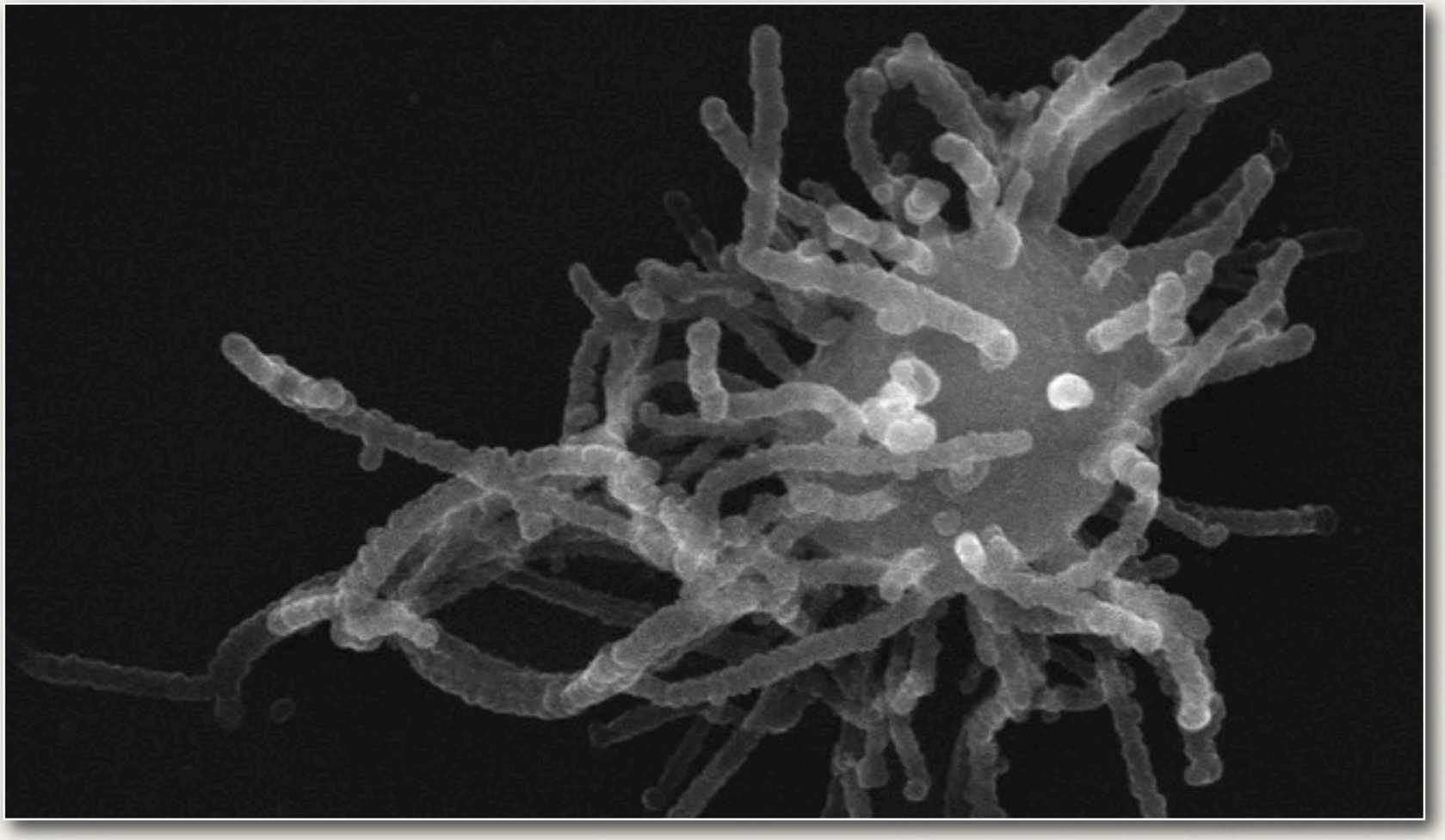
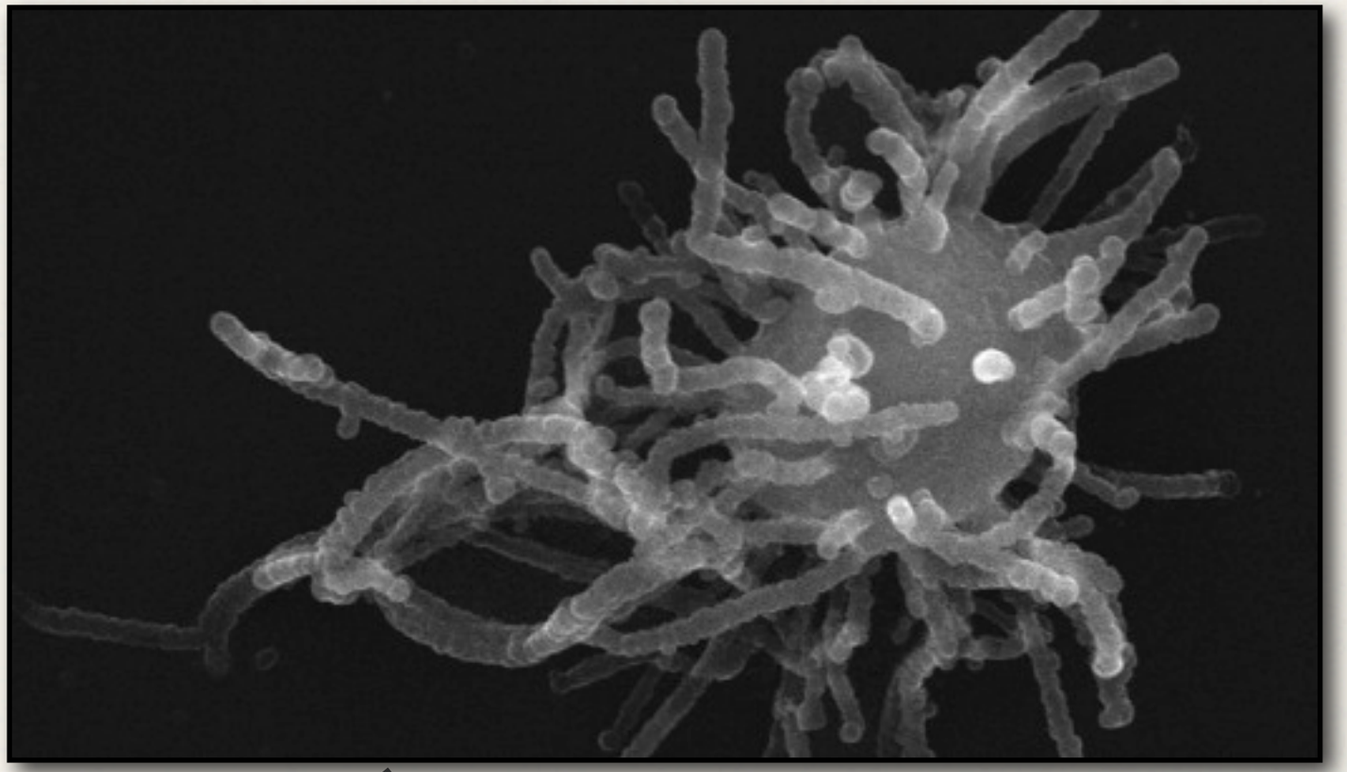


The origin of metazoan multicellularity: what comparative genomics is telling us and how we could go forward

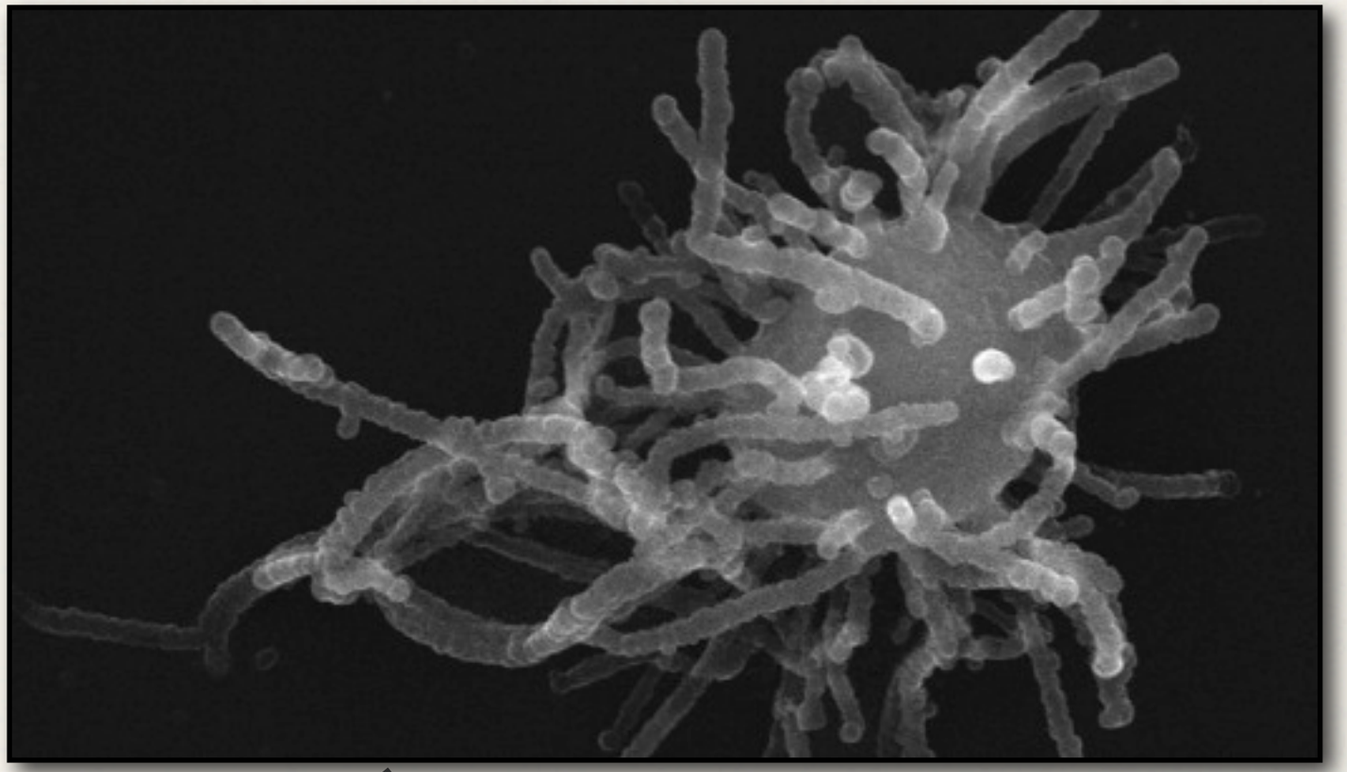
Iñaki Ruiz-Trillo, Institute of Evolutionary Biology & Universitat de Barcelona & Kitp

Cooperation And The Evolution Of Multicellularity, Kitp, 21 February 2013

Institut de Biologia Evolutiva (UPF-CSIC)



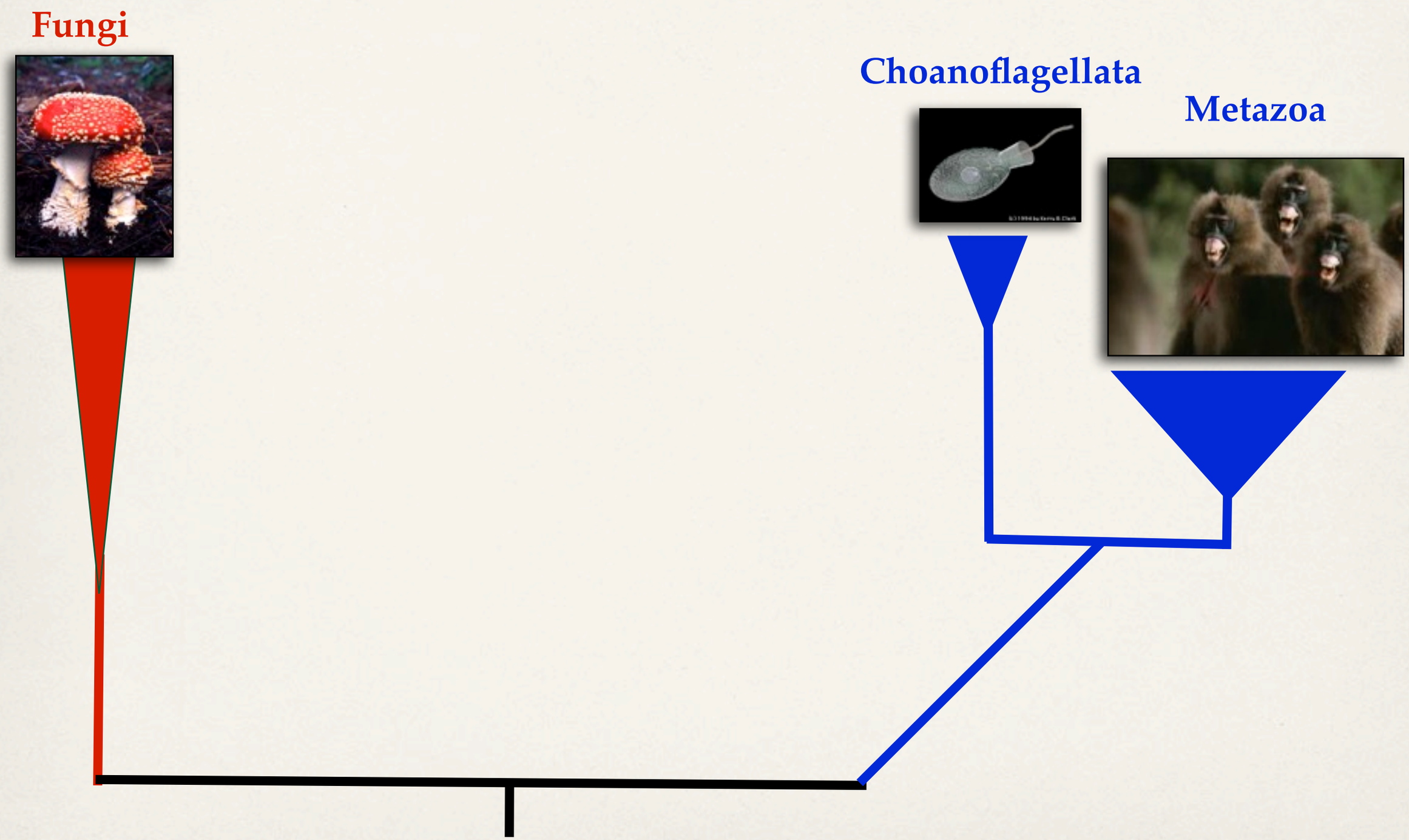
last common unicellular ancestor



last common unicellular ancestor

1. A phylogenetic framework

who are the closest living relatives of animals?



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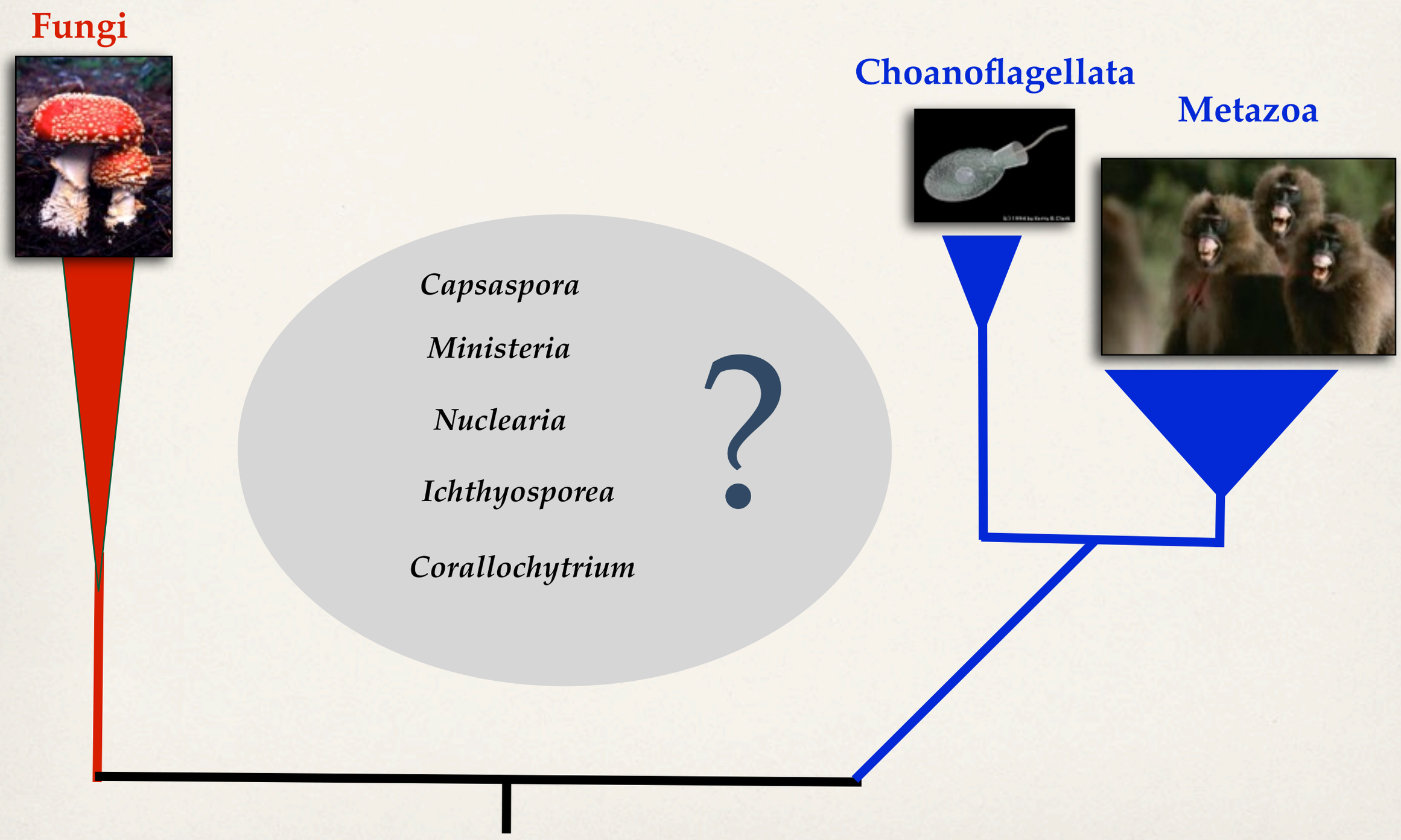
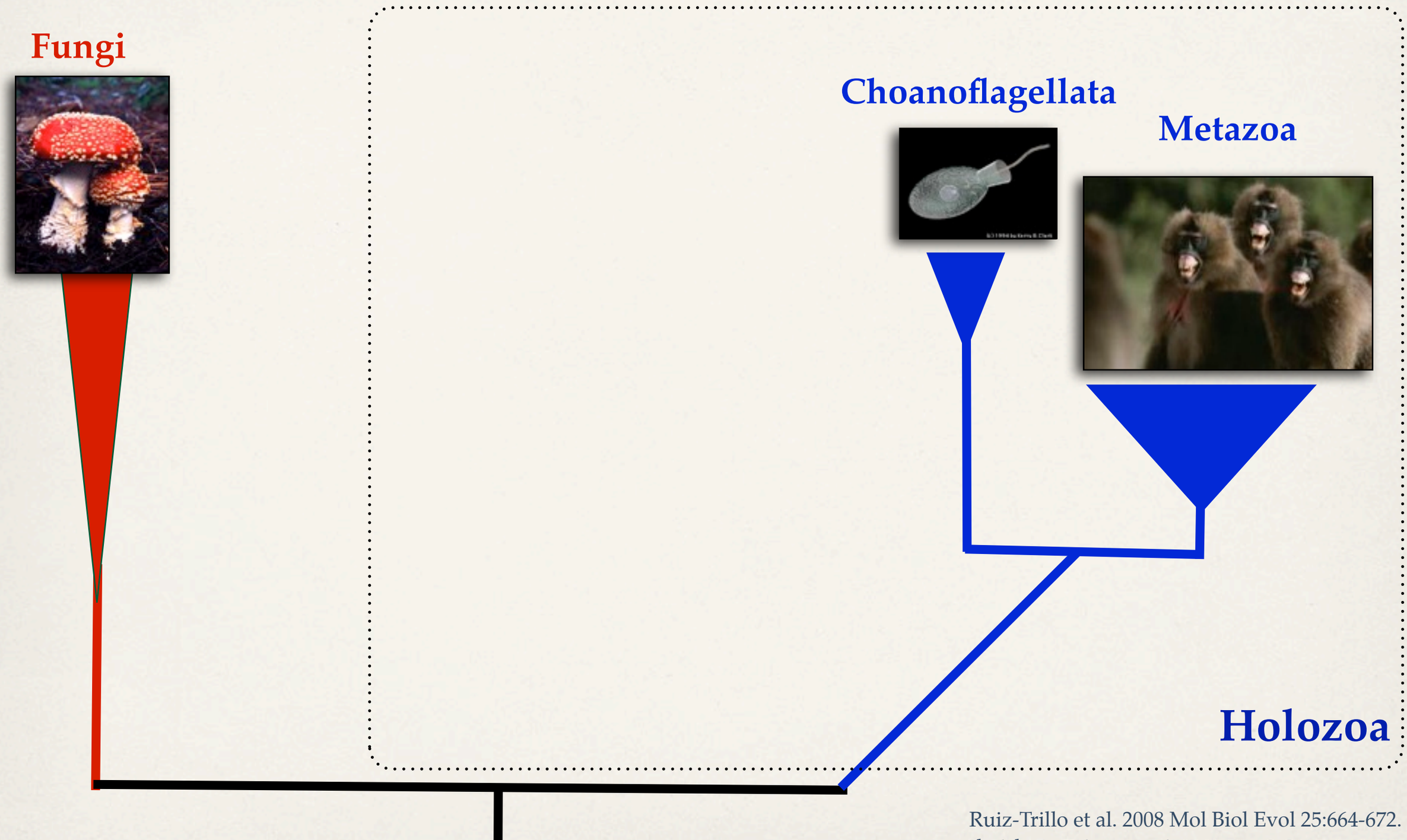


A new phylogenetic framework



Ruiz-Trillo et al. 2008 Mol Biol Evol 25:664-672.
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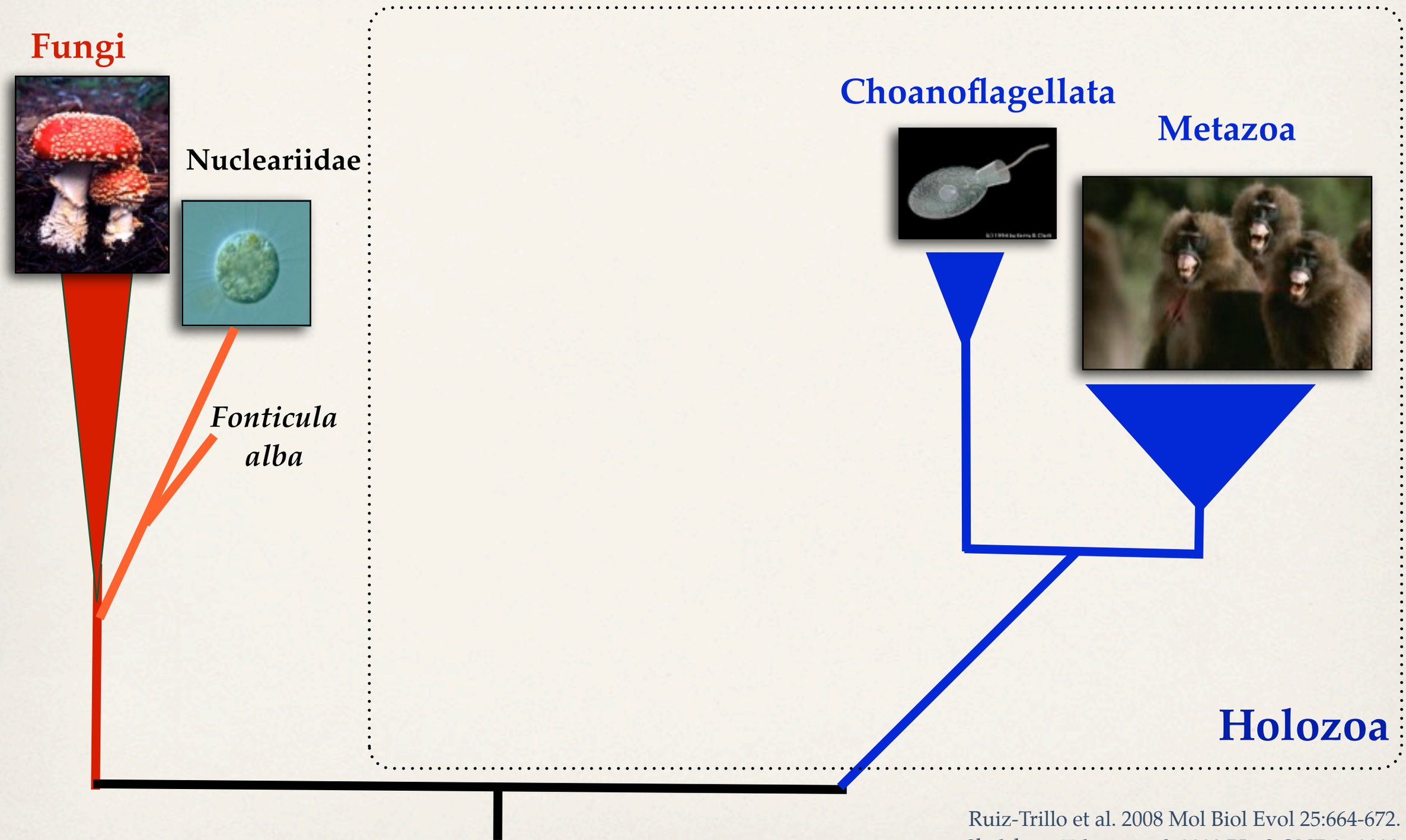
A new phylogenetic framework



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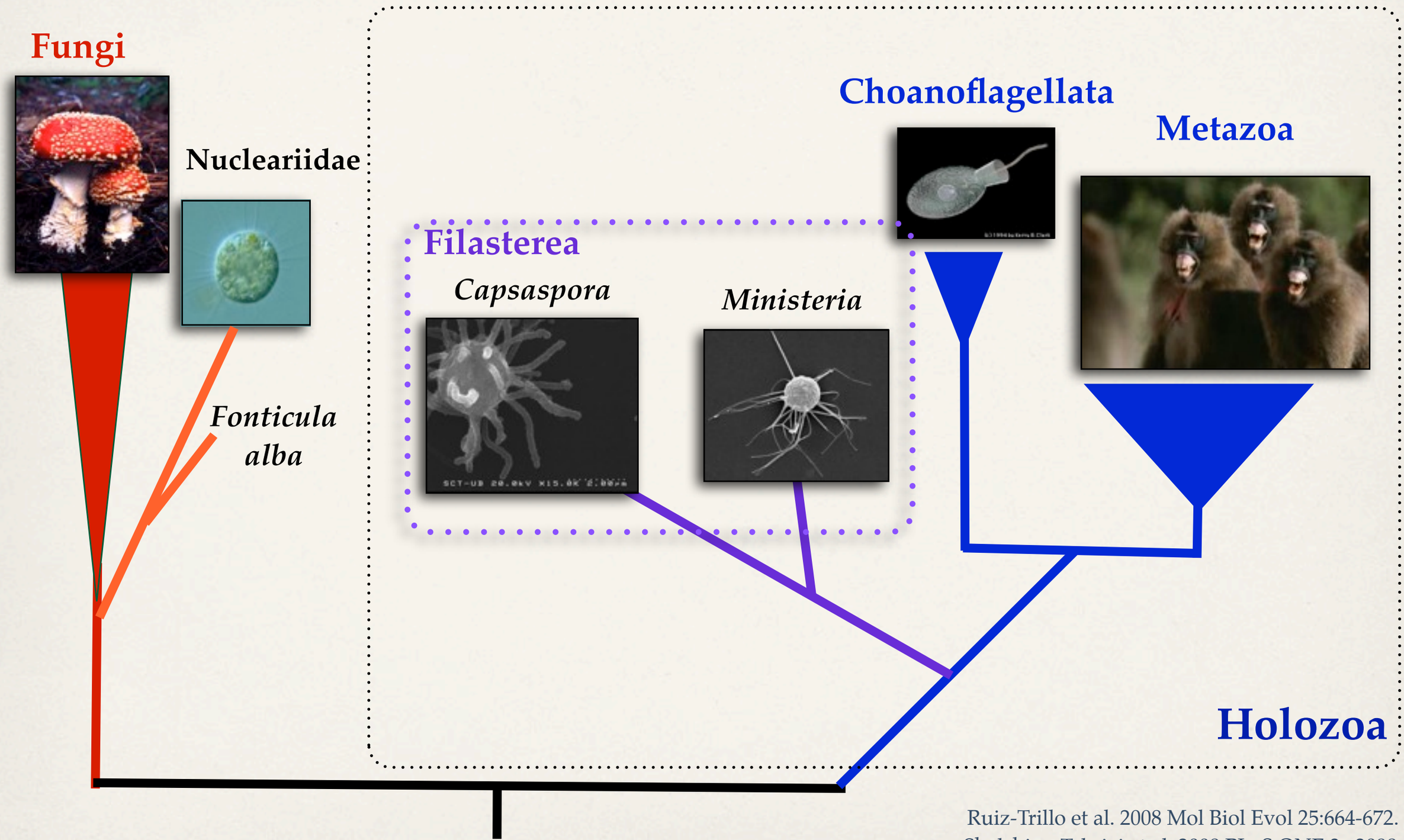

