Siphonophore Phylogeny

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Abstract

Introduction

Siphonophores are a group of 188 valid species within Hydrozoa (Cnidaria), the vast majority of which are members of the plankton (fig. 1). Siphonophores are colonial, and are composed of zooids that are each homologous to solitary animals, but are physiologically integrated (Totton and Bargmann 1965; Mackie et al. 1988; Dunn and Wagner 2006). Siphonophores differ significantly from all other colonial hydrozoans in terms of colony structure, development, and the degree to which they are functionally specialized (Beklemishev 1969; Cartwright and Nawrocki 2010). A siphonophore colony arises from a single embryo, which forms a protozooid and a growth zone from which other genetically identical zooids bud asexually (Carré 1967, 1969; Carré and Carré 1991, 1995). Each zooid arises in the same repeating species-specific pattern, and is functionally specialized for a particular task (e.g feeding, reproducing, swimming) (fig. 2) (Dunn and Wagner 2006).

Most siphonophores are planktonic, with the exception of one pleustonic species (*Physalia physalis*, Portuguese man of war) that lives at the interface of water and air, and a small clade of benthic siphonophores, the Rhodaliidae (Pugh 1983;).

Methods

This manuscript is an executable document computed directly from the data, providing an explicit and reproducible description of all findings. All scripts for the analyses are available in a git repository at https://github.com/caseywdunn/siphonophore_phylogeny_2017. The most recent commit at the time of the analysis presented here was 7cdbb6983a730c124a1e97f4be6dcf83e4de9c22.

Collecting

Collection data on all examined specimens, a description of the tissue that was sampled from the colony, collection mode, sample processing details, mRNA extraction methods, sequencing library preparation methods and sequencing details are summarized in supplementary table 1. Monterey Bay and Gulf of California specimens were collected by remotely operated underwater vehicle (ROV) or during blue-water scuba dives. *Chelophyes appendiculata* and *Hippopodius hippopus* specimens were collected in the bay of Villefranche-sur-Mer, France, during a plankton trawl on 04/13/11. Available physical vouchers have been deposited at the Museum of Comparative Zoology (Harvard University), Cambridge, MA, or had been previously deposited at the United States National Museum (Smithsonian Institution), Washington, DC. Accession numbers are given in supplementary table X. In cases where physical vouchers were unavailable we provide photographs to document species identity (table x).

Sequencing

When possible specimens were starved overnight in filtered seawater at temperatures close to ambient water temperatures at the time point of specimen collection (supplementary table 1). mRNA was extracted directly from tissue using a variety of methods (supplementary table x): Magnetic mRNA Isolation Kit (NEB, #S1550S), Invitrogen Dynabeads mRNA Direct Kit (Ambion, #61011), Zymo Quick RNA MicroPrep (Zymo #R1050), or from total RNA after Trizol (Ambion, #15596026) extraction and through purification using Dynabeads mRNA Purification Kit (Ambion, #61006)- in case of anticipated very small total RNA quantities, only a single round of bead purification was performed; or Trizol directly into the Illumina TruSeq Stranded Library Kit. Extractions were performed according to the manufacturer's instruction. Any resulting higher rRNA read counts were dealt with further downstream in the bioinformatics workflow. Libraries were prepared for sequencing using the Illumina TruSeq RNA Sample Prep Kit (Illumina, #FC-122-1001, #FC-122-1002), the Illumina TruSeq Stranded Library Prep Kit (Illumina, #RS-122-2101) or the NEBNext RNA Sample Prep Master Mix Set (NEB, #E6110S). We collected long read paired end Illumina data for *de novo* transcriptome assembly. In the case of large tissue inputs, libraries were sequenced separately for each tissue, subsequently subsampled and pooled *in silico*. Libraries were sequenced on the HiSeq 2000, 2500, and 3000 sequencing platforms (supplementary table 1). Summary statistics for expression libaries are given in Table 1.

Analysis

New data were analysed in conjunction with 13 publically available datasets, with a total number of 43 species. Sequence assembly, annotation, Maximum Likelihood (ML) phylogenetic analysis were conducted with the tool Agalma (Dunn et al. 2013), v. 1.00, and Bayesian Inference (BI) analyses were conducted using Phylobayes(Lartillot et al. 2009) v. 1.7a-mpi. Source code for all analysis steps, sequence alignments, sampled and consensus trees, and voucher information are available in a git repository https://github.com/caseywdunn/siphonophore_phylogeny_2017.

In the final analyses, we sampled 1,071 genes to generate a supermatrix with 60% occupancy and a length of 378,468 amino acids. Two outgroup species, *Atolla vanhoeffeni* and *Aegina citrea*, were removed from the final supermatrix and phylogeny due to low gene occupancy (gene sampling of 20.8% and 14.5% respectively in a 50% occupancy matrix with 2,203 genes). ML analyses were conducted on the unpartitioned supermatrix using the WAG+Γ model of amino acid substitution, and bootstrap values were estimated using 1000 replicates. BI was conducted using two different CAT models, CAT-Poisson and CAT-GTR (Lartillot and Philippe 2004). Two independent MCMC chains were run under the CAT-GTR model, and four independent MCMC chains were run under the CAT-Poisson model. The CAT-GTR and CAT-poisson models did not converge after a long CPU time, and only the results from the CAT-poisson model are included here.

Morphological character data were obtained from the literature, or from direct observation of available voucher material.

Subsequent analyses were conducted in R and integrated into this manuscript with the `knitr` package. See Supplementary Information for R package version numbers.

Hypothesis testing

Results and Discussion

Sample collecting and sequencing

Species phylogeny

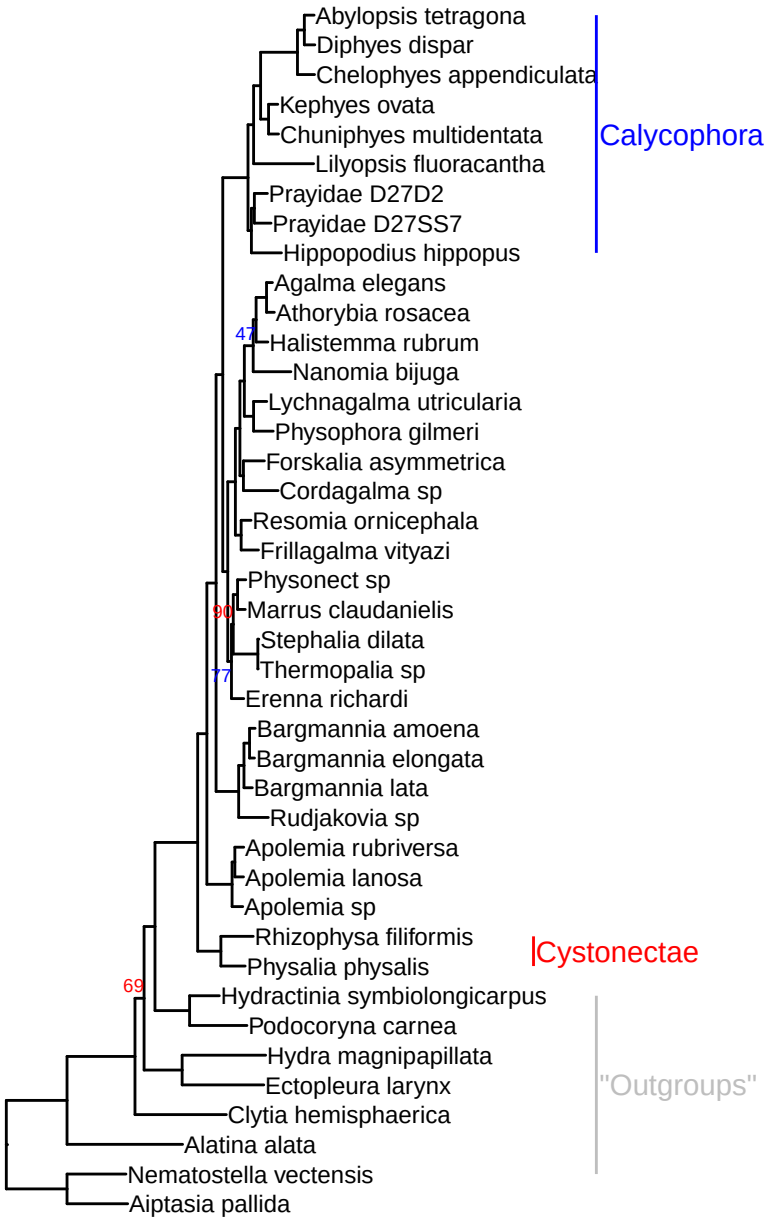


Figure 1: Phylogram of siphonophore relationships. Node labels indicate bootstrap support percent, unnumbered nodes have 100% support. The image was rendered with ggtree (Yu et al. 2016)

(XX This paragraph on sampling through Agalma analyses) The analyses presented here consider XXX siphonophore species and 8 outgroup species. This includes new data for XXX species. Summary stats on assemblies XX (Table XX). Matrix has XX genes, XX sites, and occupancy is XX.

(XX This paragraph on summarizes phylogeny runs, apart from tree topology) Maximimum likelihood analyses had 1000 replicates. We ran 4 phylobayes chains, and visual inspection of the traces indicated that a burn in of 400 trees was sufficient for all runs. This left 15847 trees in the posterior. XXConvergence...

These findings are entirely consistent with a previous analysis based on two genes (16S and 18S ribosomal RNA) (Dunn et al. 2005). Cystonectae is the sister group to the remaining siphonophores, and Calycophorae is nested within the paraphyletic "Physonectae". In addition, multiple nodes that were not resolved in the previous two-gene analysis do recieve strong support in this 1,071-gene transcriptome analysis. These findings include XXX.

Character Evolution

Siphonohores have evolved a fascinating diversity of morphological features, zooid types, life history traits, and habitats. Here we explore the evolutionary history of some of these features.

In our previous siphonophore phylogenetic analysis (Dunn et al. 2005) there were several characters left with equivocal evolutionary histories, due to unresolved relationships between physonects. With our current cladistic resolution, we were able to answer some of the questions left open:

Evolution of Monoecy

[Citation needed] noticed for the first time that some siphonophores were monoeicous and others were dioiecious. Our analyses in 2005 reconstructed this character and found a great amount of phylogenetic conservatism, with an unambiguous resolution of the MRCA (most recent common ancestor) as dioecious, and the appearance of monoecy in several taxa and clades (including Calycophorae) within the polytomy. Figure X shows the evolution of sex distribution in siphonophores under the current better-resolved tree model, and it strongly indicates that monoecy in siphonophores from a dioecious ancestor occured twice, in the branch leading to Calycophorae and in the branch leading to Agalmatids (sensu lato). There is a small probability for an alternative scenario featuring a single gain of monoecy before the split of Calycophorae, with a subsequent derived shift back to dioecy in the *Marrus-Erenna* clade.

The Evolution of Zooid Types

One of the most striking aspects of siphonophore biology is their diversity of unique zooid types. Other colonial cnidarians (such as Hydractinia) and some bryozoans (example) have been found to have up to X different zooid types [Citation1 , Citation 2]. The siphonophore genus Forskalia has 6 basic zooid types (pneumatophore, nectophore, gastrozooid, palpon, bract, and gonophore), and a total of 10 counting subtypes (4 types of bract, male & female gonophores). Diphyomorphs have more than 1 type of nectophore, while Cystonects have none. Here we reconstruct the evolutionary origins ofthe different zooid types on the present transcriptome tree.

Nectophores are retained modified medusae that Codonophora use for coordinated colony-level swimming. The nectosome is the region of the colony that develops from the nectosomal growth zone. Unlike the siphosomal growth zone, the nectosome does not bud gastrozooids, but nectophores (and in the case of *Apolemia*, also palpons). If fact, with the exception of *Physalia physalis* (which grows small nectophores near the gonodendra), siphonophore nectophores are exclusively found on the nectosome. It is possible that the MRCA of siphonophores had a nectosome, which has lost on the branch leading to Cystonects. We cannot exclude with certainty the alternative hypothesis of a nectosome-less ancestor followed by a gain of the nectosome in the branch leading to the Codonophora. The nectosome probably arose as a duplication of the siphosome, followed by functional specialization in propelling the colony. The nectosome has been lost within Codonophora in the genus *Athorybia*.

Following the colony development orientation framework presented in (citation), the nectosome can be located in a dorsal or a ventral position. Our ancestral reconstructions for this character (Supp figure) show that

a ventrally-oriented nectosome was the ancestral form in siphonophores, and that a dorsal nectosome has evolved twice independently, in the braches leading to the Agalmatidae (sensu stricto) and the branch leading to the *Bargmannia* species. All Codonophora (with the exception of *Athorybia* species) have a nectosome, but the number and subtypes of nectophores present varies greatly between species. As shown in Figure X, most Codonophora presents the ancestral nectosome with multiple nectophores of the same subtype. However, Calycophorans evolved a different system with just 2 nectophores of one type. This shift may be associated with the loss of the pneumatophore. Not all Calycophorans remained with this arrangement. The Hippopodidae returned to bearing multiple identical nectophores, many of which are inactive and serve functions of defense (like a shell to retract in) and buoyancy. As in the rest of Calycophorans, the Hippopodids only use 2 nectophores to propel the colony. Another interesting shift occurs in the branch leading to Diphyomorpha, where the 2 nectophores specialize into 2 subtypes, associated with a shift into a vertically aligned position and pointed bell shapes. The 2 types function together in a coupled hydrodynamic system that allows very fast escape responses (Mackie 1964).

Bracts are highly reduced zooids unique to siphonophores, but they are only present in the Codonophora. As with the nectosome, we have ambiguity determining whether the MRCA of siphonophores had bracts or not. The MRCA of Codonophora ahd only one bract subtype, which was lost in Hippopodidae and in *Gymnopraia lapislazula*. Bracts are functional for protection of the delicate zooids and to help maintain neutral buoyancy. Some calycophorans are able to actively exclude suphate ions in their bracts to adjust their buoyancy along the colony Bidigare, R. R., & Biggs, D. C. (1980).

The ancestral siphonophore certainly had a pneumatophore (Supp Figure X). This unique zooid fills itself with gas, which helps the colony float and maintain its orientation in the water column. Recent evidence of neural arrangement in the pneumatophore of *Nanomia bijuga* (Church, 2013) suggests it could also gather information on relative pressure changes (and thus depth changes), helping regulate geotaxis. Despite its multiple biological functions, it was lost in the Calycophorae and never gained again in that clade. Calycophorans rely on the ionic balance of their gelatinous nectophores and bracts to retain posture and neutral buoyancy.

Palpons are modified mouthless gastrozooids used for digestion and circulation of the gastrovascular fluid. They were present in the MRCA of siphonophores (Supp Figure X), retained in most species, but lost twice independently in the branches leading to *Bargmannia* and Calycophorae. These taxa might have found other avenues to effectively circulate nutrients across the colony.

