Supplementary Figure Legends

All branches in the phylogenetic trees follow the same taxonomic colour-code: Metazoa-Black (e.g. *Homo sapiens, Drosophila melanogaster, Daphnia pulex, Capitella teleta, Lottia gigantea, Nematostella vectensis, Amphimedon queenslandica*), Choanoflagellata-Green (*Monosiga brevicollis, Salpingoeca rosetta*), Filasterea-Red (*Capsaspora owczarzaki*), Fungi-Orange (e.g. *Laccaria bicolor, Saccharomyces cerevisiae, Allomyces macrogynus, Spizellomyces punctatus*), Apusozoa-Yellow (*Thecamonas trahens*), Amoebozoa-Blue (*Acanthamoeba castellanii*, *Dictyostelium discoideum*), Bikonta-Grey (*Arabidopsis thaliana*, *Ostreococcus taurii, Chlamydomonas reindhartii* (Plantae), *Trichomonas vaginalis*, *Naegleria gruberi* (Excavata), *Thalassiosira pseudonana, Tetrahymena thermophila*, *Toxoplasma gondii* (Chromalveolata)).

Fig. S1. Maximum likelihood phylogenetic tree of FERM-domain containing genes, including ERM, Merlin and FERM4A. Alignment is based on the FERM domain and contains 408 amino acid positions. The tree was inferred by RAxML using the WAG+Γ+I model of evolution. The tree is rooted using FERM4A as an outgroup. Statistical support was obtained by RAxML with 100 bootstrap replicates (BV) and Bayesian Posterior Probabilities as inferred with MrBayes. PP. Both values are shown on key branches.

Fig. S2. (A) Schematic alignment of ERM proteins. Pfam domains are highlighted. Key amino acids for binding to the membrane lipid phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), involved in regulation, are highlighted in blue. Key amino acids for actin-binding are highlighted in green. Key amino acids after Turunen et al. 1994 and Niggli et al. 2007. Taxa include Aq (*Amphimedon queenslandica*), Bf (*Branchiostoma floridae*), Co (*Capsaspora owczarzaki*), Dm (*Drosophila melanogaster*), Dp (*Daphnia pulex*), Hs (*Homo sapiens*), Mb (*Monosiga brevicollis*), Nv (*Nematostella vectensis*) and Sr (*Salpingoeca rosetta*). (B) Schematic alignment of fascin proteins, showing the four consecutive fascin protein domains. Key amino acids for protein folding are highlighted in blue. Key amino acids for actin-bundling activity are highlighted in green. Key amino acids after Sedeh et al. (2010) and Zanet et al. (2012).Taxa include Aq (*Amphimedon queenslandica*), Co (*Capsaspora owczarzaki*), Ct (*Capitella teleta*), Dm (*Drosophila melanogaster*), Dp (*Daphnia pulex*), Hs (*Homo sapiens*), Lg (*Lottia gigantea*), Mb (*Monosiga brevicollis*), Nv (*Nematostella vectensis*) and Sr (*Salpingoeca rosetta*).

Fig. S3. Maximum likelihood phylogenetic tree of formin proteins. The alignment is based on the FH2 domain and comprises 308 amino acid positions. The tree was inferred by RAxML using the WAG+Γ+I model of evolution. The tree is rooted using the midpoint-root option. Statistical support was obtained by RAxML with 100 bootstrap replicates (BV) and by BPP. Both values are shown on key branches. Domain architectures for each class are shown.Taxa include Ac (*Acanthamoeba castellanii*), Am (*Allomyces macrogynus*), Aq (*Amphimedon queenslandica*), At (*Arabidopsis thaliana*), Co (*Capsaspora owczarzaki*), Cr (*Chlamydomonas reindhartii*), Ct (*Capitella teleta*), Dd (*Dictyostelium discoideum*), Dm (*Drosophila melanogaster*), Dp

(*Daphnia pulex*), Hs (*Homo sapiens*), Lb (*Laccaria bicolor*), Lg (*Lottia gigantea*), Mb (*Monosiga brevicollis*), Ng (*Naegleria gruberi*), Nv (*Nematostella vectensis*), Ot (*Ostreococcus tauri*), Sc (*Saccharomyces cerevisiae*), Sp (*Spizellomyces punctatus*), Sr (*Salpingoeca rosetta*), Tg (*Toxoplasma gondii*), Tp (*Thalassiosira pseudonana*), Tte (*Tetrahymena thermophila*),Tt (*Thecamonas trahens*), Tv (*Trichomonas vaginalis*). Sequences used for this tree are reported in Table S1.

Fig. S4. Schematic representation of the eukaryotic tree of life showing the distribution of the different formin classes. A black dot indicates the presence of clear homologs, whereas a dashed dot indicates the presence of putative or degenerate homologs. Absence of a dot indicates that an homolog is lacking in that taxon. *Choanoflagellate Diaphanous homologs lack a clear CRIB domain. †Only two *Naegleria gruberi* proteins cluster with Diaphanous, but they don't have a canonical DRF structure (Drf_GBD-Drf_FH3-FH2-DAD). ‡Among other eukaryotes, only the excavates *Naegleria gruberi* and *Trichomonas vaginalis* have bona fide DRF proteins. [4]Defined as having the Drf_GBD-Drf_FH3-FH2-DAD domain architecture.

Fig. S5. ML of gelsolin proteins. The alignment is based on the gelsolin domains and comprises 270 amino acid positions. The tree was inferred by RAxMlLusing the WAG+Γ+I model of evolution. The tree is rooted using the midpoint-root option. Statistical support was obtained by RAxML with 100 bootstrap replicates (BV) and BPP. Both values are shown on key branches. Domain architectures for each class are shown.Taxa include Ac (*Acanthamoeba castellanii*), Am (*Allomyces macrogynus*), Aq (*Amphimedon queenslandica*), At (*Arabidopsis thaliana*), Co (*Capsaspora owczarzaki*), Ct (*Capitella teleta*), Dd (*Dictyostelium discoideum*), Dm (*Drosophila melanogaster*), Dp (*Daphnia pulex*), Hs (*Homo sapiens*), Lg (*Lottia gigantea*), Mb (*Monosiga brevicollis*), Ng (*Naegleria gruberi*), Nv (*Nematostella vectensis*), Sp (*Spizellomyces punctatus*), Sr (*Salpingoeca rosetta*), Tp (*Thalassiosira pseudonana*), Tt (*Thecamonas trahens*). Sequences used are reported in Table S1.

