A remarkable diversity of bone-eating worms with dwarf males

Robert Vrijenhoek
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Москва, Россия (23 Apr. 2013)

"La chance favorise l'esprit préparé."

(Louis Pasteur)

"Luck favors prepared minds"

Greg Rouse
South Australia Museum, Adelaide
Scripps Institution of Oceanography
ANNELID TAXONOMY

Shana Goffredi
MBARI postdoc
Occidental College, Los Angeles
BACTERIAL SYMBIOSIS

(and flexible institutions)


Monterey
California
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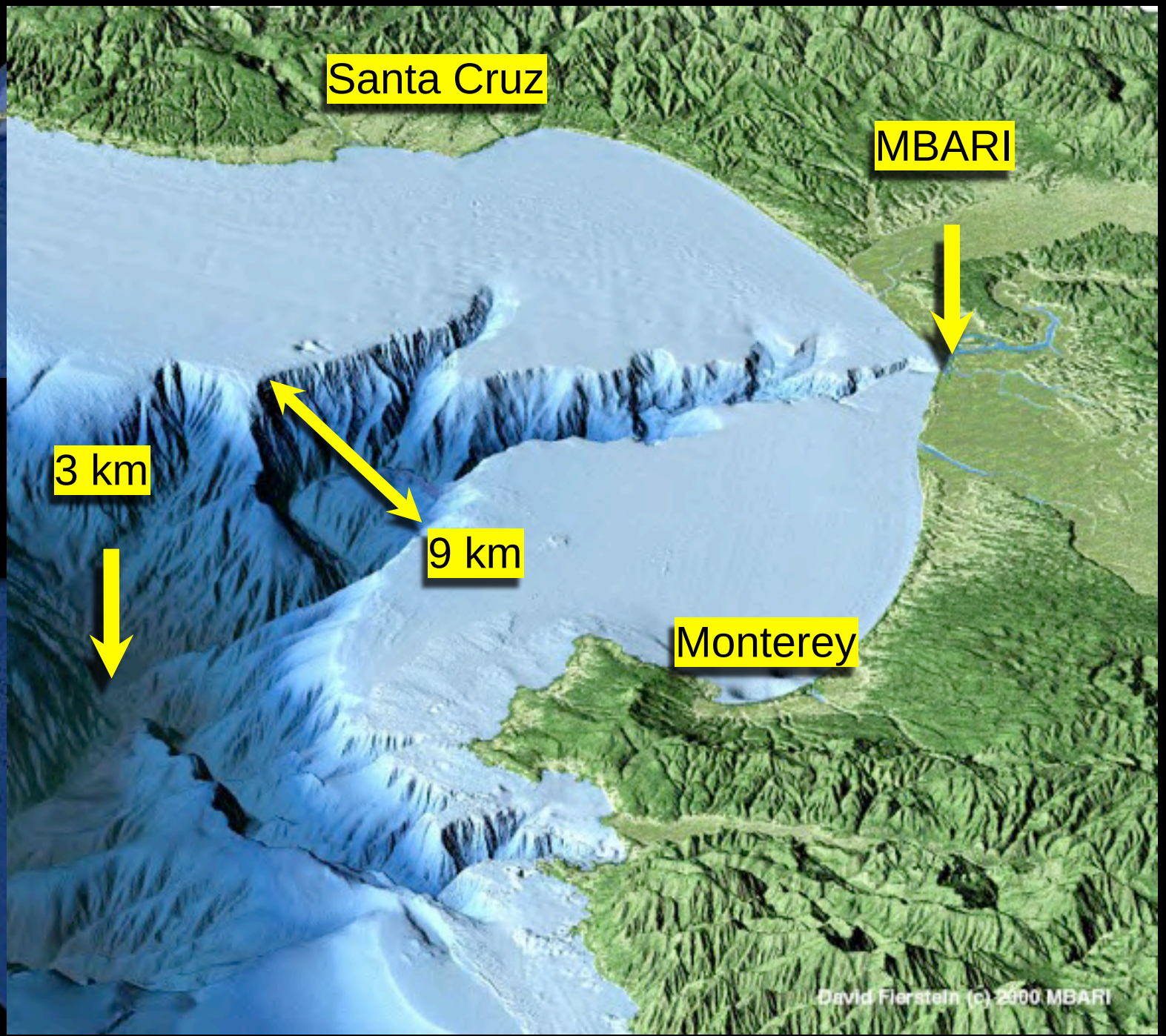
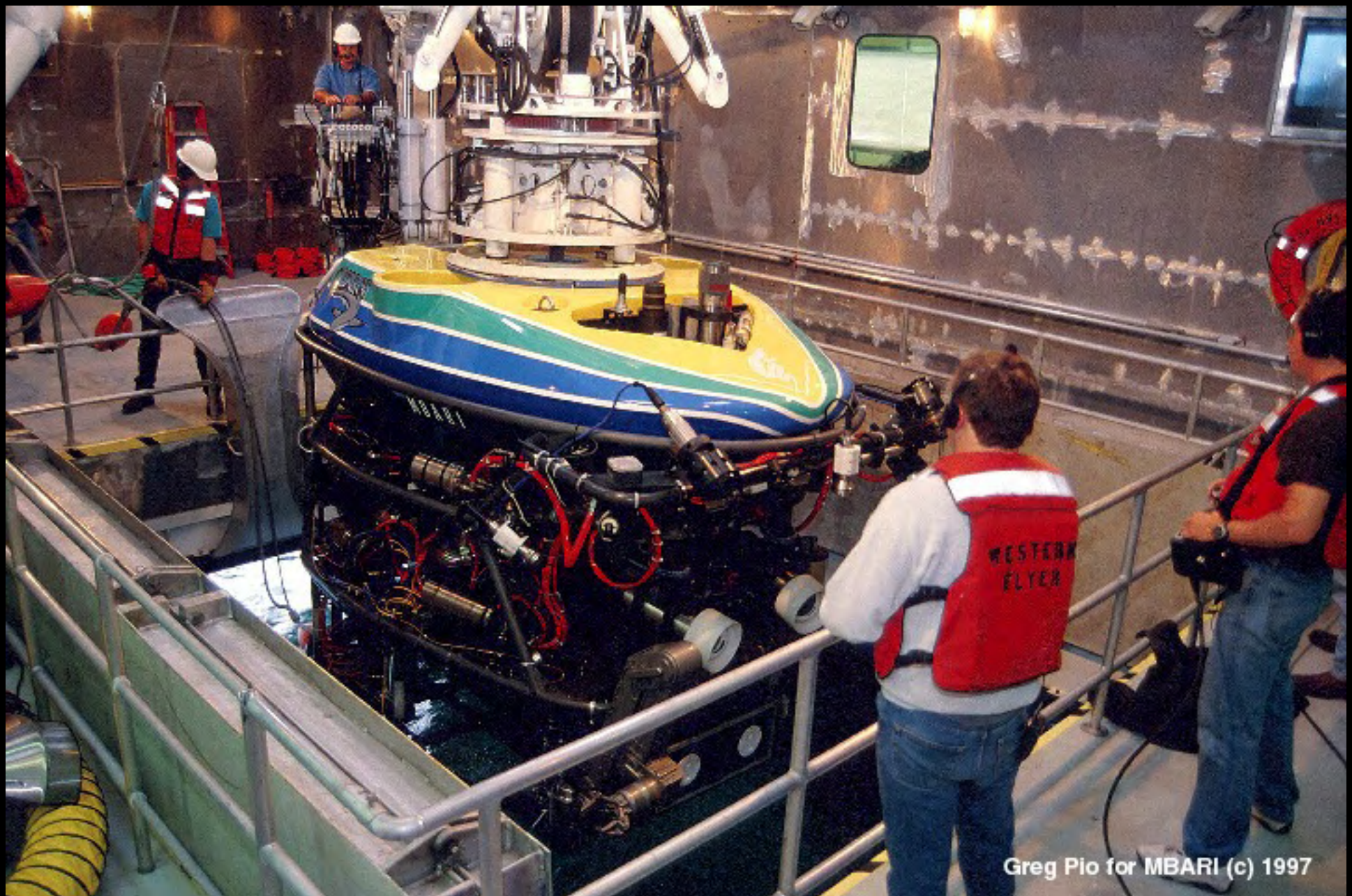
Monterey Submarine Canyon

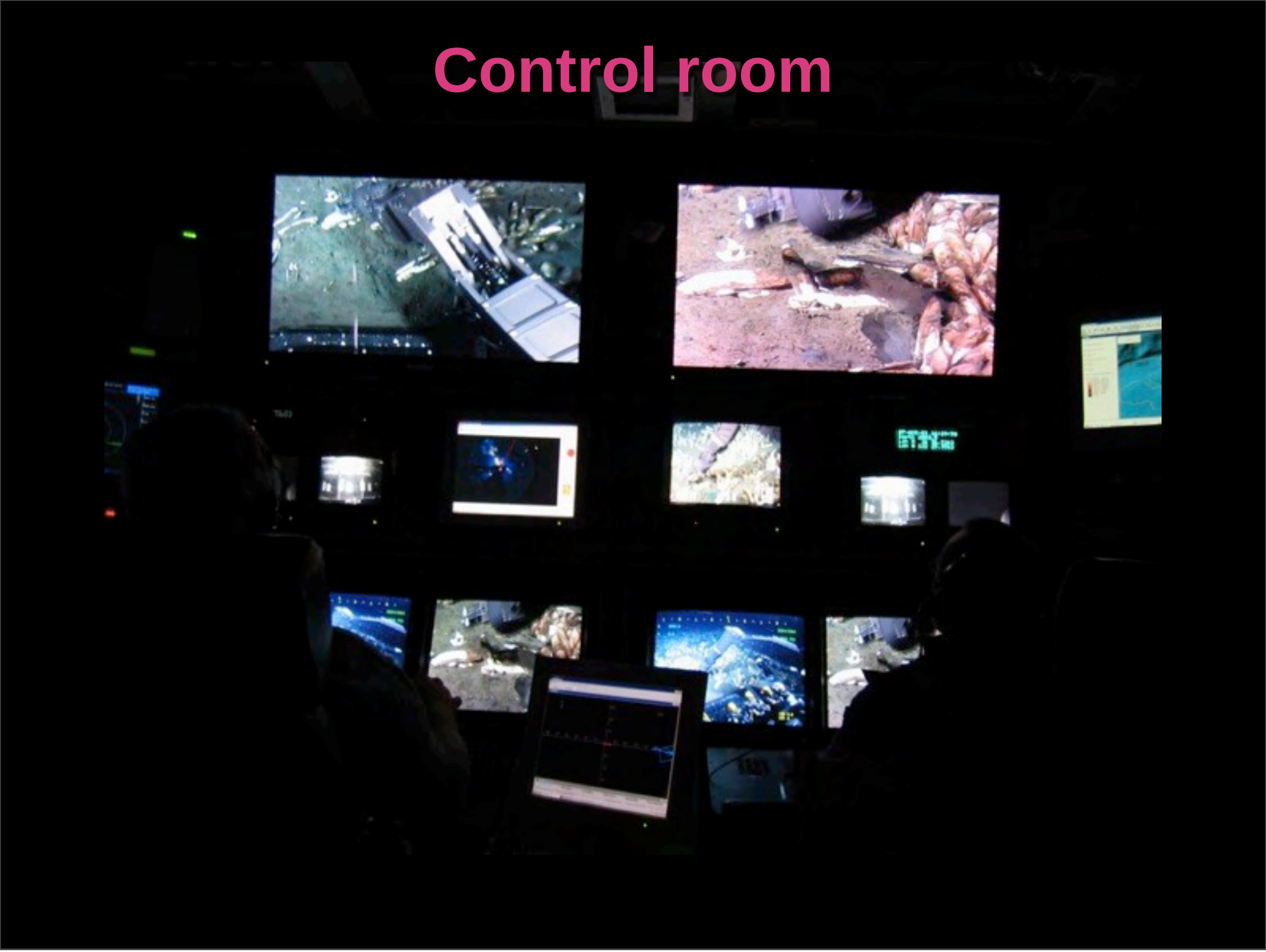


Western Flyer

ROV *Tiburon*



Control room



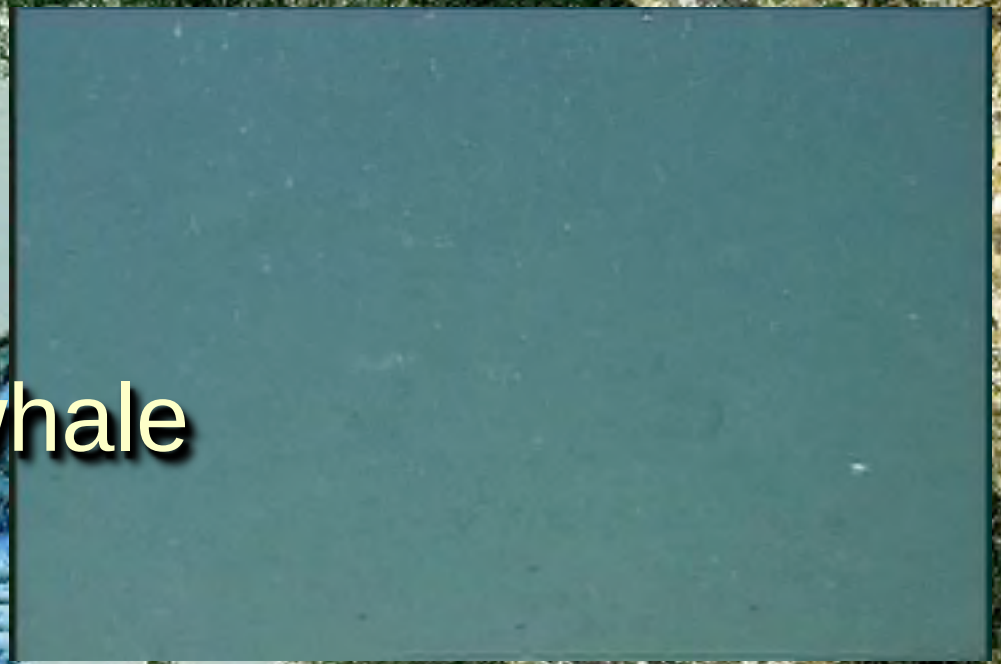
Wonderful animals to see

Sea spider (pycnogonid) eating anemone

Sampling specimens

July 2002: using sonar to search for carbonate outcroppings and chemosymbiotic clams

Santa Cruz



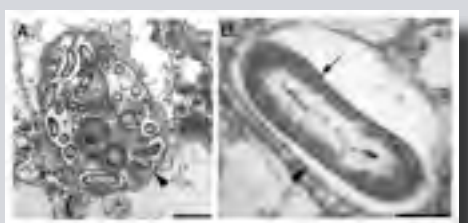
Ruby, a juvenile gray whale
9 m total length