The Gain and Loss of Tentilla

The most complex nematocyst batteries of Cnidaria can arguably be found among the siphonophores, hanging in regularly spaced tentacle side branches called tentilla. Most hydrozoans, including the clade that contains siphonophores, bear simple tentacles (tentacles with no side branches). It is still an open question whether the MRCA of Siphonophora had simple or branched tentacles. The only siphonophores genera regarded as lacking tentilla are *Physalia physalis* and *Apolemia* spp., and *Bathyphysa conifera*. Since *B. sibogae* is the only member of the *Rhizophysidae* (and of the *Bathyphysa* genus) lacking tentilla, we can safely assume this is a case of secondary loss. When we reconstrauct the evolution of this character on the current phylogeny, we find that 70% of simulations support an MRCA bearing tentilla, with two independent losses leading to *Physalia* and *Apolemia*. However, this leaves a 30% support for a simple-tentacled MRCA followed by 2 independent gains of tentilla in the branches leading to *Rhizophysidae* and *{Bargmannia, Diphyes}*.

A key issue here is how we code for absence of tentilla, especially for the case of *Physalia physalis*. The tentacles of this species, when uncoiled, show very prominent, evenly spaced, bulging buttons which contain on their ectoderm all active and functionally arranged nematocysts used by the organism for prey capture. Siphonophore tentilla are complete diverticular brachings of the tentacle ectoderm, mesoglea, and gastrovascular canal (lined by endoderm). Hessinger & Ford 1988 (in the Biology of Nematocysts) described *Physalia*'s buttons as enclosing individual fluid-filled chambers connected by narrow channels to the tentacular canal, lined by endoderm. This suggests they are not just ectodermal swellings, but probably are reduced tentilla. When we code *Physalia physalis* as tentilla bearing, the results for the character reconstraction lead to a

more robust support for a tentilla-bearing MRCA followed by a single loss of tentilla in the branch leading to *Apolemiidae* (Figure 5b).

Siphonophore tentilla present an astounding diversity of sizes, shapes, colors, and nematocyst complements, and some have been observed to rapidly uncoil in contact with prey. Future research should explore the evolutionary history of these unique structures.

The Evolution of Vertical Habitat Use

Siphonophores are abundant predators in the pelagic realm, ranging from the surface (*Physalia physalis*) to bathypelagic depths (ref , *Bargmannia sp* 3888m VARS unpublished). While there are some pleustonic (*Physalia*) and benthic (*Rhodaliidae*) siphonophores, the phylogeny suggests the siphonophore MRCA was planktonic, as most extant taxa are. Some interesting questions arise from these facts, including 1) what was the bathymetric niche of the siphonophore MRCA, and 2) how did siphonophore's vertical habitat use of the water columns evolve along the phylogeny. Our results indicate a mesopelagic MRCA, with several convergent transition events to epipelagic and bathypelagic waters. There was only a single transition to benthic lifestyle on the stem of *Rhodaliidae*.

Discussion

The strong phylogenetic signal in the characters traditinally used for taxonomic diagnostics is a positive indicator of the applicability and unambiguity of these characters.

Conclusions

Acknowledgements

This work was supported by the National Science Foundation (DEB-1256695 and the Waterman Award). Sequencing at the Brown Genomics Core facility was supported in part by NIH P30RR031153 and NSF EPSCoR EPS-1004057. Data transfer was supported by NSF RII-C2 EPS-1005789. Analyses were conducted with computational resources and services at the Center for Computation and Visualization at Brown University, supported in part by the NSF EPSCoR EPS-1004057 and the State of Rhode Island. We also thank the MBARI crews and ROV pilots for collection of the specimens.

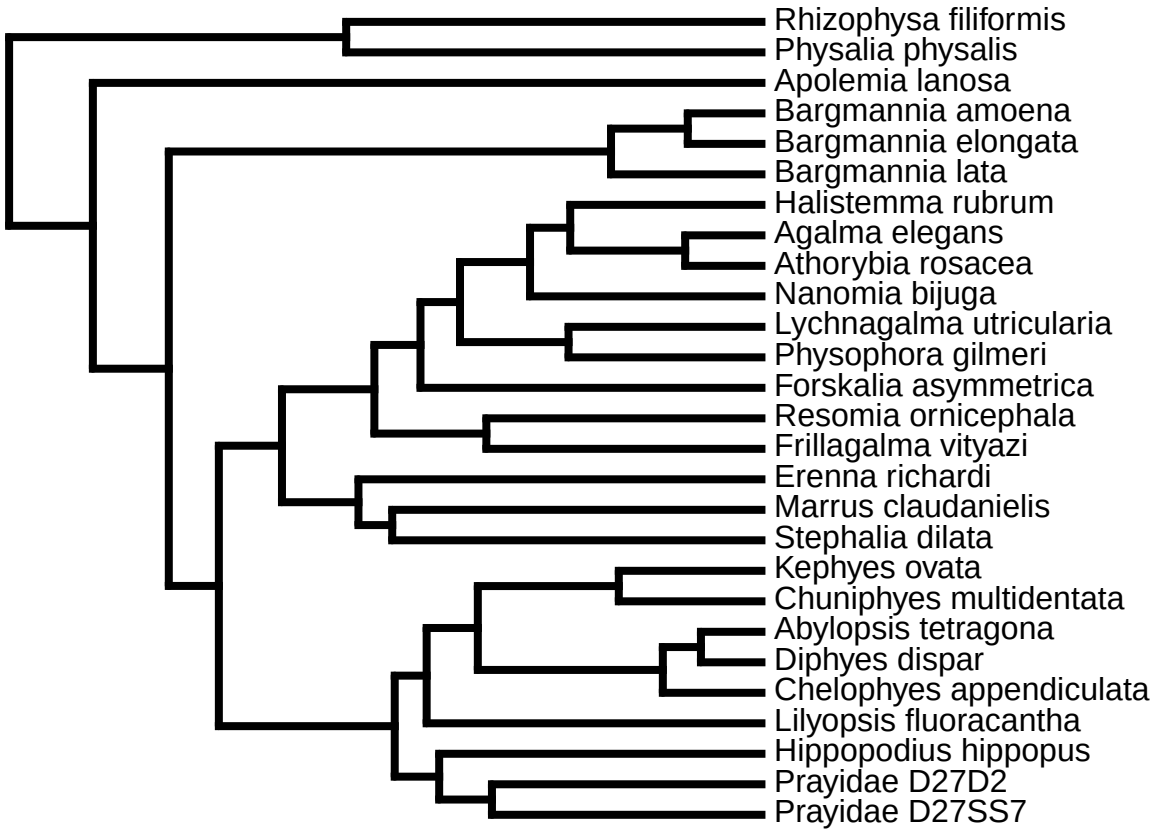
Supplementary Information

% latex table generated in R 3.4.1 by xtable 1.8-2 package % Fri Nov 3 16:00:19 2017

Table 1 (at the end of file): Summary statistics for libraries.

Supplementary Analyses

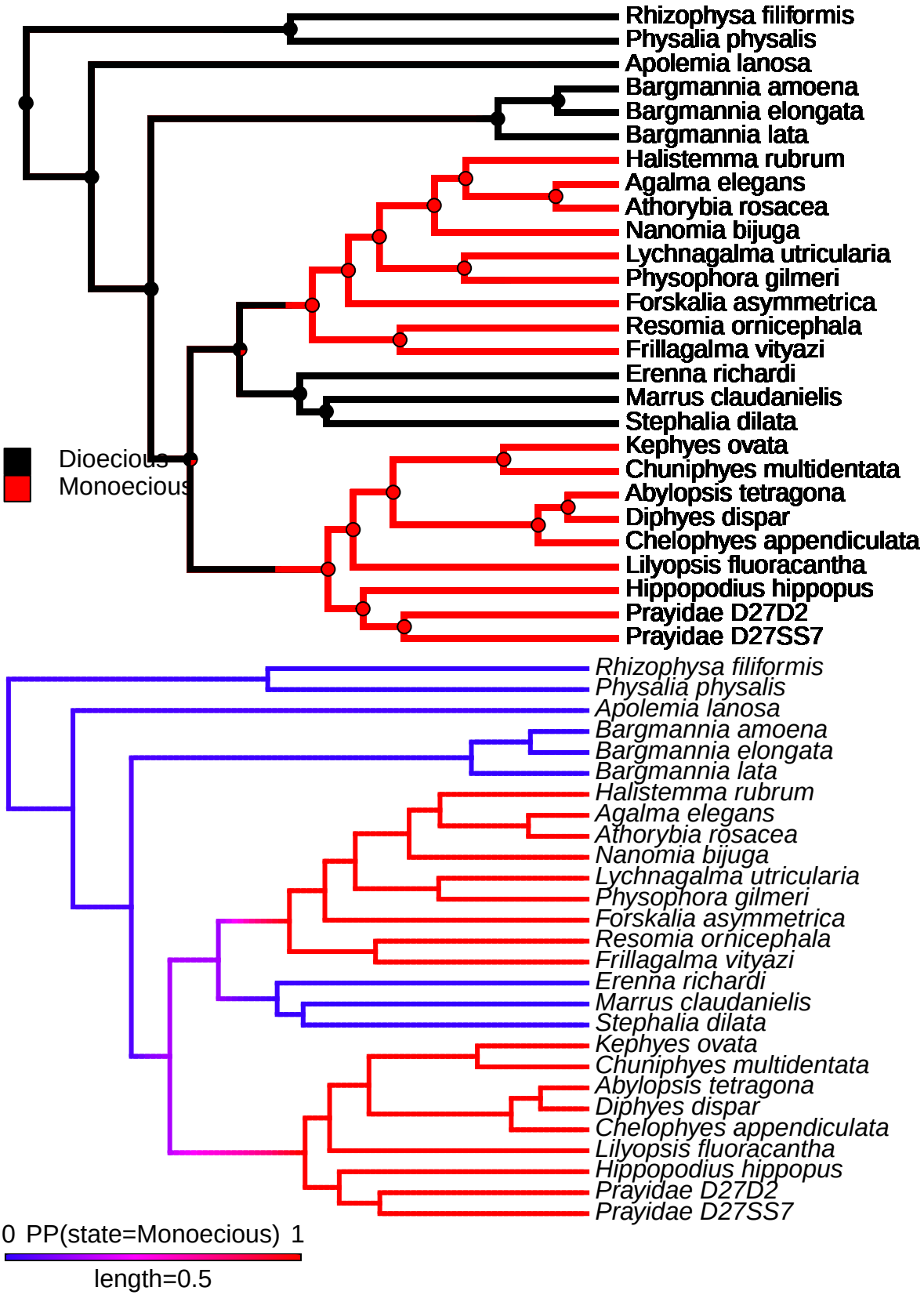
The Tree




```
## make.simmap is sampling character histories conditioned on the transition matrix
##
## Q =
##            0          1
## 0 -0.1892209  0.1892209
## 1  0.1892209 -0.1892209
## (estimated using likelihood);
## and (mean) root node prior probabilities
## pi =
##   0   1
## 0.5 0.5
```

When we reconstruct the evolutionary history of different traits in siphonophore species, we obtain the following results:

SIMMAP Sex distribution

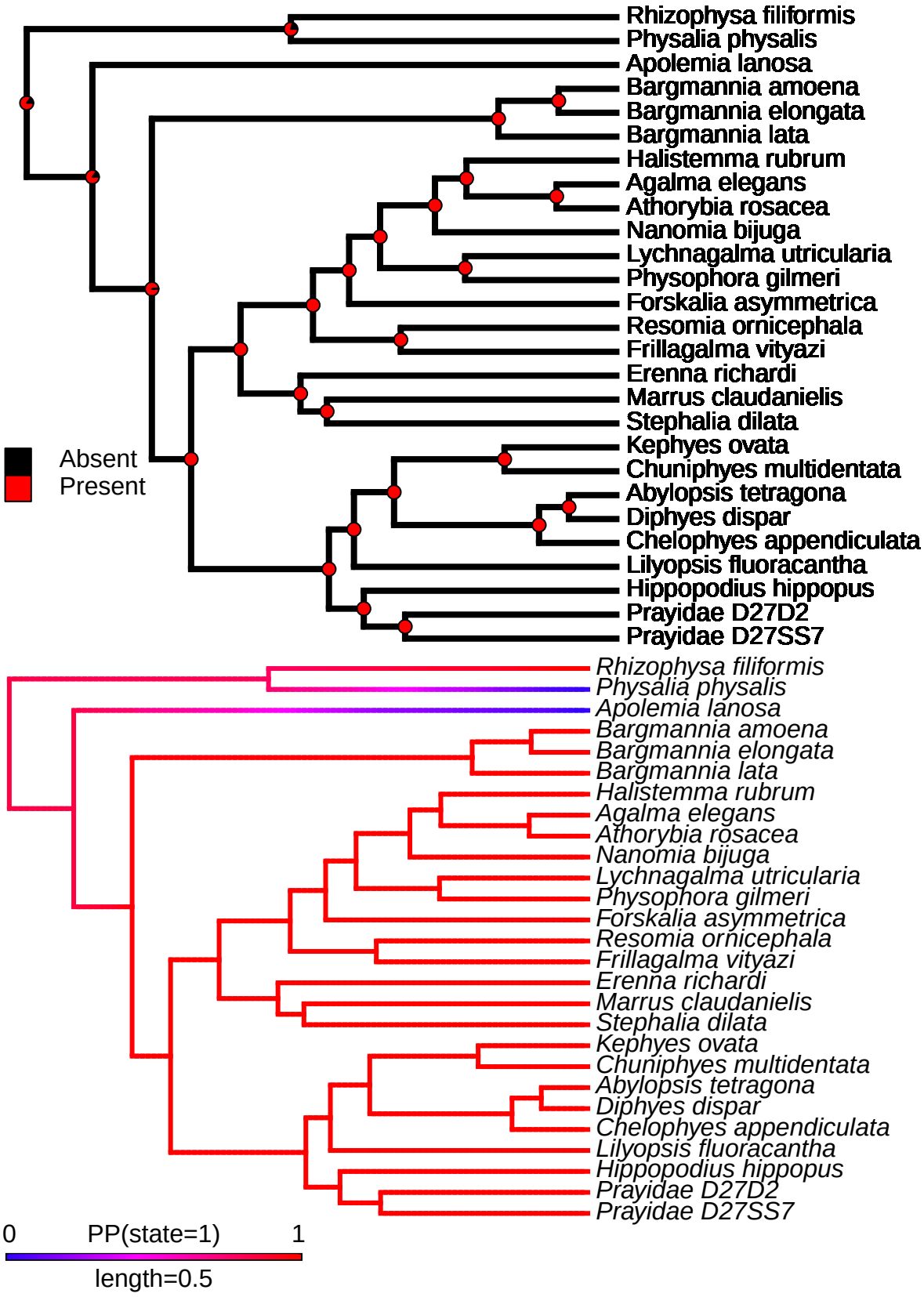


Posterior probabilities of states (0 Dioecious, 1 Monoecious).

SIMMAP Presence of palpons

Posterior probabilities of states (1 Present, 0 Absent).

