Evolution of CCA-adding Enzymes at the Origin of Metazoa

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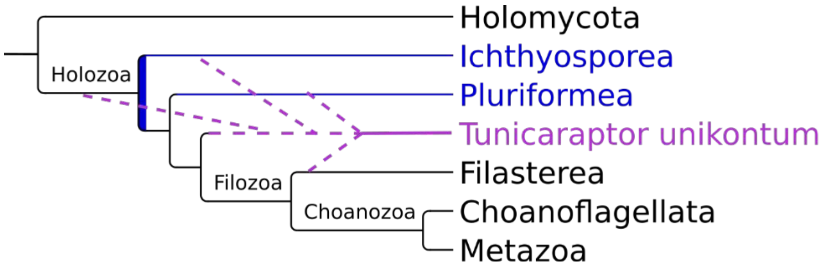
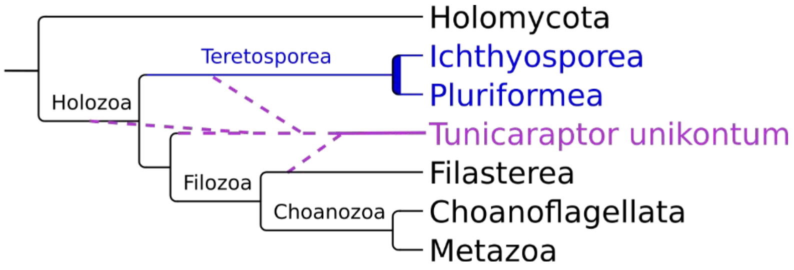
Phylogeny

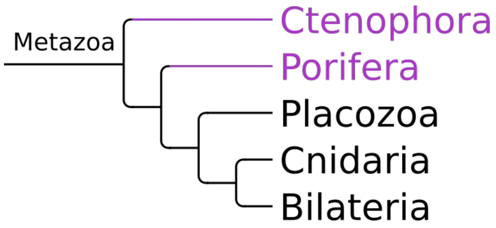
- Holozoa: Metazoa and their unicellular relatives
- holozoan phylogeny still discussed: Pluriformea as sister group to Filozoa vs. ‘Teretosporea’ hypotheses
- the unicellular species *T. unikontum* is yet to be placed in the phylogenetic tree

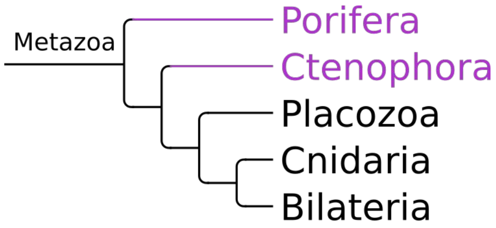




Phylogeny

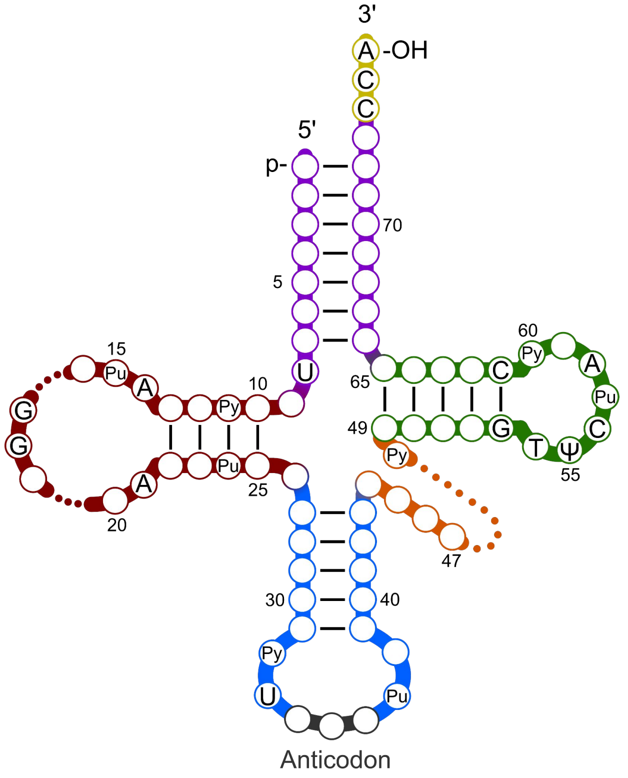
- phylogeny of early-branching Metazoa is strongly debated
- ‘Ctenophora first’ vs. ‘Porifera first’ hypotheses (comb jellies vs. sponges)
- phylogeny of unicellular Holozoa and early-branching Metazoa is based on a small set of genomes and transcriptomes
- the fast evolutionary rates of ctenophores further complicates things
- ambiguities also exist within the clades





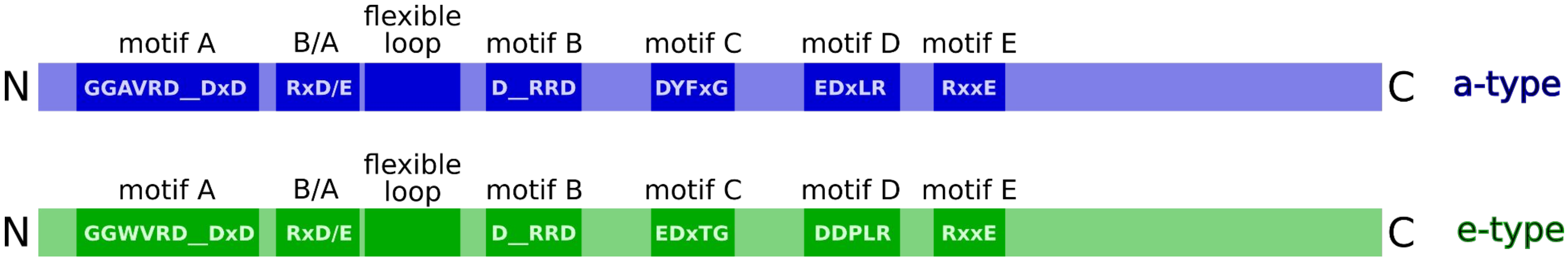
CCA-adding Enzymes

- CCA sequence is usually added post-transcriptionally by CCA-adding enzymes or CC-/A-adding enzymes
- class I: archaeal enzymes
- class II: bacterial and eukaryotic enzymes
- class II enzymes are characterized by five highly conserved catalytic core motifs



CCA-adding Enzymes

- only a few paralogs identified in eukaryotes (Choanoflagellata & Schizosaccharomyces)
- eukaryotic CCA: eCCA
- alphaproteobacterial CCA: aCCA
- differ in their conserved motifs A, C and D



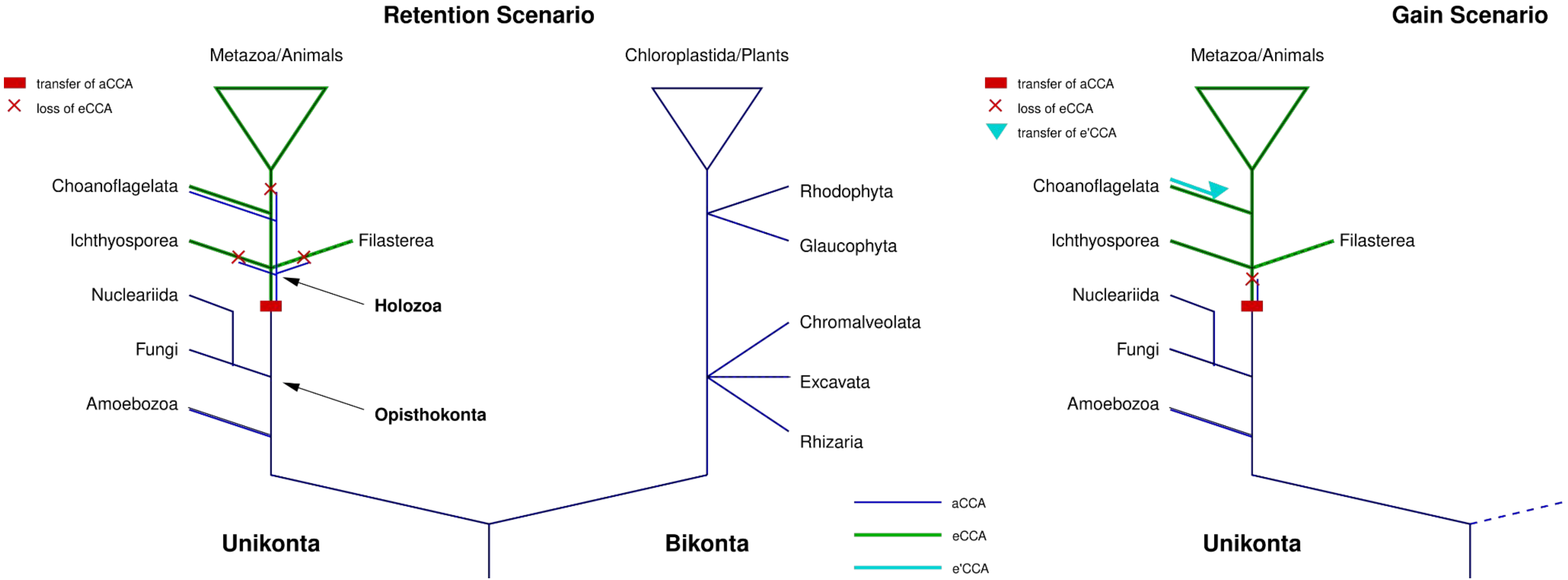
	motif A	B/A	flexible loop	motif B	motif C	motif D	motif E	
N	GGAVRD_DxD	RxD/E		D_RRD	DYFxG	EDxLR	RxxE	C a-type
N	GGWVRD_DxD	RxD/E		D_RRD	EDxTG	DDPLR	RxxE	C e-type

