Supplementary Data

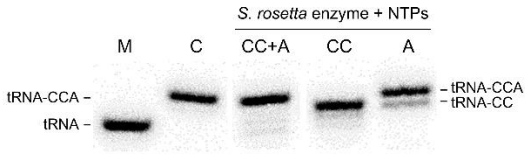

Figure S1: *S. rosetta* CC- and A-adding enzymes collaborate in CCA-addition. Equimolar amounts of *S. rosetta* CC- and A-enzyme were pooled and incubated with $tRNA^{Phe}$ without CCA-end in the presence of NTPs. As a control, both enzymes were also incubated separately. The A-adding enzyme was incubated with $tRNA^{Phe}$ carrying a CC-end, the CC-adding enzyme and the pooled enzymes were incubated with a $tRNA^{Phe}$ without CCA-end. The complete CCA-sequence is only added when both enzymes are present. M, mock incubation of $tRNA^{Phe}$ without enzymes. C, control lane with $tRNA^{Phe}$ with CCA-end as size standard.

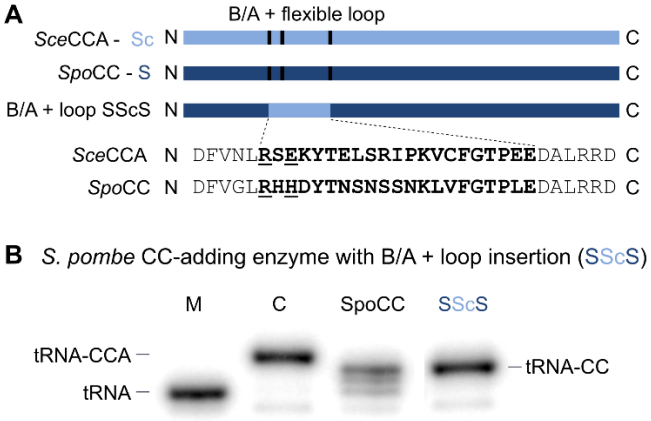

Figure S2: The flexible loop of the *S. cerevisiae* CCA-adding enzyme is not compatible with the *S. pombe* CC-adding enzyme. (A) In the CC-adding enzyme (SpoCC), both the flexible loop and the upstream located B/A motif deviate from the consensus sequence of eukaryotic e-type CCA-adding enzymes. For construction of enzyme chimeras, the B/A motif and the loop sequence from the CCA-adding enzyme of *S. cerevisiae* (Sc) were inserted into the CC-adding enzyme of *S. pombe* (S), generating chimera SScS_B/A + loop (S, S. pombe CC-adding enzyme N-terminus; Sc, B/A motif and loop of *S. cerevisiae* CCA-adding enzyme; S, *S. pombe* CC-adding enzyme C-terminus). (B) Insertion of B/A motif and loop of *S. cerevisiae* CCA-adding enzyme does not result in a restoration of A-adding activity. Obviously, this region is not compatible with the context of the *S. pombe* CC-adding enzyme due to their distant evolutionary relation. M, mock incubation of the tRNA in the absence of enzymes; C, control $tRNA^{Phe}$ with CCA end as size marker.

Protein Sequences of CC- and A-adding Enzymes of *Salpingoeca rosetta, Schizosaccharomyces pombe*

>euhChoSalros_aNtr *Salpingoeca_rosetta* EGD81016
MLLGRVACAAVLGGVSGGGCCARSVSCCCCCWSVGAATRGRPWRRPVLQLTRRNLQPTMPTSQEVKQKILGTPAV
QQLKHIFQEDHHEFRLVGGAVRDILLERWPKDYDFATTALPQQTQQLLESRGVRVVLTGLQHGTVTAVIDNVPYE
VTTLRLDHEGAEGTGPVCFTDDWKLDAERRDLTINAMSMDLDGHIFDYFDGRDHLAQEKIVFVGDPAQRIQEDFR
ILRYFRFHGKVCRHNNHEPAQIQAITDNVQGLESVSGERIWMEMSKILKTARAPELVECMQACGVLPHIQLANVE
QHHLQRLRHVHRYELEPATALTALVDDVQQFEGVAAAWRLSNAERKLGLFIIQHRDVDVCMNSAQDLLVDGINRT
YVTQLCRYQGHLDIAVAMQDWAVPEFPITGKKLIQHGLKPGPNMGRVLAALKDNWKQSRFQLGEEELLAQMDSVV
ASLS

