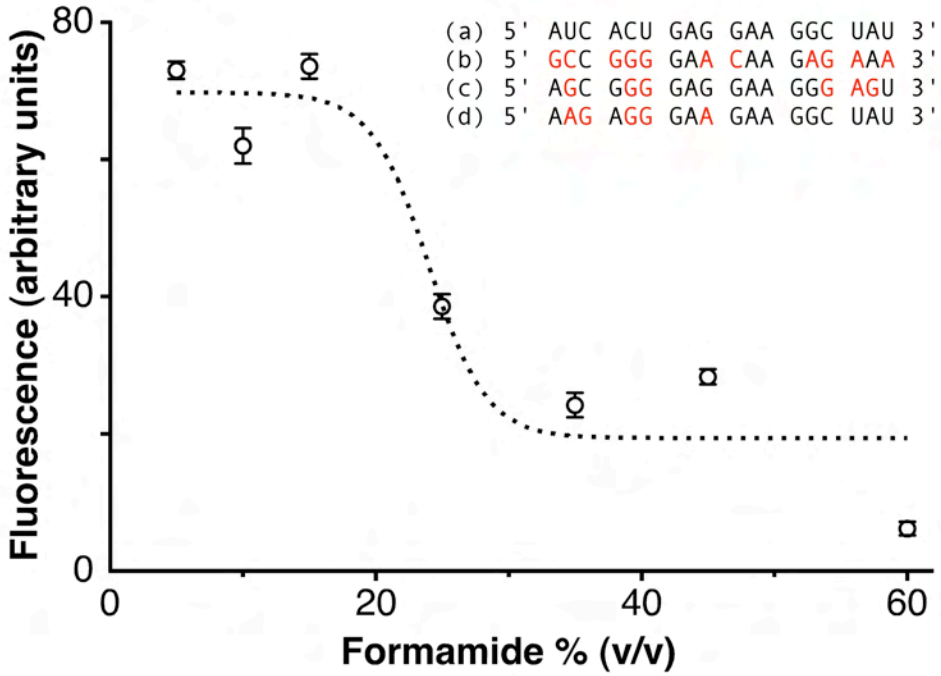
Supplemental Table S1 Phylogetic identity of different 16S rRNA gene sequence types present among 32 analyzed clones originating from a *Xenoturbella bocki* whole body clone library generated with general bacterial PCR primers.

Clone[a]	Closest relative[b]	Taxonomic affiliation	pairwise identity[b]
Xbod3b (16/32)	Zebrafish digestive tract clone aab54f12 (DQ814869)	*Alphaproteobacteria Rhizobiales*	99%
Xbod4 (1/32)	South China Sea sponge-associated clone B27 (FJ999599)	*Betaproteobacteria Burkholderiales*	99%
Xbod8 (2/32)	Drinking water treatment plant clone MF117 (FJ572671)	*Betaproteobacteria Burkholderiales*	98%
Xbod14 (1/32)	Pepper plant isolate *Achromobacter* sp. BPZ11 (GQ853553)	*Betaproteobacteria Burkholderiales*	99%
Xbod17 (7/32)	Drinking water distribution system simulator clone DSSD11 (AY328710)	*Alphaproteobacteria Rhodospirillales*	93%
Xbod23 (5/32)	Mono Lake isolate *Halomonas* sp. ML-028 (AF139994)	*Gammaproteobacteria Oceanospirillales*	99%

