Table S1 List of CSCGs (conserved single-copy genes)

CSCG	ID	Abbreviation	AA length	Protein
PF00162.14		*pgk1*	384	Phosphoglycerate kinase
PF00276.15	10140	*rplW*	92	Ribosomal protein L23
PF00281.14	10143	*rplE*	56	Ribosomal protein L5
PF00297.17	20028	*rplC*	263	Ribosomal protein L3
PF00347.18	10145	*rplF*	77	Ribosomal protein L6
PF00366.15	10140	*rpsQ*	69	Ribosomal protein S17
PF00380.14	10160	*rpsI*	121	Ribosomal protein S9/S16
PF00410.14	10144	*rpsH*	129	Ribosomal protein S8
PF00411.14	10153	*rpsK*	110	Ribosomal protein S11
PF00416.17	10152	*rpsM*	106	Ribosomal protein S13/S18
PF00466.15	20085	*rplJ*	100	Ribosomal protein L10
PF00573.17	20068	*rplD*	192	Ribosomal protein L4/L1 family
PF00750.14	20073	*argS*	354	arginine-tRNA ligase
PF01025.14	10207	*grpE*	166	GrpE
PF01795.14	20029	*mraW*	310	rRNA small subunit methyltransferase
TIGR00002	10213	*rpsP*	78	S16: ribosomal protein S16
TIGR00009	10224	*rpmB*	58	L28: ribosomal protein L28
TIGR00012	20089	*rpmC*	56	L29: ribosomal protein L29
TIGR00019	10101	*prfA*	361	peptide chain release factor 1
TIGR00029	10178	*rpsT*	87	S20: ribosomal protein S20
TIGR00043	10040	*EcybeY*	111	metalloprotein, YbeY/UPF0054 family
TIGR00059	10155	*rplQ*	112	ribosomal protein L17
TIGR00060	10146	*rplR*	114	ribosomal protein L18
TIGR00061	10183	*rplU*	101	ribosomal protein L21
TIGR00062	10182	*rpmA*	83	ribosomal protein L27
TIGR00064		*ftsY*	279	signal recognition particle-docking protein FtsY
TIGR00082	10030	*rbfA*	115	ribosome-binding factor A
TIGR00086	10122	*smpB*	144	SsrA-binding protein
TIGR00092	10045	*engD*	368	GTP-binding protein YchF, ribosome associated
TIGR00115	10124	*tig*	410	trigger factor
TIGR00116	10037	*tsf*	293	translation elongation factor Ts
TIGR00152	10084	*coaE*	188	dephospho-CoA kinase
TIGR00158	20019	*rplK*	148	ribosomal protein L9
TIGR00165	20081	*rpsR*	70	ribosomal protein S18
TIGR00166	20093	*rpsF*	95	ribosomal protein S6
TIGR00168	20024	*infC*	165	translation initiation factor IF-3
TIGR00234	10001	*tyrS*	406	tyrosine--tRNA ligase
TIGR00337	20072	*pyrG*	526	CTP synthase
TIGR00344	10130	*(alaS)*	847	alaS: alanine--tRNA ligase
TIGR00362	20001	*dnaA*	437	chromosomal replication initiator protein DnaA
TIGR00388		*glyQS*	293	glycine--tRNA ligase, alpha subunit
TIGR00389	10186	*glyR*	565	glycine--tRNA ligase

