

Title: Supplement to “Phylogenetics identifies two eumetazoan TRPM clades and an 8th TRP family, TRP soromelastatin (TRPS)”

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Supplementary Materials:

Supplemental Tables

| Phylum | Species | Source |
|-----------------|---|---------------------------|
| Cnidaria | <i>Aurelia</i> (moon jelly) | (Gold, et al. 2019) |
| | <i>Acropora digitifera</i> (coral) | (Shinzato, et al. 2011) |
| | <i>Acropora tenuis</i> (coral) | (Voolstra, et al. 2015) |
| | <i>Aiptasia</i> (sea anemone) | (Baumgarten, et al. 2015) |
| | <i>Amplexidiscus fenestrafer</i> (elephant ear anemone) | (Wang, et al. 2017) |
| | <i>Fungia</i> spp. (coral) | (Voolstra, et al. 2015) |
| | <i>Galaxea fascicularis</i> (galaxy coral) | (Voolstra, et al. 2015) |
| | <i>Goniastrea aspera</i> (stony coral) | (Voolstra, et al. 2015) |
| | <i>Pocillopora damicornis</i> (lace coral) | (Cunning, et al. 2018) |
| | <i>Porites lutea</i> (small polyp stony coral) | (Voolstra, et al. 2015) |
| | <i>Stylophora pistillata</i> (hood coral) | (Voolstra, et al. 2017) |
| Xenacoelomorpha | <i>Hofstenia miamia</i> (three-banded panther worm) | (Gehrke, et al. 2019) |
| | <i>Praesagittifera naikaiensis</i> (acoel flatworm) | (Arimoto, et al. 2019) |
| Hemichordata | <i>Ptychodera flava</i> | (Simakov, et al. 2015) |
| Chordata | <i>Petromyzon marinus</i> (sea lamprey) | (Smith, et al. 2013) |
| | <i>Eptatretus burger</i> (inshore hagfish) | PRJEB21290 |
| | <i>Chiloscyllium punctatum</i> (brownband bamboo shark) | (Hara, et al. 2018) |
| | <i>Rhincodon typus</i> (whale shark) | (Hara, et al. 2018) |
| | <i>Scyliorhinus torazame</i> (cloudy catshark) | (Hara, et al. 2018) |
| | <i>Carcharodon carcharias</i> (white shark) | (Marra, et al. 2019) |
| Nemertea | <i>Notospermus geniculatus</i> (ribbon worm) | (Luo, et al. 2018) |
| Phoronida | <i>Phoronis australis</i> (horseshoe worm) | (Luo, et al. 2018) |

Table S1. Genomes added to the initial NCBI-based sequence database.

Supplemental Figures

Fig. S1. TRPS constitutes a distinct family of TRP channel. Maximum likelihood tree for TRPM, TRPS (ced-11-like), TRPN, and TRPC sequences, for all those species in initial database that had a ced-11-like sequence. UFboot confidence is indicated by red-green color scale, with major branch values listed.

Fig. S2. Principal component analyses of pairwise sequence identity for alignment of TRPC, TRPN, TRPM, and ced-11-like (TRPS) sequences show 4 distinct clusters. Alignment restricted to transmembrane segments. 3-dimensional PCA plot extracted from Jalview and plotted in two transformed, arbitrary dimensions.

Fig. S3. Maximum likelihood tree for TRPM, TRPS (ced-11-like), TRPN, and TRPC sequences for all those species in initial database that had a ced-11-like sequence, but excluding Xenacoelomorpha and Cnidaria in order to test for effects of long-branch attraction. UFboot confidence is indicated by red-green color scale, with major branch values listed.

Fig. S4. Graph Splitting tree for TRPM, TRPS (ced-11-like), TRPN, and TRPC sequences for all those species in initial database that had a ced-11-like sequence, in order to test for effects of long-branch attraction. Edge perturbation (EP) confidence is indicated by red-green color scale, with major branch values listed.

Fig. S5. Reconciled and rearranged maximum likelihood TRPS phylogram with duplication sites (red) and UFboot branch support values listed. Branches without support values were rearranged (<95 UFboot) by NOTUNG. Individual expansion events occurred in molluscs, nematodes, tardigrades, and chelicerates. While *S. maritima* has 2 TRPS genes, this was not assumed to represent a taxon-wide duplication event due to it being the sole representative of Myriapoda.

Fig. S6. Two hypotheses concerning the loss of TRPS in Ambulacraria and Olfactores. While a monophyletic Deuterostomia has been well supported for some time (left), recent work has suggested that Ambulacraria may be a sister clade to Xenacoelomorpha (Philippe, et al. 2019) (right).

Fig. S7. Arthropod TRPS diversification, extracted from **Fig. S3**. The duplication in arthropod TRPS appears restricted to Chelicerata, and the simplest hypothesis concerning TRPS loss is that it was lost early in the evolution of Pancrustacea.

Fig. S8. Reconciled and rearranged maximum likelihood tree of TRPM sequences from Ambulacraria (bold), with TRPM sequences from Cnidaria, Xenacoelomorpha, human, and *Drosophila* for context, and TRPS sequences for rooting.

Fig. S9. Reconciled and rearranged maximum likelihood tree of TRPM sequences from Chordata (bold, excluding ray-finned fish), with TRPM sequences from Cnidaria, Xenacoelomorpha, human, and *Drosophila* for context, and TRPS sequences for rooting.

Fig. S10. Reconciled and rearranged maximum likelihood tree of TRPM sequences from Lophotrochozoa (bold), with TRPM sequences from Cnidaria, Xenacoelomorpha, human, and *Drosophila* for context, and TRPS sequences for rooting.

Fig. S11. Reconciled and rearranged maximum likelihood tree of TRPM sequences from Priapulida and Nematoda (bold), with TRPM sequences from Cnidaria, Xenacoelomorpha, human, and *Drosophila* for context, and TRPS sequences for rooting.

Fig. S12. Reconciled and rearranged maximum likelihood tree of TRPM sequences from Priapulida and Arthropoda (bold), with TRPM sequences from Cnidaria, Xenacoelomorpha, human, and *Drosophila* for context, and TRPS sequences for rooting.

Fig. S13. Reconciled and rearranged maximum likelihood tree of TRPM sequences from Ambulacraria (bold) as in **Fig. S7**, with Xenacoelomorpha removed. Ambulacrarians have both α - and β TRPMs, and saw independent expansion of β TRPM.

Fig. S14. Reconciled and rearranged maximum likelihood tree of TRPM sequences from Chordata (bold, excluding ray-finned fish) as in **Fig. S8**, with Xenacoelomorpha. Chordates have α - and β TRPMs, and both α - and β TRPMs expanded in vertebrates.

Fig. S15. Reconciled and rearranged maximum likelihood tree of TRPM sequences from Lophotrochozoa (bold) as in **Fig. S9**, with Xenacoelomorpha removed. Lophotrochozoans have both α - and β TRPMs, and saw independent expansion of β TRPM.

Fig. S16. Reconciled and rearranged maximum likelihood tree of TRPM sequences from Priapulida and Nematoda (bold) as in **Fig. S10**, with Xenacoelomorpha removed. Nematodes likely have both α - and β TRPMs, and likely saw expansion in both. However, given that *Toxocara canis* TRPMs are the only members of the nematode α TRPM clade, it is unclear if the α expansion was species-specific.

Fig. S17. Reconciled and rearranged maximum likelihood tree of TRPM sequences from Priapulida and Arthropoda (bold) as in **Fig. S11**, with Xenacoelomorpha removed. The majority of Arthropods only have α TRPM (many having only a single copy), but several chelicerates and crustaceans may have channels more distantly related to human, priapulid, and cnidarian β TRPMs.

Fig. S18. Vertebrate TRPM8 was independently lost in most vertebrate lineages, surviving only in the lobe-finned fish lineage (including tetrapods). Maximum likelihood tree of TRPM sequences from Chordata (including lancelets, tunicates, agnathans, sharks, coelacanth, tetrapods, and ray-finned fish), rooted in TRPS, with the 8 vertebrate TRPM clades labeled. Unlabeled clades are from invertebrate species. UFboot confidence is indicated by red-green color scale. Black indicates rearranged branches (<95 UFBoot).