2893 meters depth

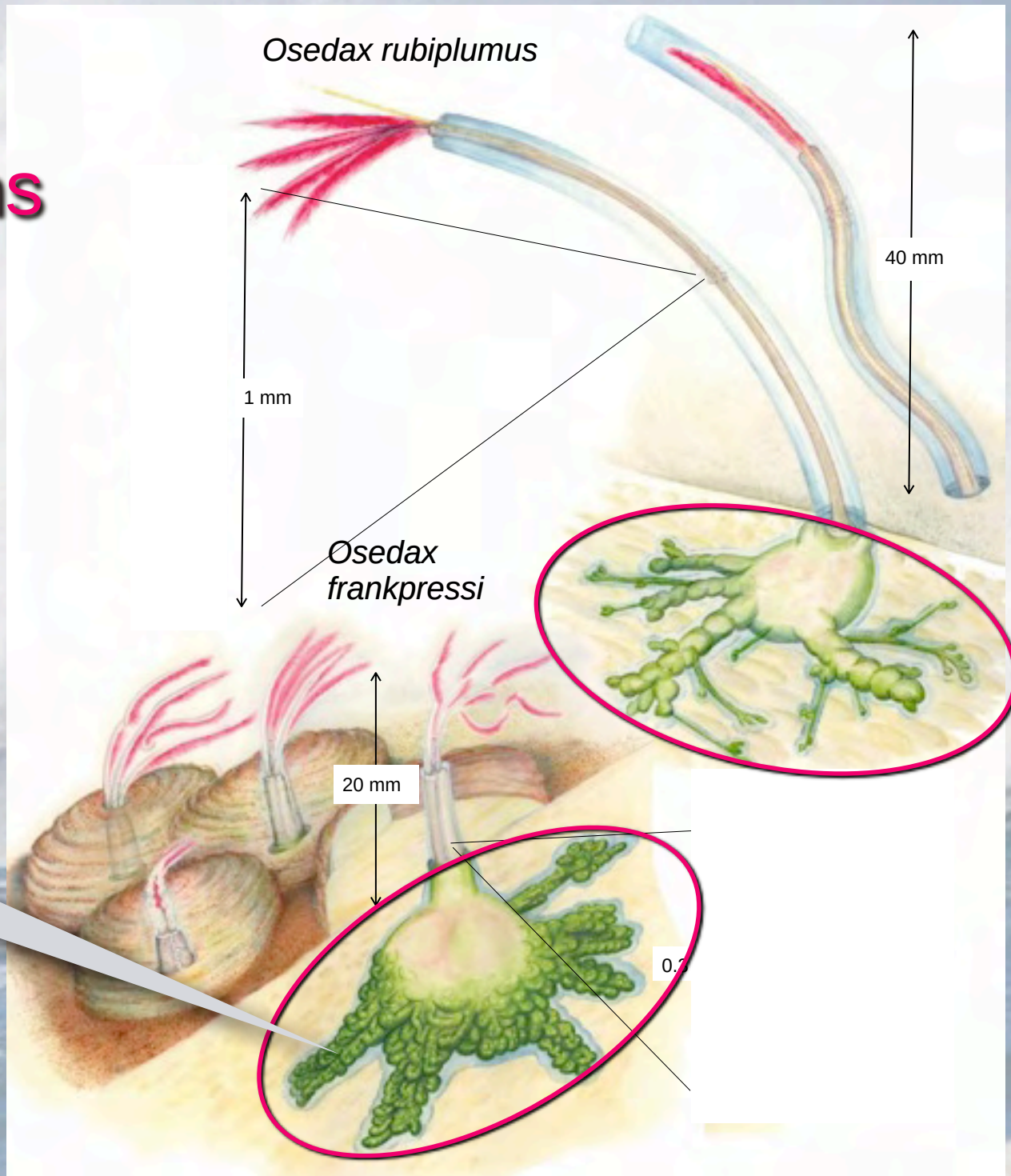
Mystery worms

Osedax: new genus of bone-eating worms with dwarf males

Rouse, Goffredi & Vrijenhoek (2004) *Science* 305: 668-671



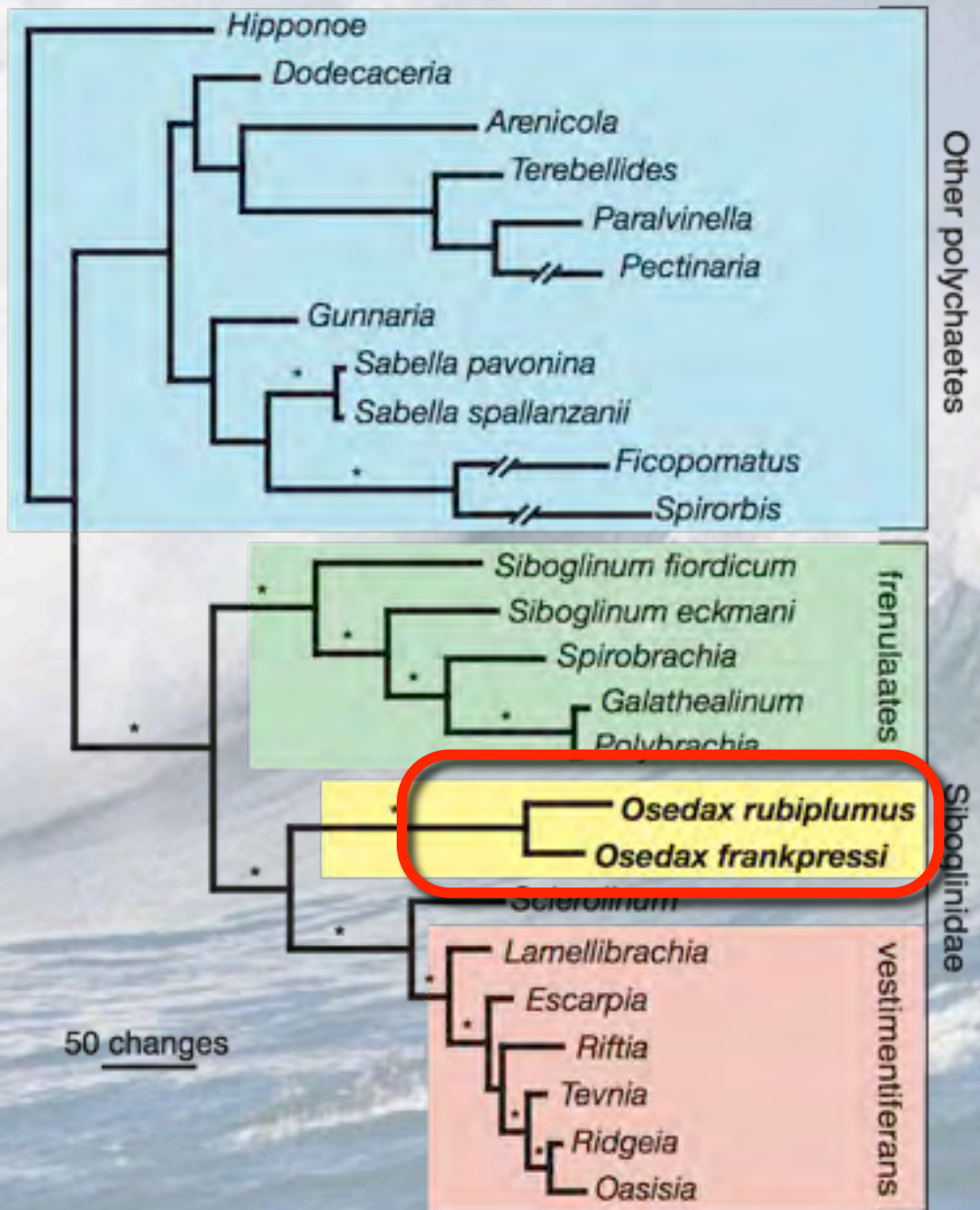
endosymbionts



Osedax are polychaete annelids

Polychaete annelids

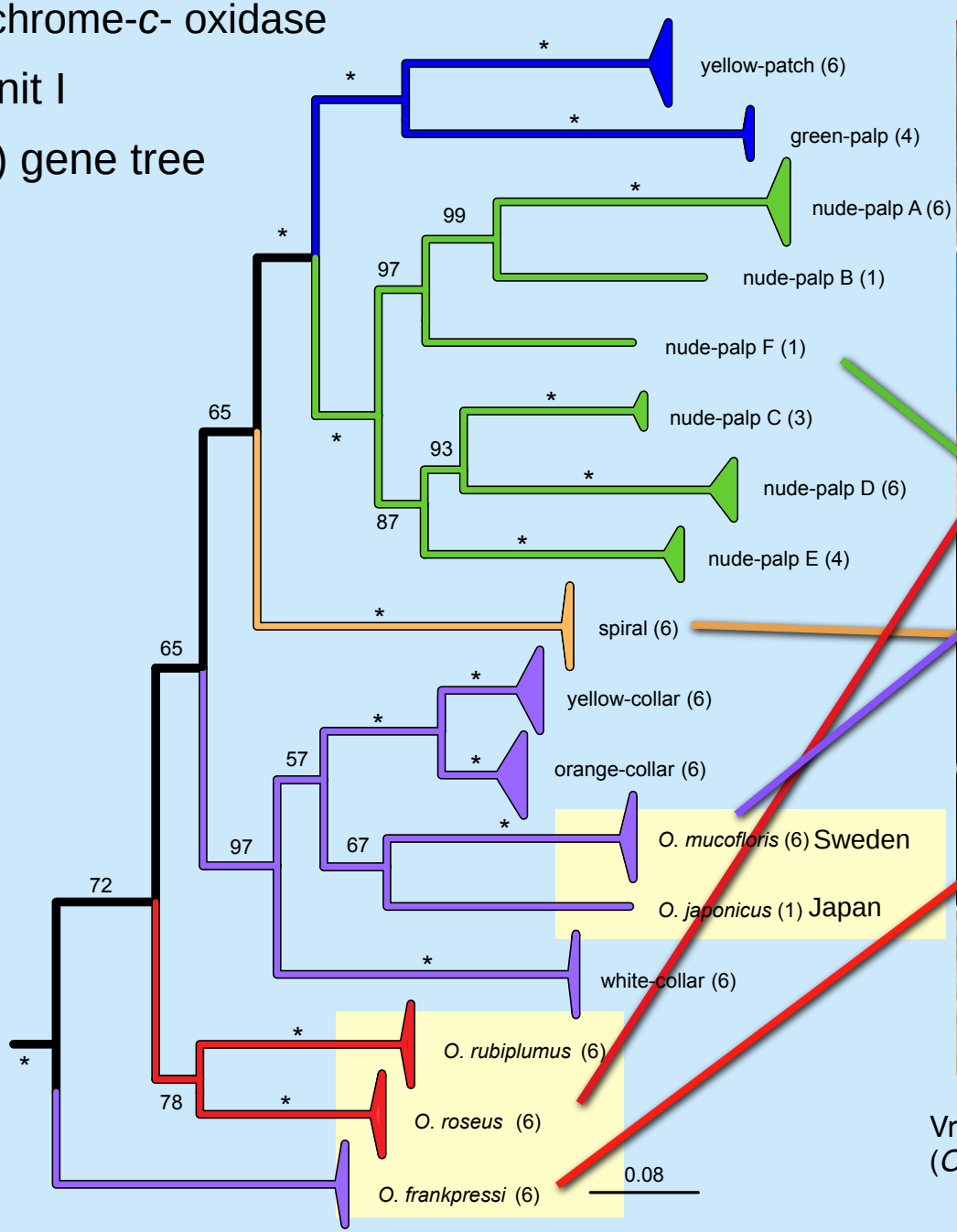
16S and 18S rRNA

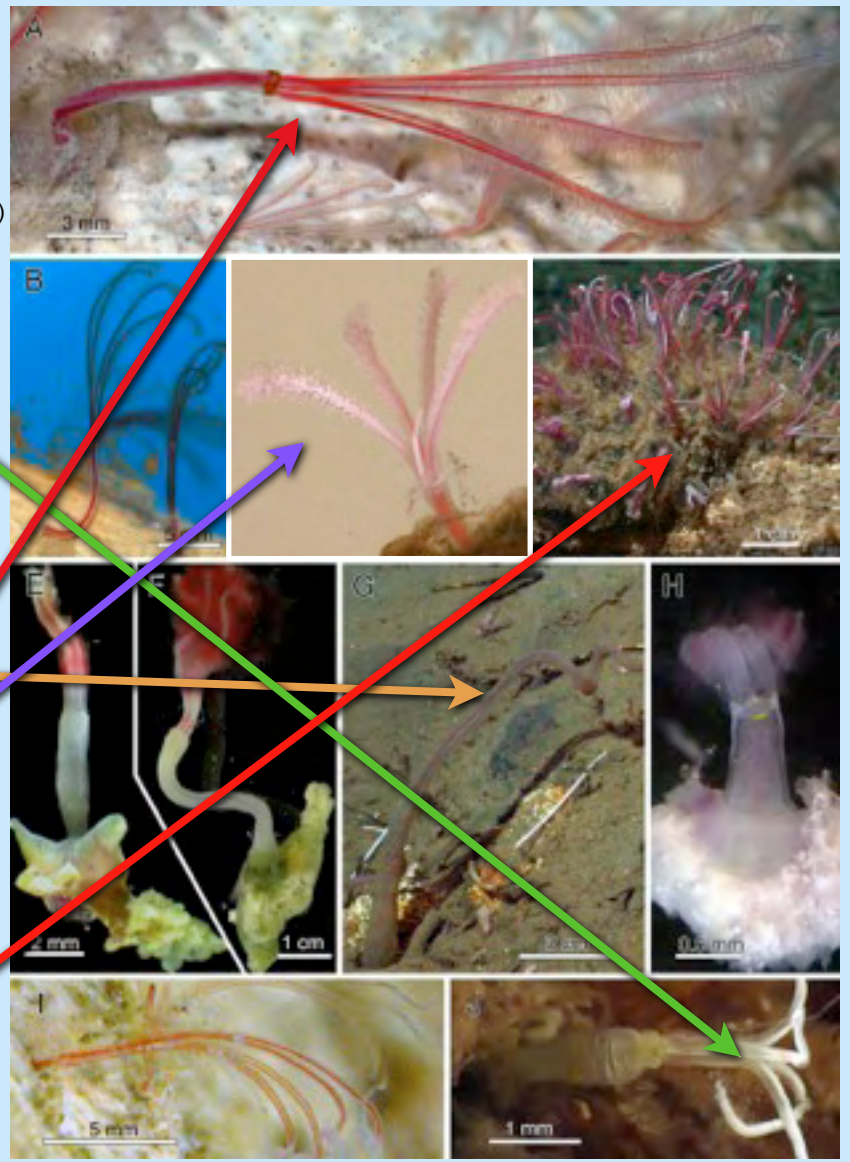


Rouse, Goffredi & Vrijenhoek (2004) *Science* 305: 668-671

Remarkable diversity of Osedax

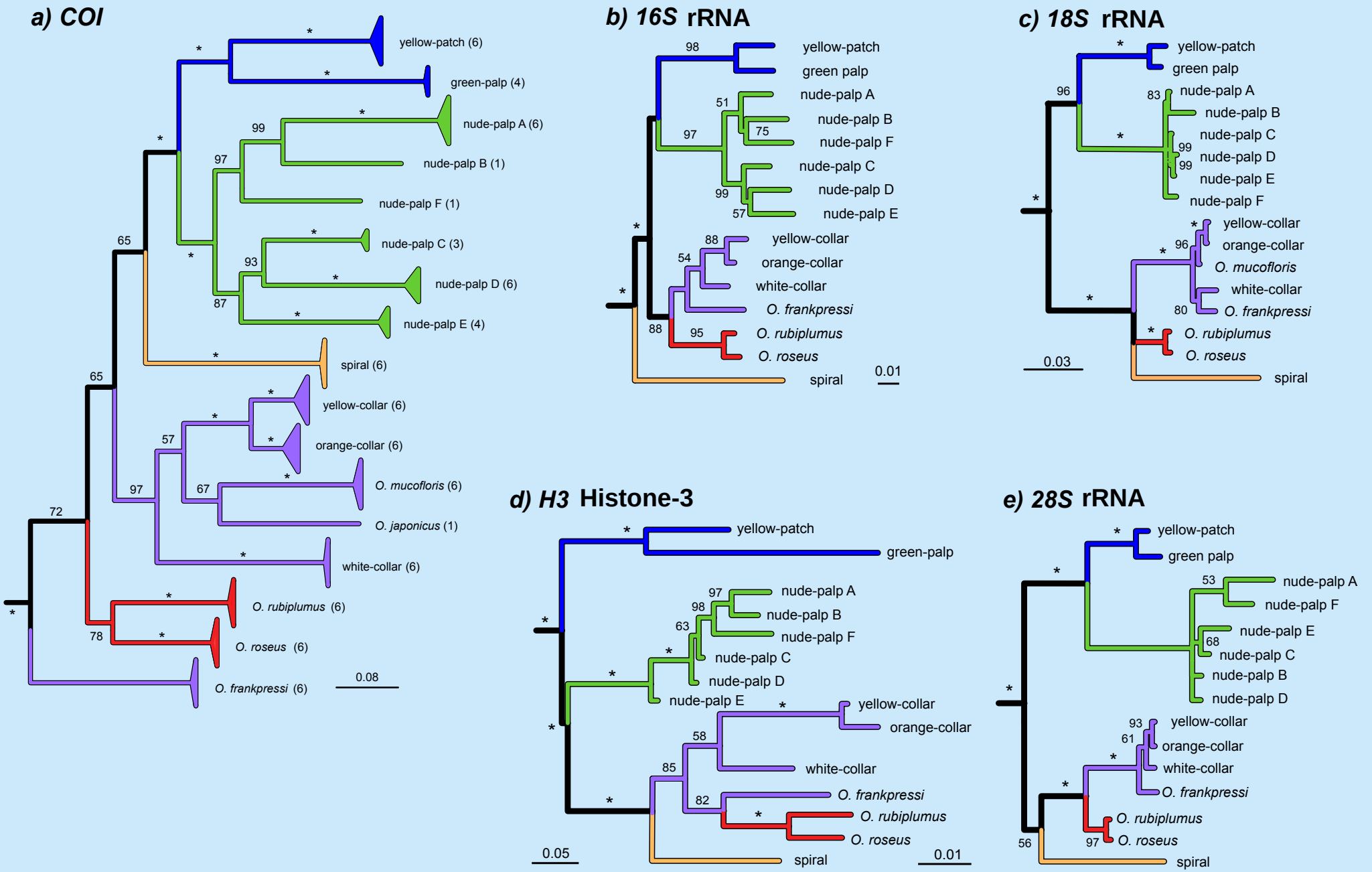
Cytochrome-*c*- oxidase subunit I (COI) gene tree





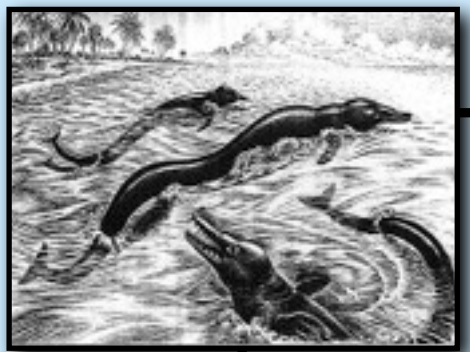
Vrijenhoek *et al.* (2010) A remarkable diversity of boneworms (*Osedax*; Siboglinidae, Annelida). *BMC-Biology* **7**, 74.

