Widespread Occurrence of an Intranuclear Bacterial Parasite in Vent and Seep Bathymodiolin Mussels [Supplementary Material]

SUPPLEMENTARY VIDEO LEGENDS

Videos. Animations show stacks of 2D images created by moving the focal plane through the z-axis of 10 µm thick sections at 0.2 µm intervals. S1: developmental stage 2; S2: developmental stage 3**;** S3: developmental stage 4**;** S4: developmental stage 6.

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SUPPLEMENTARY FIGURES

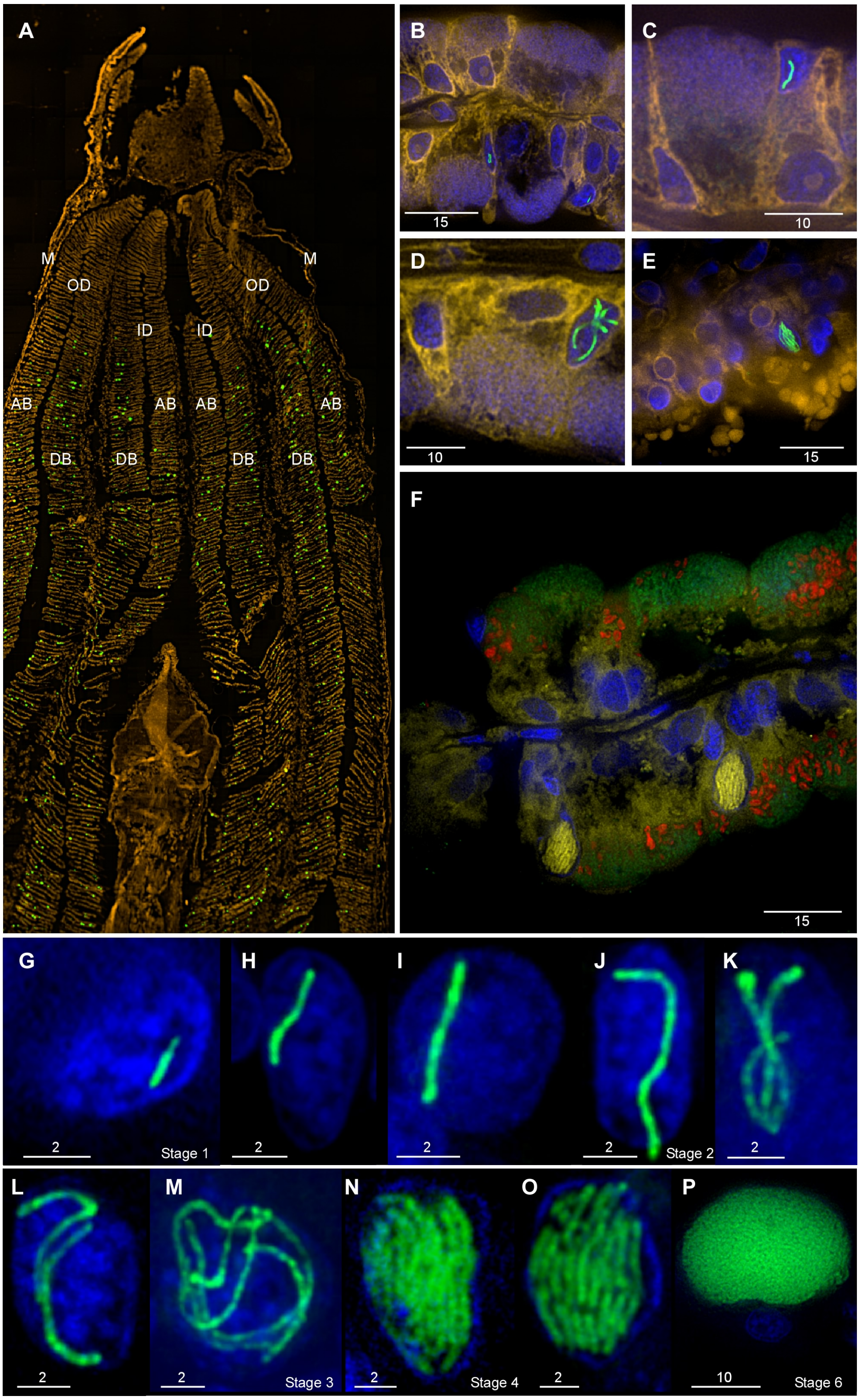

Figure S1. "*Ca*. E. bathymodioli" in various mussel tissues and developmental stages (original RGB image of Fig. 2 in main document). For figure caption see main document.

