

1 **Bone-eating *Osedax* worms lived on Mesozoic marine reptile deadfalls**

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15 **Abstract**

16 We report fossil traces of *Osedax*, a genus of siboglinid annelids that consume the  
17 skeletons of sunken vertebrates on the ocean floor, from Early-Late Cretaceous (~100  
18 million years, Myr) plesiosaur and sea turtle bones. Although plesiosaurs went extinct at  
19 the end-Cretaceous mass extinction (66 Myr), chelonioids survived the event and  
20 diversified, and thus provided sustenance for *Osedax* in the 20 Myr gap preceding the  
21 radiation of cetaceans, their main modern food source. This finding shows that marine  
22 reptile carcasses, before whales, played a key role in the evolution and dispersal of  
23 *Osedax*, and confirms that its generalist ability of colonizing different vertebrate  
24 substrates, like fishes and marine birds, besides whale bones, is an ancestral trait. A  
25 Cretaceous age for unequivocal *Osedax* trace fossils also dates back to the Mesozoic the  
26 origin of the entire siboglinid family, which includes chemosynthetic tubeworms living at  
27 hydrothermal vents and seeps, contrary to phylogenetic estimations of a late Mesozoic-  
28 Cenozoic origin (~50-100 Myr).

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31 **1. Introduction**

32 The exploration of the deep sea in the last decades has led to the discovery of many new  
33 species with unique adaptations to extreme environments, raising important questions on  
34 their origin and evolution through geological time (1, 2). *Osedax* is a genus of marine  
35 worms that colonize the bones of marine vertebrates, mostly whales, sunken to the deep  
36 sea-floor (3). It belongs to the Siboglinidae family of annelids that, as adults, lack mouth  
37 and digestive system and are nutritionally dependent on endosymbiotic bacteria (4).  
38 Among siboglinids, *Osedax* has developed a unique metazoan-bacteria symbiosis that  
39 exploits the organic material sequestered within the bones of dead vertebrates as an  
40 energy source. The posterior body of *Osedax* penetrates into the bone using root-like  
41 structures (figure 1*a-b*). The root epithelium absorbs bone collagen and lipids, which are  
42 possibly metabolized by heterotrophic symbiotic bacteria that serve for *Osedax* nutrition  
43 (3, 5). The anterior part of the body, the trunk, extends into the water and is crowned with  
44 respiratory palps (6).

45 *Osedax* is a highly speciose clade, found at depths ranging from 21-4000 m, with a near  
46 global geographic distribution (7, 8). The origins and causes of this diversity are an  
47 unresolved aspect of its biology and evolution. Molecular age estimations suggest that  
48 either *Osedax* split from its siboglinid relatives ~45 Myr ago, possibly coincident with the  
49 origins of large archeocete cetaceans during the Eocene (3, 7), or ~125 Myr ago in the  
50 Cretaceous, when it could have lived on the bones of large marine Mesozoic reptiles (7, 9).  
51 Only direct fossil evidence of the trace fossil left by *Osedax* worms can confirm which of  
52 these scenarios is correct, as it is unlikely for the soft-bodied animal itself to be

53 preserved. The oldest *Osedax* traces known to date come from ~30 Myr whale and fish  
54 bones, indicating a generalist ability to thrive on different vertebrate substrates (10, 11).  
55 Here we show that *Osedax* colonized the bones of plesiosaurs and cheloniids in the  
56 Cretaceous, validating the hypothesis of a Mesozoic origin of the clade, and provide  
57 important implications for the evolution of the entire Siboglinidae family.

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## 59 **2. Materials and Methods**

60 Traces resembling those created by recent *Osedax* species were found on (i) one isolated  
61 plesiosaur humerus from the Cenomanian (~100-93.9 Myr) Cambridge Greensand  
62 Member, UK, whose fossil fauna is mainly reworked from the top of the underlying late  
63 Albian Gault Formation (~100 Myr); and (ii) two fragmentary bones (rib and costal plate)  
64 belonging to a marine turtle, family Cheloniidae, originally referred to *Chelone camperi?*  
65 Owen 1851, collected from the Cenomanian Grey Chalk Subgroup (Burham, Kent, UK)  
66 (figure 1). Specimens are curated in the Sedgwick Museum of Earth Sciences, University  
67 of Cambridge, UK. No invertebrate remains are associated with them. The bones were  
68 investigated using micro computed tomography (CT), a well-established method that  
69 allows the morphology of subsurface bone structures to be quantitatively described,  
70 providing their three-dimensional reconstruction (12) (*see* electronic supplementary  
71 material, ESM, for details on the specimens, figure S1; geological setting; and analytical  
72 methods).

