

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 is generated asexually, and 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). Siphonophores are found at all depths - most species 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 (Totton, 1960; Pugh, 1983; Carré and Carré, 1995). They are among the most abundant gelatinous predators in the open ocean, and play an important ecological role in oceanic waters (Williams and Conway, 1981; Purcell, 1981; Pugh, 1984; Pugh et al., 1997; Pagès et al., 2001).

Siphonophores are monophyletic and nested within the Hydrodolina, although relationships among the major lineages of the Hydrodolina remain difficult to resolve (Cartwright et al., 2008; Cartwright and Nawrocki, 2010; Zapata et al., 2015). The most resolved siphonophore phylogeny to date focused on two genes (16S, 18S) from 52 siphonophore taxa, and resolved many long standing questions about siphonophore biology, including the relationships of the three historically recognised groups (Cystonectae, Physonectae, and Calycophorae) (Dunn et al., 2005). The cystonects were found to be sister to all other siphonophores, while the calycophorans were nested within physonects (the name Codonophora was given to this clade)(Dunn et al., 2005).

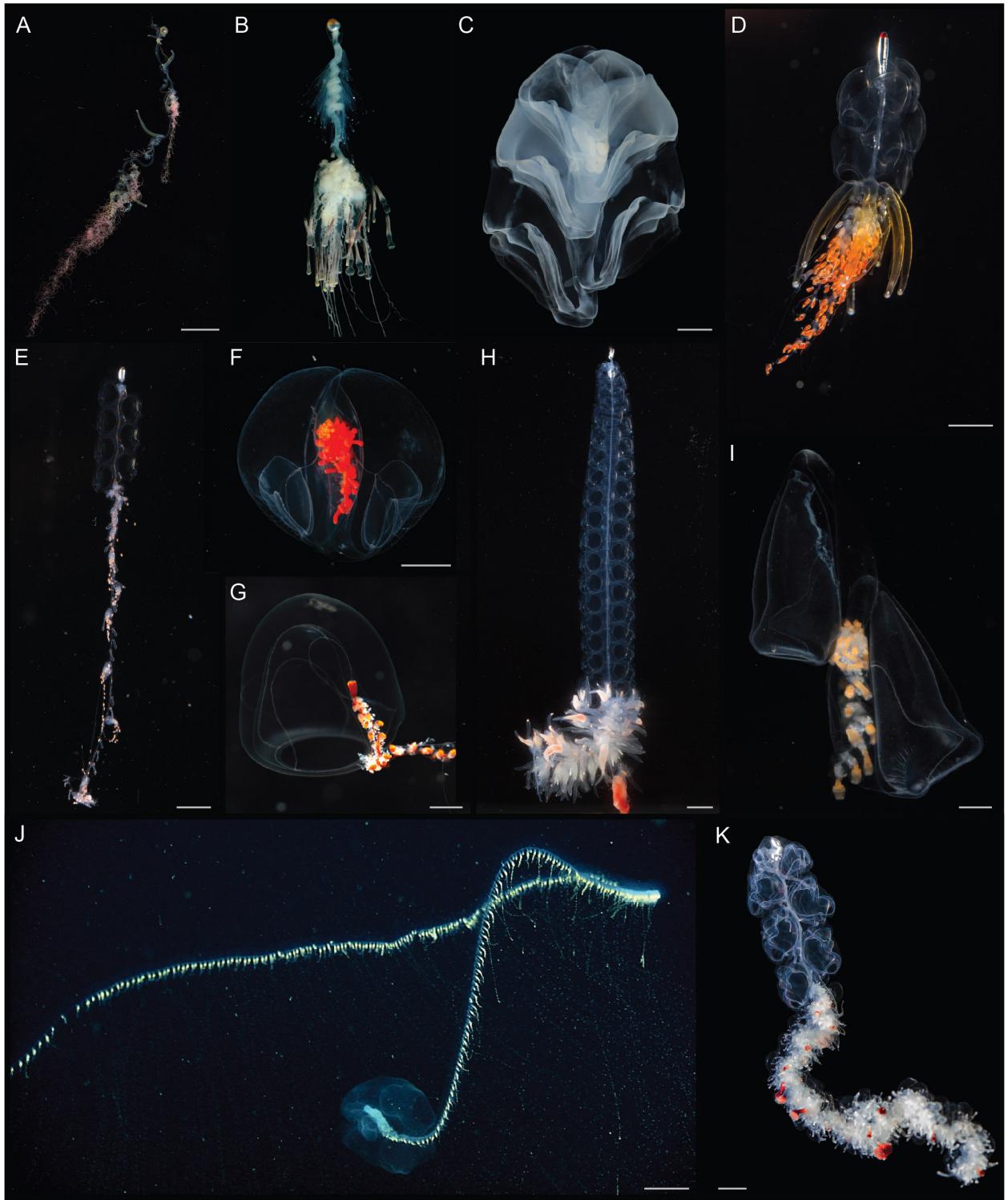


Figure 1: Photographs of representatives of the major groups of siphonophores. (A) *Rhisophysa eysenhardtii*, scale bar = 1 cm. (B) *Bathypysa conifera*, scale bar = XXX. (C) *Hippopodius hippopus*, scale bar = 5 mm. (D) *Physophora hydrostatica*, scale bar = 5 mm. (E) *Nanomia bijuga*, scale bar = 1 cm. (F) *Desmophyses haematogaster*, scale bar = 5 mm. (G) *Sphaeronectes christiansonae*, scale bar = 2 mm. (H) *Lychnagalma utricularia*, scale bar = 1 cm. (I) *Kephyes hiulcus*, scale bar = 2 mm. (J) *Praya dubia*, scale bar = 4 cm . (K) *Apolemia* sp., scale bar = 1 cm.

