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Structure Modeling of Small Subunit Ribosomal RNA (SSU rRNA) of Copper Resistant Ciliates

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Abstract.- The most suitable secondary structures of SSU rRNA of *Euplotes adiculatus lahorensis* and two new subspecies *Tetrahymena tropicalis lahorensis* subsp. nov. and *Euplotes muscicola* SBSrc subsp. nov. are being proposed based on minimum free energy. These ciliates were highly resistant to copper with minimum inhibitory concentration of 50 ppm. *T. t. lahorensis* SSU rRNA structure with 564 base pairs consists of 117 stems, while the free energy of the structure is -380.71 kcal at 37 ºC. The SS rRNA structure of *E. muscicola* SBSrc has 126 stems and 606 base pairs with -396.17 kcal free energy, whereas *E. adiculatus lahorensis* has 121 stems, 609 base pairs and the free energy of its structure is -425.23 kcal at 37 ºC. There are number of salient features located in all the three rRNA secondary structures. Most important are the sequence complementary to ribosome binding site (RBS) of mRNA and the variable regions (V1–V9). Alignments of these regions indicate noticeable variation in sequence, which may be considered as basis for classification of ciliates.

Keywords: *Euplotes* sp., Free energy minimization, Secondary structure of RNA , SSU rRNA, *Tetrahymena* sp.

INTRODUCTION

The molecular techniques based on prokaryotic small subunit ribosomal RNA genes (SSU rDNA) have significantly improved our understanding of prokaryotic ecology and evolution. These methods are powerful tools for identification as they do not require any culturing of microorganisms in the laboratory. The slow-evolving SSU rRNA is often the best marker for molecular phylogenetic analysis as it is ubiquitous with multiple copies, highly conserved sequence, and has exactly the same function in all cells. Amplification and sequencing of SSU rDNA has been extensively used to determine microbial diversity in diverse habitats (Hugenholtz *et al.*, 1998; Roesch *et al.*, 2007; Brown *et al.*, 2009). If the genome sizes for the organisms and SSU rDNA copy number for the specific taxon in the sample is known, the relative abundance of the specific taxon or group in the complex environmental sample can be determined (Fogel *et al.*, 1999).

The biochemical activity of structural RNA molecule in most fundamental cellular functions is determined by its three-dimensional (tertiary) structure. Through the comparison of various rRNA sequences it is possible to make predictions about the structure of the RNA. There are number of features of rRNA secondary structure which are distinctly different between eukaryotes and prokaryotes (Brimacombe, 1984; Chan *et al.*, 1984; Leffers and Garrett, 1984). These include both deletions and insertions of blocks of sequence. Most of the insertions involve the extension in eukaryotes of stem-and-loop structures which exist in prokaryotic SSU rRNAs (Chan *et al.*, 1984).

Generally two methods are employed; free energy minimization and comparative analysis, for the prediction of RNA secondary and tertiary structure from its primary structure. The most popular method of free energy minimization (Zuker, 1989) is based on energy parameters determined according to the nearest-neighbor model (Lu *et al.*,

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2006) with the assumption that the molecule folds into the lowest energy state. The stability of each base pair depends upon identity of its nearest neighbor. The free energy of various predicted structures is normally calculated with programming approach. In contrast, the comparative analysis method assumes conservation of secondary and tertiary structure during evolution and recommends same structure for homologous RNA sequences (Gutell *et al.,* 1994). Andronescu *et al.* (2007) combined the optical melting experimental data with database of RNA sequences with known secondary structures to get an alternative set of free energy change parameter values. Being adjacent in the genome, the large-subunit (LSU) and SSU rRNA genes could be easily amplified by PCR even from environmental DNA samples. This approach has already been used for marine planktonic bacteria (Suzuki *et al.,* 2001) and to study eukaryotic diversity in environmental samples (Marande *et al.,* 2009).

