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Marine worms (genus *Osedax*) colonize cow bones

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Bone-eating worms of the genus *Osedax* colonized and grew on cow bones deployed at depths ranging from 385 to 2893 m in Monterey Bay, California. Colonization occurred as rapidly as two months following deployment of the cow bones, similar to the time it takes to colonize exposed whalebones. Some *Osedax* females found on the cow bones were producing eggs and some hosted dwarf males in their tubes. Morphological and molecular examinations of these worms confirmed the presence of six *Osedax* species, out of the eight species presently known from Monterey Bay. The ability of *Osedax* species to colonize, grow and reproduce on cow bones challenges previous notions that these worms are 'whale-fall specialists.'

Keywords: Annelida; Polychaeta; Siboglinidae; whale-fall; cytochrome-*c*-oxidase subunit I

1. INTRODUCTION

Recent studies of submerged whale carcasses (whale-falls) have revealed a potentially worldwide distribution of bone-eating polychaete worms of the genus *Osedax* (Annelida, Siboglinidae; Rouse *et al.* 2004, in press; Glover *et al.* 2005; Dahlgren *et al.* 2006; Fujikura *et al.* 2006, 2007). Lacking a mouth and gut, *Osedax* females host heterotrophic bacterial symbionts (Oceanospirillales) in a branching 'root' system that invades the bone to extract organic compounds (Goffredi *et al.* 2005, 2007). A time-series analysis of whale carcasses deployed at varying depths in Monterey Bay, California, has revealed that exposed bones are colonized as rapidly as two months following their deposition on the seafloor (Braby *et al.* 2007). Such rapid colonization of a spatially scattered and temporally unpredictable food resource requires an immense pool of propagules, which disperse as fertilized eggs and developing larvae, though their location in the water column is presently unknown. Indeed, genetically based estimates of *Osedax* population sizes are large and comparable with those of other deep-sea polychaetes (Rouse *et al.* 2004, in press).

Osedax have been called 'whale worms' and 'whale-fall specialists' by several researchers (Glover *et al.* 2005; Dahlgren *et al.* 2006; Fujikura *et al.* 2006). Historically, the large lipid-rich bones of whales (Smith & Baco 2003) have undoubtedly provided a substantial food resource for *Osedax*, but the capacity of *Osedax* to grow and reproduce on other mammalian and possibly non-mammalian bones has not been studied experimentally. Here we report our efforts during the past 2 years to test whether *Osedax* are whale-fall specialists by deploying cow bones (an unlikely resource for marine worms) at depths ranging from 385 to 2893 m in Monterey Bay, CA.

2. MATERIAL AND METHODS

Fresh bovine femurs (20–26 cm long) with most of the flesh removed were cut in half longitudinally and suspended with cable ties from 'bone trees' constructed from 1.9 cm diameter PVC pipe (figure 1*a*). Each bone tree holding six bones was anchored in a plastic bucket filled with 7 kg of concrete. Altogether seven trees were deployed prior to the writing of this report. We used the robotic submersibles *ROV Tiburon* and *ROV Ventana* to deploy bone trees adjacent to Monterey Bay whale-falls at four depths (table 1). The biological communities found at each whale-fall at the time of these deployments were described elsewhere (Braby *et al.* 2007). The cow bones were closely scanned with high definition video (figure 1*b*) during subsequent submersible visits and sampled if *Osedax* females were present (table 1). Individual worms were dissected from the recovered bones and positively identified by their species-diagnostic mitochondrial cytochrome-*c*-oxidase subunit I (*mtCOI*) sequences. DNA extractions, PCR amplifications and DNA sequencing followed established protocols for *Osedax* (Goffredi *et al.* 2005; Braby *et al.* 2007). Unique *mtCOI* sequences produced in this study have been deposited in GenBank (table 3).

