

First record of *Ditrupa gracillima* (Annelida, Polychaeta) from the late Pliocene of Andaman and Nicobar Basin: insights on the ultrastructure, stable isotopic signature and distribution pattern

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ABSTRACT: In the present study from the late Pliocene of Neill West Coast Formation, Neil Island, serpulid tubes assignable to *Ditrupa gracillima* Grube have been identified with the help of tube morphological (light microscopy) and ultrastructural (SEM) studies. This is the first record of the species from the late Pliocene of India. The outer layer of *D. gracillima* is entirely different from other invertebrates, and the presence of Ridged Prismatic Structure (RRP) exclusively characterises this particular species. The mineralogical analysis (Raman spectroscopy and EDS) reveals a Mg-Ca skeletal for *D. gracillima*. Isotopic analysis indicates that there is a narrow range of variation in the $\delta^{18}\text{O}$ values of *D. gracillima*. The $\delta^{13}\text{C}$ of *D. gracillima* shows significant variation and displays the most ^{13}C depleted values. Based on isotopic analysis it has been inferred that the deposition of sediments took place in a shallow marine environment and *D. gracillima* thrived in the proximity of hydrocarbon seepages. The present day distributional pattern of *D. gracillima* indicates that they are mainly confined in the Indo-Pacific region.

Keywords: Neogene, marine, palaeoecology, serpulid, calcite

INTRODUCTION

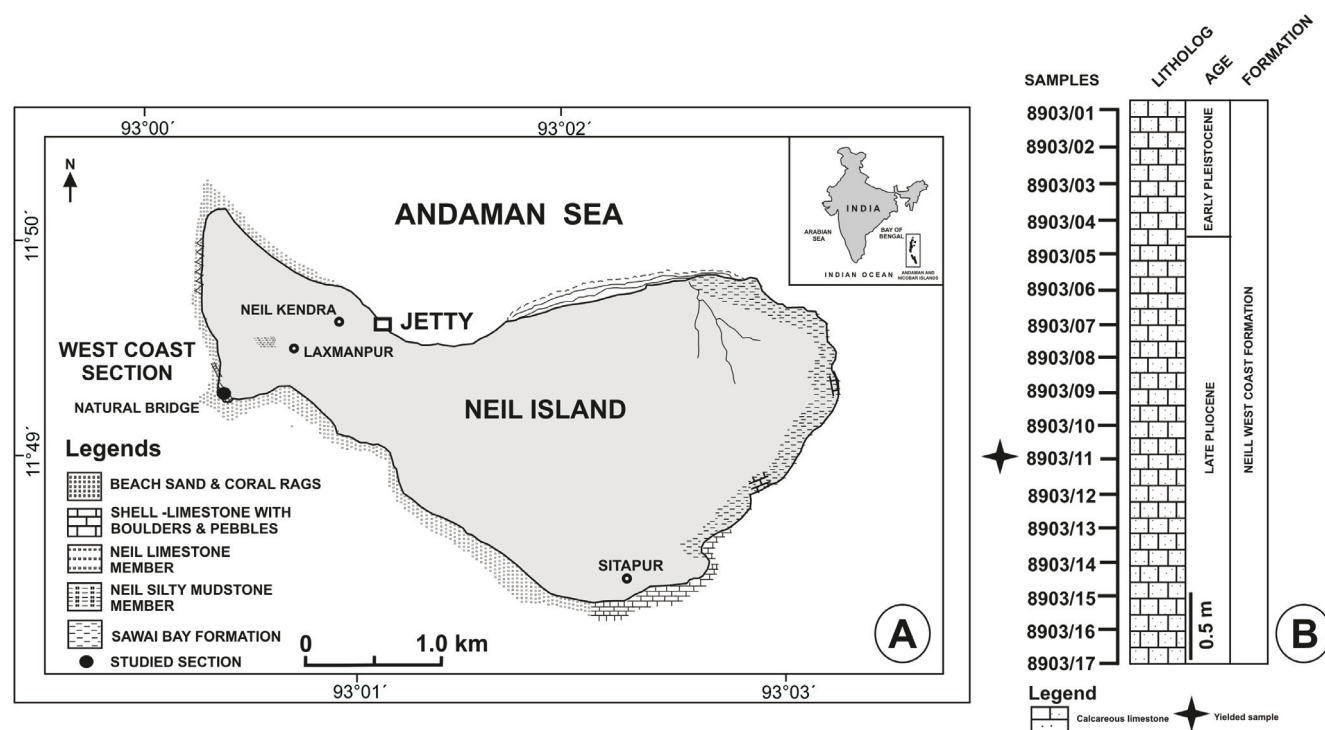
High carbon dioxide concentration in the earth's atmosphere is responsible for changes in ocean chemistry. This has attracted the palaeobiologists and geochemists dealing with marine carbonate biota and their skeletal composition to decipher probable effects on dissolution as well as calcification. Atmospheric carbon dioxide is increasing unusually during the last couple of decades (Lindsey 2020). One-third of atmospheric carbon dioxide is absorbed by ocean water and that consequently decreases pH, carbonate ion concentration and the calcium carbonate saturation. Ocean acidification can deleteriously affect the calcifying organisms. The significant studies on the impact of ocean acidification on the calcareous invertebrates have been carried out by several authors in the last two decades (Díaz-Castañeda et al. 2019 and references therein). Polychaetes are a class of annelids consisting of around 85 families belonging mainly to the marine habitats, except for ten families that live in brackish or fresh water (Benbow 2009). These worms are one of the major components of the marine food chain. However, among these annelids, calcareous tubes are known in serpulid, cirratulid, and sabellid polychaetes (Vinn et al. 2008c, 2008d; Vinn 2009).