The stability of a secondary structure is quantified as the amount of free energy released or used by forming base pairs. The more negative the free energy of a structure, the more likely is formation of that structure, because more stored energy is released. In the present study, free energy minimization approach was used to predict the secondary structure of SSU rRNAs from the three copper resistant ciliates. The variable regions were located in the structures and non-conserved residues were marked on the basis of sequence alignments.

MATERIALS AND METHODS

Genes and their source

The sequences of SSU rRNA genes were retrieved from GenBank. These were from the three ciliates previously isolated from industrial effluent containing heavy metals (Chaudhry and Shakoori, 2010, 2011a,b, 2012), *Euplotes adiculatus lahorensis* (EU103618, 1875 bp), *Tetrahymena tropicalis lahorensis* subsp. nov. (EF428128, 1752 bp), and *Euplotes muscicola* SBSrc subsp. nov. (DQ917684, 1818 bp).

Secondary structure modeling

The program RNA Draw V1.1b2 (Matzura and Wennborg, 1996) was used to predict the secondary structures of the three RNAs. This program constructs the RNA secondary structures according to energy parameters taken from Freier *et al.* (1986), Turner *et al*. (1988), and Jaeger *et al.* (1989). All the secondary structures folded according to the rules of free energy minimization with energy calculations conducted at 37°C.

