RESEARCH NOTE

REVISED Revisiting the phylogeny of phylum Ctenophora: a molecular perspective [version 2; peer review: 1 approved with reservations, 3 not approved]

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Abstract

The phylogenetic relationships of deep metazoans, specifically in the phylum Ctenophora (inside and outside the phylum), are not totally understood. Several loci (protein coding and ribosomal RNA) from organisms belonging to this phylum are currently available on public databases (e.g. GenBank). Previous studies take into account the ribosomal data and the protein data separately. In this study, we perform a meta-analysis of previously published data together. The published data of this phylum have been used in previous phylogenetic analyses inside the phylum and consist in nuclear ribosomal data, such as 18S, 5.8S, ITS1, ITS2, and protein-coding markers such as NFP (non-fluorescent protein).

Previous studies concentrate their efforts toward the analyses of ribosomal data or the protein-coding marker separately. Now we take into account these markers together for an upgrade of the phylogenetic analysis of this phylum. We also test several markers such as 28S, IPNS, Tyrosine aminotransferase and HLH domaincontaining protein for the improvement of the study. This markers were analyzed by Bayesian Inference (MrBayes) and Maximum Likelihood (Garli and RAxML), individually and concatenated, showing improvement in the orders placement and presenting new interesting relationship between the paraphyletic order Cydippida and the other ctenophores. These analyses also include sequences from undescribed species that have been reported in GenBank which improved the alignment matrices and support values of some nodes. Adding the undescribed species suggests

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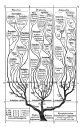
Any reports and responses or comments on the article can be found at the end of the article.

interesting and well supported clades, the posterior identification of this species would led to an improvement on the ctenophore's taxonomy.

Keywords
Ctenophora , Phylogenetic reconstruction , Ribosomal subunits , Non-fluorescent protein (GFP-like) , Bayesian Inference , Maximum Likelihood , Isopenicillin-n-synthase (IPNS)



This article is included in the Phylogenetics collection.

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REVISED **Amendments from Version 1**

We revised the manuscript and performed the following changes according the suggestions made by the referees for the last version of the manuscript:

1. Here we present the meta-analysis combining amino acid and nucleotide data to resconstruct a single tree (instead of one per dataset). As a consequence of this we re-drawed our conclusions.
2. We perform phylogenetic reconstructions using the combined dataset by Bayesian Inference and Maximum Likelihood, but for ML we used RAxML in addition to GARLI.
3. We included a new Figure 1 to replace the one in the former version.
4. Rooted trees for each analysis (RAxML, GARLI and MrBayes) have been included in Supplementary material.
5. As suggested by the reviewers we excluded IPNS as a marker for the analysis since it is a duplicated gene, and not informative for phylogenetic reconstriction. We included 2 protein coding genes (tyrosine aminotransferase and HLH domain containing protein) to the analysis to solve this problem.
6. We included to the analysis sequenced from undescribed species and other taxa not included in the previous version.

See referee reports

Introduction

Several phylogenetic hypotheses of the phylum Ctenophora based on morphological data[1], ribosomal markers[2,3], protein-coding markers[4], have been proposed, all of them through different approaches.

Due to the poor fossil record of the group, the morphological data of fossil taxa have been not enough to help to resolve this question, because it's impossible to determine which characteristics arose first between the ctenophores, for several reasons such as the poor conservation state of the available fossils[5,6] or the lack of shared characteristics between the extant ctenophores and the extinct ctenophores[7]. Also some morphological characteristic have been demonstrated to be homoplastic[1,3]. These situations slow down the process of reconstructing the phylogeny of this phylum.

There are also difficulties establishing an appropriate outgroup, due to the unknown position of this phylum inside Metazoa, many hypotheses have been suggested[8–11]. This uncertainty could bias the phylogenetic analyses if a distant outgroup is chosen (eg. highly saturated sequences, not homologous sequences available, etc), affecting directly the support values and the topology of the reconstructed tree[12].

In this study we reconfirm the paraphyly of order Cyddipida similarly to all previous studies, also we confirm the order Lobata is paraphyletic, the reasons are exposed in the results section. Nevertheless, our data don't support the paraphyly of Beroidae. Due to the fairly wide taxonomic sampling of this study resulting from the fusion of the protein-coding and ribosome data, some interesting relationships are suggested, such as the placement of Cestida and Thalassocalycida orders inside Lobata.

Methods

The ribosomal sequences were obtained from public data available on GenBank and automatically downloaded, then they were classified using python scripts. The NFP sequences was also obtained from GenBank and for certain taxa supplied by Steve Haddock via e-mail, from the Supplementary data of the reporting study of the marker[4], we only included sequences from Ctenophora. The Tyrosine aminotransferase sequences and the HLH domain-containing protein were also obtained from public data of GenBank. The accession numbers of the sequences used during this study are presented in Table 1.

Previous of the concatenated final analysis, we tested several markers such as:

1. Ribosomal markers: 18S, 5.8S, 28S, ITS1, ITS2
2. Non Fluorescent Protein (NFP)
3. Tyrosine aminotransferase
4. HLH domain-containing protein

We execute a single locus analysis for all these markers. The ribosomal sequences were aligned by MAFFT v.7.7[13] with the option –auto. The proteing coding sequences (NFP, Tyrosine aminotransferase and HLH domain-containing protein) were aligned using RevTrans2 (http://www.cbs.dtu.dk/services/RevTrans-2.0/web/)[14].