CCA-adding Enzymes

- Holozoa: aCCA through HGT, eCCA lost in most lineages
- Choanoflagellata: all have aCCA, some eCCA additionally
- subfunctionalization shown for *S. rosetta*
    - aCCA: CC-addition, mutations in the flexible loop
    - eCCA: A-addition

Evolutionary Events in Holozoa

- Retention vs. Gain Scenario to explain paralogs in Choanoflagellata

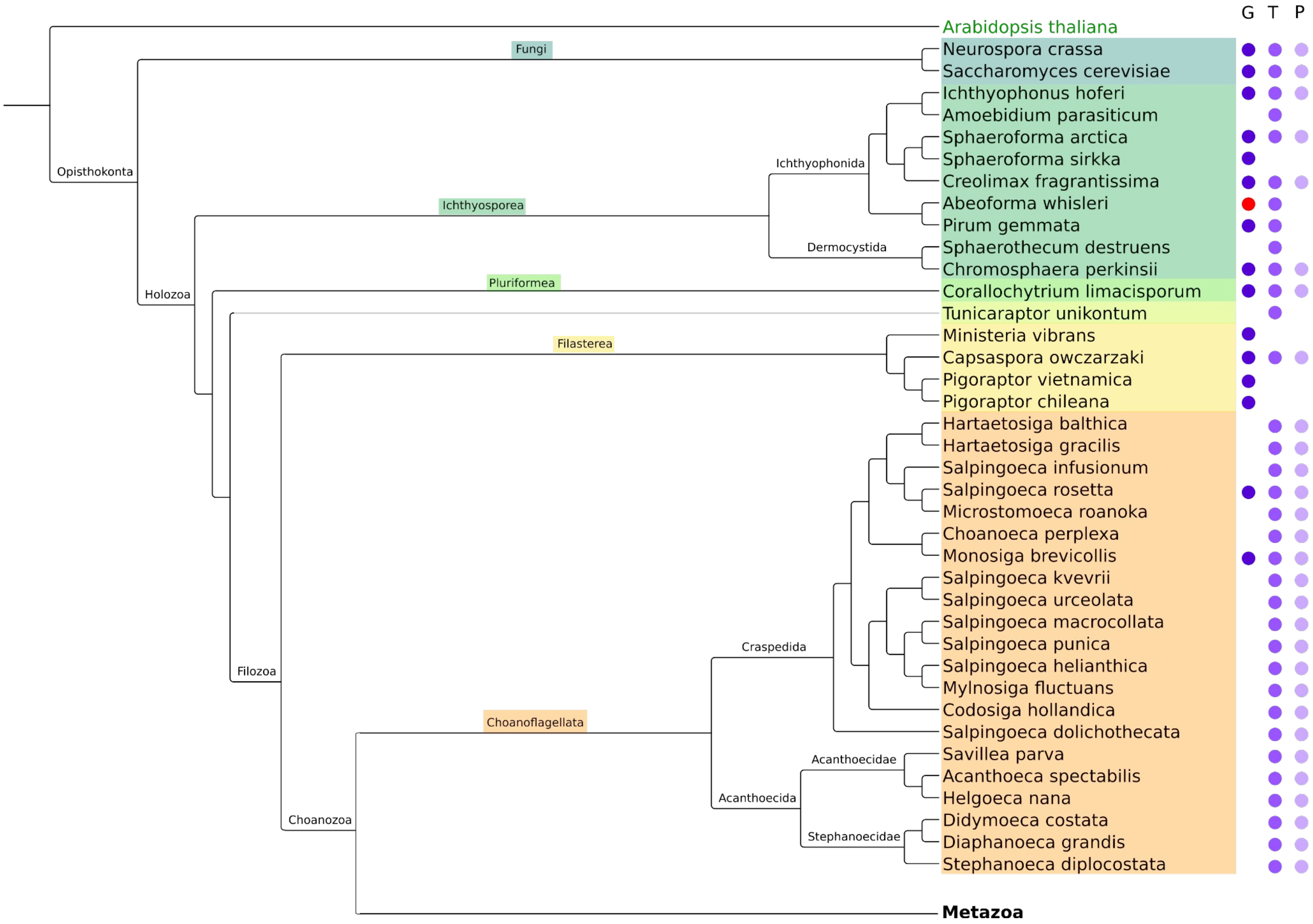


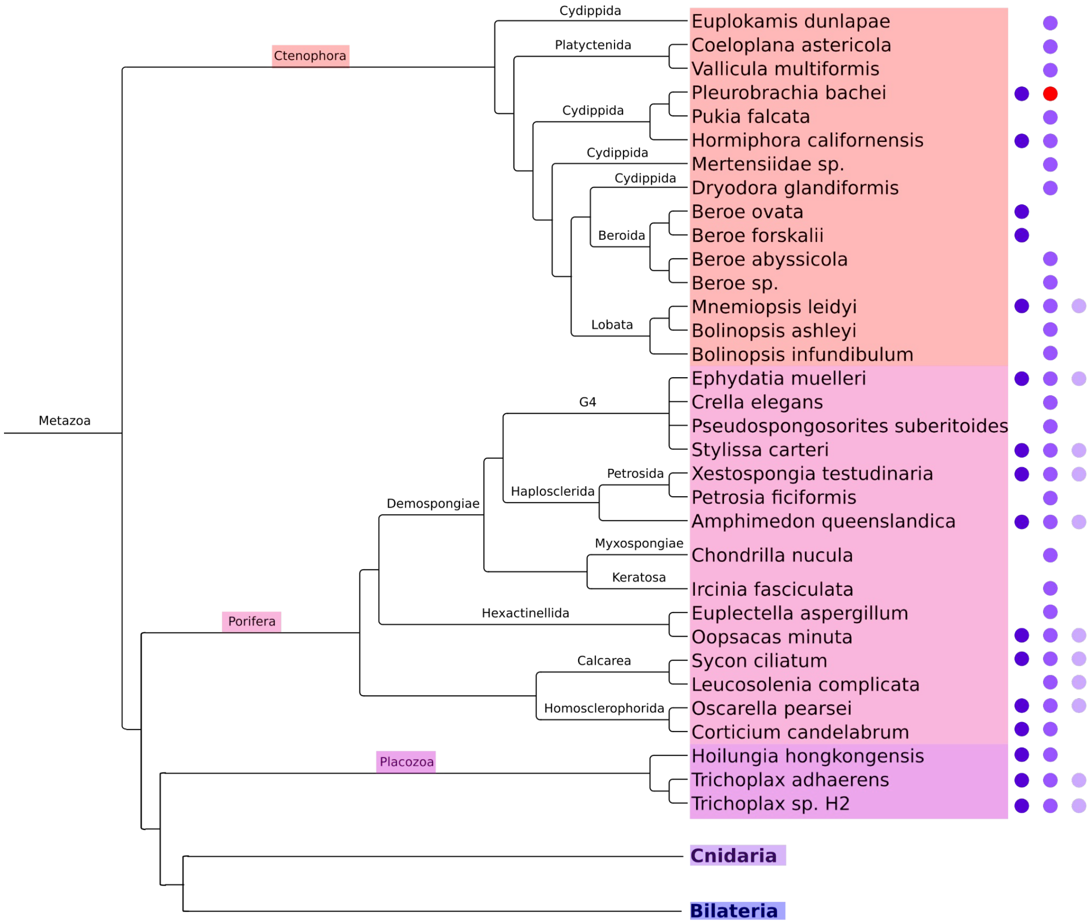
Is the occurrence of paralogs limited to our closest unicellular relatives?

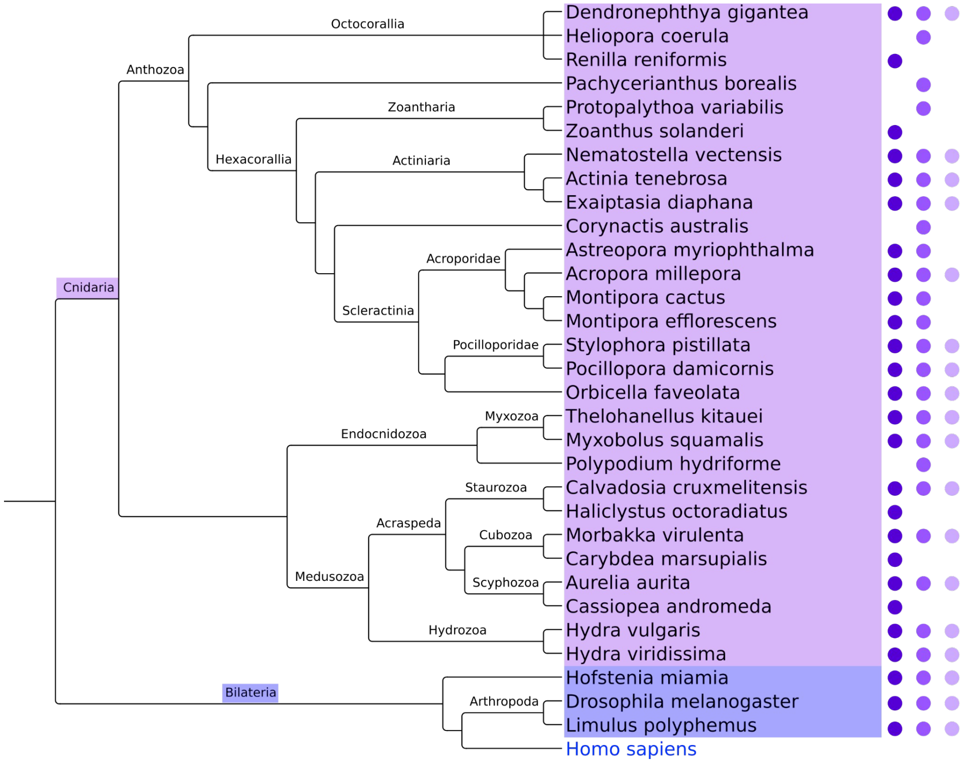
- ➢ expectation: all Metazoa possess one aCCA enzyme

