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# Inference of bryophytes paraphyly using mitochondrial genomes

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## Abstract

The present phylogenetic analysis was conducted using mitochondrial genome sequences of plants to infer evolutionary relationship of bryophytes. Paraphyly of bryophytes was shown by reconstructed trees. Liverworts are the first to diverge therefore appeared as sister to land plants in nucleotide based trees. However protein sequences based tree show mosses and liverworts to form a joint clade. Hornworts formed sister relationship with vascular plants.

## Introduction

Bryophytes (liverworts, mosses and hornworts) are the most primitive land plants and share ancestry with charophycean algae (Kenrick and Crane 1997, Renzaglia et al. 2000, Qiu et al. 2006, Finet et al. 2010). Previously several studies were conducted to resolve the relationship among bryophytes lineages (Mishler et al. 1994, Lewis et al. 1997, Nickrent et al. 2000, Nishiyama et al. 2004, Shanker et al. 2011, Shanker and Sharma 2012, Shanker 2013). However these studies provide contrasting results. As several complete mitochondrial genomes are available in public databases their sequences can be used to infer relationship among plants. Therefore this analysis was conducted to deduce bryophytes relationship using mitochondrial genome sequences.

## Materials and methods

Mitochondrial genome sequences of 18 organisms (Table 1) were downloaded from National Center for Biotechnology Information (NCBI). Protein sequences of these organelle genomes were retrieved along with corresponding coding sequences (CDS). Data set of mitochondrial orthologous protein sequences was created as suggested by Shanker et al. (2009). MUSCLE (Edgar 2004) was used to get the multiple sequence alignment (MSA) of each set of orthologous protein sequences. Corresponding CDS was aligned using PAL2NAL (Suyama et al. 2006) which

uses protein MSA to guide the nucleotide alignment. CDS alignments were assembled into concatenated nucleotide data set thus generating 23 genes alignment. Additionally concatenated protein sequences data set was also generated.

To determine the best fitting model of nucleotide substitution Mraic.pl (Nylander 2004), a PERL script, was used which showed GTRIG as the best model considering Akai Information Criterion (AIC). PHYML (Guindon and Gascuel 2003) with 500 bootstrap replicates was used to construct the maximum likelihood (ML) based phylogenetic tree. MrBayes (Huelsenbeck and Ronquist 2001) was used to create Bayesian Inference (BI) based tree. The parameters for BI were taken as given by Shanker (2013). MtRev (Adachi and Hasegawa 1996) model estimated for mitochondrial encoded proteins were used to construct protein sequences based tree using PHYML (500 bootstrap replicates). *Ostreococcus tauri*, a chlorophyte, was used to root the trees.

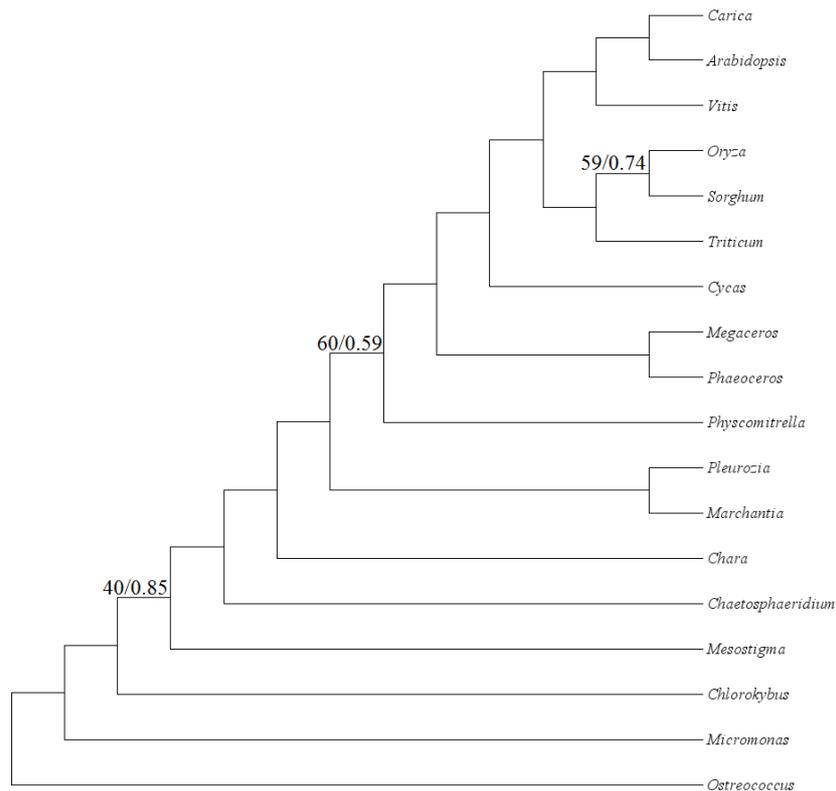
Table 1. Information of mitochondrial genomes.