Models for single locus analyses were selected with two programs: jModelTest 2.1.10[15] for nucleotide datasets (ribosomal data), and ProtTest v 3.4.2 for protein markers[16].

Single locus analyses were performed by partitions obtained with Gblocks 0.91b (http://molevol.cmima.csic.es/castresana/Gblocks_server.html)[17,18].

For phylogenetic reconstruction using the concatenated ribosomal dataset the pipeline PhyPipe was used[19] (available at: https://gitlab.com/cibiop/phypipe/).

The Bayesian inference analyses (BI) were all performed by MrBayes 3.2.6[20] and the Maximum Likelihood (ML) analyses were performed by GARLI 2.01[21] and RAxML 8.0.0[22].

NFP was previously introduced as an ortholog by 4, which function is still unknown. So we took this marker along the ribosomal data as the backbone of the alignment matrix (and the study).

Concatenation of the sequences for the study (NFP,HLH,Tyr, 18S, 5.8S, ITS1, ITS2) was performed by 2matrix 1.0 (https://github.com/nrsalinas/2matrix)[23]. This script allows the automatic concatenation of a heterogeneous matrix, and also convert the concatenated matrix to the files input of the Maximum Likelihood and Bayesian Inference programs.

Partition and selection of the models for the concatenated matrix were performed by PartitionFinder2[24], separately from Ribosomal

Table 1. Accession numbers of used sequences.

Order	Family	Species	TaxID	HLH	ITS1	ITS2	NFP	TAT	18s	5.8s
Beroida	Beroidae	Beroe abyssicola	320166		AB377608.1		AOI27767.1			AB377608.1
Beroida	Beroidae	Beroe cucumis	31167		AF293699.1	AF293695.1			AF293699.1	AF293699.1
Beroida	Beroidae	Beroe forskalii	140453	AHA51264.1	AF293698.1	AF293698.1	AOI27768.1		AF293698.1	KJ754168.1
Beroida	Beroidae	Beroe forskalii	140453	AHA51262.1						
Beroida	Beroidae	Beroe gracilis	140454						AF293696.1	
Beroida	Beroidae	Beroe ovata	10201		EF173679.1	AF293694.1			AF293694.1	AF293694.1
Beroida	Beroidae	Beroe sp. KUR20	499921		AB377601.1					
Beroida	Beroidae	Beroe sp. KUR21	499922		AB377602.1					
Cestida	Cestidae	Cestum sp. SC-2008	499920		AB377600.1					
Cestida	Cestidae	Cestum veneris	12997		KJ754165.1	KJ754165.1	AOI27770.1		AF293692.1	KJ754165.1
Cestida	Cestidae	Velamen parallelum	140491		AF293693.1	AF293693.1	AOI27791.1	AQX17837.1	AF293693.1	AF293693.1
Cydippida	-	Cydippida sp. KUR30	500091		AB377607.1					AB377607.1
Cydippida	Bathyctenidae	Bathyctena chuni	1403704	AHA51224.1				AQX17834.1		
Cydippida	Bathyctenidae	Bathyctena chuni	1403704	AHA51235.1						
Cydippida	Dryodoridae	Dryodora glandiformis	1566677				AOI27776.1			
Cydippida	Euplokamidae	Euplokamis dunlapae	1403701	AHA51355.1			AOI27777.1	AQX17839.1		
Cydippida	Euplokamidae	Euplokamis sp. G1	1303913		HF912430.1					HF912430.1
Cydippida	Euplokamidae	Euplokamis sp. SM-2011a	1128081						HE647719.2	
Cydippida	Haeckeliidae	Haeckelia beehleri	140469		AF293673.1	AF293673.1	AOI27779.1		AF293673.1	AF293673.1
Cydippida	Haeckeliidae	Haeckelia rubra	140470		AF293674.1	AF293674.1	AOI27778.1		AF293674.1	AF293674.1
Cydippida	Lampeidae	Lampea lactea	1403706				AOI27782.1	AQX17835.1		
Cydippida	Lampeidae	Lampea pancerina	1532213		KJ754169.1	KJ754169.1			KJ754155.1	KJ754169.1
Cydippida	Lampeidae	Lampea sp. WRF-2016	1897451				AOI27783.1			
Cydippida	Mertensiidae	Charistephane fugiens	140462	AHA51304.1			AOI27771.1		AF293682.1	
Cydippida	Mertensiidae	Mertensia ovum	140496		FJ668937.1				FJ668937.1	FJ668937.1
Cydippida	Mertensiidae	Mertensiidae sp. A8	1303918		HF912432.1	HF912432.1				HF912439.1
Cydippida	Mertensiidae	Mertensiidae sp. A9	1303919						HF912439.1	
Cydippida	Mertensiidae	Undescribed mertensiid sp. 2	140498						AF293680.1	
Cydippida	Mertensiidae	Undescribed mertensiid sp. 3	140499						AF293681.1	
Cydippida	Pleurobrachiidae	Hormiphora californensis	1403702	AHA51384.1			AOI27780.1	AQX17838.1		
Cydippida	Pleurobrachiidae	Hormiphora californensis	1403702	AHA51386.1						
Cydippida	Pleurobrachiidae	Hormiphora plumosa	140456		AF293676.1	AF293676.1			AF293676.1	AF293676.1
Cydippida	Pleurobrachiidae	Pleurobrachia bachei	34499		AF293677.1	AF293677.1	Haddock		AF293677.1	AF293677.1
Cydippida	Pleurobrachiidae	Pleurobrachia brunnea	1532215		KJ754163.1	KJ754163.1			KJ754154.1	KJ754163.1
Cydippida	Pleurobrachiidae	Pleurobrachia globosa	940420		HM053535.1	HM053535.1			KJ859219.1	HM053535.1
Cydippida	Pleurobrachiidae	Pleurobrachia pileus	140457		KJ754162.1	KJ754162.1			AF293678.1	KJ754162.1
Cydippida	Pleurobrachiidae	Undescribed mertensiid sp. 1	140497						AF293675.1	
Lobata	-	Undescribed Lobata sp. 4	140501		AF293686.1	AF293686.1			AF293686.1	AF293686.1
Lobata	Bathocyroidae	Bathocyroe fosteri	1566675				AOI27765.1	AQX17836.1		