3. RESULTS

To date, morphological and molecular analyses have identified eight species of *Osedax* from Monterey Bay. Their currently known depth range, distinguishing features and sequence divergence are summarized in tables 2 and 3. *Osedax rubiplumus* (Rouse *et al.* 2004) and *Osedax frankpressi* (Rouse *et al.* 2004) were the first species described in this genus, and a formal description of *Osedax* 'rosy' will soon appear (Rouse *et al.* in press). Three additional species (*Osedax* 'yellow-collar', *O.* 'orange-collar' and *O.* 'spiral') were briefly characterized by Braby *et al.* (2007) but remain unnamed. In this study, we identified two putatively new species, *Osedax* 'nude-palp-A' from 1820 m depth and *O.* 'nude-palp-B' from 2893 m depth. They are the only known *Osedax* species with four anterior palps that lack the characteristic feathery pinnules found on most *Osedax*. Instead, the palps of *O.* nude-palp-A and *O.* nude-palp-B are thin cylinders that contain two major blood vessels (figure 1*c*). Otherwise, females of the two species resemble other

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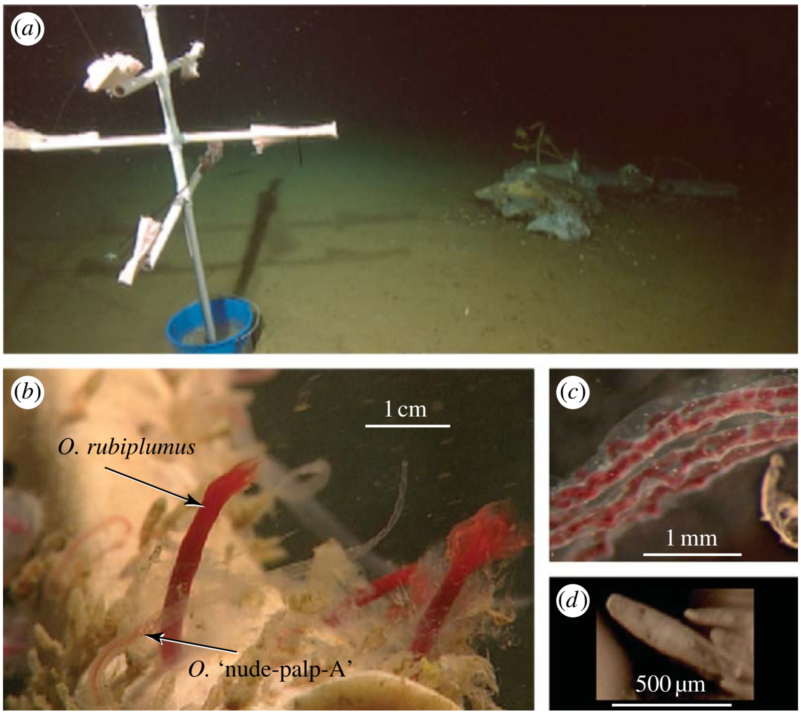

Figure 1. (*a*) Deployed 'bone tree' adjacent to whale-2893 during May 2006. (*b*) *In situ* close-up from high definition video of *Osedax rubiplumus* and *O.* nude-palp-A on cow bone adjacent to whale-1820. (*c*) Close-up of the four palps from *O.* nude-palp-A showing paired blood vessels in each palp and absence of pinnules. (*d*) Dwarf (paedomorphic) males isolated from the tube of *O. rubiplumus* specimen shown in (*b*).

known *Osedax* species in having dwarf males. Though externally similar, the *mtCOI* sequences of *O.* nude-palp-A and *O.* nude-palp-B differ by almost 18%, and they differ by at least 14% from all other known species from Monterey Bay and elsewhere (table 3). Pairwise sequence divergences among these 10 putative species greatly exceeds intraspecific levels of sequence divergence (less than 1%), though only single populations of each named species has been examined to date. We did not provide a phylogenetic analysis based on presently available *mtCOI* sequences because the resulting trees are poorly resolved at deeper nodes. The present sequences, which include the 5′-end of *mtCOI*, are provided for DNA bar coding purposes (Hebert *et al.* 2003) and intended to facilitate the identification of *Osedax* species as they are discovered.

Six out of the eight Monterey species of *Osedax* have colonized experimentally deployed cow bones (table 1) and correspond with species found on the adjacent whale-fall. *Osedax* yellow-collar and orange-collar are found on whale-385, but only yellow-collar has colonized nearby cow bones. *Osedax* rosy is abundant on whale-1018 and on adjacent cow bones. Two *Osedax* species (*O. rubiplumus* and *O. frankpressi*) occur on whale-1820 and both occur on adjacent cow bones. *Osedax* nude-palp-A has only been observed on cow bones adjacent to whale-1820. Since discovering whale-2893, a natural carcass, in 2002 we have observed *O. rubiplumus*, *O. frankpressi* and *O.* spiral living there (Braby *et al.* 2007). But *Osedax* are scarce now on its highly degraded bones, and these species have not been observed on adjacent cow bones. Nonetheless, three specimens of *Osedax* nude-palp-B were found on an adjacent cow bone. Though *Osedax* species nude-palp-A and nude-palp-B were not observed on adjacent whale-bones, these transparent worms might have been over-looked among the dense colonies of *O. rubiplumus* and *O. frankpressi* on whale-1820 and whale-2893, respectively. Both of the undescribed 'nude-palp' species were subsequently collected from whalebones following detailed video surveys of whale-1018.

