

**COMARITIVE PHYLOGENETIC ANALYSIS OF PARASITIC MERMITHID NEMATODES
BASED ON 18S rRNA GENE SEQUENCES**

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ABSTRACT

18S rRNA gene sequences of different species of the Mermithid nematodes, downloaded from NCBI and Parasite Genome Data Base were aligned and analyzed to determine the diversity in respect to the percentage of Adenine, Thymine, Guanine and Cytosine, AT content, GC content and relative melting temperature. DNA polymorphism was studied in respect to the number of polymorphic /segregating sites, number of mutations, haplotype (gene) diversity (Hd), average number of nucleotide differences (k), nucleotide diversity (π) and Tajima's D neutrality test. Fingerprinting of the nucleotide composition, heterogeneity, identity and heterozygosity were also made. The AT content ranged from 51.85 % to 55.43 % with the mean value of 53.36% having standard deviation of 1.25 and GC content ranged from 44.57 % to 48.15 % with the mean value of 46.64 % having standard deviation of 1.25. The relative melting temperature ranged from 0.36261 to 0.37123. Haplotype (gene) diversity (Hd) was 1.000. Average number of nucleotide differences (k) and nucleotide diversity (π) was 53.23 and 0.078 respectively. The value of Tajima's D was 0.30361.

KEYWORDS: 18S rRNA gene sequences, AT content (%), DNA polymorphism, fingerprinting, GC content (%) and relative melting temperature, Mermithid nematodes,

INTRODUCTION

Mermithidae Nematodes (Enoplea: Mermithida) parasitize terrestrial and aquatic invertebrates, including many insect disease vector species such as mosquitoes, Grass-hoppers and black flies during their aquatic larval stage (Poinar, 1979; Petersen, 1985). Mermithid nematodes are roundworms, which are free-living as adults and parasitic in their developing stages. Mermithid-parasite of mosquito larvae was first reported in 1927 in *Anopheles* and *Culex* larvae of Sunderban Delta region of West Bengal, India (Gajanana *et al.* 1978; Iyengar, 1927). Among the Mermithids, seven genera are identified as parasites of mosquitoes; these are *Culicimermis*, *Empidomermis*, *Hydromermis*, *Octomyomermis*, *Perutilimermis*, *Romanomermis*, and *Strelkovimermis* by Poinar (2001). Aquatic mermithids infecting mosquitoes have similar life cycles. The pre-parasitic form when invades a first or second instar mosquito larvae, parasitism is highly successful (Kobylinski *et al.*, 2012). The nematode larvae grow and develop in the haemocoel of the mosquito host and emerge from the larval or adult stage as post-parasitic forms causing the death of the host (Chandras and Rajagopalan 1979). Lethal effect of Mermithids upon mosquito vectors has opened a new prospect of using them as potent biological control agents. Mermithids are easy to apply, environmentally safe, host specific, and potential for long-term recycling in the environment, that make them attractive for mosquitocidal agents (Platzer, 2007). A very scanty literature is available regarding the phylogenetic status and molecular characterization of different Mermithid species. The present work is an attempt to study the phylogenetic analysis of different Mermithid nematodes based on the 18S rDNA gene sequences.

MATERIALS AND METHODS

18S rRNA gene sequences of different species of Mermithidae nematodes were downloaded from NCBI and Parasite Genome Data Base. The sequence data were aligned using the 'ClustalW Submission Form' (<http://www.ebi.ac.uk/clustalw/>) and analyzed by ClustalW (Thompson *et al.*, 1994). Sequences were analyzed to determine the diversity in respect to the percentage of Adenine, Thymine, Guanine and Cytosine, AT content, GC content, and relative melting temperature. Fingerprinting of the nucleotide composition, heterogeneity, identity and heterozygosity were done following Lou and Golding (2007). Relative melting temperature based on nearest-neighbour thermodynamics (Santalucia, 1998) by $T_m = \text{SumDeltaH} / \text{SumDeltaS}$, where SumDeltaH and SumDeltaS are the summation of the dinucleotide enthalpy and entropy, respectively. T_m is relative because we do not have any

information on the concentration of the hybridizing strand. DNA polymorphism was studied in respect to the number of polymorphic /segregating sites, number of mutations, haplotype (gene) diversity (Hd), average number of nucleotide differences (k), nucleotide diversity (π) and Tajima's D neutrality test (Tajima, 1989). Evolutionary distances were calculated using the method of Jukes and Cantor (Jukes and Cantor, 1969) and a Phylogenetic tree was prepared by 'neighbor-joining' method (Saitou and Nei, 1987; Tamura *et al.*, 2007).

