

# The evolution of siphonophore tentilla reveals association with diet

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## Abstract

Siphonophores have the most complex nematocyst batteries of all Cnidaria. These structures are held on the tentacles' side branches called tentilla. Tentilla serve as the principal organs for prey capture, making siphonophores an ideal system for the study of trophic specialization from an evolutionary approach. Modern comparative methods have been applied to study the evolution of siphonophore zooid types, but not yet to the unique morphological diversity of siphonophore tentilla. The primary objectives of this work are to describe the morphology and morphometrics of siphonophore tentilla and nematocysts, and identify patterns in the evolutionary history of siphonophore cnidoband and nematocyst morphologies. A phylogeny with 55 species was reconstructed using 18S and 16S gene markers, constraining the topology to be congruent with a transcriptome-based tree. Morphological characters were measured from DIC and confocal microscopy images of fixed tentacle specimens. Diet data was extracted from published sources. The functional implications of the different morphologies during prey capture were recorded using high speed video on live specimens under a stereoscopic microscope. Evolutionary transitions in siphonophore tentilla present a very low dimensionality with a single principal component. This main axis of variation aligns with total nematocyst volume per tentillum. Most nematocyst complement (cnidome) traits appear to have evolved under simple Brownian Motion (BM) and Early Burst (EB) processes, while tentillum shape and size evolution is better represented by Ornstein-Uhlenbeck (OU) models. Many tentillum and cnidome traits show stronger supports towards OU models when diet is reconstructed on the tree as selective regimes. Using phylogenetic GLS we identified traits with correlated evolutionary histories, some of which are functionally coupled during prey capture. Size and abundance of nematocysts were phylogenetic correlated with prey type ratios in the diet. These patterns could represent macroevolutionary adaptive responses to prey availability leading to predatory specialization. Understanding the evolution of the prey capture apparatus of siphonophores can help us test hypotheses about the evolution of their predatory habits.

## 1. Introduction

Siphonophores are colonial planktonic predators present in all oceans of the world.

Siphonophores bear the most complex nematocyst batteries of all cnidarians, with up to 4 types of positionally and morphologically differentiated nematocysts, combined with a spring-release mechanism for fast discharge of the structure onto the prey. Not all siphonophores bear tentilla on their tentacles, exceptions include *Apolemia* spp. and *BathypHYSA conifera*. While *Physalia physalis* has been considered to have simple tentacles, it is likely that their button-like structures are homologous to reduced tentilla (Munro et al. 2018). All tentilla are unilateral side branches of the tentacle, with epidermis, mesoglea, gastrodermis, and gastrovascular cavity. Each gastrozooid bears a single tentacle. Each tentacle serially buds tentilla from the basal region (basigaster), which develop as they are displaced further with tentacle growth. During development, tentilla grow and change shape into their final configuration while mature nematocysts formed in the basigaster migrate through the tentacle epidermis into the tentillum. Nematocysts arriving into the developing tentillum then rearrange into their final position (Skaer 1991). Tentilla are a single-use ensnaring weapon which is ingested and digested together with the prey it attached to. The cnidoband and terminal filament bear all the active nematocysts. Cnidobands range in size from 40 microns to 8 cm, they can be coiled or straight, free hanging or enclosed in an involucre (Mapstone 2014). Cnidobands always carry haploneme nematocysts, which can be isorhizas or anisorhizas. In addition, many species' cnidoband can carry a row of heteroneme

nematocysts along each side. These heteroneme nematocyst can be stenoteles, microbasic mastigophores, euryteles, or birhopaloids. In codonophorans, tentilla bear a pair of elastic strands (Totton and Bargmann 1965) derived from the wall of the gastrovascular cavity.

While zooid type evolution has been addressed in a phylogenetic framework, tentillum and nematocyst morphology has not. Since tentilla are used exclusively for prey capture, we expect their morphological diversity to have evolved in adaptive response to the evolution of different diets and prey type specializations. Earlier work by Purcell (Purcell 1984) has shown clear associations between nematocyst morphology, size, and number with prey type and copepod prey length. However, this pioneer study did not take phylogenetic relationships into account beyond grouping species by taxonomic suborder, and was limited to a few epipelagic species.

## 2. Methods

### 3.1 Phylogeny

For this phylogenetic analysis we included 55 siphonophore species and 6 outgroup cnidarian species (*Clytia hemisphaerica*, *Hydra circumcincta*, *Ectopleura dumortieri*, *Porpita porpita*, *Velella velella*, *Staurocladia wellingtoni*). The gene sequences used in this study are available online (accession numbers in Supp. Table XX). Some of these sequences were used in (Dunn et al. 2005), others were sequenced by XYZ. Sequences were aligned using MAFFT.

A Maximum Likelihood (ML) phylogeny (Figure XX) was reconstructed from 16S and 18S ribosomal rRNA genes using IQTree (Nguyen et al., 2015) with 1000 bootstraps. We used ModelFinder (Kalyaanamoorthy et al., 2017) implemented in IQTree v1.5.5. to assess relative model fit. ModelFinder selected GTR+R4 for having the lowest Bayesian Information Criterion score. A minimal set of 5 nodes were constrained to be consistent with the (Munro et al. 2018) transcriptome phylogeny strict consensus tree. In addition to the ML tree, we reconstructed a Bayesian tree with each marker as an independent partition in RevBayes. The same node constraints were applied, and the tree topology recovered was identical to the ML tree. To obtain ultrametric branch lengths for this topology, a Bayesian strict molecular clock time-tree was constructed in Rev Bayes, using a birth-death process constrained to be fully congruent with the topology recovered in the abovementioned analyses. Scripts available in Suppl. Mat. XX.

### 3.2 Microscopy

Specimens from the Monterey Bay and Gulf of California were collected by remotely operated underwater vehicles (ROV) or by blue-water SCUBA divers, following the protocols established in (Haddock and Heine 2005).

All specimens are accessioned in the Yale Peabody Museum Invertebrate Zoology Collection. Accession numbers and collection data for the specimens are available in Supplementary Table XXX. All specimens were preserved in ~4% buffered formalin in seawater.

A total of 39 siphonophore species were selected based on availability and phylogenetic representativity criteria to capture intraspecific variation. Three to five specimens from each selected species were measured in order to account for intraspecific variation. Additionally, 30 species with only a single specimen were also measured and reported in this work, but their data will not be used in the statistical analyses downstream.

Tentacles from the studied specimens were dissected, dehydrated in a series of ethanol dilutions (25% in PBS, 50% in PBS, 75% in water, 100%), cleared in methyl salicylate or d-limonene, and mounted in plastic slides (Canada balsam, Cytoseal, or Permount). Slides were imaged using laser scanning confocal microscopy and differential interference contrast (DIC) microscopy. Images were measured using Fiji-ImageJ (Schindelin et al. 2012).

The characters measured for this study are described in Supplementary Table SXX. Homology assumptions for nematocyst supertypes (heteroneme, haploneme, rhopaloneme, and desmoneme) and tentillum structures (involucrum, elastic strand, pedicle, cnidoband) were informed by positional, developmental, and morphological criteria.

Diet data for epipelagic siphonophores was compiled from published studies on visual gut content recognition (Purcell 1981), (Purcell 1984). Presence of taxonomic groups in the diet as well as percent individual composition was retrieved from these sources. For deep dwelling species, MBARI VARS (Schlining and Stout 2006) observations were used to score presence of prey taxonomic groups in annotated predation events with different siphonophore species.

### 3.3 Phylogenetic Comparative Methods

All phylogenetic statistical analyses were carried out in the programming environment R (Team 2017), using the species tree, and incorporating intraspecific variation estimated from the specimen data. Phylogenetic signal in each of the measured characters was calculated using Blomberg's K (Blomberg et al. 2003). The phylogenetic signal of multivariate character sets was calculated using the package *geomorph* (Adams et al. 2016).

Ancestral states were reconstructed using ML for continuous characters (*anc.ML* (Revell 2012)), and stochastic character mapping (*make.simmap*) for categorical characters.

Different models generating the observed data distribution given the phylogeny were fitted for each continuous character using the function *fitContinuous* (Harmon et al. 2007). The models compared were the white noise (WN) (non-phylogenetic), the Brownian Motion (BM) model of neutral divergent evolution (Martins 1996), the Early Burst (EB) model of decreasing rate of evolutionary change (Harmon et al. 2010), and the Ornstein-Uhlenbeck (OU) model of stabilizing selection around a fitted optimum state (Uhlenbeck and Ornstein 1930), (Butler and King 2004). Models were ranked in order of increasing parametric complexity (WN, BM, EB, OU). Corrected Akaike Information Criterion support scores (Sugiura 1978) were compared to the lowest (best) score, and a cutoff of 2 units was used to determine significantly better support. If the best fitting model is not significantly better than a less complex alternative, the least complex model is selected. The models selected for each character were evaluated in terms of model adequacy using the package *arbutus* (Pennell et al. 2015). Multivariate BM and OU models were also evaluated using the package *mvSLOUCH* (Bartoszek et al. 2012).

In order to explore the correlational structure among continuous characters and among their evolutionary histories, we used principal component analyses (PCA) and phylogenetic PCA (Revell 2012). In addition, we obtained the correlations between the phylogenetic independent contrasts (Felsenstein 1985) using the package *rphylip* (Revell and Chamberlain 2014). We modeled the relationship between correlated characters using phylogenetic least squares regression (PGLS) in the package *phytools* (Revell.cite). Evolutionary relationships between continuous and categorical characters were assessed using phylogenetic ANOVAs (*phyl.anova* *phytools*) and phylogenetic generalized linear mixed models (PGLMM).

#### 0.0.1 3.4 High-Speed Video

High speed footage (1000-3000 fps) of tentillum and nematocyst discharge was recorded on live siphonophore specimens using a Phantom Miro 320S camera mounted on a stereoscopic microscope. Specimens were collected by SCUBA divers and the remotely operated underwater vehicle Doc Ricketts during expeditions onboard the R/V Western Flyer. Tentillum and nematocyst discharge were elicited mechanically using a fine metallic pin. Video footage was analyzed using Phantom PCC software.

Total cnidoband discharge time (ms), average cnidoband discharge speed (mm/s), maximum cnidoband discharge speed (mm/s), heteroneme discharge speed (mm/s), haploneme discharge speed (mm/s), heteroneme shaft discharge speed (mm/s), and heteroneme filament length (microns) were measured when possible for 10 species.

### 3. Results

0 – PHYLOGENY 0.0 ML ribosomal marker phylogeny constrained by transcriptome tree with BS values at the nodes.

The topology of this tree is congruent with the results from (Dunn et al. 2005). In addition, we found *Erenna* and *Stephanomia* as sister genera in a monophyletic group.

#### 1 – DESCRIPTIVE MORPHOLOGY AND EVOLUTIONARY HISTORY

1.05 Plate with all the tentillum diversity.

1.1 Table showing each character’s (illustrating the character per row) the phylogenetic signal K with the p-value obtained by testing against 1000 simulated permutations, and the number of specimens included.