>euhChoSalros_eNtr *Salpingoeca_rosetta* EGD77004
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FTINALYYNINQDAIEDFTDTGLSDLDLGLVRTPADPLRSFKEDPLRVLRAIRYTGRYGFKLEDAARHAILDPQV
KECLQTKVSRERYGIEVDKMFKADHPLACLSLLCNLGLYDVVFHSPAEYHRDPTMQMVPFPYFPVPLRMIEPRLV
MEASLEIANRANKSIVAKAQDGDHHAVQTVLLSAFFSPLWGYICSQDKKVTHRSIVYHMLRRGICLSKASAEMIC
HMLFSAQQFAEVSRQVLKLYVEKHEEATAPLPDDQQQTQQQQQQQTQQQQQQQGDVDLGDLPADVRLAIGKAMVT
AGEHWRDAIILCDVFARHFHAFVIERYLPSALLMPWIEKSGLVGCWTWRPLLSGRDVMTILDIKGPMVGRLVQRI
SDEMLLQPRMTREECEQWLR
DNVQALMAECDVDKK

>eufAscSchpom_eNtr1 *Schizosaccharomyces_pombe* NP_588119.1
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VNTLFFNIRTEKIEDITKRGYKDLQTKVLVTPISPLQSFLEDPLRILRGIRFASRFEFTIDPSVVSAIQDPKVCK
AFEKKVSKERVGEEIEKMLKGANAKLALQLLYSTNTYQFTFDTLPAEKEFQIPKALEATESLFQSLALTFPKLMK
LSEDEKIGLWLYVALIPWSSQTVMVKKKQFYIPAIIAKDKLKLRSTYVNQLNQCCTFNPIFDELVNDTSTKNCSS
IGSLIRQLNKSWEVVFLTSVIYSCCKTPAASVSNTFSSYKSLYDFIYDKNLQNAYNMKPLLDGKQIMKNVGVKPG
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LFYNLKSKTVEDFTGKGLVDLSNKIIRTPLVADETFGDDPLRAVRCIRFATKYDFNIHEETIKGLKNPELHERLR
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Protein Sequences of newly identified tRNA Nucleotidyltransferases

Choanoflagellata:

> euhChoMonbre_a-type *Monosiga_brevicollis*
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> euhCorCorlim_a-type *Corallochytrium_limacisporum* Betat_2015_aCCA
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VGNAEKRIREDYLRILRYFRFHARIVPDPTLPGSHDEATLKALRENRHGLNRISGERIWQEMGKILTGPAAPAELRVMYDLEL
MKEIGLDQDTSVHMDHFCRMHARSHDAIACLCSLLNSPEDIEKMKKRWKFSNSEERMGMFLVTQRNKDYSLKACKDFLVDKTP
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S

> euhIchAbewhi_a-type *Abeoforma_whisleri* Betat_2015_aCCA
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GERIWTEISKIIAGNRNVNIFKTMYLCGVCESIHLPDHNRLDTLEYVSTFTLEPISLLVSLLHDLSEFENICLTWKLANTEKN
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> euhIchAmopar_a-type *Amoebidium_parasiticum* Betat_2015_aCCA
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Schizosaccharomycetes:

> eufAscProino_CCA *Protomyces_inouyei* built(genome_search,prosplign)
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DLKRGLIQTPLPPRQTFLDDPLRVLRCVRFASQFGFEIEEGTLACLAEGEVRDALDRKVVRERIGLEVYKMLRGIDPARALKT
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> eufAscTapdef_CCA *Taphrina_deformans* built(genome_search,prosplign)
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> eufAscTapwie_CCA *Taphrina_wiesneri* built(genome_search,prosplign)
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> eufAscNeoirr_CCA *Neolecta_irregularis* built(genome_search,prosplign)
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Loop sequences for e-type nucleotidyltransferase Weblogo generation

>Q94K06_Arabidopsis_thaliana
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>C1E3J8_Micromonas_sp
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>A8J6P4_Chlamydomonas_reinhardtii
DLVNLRSETYAADSRIPTMTFGTPQQDALRRD

