are planktotrophic, which may confer greater dispersal capability compared with other larval types (Billett 1991; Young and Eckelbarger 1994; Pawson and Vance 2004). *Chiridota heheva* may also be capable of reproducing year-round, as mature eggs were present in the gonads of individuals collected in May and October from the same area (Pawson and Vance 2004). Planktotrophic larvae and continuous reproduction could both facilitate the widespread distribution of this species, and we speculate that there is some level of genetic connectivity between populations.

Chiridota heheva is evidentially an opportunist that colonises sites of ephemeral organic enrichment, occurring at all three types of reducing environment—hydrothermal vents, cold seeps and organic falls. Only a few other deep-sea species, such as the polychaete *Bathylkurila guaymasensis* (Glover et al. 2005) and siboglinid annelid *Sclerolinum contortum* (Georgieva et al. 2015), have been described from all three habitat types. Organic falls, such as wood falls, are thought to provide dispersal ‘stepping stones’ connecting populations over several generations (Smith et al. 1989; Smith and Baco 2003; Yearsley and Sigwart 2011), which may facilitate *C. heheva*’s widespread distribution, and support ongoing genetic connectivity between populations. The hypothesis for dispersal stepping stones between reducing environments has been previously supported in chemosynthetic-generalist taxa (Lorion et al. 2009; Bienhold et al. 2013; Sumida et al. 2016). Such connectivity between different types of reducing environments is only possible for marginal species that are neither endemic, specialists nor chemosymbiotic, but opportunists that occupy the periphery of these habitats like *C. heheva*. Further study of taxa found at all three reducing environments could improve understanding of dispersal mechanisms and connectivity in the deep sea.

Conspecificity of *Chiridota heheva* and *Chiridota hydrothermica*

The identification of specimens from the Longqi vent field as *C. heheva* was especially surprising since, prior to this study, the species was yet to be recorded from hydrothermal-vent habitats. Preliminary examination of the specimens first associated them with the species *C. hydrothermica* (Copley et al. 2016), the only known holothurian endemic to hydrothermal vents. The morphological distinctions between *C. heheva* and *C. hydrothermica* are minor, and both species appear to occupy similar ecological niches, with broad similarities in feeding behaviour and zonation at their respective reducing habitats (Smirnov et al. 2000; Pawson and Vance 2004). Both species also have widespread distributions for species associated with specialist habitats, occupying reducing environments spanning across oceans. These strong morphological, ecological and biogeographical parallels prompt the question of whether *C. hydrothermica* and *C. heheva* may represent a single widespread species.

Chiridota heheva and *C. hydrothermica* are distinguished from other chiridotid holothurians in their habitat preference, peltato-digitate tentacle structure, shape of body wall and tentacle ossicles, and a lack of perforations to the calcareous ring for the passage of the radial nerve (Smirnov et al. 2000; Pawson and Vance 2004). The key morphological characters used to distinguish *C. heheva* from *C. hydrothermica* are in the arrangement of the tentacles and the structure of the tentacle digits. Smirnov et al. (2000) described the presence of a ventral gap in the arrangement of the tentacles in *C. hydrothermica*, a feature which is absent in *C. heheva* (Pawson and Vance 2004) and was the primary determining feature to identify the Longqi vent *C. heheva* specimens examined herein. *Chiridota heheva* also has discrete tentacle digits as opposed to the marginally fused digits in *C. hydrothermica* (Smirnov et al. 2000; Pawson and Vance 2004).