Principal component analyses were carried out separately on raw log-transformed measurements and compound morphometric characters (such as volumes and ratios). PC1 (aligned with tentillum size and nematocyst shape) explains ~64% of the variation in the tentillum morphospace, whereas PC2 (aligned with nematocyst size characters) explained ~18%.

Controlling for phylogenetic expected covariance, ~88% of the evolutionary variation is explained by shifts in size, exhibiting a low effective dimensionality.

The calycephoran morphospace is quite distinct from that of other siphonophores. A non-calycephoran (*Frillagalma vityazi*), has secondarily “invaded” this morphospace, which could indicate a case of convergence.

Most characters present a moderate yet significant phylogenetic signal, and predominantly support BM models, indicating a history of neutral constant divergence. Total nematocyst volume and cnidoband-to-heteroneme length ratio showed strongly conserved phylogenetic signals. These characters may be useful for taxonomic purposes. Bayesian Analysis of Macroevolutionary Mixtures (Rabosky et al. 2014) identified evolutionary rate regime shifts in the branches leading to the euphysonects, to clade B, and to *Cordagalma* for several characters. These shifts could be associated with adaptive responses to dietary change.

Novel phylogenetic relationships: -*Erenna* spp closely related to *Stephanomia*

1.0 Table showing for each species the mean, number of individuals measured, and standard error of every character, ordered by cnidomic index / tentillum size, and/or colored by clade.

2431.45000	127.006043	406.10000
14668.80000	1935.200000	542.15000
4360.55000	113.550000	352.60000
2759.57500	196.705104	154.75000
832.46000	34.077230	87.46000
1668.60000	0.000000	113.00000
1378.00000	0.000000	101.50000
183.82000	22.285116	46.18000
224.10000	10.933115	41.92000
2496.00000	0.000000	97.20000
12904.80000	0.000000	421.70000
4941.70000	1045.465376	170.90000
7780.40000	0.000000	252.70000
162.60000	15.475755	42.86000
121.00000	0.000000	54.50000
186.82000	11.332846	35.12000

1.2 Table showing the best AICc supported model generating the data for each character and its AICc score. Model adequacy.

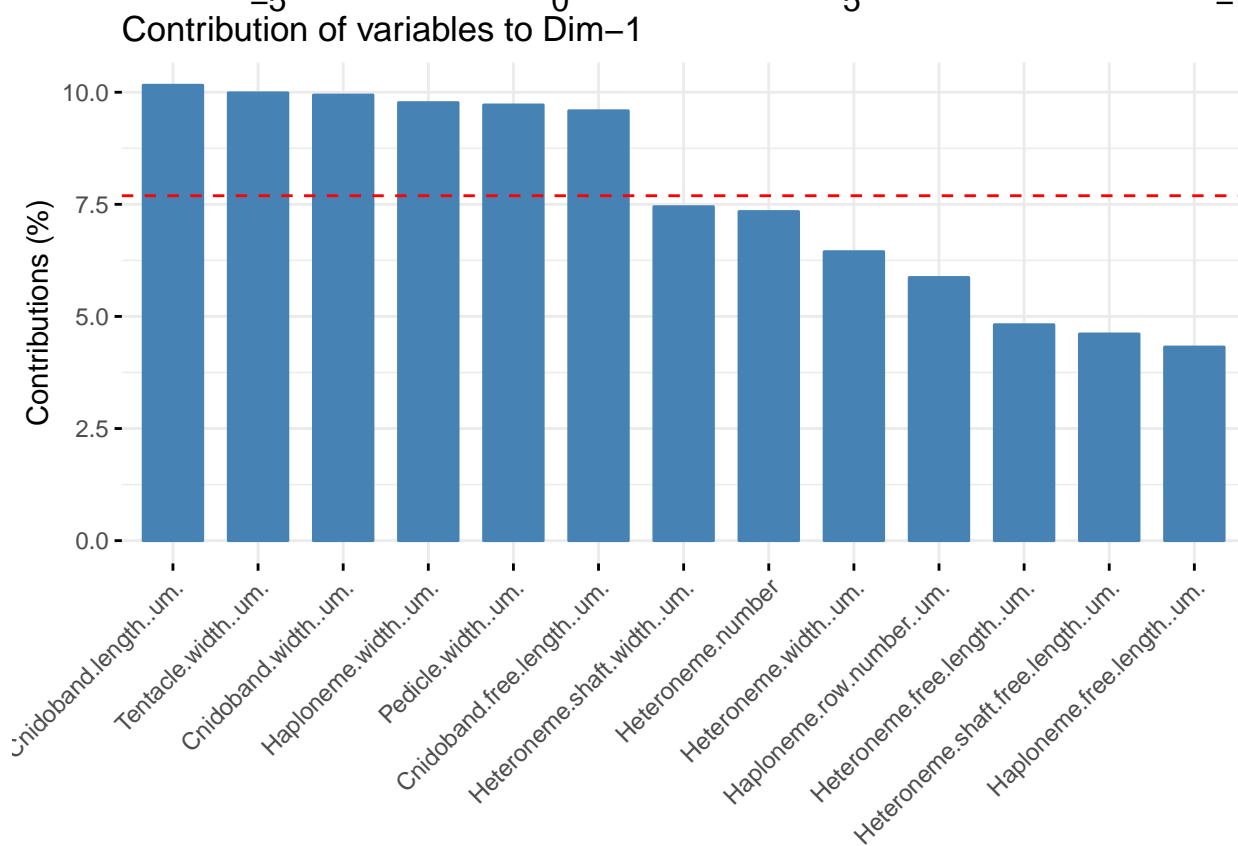
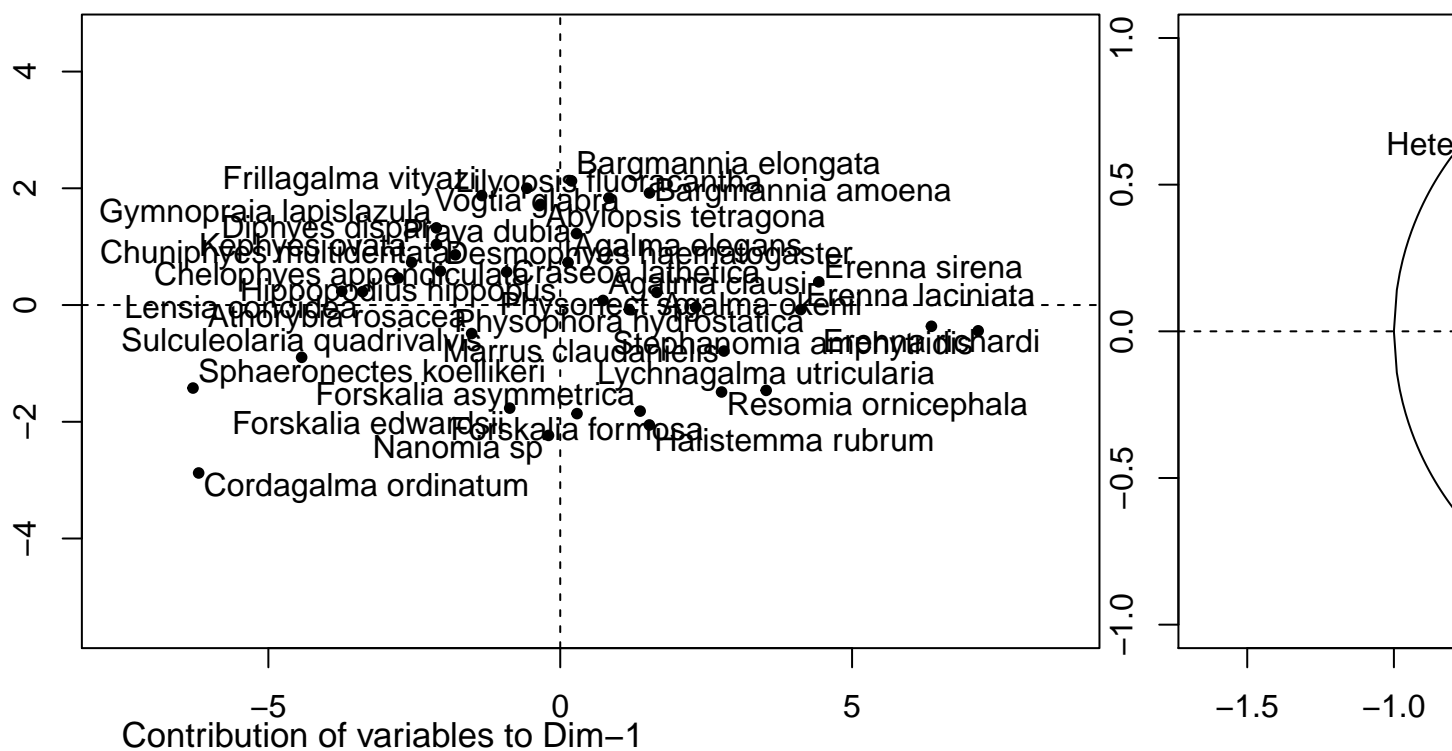
1.3 Figure showing major categorical character shifts, gains, and losses on a simplified phylogeny.

2 – COMPARATIVE ANALYSES 2.0 Confusion matrix table showing PIC\_correlations and trait correlation R2 and p-values for each pair of continuous characters.