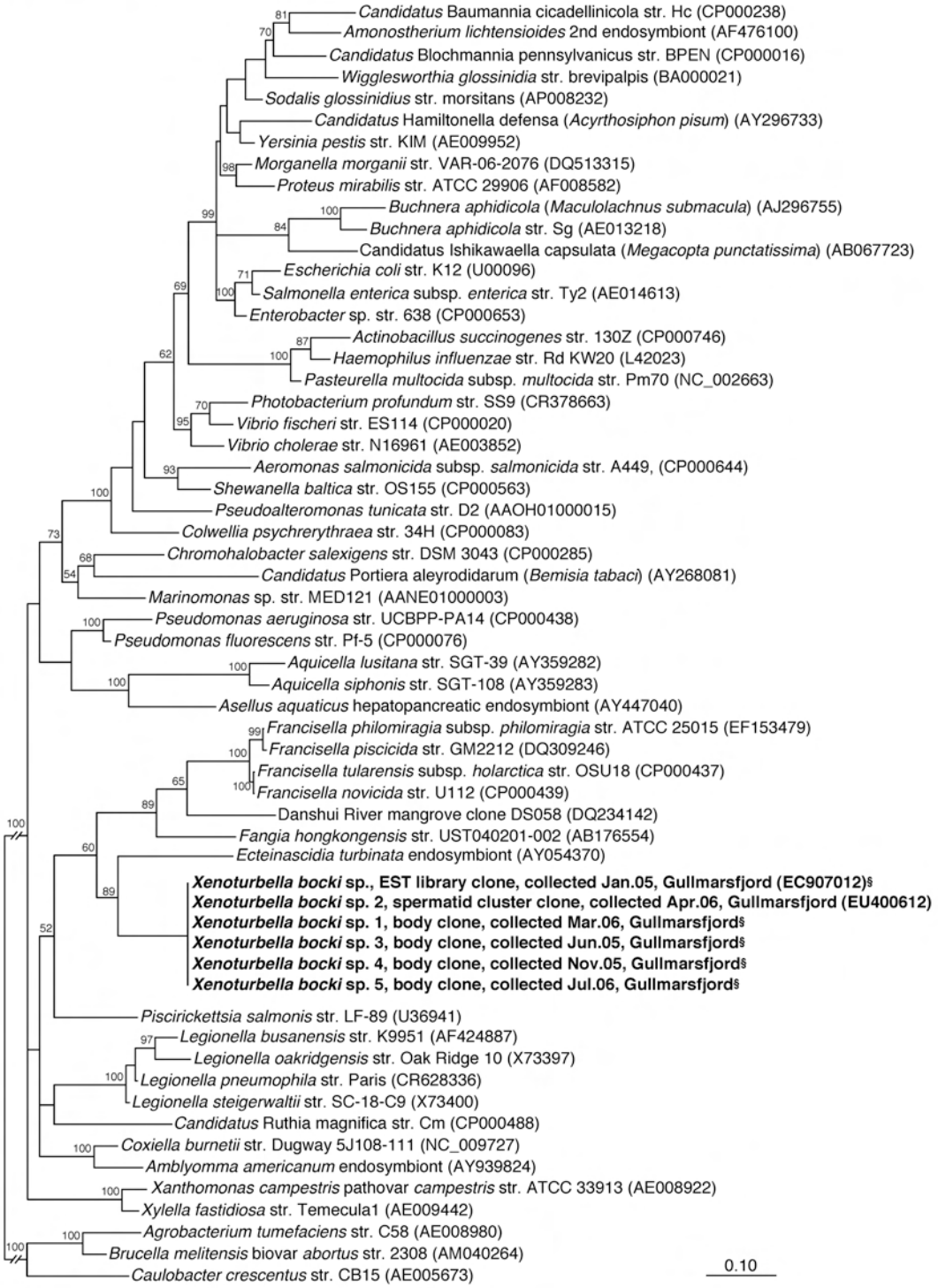
[a] Numbers in parentheses indicate the relative abundance of the respective clones.

[b] As determined by comparison against the NCBI (http://www.ncbi.nlm.nih.gov) nr nucleotide sequence database using BLAST.