The Apolemiidae are sister to all other Codonophora, however there was little resolution of deep relationships within Codonophora. The history of colony structure and budding was investigated by mapping these traits to the tree, and suggesting a complex history of zooid gain and loss. Resolving deep relationships within Codonophora is key to resolving the evolution of several character traits, including sexual systems (monoecy vesus dioecy) or the gain and loss of particular zooids, such as palpons.

Siphonophores are difficult to collect, in part because they are fragile (nets destroy all but the most robust parts of the colony), but also because many species are found in the deep sea (Youngbluth, 1984; Dunn et al., 2005). Specimen sampling for the previous phylogeny, and this new work, is enabled by modern collection techniques, including blue-water scuba diving and remotely operated vehicles (ROVs). Here we present a broadly sampled phylogenomic analysis of Siphonophora, assessing transcriptomic data from 34 siphonophore species and 9 outgroup species. Using 1071 genes shared across species, we are able to resolve deep relationships within the siphonophore phylogeny and reconstruct the evolutionary history of characters, including zooid type, life history traits, and habitat.

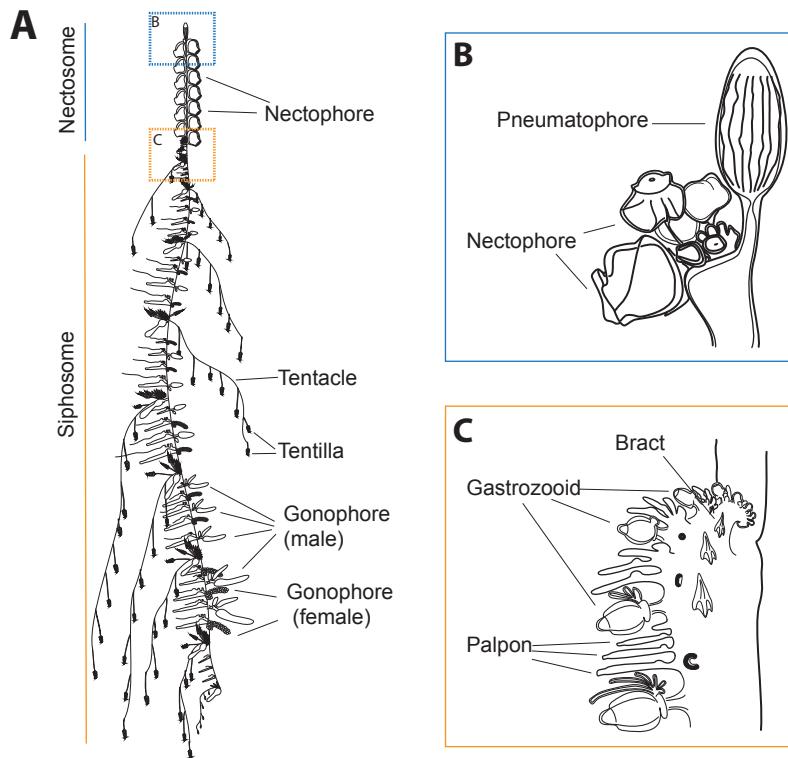


Figure 2: Schematic of the siphonophore *Nanomia bijuga*, oriented with the anterior of the colony at the top, and the ventral side to the left. Adapted from http://commons.wikimedia.org/wiki/File:Nanomia_bijuga_whole_animal_and_growth_zones.svg, drawn by Freya Goetz. (A) Overview of the whole mature colony. (B) Inset of the nectosomal growth zone with pneumatophore. A series of buds gives rise to nectophores. (C) Inset of the siphosomal growth zone. Probuds subdivide to give rise to zooids in repeating-units (cormidia). Cormidial boundaries are marked by a gastrozooid.

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 fc0c98a73e92e45ac5ad022b9a54de0f21d57596.

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. *Chelophyses 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

(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) Maximum 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 receive strong support in this 1,071-gene transcriptome analysis. These findings include XXX.

Character Evolution

Siphonophores 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 monoecious and others were dioecious. 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 5a 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 occurred 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.

