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

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12 Keywords: Siboglinidae, taphonomy, Cretaceous, marine reptile, whale-fall

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14

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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30

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.

58

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.

103

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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149

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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.

169

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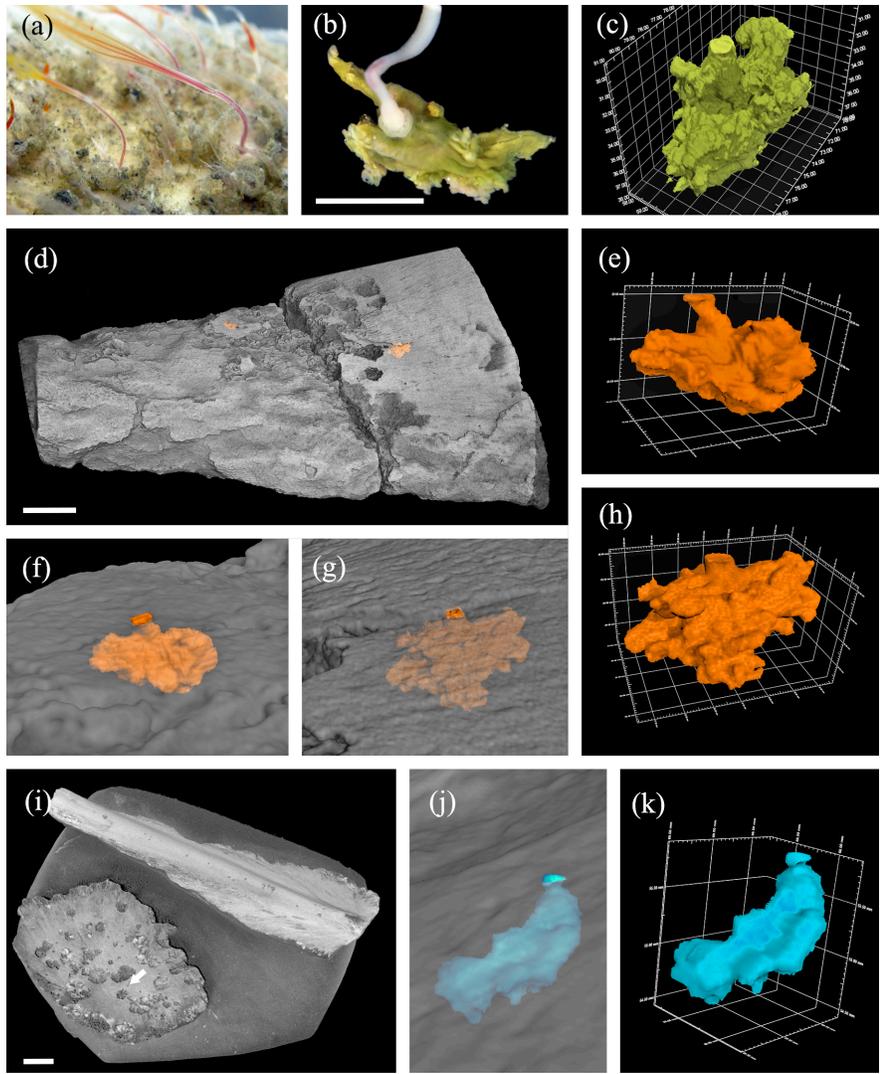
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242 **Table 1.** Quantitative morphometrics of individual *Osedax* borings in fossil Mesozoic
 243 reptile bones (this study) compared with *Osedax* borings in Cenozoic fossil bones.