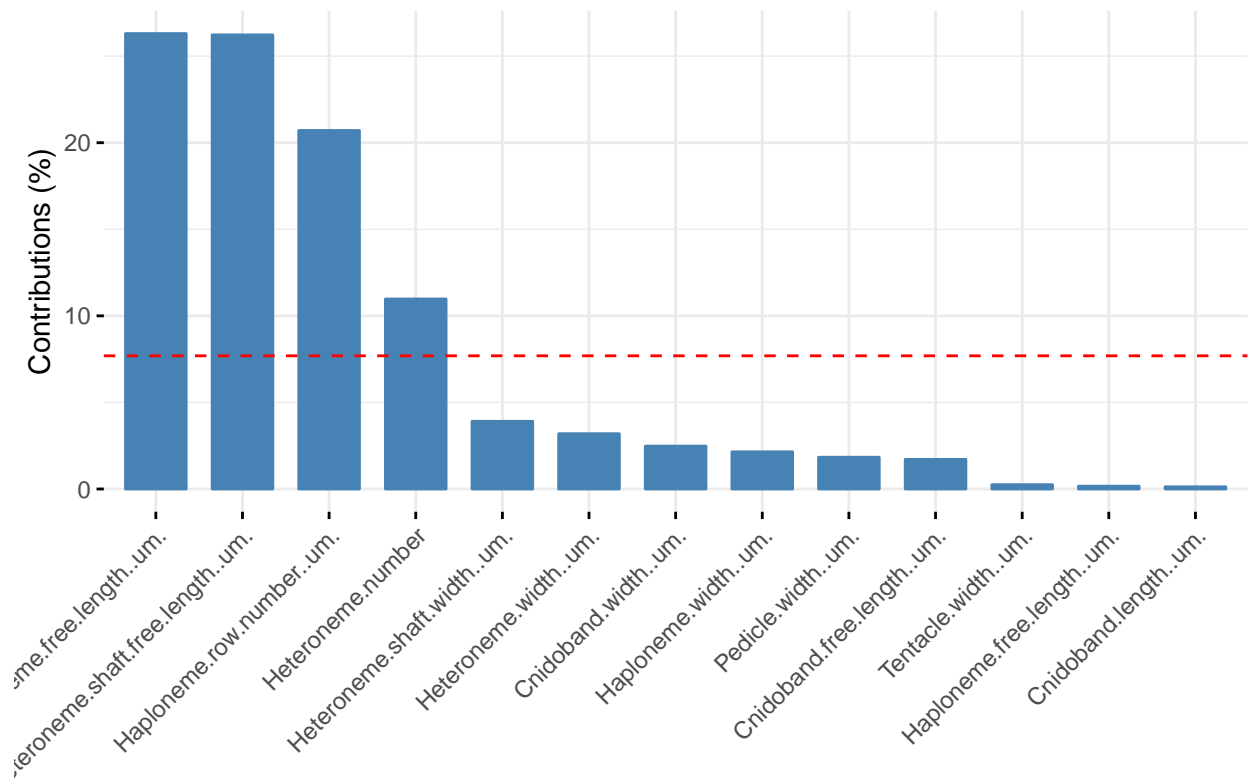
2.1 Table showing the model parameters for each significant regression.

2.3 Verbally PCA and phylogenetic PCA/PICVarA\_PCA of the characters. Phylomorphospace superimposed to the tip coordinates in PC1–PC2.

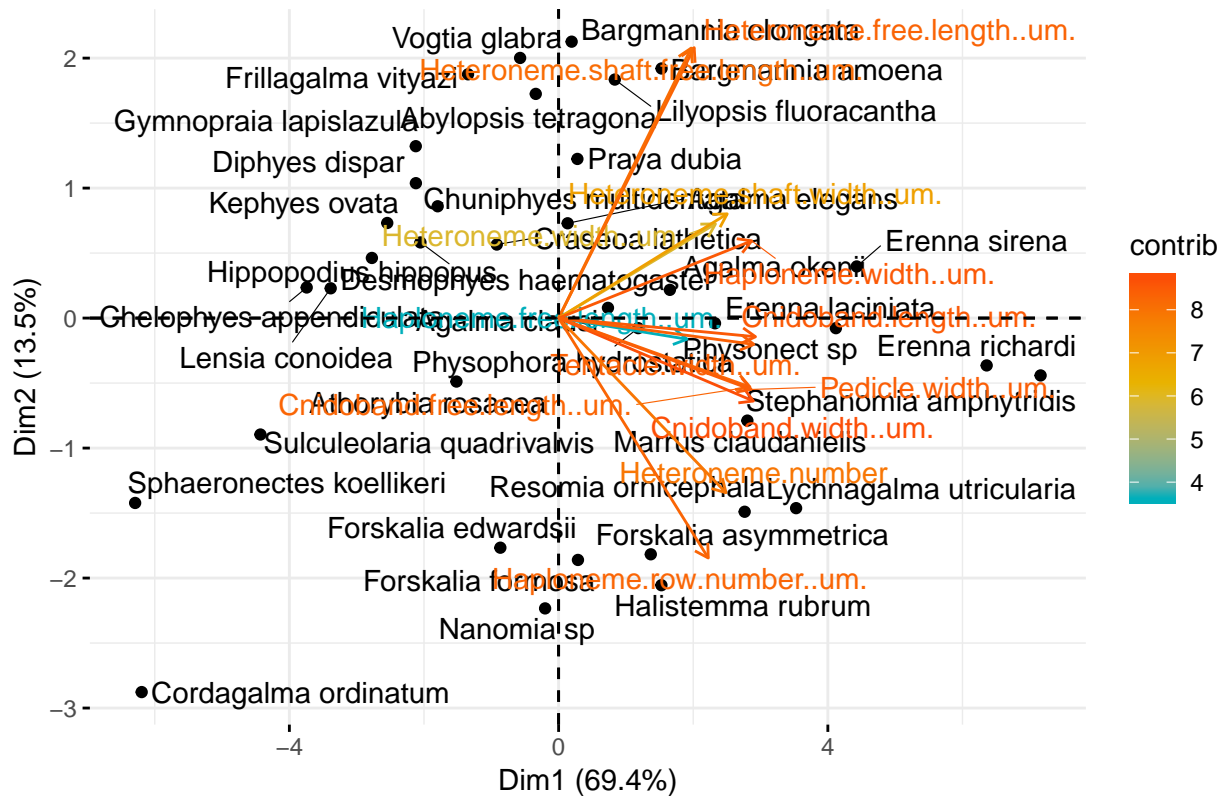
## Individuals factor map (PCA)



Contribution of variables to Dim-2



PCA – Biplot







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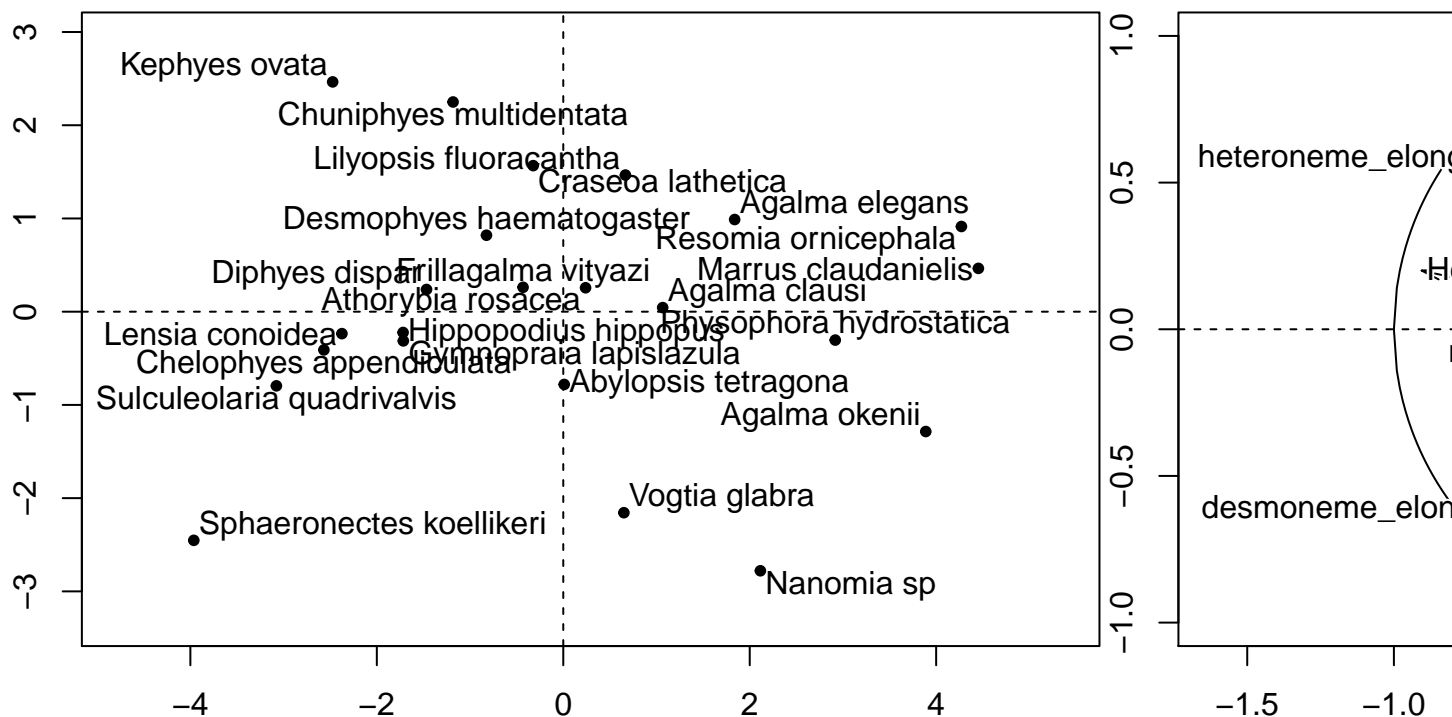
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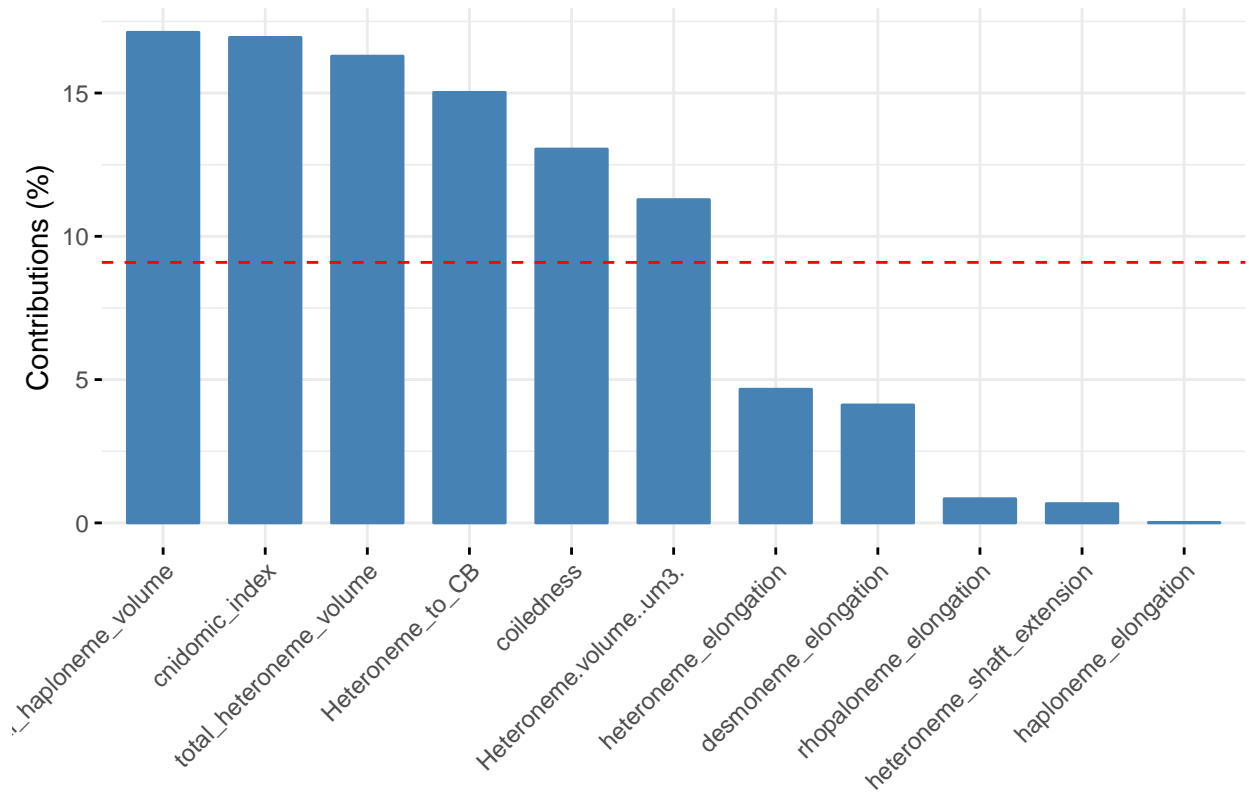
##
## Call:
## physignal(A = Pca_raw$ind$coord, phy = raw_tree)
##
##
## Observed Phylogenetic Signal (K): 0.8519
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## P-value: 0.001
##
## Based on 1000 random permutations
##
##          K PIC.variance.obs PIC.variance.rnd.mean PIC.variance.P
## Dim.1 0.9455791      12.978359      41.0430532      0.001
## Dim.2 0.9422429       2.781587       7.9229423      0.001
## Dim.3 1.0748318       1.397841       5.1998839      0.001
## Dim.4 0.3307463       1.874183       2.1591274      0.397
## Dim.5 0.2158009       1.259469       0.9173631      0.888
##
##          PIC.variance.Z
## Dim.1      -2.087373
## Dim.2      -2.379791
## Dim.3      -1.464199
## Dim.4      -0.384901
## Dim.5       1.198382

