Report

A Dwarf Male Reversal in Bone-Eating Worms

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Summary

Darwin [1] hypothesized that sexes in a species should be similar unless sexual selection, fecundity selection, or resource partitioning has driven them apart. Male dwarfism has evolved multiple times in a range of animals, raising questions about factors that drive such extreme size dimorphism [2–4]. Ghiselin [5] noted that dwarf males are more common among smaller marine animals, and especially among sedentary and sessile species living at low densities, where mates are difficult to find, or in deep-sea environments with limited energy sources. These benefits of male dwarfism apply well to *Osedax* (Annelida: Siboglinidae), bone-eating marine worms [6]. *Osedax* males, notable for extreme sexual size dimorphism (SSD), are developmentally arrested larvae that produce sperm from yolk reserves. Harems of dwarf males reside in the lumen of the tube surrounding a female. Herein, we describe *Osedax priapus* n. sp., a species that deviates remarkably by producing males that anchor into, and feed on, bone via symbiont-containing "roots," just like female *Osedax*. Phylogenetic analyses revealed *O. priapus* n. sp. as a derived species, and the absence of dwarf males represents a character reversal for this genus. Some dwarf male features are retained due to functional and morphological constraints. Since *O. priapus* n. sp. males are anchored in bone, they possess an extensible trunk that allows them to roam across the bone to contact and inseminate females. Evolutionary and ecological implications of a loss of male dwarfism are discussed.

Results and Discussion

Molecular Taxonomy and Phylogeny

Both male and female specimens of *Osedax priapus* n. sp. (Figure 1) were sequenced for DNA analysis. Mitochondrial cytochrome *c* oxidase subunit I (*COI*) sequences from ten specimens (six females and four males; Monterey Bay) showed a maximum pairwise uncorrected distance of 1.2%. Eight distinct haplotypes formed a single parsimony network (Figure 2A, rectangle) shared among the sexes.

Phylogenetic analyses of concatenated sequences from five genes recovered generally congruent phylogenies (Figure 2B).

Osedax priapus n. sp. always clustered as sister group to *O. greenpalp* and *O. yellowpatch*, designated here as clade I. The following minor topological variations were probably due to missing data (Table S1 available online): (1) arrangements of *O. mucofloris*, *O. japonicus*, *O.* orange collar, and *O.* yellow collar; (2) relationships within the "nudepalp" clade; (3) placement of *O. deceptionensis*; and (4) placement of the nudepalp clade (clade II) with respect to clade I. The Gblocked maximum parsimony analysis placed clade II (less *O. deceptionensis*) as sister to the remaining *Osedax* terminals and uniquely placed *O. deceptionensis* as sister to clade III-V species. Nevertheless, all analyses placed frenulate siboglinids as sister group to *Osedax*.

Taxonomy

Siboglinidae Caullery, 1914.
Osedax priapus new species.
(Figures 1 and S1–S6.)

Male holotype (Scripps Institution of Oceanography Benthic Invertebrate Collection [SIO-BIC] A4609) from fur seal bone, 740 m, in Monterey Submarine Canyon, California (Figure S1). Paratypes (SIO-BIC A4600–A4608 and A4610–A4616) from fur seal, an elephant seal (also Monterey Canyon, 633 m depth), and cow bones deployed at Hydrate Ridge, Oregon at 610 m.

Brief Description. Holotype (Figure 1E) in life with extensible trunk, yellow patch ventrally at anterior end, just behind prostomium. Two palps with pinnules (Figures 1A, 1B, 1D, S2A, S5F, and S6A–S6D). Seminal vesicle dorsally behind crown (Figures 1A, 1B, 1D–1F, S2C, S2H, S2I, S5A, and S6B–S6D), filled with white sperm mass (Figures 1A, 1B, 1D–1F, and S2B-S2D). Sperm free-swimming, with spirally grooved elongate head (Figures S2E and S2F) exit through anterior pore. Seminal vesicle transitions to thin sperm duct along trunk dorsal surface (Figures 1E, S6D, and S6H) to irregularly shaped testis sac (normally inside bone), 0.64 mm by 0.33mm in holotype (Figures 1E and 1F). Testis sac contains masses of developing sperm (Figures 1G and S4). Small lobe-like roots extend from testis sac (Figures 1E and 1F). Bacteriocyte layer beneath epidermis of testis sac and roots houses bacteria (Figure S4C). Bacterial 16S rDNA sequences from Monterey male and female specimens fell in the Rs1 [8] clade of symbionts and the Rs2 clade for an Oregon male specimen (GenBank accession numbers KP119598–KP119602). No chaetae or obvious segmentation in males or females. Females have four palps and a tube-like oviduct like other *Osedax* females (Figures 1H, 1I, and S5). Spawned eggs 72 by 53 μm (n = 5).