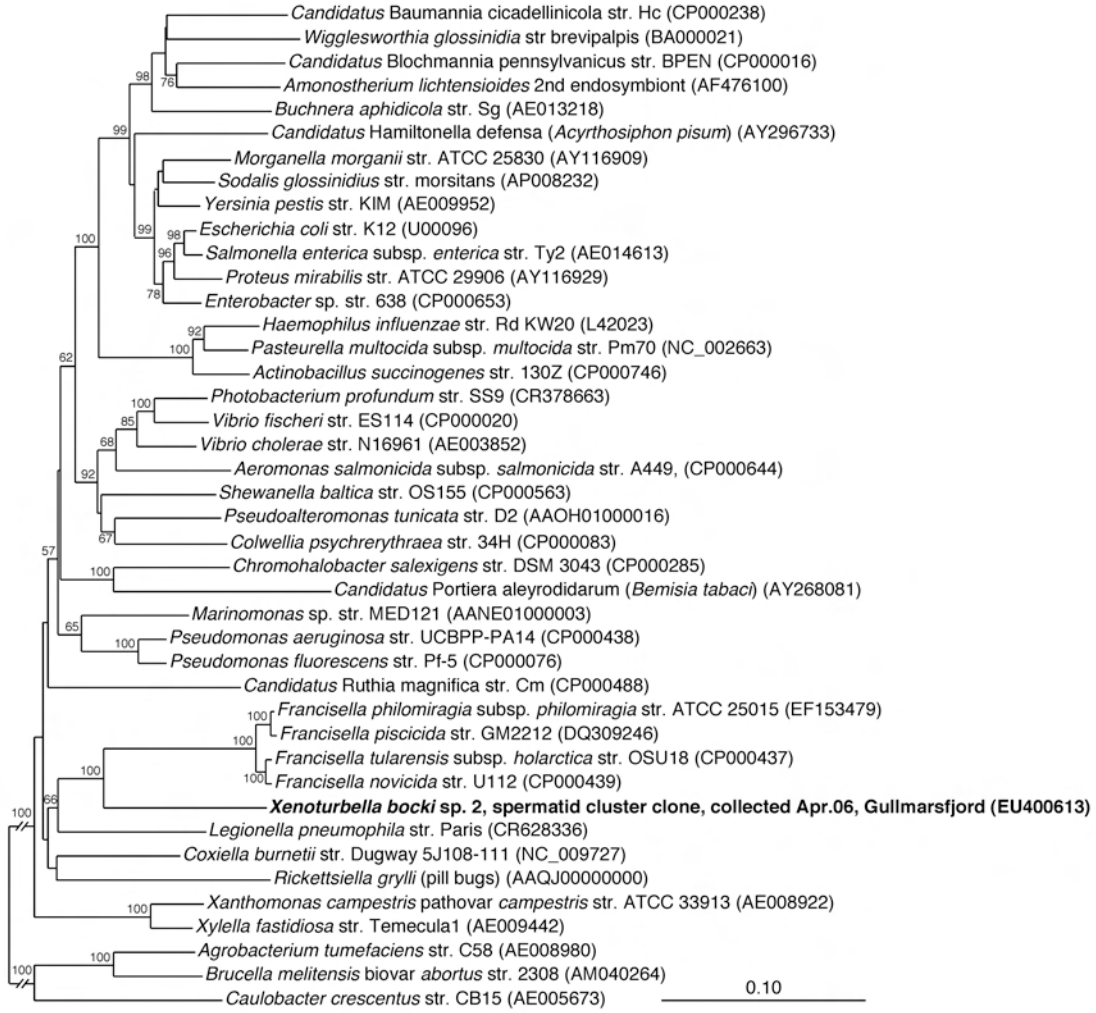


Supplemental Figure S1 Dissociation curve for probe XenoGam441 determined with transformed *E. coli* JM109(DE3) cells expressing the 16S rRNA gene of the *X. bocki* gammaproteobacterial endosymbiont. The dotted line represents a sigmoidal best fit. Error bars indicate standard errors, n = 3 random microscopic fields of view (630x) images collectively containing at least 200 hybridized cells. The insert shows an alignment of the 16S rRNA probe target region in (a) the *X. bocki* gammaproteobacterial endosymbiont, (b) the *X. bocki* chlamydial endosymbiont, (c) untransformed *E. coli* JM109(DE3) [AB305017] and (d) *Desulfovibrio* strain I8 [FJ655907]; no positive hybridizations were recorded at any formamide concentration tested for the two latter strains. Mismatches against the probe are marked in red.

