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Rapid evolution of the compact and unusual mitochondrial genome in the ctenophore, *Pleurobrachia bachei*

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Abstract

Ctenophores are one of the most basally branching lineages of metazoans with the largest mitochondrial organelles in the animal kingdom. We sequenced the mitochondrial (mtDNA) genome from the Pacific ctenophore, *Pleurobrachia bachei*. The circular mitochondrial genome is 11,016 nts, with only 12 genes, and one of the smallest metazoan mtDNA genomes recorded. The protein coding genes are intronless *cox1-3*, *cob*, *nad1*, 3, 4, 4L and 5. The *nad2* and 6 genes are represented as short fragments whereas the *atp6* gene was found in the nuclear genome. Only the large ribosomal RNA subunit and 2 tRNAs were present with possibly the small subunit unidentifiable due to extensive fragmentation. The observed unique features of this mitochondrial genome suggest that nuclear and mitochondrial genomes have evolved at very different rates. This reduced mtDNA genome sharply contrasts with the very large sizes of mtDNA found in other basal metazoans including Porifera (sponges), and Placozoa (*Trichoplax*).

Keywords

mitochondrial genome; ctenophore; basal metazoan; gene loss

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1. Introduction

Comb jellies, or ctenophores, are holoplanktonic predators, representatives of one of the most basally branching lineages of metazoans, and critical for our understanding of early animal evolution. In metazoans, there are five basal taxa, including ctenophores (Ctenophora), sponges (Porifera), cnidarians (polyps and medusas), placozoans (Placozoa, *Trichoplax*), and bilaterians. Relationships among these taxa are presently unclear, but may be further illuminated by genomic studies. The phylogenetic position of Ctenophora is still highly controversial (Hejnol et al., 2009; Philippe et al., 2009) and very little is known about their molecular organization. However, Ctenophora is the only major non-bilaterian lineage for which genomic studies are lacking.

Ctenophores are the largest animals to propel themselves by cilia. Their elongated cells that support these cilia of the comb rows have the largest mitochondrial organelles in the animal kingdom, reaching up to 8 μm in diameter (Horridge, 1964). Here, we report the first sequencing of the mitochondrial (mtDNA) genome in ctenophores from the Pacific cidipid, *Pleurobrachia bachei* A. Agassiz, 1860. Surprisingly, *Pleurobrachia* has one of the smallest and most derived animal mitochondrial genomes, suggesting an extensive degree of evolution in this ancestral lineage.

2. Materials and Methods

2.1 Animal collection and molecular work

Animals were collected from the dock at Friday Harbor Laboratories (Friday Harbor, WA) in spring-summer 2008-2010. They were maintained for up to one week in running sea water at 10°C or filtered sea water at 4°C until further use. All animals were examined for exo-parasites then washed three times in twice filtered (0.2 μm) sea water. Animals were anesthetized by incubation in isotonic MgCl_2 (337mM). Both RNA and DNA were isolated for sequencing. Genomic DNA was extracted with a genomic tip 100 (Cat# 10243, Qiagen) and buffer set (Cat #19060, Qiagen) according to the manufacture's recommendations. Incubation of between 1-10 animals was with lysis buffer and proteinase K at 50°C for 10 min followed with RNase treatment. We finally eluted the gDNA in 100 μl of MQ water. This gDNA was then used for PCR amplification or library construction for high throughput genomic sequencing. Over $20 \times$ coverage of the genome with 454 sequencing was obtained and an assembly was constructed from this sequencing data.

Initially, sequences with high similarity to *cox1* and *cob* genes were identified by BlastP in our original 454 transcriptome datasets. Next, specific primers for these predicted mitochondrial genes were designed and gDNA was amplified and sequenced. Then primers were designed to walk along the whole mitochondrial genome. Sequencing was finished when the sequence circularized. The large-scale genomic 454 sequencing validated the mitochondrial genome with more than $20 \times$ physical coverage, and a single scaffold representing the entire mitochondrial sequence.