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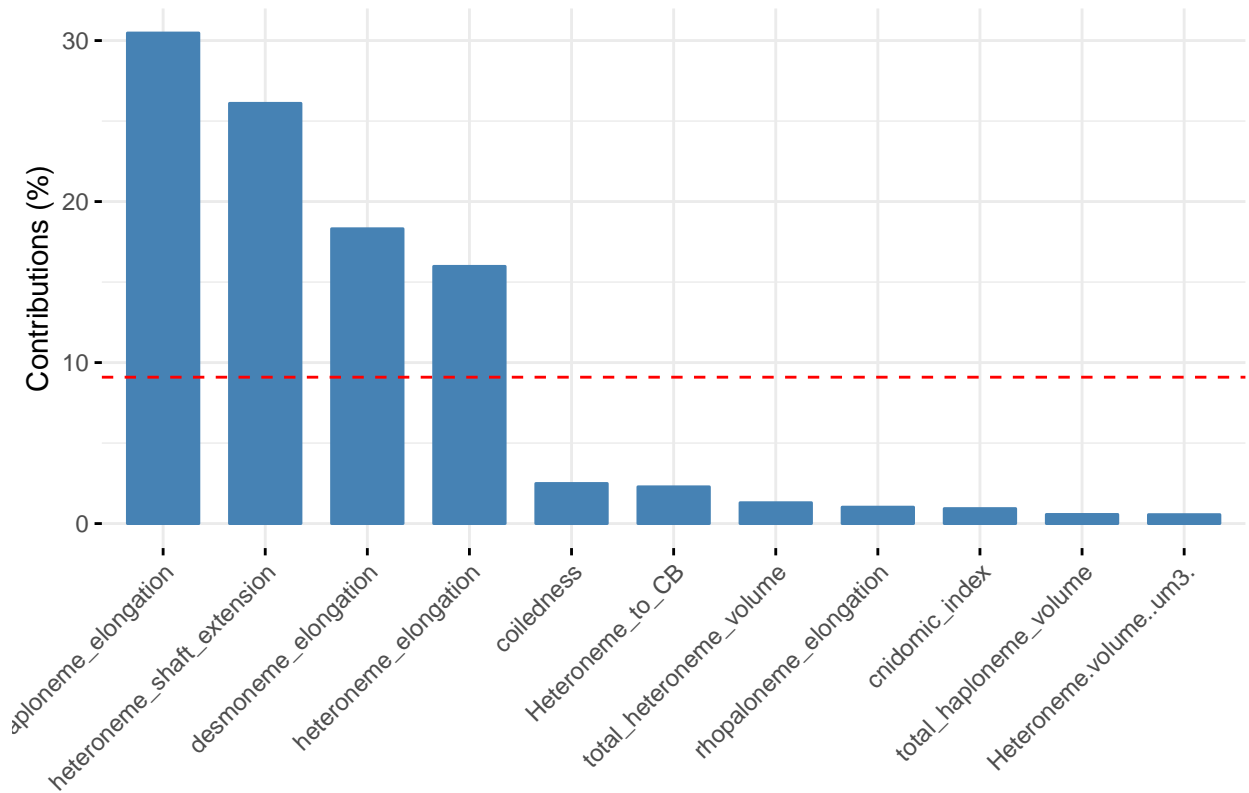
### Individuals factor map (PCA)



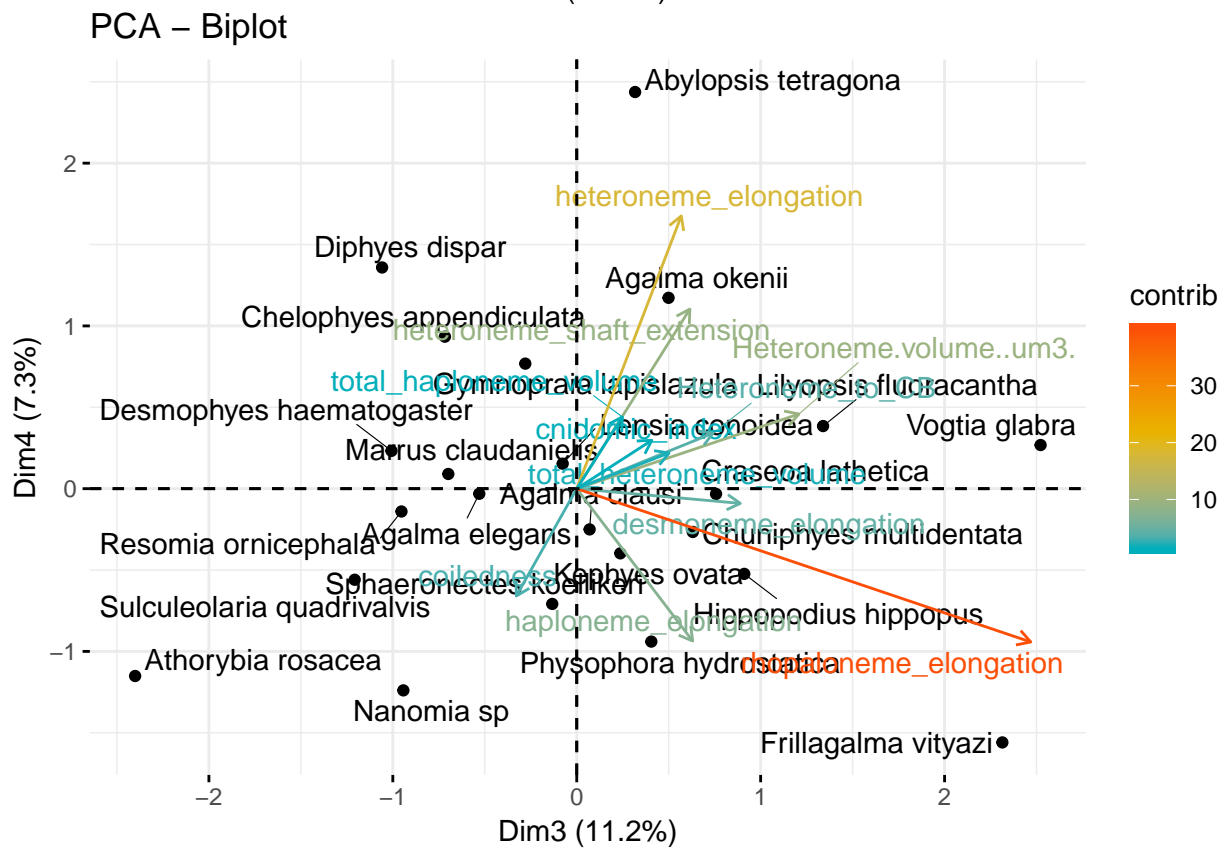
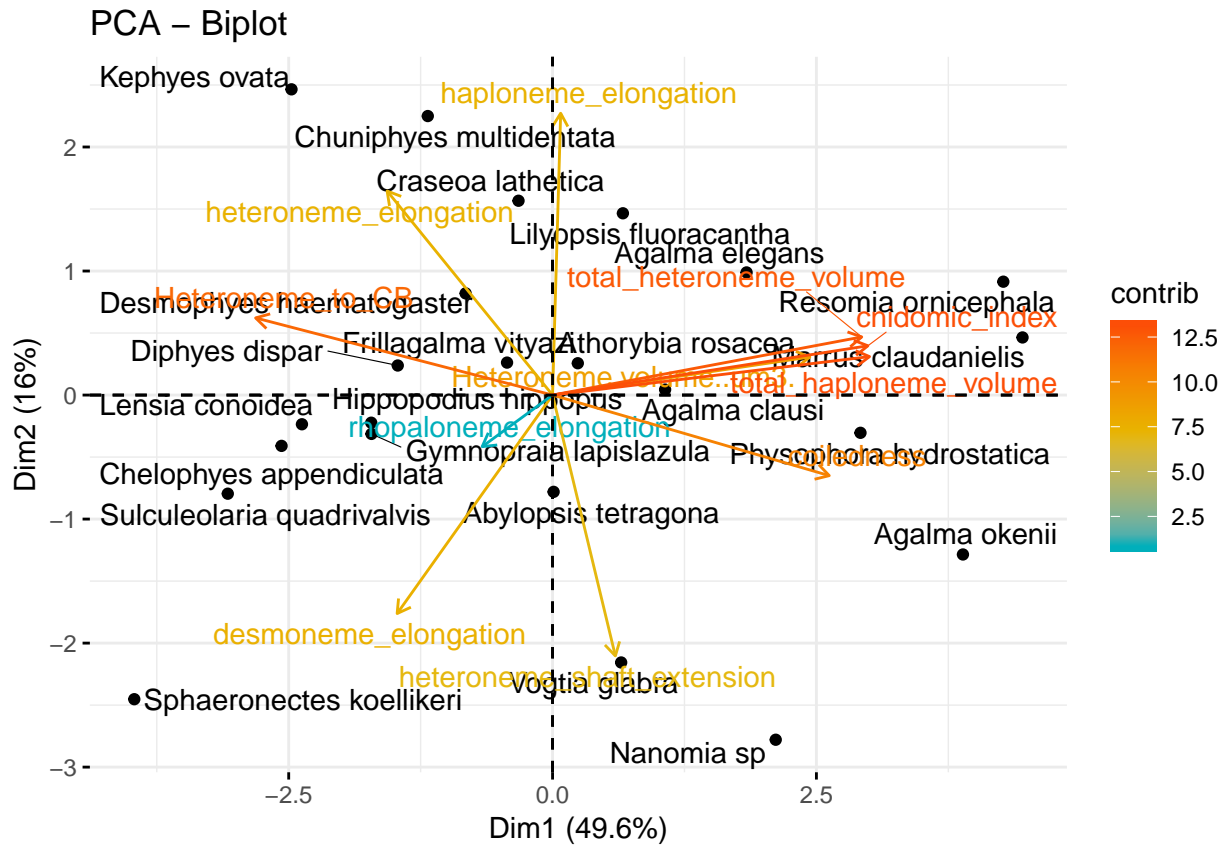
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Contribution of variables to Dim-2









