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Identification of a New Copper Resistant Protist Isolate *Tetrahymena* RT-1 subsp. nov.

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Abstract.- Ciliates are among the most divergent eukaryotic organisms and yet remained uncharacterized at the molecular level. Historically, classification of phylum protozoa was done on the basis of classical morphological criteria, which proved to be labile and less effectual. In this situation, molecular methods targeting ribosomal DNA, particularly small subunit ribosomal DNA (SS rDNA), might endow with solid basis for classification as already proved for bacteria and archaea. A new isolated ciliate, *Tetrahymena* RT-1, was found to be copper resistant and the current study presents its physical characteristics, phylogenetic analysis and comparison with other closely related species of the genus. The alignment of the sequences was done using CLUSTAL W and homologues were selected from result of BLASTP search of GenBank. *T. tropicalis* and *T. mobilis* were found to have 98.6% sequence similarity with *Tetrahymena* RT-1. By sequence alignment, basepair differences with these and other closely related species were observed specially in two regions, *viz.*, 484-756 and 1325-1672 bp. In addition to T→A, A→T and G→T transversions common to other species of the genus *Tetrahymena*, certain specific transversions *viz.*, A→C, C→A and G→C were also observed in *Tetrahymena* RT-1. Phylogenetic analysis revealed its grouping with other members of the *borealis* rather than *australis* group while SS rDNA sequence homology and comparison seems insufficient for definite identification. On the basis of sequence homology, the isolate *Tetrahymena* RT-1 is suggested to be a new subspecies of *Tetrahymena tropicalis* and named as *Tetrahymena tropicalis lahorensis* subsp. nov.

Key words: *Tetrahymena tropicalis lahorensis*, copper resistant ciliate, SS rRNA, ribotyping.

INTRODUCTION

Identification of protozoa by classical morphological criteria is tremendously tedious and requires exceptional knowledge and skills. Moreover, the soundness and validity of classical identification is "questioned" in view of the fact that many of the "species" show a substantial morphological plasticity (Dehority, 1994). The introduction of modern molecular methods based on DNA analysis and fingerprints, principally the methods targeted at ribosomal RNA operon present precise and accurate insight into similarity studies of microorganisms. While there are numerous examples of application of molecular methods for identification of bacteria (Kampher *et al*., 2006; Lindh *et al*., 2005), there are also papers (Lynn *et al*., 2000: Regensbogenova *et al*., 2004) which deal with identification of protozoa. Ciliates are among the most divergent eukaryotic organisms/protozoa yet characterized at the molecular level.

According to Ye and Romero (2002) all species of genus *Tetrahymena* are morphologically very similar. Corliss (1970) distinguished three morphological species complexes: the *patula* complex with species that experience microstome-macrostome transformation; the *pyriformis* complex amid smaller, bacterivorous species and less somatic kinetics; and the *rostrata* complex with larger histophagous or parasitic species, the ability to form resting cysts, and more somatic kinetics. More lately, gene sequences of ribosomal DNA (rDNA) and histones have been used to decide relationships among *Tetrahymena* species. It is revealed by the phylogenies based on these sequences that there is little divergence between the *Tetrahymena* species (Preparata *et al*., 1989; Brunk *et al*., 1990; Jerome and Lynn, 1996). By phylogenetic analysis two main clusters the *australis* and the *borealis* group can be alienated. To infer a stable topology for most *Tetrahymena* species there are adequate differences between the sequences in the small subunit ribosomal DNA (SS rDNA), but usually, genetic distances for the SS rDNA among the species within those two clusters are very minute. The conclusions of Kypke *et al.* (2001) supported the actuality that parallel evolution of histophagy is present from a

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bacteriovorous ancestor within the genus *Tetrahymena*.

In the present study the SS rDNA of a new copper resistant isolate (tentatively identified as a member of the genus *Tetrahymena* on microscopic observation) *Tetrahymena* RT-1 is being compared and phylogenetic analysis is being done with other species of the genus.

MATERIALS AND METHODS

Culture maintenance and purification

Tetrahymena RT-1, isolated from the industrial effluents of District Kasur, was found to be copper resistant. The minimum inhibitory concentration (MIC) of copper was found to be 60 mg/L (942 μM). The organism showed good growth in Bold-basal medium at pH 7.3-7.6 at 28±2 °C. For culture purification of the organism, antibiotics, i.e. ampicillin (25 μg ml^{-1}), chloramphenicol (35 μg ml^{-1}) and gentamicin (25 μg ml^{-1}) were used to prevent the bacterial growth. Culture was plated on potato dextrose agar (PDA) and no growth appeared on the fungal medium. Axenic culture of protozoa was made according to Shakoori *et al*. (2004). The growth of *Tetrahymena* was followed by counting the number of ciliates under microscope.