Remarks. *Osedax priapus* n. sp. is the only *Osedax* with males that approach female sizes (Table 1), have palps (though only two), and grow bone-penetrating roots. Both sexes host symbiotic bacteria, unlike the dwarf males [10]. Time-lapse photos of a live *O. priapus* n. sp. male showed it extending from a contracted length of 2mm out to 15 mm (Figure S5), thus presumably making it capable of delivering sperm bundles to nearby females. Though unobserved, females may have similar range of extensibility.

Etymology. Latin *Priapus* or Greek *Priapos* (Πριαπος) for the god of procreation and personification of the phallus.
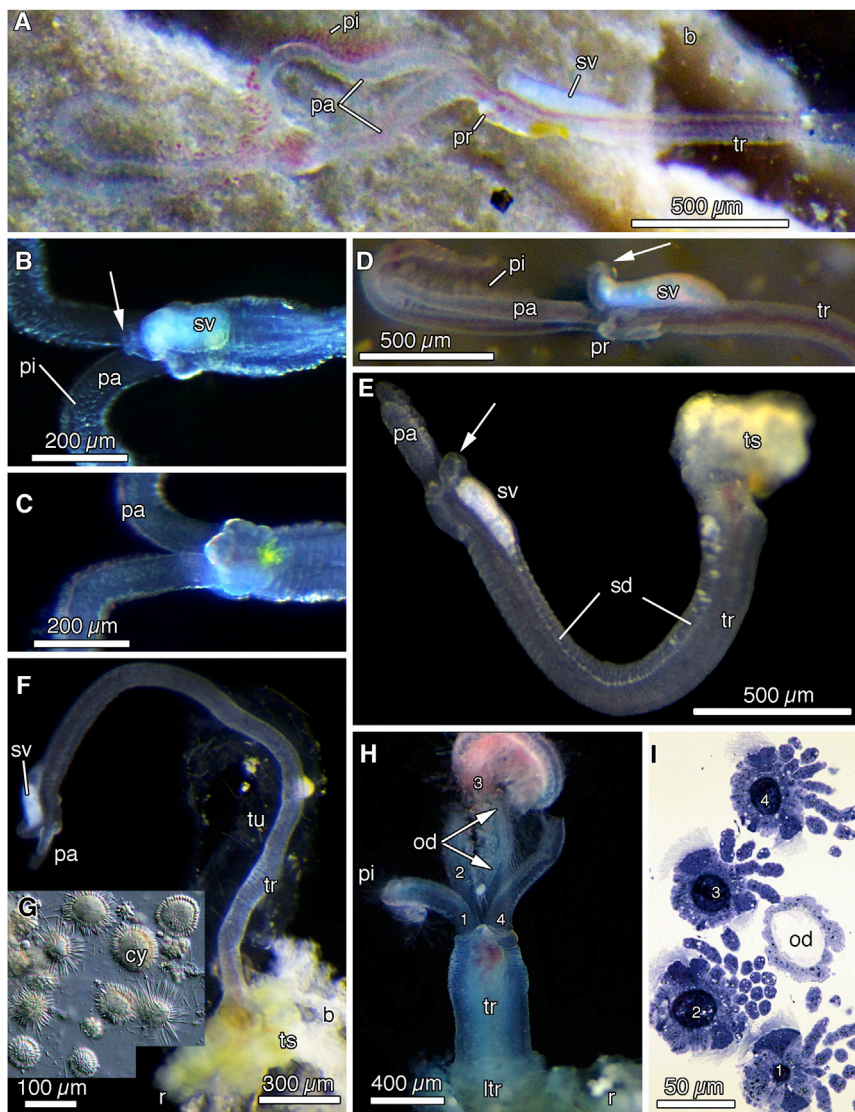
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Figure 1. *Osedax priapus* n. sp. Male and Female Anatomy

(A) Lateral view of anterior trunk and pair of palps of a male (paratype, SIO-BIC A4613), photographed in situ on bone (b). The anteriodorsal seminal vesicle (sv) is swollen with sperm. The small ventral prostomium (pr) has yellow pigment and precedes the two palps (pa).