>Q42867_Lupinus_albus
DFVNLRSEEYTDNSRIPSMQRFGTPEEDAYRRD

>Q2QX33_Oryza_sativa_subsp_japonica
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>A4RUG6_Ostreococcus_lucimarinus
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>Q01CL7_Ostreococcus_tauri
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>A9RJR2_Physcomitrella_patens
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>A7QGJ5_Vitis_vinifera
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>B9MW94_Populus_trichocarpa
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>B9T6L0_Ricinus_communis
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>Q74ZT0_Ashbya_gossypii
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>Q9P4S5_Candida_glabrata
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>Q6CSU9_Kluyveromyces_lactis
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>A7TSN1_Vanderwaltozyma_polyspora
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>A3LSZ1_Pichia_stipitis
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>Q2H0X0_Chaetomium_globosum
DFVNLRRETYTESSRNPVVEFGTAEEDALRRD

>A1C7Z8_Aspergillus_clavatus
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>A6SEN3_Botryotinia_fuckeliana
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>A7ESI2_Sclerotinia_sclerotiorum
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>A9CRH2_Enterocytozoon_bieneusi
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>Q55XT2_Cryptococcus_neoformans
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>A8Q3K4_Malassezia_globosa
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>P21269_Saccharomyces_cerevisiae
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>A4RHL3_Magnaporthe_grisea
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>Q4WZK0_Aspergillus_fumigatus
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>A2QJY3_Aspergillus_niger
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>Q2UB09_Aspergillus_oryzae
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>Q0CVM0_Aspergillus_terreus
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>Q1DJQ5_Coccidioides_immitis
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>Q5AWL8_Emericella_nidulans
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>A6R867_Ajellomyces_capsulata
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>Q0UH04_Phaeosphaeria_nodorum
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>B0CQW9_Laccaria_bicolor
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>Q4YRW2_Plasmodium_berghei
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>A0BX57_Paramecium_tetraurelia
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>Q8IIG2_Plasmodium_falciparum
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Loop sequences for a-type nucleotidyltransferase Weblogo generation

>euhIchSphdes [Sphaerothecum_destruens_NA]
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>euhFilMinvib [Ministeria_vibrans_NA]
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>euhCorCorlim [Corallochytrium_limacisporum_NA]
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>euhIchCrefra [Creolimax_fragrantissima_NA]
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>euhMetDromel [AAF51964_Drosophila_melanogaster]
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>euhIchAbewhi [Abeoforma_whisleri_NA]
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>euhIchPirgem [Pirum_gemmata_NA]
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>euhMetStrpur [XP_784774_Strongylocentrotus_purpuratus]
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>euhChoMonbre [EDQ84681_Monosiga_brevicollis]
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>euhMetNemvec [XP_001637889_Nematostella_vectensis]
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>AniLupus[533758_anis_lupus_familiaris_(dog)]
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>IxoScap[B7Q4B3_Ixodes_scapularis]
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>BOVIN[Q1RML6_BOVIN]
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>RatNor[Q4VBH2_RAT]
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>MacMula[XP_001100673_1_Macaca_mulatta]
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>TaeGutt[XP_002186976_Taeniopygia_guttata]
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>MonDome[1_XP_001374161_Monodelphis_domestica]
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>MusMusc[Q8K1J6_Mus_musculus]
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>OrnAnat[XP_001506360_1_Ornithorhynchus_anatinus]
EITTLRIDVMTDGRHAEVEFTTDWEKDAGRRD