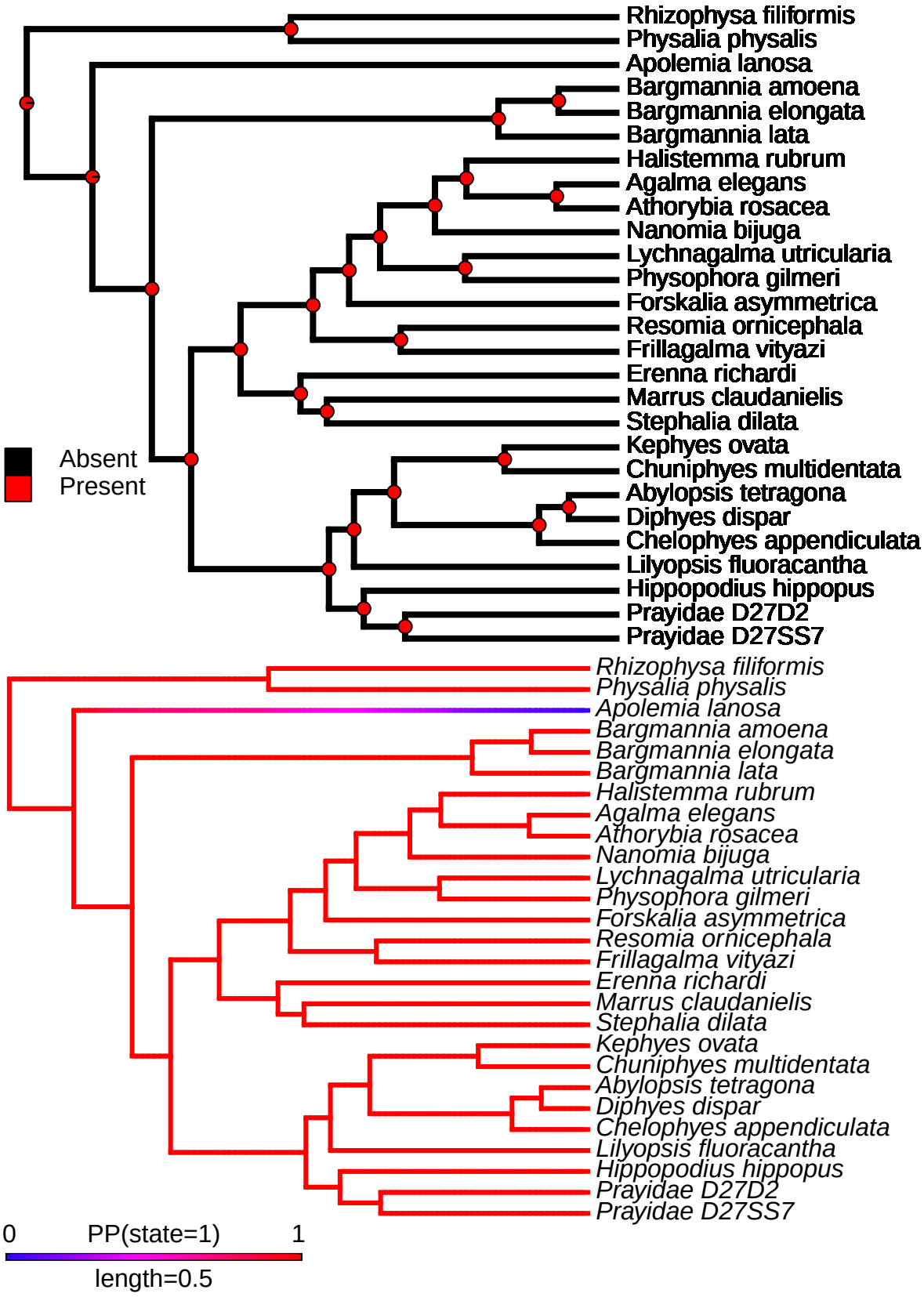
SIMMAP Presence of tentilla



Posterior probabilities of states (1 Present, 0 Absent).

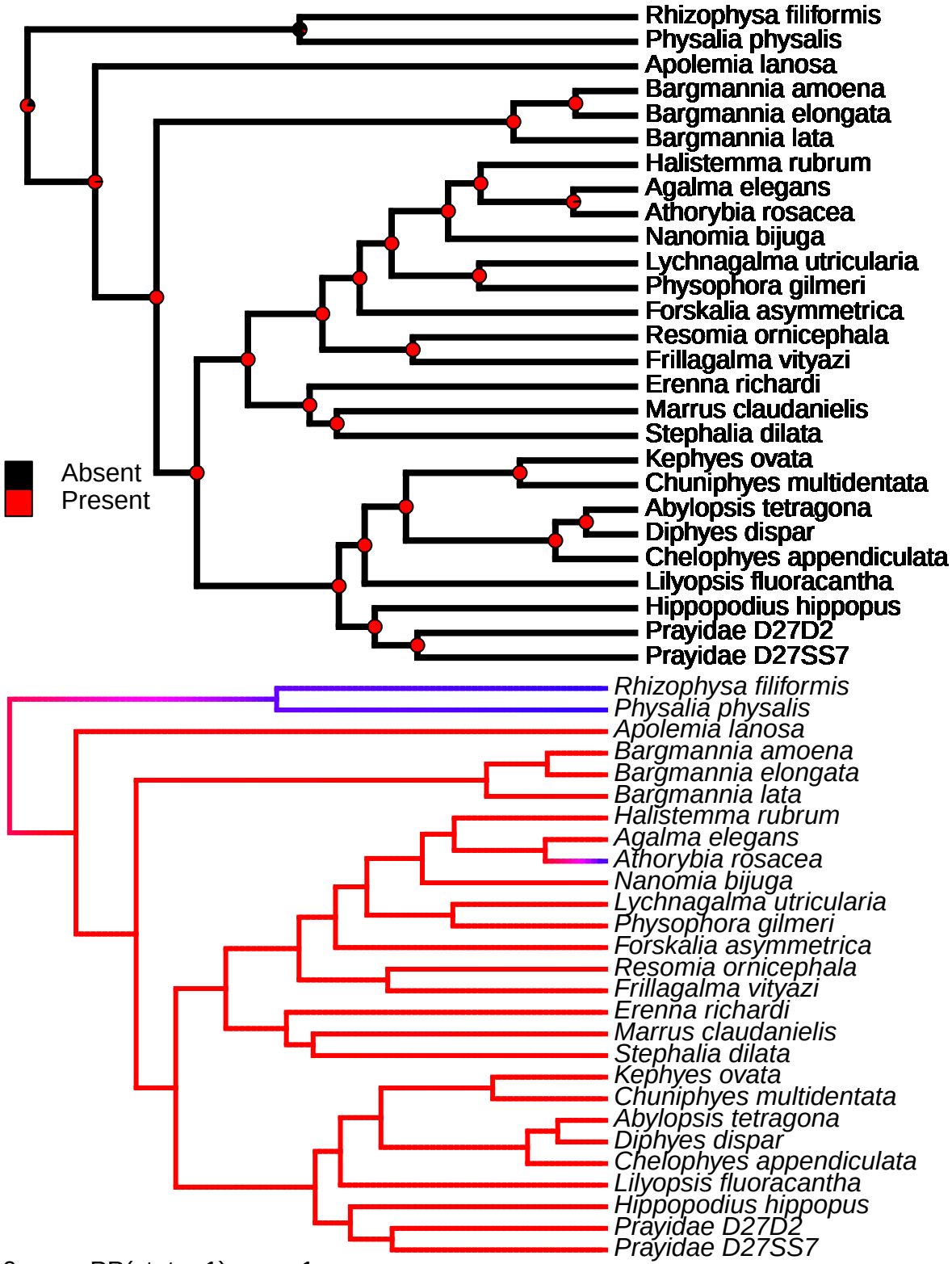
Physalia physalis may have reduced tentilla, which would indicate there was only one loss of tentilla at the branch leading to *Apolemia*.

SIMMAP Presence of tentilla - *Physalia* corrected



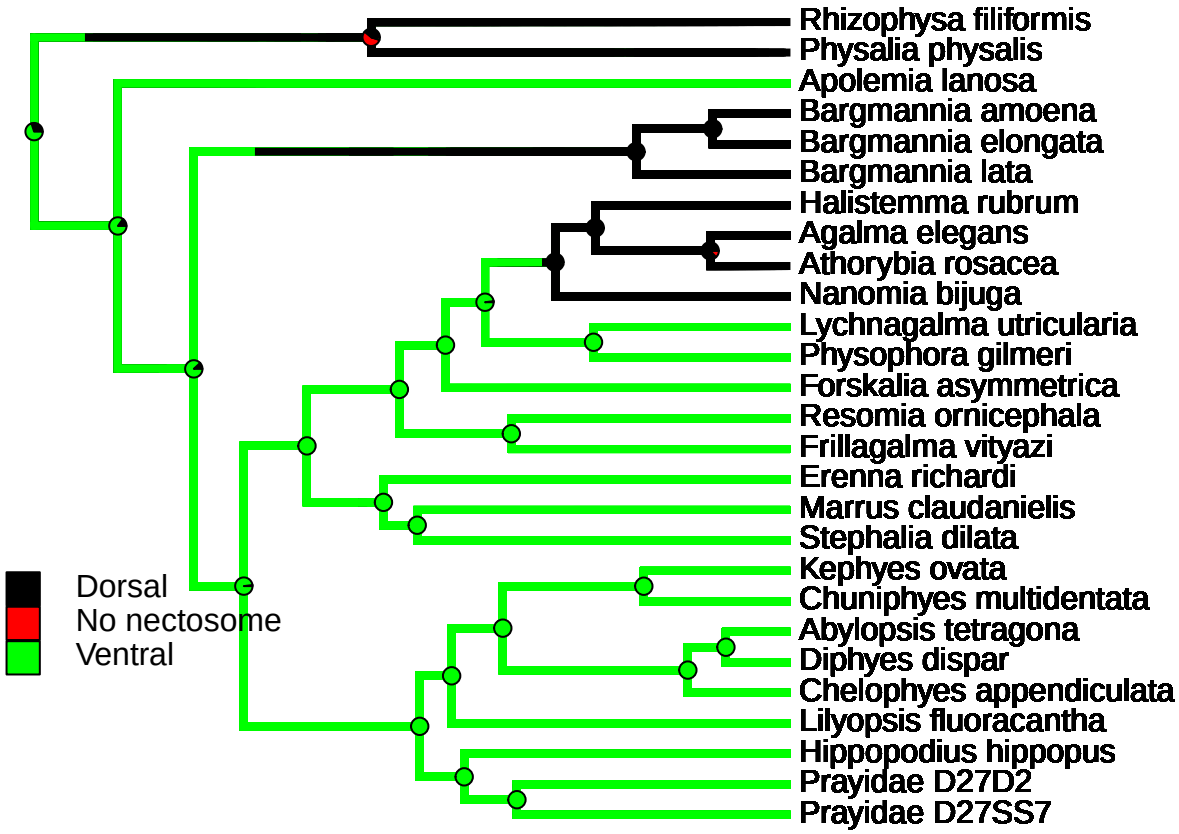
Posterior probabilities of states (1 Present, 0 Absent).

SIMMAP Presence of Nectophores

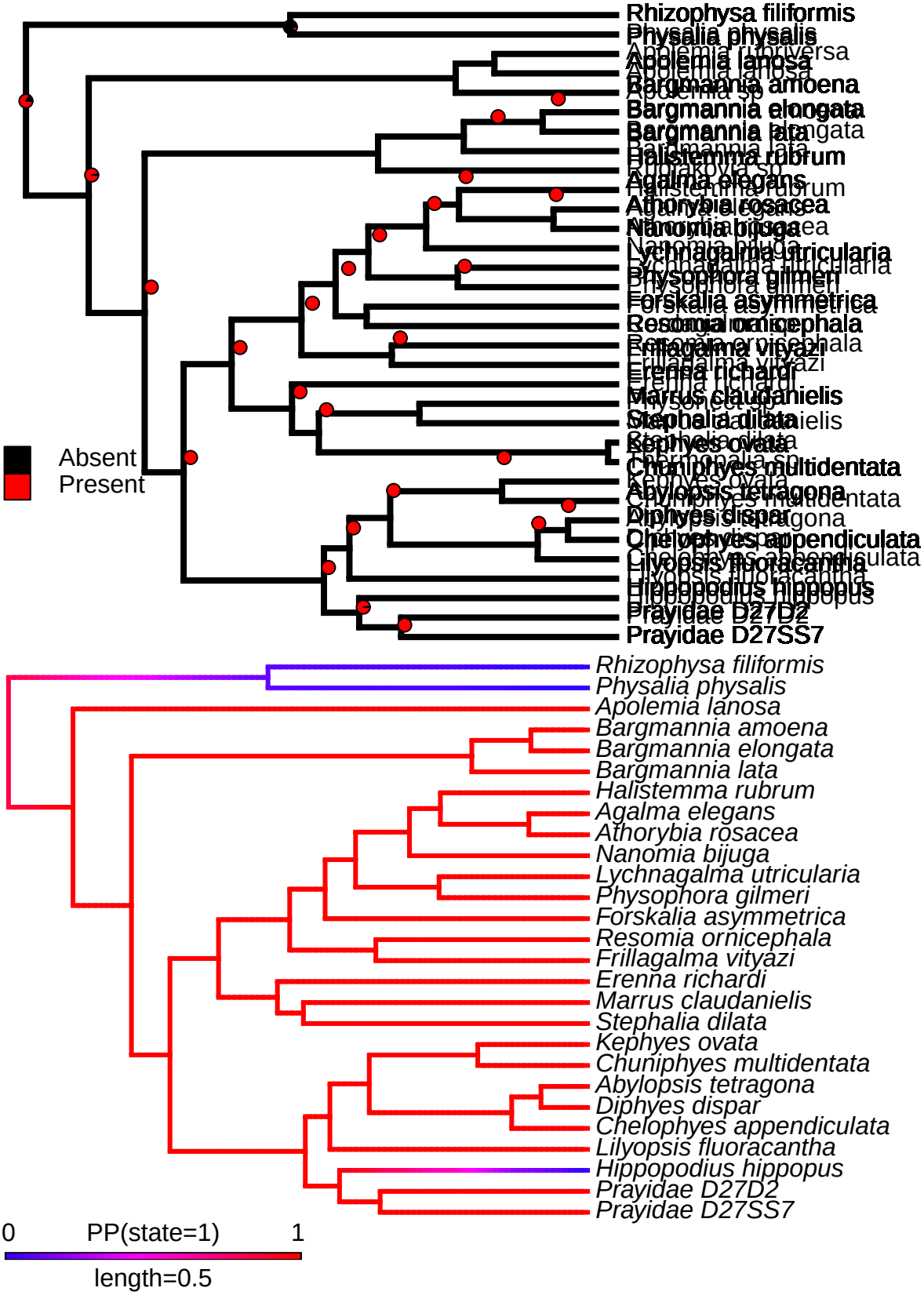


Posterior probabilities of states (1 Present, 0 Absent).