Multiple gene genealogies

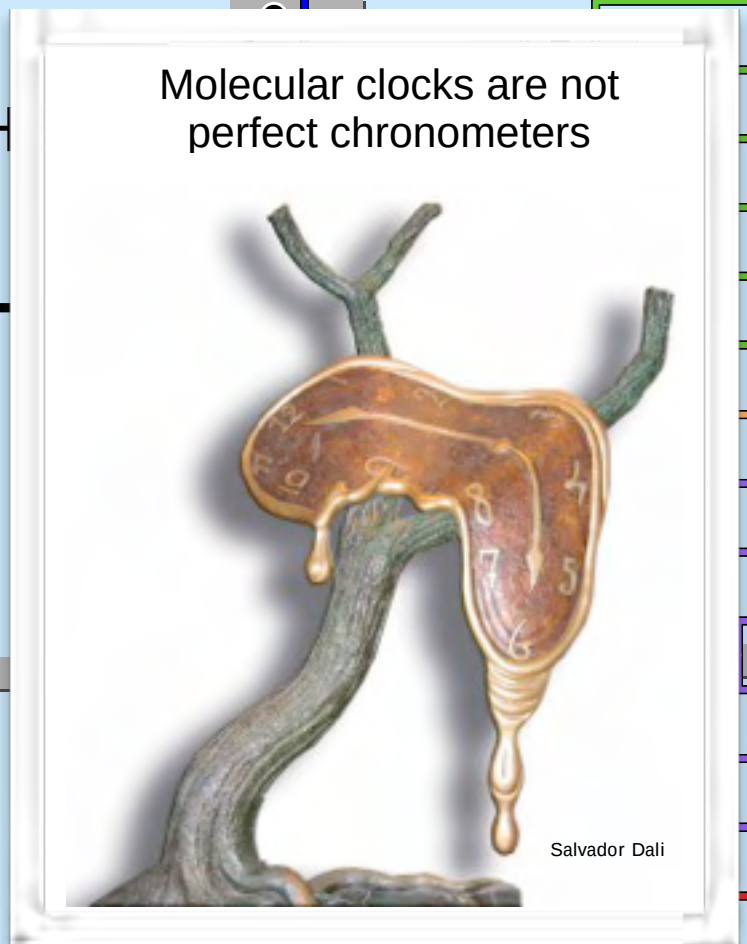


Multigene phylogeny

COI, 16S, 18S, 28S, H



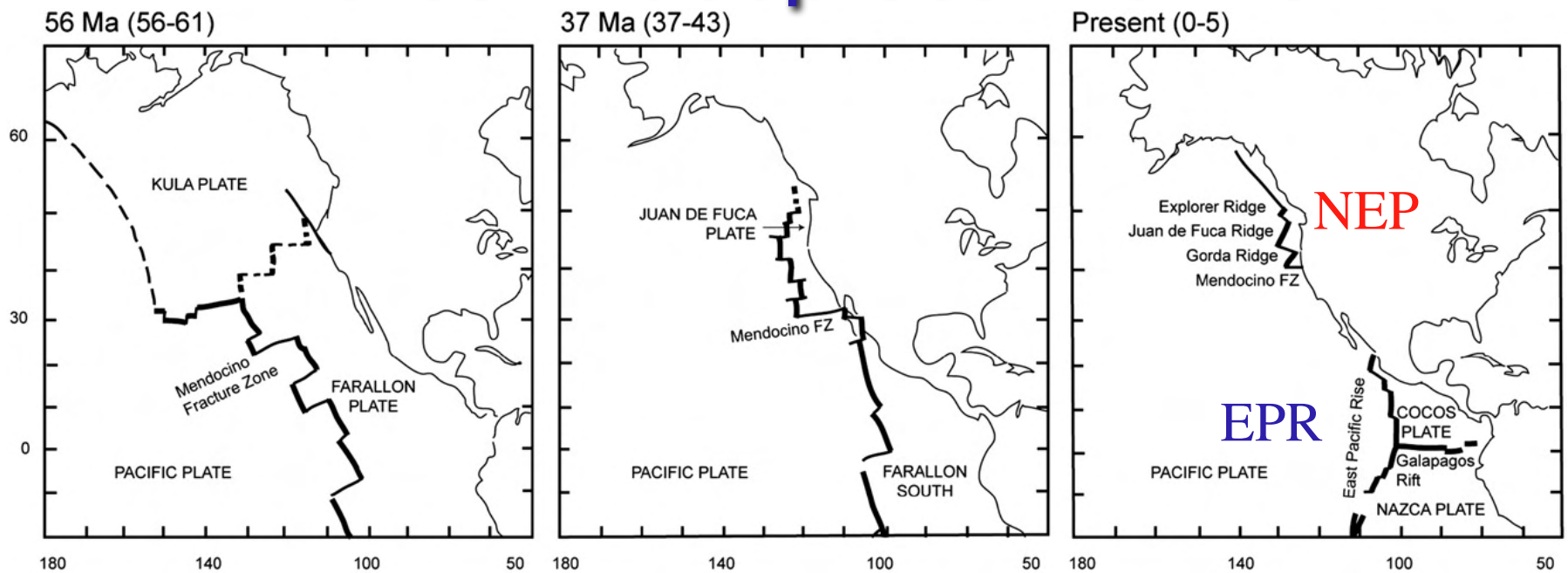
Eocene *Basilosaurus*



fast rate of *COI* sequence divergence based on vicariance of shrimp across Isthmus of Panama

Vrijenhoek *et al.* (2010) A remarkable diversity of boneworms (*Osedax*; Siboglinidae, Annelida). *BMC-Biology* **7**, 74.

Vicariance and slower *COI* substitution rates in deep-sea worms



$T \approx 28.5$ Myr

Fig. 3. Cenozoic history of eastern Pacific ridge systems (modified from Atwater, 1989).

$r = d/2T$

EPR species	NEP species	*d* %	*r* (%)/Myr
Amphisamytha galapagensis	*A.* new sp.	14.2	0.249
Paralvinella grassleii	*P. palmiformis*	7.6	0.133
Oasisia alvinae	*Ridgeia piscesae*	13.1	0.230
Lepetodrilus tevnianus	*L fucensis*	15.7	0.275
	L. gordensis	16.1	0.282
		mean:	0.234 ± 0.054

Multigene phylogeny

COI, 16S, 18S, 28S, H3

Cretaceous Plesiosaur

O. yellow-patch
O. green-palp
I 600–1800 m

O. nude-palp A
O. nude-palp B
O. nude-palp F
absence of pinnules
O. nude-palp C
O. nude-palp D
O. nude-palp E
II > 1000 m

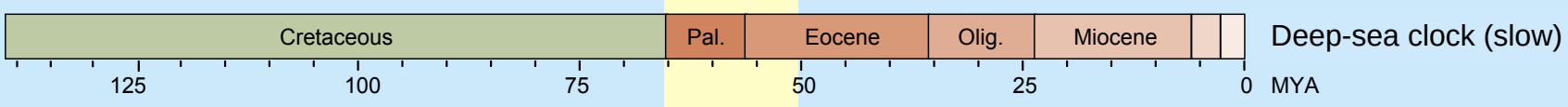
absence of palps
O. spiral
III 3000 m

O. frankpressi
O. white-collar
O. orange-collar
O. yellow-collar
O. japonicus
O. mucofloris
IV > 1000 m
25–1000 m
Salvador Dali

O. roseus
O. rubiplumus
V > 1000 m

S. brattstromi

based on *COI* divergence of deep-sea annelids with Farallon-Pacific vicariance



Vrijenhoek *et al.* (2010) A remarkable diversity of boneworms (*Osedax*; Siboglinidae, Annelida). *BMC-Biology* **7**, 74.

Fossil evidence

PNAS | **May 11, 2010** | vol. 107 | no. 19

Fossil traces of the bone-eating worm *Osedax* in early Oligocene whale bones

Steffen Kiel[a,1], James L. Goedert[b], Wolf-Achim Kahl[a], and Greg W. Rouse[c]

[a]Institut für Geowissenschaften, Christian-Albrechts-Universität, 24118 Kiel, Germany; [b]Burke Museum, University of Washington, Seattle, W and [c]Scripps Institution of Oceanography, University of California, La Jolla, CA 92093

Edited by Robert C. Vrijenhoek, Monterey Bay Aquarium Research Institute, Moss Landing, CA, and accepted by the Editorial Board March 19, for review February 22, 2010)

Osedax **is a recently discovered group of siboglinid annelids that consume bones on the seafloor and whose evolutionary origins have been linked with Cretaceous marine reptiles or to the post-**

process. The dorsal side of this skull had corroded away fossilized, likely due to *Osedax*.

The traces that we attribute to *Osedax* start as bore

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Naturwissenschaften
DOI 10.1007/s00114-010-0740-5

SHORT COMMUNICATION

Osedax borings in fossil marine bird bones

Steffen Kiel · Wolf-Achim Kahl · James L. Goedert

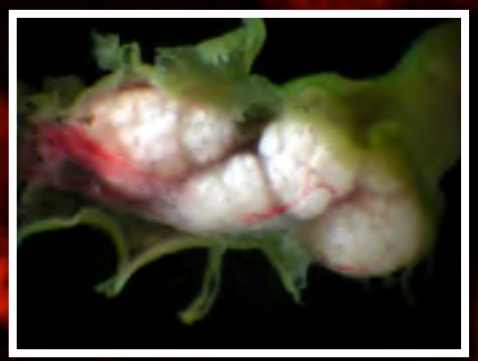
Bone from an Oligocene penguin-like member of the Plotopteridae. Riddled with holes (arrows) made by *Osedax*.

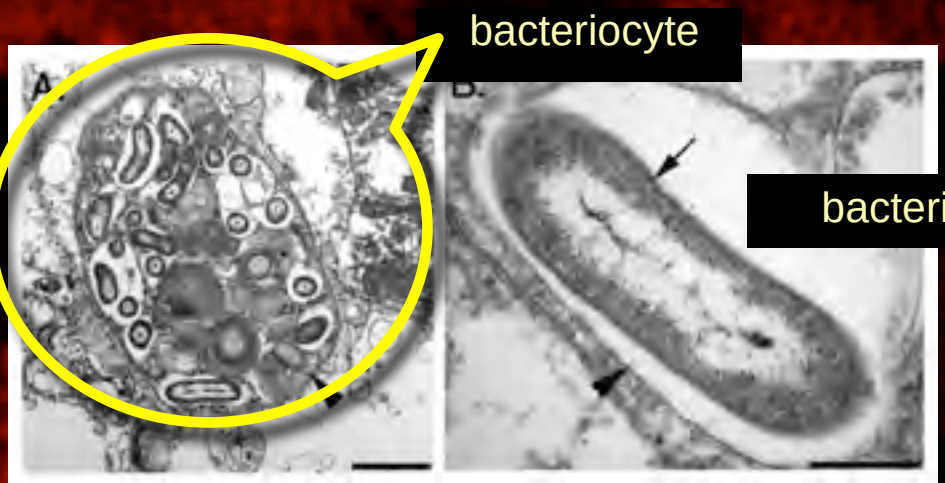
G. Hundertmark/Göttingen Univ.

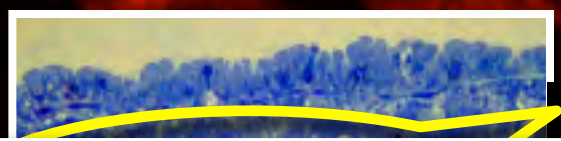
Heterotrophic endosymbionts

Goffredi et al. (2004) Environmental Microbiology 7:1369-1378.

Goffredi et al. (2007). Applied and Environmental Microbiology 73:2314-2323.



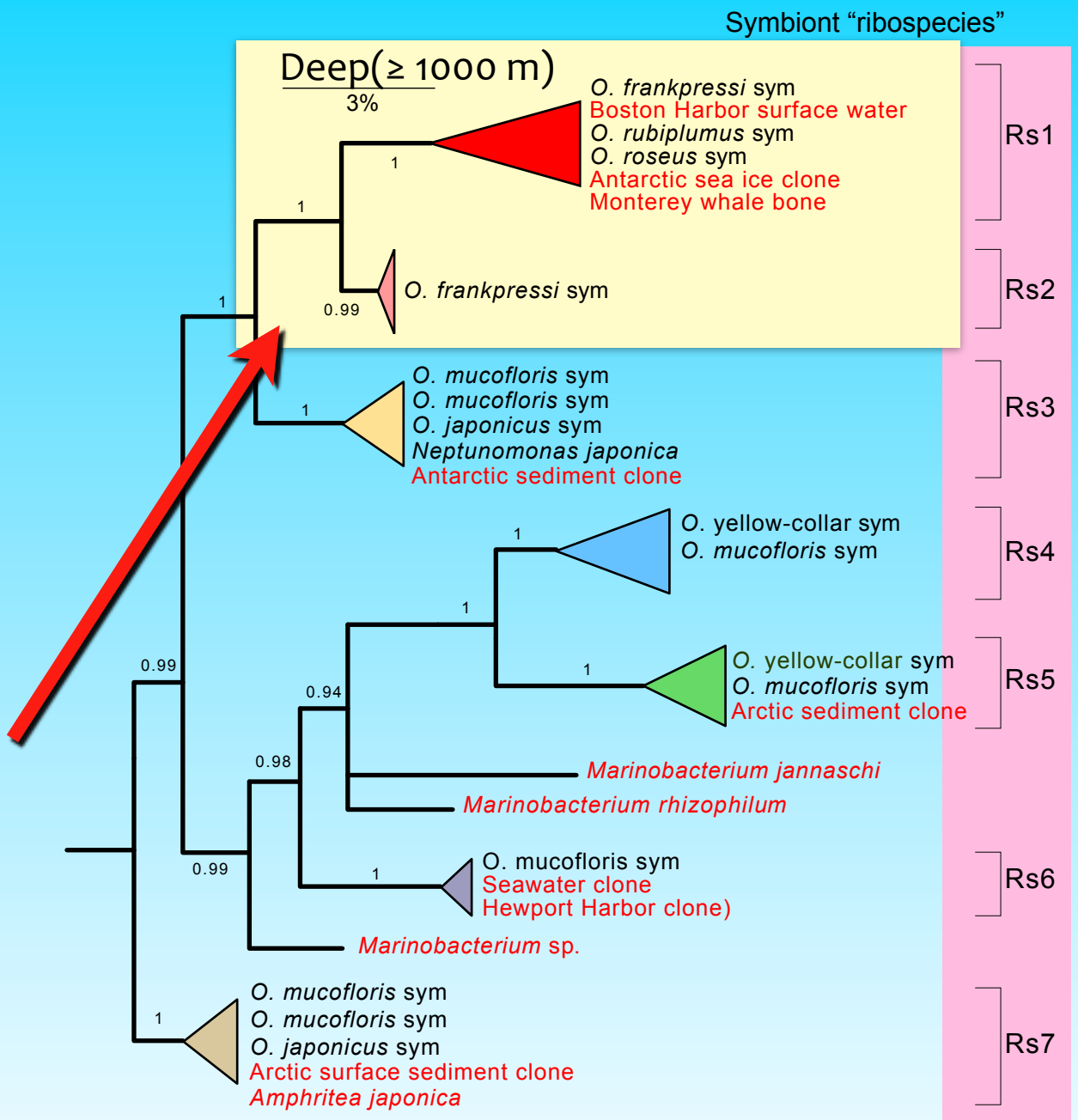




- **Oceanospirillales verified by 16S rRNA**
- ***Osedax* concentrate bacterial lipids**
- ***Osedax* obtain carbon from photic zone, verified by stable isotopes**
- **Symbiont grown on collagen/cholesterol medium**
- **Symbiont genomes now annotated**