>XenTrop[Q6P873_Xenopus_tropicalis]
EITTLRVDVQTDGRHAEVEFTTDWETDAERRD

>DanReri[Q6IQR7_Danio_rerio]
EVTTLRVDVQTDGRHAEVEFTTDWQKDAERRD

>TetNigr[Q4SR02_Tetraodon_nigroviridis]
EVTTLRVDVQTDGRHAEVEFTTDWQKDAERRD

>NemVect[A7RRV1_Nematostella_vectensis]
EVTTLRIDVETDGRHAKVKFTNDWQLDAERRD

>AedAegy[Q17N51_Aedes_aegypti]
EITTLRIDAVTDGRHAEVIHTTDWLLDANRRD

>AnoGambi[Q7QCF2_Anopheles_gambiae]
EITTLRIDAITDGRHAEVIHTTDWLLDANRRD

>CulQuin[B0X1I4_Culex_quinquefasciatus]
EITTLRIDAVTDGRHAEVIHTKDWLLDANRRD

>CaeEleg[Q93795_Caenorhabditis_elegans]
EITTLRVDIVCDGRRAQVEYTTDWQLDANRRD

>TriCast[XP_969345_Tribolium_castaneum]
EVTTLRIDVVTDGRRAEVQFTTDWLLDALRRD

Loop sequences of a-type enzymes from arbitrarily chosen Choanoflagellata

>*Hartaetosiga_balthica*
EITTLRLDHSINGVAMETQCFTDDWELDALRRD

>*Salpingoeca_kvevrii*
EISTLRIDPGNLAAVDEEDSEAPWRADAACRD

>*Microstomoeca_roanoka*
EVTTLRLDEHTCEDTSKEKAPQADEQIIFTENWKLDAERRD

>*Salpingoeca_punica*
EITTLRTDENDSAPDRTFLLDAKCRD

>*Salpingoeca_rosetta*
EVTTLRLDHEGAEGTGPVCFTDDWKLDAERRD

Abbreviations used in Figure 2 and 3

Abbreviation	Organism	Accession number
bAquAquaeo_A	*Aquifex aeolicus*	NP_213288
bAquAquaeo_CC	*Aquifex aeolicus*	NP_214480
bBciBachal_A	*Bacillus halodurans*	NP_243747
bBciBachal_CC	*Bacillus halodurans*	NP_242550
bCyaSyysp_A	*Synechocystis sp.*	NP_441479
bCyaSyysp_CC	*Synechocystis_sp.*	NP_442458
bDeiDeirad_A	*Deinococcus radiodurans*	NP_294707
bDeiDeirad_CC	*Deinococcus radiodurans*	NP_294915
bDeiThethe_A	*Thermus thermophilus*	YP_144097
bDeiThethe_CC	*Thermus thermophilus*	YP_144191
bSpiBorbur_CCA	*Borrelia burgdorferi*	ZP_03086907
bTheFernod_CCAIIa	*Fervidobacterium nodosum*	A7HLG0
bTheThemar_CCAIIa	*Thermotoga maritima*	Q9WZH4
baEhrcha_CCA	*Ehrlichia chaffeensis*	Q2GF83
baRhilot_CCA	*Rhizobium loti*	Q98HS9
baRicpro_CCA	*Rickettsia prowazekii*	Q9ZEC8
bdGeosul_A	*Geobacter sulfurreducens*	NP_952632
bdGeosul_CC	*Geobacter sulfurreducens*	NP_953233
bgEsccol_CCA	*Escherichia_coli*	P06961
bgWigglo_CCA	*Wigglesworthia glossinidia*	Q8D2W4
ebChlAratha_CCA	*Arabidopsis thaliana*	Q94K06
ebChlLupalb_CCA	*Lupinus albus*	AAB03077.1
ebChlPhypat_CCA	*Physcomitrella patens*	A9RJR2
eufAscAspnid_CCA	*Aspergillus nidulans*	KZN89459
eufAscCangla_CCA	*Candida glabrata*	XP_449283.1
eufAscKlulac_CCA	*Kluyveromyces lactis*	AAG00316
eufAscNeoirr_CCA	*Neolecta irregularis*	NA
eufAscNeucra_CCA	*Neurospora crassa*	XP_962692
eufAscPnecar_CCA	*Pneumocystis carinii*	XP_018225359.1
eufAscPnejir_CCA	*Pneumocystis jirovecii*	XP_018230996.1
eufAscPnemur_CCA	*Pneumocystis murina*	XP_007874977.1
eufAscProino_CCA	*Protomyces inouyei*	NA
eufAscProlac_CCA	*Protomyces lactucaedebilis*	ORY85595
eufAscSaccer_CCA	*Sacharomyces cerevisiae*	NP_011095
eufAscSaicom_CCA	*Saitoella complicata*	XP_019025108.1
eufAscSchcry_A	*Schizosaccharomyces cryophilus*	EPY50617_Scry/1489
eufAscSchcry_CC	*Schizosaccharomyces cryophilus*	EPY50393_Scry/1538
eufAscSchjap_A	*Schizosaccharomyces japonicus*	EEB06657_Sjap/1487
eufAscSchjap_CC	*Schizosaccharomyces japonicus*	EEB07205_Sjap/1526
eufAscSchoct_A	*Schizosaccharomyces octosporus*	EPX70971_Soct/1489
eufAscSchoct_CC	*Schizosaccharomyces octosporus*	EPX73121_Soct/1536
eufAscSchpom_A	*Schizosaccharomyces pombe*	NP_588119.1
eufAscSchpom_CC	*Schizosaccharomyces pombe*	NP_594651.2
eufAscTapdef_CCA	*Taphrina deformans*	NA