Order	Family	Species	TaxID	HLH	ITS1	ITS2	NFP	TAT	18s	5.8s
Lobata	Bolinopsidae	Bolinopsis infundibulum	140455		EF175465.1		AOI27769.1	AQX17833.1	AF293687.1	
Lobata	Bolinopsidae	Bolinopsis sp.	51107			U65480.1				U65480.1
Lobata	Bolinopsidae	Mnemiopsis leidyi	27923		AF293700.1	AF293700.1	Haddock		AF293700.1	KJ754164.1
Lobata	Eurhamphaeidae	Deiopea kaloktenota	1532218		KJ754167.1	KJ754167.1	AOI27775.1		KJ754160.1	KJ754167.1
Lobata	Eurhamphaeidae	Kiyohimea sp. WRF-2015	1649256				AOI27781.1			
Lobata	Lampoctenidae	Lampocteis cruentiventer	127145				AOI27784.1		KF202290.1	
Lobata	Leucotheidae	Leucothea multicornis	1532214		KJ754166.1	KJ754166.1			KJ754159.1	KJ754166.1
Lobata	Leucotheidae	Leucothea pulchra	140477		AF293688.1	AF293688.1	AOI27785.1		AF293688.1	AF293688.1
Lobata	Ocyropsidae	Ocyropsis crystallina crystallina	140481		AF293690.1	AF293690.1			AF293690.1	AF293690.1
Lobata	Ocyropsidae	Ocyropsis crystallina guttata	140482		AF293691.1	AF293691.1			AF293691.1	AF293691.1
Lobata	Ocyropsidae	Ocyropsis maculata	140483		AF293689.1	AF293689.1	AOI27788.1		AF293689.1	AF293689.1
Platyctenida	Coeloplanidae	Coeloplana anthostella	1037658		HQ435812.1				HQ435810.1	
Platyctenida	Coeloplanidae	Coeloplana bannwarthii	140474		AF293683.1	AF293683.1			AF293683.1	AF293683.1
Platyctenida	Coeloplanidae	Coeloplana bocki	1017162		HQ435814.1				HQ435813.1	
Platyctenida	Coeloplanidae	Coeloplana sp. PS-2014	1532212		KJ754170.1	KJ754170.1				KJ754170.1
Platyctenida	Coeloplanidae	Vallicula multiformis	140489		AF293684.1	AF293684.1			AF293684.1	AF293684.1
Platyctenida	Lyroctenidae	Lyrocteis sp. LMC-2016	1919245						KY026603.1	
Thalassocalycida	Thalassocalycidae	Thalassocalyce inconstans	140487	AHA51435.1			AOI27790.1		AF293685.1	
Thalassocalycida	Thalassocalycidae	Thalassocalyce sp. KUR23	499924		AB377604.1					
Thalassocalycida	Thalassocalycidae	Thalassocalyce sp. KUR22	499923		AB377603.1					AB377603.1
-	-	Ctenophora sp. L2 WRF-2015	1651134				AOI27786.1			
-	-	Ctenophora sp. L1 WRF-2015	1651133				AOI27787.1			
-	-	Ctenophora sp. B WRF-2014	1567048				AOI27766.1			
-	-	Ctenophora sp. C WRF-2014	1567049				AOI27772.1			
-	-	Ctenophora sp. K WRF-2015	1651141				AOI27773.1			
-	-	Ctenophora sp. M WRF-2015	1651132				AOI27789.1			
-	-	Ctenophora sp. P WRF-2015	1651142				AOI27774.1			
-	-	Ctenophora sp. T WRF-2014	1567052				AOI27792.1			

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data and protein-coding markers. The best scheme files are available inside the Supplementary data.

The models used for protein-coding markers in all analyses are: For NFP, JJT+I+G[25]; for Tyrosine amino-transferase, LG+G[26], and for HLH, VT+I[27].

The set of parameters for Bayesian Inference analysis are reported in the Supplementary data inside the NEXUS file, this analysis were performed in CIPRES[28], we used 8 MCMC with 10'000.000 generations by duplicate, this allows an optimal performance of the analysis. For 18S and 5.8S the analysis was performed with HKY+I+G[29], for ITS1 and ITS2 with SYM+G[30].