Serpulidae is a large family of sedentary benthic polychaete worms which secretes and live within calcareous tubes. This family includes the subfamilies: Spiborbinae, Filigraninae, and Serpulinae (ten Hove and Kupriyanova 2009). Serpulids are common members of marine hard-substratum communities present in a wide variety of aquatic systems that may also have salinities up to 55‰ with world-wide distribution (Rouse and Pleijel 2001). At present, there are approximately 343 species belonging to 74 genera (Lehrke et al. 2007). Most of the Recent species of serpulids live in marine environments. The larval

stages of these organisms are (semi) benthic to planktonic and after settling to hard substrate juvenile serpulids start to build calcareous tubes. In most of the serpulids, tubes of adult specimens are attached to a hard substratum with an exception in case of the genus *Ditrupa*. In *Ditrupa*, the unattached tubes occur in a soft substrate (ten Hove and van der Hurk 1993).

It is difficult to identify the fossil serpulid tubes because they have only a few characteristic features. In the process of fossilisation, the soft body, and chitinous parts of the polychaetes are lost. So, the tubes are the only way to identify the species (ten Hove and van der Hurk 1993). Serpulids possess the most advanced biomineralisation system among the annelids (Vinn et al. 2009; Vinn and Kupriyanova 2011; Vinn and ten Hove 2011; Vinn 2011; Vinn 2013a; Chan et al. 2015). They possess very diverse tube ultrastructures which often are specific to particular species (Vinn 2008; Vinn and Furrer 2008; Vinn 2013b, 2013c).

The knowledge on these tubes in the Indian subcontinent is still scarce due to few fossil records. Polychaete fossils have been reported earlier from different horizons of India ranging from Cambrian to Recent (d'Archiac and Heime 1853; Stoliczka 1872-73; Bose 1884; Waagen 1886; Kossmat 1897; Verma 1969). Various species of polychaetan affinity have been described; however, most of them are from the late Cretaceous of southern India (Chiplonkar and Tapaswi 1973; Chiplonkar and Ghare 1977; Tapaswi 1980). From the early Eocene (Khiala Formation) of Jaisalmer Basin, Rajasthan Ghosh (1987) described well-preserved serpulid framestone. Serpulid beds also have been identified in the middle Eocene-Oligocene carbonate sediments of Kutch (Banerjee et al. 2018). Reuter et al. (2011) reported the occurrence of serpulids in the faunal assemblage of



TEXT-FIGURE 1

A. Geological Map of Neil Island, Andaman and Nicobar Islands (modified after Sharma and Srinivasan 2007). B. Lithology of the studied section.

the Burdigalian sediments of Quilon Formation, Kerala. From the Miocene of Kachchh (stratotype section of Vinjhan Formation) serpulid tubes have been reported by Ippolitov and Desai (2015). The serpulid tubes of the genus *Ditrupa* were first reported in India from the Cretaceous sediments (Chiplonkar and Tapaswi 1973). *Ditrupa* was also reported from the Palaeocene of Pondicherry (Kaye 1840), Upper Cretaceous of Uttattur and Trichinopoly groups, Tiruchirappalli (Chiplonkar and Tapaswi 1973), south India and late Cretaceous nodular limestone of Bagh Beds (Chiplonkar 1974), central India.

The skeletal material of most invertebrates is formed by calcium carbonate (CaCO_3) that is secreted by the organisms (Taylor 2008). These organisms can secrete three mineral polymorphs of CaCO_3 i.e., calcite, aragonite, and vaterite. Amongst these, calcite and aragonite are commonly available, whereas, vaterite is a rare biomineral. The mineral composition of the serpulid tubes may be calcitic, aragonitic or a combination of both these minerals (Lowenstam 1954; Bornhold and Milliman 1973; Simkiss and Wilbur 1989; Vovelle et al. 1991; Vinn et al. 2008a, 2008b, 2008d). The evolution of mineralogical composition of serpulid tubes is still poorly known. For understanding the biomineralization of serpulid tubes, Raman spectroscopy may have more advantages over several other methods. It is relatively rapid and non-destructive since the material is not consumed and subsequently the same specimen can be used for XRD, SEM and other studies.