Morphological variability may be a product of phenotypic plasticity related to differing environmental conditions in seeps, vents and organic falls (Vrijenhoek 2009; Chen et al. 2019). A comparable example is the vestimentiferan tubeworm *Ridgeia piscesae*, with morphology ranging from short-fat to long-skinny forms that were originally described as separate species (Jones 1985), but later recognised as ecomorphotypes at corresponding temperatures and hydrothermal-vent fluid fluxes (Southward et al. 1995; Carney et al. 2002; Vrijenhoek 2009; Tunnicliffe et al. 2014). The large, lobe-like tentacles of *C. hydrothermica* from vents in the Manus Basin and along the SEPR may be adapted to facilitate switching between suspension and deposit feeding (Smirnov et al. 2000), so it is likely that the more digitated tentacles observed in the West-Atlantic cold-seep populations of *C. heheva* (Pawson and Vance 2004) are also adapted to facilitate particular feeding methods that may be habitat- or

location-specific. Such distinctions are within the scope of normal intraspecific plasticity known for shallow-water holothurians (McKenzie 1991); however, further morphological and in situ evidence would be required to confirm this morphological heterogeneity as environmentally mediated plasticity.

The tentacle structures, observed feeding behaviour and isotopic data for *C. heheva* and *C. hydrothermica* all suggest that these species can exploit various food resources at reducing environments, from sediment to suspended material to wood fragments (Smirnov et al. 2000; Pawson and Vance 2004). Furthermore, both species tolerate enriched toxic compounds accumulated in the food web by trophic magnification (Erickson et al. 2009; Olu et al. 2009; Carney 2010; Copley et al. 2016). Both *C. heheva* and *C. hydrothermica* also have similar zonation preferences, generally occurring at the periphery of the reducing environments that they inhabit to exploit the abundant organic matter (Smirnov et al. 2000; Copley et al. 2016, Sun et al. 2020), although chiridotids at the REGAB Pockmark were observed among vesicomid and mytilid bivalves (Olu et al. 2009). These traits are indicative of the adaptable and opportunistic lifestyle characterised by both *C. heheva* and *C. hydrothermica*.

Based on this evidence, we hypothesise that *C. heheva* may represent a junior synonym of *C. hydrothermica*. Unfortunately, we were not able to test this hypothesis during the present study owing to the rarity of specimens and difficulty to extract DNA for phylogenetic analysis from historical material. We obtained tissue snips from *C. hydrothermica* specimens, including paratype material from the Zoological Museum of the Zoological Institute of the Russian Academy of Sciences; however, DNA extraction and amplification of target genes was unsuccessful, and although we identified a number of other *Chiridota* specimens in global collections, this material was not preserved for DNA extraction. We recommend that the collection of new specimens, including from type localities, is essential to further study the phylogenetic relationship of *Chiridota* at reducing environments and to investigate the possible synonymy of *C. heheva* and *C. hydrothermica* presented here.

Identification by imagery and observational notes

As well as the records identified here and in the literature based on detailed study of specimens, numerous other observations of chiridotid holothurians attributed to *Chiridota* sp. have been recorded at global deep-sea reducing environments (Table 3). These taxonomically unverified records add ten locations to the known distribution of *Chiridota* at reducing environments (Fig. 1), including hydrothermal vents on the Central and Southeast Indian Ridges and in the Lau Basin, asphalt mounds with active seepage in the Gulf of Mexico and on the Angolan margin, and a wood fall in the South

China Sea (Hashimoto et al. 2001; Podowski et al. 2010; Nakamura et al. 2012; Jones et al. 2014; Sen et al. 2016; Watanabe and Beedesssee 2015; C. Chen pers. comm. 2019; Gerdes et al. 2019; MacDonald et al. 2020; Sun et al. 2020; Wegener et al. 2020). In situ imagery from ROV and human occupied vehicles (HOV) is central to capturing these additional distribution records, highlighting the importance of these technologies for deep-sea biodiversity research (Sigwart et al. 2019). Furthermore, photographs and video footage of species in vivo reveal important morphological features, as well as ecological and behavioural observations that enrich species descriptions. For example, the papillae of *Chiridota* spp. can be inconspicuous in preservation, whereas in situ imagery reveals the longitudinal patterns of papillae along the body wall of the specimens (Fig. 4; Smirnov et al. 2000; Pawson and Vance 2004).