SUPPLEMENTARY TABLES

Table S1. Closest relatives of "*Candidatus* Endonucleobacter bathymodioli" (Logatchev)

Uncultured gamma proteobacterium or isolate	Identities [%]	Sequence length [bp]	Associated with	Taxon or species	Accession Number
clone B6F4	98.8	415	hydrothermal fluid	-	AM268617
clone Dd-spF-A85	97.2	389	marine sponge	*Discodermia dissoluta* [a]	AY897105
clone nerCTABwin06-4*	96.6	684	polychaete worm (gut)	*Nereis succinea* [b]	EU797581
clone sedCTABwin06-1*	96.0	705	marine sediment	-	EU797583
clone CD14B9 and 46 other clones	95-97	212-616	coral	*Diploria strigosa* [a]	AF442025
clone Fiji7-A3	95.4	479	seawater	-	DQ670587
clone C19; clone CRNA5	94.5; 95.1	1489; 413	colonial ascidian	*Cystodytes dellechiajei* [b]	DQ884169; DQ884160
*Spongiobacter nickelotolerans**	94.9	1492	marine sponge	-	AB205011
*Kistomonas amurensis**	94.6	1343	seastar	*Asterias amurensis*	EU599216
*Endozoicomonas elysicola**	94.4	1437	sea slug	*Elysia ornata* [b]	AB196667
clone H1	94.4	531	sea urchin	*Tripneustes gratilla*	AM495252
clone *Spongiobacter* sp. ME19 and 5 other clones	92-94	1502-1527	*coral*	*Muricea elongata* [b]	DQ917863
clone HOC27; clone HOC2	94.0	1473	marine sponge	*Halichondria okadai* [b]	AB054161; AB054136
clone PDA-OUT2; clone PDA-OUT3	93.6; 94.0	1500; 1503	coral	*Pocillopora damicornis* [b]	AY700600; AY700601
clone CN34	93.9	1448	marine sponge	*Chondrilla nucula*	AM259915
clone s1uc48	93.9	682	coral	*Oculina patagonica* [b]	DQ416306
clone H262*	93.9	703	marine sponge; sea slug	Dictyoceratida, Thorectidae [b]; Pleurobranchidae [b]	AY370006
clone H425*	93.9	740	marine sponge	Halichondrida, Axinellidae [b]; Halichondrida, Halichondriidae [b]	AY370008
clone C23	93.4	1486	colonial ascidian	*Cystodytes dellechiajei* [b]	DQ884170
clone HstpL43	93.4	1024	sea grass	*Halophila stipulacea* [b]	AF159674
clone MOLA 531*	93.3	1423	marine sponge	*Petrosia ficiformis*	AM990755
clone Gamma7	93.2	919	fish	*Salmo salar* [c]	AY494615
clone 1C227608	92.9	1420	seawater	-	EU799933
clone CD207E01 and 8 other clones	89.3-92.7	865-983	coral	*Montastraea annularis* [b]	DQ200605
clone NWCu007	92.2	895	marine sponge	*Rhopaloeides odorabile* [b]	AF313496
clone *Spongiobacter* sp. EC22; clone *Spongiobacter* sp. EC121	92.0; 91.8	1504; 1507	octocoral	*Erythropodium caribaeorum* [b]	DQ889931; DQ889891
nuclear inclusion X (NIX)	91.1	695	clam	*Siliqua patula* [d]	M94380-82
clone *Spongiobacter* sp. BME76	90.9	1512	coral	*Muricea elongata* [b]	DQ917830
clone OTU 6 and 7 other clones	88.1-90.8	1296-1298	coral	*Alcyonium antarcticum* [b]	DQ312244
*Zooshikella ganghwensis**	90.6	1441	marine sediment		AY130994
Oceanrickettsia ariakensis; R6; *O. ariakensis*; R54	87.8	1300; 1304	oyster	*Crassostrea ariakensis*	DQ118733; DQ123914

[a] Clones obtained from healthy specimens; [b] Condition of specimens not stated; [c] Clone obtained from specimen infected with amoebic gill disease; [d] bacterium involved in massive mortalities of the Pacific razor clam *Siliqua patula*; * isolates

Table S2. Nucleotide differences of dominant and minor "*Ca.* E. bathymodioli" 16S rRNA sequences

Specimen	Location		# examined [a]	# infected [b]	# full sequences [c]					nucleotide position [d]		Probe [e]	Acc. No	FISH [f]
					I	**II**	**III**	**IV**	**total**					
B. brooksi	AT, AL, CH	GoM	4	4	8	8	5	6	**27**			o	FM162182-FM162184	+
B. heckerae	WF, CH	GoM	3	1	5	-	-	na	**5**			o	FM162185	+
"B." childressi	AL, MC	GoM	2	1	-	2	na	na	**2**			o	FM244838	+
***B.* sp.**	Lilliput	MAR	1	1	4	na	na	na	**4**			y	FM162186	/
B. azoricus	Menez Gwen	MAR	3	1	1	-	-	na	**1**			o	FM162187	+
B. puteoserpentis	Logatchev	MAR	4	3	-	3	3	3	**9**	474				+
dominant phylotype					-	1	2	3	6	**G**		x,y,z	FM162188	
minor phylotype					-	2	1	-	3	**A**		x,y,z	FM162189	
***B.* sp.**	Wideawake	MAR	4	4	8	6	8	8	**30**	474	1023			+
dominant phylotype					8	4	5	-	17	**A**	**A**	y,z	FM162190	
minor phylotype I					-		1	8	9	**G**	**A**	y,z	FM162191	
minor phylotype II					-	2	1	-	3	**G**	**G**	y,z	FM162192	
minor phylotype III					-	-	1	-	1				FM162193	
B.* aff. *thermophilus	German Flats	PAR	4	3	6	8	8	-	**22**	137	184			+
dominant phylotype					5	7	7	-	19	**C**	**C**	o	FM162194	
minor phylotype					1	1	1	-	3	**T**	**T**	o	FM162195	