RESULTS AND DISCUSSION

Different species of Mermithid nematodes and their GenBank accession numbers of the 18S rRNA gene sequences have been shown in Table 1.

Table 1: GenBank-accession numbers and nucleotide base composition of 18S rRNA gene sequences of different Mermithid nematodes

Name of the species with	GenBank-accession numbers	Adenine (Mol %)	Thymine (Mol %)	Guanine (Mol %)	Cytosine (Mol %)
Mermithid sp.JH 2004	(AY284743)	465	454	419	320
Mermithid sp. JLR 2009	(FJ516757)	182	183	179	140
Mermithidae sp.KCK-2013	(KC2433121)	198	205	196	157
Mermithidae sp.TB-2009	(FJ605514)	207	204	207	163
Mermithidae sp. B-AV 2003	(AY374416)	212	201	210	165
Mermithidae sp.A-AV-2003	(AY374415)	226	220	206	166
Mermithidae sp. C-AV-2003	(AY374417)	210	211	219	172

Table 2 : Length of DNA (bp), Molecular weight(Da), AT content (%), GC content (%) and relative melting temperature of 18S rRNA gene sequence of Different Mermithid nematodes

Species	Length of DNA (bp)	Molecular weight (Da)	AT Content (%)	GC Content(%)	Relative Tm(%)
Mermithid sp.JH 2004(AY284743)	1658	1006405.00	55.43	44.57	0.37123
Mermithid sp. JLR-2009(FJ516757)	684	415449.00	53.36	46.64	0.36261
Mermithidae sp.KCK-2013(KC2433121)	756	459183.00	53.31	46.69	0.36332
Mermithidae sp.TB-2009(FJ605514)	781	474457.00	52.62	47.38	0.36369
Mermithidae sp. B-AV-2003 (AY374416)	788	478739.00	52.41	47.59	0.36383
Mermithidae sp.A-AV-2003(AY374415)	818	496670.00	54.52	45.48	0.36366
Mermithidae sp. C-AV-2003 (AY374417)	812	493395.00	51.85	48.15	0.36415

Nucleotide base composition (Mole %) of the sequences of different species has been depicted in Table 1 and Fig 1. Lengths of DNA, molecular weight, AT content (%), GC content (%) and relative melting temperature of 18S rRNA gene sequences have been shown in Table 2. Pair wise comparisons for 18S rRNA gene sequences based on the number of nucleotide differences between the two sequences (N.Diff), Raw D (N.Diff divided by Sequence Length) and Jukes and Cantor's distance (JC) were shown in Table 3. Molecular weight of DNA molecules ranged from 415449.00 Da to 1006405.00 Da.

Table 3: Pairwise comparisons for 18S rRNA gene sequences of different Mermithid nematodes

Pair	N.Diff	Raw D	JC
FJ516757 vs AY374415	1016	0.6026	1.2202
FJ516757 vs AY284743	1025	0.6079	1.2479
FJ516757 vs KC2433121	1056	0.6263	1.3519
FJ516757 vs AY374416	1063	0.6305	1.3775
FJ516757 vs FJ605514	1059	0.6281	1.3627
FJ516757 vs AY374417	1088	0.6453	1.4768
AY374415 vs AY284743	918	0.5445	0.9709
AY374415 vs KC2433121	988	0.5860	1.1402
AY374415 vs AY374416	961	0.5700	1.0703
AY374415 vs FJ605514	975	0.5783	1.1057
AY374415 vs AY374417	968	0.5741	1.0878
AY284743 vs KC2433121	984	0.5836	1.1294
AY284743 vs AY374416	964	0.5718	1.0777
AY284743 vs FJ605514	960	0.5694	1.0678
AY284743 vs AY374417	977	0.5795	1.1109
KC2433121 vs AY374416	991	0.5878	1.1483
KC2433121 vs FJ605514	995	0.5902	1.1594
KC2433121 vs AY374417	1019	0.6044	1.2294
AY374416 vs FJ605514	983	0.5830	1.1267
AY374416 vs AY374417	1002	0.5943	1.1791
FJ605514 vs AY374417	989	0.5866	1.1429

N.Diff : the number of nucleotide differences between the two sequences.