Data

- proteome, transcriptome and genome data of 102 holozoan species
  - 58 genomes
  - 91 transcriptomes
  - 59 proteomes
- reference protein sequences:
  aCCA: *Homo sapiens*
  eCCA: *Arabidopsis thaliana*


G
T
P
Arabidopsis thaliana
Fungi
Neurospora crassa
Saccharomyces cerevisiae
Opisthokonta
Ichthyosporea
Ichthyophonida
Dermocystida
Ichthyophonus hoferi
Amoebidium parasiticum
Sphaeroforma arctica
Sphaeroforma sirkka
Creolimax fragrantissima
Abeoforma whisleri
Pirum gemmata
Sphaerothecum destruens
Chromosphaera perkinsii
Holozoa
Pluriformea
Corallochytrium limacisporum
Tunicaraptor unikontum
Filasterea
Ministeria vibrans
Capsaspora owczarzaki
Pigoraptor vietnamica
Pigoraptor chileana
Filozoa
Choanoflagellata
Craspedida
Hartaetosiga balthica
Hartaetosiga gracilis
Salpingoeca infusionum
Salpingoeca rosetta
Microstomoeca roanoka
Choanoeca perplexa
Monosiga brevicollis
Salpingoeca kvevrii
Salpingoeca urceolata
Salpingoeca macrocollata
Salpingoeca punica
Salpingoeca helianthica
Mylnosiga fluctuans
Codosiga hollandica
Salpingoeca dolichothecata
Acanthoecida
Acanthoecidae
Savillea parva
Acanthoeca spectabilis
Helgoeca nana
Stephanoecidae
Didymoeca costata
Diaphanoeca grandis
Stephanoeca diplocostata
Choanozoa
Metazoa


Metazoa
Ctenophora
Cydippida
Platyctenida
Cydippida
Cydippida
Cydippida
Beroida
Lobata
Euplokamis dunlapae
Coeloplana astericola
Vallicula multiformis
Pleurobrachia bachei
Pukia falcata
Hormiphora californensis
Mertensiidae sp.
Dryodora glandiformis
Beroe ovata
Beroe forskalii
Beroe abyssicola
Beroe sp.
Mnemiopsis leidyi
Bolinopsis ashleyi
Bolinopsis infundibulum
Porifera
Demospongiae
G4
Petrosida
Haplosclerida
Myxospongiae
Keratosa
Hexactinellida
Calcarea
Homoscleromorphida
Ephydatia muelleri
Crella elegans
Pseudospongosorites suberitoides
Stylissa carteri
Xestospongia testudinaria
Petrosia ficiformis
Amphimedon queenslandica
Chondrilla nucula
Ircinia fasciculata
Euplectella aspergillum
Oopsacas minuta
Sycon ciliatum
Leucosolenia complicata
Oscarella pearsei
Corticium candelabrum
Placozoa
Hoilungia hongkongensis
Trichoplax adhaerens
Trichoplax sp. H2
Cnidaria
Bilateria

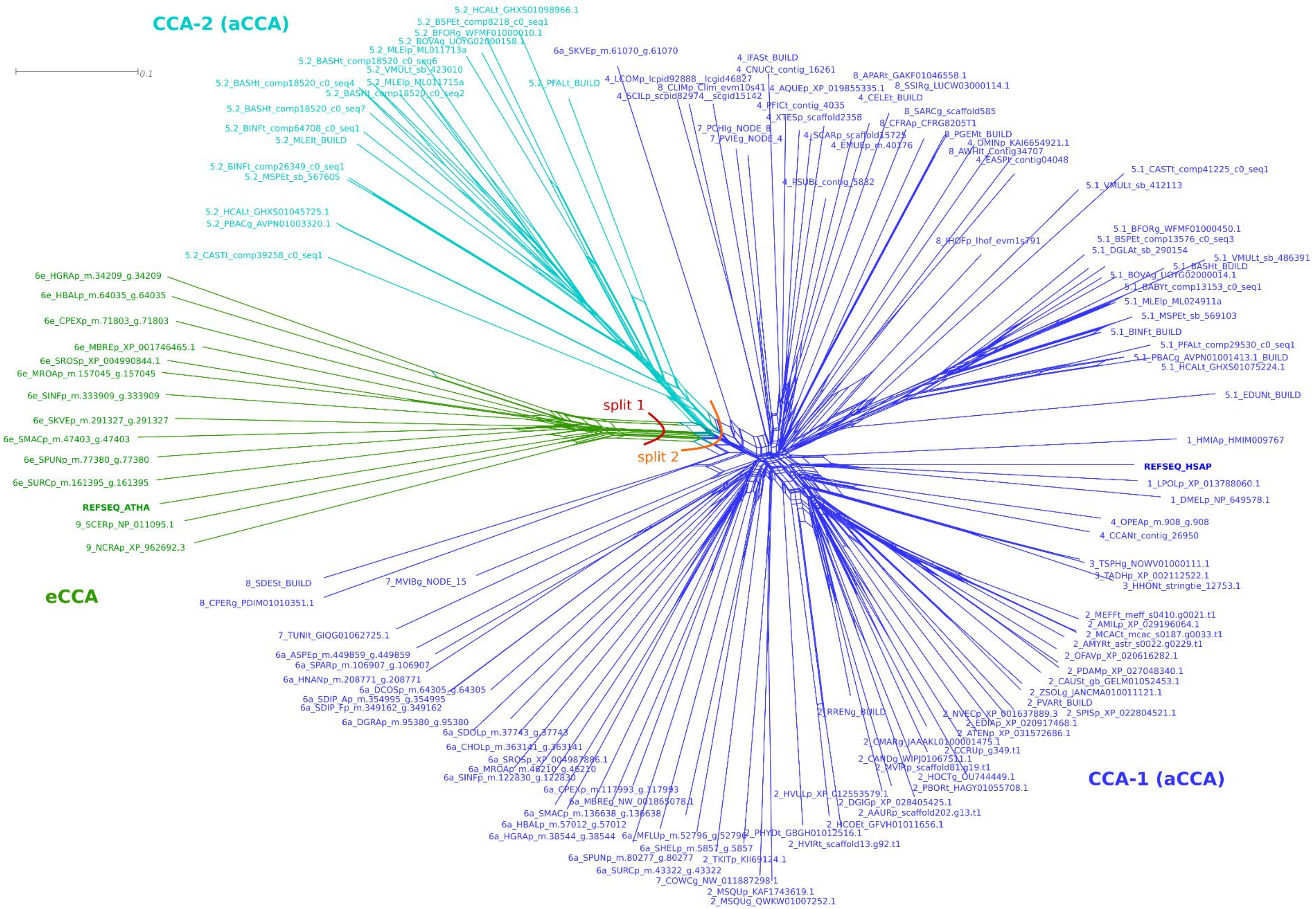

Octocorallia
Anthozoa
Zoantharia
Hexacorallia
Actiniaria
Acroporidae
Cnidaria
Scleractinia
Pocilloporidae
Myxozoa
Endocnidozoa
Staurozoa
Acraspeda
Cubozoa
Medusozoa
Scyphozoa
Hydrozoa
Bilateria
Arthropoda
Dendronephthya gigantea
Heliopora coerula
Renilla reniformis
Pachycerianthus borealis
Protopalythoa variabilis
Zoanthus solanderi
Nematostella vectensis
Actinia tenebrosa
Exaiptasia diaphana
Corynactis australis
Astreopora myriophthalma
Acropora millepora
Montipora cactus
Montipora efflorescens
Stylophora pistillata
Pocillopora damicornis
Orbicella faveolata
Thelohanellus kitauei
Myxobolus squamalis
Polypodium hydriforme
Calvadosia cruxmelitensis
Haliclystus octoradiatus
Morbakka virulenta
Carybdea marsupialis
Aurelia aurita
Cassiopea andromeda
Hydra vulgaris
Hydra viridissima
Hofstenia miamia
Drosophila melanogaster
Limulus polyphemus
Homo sapiens

Workflow

- identify candidate sequences
- transcriptome: translate candidates
- genome: splice site prediction (unicellular Holozoa) or gene prediction using clade specific parameters (early-branching Metazoa)
- check for contaminations
- iterate using clade specific protein sequence if only partial sequences/no candidates found
- align candidates, generate phylogenetic network
- classify candidates using sequence similarity, common motifs and the phylogenetic network

Expectations

- one aCCA enzyme in all holozoan species
- additional eCCA sequence in Choanoflagellata
- clear separation of eCCA sequences from Choanoflagellata and aCCA sequences from all holozoan lineages