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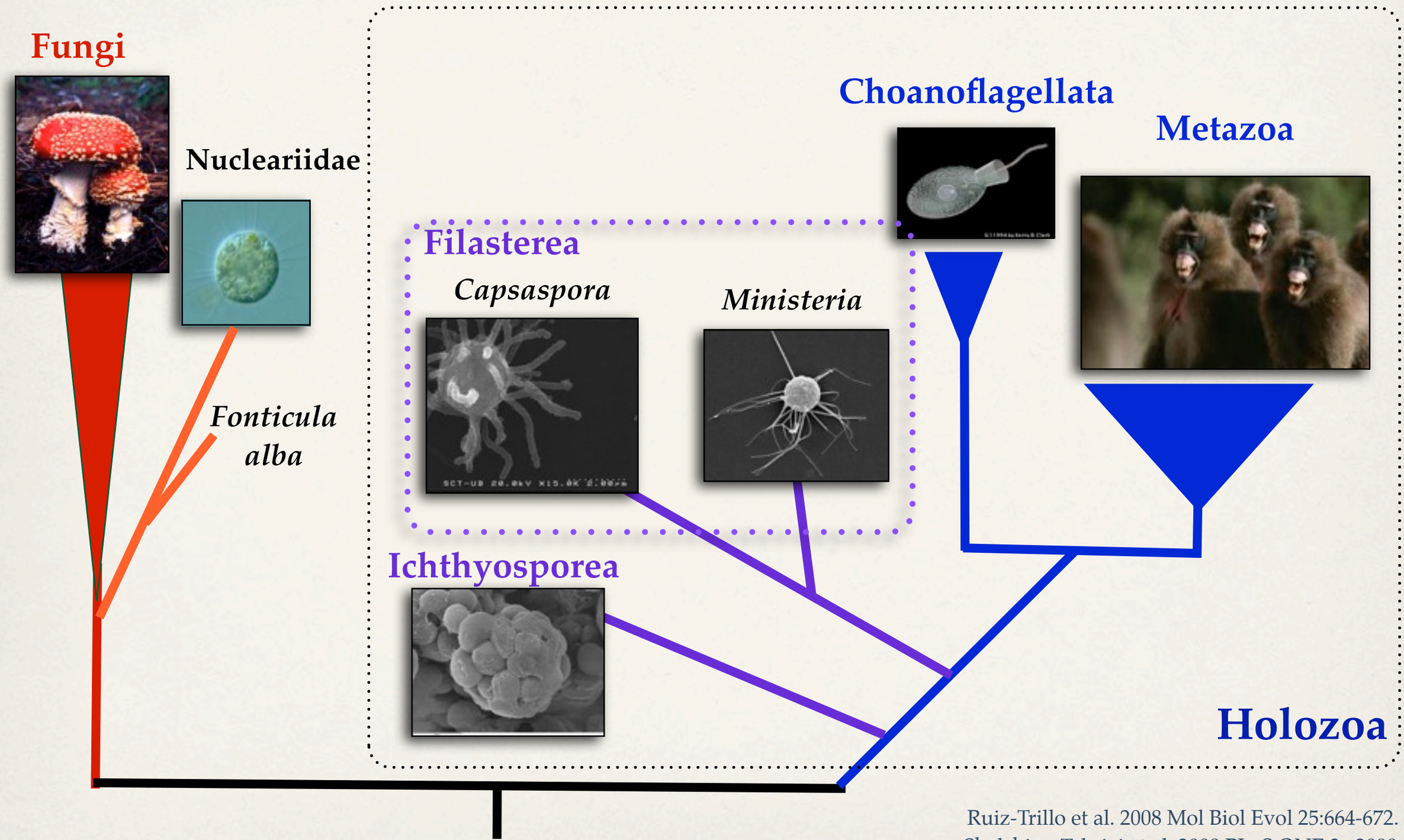

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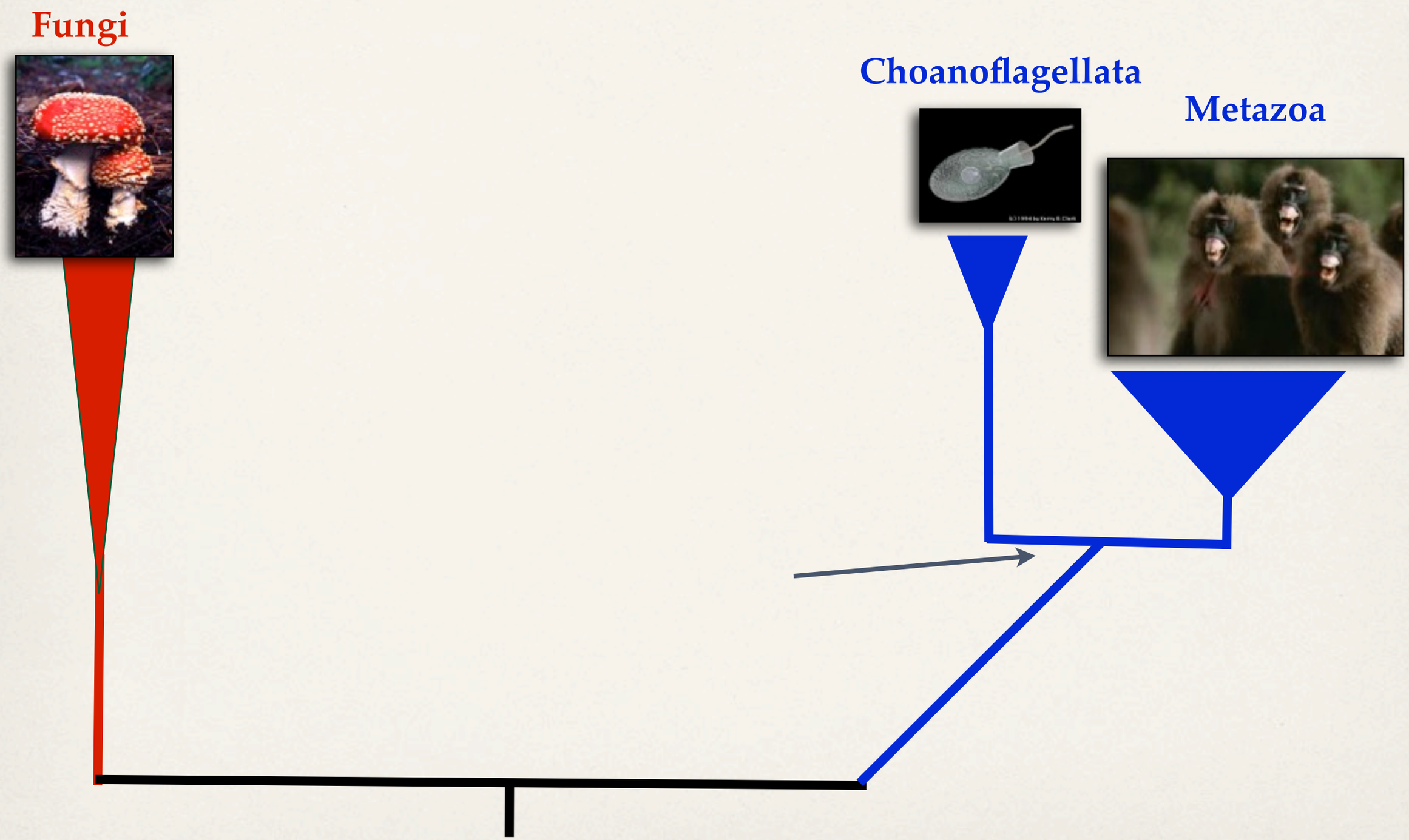
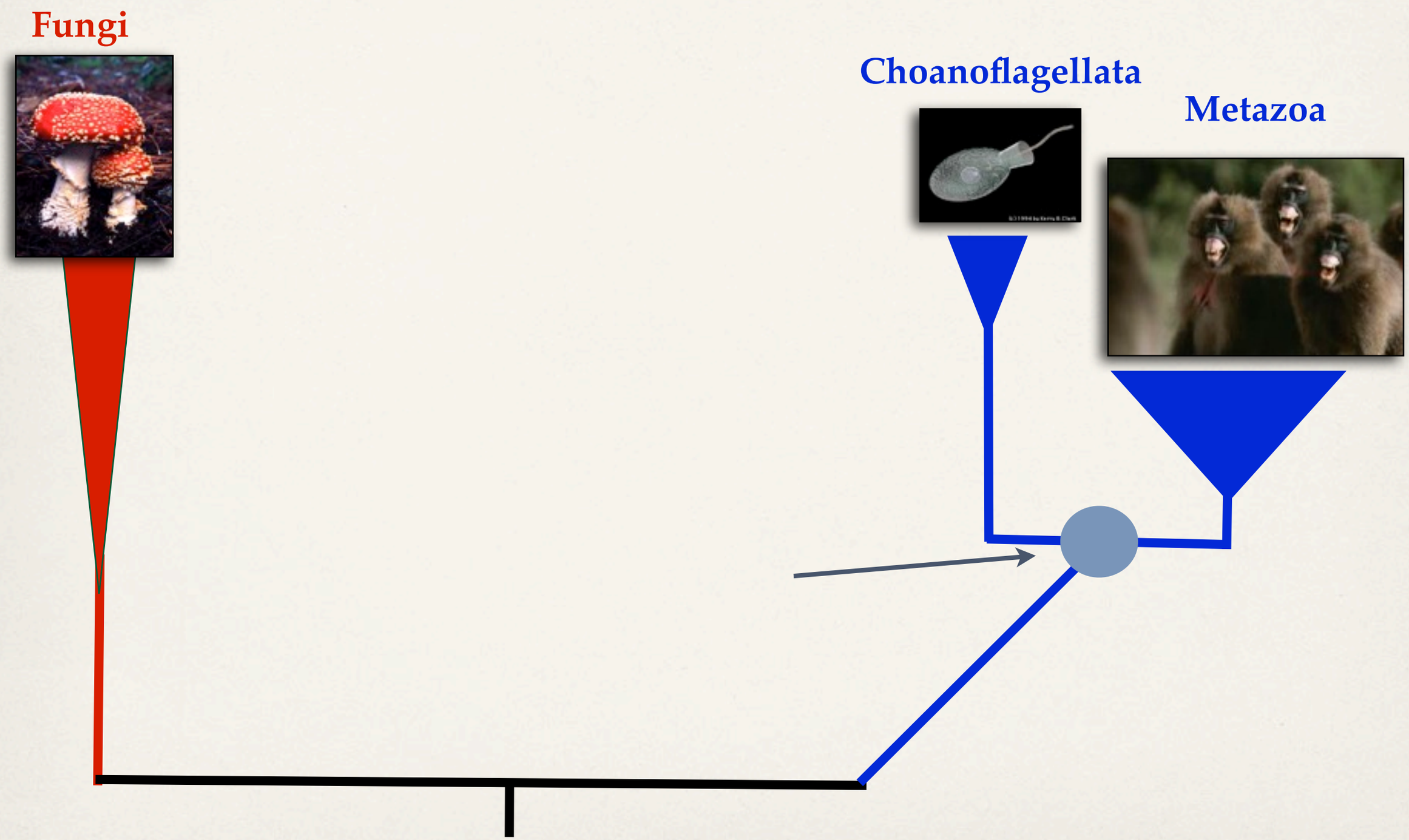
2. Genome data

M. brevicollis genome



King et al. 2008 Nature 451:783-788.

M. brevicollis genome



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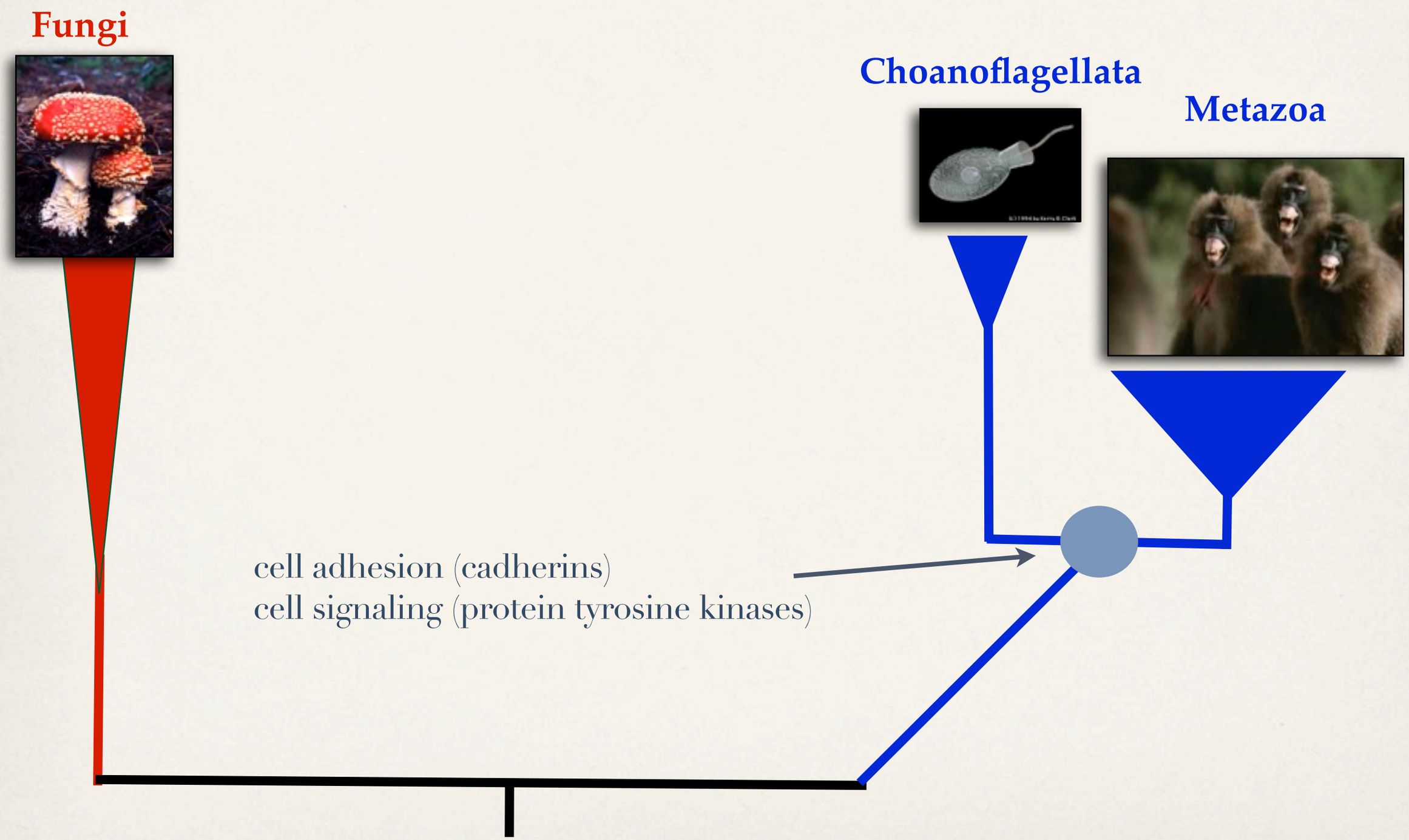

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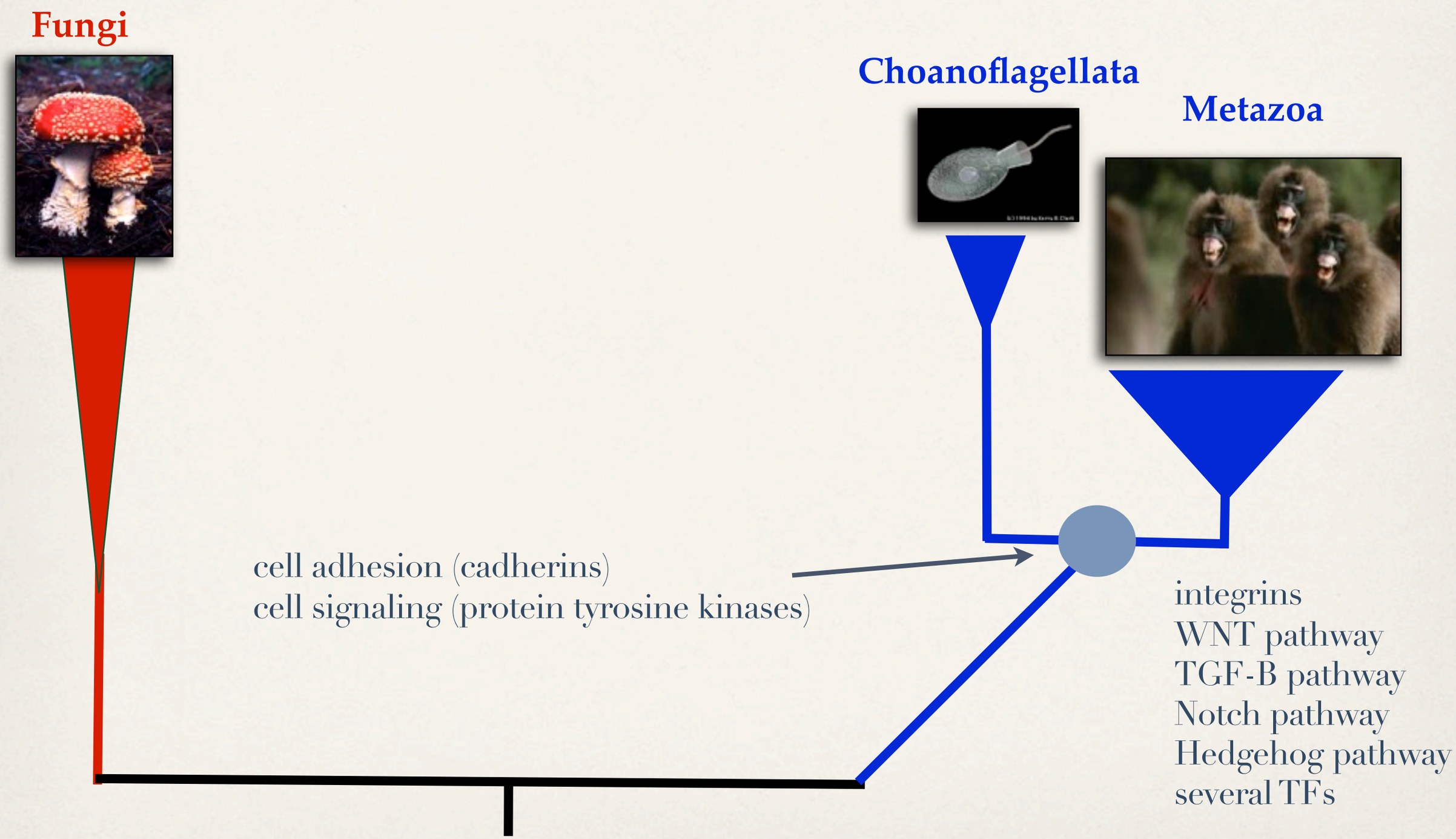
M. brevicollis genome



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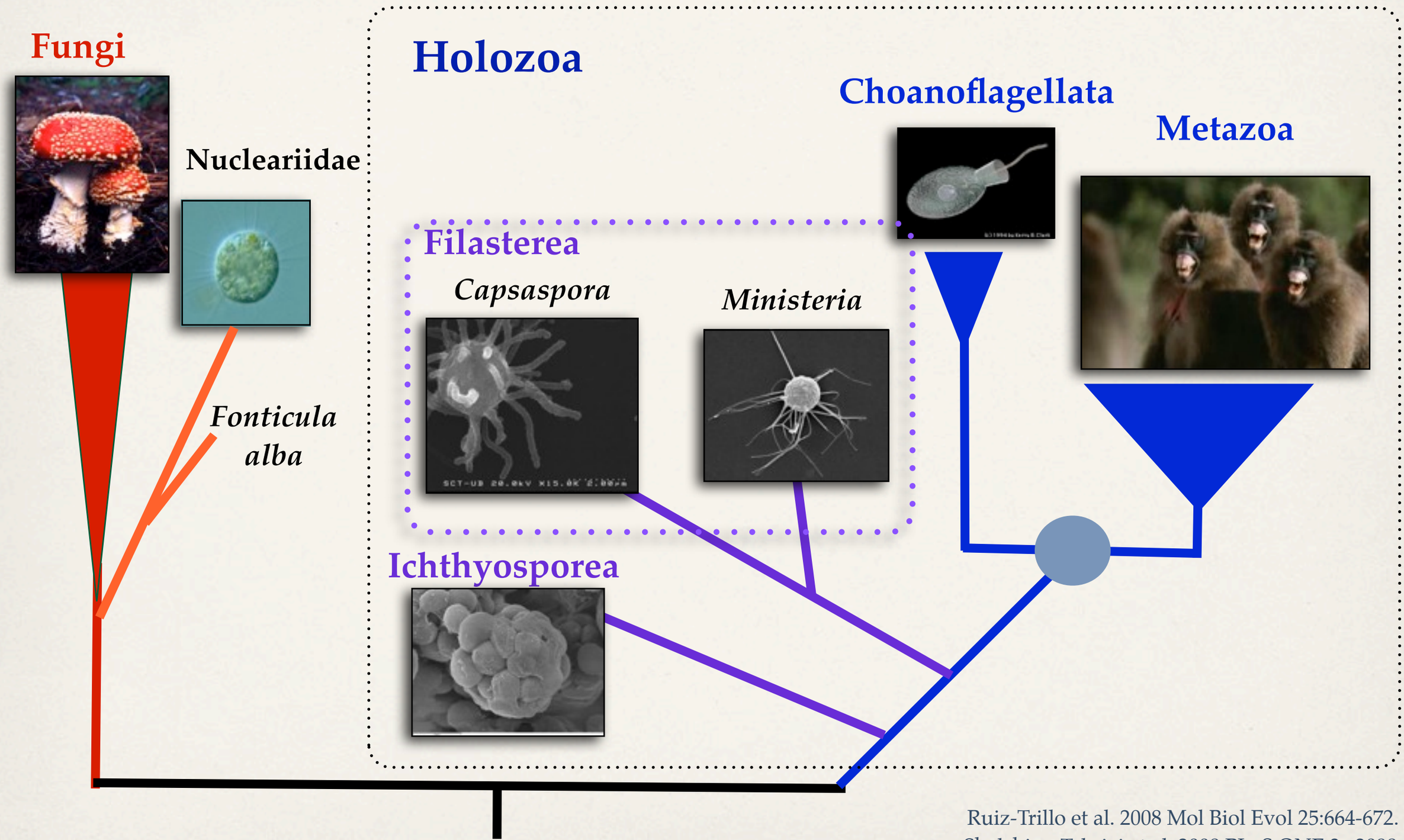
Unicellular relatives of animals: not a game of one



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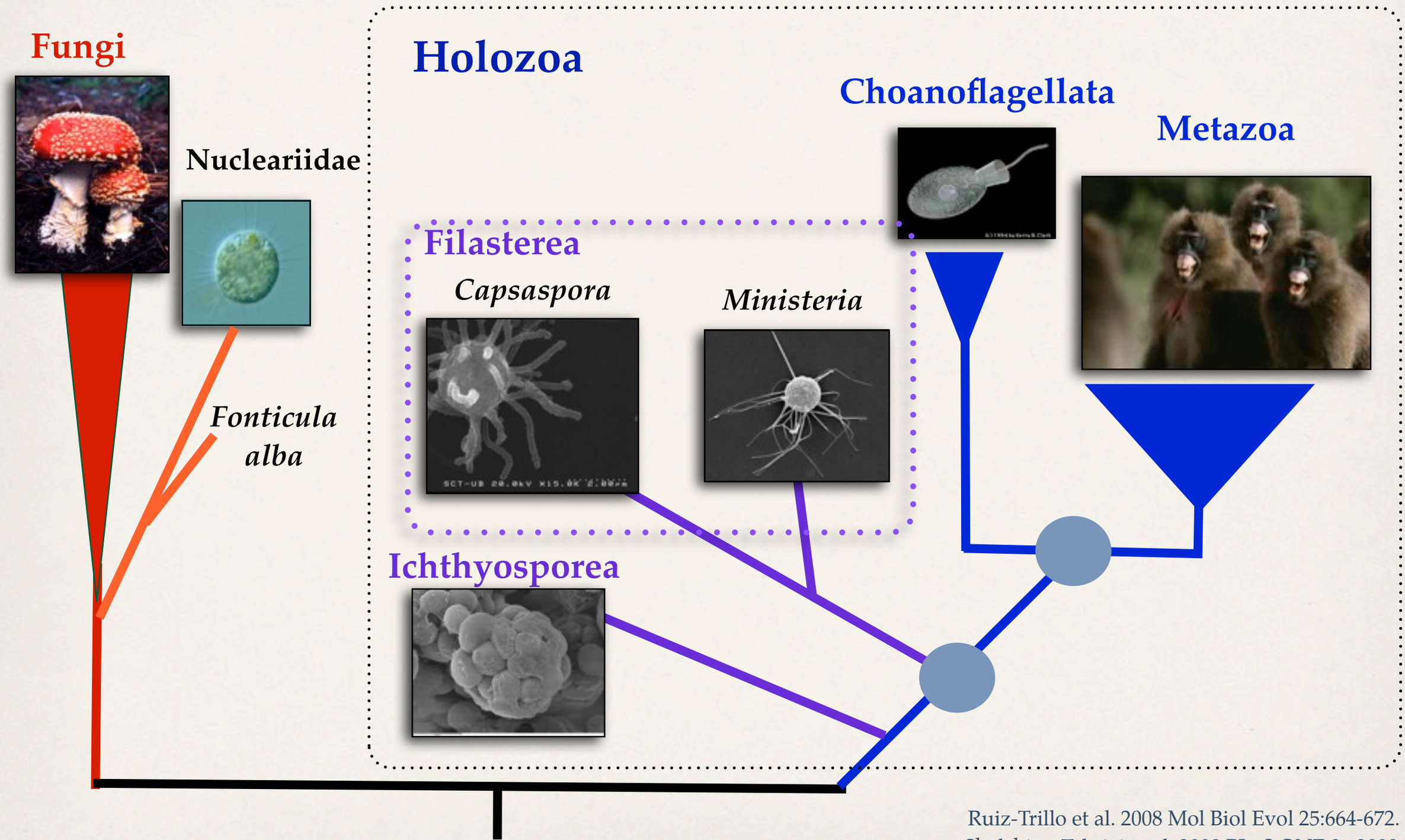