Supplemental Data (separate file)

Data S1. Table indicating presence of Nudix and SLOG domains across the TRPS sequence database.

Data S2. GO terms for *C. elegans* TRPS (ced-11) co-expression gene network, with enriched term highlighted.

Data S3. GO terms for *C. elegans* TRPM (gon-2, gtl-1, gtl-2) co-expression gene network, with enriched term highlighted.

Additional Data Deposition

The TRPN, TRPC, TRPM, and TRPS sequence databases have been deposited on Dryad in the FASTA format (doi:10.5061/dryad.kwh70rz03).

Supplemental References

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Tree scale: 1

TRP Family

- TRPS (sorumelastatin)
- TRPM (melastatin)
- TRPN (no mechanoreceptor potential C)
- TRPC (canonical)

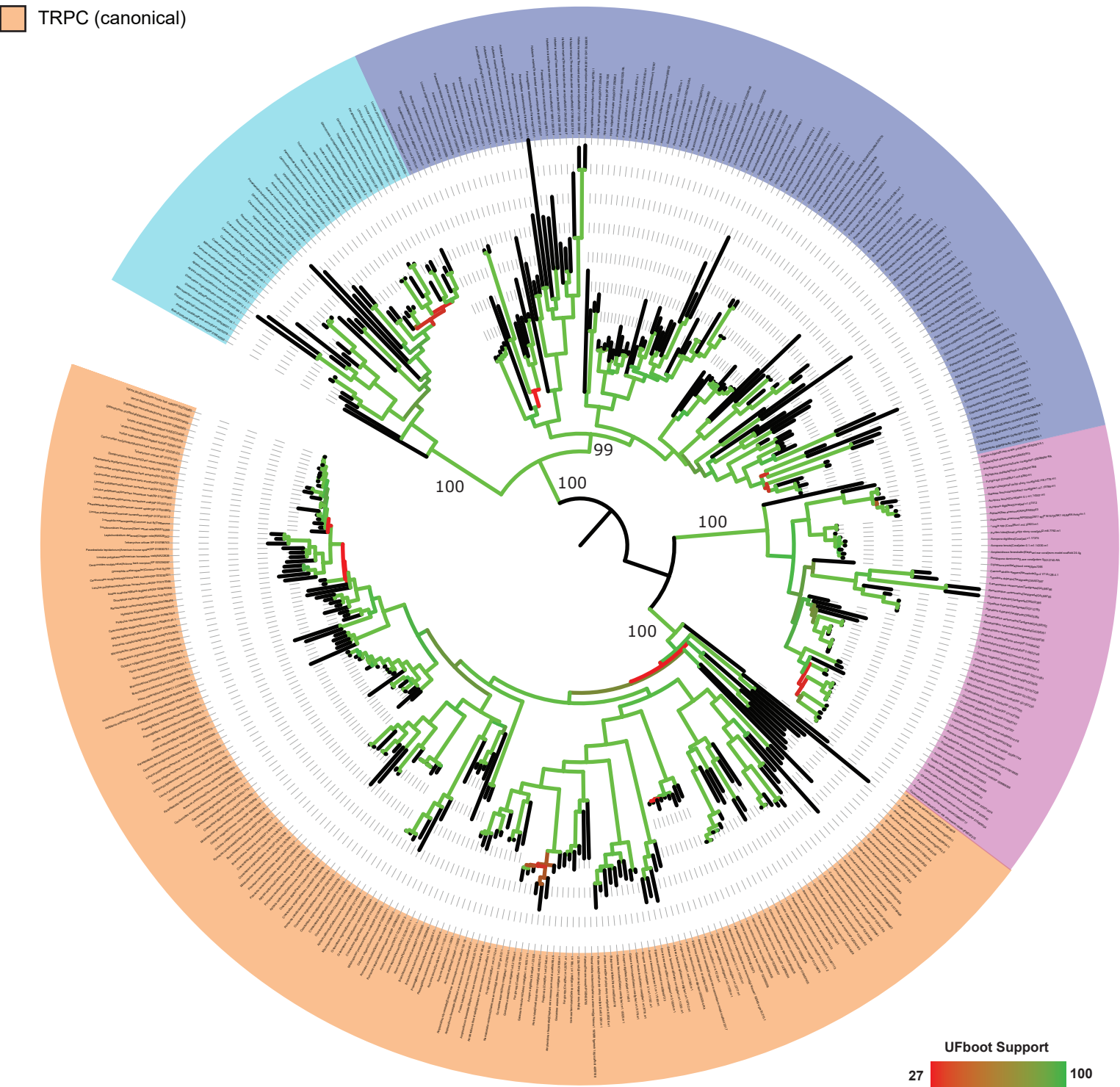


Figure S1

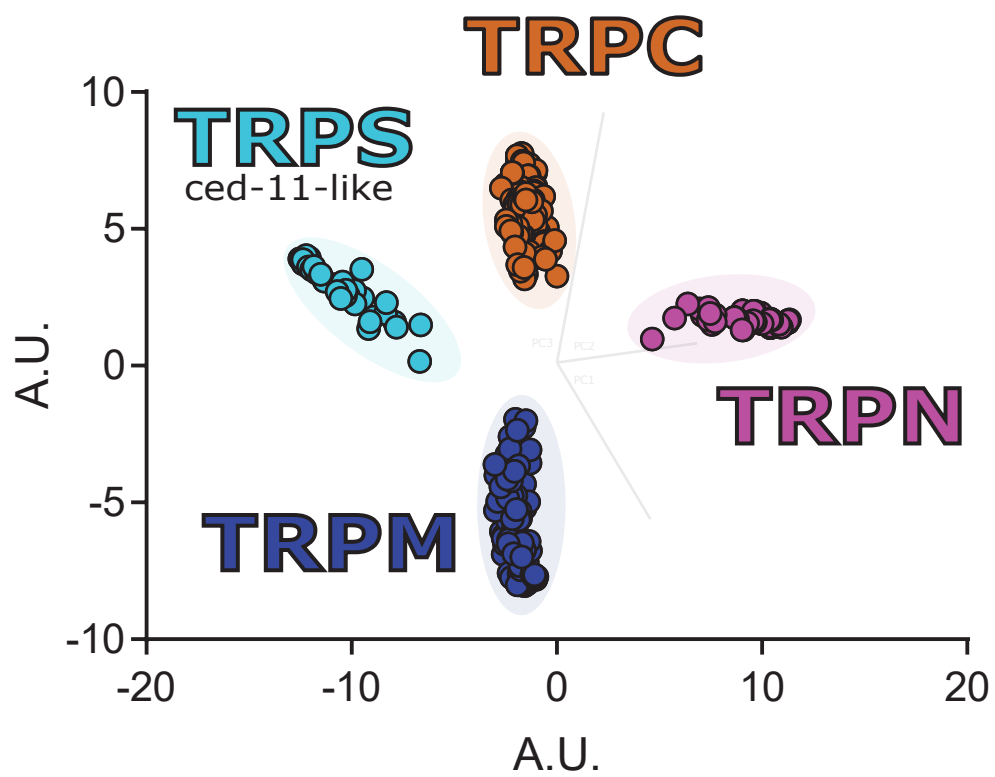




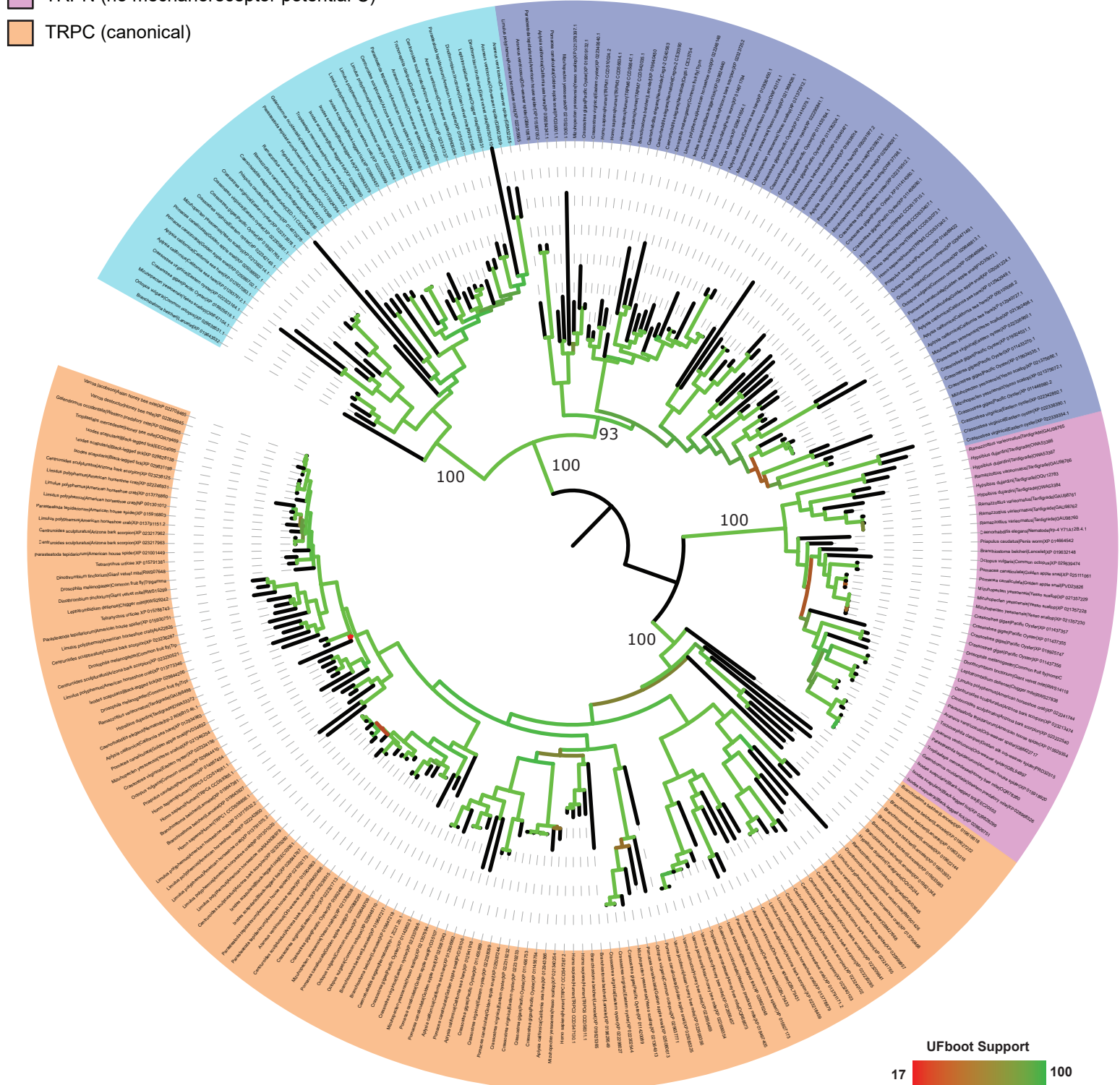


Figure S2

TRP Family

-  TRPS (soromelastatin)
-  TRPM (melastatin)
-  TRPN (no mechanoreceptor potential C)
-  TRPC (canonical)



TRP Family

- TRPS (soromelastatin)
- TRPM (melastatin)
- TRPN (no mechanoreceptor potential C)
- TRPC (canonical)