73

## 74 **3. Results**

75 Digital removal of the matrix overlying the plesiosaur humerus revealed that *Osedax*  
76 bioerosion is concentrated in the center of the bone (figure 1*d*). Two intact individual  
77 borings were identified on the periphery of the bioeroded area (figure 1*d*). The borings  
78 consist of circular surficial openings (diameter 0.56 & 0.36 mm) with a uniform tube  
79 section that expands to an irregularly shaped chamber inside the bone (figure 1, figure S2,  
80 Table 1). Similar borings were identified on the cheloniid fragments (figure 1*i-k*, figure  
81 S3). The costal plate, even if heavily eroded, shows some intact borings. The largest  
82 example consists of a small circular surface opening (diameter 0.20 mm) with a laterally  
83 expansive, but shallow subsurface chamber (figure 1*j-k*). The rib shows 15 small  
84 subcircular holes that extend as long tubes into the bone, terminating in expanded  
85 irregular chambers (figure S3). These chambers are generally smaller than those  
86 identified on the plesiosaur skeleton (Table 1).

87 *Osedax* often colonize bones in such dense aggregations that the cavities formed by their  
88 root systems merge together under the bone surface (13). Therefore only cavities with a  
89 single borehole reflect the shape of the root system of an individual animal and are of  
90 particular diagnostic value (13). The tube section represents the trunk of *Osedax*, partially  
91 embedded within bone matrix in modern specimens (14), whereas the chamber represents  
92 the hole left by the ovisac and root structure (figure 1*b-c*). Although modern *Osedax*  
93 borings display a diverse range of morphologies (12), the combination of a narrow  
94 opening with laterally-expansive irregular subsurface chambers are diagnostic features of  
95 *Osedax* activity (13). Once buried, larger borings may collapse, leaving behind rounded  
96 pockmarks in the bones, as shown in our samples (figure 1*d,i*). The intact borings  
97 identified here are relatively small, but their sizes are within the range of known *Osedax*

98 borings (12) and consistent with other fossil *Osedax* traces (Table 1). In modern *Osedax*,  
99 the morphology of the borings of a single species is consistent in the same bone but  
100 changes between bone types (12), suggesting that each of the fossil bones in this study  
101 was colonized by a single species, although it is not possible to estimate the total number  
102 of species.

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#### 104 **4. Discussion**

105 Together with chemosynthesis-based associations of molluscs found on Late Cretaceous  
106 plesiosaurs (15), our discovery confirms that vertebrate deadfall communities developed  
107 several times in the geological past, not only on whale-falls, and had a key role in the  
108 dispersal and evolution of specialized fauna (16). Plesiosaurs were a group of diverse,  
109 cosmopolitan marine reptiles fully secondary adapted to aquatic life, with largest species  
110 reaching up to 15 meters in length, a size comparable to that of modern sperm whales  
111 (17). Whereas they could have contributed to the evolution and dispersal of *Osedax*  
112 during the Cretaceous, they went extinct at the Cretaceous-Paleogene boundary, together  
113 with other large marine reptiles such as mosasaurs, leaving an almost 20 Myr gap for  
114 *Osedax* to survive before the evolution of whales (figure 3). Our finding of *Osedax* traces  
115 also on Late Cretaceous sea turtles reinforces the hypothesis of *Osedax* survival and  
116 diversification through the Paleogene, as suggested by phylogenetic analyses (7).  
117 Differently from other marine reptiles originated in the Mesozoic, sea turtles show their  
118 highest diversity during the Late Cretaceous–Early Paleogene, when they were  
119 represented by a large number of taxa with diverse ecological adaptations to aquatic life  
120 (18). Thereafter, sea turtles, together with teleost fishes, whose most modern clades

121 diversified during the Paleogene (19), might have provided sustenance for *Osedax*  
122 through this time interval. The finding of bone-eating limpets (*Osteopelta*), typical of  
123 whale-falls, on an Eocene leatherback turtle (20), supports the hypothesis that sea turtles  
124 sustained organisms later adapted to live on whale bones.