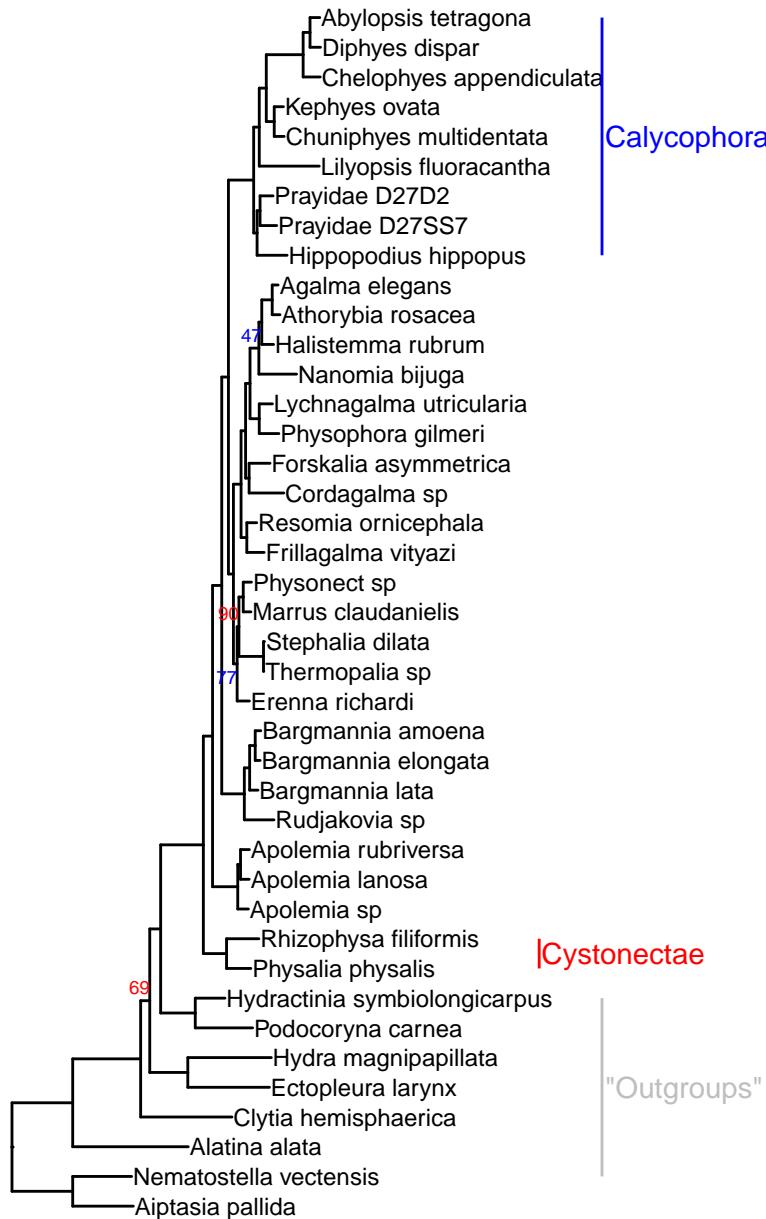


Figure 3: Phylogram of siphonophore relationships. Node labels indicate bootstrap support percent, unnumbered nodes have 100% support. The image was rendered with ggtree (Yu et al., 2016)