(B) Dorsal view of same male as in (A), showing seminal vesicle and opening (arrow). Pinnules (pi) line the dorsal sides of the palps.

(C) Ventral view of same male as in (A), showing pointed prostomium and yellow pigment patch. Ventrally, the palps are smooth.

(D) Lateral view of male anterior (specimen lost). An arrow marks the opening for the seminal vesicle.

(E) Whole male dissected from bone (holotype, SIO-BIC A4609) showing sperm duct (sd) connecting testis sac and seminal vesicle. An arrow marks the opening for the seminal vesicle.

(F) Lateral view of a whole male partially dissected from bone (paratype, SIO-BIC A4603). The testis sac (ts) is surrounded by green tissue, as in female *Osedax*. Note the extended trunk (tr) surrounded by transparent tube (tu). Palps were broken in this specimen.

(G) Interference contrast micrograph of spermatids from the testis sac of the holotype.

(H) Dorsal view of the female (paratype, SIO-BIC A4614) showing the oviduct (with oocyte) among the four palps with pinnules oriented dorsally.

(I) Semithin (1 μm) transverse section through the crown of a female (paratype, SIO-BIC A4607 from Monterey) showing the four palps with pinnules oriented dorsally and the central (dorsal) oviduct, typical of most other female *Osedax*.

See also Figures S1–S6.

The AU test showed this constrained tree (−ln L 33849.96303) was significantly worse (p < 0.05) than the unconstrained tree (−ln L 33782.99460).

Analysis of female body size (Figure 3A) indicated that larger and smaller females arose independently from medium-sized ancestor several times, although other scenarios are possible. For instance, the MRCA of clade I (*O. priapus* n. sp. plus *O.* green-palp and *O.* yellowpatch) might have been medium sized, but the proportional likelihood was only 43%. Missing female size data, especially for many nudepalp species, were largely responsible for ambiguities. Unlike the extreme sexual size dimorphism (SSD) of other species, *O. priapus* n. sp. males are approximately one-third of the female volume. Interestingly, *Osedax priapus* n. sp. produces relatively small females (Table 1).

Osedax Body Sizes

Outgroups and siboglinid relatives of *Osedax* have similar-sized sexes [11, 12]. Table 1 documents body-size variation of *Osedax* species examined to date. Dwarf males were previously reported for *O. rubiplumus*, *O. frankpressi*, *O. roseus*, *O.* "spiral," *O.* orange collar, and *O. antarcticus* [6, 13–16] and, although initially not found, are now also known for *O. mucofloris* and *O. japonicus* [17, 18]. Herein, we document dwarf males in five additional species of *Osedax*: *O.* whitecollar, *O.* nudepalpE, *O.* nudepalpG, *O.* yellowcollar, and *O.* yellowpatch (Figure S7). Males are unknown for nine other known species.

Transformation of male and female sizes onto the maximum-likelihood tree topology revealed that the most recent common ancestor (MRCA) of *Osedax* probably produced dwarf males (Figure 3; proportional likelihood ≥ 95%). Thus, the bone-eating adult males of *Osedax priapus* n. sp. represent a reversal of the pedomorphic dwarf condition. A single-most-parsimonious reconstruction for this character (not shown) resulted in the same evolutionary pattern. We assessed whether the absence of dwarf males is ancestral for *Osedax* by constraining *O. priapus* n. sp. as sister group to other *Osedax*.

Atavism, but Constrained by Dwarf Ancestry

Our results suggest that the MRCA of *Osedax* had extreme SSD, with *O. priapus* n. sp. showing a reversal from this condition (Figure 3). This is one of the first times that the loss of dwarf males has been demonstrated among animals. Where phylogenetic studies have been done on other animal groups, the occurrence of dwarfs is arguably derived, e.g., in echiuran annelids [19]. Often, dwarf males have appeared independently multiple times in a clade, as in anglerfish [20], barnacles [21, 22], and, arguably, in xylotrophic bivalves

A

TCS COI network for *O. priapus*

♀ (magenta box)
♂ (blue box)

Osedax priapus n.sp.