125 Our finding has also important implications for the debated age of the entire Siboglinidae  
126 family and their evolutionary leap from conventional heterotrophic annelids to  
127 specialized forms adapted to extreme chemosynthetic environments, such as  
128 hydrothermal vents, cold seeps and whale-falls (4). Molecular data indicate a late  
129 Mesozoic or Cenozoic (~50-100 Myr) origin for the siboglinids (1, 21), whereas their  
130 fossil record goes back to the Mesozoic or even to the Paleozoic (22). The Cretaceous  
131 *Osedax* traces presented here provide a firm calibration point for the molecular clock of  
132 the siboglinid phylogenetic tree, placing a common siboglinid ancestor as far back as the  
133 mid-Cretaceous, and the origin of the Siboglinidae family even earlier still. This adds to  
134 the growing evidence that the Cretaceous was a key period for the evolution of modern  
135 chemosynthetic communities (1, 23).

136 The evolution of *Osedax* has been viewed as an extreme adaptation to deep-sea  
137 environments since most species of *Osedax* have been discovered at bathyal depths, but  
138 shallower species have been documented from the Antarctic, Japan and the Northeast  
139 Atlantic (24). *O. mucofloris* has been found at multiple sites from 30-125 m depth and is  
140 the only species known from the North Atlantic (14). Our findings show that *Osedax*  
141 species occupied similarly shallow waters (*see* Geological setting in ESM) in this region  
142 early on in the clade's evolutionary history. The increasing evidence for *Osedax* in shelf-  
143 depth settings combined with their propensity to rapidly consume a wide range of

144 vertebrate skeletons, suggests that *Osedax* may have had a significant negative effect on  
145 the preservation of marine vertebrates in the fossil record. The true extent of this “*Osedax*  
146 effect”, previously hypothesized only for the Cenozoic (10), now needs to be assessed for  
147 Cretaceous marine vertebrates.

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153 **Author Contributions.** SD designed the research. NDH analyzed the micro-CT scan  
154 images and created the 3D reconstructions. SD and NDH wrote the paper.

155 **Data accessibility.** Data for this study are available at Dryad (doi:10.5061/dryad.k4d0q).

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158 **Competing interests.** The authors declare that they have no competing interests.

159

160 **Figure Legends**

161 **Figure 1.** Modern and fossil *Osedax* borings. (a) *Osedax* trunk and palps emerging from  
162 whale bone. (b) *Osedax* ovisac and root tissue. (c) CT reconstruction of a modern boring  
163 created by *O. frankpressi*. (d) CT reconstruction of plesiosaur bone (semi-transparent),  
164 with two *Osedax* borings reconstructed in orange. (e-f) Boring-1 digitally dissected and  
165 close up in situ. (g-h) Boring-2 digitally dissected and close up in situ. (i) CT  
166 reconstruction of cheloniid bones. Arrow indicates borings in *j* & *k*. (*j-k*) Boring in  
167 cheloniid costal plate. (c) modified from Higgs et al. (12). Scale bars are 1 cm and scale  
168 meshes have spacing of 1 mm.

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170 **Figure 2.** Stratigraphic range of marine vertebrates (Cetacea, Plotopteridae, teleost fishes,  
171 Plesiosauria, Chelonioidea) associated with modern and fossil evidence of *Osedax* worms.  
172 Molecular age estimations of the origin of the *Osedax* clade from Vrijenhoek et al. (7).  
173 Data on modern and fossil occurrence of *Osedax* from Smith et al. (8); Kiel et al. (10, 11,  
174 25), Higgs et al. (13).