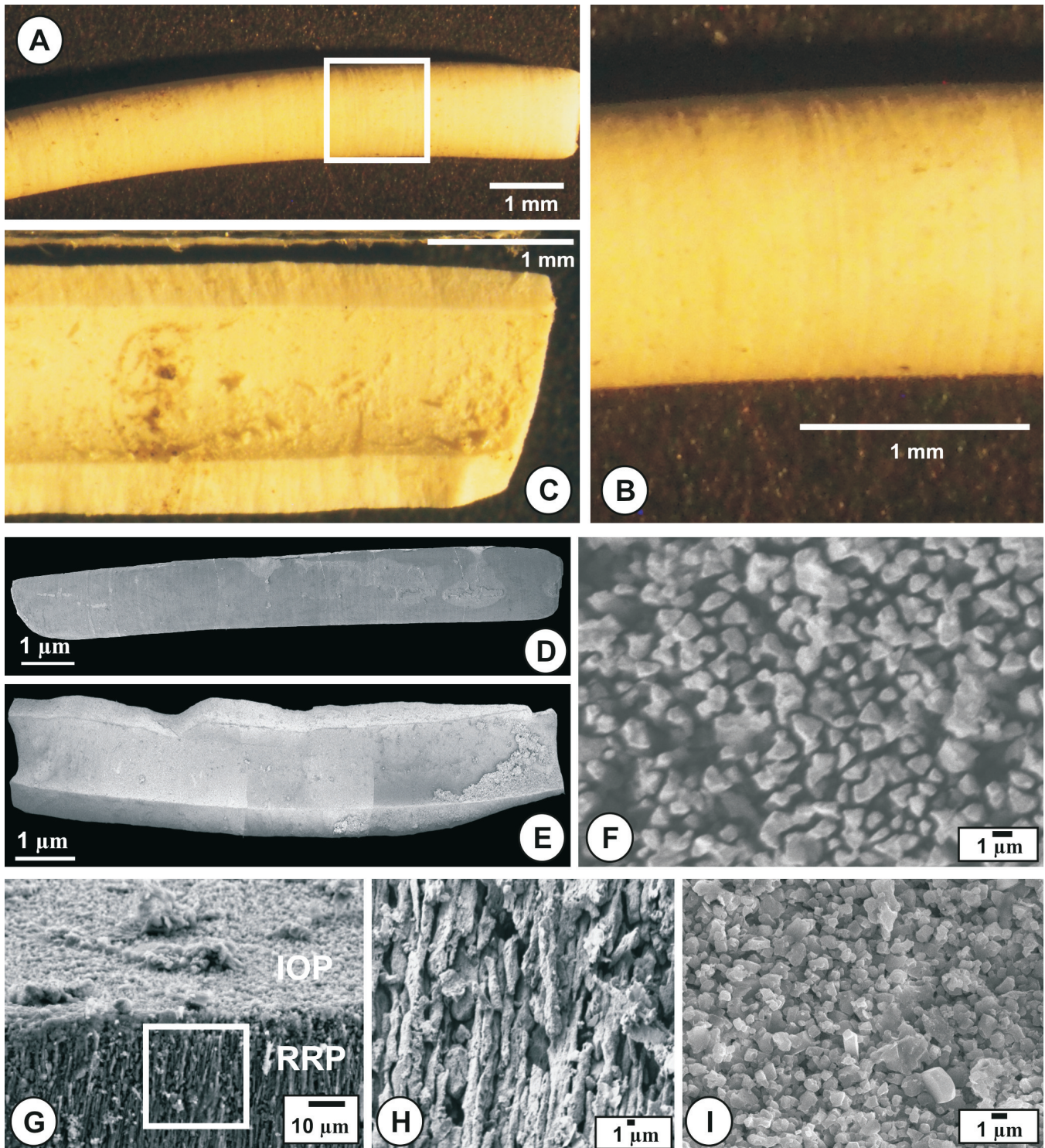
Recently a late Pliocene age has been determined for the samples that yielded serpulid tubes from the Neill West Coast Formation, Neil Island, Andaman and Nicobar Basin based on the planktonic foraminiferal assemblage that is dominated by

Globigerinoides ruber, *Globorotalia rubescens* and *Trilobatus* sp. (Dey et al. 2019).

The aims of this contribution are: (1) to record serpulid tubes assignable to *Ditrupa gracillima* for the first time from the late Pliocene sediments of Andaman and Nicobar Islands, (2) to analyse the ultrastructure and mineralogy of *Ditrupa* tubes and (3) to understand the palaeoecology of *Ditrupa* based on biotic and isotopic signatures.

Geological Settings

Andaman and Nicobar group of islands represents the subaerial part of the Arakan-Yoma Range that extends from Myanmar towards the north and to Java to the south. This islandic chain exposes an imbricate stack of rock strata that are interesting in the view of several geological and palaeontological studies. The structural complexity of these deposited sediments in different marine realms is stratigraphically significant and the imbedded fossils are important for deciphering the depositional environment. Predominantly deep water and occasionally shallow marine sediments of Neogene and Quaternary are well exposed in the Ritchie's Archipelago of Andaman and Nicobar group of islands. Neil Island of Ritchie's Archipelago exhibits well preserved marine micro and macrofossils. The outcrops present in this island ranges from late Miocene to early Pleistocene (Sharma and Srinivasan 2007). This study has been carried out on the samples collected from the west coast of Neil Island (text-fig. 1A). Lithostratigraphically, the outcrop belongs to Neill West Coast Formation and chronostratigraphically to the Taipan Regional Stage (late Pliocene). Before the present study, a number of micropalaeontological work on different as-