Analysis of nucleotide composition was performed with in-house perl scripts. Predictions for tRNAs were performed with tRNAScan-SE (Lowe and Eddy, 1997; Schattner et al.,

2005), ARWEN <http://130.235.46.10/ARWEN/> and MITOS, a free web server at <http://mitos.bioinf.uni-leipzig.de>.

2.2 Phylogenomic analyses

Mitochondrial genome processing—Complete mitochondrial genomes were downloaded from NCBI and amino acid sequences for the 13 protein-coding genes found in most metazoan mitochondrial genomes and were parsed into individual fasta files using the mitobank Perl script. All protein-coding genes from the *P. bachei* mitochondrion were used in the analysis.

Orthology assignment and dataset assembly—All OGs were then trimmed using gblocks (b1 = half the number of sequences in alignment plus one, b2 = b1, b3 = 8, b4 = 2, b5 = h) to remove regions with ambiguous the alignment or little to no phylogenetic signal. A final data matrix of all 13 protein-coding genes was assembled using FASconCAT.

Phylogenetic analyses—Phylogenetic analyses were conducted using ML in RAxML 7.2.7 and BI in PhyloBayes 2.3 (Fig. 1S on the Alabama Supercomputer Authority Dense Memory Cluster (<http://www.asc.edu/>). For ML analyses, the CAT + WAG +F model was used. Topological robustness (i.e., nodal support) for all ML analyses was assessed with 100 replicates of nonparametric bootstrapping. For the BI analysis, the CAT model was employed to account for site-specific rate heterogeneity. BI analyses were conducted with five parallel chains run for 15,000 cycles each, with the first 5,000 trees discarded as burn-in. A 50% majority rule consensus tree was computed from the remaining 10,000 trees from each chain. Topological robustness was assessed using posterior probabilities. Species, abbreviations and their accession numbers used in phylogenetic analysis can be found in Supplementary Material 1S.

3. Results

3.1 Features of the mitochondrial genome from *P. bachei*

Using standard molecular biology techniques complimented with 454 and Illumina sequencing technology, we sequenced the complete mitochondrial genome of *P. bachei* at >20 X coverage. To verify the assembled mtDNA genome, we then PCR amplified, cloned and Sanger sequenced the mtDNA genome to validate the overall assembly as a single scaffold. The circular mtDNA genome of *Pleurobrachia* (Acc # JN392469) is 11,016 nts in length (Fig. 1). Thus, *P. bachei* has the most compact metazoan mitochondrial genome ever reported.

This compact mtDNA genome is comprised of just 9 intronless protein-coding genes (versus 13 protein coding genes in a majority of animals (Chan, 2006)), 1 large ribosomal RNA gene and 2 tRNA genes (Fig. 1 and Table 1). The nine protein coding genes encode for the following: cytochrome oxidase subunit I, cytochrome oxidase subunit II, cytochrome oxidase subunit III, NADH dehydrogenase subunit 1, NADH dehydrogenase subunit 3, NADH dehydrogenase subunit 4, NADH dehydrogenase subunit 4L, NADH dehydrogenase subunit 5 and cytochrome b. All use AUG as a start codon except for NAD1, which uses AUU; and all protein coding genes use UAA as a stop codon. The *nad2* and 6 genes are

found as incomplete fragments (<50nt identifiable fragments) in the mitochondrial genome and have not been identified in the nuclear genome either. Both *nad2* and *6* genes have low homology to other mitochondrial proteins. Areas where fragments were identified are marked in light pink Fig. 1.

No *atpase* subunits 6, 8 or 9 were detected in the *P. bachei* mt genome. However, we identified and cloned a complete *atp6* gene from the nuclear genome and found that it contains four introns (Table 1S and see alignments in 3S Supplementary Material). The mitochondrial large ribosomal subunit is small in size and highly derived, whereas the small ribosomal subunit is not detected, possibly due to extensive unidentifiable fragmentation, or is not present.

There are 4 canonical codons reported for other animal mt genomes that were not used in *P. bachei*'s mtDNA -- Arg; CGA, Arg; CGG, Pro; CCG and Stop; UAG (see Table 2S in Supplementary Material, and analysis using the Invertebrate Mitochondrial Code). In most metazoan mitochondrial genomes the UGA codon is a Tryptophan residue (Trp), however the UGA codon is undetermined in *P. bachei*'s mitochondrial genome. Based on alignments with other conserved proteins, UGA has the potential to encode different amino acids (Table 1 and Table 3S in Supplementary Material).