Diversity of endosymbionts infecting *Osedax* species worldwide

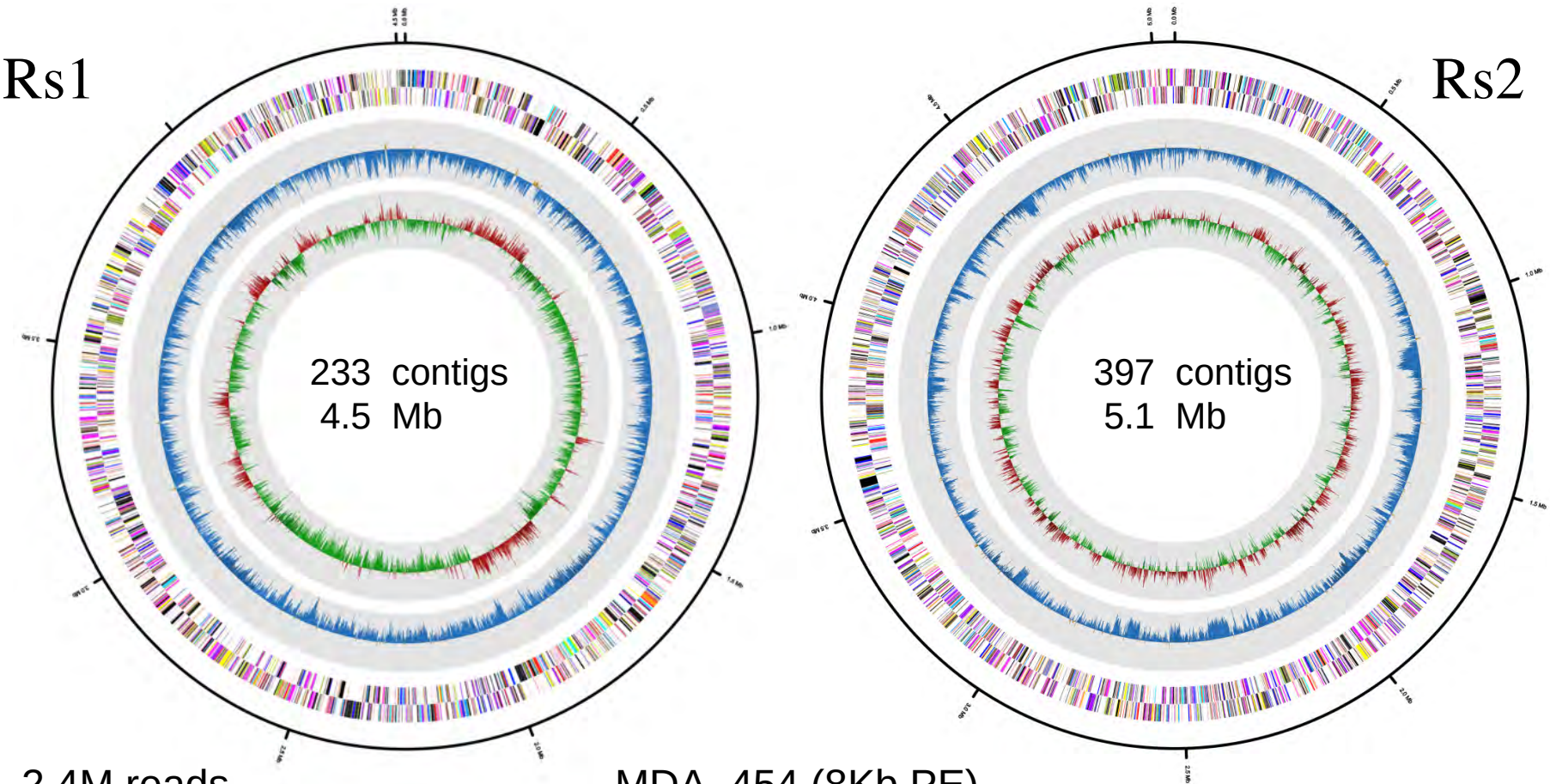
- Aposymbiotic worm larvae infected after settling on bones.
- 7 symbiont "ribospecies" infect *Osedax* (red = free-living bacteria).
- Symbiont "ribospecies" stratified by depth: yellow >1000 m
- Rs1 and Rs2 show succession as carcass decays



BioMed Central

Salathé and Vrijenhoek, 2012. *BMC Evolutionary Biology* 2012, 12:189

Osedax symbiont draft genome maps



2.4M reads	MDA_454 (8Kb PE)	
62 M reads	MDA_Illumina (100bp PE)	58 M reads
61 M reads	Nextera_Illumina (150bp PE)	
1-180 kb (avg 19 kb)	Contig size range	1-76 kb (avg 13 kb)
90%	Completeness	94%

from: Goffredi et al. manuscript in preparation

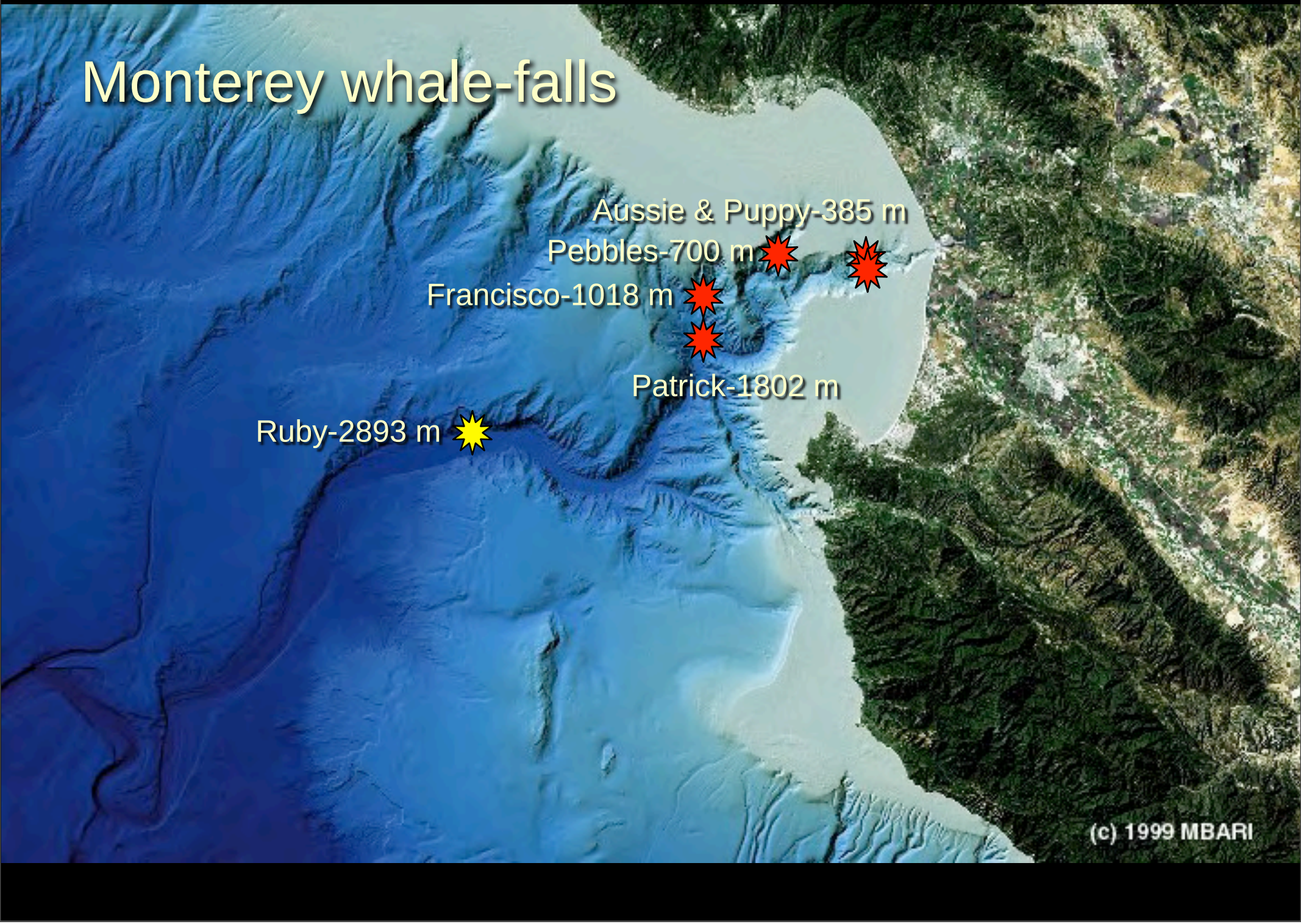
Functional comparisons of Rs1 and Rs2

Symbiont functions:
- synthesis of B-vitamins/amino acids
- degradation of branched amino acids, etc.

Differences between Rs1 and Rs2:
- Rs2 dominates later in whale-fall succession
- Rs2 has genes for sulfur metabolism (detoxification?)
- more genes involved in heme uptake
- differences for genes involved in capsule formation, chemotaxia, etc.

from: Goffredi et al. manuscript in preparation

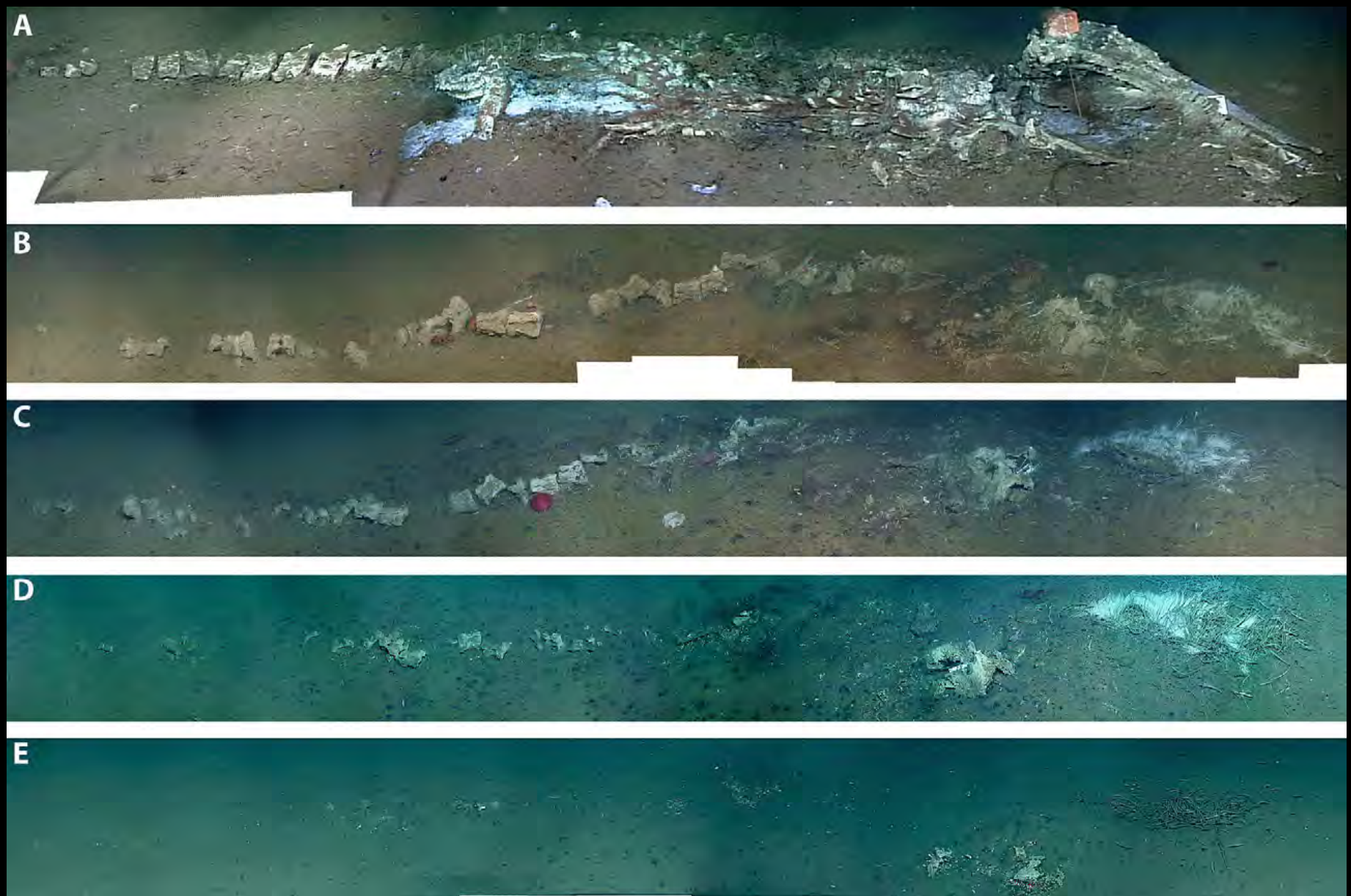
Experimental whale-falls


Monterey whale-falls
Aussie & Puppy-385 m
Pebbles-700 m
Francisco-1018 m
Patrick-1802 m
Ruby-2893 m
(c) 1999 MBARI

Francisco deployed 5 Oct. 2004

remains on 5 Jan. 2005

Decomposition of whale-2893

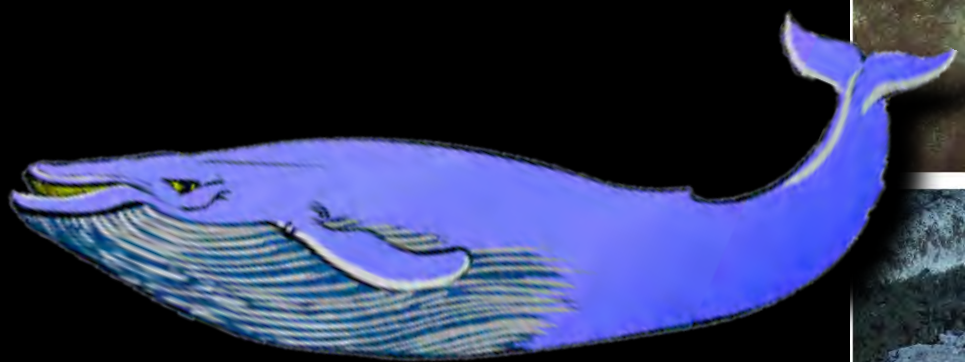


Lundsten et al. Deep-Sea Research I 57 (2010) 1573–1584

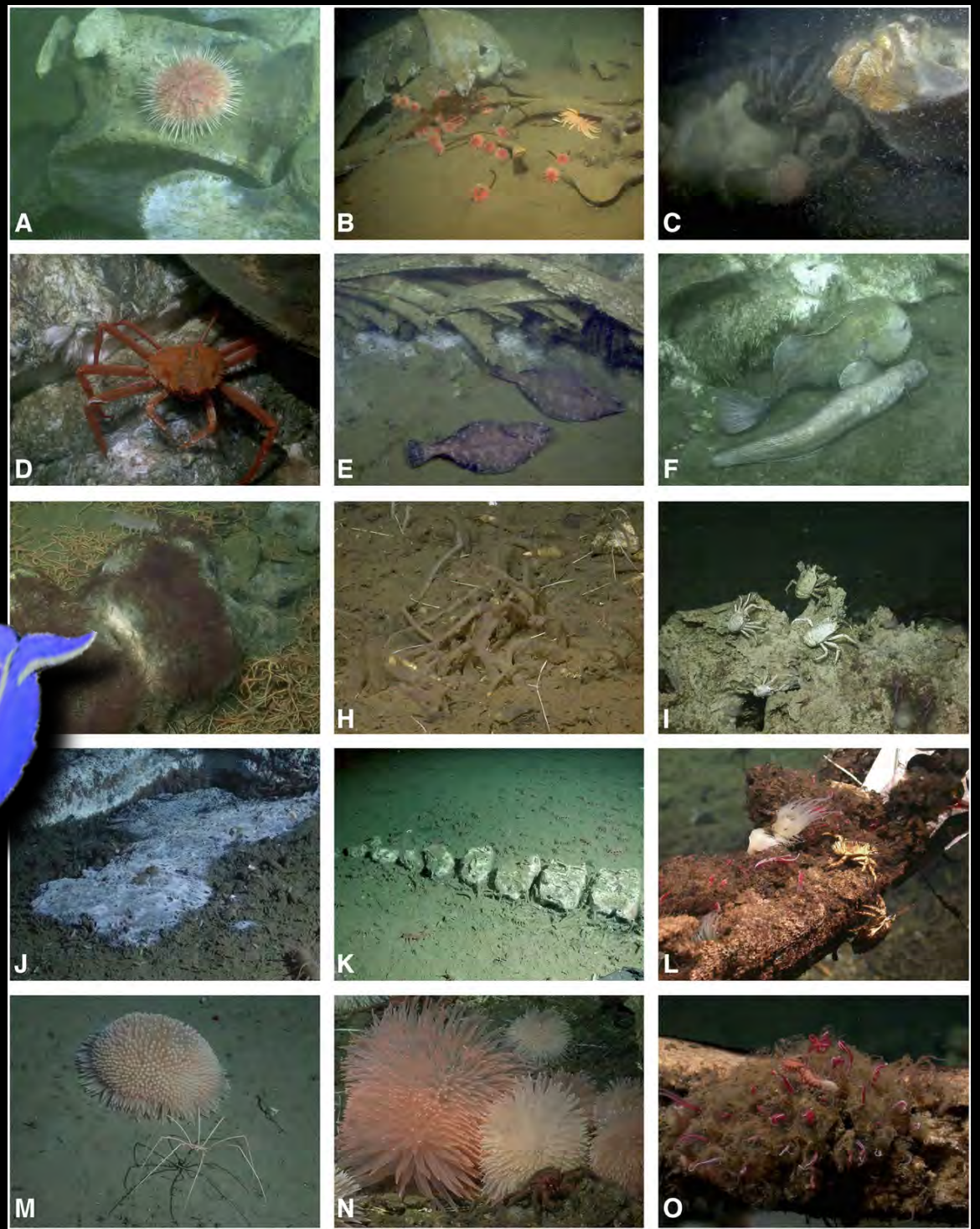
Megafauna at Monterey whale-falls

Mostly background fauna

Lundsten et al. Deep-Sea Research I 57 (2010) 1573–1584

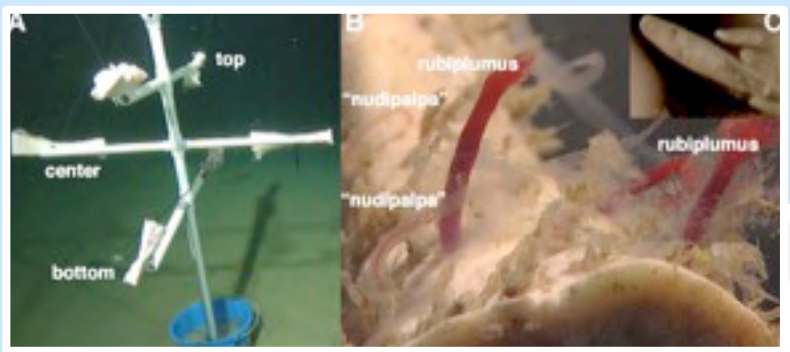


Whale-falls
407 species
21 endemic species
Smith & Baco (2003)

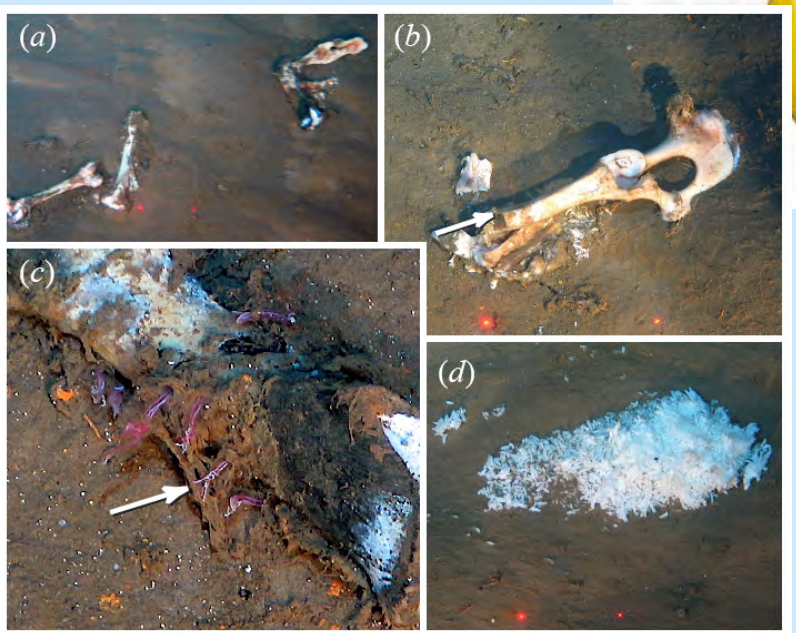


Are *Osedax* are not “whale-fall specialists"?