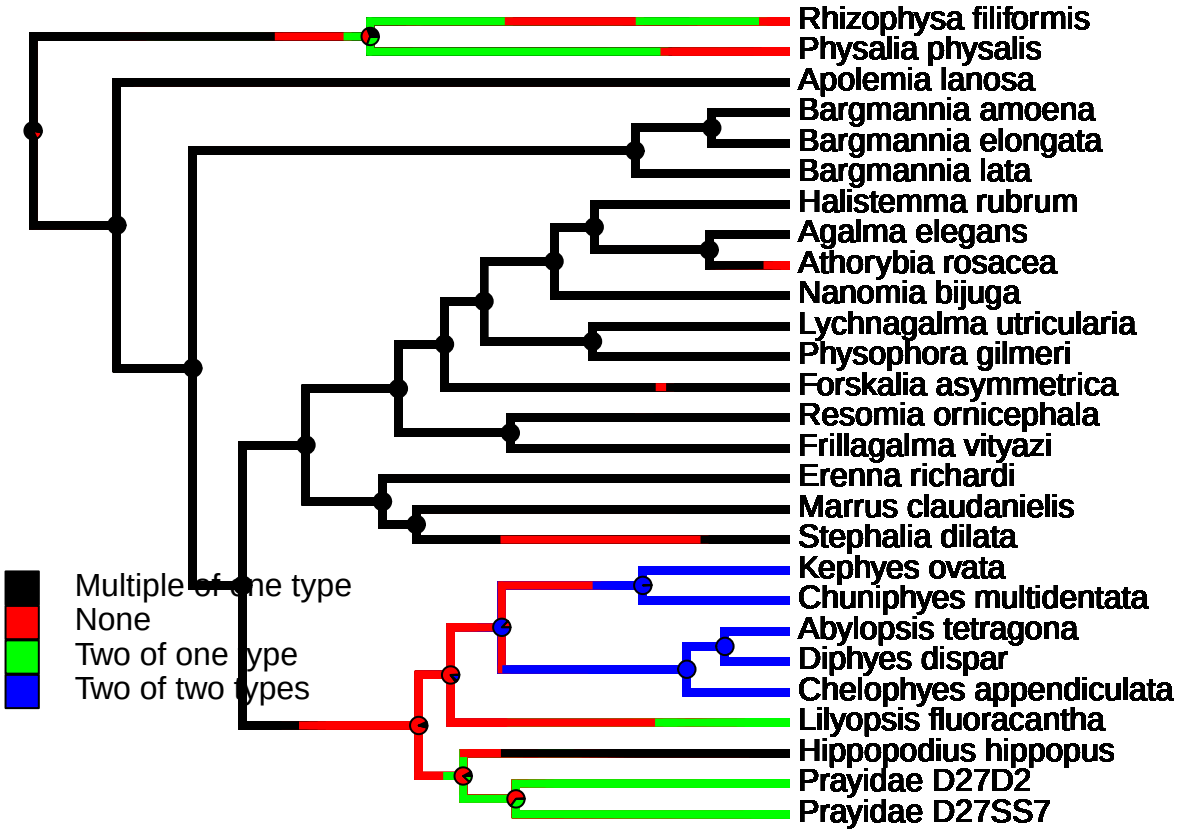
SIMMAP Position of Nectosome



SIMMAP BRACTS:

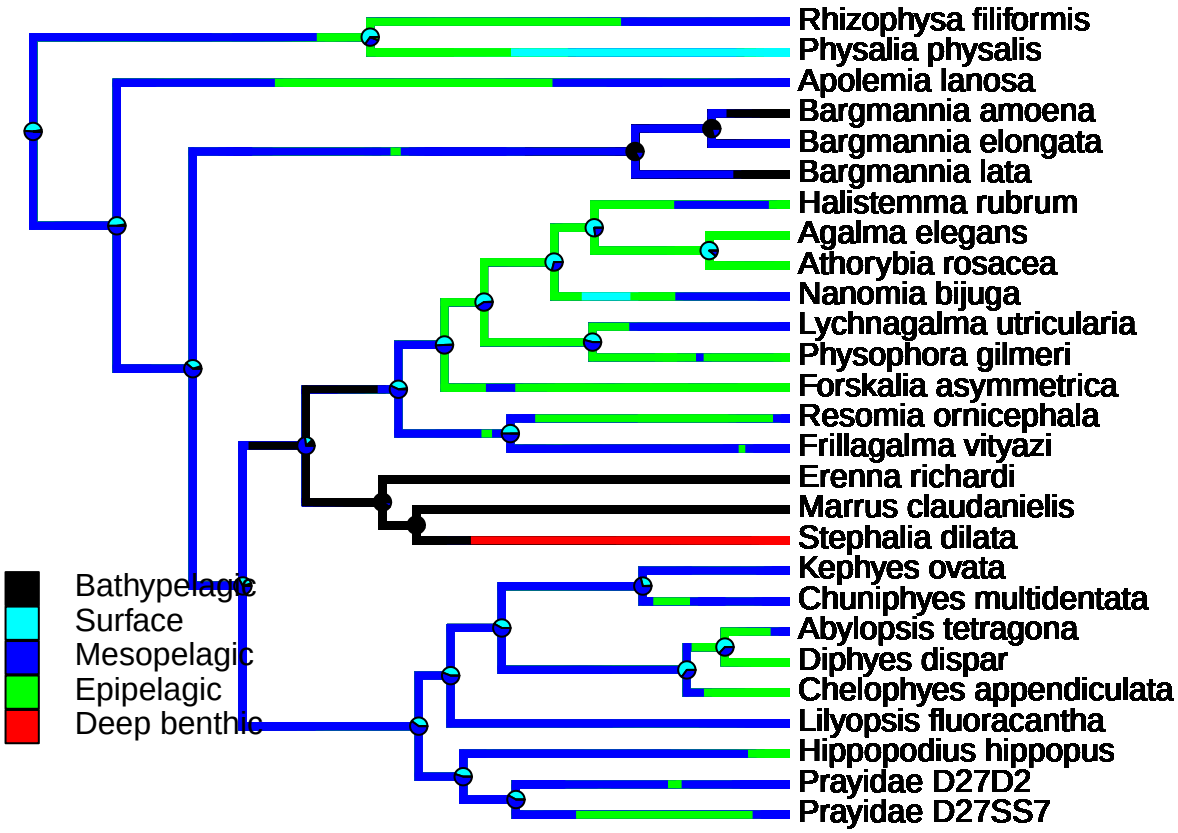


SIMMAP Nectophore number and types



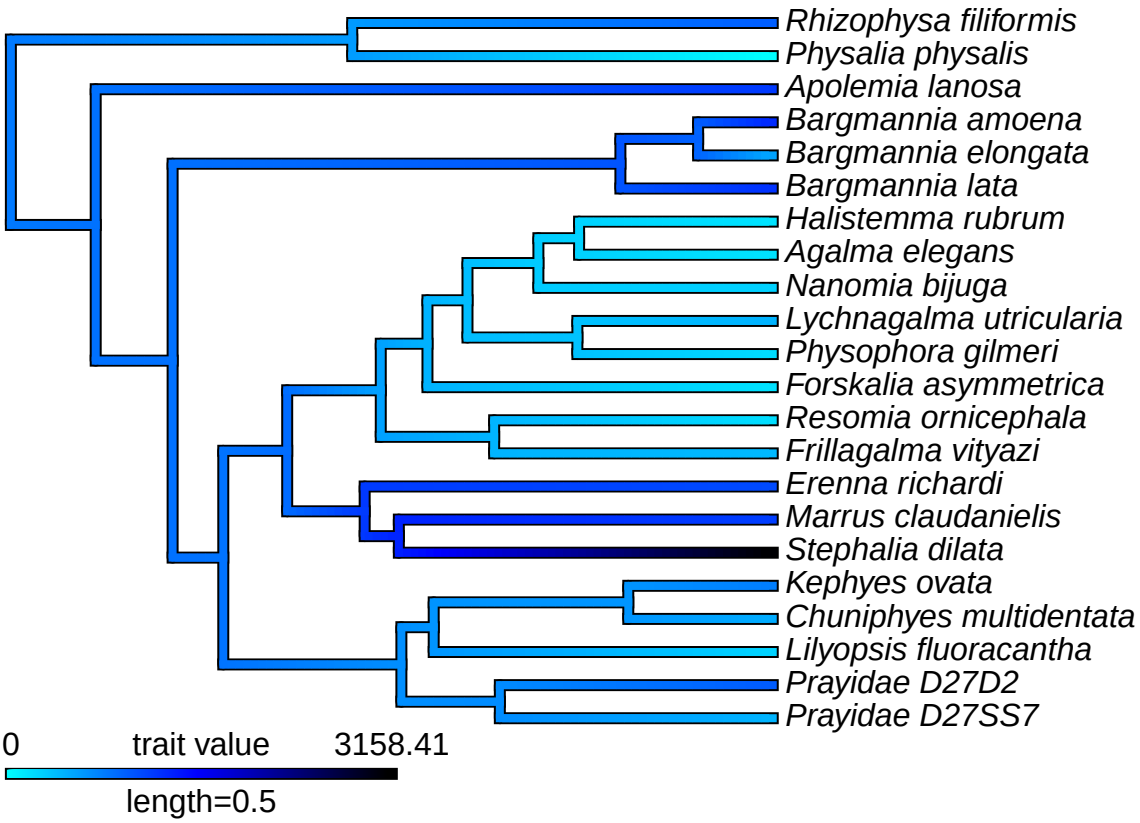
Posterior probabilities of states (1 Present, 0 Absent).It is not clear whether or not the stem group of siphonophores had a pneumatophore.

SIMMAP Habitat

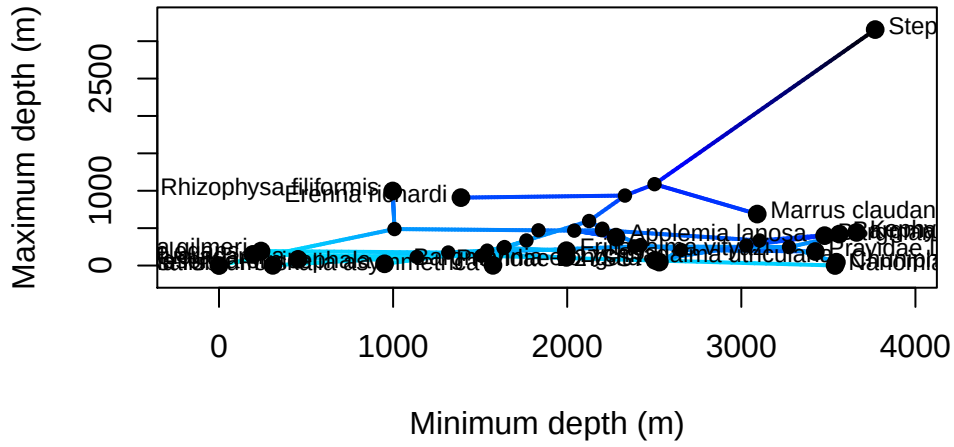


Continuous traits: Brownian motion reconstructions

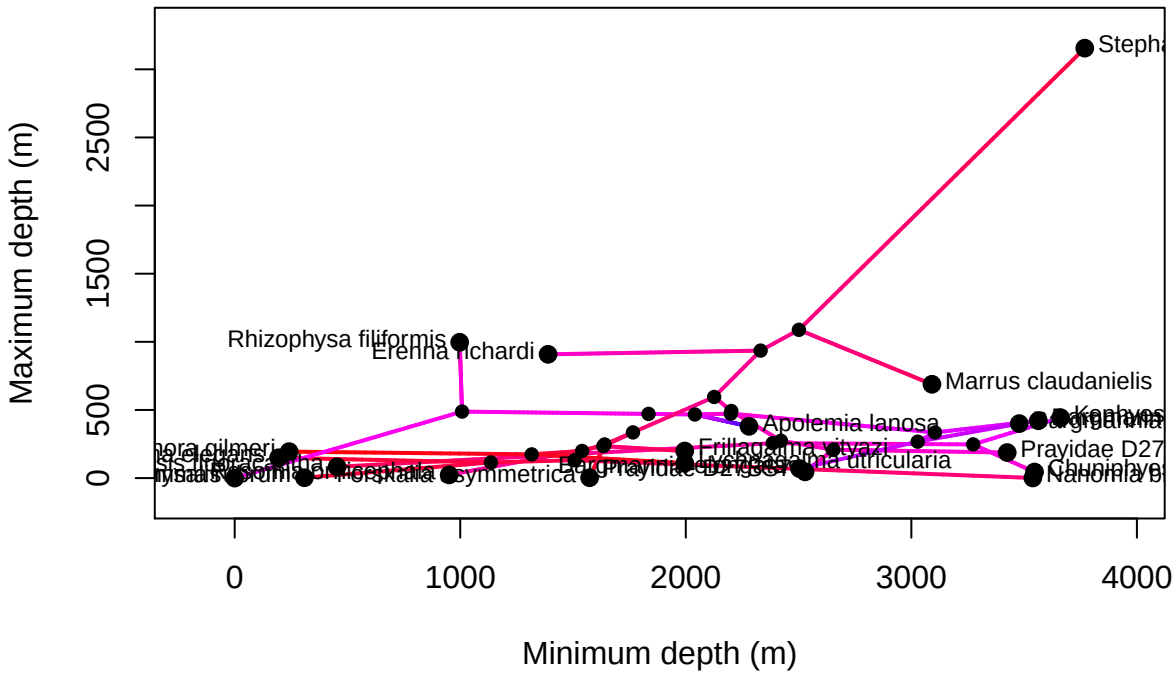
Median depth reconstruction:



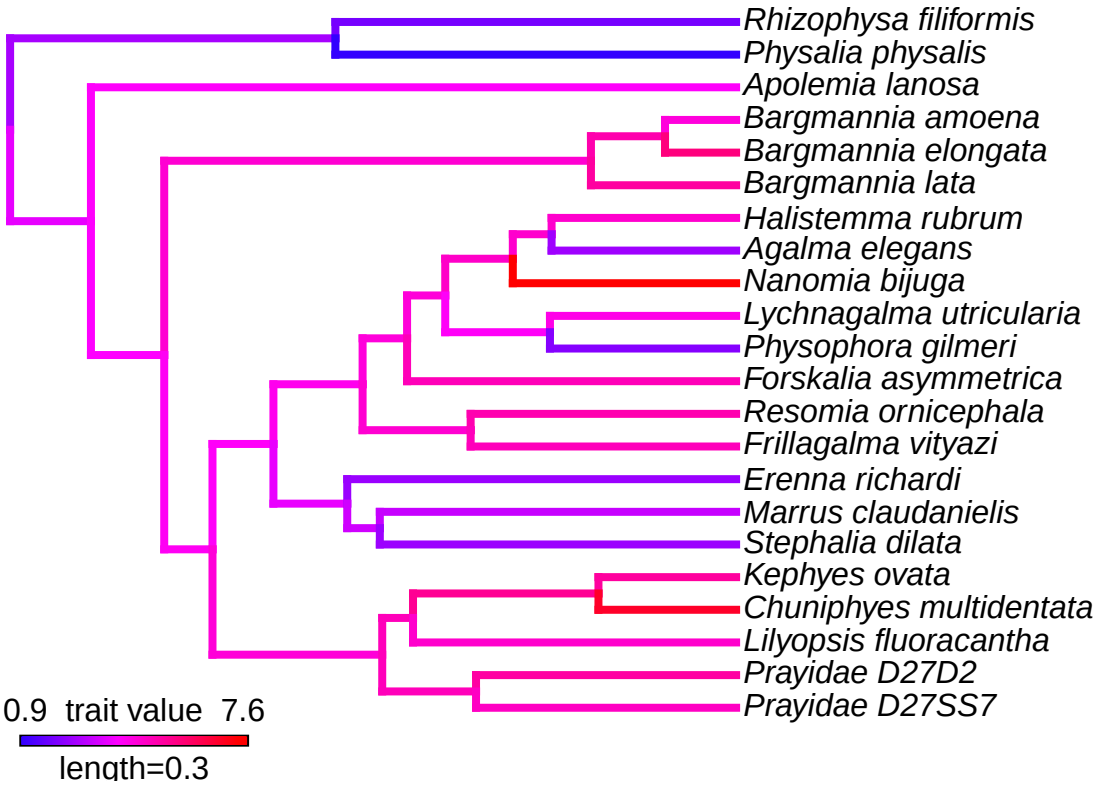
Bathymetrics phylomorphospace



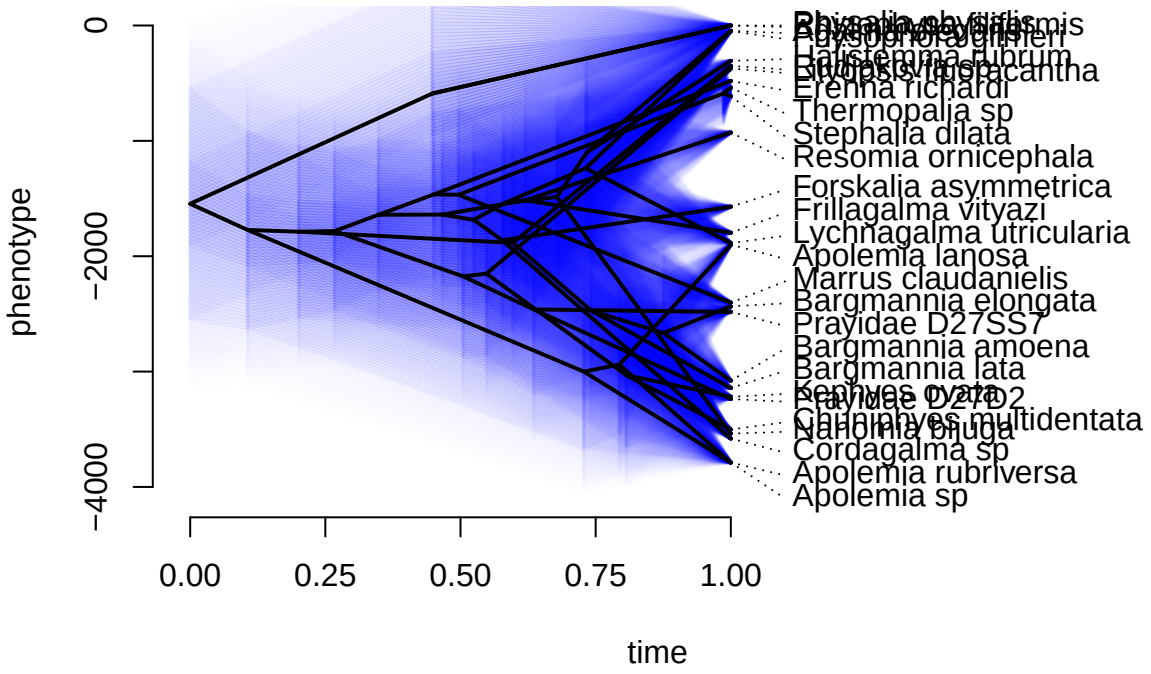
Bathymetrics phylomorphospace with time from root as color



Abundance of each taxon in the Monterey Bay sampling site

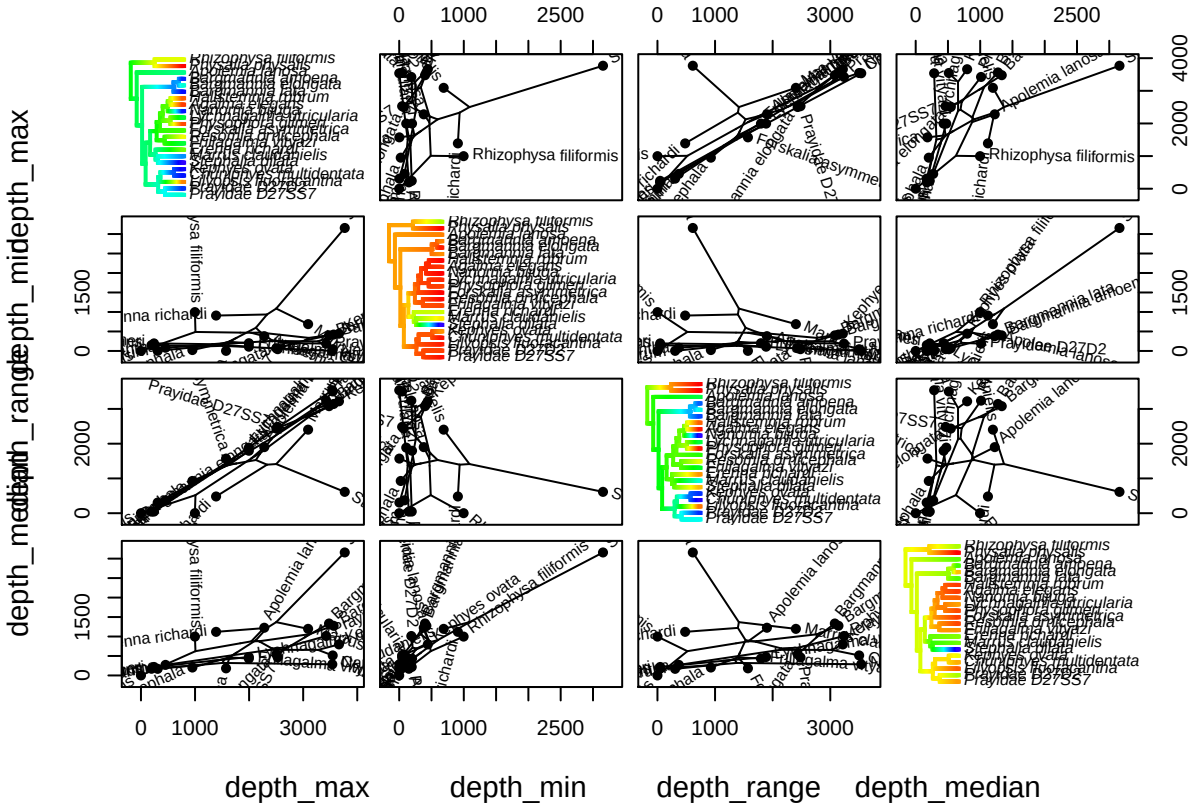


Uncertainty inclusive traitgram for depth range



Bathymetrics scattergram array

```
## Computing multidimensional phylogenetic scatterplot matrix...
```



Distribution of sex across siphonophore taxa

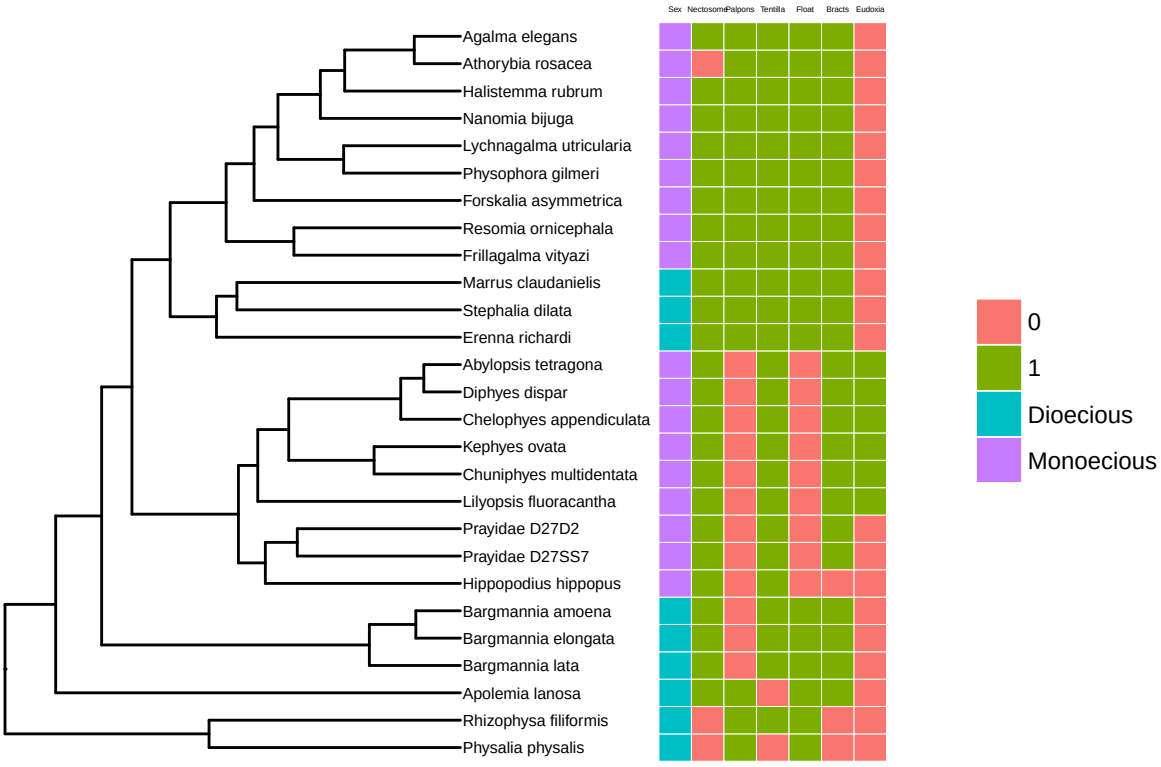
Tentilla presence across siphonophore taxa

Palpon presence across siphonophore taxa

Overview grid of main binary traits

```
##                                Sex Nectosome Palpons Tentilla
## Prayidae D27SS7          Monoecious         1       0        1
## Prayidae D27D2           Monoecious         1       0        1
## Hippopodius hippopus     Monoecious         1       0        1
## Lilyopsis fluoracantha   Monoecious         1       0        1
## Chelophyes appendiculata Monoecious         1       0        1
## Diphyes dispar           Monoecious         1       0        1
## Abylopsis tetragona      Monoecious         1       0        1
## Chuniphyes multidentata  Monoecious         1       0        1
## Kephyes ovata            Monoecious         1       0        1
## Stephalia dilata          Dioecious         1       1        1
## Marrus claudanielis       Dioecious         1       1        1
## Erenna richardi           Dioecious         1       1        1
## Frillagalma vityazi      Monoecious         1       1        1
## Resomia ornicephala      Monoecious         1       1        1
## Forskalia asymmetrica    Monoecious         1       1        1
## Physophora gilmeri       Monoecious         1       1        1
## Lychnagalma utricularia  Monoecious         1       1        1
## Nanomia bijuga           Monoecious         1       1        1
## Athorybia rosacea        Monoecious         0       1        1
## Agalma elegans           Monoecious         1       1        1
## Halistemma rubrum        Monoecious         1       1        1
## Bargmannia lata           Dioecious         1       0        1
## Bargmannia elongata       Dioecious         1       0        1
## Bargmannia amoena         Dioecious         1       0        1
## Apolemia lanosa           Dioecious         1       1        0
## Physalia physalis         Dioecious         0       1        0
## Rhizophysa filiformis     Dioecious         0       1        1
##                          Pneumatophore Bracts Eudoxia
## Prayidae D27SS7                      0      1       0
## Prayidae D27D2                       0      1       0
## Hippopodius hippopus                 0      0       0
## Lilyopsis fluoracantha               0      1       1
## Chelophyes appendiculata             0      1       1
## Diphyes dispar                       0      1       1
## Abylopsis tetragona                  0      1       1
## Chuniphyes multidentata              0      1       1
## Kephyes ovata                        0      1       1
## Stephalia dilata                     1      1       0
## Marrus claudanielis                  1      1       0
## Erenna richardi                      1      1       0
## Frillagalma vityazi                  1      1       0
## Resomia ornicephala                  1      1       0
## Forskalia asymmetrica                1      1       0
```

```
## Physophora gilmeri                1       1       0
## Lychnagalma utricularia           1       1       0
## Nanomia bijuga                    1       1       0
## Athorybia rosacea                 1       1       0
## Agalma elegans                    1       1       0
## Halistemma rubrum                 1       1       0
## Bargmannia lata                   1       1       0
## Bargmannia elongata               1       1       0
## Bargmannia amoena                 1       1       0
## Apolemia lanosa                   1       1       0
## Physalia physalis                 1       0       0
## Rhizophysa filiformis             1       0       0
```



Phylogenetic signal in the character data

Agnostic branch length tree generation:

Phylogenetic signal in Binary Traits

```
##                         K PIC.variance.obs PIC.variance.rnd.mean
## Nectosome      0.6773278       0.26773672             0.3507614
## Palpons        3.3888711       0.10615819             0.8624120
## Tentilla       2.1806411       0.07424251             0.2434057
## Pneumatophore 3.9000634       0.08521926             0.7768665
##                PIC.variance.P PIC.variance.Z
## Nectosome               0.319     -0.6680884
## Palpons                 0.001     -4.2989067
## Tentilla                0.003     -1.5642236
```

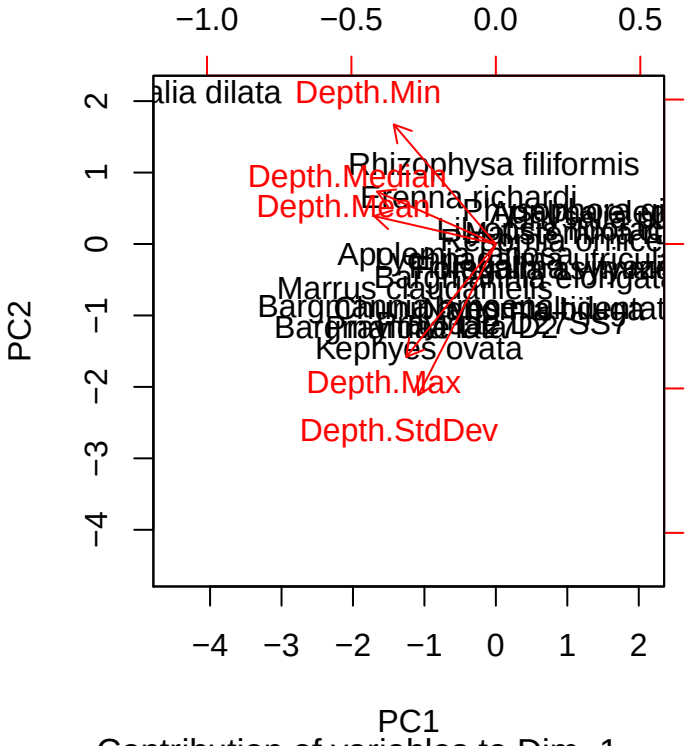
```
## Pneumatophore          0.001     -4.0370321
```