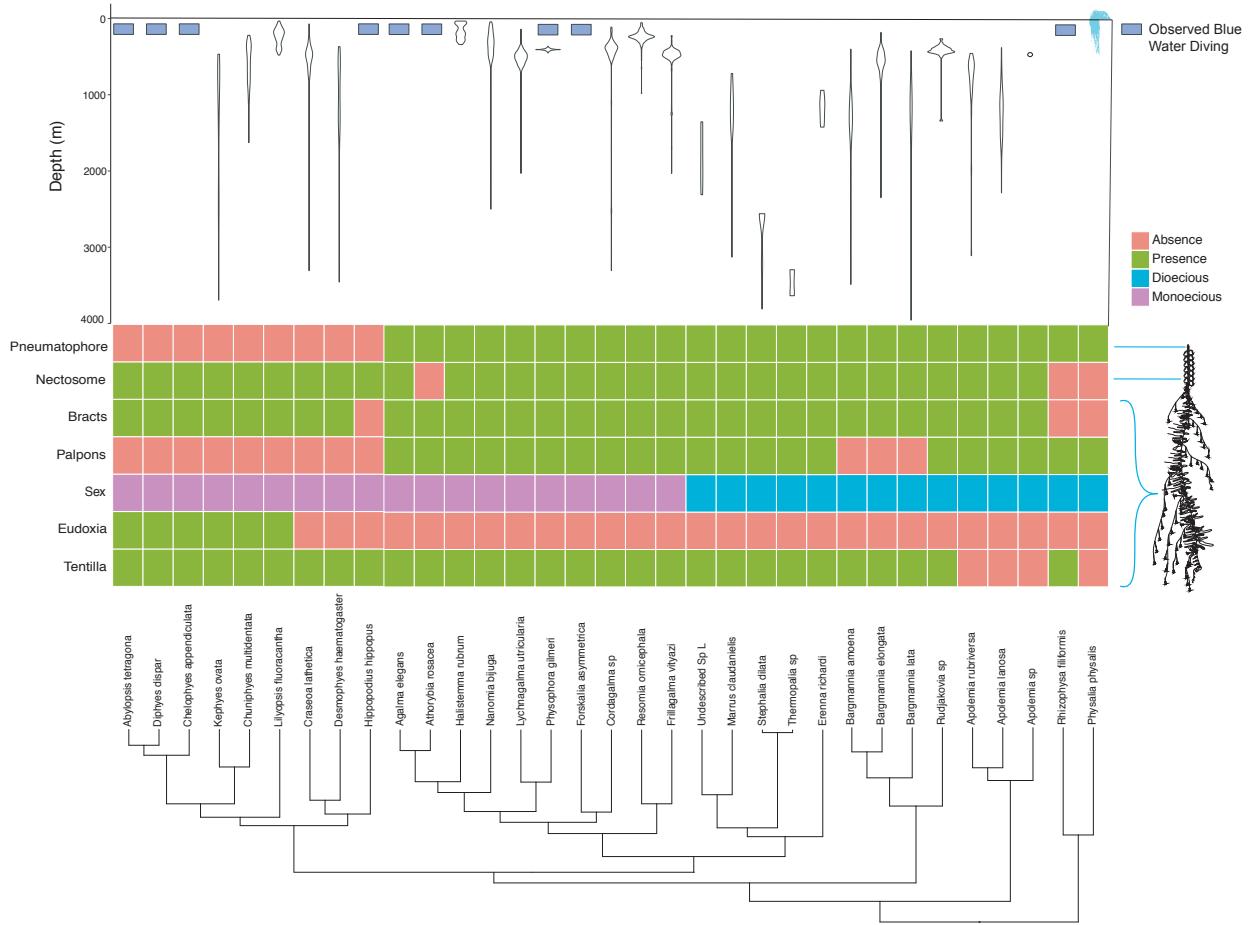


Figure 4: Figure 4. Siphonophore phylogeny showing the distribution of the main anatomical characters and the bathymetric distributions of the different species.

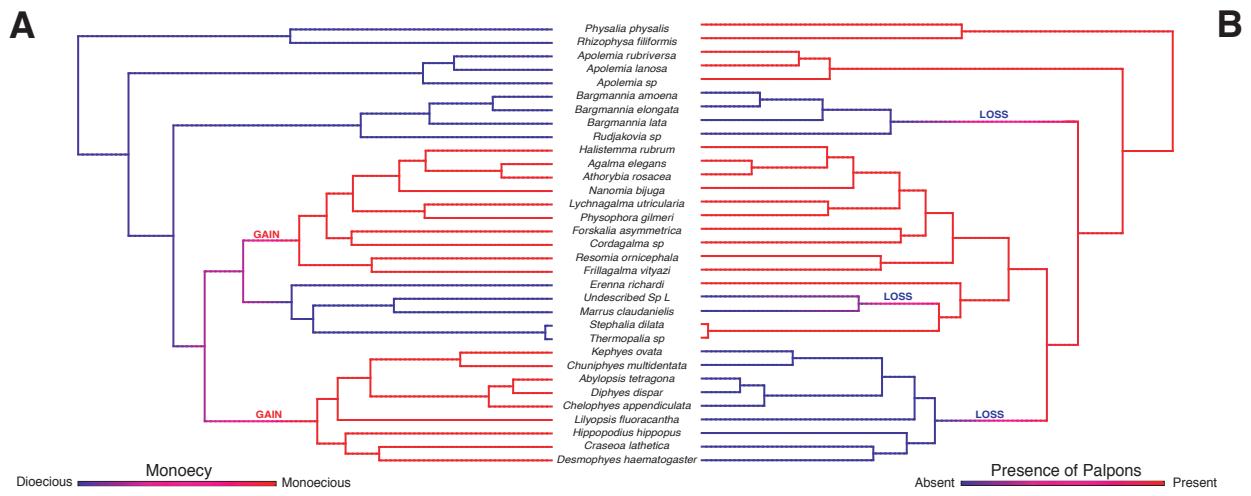


Figure 5: Figure 5. Stochastic mapping reconstruction of the evolutionary history of A) palpon zooids, and B) distribution of sexual zooids on the colonies.

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 of the 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). In 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 branches 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 had only one bract subtype, which was lost in Hippopodidae, *Physophora hydrostatica* (however, they are present in its sister species, *P. gilmeri* included in the present phylogeny), 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 sulphate ions in their bracts to adjust their buoyancy along the colony (Bidigare & Biggs, 1980).

The ancestral siphonophore certainly had a pneumatophore, since both Cystonects and most Codonophorans possess one (Figure 4). 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 (Figure 5b), retained in most species, but lost three times

independently in the branches leading to *Pyrostephidae* (represented here by the genera *Bargmannia* and *Rudjakovia*), in Calycocephorans, and in *Marrus claudanielis*. 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. conifera* 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 reconstruct 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 branchings 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 reconstruction lead to a more robust support for a tentilla-bearing MRCA followed by a single loss of tentilla in the branch leading to *Apolemiidae* (Supp Figure).

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 , Figure 4). 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 traditionally used for taxonomic diagnostics is a positive indicator of the applicability and unambiguity of these characters.