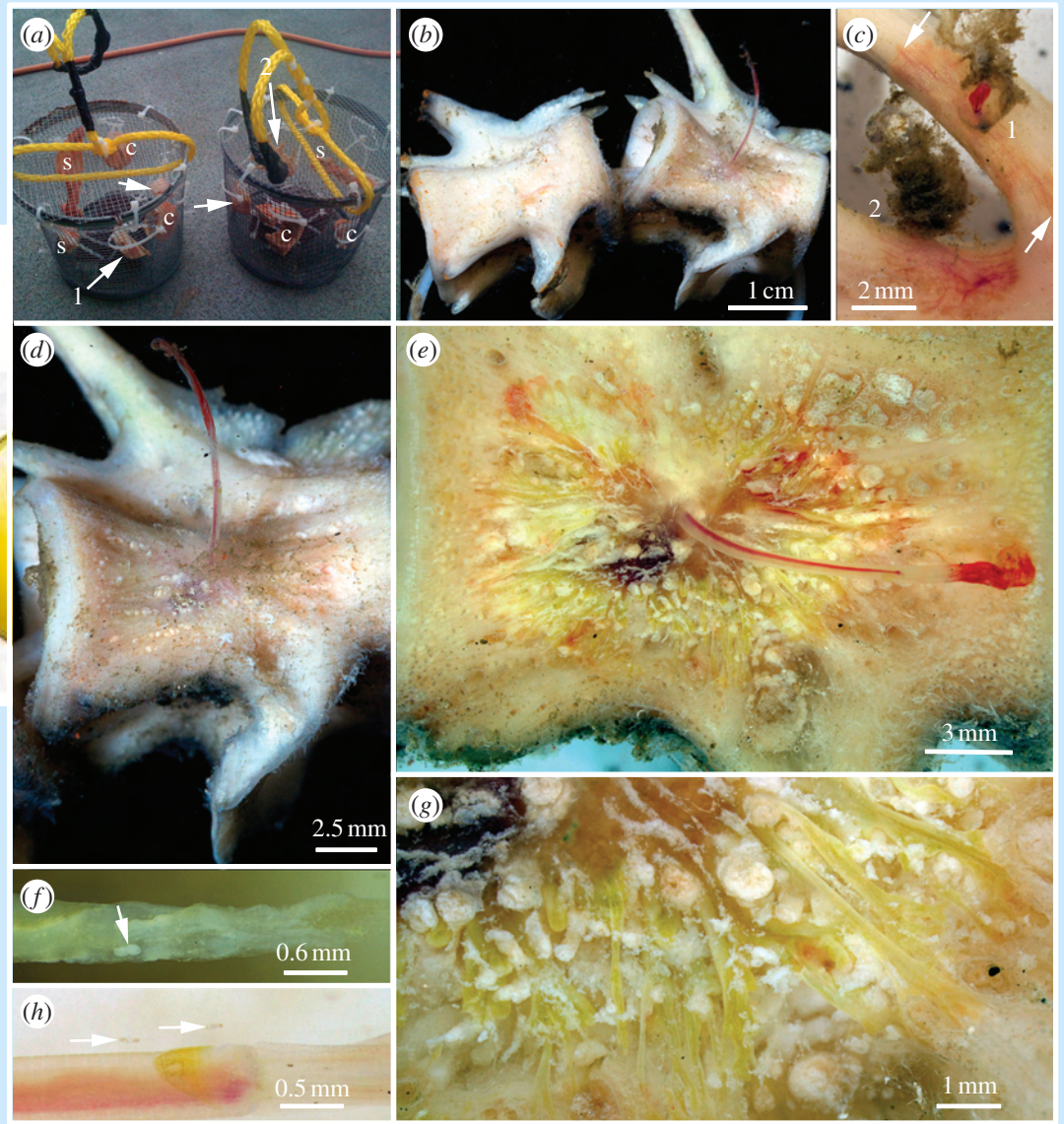
Glover *et al.* 2005. *Proc. R. Soc. B.* **272**:2587-2592; **Fujikura *et al.*. 2006.** *Zoolog. Sci.* **23**:733-740; **Dahlgren *et al.* 2006.** *Cah. Biol. Mar.* **47**:1-4.



cow bones Jones *et al.* 2008. *Proc. R. Soc. B.* 275:387-391.



galley waste Vrijenhoek *et al.* (2008). *Proc. R. Soc. B.* 275:1961-1963.

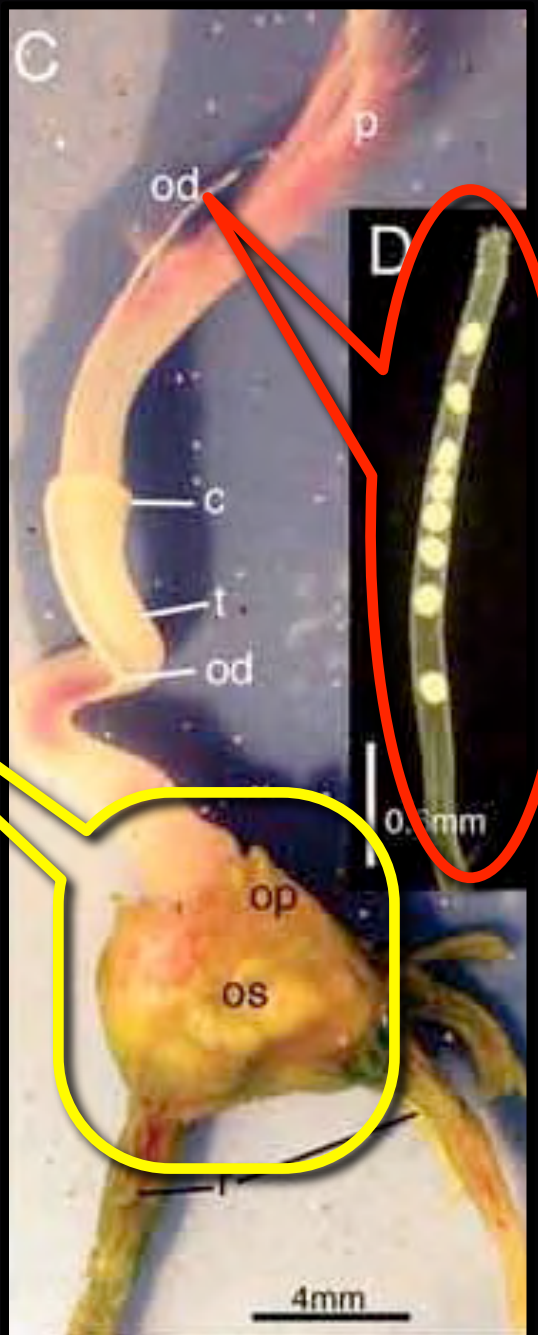


fish bones Rouse *et al.* (2011) *Biology Letters*, *Biology Letters* 2011, **7**(5):736-739..

Sexual reproduction in Osedax

O. rubiplumus female

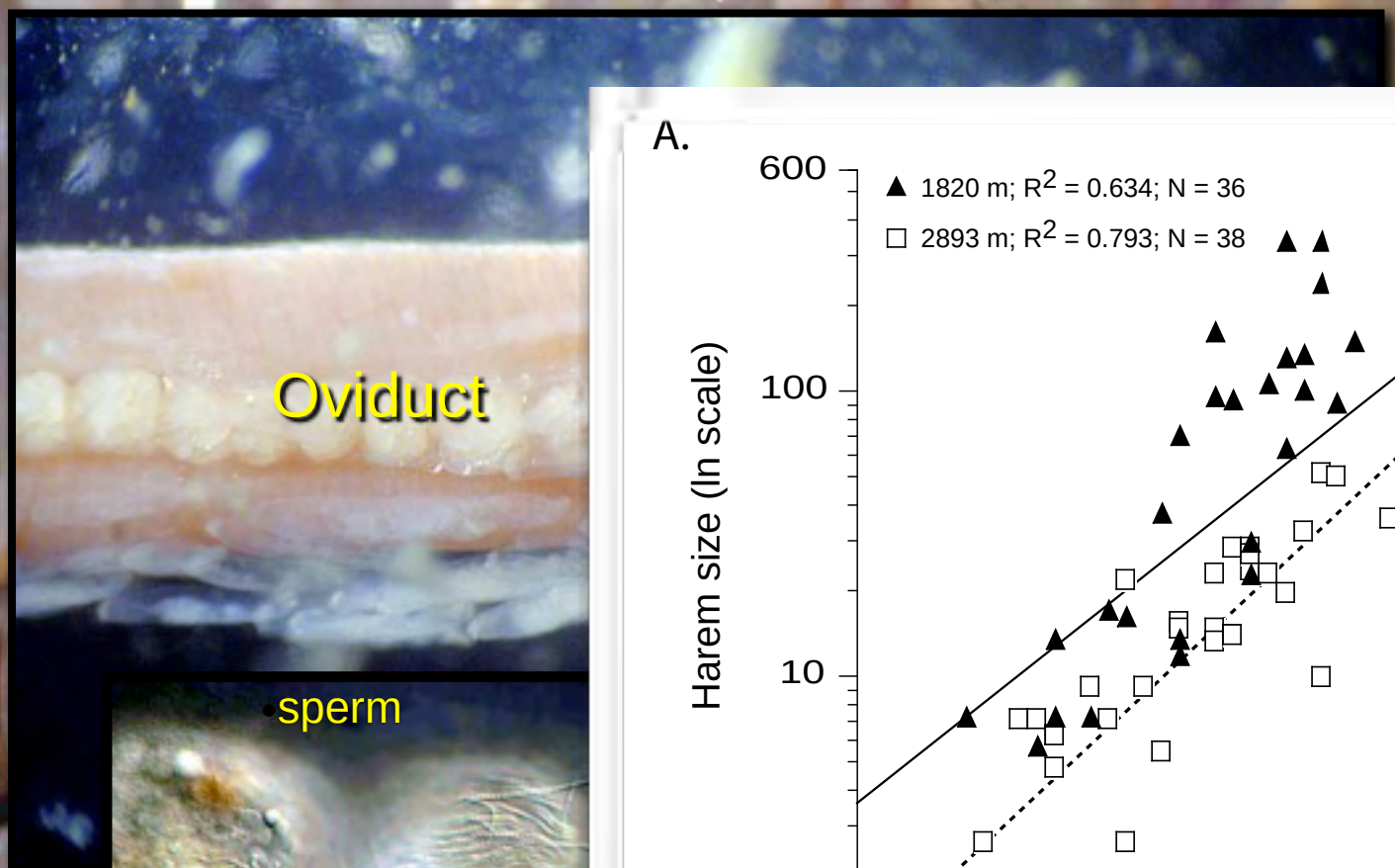
- eggs



- oviduct
- ovisac

O. rubiplumus spawning (1802 m)

Where are the males?

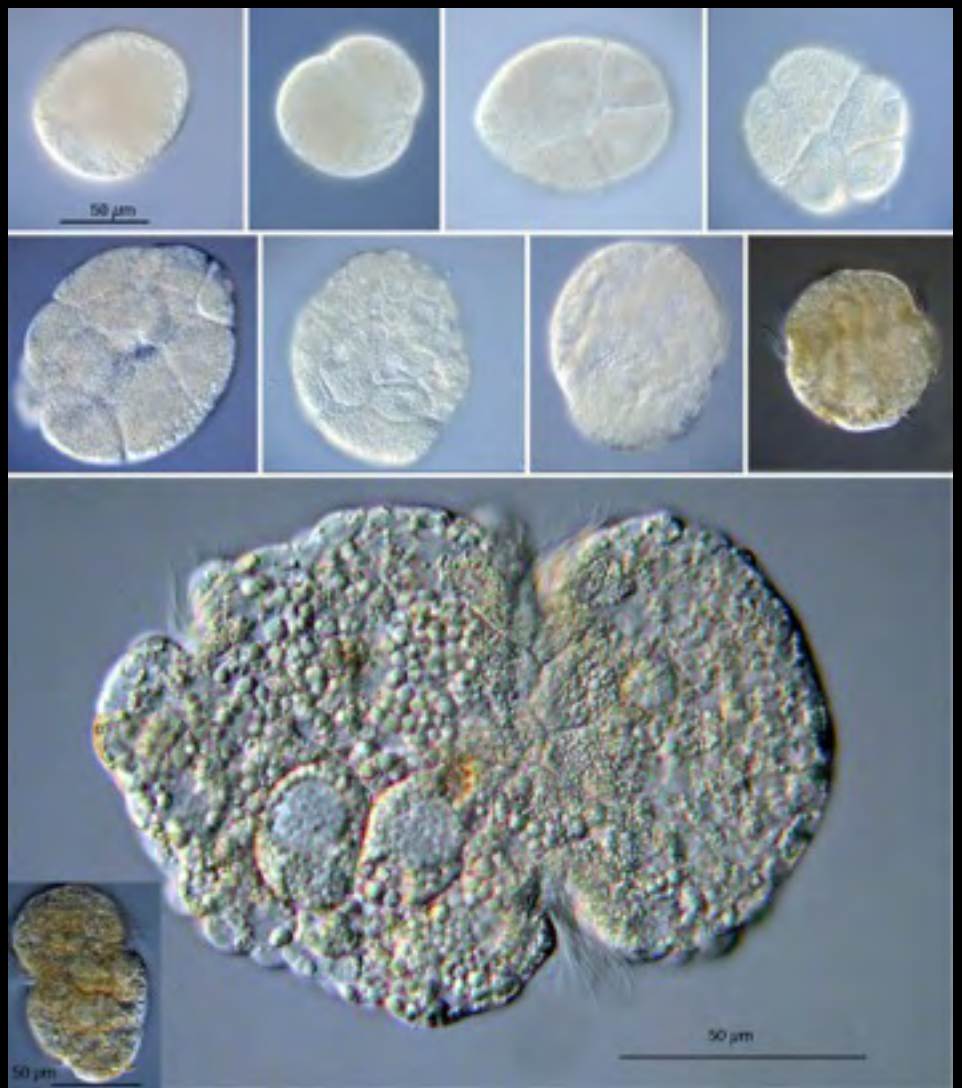


Osedax frankpressi male

Green=Phallodin= Actin = Muscle
Blue = Tubulin = Cilia, Nerves, Sperm tails
Pink = Serotonin = Nerves
Beige glubular structures = yolk

Worsaae K, Rouse G (2010) The simplicity of males: Dwarf males of four species of *Osedax* (Siboglinidae; Annelida) investigated by confocal laser scanning microscopy. *Journal of Morphology* **271**, 127-142

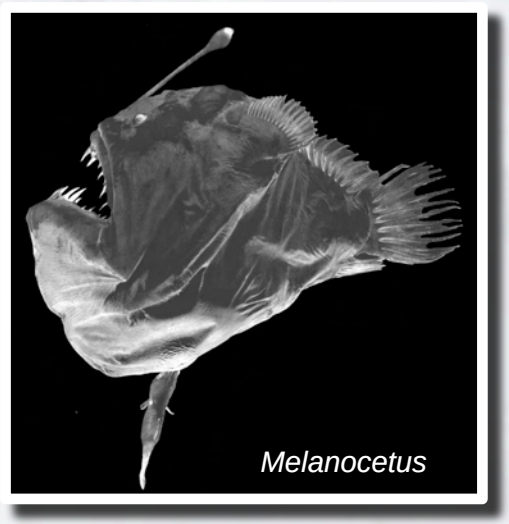
Osedax roseus development



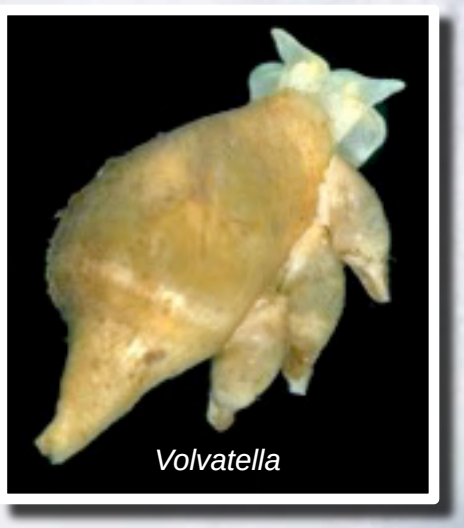
Rouse, Wilson, Johnson, Goffredi, Smart, Widmer, Young & Vrijenhoek (2009) Marine Biology **156: 395-405**

Why produce dwarf males?