As well as enriching species descriptions with observations of morphological traits prior to collection, ROV and HOV footage can also enhance our understanding of real variability for species delimitation. The in situ off-white colour of the Longqi vent *C. heheva*, for example, does not conform to the purple colouring of *C. heheva* at the Haima cold seep or the Cayman Rise wood fall (Fig. 2). Colour was one of the features which was originally used to morphologically distinguish *C. heheva* from *C. hydrothermica* (Pawson and Vance 2004); yet, colour polymorphism is a feature of many holothurian taxa (Marcus 1983) and may be attributable to different morphotypes in conspecific populations of *Chiridota*. The specimens examined in this study also varied in size, from ~50 to 300 mm in preservation. It is unclear whether or how much of this variability can be attributed to habitat type, sampling bias, specimen preservation or the age of the animal.

In situ imagery should not be used alone in deep-sea taxonomic identification, as it does not provide the opportunity to test species attribution. Furthermore, key diagnostic features of species, such as tentacle structure in *Chiridota*, are not always discernible from ROV footage. Despite this, in situ imagery represents an important component for accurate and fully comprehensive specimen identification and should be employed as a complimentary feature to all future studies of deep-sea fauna (Macreadie et al. 2018).

Conclusion

Echinoderms are often disregarded or noted only parenthetically in ecological studies of deep-sea reducing environments, and yet holothurians may be among the most common species inhabiting the periphery of these ecosystems (Watanabe and Beedesssee 2015). The present study highlights the importance of holothurians to advance understanding of connectivity and

speciation at insular deep-sea habitats. *Chiridota heheva* was originally described as a species endemic only to cold seeps and wood falls in the Northwest Atlantic, Caribbean Sea and Gulf of Mexico (Pawson and Vance 2004). On the contrary, *C. heheva* is, in fact, a widespread, globally cosmopolitan species that can opportunistically occupy any deep-sea reducing habitat, including hydrothermal vents.

Acknowledgements We thank Chong Chen for sparking new collaborations for this study and providing a new record of *Chiridota* sp. at reducing environments. We thank Andrey Gebruk, Dieter Fiege and Yadong Zhou for loaning additional specimen material to support this study. Thanks also to colleagues at the University of Southampton, National Oceanography Centre, Southampton, and Queen's University Belfast for their support and stimulating discussion. Southwest Indian Ridge specimens were collected by research cruise JC67, and we thank the Master and ship's company, UK National Marine Facilities technicians, GEOMAR Kiel6000 ROV team, and science team colleagues aboard the RRS *James Cook*. We thank David Shale for JC67 specimen photography. We would also like to thank the pilots and crews of the HOV *Shenhaiyongshi* and RV *Tansuoyihao* for their professional services during the cruise TS07. Ifremer specimens from the Gulf of Guinea were sampled during the RV *Pourquoi pas?* WACS cruise, using ROV *Victor 6000*. This research also used samples provided by the Ocean Exploration Trust's *Nautilus* Exploration Program, Cruise NA034. We thank Gustav Paulay and an anonymous reviewer for providing valuable comments to improve this manuscript.

Funding EAT is supported by a studentship awarded by the Faculty of Medicine, Health and Life Sciences, Queen's University Belfast. This study was partially funded by a Systematics Research Fund grant from the Systematics Association and Linnean Society, awarded to EAT. DA has received funding from the European Union's Horizon 2020 Research and Innovation Program under the Marie Skłodowska-Curie grant agreement number 747946. Research cruise JC67 was funded by NERC grant NE/H012087/1 to JTC. JDS is supported by the Hong Kong Branch of Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou). RL and HZ are supported by the National Key Research and Development Program of China (2016YFC0304905).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national and/or institutional guidelines for animal testing, animal care and use of animals were followed by the authors.

Sampling and field studies All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements, if applicable. The study is compliant with CBD and Nagoya protocols.