TIGR00392	20033	*ileS*	861	isoleucine--tRNA ligase
TIGR00396	10059	*leuS*	843	leucine--tRNA ligase
TIGR00409	10195	*proS*	568	proS fam II: proline--tRNA ligase
TIGR00414	20006	*serS*	418	serine--tRNA ligase
TIGR00418	20023	*thrS*	565	threonine--tRNA ligase
TIGR00420		*trmU*	351	tRNA (5-methylaminomethyl-2-thiouridylate)-methylase
TIGR00422	10180	*valS*	863	valine--tRNA ligase
TIGR00435	20015	*cysS*	466	cysteine--tRNA ligase
TIGR00436	10067	*era*	270	GTP-binding ribosome 16S and 30S maturation protein
TIGR00442	10127	*hisS*	406	histidine--tRNA ligase
TIGR00459	10128	*aspS*	586	aspartate--tRNA ligase
TIGR00460		*fmt*	315	methionyl-tRNA formyltransferase
TIGR00468	20026	*pheS*	324	phenylalanine--tRNA ligase, alpha subunit
TIGR00472	20027	*pheT*	798	pheT bact: phenylalanine--tRNA ligase, beta subunit
TIGR00487	10031	*infB*	587	translation initiation factor IF-2
TIGR00496	10034	*frr*	176	ribosome recycling factor
TIGR00575	10100	*ligA*	652	DNA ligase, NAD-dependent
TIGR00631		*uvrB*	658	uvrb: excinuclease ABC subunit B
TIGR00663	20002	*dnaN*	367	DNA polymerase III, beta subunit
TIGR00775	10150	*nhaD*	420	Na^+/H^+ antiporter, NhaD family
TIGR00810	10117	*secG*	73	preprotein translocase, SecG subunit
TIGR00855	20084	*rplL*	125	ribosomal protein L7/L12
TIGR00922	20018	*nusG*	172	transcription termination/antitermination factor
TIGR00952	10028	*rpsO*	86	ribosomal protein S15
TIGR00959		*ffh*	428	ffh: signal recognition particle protein
TIGR00963	10115	*secA*	787	preprotein translocase, SecA subunit
TIGR00964		*secE*	57	preprotein translocase, SecE subunit
TIGR00967	10149	*secY*	414	preprotein translocase, SecY subunit
TIGR00981	10096	*rpsL*	124	ribosomal protein S12
TIGR01009	20063	*rpsC*	212	ribosomal protein S3
TIGR01011	10038	*rpsB*	225	ribosomal protein S2
TIGR01017	10002	*rpsD*	200	ribosomal protein S4
TIGR01021	10147	*rpsE*	156	ribosomal protein S5
TIGR01024	10054	*rplS*	114	ribosomal protein L19
TIGR01029	10097	*rpsG*	154	ribosomal protein S7
TIGR01030	10179	*rpmH*	44	ribosomal protein L34
TIGR01031	20028	*rpmF*	56	ribosomal protein L32
TIGR01032	20050	*rplT*	114	ribosomal protein L20
TIGR01044	20064	*rplV*	103	ribosomal protein L22
TIGR01049	20098	*rpsJ*	99	ribosomal protein S10
TIGR01050	20065	*rpsS*	92	ribosomal protein S19
TIGR01059	20004	*gyrB*	639	DNA gyrase, B subunit

TIGR01063	20005	*gyrA*	800	DNA gyrase, A subunit
TIGR01066	10159	*rplM*	141	ribosomal protein L13
TIGR01067	10141	*rplNA*	122	ribosomal protein L14
TIGR01071	10148	*rplO*	144	ribosomal protein L15
TIGR01079	10142	*rplX*	104	ribosomal protein L24
TIGR01164	20062	*rplP*	126	ribosomal protein L16
TIGR01169	20020	*rplA*	227	ribosomal protein L1
TIGR01171	20066	*rplB*	275	ribosomal protein L2
TIGR01391	10185	*dnaG*	414	DNA primase
TIGR01393	10055	*lepA*	595	GTP-binding protein LepA
TIGR01632	20019	*rplK*	140	ribosomal protein L11
TIGR01953	10032	*nusA*	340	transcription termination factor NusA
TIGR02012	20044	*recA*	321	protein RecA
TIGR02013	20071	*rpoB*	1238	DNA-directed RNA polymerase, beta subunit
TIGR02027	10154	*rpoA*	298	DNA-directed RNA polymerase, alpha subunit
TIGR02191			219	RNaseIII: ribonuclease III
TIGR02350	10208	*dnaK*	596	chaperone protein DnaK
TIGR02386	20070	*rpoC*	1147	DNA-directed RNA polymerase, beta' subunit
TIGR02397	20007	*dnaX*	355	DNA polymerase III, subunit gamma and tau
TIGR02432		*tilS*	189	lysidine TilS N: tRNA(Ile)-lysidine synthetase
TIGR02729	10123	*obgE*	329	Obg CgtA: Obg family GTPase CgtA
TIGR03263	20054	*gmk*	180	guanylate kinase
TIGR03594	20049	*der*	432	ribosome-associated GTPase EngA

The gene IDs were referred to the annotation of "*Ca.* Spiroplasma holothuricola" strain MT37 in MicroScope platform under accession number 9445. The shaded CSGCs could not be identified in the genome. The first number of gene IDs denotes the chromosome where the gene is located.

Table S2 Comparison of KEGG pathways.

KEGG ko	Pathway	SH	BA	ME	ND	SD	SM	MBG
ko01230	Biosynthesis of amino acids	4	62	25	10	20	42	12
ko00010	Glycolysis / Gluconeogenesis	1	14	14	1	27	8	22
ko00020	Citrate cycle (TCA cycle)	0	5	7	0	5	9	2
ko00500	Starch and sucrose metabolism	2	4	2	0	35	0	33
ko00520	Amino sugar and nucleotide sugar metabolism	2	8	5	0	18	0	29
ko00190	Oxidative phosphorylation	1	18	15	2	1	0	4
ko00564	Glycerophospholipid metabolism	1	1	1	0	8	0	10
ko00230	Purine metabolism	20	29	20	5	32	7	28
ko00240	Pyrimidine metabolism	21	29	19	6	32	8	31
ko00550	Peptidoglycan biosynthesis	0	13	7	0	1	0	0
ko00760	Nicotinate and nicotinamide metabolism	3	5	0	0	7	0	7
ko00770	Pantothenate and CoA biosynthesis	2	9	3	0	5	5	3
ko00780	Biotin metabolism	0	6	4	0	0	0	0
ko00900	Terpenoid backbone biosynthesis	0	9	1	0	8	0	0
ko00983	Drug metabolism - other enzymes	6	1	0	0	9	0	5
ko03010	Ribosome	40	54	53	36	50	49	49
ko00970	Aminoacyl-tRNA biosynthesis	22	23	22	8	27	7	24
ko03060	Protein export	3	11	7	0	8	3	6
ko02010	ABC transporters	7	4	9	0	14	0	10
ko02060	Phosphotransferase system (PTS)	0	5	5	0	21	0	30