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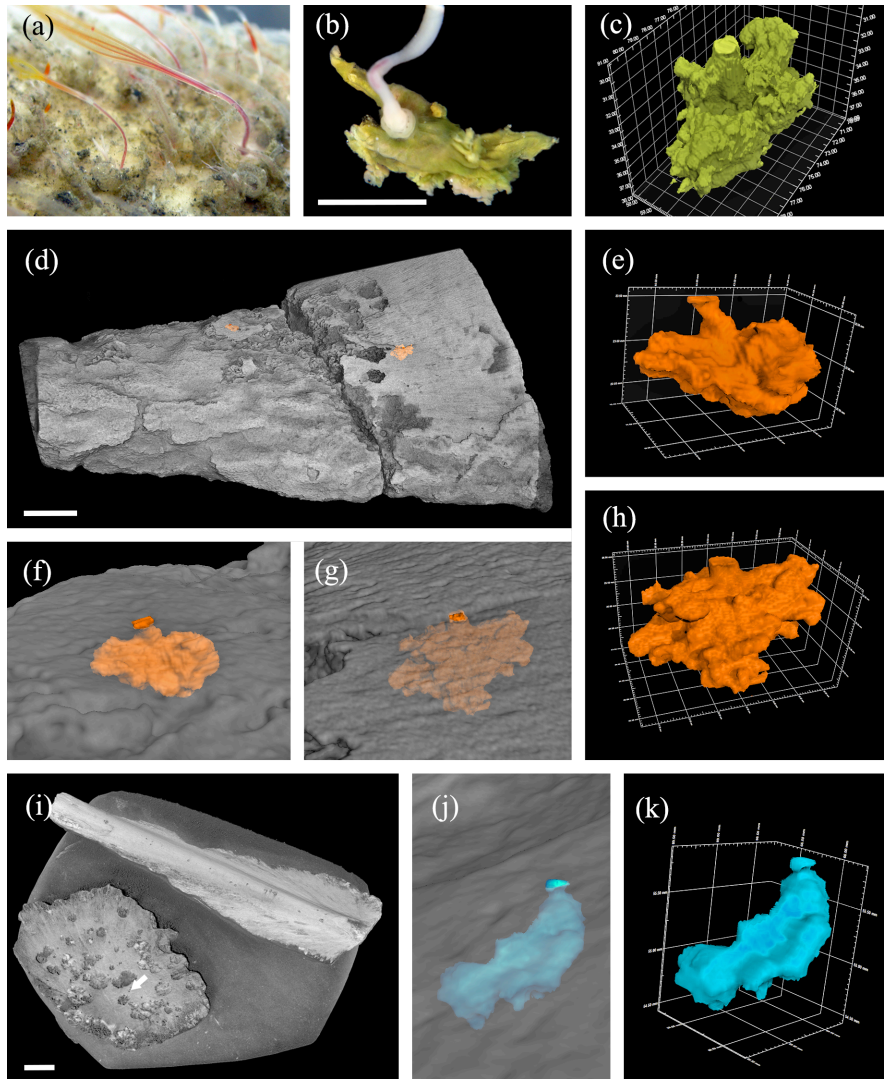
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242 **Table 1.** Quantitative morphometrics of individual *Osedax* borings in fossil Mesozoic  
 243 reptile bones (this study) compared with *Osedax* borings in Cenozoic fossil bones.