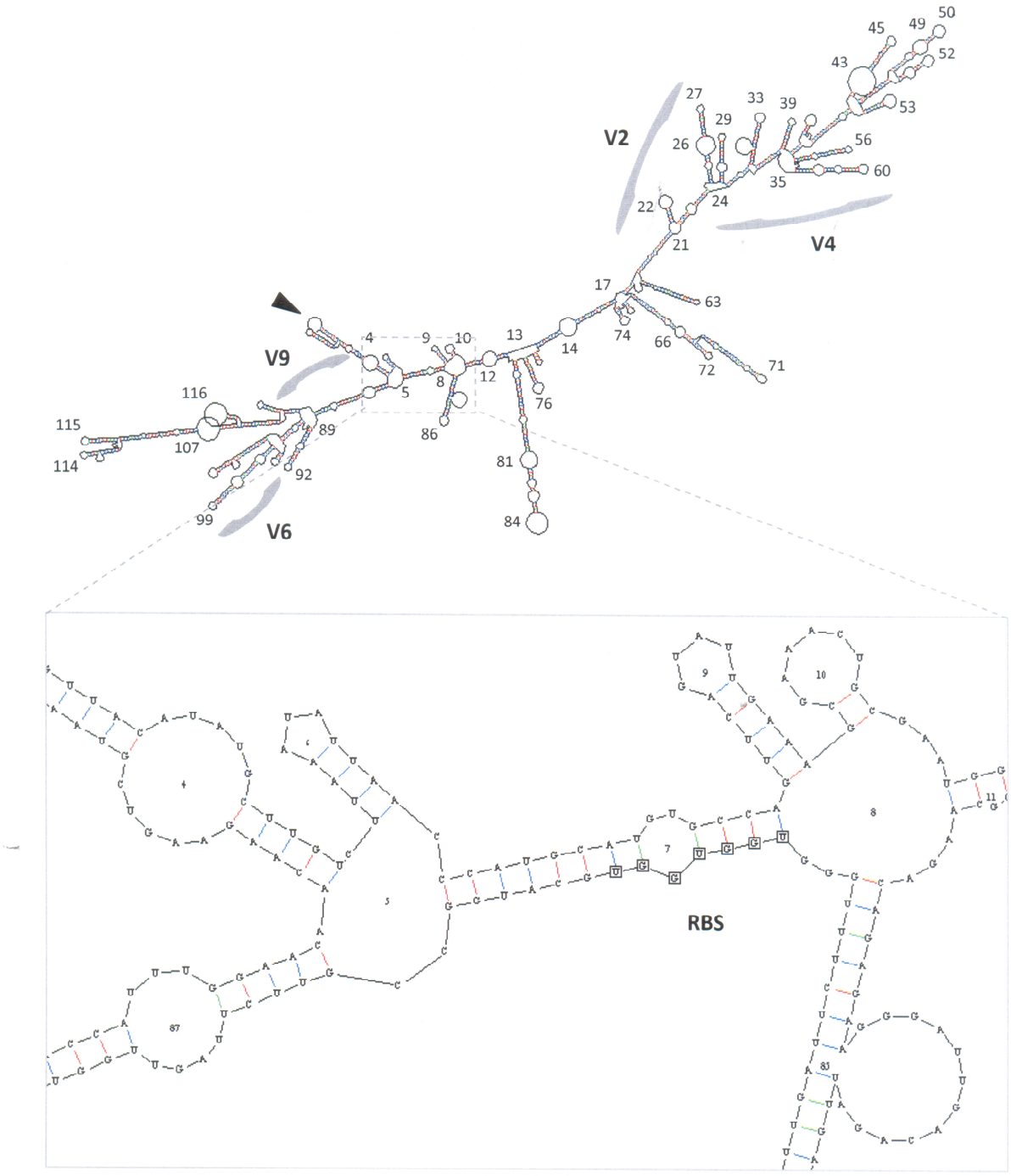
Variable regions and sequence alignment

The hyper-variable regions (V1–V9) in SSU rRNAs were located according to Lee and Gutell (2012) SSU rRNA comparative secondary structure of *T. thermophila*. Pairwise and multiple sequence alignments were made with the CLUSTAL W (Aiyar, 2000).

RESULTS AND DISCUSSION

Figures 1, 2 and 3 show the secondary structures of SSU rRNA of *T. tropicalis* subsp. *lahorensis*, *Euplotes muscicola* SBSrc and *Euplotes adiculatus* subsp. *lahorensis*, respectively. RNA is transcribed in cells as single strands of nucleic acids (ribose) where intra-strand base pairing produces specific three-dimensional structures. The RNA secondary structure provides a framework to understand the actual three-dimensional structure, which in turn, confers its biochemical function within the cell. In RNA, adenine and uracil pair (AU) by forming a double hydrogen bond, and guanine and cytosine pair (GC) by a triple hydrogen bond; additionally, G and U can form a single hydrogen bond base pair. These hydrogen bonds within the molecule lead to several recognizable "domains" of secondary structure like hairpin loops, double helices, bulges and internal loops. Among all the predicted secondary structure of SSU rRNA, the one with more negative free energy is considered more stable as more stored energy is released for its formation (Zuker, 1989). Based on this fact the most suitable secondary structures of SS rRNA of three ciliates, highly resistant to copper are being proposed.

As indicated in Figure 1, the secondary structure of *T. t. lahorensis* SSU rRNA consisted of 117 terminal and internal loops and 564 base pairs. The free energy of this structure was -380.71 kcal at 37ºC. The SS rRNA structure of *Euplotes muscicola*



Fig, 1. Secondary Structure of SSU rRNA of *T. tropicalis* subsp. *lahorensis* showing base-pairs and several recognizable domains like hairpin loops, bulges, and internal loops. The numbers show terminal and internal loops. Arrow head indicates sequence start point. The positions of variable regions V2, V4, V6, and V9 are shown by gray brackets. The region containing RBS has been enlarged.