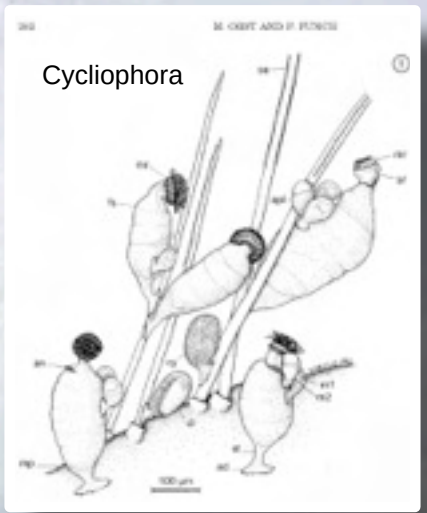
ceratioid anglerfish



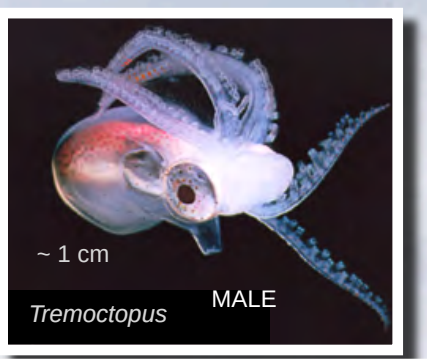
sea slugs



Cycliophora



Tremoctopus



barnacles, silk spiders, mosses, etc.

Michael Ghiselin

- Dwarf males are favored when females are sedentary, and hard to find (e.g., in deep-sea environments)
- Typically due to accelerated development or early maturation (e.g., paedomorphosis)
- Reduces sexual competition for limited nutrients and space.

(1974) **The Economy of Nature and the Evolution of Sex.** University of California Press.; 1974.

Dwarf males and environmental sex-determination (ESD) in *Bonellia viridens*

Dwarf males:

- paedomorphosis
- commensals
- androecium
- male-biased sex ratio
  3 to 4 ♂♂ per ♀
- bonellin
  green pigment = hormone that arrests development of larvae

Baltzer, F. 1934. Biological Bulletin of the Marine Biology Laboratory, Woods Hole 10:101-108.

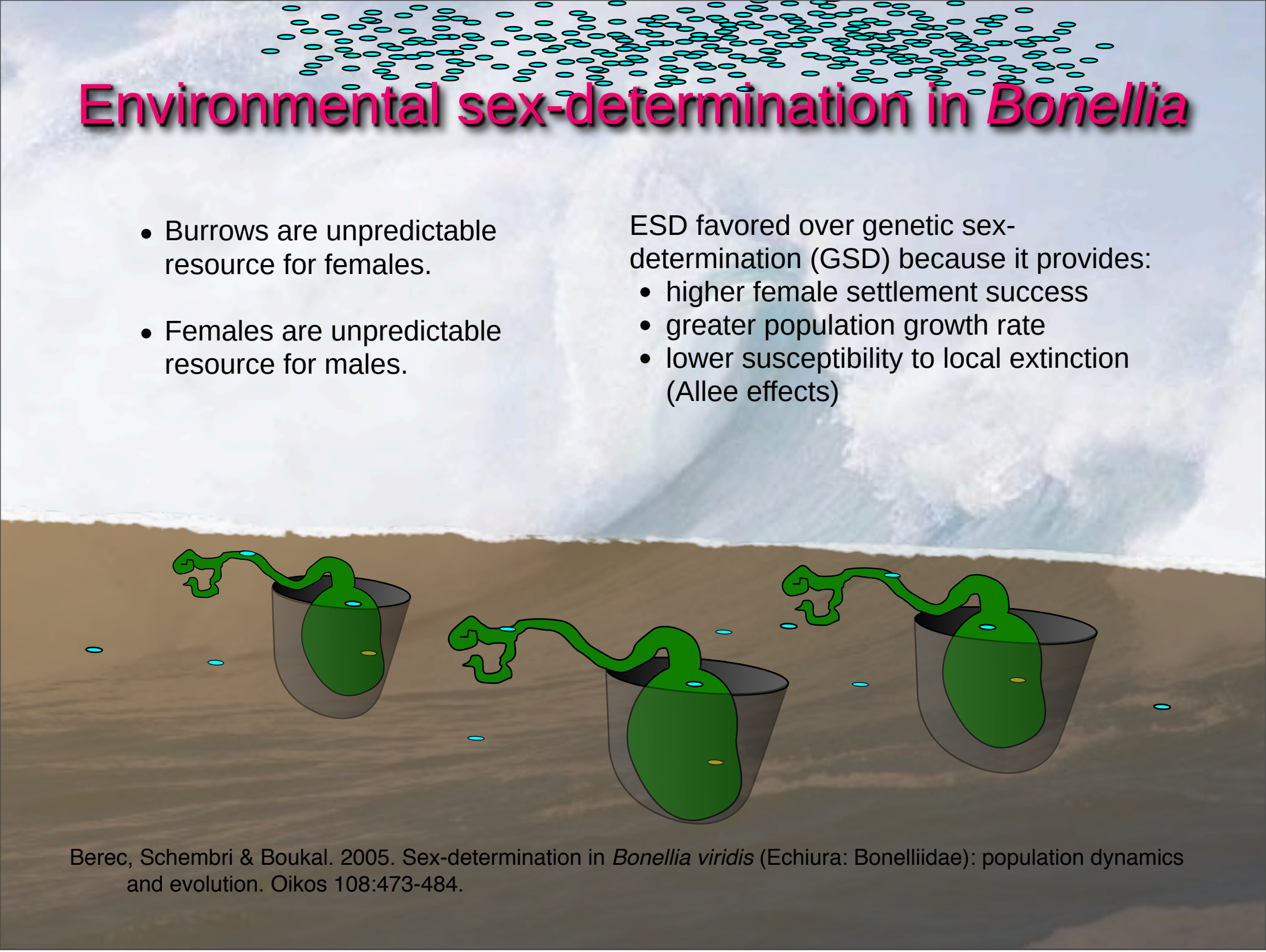
Jaccarini *et al*. 1983. Journal of Experimental Marine Biology and Ecology 66:25-40.

Environmental sex-determination in *Bonellia*

- Burrows are unpredictable resource for females.
- Females are unpredictable resource for males.

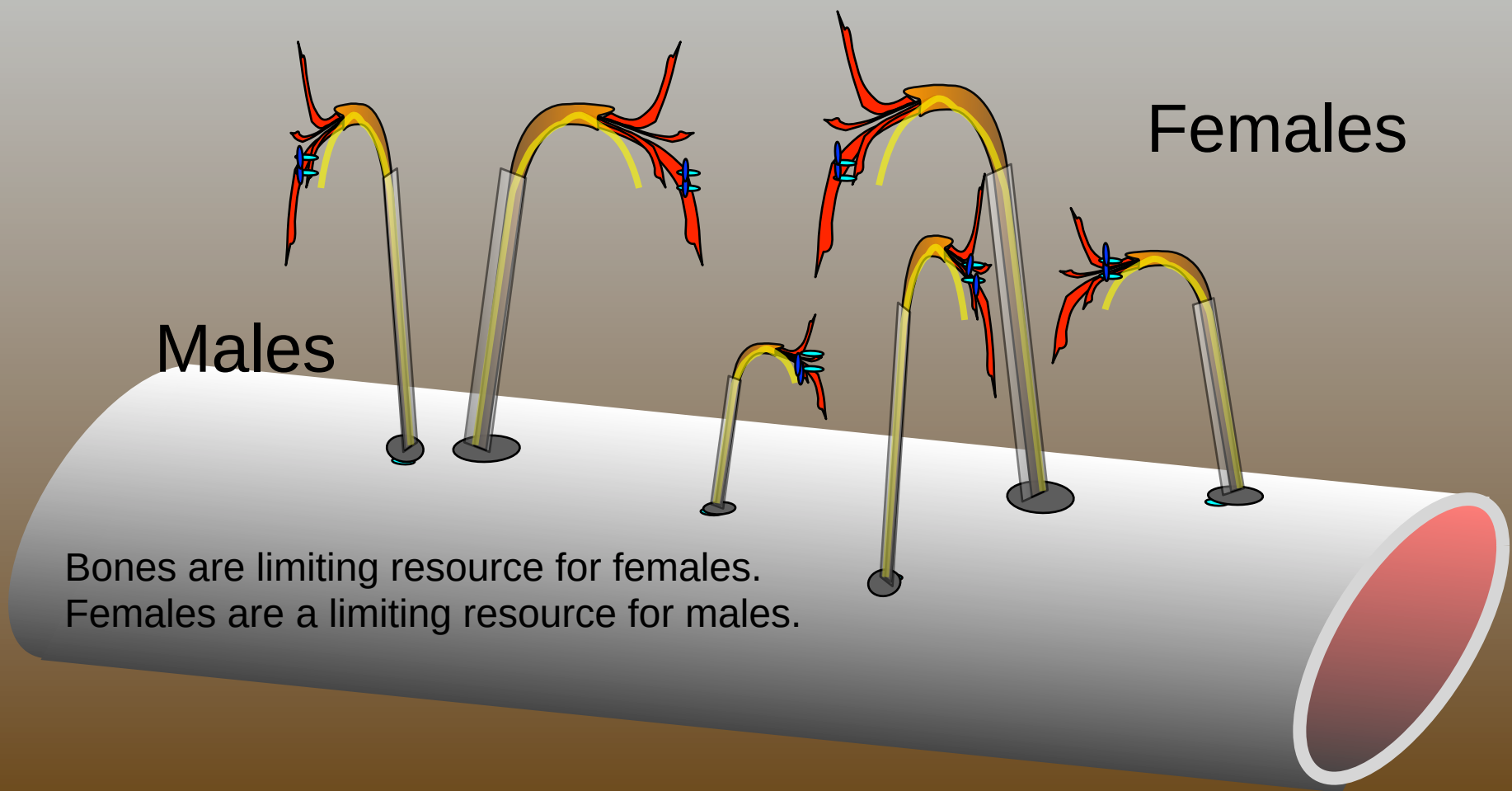
ESD favored over genetic sex-determination (GSD) because it provides:

- higher female settlement success
- greater population growth rate
- lower susceptibility to local extinction (Allee effects)



Berec, Schembri & Boukal. 2005. Sex-determination in *Bonellia viridis* (Echiura: Bonelliidae): population dynamics and evolution. Oikos 108:473-484.

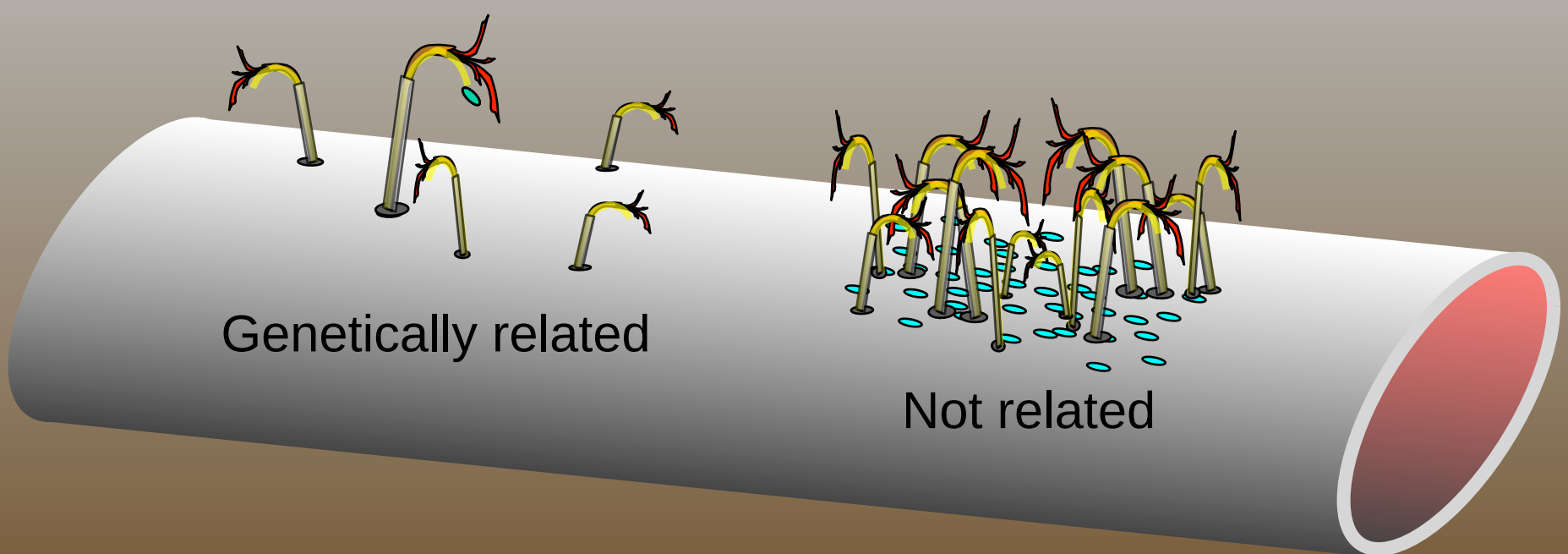
Bonellia model applied to *Osedax*



How do *Osedax* females colonize bones?

A few larvae colonize, followed by local reproduction and recruitment

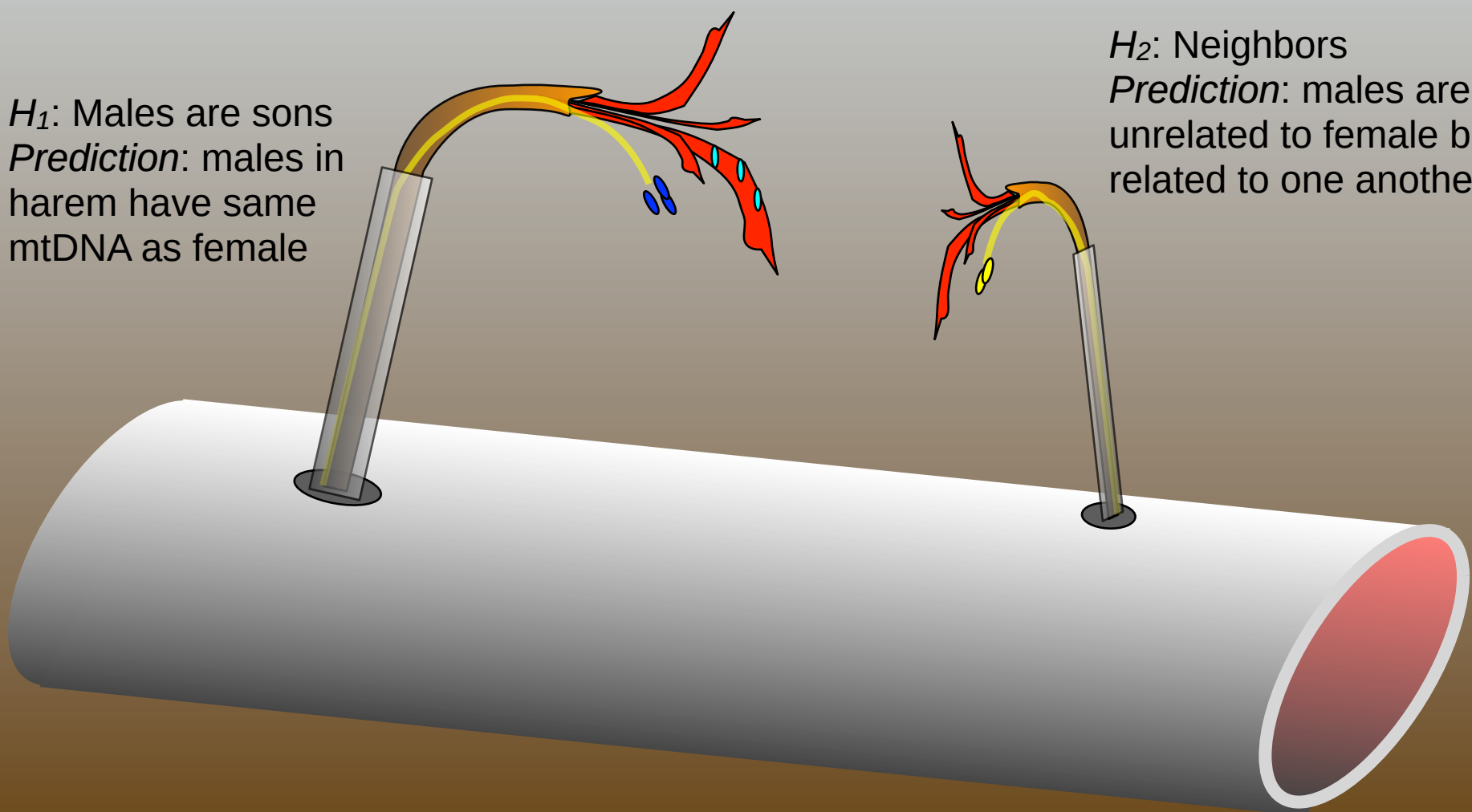
Numerous colonists from a large pool of larvae



How are males recruited?