CCA-2 (aCCA)
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5.2_BOVAg_UOYG02000158.1
5.2_MLEIp_ML011713a
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4_CNUCt_contig_16261
4_LCOMp_lcpid92888__lcgid46827
8_CLIMp_Clim_evm10s41
4_AQUEp_XP_019855335.1
4_SCILp_scpid82974__scgid15142
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4_PFICt_contig_4035
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8_SARCg_scaffold585
8_CFRAp_CFRG8205T1
7_PCHIg_NODE_8
7_PVIEg_NODE_4
4_SCARp_scaffold15725
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8_PGEMt_BUILD
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4_EASPt_contig04048
4_PSUBt_contig_5832
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2_PBORt_HAGY01055708.1
CCA-1 (aCCA)
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2_HCOEt_GFVH01011656.1
2_PHYDt_GBGH01012516.1
2_HVIRt_scaffold13.g92.t1
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6a_SPUNp_m.80277_g.80277
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6a_HBALp_m.57012_g.57012
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6a_DCOSp_m.64305_g.64305
6a_HNANp_m.208771_g.208771
6a_SPARp_m.106907_g.106907
6a_ASPEp_m.449859_g.449859
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8_SDESt_BUILD
7_MVIBg_NODE_15
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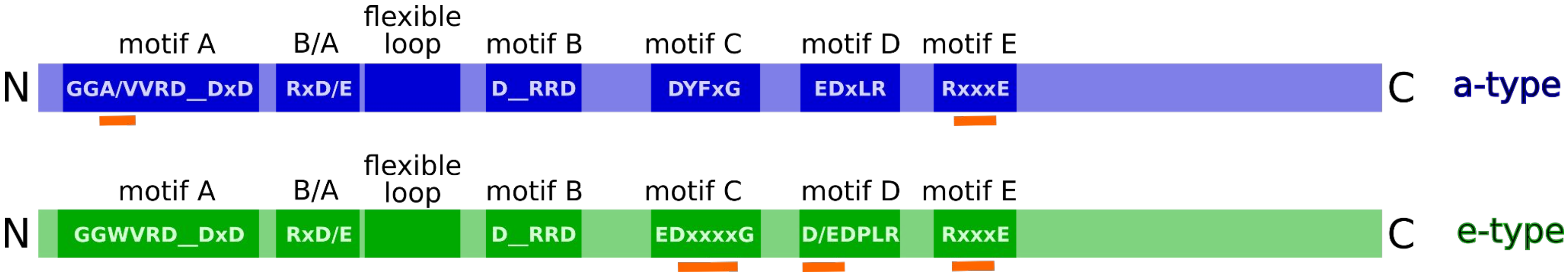
6e_CPEXp_m.71803_g.71803
6e_HBALp_m.64035_g.64035
6e_HGRAp_m.34209_g.34209
split 1
split 2

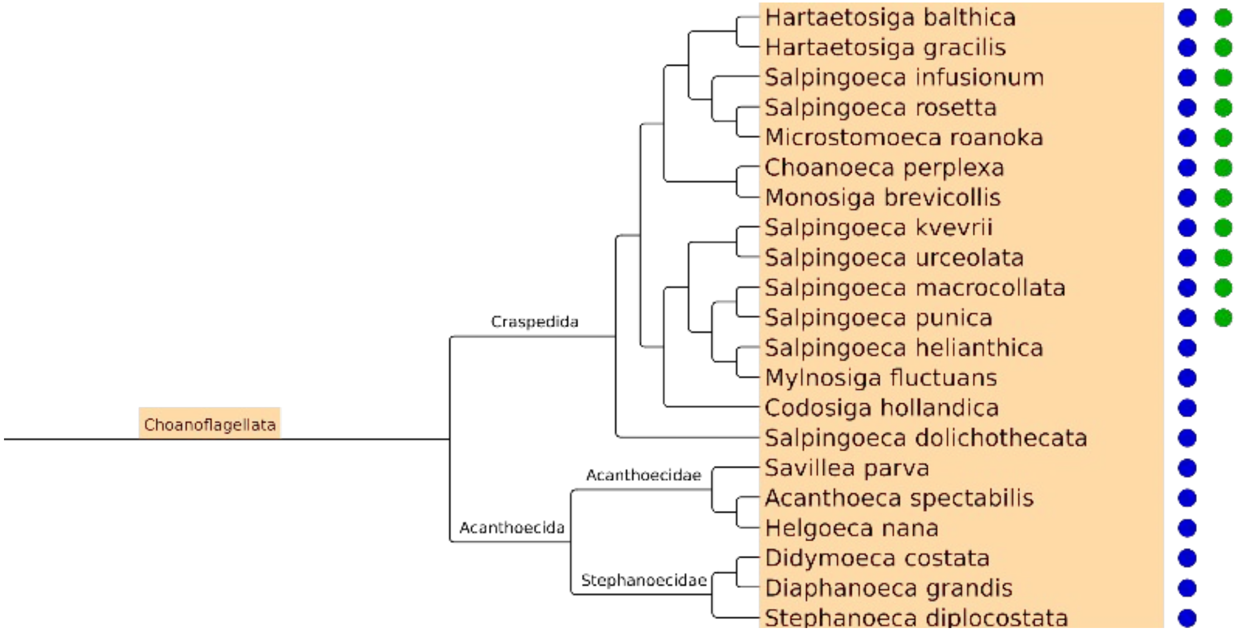
Motifs

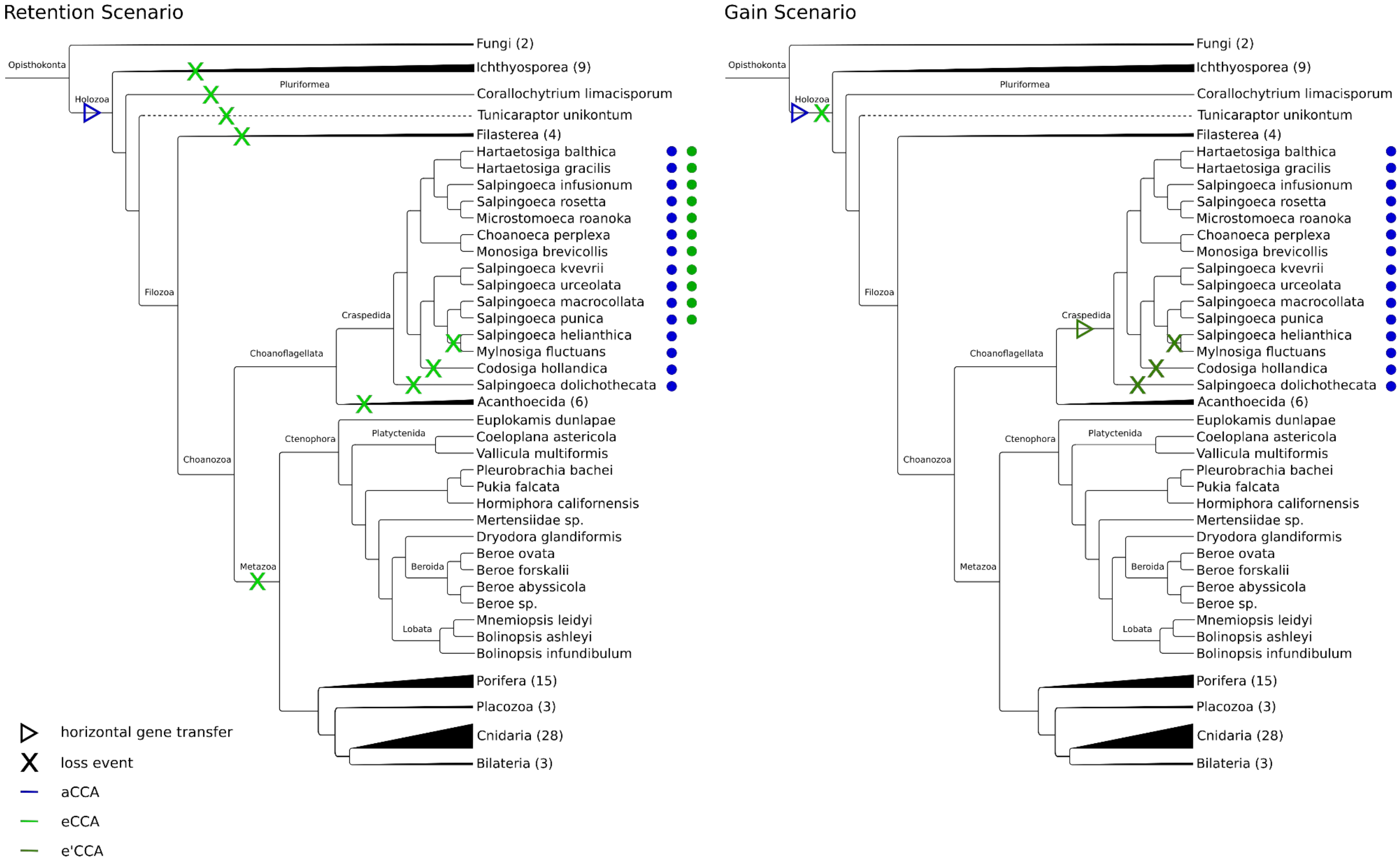
- motifs A, C, D and E need revision
- strict pattern search failed to classify eCCA sequences