Fig. S6. Maximum likelihood phylogenetic tree of WASP proteins. The alignment comprises 139 amino acid positions. The tree was inferred by RAxML using the WAG+Γ+I model of evolution. The tree is rooted using the midpoint-root option. Statistical support was obtained by RAxML with 100 bootstrap replicates (BV) and BPP. Both values are shown on key branches. Domain architectures for each class are shown. Sequences used are reported in Table S1.

Fig. S7. Maximum likelihood phylogenetic tree of RhoGTPase proteins from unikont taxa. The alignment is based on the Ras domain and comprises 153 amino acid positions. The tree was inferred by RAxML using the WAG+Γ+I model of evolution. The tree is rooted using the MIRO GTPases as an outgroup. Statistical support was obtained by RAxML with 100 bootstrap replicates (BV) and BPP. Both values are shown on key branches. Domain architectures for each class are shown. Taxa include Ac (*Acanthamoeba castellanii*), Am (*Allomyces macrogynus*), Aq (*Amphimedon queenslandica*), Co (*Capsaspora owczarzaki*), Ct (*Capitella teleta*), Dd (*Dictyostelium discoideum*), Dm (*Drosophila melanogaster*), Dp (*Daphnia pulex*), Hs (*Homo sapiens*), Lb (*Laccaria bicolor*), Lg (*Lottia gigantea*), Mb (*Monosiga brevicollis*), Nv (*Nematostella*

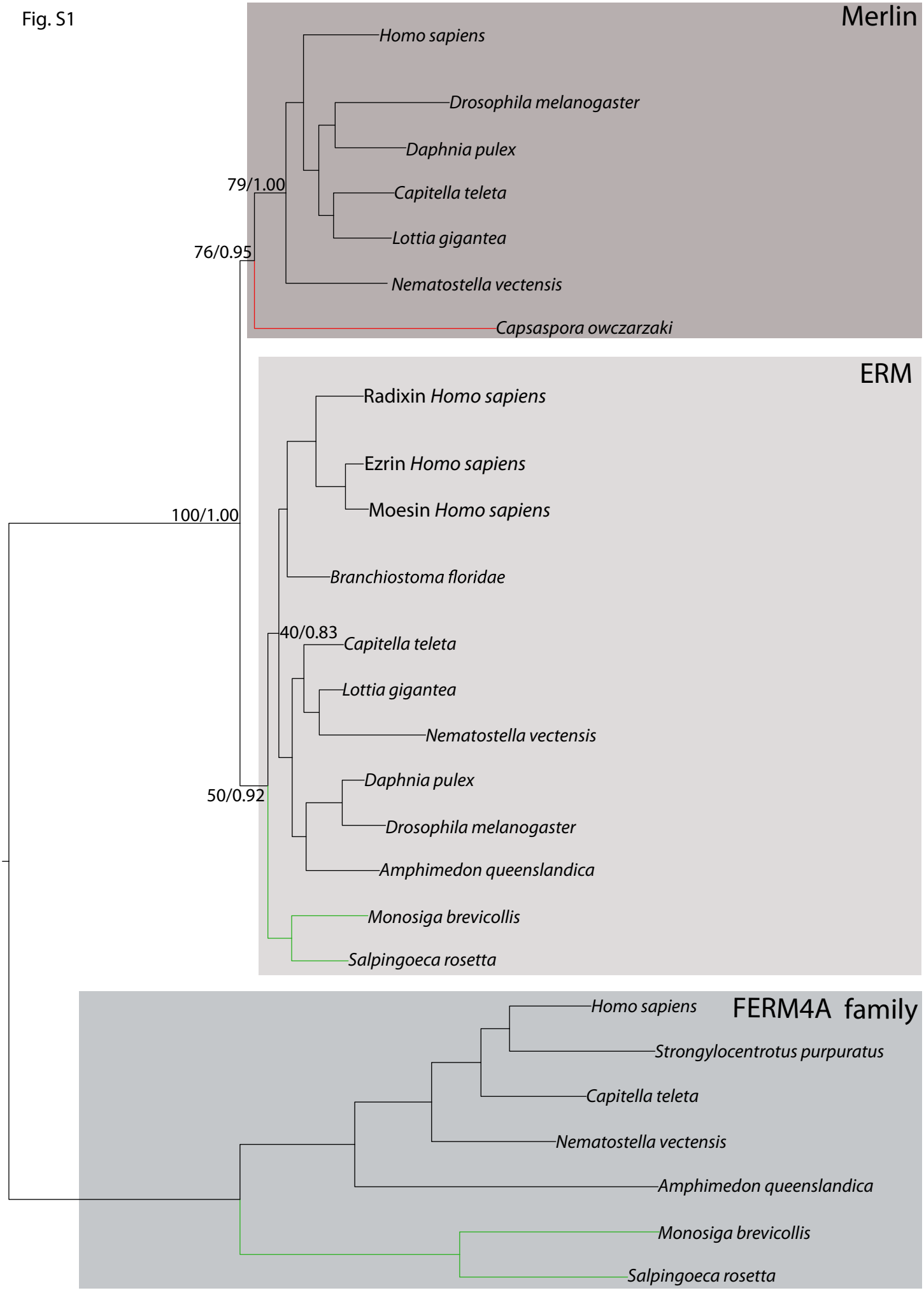
vectensis), Sc (*Saccharomyces cerevisiae*), Sp (*Spizellomyces punctatus*), Sr (*Salpingoeca rosetta*), Tt (*Thecamonas trahens*). Newly classified sequences are reported in Table S1.

Fig. S8. Maximum likelihood phylogenetic tree of abLIM and other LIM-domain containing proteins. The alignment comprises 239 amino acid positions. The tree was inferred by RAxML using the WAG+Γ+I model of evolution. The tree is rooted using the midpoint-root option. Statistical support was obtained by RAxML with 100 bootstrap replicates (BV) and BPP. Both values are shown on key branches. Sequences used are reported in Table S1.

Fig. S9. Maximum likelihood phylogenetic tree of IMD-domain containing proteins. The alignment is based on the IMD domain and comprises 180 amino acid positions. The tree was inferred by RAxML using the WAG+Γ+I model of evolution. The tree is rooted using the midpoint-root option. Statistical support was obtained by RAxML with 100 bootstrap replicates (BV) and BPP. Both values are shown on key branches. Domain architectures for each class are shown. Sequences used are reported in Table S1.

Table S1. Sequences used in study.