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## Call:
## physignal(A = Pca_compound$ind$coord, phy = compound_tree)
##
##
## Observed Phylogenetic Signal (K): 0.5739
##
## P-value: 0.082
##
## Based on 1000 random permutations
##
##           K PIC.variance.obs PIC.variance.rnd.mean PIC.variance.P
## Dim.1 0.8342357          9.083523          17.108620          0.017
## Dim.2 0.6217507          3.991989           5.500554          0.207
## Dim.3 0.3672947          4.772328           3.880531          0.772
## Dim.4 0.4172393          2.680021           2.511400          0.672
## Dim.5 0.2648588          3.720068           2.211501          0.971
##
##           PIC.variance.Z
## Dim.1      -1.6057688
## Dim.2      -0.8474386
## Dim.3       0.6756166

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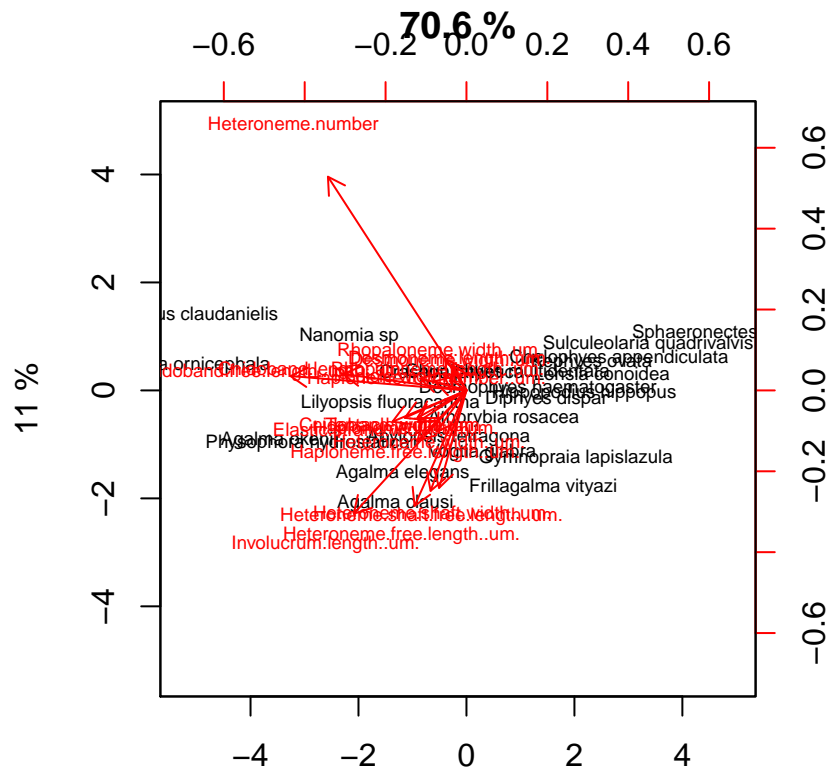
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## Dim.4      0.1921810
## Dim.5      2.3886666

## Importance of components:
##           PC1      PC2      PC3      PC4
## Standard deviation  3.4542643 1.3658524 0.95974198 0.91568205
## Proportion of Variance 0.7061902 0.1104125 0.05451545 0.04962494
## Cumulative Proportion 0.7061902 0.8166027 0.87111818 0.92074312
##           PC5      PC6      PC7      PC8
## Standard deviation  0.68283759 0.52010548 0.47043746 0.393828803
## Proportion of Variance 0.02759596 0.01601008 0.01309828 0.009179638
## Cumulative Proportion 0.94833907 0.96434915 0.97744743 0.986627072
##           PC9      PC10      PC11      PC12
## Standard deviation  0.259997609 0.246831176 0.225725900 0.122463187
## Proportion of Variance 0.004000823 0.003605875 0.003015598 0.000887609
## Cumulative Proportion 0.990627895 0.994233769 0.997249367 0.998136976
##           PC13      PC14      PC15      PC16
## Standard deviation  0.1094034873 0.0939535939 0.0640202947 0.0562356024
## Proportion of Variance 0.0007083908 0.0005224412 0.0002425749 0.0001871687
## Cumulative Proportion 0.9988453668 0.9993678080 0.9996103829 0.9997975517
##           PC17      PC18      PC19
## Standard deviation  0.0431552042 3.850235e-02 8.706778e-03
## Proportion of Variance 0.0001102242 8.773746e-05 4.486684e-06
## Cumulative Proportion 0.9999077759 9.999955e-01 1.000000e+00

## Importance of components:
##           PC1      PC2      PC3      PC4
## Standard deviation  3.4542643 1.3658524 0.95974198 0.91568205
## Proportion of Variance 0.7061902 0.1104125 0.05451545 0.04962494
## Cumulative Proportion 0.7061902 0.8166027 0.87111818 0.92074312
##           PC5      PC6      PC7      PC8
## Standard deviation  0.68283759 0.52010548 0.47043746 0.393828803
## Proportion of Variance 0.02759596 0.01601008 0.01309828 0.009179638
## Cumulative Proportion 0.94833907 0.96434915 0.97744743 0.986627072
##           PC9      PC10      PC11      PC12
## Standard deviation  0.259997609 0.246831176 0.225725900 0.122463187
## Proportion of Variance 0.004000823 0.003605875 0.003015598 0.000887609
## Cumulative Proportion 0.990627895 0.994233769 0.997249367 0.998136976
##           PC13      PC14      PC15      PC16
## Standard deviation  0.1094034873 0.0939535939 0.0640202947 0.0562356024
## Proportion of Variance 0.0007083908 0.0005224412 0.0002425749 0.0001871687
## Cumulative Proportion 0.9988453668 0.9993678080 0.9996103829 0.9997975517
##           PC17      PC18      PC19
## Standard deviation  0.0431552042 3.850235e-02 8.706778e-03
## Proportion of Variance 0.0001102242 8.773746e-05 4.486684e-06
## Cumulative Proportion 0.9999077759 9.999955e-01 1.000000e+00

```



## Importance of components:

	PC1	PC2	PC3	PC4
## Standard deviation	5.3037419	1.28415066	0.78070613	0.514351156
## Proportion of Variance	0.9086436	0.05326731	0.01968811	0.008545712
## Cumulative Proportion	0.9086436	0.96191093	0.98159904	0.990144749

	PC5	PC6	PC7	PC8
## Standard deviation	0.397043104	0.266580623	0.1714579117	0.1478522695
## Proportion of Variance	0.005092185	0.002295546	0.0009496069	0.0007061302
## Cumulative Proportion	0.995236934	0.997532479	0.9984820864	0.9991882166

	PC9	PC10	PC11
## Standard deviation	0.1266825741	0.0895589599	0.0325854078
## Proportion of Variance	0.0005183971	0.0002590878	0.0000342985
## Cumulative Proportion	0.9997066137	0.9999657015	1.0000000000

## Importance of components:

	PC1	PC2	PC3	PC4
## Standard deviation	5.3037419	1.28415066	0.78070613	0.514351156
## Proportion of Variance	0.9086436	0.05326731	0.01968811	0.008545712
## Cumulative Proportion	0.9086436	0.96191093	0.98159904	0.990144749

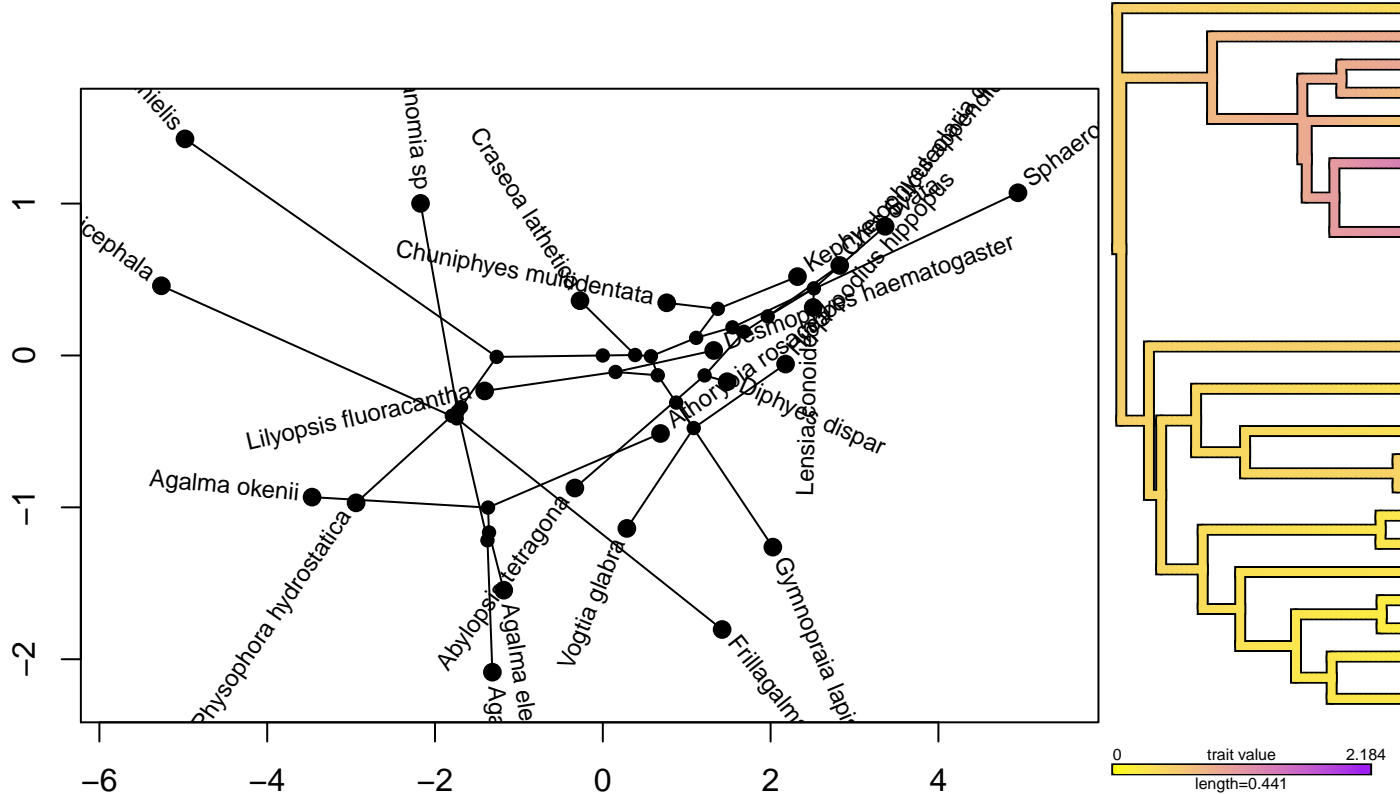
  

	PC5	PC6	PC7	PC8
## Standard deviation	0.397043104	0.266580623	0.1714579117	0.1478522695
## Proportion of Variance	0.005092185	0.002295546	0.0009496069	0.0007061302
## Cumulative Proportion	0.995236934	0.997532479	0.9984820864	0.9991882166

	PC9	PC10	PC11
## Standard deviation	0.1266825741	0.0895589599	0.0325854078
## Proportion of Variance	0.0005183971	0.0002590878	0.0000342985
## Cumulative Proportion	0.9997066137	0.9999657015	1.0000000000

