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18S rRNA Approach for Identification of *Chara L.* Species

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The study focused on the molecular phylogenetics for the identification of genus Chara species through 18S rRNA genes. Genus Chara sample is collected in the freshwater streams of Talakona region, Chittoor district, Andhra Pradesh and DNA was isolated. The 18S rRNA, gene sequence was used to carry out BLAST with the 'nr' database of NCBI GenBank database. First ten sequences were selected based on maximum identity score using multiple alignment software program Clustal W. Ribosomal Database Project was used for generating distance matrix and Molecular Evolutionary Genetics Analysis 6 Software was used for constructing the phylogenetic tree. Based on the maximum identifying score Chara species was identified as Chara foetida.

Keywords: Chara foeitda; Identification; Molecular phylogenetics; Phylogenetic tree.

Charophyceae is macroscopic algae, habit in both fresh and brackish water, upright stem like structure with branches, in addition to whorl of secondary branches[15]. These members are in close relation to higher plants due to the presence of similar pigmentation and reproductive structures[4]. They are used as research models in biotechnology, genetic engineering, biochemistry, cell cycle and physiology[5]. Algae have the great importance in ecological and economical context. Hence the availability of systematic details, diversity and distribution is essential for future research work. Systematics deals with identification of plant and evolutionary relationships[3]. They are studied by the classical and molecular methods. In classical method the plants are identified based on the morphological characteristics and inmolecular method plant are identified with the DNA sequence[1]. Identification of organism through molecular phylogenetic study can be done in short gene from a uniform locality on a genome. Each organism has different type of DNA barcode, so it has the great importance in the taxonomy, evolution and phylogenetical arrangement[2]. The first step of the Molecular phylogenetic studies is to select the suitable primers, amplified the barcode region PCR. In plant species which show similar morphology, differences can be identified through molecular phylogenetic study[12]. Sometimes it is difficult to identify the *Chara* speciesin which do not bear sex organs[15]. In such conditions the Molecular phylogenetic study helps in the identification of Organism. Anyone who has the Basic knowledge on DNA technology is adequate to identify the organism. Molecular phylogenetic study is not the only solution for all taxonomical problems, but it provides the effective nomenclature[10] and identification for the unknown or un recognized

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species. The organisms which are not identified in the classical methods can be identified by the molecular phylogenetic study.

MATERIAL AND METHODS

Collection and Culture of *Chara* species

Chara sample was collected from the fresh water streams of Talakona region, Chittoor district, Andhra Pradesh. The sample was isolated from other associated algae, cleaned thoroughly with the water[19]. *Chara* sample was cultured in *in vivo* condition and named as CH9.

Molecular Phylogenetic

DNA was isolated from the samples with DNeasy plant Mini Kit(Quiagen, Hilden, Germany). 1.0 % agarose gel was used for quality assessment, a high-molecular weight single band DNA has been identified.

CDMFP and CDMRP primers were used for amplifying the fragments of 18S rRNA gene. 700 bp a single discrete PCR amplicon band identified in the Sample when resolved in agarose gel. CDMFP and CDMRP primers are carried out the DNA sequencing reaction. Forward and reverse PCR amplicon was used in BDT v3.1 Cycle (sequencing kit on ABI 3730xl)on Genetic analyzer for eliminating contaminants.

Aligner software used for generating Consensus sequence of 18S rRNA from forward and reverse sequence data. The 18S rRNA gene sequence was used to carry out BLAST with the 'nr' database of NCBI GenBank database. Ten sequences are selected based on the Maximum identity score and multiple alignment software program Clustal W was used for alignment. Ribosomal Database Project was used for generating distance matrix. Molecular Evolutionary Genetics Analysis 6 software was used for constructing the phylogenetic tree.

RESULTS

Morphological Characters

Plant is 30 cm long, 620 µm wide and 6-9 whorls branch lets,incurved, terminal cell with 3 projections.

Collection number: Talakona surrounding areas.