Fig. S1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76/0.95
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Capsaspora owczarzaki
ERM
Radixin Homo sapiens
Ezrin Homo sapiens
100/1.00
Moesin Homo sapiens
Branchiostoma floridae
40/0.83
Capitella teleta
Lottia gigantea
Nematostella vectensis
Daphnia pulex
50/0.92
Drosophila melanogaster
Amphimedon queenslandica
Monosiga brevicollis
Salpingoeca rosetta
FERM4A family
Homo sapiens
Strongylocentrotus purpuratus
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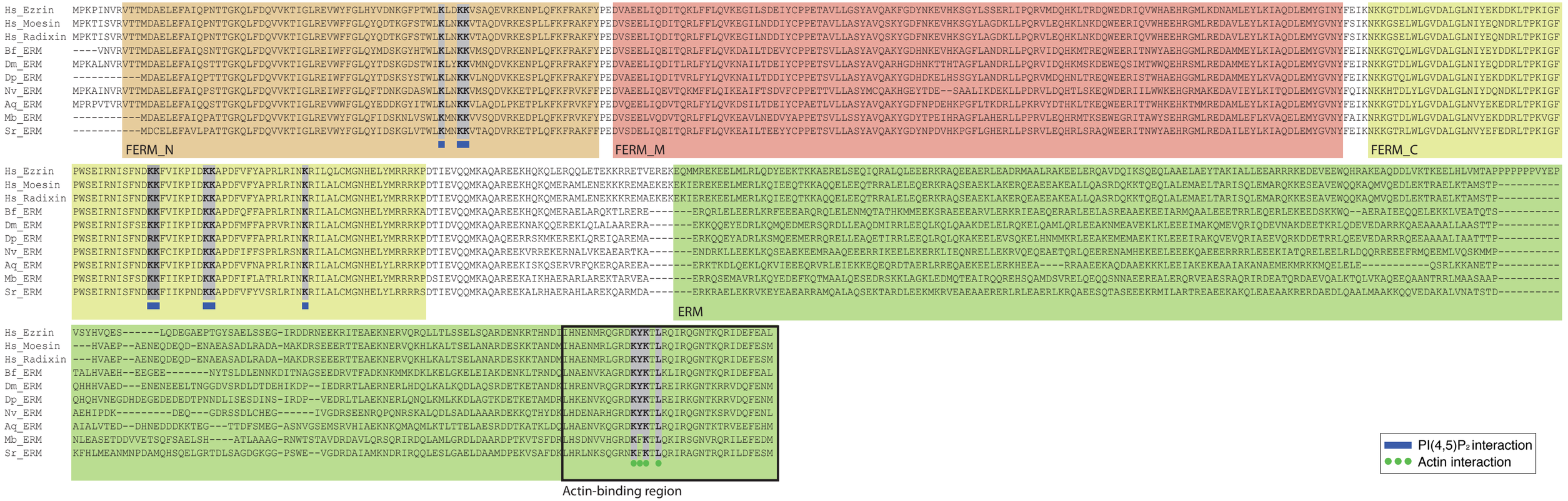
Nematostella vectensis
Amphimedon queenslandica
Monosiga brevicollis
Salpingoeca rosetta
0.4

A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Hs_Moesin
Hs_Radixin
Bf_ERM
Dm_ERM
Dp_ERM
Nv_ERM
Aq_ERM
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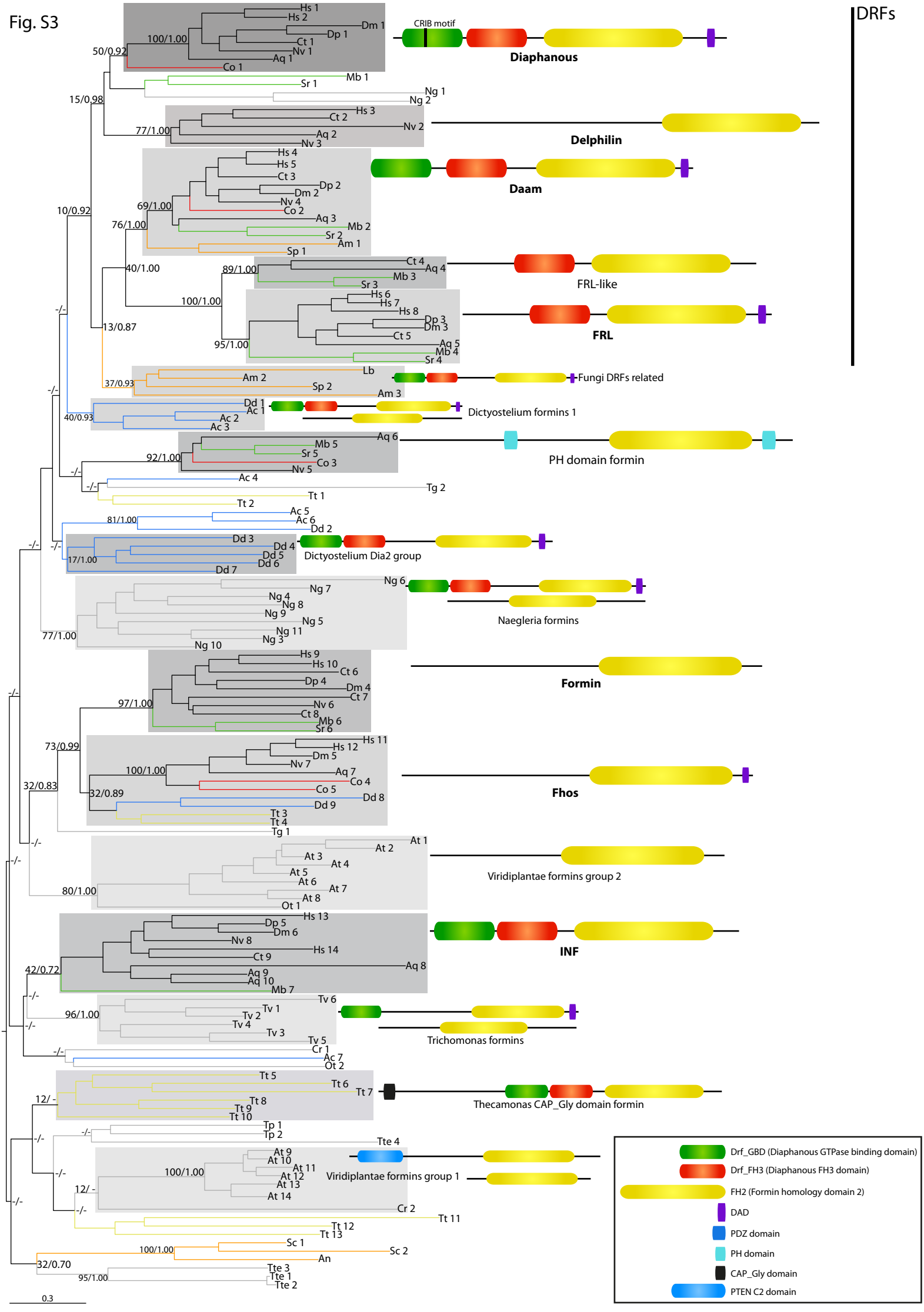
FERM_N
FERM_M
FERM_C
ERM
Actin-binding region
PI(4,5)P₂ interaction
Actin interaction