RAxML analysis was performed in CIPRES[28] with 20 independent maximum likelihood analyses and 10.000 bootstrap iterations (pseudoreplicates) for nucleotide partitions, and the model used in this analysis was GTR+G+I[31]. The importance of invariant proportion executing an analysis with RAxML in this specific dataset is explained in Discussion section.

GARLI analysis was performed with 10 independent maximum likelihood analyses and 1004 bootstrap iterations (pseudoreplicates) for nucleotide partitions we used more specific models. For 18S we used TrN+I+G[32]; for 5.8S, TIMef+I+G; for ITS1, TIMef+G, and for ITS2, TVMef+G. These models were indicated by jModeltest 2.1.10[15].

The models were all selected by BIC criterion. In this study we did not used an outgroup.

In order to obtain a more complete matrix, we fused sequences from few species of the same genus into a single record, for example, we fused *Hormiphora plumosa* and *Hormiphora californiensis* into a single *Hormiphora sp.*; *Bolinopsis sp.* and *Bolinopsis infundibulum* into a single *Bolinopsis sp.* and *Lampea lactea* and *Lampea pancerina* into a single *Lampea sp.* These fused species allow an improvement of the alignment matrix and the phylogenetic reconstruction. Also few species were duplicated, such as *Beroe forskalii, Bathyctena chuni, Hormiphora sp.*, because the HLH marker presented variation amongst the species and was not possible to obtain a consensus. We confirm the monophyly of this variation through a single locus analysis, as mentioned before.

Results

Single locus analyses

According to[3] partitions obtained by Gblocks did not improve the analysis for ribosomal markers, also it did not improve the analysis for Tyrosine aminotransferase. On the other hand, for NFP and HLH domain-containing protein the bootstrap values and posterior probability improved with partitioned analysis (see Supplementary data), unfortunately this marker didn't improve the final alignment matrix results.

We found that trees reconstructed using 28S, IPNS, and the other domain-containing proteins, presented several incongruences between them and the other markers. Ribosomal markers, Tyrosine aminotransferase, Non-fluorescent protein and HLH domain-containing protein did not present any strong incongruence amongst them. For that reason those markers were chosen for the concatenated analysis (protein sequences + nucleotide sequences).

Combined dataset

The tree reconstructed from the combined dataset (protein + ribosomal DNA) is presented on Figure 1. The results from both Maximum Likelihood analyses (RAxML and GARLI) for the combined dataset (protein + ribosomal DNA) are similar, except in the specific relationships between Eurhamphaeidae + Cestida + Leucotheidae + Bolinopsidae. RAxML results matches with Mr-Bayes results, but three nodes of the analysis have low posterior probability (BI) or low bootstrap values (ML). RAxML analysis shows a clade composed by Eurhamphaeidae and Leucotheidae, and other clade composed by Cestida and Bolinopsidae. Whereas GARLI analysis shows Eurhamphaeidae as sister taxa of Leucotheidae, Cestida and Bolinopsidae. RAxML results are similar to 3. All of our analyses show Cestida within Lobata with high bootstrap values and posterior probability, defining Lobata as a clade composed by Leucotheidae, Eurhampaheidae, Bolinopsidae and Ocyropsidae. Bathocyroidae and Lampoctenidae families have an uncertain position between Lobata and the clade composed by *Beroe* sp. and Haeckelidae.

Our analysis support a clade including Thalassocalycida and Lampoctenidae but the position of this clade remains controversial due to the lack of high bootstrap values. The family Bathocyroidae forms a clade with Dryodoridae family, but this clade has a low posterior probability and low bootstrap values, so for now it is not accurate to set hypothesis around this result, this results is similar to obtained by 4. Also the position of *Dryodora glandiformis* is still undetermined, in ML analysis this family could group with even Pleurobrachidae, due to the low bootstrap values of nodes between Lobata and Pleurobrachidae and with BI analysis with all lobates. Undescribed species T forms a good supported clade with Bathocyroidae. Further studies may focus in describing this taxon for morphological purposes. We executed a *rogue taxa* analysis through RogueNaRok[33], we found that *Beroe ovata, Beroe cucumis, Beroe gracilis, Lampocteis cruentiventer, Dryodora glandiformis, UCS4, Llyria B, and Lyrocteis sp.* were *rogue taxa* during this analysis, the low bootstrap values could be related to this.

The relationship of Bathyctenidae family (Represented by *Bathyctena chuni*) with the Mertensiidae family and the Platyctenida order remains unclear, this family shows affinity to this clade, also in several times forms a clade with two undescribed Mertensiids (A9 and undescribed sp3), this two taxa are excluded of the family Mertensiidae (Represented by *Mertensia ovum*, *Charistephane fugiens*).

The identity of spB remains unclear. species spC forms a good supported clade with Lampeidae by both methods.