♂
♀♂
♀♂♂
♀♂
♀♂
♂
♂ (Oregon)
♂
?
?

I

green palp
yellow patch

O. deceptionensis

nudepalpC
nudepalpD
nudepalpE
O. crouchi
O. antarcticus
nudepalpA
nudepalpG
nudepalpB
O. nordenskjoeldi
nudepalpF
O. rogersi

II

spiral

III

O. frankpressi
MB16
white collar
O. mucofloris
O. japonicus
orange collar
yellow collar

IV

MB17
O. roseus
O. rubiplumus

V

Siboglinum

Oligobrachia

Lamellibrachia
Riftia
Sclerolinum b
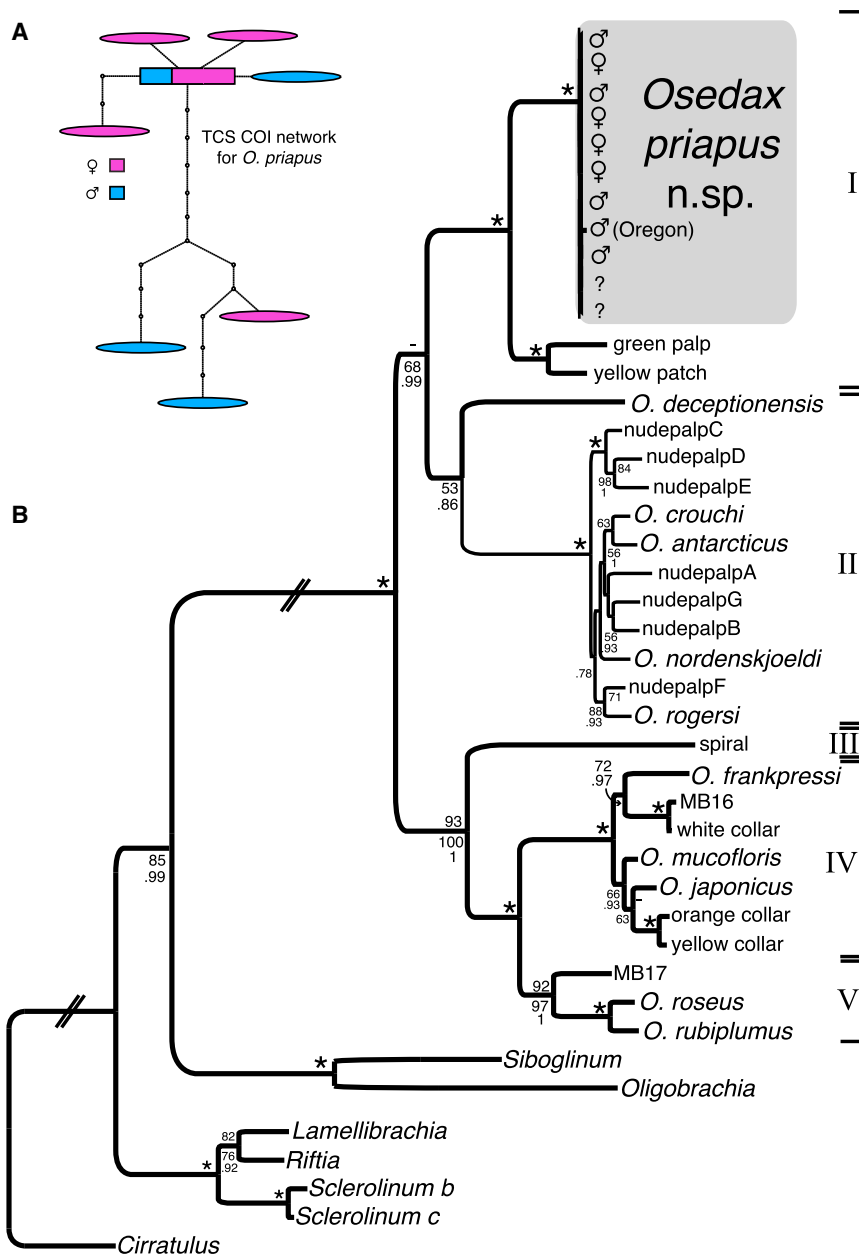*Sclerolinum c*