Colonization and growth of *Osedax* on the deployed cow bones occurred rapidly at two depths. *Osedax* rosy was found 56 days post-deployment near whale-1018 (table 1) but the sampled worms did not contain eggs. Three months later, 154 days post-deployment, the worms had matured, as three individuals of *Osedax* rosy contained eggs. Similarly, a mature *O. rubiplumus* female (154 days post-deployment and adjacent to whale-1820) had eggs in her oviduct and a harem of dwarf (paedomorphic) males in her tube (figure 1*d*) that are a notable characteristic of this siboglinid genus (Rouse *et al.* 2004, in press). Colonization of cow bones at the shallowest site was markedly slower, about 1 year, but *Osedax* colonization of whale-385 was also slow, a probable consequence of frequent disturbance due to intense sediment flows in this part of Monterey Canyon (Braby *et al.* 2007). Colonization of cow bones near the deepest carcass, whale-2893 m, also was slow.

4. DISCUSSION

By considering *Osedax* worms as whale-fall specialists, Glover *et al.* (2005, p. 2591) concluded that the size of '*Osedax* habitat is dependant on the current population densities of cetaceans that are known to create lipid-rich whalebone reefs.' Large whalebones have the capacity to provide habitats that may persist for decades (Smith & Baco 2003; Schuller *et al.* 2004). But here we report on six *Osedax* species that can grow on smaller mammalian bones with significantly lower lipid contents than intact whalebones (Evershed *et al.* 1995; Goffredi *et al.* 2007). Each cow bone used for this study was cut longitudinally prior to deployment, and the exposed marrow had been

Table 1. *Osedax* species found on cow bones in Monterey Bay, CA.

whale-depth (m)	bone tree deployed	prior visits in days[a]	bone recovered	days to recovery	dive[b]	latitude N	longitude W	proximity to carcass (m)	*Osedax* species (no. of worms)[c]
whale-385	25 Jan 2006	120, 272	11 Jan 2007	351	T1070	36.79019928	121.8872376	1.2	0
	25 May 2006	128, 231	4 Jun 2007	375	V3014	36.79017639	121.8872681	0.8	yellow-collar (7)
whale-1018	9 Nov 2005	0	4 Jan 2006	56	T931	36.77140427	122.0829163	1.7	rosy (2)
	25 May 2006	56, 63, 78	25 Oct 2006	153	T1049	36.77149963	122.0830536	2.4	rosy (3)
		79, 153	12 Jan 2007	233	T1072	36.77149963	122.0830536	2.4	0
whale-1820	23 May 2006	0	24 Oct 2006	154	T1048	36.70830154	122.1050644	0.9	nude-palp-A (1)
									nude-palp-A (3)
	12 Jan 2007	0	7Aug 2007	207	T1119	36.70837900	122.1052120	1.7	*rubiplumus* (2)
									frankpressi (1)
whale-2893	24 May 2006	0	10 Jan 2007	231	T1069	36.61341300	122.4354320	9.2	nude-palp-B (3)

[a] Days post-deployment that bones were observed prior to recovery.
[b] Submersible dive numbers: T indicates *ROV Tiburon*; V indicates *ROV Ventana*.
[c] Zero (0) indicates no *Osedax* detected on recovered bone; negative sign (−) indicates none seen in video.

Table 2. Described and undescribed *Osedax* species found in Monterey Bay, California.

Osedax species	depths (m)	size[a]	palps (colour)	pinnules (orientation)	roots	references
rubiplumus	1820–2893	59	4 (brilliant red)	yes (outward)	long, branched	Rouse *et al.* (2004)
frankpressi	1820–2893	23	4 (red with white stripe)	yes (inward)	robust, lobate	Rouse *et al.* (2004)
rosy	633–1820	24	4 (red)	yes (outward)	long, branched	Rouse *et al.* (in press)
yellow-collar	385–1018	~18	4 (pale)	yes (in & out)	robust, lobate	Braby *et al.* (2007)
orange-collar	385	~18	4 (pale)	yes (undefined)	robust, lobate	Braby *et al.* (2007)
spiral	2893	~25	none	no	filamentous	Braby *et al.* (2007)
nude-palp-A	1820	~25	4 (red)	no	unknown	this report
nude-palp-B	2893	~25	4 (red)	no	unknown	this report

[a] Maximum length of trunk and crown (mm) when preserved.