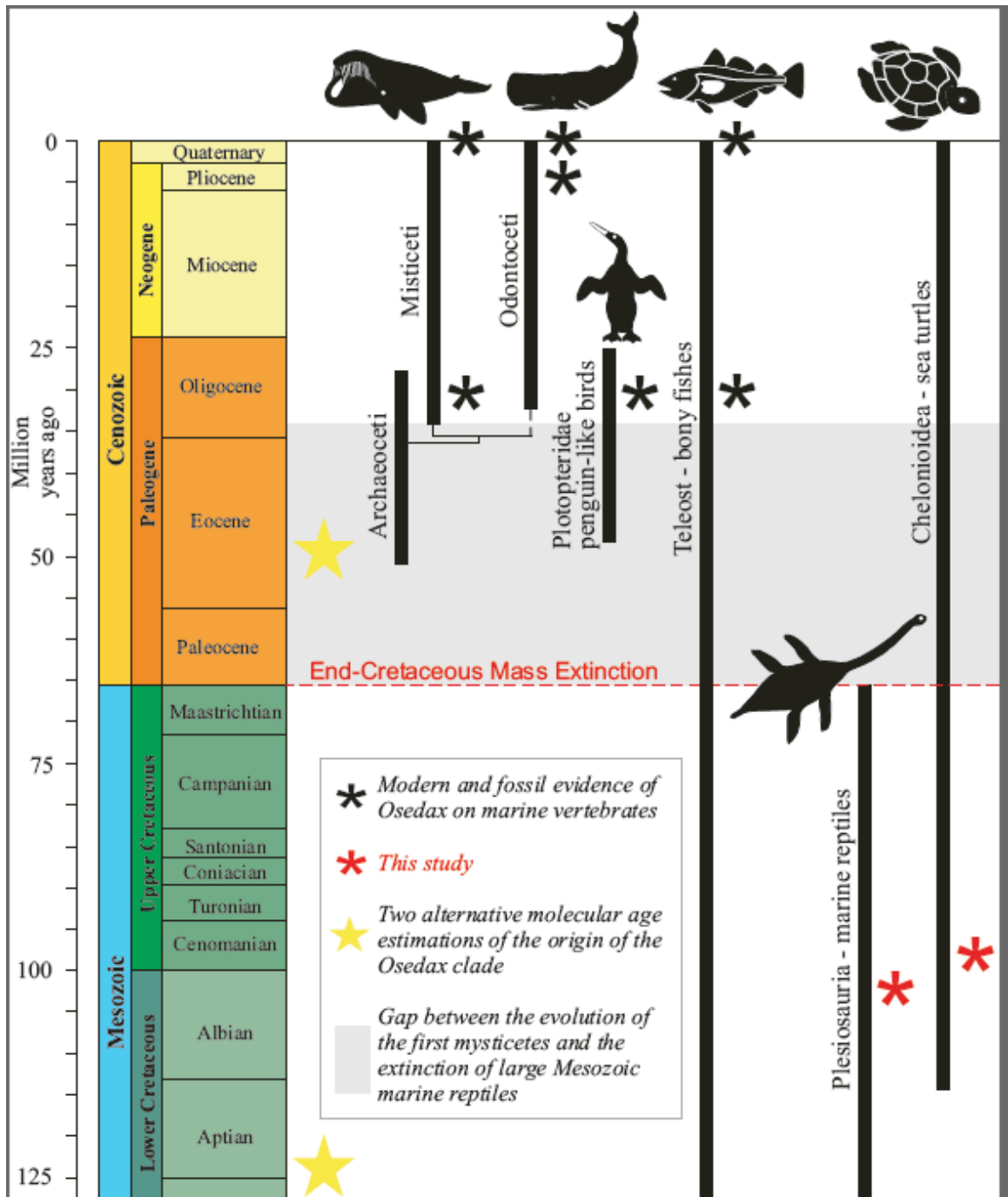
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Fossil bone	Age	Aperture diameter (mm)	Length (mm)	Width (mm)	Depth (mm)	Volume (mm <sup>3</sup> )	Surface Area (mm)	Source
Plesiosaur humerus (1)	Albian	0.56	3.50	2.30	1.93	2.70	29.78	This study
Plesiosaur humerus (2)	Albian	0.36	2.04	1.31	1.20	0.65	6.32	This study
Cheloniid rib (1)	Cenomanian	0.83	2.44	1.69	0.94	0.54	2.88	This study
Cheloniid rib (2)	Cenomanian	0.53	1.19	1.19	0.88	0.14	2.57	This study
Cheloniid rib (3)	Cenomanian	0.18	0.62	0.62	0.62	0.07	1.17	This study
Cheloniid costal plate (7)	Cenomanian	0.20	1.25	0.51	1.44	0.23	3.21	This study
Whale bone	Oligocene	0.10–0.45	-	-	1.7	-	-	Ref. 10
Avian bone	Oligocene	<0.3-1.5	-	-	-	-	-	Ref. 25
Whale teeth	Oligocene	max 0.50	max 3.00	-	2.3	-	-	Ref. 11
Fish bone	Oligocene	~0.1	-	6.5	2	-	-	Ref. 11
Whale bone	Pliocene	0.50–0.71	-	-	2.3–4.5	-	-	Ref. 13



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Figure 1



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FIGURE 2