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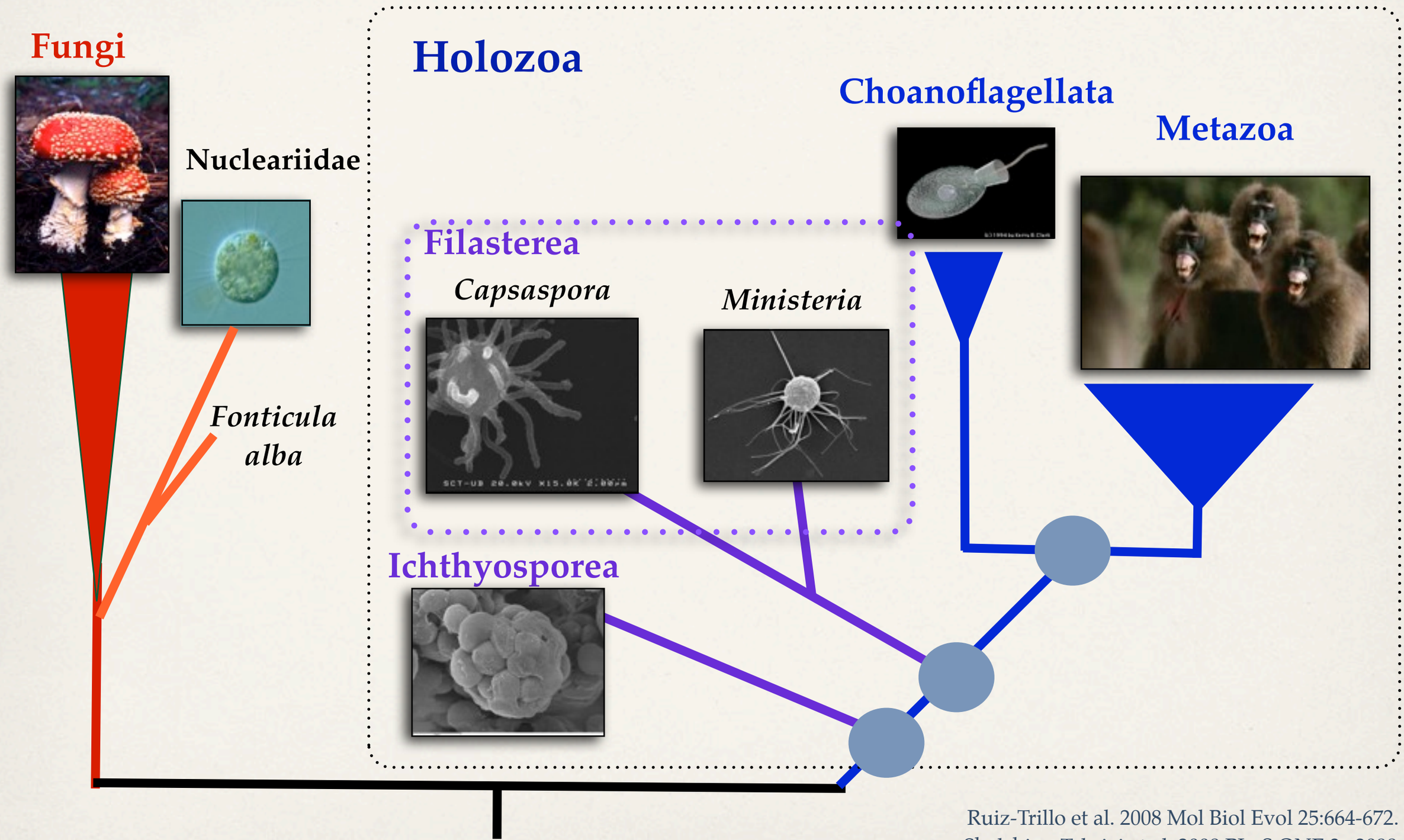

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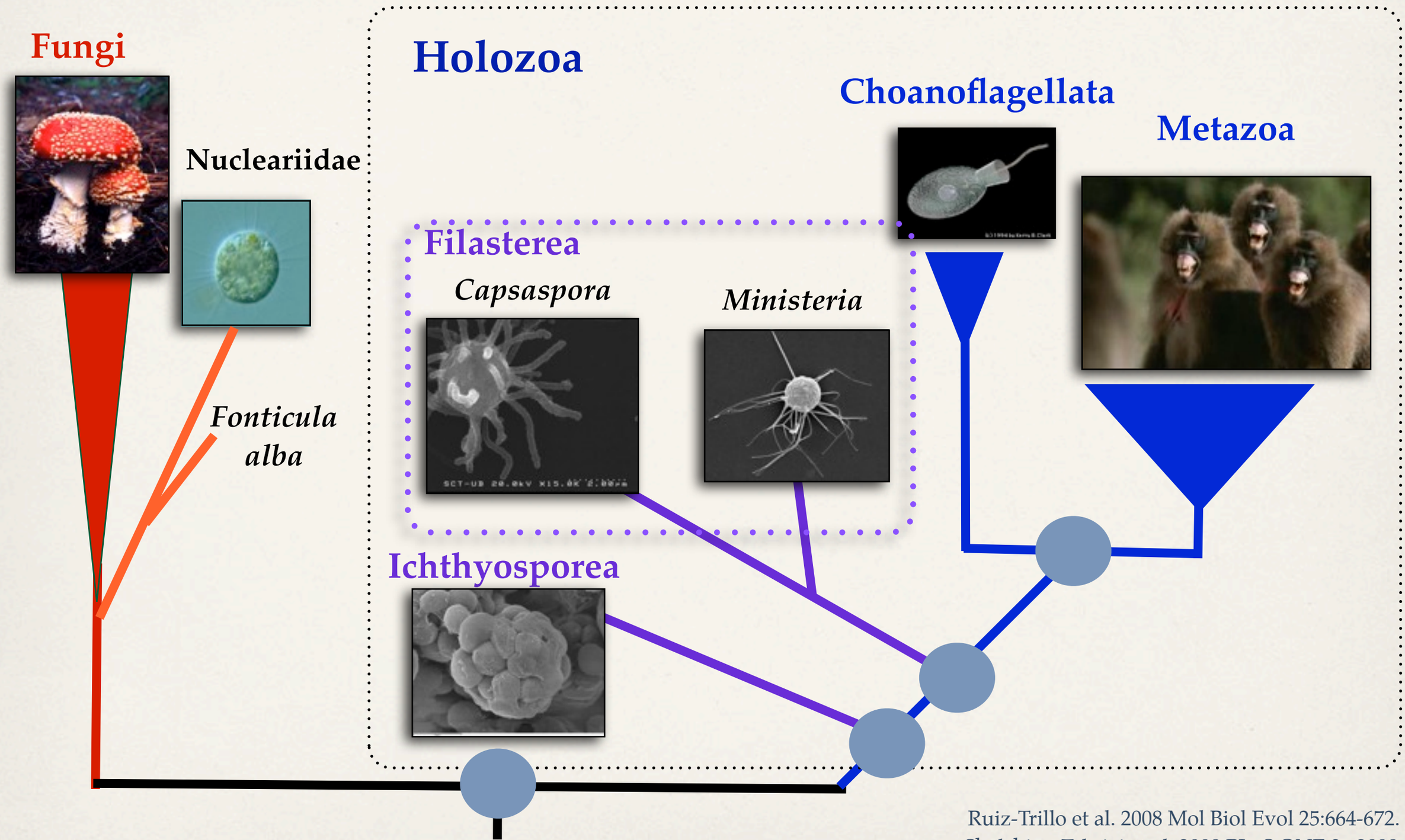

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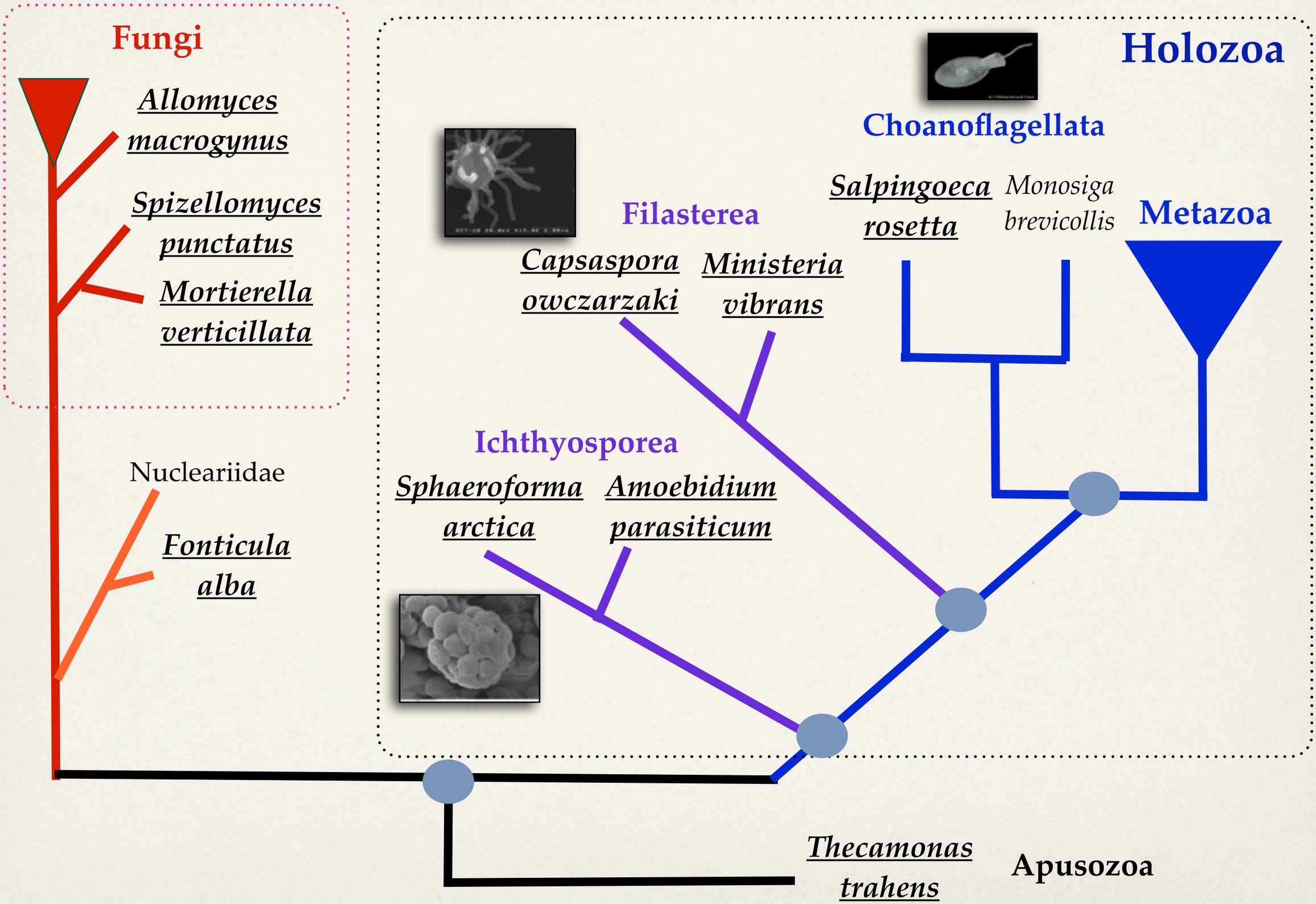
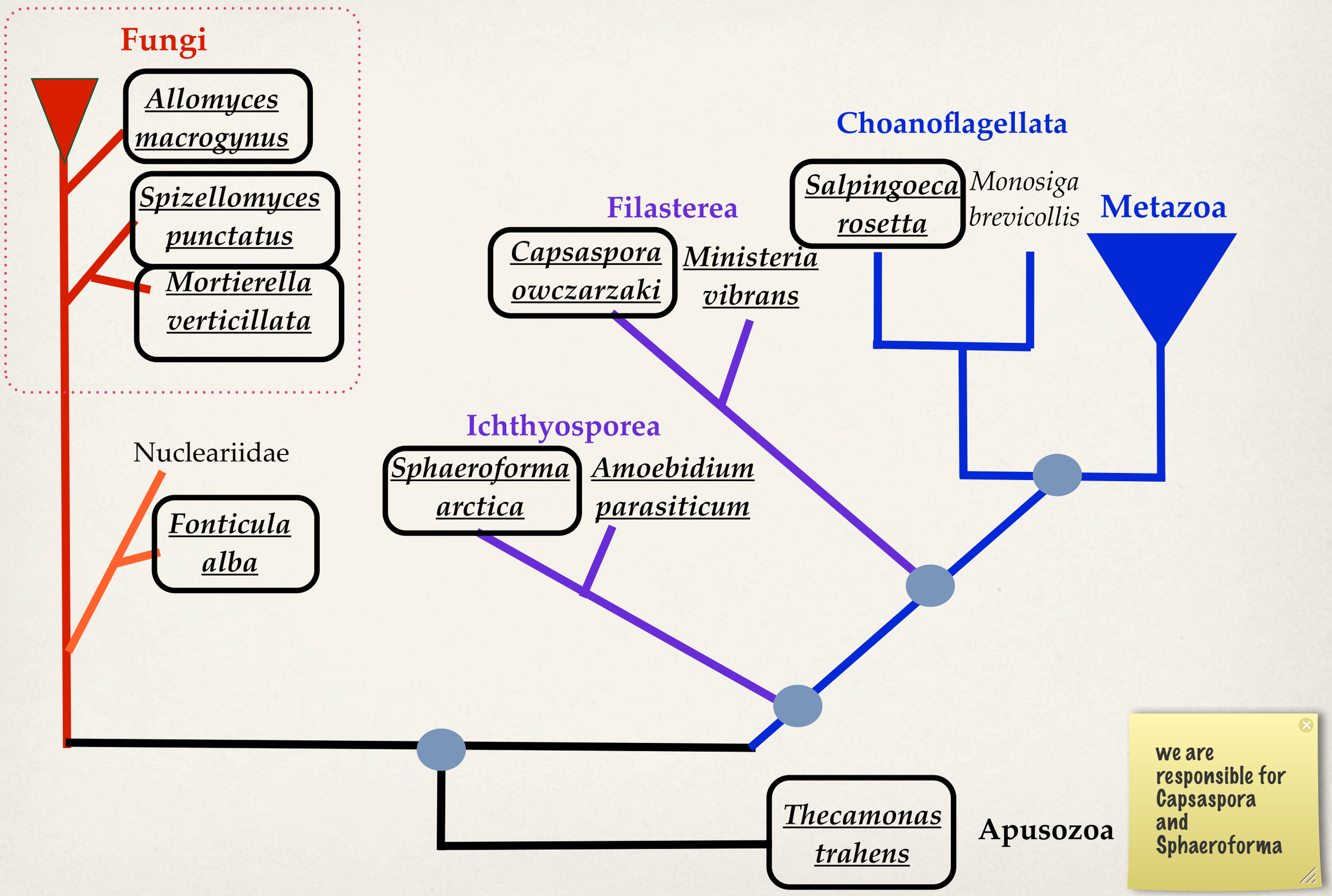
UNICORN project



Ruiz-Trillo et al. 2007. Trends in Genetics 23:113-118.

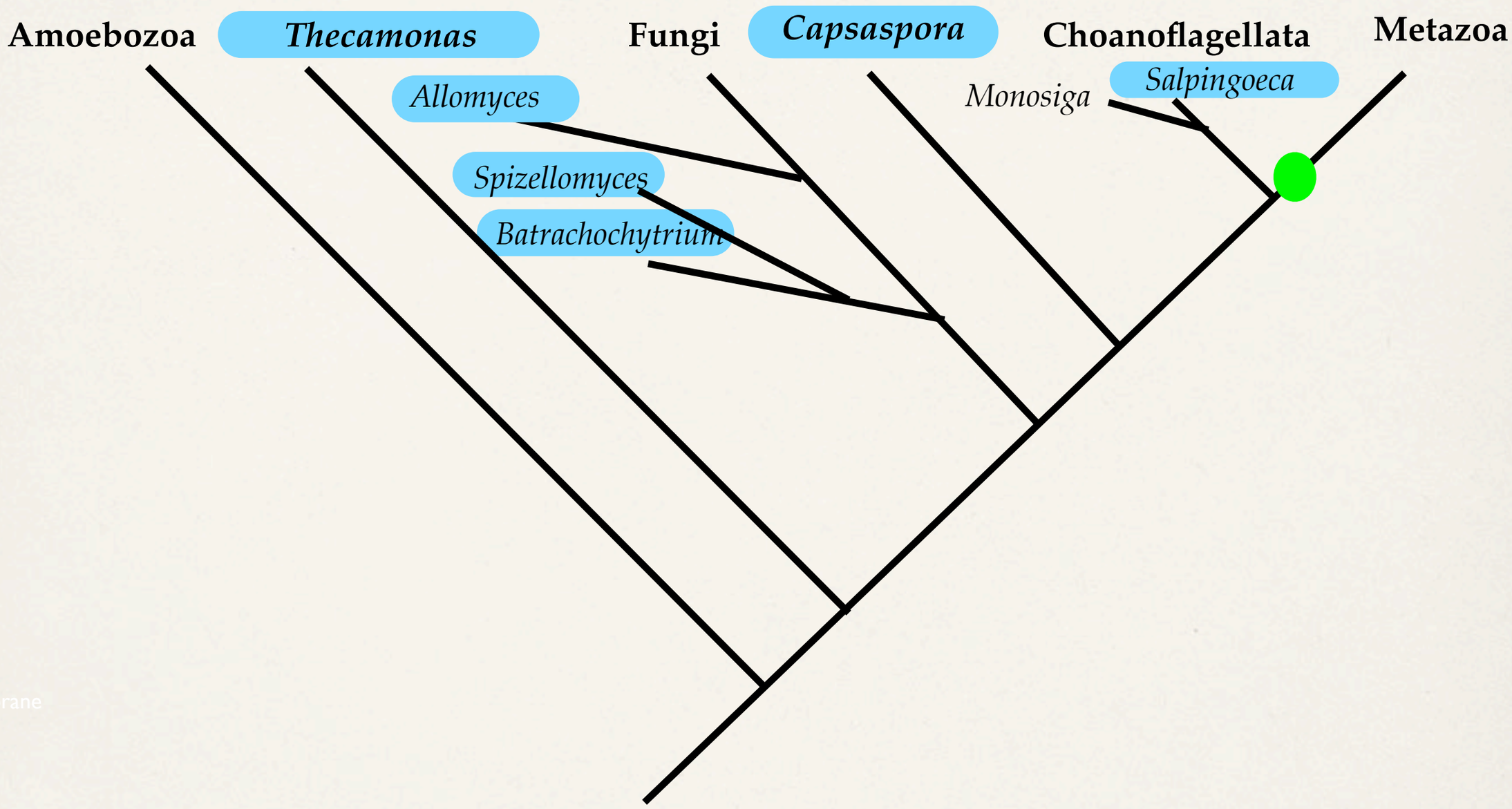
UNICORN: sequenced genomes (August 2012)



we are responsible for Capsaspora and Sphaeroforma

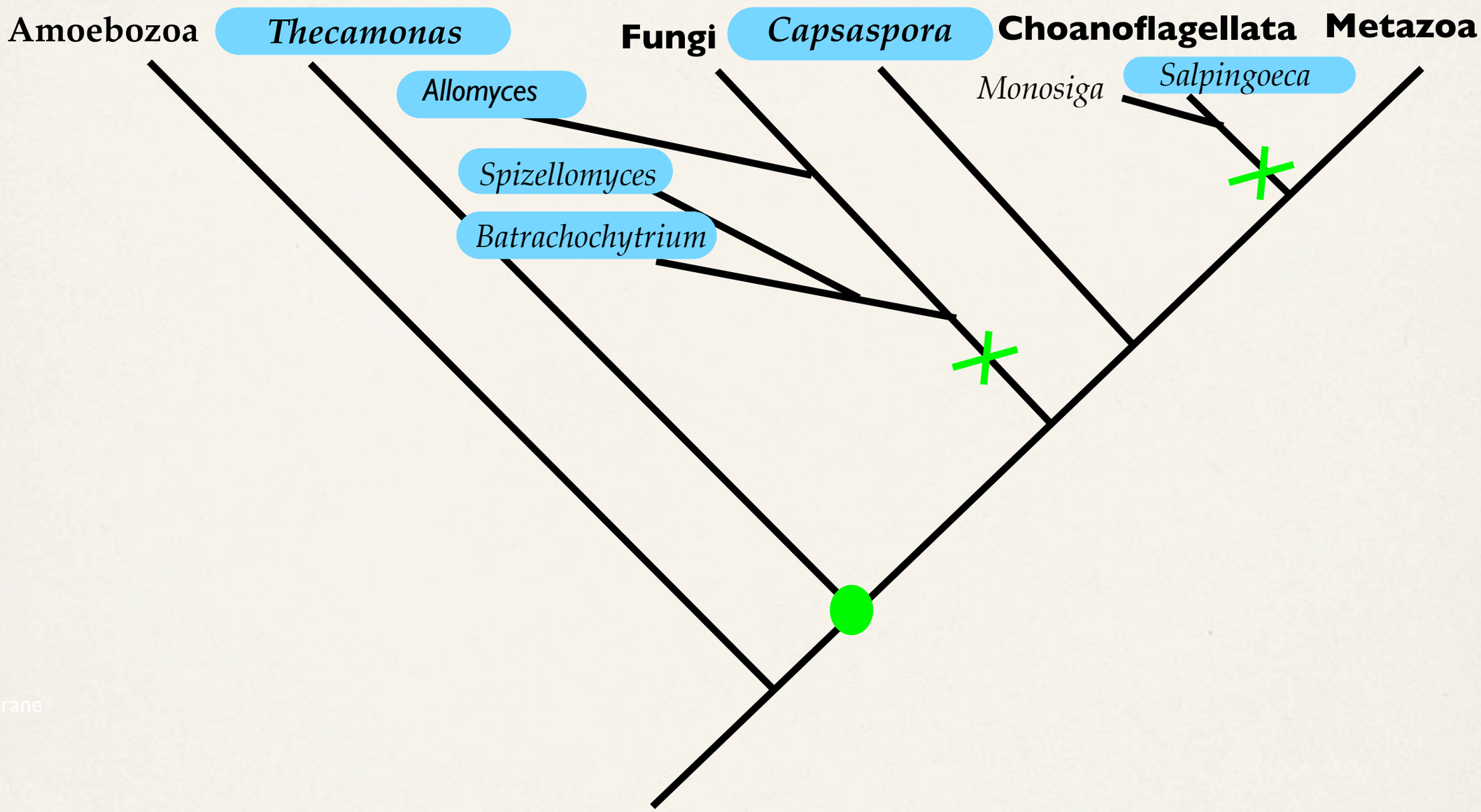
Ruiz-Trillo et al. 2007. Trends in Genetics 23:113-118.

Integrin-mediated adhesion system: pre-UNICORN view



membrane

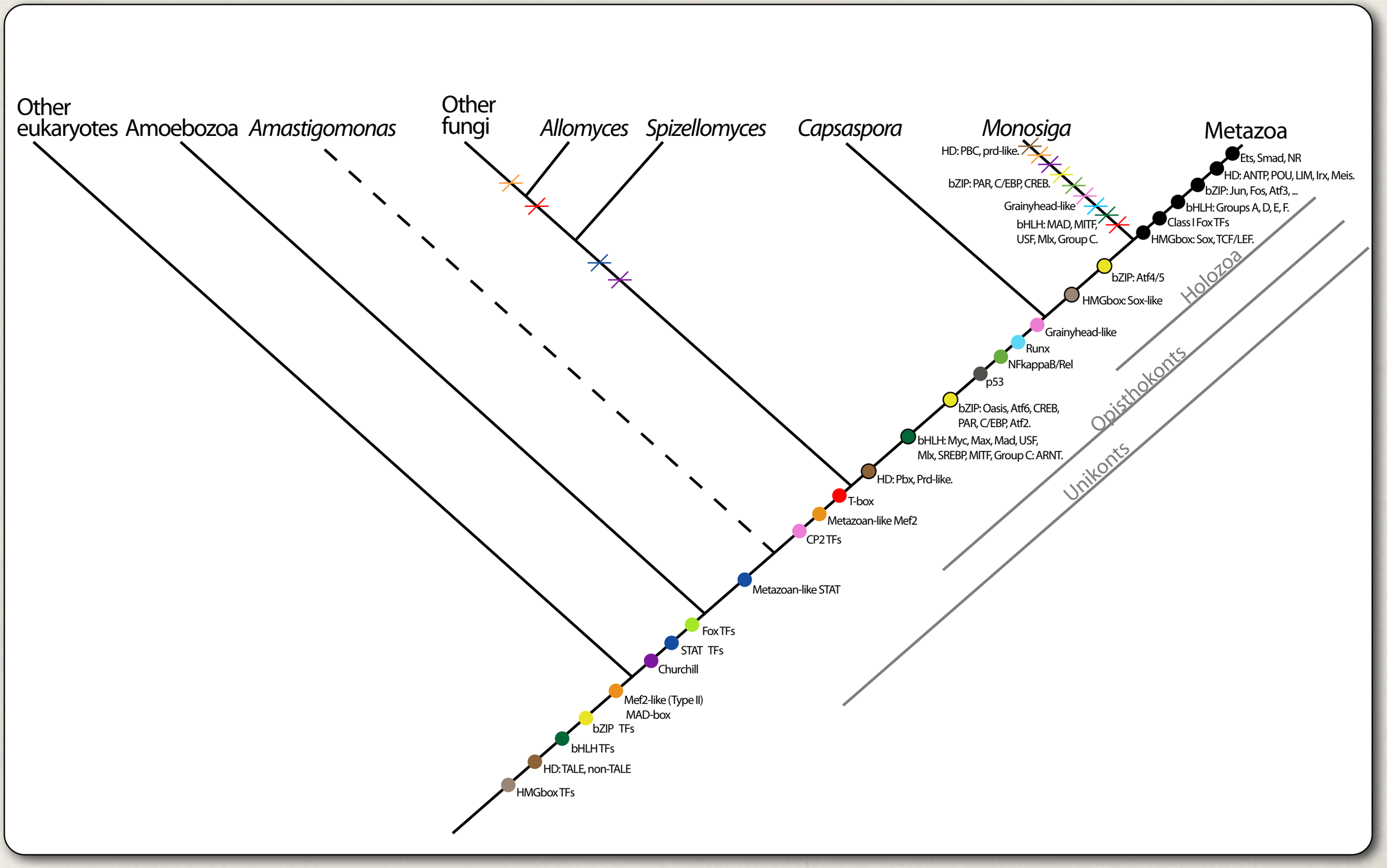
Integrin-mediated adhesion system: post-UNICORN view



membrane

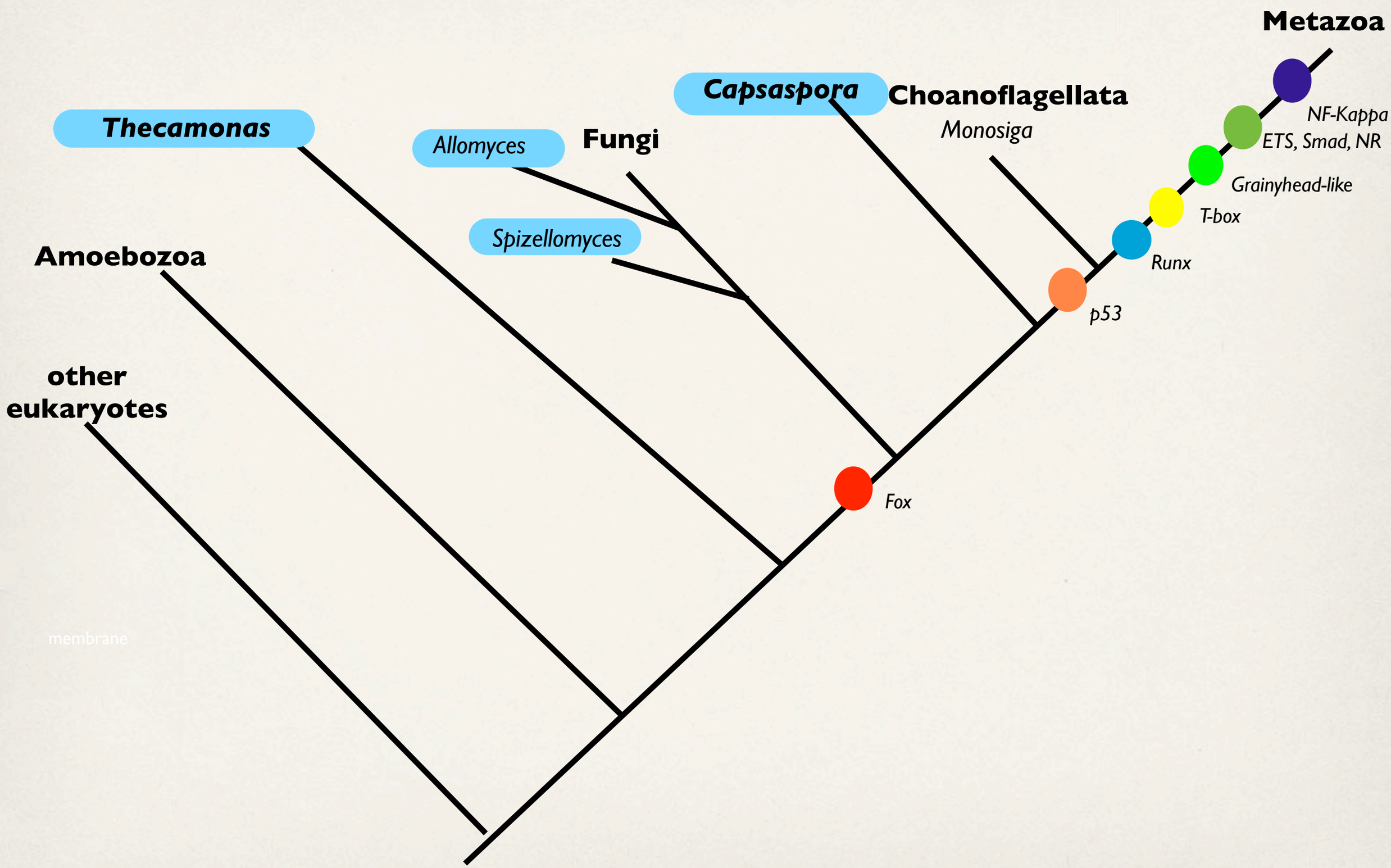
Sebé-Pedrós et al. 2010. PNAS 107:10142-10147.