Distribution: First report from Andhra Pradesh[6]

Molecular Phylogenetic

*Chara*species shows 91.16% homology to the *Charafoeitda.* So, *Chara* sample was identified as *Chara foeitda*

Fig. 1. *Chara foeitda*

Table 1. Sequences producing significant alignment

Description	Maxscore	Totalscore	Querycover	Evalue	Ident	Accession
C.foetida	968	968	99%	0	91.16%	X70704.1
Chara andina	965	965	99%	0	91.02%	AF032724.1
Chara globularis voucher NY:02282230	963	963	99%	0	91.02%	KR080214.1
Chara braunii voucher NY:02282229	963	963	99%	0	91.02%	KR080213.1
Chara intermedia , isolate TK31	963	963	99%	0	91.02%	HF913651.2
Chara globularis , isolate GJ29	963	963	99%	0	91.02%	HF913643.2
Chara aspera , isolate BR02	963	963	99%	0	91.02%	HF913640.2
Chara australis strain X-067	963	963	99%	0	91.02%	AY823707.1
Chara globularis	963	963	99%	0	91.02%	Y16465.1
Chara polyacantha	963	963	99%	0	91.02%	AF032742.1

Maximum Likelihood method (Tamura-Neimodel) are used for the evolutionary history[16,17] CH9 sample tree shows highest log likelihood at (-1309.77). 11 nucleotide sequences are involvedin analysis and Codon positions included were 1st+2nd+3rd+Noncoding.In *Chara foetida* total of 737 positions in the final data set. Evolutionary analyses were conducted in MEGA 6.

DISCUSSION

The taxonomical studies based on the morphology sometimes may leads to the

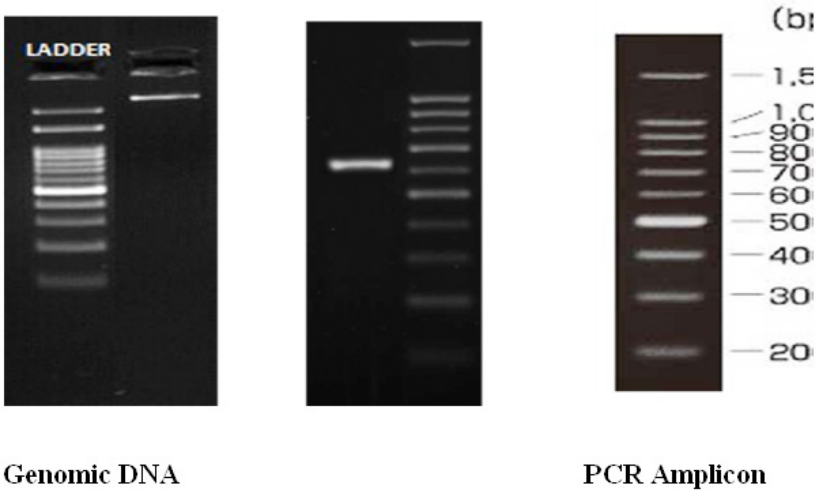

Fig. 2. Genomic DNA and Amplicon QC data

CDMRP Primer

CH9_CDMRP-D04 - Notepad
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>CH9_CDMRP-D04.ab1
AATTGCAATGCTCTATCCCCGTCACGATGAACTTTGAAAAGGTTACCCGAGCCTTTCGACCCAGGCAAGCAACTCGTTGG
ATTCATCAGTGTAGCGCGCGTGCGGCCCAGAACATCTGAGGGCATCACAGACCTGTTATTGCCTCAAACTTCCGTCGGCT
AGTCGTCCAACACCCCCTCAAAGAAGCTGCGCACACGACGGAAAGGTCCCCGGGTATCTATTTAGCAGGCTGAGGTCTCG
TTCGTTAACGGAATTAACCAGACAAATCACTCCACCAACTAAGAACGGGCATGCACCACCACCCATAGCAATCAAGAAAG
ATCTCTCAATCTGTCAATCCTTGGTATGTCTGGACCTGGTGAGTTTCCCCGTGTTGAGTCAAATTCGCCGCGCGCTCCAC
GGGTGGGGGTGCCCTTCAGTCAATTCCTTTAAGTTTCAGCCGTGCGACCATACTCCCCCCGGAACCCGTAAACTTTTATT
TCTGCACGGGGTGCTGGCAGAGTCATCCAATAGACATCCGTCCAATCTCTAGTCGGCATCGTTGTATGGTAGACACTAGG
ACGGTATCTGATCGTCTTCGATCCCCCATCTTTCTTTCTAGATTAAAAGAGAAACGTCCTTGGCAAGTGCTCTCACAGA
AGTTTCTCTCATCCATAAATCCAATATTTCCCCCCTAAAAAAAAAAA
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CDMFP Primer