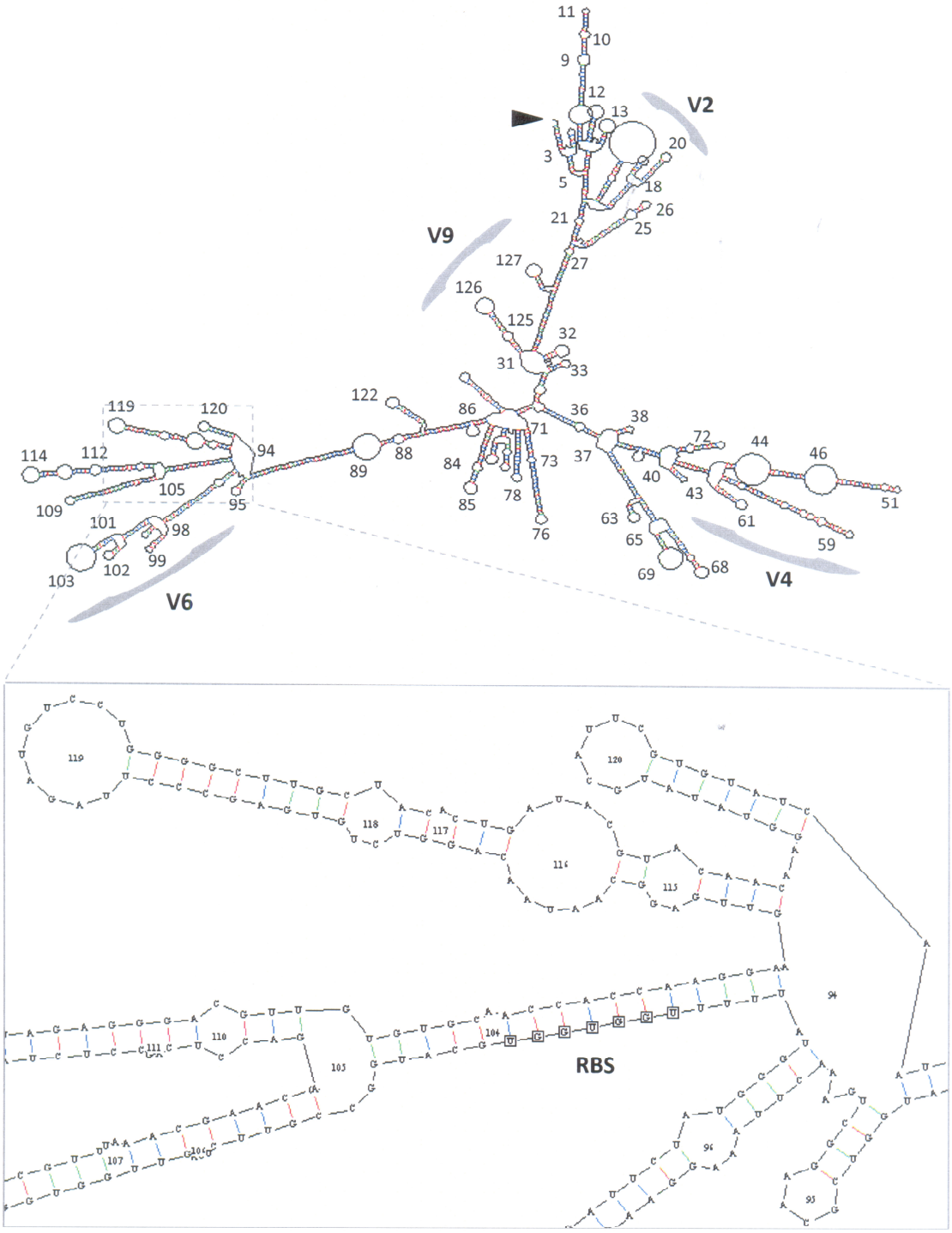


Fig. 2. Secondary Structure of SSU rRNA of *E. muscicola* SBSrc showing base-pairs and several recognizable domains like hairpin loops, bulges, and internal loops. The numbers show terminal and internal loops. Arrow head indicates sequence start point. The positions of variable regions V2, V4, V6, and V9 are shown by gray brackets. The region containing RBS has been enlarged.