B

Hs
Dm
Dp
Lg
Ct
Nv
Aq
Sr
Sr_2
Mb
Mb_2
Co
Fascin
Fascin
Fascin
Fascin
Essential for protein folding
Essential for bundling activity

Fig. S2

Fig. S3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Delphilin
Daam
FRL-like
FRL
Fungi DRFs related
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PH domain formin
Dictyostelium Dia2 group
Naegleria formins
Formin
Fhos
Viridiplantae formins group 2
INF
Trichomonas formins
Thecamonas CAP_Gly domain formin
Viridiplantae formins group 1
Drf_GBD (Diaphanous GTPase binding domain)
Drf_FH3 (Diaphanous FH3 domain)
FH2 (Formin homology domain 2)
DAD
PDZ domain
PH domain
CAP_Gly domain
PTEN C2 domain
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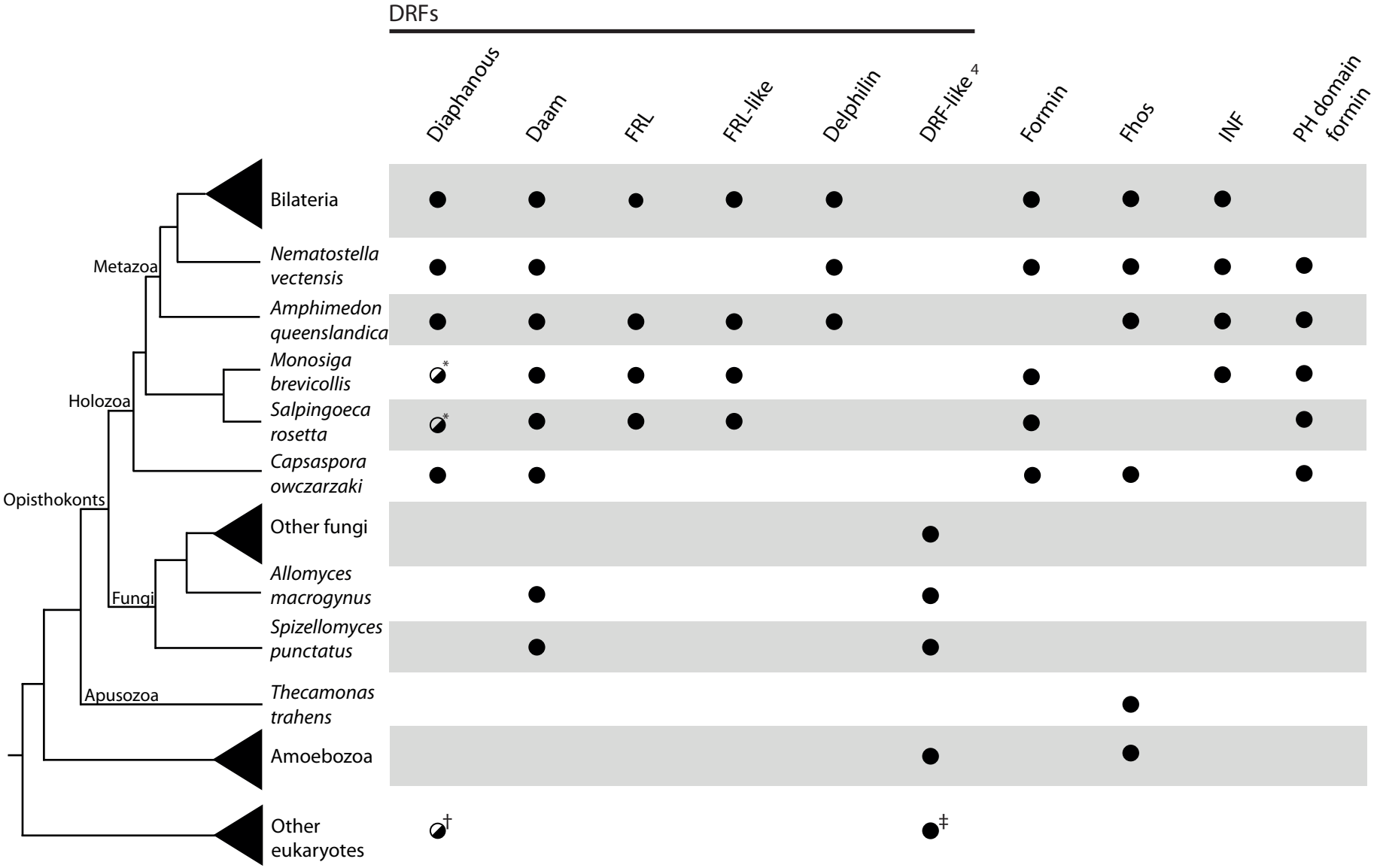
Fig. S4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FRL
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Delphilin
DRF-like 4
Formin
Fhos
INF
PH domain formin
Bilateria
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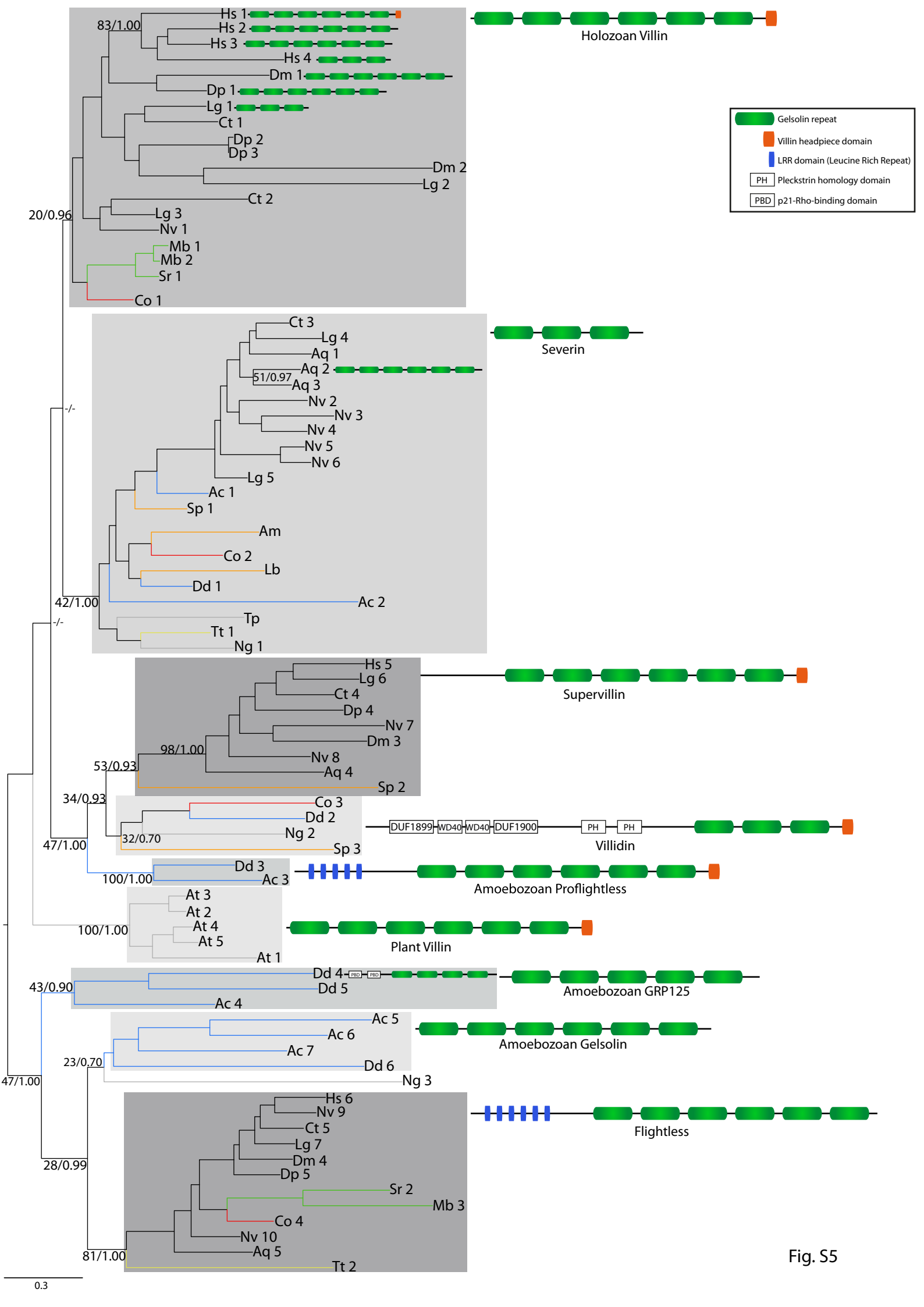
Amphimedon queenslandica
Monosiga brevicollis
Salpingoeca rosetta
Capsaspora owczarzaki
Other fungi
Allomyces macrogynus
Spizellomyces punctatus