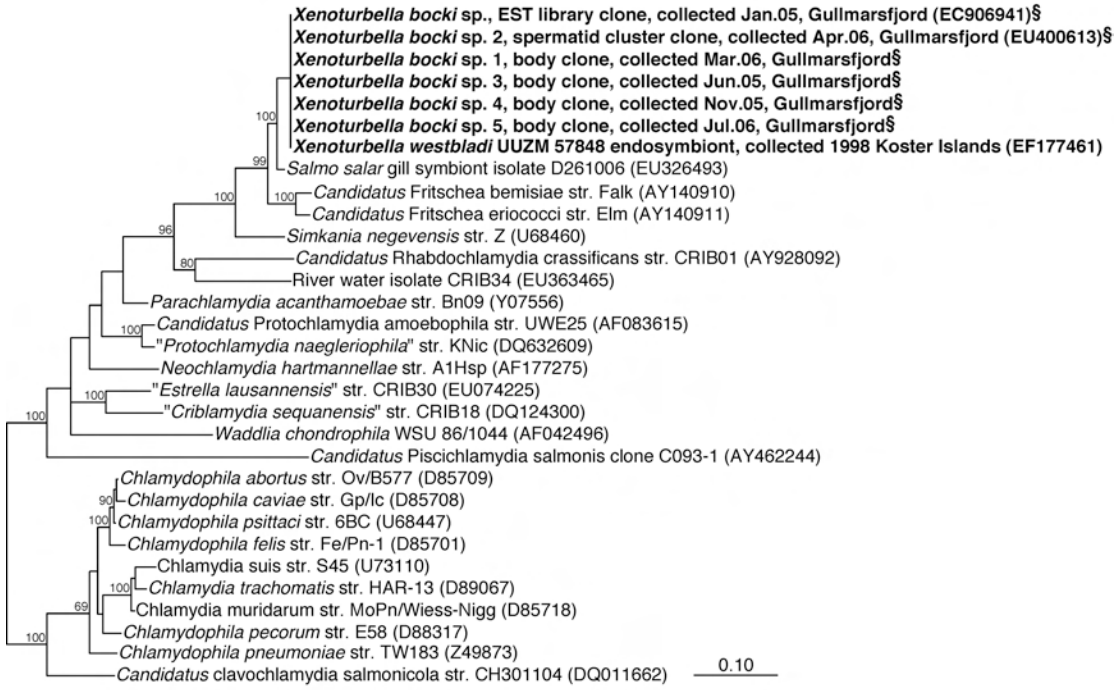
(A) 16S rRNA


Candidatus Baumannia cicadellinicola str. Hc (CP000238)
Amonostherium lichtensioides 2nd endosymbiont (AF476100)
Candidatus Blochmannia pennsylvanicus str. BPEN (CP000016)
Wigglesworthia glossinidia str. brevipalpis (BA000021)
Sodalis glossinidius str. morsitans (AP008232)
Candidatus Hamiltonella defensa (*Acyrthosiphon pisum*) (AY296733)
Yersinia pestis str. KIM (AE009952)
Morganella morganii str. VAR-06-2076 (DQ513315)
Proteus mirabilis str. ATCC 29906 (AF008582)
Buchnera aphidicola (*Maculolachnus submacula*) (AJ296755)
Buchnera aphidicola str. Sg (AE013218)
Candidatus Ishikawaella capsulata (*Megacopta punctatissima*) (AB067723)
Escherichia coli str. K12 (U00096)
Salmonella enterica subsp. *enterica* str. Ty2 (AE014613)
Enterobacter sp. str. 638 (CP000653)
Actinobacillus succinogenes str. 130Z (CP000746)
Haemophilus influenzae str. Rd KW20 (L42023)
Pasteurella multocida subsp. *multocida* str. Pm70 (NC_002663)
Photobacterium profundum str. SS9 (CR378663)
Vibrio fischeri str. ES114 (CP000020)
Vibrio cholerae str. N16961 (AE003852)
Aeromonas salmonicida subsp. *salmonicida* str. A449, (CP000644)
Shewanella baltica str. OS155 (CP000563)
Pseudoalteromonas tunicata str. D2 (AAOH01000015)
Colwellia psychrerythraea str. 34H (CP000083)
Chromohalobacter salexigens str. DSM 3043 (CP000285)
Candidatus Portiera aleyrodidarum (*Bemisia tabaci*) (AY268081)
Marinomonas sp. str. MED121 (AANE01000003)
Pseudomonas aeruginosa str. UCBPP-PA14 (CP000438)
Pseudomonas fluorescens str. Pf-5 (CP000076)
Aquicella lusitana str. SGT-39 (AY359282)
Aquicella siphonis str. SGT-108 (AY359283)
Asellus aquaticus hepatopancreatic endosymbiont (AY447040)
Francisella philomiragia subsp. *philomiragia* str. ATCC 25015 (EF153479)
Francisella piscicida str. GM2212 (DQ309246)
Francisella tularensis subsp. *holarctica* str. OSU18 (CP000437)
Francisella novicida str. U112 (CP000439)
Danshui River mangrove clone DS058 (DQ234142)
Fangia hongkongensis str. UST040201-002 (AB176554)
Ecteinascidia turbinata endosymbiont (AY054370)
***Xenoturbella bocki* sp., EST library clone, collected Jan.05, Gullmarsfjord (EC907012)§**
***Xenoturbella bocki* sp. 2, spermatid cluster clone, collected Apr.06, Gullmarsfjord (EU400612)**
***Xenoturbella bocki* sp. 1, body clone, collected Mar.06, Gullmarsfjord§**
***Xenoturbella bocki* sp. 3, body clone, collected Jun.05, Gullmarsfjord§**
***Xenoturbella bocki* sp. 4, body clone, collected Nov.05, Gullmarsfjord§**
***Xenoturbella bocki* sp. 5, body clone, collected Jul.06, Gullmarsfjord§**
Piscirickettsia salmonis str. LF-89 (U36941)
Legionella busanensis str. K9951 (AF424887)
Legionella oakridgensis str. Oak Ridge 10 (X73397)
Legionella pneumophila str. Paris (CR628336)
Legionella steigerwaltii str. SC-18-C9 (X73400)
Candidatus Ruthia magnifica str. Cm (CP000488)
Coxiella burnetii str. Dugway 5J108-111 (NC_009727)
Amblyomma americanum endosymbiont (AY939824)
Xanthomonas campestris pathovar *campestris* str. ATCC 33913 (AE008922)
Xylella fastidiosa str. Temecula1 (AE009442)
Agrobacterium tumefaciens str. C58 (AE008980)
Brucella melitensis biovar *abortus* str. 2308 (AM040264)
Caulobacter crescentus str. CB15 (AE005673)
0.10