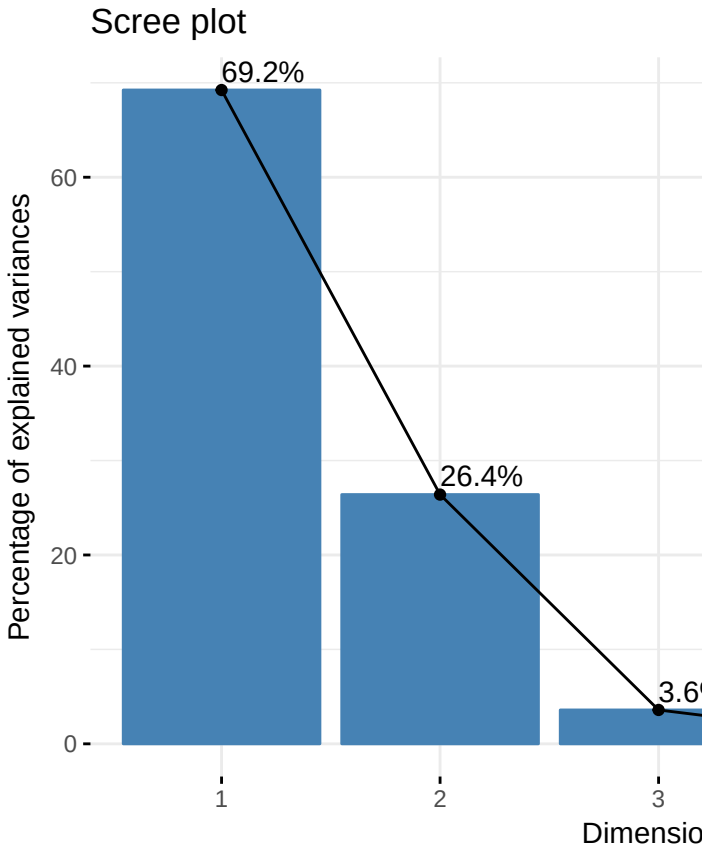
Phylogenetic signal in VARS bathymetrics

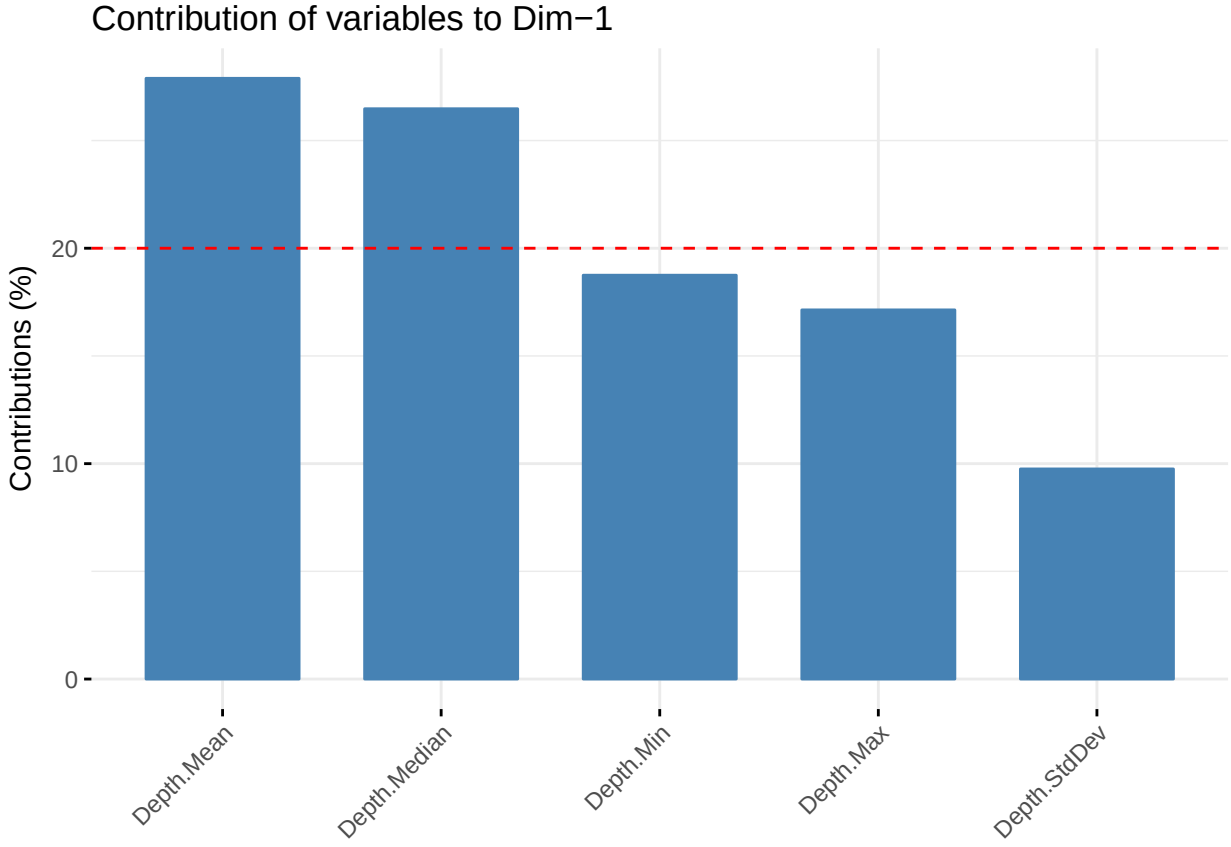
```
##
## Call:
## physignal(A = dpruned_data[, c(14, 15, 16, 18, 19)], phy = dpruned_tree)
##
##
##
##
## Observed Phylogenetic Signal (K): 0.7424
##
## P-value: 0.039
##
## Based on 1000 random permutations
```

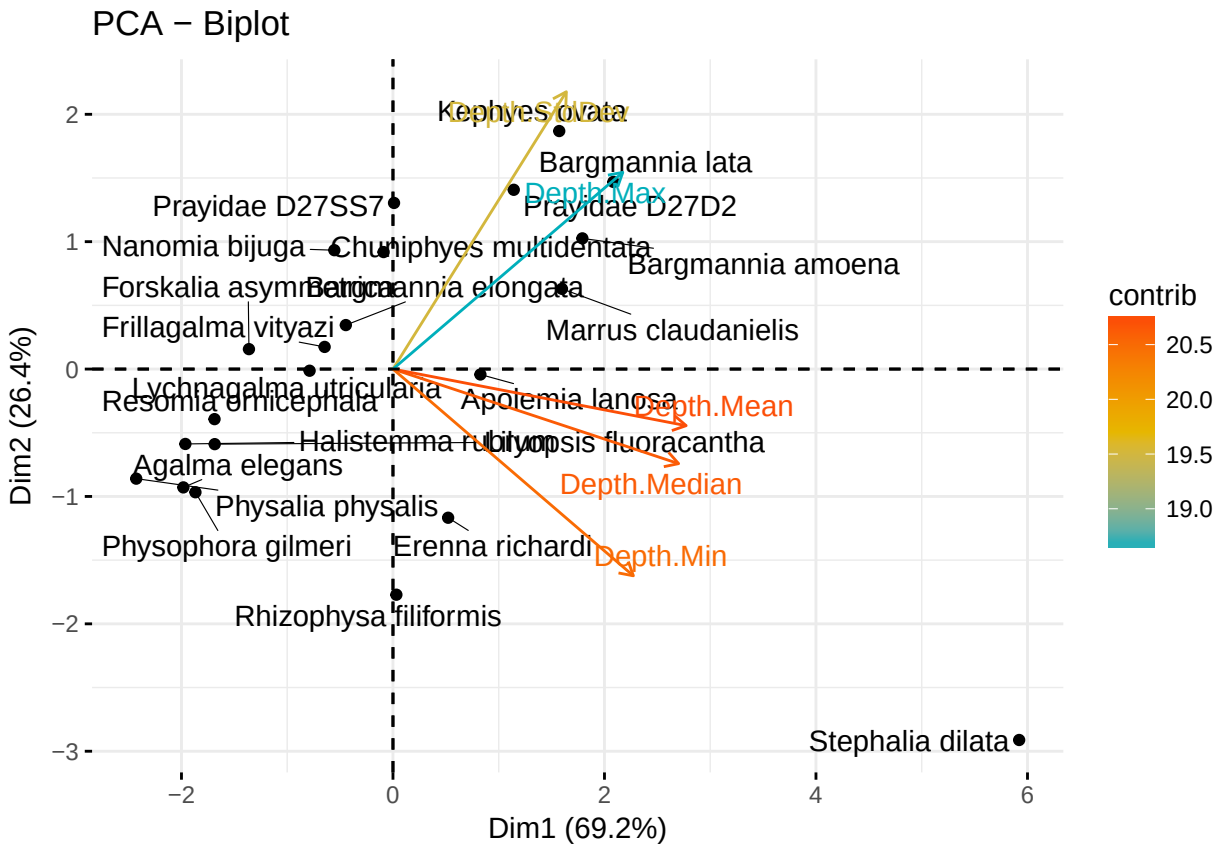
```
##                      K PIC.variance.obs PIC.variance.rnd.mean
## Depth.Median 0.7830816         797529.1             1276559.4
## Depth.Mean   0.7388410         941754.2             1453919.6
## Depth.StdDev 0.5929636         136382.4              164134.2
## Depth.Max    0.7181495        3265732.2             4754543.8
## Depth.Min    0.8396274         723182.9             1258465.5
##              PIC.variance.P PIC.variance.Z
## Depth.Median          0.065     -1.0427676
## Depth.Mean            0.057     -1.0214493
## Depth.StdDev          0.237     -0.7314187
## Depth.Max             0.059     -1.4636342
## Depth.Min             0.091     -0.9867170
```

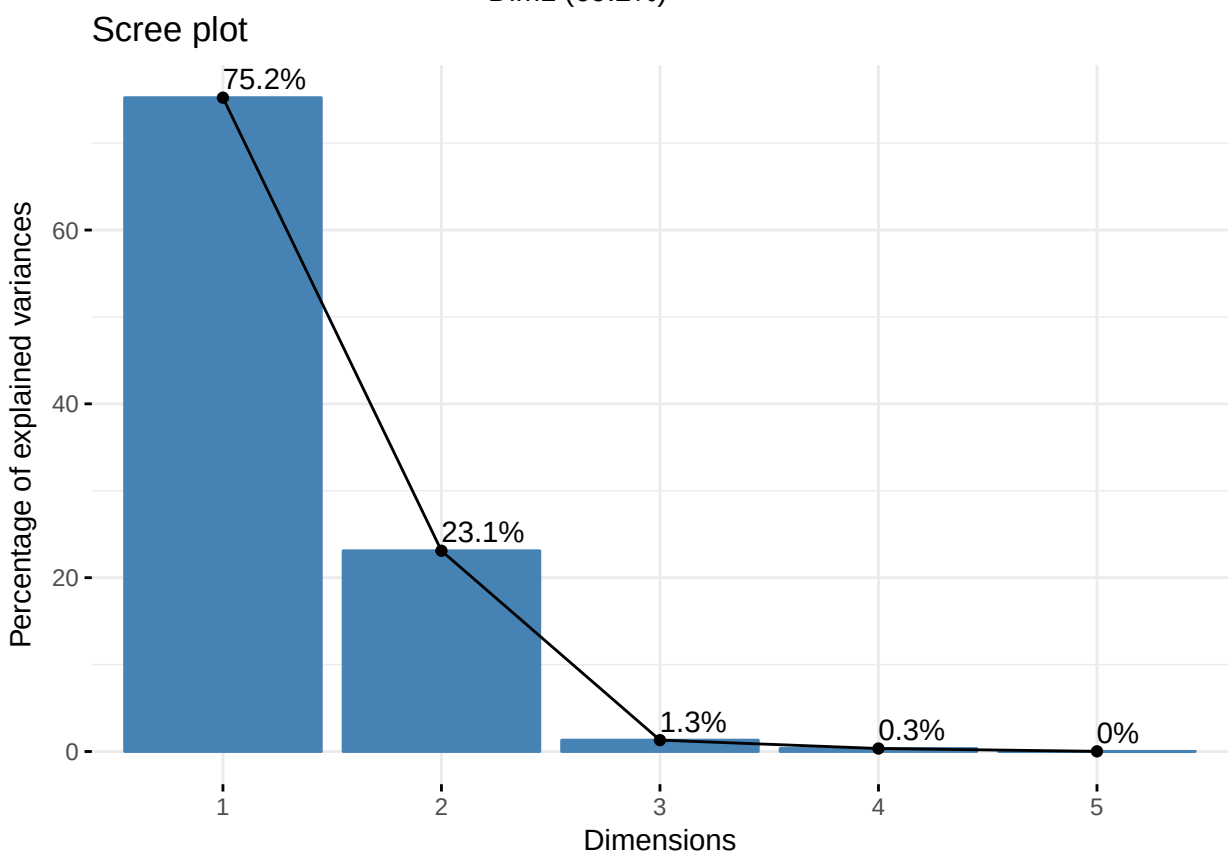
Phylogenetic PCA for bathymetrics

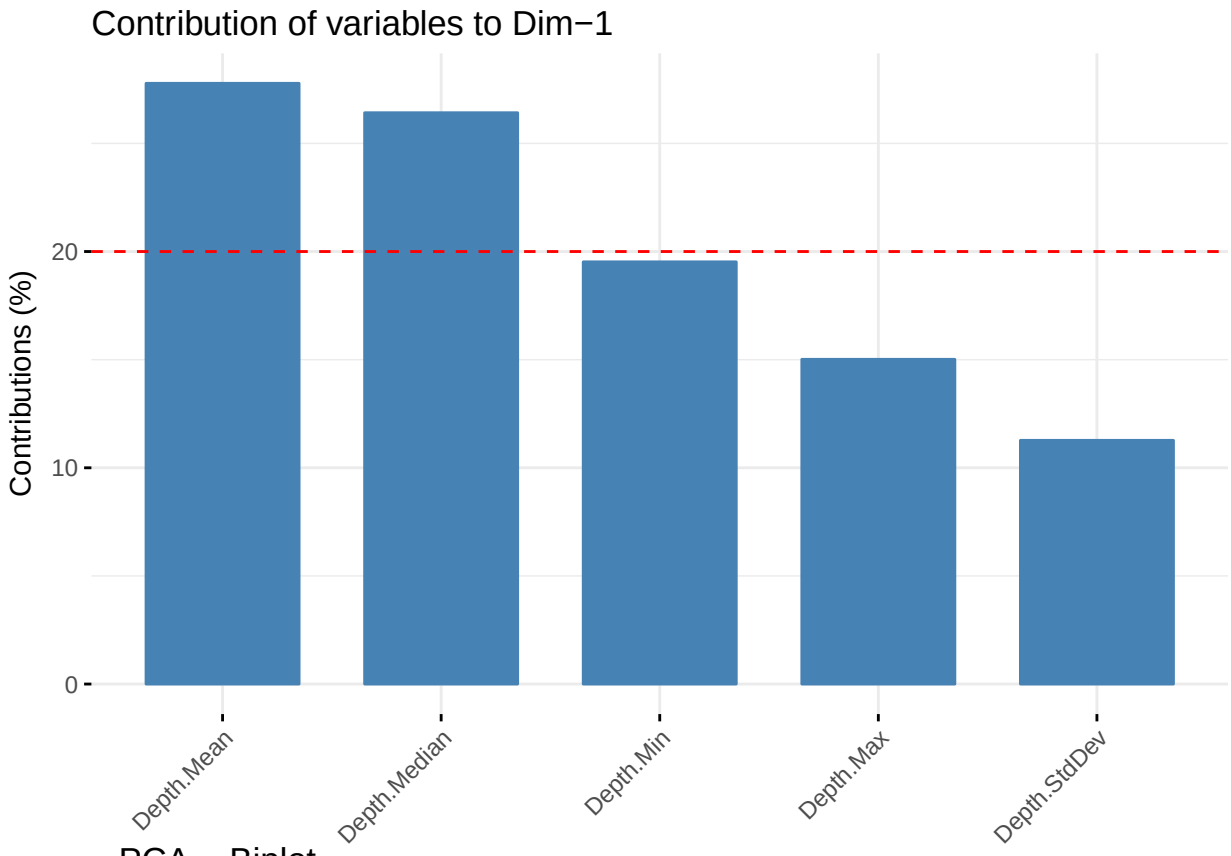

−1.0
−0.5
0.0
0.5
alia dilata
Depth.Min
Depth.Median
Depth.Mean
Rhizophysa filiformis
Eudoxoides spiralis
Kephyes ovata
Depth.Max
Depth.StdDev
PC2
PC1
2
1
0
−1
−2
−3
−4
0.5
0.0
−0.5
−1.0