Metazoan Transcription Factors (TFs)

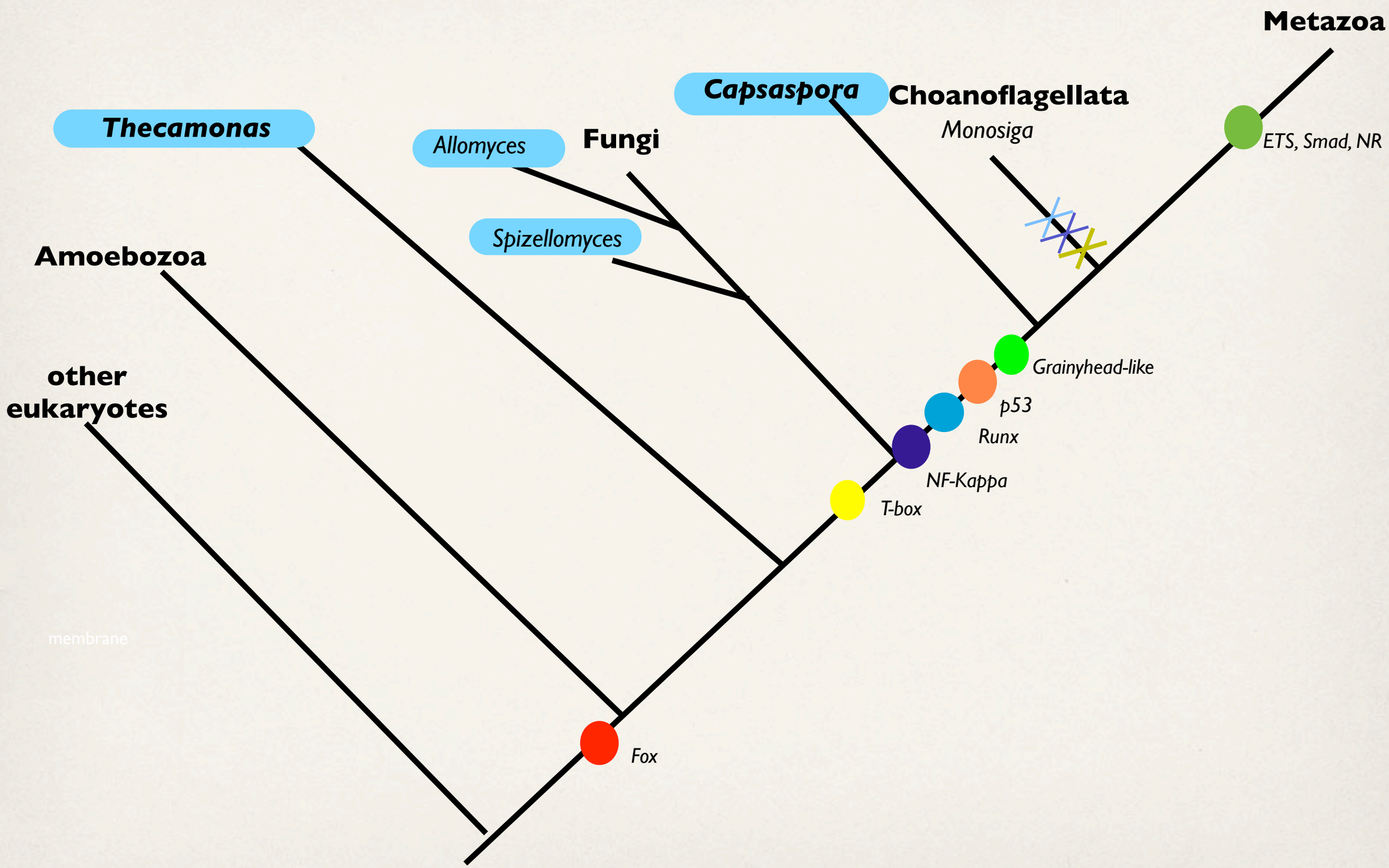


Sebé-Pedrós et al. 2011. Mol. Biol. Evol. 28:1241-54

Metazoan Transcription Factors: pre-UNICORN view

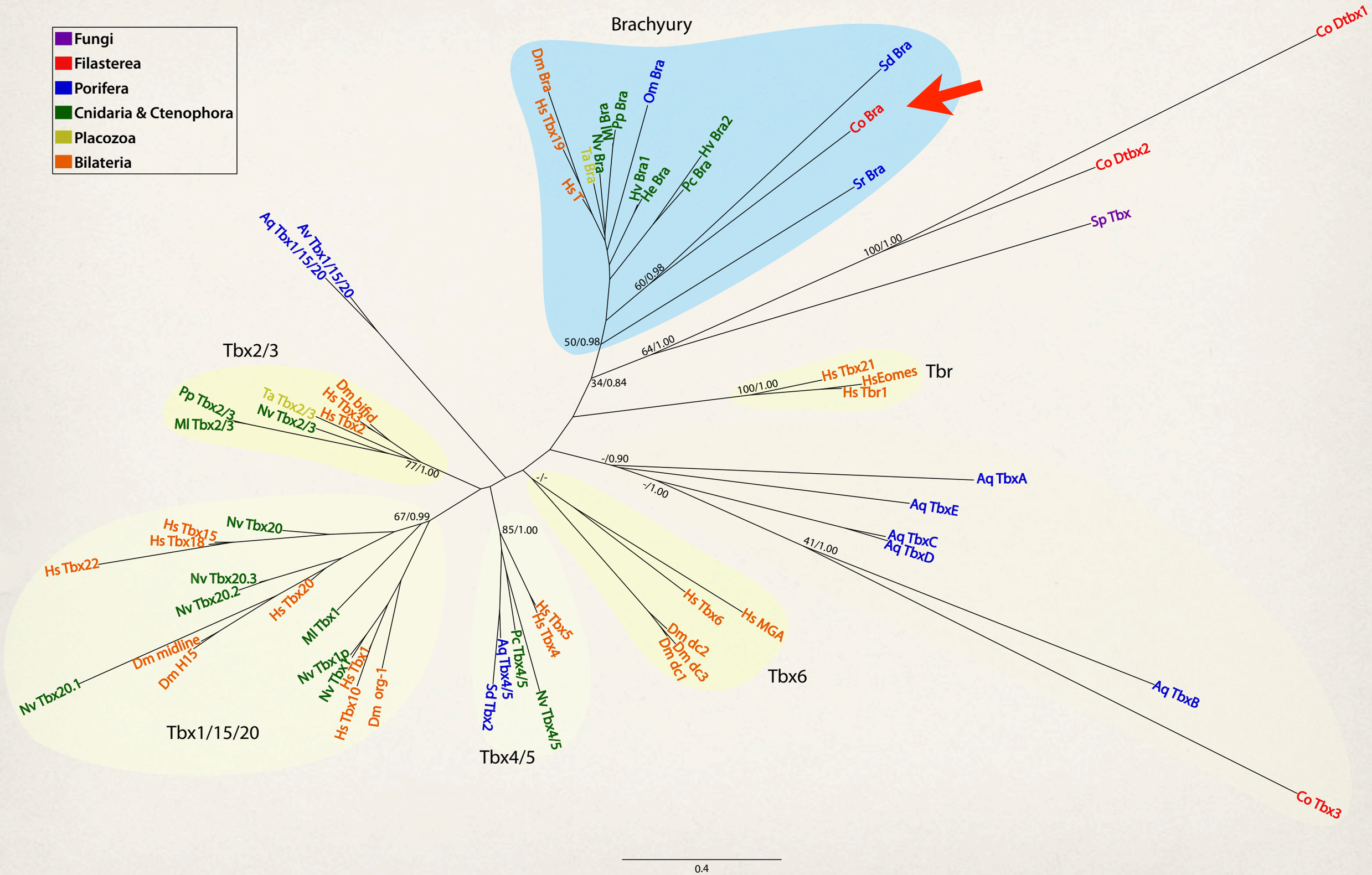


Metazoan Transcription Factors: post-UNICORN view



Sebé-Pedrós et al. 2011. Mol. Biol. Evol. 28:1241-54

Capsaspora has a *Brachyury gen*



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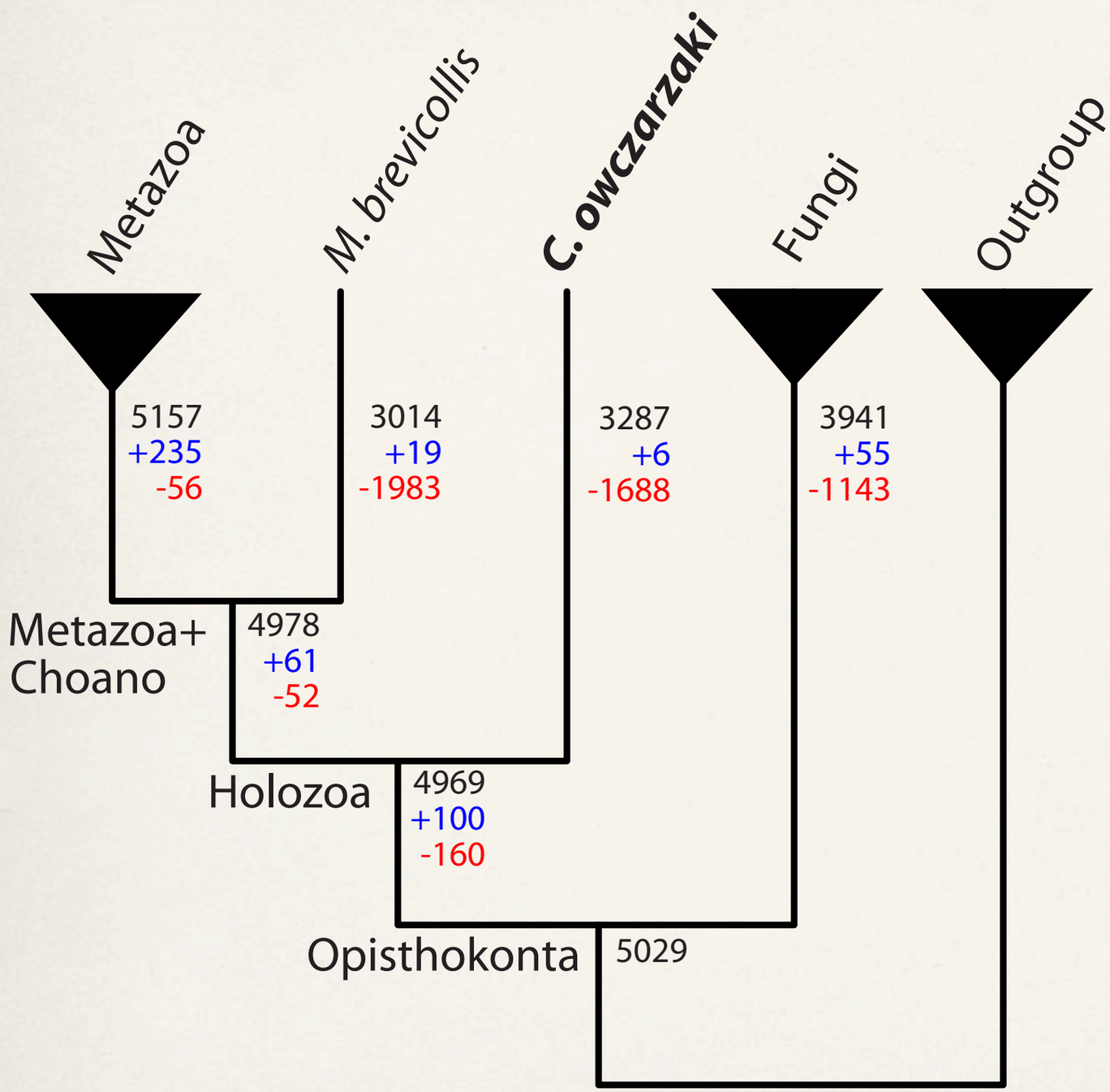
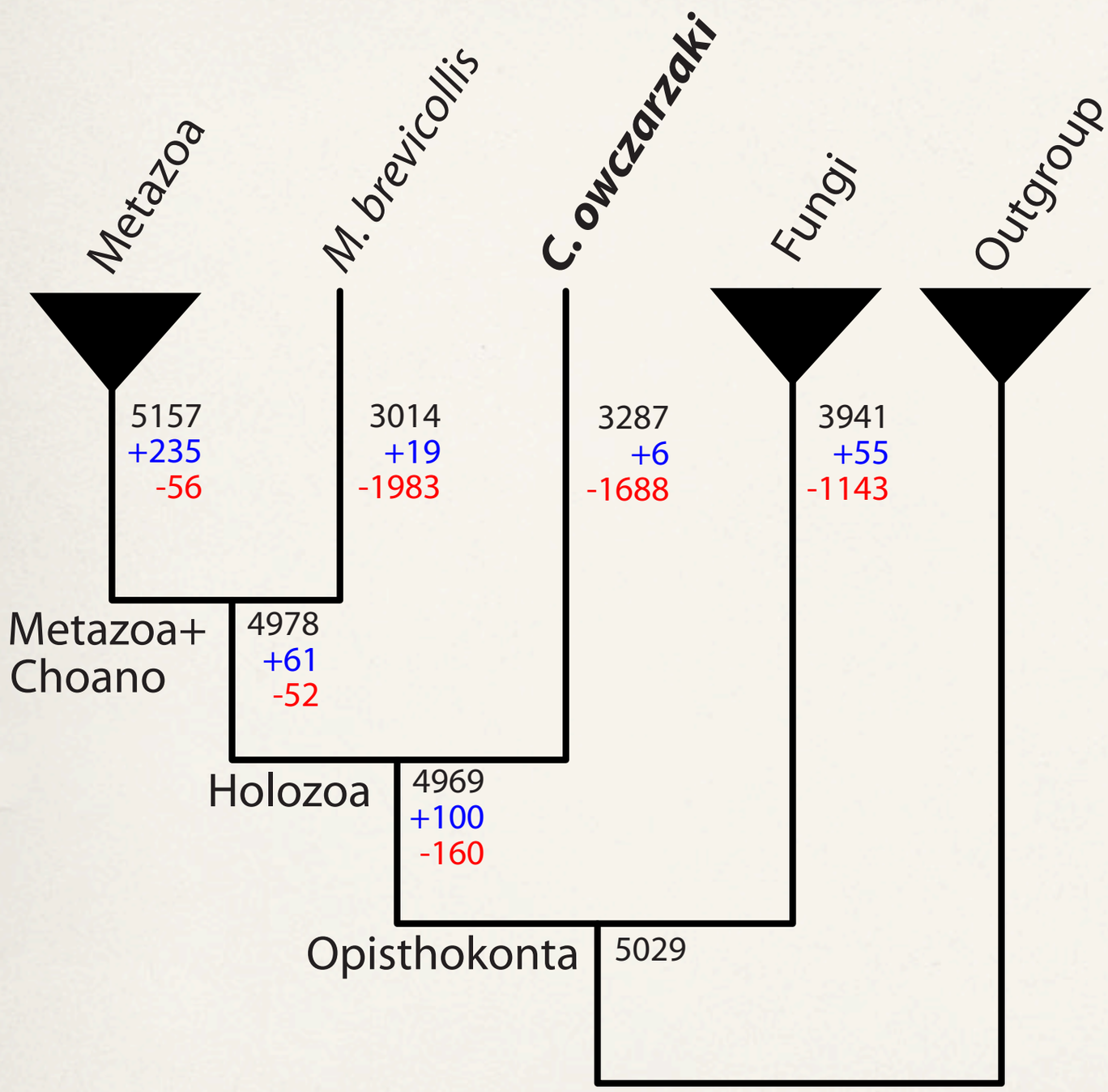
PFAM gain/loss



PFAM gain/loss



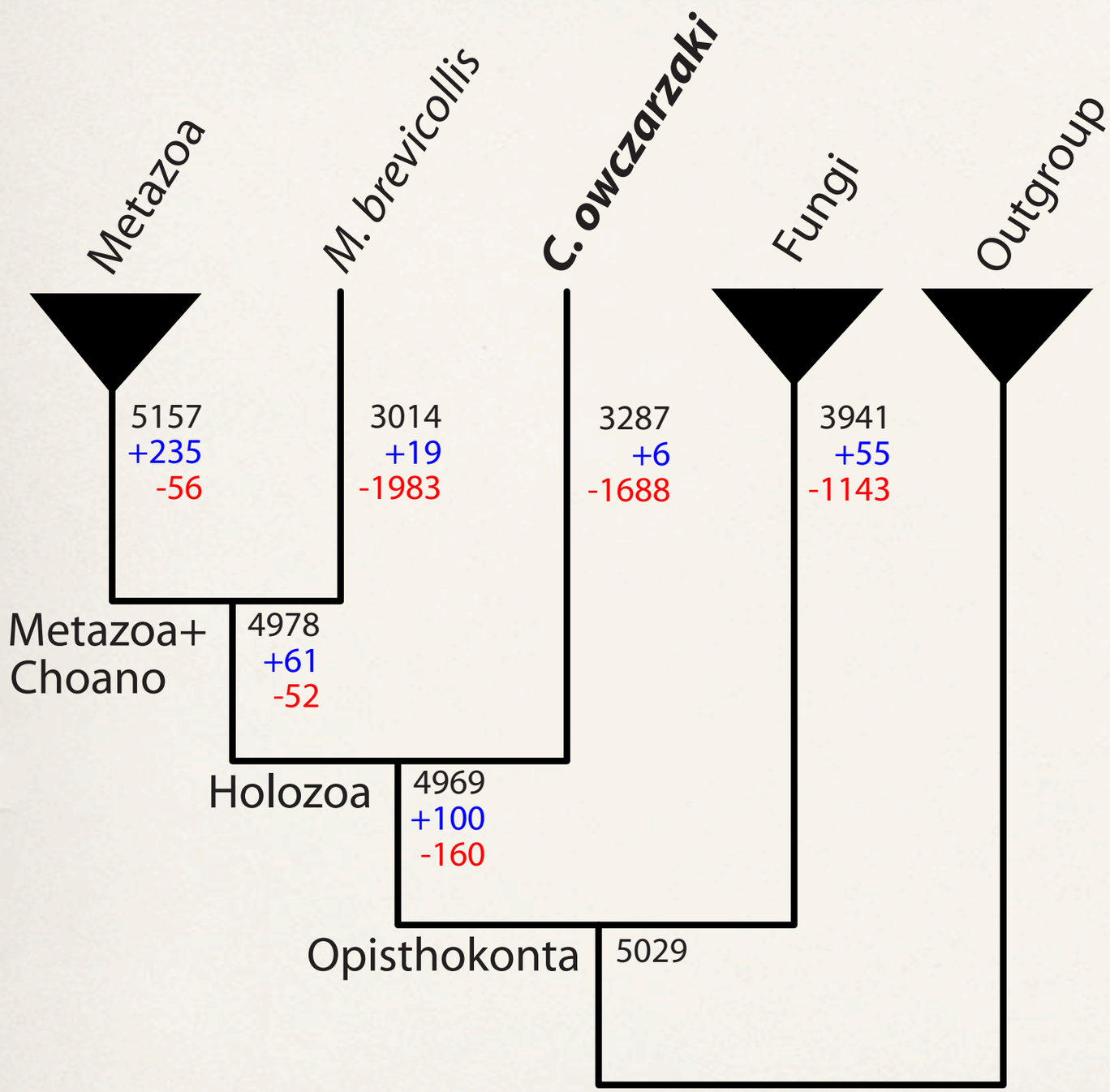
Gains (gene ontology, > 7 domains**)**

Holozoa

Signal transducer activity

Transcription regulatory region DNA binding

PFAM gain/loss



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Metazoa

Extracellular region

Transcription regulatory region DNA binding

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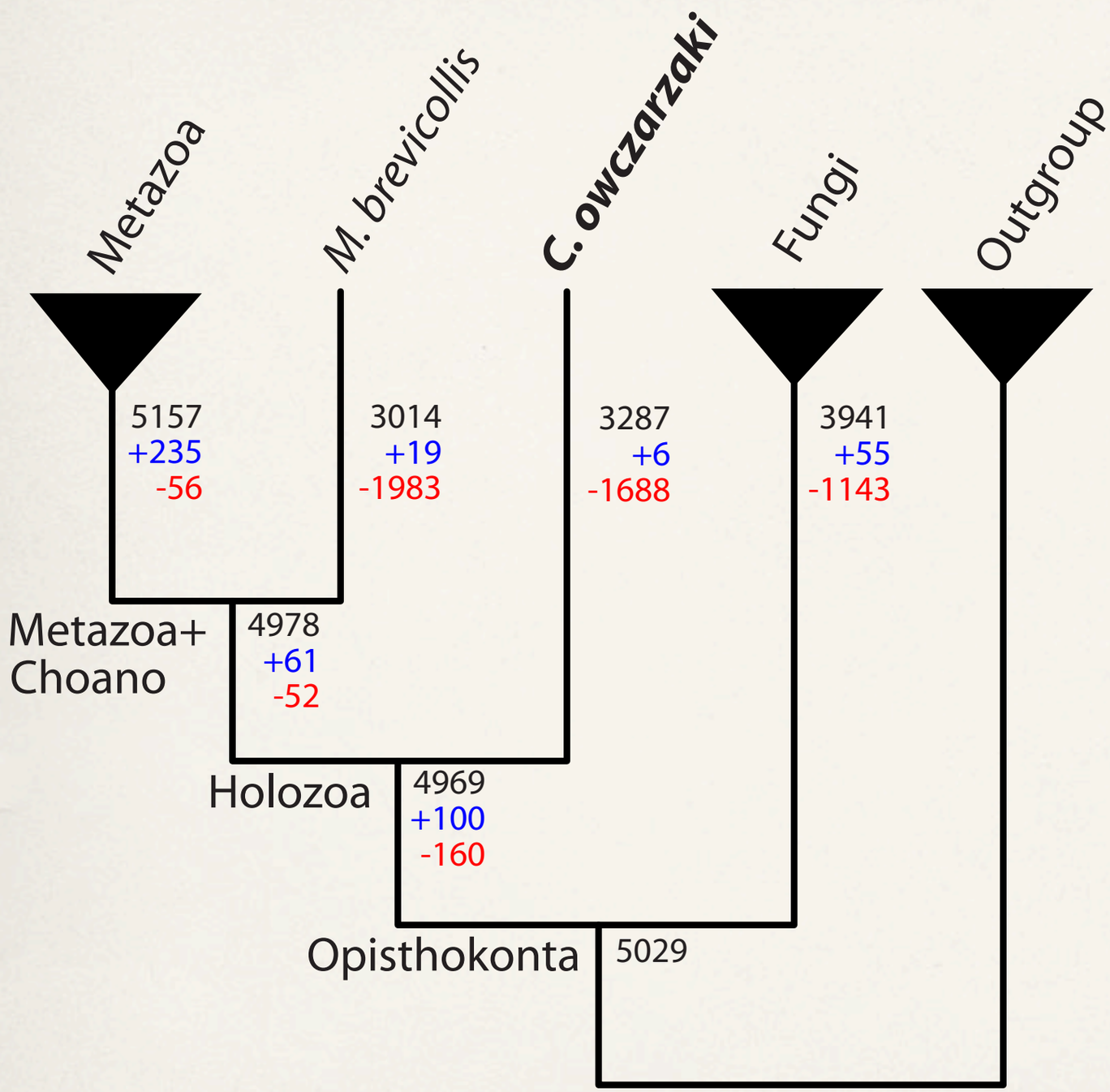


Gains (gene ontology, > 7 domains**)**

Holozoa

Signal transducer activity

Transcription regulatory region DNA binding

Metazoa

Extracellular region

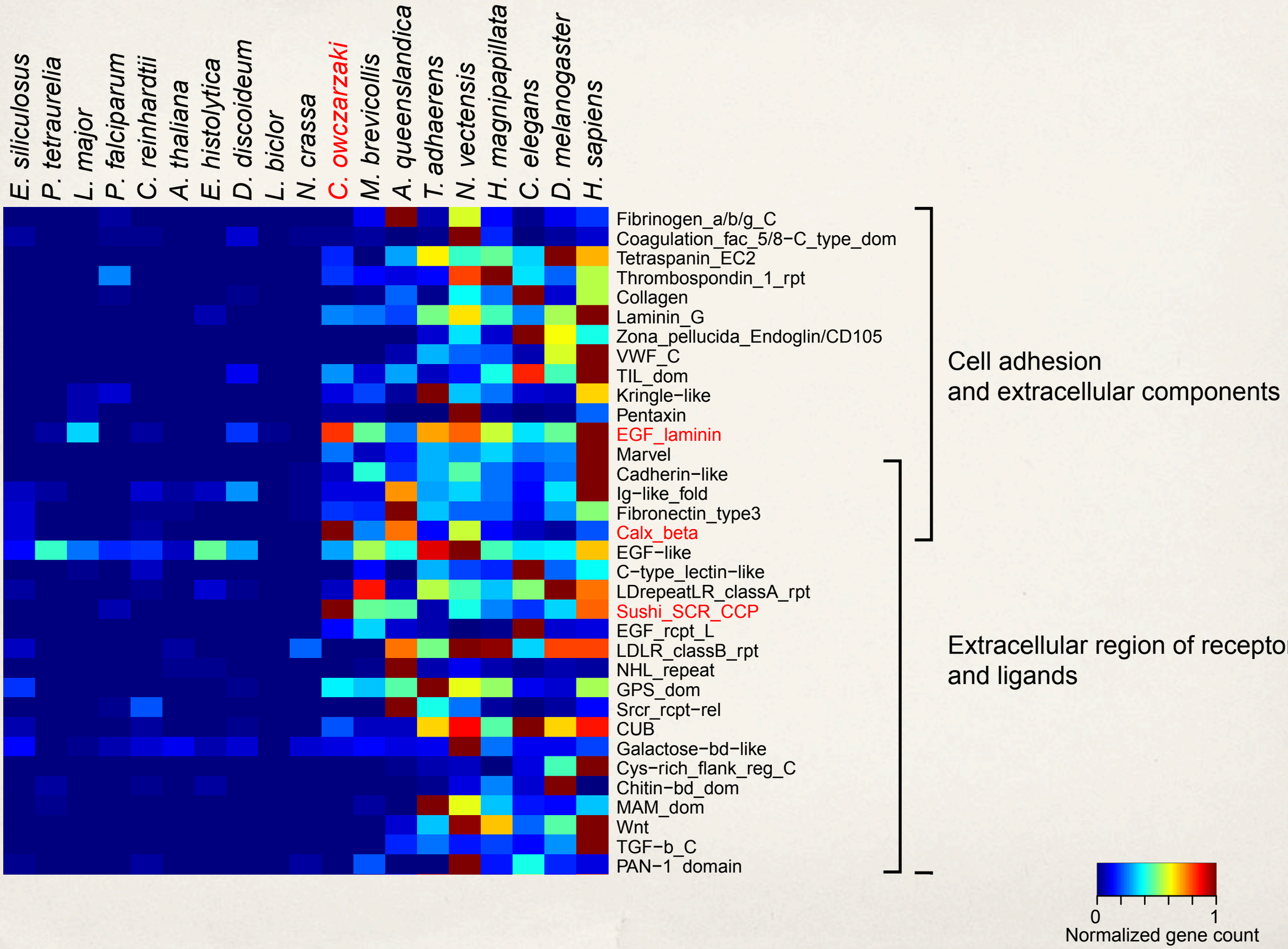
Transcription regulatory region DNA binding