SH: “*Ca.* Spiroplasma holothuricola”; BA: *Buchnera aphidicola*; ME: “*Ca.* Moranella endobia”; ND: “*Ca.* Nasuia deltocephalinicola”; SD: *Spiroplasma diminutum*; SM: “*Ca.* Sulcia muelleri”; MBG: *Mycoplasma* sp. Bg1.

Table S3 List of core genes

Gene	Syn3.0	Spiroplasma	Buchnera
dnaA	MMSYN1 0001	MT37 v2 20001	BCc 004
dnaE	MMSYN1 0612	MT37 v2 10004	BCc 148
dnaQ	≠	≠	BCc 156
dnaB	MMSYN1 0609	MT37 v2 20011	BCc 359
dnaG	MMSYN1 0406	MT37 v2 10185	BCc 035
dnaN	MMSYN1 0002	MT37 v2 20002	BCc 003
dnaX	MMSYN1 0047	MT37 v2 20007	BCc 300
polC	MMSYN1 0303	MT37 v2 10033	≠
gyrA	MMSYN1 0007	MT37 v2 20005	BCc 116
gyrB	MMSYN1 0006	MT37 v2 20004	BCc 002
holA	MMSYN1 0826	MT37 v2 10175	BCc 275
holB	MMSYN1 0044	MT37 v2 20030	BCc 219
ligA	MMSYN1 0690	MT37 v2 10100	BCc 039
hup	MMSYN1 0350	MT37 v2 20048	BCc 084
ssb	(MMSYN1 0558)	MT37 v2 20082	BCc 357
nth	≠	≠	BCc 362
polA	MMSYN1 0097	MT37 v2 10218	BCc 265
rpoA	MMSYN1 0645	MT37 v2 10154	BCc 316
rpoB	MMSYN1 0804	MT37 v2 20071	BCc 019
rpoC	MMSYN1 0803	MT37 v2 20070	BCc 018
rpoD	MMSYN1 0407	MT37 v2 10184	BCc 034
greA	MMSYN1 0253	MT37 v2 10204	BCc 237
nusA	MMSYN1 0300	MT37 v2 10032	BCc 232
nusB	MMSYN1 0107	MT37 v2 10092	BCc 285
nusG	MMSYN1 0840	MT37 v2 20018	BCc 024
deaD	MMSYN1 0410	0	BCc 226
groL	0	0	BCc 011
groS	0	0	BCc 010
dnaK	MMSYN1 0542	MT37 v2 10208	BCc 096
dnaJ	MMSYN1 0541	MT37 v2 10209	BCc 095
grpE	MMSYN1 0543	MT37 v2 10207	BCc 158
mnmA	MMSYN1 0387	0	BCc 166
mnmE	MMSYN1 0081	promiscuous	BCc 008
mnmG	MMSYN1 0885	0	BCc 001
trmN	MMSYN1 0043	MT37 v2 10100	BCc 112
tsaD	MMSYN1 0079	MT37 v2 10065	BCc 037
tsaE	MMSYN1 0270	MT37 v2 10061	BCc 235
pth	MMSYN1 0832	N-terminal(MT37 v2 20089)	BCc 123
rnpA	MMSYN1 0909	MT37 v2 20021	BCc 006
icsS	MMSYN1 0441	0	BCc 391
orn	≠	≠	BCc 373
nrnA	MMSYN1 0139	MT37 v2 10139	≠