Raw D :N.Diff divided by Sequence Length and JC :Jukes and Cantor's distance

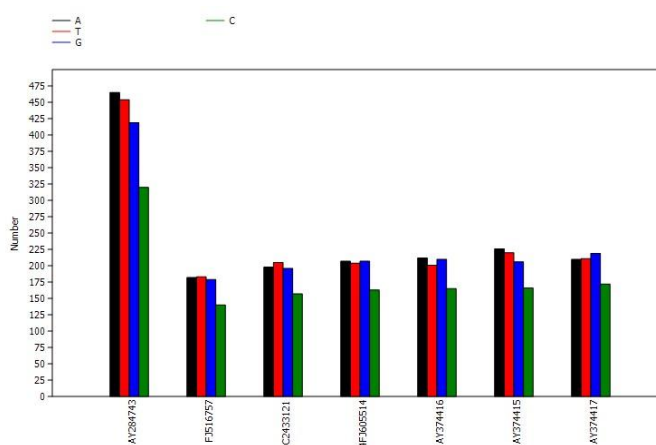


Figure 1. Bar diagram of the number of Adenine(A), Thymine(T), Guanine(G) and Cytosine(C) in 18S rRNA gene sequences of different Mermithid nematodes.

The AT content ranged from 51.85 % to 55.43 % with the mean value of 53.36% having standard deviation of 1.25 and GC content ranged from 44.57 % to 48.15 % with the mean value of 46.64 % having standard deviation of 1.25. The relative melting temperature ranged from 0.36261 to 0.37123.