Overall nucleotide composition is 23% G+C. All genes are transcribed unidirectionally, except for the tRNA-G, from the coding strand, which has an asymmetrical nucleotide composition of 52.3% T, 24.4% A, 13.1% C and 10.2 % G. The genome composition is 76% coding (protein, rRNA and tRNA) and 24% non-coding. No other open reading frames (ORF) were identified.

3.2 Ctenophore mitochondrial genome reflects high rate of nucleotide substitution

Phylogenomic analysis of mt genomes was performed with 94 species from all major animal clades whose conserved protein coding sequences were concatenated (species used, accession numbers for mtDNA genomes and methodological details are described in Supplementary Material 1S). Fig. 2 depicts the tree obtained by maximum likelihood (ML) in RAxML 7.2.7 (see additional trees Figs. 1S and 2S in the Supplementary Material). Phylogenetic placement of *P. bachei* is problematic because of the elevated rate of base substitution (i.e., long-branch attraction). Indeed, this particular position looks to be an artifact of the long-branch attraction. Evident gene loss plus no unknown ORF found in *P. bachei* mtDNA further support that the mt genome in the ctenophore lineage has been subject to rapid evolution compared to other animals sequenced so far.

4. Discussion

4.1 Mitochondrial genome comparison reveals multiple gene loss

The recent advent of convenient, cheaper and fast sequencing capabilities has facilitated progress in the field, with 2406 animal mt genomes having been sequenced by Sept 2011. The majority of animals sequenced so far have a 'typical' 15, 000-18,000 nt mtDNA genome with 37 genes: 13 protein subunits in the respiratory complexes I, III, IV and V, 22 mitochondrial tRNA (20 standard amino acids, plus an extra gene for leucine and serine),

and 2 rRNA (Chan, 2006). Most sequenced mtDNA genomes are from bilaterian animals (especially vertebrates and arthropods), leaving the basal metazoans under represented. There are 5 mt genomes from Placozoans (likely representing different lineages of *Trichoplax*), 42 mt genomes from sponges and 45 mt genomes from Cnidaria. Thus, Ctenophora is the major basal metazoan lineage lacking any information about mtDNA genomes. Here we report one of the smallest, 11,016 nts, of all metazoan mtDNA genomes from the ctenophore *Pleurobrachia bachei*.

The *P. bachei* gene complement described here contrasts even with all other basal metazoan genomes that typically have larger sizes and more genes than bilaterian animals (see Table 2). Indeed, the largest mtDNA genome has been reported in the placozoan, *Trichoplax adhaerens*, at 43,079 nts and 47 genes (Dellaporta et al., 2006; Signorovitch et al., 2007); representatives of choanoflagellates, sister group to Metazoa, and other unicellular Opisthokonta (e.g. *Capsaspora*) and fungi possess even larger genomes (Burger et al., 2003; Burger and Lang, 2003; Lang et al., 2002). Therefore, there is a recognized trend for significant mitochondrion gene loss in the course of animal evolution and a reduction of the mt genome size compared to a common ancestor of all metazoans. The fact that *P. bachei* has the most compact mtDNA genome can be interpreted as a highly derived feature.

The mtDNA genome of *P. bachei* encodes only 9 protein coding genes - the smallest complement of protein coding genes found in any metazoan mitochondrial genome to date. A complete *atp6* gene is present in the nuclear genome, suggesting secondary relocation of its function to the nucleus. However, it is not clear if the two fragmented genes *nad2* and *6*, as well as the short *nad3* and *4L*, encode for functional proteins. There are no recognized complements for these *nad* genes, or the missing mtDNA *atp8* gene, in the nuclear genome.