(B) 23S rRNA


Candidatus Baumannia cicadellinicola str. Hc (CP000238)
Wigglesworthia glossinidia str brevipalpis (BA000021)
Candidatus Blochmannia pennsylvanicus str. BPEN (CP000016)
Amonostherium lichtensioides 2nd endosymbiont (AF476100)
Buchnera aphidicola str. Sg (AE013218)
Candidatus Hamiltonella defensa (*Acyrthosiphon pisum*) (AY296733)
Morganella morganii str. ATCC 25830 (AY116909)
Sodalis glossinidius str. morsitans (AP008232)
Yersinia pestis str. KIM (AE009952)
Escherichia coli str. K12 (U00096)
Salmonella enterica subsp. *enterica* str. Ty2 (AE014613)
Proteus mirabilis str. ATCC 29906 (AY116929)
Enterobacter sp. str. 638 (CP000653)
Haemophilus influenzae str. Rd KW20 (L42023)
Pasteurella multocida subsp. *multocida* str. Pm70 (NC_002663)
Actinobacillus succinogenes str. 130Z (CP000746)
Photobacterium profundum str. SS9 (CR378663)
Vibrio fischeri str. ES114 (CP000020)
Vibrio cholerae str. N16961 (AE003852)
Aeromonas salmonicida subsp. *salmonicida* str. A449, (CP000644)
Shewanella baltica str. OS155 (CP000563)
Pseudoalteromonas tunicata str. D2 (AAOH01000016)
Colwellia psychrerythraea str. 34H (CP000083)
Chromohalobacter salexigens str. DSM 3043 (CP000285)
Candidatus Portiera aleyrodidarum (*Bemisia tabaci*) (AY268081)
Marinomonas sp. str. MED121 (AANE01000003)
Pseudomonas aeruginosa str. UCBPP-PA14 (CP000438)
Pseudomonas fluorescens str. Pf-5 (CP000076)
Candidatus Ruthia magnifica str. Cm (CP000488)
Francisella philomiragia subsp. *philomiragia* str. ATCC 25015 (EF153479)
Francisella piscicida str. GM2212 (DQ309246)
Francisella tularensis subsp. *holarctica* str. OSU18 (CP000437)
Francisella novicida str. U112 (CP000439)
***Xenoturbella bocki* sp. 2, spermatid cluster clone, collected Apr.06, Gullmarsfjord (EU400613)**
Legionella pneumophila str. Paris (CR628336)
Coxiella burnetii str. Dugway 5J108-111 (NC_009727)
Rickettsiella grylli (pill bugs) (AAQJ00000000)
Xanthomonas campestris pathovar *campestris* str. ATCC 33913 (AE008922)
Xylella fastidiosa str. Temecula1 (AE009442)
Agrobacterium tumefaciens str. C58 (AE008980)
Brucella melitensis biovar *abortus* str. 2308 (AM040264)
Caulobacter crescentus str. CB15 (AE005673)
0.10

(C) 16S rRNA



Supplemental Figure S2 Phylogeny of the *Xenoturbella* endosymbionts (bold) as inferred by Bayesian analysis. (A, B) Position of the gammaproteobacterial endosymbiont among members of the class *Gammaproteobacteria* based on 16S rRNA (A) and 23S rRNA (B) gene sequences; the trees were rooted arbitrarily by three alphaproteobacterial taxa. (C) 16S rRNA gene sequence-based position of the chlamydial endosymbiont among members of the class *Chlamydia*. Bars, 10% estimated sequence divergence. Numbers indicate neighbour-joining-based bootstrap percentage values. §Short sequence added to the tree by the parsimony method.