Conclusions

Acknowledgements

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Supplementary Information

Agalma analysis

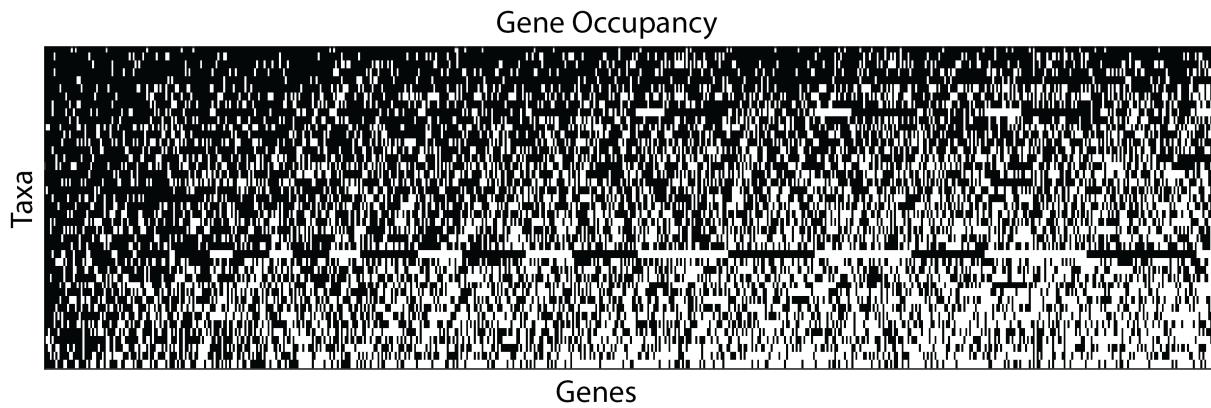


Figure 6: 60% gene occupancy matrix for 41 species across 1,071 genes. Genes and species are sorted by sampling, the best sampled shown in the upper left.

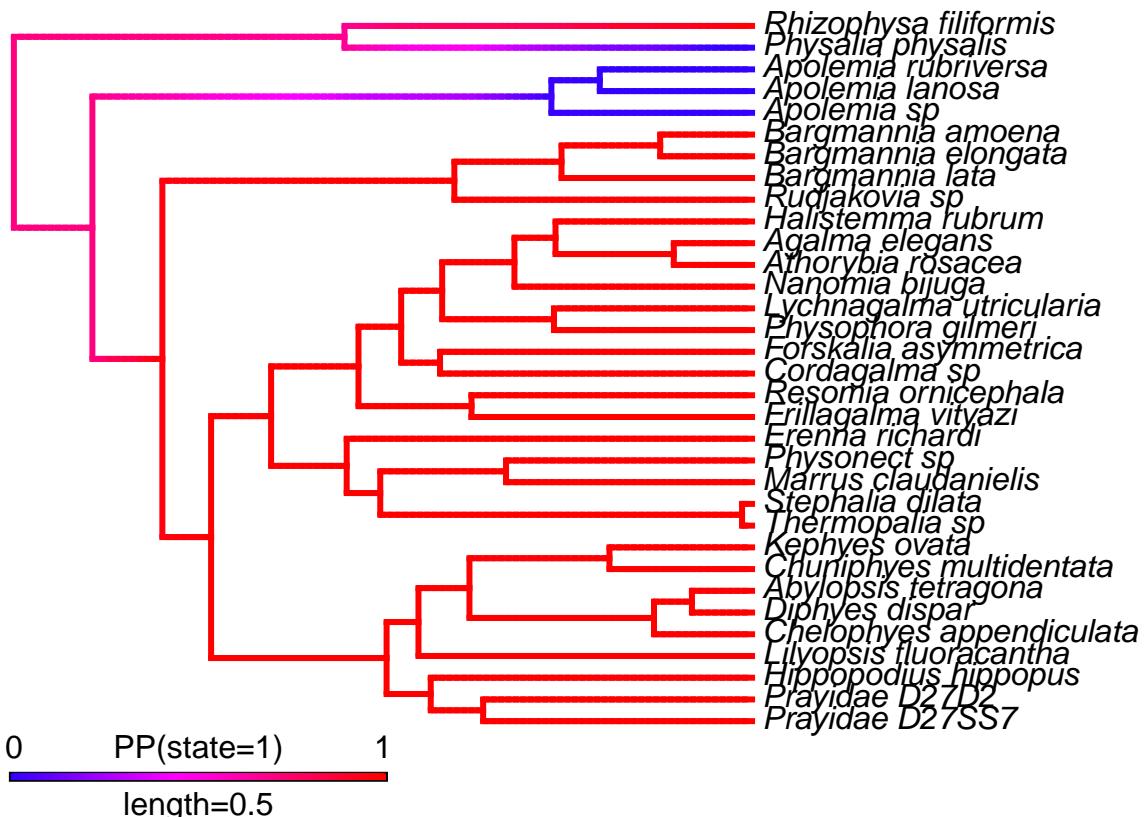
Species	Mean in- sert size (bp)	Insert size sd	# read pairs	% kept after rRNA re- moval	% kept after rRNA as- sem- bly	Adapter fails	Quality fails	Base Comp. fails	Total trans- cripts	Coding trans- cripts
<i>Marrus clau- danielis</i>	253.03	53.30	35866092	96.80	62.10	113298	15594637	4981676	105439	22511
<i>Apolemia rubriversa</i>	290.81	80.64	19228660	91.70	77.80	40594	5179800	516100	87701	17540
<i>Chuniphyes multidentata</i>	284.46	35.74	17999147	77.00	74.30	24263	4941872	335297	84341	22084
<i>Apolemia sp</i>	167.41	53.46	16761717	81.30	79.40	1332063	3062035	41943	51470	14752
<i>Apolemia lanosa</i>	167.74	55.30	18444476	96.40	80.90	923922	3882968	439569	70184	15579
<i>Bargmannia elongata</i>	271.43	40.17	40008913	79.80	78.50	342825	8084832	2017709	152019	23661
<i>Diphyes dis- par</i>	223.14	58.73	80000000	78.70	63.40	14412120	21322624	690159	210406	50868
<i>Aiptasia pall- ida</i>	183.18	53.88	74558341	98.40	79.30	210730	23826421	307520	83950	33523