TEXT-FIGURE 2

Ditrupa gracillima A. Tusk shaped tube under light microscope (BSIP41930/a). B. Enlarged view of tube showing growth lines in the inset of Fig. 2A. C. Inner surface of the fractured tube showing growth lines under light microscope (BSIP 41930/b). D. SEM of tube showing the outer surface showing the growth lines (BSIP 41930/a). E. SEM of fractured inner surface of tube showing the growth lines (BSIP 41930/b). F. Magnified view of large prismatic crystals (RRP) in the outer part of the wall under SEM (BSIP 41930/b). G. Confluence of outer (RRP) and inner (IOP) surface under SEM (BSIP 41930/b). H. Longitudinal section of RRP external layer in the inset of Fig. 2G. I. Magnified view of inner crisscross (IOP) layer under SEM (BSIP 41930/b).

pects have been carried out from this rock horizon of Neil Island (Singh and Vimal 1974, 1976; Srinivasan and Azmi 1976a, 1976b; Kishore et al. 2015; Chakraborty et al. 2017; Dey et al. 2019) but this is the novel contribution on polychaete tubes that includes ultrastructural studies and palaeoenvironmental interpretation determined by isotopic analysis.

MATERIALS AND METHODS

The serpulid tubes isolated in the present study were collected from the type locality of Neill West coast Formation located at the west coast of Neil Island near the Natural Bridge (11°49'48.80" N, 93°00'54.50" E). The outcrop is about 11 m thick; however, only a thickness of ~4 m from the base is accessible owing to huge steepness and dense forest cover. Seventeen samples (BSIP 8903/01–8903/17) were collected from the measured accessible part of the outcrop (text-fig. 1B), however, only one sample (BSIP 8903/11) yielded the serpulid tubes. Lithologically it is composed of yellowish fragile calcareous limestone, occasionally lumpy and with concretions (text-fig. 1B).

Sediments were disaggregated using concentrated hydrogen peroxide (~35%) and heated for ten minutes on a hotplate. Later on, these treated sediments were kept in an ultrasonicator (about 10 minutes) for the removal of any organic matter. The samples were kept overnight and washed thoroughly with distilled water. The processed samples were then studied under an Olympus SZX16 Stereo zoom microscope. The specimens were isolated and kept in micropalaeontological slides for further study. No complete worm tubes were found; however, the lack of diagenetic alteration of many of the tube fragments enabled their morphology to be assessed in detail. Microphotographs were taken by a digital camera (Olympus OM-D E-M10) attached to the microscope. For detail analyses of its ultrastructure all the isolated specimens were dehydrated in ethanol, critical point-dried and covered with a 20 µm gold coating and examined under a JEOL JSM 7610f Field Emission Electron Scanning Microscopy (FESEM) at Birbal Sahni Institute of Palaeosciences (BSIP), Lucknow, India. The studied samples are housed at the museum of BSIP.

The world maps showing the distribution of identified serpulid genus (extinct and extant) and species (extant) have been created using the Free and Open Source QGIS 3.10 software (<http://www.qgis.org/>) with the GBIF (Global Biodiversity Information Facility) plugin (<http://www.gbif.org/>).

The elemental composition of the identified tubes was determined using an E-DAX detector attached to a JEOL JSM 7610f FESEM. In addition to this, the mineralogical composition of the tube fragments was also analysed using Laser Raman spectroscopy to determine the form of calcium carbonate present in the tubes. The intact tube was studied, and the inner surface of the tube was also analysed by splitting a tube into two halves. Spectroscopic data were obtained using a RENISHAW InVia Raman Microscope with macro-Raman and confocal micro-Raman capabilities at BSIP. A Renishaw Diode laser (785 nm) provided the spectra for the analyses. The 785 nm laser excitation coverage from 100 to 4000 cm⁻¹ was obtained that contains all the major Raman bands of calcite and aragonite. For this analysis, the specimens were centred in the path of the laser beam projected through the microscope attached to the instrument. Point spectra were obtained from various parts of the interior and exterior surfaces of the selected tubes.

For carbon and oxygen isotopic measurement in the carbonate samples, ~300–500 µg of powder samples as well as carbonate standards (IAEA-603, NBS 18, Carrara Marble; n=4 each) were kept into individual screw-capped glass vials. After flushing with Helium gas, 100% orthophosphoric acid (H₃PO₄) was injected into each vial which was kept at 72°C temperature bath for two hours. The evolved CO₂ was purified by Nafion tube and Pora pack column in Gas Bench and allowed into Continuous Flow Isotope Ratio Mass Spectrometer (CFIRMS, MAT 253) for isotopic analysis. Each measurement comprised three pulses of reference, followed by six pulses of sample CO₂ gas. All samples including standards were measured with respect to the calibrated tank gas, and the tank reference gas was calibrated by using IAEA-603. Isotopic data have been further corrected using linear calibration with two international standards (IAEA-603 and NBS 18). The isotopic data have been reported against VPDB with a precision of ±0.1‰ (1σ) for both δ¹⁸O and δ¹³C values. All the carbonate samples were measured at the BSIP stable isotope facility.