Mid-root point and Fossil studies

In this study, we do not include an outgroup, as consequence of it, we used mid-root point method for rooting topologies using

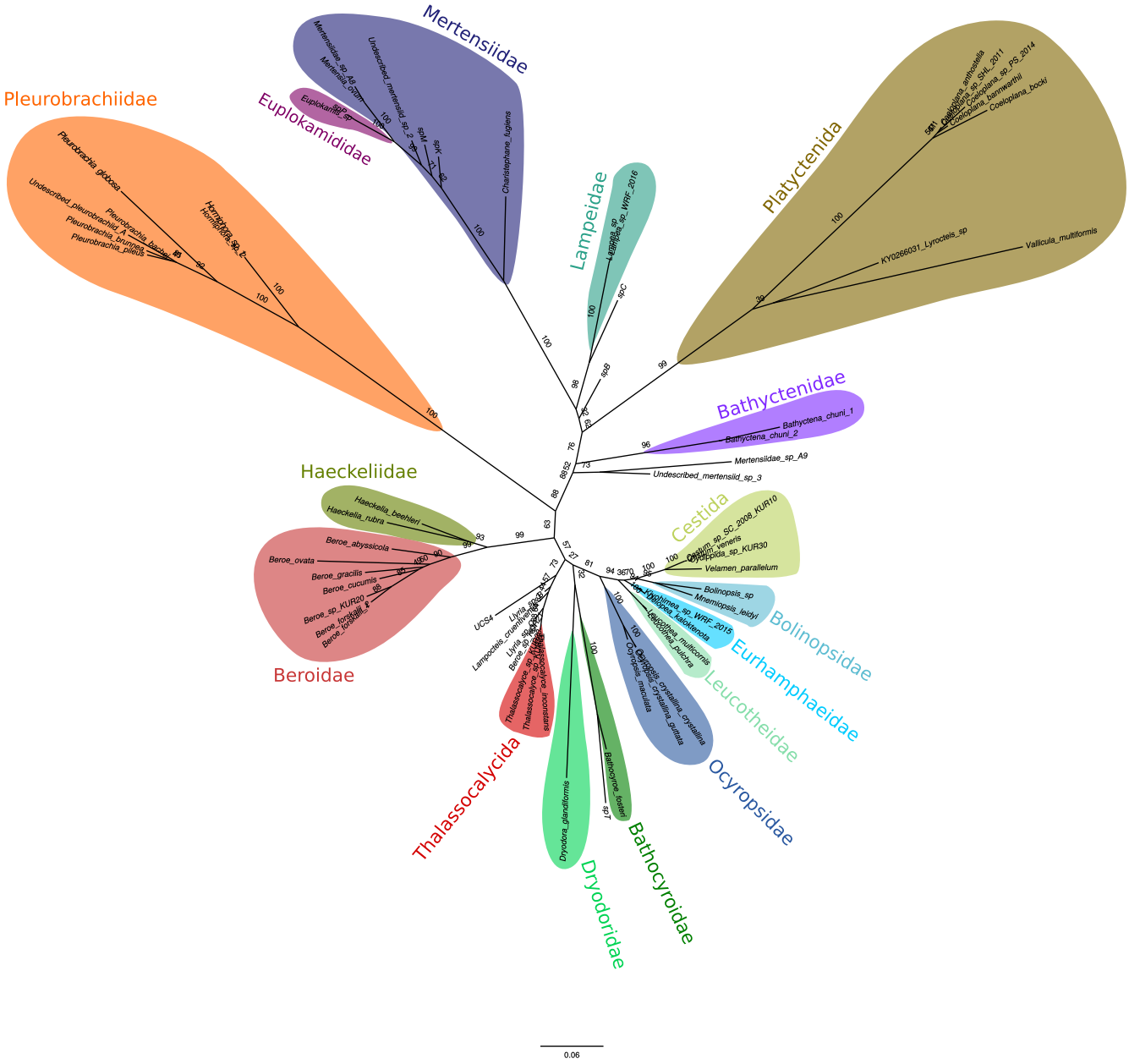

Figure 1. Unrooted tree reconstructed with RAxML for the combined dataset (NF, HLH, Tyr, 18S, 5.8S, ITS1, ITS2). Support values of nodes (bootstrap values) are shown on tree branches.

Figtree v.1.4[34]. Rooted trees for each analysis (RAxML, GARLI and MrBayes) are in the Supplementary material section.

By Mid-root point method, the topologies were splited in two major clades, one composed of Lyroctenidae+Coeloplanidae+Mertensiidae+Lampeidae+Bathyctenidae and one composed of Pleurobrachiidae+Haeckelidae+*Beroe* sp.+Cestida+Lobata+Dryodoriade+Thalassocalycida. These results are similar to 3. Both major clades with high bootstrap values(RAxML, 90 for both clades and GARLI, 84 for both clades) and for Mrbayes 0.96 as posterior probability for both clades.

This study also present interesting similarity to morphological study presented by Ou in 7, were the extant ctenophores (Excluding Beroida) are splited into two major clades, in one of the clades, Cyddipida+Platyctenida and other clade presenting Lobata+Cestida+Thalassocalycida+Ganeshida. Setting the paraphyly of Cyddipida would be interesting improve this morphological study, due to the similarities (until certain point) that the study present with the current. Also[7] study present Beroe as the most basal inside the extant Ctenophora, this study, as previous[3], denies the basal position of Beroida, also denies the Beroida as a paraphyletic group[3].