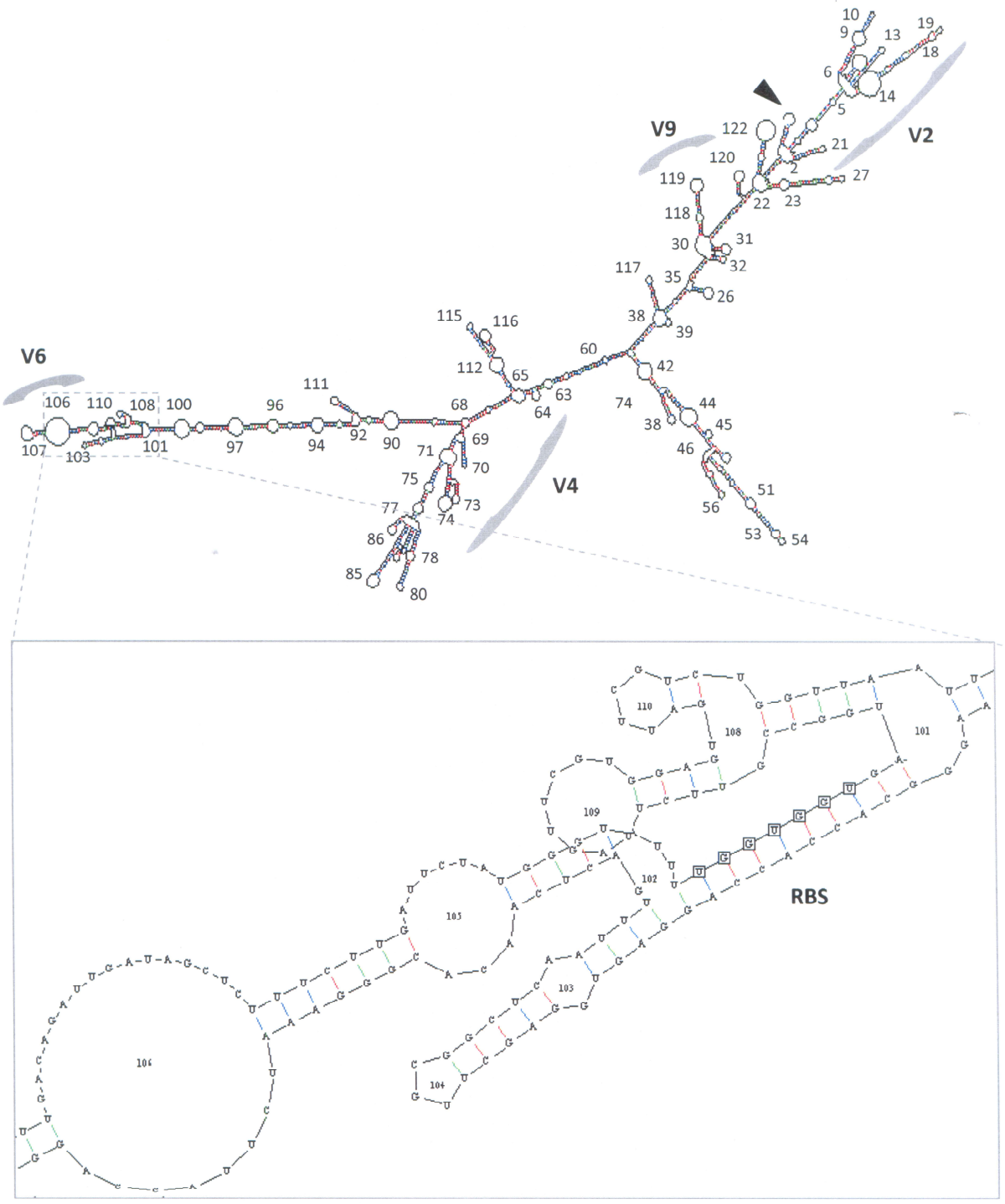


Fig. 3. Secondary Structure of SSU rRNA of *E. adiculatus* subsp. *lahorensis* showing base-pairs and several recognizable domains like hairpin loops, bulges, and internal loops. The numbers show terminal and terminal and internal loops. Arrow head indicates sequence start point. The positions of variable regions V2, V4, V6, and V9 are shown by gray brackets. The region containing RBS has been enlarged.