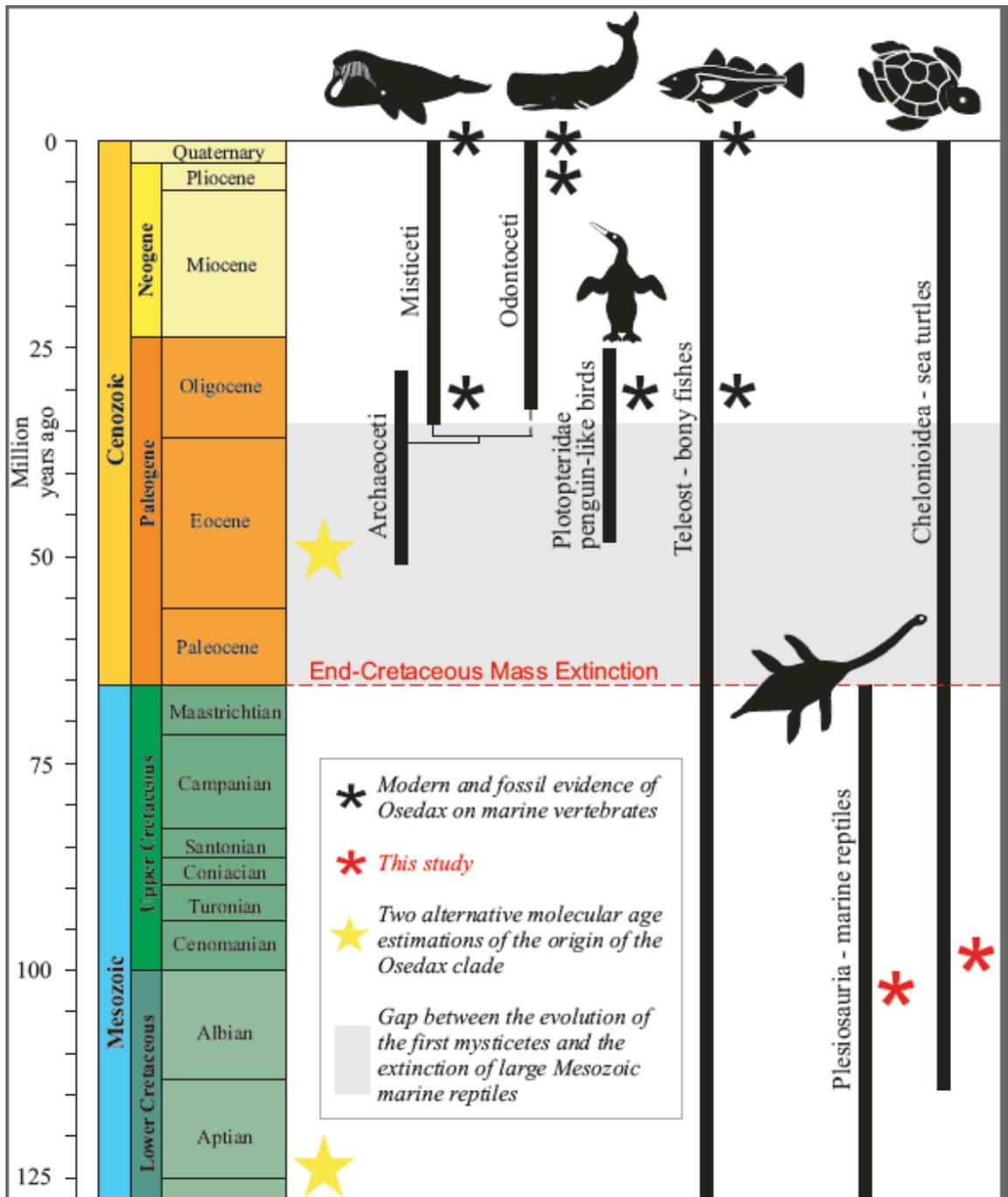
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Fossil bone	Age	Aperture diameter (mm)	Length (mm)	Width (mm)	Depth (mm)	Volume (mm ³)	Surface Area (mm)	Source
Plesiosaur humerus (1)	Albian	0.56	3.50	2.30	1.93	2.70	29.78	This study
Plesiosaur humerus (2)	Albian	0.36	2.04	1.31	1.20	0.65	6.32	This 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