Distribution of CCA-adding Enzymes

- ➢ eCCA in Craspedida clade 1 and clade 2




Retention Scenario
Opisthokonta
Holozoa
Pluriformea
Filozoa
Choanozoa
Choanoflagellata
Craspedida
Metazoa
Ctenophora
Platyctenida
Beroida
Lobata
Fungi (2)
Ichthyosporea (9)
Corallochytrium limacisporum
Tunicaraptor unikontum
Filasterea (4)
Hartaetosiga balthica
Hartaetosiga gracilis
Salpingoeca infusionum
Salpingoeca rosetta
Microstomoeca roanoka
Choanoeca perplexa
Monosiga brevicollis
Salpingoeca kvevrii
Salpingoeca urceolata
Salpingoeca macrocollata
Salpingoeca punica
Salpingoeca helianthica
Mylnosiga fluctuans
Codosiga hollandica
Salpingoeca dolichothecata
Acanthoecida (6)
Euplokamis dunlapae
Coeloplana astericola
Vallicula multiformis
Pleurobrachia bachei
Pukia falcata
Hormiphora californensis
Mertensiidae sp.
Dryodora glandiformis
Beroe ovata
Beroe forskalii
Beroe abyssicola
Beroe sp.
Mnemiopsis leidyi
Bolinopsis ashleyi
Bolinopsis infundibulum
Porifera (15)
Placozoa (3)
Cnidaria (28)
Bilateria (3)
Gain Scenario
Opisthokonta
Holozoa
Pluriformea
Filozoa
Choanozoa
Choanoflagellata
Craspedida
Metazoa
Ctenophora
Platyctenida
Beroida
Lobata
Fungi (2)
Ichthyosporea (9)
Corallochytrium limacisporum
Tunicaraptor unikontum
Filasterea (4)
Hartaetosiga balthica
Hartaetosiga gracilis
Salpingoeca infusionum
Salpingoeca rosetta
Microstomoeca roanoka
Choanoeca perplexa
Monosiga brevicollis
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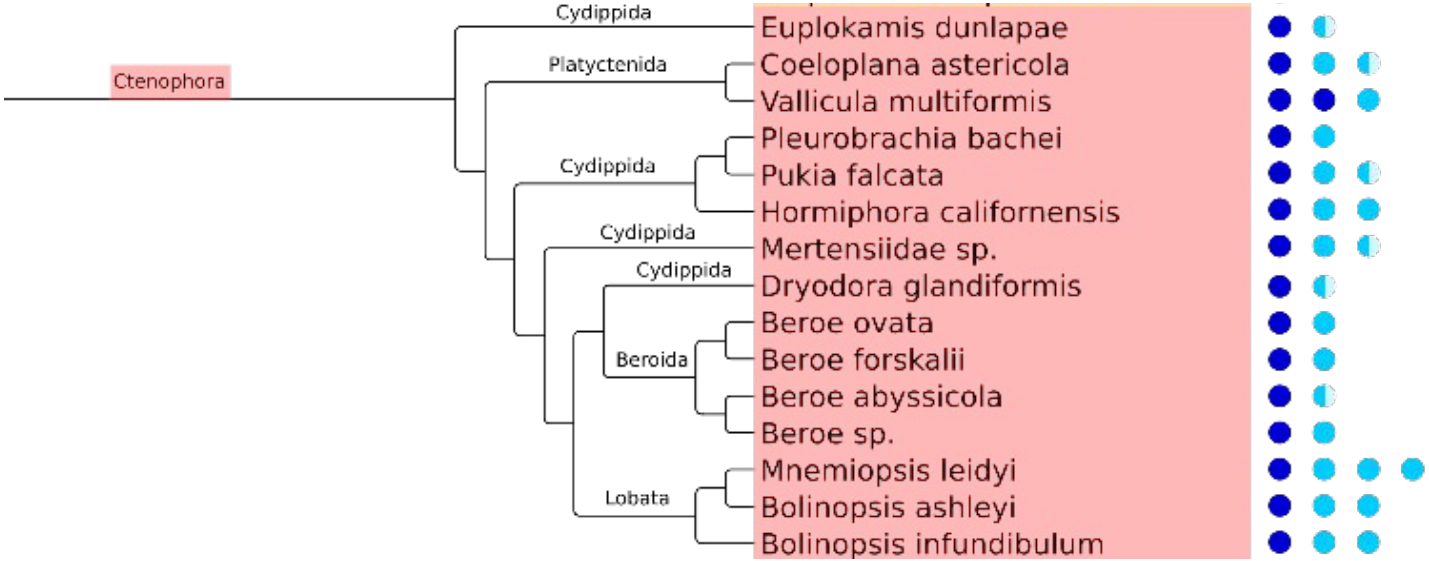
Vallicula multiformis
Pleurobrachia bachei
Pukia falcata
Hormiphora californensis
Mertensiidae sp.
Dryodora glandiformis
Beroe ovata
Beroe forskalii
Beroe abyssicola
Beroe sp.
Mnemiopsis leidyi
Bolinopsis ashleyi
Bolinopsis infundibulum
Porifera (15)
Placozoa (3)
Cnidaria (28)
Bilateria (3)
horizontal gene transfer
loss event
aCCA
eCCA
e'CCA