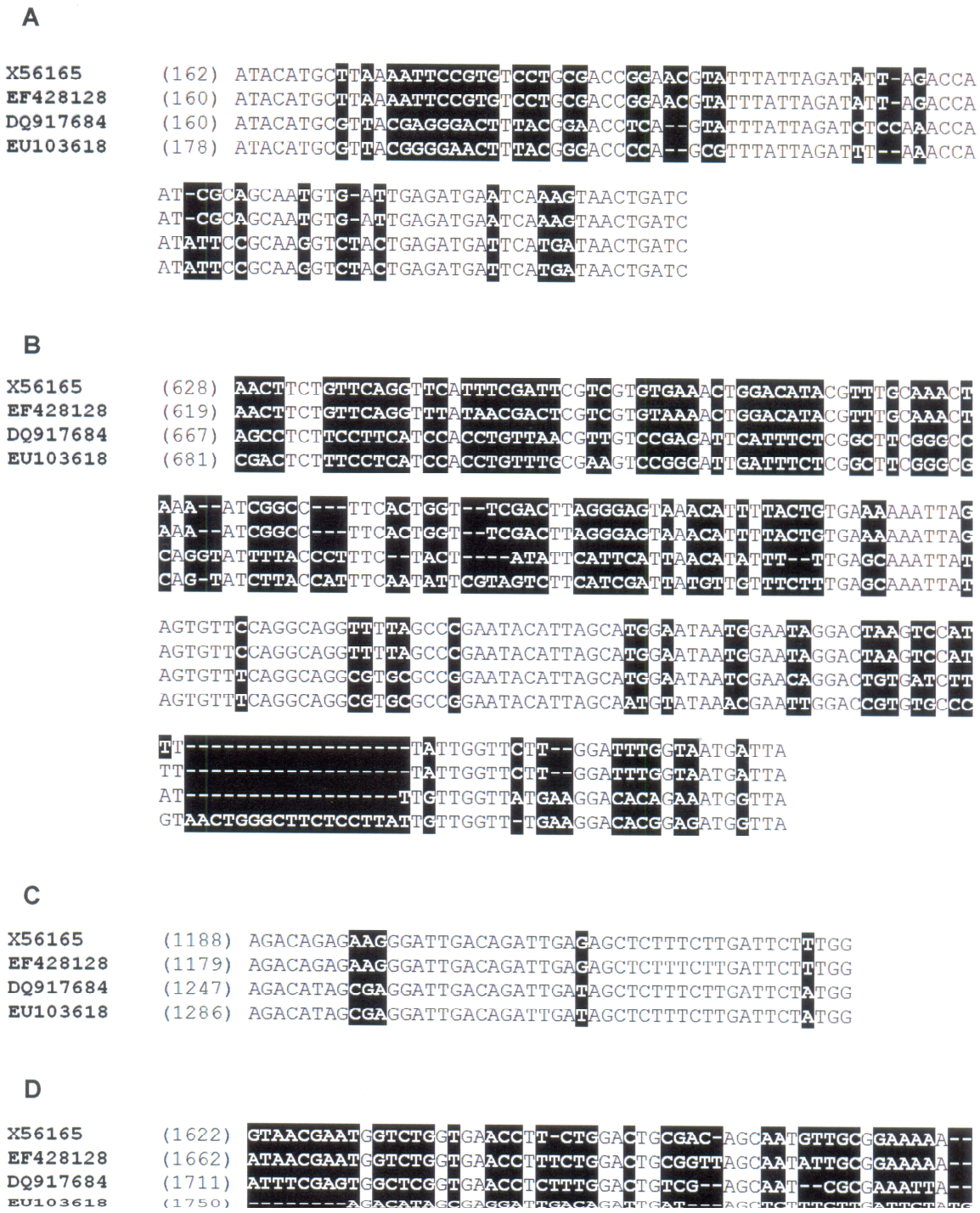


Fig. 4. Sequence alignments of various variable regions of SSU rRNAs. A, V2; B, V4; C, V6; D, V9. The non-conserved residues are highlighted in black. Numbers within parentheses indicate number of residues between protein amino terminal and aligned block. The accession numbers (from top to bottom) correspond to SSU rRNAs from the following species: *T. thermophila*, *T. tropicalis* subsp. *lahorensis*, *E. muscicola* SBSrc, *E. adiculatus* subsp. *lahorensis*.

SBSrc has 126 stems and 606 base pairs with -396.17 kcal free energy at 37ºC (Fig. 2), whereas *Euplotes adiculatus lahorensis* contains 121 stems and 609 base pairs in secondary structure of its SS rRNA (Fig. 3) and the free energy of this structure is -425.23 kcal at 37ºC.

There are number of salient features in all the three rRNA secondary structures. Most important is the sequence (UGGUGGU) complementary to ribosome binding site (RBS) of mRNA. Protein synthesis in all organisms is regulated by the structure and sequence of 5′ untranslated region (UTR) of the mRNA transcript. The RBS promotes efficient and accurate translation of mRNA and lies within the short 5′ UTR. The purine rich RBS promotes efficient and accurate translation of mRNA. Activity of a RBS can be influenced by the length and nucleotide composition of the spacer separating the RBS and the initiator AUG. In Figures 1, 2 and 3, the region containing RBS is enlarged to show its exact position within the structure.

Concurrently, being highly conserved and functionally equivalent in all known organisms, RNA sequence still contains non-conserved regions called the variable regions. Petrov *et al.