CH9_CDMFP-D03 - Notepad
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```
>CH9_CDMFP-D03.ab1
TCAAGAACGAAAGTTGGGGGATCGAAGACGATCAGATACCGTCCTAGTCTCAACCATAAACGATGCCGACTAGGGATTGG
CGGATGTCTATTGGATGACTCTGCCAGCACCTTGTGAGAAATCAAAGTTTACGGGTTCCGGGGGGAGTATGGTCGCAAGG
CTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTC
ACCAGGTCCAGACATAGCAAGGATTGACAGATTGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTA
GTGGGTGAAGTGATATGTCTGGTTAATTCCGTTAACGAACGAGACCTCACCCTCCTAAATAGCTACCCGGCGATCTTTCG
TCGTGGCCATCTTCTTAGAAGGACTGTTGGACAACTAGCCAACGTAAGTTTGAGGCACTAGCAGGTCTGTGATGCCCTTA
TATGTGCTGGGCCGCACGCGCGCTACTACTGATGAATCCAACTGAGTTTGTCTTGCCGTGGGGTCGAGAAGGCCTCGGAG
TAATCCTTGTTCAAGGTCTCATCGTGGACGAGGGATAGAATTGTTTGTAATTTATTCGCTCTTGAACGAATGAATTGCCT
AAGTAAGGCAGTGAGTCAATCAGGCTTGCGC
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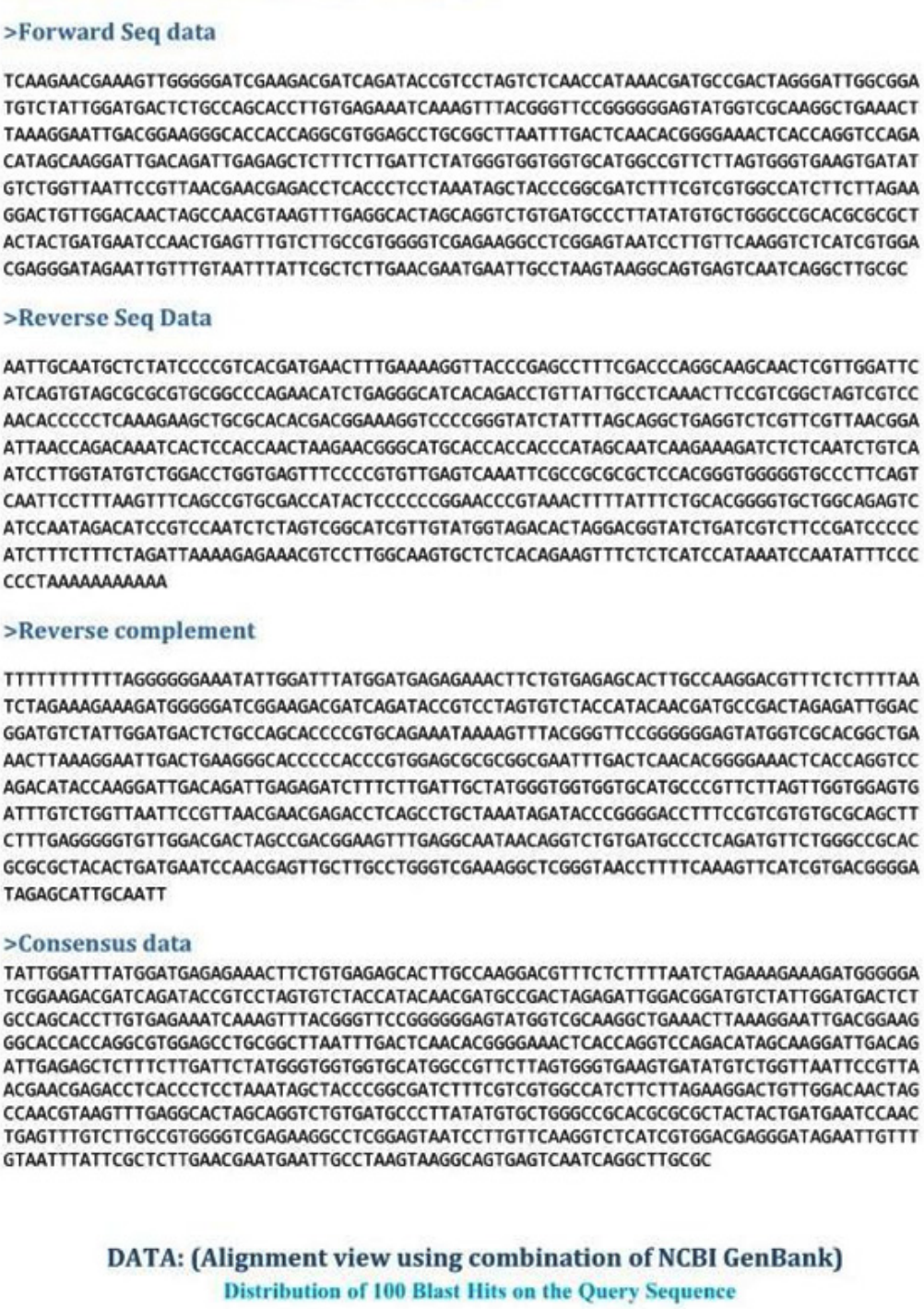