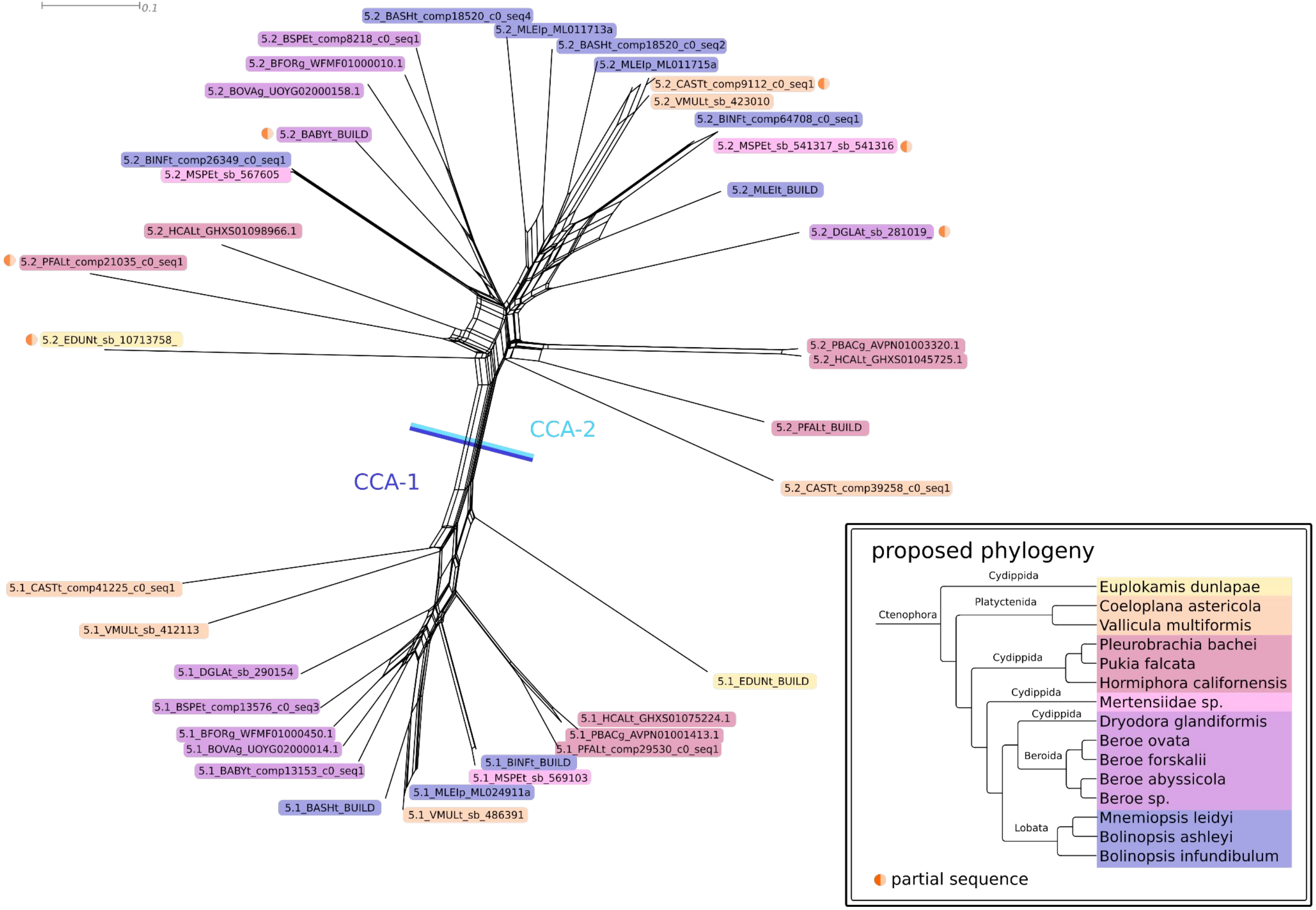
Distribution of CCA-adding Enzymes

- multiple a-type enzymes in Ctenophora



Ctenophore Sequences

- multiple sequences found in Ctenophora, all clearly classified as aCCA
- significant differences separating them into two distinct groups
  →CCA-1 and CCA-2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5.2_BSPEt_comp8218_c0_seq1
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5.2_MLEIp_ML011715a
5.2_BOVAg_UOYG02000158.1
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5.2_VMULt_sb_423010
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5.2_BABYt_BUILD
5.2_MSPEt_sb_541317_sb_541316
5.2_BINFt_comp26349_c0_seq1
5.2_MSPEt_sb_567605
5.2_MLEIt_BUILD
5.2_HCALt_GHXS01098966.1
5.2_DGLAt_sb_281019_
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CCA-2
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CCA-1
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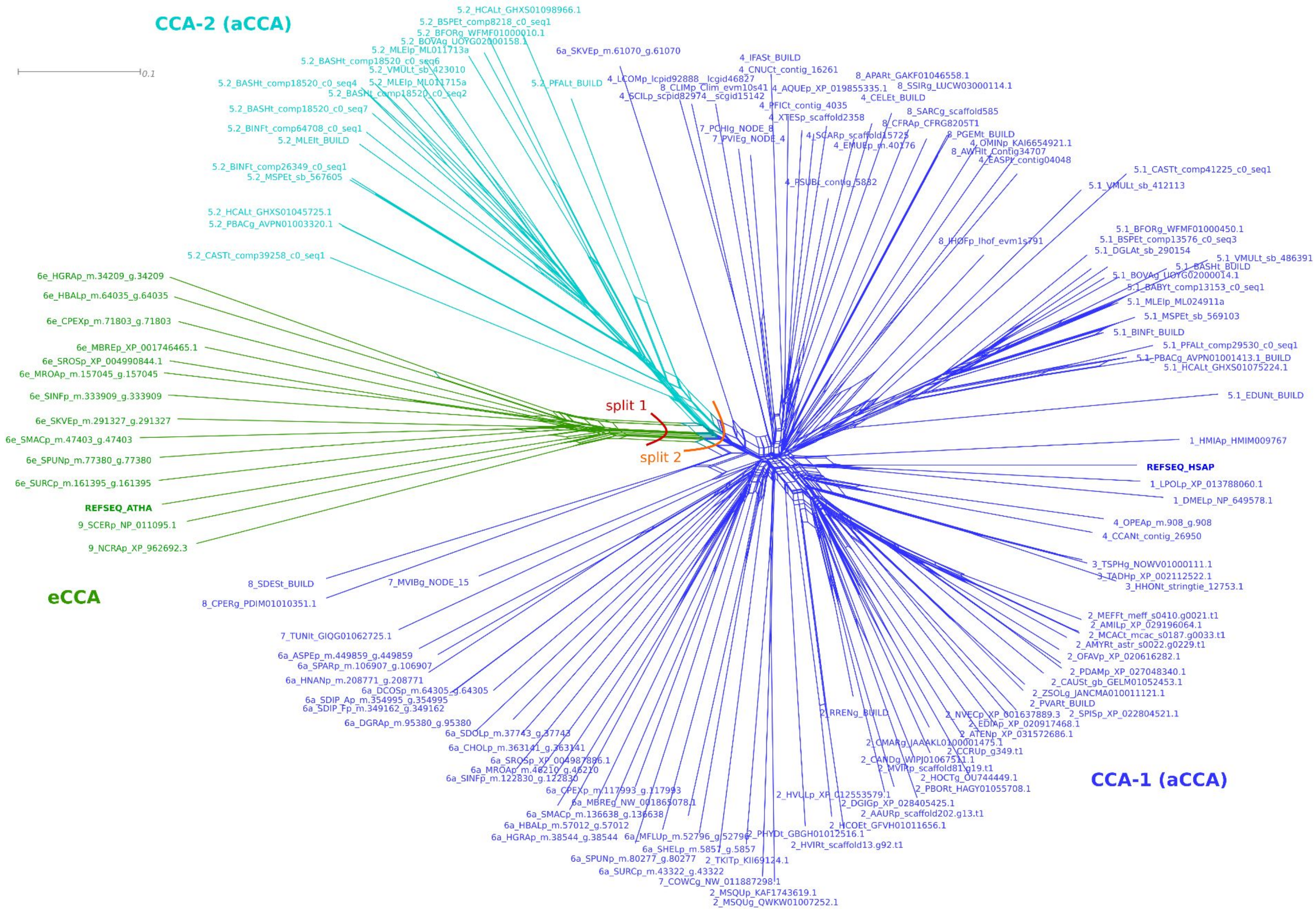
5.1_MSPEt_sb_569103
5.1_MLEIp_ML024911a
5.1_BASHt_BUILD
5.1_VMULt_sb_486391
proposed phylogeny
Ctenophora
Cydippida
Platyctenida
Cydippida
Cydippida
Cydippida
Beroida
Lobata
Euplokamis dunlapae
Coeloplana astericola
Vallicula multiformis
Pleurobrachia bachei
Pukia falcata
Hormiphora californensis
Mertensiidae sp.
Dryodora glandiformis
Beroe ovata
Beroe forskalii
Beroe abyssicola
Beroe sp.
Mnemiopsis leidyi
Bolinopsis ashleyi
Bolinopsis infundibulum
◐ partial sequence


CCA-2 (aCCA)
0.1
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5.2_BOVAg_UOYG02000158.1
5.2_MLEIp_ML011713a
5.2_BASHt_comp18520_c0_seq6
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2_MSQUg_QWKW01007252.1

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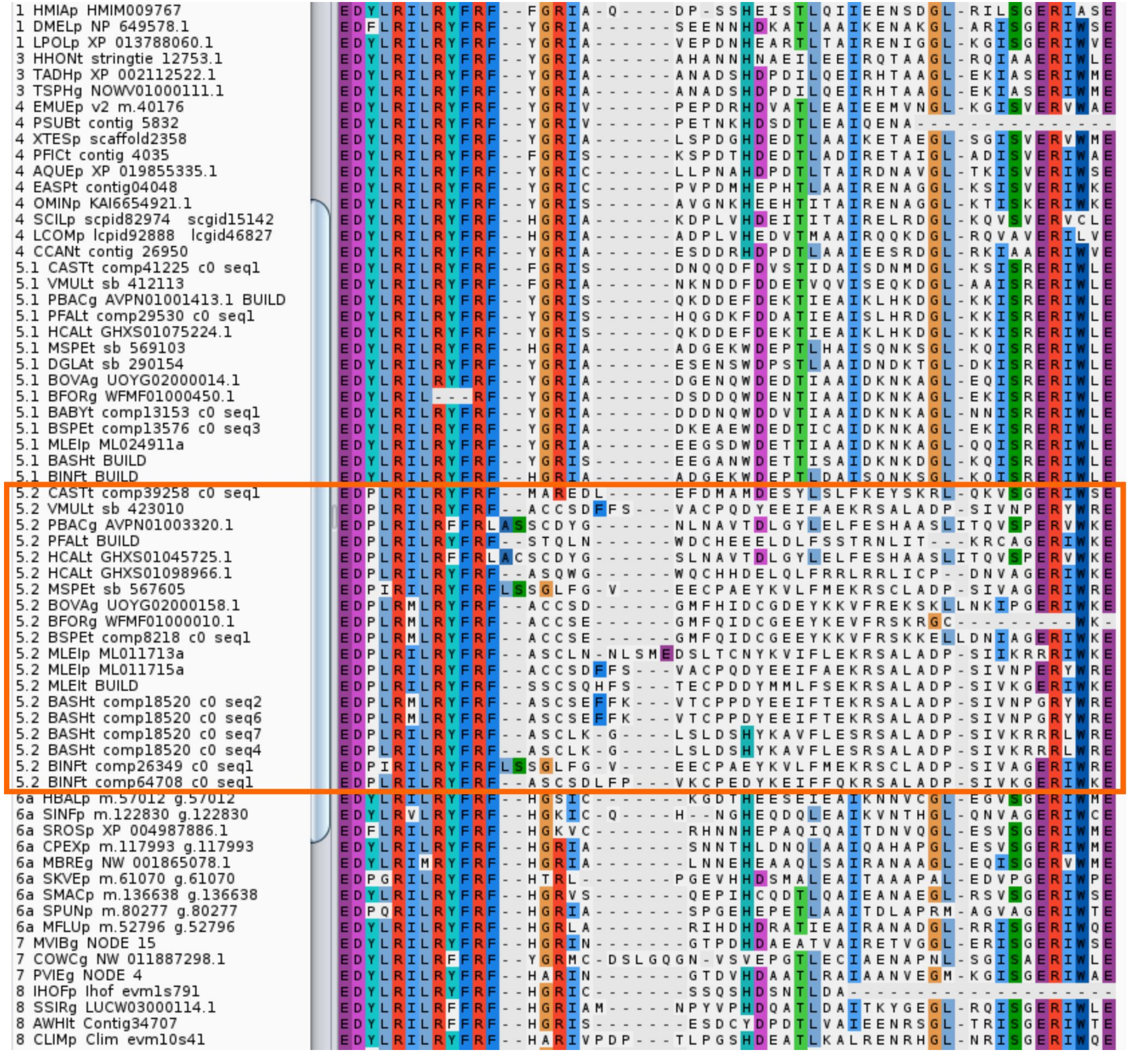