RESULTS

Systematic Palaeontology

Phylum ANNELIDA Lamarck 1809

Class POLYCHAETA Grube 1850

Order SABELLIDA Latreille 1825

Family SERPULIDAE Rafinesque 1815

Genus *Ditrupa* Berkeley 1835

Type Species: *Ditrupa arietina* Müller 1776

Ditrupa gracillima Grube 1878

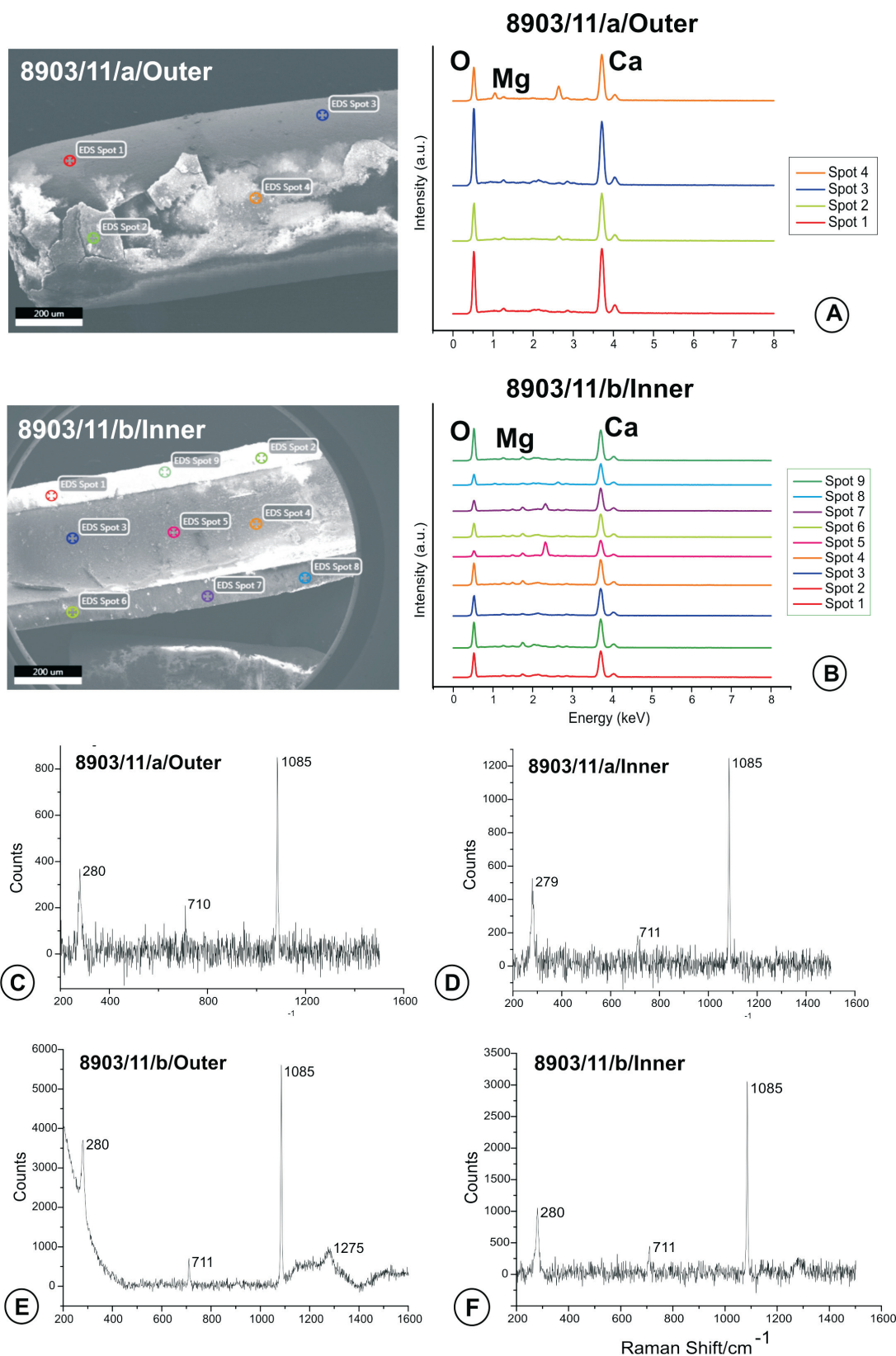
Text-Figure 2A–I

Description: It is characterised by a distinct calcitic exoskeleton in the form of a tusk-shaped tube (text-fig. 2A). Tubes free, circular in cross-section, open at both ends, slender, maximum available length of the tubes varies from 9 to 10 mm, and the diameter ranges from 1.7 to 1.9 mm. Outer and inner surfaces of the tube more or less smooth, bright white to pale yellowish in colour (text-fig. 2A–C), texture with fine growth lines (text-fig. 2B–E).

The ultrastructural studies reveal two calcitic layers. Scanning Electron Microscopy shows more or less smooth surface, and large prismatic crystals in outer part of wall (text-fig. 2F) and inner criss-cross layer (text-fig. 2G). The cross-section of these prismatic structures depicts multi-angular crystallite habits of trigonal symmetry. The diameter of each prism varies from 0.526–1.141 µm (text-fig. 2F). The prisms have regular ridges in between, and the longitudinal sections of the tubes show the external calcitic regular ridged prismatic (RRP) layer (text-fig. 2G–H). The fractured surface of the tubes in the longitudinal section of irregularly oriented prismatic (IOP) layer shows needles embedded in the matrix (text-fig. 2I). At the contact of the two layers of the tube, some of these IOP needles are inserted in the RRP layer (text-fig. 2G).