Discussion

Next steps for the resolving of the phylogeny of this group is to determine who is the most basal branch inside the Ctenophora, making possible and reconstruction of ancestral characters. Inside the upper clade formed by Pleurobrachiidae, Haeckelidae, Beroida, Thalassocalycida, Dryodoridae(with low support values), Lobata and Cestida, could be very crucial the reconstruction of ancestral characters for the understanding of the plasticity of the characteristic inside this group. This could only achieved by setting a good outgroup for this group.

We strongly recommend for further studies, the identification and posterior description for undescribed species. Also an enrichment of the aligment matrix produced by this study, through sequencing crucial markers such as 18s, ITS1 and NFP, which played an important role in the reconstruction of the phylogeny presented in this study.

Also, we recommend for further studies an extensive sampling of groups like Pleurobrachidae, in an attempt to collapse the long branches presented in previous studies[3] and the present study, genus as *Tinerfe*, which present morphological similarities with families as Haeckeliidae[1]. More sampling outside groups such as Lobata would allow an improvement for further studies.

The proportion of invariant sites, plays an important role in the analysis of Ribosomal data. During the analysis for this paper, we noticed that the absence of this feature in the analysis in RAxML, forms a clade composed by Beroe and Pleurobrachidae as sister taxa of Lobata, Cestida and Thalassocalycida, this clade was of course with an extremely low bootstrap value; the presence of this feature presents Pleurobrachidae as the sister taxa of Beroe, Lobata, Cestida and Thalassocalycida (Presented in suplementary data). So the absence or presence of this feature during the analysis should be relevant.

Data availability

The raw data used for this project are available in Zenodo, DOI 10.5281/zenodo.838689 (Arteaga-Figueroa *et al.*, 2016).

Author contributions

LAAF conceived the study, performed the sequence compilation and literature revision. LAAF, VSB and NDFS carried out the phylogenetic reconstructions and analysed the results. All authors were involved in writing the manuscript and have agreed to its final content.

Competing interests

No competing interests were disclosed.

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We specially thank Sergio Pulido-Tamayo for stimulating discussions and critical review of the manuscript, Juan F. Díaz-Nieto, Javier C. Alvarez and Diana Rincón T. for their guidance and valuable comments. We also want to thank Lizette I. Quan-Young and Steve Haddock for providing useful bibliography and sequences for this analysis, respectively. Also, we thank to Derrick Zwickl for the comments about model configuration on GARLI.

Supplementary material

Supplementary File 1. Rooted tree for the combined dataset (protein + ribosomal DNA) reconstructed by BI using MrBayes. The tree was rooted by midpoint root method and support values (posterior probabilities) are shown on tree nodes.

Click here to access the data.

Supplementary File 2. Rooted tree for the combined dataset (protein + ribosomal DNA) reconstructed by ML using RAxML. The tree was rooted by midpoint root method and support values (bootstrap values) are shown on tree nodes.

Click here to access the data.

Supplementary File 3. Rooted tree for the combined dataset (protein + ribosomal DNA) reconstructed by ML using GARLI. The tree was rooted by midpoint root method and support values (bootstrap values) are shown on tree nodes.

Click here to access the data.

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Open Peer Review

Current Peer Review Status: ? ✗ ✗ ✗

Version 2

Reviewer Report 08 September 2017

https://doi.org/10.5256/f1000research.13364.r25191

✗ **Steven H.D. Haddock**
Monterey Bay Aquarium Research Institute, Moss Landing, CA, USA

This paper still does not meet a basic standard to qualify as original research.

They have removed one set of gene sequences, and added two others gleaned from Genbank. These last two sets have low species coverage and have been published. These two sequence alignments are posted as part of the zenodo supplement without the original genbank accession numbers or other source information, making it unclear that these were posted by another lab.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 21 Sep 2017

Luis Alfonso Arteaga-Figueroa, Universidad EAFIT, Medellin, Colombia

Good day Mr. Haddock,

I am writing you regarding your review in our research note. It's important to outline that the main objective of this research note was to revisit the phylogenetic reconstruction (mostly methods) for this group, using available sequences in the GenBank. It is a meta-analysis, as the reviewers said previously. We are not reporting nothing new but our methods for this specific set of sequences.

Research note is defined as: "Research Notes include single-finding papers that can be reported with one or two illustrations (figures/tables), descriptions of unexpected observations, and lab protocols. Posters from conferences or internal meetings may be

summarized as Research Notes. In many cases, some additional detail, particularly in the methods, description of the results, and/or discussion/conclusions will be required to make sure that readers (and referees) have enough information to understand the description of the work". The phylogenetic reconstruction is basically a bioinformatic lab recipe, so here we report the results of our "lab protocol".

For the second version we took the comments of the reviewers (including yours) and we tried to accomplish all of them. One of your comments was about IPNS1, a duplicated gene in Table 1 (I must comment that in the supplementary data there was not a Hormiphora IPNS gene):
"There is some confusion because there is no Hormiphora IPNS gene in their [our] dataset, yet it is listed in the table of genes and that species is present in the tree, apparently based on a GFP-like gene that was found. Furthermore, the IPNS genes are not single-copy[2], so are not reliable for phylogeny building."

We so, reevaluated the accuracy of the phylogenetic reconstruction of the protein tree of the first version and we decided to remove it. It was not a random exercise. In fact, we also removed 28s information for the same reasons, we decided that it wasn't suitable for the phylogenetic reconstruction through single locus phylogenetic reconstruction.