[a] number of examined individuals; [b] number of infected individuals; [c] number of full sequences per individual (I-IV); [d] position based on *Escherichia coli* numbering; [e] probe specificity: x,y,z – probes Bnix64 (x), Bnix643 (y), and Bnix1249 (z) match their target specifically (no mismatches), probe Bnix64 is applicable only to "*Ca.* E. bathymodioli" from *B. puteoserpentis* (Logatchev) and *"B." childressi* as this part of the 16S rRNA sequence is not known from the other phylotypes, o – either one of the probes y and z has one single mismatch; [f] results of gill tissue analysis by fluorescence in situ hybridization (FISH); Abbreviations: AT – Atwater Valley, AL – Alaminos Canyon, CH – Chapopote, MC – Mississippi Canyon, WF – West Florida Escarpment, GoM – Gulf of Mexico, MAR – Mid-Atlantic Ridge, PAR – Pacific-Antarctic Ridge; na – not applicable, + positive FISH result, / negative FISH result

Table S3. Intranuclear bacteria described from protists

Protist taxon *Host species*	Protist super group and higher ranking taxa Designation of bacteria inside nuclei	Shape	Phylogeny	Reference
Ciliophora	**Chromalveolata: Alveolata**			
Paramecium spp. and numerous other ciliate genera	endonuclear bacteria; (*Holospora* spp. [9 species] [a, c], *Caedibacter caryophilus* [d], *C. macronucleorum* [c], *Nonospora macronucleata* [c], and several other but unnamed endonuclear bacteria)	rods	Alpha	(Görtz, 1983, 1986; Görtz and Brigge, 1998; Görtz, 2001; Fokin, 2004; Görtz, 2006)
Dinoflagellata	**Chromalveolata: Alveolata: Dinozoa**			
Gyrodinium instriatum	endonucleoplasmic bacteria (unnamed) [a, d]	rods	Beta	(Silva and Franca, 1985; Alverca et al., 2002; Biegala et al., 2002)
Gymnodinium splendens; Glenodinium foliaceum	endonuclear bacteria (unnamed) [a, c]	rods	nd	(Silva, 1978; Silva and Franca, 1985)
Euglenida	**Excavata: Euglenozoa**			
Euglena spirogyra; Strombomonas conspersa; Trachelomonas oblonga; Lepocinclis ovum; Hyalophacus ocellatus	endonuclear bacteria (unnamed) [d]	rods	nd	(Leedale, 1969)
Euglena hemichromata	endonuclear bacteria (unnamed) [a, b, c]	rods	nd	(Shin et al., 2003)
Euglena deses	*Caryococcus hypertrophicus* [b]	cocci	nd	(Dangeard, 1902, 1933; Kirby, 1941, 1944)
Trachelomonas sp.	electron dense bodies [e]	rods	nd	(Ueda, 1960)
Peranema trichophorum	rod-shaped bodies [a, e]	rods	nd	(Roth, 1959)
Trichonymphida	**Excavata: Parabasalia**			
Trichonympha seapiculae	nuclear parasites	spherical bodies	nd	(Kirby, 1941)
Trichonympha corbula	*Caryococcus nucleophagus* [c]; *Caryococcus cretus* [c]	cocci	nd	(Kirby, 1944)
Trichonympha peplophora	*Caryococcus invadens* [c]	cocci	nd	(Kirby, 1944)
Trichonympha chattoni	*Caryococcus dilatator* [a, b, c]	cocci	nd	(Kirby, 1944)
Staurojoenina assimilis	intranuclear symbionts [a, c]	cocci	nd	(Dolan et al., 2004)
Cristamonadida	**Excavata: Parabasalia**			
Caduceia versatilis	intranuclear bacteria [a, e]	cocci	nd	(Tamm and Tamm, 1974; d'Ambrosio et al., 1999)
Spirotrichonymphida	**Excavata: Parabasalia**			
Holomastigotoides hemygymnum	bactéries dans le noyau [e]	rods	nd	(Hollande and Carruette-Valentin, 1971)

[a] bacteria cause hypertrophy of nuclei; [b] intranuclear bacteria are associated with the disappearance of chromatin; [c] obligate intranuclear; [d] facultative intranuclear; [e] unclear if obligate or facultative in the nucleus; nd – not determined