Thecamonas trahens
Amoebozoa
Other eukaryotes
Metazoa
Holozoa
Opisthokonts
Fungi
Apusozoa
*
*
†
‡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Hs 4
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Dm 2
Lg 2
Ct 2
Lg 3
Nv 1
Mb 1
Mb 2
Sr 1
Co 1
83/1.00
20/0.96
Holozoan Villin
Gelsolin repeat
Villin headpiece domain
LRR domain (Leucine Rich Repeat)
PH Pleckstrin homology domain
PBD p21-Rho-binding domain
Ct 3
Lg 4
Aq 1
Aq 2
Aq 3
51/0.97
Nv 2
Nv 3
Nv 4
Nv 5
Nv 6
Lg 5
Ac 1
Sp 1
Am
Co 2
Lb
Dd 1
Ac 2
Tp
Tt 1
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42/1.00
-/-
Severin
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Lg 6
Ct 4
Dp 4
Nv 7
Dm 3
Nv 8
Aq 4
Sp 2
98/1.00
53/0.93
Supervillin
34/0.93
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Dd 2
Ng 2
Sp 3
32/0.70
47/1.00
DUF1899
WD40
WD40
DUF1900
PH
PH
Villidin
Dd 3
Ac 3
100/1.00
Amoebozoan Proflightless
At 3
At 2
At 4
At 5
At 1
100/1.00
Plant Villin
Dd 4
Dd 5
Ac 4
43/0.90
Amoebozoan GRP125
Ac 5
Ac 6
Ac 7
Dd 6
23/0.70
Amoebozoan Gelsolin
Ng 3
47/1.00
Hs 6
Nv 9
Ct 5
Lg 7
Dm 4
Dp 5
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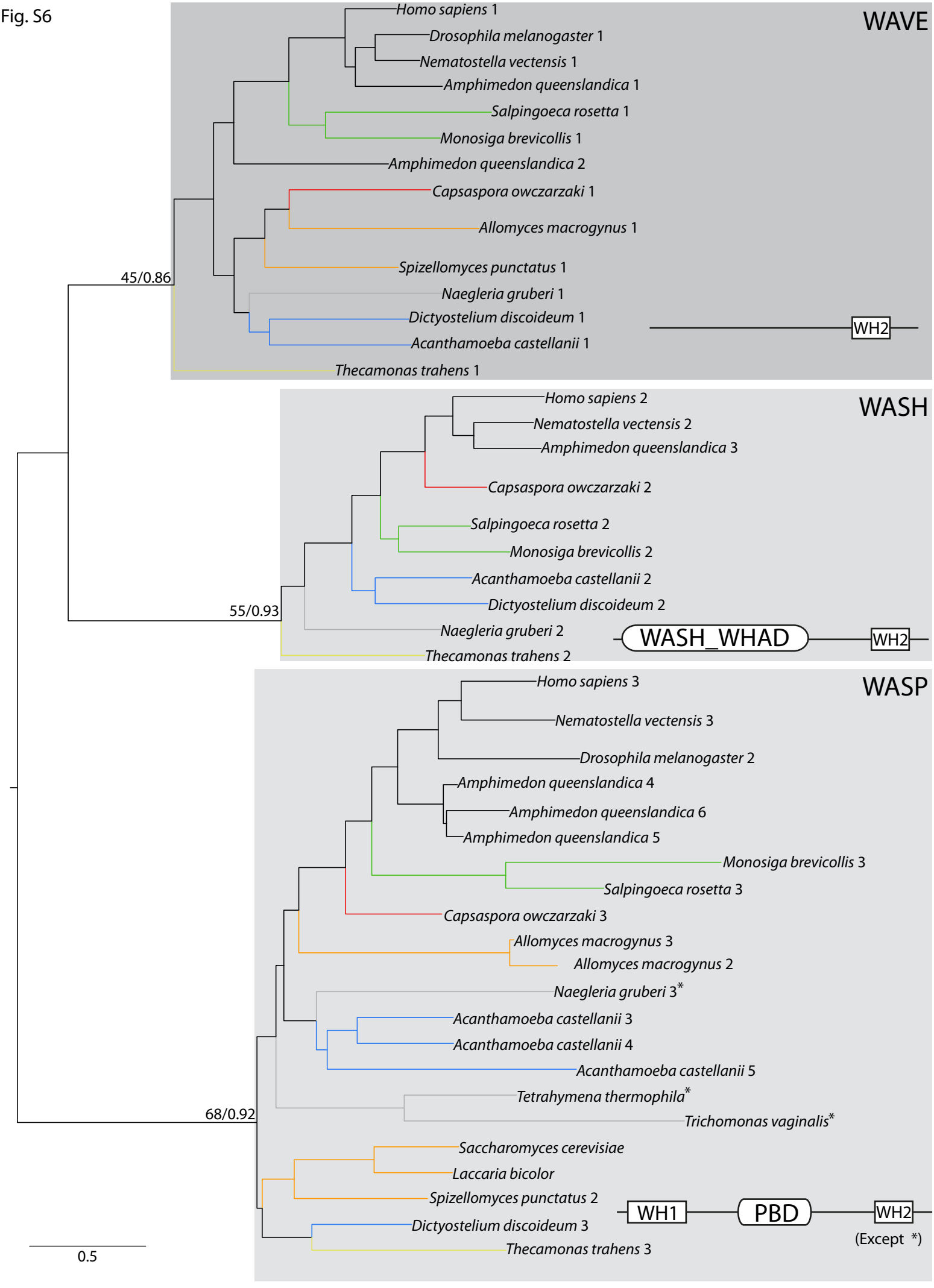
Fig. S5