And I must assure that the inclusion of the HLH domain containing protein and Tyrosine aminotransferase wasn't either a random exercise. We executed many single locus for the great variety of the reported sequences from Ctenophora in the GenBank, for example, we determined that the other domain-containing proteins were not suitable for the phylogenetic reconstruction, as the photoprotein reported also in Francis 2015 [2]. But when we tested Tyrosine aminotransferase and HLH domaing containing protein, we observed that it didn't present incongruences with ribosomal data and NFP. One of my biggest concerns was about how this could bias the phylogenetic reconstruction because they have low species coverage.

So my team and I, we reviewed about how missing data would bias the phylogenetic. We found a paper, Wiens, 2006 [1]. One of the paper's conclusion was: "Recent simulations show that there is little evidence to support excluding taxa based simply on the amount or proportion of missing data that they bear. The placement of highly incomplete taxa in a phylogeny can be resolved with perfect accuracy (based on simulations) and with strong support statistical support (based on empirical analyses)". So we "gleaned" the sequences from the GenBank and included in the final analysis, because, through this we improved the analysis.

In the supplementary data for this version 2 there are no accession numbers, they are presented in Table 1 of the research note. The objective of the supplementary data is that anyone can reproduce our results. Nowhere in the paper is not mentioned that we are reporting a sequence, as previously said, this is a meta-analysis, a bioinformatic lab exercise.

Best,
Luis Alfonso.

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Competing Interests: No competing interests were disclosed.

Version 1

Reviewer Report 03 April 2017

https://doi.org/10.5256/f1000research.11235.r19434

D Timothy J. Littlewood
Department of Life Sciences, Natural History Museum, London, UK

Unfortunately I must concur with the major concerns highlighted by other reviewers.

The data set is essentially a reassessment (meta-analysis) of previously published data. The phylogeny is functionally rooted against an in-group taxon without explanation. The analyses could have been improved by combining amino acid and nucleotide data (rather than solely treating these data separately). Due reference to similar articles from which these data have been derived was omitted. Other key references are missing. There is confusion within the article over the utility of some multi-copy genes and so interpretation and veracity of results is compromised.

In combination and considering the lack of sufficient novelty of data, approach or interpretation the publication falls short of achieving its goal. The phylogeny is revisited and with some investment of time from the authors, but little additional clarity and few insights are forthcoming to merit acceptance in its current state.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for

reasons outlined above.

Reviewer Report 28 February 2017

https://doi.org/10.5256/f1000research.11235.r20287

Kevin M. Kocot
Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL, USA

This study is a metaanalysis of available ctenophore sequence data. My thoughts largely echo those of the previous two reviewers.

The methods seem reasonable (with the exception of the methodological problems with analysis of the Homiphora IPNS gene raised by Steve Haddock) but rooting with Beroida as the outgroup is inappropriate as no molecular studies have supported this in the past, key references are absent, and the English of the manuscript needs significant improvement.

Available ctenophore transcriptome data could be used to expand sampling of the protein-coding genes. If that were done, a concatenated analysis of all of the markers used with only taxa sampled for 18S (so all taxa overlap for at least part of the alignment) with the addition of appropriate outgroups would be an interesting improvement.

Competing Interests: I am also actively studying the evolution of Ctenophora but I can honestly say that this does not impact my view on the present work.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 13 February 2017

https://doi.org/10.5256/f1000research.11235.r20040

Steven H.D. Haddock
Monterey Bay Aquarium Research Institute, Moss Landing, CA, USA

This article performs a meta-analysis of molecular phylogenetics within the Ctenophores using

published sequences. I have previously had amiable correspondence with the authors and sent them data. Although the protein-coding sequences and some of the ribosomal RNA data came from my lab, I nonetheless feel I can give an unbiased assessment of their subsequent use.

Regrettably, I do not see enough original intellectual contribution or additional scientific value to justify its publication. At best, it is a minor contribution, in which case the interpretation needs to be improved, and at worst, a good portion of it is a re-publishing of work already published by other authors (Simion [1] and Podar [4] in particular).

A large part of the analysis is building trees using previously published ribosomal RNA datasets. This recapitulation does not add anything to the discussion of ctenophore internal relationships, and in fact, by rooting the tree with Beroe, they obscure the true evolution of the group as shown repeatedly since at least 2001 [4]. They also fail to cite Simion, *et al.* [1], which is the source of some of the data. Merely adding that citation would not solve the fundamental issue, which is that there is no added value to their re-building of the same phylogeny.

The "novel" aspect of the paper is building trees based on two protein-coding genes which were also published previously in two separate papers [2,3]. The trees based on their [our] protein datasets do not give any additional insights into ctenophore relationships except in that some species are present in those trees that are not represented in the 18S phylogeny. This taxonomic coverage does not reveal any particular insight. These data also already appeared in trees (albeit not limited to ctenophores only) in the original publication.

There is some confusion because there is no Hormiphora IPNS gene in their [our] dataset, yet it is listed in the table of genes and that species is present in the tree, apparently based on a GFP-like gene that was found. Furthermore, the IPNS genes are not single-copy[2], so are not reliable for phylogeny building.

There is a misspelling of Bathyctena in Table 1 and 2 and of Lampocteis in Table 2.

In summary, two gene trees, of which one gene which was found to be absent in a ctenophore lineage, does not seem to be sufficient basis for a paper. The title itself is a vast overstatement of the content of this study.