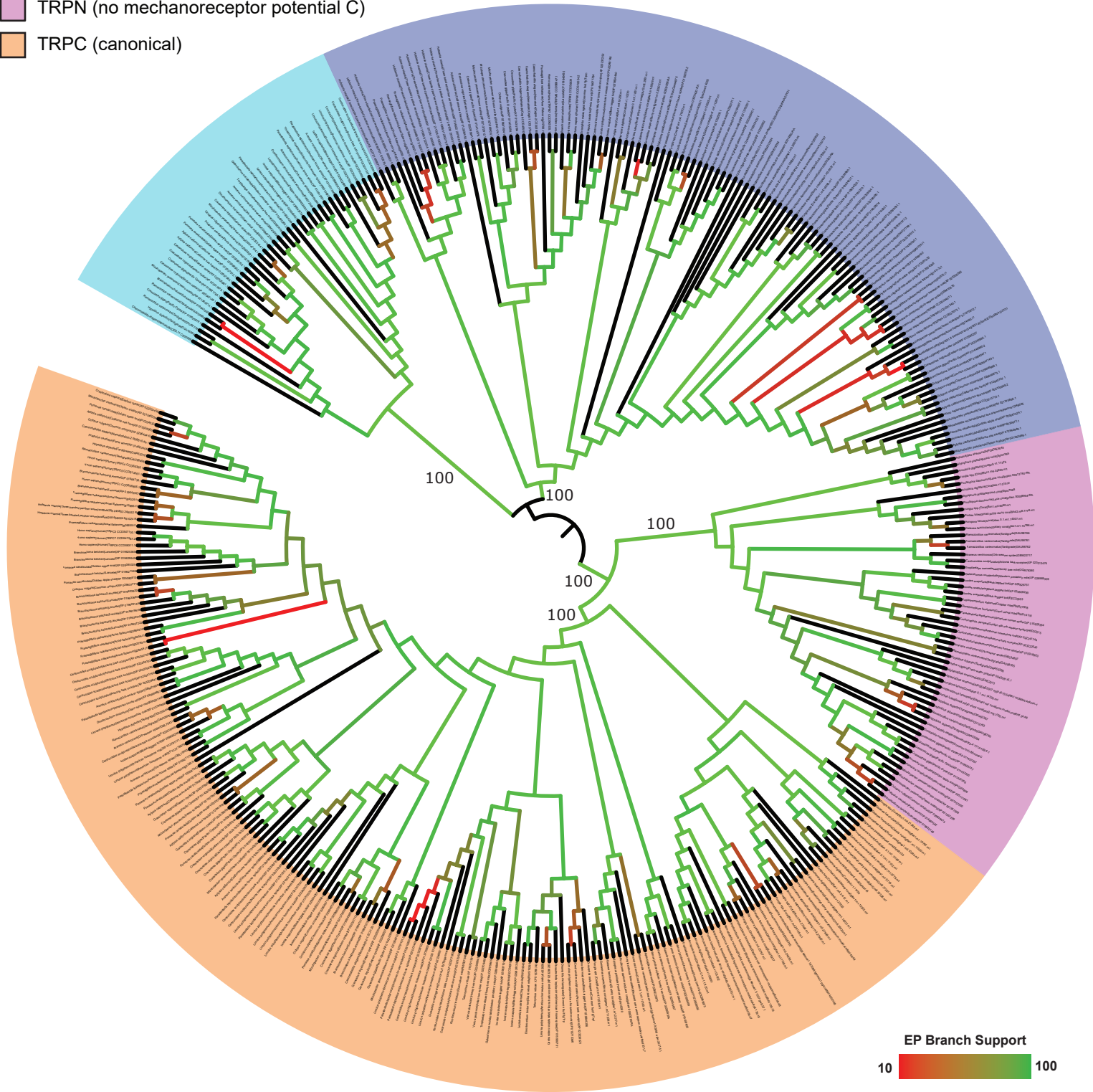


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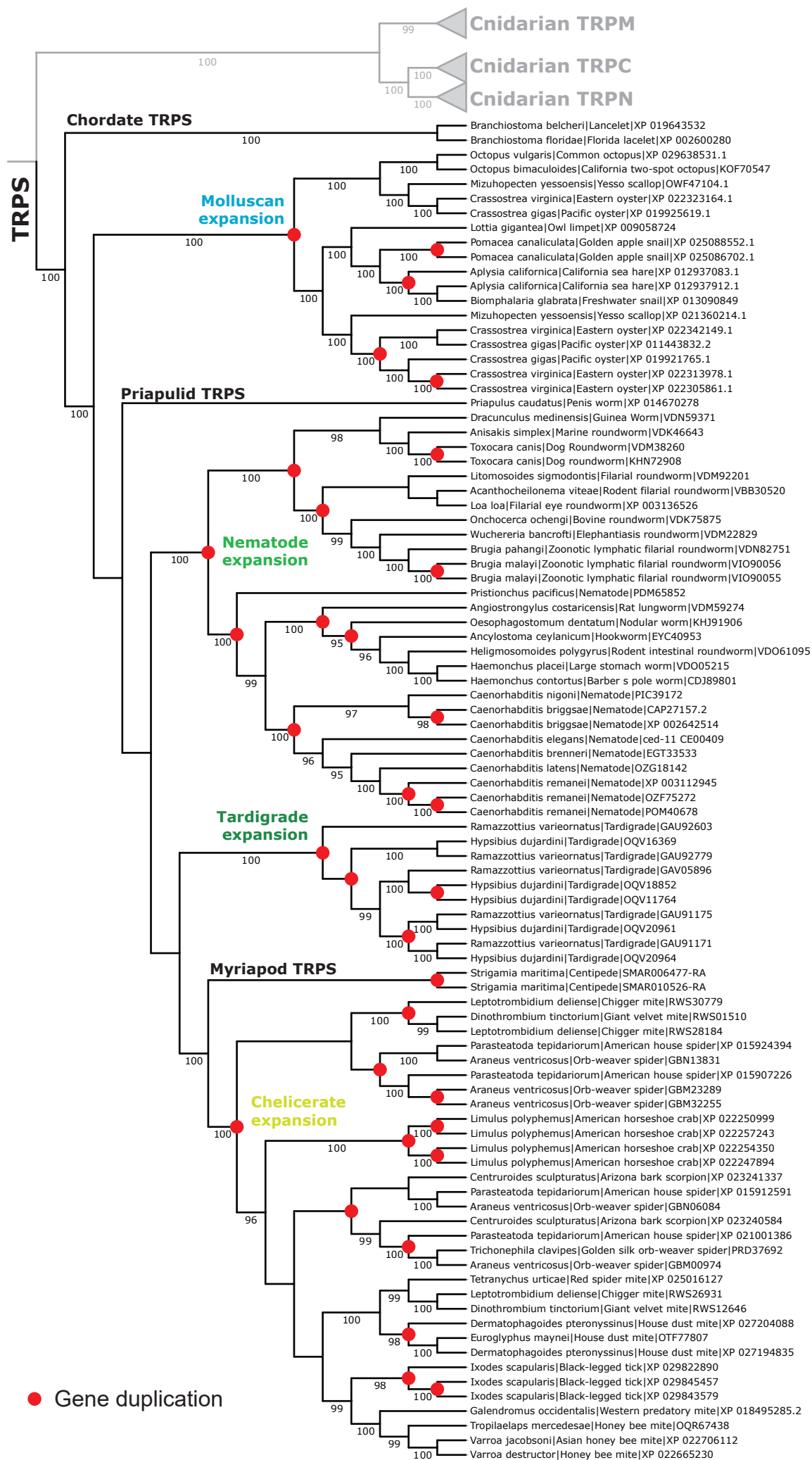
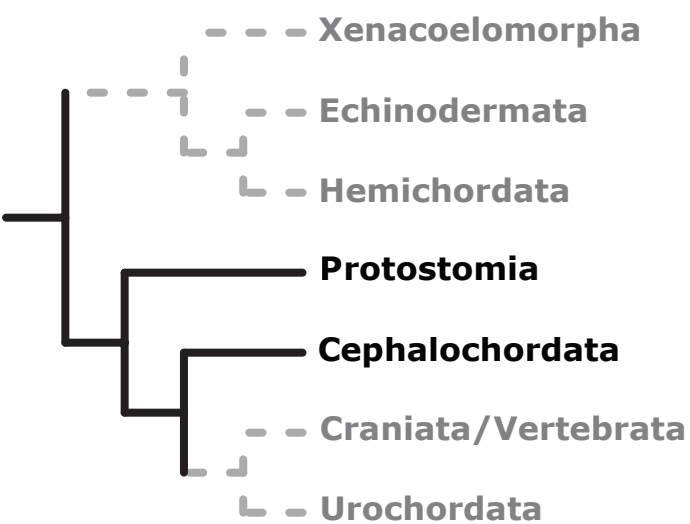
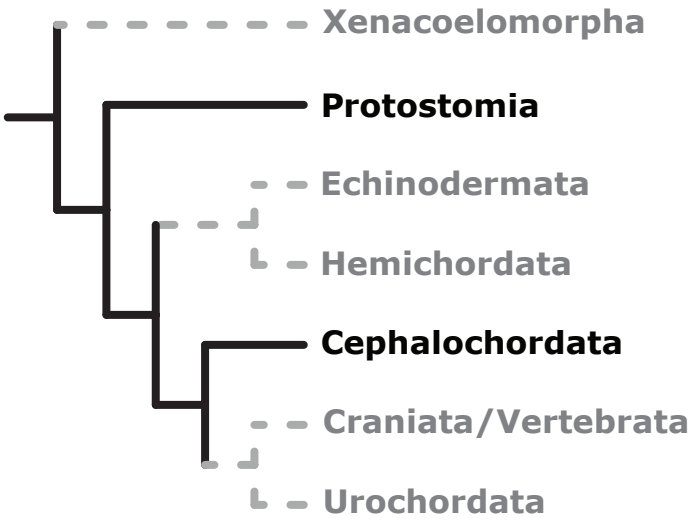