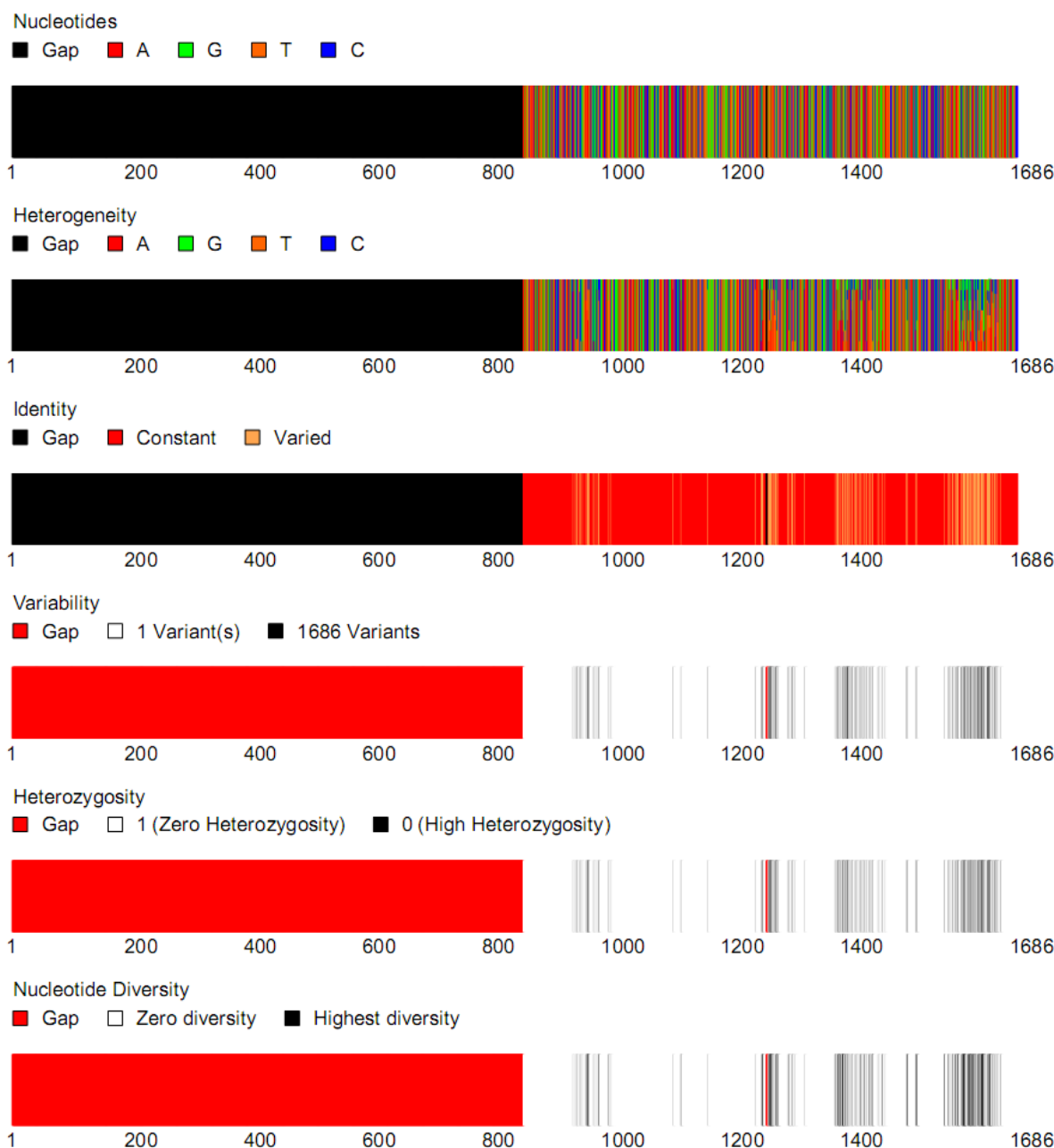


Fig 2. Fingerprint of nucleotide composition, heterogeneity, variability, heterozygosity and nucleotide diversity of 18S rRNA gene sequences of different Mermithid nematodes.

The fingerprint of nucleotide composition, heterogeneity, identity and heterozygosity of aligned 18s rRNA gene sequences of Mermithid nematodes have been shown in Fig 2. It has been observed that the number of polymorphic /segregating sites(S) was 124. Total number of mutations (Eta) was 145. Haplotype (gene) diversity (Hd) was found to be 1.000. Variance and standard deviation of haplotype diversity was 0.005 and 0.076 respectively. Average number of nucleotide differences (k) and nucleotide diversity (π) have been calculated to be 53.23 and 0.078 respectively. Theta (per site) from Eta was 0.08768. Theta (per site) from S(Theta-W) was 0.074.