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Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 08 February 2017

https://doi.org/10.5256/f1000research.11235.r19979

?

Martin Dohrmann
Department of Earth & Environmental Sciences & GeoBio-Center, Ludwig-Maximilians-Universität München, Munich, Germany

Introduction

1st paragraph:

- "deep metazoans" is an odd term; also "Parazoa" is no longer accepted as a valid name. All 4 taxa are clearly metazoans; they are best summarized as "non-bilaterian metazoans".

- The citation after the 1st sentence is from 1999; this should be replaced with something more recent as a lot of research in this area has been done since then.

- While the statement of the 1st sentence is true, reconstructing the phylogenetic relationships within Ctenophora does not help much to solve these issues, i.e. finding the position of Ctenophora in the animal tree of life is a separate issue that this study is unable to address.

- The "previous work" cited in the 2nd sentence is very old. There is a study from 2015 (Simion *et al.*, Zoology 118: 102-114) that also reconstructed internal relationships of Ctenophora based on multigene analyses. It is crucial that the authors interpret their results in light of that study. It is actually quite puzzling that the paper is not cited, especially because the authors used sequences originally reported in Simion *et al.* (2015).

- The statement in the following sentence is highly debatable. As long as the phylogenetic position of ctenophores is not resolved (see e.g. Dohrmann & Wörheide 2013 Integr. Comp. Biol. 53: 503-511; Pisani *et al.* 2015 PNAS 112: 15402-15407), it is totally unclear how relevant they are to answering these questions.

- In the next sentence, "previous studies" should be replaced with "ctenophores".

- The following 2 sentences suggest that this paper represents the first multilocus analysis

addressing internal phylogeny of Ctenophora. As mentioned above, this is not true. In this study, the authors used 2 protein-coding genes and the 28S gene in addition to the 18S and ITS/5.8S markers already used by Simion *et al.* (2015). This is what sets their study apart from the previous paper, and this has to be clearly communicated. The paper should focus on discussing differences to the results of Simion *et al.* in light of expanding the set of markers (but also addressing the different taxon sampling in the 2 studies).

- The abbreviation "MLSA" is introduced for "multilocus analysis" – what does the "S" stand for? Maybe it should read "multilocus sequence analysis"?

2nd paragraph:

- "ribosomal genes" should read "ribosomal RNA genes" (also elsewhere in the MS), since there are also genes coding for ribosomal proteins.

- "ortholog" should be replaced with "protein-coding" (also elsewhere in the MS), since ribosomal RNA genes are also orthologs.

3rd paragraph:

- I think the taxonomic overlap between the protein-coding and the ribosomal RNA datasets is sufficient to conduct a combined analysis, to infer a tree based on all the evidence simultaneously. As far as I recall, using mixed nucleotide and amino-acid data is possible with RAxML and MrBayes; alternatively, the protein-coding partition could be analyzed on nucleotide level (possibly excluding 3rd codon positions if they are oversaturated).

Methods

- It is unclear how ambiguously alignable regions were treated. These have to be excluded prior to analysis, but a quick glance at the concatenated matrices provided in the data supplement (concat_matrix and concat_prot_corrected) suggests otherwise. Difficult-to-align regions can bias phylogenetic inference, so this is an important point to address.

- Information about the lengths of loci and concatenated alignments should be given.

- Information about how the trees were rooted should be given in this section. In the Results section it is mentioned that Beroida was used as the outgroup to all other ctenophores. However, this is poorly justified. For example, Simion *et al.* (2015) found this group deeply nested within ctenophores. In general, I suggest following closely the methodological protocol of Simion *et al.* to make the 2 studies truly comparable.

3rd paragraph:

- Replace "is" with "are"

- The accession numbers are buried in some text files in the data supplement, which is quite inconvenient for the reader. I suggest providing them directly in Tables 1 and 2.

7th paragraph:

- Information about the substitution models used has to be provided here. I highly recommend using partitioning by gene and incorporating secondary structure information, for full comparability with Simion *et al.* (secondary structure models are available both in RAxML and MrBayes).

Results, Discussion

- These sections have to be rewritten after reanalysis of the data and comparison with Simion *et al.* (2015).

References

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Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 12 Feb 2017

Nicolás D. Franco-Sierra, Universidad EAFIT, Colombia

Thanks for your comments and suggestions on our work, they are really helpful to improve our analysis. We agree with the observations you pointed out above and we are currently working on a revised version of our manuscript.

We sincerely apologize for not including the respective comparison with the work performed by Simion *et al* (2015). We are working on the readjustments in order to make our analysis fully comparable with Simion *et al* (2015).

Competing Interests: No competing interests were disclosed.

Comments on this article

Version 1

Reader Comment 05 Jan 2017

Paul Simion, Université Montpellier, France

Dear authors,

While I am always glad to see new studies on ctenophore phylogeny, I am very surprised that you did not cite Simion *et al.* 2014 (of which I am the first author) for two reasons :

1. You used all the data sequenced in that study.
2. Both study are very similar in topic and design, and should therefore be compared.

Please find a link to the study :
http://www.sciencedirect.com/science/article/pii/S0944200614000816

Sincerely,
Paul Simion

Competing Interests: No competing interests were disclosed.