Data availability Molecular data deposited in GenBank: <https://www.ncbi.nlm.nih.gov/genbank/>

Author contribution statement EAT, JTC and JDS conceived and designed research. RL, DA, JTC, KO and HZ contributed new specimens. EAT, RL, AG and HW collected data. EAT, SJH and JDS analysed data. All authors contributed to writing and have approved the manuscript.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Arndt A, Marquez C, Lambert P, Smith MJ (1996) Molecular phylogeny of eastern Pacific sea cucumbers (Echinodermata: Holothuroidea) based on mitochondrial DNA sequence. *Mol Phylogenet Evol* 6: 425–437. <https://doi.org/10.1006/MPEV.1996.0091>
- Audzijonyte A, Krylova EM, Sahling H, Vrijenhoek RC (2012) Molecular taxonomy reveals broad trans-oceanic distributions and high species diversity of deep-sea clams (Bivalvia: Vesicomidae: Pliocardiinae) in chemosynthetic environments. *Syst Biodivers* 10: 403–415. <https://doi.org/10.1080/14772000.2012.744112>
- Bienhold C, Ristova PP, Wenzhöfer F, Dittmar T, Boetius A (2013) How deep-sea wood falls sustain chemosynthetic life. *PLoS One* 8: e53590. <https://doi.org/10.1371/journal.pone.0053590>
- Billett DSM (1991) Deep-sea holothurians. *Oceanogr Mar Biol Annu Rev* 29:259–317
- Borrero-Pérez GH, Dueñas LF, León J, Puentes V (2020) Deep-sea holothurians (Echinodermata, Holothuroidea) from the Colombian Southern Caribbean Sea. *Check List* 16:535–551. <https://doi.org/10.15560/16.3.535>
- Carney RS (2010) Stable isotope trophic patterns in echinoderm megafauna in close proximity to and remote from Gulf of Mexico lower slope hydrocarbon seeps. *Deep Sea Res Part II Top Stud Oceanogr* 57:1965–1971. <https://doi.org/10.1016/J.DSR2.2010.09.027>
- Carney SL, Peoples JR, Fisher CR, Schaeffer SW (2002) AFLP analyses of genomic DNA reveal no differentiation between two phenotypes of the vestimentiferan tubeworm, *Ridgeia piscesae*. *Cah Biol Mar* 43:363–366
- Chapman ASA, Beaulieu SE, Colaço A et al (2019) sFDvent: a global trait database for deep-sea hydrothermal-vent fauna. *Glob Ecol Biogeogr* 28:1538–1551. <https://doi.org/10.1111/geb.12975>
- Charif D, Lobry JR (2007) SeqinR 1.0-2: a contributed package to the R project for statistical computing devoted to biological sequences retrieval and analysis. In: Bastolla U, Porto M, Roman HE, Vendruscolo M (eds) *Structural approaches to sequence evolution*. Springer, Berlin, pp 207–232
- Chen C, Watanabe HK, Nagai Y et al (2019) Complex factors shape phenotypic variation in deep-sea limpets. *Biol Lett* 15:20190504. <https://doi.org/10.1098/rsbl.2019.0504>
- Cherbonnier G (1955) Holothuriers récoltés en Océan française par G. Ranson en 1952. *Bulletin Muséum National Histoire Naturelle Paris* 27:380–386
- Copley JT, Marsh L, Glover AG et al (2016) Ecology and biogeography of megafauna and macrofauna at the first known deep-sea hydrothermal vents on the ultraslow-spreading Southwest Indian Ridge. *Sci Rep* 6:39158. <https://doi.org/10.1038/srep39158>
- Desbruyères D, Alayse-Danet A-M, Ohta S, the Scientific Parties of BIOLAU and STARMER Cruises (1994) Deep-sea hydrothermal communities in Southwestern Pacific back-arc basins (the North Fiji