Remarks: The present specimens resemble *Ditrupa gracillima* described by Vinn et al. (2008a) from the middle Miocene of Australia (Specimen: ZMA V. Pol. 3558, Vinn et al. 2008b, text-fig. 5E).

Stratigraphic range: Stratigraphically the genus *Ditrupa* ranges from ?early Jurassic (Pliensbachian, ~190 Ma) (Rosenkrantz 1942) and *D. gracillima* ranges from Miocene to Recent (ten Hove and Smith 1990; Vinn et al. 2008b).



TEXT-FIGURE 3

A-B. EDX spectra of outer and inner layers of *Ditrupa gracillima* tubes at random spots. C-F. Diagnostic calcite peaks obtained from Raman spectra of *Ditrupa gracillima*.

TABLE 1
Oxygen and carbon isotopic values of the serpulid tubes.

Analysed Samples	$\delta^{13}\text{C}$ values (‰; VPDB)	$\delta^{18}\text{O}$ values (‰; VPDB)
8903/11/FM 3	-14.78	1.02
8903/11/FM 5	-8.70	1.11
8903/11/FM 6	-6.04	1.04
8903/11/FM 8	-10.91	0.87
8903/11/FM 9	-8.07	0.94
8903/11/FM10	-17.54	0.86
8903/11/FM11	-15.29	-0.66

Occurrence: This species is mostly tropical and at present widely distributed in the Indo-West Pacific region.

Specimen Repositories: Museum of BSIP, Lucknow, Specimen numbers 41930/a and 41930/b.

Associated biota: The analysed samples that yielded the polychaete tubes are associated with shells of sea urchins, bivalves and gastropods, planktonic foraminifers (Dey et al. 2019), and benthic calcareous algae and foraminifera in thin sections (Chakraborty et al. 2017).

Mineralogical composition of tubes

The main objective for EDX analysis was to infer the mineralogy of *D. gracillima*. Presence of Magnesium (Mg) or Strontium (Sr) is used as proxies to denote whether the tubes are calcitic or aragonitic in nature (Kupriyanova et al. 2014). The EDX analysis of all the tubes detected most prominent Calcium (Ca) and low Magnesium (Mg) peak suggesting Magnesian calcitic composition (text-fig. 3A-B). The Raman spectral analysis also shows the same calcitic nature of the tubes on the different spots in the outer (text-fig. 3C, 3E) and inner (text-fig. 3D, 3F) layers of the tubes. The results indicate that both the outer and inner layers of the tubes are made up of calcite as biomineral (text-fig. 3C-F). As opined by Taylor et al. (2010) and Kupriyanova et al. (2014) the peaks of molecular and lattice vibrational modes are very well separated in the Raman spectra for calcite and aragonite. The high-frequency band that corresponds to the vibrational modes of carbonate anion (CO_3^{2-}) is very clear and closely placed at $\sim 1085\text{ cm}^{-1}$ (text-fig. 3C-F). This peak is similar for both the polymorphs of CaCO_3 (calcite and aragonite) that makes difficulty in differentiating. The other two bands at ~ 710 and $\sim 283\text{ cm}^{-1}$ (text-fig. 3C-F) for calcite are completely different from the aragonite. These analyses provide an unequivocal determination of the tube mineralogy as calcite.

$\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values of *Ditrupa gracillima*

The $\delta^{18}\text{O}$ values of *D. gracillima* vary from -0.66 to 1.1‰ (Table 1) and covering 1.8‰ variations with an average value of 0.7‰. On the other hand, $\delta^{13}\text{C}$ values show large variations and range from -17.5 to -6.0‰ (Table 1) with an average value of -11.5‰.