>Forward Seq data

TCAAGAACGAAAGTTGGGGGATCGAAGACGATCAGATACCGTCCTAGTCTCAACCATAAACGATGCCGACTAGGGATTGGCGGA
TGTCTATTGGATGACTCTGCCAGCACCTTGTGAGAAATCAAAGTTTACGGGTTCCGGGGGGGAGTATGGTCGCAAGGCTGAAACT
TAAAGGAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGA
CATAGCAAGGATTGACAGATTGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTGGGTGAAGTGATAT
GTCTGGTTAATTCCGTTAACGAACGAGACCTCACCCTCCTAAATAGCTACCCGGCGATCTTTCGTCGTGGCCATCTTCTTAGAA
GGACTGTTGGACAACTAGCCAACGTAAGTTTGAGGCACTAGCAGGTCTGTGATGCCCTTATATGTGCTGGGCCGCACGCGCGCT
ACTACTGATGAATCCAACTGAGTTTGTCTTGCCGTGGGGTCGAGAAGGCCTCGGAGTAATCCTTGTTCAAGGTCTCATCGTGGA
CGAGGGATAGAATTGTTTGTAATTTATTCGCTCTTGAACGAATGAATTGCCTAAGTAAGGCAGTGAGTCAATCAGGCTTGCGC

>Reverse Seq Data

AATTGCAATGCTCTATCCCCGTCACGATGAACTTTGAAAAGGTTACCCGAGCCTTTCGACCCAGGCAAGCAACTCGTTGGATTC
ATCAGTGTAGCGCGCGTGCGGCCCAGAACATCTGAGGGCATCACAGACCTGTTATTGCCTCAAACTTCCGTCGGCTAGTCGTCC
AACACCCCCTCAAAGAAGCTGCGCACACGACGGAAAGGTCCCCGGGTATCTATTTAGCAGGCTGAGGTCTCGTTCGTTAACGGA
ATTAACCAGACAAATCACTCCACCAACTAAGAACGGGCATGCACCACCACCCATAGCAATCAAGAAAGATCTCTCAATCTGTCA
ATCCTTGGTATGTCTGGACCTGGTGAGTTTCCCCGTGTTGAGTCAAATTCGCCGCGCGCTCCACGGGTGGGGGTGCCCTTCAGT
CAATTCCTTTAAGTTTCAGCCGTGCGACCATACTCCCCCCGGAACCCGTAAACTTTTATTTCTGCACGGGGTGCTGGCAGAGTC
ATCCAATAGACATCCGTCCAATCTCTAGTCGGCATCGTTGTATGGTAGACACTAGGACGGTATCTGATCGTCTTCCGATCCCCC
ATCTTTCTTTCTAGATTAAAAGAGAAACGTCCTTGGCAAGTGCTCTCACAGAAGTTTCTCTCATCCATAAATCCAATATTTCCC
CCCTAAAAAAAAAAA