Cirratulus

B

Figure 2. *Osedax* Phylogenetic Analyses
(A) *COI* haplotype network for ten *Osedax priapus* n. sp. individuals from Monterey Bay.
(B) *Osedax* multigene phylogeny with *Cirratulus cirratus* as outgroup. The RAxML tree was based on MAFFT-aligned, Gblocked, and partitioned data set of five gene segments. The maximum parsimony jackknife support (JS), maximum likelihood bootstrap support (BS), and Bayesian posterior probability (PP) are listed (vertically) at each node. Asterisks indicate BS ≥ 95% and PP ≥ 0.99. Missing values indicate BS < 50% and PP < 0.70. Five major *Osedax* clades are distinguished, following Vrijenhoek et al. [7].

males of other *Osedax* species reside in a female's tube and have free sperm in an anterior seminal vesicle [14, 32]. In contrast, other siboglinids package their sperm [33, 34]. Like the dwarfs, *O. priapus* males still store free sperm (Figure S2) in a seminal vesicle in the head. The free sperm are unlikely to swim well in seawater [35], constraining *O. priapus* males to direct sperm transfer, which is accomplished by their extensible trunk that functions as a penis. Placement of the large anterodorsal seminal vesicle appears to preclude development of the dorsal palps, as they would likely prevent efficient sperm transfer to females. Most female *Osedax*, including *O. priapus* (though not *O. spiral*), have four palps, but males of *O. priapus* only have two ventral palps. Juvenile *O. japonicus* (Figures 1g–1k in [18]) show initial appearance and development of ventral palps while the dorsal palps are buds. We hypothesize that the anterodorsal sperm vesicle precludes development of the dorsal palps.

Sexual Conflict
Osedax male dwarfism eliminates competition with females over food and space, namely scattered bones on the ocean floor. Avoiding sexual conflicts over resources generally allows females to attain greater body sizes and fecundities [5, 36, 37], as seen in most *Osedax* species. Thus, fertility selection should favor fast-growing females that rapidly exploit sunken bones, whereas natural selection favors the evolution of nonfeeding dwarf males [9]. However, *Osedax priapus* n. sp. males can achieve sizes similar to females only because they compete with them to occupy and exploit bones as a food resource.

This raises the obvious question as to why extreme SSD is lost in *O. priapus* n. sp. yet has not occurred in other *Osedax* species. Vrijenhoek et al. [9] proposed that extreme SSD arose in *Osedax* primarily in accordance with arguments presented by Ghiselin [5]. In particular, Ghiselin suggested that male dwarfism is more likely to evolve when most or all of the following factors are in place: (1) males don't compete directly for females, (2) a sessile lifestyle, (3) limited resources (i.e., bones), and (4) it is difficult to find a mate. Small males with

[23–25]. Orb-web spiders (Nephilidae) show marked SSD, females being much larger than males, with a complex history of increases and decreases in size for both sexes [26]. However, the loss of dwarf males in *O. priapus* n. sp. is more than simple size change. It signifies a release from pedomorphosis and a reversion to the ancestral siboglinid condition of little SSD. Dollo's law claims that lost complex traits do not reappear because the responsible genes will, over time, mutate and become nonfunctional [27]. Yet, notable examples exist (reviewed in [28, 29]). For instance, parthenogenetic orabatid mites gave rise to sexually reproducing species [30], and squamate reptiles that evolved viviparity early in their evolution show multiple reversions to oviparity [31].