On a smaller scale, trends of gene loss within mtDNA can be observed in other animal groups (Table 2). For example in sponges, *Plakina trilopha*'s mitochondrial genome is missing the *nad4L* gene (Gazave et al., 2010) and the *Sympagella nux* mtDNA genome lost both *atp8* and *nad6* (Haen et al., 2007). In metazoans, *atp8* is the smallest mitochondrial encoded protein, being only 50-65 amino acid residues long, and only about a half dozen of these amino acid residues are well conserved across animal mtDNAs. Among the animal groups that have been sampled, *atp8* has been independently lost from the mtDNA genomes in some bivalve molluscs (Ren et al., 2010) but is found in others (e.g. in *Mytilus* see (Breton et al., 2010); (Smietanka et al., 2010)), secernentean nematodes (Lavrov and Brown, 2001), and platyhelminths (Le et al., 2000)). All 5 species of chaetognaths studied so far have also lost both their *atp6* and *8* genes (Faure and Casanova, 2006; Helfenbein et al., 2004; Miyamoto et al., 2010). Interestingly, even a very conservative organization of mtDNA genomes in land vertebrates can be subject to extensive gene loss, as reported for certain amphibians. For example, the mtDNA genome of the tree frog, *Polypedates megacephalus*, lost both *atp8* and *nad5* genes (Zhang et al., 2005a; Zhang et al., 2005b), see details in Table 2. Of course, there is a possibility of extensive mitochondrial RNA editing for *nad2*, *nad3*, *nad4L*, and *nad6* (Vanfleteren and Vierstraete, 1999), resulting in the lack of the recognized sequences. Complete genome sequencing is required to identify these components in the nucleus. However, all currently available 454 data suggest that this possibility is unlikely.

The dynamic evolutionary nature of mt genomes is also supported by their high variability of total genes and tRNA genes across Metazoa. For example porifera's gene content ranges from 44 genes to 18 genes. Gene loss particularly for the tRNA genes appears to be common in cnidarians such as the sea anemone, *Nematostella*, as well as *Sarcophyton glaucum*, *Renilla kolikeri*, and *Metridium senile* which contain only 2 tRNA genes (Medina et al., 2006).

The presence of only 2 tRNAs and 1 rDNA in *P. bachei*'s mtDNA genome suggest gene loss was not restricted to protein coding genes. By comparison, chaetognaths lost all but one of their tRNA genes (Faure and Casanova, 2006; Helfenbein et al., 2004; Miyamoto et al., 2010). Interestingly, some unicellular eukaryotes such as apicomplexans and trypanosomatids encode no tRNAs in their mtDNA genomes. Plants still preserve some tRNAs from a common eukaryote ancestor, and they import multiple tRNAs from the cytosol into their mitochondria (see review (Schneider, 1994)). Similar mechanisms might operate in the ctenophore *P. bachei* to support mitochondrial functions.

In summary, further transcriptome and genomic information from *P. bachei* and related species is needed to reconstruct the unique evolutionary history and functional adaptations in the mitochondrion of ctenophores. As recently shown for human mitochondria, direct transcriptional profiling is vital to decipher and validate the functional role of unconserved RNA or fragmented protein coding genes (Mercer et al., 2011). It is possible that some 'missing' components of the *Pleurobrachia* mt genome complement (e.g. srRNA) are highly fragmented beyond recognition and/or functionally not required. At this moment we posit that the RNA/protein content of the mtDNA genome in ctenophores is the smallest among animals and therefore dependent on import from the nuclear genome.