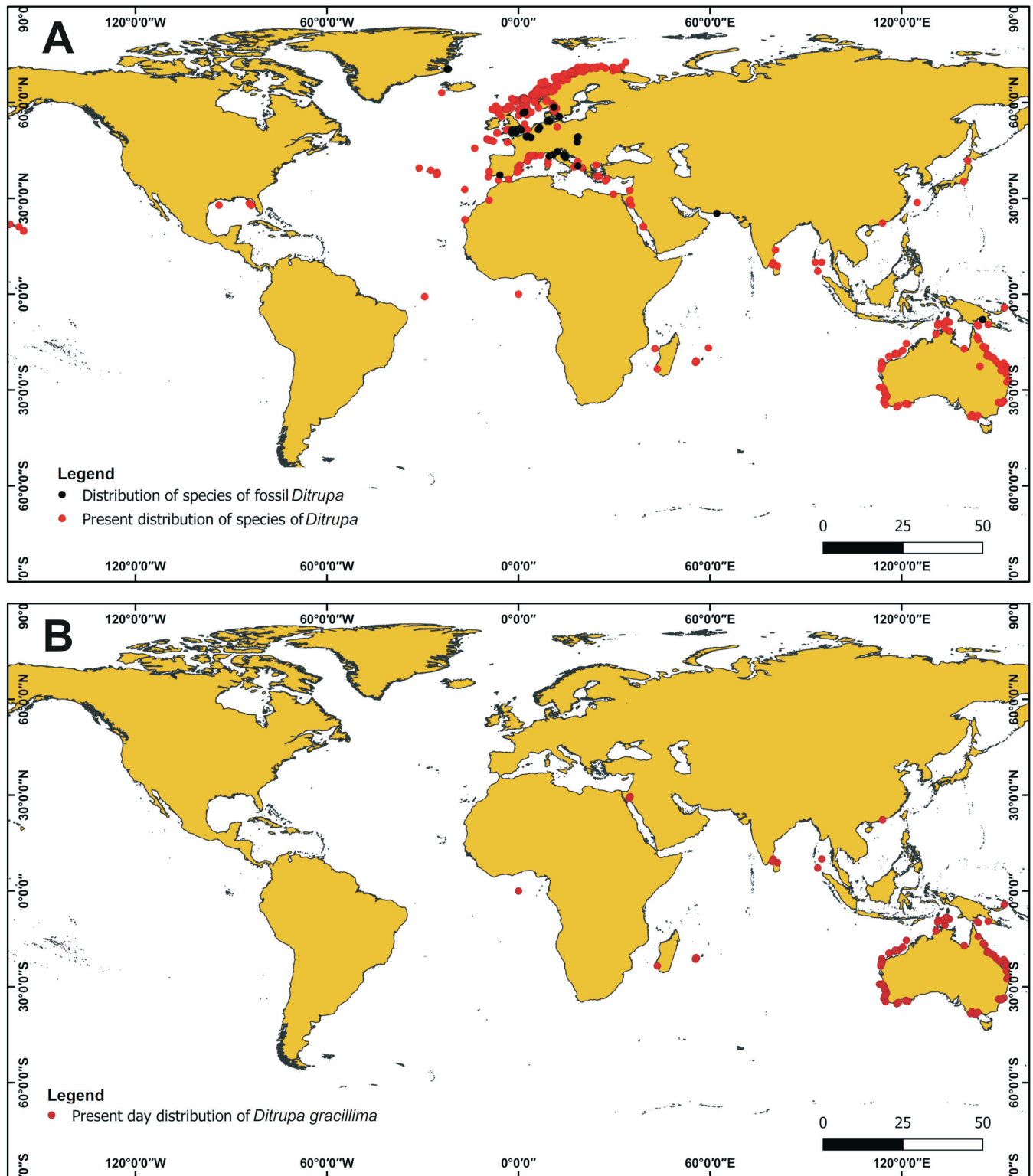
DISCUSSION

Serpulids are highly successful group of organisms that are geographically widely distributed and have wide bathymetric distribution, from the intertidal to the abyssal zones (Vinn et al.

2013). Serpulids appeared in the Permian and diverse tube microstructures are known since the Jurassic (Vinn et al. 2008b, 2013; Ippolitov et al. 2014; Sanfilippo et al. 2017). Kupriyanova et al. (2006) traced the evolutionary history of serpulids based on both morphological and molecular data. According to the evolutionary history of serpulids (Annelida, Polychaeta) based on 18S rDNA analysis, the family Serpulidae stands as a monophyletic group and within this there are four monophyletic clades referred as Protula-group, Serpula-group, Pomatoceros-group, and Spirorbinae-group (Lehrke et al. 2007). Based on these studies, the genus *Ditrupa* has been assigned in the *Pomatoceros*-group (Kupriyanova et al. 2006; Lehrke et al. 2007). This genus is represented by two extant species viz., *D. arietina* and *D. gracillima* and ten extinct species (Accessed through: World Register of Marine Species (WoRMS) at: <http://www.marinespecies.org/aphia.php?p=taxdetails&id=129561> on 2020-01-22). The extinct and extant *Ditrupa* is distributed mostly in all parts of the world (text-fig. 4A); however, the extant *D. gracillima* is mostly restricted in the Indo-Pacific region (text-fig. 4B).

The ultrastructural study shows that both *D. gracillima* and *D. arietina* are very similar. These two species only can be differentiated by their outer tube morphology (ten Hove and Smith 1990). The outer tube layer in *D. gracillima* is not as hyaline as in *D. arietina*, whereas, the inner tube layer is opaque for both the species (ten Hove and Smith 1990). Several studies on *D. arietina* have been carried out on its microstructure and geochemical aspects, but the present contribution is one of the first comprehensive studies on *D. gracillima*. ten Hove and Smith (1990) mainly concentrated their studies on the morphology of recent and some fossil specimens of *D. gracillima*. Since fossil specimens do not possess all the characters that are evident in a recent specimen, it is complicated to identify the fossil specimens. The present study has enhanced the potentiality for identifying fossil polychaete tubes and their palaeogeographical distribution in time and space. Most of the recent serpulid tubes have irregularly oriented prismatic structure (IOP) which occurs in 60% of serpulid species (Vinn et al. 2008b) and it is also common in fossil serpulids (Vinn 2007; Vinn et al. 2008b, 2012; Vinn et al. 2014; Kupriyanova et al. 2014). The presently studied tubes are identified as *D. gracillima* as the outer tube is not hyaline, and they possess IOP and RRP layers as observed in the ultrastructural studies. Similar features were also observed earlier by ten Hove and Smith (1990).