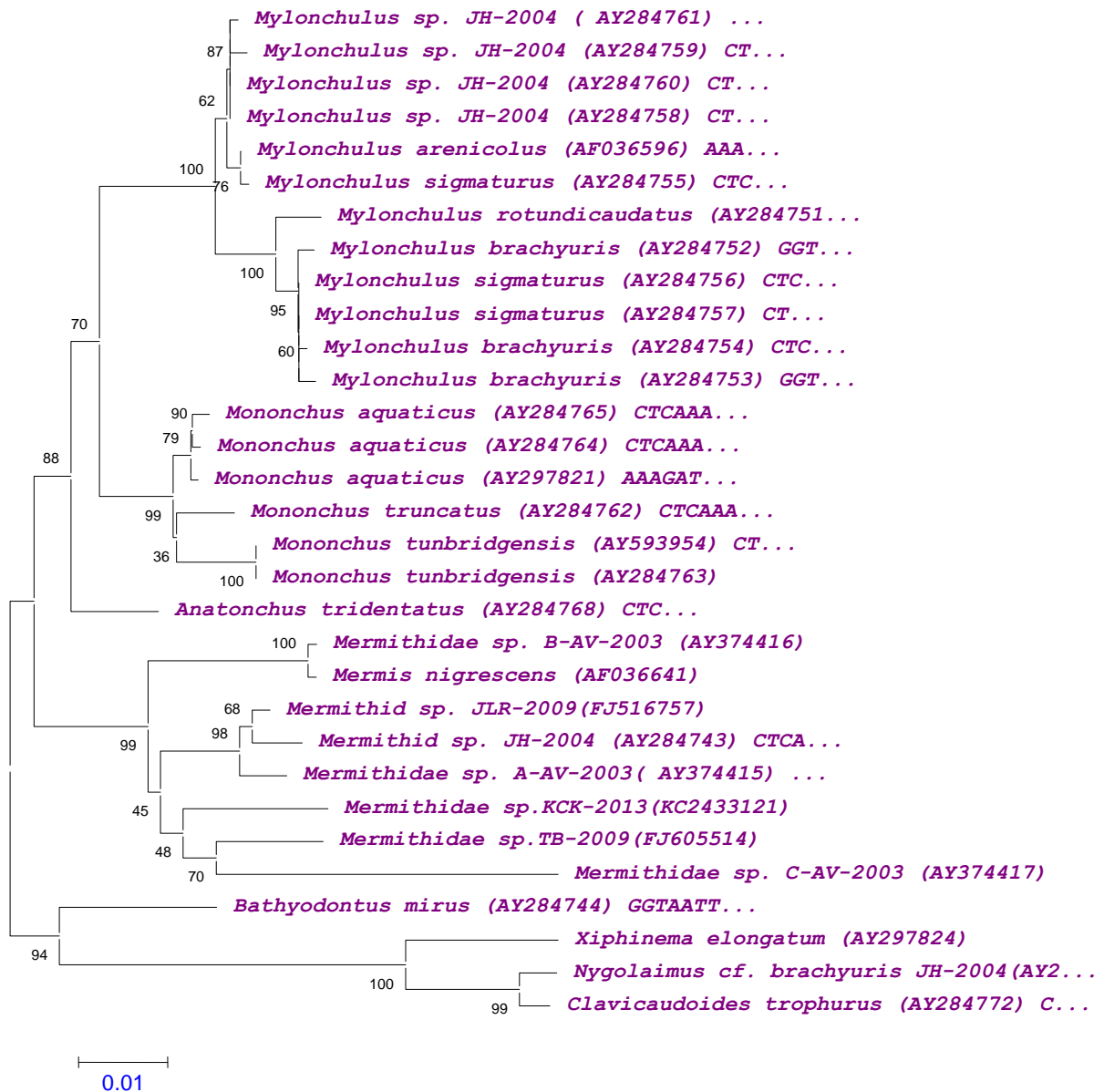


Fig.3. Neighbour-joining tree based on the 18S rRNA gene sequences of different Mermithid nematodes.

The value of Tajima's D has been found to be 0.30361. Four species of Mermithid nematode parasites of black flies from Northeast America were distinguished using discriminatory PCR of COI and 18S rDNA (St-Onge *et al.* 2008). In a study on phylogenetic analysis based on partial sequence of the 18S ribosomal DNA barcode by Crainey *et al.* (2009), it has been showed that Mermithid nematode *Isomermis lairdi* was grouped in a monophyletic group with *Gastromermis viridis* Welch and *Isomermis wisconsinensis* Welch. Phylogenetic tree based on the aligned 18S rRNA gene sequences of different species of the Mermithidae nematodes revealed that the cluster containing *Mermithidae* sp. (AY374416) and *Mermis nigrescens* branched with the cluster containing other Mermithid species with 99% bootstrap value. The cluster containing *Mermithid* sp. JLR-2009(FJ516757), *Mermithid* sp. JH-2004 (AY284743) and *Mermithidae* sp. A-AV-2003 (AY374415) branched with the cluster containing *Mermithidae* sp.KCK-2013(KC2433121), *Mermithidae* sp.TB-2009(FJ605514) and *Mermithidae* sp. C-AV-2003 (AY374417) with 45%

bootstrap value. The cluster containing *Mermithid* sp. JLR-2009(FJ516757) and *Mermithid* sp. JH-2004 (AY284743) branched with *Mermithidae* sp. A-AV-2003 (AY374415) with 98% bootstrap value. *Mermithidae* sp. TB-2009(FJ605514) branched with *Mermithidae* sp. C-AV-2003 (AY374417) with 70 % bootstrap value (Fig 3). It is well known that parasitism is species-specific and a particular species of Mermithid nematodes having specific molecular features will be able to act as a biocontrol agent on a particular mosquito species.

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