Stephalia	di-	267.82	43.33	30953585	98.00	63.90	86068	15731787	1435119	107925	23984
Physalia	physalis	233.10	54.37	36481773	96.60	88.50	18553	5491979	259497	74994	23705
Bargmannia	amoena	161.57	53.77	20195498	84.60	80.10	1049110	3996085	274520	66726	17975
Frillagalma	vityazi	283.11	44.91	79902051	54.00	70.40	668400	13591301	5558767	181508	29293
Alatina	alata	173.96	60.33	96259870	52.10	77.90	1236251	15771112	468662	166584	28743
Clytia	hemisphaerica									11476	6642
Ectopleura	larynx	229.05	37.75	109024653	95.90	74.60	432309	40629127	897590	84034	28015
Athorybia	rosacea	221.16	93.65	28696930	99.90	86.90	691916	4026467	633700	100839	24543
Forskalia	asymmetrica	259.42	35.04	25275184	58.90	65.10	41922	7463840	841930	82483	18419
Prayidae	D27D2	267.32	45.59	38233199	85.20	66.40	59773	15338562	1878732	144909	28065
Lilyopsis	fluoracantha	259.39	39.08	51855968	76.50	65.30	72416	19809124	1388875	114662	29854
Hydractinia	symbiolongicarpus	175.22	57.84	60462724	49.80	85.20	162430	6678373	124392	71450	24639
Rhizophysa	filiformis	269.74	46.52	26937827	92.50	65.30	66010	13238701	361376	110109	24833
Agalma	elegans	238.94	57.54	40007833	99.60	86.00	31957	7177806	748977	122053	26601
Chelophyes	appendiculata	275.93	39.43	21103284	89.00	74.20	63548	6777022	344708	110612	32309
Prayidae	D27SS7	303.39	50.83	25233164	66.70	65.60	39061	8616289	347189	94917	24211
Rudjakovia	sp	213.88	89.71	20582477	99.10	79.70	489520	3936086	1546686	91695	24184
Abylopsis	tetragona	292.16	38.32	21575176	73.30	73.80	38764	5759144	370604	102946	25268
Nematostella	vectensis									26511	18080
Hippopodius	hippopus	266.99	45.11	35638254	84.50	66.30	56152	15490291	535026	137341	35031
Physophora	gilmeri	214.97	93.78	19847377	99.30	81.30	936617	3464079	1007839	52639	17381
Bargmannia	lata	166.71	62.80	16984483	98.70	71.10	3118496	4227140	868584	59051	16451
Nanomia	bijuga	296.69	44.20	39983229	85.70	75.50	155093	10854240	795226	165792	28927
Erenna	richardii	291.53	36.76	29776547	64.20	66.60	39775	8958200	1245523	89423	18236
Physonect	sp	261.09	35.96	38243543	24.30	66.70	12494	4517982	274838	57746	12596
Thermopalnia	sp	201.29	84.79	19074319	99.70	85.40	736393	2809499	531359	50572	16913
Cordagalma	sp	289.42	37.89	20416265	62.40	77.30	22069	3875945	387383	88468	22604