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1 LPOLp XP 013788060.1                EDYLRILRYFRF--YGRIA------VEPDNHEARTLTAIRENIGGL-KGISGERIWVE
3 HHONt stringtie 12753.1            EDYLRILRYFRF--YGRIA------AHANNHNAEILEEIRQTAAGL-RQIAAERIWLE
3 TADHp XP 002112522.1                EDYLRILRYFRF--YGRIA------ANADSHDPDILQEIRHTAAGL-EKIASERIWME
3 TSPHg NOWV01000111.1                EDYLRILRYFRF--YGRIA------ANADSHDPDILQEIRHTAAGL-EKIASERIWME
4 EMUEp v2 m.40176                   EDYLRILRYFRF--YGRIV------PEPDRHDVATLEAIEEMVNGL-KGISVERVWAE
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4 SCILp scpid82974  scgid15142      EDYLRILRYFRF--HGRIA------KDPLVHDEITITAIRELRDGL-KQVSVERVCLE
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5.1 CASTt comp41225 c0 seq1         EDYLRILRYFRF--FGRIS------DNQQDFDVSTIDAISDNMDGL-KSISRERIWLE
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5.1 PBACg AVPN01001413.1 BUILD      EDYLRILRYFRF--YGRIS------QKDDEFDEKTIEAIKLHKDGL-KKISRERIWLE
5.1 PFALt comp29530 c0 seq1         EDYLRILRYFRF--YGRIS------HQGDKFDDATIEAISLHRDGL-KKISRERIWLE
5.1 HCALt GHXS01075224.1            EDYLRILRYFRF--YGRIS------QKDDEFDEKTIEAIKLHKDGL-KKISRERIWLE
5.1 MSPEt sb 569103                 EDYLRILRYFRF--HGRIA------ADGEKWDEPTLHAISQNKSGL-KQISRERIWLE
5.1 DGLAt sb 290154                 EDYLRILRYFRF--YGRIA------ESENSWDPSTLAAIDNDKTGL-DKISRERIWLE
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5.1 BFORg WFMF01000450.1            EDYLRIL---RF--YGRIA------DSDDQWDENTIAAIDKNKAGL-EKISRERIWLE
5.1 BABYt comp13153 c0 seq1         EDYLRILRYFRF--YGRIA------DDDNQWDDVTIAAIDKNKAGL-NNISRERIWLE
5.1 BSPEt comp13576 c0 seq3         EDYLRILRYFRF--YGRIA------DKEAEWDEDTICAIDKNKAGL-EKISRERIWLE
5.1 MLEIp ML024911a                 EDYLRILRYFRF--YGRIA------EEGSDWDETTIAAIDKNKAGL-QQISRERIWLE
5.1 BASHt BUILD                     EDYLRILRYFRF--YGRIS------EEGANWDETTISAIDKNKDGL-KQISRERIWLE
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5.2 PFALt BUILD                     EDPLRILRYFRF--STQLN------WDCHEEELDLFSSTRNLIT---KRCAGERIWKE
5.2 HCALt GHXS01045725.1            EDPLRILRFFRLACSCDYG------SLNAVTDLGYLELFESHAASLITQVSPERVWKE
5.2 HCALt GHXS01098966.1            EDPLRILRYFRF--ASQWG------WQCHHDELQLFRRLRRLICP--DNVAGERIWKE
5.2 MSPEt sb 567605                 EDPIRILRYFRFLSSGLFG-V----EECPAEYKVLFMEKRSCLADP-SIVAGERIWRE
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5.2 BFORg WFMF01000010.1            EDPLRMLRYFRF--ACCSE------GMFQIDCGEEYKEVFRSKRGC---------WK-
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5.2 MLEIp ML011715a                 EDPLRILRYFRF--ACCSDFFS---VACPQDYEEIFAEKRSALADP-SIVNPERYWRE
5.2 MLEIt BUILD                     EDPLRILRYFRF--SSCSQHFS---TECPDDYMMLFSEKRSALADP-SIVKGERIWKE
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5.2 BASHt comp18520 c0 seq6         EDPLRMLRYFRF--ASCSEFFK---VTCPPDYEEIFTEKRSALADP-SIVNPGRYWRE
5.2 BASHt comp18520 c0 seq7         EDPLRILRYFRF--ASCLK-G----LSLDSHYKAVFLESRSALADP-SIVKRRRLWRE
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5.2 BINFt comp26349 c0 seq1         EDPIRILRYFRFLSSGLFG-V----EECPAEYKVLFMEKRSCLADP-SIVAGERIWRE
5.2 BINFt comp64708 c0 seq1         EDPLRILRYFRF--ASCSDLFP---VKCPEDYKEIFFQKRSALADP-SIVKGERIWKE
6a HBALp m.57012 g.57012            EDYLRILRYFRF--HGSIC-------KGDTHEESEIEAIKNNVCGL-EGVSGERIWME
6a SINFp m.122830 g.122830          EDYLRVLRYFRF--HGKIC-Q----H--NGHEQDQLEAIKVNTHGL-QNVAGERIWCE
6a SROSp XP 004987886.1             EDFLRILRYFRF--HGKVC-------RHNNHEPAQIQAITDNVQGL-ESVSGERIWME
6a CPEXp m.117993 g.117993          EDYLRILRYFRF--HGRIA-------SNNTHLDNQLAAIQAHAPGL-ESVSGERIWME
6a MBREg NW 001865078.1             EDYLRIMRYFRF--HGRIA-------LNNEHEAAQLSAIRANAAGL-EQISGERVWME
6a SKVEp m.61070 g.61070            EDPGRILRYFRF--HTRL-------PGEVHHDSMALEAITAAAPAL-EDVPGERIWPE
6a SMACp m.136638 g.136638          EDYLRILRYFRF--HGRVS-------QEPIHCQDTLQAIEANAEGL-RSVSGERIWSE
6a SPUNp m.80277 g.80277            EDPQRILRYFRF--HGRIA-------SPGEHEPETLAAITDLAPRM-AGVAGERIWTE
6a MFLUp m.52796 g.52796            EDYLRILRYFRF--HGRLA-------RIHDHDRATIEAIRANADGL-RRISGERIWQE
7 MVIBg NODE 15                     EDYLRILRYFRF--HGRIN-------GTPDHDAEATVAIRETVGGL-ERISGERIWSE
7 COWCg NW 011887298.1              EDYLRILRFFRF--YGRMC-DSLGQGN-VSVEPGTLECIAENAPNL-SGISAERIWLE
7 PVIEg NODE 4                      EDYLRILRYFRF--HARIN-------GTDVHDAATLRAIAANVEGM-KGISGERIWAE
8 IHOFp Ihof evm1s791               EDYLRILRYFRF--HGRIC-------SSQSHDSNTLDA-------------------
8 SSIRg LUCW03000114.1              EDYLRILRFFRF--HGRIAM-----NPYVPHDQATLDAITKYGEGL-RQISGERIWLE
8 AWHIt Contig34707                 EDYLRILRFFRF--HGRIS-------ESDCYDPDTLVAIEENRSGL-TRISGERIWTE
8 CLIMp Clim evm10s41               EDYLRILRYFRF--HARIVPDP---TLPGSHDEATLKALRENRHGL-NRISGERIWQE
```

Ctenophore Sequences

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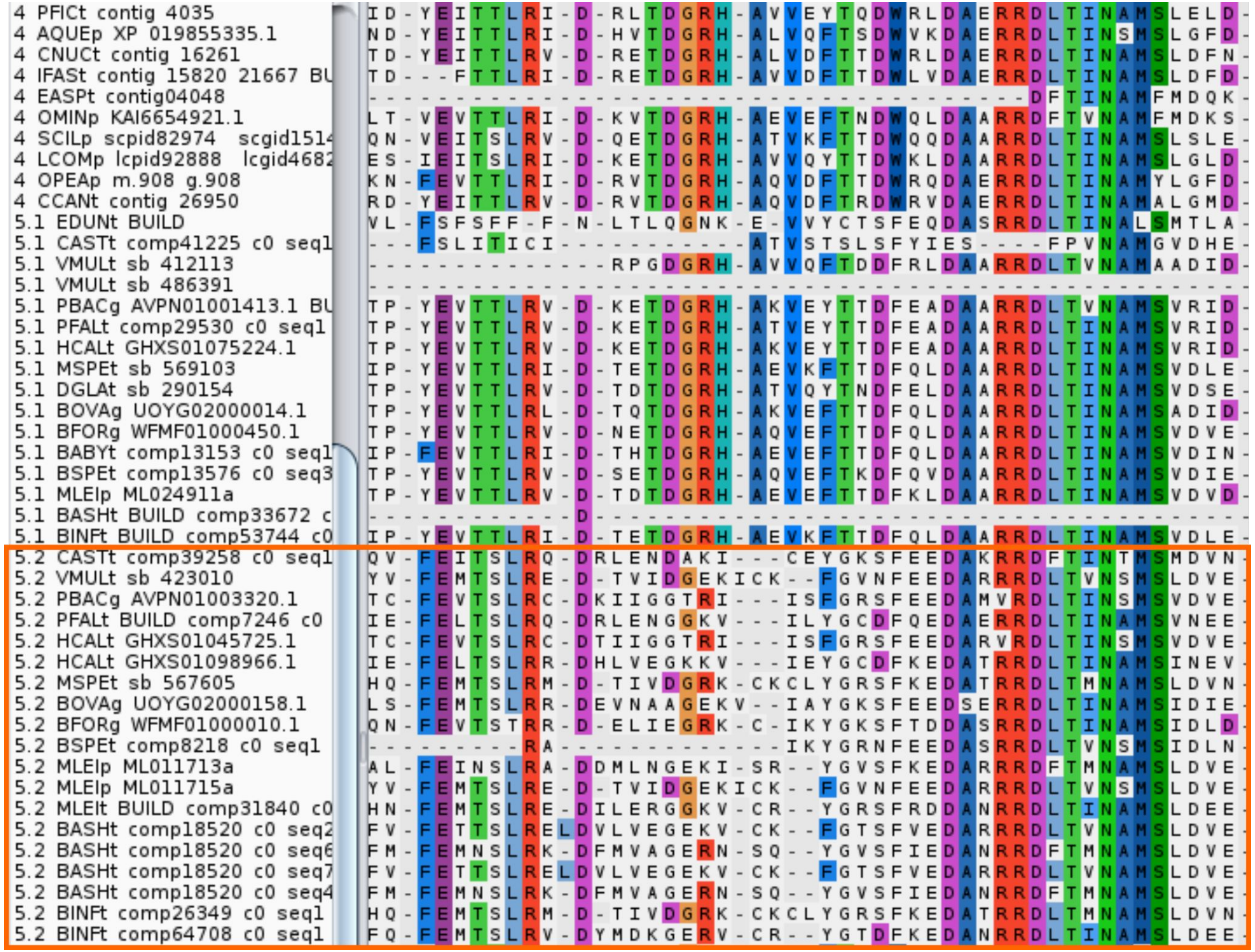
    →indicative of structural changes
  - point mutations in the flexible loop
  - exon counts
- structural and functional differences likely, subfunctionalization possible (CCA-1 A-addition, CCA-2 CC-addition)



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4 IFASt contig 15820 21667 BU    TD---FTTLRI-D-RETDGRH-AVVDFTTDWLVDAERRDLTINAMSLDFD-
4 EASPt contig04048              --------------------------------------DFTINAMFMDQK-
4 OMINp KAI6654921.1             LT-VEVTTLRI-D-KVTDGRH-AEVEFTNDWQLDAARRDFTVNAMFMDKS-
4 SCILp scpid82974  scgid1514    QN-VEITSLRV-D-QETDGRH-ATVKFTTDWQQDAARRDLTINAMSLSLE-
4 LCOMp lcpid92888  lcgid4682    ES-IEITSLRI-D-KETDGRH-AVVQYTTDWKLDAARRDLTINAMSLGLD-
4 OPEAp m.908 g.908              KN-FEVTTLRI-D-RVTDGRH-AQVDFTTDWRQDAERRDLTINAMYLGFD-
4 CCANt contig 26950             RD-YEITTLRV-D-RVTDGRH-AQVDFTRDWRVDAERRDLTINAMALGMD-
5.1 EDUNt BUILD                  VL-FSFSFF-F-N-LTLQGNK-E-VVYCTSFEQDASRRDLTINALSMTLA-
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5.1 VMULt sb 412113              --------------RPGDGRH-AVVQFTDDFRLDAARRDLTVNAMAADID-
5.1 VMULt sb 486391              ---------------------------------------------------
5.1 PBACg AVPN01001413.1 BU      TP-YEVTTLRV-D-KETDGRH-AKVEYTTDFEADAARRDLTVNAMSVRID-
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5.1 HCALt GHXS01075224.1         TP-YEVTTLRV-D-KETDGRH-AKVEYTTDFEADAARRDLTINAMSVRID-
5.1 MSPEt sb 569103              IP-YEVTTLRI-D-TETDGRH-AEVKFTTDFQLDAARRDLTINAMSVDLE-
5.1 DGLAt sb 290154              TP-YEVTTLRV-D-TDTDGRH-ATVQYTNDFELDAARRDLTINAMSVDSE-
5.1 BOVAg UOYG02000014.1         TP-YEVTTLRL-D-TQTDGRH-AKVEFTTDFQLDAARRDLTINAMSADID-
5.1 BFORg WFMF01000450.1         TP-YEVTTLRV-D-NETDGRH-AQVEFTTDFQLDAARRDLTINAMSVDVE-
5.1 BABYt comp13153 c0 seql      IP-FEVTTLRI-D-THTDGRH-AEVEFTTDFQLDAARRDLTINAMSVDIN-
5.1 BSPEt comp13576 c0 seq3      TP-YEVTTLRV-D-SETDGRH-AQVEFTKDFQVDAARRDLTINAMSVDIE-
5.1 MLEIp ML024911a              TP-YEVTTLRV-D-TDTDGRH-AEVEFTTDFKLDAARRDLTINAMSVDVD-
5.1 BASHt BUILD comp33672 c      ------------D--------------------------------------
5.1 BINFt BUILD comp53744 c0     IP-YEVTTLRI-D-TETDGRH-AEVKFTTDFQLDAARRDLTINAMSVDLE-
5.2 CASTt comp39258 c0 seql      QV-FEITSLRQ-DRLENDAKI---CEYGKSFEEDAKRRDFTINTMSMDVN-
5.2 VMULt sb 423010              YV-FEMTSLRE-D-TVIDGEKICK--FGVNFEEDARRRDLTVNSMSLDVE-
5.2 PBACg AVPN01003320.1         TC-FEVTSLRC-DKIIGGTRI---ISFGRSFEEDAMVRDLTINSMSVDVE-
5.2 PFALt BUILD comp7246 c0      IE-FELTSLRQ-DRLENGGKV---ILYGCDFQEDAERRDLTINAMSVNEE-
5.2 HCALt GHXS01045725.1         TC-FEVTSLRC-DTIIGGTRI---ISFGRSFEEDARVRDLTINSMSVDVE-
5.2 HCALt GHXS01098966.1         IE-FELTSLRR-DHLVEGKKV---IEYGCDFKEDATRRDLTINAMSINEV-
5.2 MSPEt sb 567605              HQ-FEMTSLRM-D-TIVDGRK-CKCLYGRSFKEDATRRDLTMNAMSLDVN-
5.2 BOVAg UOYG02000158.1         LS-FEMTSLRR-DEVNAAGEKV--IAYGKSFEEDSERRDLTINAMSIDIE-
5.2 BFORg WFMF01000010.1         QN-FEVTSTRR-D-ELIEGRK-C-IKYGKSFTDDASRRDLTINAMSIDLD-
5.2 BSPEt comp8218 c0 seql       ---------RA-------------IKYGRNFEEDASRRDLTVNSMSIDLN-
5.2 MLEIp ML011713a              AL-FEINSLRA-DDMLNGEKI-SR--YGVSFKEDARRRDFTMNAMSLDVE-
5.2 MLEIp ML011715a              YV-FEMTSLRE-D-TVIDGEKICK--FGVNFEEDARRRDLTVNSMSLDVE-
5.2 MLEIt BUILD comp31840 c0     HN-FEMTSLRE-DILERGGKV-CR--YGRSFRDDANRRDLTINAMSLDEE-
5.2 BASHt comp18520 c0 seq2      FV-FETTSLRELDVLVEGEKV-CK--FGTSFVEDARRRDLTVNAMSLDVE-
5.2 BASHt comp18520 c0 seq6      FM-FEMNSLRK-DFMVAGERN-SQ--YGVSFIEDANRRDFTMNAMSLDVE-
5.2 BASHt comp18520 c0 seq7      FV-FETTSLRELDVLVEGEKV-CK--FGTSFVEDARRRDLTVNAMSLDVE-
5.2 BASHt comp18520 c0 seq4      FM-FEMNSLRK-DFMVAGERN-SQ--YGVSFIEDANRRDFTMNAMSLDVE-
5.2 BINFt comp26349 c0 seql      HQ-FEMTSLRM-D-TIVDGRK-CKCLYGRSFKEDATRRDLTMNAMSLDVN-
5.2 BINFt comp64708 c0 seql      FQ-FEMTSLRV-DYMDKGERV-CR--YGTDFKEDANRRDLTINAMSLDEE-
```