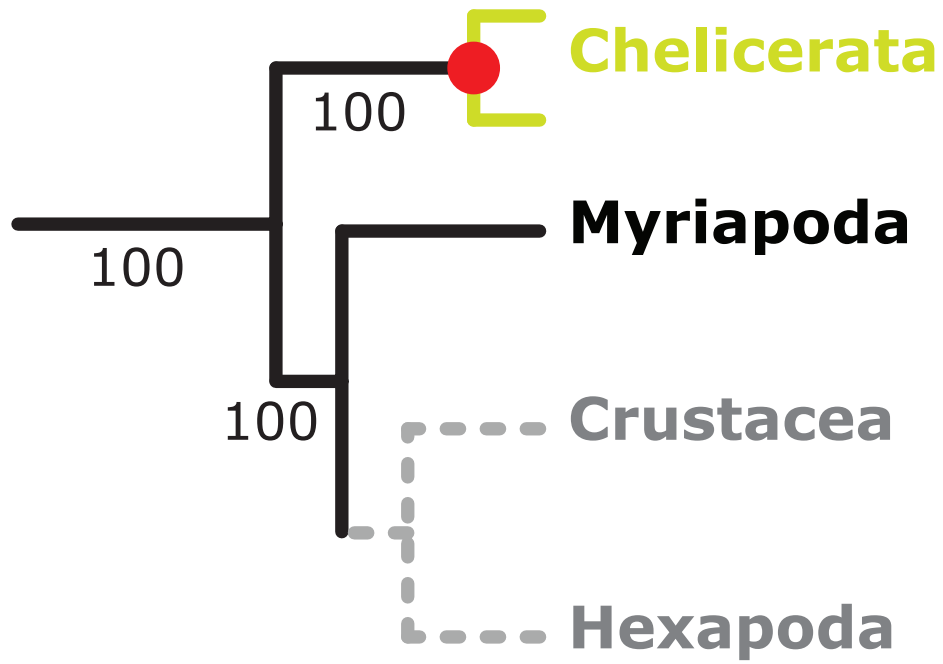
Figure S5

Monophyletic Deuterostomia Hypothesis

Xenambulacraria Hypothesis



Arthropoda



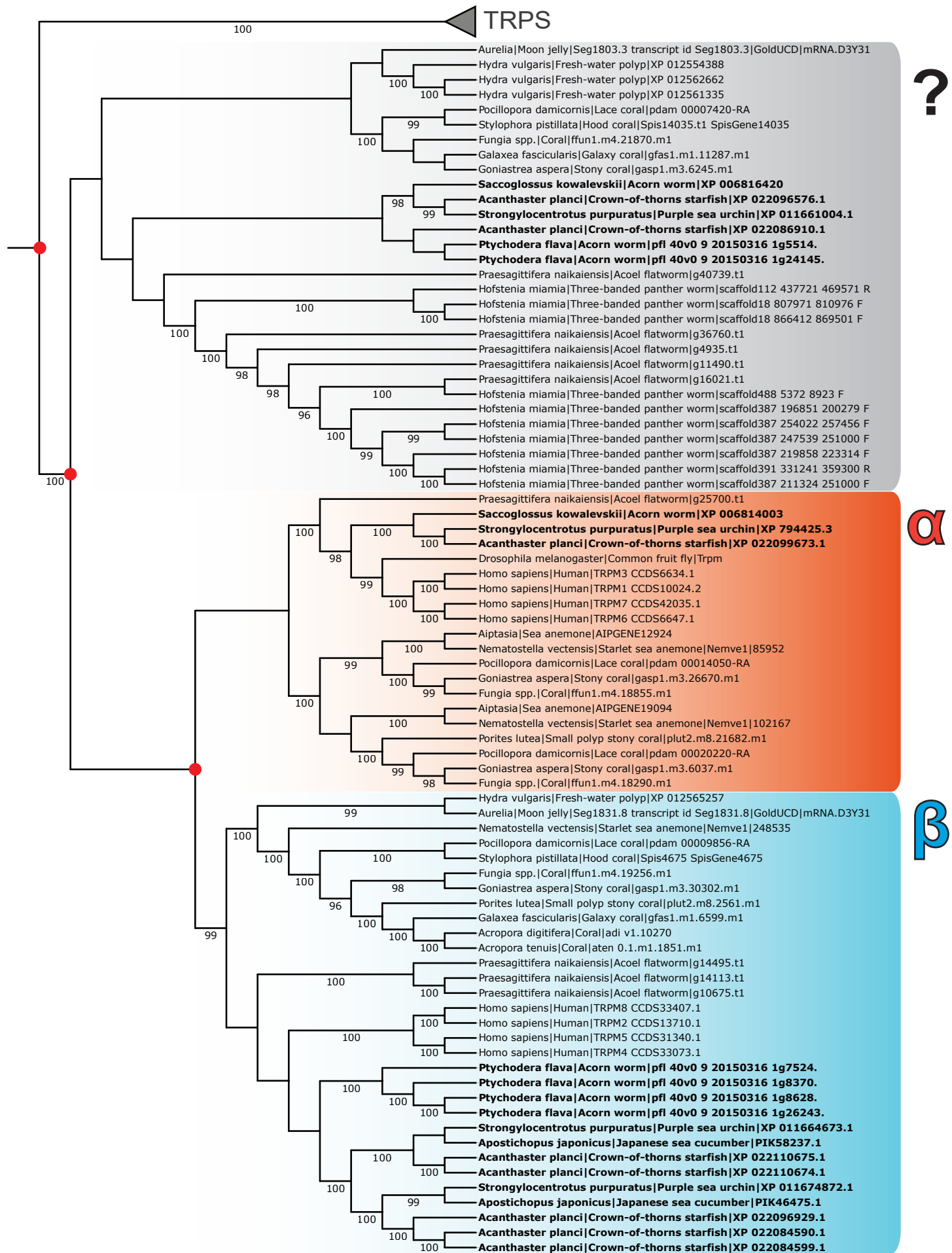
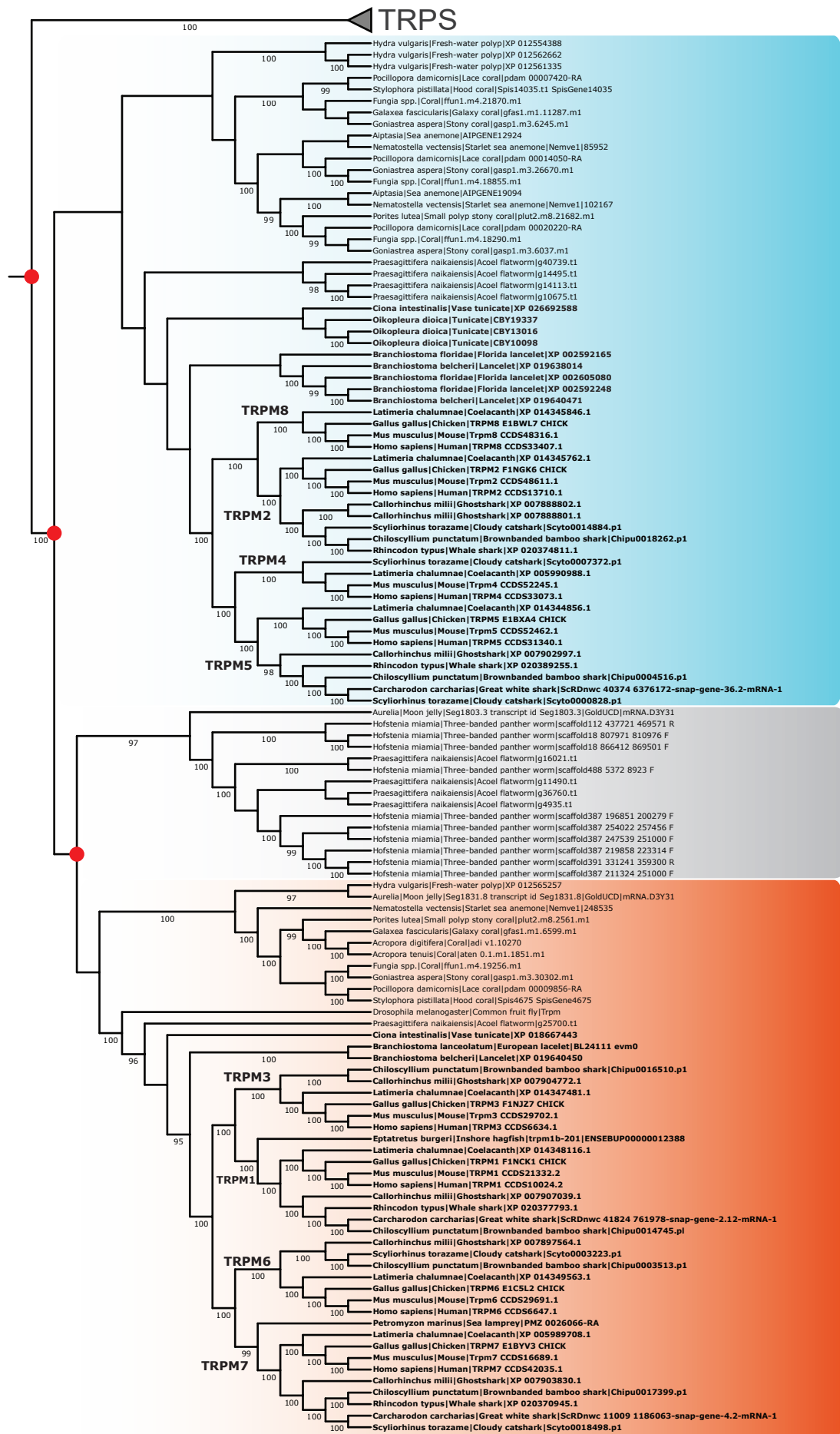