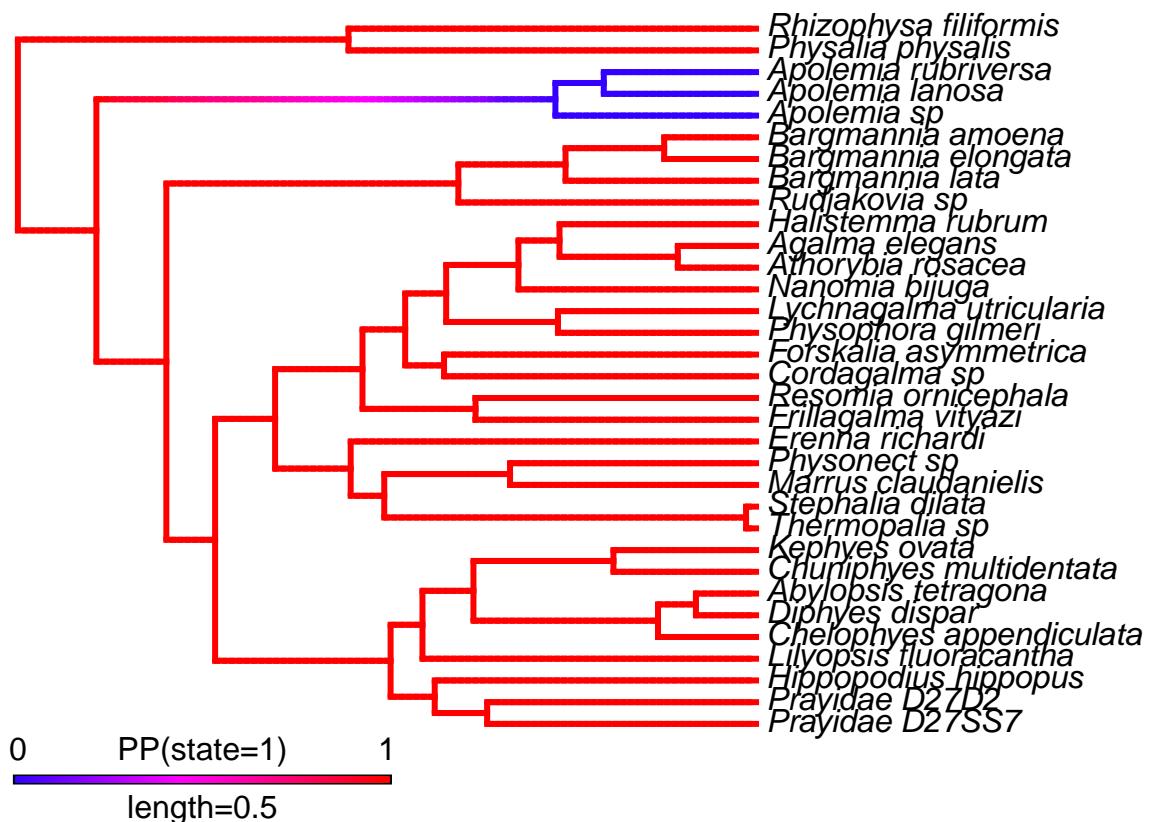
Lychnagalma	266.38	50.25	35833131	63.90	65.70	60019	11691276	714049	95708	21057
utricularia										
Kephyses	243.52	61.49	42044676	99.20	85.40	12567	7982239	496206	146394	31286
ovata										
Hydra magni-									17741	12581
papillata										
Resomia orni-	258.55	36.28	42998185	68.50	65.10	47093	14776612	1429610	103682	22777
cephala										
Halistemma	224.84	100.91	26331113	97.90	85.10	439185	4294857	714798	87526	25351
rubrum										
Podocoryna	173.51	52.58	46216536	72.80	74.10	121866	13195545	142954	109584	45849
carnea										

The assembly summary statistics for all included taxa, showing mean insert size, and the percentage of reads kept after pipeline steps.