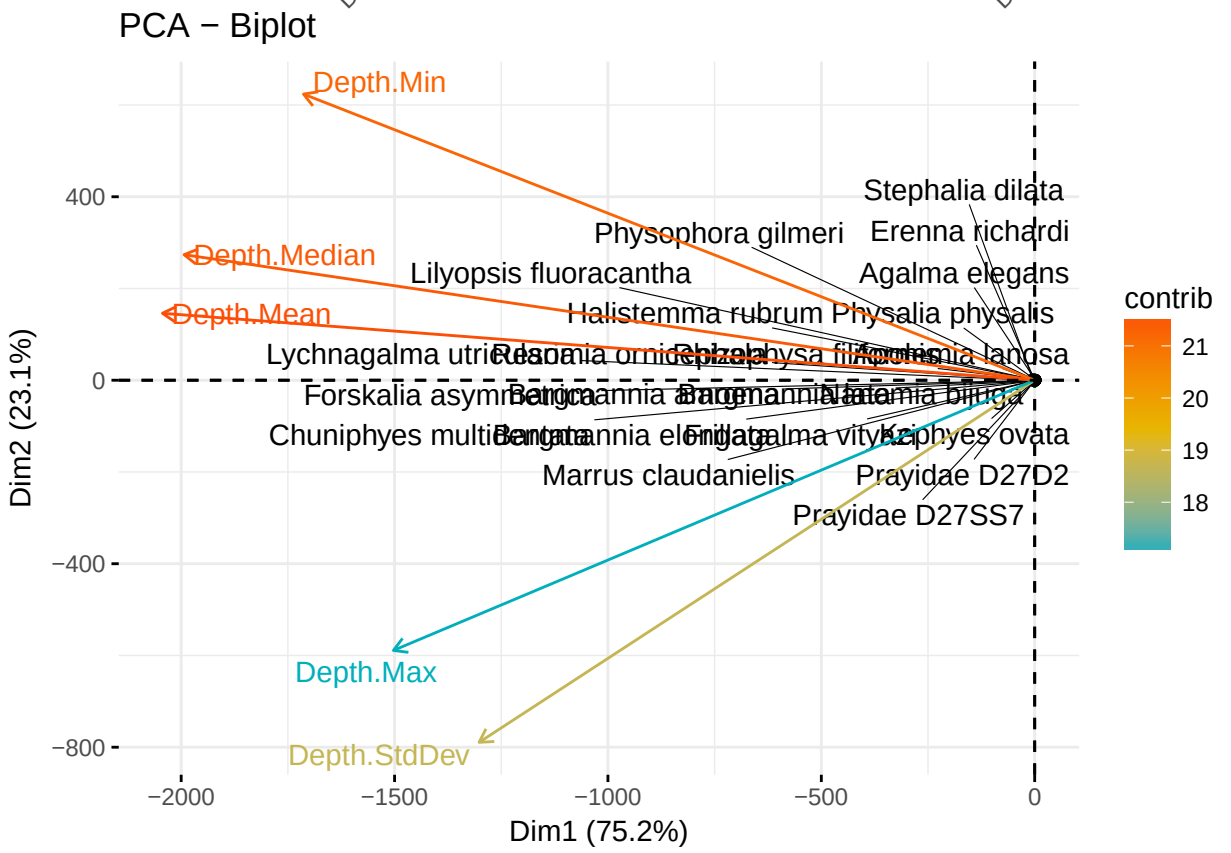

Scree plot
69.2%
26.4%
Percentage of explained variances
60
40
20
0
1
2
3
Dimensio

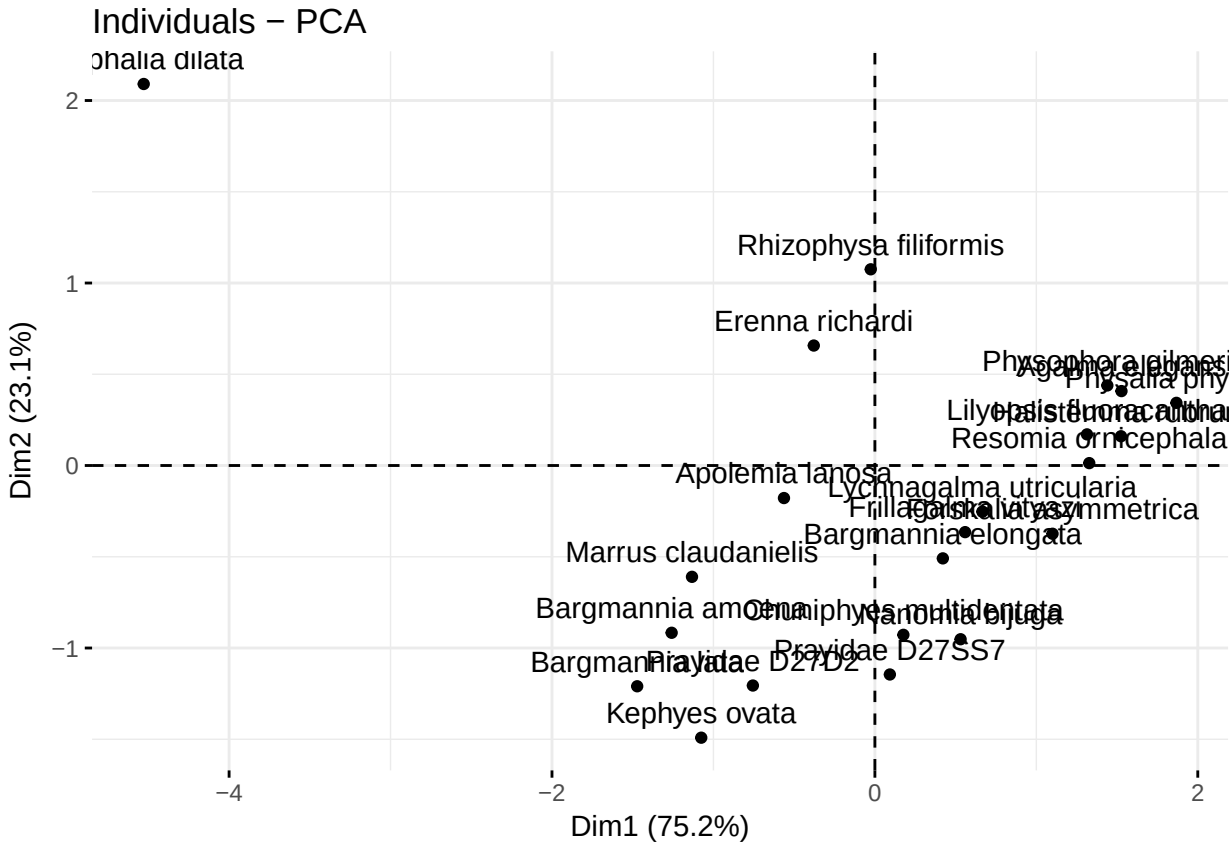

Contribution of variables to Dim−1
Contributions (%)
20
10
0
Depth.Mean
Depth.Median
Depth.Min
Depth.Max
Depth.StdDev


PCA − Biplot
Kephyes ovata
Bargmannia lata
Depth.Max
Prayidae D27SS7
Prayidae D27D2
Nanomia bijuga
Chuniphyes multidentata
Bargmannia amoena
Forskalia asymmetrica
Bargmannia elongata
Frillagalma vityazi
Marrus claudanielis
Lychnagalma utricularia
Resomia ornicephala
Apolemia lanosa
Depth.Mean
Halistemma rubrum
Diphyopsis fluoracantha
Agalma elegans
Depth.Median
Physalia physalis
Physophora gilmeri
Erenna richardi
Depth.Min
Rhizophysa filiformis
Stephalia dilata
contrib
20.5
20.0
19.5
19.0
Dim2 (26.4%)
Dim1 (69.2%)
2
1
0
−1
−2
−3
−2
0
2
4
6


Scree plot
75.2%
23.1%
1.3%
0.3%
0%
Percentage of explained variances
Dimensions
60
40
20
0
1
2
3
4
5


Contribution of variables to Dim−1
Contributions (%)
20
10
0
Depth.Mean
Depth.Median
Depth.Min
Depth.Max
Depth.StdDev


PCA − Biplot
Depth.Min
Depth.Median
Depth.Mean
Depth.Max
Depth.StdDev
Stephalia dilata
Physophora gilmeri
Erenna richardi
Lilyopsis fluoracantha
Agalma elegans
Halistemma rubrum
Physalia physalis
Lychnagalma utricularia
Forskalia asymmetrica
Chuniphyes multidentata
Chuniphyes ovata
Marrus claudanielis
Prayidae D27D2
Prayidae D27SS7
contrib
21
20
19
18
Dim2 (23.1%)
400
0
−400
−800
−2000
−1500
−1000
−500
0
Dim1 (75.2%)



Phylogenetic ANOVA for depth

Each phylogenetic ANOVA compares variance of depth (continuous variable) within and between groups clustered by their categorical biological traits.

```
## [1] "Depth vs Sex Distribution"

## $F
## [1] 10.48139
##
## $Pf
## [1] 0.081
##
## $T
##            Dioecious Monoecious
## Dioecious   0.000000   3.237497
## Monoecious -3.237497   0.000000
##
## $method
## [1] "holm"
##
## $Pt
##            Dioecious Monoecious
## Dioecious      1.000      0.081
## Monoecious     0.081      1.000

## [1] "Depth vs Nectosome"
```

```
## $F
## [1] 0.2358588
##
## $Pf
## [1] 0.784
##
## $T
##          0         1
## 0 0.000000 -0.485653
## 1 0.485653  0.000000
##
## $method
## [1] "holm"
##
## $Pt
##       0     1
## 0 1.000 0.784
## 1 0.784 1.000
```

```
## [1] "Depth vs Nectophore Types and Number"
```

```
## $F
## [1] 0.156464
##
## $Pf
## [1] 0.982
##
## $T
##                       Multiple of one type None One of one type
## Multiple of one type                   NA   NA              NA
## None                                   NA   NA              NA
## One of one type                        NA   NA              NA
## Two of one type                        NA   NA              NA
## Two of two types                       NA   NA              NA
##                       Two of one type Two of two types
## Multiple of one type              NA               NA
## None                              NA               NA
## One of one type                   NA               NA
## Two of one type                   NA               NA
## Two of two types                  NA               NA
##
## $method
## [1] "holm"
##
## $Pt
##                       Multiple of one type None One of one type
## Multiple of one type                   NA   NA              NA
## None                                   NA   NA              NA
## One of one type                        NA   NA              NA
## Two of one type                        NA   NA              NA
## Two of two types                       NA   NA              NA
##                       Two of one type Two of two types
## Multiple of one type              NA               NA
## None                              NA               NA
## One of one type                   NA               NA
```

```
## Two of one type                   NA               NA
## Two of two types                  NA               NA
```

```
## [1] "Depth vs Nectosome Position"
```

```
## $F
## [1] 0.237949
##
## $Pf
## [1] 0.909
##
## $T
##             Dorsal      None    Ventral
## Dorsal   0.0000000 0.2240508 -0.4988957
## None    -0.2240508 0.0000000 -0.5640384
## Ventral  0.4988957 0.5640384  0.0000000
##
## $method
## [1] "holm"
##
## $Pt
##         Dorsal None Ventral
## Dorsal       1    1       1
## None         1    1       1
## Ventral      1    1       1
```

```
## [1] "Depth vs Palpons"
```

```
## $F
## [1] 0.05567054
##
## $Pf
## [1] 0.888
##
## $T
##            0         1
## 0  0.0000000 0.2359461
## 1 -0.2359461 0.0000000
##
## $method
## [1] "holm"
##
## $Pt
##       0     1
## 0 1.000 0.888
## 1 0.888 1.000
```

```
## [1] "Depth vs Tentilla"
```

```
## $F
## [1] 0.06009463
##
## $Pf
## [1] 0.856
##
## $T
##           0          1
```

```
## 0 0.0000000 -0.2451421
## 1 0.2451421  0.0000000
##
## $method
## [1] "holm"
##
## $Pt
##       0     1
## 0 1.000 0.856
## 1 0.856 1.000
```

```
## [1] "Depth vs Pneumatophore"
```

```
## $F
## [1] 0.1796993
##
## $Pf
## [1] 0.817
##
## $T
##           0          1
## 0 0.0000000 -0.4239096
## 1 0.4239096  0.0000000
##
## $method
## [1] "holm"
##
## $Pt
##       0     1
## 0 1.000 0.817
## 1 0.817 1.000
```