- and Lau Basins): composition, microdistribution and food web. *Mar Geol* 116:227–242. [https://doi.org/10.1016/0025-3227\(94\)90178-3](https://doi.org/10.1016/0025-3227(94)90178-3)
- Desbruyères D, Segonzac M, Bright M (2006) Handbook of deep-sea hydrothermal vent fauna. Biologiezentrum der Oberösterreichische Landesmuseen, Linz
- Eilertsen MH, Georgieva MN, Kongsrud JA et al (2018) Genetic connectivity from the Arctic to the Antarctic: *Sclerolinum contortum* and *Nicomache lokii* (Annelida) are both widespread in reducing environments. *Sci Rep* 8:4810. <https://doi.org/10.1038/s41598-018-23076-0>
- Erickson KL, Macko SA, Van Dover CL (2009) Evidence for a chemoautotrophically based food web at inactive hydrothermal vents (Manus Basin). *Deep Sea Res Part II Top Stud Oceanogr* 56:1577–1585. <https://doi.org/10.1016/J.DSR2.2009.05.002>
- Eschscholtz F (1829) Zoologischer Atlas, enthaltend Abbildungen und Beschreibungen neue Thierarten während der Flottcapitains von Kotzebue zweiter reise um die Welt 1823-26. Reimer, Berlin
- Galkin SV (1997) Megafauna associated with hydrothermal vents in the Manus Back-Arc Basin (Bismarck Sea). *Mar Geol* 142:197–206. [https://doi.org/10.1016/S0025-3227\(97\)00051-0](https://doi.org/10.1016/S0025-3227(97)00051-0)
- Galley EA, Tyler PA, Smith CR, Clarke A (2008) Reproductive biology of two species of holothurian from the deep-sea order Elasipoda, on the Antarctic continental shelf. *Deep Sea Res Part II Top Stud Oceanogr* 55:2515–2526. <https://doi.org/10.1016/J.DSR2.2008.07.002>
- Georgieva MN, Wiklund H, Bell JB et al (2015) A chemosynthetic weed: the tubeworm *Sclerolinum contortum* is a bipolar, cosmopolitan species. *BMC Evol Biol* 15:280. <https://doi.org/10.1186/s12862-015-0559-y>
- Gerdes K, Martínez Arbizu P, Schwarz-Schampera U, Schwentner M, Kihara TC (2019) Detailed mapping of hydrothermal vent fauna: a 3D reconstruction approach based on video imagery. *Front Mar Sci* 6:96. <https://doi.org/10.3389/fmars.2019.00096>
- Glover AG, Goetze E, Dahlgren TG, Smith CR (2005) Morphology, reproductive biology and genetic structure of the whale-fall and hydrothermal vent specialist, *Bathylkurila guaymasensis* Pettibone, 1989 (Annelida: Polynoidae). *Mar Ecol* 26:223–234. <https://doi.org/10.1111/j.1439-0485.2005.00060.x>
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98
- Hashimoto J, Ohta S, Gamo T et al (2001) First hydrothermal vent communities from the Indian Ocean discovered. *Zool Sci* 18:717–721. <https://doi.org/10.2108/zsj.18.717>
- Hecker B (1985) Fauna from a cold-sulfur-seep in the Gulf of Mexico: comparison with hydrothermal vent communities and evolutionary implications. *Bull Biol Soc Washingt* 6:465–473
- Herdendorf CE (1995) Science on a deep-ocean shipwreck. *Ohio J Sci* 95:4–212
- Janies DA, Voight JR, Daly M (2011) Echinoderm phylogeny including *Xyloplax*, a progenetic asteroid. *Syst Biol* 60:420–438. <https://doi.org/10.1093/sysbio/syr044>
- Johnson SB, Warén A, Vrijenhoek RC (2008) DNA barcoding of *Lepetodrilus* limpets reveals cryptic species. *J Shellfish Res* 27:43–51. [https://doi.org/10.2983/0730-8000\(2008\)27\[43:DBOLLR\]2.0.CO;2](https://doi.org/10.2983/0730-8000(2008)27[43:DBOLLR]2.0.CO;2)
- Jones DOB, Walls A, Clare M et al (2014) Asphalt mounds and associated biota on the Angolan margin. *Deep Res Part I Oceanogr Res Pap* 94:124–136. <https://doi.org/10.1016/j.dsr.2014.08.010>
- Jones M (1985) On the Vestimentifera, new phylum: six new species, and other taxa, from hydrothermal vents and elsewhere. *Bull Biol Soc Washingt* 6:117–158
- Knowlton N (1993) Sibling species in the sea. *Annu Rev Ecol Syst* 24:189–216