H_0: Common larval pool.
Prediction: Males and females are equivalent samples of total diversity

H_1: Males are sons
Prediction: males in harem have same mtDNA as female



H_2: Neighbors
Prediction: males are unrelated to female but related to one another

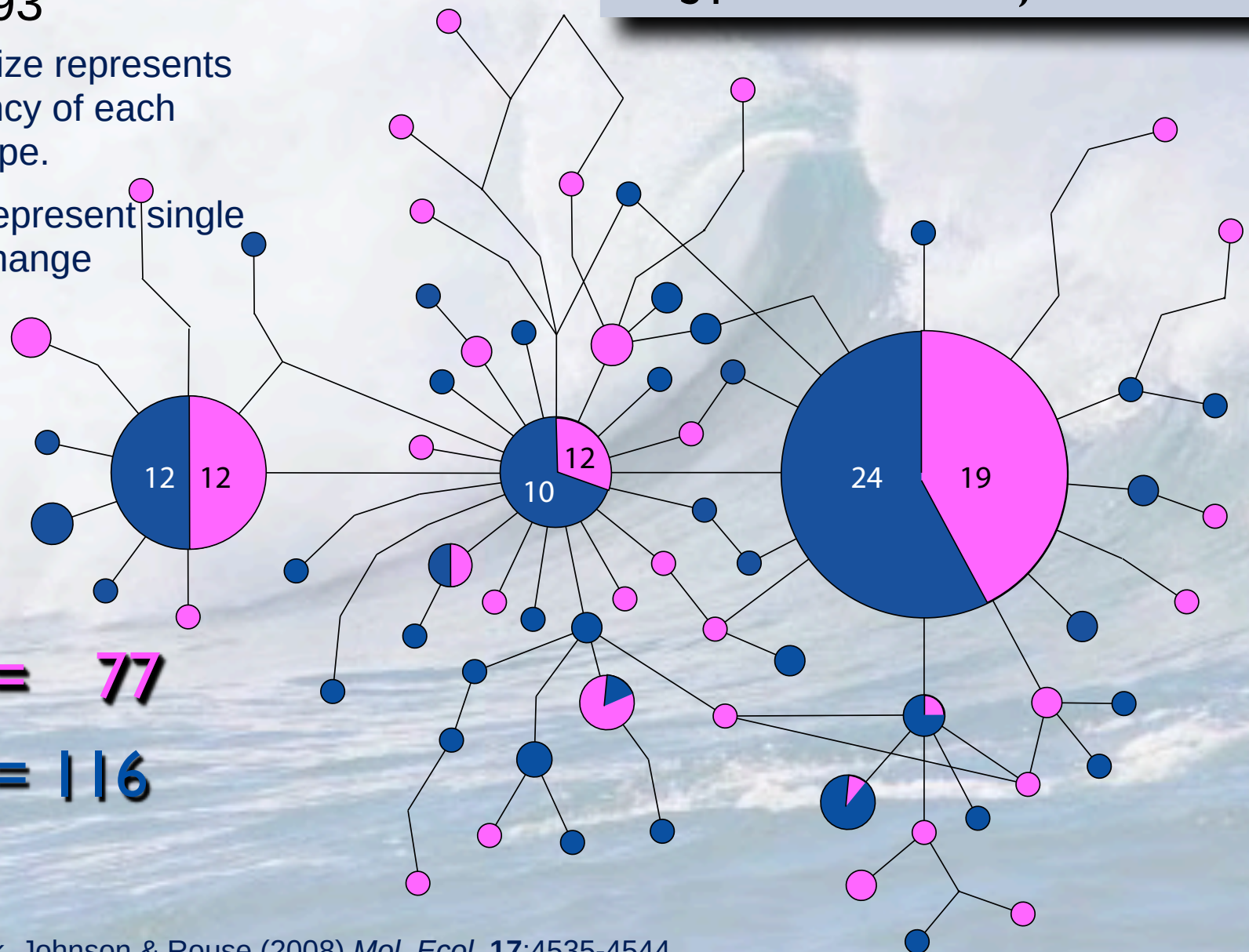
Decompose into ♂♂ vs. ♀♀

999 bp mtCOI

N = 193

- Node size represents frequency of each haplotype.
- Lines represent single base change

$F_{ST} = 0.0126; P = 0.405$



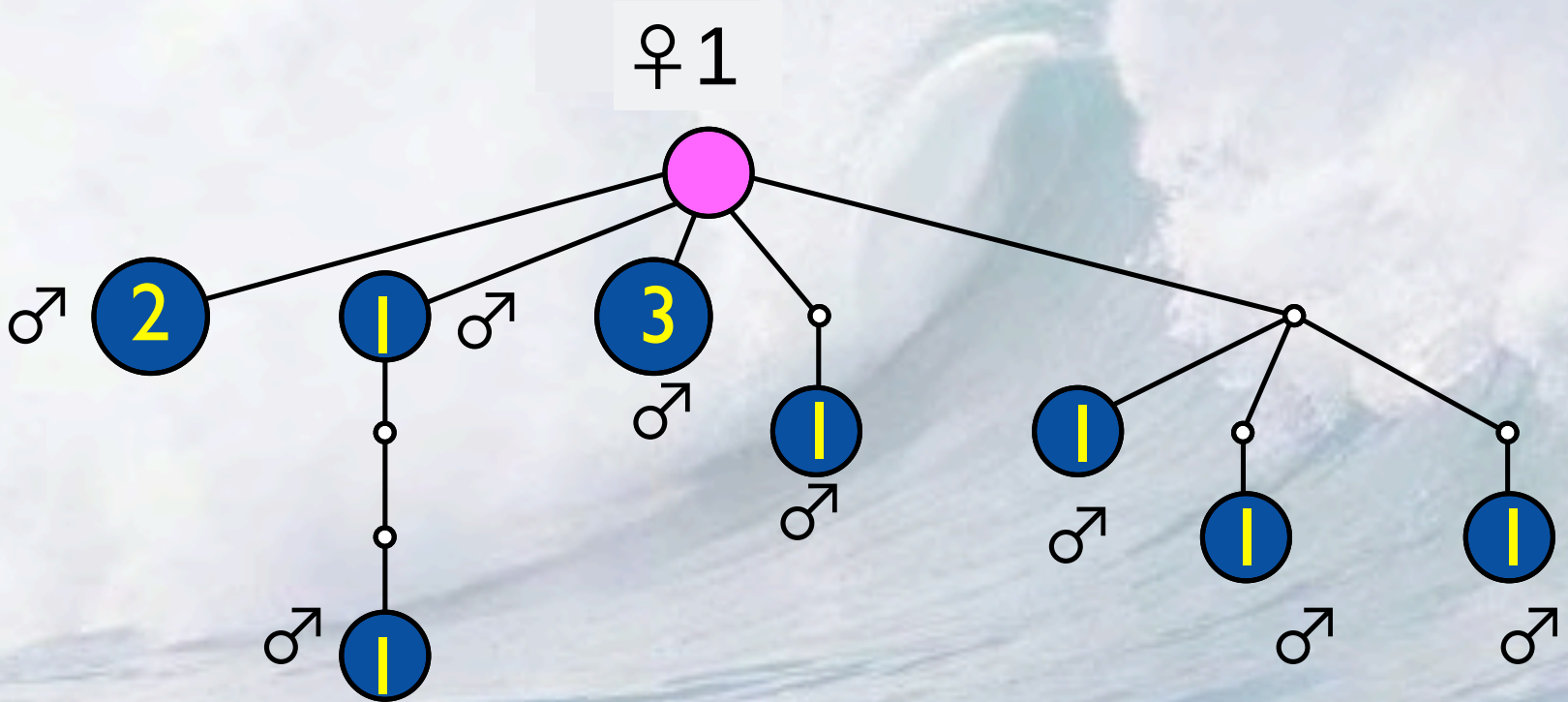
$N_♀ = 77$

$N_♂ = 116$

Vrijenhoek, Johnson & Rouse (2008) *Mol. Ecol.* **17**:4535-4544.

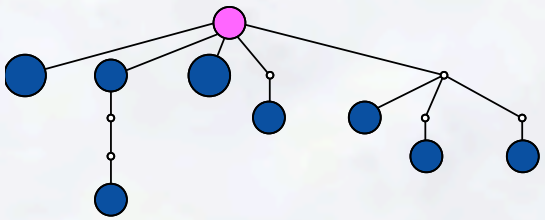
Osedax rubiplumus: male harem # 1

11 ♂♂ were MT-typed



Osedax rubiplumus: male harems 1–10

♀# 1



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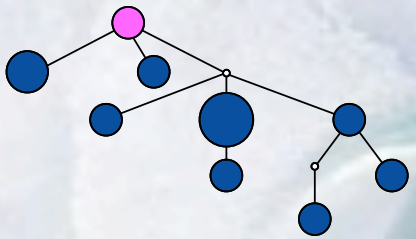

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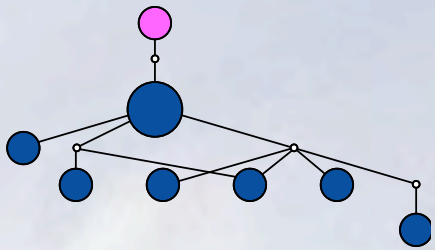

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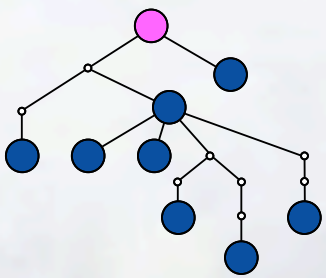
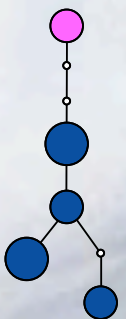
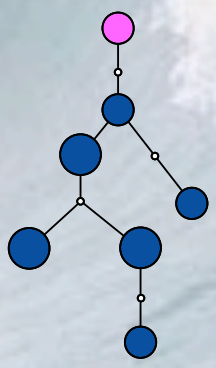


♀#5



♀#6

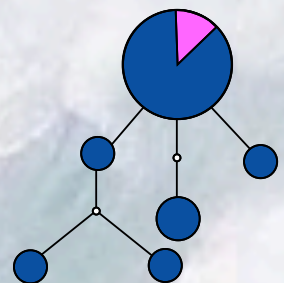


Osedax rubiplumus: male harems 1–10

$$\Pr(n) = \frac{N!}{n!(N-n)!} q_i^n (1-q_i)^{N-n}$$

♀	n	N	q_i	Pr(n)
6	1	7	0.026	0.155
7	1	9	0.124	0.387
8	1	9	0.223	0.267
9	1	10	0.223	0.230
10	4	10	0.223	0.114

♀# 7



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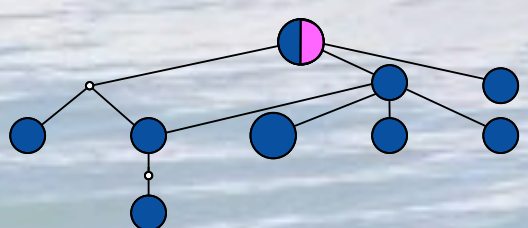
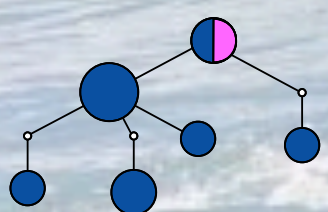
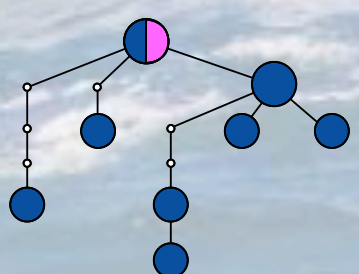


♀# 9



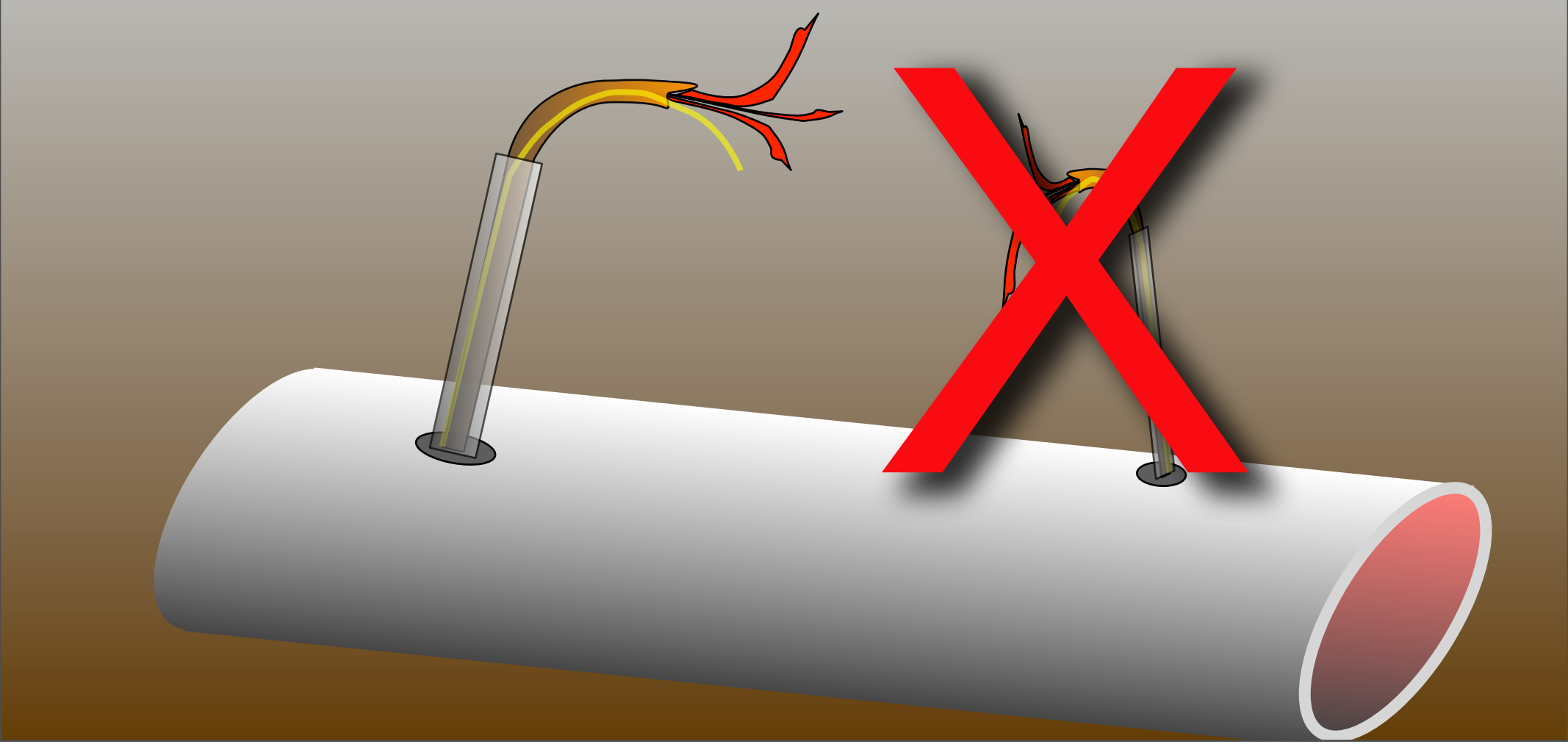
♀#10



Neighbor hypothesis

$F_{ST} = 0.0111; P = 0.501$

No significant co-ancestry among males in harems



How are males acquired?