```
## Warning in phylomorphospace(raw_tree, PPCA_raw$S): X has more than 2
## columns. Using only the first 2 columns.
```



2.4 Table showing BAMM-analyzed number of regime shifts in the rates of evolution of each character.

Out of 29 characters, 11 did not significantly ( $\Delta \text{AICc} > 2$ ) support a phylogenetic model. From the remaining 18, Brownian Motion was supported for 17 of them. Only haploneme nematocyst length had unequivocal support for Early Burst.

### 3 – RELATIONSHIPS WITH DIET / FUNCTION / Hypothesis testing

3.0 Phylogeny with the tips mapped with the literature dietary associations, and internal nodes showing SIMMAP reconstructed states.

```
##           [,1]
## Copepod    -0.8080130
## Crustacean  1.2677320
## Fish       0.3333011
## Mixed     -0.7930201
## attr(,"scaled:center")
## [1] 0.25
## attr(,"scaled:scale")
## [1] 0.04963549
## [1] "Crustacean"
##           [,1]
## Copepod    -0.5351944
## Crustacean  1.4987748
## Fish       -0.4430773
## Mixed     -0.5205031
## attr(,"scaled:center")
## [1] 0.25
## attr(,"scaled:scale")
## [1] 0.07105532
## [1] "Crustacean"
##           [,1]
## Copepod    -0.4288748
## Crustacean  1.4981107
## Fish       -0.5416261
## Mixed     -0.5276098
## attr(,"scaled:center")
## [1] 0.25
## attr(,"scaled:scale")
## [1] 0.07677227
## [1] "Crustacean"
##           [,1]
## Copepod    -0.1818167
## Crustacean  1.4595679
## Fish       -0.7395739
## Mixed     -0.5381772
## attr(,"scaled:center")
## [1] 0.25
## attr(,"scaled:scale")
## [1] 0.0802803
## [1] "Crustacean"
##           [,1]
## Copepod     0.7722535
## Crustacean  0.8736945
## Fish       -1.2009358
## Mixed     -0.4450121
## attr(,"scaled:center")
```

```

## [1] 0.25
## attr("scaled:scale")
## [1] 0.08117332
## [1] "Crustacean"
##           [,1]
## Copepod    0.7065757
## Crustacean 0.8137313
## Fish       -1.3445016
## Mixed      -0.1758054
## attr("scaled:center")
## [1] 0.25
## attr("scaled:scale")
## [1] 0.08661124
## [1] "Crustacean"
##           [,1]
## Copepod    0.9704528
## Crustacean 0.2328527
## Fish       -1.4016618
## Mixed      0.1983563
## attr("scaled:center")
## [1] 0.25
## attr("scaled:scale")
## [1] 0.0864187
## [1] "Copepod"
##           [,1]
## Copepod    -1.10536223
## Crustacean 1.30040284
## Fish       -0.28205537
## Mixed      0.08701476
## attr("scaled:center")
## [1] 0.25
## attr("scaled:scale")
## [1] 0.05855868
## [1] "Crustacean"
##           [,1]
## Copepod    -0.6584028
## Crustacean 0.9063961
## Fish       -1.0497940
## Mixed      0.8018007
## attr("scaled:center")
## [1] 0.25
## attr("scaled:scale")
## [1] 0.1146443
## [1] "Crustacean"
##           [,1]
## Copepod    -0.6406158
## Crustacean 0.8476353
## Fish       -1.0652003
## Mixed      0.8581808
## attr("scaled:center")
## [1] 0.25
## attr("scaled:scale")
## [1] 0.1163184
## [1] "Mixed"

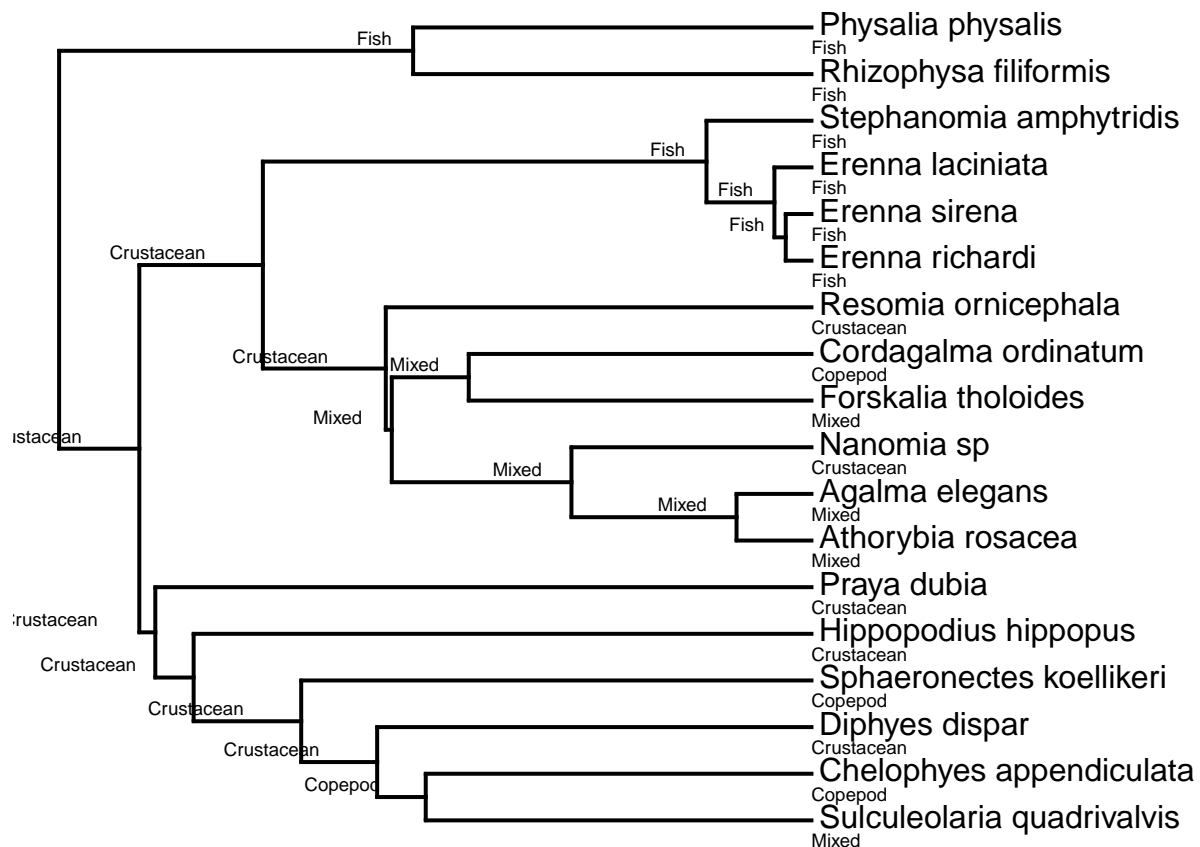
```

```

##           [,1]
## Copepod    -0.8107895
## Crustacean  0.5776793
## Fish       -0.8788687
## Mixed      1.1119788
## attr("scaled:center")
## [1] 0.25
## attr("scaled:scale")
## [1] 0.1964242
## [1] "Mixed"
##           [,1]
## Copepod    -0.5044886
## Crustacean -0.4902915
## Fish       -0.5051847
## Mixed      1.4999647
## attr("scaled:center")
## [1] 0.25
## attr("scaled:scale")
## [1] 0.4843687
## [1] "Mixed"
##           [,1]
## Copepod    -0.08330234
## Crustacean  0.07687002
## Fish       -1.21889902
## Mixed      1.22533134
## attr("scaled:center")
## [1] 0.25
## attr("scaled:scale")
## [1] 0.1079133
## [1] "Mixed"
##           [,1]
## Copepod    -0.5009666
## Crustacean -0.4990274
## Fish       1.4999995
## Mixed      -0.5000055
## attr("scaled:center")
## [1] 0.25
## attr("scaled:scale")
## [1] 0.4838411
## [1] "Fish"
##           [,1]
## Copepod    -0.5000053
## Crustacean -0.4999947
## Fish       1.5000000
## Mixed      -0.5000000
## attr("scaled:center")
## [1] 0.25
## attr("scaled:scale")
## [1] 0.4998351
## [1] "Fish"
##           [,1]
## Copepod    -0.5000003
## Crustacean -0.4999997
## Fish       1.5000000

```

```
## Mixed      -0.5000000
## attr(,"scaled:center")
## [1] 0.25
## attr(,"scaled:scale")
## [1] 0.4999777
## [1] "Fish"
##           [,1]
## Copepod   -0.5242174
## Crustacean -0.4512104
## Fish       1.4991178
## Mixed     -0.5236900
## attr(,"scaled:center")
## [1] 0.25
## attr(,"scaled:scale")
## [1] 0.2234625
## [1] "Fish"
```



3.1 Table showing quantitative and categorical characters association effect size with each prey type in the diet.

Nematocyst morphology appears to be associated to prey type presence in diet. Phylogenetic regression with frequencies of prey types (in 8 species) revealed that decapod larvae consumption is associated to many traits, while fish larvae specialization is only associated to more rounded nematocyst shapes with stubbier shafts, and copepod specialization is mainly associated to fewer nematocyst and thinner tentacles. Transitions to fish and copepod specialization correlate with regime shifts in the rates for those characters (detected with BAMM).