Figure S8



β

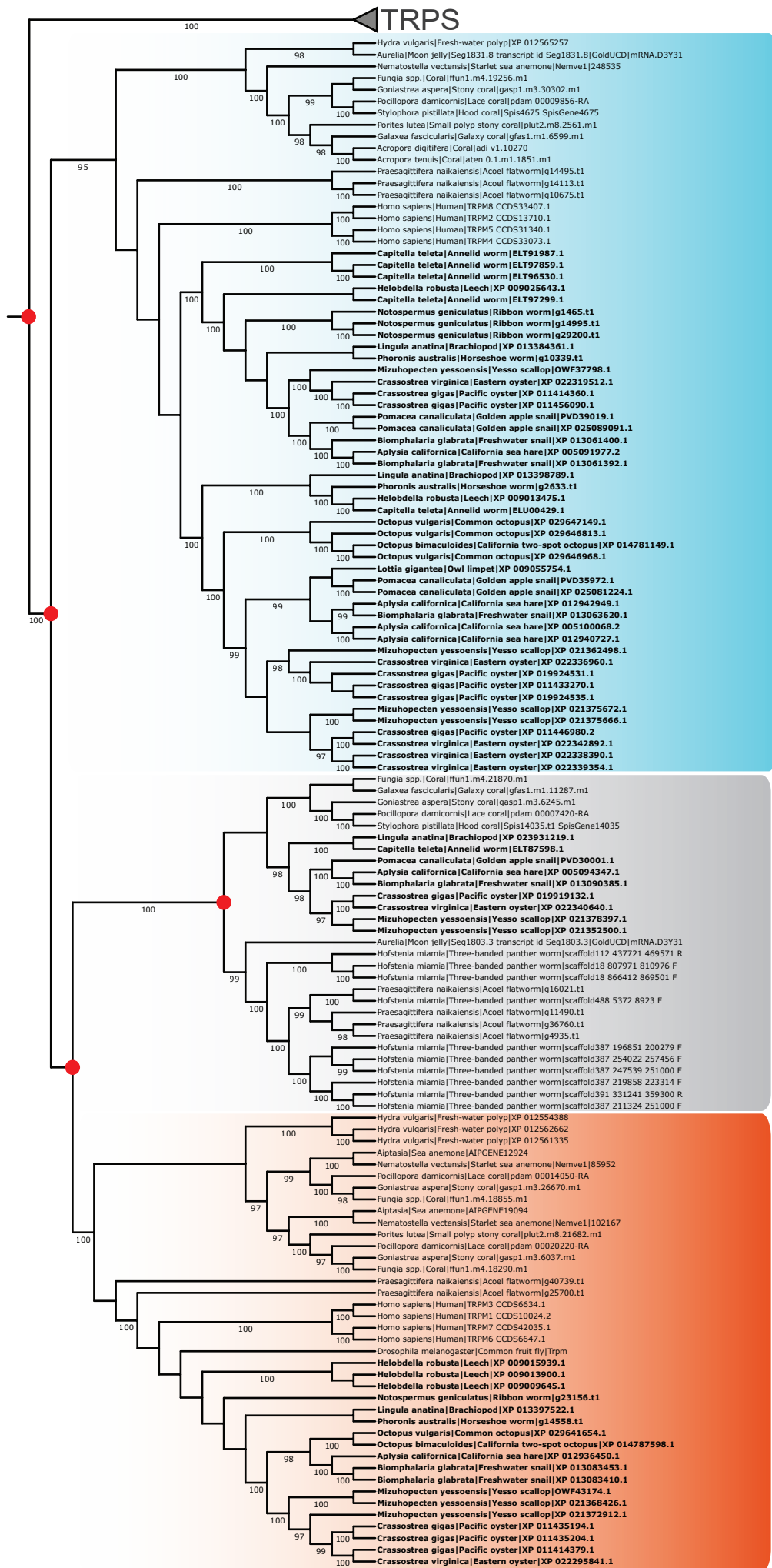
?

α

Duplications before
Cnidaria-Bilateria split

Figure S9

TRPS



β

?