Loss

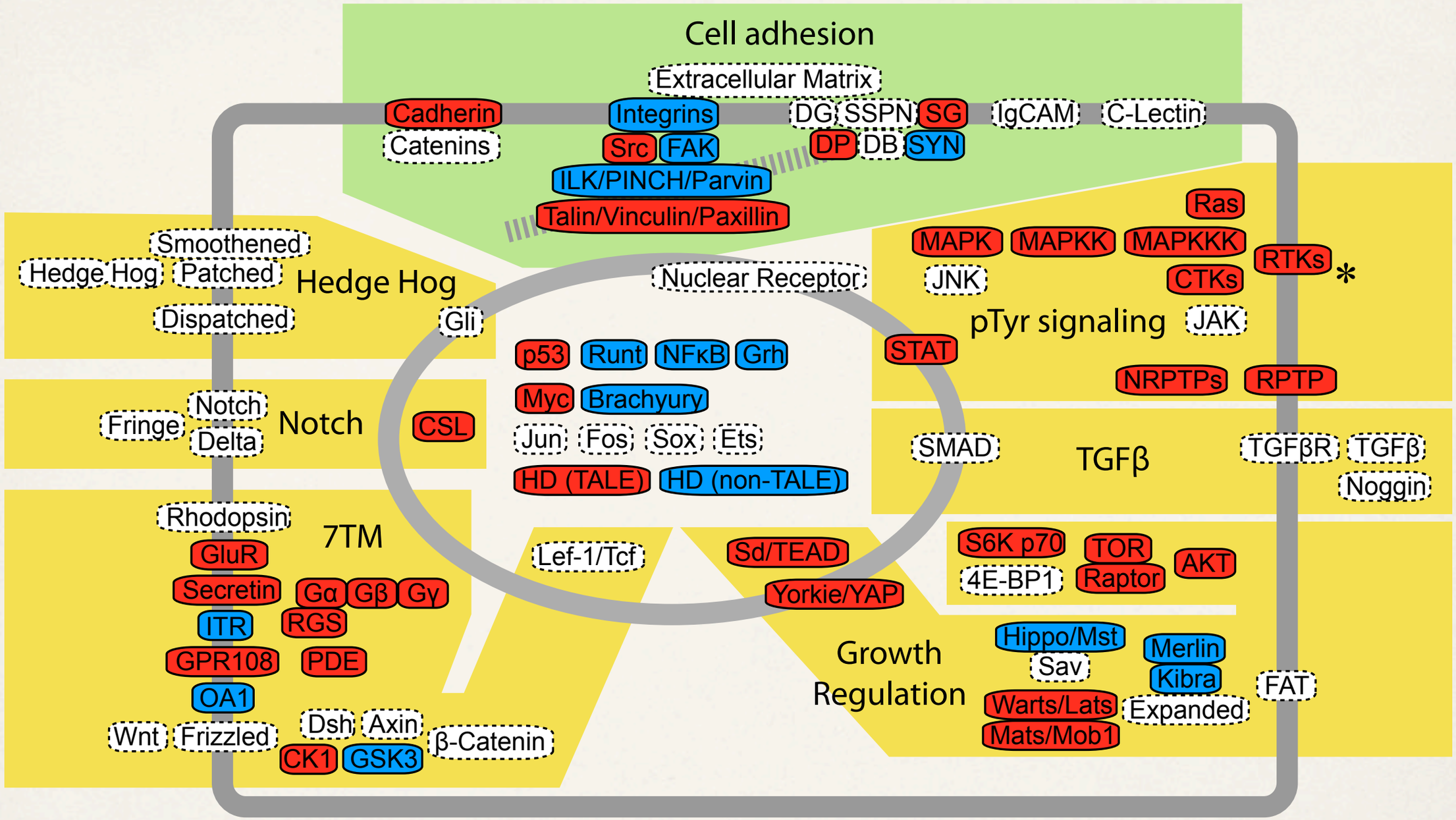
M. brevicollis

Regulation of transcription, DNA-dependent

gene expansions

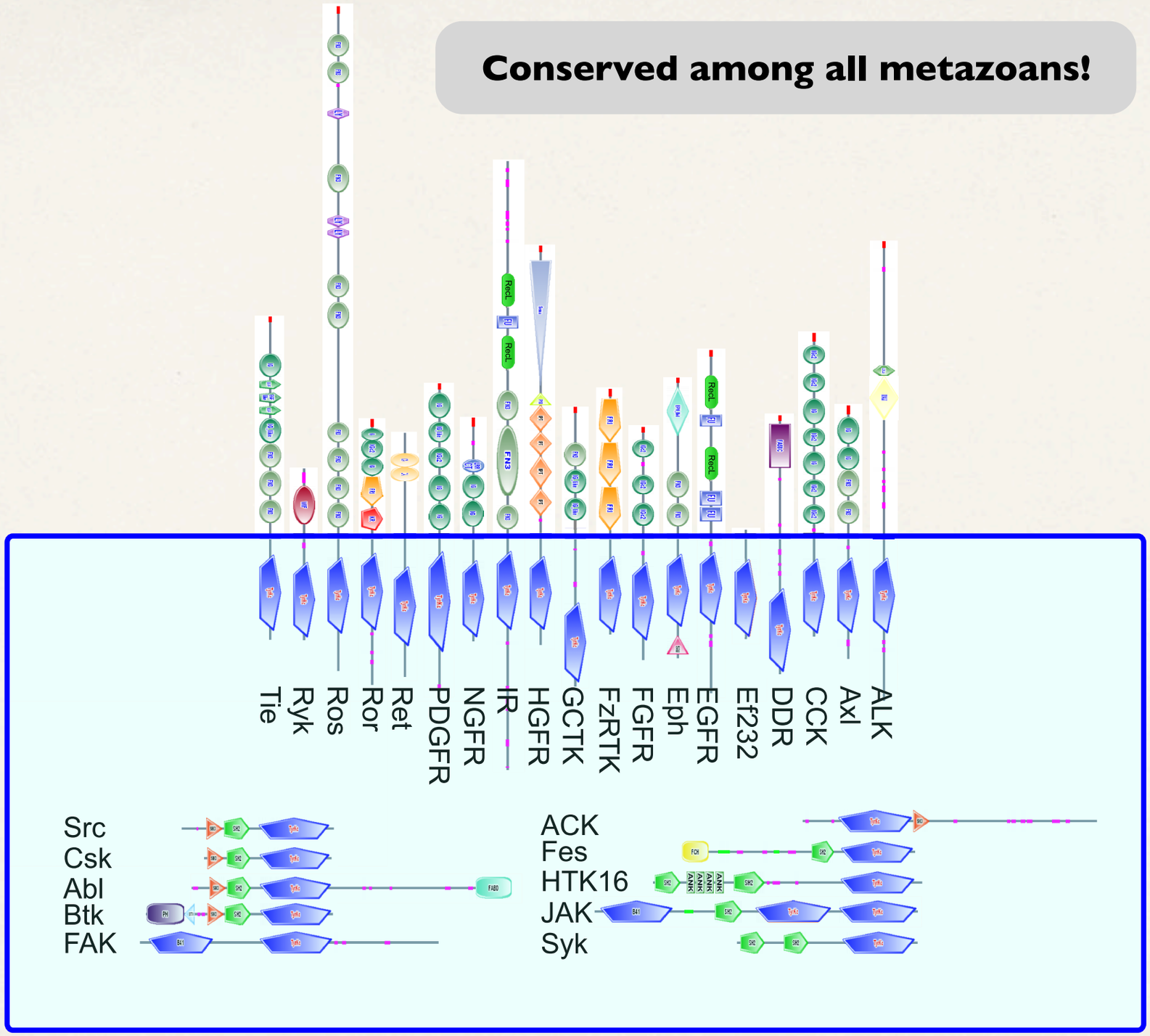


Capsaspora cell



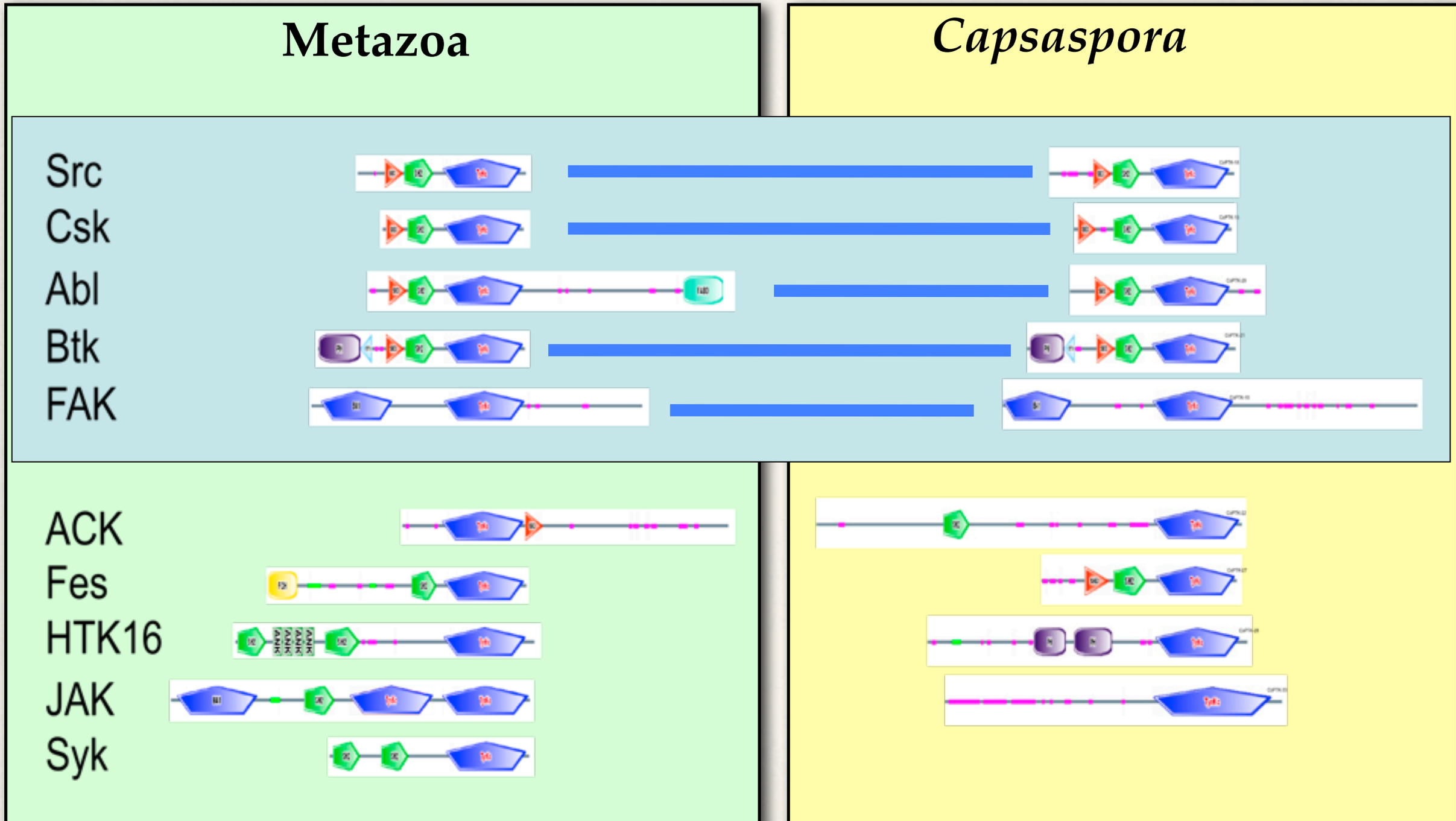
Protein Tyrosine Kinases-Metazoan PTKs

Conserved among all metazoans!



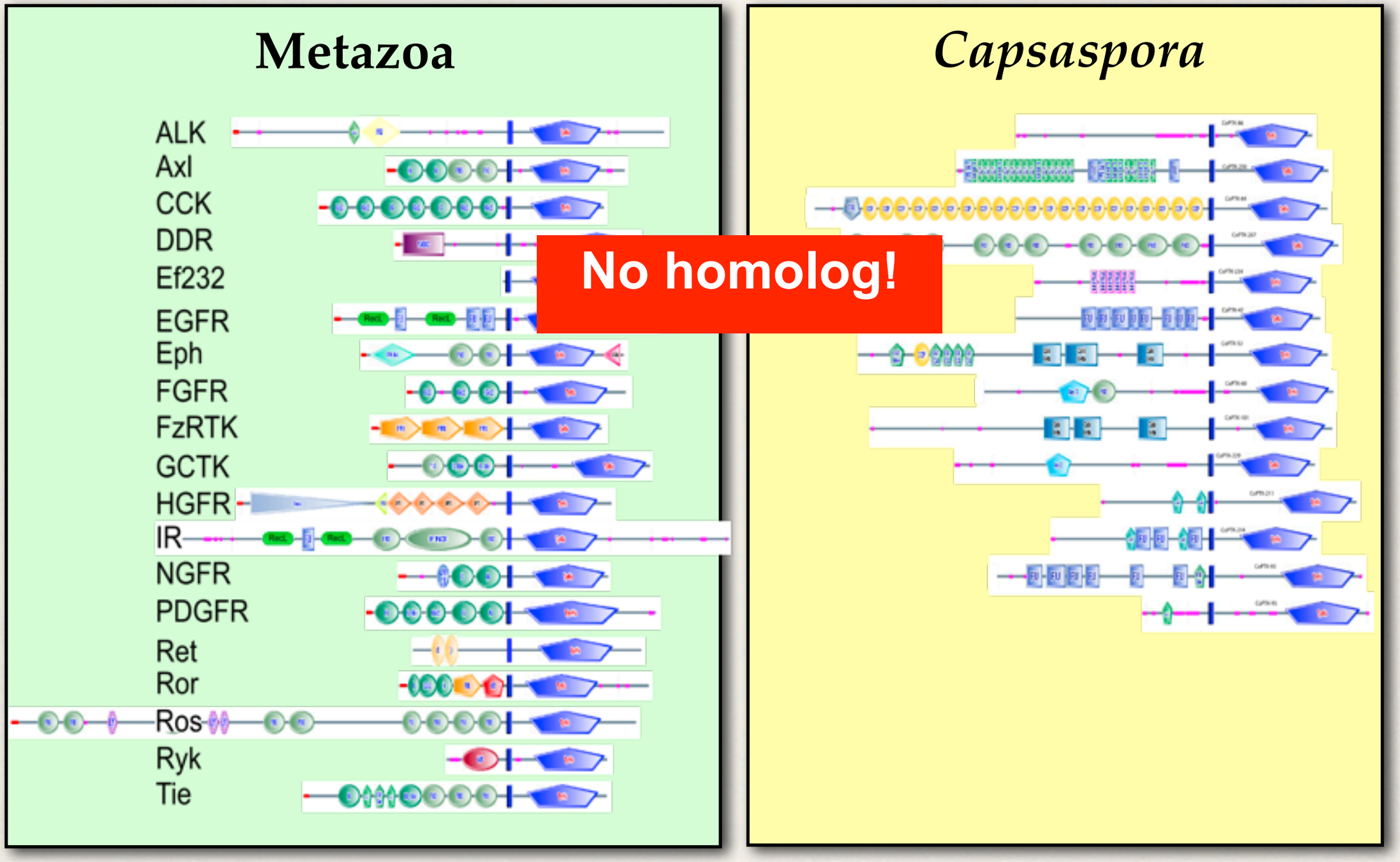
Choanoflagellates have hundreds of PTKs

PTKs-Cytosolic PTKs



H. Suga et al., Science Signaling 5, ra35 (2012).

PTKs-Receptor PTKs



H. Suga et al., Science Signaling 5, ra35 (2012).

PTKs-lineage-specific divergence of RPTKs

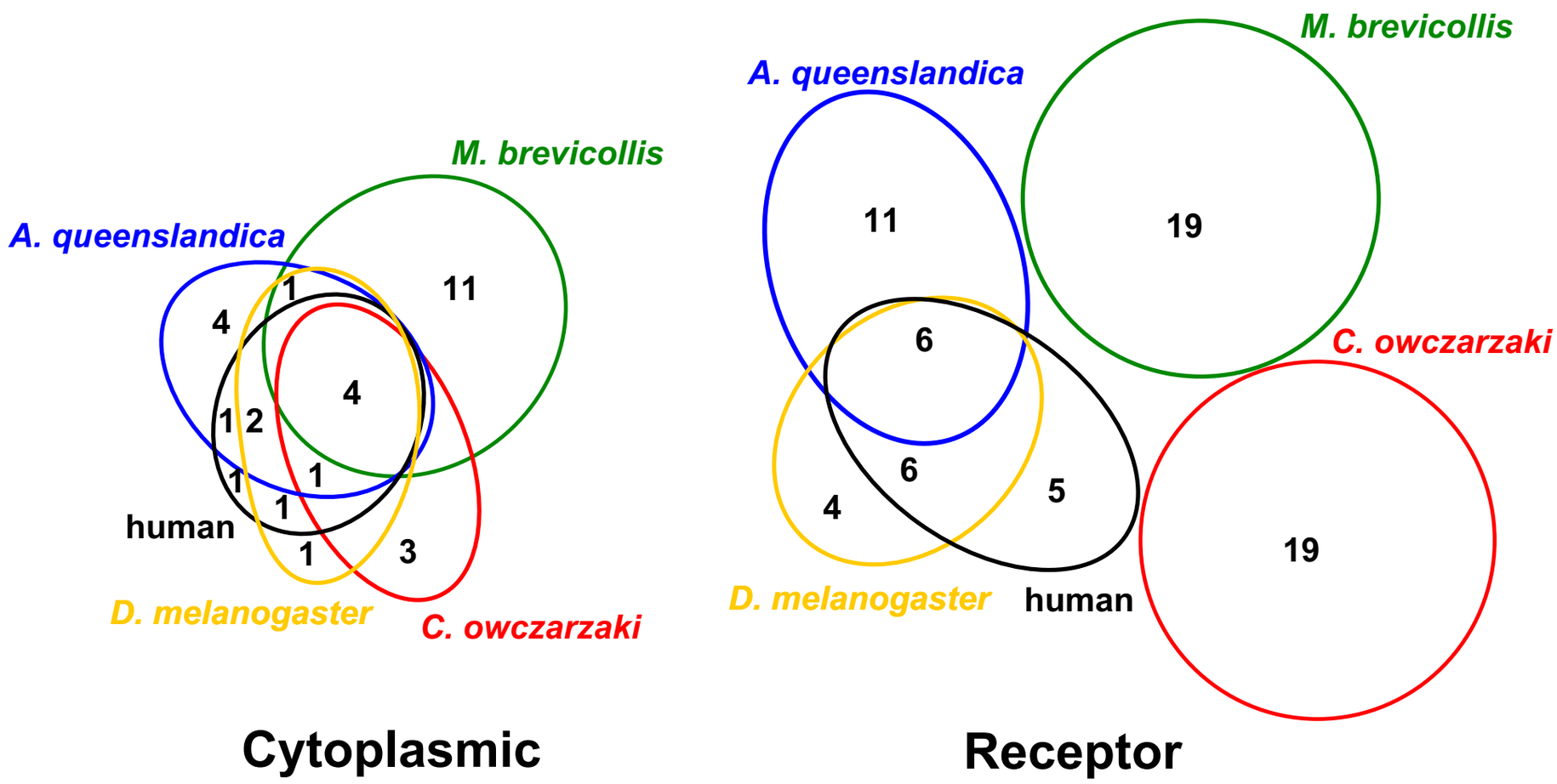
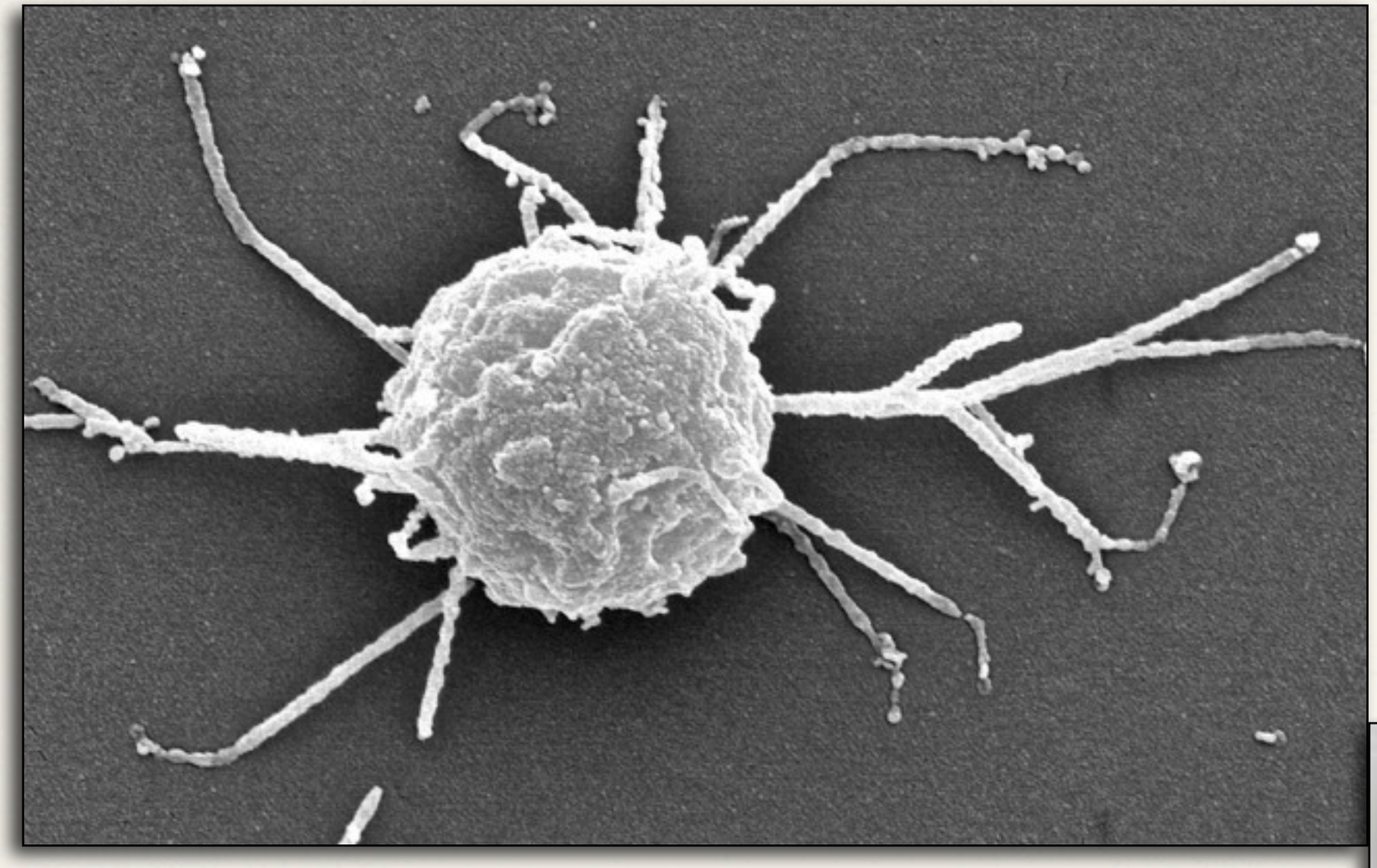


Fig. 5. Suga et al.

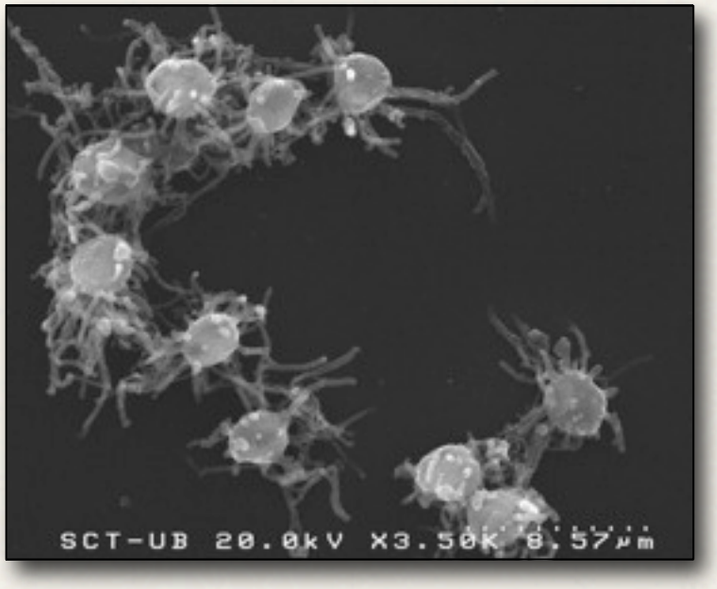
H. Suga et al., Science Signaling 5, ra35 (2012).