* (2014) identified nine variable regions (V1–V9) in SSU rRNA of *E. coli*. In addition, Lee and Gutell (2012) described these hyper-variable regions in SSU rRNA comparative secondary structure of *T. thermophila*. Analogous to these regions, variable regions were located in secondary structures of all the three ciliates described in this report as shown in Figures 1, 2, and 3. Sequence identity with SSU rRNA of *T. thermophila* (X56165) in various variable regions is shown in the Table I. The sequence alignment of V2, V4, V6, and V9 is shown in Figure 4 (A–D), comparing these with corresponding variable regions of *T. thermophila* sequence (X56165). The V2 variable region is located near 5′ terminal of the sequence. *E. adiculatus lahorensis* contains insertion sequence (AACTGGGCTTCTCCTTAT) in the second half of V4 region (Fig. 4B). The V6 region contains just few non-conserved residues (Fig. 4C). The V9 region (Neefs *et al.*, 1990) is present near 3′ end of the sequence. Hudson and Adlard (1996) employed sequence of this region to analyze the genetic status of *Hematodinium*-like organisms. This region was found highly-variable, especially for *E. adiculatus lahorensis* (Fig. 4D).

Table I.- Sequence identity with SSU rRNA of *T. thermophila* (X56165) in various variable regions. The accession numbers (from top to bottom) correspond to SSU rRNA of *T. tropicalis* subsp. *lahorensis*, *E. muscicola* SBSrc and *E. adiculatus* subsp. *lahorensis*.

	Sequence identity (%)			
	V2	V4	V6	V9
EF428128	100	97.6	100	100
DQ917684	64.3	55.9	87.5	64.2
EU103618	64.0	51.2	87.5	30.5

In conclusion, the models presented here on the basis of free energy minimization indicate hyper-variable regions in the SSU rRNA secondary structures of the three copper-resistant ciliates. The sequence alignment revealed that V2, V4 and V9 regions contain non-conserved residues and the classification of ciliates may be further improved by taking these regions into consideration.

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