eufAscTapfla_CCA	*Taphrina flavorubra*	NA
eufAscTappop_CCA	*Taphrino populina*	NA
eufAscTapwie_CCA	*Taphrina wiesneri*	NA
euhCorCorlim_a-type	*Corallochytrium limacisporum*	NA
euhFilCapowc_a-type	*Capsaspora owczarzaki*	Cowc_CAOG_04900
euhIchAbewhi_a-type	*Abeoforma whisleri*	NA
euhIchAmopar_a-type	*Amoebidium_parasiticum*	NA
euhIchCrefra_a-type	*Creolimax fragrantissima*	CFRG8205T1
euhMetCaeele_CCA	*Caenorhabditis elegans*	Q93795
euhMetDromel_CCA	*Drosophila melanogaster*	AAF51964
euhMetHomsap_CCA	*Homo sapiens*	BAB70662
euhMetStrpur_CCA	*Strongylocentrotus purpuratus*	XP_787015
euhChoAcaspe_a-type	*Acanthoeca spectabilis*	lcl|GGPA01036680.1
euhChoChoper_a-type	*Choanoeca perplexa*	lcl|GGOP01021473.1
euhChoCodhol_a-type	*Codosiga hollandica*	lcl|GGOV01001436.1
euhChoDiagra_a-type	*Diaphanoeca grandis*	lcl|GGPB01030090.1
euhChoDidcos_a-type	*Didymoeca costata*	lcl|GGOQ01024127.1
euhChoHarbal_a-type	*Hartaetosiga balthica*	lcl|GGOO01006806.1
euhChoHargra_a-type	*Hartaetosiga gracilis*	lcl|GGOU01008309.1
euhChoHelnan_a-type	*Helgoeca nana*	lcl|GGOR01029434.1
euhChoMicroa_a-type	*Microstomoeca roanoka*	lcl|GGON01002325.1
euhChoMonbre_a-type	*Monosiga brevicollis*	NA
euhChoMylflu_a-type	*Mylnosiga fluctuans*	lcl|GGOI01002178.1
euhChoSaldol_a-type	*Salpingoeca dolichothecata*	lcl|GGOK01027531.1
euhChoSalhel_a-type	*Salpingoeca helianthica*	lcl|GGOJ01023652.1
euhChoSalinf_a-type	*Salpingoeca infusionum*	lcl|GGOW01010160.1
euhChoSalkve_a-type	*Salpingoeca kvevrii*	lcl|GGOX01015633.1
euhChoSalmac_a-type	*Salpingoeca macrocollata*	lcl|GGOT01013341.1
euhChoSalpun_a-type	*Salpingoeca punica*	lcl|GGOZ01014644.1
euhChoSalros_a-type	*Salpingoeca rosetta*	EGD81016
euhChoSalurc_a-type	*Salpingoeca urceolata*	lcl|GGOY01009706.1
euhChoSavpar_a-type	*Savillea parva*	lcl|GGOL01014571.1
euhChoStedip_a-type	*Stephanoeca diplocostata*	lcl|GGOM01014527.1
euhChoChoper_e-type	*Choanoeca perplexa*	lcl|GGOP01015435.1
euhChoHarbal_e-type	*Hartaetosiga balthica*	lcl|GGOO01012516.1
euhChoHargra_e-type	*Hartaetosiga gracilis*	lcl|GGOU01003296.1
euhChoMicroa_e-type	*Microstomoeca roanoka*	lcl|GGON01033848.1
euhChoMonbre_e-type	*Monosiga brevicollis*	EDQ88852
euhChoSalinf_e-type	*Salpingoeca infusionum*	lcl|GGOW01025095.1
euhChoSalkve_e-type	*Salpingoeca kvevrii*	lcl|GGOX01014948.1
euhChoSalmac_e-type	*Salpingoeca macrocollata*	lcl|GGOT01008786.1
euhChoSalpun_e-type	*Salpingoeca punica*	lcl|GGOZ01000957.1
euhChoSalros_e-type	*Salpingoeca rosetta*	EGD77004
euhChoSalurc_e-type	*Salpingoeca urceolata*	lcl|GGOY01035099.1