The outer layer of calcareous animals may be made up of aragonite, calcite, or it may be a mixture of both mineral phases (Taylor et al. 2010). The mineral composition of all calcareous polychaete tubes is not known. All Serpulid tubes are made up of CaCO_3 . Amongst the two mineral polymorphs of CaCO_3 , calcite is the most stable biomineral in serpulids (Kupriyanova et al. 2014). The high-frequency region of the spectral band obtained from the studied specimens clearly shows the dominance of vibrational mode of carbonate anion CO_3^{2-} with a major peak at $\sim 1085\text{ cm}^{-1}$. This peak certifies that the shell is made up of calcite. The low-frequency Raman band was observed at $\sim 280\text{ cm}^{-1}$. This also determines that both the outer and inner surface of the tube is composed of calcite. In all the EDX analyses, Strontium (Sr) was not detectable, but Mg was present. This indicates that the studied tubes are composed of calcite, and that was also opined by Taylor et al. (2010). Altogether, the elemental analyses corroborate the calcitic mineralogy of the studied



TEXT-FIGURE 4
A. Global distribution of extinct and extant *Ditrupa*. B. Present day distribution of *Ditrupa gracillima*.

serpulid tubes determined by Laser Raman spectroscopy and Energy-dispersive X-ray spectroscopy. Sometimes *Ditrupa* tubes are misidentified as a scaphopod mollusc, but calcite mineralogy of the tubes confirms that these tubes are not of Scaphopoda which are composed of aragonite (Bøggild 1930).

Our isotopic study of *Ditrupa gracillima* is important to understand its ecology. This study reveals that the identified *Ditrupa gracillima* tubes is characterized by the most ^{13}C -depleted values (up to -17.5‰ , Table 1) but nearly constant and higher $\delta^{18}\text{O}$ values. It further indicates that the isotopic signals are virtually unaffected during early diagenesis and contain pristine isotopic signals. Therefore, oxygen isotope signals preserved in the *D. gracillima* tubes reflect the $\delta^{18}\text{O}$ of marine waters in which these organisms precipitated their shells in isotopic equilibrium and ^{13}C value provide information regarding the sources of carbon into marine water. Study suggests that the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ value of recent *Ditrupa arietina* tubes are characterised by normal marine isotopic values which ranges from $+1.48$ to $+1.80\text{‰}$ and from $+3.24$ to $+3.54\text{‰}$, respectively (Aguirre et al. 2015). The $\delta^{18}\text{O}$ values in the present study shows a close similarity with these modern isotopic values, which further suggests typical marine water signature. Overall, the $\delta^{18}\text{O}$ value shows $\sim 1.8\text{‰}$ variations within *Ditrupa* that suggest a change in marine water isotopic composition.

On the other hand, the identified *Ditrupa* tube shows very low $\delta^{13}\text{C}$ values (Table 1). These lower $\delta^{13}\text{C}$ values are similar to the earlier reports from the upper Miocene heterozoan carbonates of Seville, southern Spain (Aguirre et al. 2015) and Santa Monica Basin, California (Georgieva et al. 2019). Such abnormal lower $\delta^{13}\text{C}$ values in the studied *Ditrupa* suggest an extra supply of light carbon (^{12}C) in the water column probably due to cold seeps (Aharon et al. 1992; Valentine et al. 2001; Charlou et al. 2003; Lietard and Pierre 2009). Organisms living near to the hydrocarbon seepage might incorporate available lighter carbon and precipitate their carbonate skeletons directly from the seawater. In this condition, the organism living in the proximity of hydrocarbon seepages will be characterised by anomalously negative $\delta^{13}\text{C}$ values due to the influence of the oxidised methane fluids released into the water column.

At present polychaete annelids commonly live in the marine to brackish environment (Yang et al. 2012). Most of them are found generally at the shallower part of the ocean; however, some also can thrive in deeper water (Kupriyanova and Nishi 2010; Vinn et al. 2013). In addition, some euryhaline species can live in brackish water, and a few species live in freshwater, too (Yang et al. 2012). Mostly polychaetes prefer the benthic habitat. In the modern oceans, serpulids are one of the dominant groups amongst the benthic biota. They live in the tidal zone up to 950 m water depth; however, majority of polychaetes dwell in 3 to 65 m shallow water (ten Hove and Kupriyanova 2009; Kupriyanova and Nishi 2011; Yang et al. 2012). The present study area also documents other benthic organisms which denote that the deposition was in the shallower part of the ocean. The source of calcium that is utilised by the serpulids is at least in part from the adjacent water (Vinn et al. 2008b). The ultrastructure does not depict any difference in different waters like marine, brackish and fresh (Vinn et al. 2008b), however, isotopic analysis provides, a clue that the *D. gracillima* recorded herein thrived in a shallow marine environment.

CONCLUSIONS

- The tube morphological features (light microscopic) and their ultrastructure (SEM) have been utilised for taxonomic analysis in identifying the serpulid tubes.
- The ultrastructural studies (both RRP and IOP structures) clearly identify the serpulid tubes as *Ditrupa gracillima*.
- The mineralogical studies (Raman spectroscopic and EDS analyses) of these serpulid tubes also reconfirm its affinity as both these studies envisage calcite mineralogy.
- The $\delta^{13}\text{C}$ values of the *Ditrupa gracillima* tubes indicate that most likely these organisms living in the proximity of hydrocarbon seepages and might precipitate their shells incorporating light carbon isotopes (i.e., ^{12}C) in their carbonate skeletons directly from the seawater. The isotopic analysis also provides an indication that the identified *D. gracillima* tubes thrived in the shallower part of the ocean.

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