Fig. S6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Amphimedon queenslandica 2
Capsaspora owczarzaki 1
Allomyces macrogynus 1
Spizellomyces punctatus 1
Naegleria gruberi 1
Dictyostelium discoideum 1
Acanthamoeba castellanii 1
Thecamonas trahens 1
WH2
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WASH
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Capsaspora owczarzaki 2
Salpingoeca rosetta 2
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Acanthamoeba castellanii 2
Dictyostelium discoideum 2
Naegleria gruberi 2
Thecamonas trahens 2
WASH_WHAD
WH2
55/0.93
WASP
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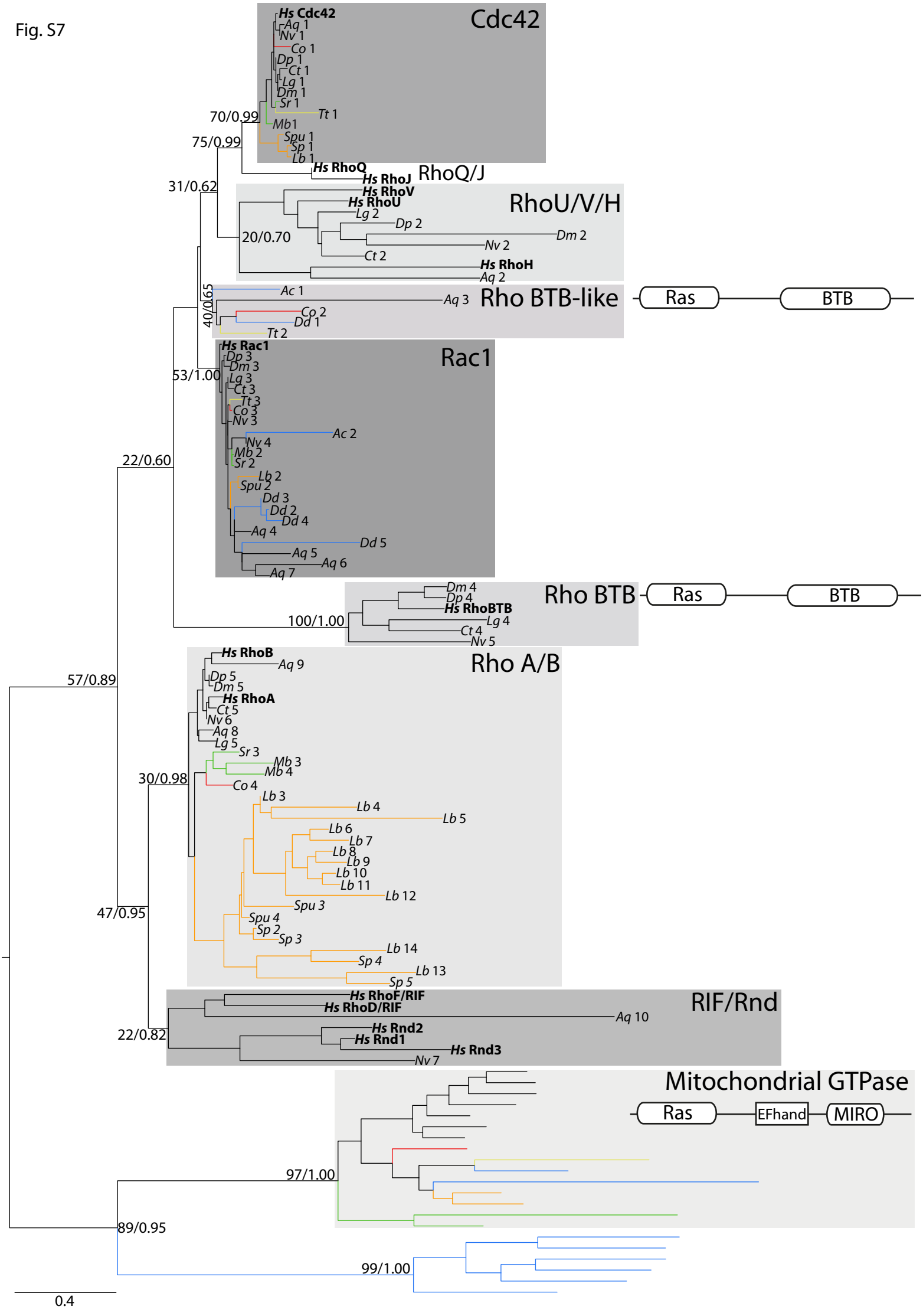
Nematostella vectensis 3
Drosophila melanogaster 2
Amphimedon queenslandica 4
Amphimedon queenslandica 6
Amphimedon queenslandica 5
Monosiga brevicollis 3
Salpingoeca rosetta 3
Capsaspora owczarzaki 3
Allomyces macrogynus 3
Allomyces macrogynus 2
Naegleria gruberi 3*
Acanthamoeba castellanii 3
Acanthamoeba castellanii 4
Acanthamoeba castellanii 5
Tetrahymena thermophila*
Trichomonas vaginalis*
68/0.92
Saccharomyces cerevisiae
Laccaria bicolor
Spizellomyces punctatus 2
Dictyostelium discoideum 3
Thecamonas trahens 3
WH1
PBD
WH2
(Except *)
0.5