Note in Proof—While this paper was under review the *Mnemiopsis leidyi* mitochondrial genome was published (Pett et al., 2011). The findings in these two studies are in close accordance. The just released *Mnemiopsis* mt genome is similar to the *Pleurobrachia* genome (Both mt genomes are extremely AT rich and have high rates of substitution) but has several differences that can be summarized as follows. (i) the *Pleurobrachia* genome still has 2 tRNA's, where as there are none in the *Mnemiopsis* mt genome; (ii) *Mnemiopsis* has a small and large mt rRNA, whereas the small mt rRNA was unidentifiable in *Pleurobrachia*; (iii) *nad2* is recognized as incomplete fragment in *Pleurobrachia* but is present as a full coding sequence in *Mnemiopsis*; (iv) there is a possibility that *atp6* has been transferred to the nuclear genome prior to the divergence of both species but additional analysis of other ctenophore mt genomes would be desirable to resolve the reported unique features of their organization and evolution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Breton S, Stewart DT, Hoeh WR. Characterization of a mitochondrial ORF from the gender-associated mtDNAs of *Mytilus* spp. (Bivalvia: Mytilidae): identification of the “missing” ATPase 8 gene. *Mar Genomics*. 2010; 3:11–18. [PubMed: 21798192]
- Burger G, Forget L, Zhu Y, Gray MW, Lang BF. Unique mitochondrial genome architecture in unicellular relatives of animals. *Proc Natl Acad Sci U S A*. 2003; 100:892–897. [PubMed: 12552117]
- Burger G, Lang BF. Parallels in genome evolution in mitochondria and bacterial symbionts. *IUBMB Life*. 2003; 55:205–212. [PubMed: 12880200]
- Chan DC. Mitochondria: dynamic organelles in disease, aging, and development. *Cell*. 2006; 125:1241–1252. [PubMed: 16814712]
- Dellaporta SL, Xu A, Sagasser S, Jakob W, Moreno MA, Buss LW, Schierwater B. Mitochondrial genome of *Trichoplax adhaerens* supports placozoa as the basal lower metazoan phylum. *Proc Natl Acad Sci U S A*. 2006; 103:8751–8756. [PubMed: 16731622]
- Faure E, Casanova JP. Comparison of chaetognath mitochondrial genomes and phylogenetical implications. *Mitochondrion*. 2006; 6:258–262. [PubMed: 16949352]
- Gazave E, Lapebie P, Renard E, Vacelet J, Rocher C, Ereskovsky AV, Lavrov DV, Borchellini C. Molecular phylogeny restores the supra-generic subdivision of homoscleromorph sponges (Porifera, Homoscleromorpha). *PLoS One*. 2010; 5:e14290. [PubMed: 21179486]
- Haen KM, Lang BF, Pomponi SA, Lavrov DV. Glass sponges and bilaterian animals share derived mitochondrial genomic features: a common ancestry or parallel evolution? *Mol Biol Evol*. 2007; 24:1518–1527. [PubMed: 17434903]
- Hejnal A, Obst M, Stamatakis A, Ott M, Rouse GW, Edgecombe GD, Martinez P, Baguna J, Bailly X, Jondelius U, Wiens M, Muller WE, Seaver E, Wheeler WC, Martindale MQ, Giribet G, Dunn CW. Assessing the root of bilaterian animals with scalable phylogenomic methods. *Proc Biol Sci*. 2009; 276:4261–4270. [PubMed: 19759036]
- Helfenbein KG, Fourcade HM, Vanjani RG, Boore JL. The mitochondrial genome of *Paraspadella gotoi* is highly reduced and reveals that chaetognaths are a sister group to protostomes. *Proc Natl Acad Sci U S A*. 2004; 101:10639–10643. [PubMed: 15249679]
- Horridge GA. The Giant Mitochondria of Ctenophore Comb-Plates. *Quarterly Journal of Microscopical Science*. 1964; s3:301–310.
- Lang BF, O’Kelly C, Nerad T, Gray MW, Burger G. The closest unicellular relatives of animals. *Curr Biol*. 2002; 12:1773–1778. [PubMed: 12401173]
- Lavrov DV, Brown WM. *Trichinella spiralis* mtDNA: a nematode mitochondrial genome that encodes a putative ATP8 and normally structured tRNAs and has a gene arrangement relatable to those of coelomate metazoans. *Genetics*. 2001; 157:621–637. [PubMed: 11156984]
- Le TH, Blair D, Agatsuma T, Humair PF, Campbell NJ, Iwagami M, Littlewood DT, Peacock B, Johnston DA, Bartley J, Rollinson D, Herniou EA, Zarlenga DS, McManus DP. Phylogenies inferred from mitochondrial gene orders—a cautionary tale from the parasitic flatworms. *Mol Biol Evol*. 2000; 17:1123–1125. [PubMed: 10889225]
- Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res*. 1997; 25:955–964. [PubMed: 9023104]
- Medina M, Collins AG, Takaoka TL, Kuehl JV, Boore JL. Naked corals: skeleton loss in Scleractinia. *Proc Natl Acad Sci U S A*. 2006; 103:9096–9100. [PubMed: 16754865]
- Mercer TR, Neph S, Dinger ME, Crawford J, Smith MA, Shearwood AM, Haugen E, Bracken CP, Rackham O, Stamatoyannopoulos JA, Filipovska A, Mattick JS. The human mitochondrial transcriptome. *Cell*. 2011; 146:645–658. [PubMed: 21854988]