3. Functional analyses-model organism

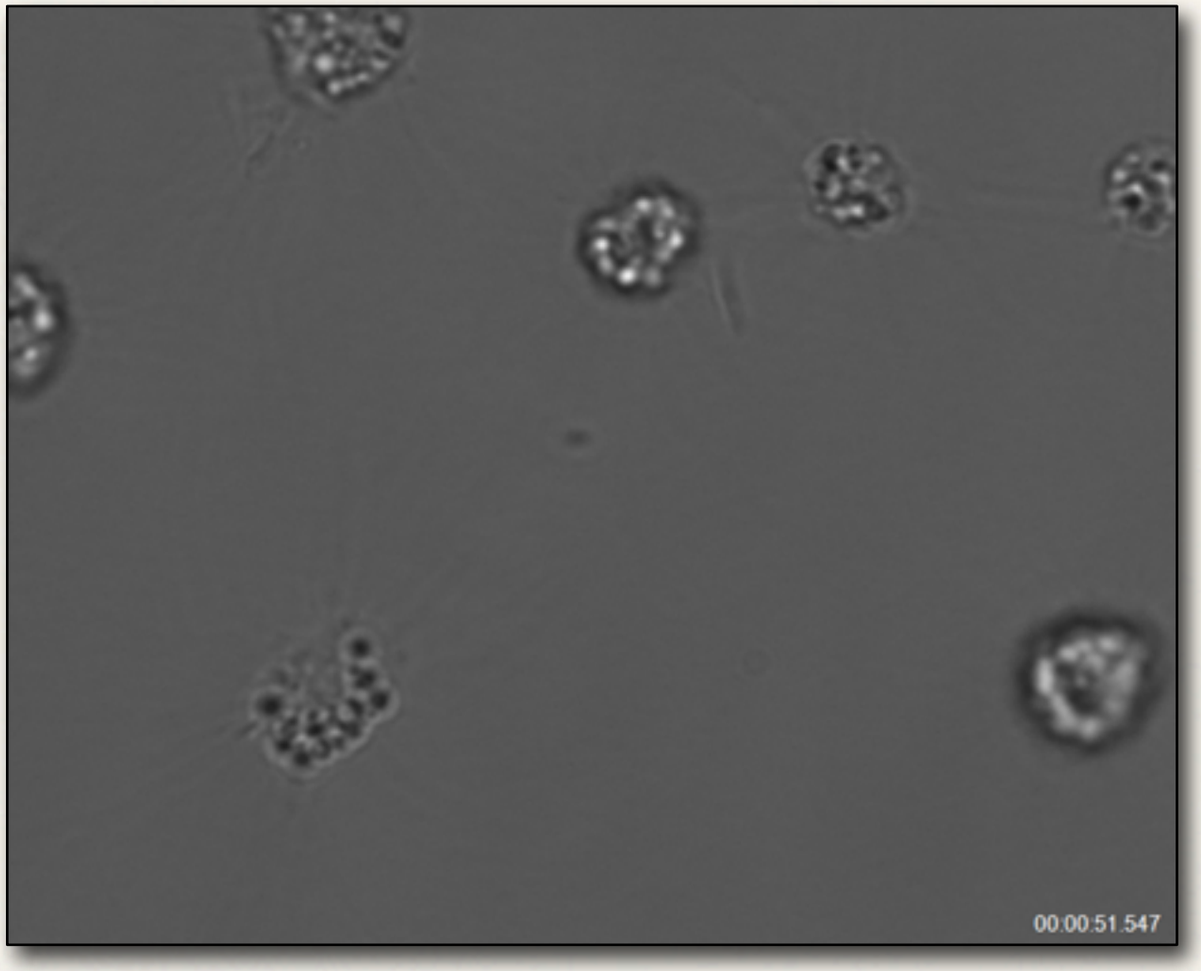
Filasterea: *Capsaspora owczarzaki*

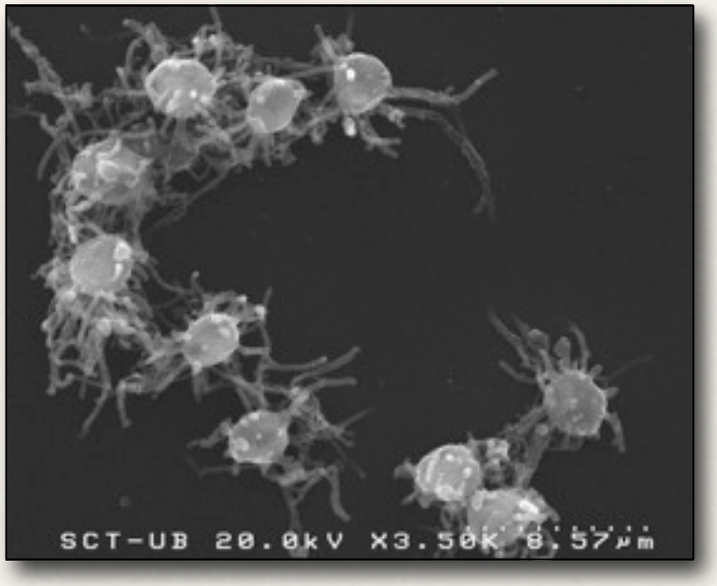


Capsaspora owczarzaki attached/filopodial stage

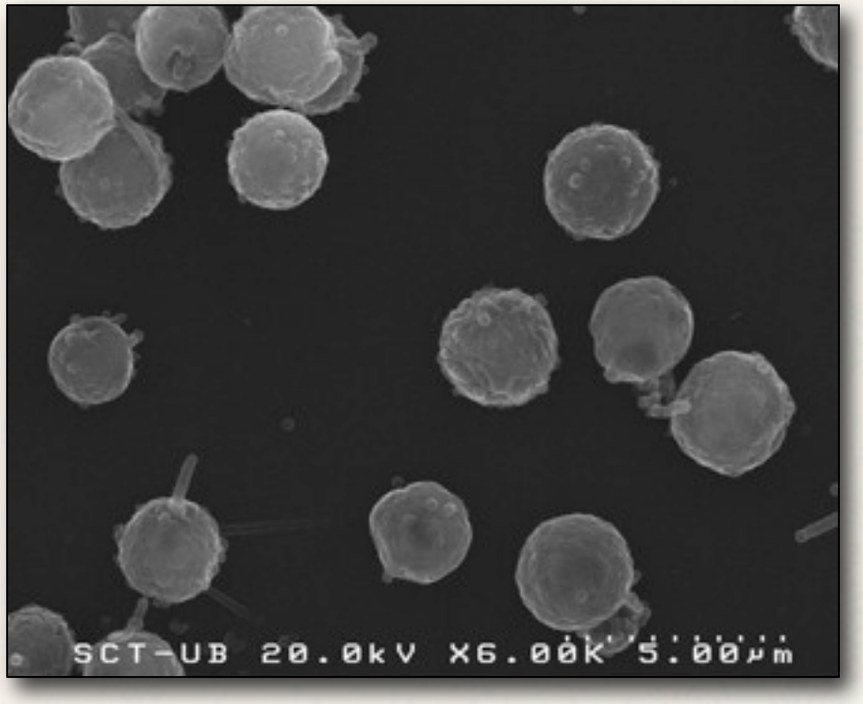


Capsaspora owczarzaki attached/filopodial stage

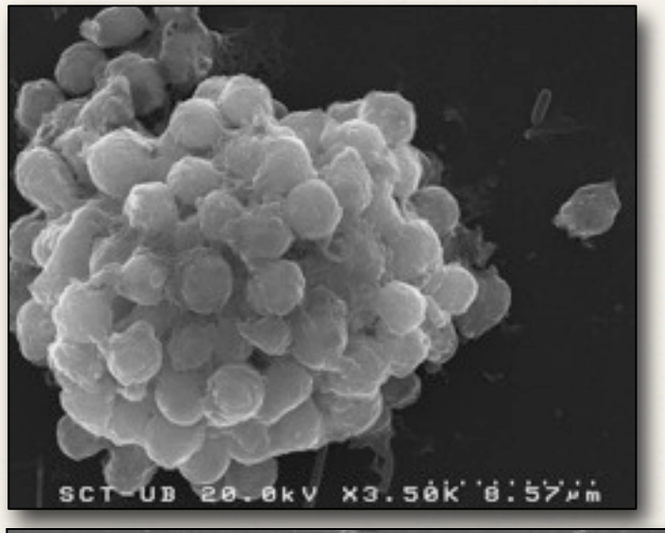


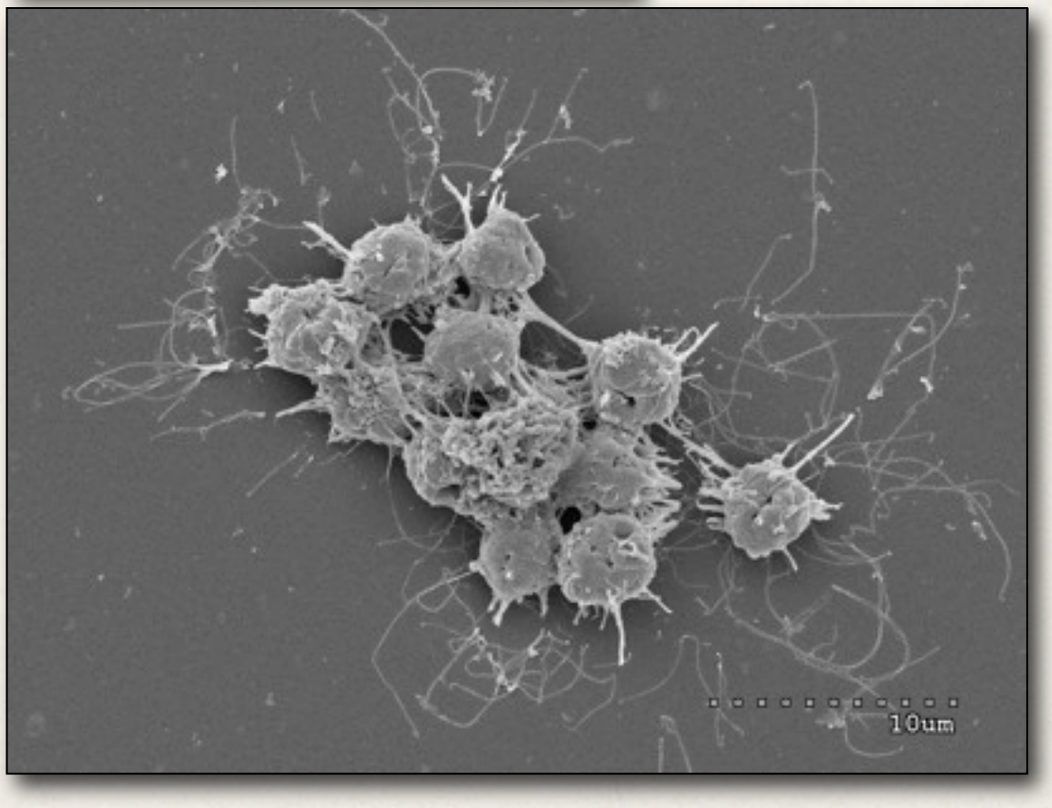


Capsaspora owczarzaki cystic/floating stage

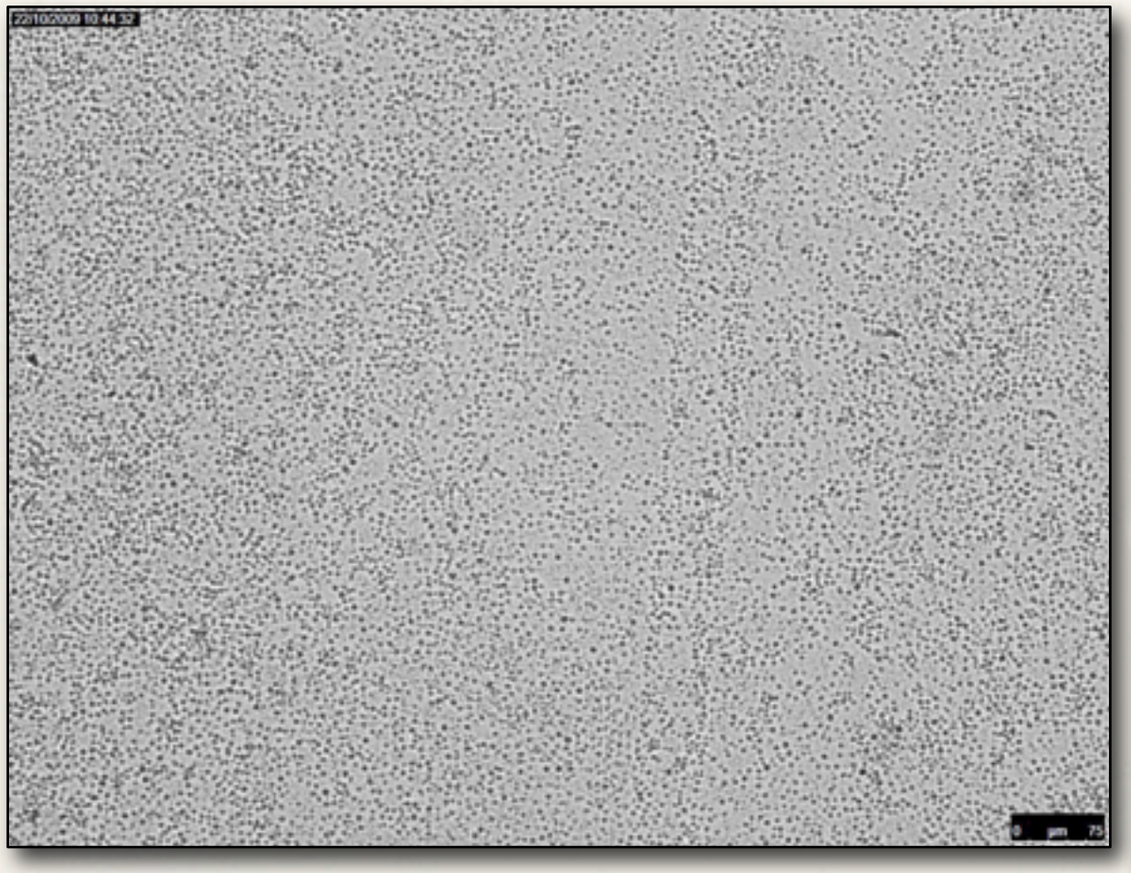


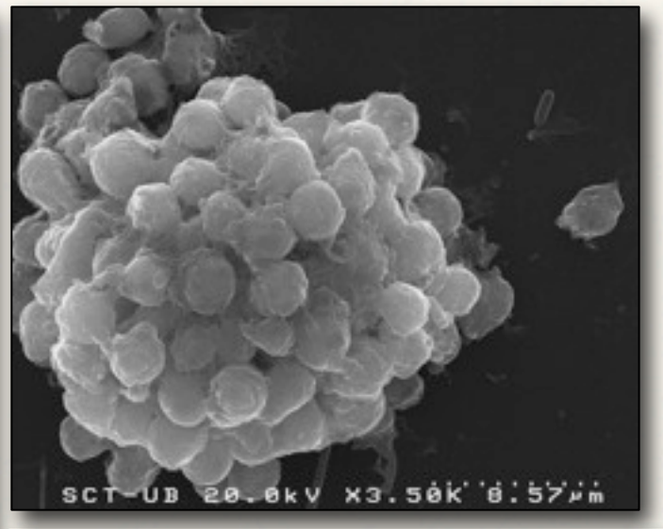
Capsaspora owczarzaki aggregative stage

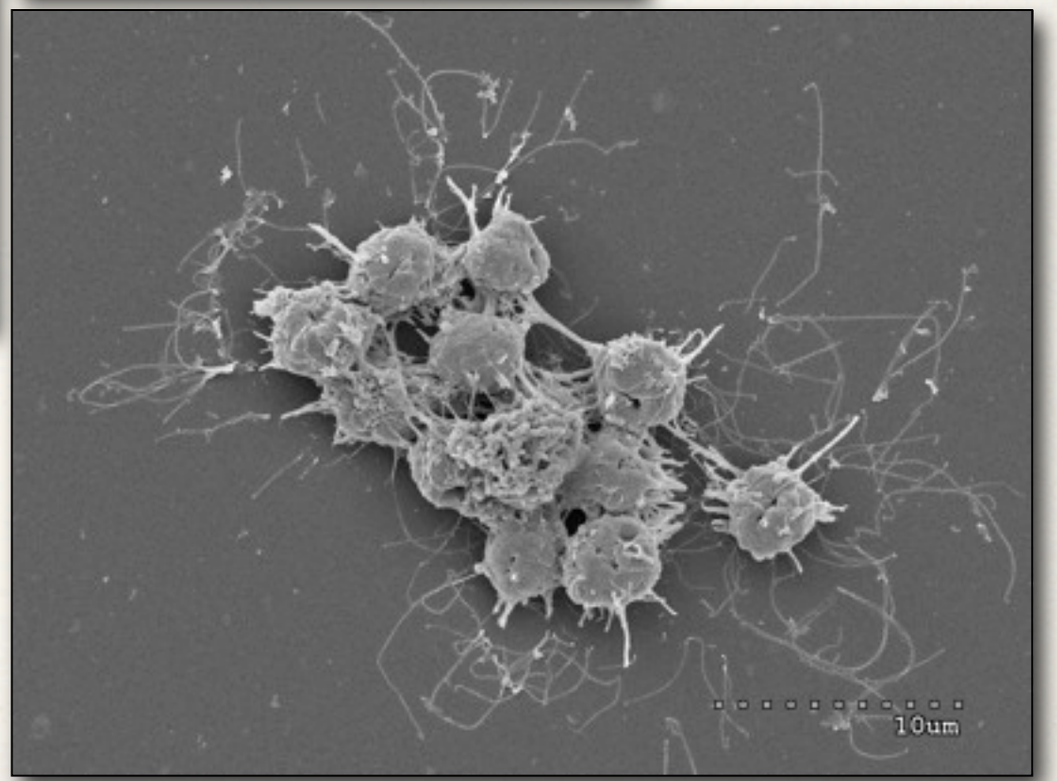




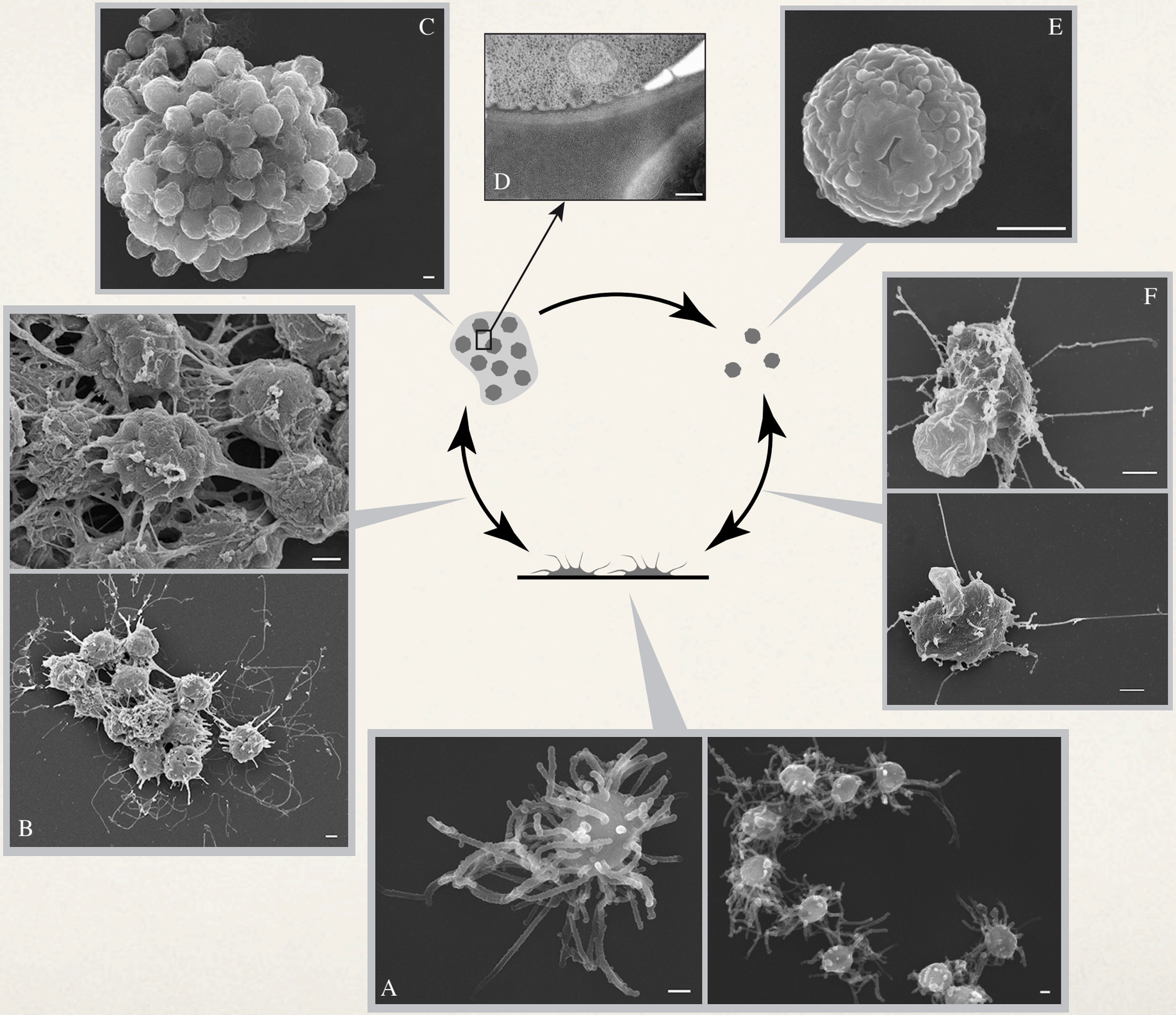
Capsaspora owczarzaki aggregative stage







Capsaspora owczarzaki life cycle



GO:0003700 sequence-specific DNA binding transcription factor activity (M)
GO:0005768 endosome (C)
GO:0005484 SNAP receptor activity (M)
GO:0016236 macroautophagy (B)
GO:0031201 SNARE complex (C)
GO:0015991 ATP hydrolysis coupled proton transport (B)
GO:0015144 carbohydrate transmembrane transporter activity (M)
GO:0017156 calcium ion-dependent exocytosis (B)
GO:0004713 protein tyrosine kinase activity (M)

GO:0015629 actin cytoskeleton (C)
GO:0003774 motor activity (M)
GO:0006520 amino acid metabolic process (B)
GO:0003779 actin binding (M)
GO:0009062 fatty acid catabolic process (B)
GO:0005681 spliceosomal complex (C)
GO:0000398 mRNA splicing, via spliceosome (B)
GO:0007155 cell adhesion
GO:0005874 microtubule (C)
GO:0009060 aerobic respiration (B)
GO:0005657 replication fork (C)
GO:0040010 positive regulation of growth rate (B)
GO:0006261 DNA-dependent DNA replication (B)
GO:0006270 DNA replication initiation (B)
GO:0042575 DNA polymerase complex (C)
GO:0046356 acetyl-CoA catabolic process (B)
GO:0030055 cell-substrate junction (C)
GO:0015986 ATP synthesis coupled proton transport (B)

Floating vs Aggregative(1630/1466)

ribosome (GO:0005840) (C)
histone acetylation (GO:0016573) (B)
histone methyltransferase complex (GO:0035097) (C)
myosin complex (GO:0016459) (C)

GO:0015991 ATP hydrolysis coupled proton transport (B)
GO:0005484 SNAP receptor activity (M)
GO:0016236 macroautophagy (B)
GO:0031201 SNARE complex (C)
GO:0003700 sequence-specific DNA binding transcription factor activity (M)

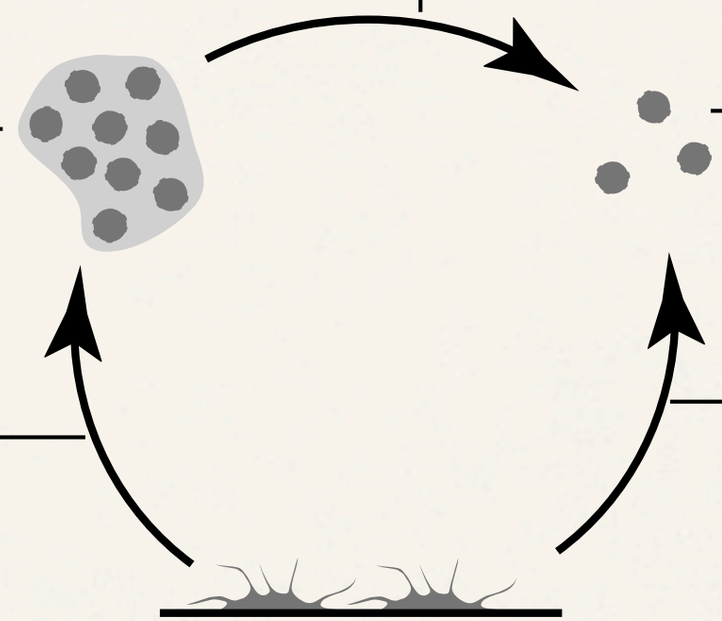
GO:0006631 fatty acid metabolic process (B)
GO:0009083 branched-chain amino acid catabolic process (B)
GO:0015629 actin cytoskeleton (C)
GO:0005759 mitochondrial matrix (C)
GO:0003774 motor activity (M)
GO:0045261 proton-transporting ATP synthase complex, catalytic core F(1) (C)
GO:0009060 aerobic respiration (B)
GO:0046356 acetyl-CoA catabolic process (B)
GO:0051258 protein polymerization (B)
GO:0005840 ribosome (C)
GO:0006414 translational elongation (B)
GO:0006270 DNA replication initiation (B)
GO:0031105 septin complex (C)
GO:0034061 DNA polymerase activity (M)
GO:0000084 S phase of mitotic cell cycle (B)

Floating vs all (1142/782/232)

GO:0005874 microtubule (C)
GO:0007091 mitotic metaphase/anaphase transition (B)
GO:0007076 mitotic chromosome condensation (B)
GO:0030055 cell-substrate junction (C)
GO:0003777 microtubule motor activity (M)
GO:0005925 focal adhesion (C)
GO:0031012 extracellular matrix (C)

GO:0003700 sequence-specific DNA binding transcription factor activity (M)
GO:0004672 protein kinase activity (M)

Aggregative vs all (233/166/0)



Floating vs Adherent (1711/1516)

GO:0015991 ATP hydrolysis coupled proton transport (B)
GO:0031201 SNARE complex (C)
GO:0005085 guanyl-nucleotide exchange factor activity (M)
GO:0008219 cell death (B)
GO:0005484 SNAP receptor activity (M)
GO:0003700 sequence-specific DNA binding transcription factor activity (M)
GO:0006888 ER to Golgi vesicle-mediated transport (B)
GO:0048193 Golgi vesicle transport (B)
GO:0016236 macroautophagy (B)
GO:0017157 regulation of exocytosis (B)

GO:0005840 ribosome (C)
GO:0006414 translational elongation (B)
GO:0015629 actin cytoskeleton (C)
GO:0006084 acetyl-CoA metabolic process (B)
GO:0016459 myosin complex (C)
GO:0003774 motor activity (M)
GO:0005739 mitochondrion (C)
GO:0006631 fatty acid metabolic process (B)
GO:0006754 ATP biosynthetic process (B)
GO:0006270 DNA replication initiation (B)
GO:0006323 DNA packaging (B)

Aggregative vs Adherent (679/675)

GO:0005925 focal adhesion (C)
GO:0043235 receptor complex (C)
GO:0007160 cell-matrix adhesion (B)
GO:0008092 cytoskeletal protein binding (M)
GO:0007229 integrin-mediated signaling pathway (B)
GO:0007166 cell surface receptor linked signal transduction (B)
GO:0007267 cell-cell signaling (B)
GO:0031012 extracellular matrix (C)
GO:0005178 integrin binding (M)

GO:0004930 G-protein coupled receptor activity (M)
GO:0003700 sequence-specific DNA binding transcription factor activity (M)
GO:0004713 protein tyrosine kinase activity (M)
GO:0007186 G-protein coupled receptor protein signaling pathway (B)

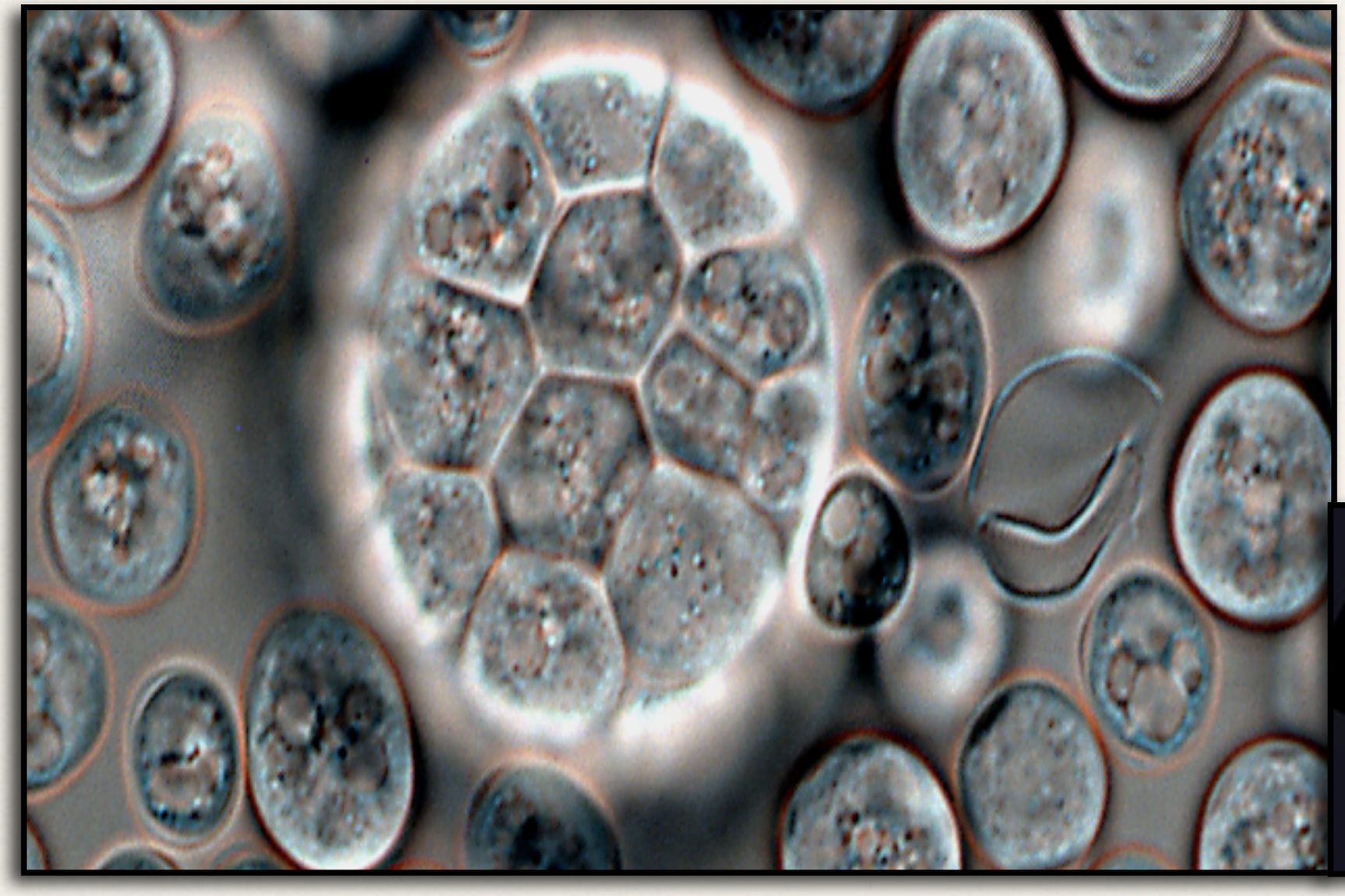
Adherent vs all (396/166/441)

GO:0046034 ATP metabolic process (B)
GO:0038023 signaling receptor activity (M)
GO:0004713 protein tyrosine kinase activity (M)
GO:0008652 cellular amino acid biosynthetic process (B)
GO:0003700 sequence-specific DNA binding transcription factor activity (M)

GO:0008287 protein serine/threonine phosphatase complex (C)
GO:0032147 activation of protein kinase activity (B)
GO:0005765 lysosomal membrane (C)

GO:0006493 protein O-linked glycosylation (B)
GO:0004672 protein kinase activity (M)

Ichthyosporea: *Sphaeroforma arctica*



Jostensen et al. 2002 European Journal of Protistology 38:93-104.