Fig. S7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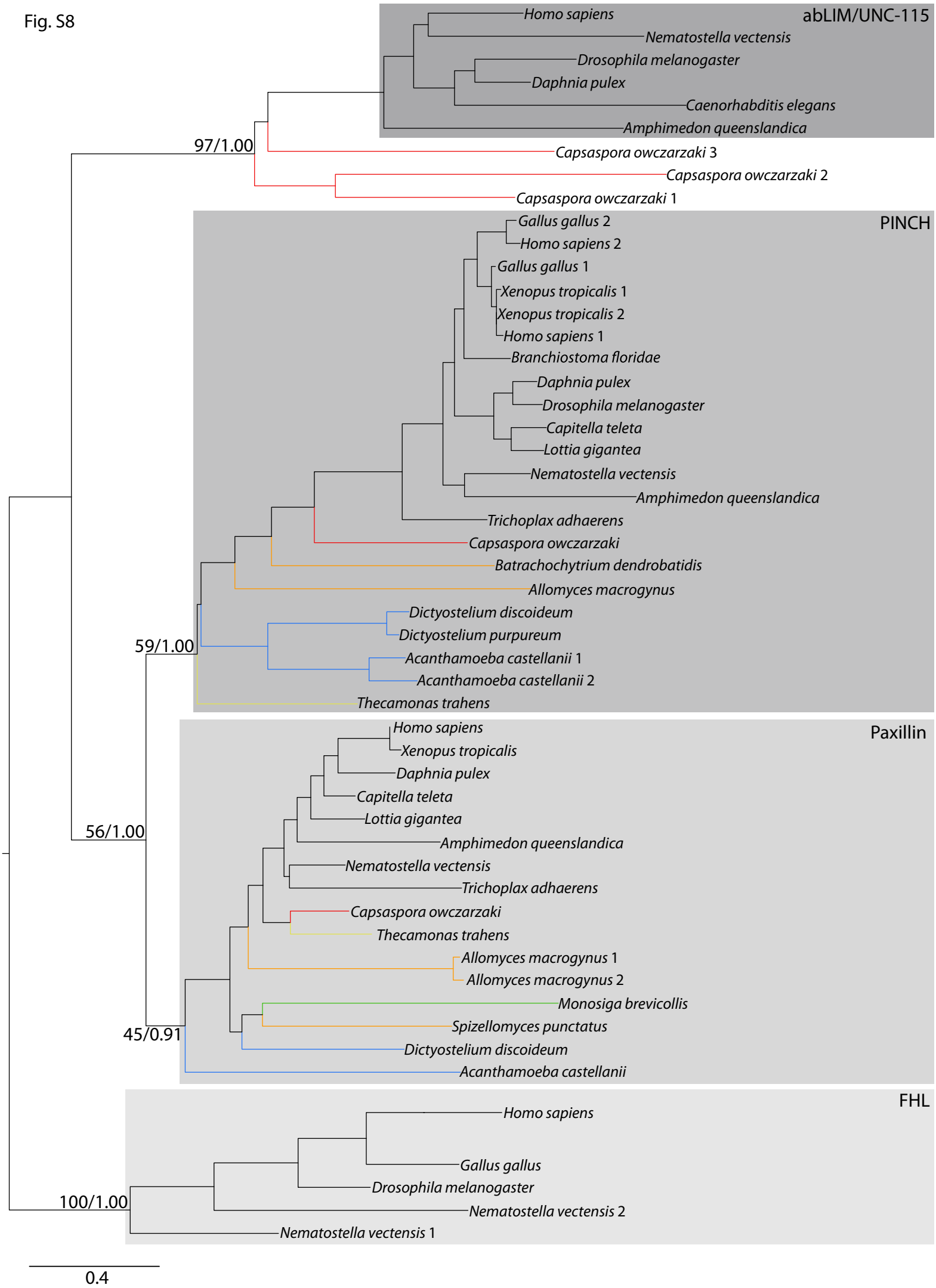
Sp 1
Lb 1
70/0.99
75/0.99
Hs RhoQ
Hs RhoJ
RhoQ/J
31/0.62
RhoU/V/H
Hs RhoV
Hs RhoU
Lg 2
Dp 2
Dm 2
Nv 2
Ct 2
Hs RhoH
Aq 2
20/0.70
Rho BTB-like
Ac 1
Aq 3
Co 2
Dd 1
Tt 2
40/0.65
Ras
BTB
Rac1
Hs Rac1
Dp 3
Dm 3
Lg 3
Ct 3
Tt 3
Co 3
Nv 3
Ac 2
Nv 4
Mb 2
Sr 2
Lb 2
Spu 2
Dd 3
Dd 2
Dd 4
Aq 4
Dd 5
Aq 5
Aq 6
Aq 7
53/1.00
22/0.60
Rho BTB
Dm 4
Dp 4
Hs RhoBTB
Lg 4
Ct 4
Nv 5
100/1.00
Rho A/B
Hs RhoB
Aq 9
Dp 5
Dm 5
Hs RhoA
Ct 5
Nv 6
Aq 8
Lg 5
Sr 3
Mb 3
Mb 4
Co 4
Lb 3
Lb 4
Lb 5
Lb 6
Lb 7
Lb 8
Lb 9
Lb 10
Lb 11
Lb 12
Spu 3
Spu 4
Sp 2
Sp 3
Lb 14
Sp 4
Lb 13
Sp 5
30/0.98
57/0.89
47/0.95
RIF/Rnd
Hs RhoF/RIF
Hs RhoD/RIF
Aq 10
Hs Rnd2
Hs Rnd1
Hs Rnd3
Nv 7
22/0.82
Mitochondrial GTPase
Ras
EFhand
MIRO
97/1.00
89/0.95
99/1.00
0.4