α

Duplications before
Cnidaria-Bilateria split

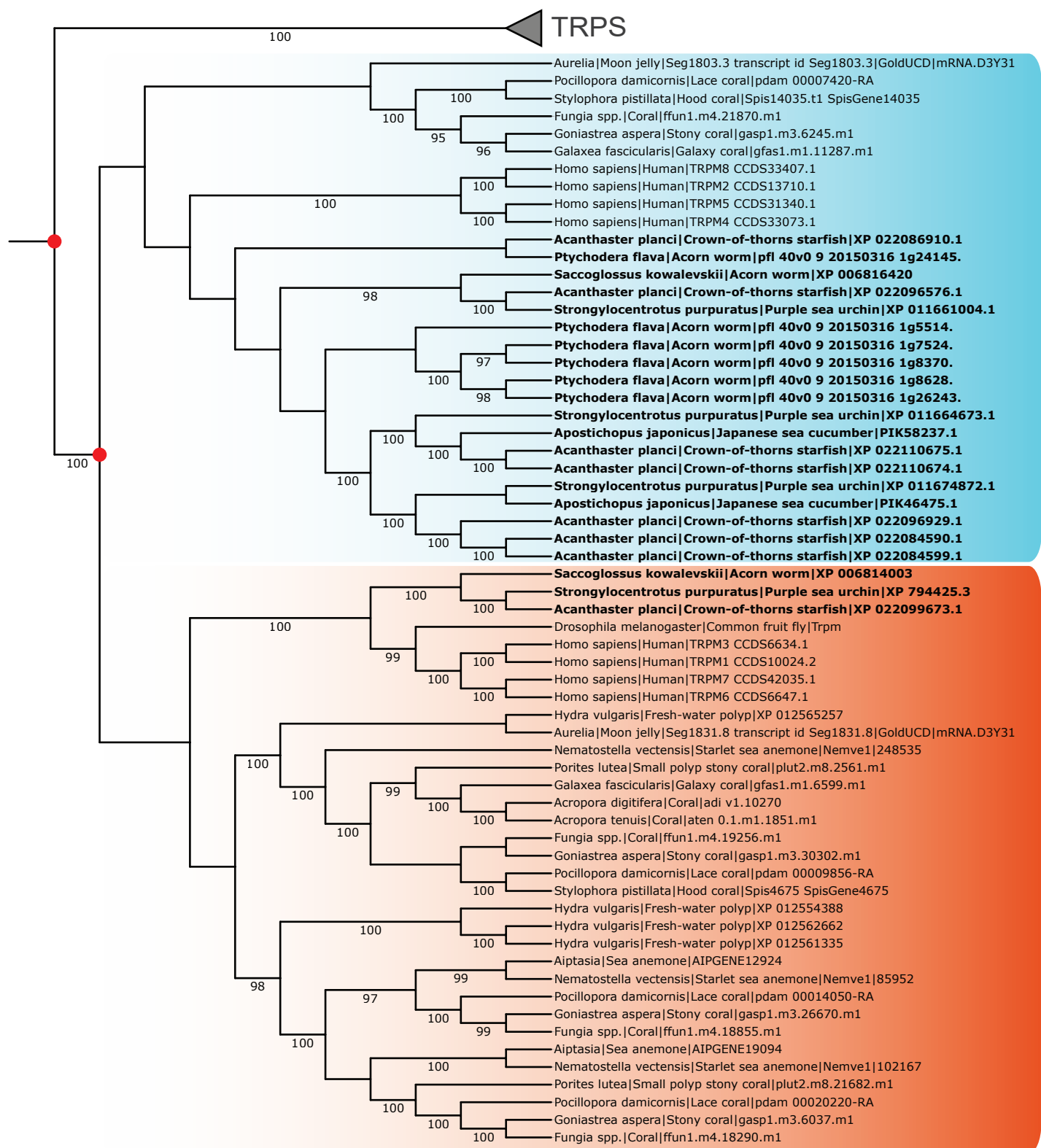
Figure S10





Duplications before
Cniadaria-Bilateria split

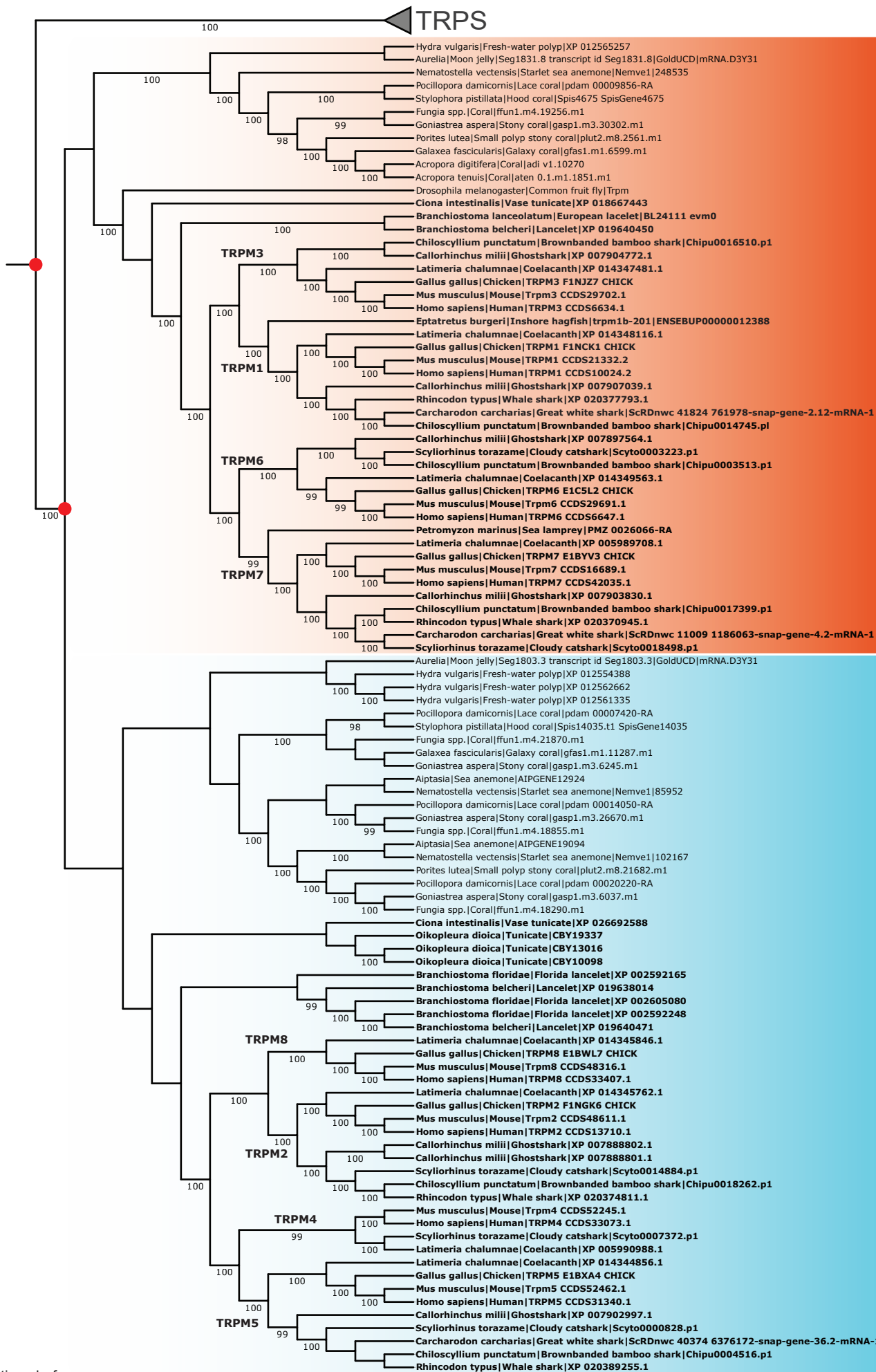