pnpA	0	MT37 v2 10200	BCc 227
rne	≠	≠	0
rnjA	MMSYN1 0600	MT37 v2 20053	≠
rnjB	MMSYN1 0257	MT37 v2 10199	≠
rnc	MMSYN1 0418	≠	BCc 163
rny	MMSYN1 0359	MT37 v2 20043	≠
rnr	MMSYN1 0775	0	BCc 168
infA	MMSYN1 0649	MT37 v2 94097	BCc 195
infB	MMSYN1 0297	MT37 v2 10031	BCc 231
infC	MMSYN1 0200	MT37 v2 20024	BCc 079
fusA	MMSYN1 0150	MT37 v2 10098	BCc 344
tufA	MMSYN1 0151	MT37 v2 10040	BCc 343
tsf	MMSYN1 0539	MT37 v2 10037	BCc 143
prfA	MMSYN1 0141	MT37 v2 10101	BCc 111
prfB	0	0	BCc 269
frr	MMSYN1 0536	MT37 v2 30027	BCc 145
fmt	MMSYN1 0390	0	BCc 314
def	MMSYN1 0201	0	BCc 313
lepA	MMSYN1 0285	MT37 v2 10055	BCc 165
efp	MMSYN1 0391	0	BCc 012
map	MMSYN1 0650	MT37 v2 10151	BCc 141
alaS	MMSYN1 0163	MT37 v2 10130	BCc 254
asnS	MMSYN1 0076	MT37 v2 10104	BCc 221
aspS	MMSYN1 0287	MT37 v2 10128	BCc 196
argS	MMSYN1 0535	MT37 v2 20073	BCc 152
cysS	MMSYN1 0837	MT37 v2 20015	BCc 306
glnS	0	0	BCc 263
gltX	MMSYN1 0126	MT37 v2 20080	BCc 042
glyS	≠	≠	BCc 085
glyQ	≠	≠	BCc 086
glyQS	MMSYN1 0405	MT37 v2 10186	≠
hisS	MMSYN1 0288	MT37 v2 10127	BCc 177
ileS	MMSYN1 0519	MT37 v2 20033	BCc 093
leuS	MMSYN1 0634	MT37 v2 10059	BCc 274
lysS	MMSYN1 0064	MT37 v2 20060	BCc 270
metG	MMSYN1 0012	MT37 v2 20021	BCc 072
pheS	MMSYN1 0529	MT37 v2 20026	BCc 082
pheT	MMSYN1 0528	MT37 v2 20027	BCc 083
proS	MMSYN1 0282	MT37 v2 10195	BCc 149
serS	MMSYN1 0061	MT37 v2 20006	BCc 193
thrS	MMSYN1 0222	MT37 v2 20023	BCc 078
trpS	MMSYN1 0308	MT37 v2 10165	BCc 350
tyrS	MMSYN1 0613	MT37 v2 10001	BCc 075
valS	MMSYN1 0260	MT37 v2 10180	BCc 223

gatA	MMSYN1 0688	MT37 v2 10137	0
gatB	MMSYN1 0687	MT37 v2 10138	0
gatC	MMSYN1 0689	?	0
rpsA	0	0	BCc 190
rpsB	MMSYN1 0540	MT37 v2 10038	BCc 142
rpsC	MMSYN1 0665	MT37 v2 20063	BCc 335
rpsD	MMSYN1 0238	MT37 v2 10002	BCc 317
rpsE	MMSYN1 0654	MT37 v2 10147	BCc 324
rpsF	MMSYN1 0027	0	BCc 370
rpsG	MMSYN1 0149	MT37 v2 10097	BCc 345
rpsH	MMSYN1 0657	MT37 v2 10144	BCc 327
rpsI	MMSYN1 0637	MT37 v2 10160	BCc 242
rpsJ	MMSYN1 0672	MT37 v2 20098	BCc 342
rpsK	MMSYN1 0646	MT37 v2 10153	BCc 318
rpsL	MMSYN1 0148	MT37 v2 10096	BCc 346
rpsM	MMSYN1 0647	MT37 v2 10152	BCc 319
rpsN	MMSYN1 0658	?	BCc 328
rpsO	MMSYN1 0294	MT37 v2 10028	BCc 228
rpsP	MMSYN1 0362	MT37 v2 10213	BCc 246
rpsQ	MMSYN1 0662	MT37 v2 10140	BCc 332
rpsR	MMSYN1 0025	MT37 v2 20081	BCc 369
rpsS	MMSYN1 0667	MT37 v2 20065	BCc 337
rpsT	MMSYN1 0082	MT37 v2 10178	BCc 094
rpsU	MMSYN1 0482	0	0
rplA	MMSYN1 0809	MT37 v2 20020	BCc 022
rplB	MMSYN1 0668	MT37 v2 20066	BCc 338
rplC	MMSYN1 0671	MT37 v2 20028	BCc 341
rplD	MMSYN1 0670	MT37 v2 20068	BCc 340
rplE	MMSYN1 0659	MT37 v2 10143	BCc 329
rplF	MMSYN1 0656	MT37 v2 10145	BCc 326
rplG	MMSYN1 0298	0	0
rplI	MMSYN1 0833	0	BCc 368
rplJ	MMSYN1 0807	MT37 v2 20085	BCc 021
rplK	MMSYN1 0810	MT37 v2 20019	BCc 023
rplL	MMSYN1 0806	MT37 v2 20084	BCc 020
rplM	MMSYN1 0638	MT37 v2 10159	BCc 243
rplN	MMSYN1 0661	MT37 v2 10141	BCc 331
rplO	MMSYN1 0653	MT37 v2 10148	BCc 322
rplP	MMSYN1 0664	MT37 v2 20062	BCc 334
rplQ	MMSYN1 0644	MT37 v2 10155	BCc 315
rplR	MMSYN1 0655	MT37 v2 10146	BCc 325
rplS	MMSYN1 0365	MT37 v2 10054	BCc 249
rplT	MMSYN1 0198	MT37 v2 20050	BCc 081
rplU	MMSYN1 0501	MT37 v2 10183	BCc 239