yes

Common larval pool
Prediction: [illegible] and fe[illegible] are equivalent samples of total diversity

H_1: Males are sons
Prediction: males in harem have same mtDNA as female

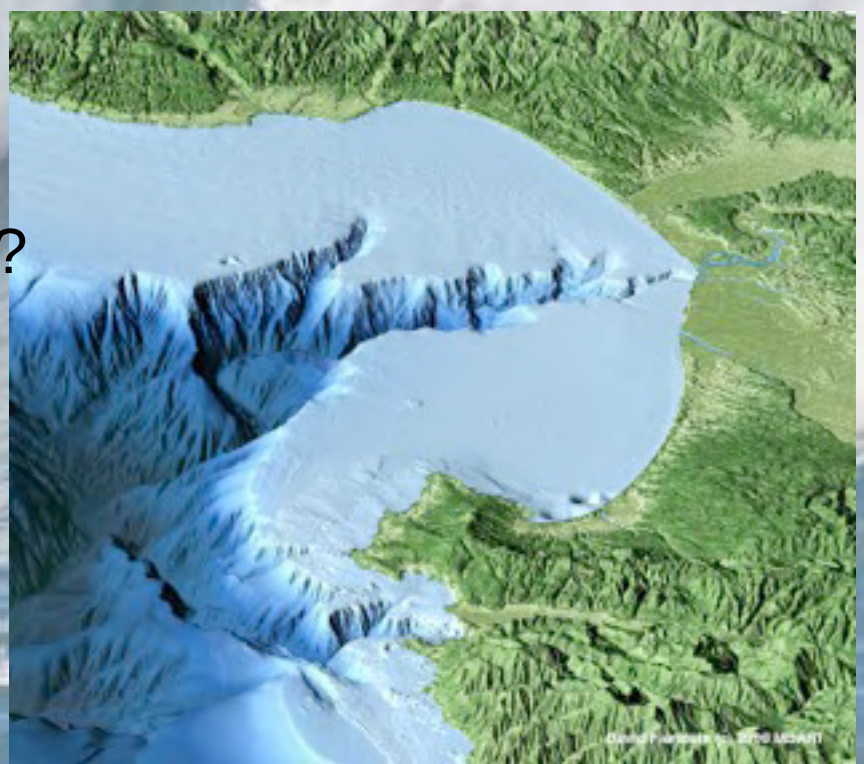
X

H_2: Neighbors
Prediction: Sons unrelated to female but related to one another

X

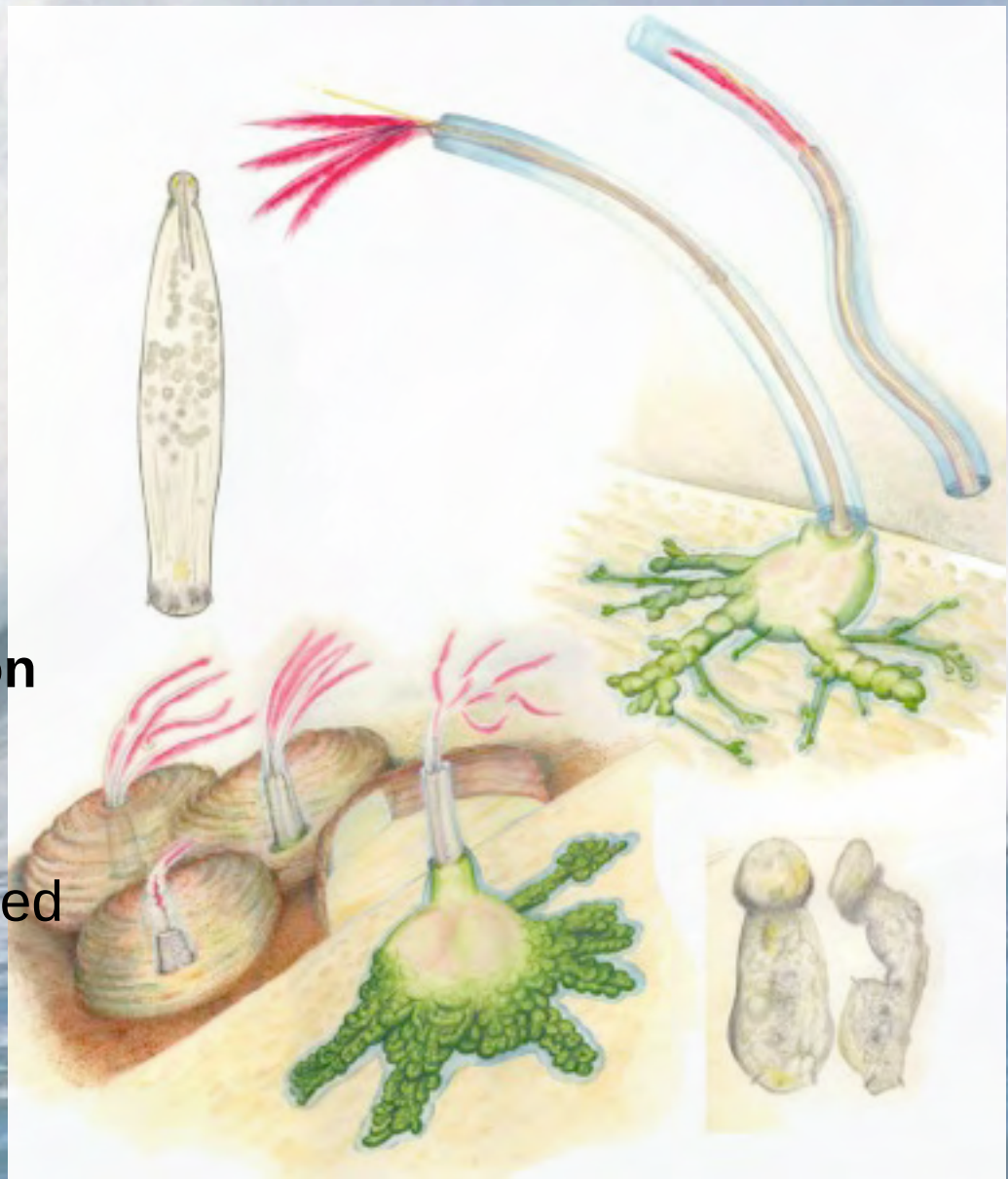
Larval pool hypothesis

- **Is depth of whale carcass relevant (1820 vs. 2893 m)? NO**
- **Is time (date of sample) relevant? NO**
- **CONCLUSION: males and females drawn equivalently from vast larval pool**
- **How vast is the pool?**
  - ✶ Monterey Bay?
  - ✶ Western N. American margin?
  - ✶ Pacific Ocean?
    - *O. rubiplumus, O. roseus and O. nudepalp-C* also found off Japan
    - *O.* cf. *frankpressi* off Papua N. Guinea



Osedax and resolution of sexual conflicts

- **Extreme sexual dimorphism**
  - subdivision of roles:
    - females obtain symbionts grow roots, feed, and make eggs
    - males move, make sperm and die
  - reduced food competition:
    - non-feeding dwarf males
  - habitat is hard to find
  - arrested male development
    - paedomorphosis
- **Environmental sex-determination**
  - larvae have little control over environment in which to settle
  - male and female fitness influenced by environment:
    - females need bones
    - males need females



Thanks to collaborators:

Shannon Johnson, MBARI

Shana Goffredi, Occidental College

Greg Rouse, UCSD/Scripps

Sigrid Katz, UCSD/Scripps

Joe Jones, U. South Carolina

Caren Braby, Oregon Fish & Wildlife Dept.

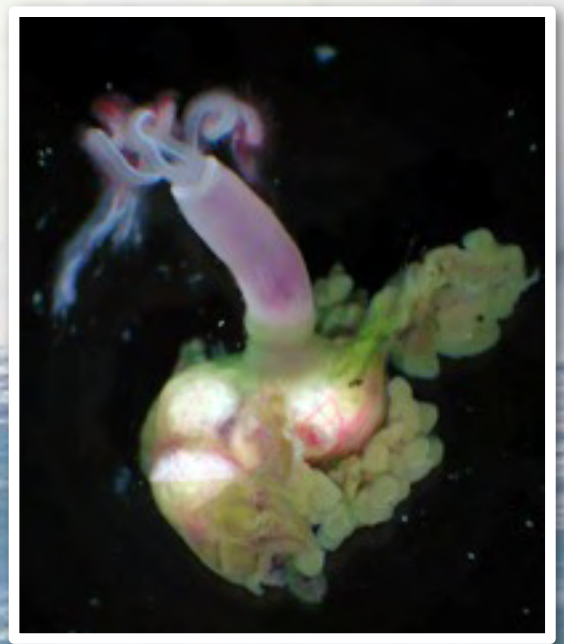
Rahel Saláthe, Penn State

POSTSCRIPT

Dynasty Foundation

POSTSCRIPT:

Do male and female reporters see the same things in a story?



National Geographic magazine (February 2005)

THE SCIENCE OF THINGS

Who Knew?

BIOLOGY

Ladies' Lunch

It's strictly the females who line up at this buffet

Robert Vrijenhoek went looking for some clams and instead discovered something that belongs in the Hall of Very Weird Animals.

He was on a research ship floating 20 miles off the coast of California, above Monterey Canyon. It was February 6, 2002, and 9,000 feet below, a robotic submersible surveyed the seafloor, desolate but for a little algae and the occasional clump of grass or rogue plastic bag. Suddenly the camera glimpsed the carcass of a whale colonized by worms with red, feathery protrusions. The sub nabbed some bones for a closer look.

The worms looked a bit like the tube worms that live around deep-sea vents, only they were smaller. Vrijenhoek named [illegible] "bone devourer." They'd been feeding on the ribs of a 30-foot-long gray whale that had sunk to the depths of the canyon (such a carcass is called a whale fall). "If there's something to eat, somebody will find a way to eat it," Vrijenhoek says. All of these *Osedax* scavengers, however, turned out to be females. Where were the males?

The mystery took two years to solve. An Australian researcher named Greg Rouse identified microscopic "sperm packages" inside the female worms' tubes. Further inquiry revealed that the packages were the males, little sperm factories living off blobs of yolk. "They just sit there giving sperm to the female until their yolk runs out," Rouse says.

Sexual dimorphism—where males and females exist in different forms—is common in the natural world. In humans, males are just slightly bigger than females. In some anglerfish species, on the other hand, the male is comically petite, attaching himself to the female and withering away, leaving only his testes.

Vrijenhoek says he knows of no sexual dimorphism as extreme as in *Osedax*. The males live their whole itty-bitty lives inside the tubes of the females, servicing their reproductive needs in an otherwise thankless existence.

Obviously this is, for some of us, a cautionary tale. Ladies, don't get any ideas. —*Joel Achenbach*

WASHINGTON POST STAFF WRITER

Arrested Development

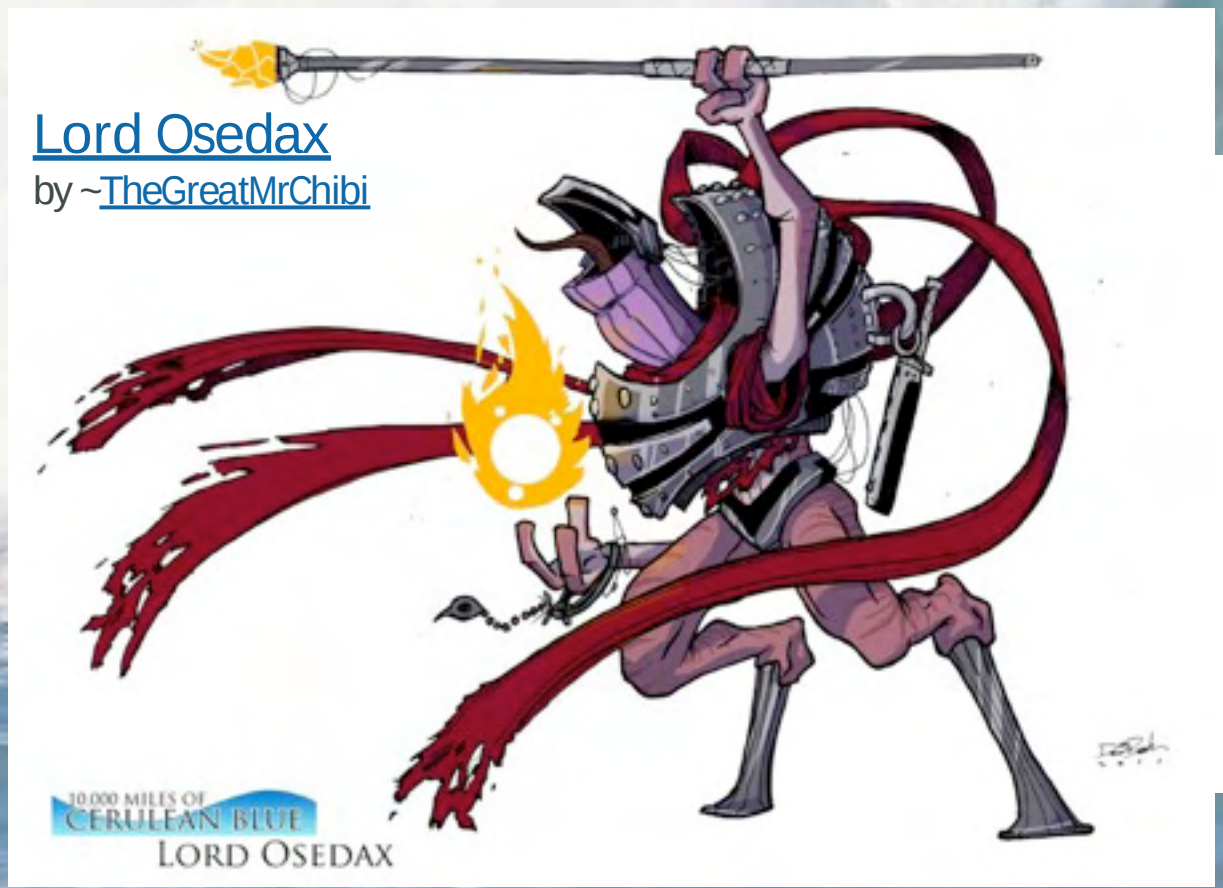
[illegible]somes, right? Not always. Environment, not genetics, determines sex in some species. Researchers postulate that *Osedax* begin as unsexed floating larvae. If they land away from other females feeding on a whale carcass, they become female. If they land on a female worm, their development is arrested, and they live ever after as male sperm donors inside the female. —*Heidi Schultz*

WEBSITE EXCLUSIVE For more on *Osedax*, and for links to Joel Achenbach's work, go to Resources at nationalgeographic.com/magazine/0502.

Google : *Osedax*

91,100 hits

(9 April 2013)

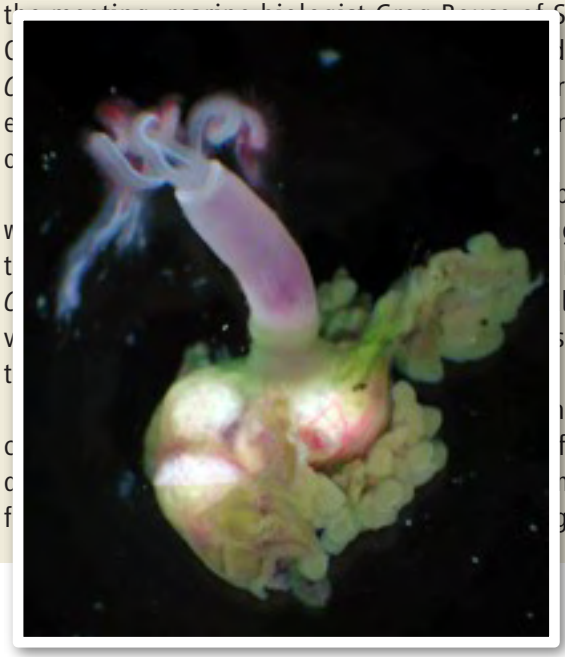


Lord Osedax is a 1,000 year old tube worm that feeds on the souls of drowned sailors. When an A'Apuan raiding party accidentally stumbled into his grotto, he devoured them, and now rides on top of one of their numbers animated corpses.

Whale Worm Sperm Factories

Five years ago, research
they found on the floo
underwater. The carcass
filled tubeworms, called
ible worms were all fema
that live in the female's
Now, biologists have ta
that they have a distinct
to mature, except with r

Since 2002, Robert
Aquarium Research Institute in Moss Landing, California
eral more whale falls, some of which are less than 100



lete this resource as they grow.
perm at different stages of development
. Rouse discovered a unique sperm duct
male's head. His studies indicate that
t the worm's head, migrate toward the
again before being released.
ed that as females age, they accumulate
sed on worms collected during recent
this view. The work suggests that imma-
come female, whereas those that settle
males—a pattern known in at least one

le worm is exciting, says marine biolo-
n University in Alabama: "It reminds us
sting discoveries that await [us] in the

–E.P.

Elizabeth Penessi

Published by AAAS

The *Osedax*

Discovery News 29 Jan 2010