- Miyamoto H, Machida RJ, Nishida S. Complete mitochondrial genome sequences of the three pelagic chaetognaths *Sagitta nage*, *Sagitta decipiens* and *Sagitta enflata*. *Comp Biochem Physiol Part D Genomics Proteomics*. 2010; 5:65–72. [PubMed: 20374943]
- Pett W, Ryan JF, Pang K, Mullikin JC, Martindale MQ, Baxevanis AD, Lavrov DV. Extreme mitochondrial evolution in the ctenophore *Mnemiopsis leidyi*: Insight from mtDNA and the nuclear genome. *Mitochondrial DNA*. 2011; 22:130–142. [PubMed: 21985407]
- Philippe H, Derelle R, Lopez P, Pick K, Borchellini C, Boury-Esnault N, Vacelet J, Renard E, Houliston E, Queinnec E, Da Silva C, Wincker P, Le Guyader H, Leys S, Jackson DJ, Schreiber F, Erpenbeck D, Morgenstern B, Worheide G, Manuel M. Phylogenomics revives traditional views on deep animal relationships. *Curr Biol*. 2009; 19:706–712. [PubMed: 19345102]
- Ren J, Shen X, Jiang F, Liu B. The mitochondrial genomes of two scallops, *Argopecten irradians* and *Chlamys farreri* (Mollusca: Bivalvia): the most highly rearranged gene order in the family Pectinidae. *J Mol Evol*. 2010; 70:57–68. [PubMed: 20013337]
- Schattner P, Brooks AN, Lowe TM. The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. *Nucleic Acids Res*. 2005; 33:W686–689. [PubMed: 15980563]
- Schneider A. Import of RNA into mitochondria. *Trends Cell Biol*. 1994; 4:282–286. [PubMed: 14731591]
- Signorovitch AY, Buss LW, Dellaporta SL. Comparative genomics of large mitochondria in placozoans. *PLoS Genet*. 2007; 3:e13. [PubMed: 17222063]
- Smietanka B, Burzynski A, Wenne R. Comparative genomics of marine mussels (*Mytilus* spp.) gender associated mtDNA: rapidly evolving atp8. *J Mol Evol*. 2010; 71:385–400. [PubMed: 20931184]
- Vanfleteren JR, Vierstraete AR. Insertional RNA editing in metazoan mitochondria: the cytochrome b gene in the nematode *Teratocephalus lirellus*. *RNA*. 1999; 5:622–624. [PubMed: 10334332]
- Zhang P, Zhou H, Chen YQ, Liu YF, Qu LH. Mitogenomic perspectives on the origin and phylogeny of living amphibians. *Syst Biol*. 2005a; 54:391–400. [PubMed: 16012106]
- Zhang P, Zhou H, Liang D, Liu YF, Chen YQ, Qu LH. The complete mitochondrial genome of a tree frog, *Polypedates megacephalus* (Amphibia: Anura: Rhacophoridae), and a novel gene organization in living amphibians. *Gene*. 2005b; 346:133–143. [PubMed: 15716017]

Abbreviations

mtDNA genome Mitochondrial genome

Fig. 1. Genomic map of the *P. bachei* mitochondrial genome

Gray areas are noncoding regions, dotted lines are tRNAs. Gene and gene product abbreviations are the following; *cox 1-3* cytochrome c oxidase subunits, *nad 1,3,4,4L* and *5* NADH dehydrogenase subunits, *cob* cytochrome b apoprotein, *lrRNA* large ribosomal RNA. The light pink bars represent the areas where the highly fragmented *nad2* and *6* sequences are located.