- Kojima S, Segawa R, Ohta S (1995) Molecular evidence that *Calyptogena laubieri* (Bivalvia : Vesicomysidae) is a valid species. *Venus* 54:153–156. https://doi.org/10.18941/venusjmm.54.2_153
- Lorion J, Duperron S, Gros O, Cruaud C, Samadi S (2009) Several deep-sea mussels and their associated symbionts are able to live both on wood and on whale falls. *Proc R Soc B Biol Sci* 276:177–185. <https://doi.org/10.1098/rspb.2008.1101>
- MacDonald IR, Gaytan-Caballero A, Escobar-Briones E (2020) The asphalt ecosystem of the southern Gulf of Mexico: abyssal habitats across space and time. In: Murawski SA, Ainsworth CH, Gilbert S et al (eds) Scenarios and responses to future deep oil spills. Springer International Publishing, Cham, pp 132–146
- Macreadie PI, McLean DL, Thomson PG et al (2018) Eyes in the sea: unlocking the mysteries of the ocean using industrial, remotely operated vehicles (ROVs). *Sci Total Environ* 634:1077–1091. <https://doi.org/10.1016/J.SCITOTENV.2018.04.049>
- Marcus NH (1983) Phenotypic variability in echinoderms. In: Jangoux M, Lawrence JM (eds) Echinoderm studies 1. A. A. Balkema, Rotterdam, pp 19–37
- McKenzie JD (1991) The taxonomy and natural history of north European dendrochirote holothurians (Echinodermata). *J Nat Hist* 25:123–171. <https://doi.org/10.1080/00222939100770091>
- Miller AK, Kerr AM, Paulay G et al (2017) Molecular phylogeny of extant Holothuroidea (Echinodermata). *Mol Phylogenet Evol* 111:110–131. <https://doi.org/10.1016/j.ympev.2017.02.014>
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments workshop (GCE). New Orleans, LA, pp 1–8
- Nakamura K, Watanabe H, Miyazaki J et al (2012) Discovery of new hydrothermal activity and chemosynthetic fauna on the Central Indian ridge at 18°–20°S. *PLoS One* 7:e32965. <https://doi.org/10.1371/journal.pone.0032965>
- Olu K, Caprais JC, Galéron J et al (2009) Influence of seep emission on the non-symbiont-bearing fauna and vagrant species at an active giant pockmark in the Gulf of Guinea (Congo–Angola margin). *Deep Sea Res Part II Top Stud Oceanogr* 56:2380–2393. <https://doi.org/10.1016/J.DSR2.2009.04.017>
- Olu K, Cordes EE, Fisher CR et al (2010) Biogeography and potential exchanges among the Atlantic equatorial belt cold-seep faunas. *PLoS One* 5:e11967. <https://doi.org/10.1371/journal.pone.0011967>
- Olu-Le Roy K, Caprais J-C, Fifiis A et al (2007) Cold-seep assemblages on a giant pockmark off West Africa: spatial patterns and environmental control. *Mar Ecol* 28:115–130. <https://doi.org/10.1111/j.1439-0485.2006.00145.x>
- Pawson DL, Gage JD, Belyaev GM, Mironov AN, Smimov AV (2003) The deep sea synaptid *Protankyra brychia* (Echinodermata: Holothuroidea) and its near- surface dwelling planktotrophic larva, *Auricularia nudibranchiata*. *Sarsia* 88:159–174. <https://doi.org/10.1080/00364820310001165>
- Pawson DL, Vance DJ (2004) *Chirodota heheva*, new species, from western Atlantic deep-sea cold seeps and anthropogenic habits (Echinodermata: Holothuroidea: Apodida). *Zootaxa* 534:1–12
- Pawson DL, Vance DJ (2005) *Rynkatorpa felderi*, new species, from a bathyal hydrocarbon seep in the northern Gulf of Mexico (Echinodermata: Holothuroidea: Apodida). *Zootaxa* 1050:15–20
- Podowski E, Ma S, Luther G, Wardrop D, Fisher CR (2010) Biotic and abiotic factors affecting distributions of megafauna in diffuse flow on andesite and basalt along the Eastern Lau Spreading Center, Tonga. *Mar Ecol Prog Ser* 418:25–45. <https://doi.org/10.3354/meps08797>
- Purcell S, Samyn Y, Conand C (2012) Commercially important sea cucumbers of the world. FAO Species Catalogue For Fisheries Purposes No. 6. FAO, Rome
- Qiagen (2006) DNeasy blood & tissue handbook. Qiagen, Hombrechtikon