Figure S12



● Duplications before
Cnidaria-Bilateria split

β

α



α

β

Duplications before
Cnidaria-Bilateria split

Figure S14

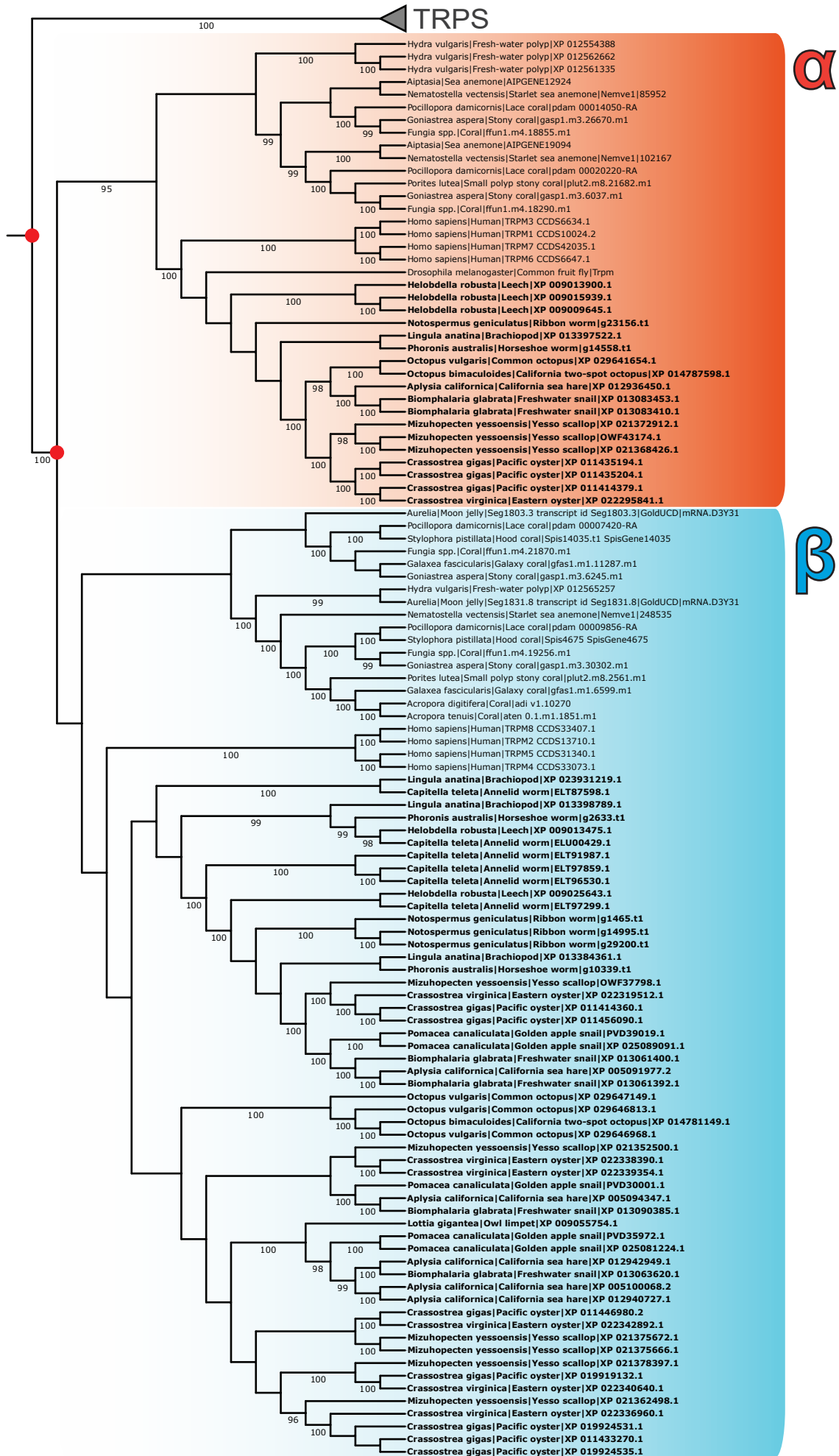


Figure S15