Growth in different media

The growth curves of *Tetrahymena* were determined in different media *i.e.* LB [2% (w/v) proteose peptone and 0.1% (w/v) Bacto yeast extract], wheat and rice grain medium (one boiled rice and wheat grain in 10 ml of distilled water) and Bold-basal salt medium [$NaNO_3$ (0.25 g l^{-1}), $CaCl_2.H_2O$ (0.025 g l^{-1}), $MgSO_4.7H_2O$ (0.075 g l^{-1}), K_2HPO_4 (0.075 g l^{-1}), KH_2PO_4 (0.175 g l^{-1}), NaCl (0.025 g l^{-1}), EDTA (0.05 g l^{-1}), KOH (0.031 g l^{-1}), $FeSO_4.7H_2O$ (0.04 g l^{-1}), H_2SO_4 (0.001 M), H_3BO_3 (0.01142 g l^{-1}), $ZnSO_4.7H_2O$ (0.00881 g l^{-1}), $MnCl_2.4H_2O$ (0.00144 g l^{-1}), MoO_3 (0.00071 g l^{-1}), $CuSO_4.5H_2O$ (0.00157 g l^{-1}) and $Co(NO_3)_2.6H_2O$ (0.00049 g l^{-1})], diluted 1:1000 with distilled water, with 5-7 wheat grains (Shakoori *et al.*, 2004). Glucose as carbon source was added as 1 g l^{-1} in Bold-basal salt medium. The pH of each medium was adjusted at 7.3-7.6 and kept at room temperature (27±2 °C) in normal day light.

Effect of copper and copper uptake ability

To study the effect of copper on growth of *Tetrahymena* RT-1 and copper uptake, treated set of culture comprised of three sterilized 500 ml flasks with 100 ml of Bold basal salt medium (pH 7.2, containing 0.1 g glucose and 20 μg/ml Cu^{2+}) was inoculated with log phase growing culture and incubated at 28±2°C. In control set of flasks no copper stress was given to the culture. Samples were taken and observed under light microscope to count the number of organisms. Each sample was centrifuged at 6471 x g (Beckman Coulter Allegra™ 25R Centrifuge) and copper concentration in supernatant was determined by Atomic Absorption Spectrophotometer (Thermo Unicam- SOLAAR) at 324.8nm wavelength using air-acetylene flame.

SS rRNA gene isolation and cloning

For amplification of SS rRNA gene of *Tetrahymena* RT-1, a single protozoan cell was picked from *in vitro* culture under the microscope, washed twice in drop of sterile water and put into 50 μl of reaction mix containing 0.04 mM of each deoxynucleoside triphosphate, 50 p mol of each primer, PCR reaction buffer (Fermentas) and 0.5 U of Taq DNA polymerase (Fermentas). Primers based on conserved regions in eukaryotic18S rRNA genes and the reaction conditions were the same as described by Regensbogenova *et al*. (2004). *E. coli* DH5α cells were transformed with the amplified SS rDNA gene cloned in pTZ57R/T. Positively screened clones were sequenced with M13 forward, M13 reverse, one forward and one reverse internal universal 18S primers (Elwood *et al.*, 1985), forward internal primer 5'GCGAGGAACAATGGGAGGGC and reverse internal primer 5'CCKCCTTCAAGATTCAYAATTTC, using automated DNA sequencer.

Phylogenetic analysis

The alignment of the sequences was done using CLUSTAL W (Thompson *et al.*, 1994). Homologues were selected from result of BLASTP search of GenBank. Nucleotide sequences of other related *Tetrahymena* species are available from GenBank under following accession numbers: *Tetrahymena australis* X56167 (Sogin *et al.*, 1986);

Tetrahymena bergeri AF364039 (Kypke *et al*., 2001); *Tetrahymena borealis* M98020 (Sogin *et al*., 1986); *Tetrahymena mobilis* AF364040 (Kypke *et al*., 2001); *Tetrahymena patula* X56174 (Sogin *et al*., 1986); *Tetrahymena pigmentosa* M26358 (Sogin *et al*., 1986); *Tetrahymena pyriformis* X56171 (Sogin *et al*., 1986); *Tetrahymena thermophila* M10932 (Spangler and Blackburn, 1985); and *Tetrahymena tropicalis* X56168 (Sogin *et al*., 1986). Since some species of *Tetrahymena* show identical SS rDNA sequences, e.g. *T. sestosa* has indistinguishable SS rDNA sequence with *T. pyriformis* and *T. rostrata* shows only one mismatch in its SS rRNA gene sequence to *T. canadensis* and *T. borealis* (Kypke *et al*., 2001), not all sequenced *Tetrahymena* species were incorporated in the phylogenetic analysis.