- Reid WDK, Wigham BD, McGill RAR, Polunin NVC (2012) Elucidating trophic pathways in benthic deep-sea assemblages of the Mid-Atlantic Ridge north and south of the Charlie-Gibbs Fracture Zone. *Mar Ecol Prog Ser* 463:89–103. <https://doi.org/10.3354/meps09863>
- Reid WDK, Sweeting CJ, Wigham BD et al (2013) Spatial differences in east scotia ridge hydrothermal vent food webs: influences of chemistry, microbiology and predation on trophodynamics. *PLoS One* 8: e65553. <https://doi.org/10.1371/journal.pone.0065553>
- Rogers AD, Tyler PA, Connelly DP et al (2012) The discovery of new deep-sea hydrothermal vent communities in the Southern Ocean and implications for biogeography. *PLoS Biol* 10:e1001234. <https://doi.org/10.1371/journal.pbio.1001234>
- Sen A, Kim S, Miller AJ et al (2016) Peripheral communities of the Eastern Lau Spreading Center and Valu Fa Ridge: community composition, temporal change and comparison to near-vent communities. *Mar Ecol* 37:599–617. <https://doi.org/10.1111/maec.12313>
- Sigwart JD, Garbett A (2018) Biodiversity assessment, DNA barcoding, and the minority majority. *Integr Comp Biol* 58:1146–1156. <https://doi.org/10.1093/icb/icy076>
- Sigwart JD, Wicksten MK, Jackson MG, Herrera S (2019) Deep-sea video technology tracks a monoplacophoran to the end of its trail (Mollusca, Tryblidia). *Mar Biodivers* 49:825–832. <https://doi.org/10.1007/s12526-018-0860-2>
- Smirnov AV (1998) On the classification of the apodid holothurians. In: Mooi R, Telford M (eds) *Echinoderms*: San Francisco. A. A. Balkema, Rotterdam, pp 265–274
- Smirnov AV, Gebruk AV, Galkin SV, Shank T (2000) New species of holothurian (Echinodermata: Holothuroidea) from hydrothermal vent habitats. *J Mar Biol Assoc United Kingdom* 80:321–328. <https://doi.org/10.1017/S0025315499001897>
- Smith CR, Baco AR (2003) Ecology of whale falls at the deep-sea floor. *Oceanogr Mar Biol* 41:311–354
- Smith CR, Kukert H, Wheatcroft RA, Jumars PA, Deming JW (1989) Vent fauna on whale remains. *Nature* 341:27–28. <https://doi.org/10.1038/341027a0>
- Southward EC, Tunnicliffe V, Black M (1995) Revision of the species of *Ridgeia* from northeast Pacific hydrothermal vents, with a redescription of *Ridgeia piscesae* Jones (Pogonophora: Obturata = Vestimentifera). *Can J Zool* 73:282–295. <https://doi.org/10.1139/z95-033>
- Sumida PYG, Alfaro-Lucas JM, Shimabukuro M et al (2016) Deep-sea whale fall fauna from the Atlantic resembles that of the Pacific Ocean. *Sci Rep* 6:22139. <https://doi.org/10.1038/srep22139>
- Sun J, Zhou Y, Chen C et al (2020) Nearest vent, dearest friend: biodiversity of Tiancheng vent field reveals cross-ridge similarities in the Indian Ocean. *R Soc Open Sci* 7:200110. <https://doi.org/10.1098/rsos.200110>
- Tunnicliffe V (1992) The nature and origin of the modern hydrothermal vent fauna. *Palaos* 7:338. <https://doi.org/10.2307/3514820>