Table 3. Mean sequence divergence within (diagonal) and between (lower left) described and undescribed *Osedax* species. (Total DNA sample sizes including redundant sequences (N) and GenBank accession numbers for unique sequences are given for each species. Published DNA sequences from *Osedax japonicus* from Japan and *O. mucofloris* from Sweden are considered for comparative purposes.)

Osedax species	*rubi.*	*fran.*	*muco.*	*japo.*	rosy	yell.	oran.	spir.	nudA.	nudB.	*N*	GenBank acc. nos.[a]
rubiplumus	0.39										32	DQ996616-20, *EU223297-311*
frankpressi	18.38	0.50									16	DQ996621, *EU223312-16*
mucofloris	20.73	17.28	0.55								10	AY827562-68
japonicus	20.17	19.20	12.95	n.c.							1	AB259569
rosy	20.01	19.27	16.77	14.32	0.11						25	DQ996625-28, *EU032469-84*, *EU223317-19*
yellow-collar	19.33	17.09	14.99	10.74	16.34	0.33					34	DQ996629-38, DQ996640, *EU223320-38*
orange-collar	22.41	15.87	16.84	11.67	21.28	6.10	0.35				21	DQ996639, DQ996641-43, *EU223339-55*
spiral	25.32	24.71	22.42	22.76	23.63	23.84	27.07	0.00			5	DQ996622-24
nude-palp-A	19.13	19.43	16.14	17.07	15.64	13.94	18.86	24.06	0.41		4	*EU223356-59*
nude-palp-B	28.52	17.99	24.72	18.98	14.90	19.14	18.96	25.85	17.90	n.c.	1	*EU236218*

[a] Sequences new to this study are reported in italics.

removed by the time we sampled the bones several months later. All the *Osedax* that we found on cow bones were growing in hard lamellar bone. Clearly, the heterotrophic bacterial endosymbionts hosted by *Osedax* are capable of extracting diverse carbon sources, dominated by collagen and cholesterol, from hard bone (Goffredi *et al.* 2005). The *Osedax* root tissues housing these Oceanospirillales bacteria produce proteolytic enzymes that are capable of hydrolysing collagen, a resource that is otherwise difficult to digest (Goffredi *et al.* 2007). Furthermore, these bacteria can be maintained in cultures enriched in collagen and cholesterol (S. Goffredi 2007, personal communication). Thus, given our current knowledge of these nutritional characteristics, there is no reason to believe that *Osedax* should be restricted to large lipid-rich whalebones.

Females belonging to two of the *Osedax* species found growing on deployed cow bones were producing eggs, and one female had accumulated a harem of dwarf males, a general characteristic of *Osedax* (Rouse *et al.* in press). It is probable, therefore, that a variety of mammalian bones provide sufficient sources of nutrition for the growth and reproduction of *Osedax*. A broad range of small cetacean and pinniped bones probably contribute to the maintenance of *Osedax* populations in Monterey Bay (e.g. common dolphins, *Delphinus delphis*, harbour porpoises, *Phocoena phocoena*, northern elephant seals, *Mirounga angustirostris*, sea lions, *Zalophus californianus*, and harbour seals, *Phoca vitulina*). Cows and other terrestrial quadrupeds probably do not provide regular food sources for *Osedax*, but native and domesticated ungulates are abundant in the flood plains of coastal rivers and their carcasses will probably be found in storm debris that settles in the submarine canyons. Finally, we cannot exclude the possibility that large fish bones and shark cartilage might also provide sources of collagen to support some fast-growing *Osedax* species. A problem with small bones and cartilage is that they are likely to be buried in sediments and unavailable to most species of *Osedax*; however, *O.* spiral may be an exception as it has only been found rooted in buried whalebone fragments (Braby *et al.* 2007). Clearly, similar experiments with a wide variety of vertebrate bones and carcasses are suggested by the present results, and we plan to carry them out during the next several years.

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