>Reverse complement

TTTTTTTTTTAGGGGGGAAATATTGGATTTATGGATGAGAGAAACTTCTGTGAGAGCACTTGCCAAGGACGTTTCTCTTTTAA
TCTAGAAAGAAAGATGGGGGATCGGAAGACGATCAGATACCGTCCTAGTGTCTACCATACAACGATGCCGACTAGAGATTGGAC
GGATGTCTATTGGATGACTCTGCCAGCACCCCGTGCAGAAATAAAAGTTTACGGGTTCCGGGGGGGAGTATGGTCGCACGGCTGA
AACTTAAAGGAATTGACTGAAGGGCACCCCCACCCGTGGAGCGCGCGGCGAATTTGACTCAACACGGGGAAACTCACCAGGTCC
AGACATACCAAGGATTGACAGATTGAGAGATCTTTCTTGATTGCTATGGGTGGTGGTGCATGCCCGTTCTTAGTTGGTGGAGTG
ATTTGTCTGGTTAATTCCGTTAACGAACGAGACCTCAGCCTGCTAAATAGATACCCGGGGACCTTTCCGTCGTGTGCGCAGCTT
CTTTGAGGGGGTGTTGGACGACTAGCCGACGGAAGTTTGAGGCAATAACAGGTCTGTGATGCCCTCAGATGTTCTGGGCCGCAC
GCGCGCTACACTGATGAATCCAACGAGTTGCTTGCCTGGGTCGAAAGGCTCGGGTAACCTTTTCAAAGTTCATCGTGACGGGGA
TAGAGCATTGCAATT

>Consensus data

TATTGGATTTATGGATGAGAGAAACTTCTGTGAGAGCACTTGCCAAGGACGTTTCTCTTTTAATCTAGAAAGAAAGATGGGGGA
TCGGAAGACGATCAGATACCGTCCTAGTGTCTACCATACAACGATGCCGACTAGAGATTGGACGGATGTCTATTGGATGACTCT
GCCAGCACCTTGTGAGAAATCAAAGTTTACGGGTTCCGGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAG
GGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACATAGCAAGGATTGACAG
ATTGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTGGGTGAAGTGATATGTCTGGTTAATTCCGTTA
ACGAACGAGACCTCACCCTCCTAAATAGCTACCCGGCGATCTTTCGTCGTGGCCATCTTCTTAGAAGGACTGTTGGACAACTAG
CCAACGTAAGTTTGAGGCACTAGCAGGTCTGTGATGCCCTTATATGTGCTGGGCCGCACGCGCGCTACTACTGATGAATCCAAC
TGAGTTTGTCTTGCCGTGGGGTCGAGAAGGCCTCGGAGTAATCCTTGTTCAAGGTCTCATCGTGGACGAGGGATAGAATTGTTT
GTAATTTATTCGCTCTTGAACGAATGAATTGCCTAAGTAAGGCAGTGAGTCAATCAGGCTTGCGC

DATA: (Alignment view using combination of NCBI GenBank)