Fig. S8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Amphimedon queenslandica
97/1.00
Capsaspora owczarzaki 3
Capsaspora owczarzaki 2
Capsaspora owczarzaki 1
PINCH
Gallus gallus 2
Homo sapiens 2
Gallus gallus 1
Xenopus tropicalis 1
Xenopus tropicalis 2
Homo sapiens 1
Branchiostoma floridae
Daphnia pulex
Drosophila melanogaster
Capitella teleta
Lottia gigantea
Nematostella vectensis
Amphimedon queenslandica
Trichoplax adhaerens
Capsaspora owczarzaki
Batrachochytrium dendrobatidis
Allomyces macrogynus
Dictyostelium discoideum
Dictyostelium purpureum
59/1.00
Acanthamoeba castellanii 1
Acanthamoeba castellanii 2
Thecamonas trahens
Paxillin
Homo sapiens
Xenopus tropicalis
Daphnia pulex
Capitella teleta
Lottia gigantea
56/1.00
Amphimedon queenslandica
Nematostella vectensis
Trichoplax adhaerens
Capsaspora owczarzaki
Thecamonas trahens
Allomyces macrogynus 1
Allomyces macrogynus 2
Monosiga brevicollis
Spizellomyces punctatus
45/0.91
Dictyostelium discoideum
Acanthamoeba castellanii
FHL
Homo sapiens
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100/1.00
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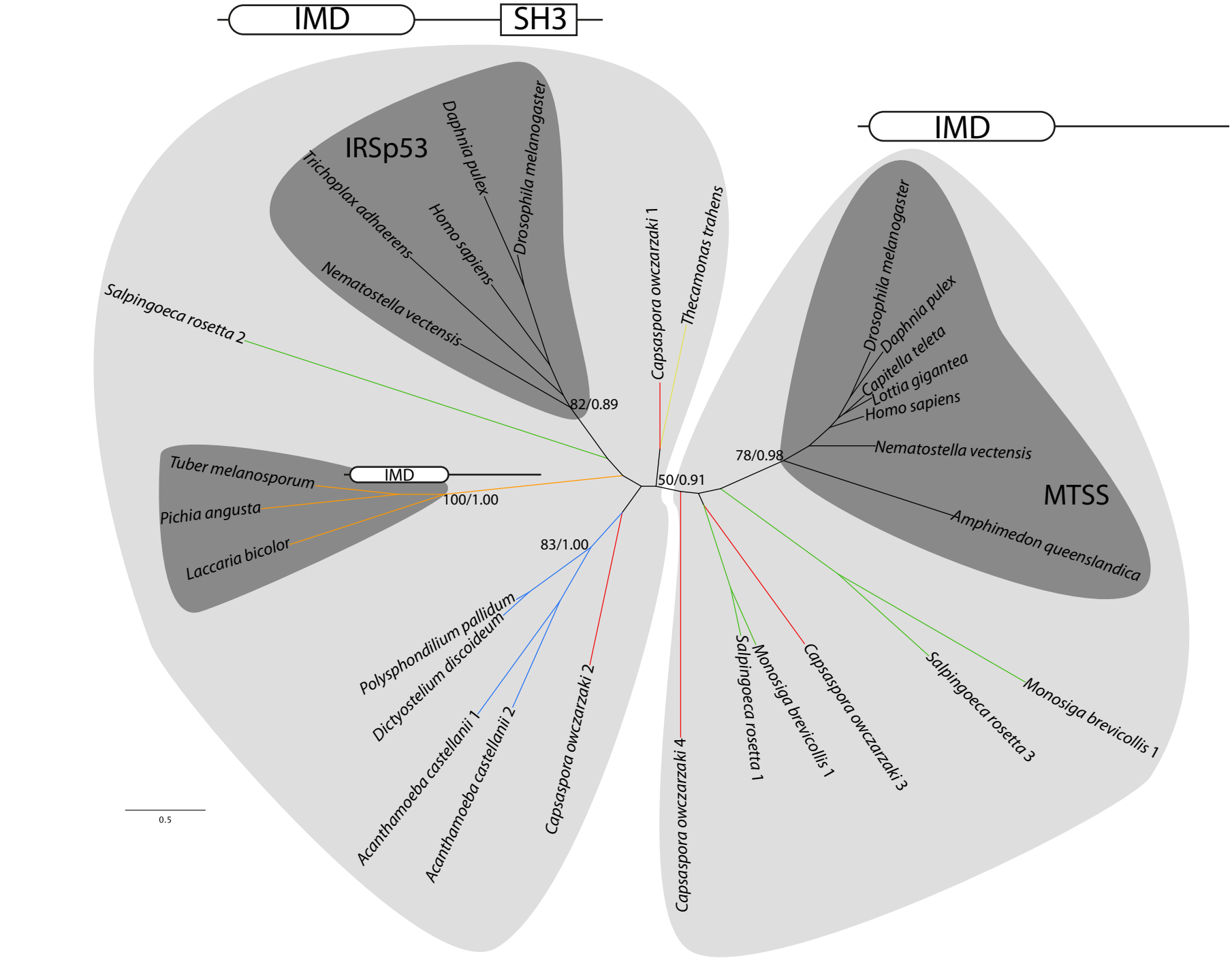
0.4

Fig. S9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Thecamonas trahens
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IMD
Tuber melanosporum
Pichia angusta
Laccaria bicolor
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50/0.91
83/1.00
Polysphondilium pallidum
Dictyostelium discoideum
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Acanthamoeba castellanii 2
Capsaspora owczarzaki 2
IMD
Drosophila melanogaster
Daphnia pulex
Capitella teleta
Lottia gigantea
Homo sapiens
Nematostella vectensis
78/0.98
MTSS
Amphimedon queenslandica
Capsaspora owczarzaki 4
Salpingoeca rosetta 1
Monosiga brevicollis 1
Capsaspora owczarzaki 3
Salpingoeca rosetta 3
Monosiga brevicollis 1
0.5