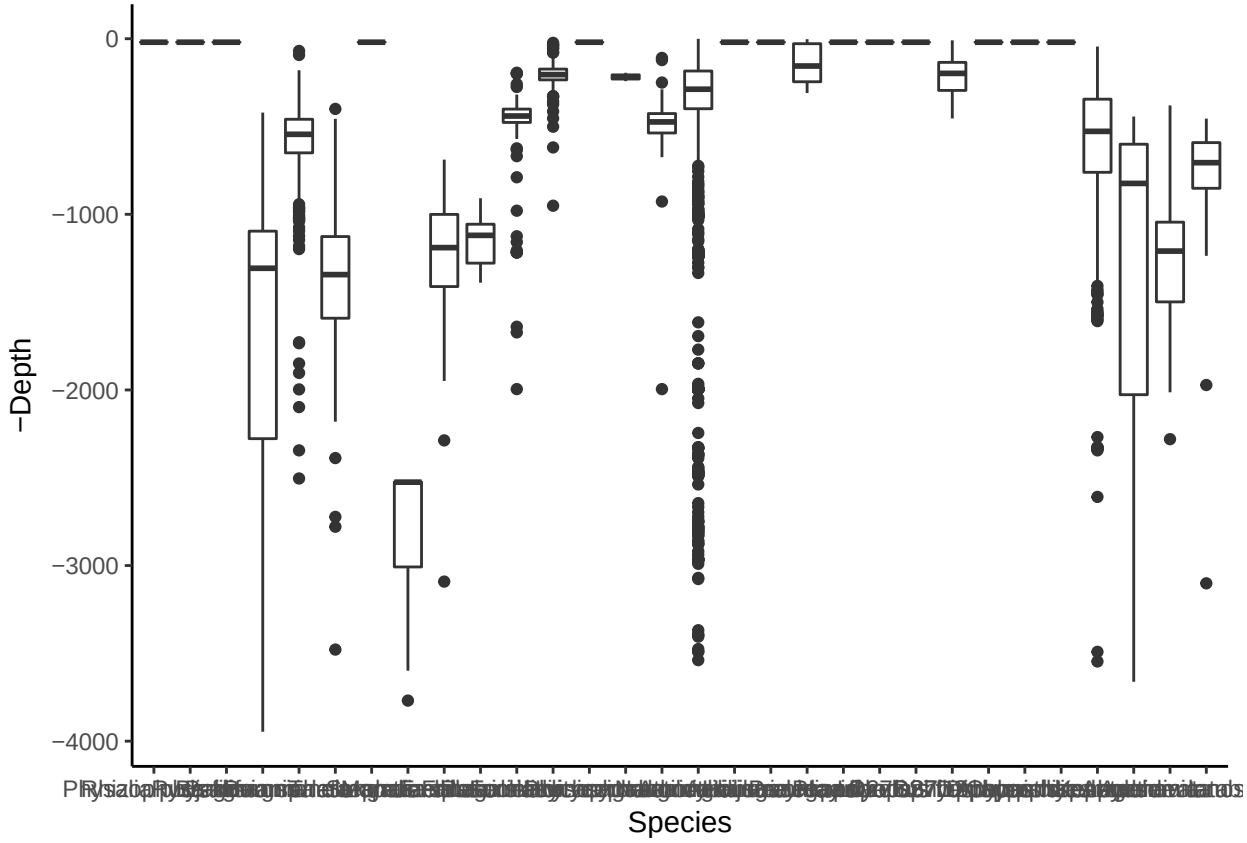
PGLS - Sex distribution

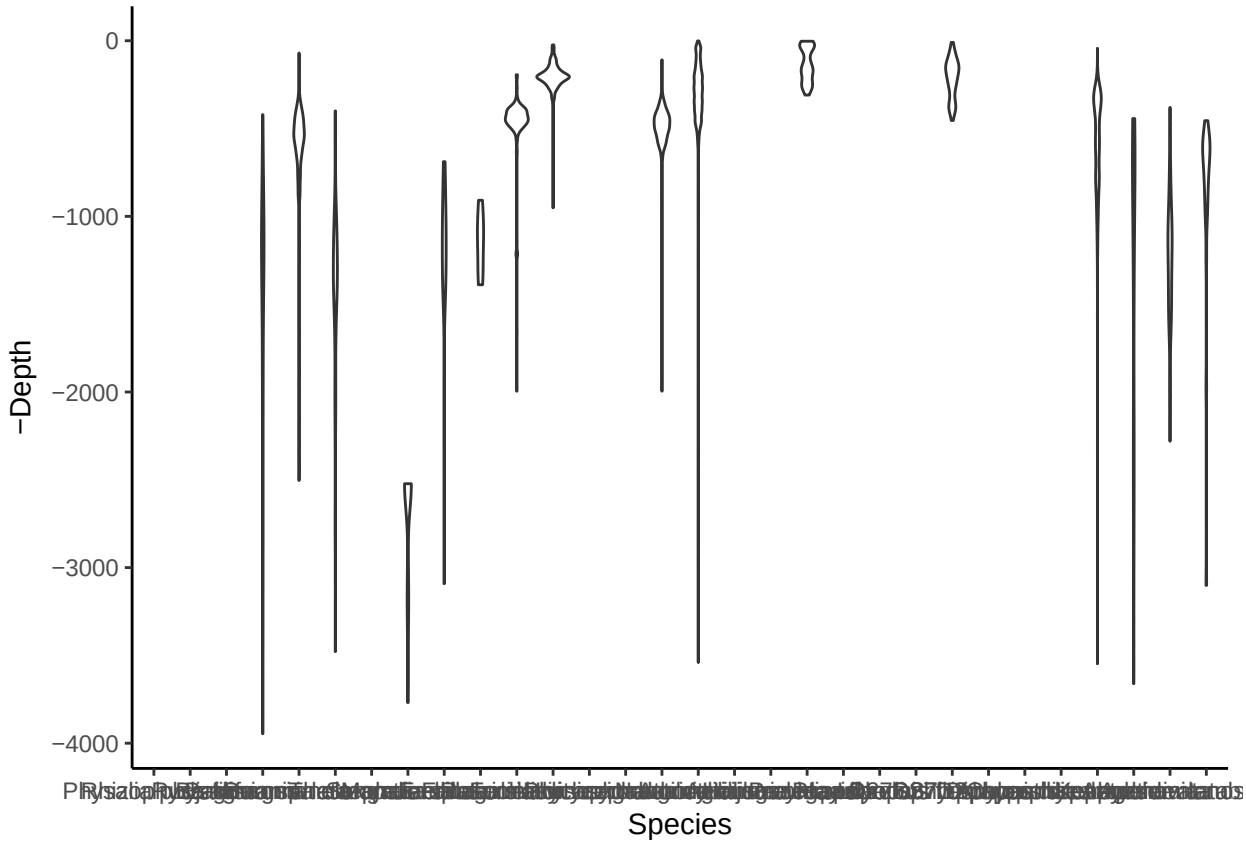
```
## ------------------------------------------------------------------
## | **Warning:                                                     |
## |   User reports suggest that this method may frequently      |
## |   fail to find the ML solution. Please use with caution.    |
## ------------------------------------------------------------------
```

```
## Call:
## phylolm(formula = depth_median ~ dp_sex, data = as.data.frame(cbind(depth_median,
##     dp_sex)), phy = dpruned_tree, model = "BM", measurement_error = TRUE,
##     boot = 100)
##
##    AIC logLik
##  347.7 -169.9
##
## Parameter estimate(s) using ML:
## sigma2: 217361.9
## sigma2_error: 142822
##
## Coefficients:
## (Intercept)      dp_sex
##    192.7735    958.4204
```

```
## Generalized least squares fit by REML
##   Model: dp_sex ~ depth_median
##   Data: NULL
##   Log-restricted-likelihood: -20.52555
##
## Coefficients:
##  (Intercept) depth_median
## 0.0978229509 0.0004267556
##
## Degrees of freedom: 22 total; 20 residual
## Residual standard error: 0.4176998
```

VARS data barplot code





Software versions

This manuscript was computed on Tue Nov 21 16:05:26 2017 with the following R package versions.

```
R version 3.4.1 (2017-06-30)
Platform: x86_64-apple-darwin15.6.0 (64-bit)
Running under: macOS Sierra 10.12.2

Matrix products: default
BLAS: /Library/Frameworks/R.framework/Versions/3.4/Resources/lib/libRblas.0.dylib
LAPACK: /Library/Frameworks/R.framework/Versions/3.4/Resources/lib/libRlapack.dylib

locale:
[1] en_GB.UTF-8/en_GB.UTF-8/en_GB.UTF-8/C/en_GB.UTF-8/en_GB.UTF-8

attached base packages:
[1] grid      parallel  stats     graphics  grDevices utils     datasets
[8] methods   base

other attached packages:
 [1] bindrcpp_0.2     phylolm_2.5      geomorph_3.0.5   rgl_0.98.1
 [5] adephylo_1.1-10  ade4_1.7-8       phylobase_0.8.4  geiger_2.0.6
 [9] phangorn_2.2.0   phytools_0.6-20  picante_1.6-2    nlme_3.1-131
[13] vegan_2.4-4      lattice_0.20-35  permute_0.9-4    ape_4.1
[17] hutan_0.5.0      FactoMineR_1.38  factoextra_1.0.5 gridExtra_2.3
[21] seriation_1.2-2  fields_9.0       maps_3.2.0       spam_2.1-1
```

```
[25] dotCall64_0.9-04 ggtree_1.8.2     treeio_1.0.2     cowplot_0.8.0
[29] xtable_1.8-2     jsonlite_1.5     knitr_1.17       digest_0.6.12
[33] magrittr_1.5     forcats_0.2.0    stringr_1.2.0    dplyr_0.7.4
[37] purrr_0.2.3      readr_1.1.1      tidyr_0.7.1      tibble_1.3.4
[41] ggplot2_2.2.1    tidyverse_1.1.1

loaded via a namespace (and not attached):
  [1] readxl_1.0.0            uuid_0.1-2
  [3] backports_1.1.1         fastmatch_1.1-0
  [5] plyr_1.8.4              igraph_1.1.2
  [7] lazyeval_0.2.0          sp_1.2-5
  [9] splines_3.4.1           rncl_0.8.2
 [11] foreach_1.4.3           htmltools_0.3.6
 [13] viridis_0.4.0           gdata_2.18.0
 [15] cluster_2.0.6           gclus_1.3.1
 [17] modelr_0.1.1            gmodels_2.16.2
 [19] prettyunits_1.0.2       jpeg_0.1-8
 [21] colorspace_1.3-2        rvest_0.3.2
 [23] ggrepel_0.7.0           haven_1.1.0
 [25] bindr_0.1               survival_2.41-3
 [27] iterators_1.0.8         glue_1.1.1
 [29] registry_0.3            gtable_0.2.0
 [31] seqinr_3.4-5            kernlab_0.9-25
 [33] prabclus_2.2-6          DEoptimR_1.0-8
 [35] scales_0.5.0            mvtnorm_1.0-6
 [37] DBI_0.7                 Rcpp_0.12.13
 [39] plotrix_3.6-6           viridisLite_0.2.0
 [41] progress_1.1.2          spdep_0.6-15
 [43] flashClust_1.01-2       foreign_0.8-69
 [45] subplex_1.4-1           bold_0.5.0
 [47] mclust_5.3              deSolve_1.20
 [49] stats4_3.4.1            animation_2.5
 [51] htmlwidgets_0.9         httr_1.3.1
 [53] gplots_3.0.1            fpc_2.1-10
 [55] modeltools_0.2-21       pkgconfig_2.0.1
 [57] reshape_0.8.7           XML_3.98-1.9
 [59] flexmix_2.3-14          deldir_0.1-14
 [61] nnet_7.3-12             crul_0.4.0
 [63] tidyselect_0.2.0        labeling_0.3
 [65] rlang_0.1.2             reshape2_1.4.2
 [67] munsell_0.4.3           cellranger_1.1.0
 [69] tools_3.4.1             broom_0.4.2
 [71] evaluate_0.10.1         yaml_2.1.14
 [73] robustbase_0.92-7       caTools_1.17.1
 [75] dendextend_1.5.2        mime_0.5
 [77] whisker_0.3-2           taxize_0.9.0
 [79] adegenet_2.1.0          leaps_3.0
 [81] xml2_1.1.1              compiler_3.4.1
 [83] curl_2.8.1              clusterGeneration_1.3.4
 [85] RNeXML_2.0.7            stringi_1.1.5
 [87] highr_0.6               trimcluster_0.1-2
 [89] Matrix_1.2-11           psych_1.7.8
 [91] msm_1.6.4               LearnBayes_2.15
 [93] combinat_0.0-8          data.table_1.10.4
```

```
 [95] bitops_1.0-6            httpuv_1.3.5
 [97] R6_2.2.2                TSP_1.1-5
 [99] KernSmooth_2.23-15      codetools_0.2-15
[101] boot_1.3-20             MASS_7.3-47
[103] gtools_3.5.0            assertthat_0.2.0
[105] rprojroot_1.2           mnormt_1.5-5
[107] diptest_0.75-7          mgcv_1.8-22
[109] expm_0.999-2            hms_0.3
[111] quadprog_1.5-5          coda_0.19-1
[113] class_7.3-14            rmarkdown_1.6
[115] rvcheck_0.0.9           ggpubr_0.1.5
[117] shiny_1.0.5             numDeriv_2016.8-1
[119] scatterplot3d_0.3-40    lubridate_1.6.0
```

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Species	Mean insert size (bp)	Insert size standard deviation	Number of read pairs	Percent kept after rRNA removal	Percent kept after assembly	Adapter fails	Quality fails	Base Composition fails	Total assembled transcripts	Coding transcripts
Marrus claudanielis	253.03	53.30	35866092	96.8	62.1	113298	15594637	4981676	105439	22511
Apolemia rubriversa	290.81	80.64	19228660	91.7	77.8	40594	5179800	516100	87701	17540
Chuniphyes multidentata	284.46	35.74	17999147	77.0	74.3	24263	4941872	335297	84341	22084
Apolemia sp	167.41	53.46	16761717	81.3	79.4	1332063	3062035	41943	51470	14752
Apolemia lanosa	167.74	55.30	18444476	96.4	80.9	923922	3882968	439569	70184	15579
Bargmannia elongata	271.43	40.17	40008913	79.8	78.5	342825	8084832	2017709	152019	23661
Diphyes dispar	223.14	58.73	80000000	78.7	63.4	14412120	21322624	690159	210406	50868
Aiptasia pallida	183.18	53.88	74558341	98.4	79.3	210730	23826421	307520	83950	33523
Stephalia dilata	267.82	43.33	30953585	98.0	63.9	86068	15731787	1435119	107925	23984
Physalia physalis	233.10	54.37	36481773	96.6	88.5	18553	5491979	259497	74994	23705
Bargmannia amoena	161.57	53.77	20195498	84.6	80.1	1049110	3996085	274520	66726	17975
Frillagalma vityazi	283.11	44.91	79902051	54.0	70.4	668400	13591301	5558767	181508	29293
Alatina alata	173.96	60.33	96259870	52.1	77.9	1236251	15771112	468662	166584	28743
Clytia hemisphaerica									11476	6642
Ectopleura larynx	229.05	37.75	109024653	95.9	74.6	432309	40629127	897590	84034	28015
Athorybia rosacea	221.16	93.65	28696930	99.9	86.9	691916	4026467	633700	100839	24543
Forskalia asymmetrica	259.42	35.04	25275184	58.9	65.1	41922	7463840	841930	82483	18419
Prayidae D27D2	267.32	45.59	38233199	85.2	66.4	59773	15338562	1878732	144909	28065
Lilyopsis fluoracantha	259.39	39.08	51855968	76.5	65.3	72416	19809124	1388875	114662	29854
Hydractinia symbiolongicarpus	175.22	57.84	60462724	49.8	85.2	162430	6678373	124392	71450	24639
Rhizophysa filiformis	269.74	46.52	26937827	92.5	65.3	66010	13238701	361376	110109	24833
Agalma elegans	238.94	57.54	40007833	99.6	86.0	31957	7177806	748977	122053	26601
Chelophyes appendiculata	275.93	39.43	21103284	89.0	74.2	63548	6777022	344708	110612	32309
Prayidae D27SS7	303.39	50.83	25233164	66.7	65.6	39061	8616289	347189	94917	24211
Rudjakovia sp	213.88	89.71	20582477	99.1	79.7	489520	3936086	1546686	91695	24184
Abylopsis tetragona	292.16	38.32	21575176	73.3	73.8	38764	5759144	370604	102946	25268
Nematostella vectensis									26511	18080
Hippopodius hippopus	266.99	45.11	35638254	84.5	66.3	56152	15490291	535026	137341	35031
Physophora gilmeri	214.97	93.78	19847377	99.3	81.3	936617	3464079	1007839	52639	17381
Bargmannia lata	166.71	62.80	16984483	98.7	71.1	3118496	4227140	868584	59051	16451
Nanomia bijuga	296.69	44.20	39983229	85.7	75.3	155093	10854240	795226	165792	28927
Erenna richardi	291.53	36.76	29776547	64.2	66.6	39775	8958200	1245523	89423	18236
Physonect sp	261.09	35.96	38243543	24.3	66.7	12494	4517982	274838	57746	12596