S. No.	Organisms	Mitochondrial genome	
		Accession No.	Sequences
1.	<i>Chaetosphaeridium globosum</i>	NC_004118	46
2.	<i>Chara vulgaris</i>	NC_005255	46
3.	<i>Chlorokybus atmophyticus</i>	NC_009630	58
4.	<i>Mesostigma viride</i>	NC_008240	41
5.	<i>Micromonas</i> sp. RCC299	NC_012643	39
6.	<i>Ostreococcus tauri</i>	NC_008290	43
7.	<i>Phaeoceros laevis</i>	NC_013765	38
8.	<i>Megaceros aenigmaticus</i>	NC_012651	48
9.	<i>Marchantia polymorpha</i>	NC_001660	76
10.	<i>Pleurozia purpurea</i>	NC_013444	69
11.	<i>Physcomitrella patens</i> subsp. patens	NC_007945	42
12.	<i>Cycas taitungensis</i>	NC_010303	39
13.	<i>Arabidopsis thaliana</i>	NC_001284	117
14.	<i>Carica papaya</i>	NC_012116	39
15.	<i>Triticum aestivum</i>	NC_007579	39
16.	<i>Sorghum bicolor</i>	NC_008360	32
17.	<i>Oryza sativa</i> Indica Group	NC_007886	54
18.	<i>Vitis vinifera</i>	NC_012119	74

## Results and discussion

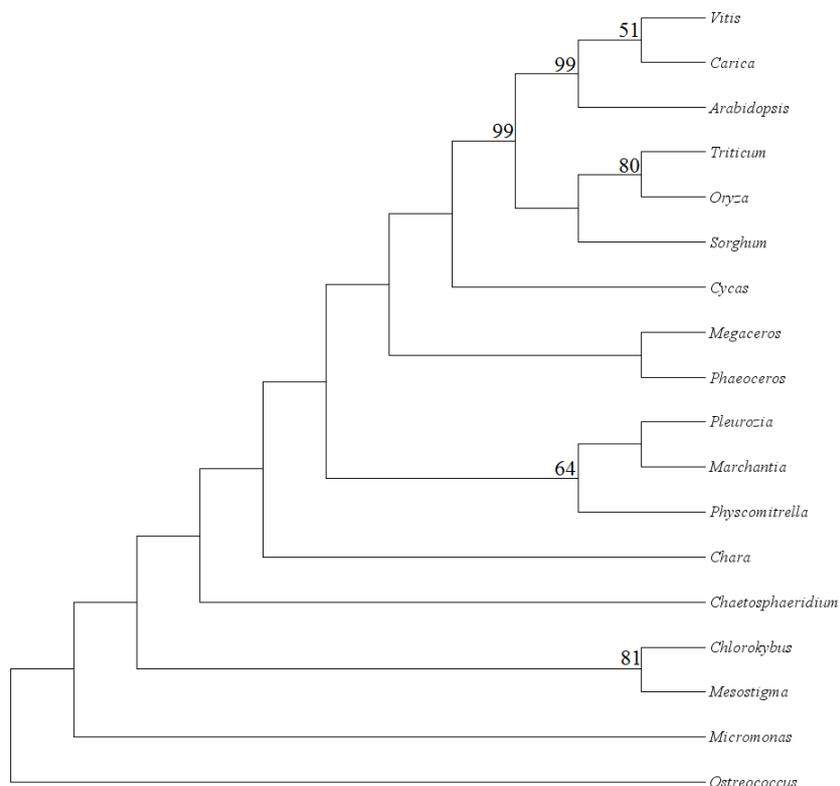
Due to availability of mitochondrial genome sequences of plants in NCBI database phylogenetic analysis to infer relationship of bryophytes was performed using both nucleotide and corresponding protein sequences. Nucleotide data from mitochondrial genomes showed paraphyly of bryophytes (Fig. 1) with same placement of bryophytes lineages as inferred by chloroplast data (Shanker 2013). The divergence of mosses and hornworts shows Bootstrap support (BS) values and Bayesian posterior probability (PP) support values of 60% and 0.59, respectively (Fig. 1).

However the tree constructed using concatenated orthologous protein sequences showed sister relationship between mosses and liverworts with 64% BS support (Fig. 2). The paraphyly of bryophytes inferred in this analysis is in strong agreement with several earlier studies (Kenrick and Crane 1997, Qiu et al. 1998, Renzaglia et al. 2000, Qiu et al. 2006, Shanker 2013).



**Figure 1.** Bryophytes phylogeny inferred from mitochondrial nucleotide data set. Bootstrap support values (<100%) and Bayesian posterior probability (<1) are shown on the internal nodes.

Contrary to this several earlier studies supported monophyly of bryophytes (Nishiyama et al. 2004, Goremykin and Hellwig 2005, Shanker et al. 2011). These studies may suffer from limitation of taxon or character sampling (Qiu 2008). The findings in present analysis can be further strengthened by adding more bryophytes taxa.



**Figure 2.** Maximum likelihood phylogeny inferred from mitochondrial protein sequences data set. Bootstrap support values (<100%) are shown on the internal nodes.

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