Distribution of 100 Blast Hits on the Query Sequence

Fig. 3. Sanger Seq Chromatogram data file

Table 2. Distance matrix

	1	2	3	4	5	6	7	8	9	10
1.CH9										
2.X70704.1	0.075									
3.AF032724.1	0.075	0.003								
4. KR080214.1	0.077	0.004	0.004							
5.KR080213.1	0.080	0.007	0.004	0.009						
6.HF913651.2	0.078	0.003	0.003	0.004	0.007					
7.HF913651.2	0.077	0.004	0.004	0.000	0.009	0.0004				
8.HF913640.2	0.077	0.001	0.001	0.003	0.006	0.001	0.003			
9.AY823707.1	0.079	0.009	0.006	0.007	0.004	0.009	0.007	0.007		
10.Y16465.1	0.077	0.004	0.004	0.000	0.009	0.004	0.000	0.003	0.007	
11.AF032742.1	0.077	0.001	0.001	0.003	0.006	0.001	0.003	0.000	0.007	0.003

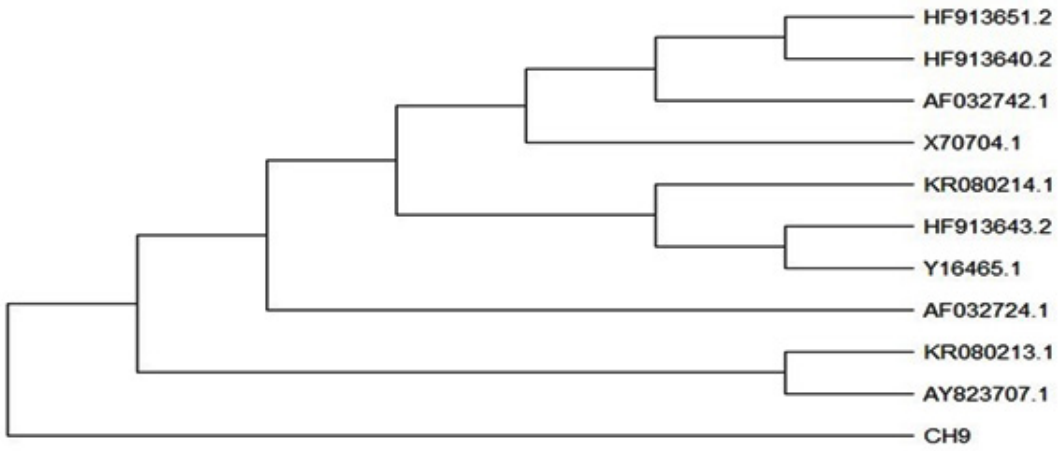

Fig. 4. Phylogenetic Tree

misidentification[11]. To overcome to this, molecular approaches are used. The 18s RNA, matK, ITS2 and rbcl genes are most widely used and effective methods in Molecular phylogenetic[13].

The class Charophyceae members are mostly benthic in nature, complex structure. The identification of these members is difficult due to the presence of phenotypic plasticity. The present study identified the macroscopic algae *Chara* with molecular phylogenetic technique. *Chara* species is identified by sequencing of 18s RNA gene analysis, namely *Chara foeitda.* The sample code CH9 shows 91.16% similarity to the *Chara foeitda*.

Monique Turmel*et al* (2006*)* studied the atpB, rbcl, 18S rRNA and mitochondria (nad5) of *Chara vulgaris* and it showed closest relation to the green plants of land[18]. Hidetoshi identified the *Chara globularis* with molecular phylogenetic based on the rbcl gene sequencing[7]. Schneider *et al* identified the 14 species of Chara with barcodes like ITS2, Mat K and rbcl genes from Europe[14]. Jacek& Michal studied the *Chara globularis* Var. tenuispina and *Chara globularis* phylogenetic relationship[8]. Based on the atpB, matK and rbcl sequences and identified C. tenuispina distinct species from *Chara globularis*[9]. The present study is one of the pioneer attempt to the molecular phylogenetic for Charophyceae of India.

CONCLUSION

Chara species are identified *as Chara foetida* based on the molecular phylogenetic. For the identification 18S rRNA sequence was used to carry out BLAST with the 'nr' database of NCBI GenBank database. First ten sequences were selected based on maximum identity score using multiple alignment software program Clustal W. Ribosomal Database Project was used for generating distance matrix and Molecular Evolutionary Genetics Analysis 6 Software was used for constructing the phylogenetic tree. Based on the maximum identifying score *Chara* species was identified as *Chara foetida.*

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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