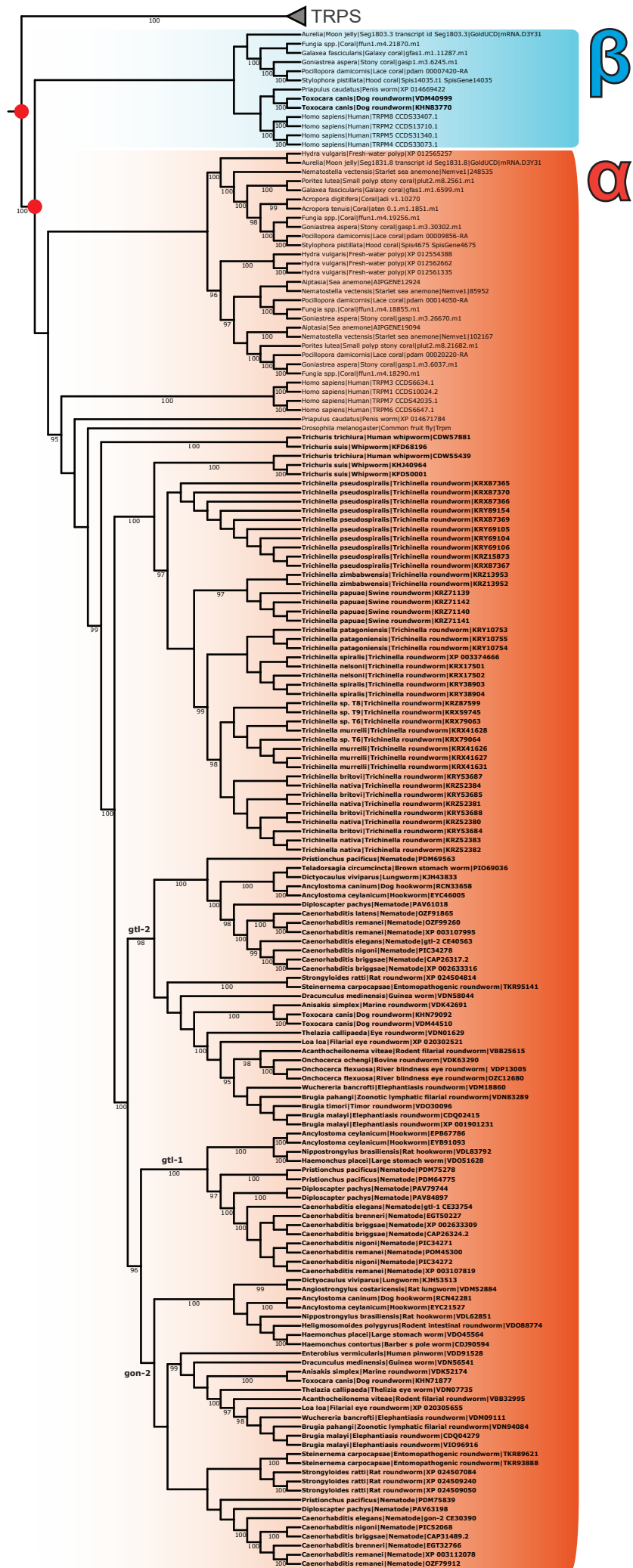
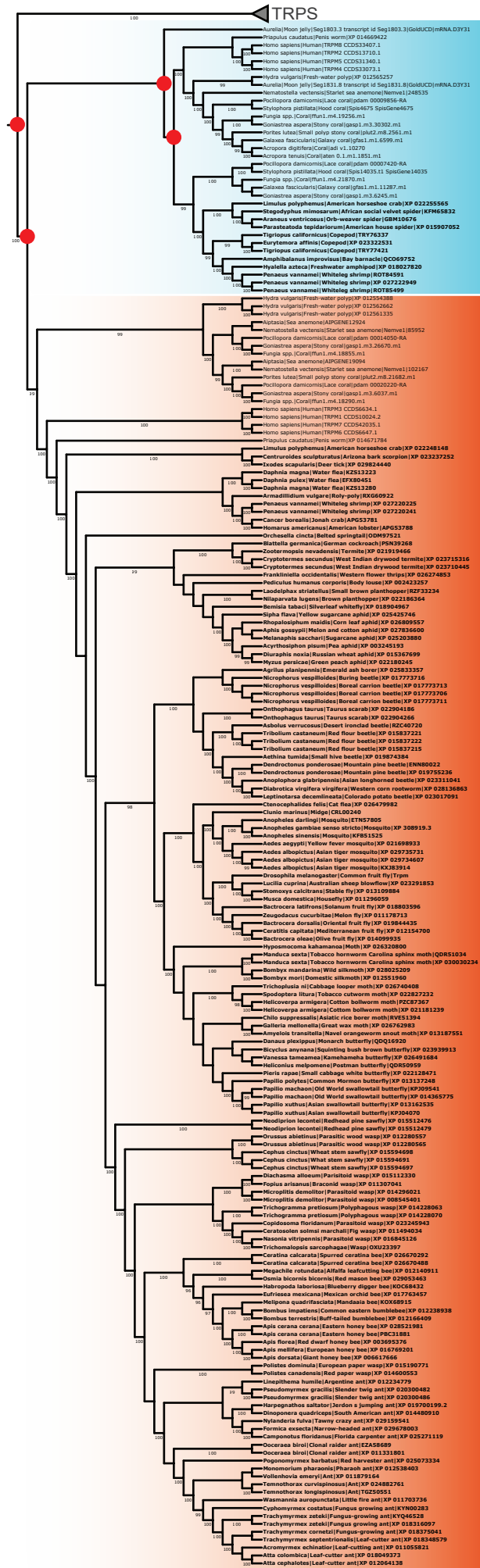


Figure S16



Duplications before
Cnidaria-Bilateria split

Figure S17

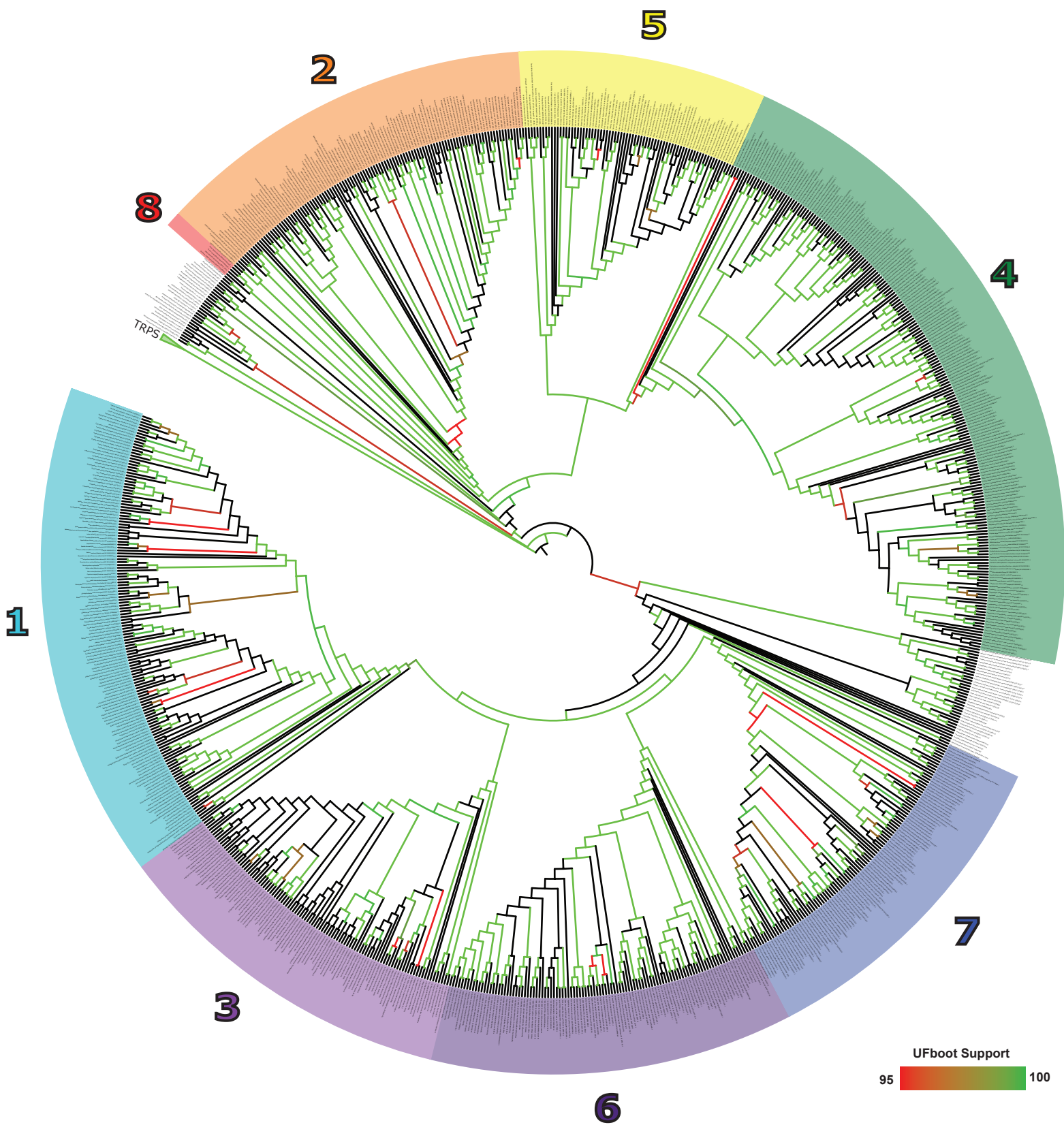


Figure S18