Fig 2. Phylogenomic analysis of concatenated proteins in animal mitochondrial genomes
 For methods see 2S Supplementary Material, which also includes a list of all accession numbers and abbreviations for each species used in the tree. Only major branches show bootstrap support. This tree is also shown in Fig. 1S and Fig. 2S as a cladograms. The ctenophore mt genome is likely a subject of rapid evolution and the position of *Pleurobrachia* in the tree might reflect the long-branch attraction.

Table 1

Genes identified in the mitochondrial genome of *P. bachei*.

Gene	Position	Stand	Start	Stop	Length (bp)	Coding (aa)	UGA
<i>cox 1</i>	1-1464	+	ATG	TAA	1464	484	(4) G, T, A
<i>cox 2</i>	1585-2166	+	ATG	TAA	582	193	(1) A
tRNA-G (ACC)	2483-2544	-			62		
tRNA-P (TGG)	3220-3277	+			58		
<i>nad4L</i>	3344-3523	+	ATG	TAA	180	60	(1) I
<i>trRNA</i>	3524-3940	+			416		
<i>cox 3</i>	3979-4719	+	ATG	TAA	741	246	(3) S, T
<i>nad4</i>	5590-6837	+	ATG	TAA	1248	415	(2) G, L
<i>cob</i>	7035-8081	+	ATG	TAA	1047	348	(6) S, E, P, S, G, L
<i>nad5</i>	8101-9597	+	ATG	TAA	1497	498	(11) I, G, T, S, M, P
<i>nad1</i>	9588-10436	+	ATT	TAA	849	282	(3) L, V
<i>nad3</i>	10728-10928	+	ATG	TAA	210	70	0

Table 2

Summary of mtDNA genome features across various Metazoa and the Choanoflagellate *Monosiga*.

Phylum	Species*	Accession Number	mtDNA genome Size (nt)	% Coding + RNA	# Protein Coding genes	# rRNA/rRNA	nad gene loss	Presence of:			
								atp6	atp8	atp9	Ribosomal Proteins (others)
Choanoflagellata	<i>Monosiga brevicollis</i>	NC_004309	76,568	41%	32	25/2		+	+	+	+
Ctenophora	<i>Pleurobrachia bachei</i>	JN392469	11,016	71%	9	2/1	nad2,6	-	-	-	-
Porifera	<i>Lubomirskia baicalensis</i>	NC_013760	28,958	43%	14	25/2		+	+	+	-
	<i>Oscarella carmela</i>	NC_009090	20,327	65%	15	27/2		+	+	+	mtb
	<i>Plakina trilopha</i>	NC_014852	20,427	60%	12	3/2	nad4L	+	+	-	-
	<i>Ircinia strobilina</i>	NC_013662	16,414	73%	14	2/2		+	+	+	-
Cnidaria	<i>Ephydatia muelleri</i>	NC_010202	23,929	52%	14	25/2		+	+	+	-
	<i>Nematostella</i> sp.	NC_008164	16,389	72%	17	2/2		+	+	-	-
	<i>Astrangia</i> sp.	NC_008161	14,853	77%	17	2/2		+	+	-	-
Placozoa	<i>Trichoplax adhaerens</i>	NC_008151	43,079	27%	20	24/3		+	-	-	8 Hypothetical
	<i>Placozoon</i> sp.BZ2423	NC_008834	36,699	46%	14	24/3		+	-	-	2 Hypothetical
	<i>Placozoon</i> sp.BZ10101	NC_008832	32,661	45%	14	24/3		+	-	-	2 Hypothetical
Cheatoznatha	<i>Sagitta enflata</i>	NC_013814	12,631	73%	11	1/2		-	-	-	-
Mollusca	<i>Aphysia californica</i>	NC_005827	14,117	76%	13	22/2		+	+	-	-
	<i>Mytilus edulis</i>	NC_006161	16,740	66%	12	23/2		+	+	-	-
Chordata	<i>Homo sapiens</i>	NC_012920	16,569	68%	13	22/2		+	+	-	-
	<i>Polypedates megacephalus</i>	NC_006408	16,702	56%	11	22/2	nad5	+	-	-	-