Atavism is incomplete in *O. priapus* males, however. Although resembling females, the males exhibit clear differences stemming from their ancestry as dwarfs. The dwarf

Table 1. Body Sizes of Male and Female *Osedax*

Osedax	Female Volume (mm^3)	Female Size	Male Volume (mm^3)	SSD Ratio[a]
antarcticus	18.13 ("mean")	M	0.012 (n = 1)	1,510
crouchi	? palps and trunk only	?	?	?
deceptionensis	0.40 (n = 1)	S	?	?
frankpressi	114.25 (n = 3)	L	0.001056 (n = 10)	108,191
japonicus	15.39	M	0.000314	49,012
mucofloris	9.30 (n = 2)	M	?	?
nordenskjoeldi	? palps and trunk only	?	?	?
rogersi	? palps only	?	?	?
roseus	14.64 (n = 3)	M	0.000277 (n = 10)	52,852
rubiplumus	202.00 (n = 2)	L	0.02 (max)[b]	10,100
rubiplumus	202.00 (n = 2)	L	0.0029 (min)[b]	69,655
MB16	no specimens	?	?	?
MB17	66.70 (n = 1)	L	?	?
nudepalpA	3.60 (n = 3)	M	?	?
nudepalpB	? palps only	?	?	?
nudepalpC	? palps only	?	?	?
nudepalpD	6.24 (n = 2)	M	?	?
nudepalpE	1.27 (n = 3)	S	0.00374 (n = 1)	339
nudepalpF	34.03 (n = 1)	M	?	?
nudepalpG	0.80 (n = 1)	S	0.00223 (n = 1)	360
greenpalps	4.06 (n = 2)	M	?	?
orangecollar	12.47 (n = 3)	M	0.00062 (n = 2)	20,112
spiral[c]	55.10 (n = 2)	L	0.001209 (n = 6)	45,492
yellowcollar	9.44 (n = 3)	M	0.00068 (n = 1)	13,882
yellowpatch	4.33 (n = 3)	M	0.0015 (n = 1)	2,884
whitecollar	3.37 (n = 2)	M	0.0022 (n = 1)	1,532
priapus n. sp.	0.86 (n = 5)	S	0.267 (n = 7)	3.2

Volumes were obtained from fixed and typically contracted specimens). No voucher material exists for *Osedax* MB16. Estimates for female volumes for *O. antarcticus*, *O. deceptionensis O. mucofloris*, and *O. japonicus* are from the descriptions. Female body size categories are based on body volume estimates L $\geq$ 50 mm^3; M = 3–50 mm^3 and S $\leq$ 2 mm^3. See also Figure S7.
[a]SSD ratio of female to male volumes.
[b]*Osedax rubiplumus* males grow and expand in volume. Values for the volume of largest males and for smallest males with sperm are used here (e.g, Figure 1D in [9]).
[c]Female trunk volume only.

precocious sexual maturity via pedomorphosis are often advantageous under these circumstances [5]. All of these factors are generally found with in *Osedax*, variation exists, especially in body size (Table 1), which might explain the extraordinary reversal of SSD in *O. priapus* n. sp.

A sessile life history, difficulty in finding a mate, and an absence of male-male competition probably hold for *O. priapus*, and sunken bones are most likely a limited resource. *Osedax* species with larger females can rapidly occupy the bones and block further access to settling larvae with their plumes (e.g., *O. roseus* [13]). However, the small size of *O. priapus* females (Table 1 and Figure S4) might result in less competition for space, allowing the evolution of bone-eating males. By feeding and growing, a male can produce more sperm than a dwarf that is limited by maternal yolk supply. Also, a bone-eating adult male can access multiple females, whereas a dwarf male is confined to a single female's tube. Notably, *O. priapus* still show a degree of SSD, as the males have only one-third the volume of females (Table 1); thus, they might not compete as much for bone as females. Nonetheless, other *Osedax* species also have relatively small females and yet retain dwarf males (Table 1). Special

circumstances might have favored a "release" from dwarfism in *O. priapus*, and further knowledge about the ecology and life history across *Osedax* will be valuable. For instance, we do not know the longevity of *O. priapus* n. sp. females with regard to other *Osedax*. If they have much shorter lifespans, they would not be able to effectively recruit larvae to make dwarf male harems. Also, *O. priapus* n. sp. has the smallest eggs of any *Osedax*, and it is the maternally provided yolk that dwarf males need to make sperm, which would be limited if *O. priapus* n. sp. had dwarf males. Also, *O. priapus* n. sp. has only been found to date on small bones and may be a specialist on these ephemeral habitats, which would favor males and females being present concurrently.