- Tunnicliffe V, St. Germain C, Hilário A (2014) Phenotypic variation and fitness in a metapopulation of tubeworms (*Ridgeia piscesae* Jones) at hydrothermal vents. *PLoS One* 9:e110578. <https://doi.org/10.1371/journal.pone.0110578>
- Turner PJ, Ball B, Diana Z et al (2020) Methane seeps on the US Atlantic margin and their potential importance to populations of the commercially valuable deep-sea red crab, *Chaceon quinque-dens*. *Front Mar Sci* 7:75. <https://doi.org/10.3389/fmars.2020.00075>
- Van Dover CL, Aharon P, Bernhard JM et al (2003) Blake ridge methane seeps: characterization of a soft-sediment, chemosynthetically based ecosystem. *Deep Sea Res Part I Oceanogr Res Pap* 50:281–300. [https://doi.org/10.1016/S0967-0637\(02\)00162-0](https://doi.org/10.1016/S0967-0637(02)00162-0)
- Van Dover CL, German CR, Speer KG, Parson LM, Vrijenhoek RC (2002) Evolution and biogeography of deep-sea vent and seep invertebrates. *Science* 295:1253–1257
- von Cosel R, Olu K (2009) Large Vesicomidae (Mollusca: Bivalvia) from cold seeps in the Gulf of Guinea off the coasts of Gabon, Congo and northern Angola. *Deep Sea Res Part II Top Stud Oceanogr* 56:2350–2379. <https://doi.org/10.1016/J.DSR2.2009.04.016>
- Vrijenhoek RC (2009) Cryptic species, phenotypic plasticity, and complex life histories: assessing deep-sea faunal diversity with molecular markers. *Deep Sea Res Part II Top Stud Oceanogr* 56:1713–1723. <https://doi.org/10.1016/J.DSR2.2009.05.016>
- Vrijenhoek RC, Schutz SJ, Gustafson RG, Lutz RA (1994) Cryptic species of deep-sea clams (Mollusca: Bivalvia: Vesicomidae) from hydrothermal vent and cold-water seep environments. *Deep Sea Res Part I Oceanogr Res Pap* 41:1171–1189. [https://doi.org/10.1016/0967-0637\(94\)90039-6](https://doi.org/10.1016/0967-0637(94)90039-6)
- Watanabe H, Beedesse G (2015) Vent Fauna on the Central Indian Ridge. In: *Subseafloor biosphere linked to hydrothermal systems*. Springer Japan, Tokyo, pp 205–212
- Wegener G, Knittel K, Bohrmann G, Schubotz F (2020) Benthic deep-sea life associated with asphaltic hydrocarbon emissions in the southern Gulf of Mexico. In: Murawski SA, Ainsworth CH, Gilbert S et al (eds) *Scenarios and responses to future deep oil spills*. Springer International Publishing, Cham, pp 101–123
- Yearsley JM, Sigwart JD (2011) Larval transport modeling of deep-sea invertebrates can aid the search for undiscovered populations. *PLoS One* 6:e23063. <https://doi.org/10.1371/journal.pone.0023063>
- Young CM, Eckelbarger KJ (1994) *Reproduction, larval biology, and recruitment of the deep-sea benthos*. Columbia University Press, New York
- Zhou Y, Zhang D, Zhang R et al (2018) Characterization of vent fauna at three hydrothermal vent fields on the Southwest Indian Ridge: implications for biogeography and interannual dynamics on ultraslow-spreading ridges. *Deep Res Part I Oceanogr Res Pap* 137:1–12. <https://doi.org/10.1016/j.dsr.2018.05.001>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.