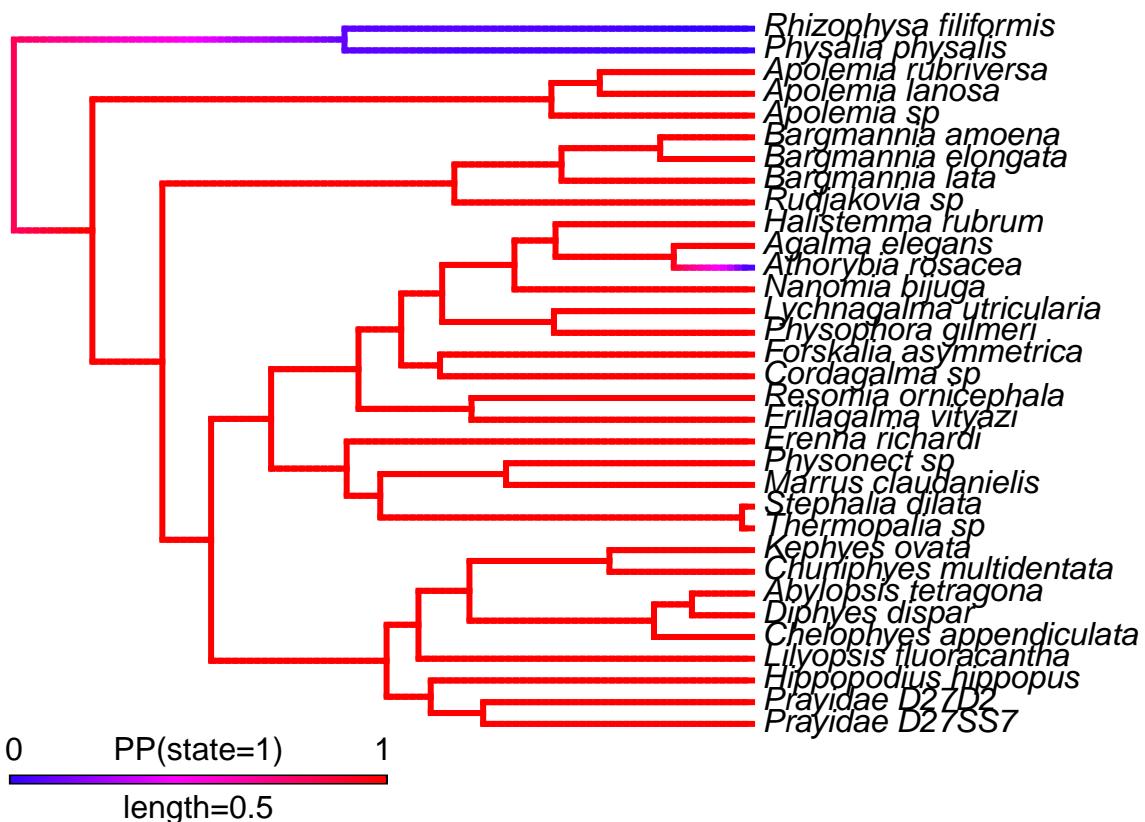
SIMMAP Presence of tentilla

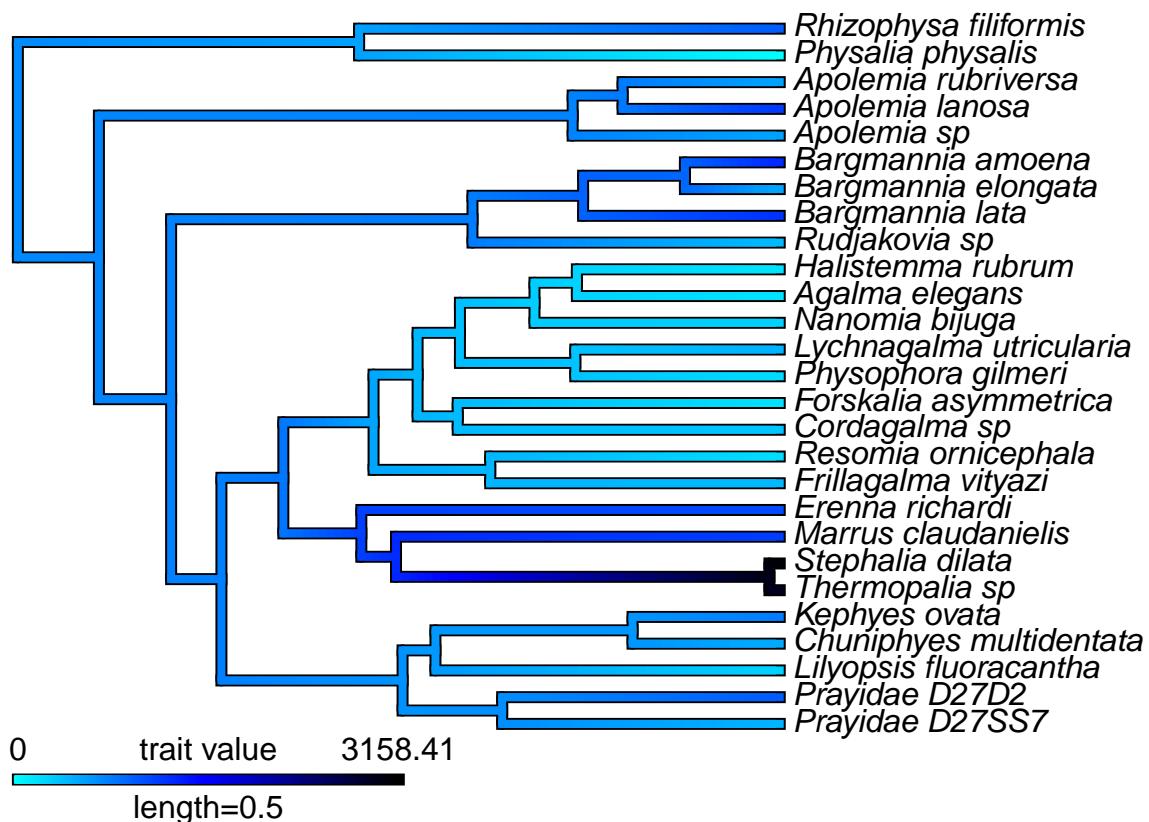


SIMMAP Presence of tentilla - *Physalia* corrected



SIMMAP Presence of Nectosome





Phylogenetic signal in Binary Traits

	K	PICvar obs	PICvar rnd	P-value	Z-score
Nectosome	0.7576982	0.20867575	0.4509123	0.2465	-0.5781193
Palpons	2.5304645	0.13420510	1.3415077	0.0010	-2.0010797
Tentilla	2.9207926	0.06470251	0.5832560	0.0010	-1.1126360
Pneumatophore	3.9936316	0.06932454	1.0899517	0.0010	-1.7503710
Bracts	1.8939427	0.08917691	0.4722325	0.0020	-0.8714136

Supplementary table x. Phylogenetic signal in the binary traits, including Blomberg's K statistic, the mean observed PIC variance, the random variance of PICs, p-value of the comparison of observed and random variance, and the z-score.

Software versions

This manuscript was computed on Fri Nov 24 21:55:19 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: OS X El Capitan 10.11.3

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_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8

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

other attached packages:
[1] bindr_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_1.39 factoextra_1.0.5  gridExtra_2.3
[21] seriation_1.2-2 fields_9.0      maps_3.2.0       spam_2.1-1
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loaded via a namespace (and not attached):
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