Environmental Sex Determination or Genetic Sex Determination?
Sex determination in annelids is generally thought to be genetic (GSD) [38–40]. Environmental sex determination (ESD) and male dwarfism occur in some echiuran annelids [41], prompting a similar model for *Osedax*, in which larvae that settle on bone mature as females, whereas larvae that settle on females arrest development and transform into males [6]. Various lines of evidence are consistent with ESD for *Osedax* [9, 13]. A study of development in *O. japonicus* was interpreted as support for ESD [18], where larvae were added to aquaria with females living on bones. Some larvae settled on females and metamorphosed into dwarf males. Intriguingly, Miyamoto et al. (Figure 1b in [18]) clearly illustrate a dwarf male, full of spermatids, that they identified as an "early juvenile … [that can] … crawl on bone." If a dwarf male was indeed found crawling on bone, rather than a female, a role for GSD may still exist in *Osedax*, as it apparently does in *Bonellia* [41]. The present discovery of nondwarf males in *O. priapus* further complicates our understanding of sex determination in *Osedax*. Further study is warranted, and a good first step may be a karyotype analysis of males and females.

Variable Sexual Size Dimorphism in *Osedax*
Here we provide for the first time extensive data on the SSD across *Osedax* (Table 1 and Figure 3). Female *O. frankpressi*, which are more than 100,000 times the volume of males, represent one of the greatest instances of SSD found in animals to date. Comparable candidates for such extreme SSD include a blanket octopus (*Tremoctopus violaceus*) with a body size differential of 40,000 in favor of females [42], and an anglerfish (*Ceratias holboelli*) in which females can weigh half a million times more than the male before he fuses with her and becomes parasitic [43]. We also show here data for five species in which females are >20,000 larger than males. Other *Osedax* show much less SSD. The nudepalp clade shows the least SSD, apart from *O. priapus*, with males ranging from 339–1,510 times smaller than females. In part, this is because the males are larger than most other *Osedax*. This is shown here for *O.* nudepalpE, which reaches 400 μm in length (Figure S7F), a size also found for males of *O. antarcticus* [16].

As well as the clear evidence from the five-gene phylogeny, bone-eating *O. priapus* n. sp. males show signs of dwarf male ancestry in their reproductive system. We also document the occurrence of dwarf males and the variability in adult body size for females and males across *Osedax*. Our explanation for the loss of extreme SSD in *O. priapus* n. sp. lies in a decrease in competition for habitat, i.e., bone. *Osedax priapus* n. sp. has among the smallest females of all *Osedax* species
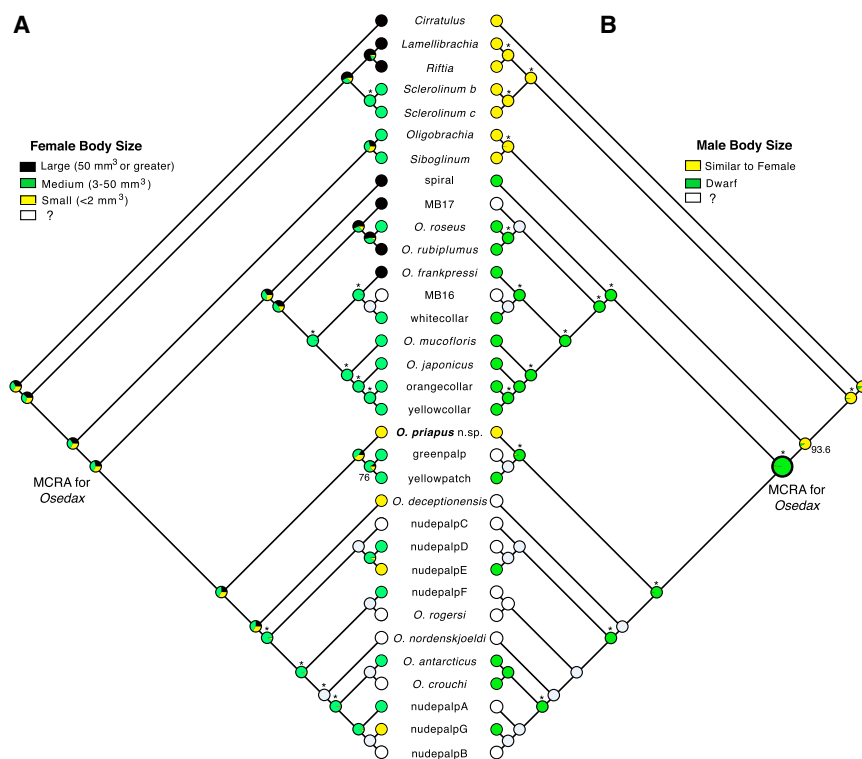
A

B

Female Body Size
- ■ Large (50 mm³ or greater)
- ■ Medium (3-50 mm³)
- ■ Small (<2 mm³)
- □ ?