```
## Warning in cbind(CH_I, CH_J): number of rows of result is not a multiple of
```

[illegible]





[illegible]



[illegible]

[illegible]

[illegible]

[illegible]

[illegible]



```

## vector length (arg 2)

## Warning in cbind(CH_I, CH_J): number of rows of result is not a multiple of
## vector length (arg 2)

## Warning in cbind(CH_I, CH_J): number of rows of result is not a multiple of
## vector length (arg 2)

## Warning in cbind(CH_I, CH_J): number of rows of result is not a multiple of
## vector length (arg 2)

## Warning in cbind(CH_I, CH_J): number of rows of result is not a multiple of
## vector length (arg 2)

## Warning in cbind(CH_I, CH_J): number of rows of result is not a multiple of
## vector length (arg 2)

## Warning in cbind(CH_I, CH_J): number of rows of result is not a multiple of
## vector length (arg 2)

## Warning in cbind(CH_I, CH_J): number of rows of result is not a multiple of
## vector length (arg 2)

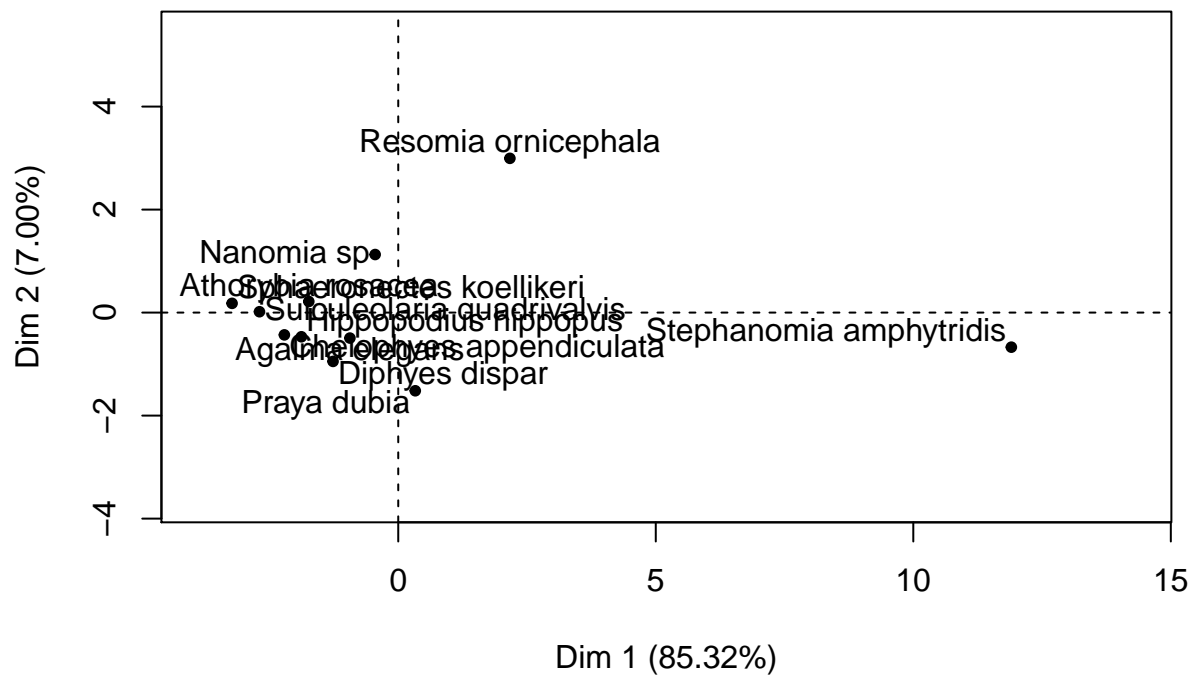
## Warning in cbind(CH_I, CH_J): number of rows of result is not a multiple of
## vector length (arg 2)

## [1] "Heteroneme.free.length..um. Relative decapod consumption 0.0150911316184195"
## [1] "Heteroneme.width..um. Relative copepod consumption 0.0154101615496608"
## [1] "Heteroneme.volume..um3. Relative copepod consumption 0.0273449183732837"
## [1] "Heteroneme.volume..um3. Relative decapod consumption 0.0162918965213711"
## [1] "Heteroneme.shaft.free.length..um. Relative decapod consumption 0.0375748337864496"
## [1] "Heteroneme.shaft.width..um. Relative copepod consumption 0.0366644014228334"
## [1] "Heteroneme.shaft.width..um. Relative decapod consumption 0.0110425153430764"
## [1] "Heteroneme.number Relative copepod consumption 0.00194568725251434"
## [1] "Heteroneme.number Relative crustacean consumption 0.00383297561996947"
## [1] "Heteroneme.number Relative decapod consumption 0.00227285574600831"
## [1] "Heteroneme.number Relative mollusc consumption 0.00457868496734718"
## [1] "Heteroneme.number Relative ostracod consumption 0.00787906233695161"
## [1] "Haploneme.width..um. Relative copepod consumption 0.0421081662955249"
## [1] "Desmoneme.length..um. Relative decapod consumption 0.0346469607675098"
## [1] "Cnidoband.free.length..um. Relative copepod consumption 0.0423568435044045"
## [1] "Cnidoband.free.length..um. Relative decapod consumption 0.00758244919964879"
## [1] "Cnidoband.width..um. Relative copepod consumption 0.0122225326887304"
## [1] "Haploneme.row.number..um. Relative copepod consumption 0.000958844820415622"
## [1] "Haploneme.row.number..um. Relative fish consumption 5.03339200134134e-05"
## [1] "Haploneme.row.number..um. Relative crustacean consumption 0.000168074352145753"
## [1] "Haploneme.row.number..um. Relative decapod consumption 1.57183006366095e-05"
## [1] "Haploneme.row.number..um. Relative chaetognath consumption 5.61183590053188e-05"

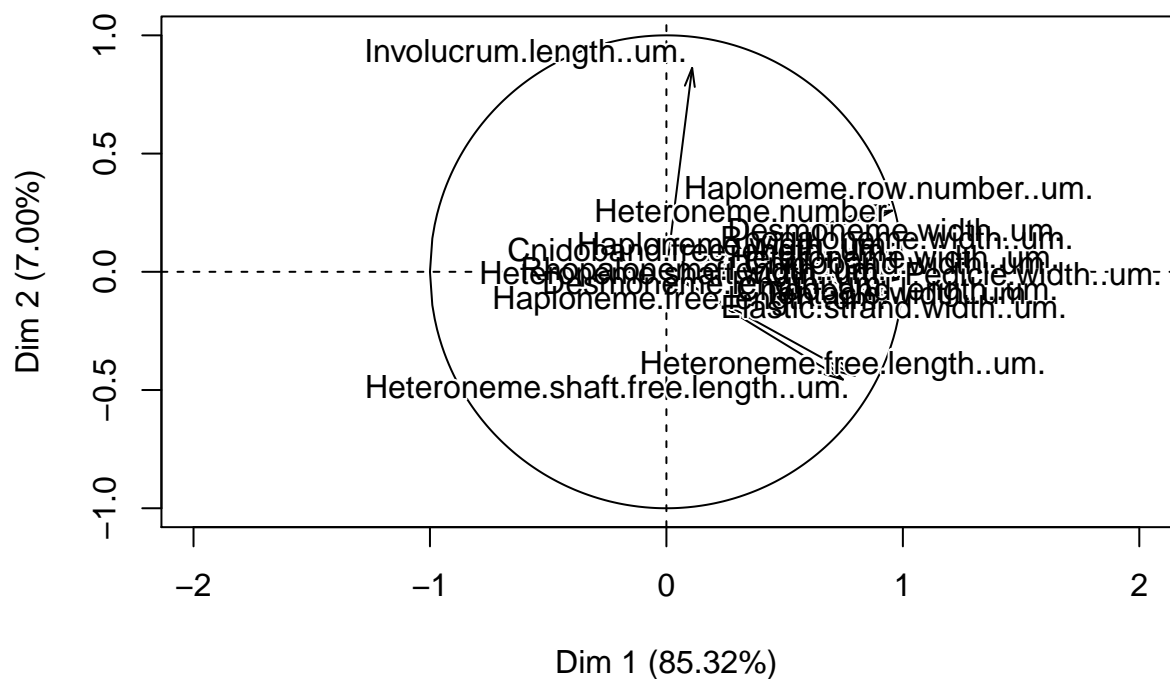
```

```
## [1] "Haploneme.row.number..um. Relative mollusc consumption 0.00397445311597162"
## [1] "Haploneme.row.number..um. Relative amphipod consumption 0.00164780631313967"
## [1] "Tentacle.width..um. Relative copepod consumption 0.00635688714230029"
## [1] "Involucrum.length..um. Relative copepod consumption 0.0449702570609739"
## [1] "Involucrum.length..um. Relative decapod consumption 0.0198097983058508"
## [1] "coiledness Relative copepod consumption 0.0183035862923914"
## [1] "heteroneme_elongation Relative crustacean consumption 0.0158687156010087"
## [1] "heteroneme_elongation Relative chaetognath consumption 0.0274790547180701"
## [1] "heteroneme_shaft_extension Relative fish consumption 0.0176572602781742"
## [1] "heteroneme_shaft_extension Relative chaetognath consumption 0.0213300789019559"
## [1] "Heteroneme_to_CB Relative copepod consumption 0.0128876533556811"
## [1] "Heteroneme_to_CB Relative decapod consumption 0.00235079129249794"
## [1] "Heteroneme_to_CB Relative chaetognath consumption 0.00388259952739823"
## [1] "Heteroneme_to_CB Relative ostracod consumption 0.027767744402016"
## [1] "Heteroneme_to_CB Relative amphipod consumption 0.0494215095388725"
## [1] "total_heteroneme_volume Relative copepod consumption 0.0252900982780285"
## [1] "cnidomic_index Relative copepod consumption 0.0465172991019794"
```

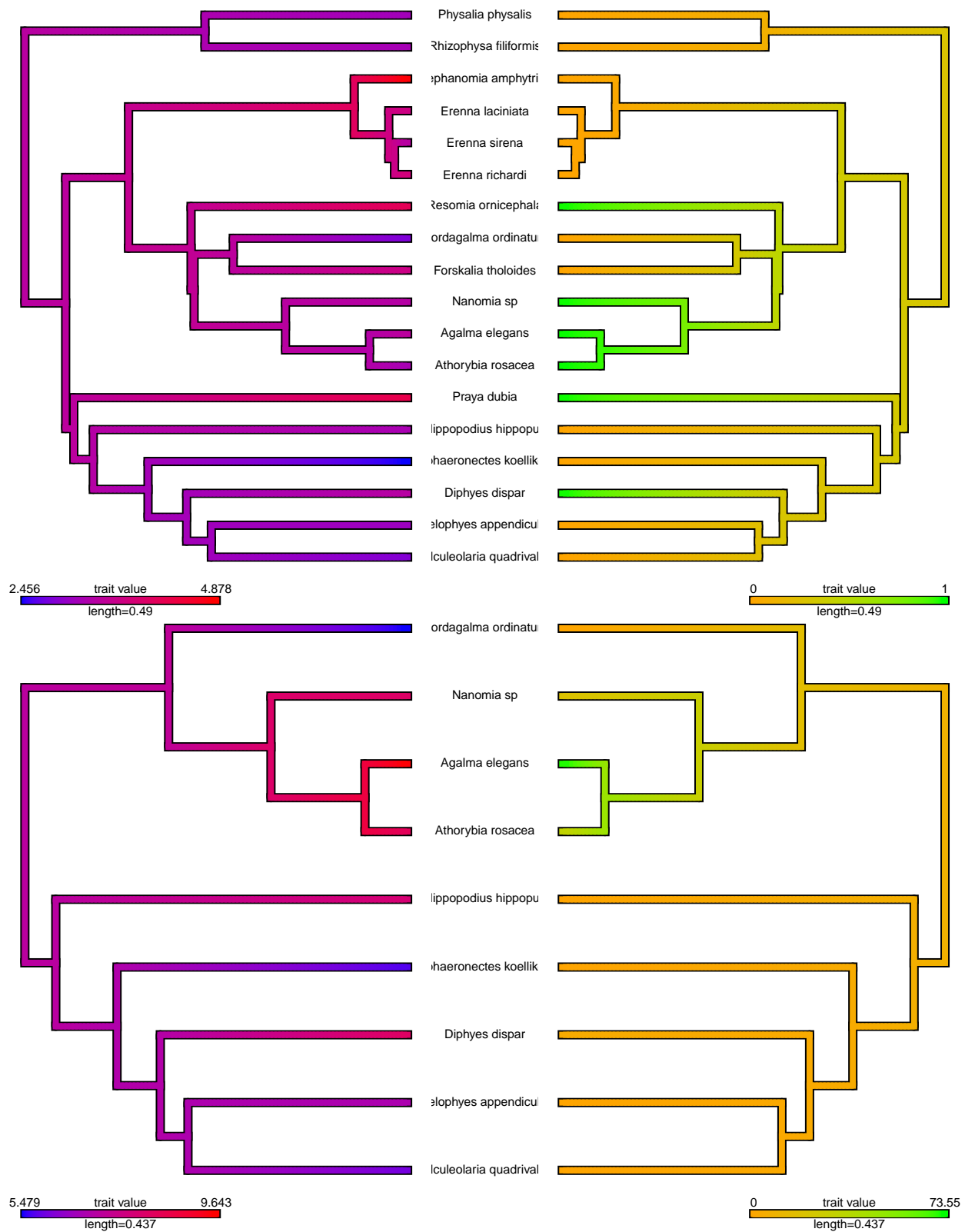
### Individuals factor map (PCA)



## Variables factor map (PCA)