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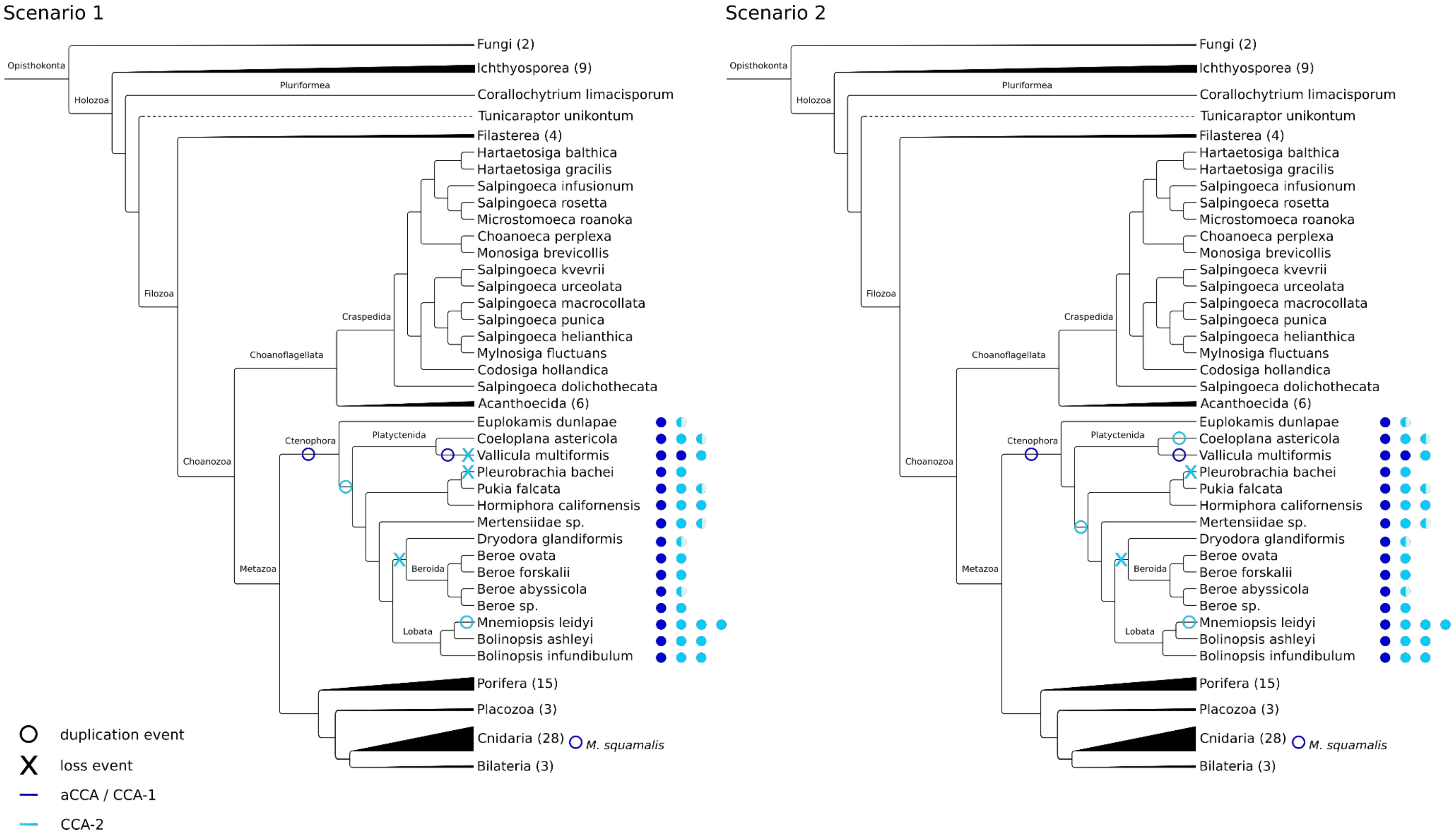
Scenario 1

Scenario 2