Cirratulus
Lamellibrachia
Riftia
Sclerolinum b
Sclerolinum c
Oligobrachia
Siboglinum
spiral
MB17
O. roseus
O. rubiplumus
O. frankpressi
MB16
whitecollar
O. mucofloris
O. japonicus
orangecollar
yellowcollar
O. priapus n.sp.
greenpalp
yellowpatch
O. deceptionensis
nudepalpC
nudepalpD
nudepalpE
nudepalpF
O. rogersi
O. nordenskjoeldi
O. antarcticus
O. crouchi
nudepalpA
nudepalpG
nudepalpB

Male Body Size
- ■ Similar to Female
- ■ Dwarf
- □ ?

MCRA for *Osedax*

MCRA for *Osedax*

76

93.6

Figure 3. Transformation of Female and Male Body Sizes in *Osedax*

Body sizes were traced on tree topology extracted from multigene phylogeny for *Osedax* (from Figure 2B). *Osedax priapus* n. sp. sequences were reduced to a single terminal branch. The colored circles at each node indicate the marginal probabilities for each character state; asterisks at a node indicate that proportional likelihood of a dominant character state was 95% or greater.

(A) Female body sizes (large, medium, and small). The plesiomorphic condition for *Osedax* females is ambiguous, as large and small body sizes appear to have arisen several times independently from a medium-sized ancestor. Further data on the body size of other species are needed to resolve the ambiguities.

(B) Male body sizes (dwarf and nondwarf). The presence of pedomorphic dwarf males is plesiomorphic for *Osedax* (enlarged node, labeled most recent common ancestor, MRCA), whereas the large adult males of *Osedax priapus* n. sp. constitute a character reversal and loss of extreme sexual size dimorphism.

See also Figure S7.

discovered to date. We hypothesize that this reduction allowed greater access to smaller more ephemeral bones and lessened the selective pressures that favored the evolution of dwarf males in the genus. Advantages for male *Osedax* to become bone eaters include (1) not being sperm limited by maternal yolk provisions, (2) not being limited to a single mate, and (3) arriving and developing simultaneously with females, which avoids the risk of arriving too late on ephemeral bones.

Experimental Procedures

Collection details are in the Supplemental Information, as are light microscopy, histology, and confocal laser scanning microscopy protocols, which were published previously [13, 14]. Specimens are lodged at the Benthic Invertebrate Collection at SIO. Nuclear 18S rDNA, 28S rDNA, and Histone-3 genes and mitochondrial *COI* and 16S rDNA genes were sequenced for the new species, and other specimens are available in GenBank under accession numbers KP119554–KP119597 (Table S1). DNA sequencing was as previously reported [7]. Eleven *Osedax priapus* specimens were sequenced, ten from Monterey Bay and one (male) from Oregon. We also obtained sequences for five genes from a previous unknown species, *Osedax* "nudepalpG," and added new sequences for *Osedax* MB16 and MB17 from Monterey [8]. Data analysis largely followed previous studies; see the Supplemental Experimental Procedures.

Accession Numbers

The GenBank accession numbers for *Osedax* and other annelids (44 new sequences) and their bacterial symbionts (five new sequences) reported in this paper are KP119554–KP119602. Details are in Table S1.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, detailed systematic description, seven figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.11.032.

Author Contributions

G.W.R. discovered the new species and conducted the photography, microscopy, and data analyses. N.G.W. generated most of the new DNA sequences. K.W. performed the confocal microscopy. R.C.V. provided the critical specimens and, with G.W.R., composed the evolutionary context of this study. All authors participated in writing the manuscript.

Acknowledgments

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