Ichthyosporea: *Sphaeroforma arctica*

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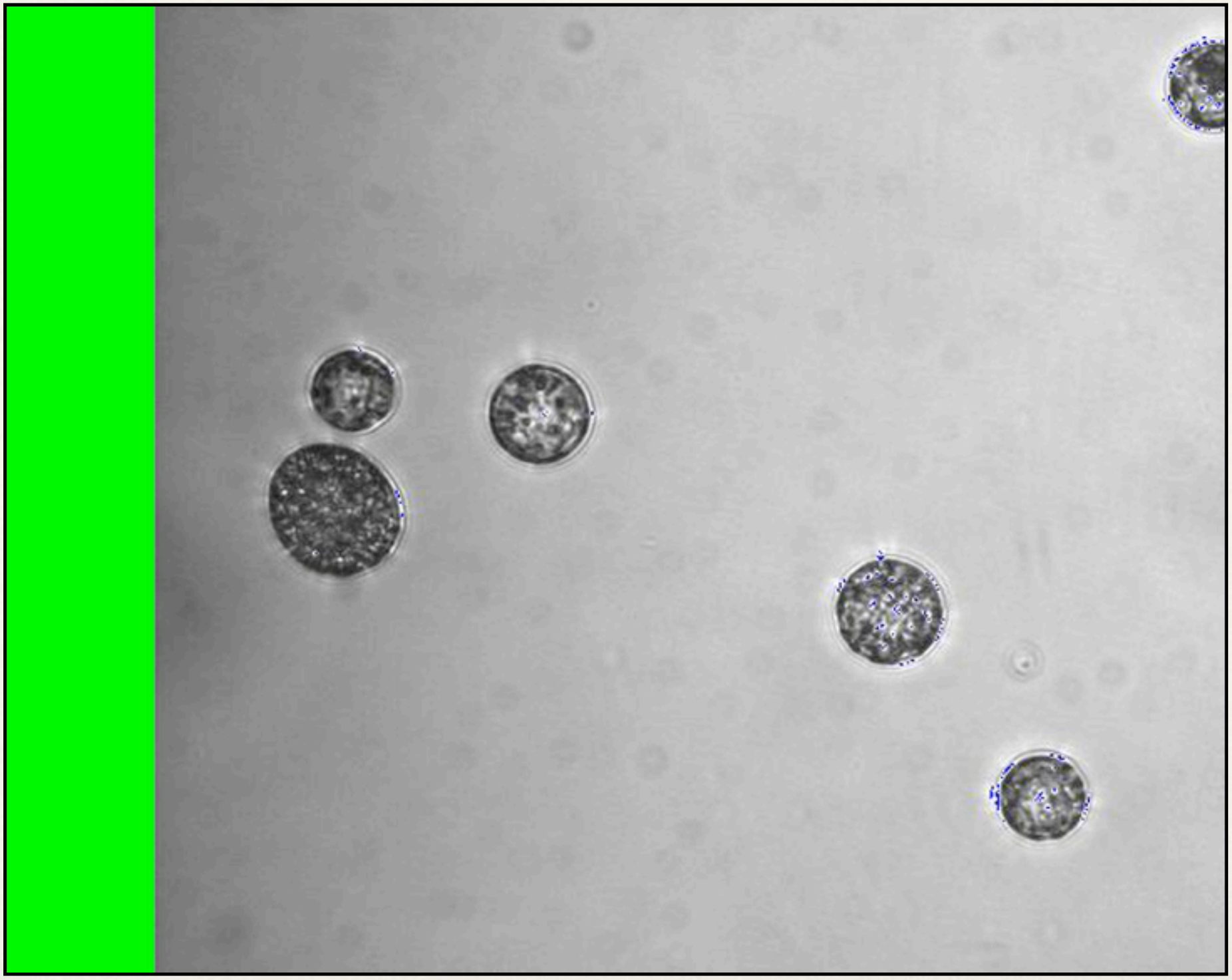


transgenesis tools failed

Brachyury and T-box-rescue in Xenopus embryos

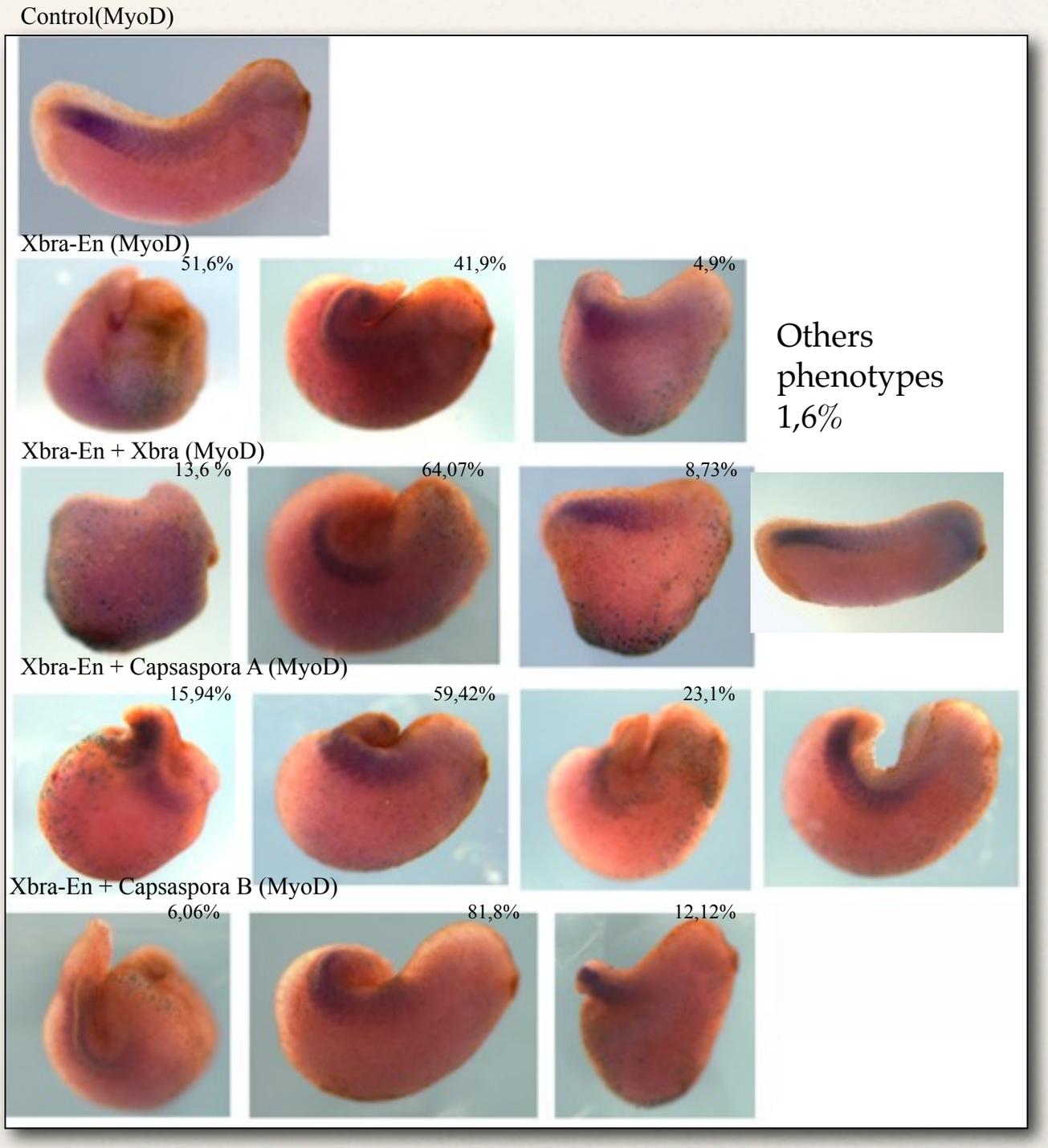
in collaboration with José Luis Gómez-Skarmeta (Sevilla)

Mutant →

Mutant +*XenopusBrachyury* →

Mutant +*CapsasporaBrachyury* →

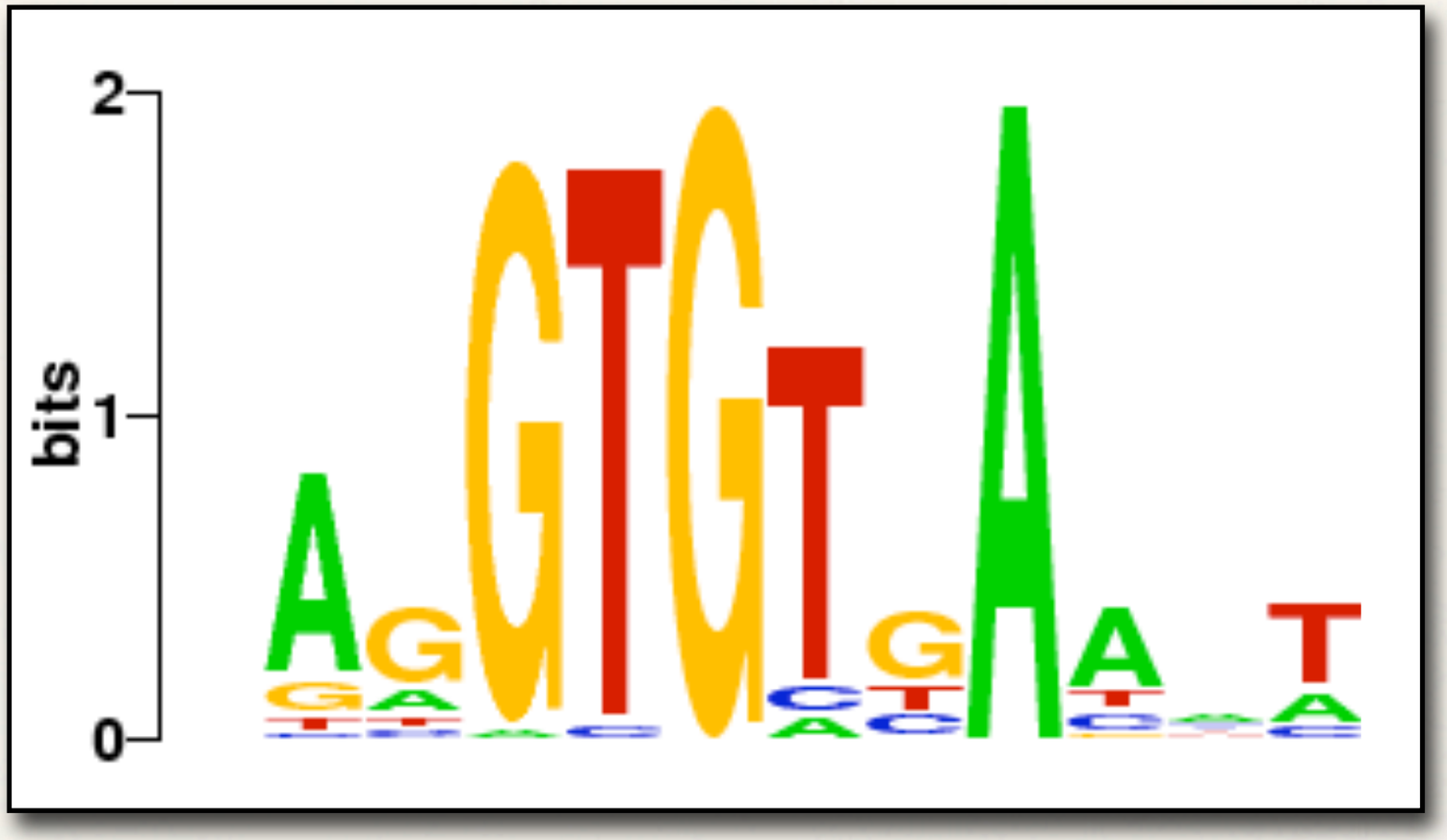
Mutant +*CapsasporaTbox3* →



Others phenotypes 1,6%

Capsaspora Brachyury consensus binding-site

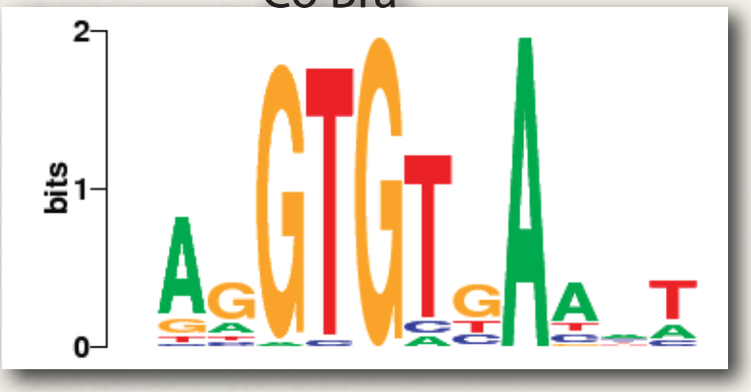
(PBM, Protein Binding Microarray, in collaboration with Tim Hughes, Toronto)



Capsaspora Brachyury consensus binding-site

(PBM, Protein Binding Microarray, in collaboration with Tim Hughes, Toronto)

Co Bra



Mouse T

Mouse Eomes

Mouse Tbx1

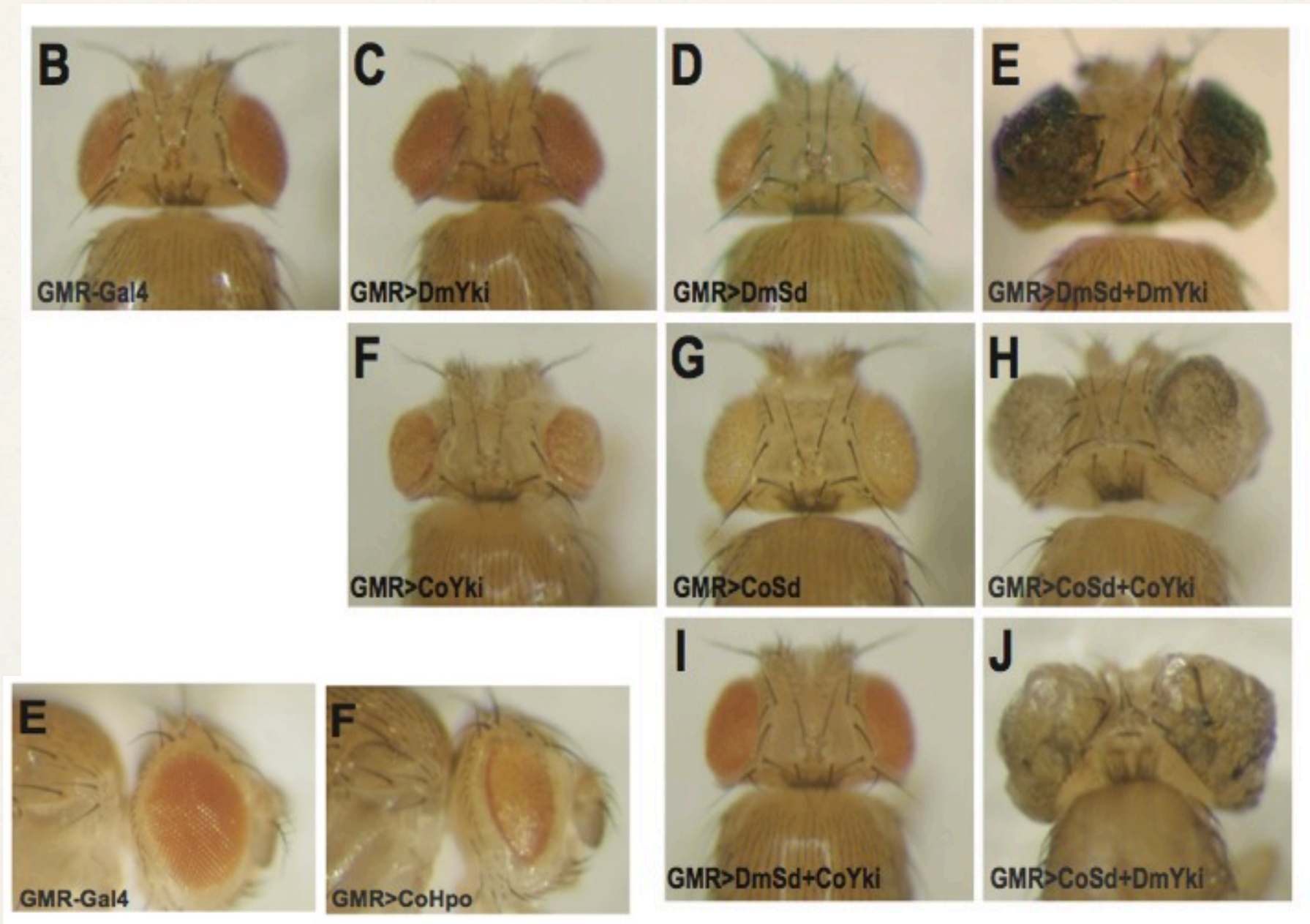
Mouse Tbx2

Mouse Tbx4

long-term goal: to perform ChipSeq

Capsaspora Hippo/Yap pathway

(in collaboration with Doujia Pan, Howard Hughes Medical Institute, Baltimore)



Sebé-Pedrós et al. 2012. Cell Reports doi:10.1016/j.cel- rep.2011.11.004

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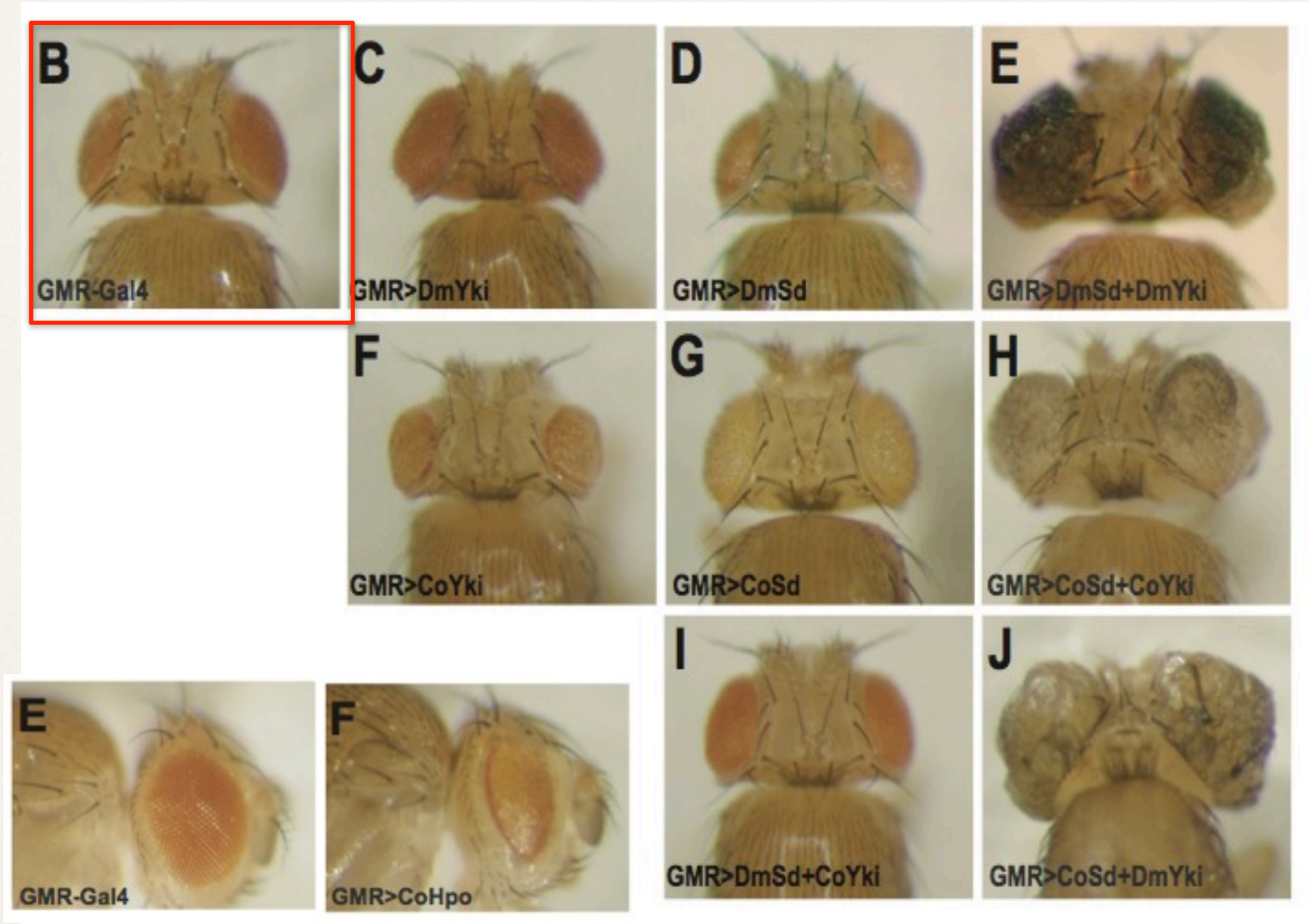

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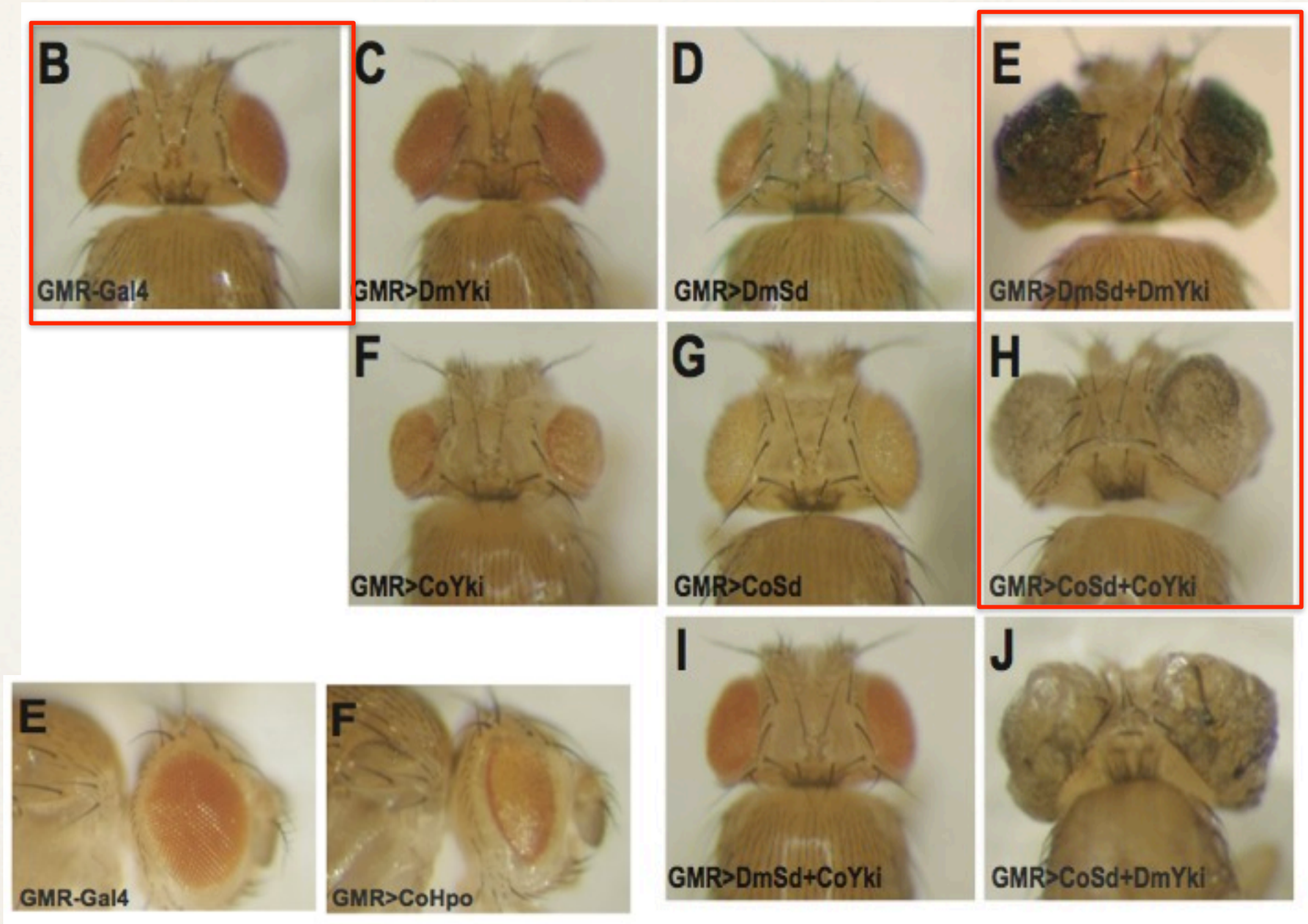

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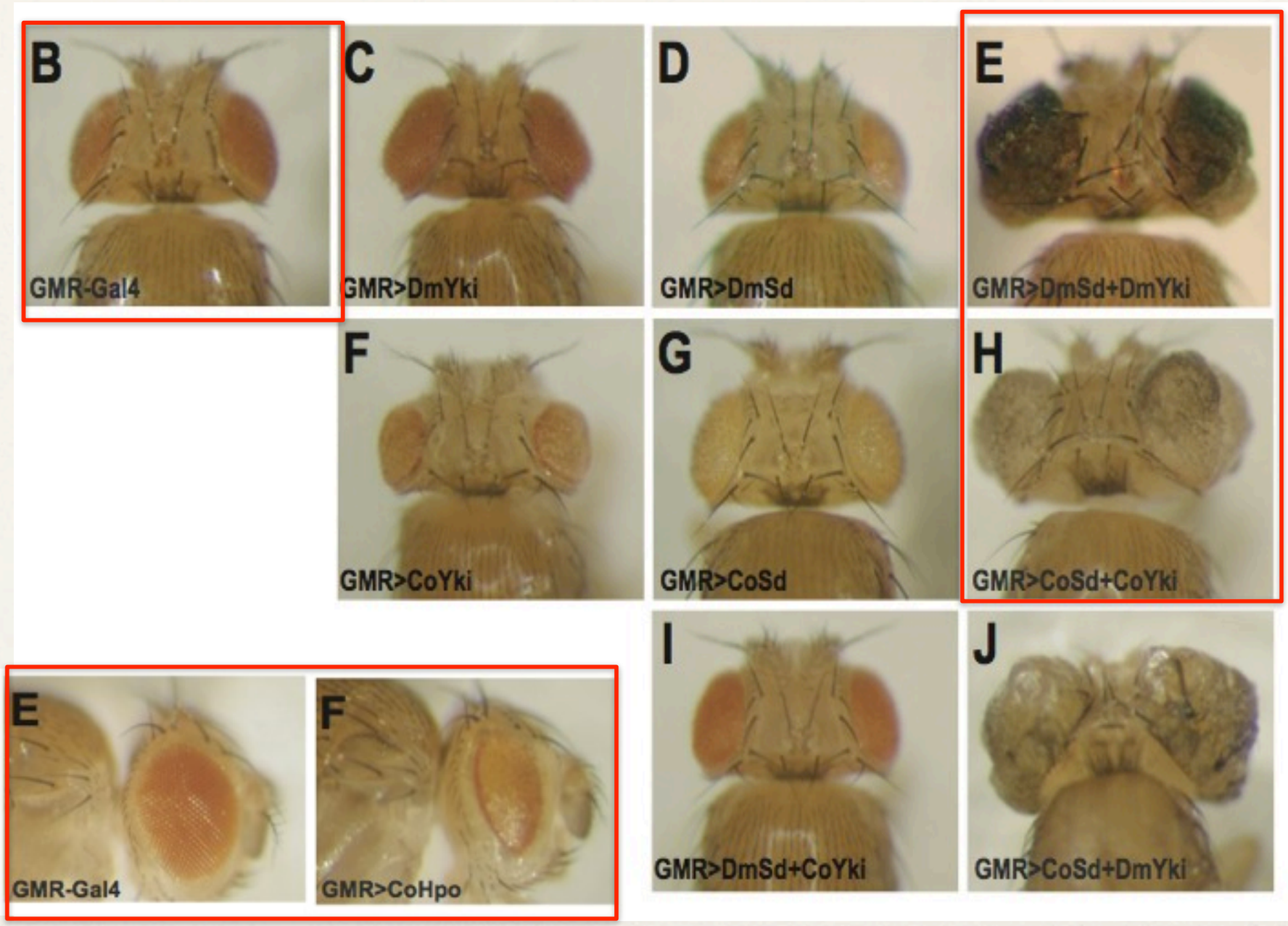
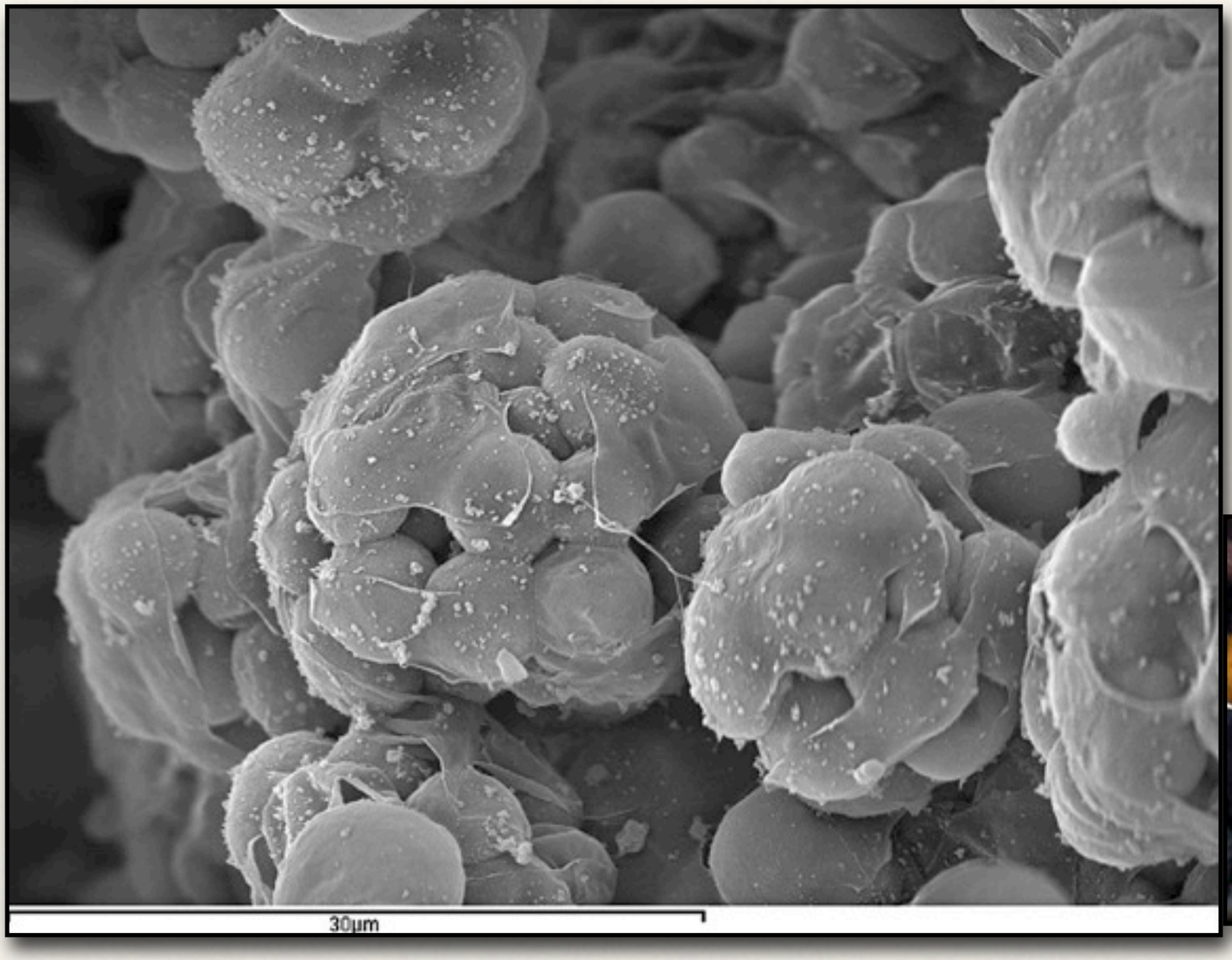