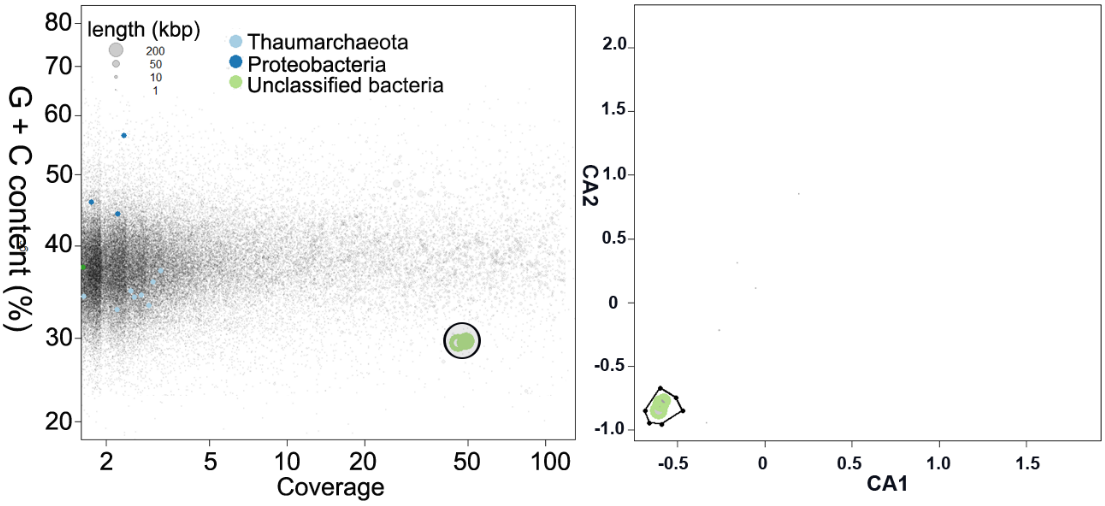
rplV	MMSYN1 0666	MT37 v2 20064	BCc 336
rplW	MMSYN1 0669	MT37 v2 10140	BCc 339
rplX	MMSYN1 0660	MT37 v2 10142	BCc 330
rplY	0	0	BCc 088
rpmA	MMSYN1 0499	MT37 v2 10182	BCc 240
rpmB	MMSYN1 0422	MT37 v2 20033	BCc 052
rpmC	MMSYN1 0663	MT37 v2 20089	BCc 333
rpmD	0	0	BCc 323
rpmE	MMSYN1 0137	MT37 v2 20083	BCc 375
rpmF	MMSYN1 0526	MT37 v2 20028	0
rpmG	0	0	BCc 051
rpmI	MMSYN1 0199	MT37 v2 20049	BCc 080
rpmJ	MMSYN1 0648	Very short	BCc 320
secA	MMSYN1 0095	MT37 v2 10115	BCc 130
secE	MMSYN1 0839	0	BCc 025
secDF	MMSYN1 0412	0	0
secY	MMSYN1 0650	MT37 v2 10149	BCc 321
ffh	MMSYN1 0360	0	BCc 245

The background colors indicate different functional groups. ≠: unnecessary, may be replaced by other genes; ?: uncertain. The annotation of "*Ca.* Spiroplasma holothuricola" genome was performed in the MicroScope platform under accession number 9445.

Figure S1. Sampling of the sea cucumber in the Mariana trench.