```
## [1] "Copepod diet"
## [1] 0.085
## [1] "Fish diet"
## [1] 0.378
## [1] "Amphipod diet"
## [1] 0.357
## [1] "Polychaete diet"
## [1] 0.665
## [1] "Mollusc diet"
## [1] 0.547
## [1] "Decapod diet"
## [1] 0.859
## [1] "Crustacean diet"
## [1] 0.001
## [1] "Chaetognath diet"
## [1] 0.448
## [1] "Ostracod diet"
## [1] 0.509
```



3.2 Table showing the dAICc of OU model fitting versus white\_noise and BM using reconstructed diets as regimes in a 16 spp tree. Evaluate model adequacy of OU fits.

When I mapped trait evolution onto a diet SIMMAP regime tree, OU models using the reconstructed states

as regimes fitted significantly better ( $dAICc > 2$ ) than BM (null models) for cnidoband coiledness, heteroneme shaft shape, haploneme length, haploneme row number, desmoneme length, and desmoneme width.

```
## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.
## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.
## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.
## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.
## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.
## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.
## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.
## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.
## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.
## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.
## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.
```

```
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.
```

[illegible]





```
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well

## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.
```

```

## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Warning: You might not have enough data to fit this model well
## Initializing...
## Finished. Begin thorough search...
## Finished. Summarizing results.

## Reading event datafile:  BAMB/Cnidoband_free_length/event_data.txt
##      .....
## Read a total of 5 samples from posterior
##
## Discarded as burnin: GENERATIONS < 0
## Analyzing 5 samples from posterior
##
## Setting recursive sequence on tree...
##
## Done with recursive sequence

##
## Analyzed 5 posterior samples
## Shift posterior distribution:
##
##           0           0.2
##           1           0.6
##           2           0.2
##

```

```
## Compute credible set of shift configurations for more information:
## See ?credibleShiftSet and ?getBestShiftConfiguration

## [1] "Copepod diet"
## [1] "Fish diet"
## [1] "Amphipod diet"
## [1] "Polychaete diet"
## [1] "Mollusc diet"
## [1] "Decapod diet"
## [1] "Crustacean diet"
## [1] "Chaetognath diet"
## [1] "Ostracod diet"
```

3.3 Interpret qualitatively the kinematic implications (high speed video) of the different regions of the morphospace.

High-speed video of cnidoband and nematocyst discharge revealed the roles of the different tentillum substructures in the mechanics of discharge. Moreover, it shows important differences in the discharge dynamics of species with different tentillum morphologies.

Videos have shown the double role of the elastic strands in initiating and controlling cnidoband discharge. A few milliseconds before cnidoband discharge, the distal attachment of the cnidoband (at the base of the terminal filament) appears to unwind to the point of a tension-release threshold. This observation indicates there might be spring mechanism driving the discharge process.

Euphysonect tentilla discharge considerably faster than Calycophoran tentilla. Larger heteronemes discharge faster than small ones, giving Euphysonects a faster heteroneme discharge speed than most calycophorans. On the contrary, Calycophoran haploneme tubules fire faster than those of Euphysonects.

The more extreme the coiledness index, the more unpredictable the trajectory of the cnidoband during discharge.

Story: low dimensionality, cost-reward-driven, dynamic specialization.

## 0.1 4. Discussion

A particularly interesting case is that of *Cordagalma ordinatum*, a specialized copepod eater (Purcell 1981), which is sister to the generalist genus *Forskalia*. *Cordagalma*'s tentilla strongly resemble the larval (protozooid exclusive) tentilla of *Forskalia* spp. This could indicate a case of paedomorphosis as a pathway to predatory specialization.

The calycophoran morphospace is quite distinct from that of other siphonophores, revealing a potential reaction line in the 2 PCs (Figure XX). A non-calycophoran (*Frillagalma vityazi*), has secondarily occupied this morphospace, which could indicate a case of convergence.

The new phylogeny delivered by this chapter will become a fundamental resource for subsequent studies of siphonophore evolution. In addition, a broad and detailed characterization of tentilla morphology and its phylogenetic distribution provide complementary tools for taxonomy, identification, and morphological delimitation.

## 0.2 5. Conclusions

The genera *Erenna* and *Stephanomia* form a monophyletic clade characterized by bearing extremely large tentilla.

### 0.3 Acknowledgements

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### References

- Adams D.C., Collyer M., Kaliontzopoulou A., Sherratt E. 2016. Geomorph: Software for geometric morphometric analyses.
- Bartoszek K., Pienaar J., Mostad P., Andersson S., Hansen T.F. 2012. A phylogenetic comparative method for studying multivariate adaptation. *Journal of Theoretical Biology*. 314:204–215.
- Blomberg S.P., Garland T., Ives A.R. 2003. Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution*. 57:717–745.
- Butler M.A., King A.A. 2004. Phylogenetic comparative analysis: A modeling approach for adaptive evolution. *The American Naturalist*. 164:683–695.
- Dunn C.W., Pugh P.R., Haddock S.H. 2005. Molecular phylogenetics of the siphonophora (cnidaria), with implications for the evolution of functional specialization. *Systematic biology*. 54:916–935.
- Felsenstein J. 1985. Phylogenies and the comparative method. *The American Naturalist*. 125:1–15.
- Haddock S.H., Heine J.N. 2005. Scientific blue-water diving. California Sea Grant College Program.
- Harmon L.J., Losos J.B., Jonathan Davies T., Gillespie R.G., Gittleman J.L., Bryan Jennings W., Kozak K.H., McPeck M.A., Moreno-Roark F., Near T.J., others. 2010. Early bursts of body size and shape evolution are rare in comparative data. *Evolution: International Journal of Organic Evolution*. 64:2385–2396.
- Harmon L.J., Weir J.T., Brock C.D., Glor R.E., Challenger W. 2007. GEIGER: Investigating evolutionary radiations. *Bioinformatics*. 24:129–131.
- Mapstone G.M. 2014. Global diversity and review of siphonophorae (cnidaria: Hydrozoa). *PLoS One*. 9:e87737.
- Martins E.P. 1996. Phylogenies, spatial autoregression, and the comparative method: A computer simulation test. *Evolution*. 50:1750–1765.
- Munro C., Siebert S., Zapata F., Howison M., Serrano A.D., Church S.H., Goetz F.E., Pugh P.R., Haddock S.H., Dunn C.W. 2018. Improved phylogenetic resolution within siphonophora (cnidaria) with implications for trait evolution. *Molecular Phylogenetics and Evolution*.
- Pennell M.W., FitzJohn R.G., Cornwell W.K., Harmon L.J. 2015. Model adequacy and the macroevolution of angiosperm functional traits. *The American Naturalist*. 186:E33–E50.
- Purcell J. 1981. Dietary composition and diel feeding patterns of epipelagic siphonophores. *Marine Biology*. 65:83–90.
- Purcell J.E. 1984. The functions of nematocysts in prey capture by epipelagic siphonophores (coelenterata, hydrozoa). *The Biological Bulletin*. 166:310–327.
- Rabosky D.L., Grundler M., Anderson C., Title P., Shi J.J., Brown J.W., Huang H., Larson J.G. 2014. BAMM tools: An r package for the analysis of evolutionary dynamics on phylogenetic trees. *Methods in Ecology and Evolution*. 5:701–707.
- Revell L.J. 2012. Phytools: An r package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*. 3:217–223.
- Revell L.J., Chamberlain S.A. 2014. Rphylip: An r interface for phylip. *Methods in Ecology and Evolution*.

5:976–981.

Schindelin J., Arganda-Carreras I., Frise E., Kaynig V., Longair M., Pietzsch T., Preibisch S., Rueden C., Saalfeld S., Schmid B., others. 2012. Fiji: An open-source platform for biological-image analysis. *Nature methods*. 9:676.

Schlining B., Stout N.J. 2006. MBARI’s video annotation and reference system. *OCEANS 2006*.:1–5.

Skaer R. 1991. Remodelling during the development of nematocysts in a siphonophore. *Hydrobiologia*. 216:685–689.

Sugiura N. 1978. Further analysts of the data by akaike’s information criterion and the finite corrections: Further analysts of the data by akaike’s. *Communications in Statistics-Theory and Methods*. 7:13–26.

Team R.C. 2017. R: A language and environment for statistical computing. vienna, austria: R foundation for statistical computing; 2017.

Totton A.K., Bargmann H.E. 1965. A synopsis of the siphonophora. *British Museum (Natural History)*.

Uhlenbeck G.E., Ornstein L.S. 1930. On the theory of the brownian motion. *Physical review*. 36:823.