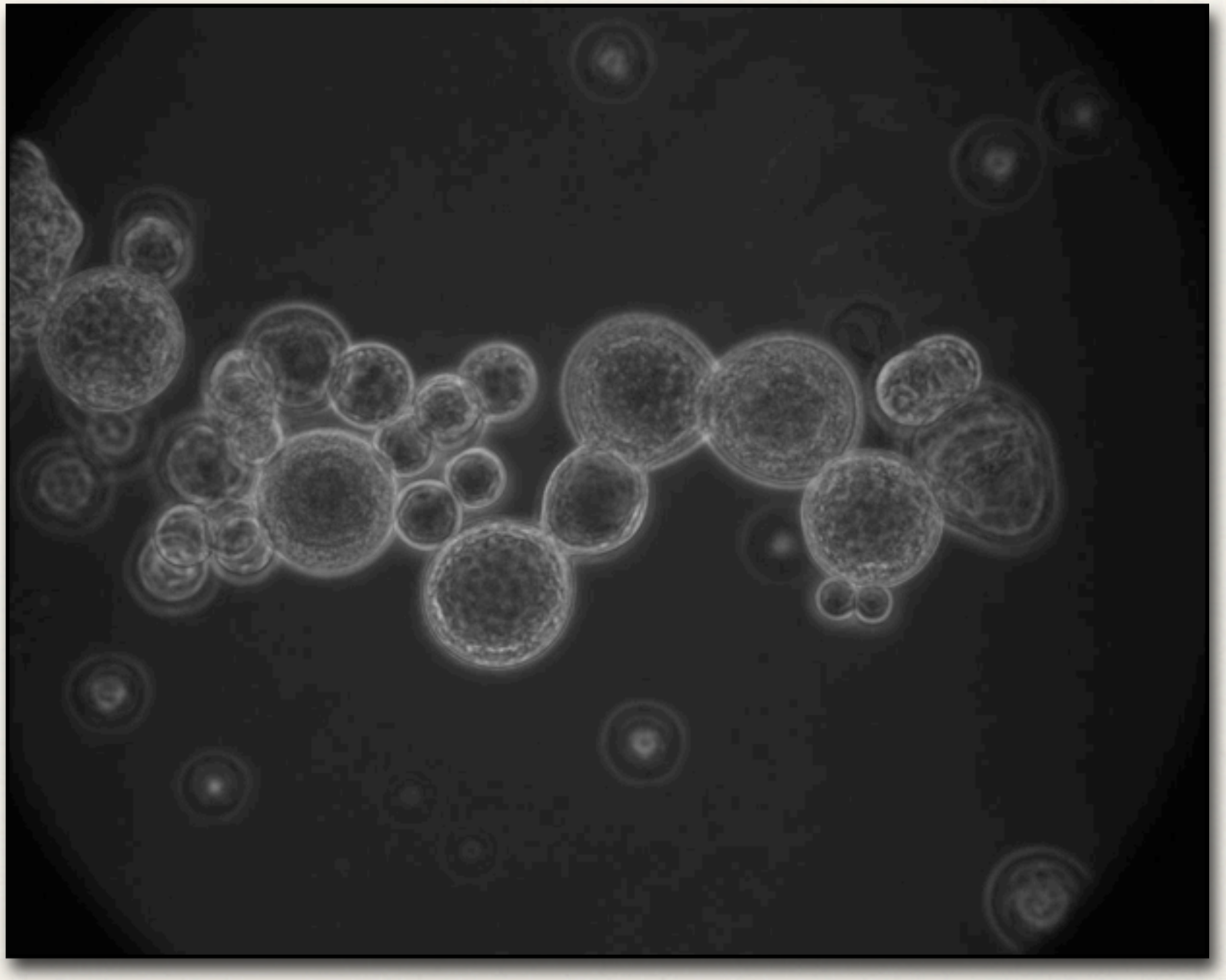
Sebé-Pedrós et al. 2012. Cell Reports doi:10.1016/j.cel- rep.2011.11.004

Our new baby, the ichthyosporean *Creolimax fragrantissima*



Marshall et al. 2008. Protist 159:415-433.

Our new baby, the ichthyosporean *Creolimax fragrantissima*

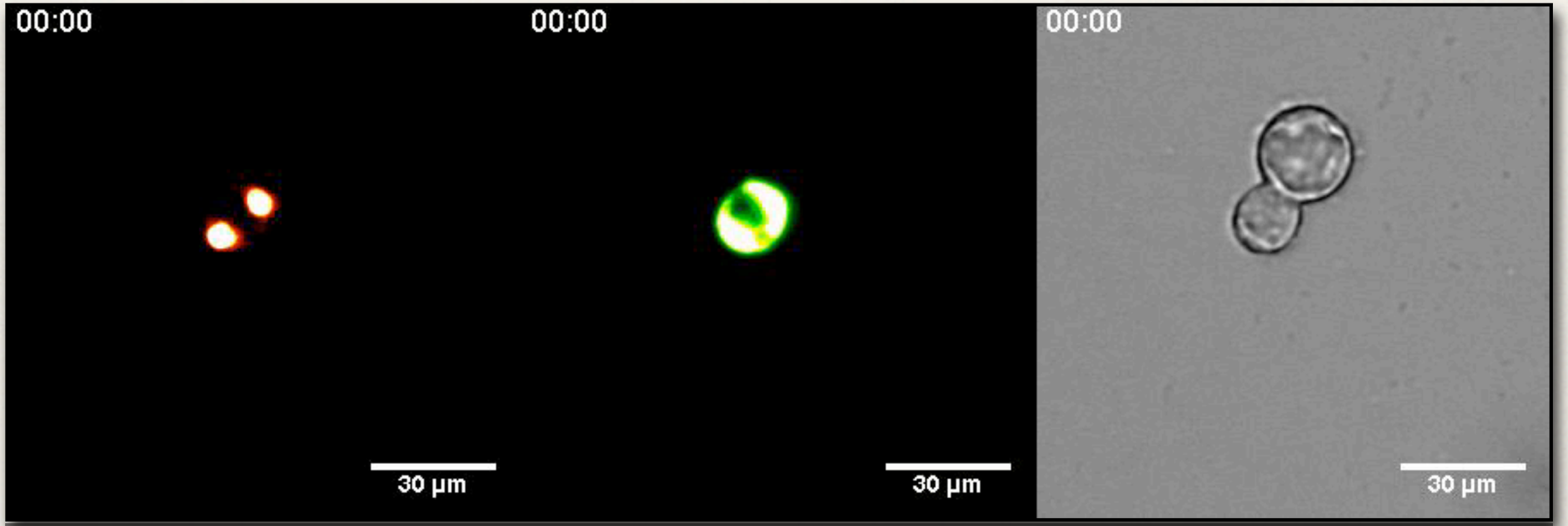


First transformation in an unicellular relative of Metazoa!!!!!

beta-tubulin-promoter *Creolimax*+*S.arctica*-histone +mcherry

beta-tubulin-promoter *Creolimax* +venus

First transformation in an unicellular relative of Metazoa!!!!!



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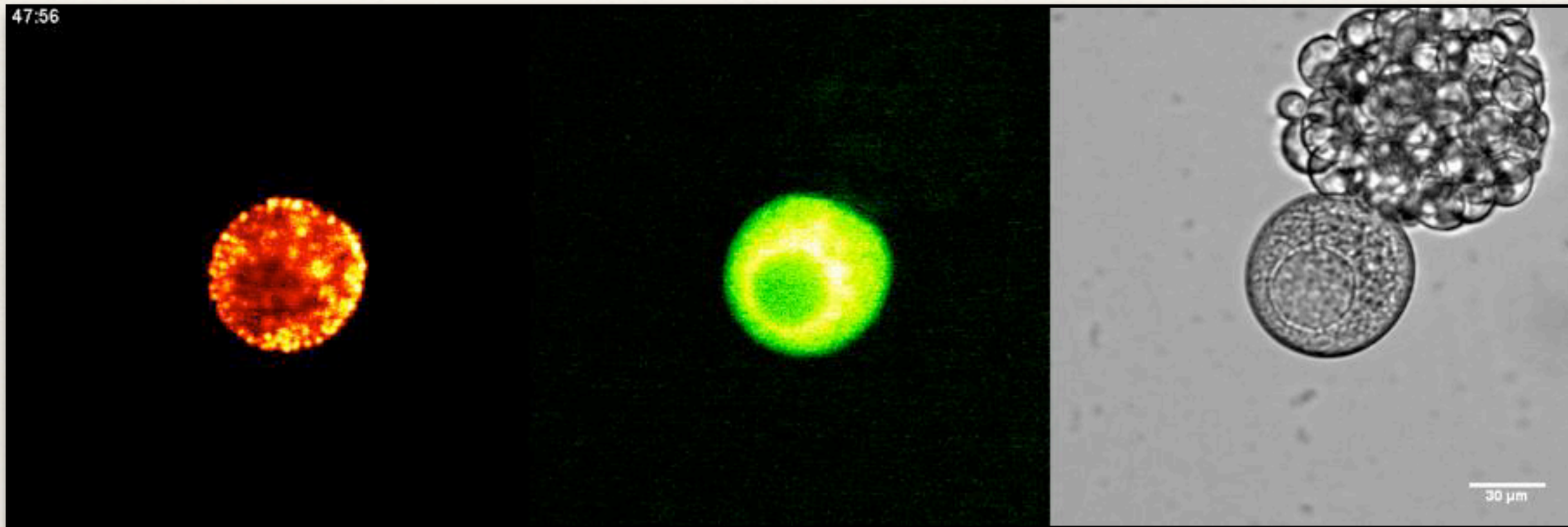
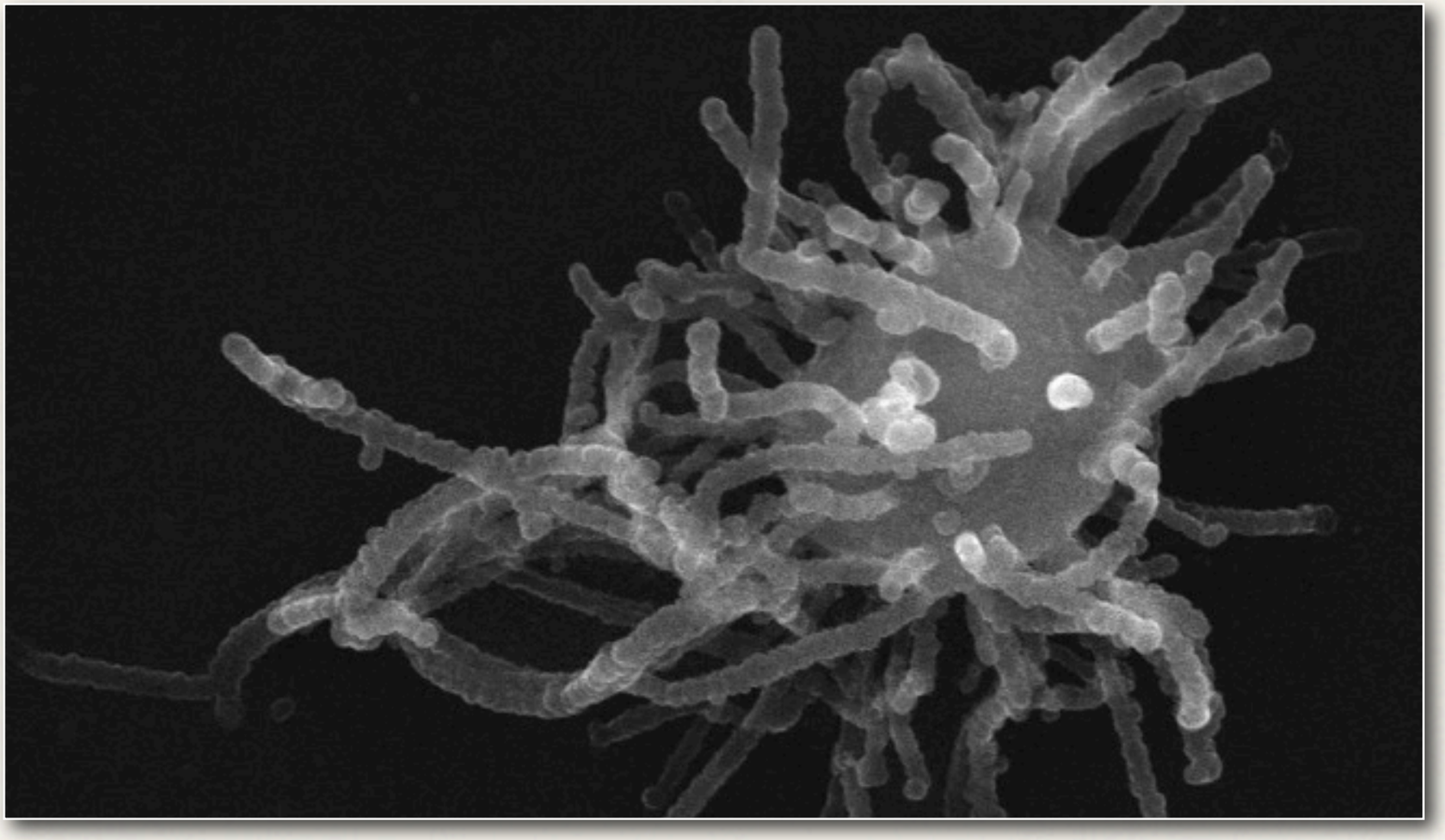


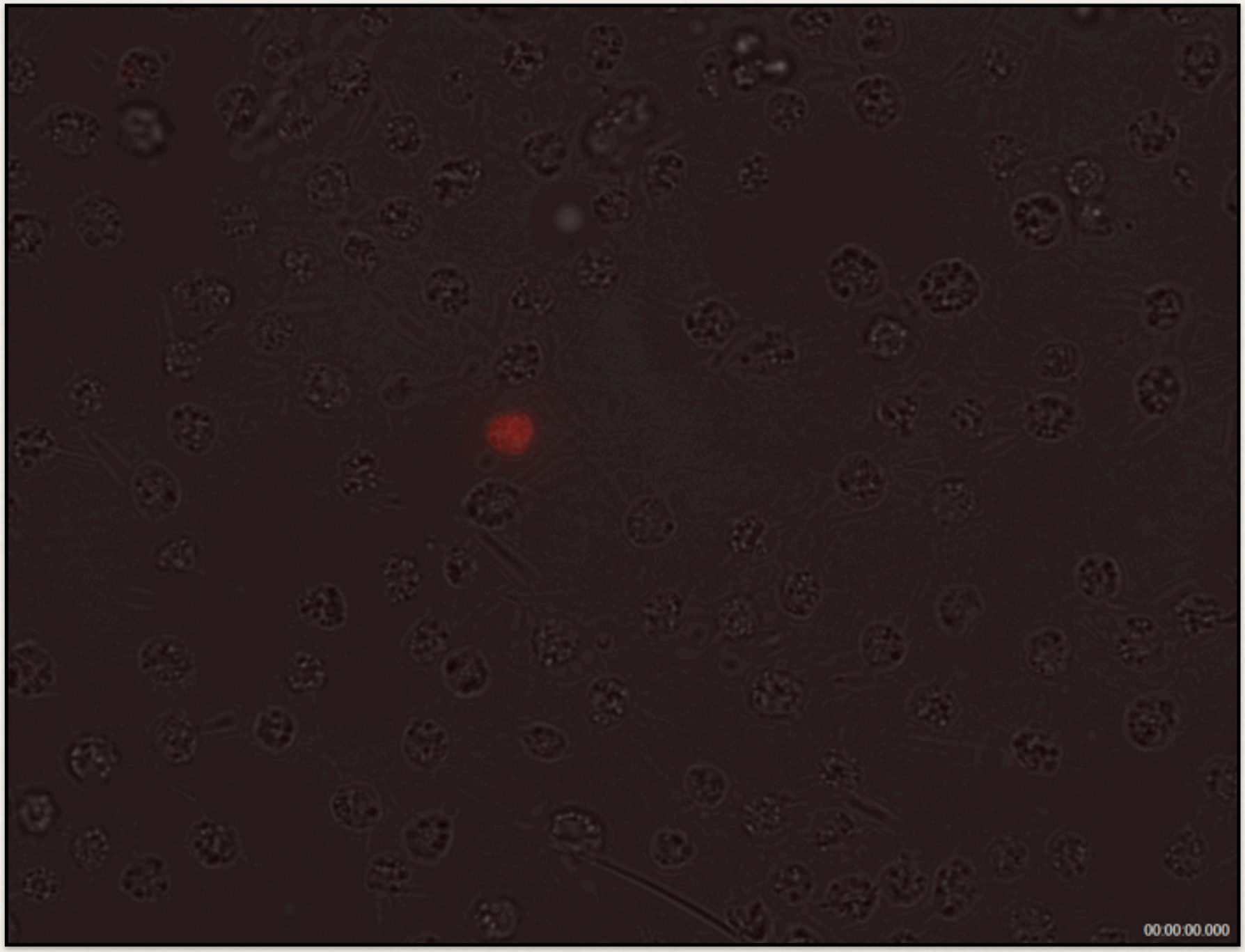
beta-tubulin-promoter *Creolimax*+*S.arctica*-histone +mcherry

beta-tubulin-promoter *Creolimax* +venus

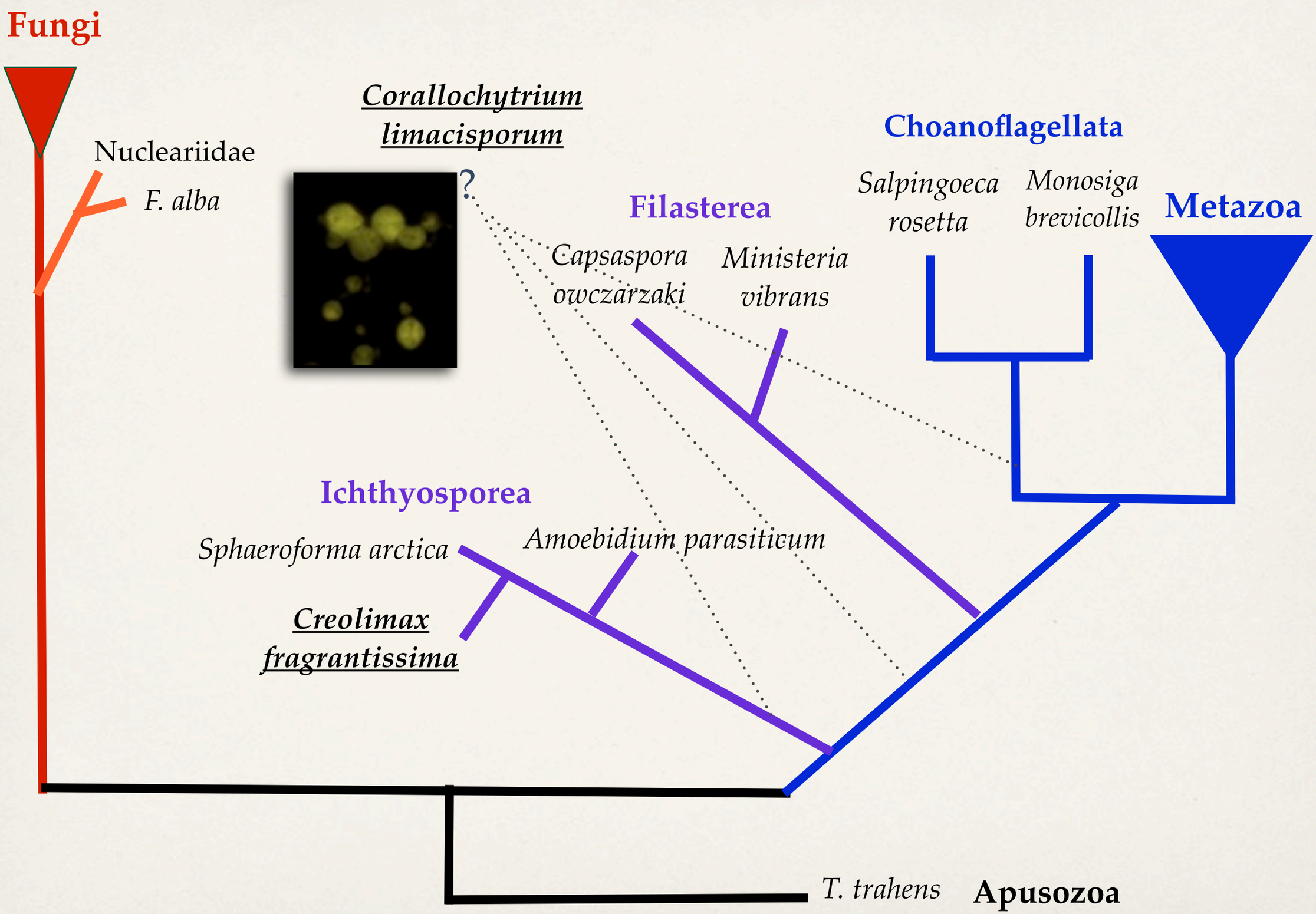
Capsaspora owczarzaki



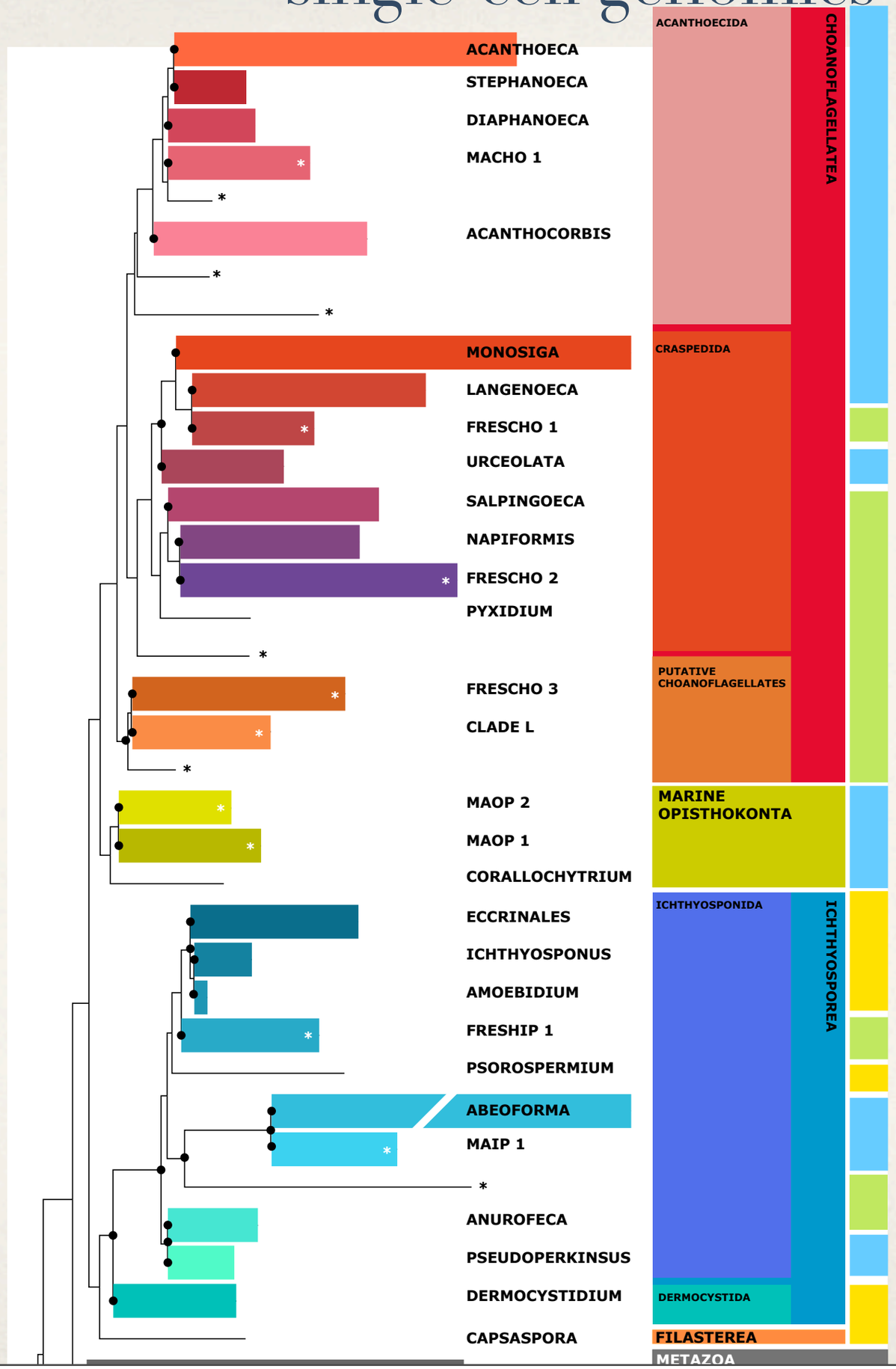
and three months ago...



more genomes?



single-cell genomics



Acknowledgments

http://www.multicellgenome.com

Our collaborators: Broad Institute team, Franz Lang, Andrew J. Roger, Nicole King, Kamran Shalchian-Tabrizi, Maja Adamska, Todd Miller, Gerard Manning, Jose Luis Gómez-Skarmeta, Doujia Pan.

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