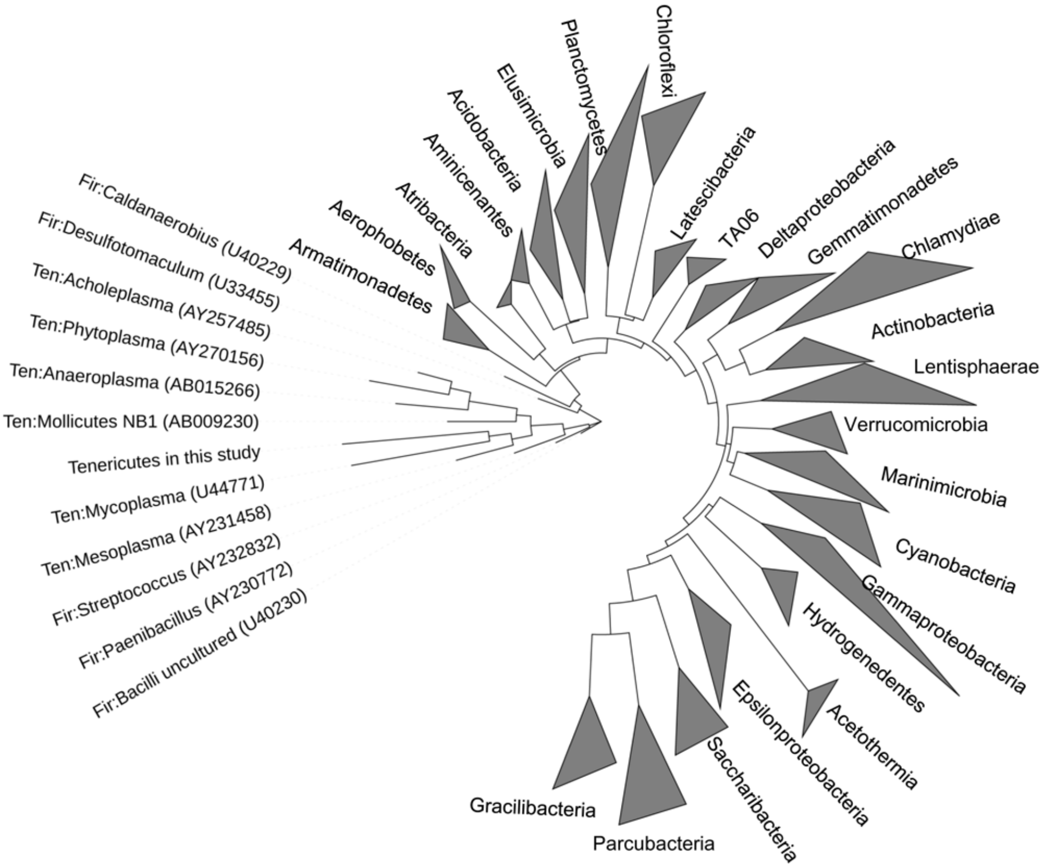
The sea cucumber was captured by a manned submersible at the southern slope of Challenger Deep (142°23’, 10°89’) at depth of 6140m. The inlet map was adapted from Google Maps.

Figure S2. Binning of a genome from an unclassified bacterium



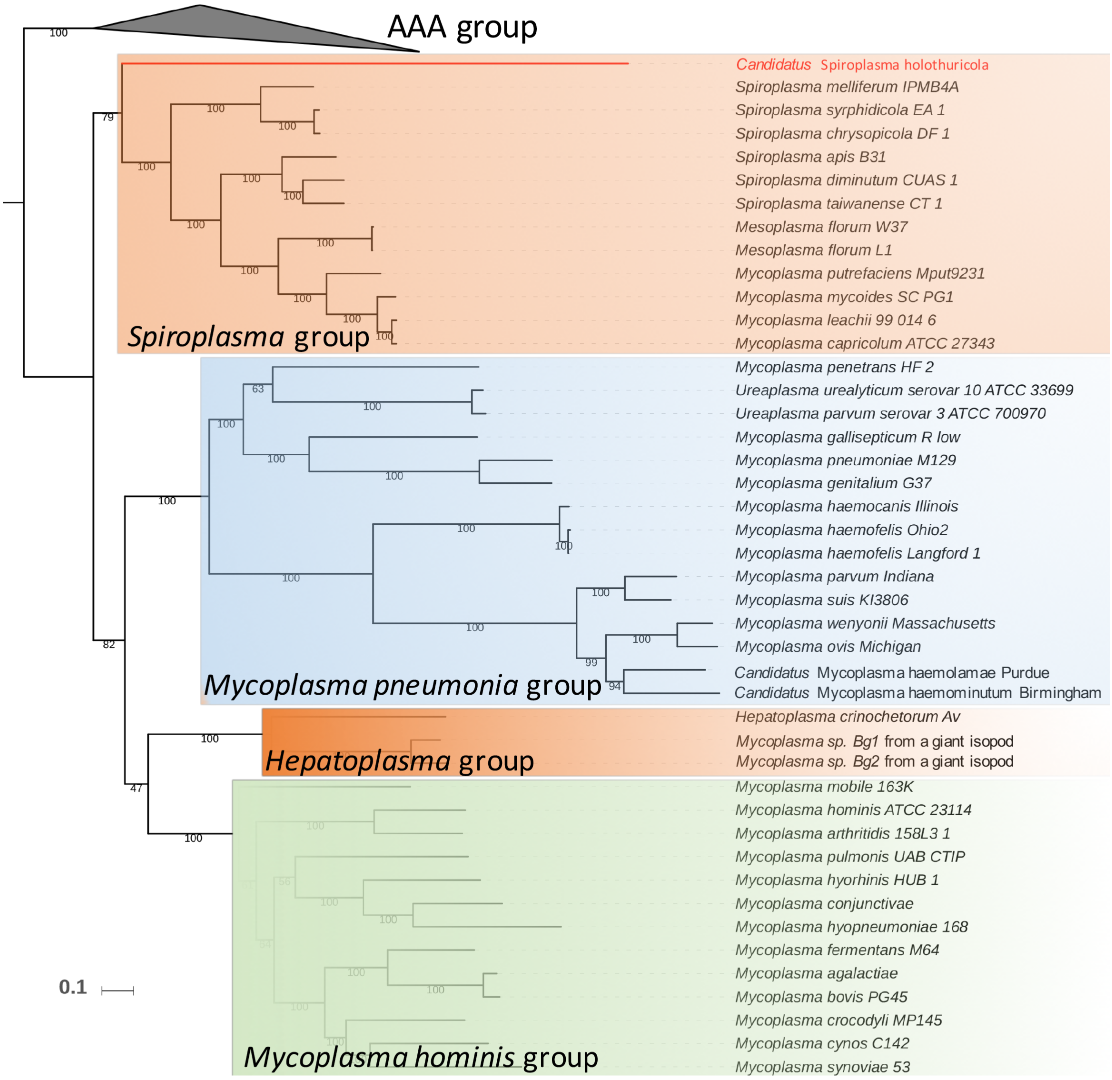
The assembled contigs for the metagenome from the hindgut were firstly separated with respect to their different G + C contents and coverage levels (X). Correspondence analysis (CA) further grouped the contigs based on their tetranucleotide frequencies. The contigs containing CSCGs for taxonomic assignment were colored to indicate their phyla. Three green contigs from unclassified bacteria were circled. The three contigs were manually checked for completeness. Two circular chromosomes were formed.

Figure S3. Phylogenetic position of "*Candidatus* Spiroplasma holothuricola" in Bacteria



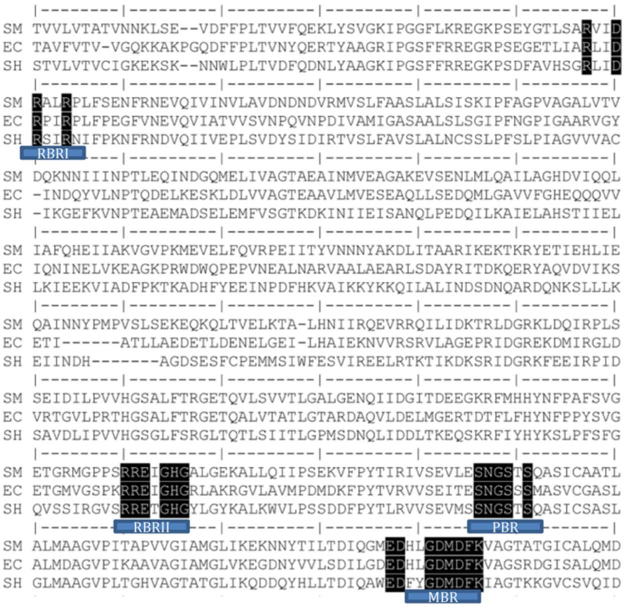
Representative 16S rRNA sequences for 27 bacterial phyla were used to construct a Maximum-likelihood tree. The sequences were mostly from different classes within a given phylum. Ten: Tenericutes; Fir: Firmicutes.

Figure S4. Phylogenetic relationships with Tenericutes groups



The maximal-likelihood tree was constructed using concatenated conserved proteins.

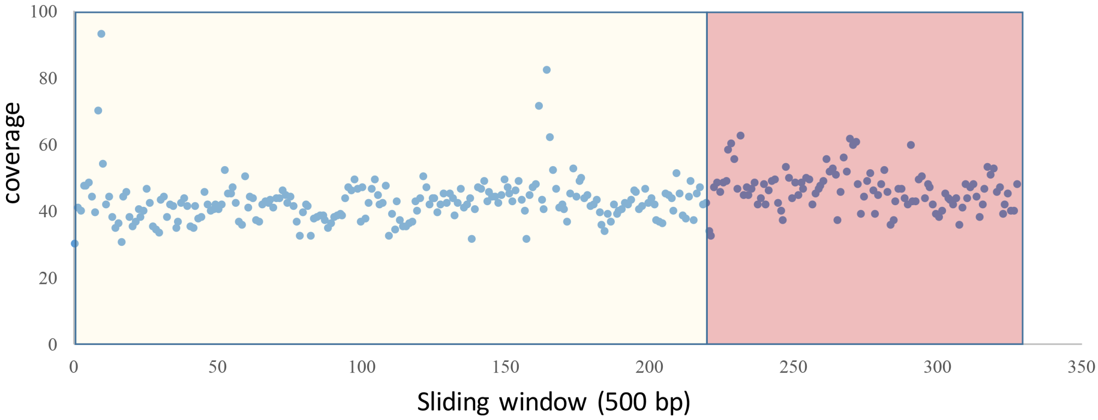
Figure S5. Alignment of PNPase proteins



```
   |--------|---------|---------|---------|---------|---------|
SM TVVLVTATVNNKLSE--VDFFPLTVVFQEKLYSVGKIPGGFLKREGKPSEYGTLSARVID
EC TAVFVTV-VGQKKAKPGQDFFPLTVNYQERTYAAGRIPGSFFRREGRPSEGETLIARLID
SH STVLVTVCIGKEKSK--NNWLPLTVDFQDNLYAAGKIPGSFFRREGKPSDFAVHSGRLID
   |--------|---------|---------|---------|---------|---------|
SM RALRPLFSENFRNEVQIVINVLAVDNDNDVRMVSLFAASLALSISKIPFAGPVAGALVTV
EC RPIRPLFPEGFVNEVQVIATVVSVNPQVNPDIVAMIGASAALSLSGIPFNGPIGAARVGY
SH RSIRNIFPKNFRNDVQIIVEPLSVDYSIDIRTVSLFAVSLALNCSSLPFSLPIAGVVVAC
   |--------|---------|---------|---------|---------|---------|
SM DQKNNIIINPTLEQINDGQMELIVAGTAEAINMVEAGAKEVSENLMLQAILAGHDVIQQL
EC -INDQYVLNPTQDELKESKLDLVVAGTEAAVLMVESEAQLLSEDQMLGAVVFGHEQQQVV
SH -IKGEFKVNPTEAEMADSELEMFVSGTKDKINIIEISANQLPEDQILKAIELAHSTIIEL
   |--------|---------|---------|---------|---------|---------|
SM IAFQHEIIAKVGVPKMEVELFQVRPEIITYVNNNYAKDLITAARIKEKTKRYETIEHLIE
EC IQNINELVKEAGKPRWDWQPEPVNEALNARVAALAEARLSDAYRITDKQERYAQVDVIKS
SH LKIEEKVIADFPKTKADHFYEEINPDFHKVAIKKYKKQILALINDSDNQARDQNKSLLLK
   |--------|---------|---------|---------|---------|---------|
SM QAINNYPMPVSLSEKEQKQLTVELKTA-LHNIIRQEVRRQILIDKTRLDGRKLDQIRPLS
EC ETI------ATLLAEDETLDENELGEI-LHAIEKNVVRSRVLAGEPRIDGREKDMIRGLD
SH EIINDH-------AGDSESFCPEMMSIWFESVIREELRTKTIKDKSRIDGRKFEEIRPID
   |--------|---------|---------|---------|---------|---------|
SM SEIDILPVVHGSALFTRGETQVLSVVTLGALGENQIIDGITDEEGKRFMHHYNFPAFSVG
EC VRTGVLPRTHGSALFTRGETQALVTATLGTARDAQVLDELMGERTDTFLFHYNFPPYSVG
SH SAVDLIPVVHGSGLFSRGLTQTLSIITLGPMSDNQLIDDLTKEQSKRFIYHYKSLPFSFG
   |--------|---------|---------|---------|---------|---------|
SM ETGRMGPPSRREIGHGALGEKALLQIIPSEKVFPYTIRIVSEVLESNGSTSQASICAATL
EC ETGMVGSPKRREIGHGRLAKRGVLAVMPDMDKFPYTVRVVSEITESNGSSSMASVCGASL
SH QVSSIRGVSRRETGHGYLGYKALKWVLPSSDDFPYTLRVVSEVMSSNGSTSQASICSASL
   |--------|---------|---------|---------|---------|---------|
SM ALMAAGVPITAPVVGIAMGLIKEKNNYTILTDIQGMEDHLGDMDFKVAGTATGICALQMD
EC ALMDAGVPIKAAVAGIAMGLVKEGDNYVVLSDILGDEDHLGDMDFKVAGSRDGISALQMD
SH GLMAAGVPLTGHVAGTATGLIKQDDQYHLLTDIQAWEDFYGDMDFKIAGTKKGVCSVQID
```

The alignment shows the four conserved domains in PNPase proteins from *Spiroplasma melliferum* (SM), *Escherichia coli* strain K12 (EC) and "*Ca.* Spiroplasma holothuricola" (SH).

Figure S6. Genomic variations as shown by different levels of the contigs.



The two contigs in the draft genome were framed in different colors. In every 500bp, coverage by Illumina reads was estimated in the sliding window that was moved 250bp in each step. Average coverage was indicated by a red dashed line at 44.5 with a standard deviation of 11.8. The genomic regions with a relative low coverage were not perfectly aligned by the reads, indicating the presence of genetic variations or gene depletions among the individual spiroplasmas dwelling in hindgut of the sea cucumber.