The two ophryoglenid species *Ichthyophthirius multifiliis* U17354 (Wright and Lynn, 1995) and *Ophryoglena cantenula* U17355 (Wright and Lynn, 1995) and the tetrahymenid species *Colpidium campylum* X56532 (Greenwood *et al*., 1991) and *Glaucoma chattoni* X56533 (Greenwood *et al.*, 1991) were selected as out-group species to test relationships within the genus *Tetrahymena* (Ye and Romero, 2002). The out-group species in sequence identity matrix just explain percentage similarity of these out-groups with other sequences devoid of affecting their mutual percentage identity.

The phylogenetic tree was constructed using MEGA 3.1 (Kumar *et al*., 2004) and genetic distances were calculated with the DNADIST program of the PHYLIP package, ver. 3.51c (Felsenstein, 1993) based on the Kimura 2-parameter model (Kimura, 1980). To construct sequence identity matrix CLUSTALX program was used and this matrix showed percentage sequence similarity among SS rRNA gene sequences of different *Tetrahymena* species. The alignment was pair-wise, calculated by using unit gap penalty of 0% and an open gap penalty of 100%. After discarding unknown bases, similarity matrix was calculated with a gap penalty of 0%. The programs FITCH (Fitch- Margoliash least squares method [Fitch and Margoliash, 1967]) and NEIGHBOR (neighbor-joining method [Saitou and Nei, 1987]) of this package were used to build distance trees. Using the same software package, bootstrap analysis was performed to test the statistical reliability and trustworthiness of the topology of the neighbor-joining tree with 1000 bootstrap re-samples of the data.

RESULTS AND DISCUSSION

Microscopic observation

Microscopic observation revealed *Tetrahymena* RT-1 to be pear-shaped having uniform body cilia and a few caudal cilia (Fig. 1). The uniform body cilia called kinetics appeared as parallel lines of dots (basal bodies) running down the length of the cell surface. Oral apparatus, located on the anterior end of the cell, was seen as a small cavity in the ventral view. Buccal cavity was clearly visible at the bottom of the ciliate RT-1.

Fig.1. Microscopic image of RT-1. On ribotyping the isolate was identified as *Tetrahymena tropicalis lahorensis.*

Growth curves

Gradual increase in the number of cells in each medium was observed while maximum growth was noted on day 2nd (Fig. 2). The number of cells increased from 0.4 x 10^5 cells/ml at the time of inoculation to 36 x 10^5 cells/ml (90 fold) in Bold-Basal salt medium, from 0.38 x 10^5 to 22 x 10^5 cells/ml (57.8 fold) in LB medium, and from 0.41 x 10^3 to 7.36 x 10^3 cells/ml (30.3 fold) in wheat and rice grain medium on 2nd day. Thus *Tetrahymena* RT-1 can be successfully grown in Bold-basal salt medium.

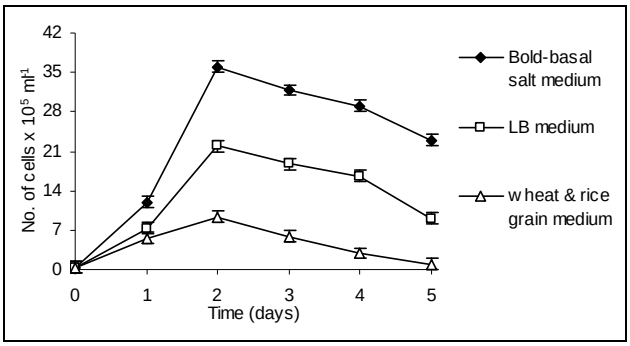

Fig. 2. Growth curve of *Tetrahymena* RT-1 in different media at pH 7.2-7.5 and 28 ± 2°C. ♦, Bold basal salt medium; □, LB medium; and Δ, wheat and rice grain medium.

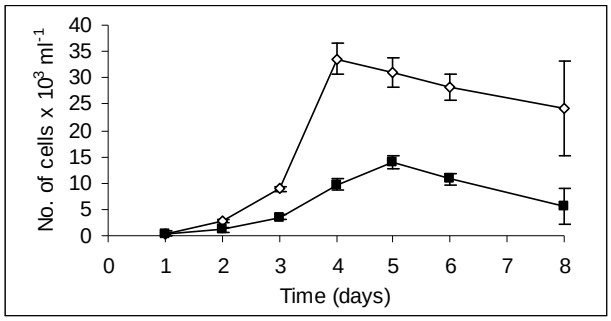

Fig. 3. Growth curve of *Tetrahymena* RT-1 in medium containing 20µg/ml Cu^{2+}. ◊ Control and ■ Treated.

The growth of *Tetrahymena* RT-1 was adversely affected in the presence of copper (20 µg/ml) (Fig. 3). There was 56% reduction in cell population of *Tetrahymena* RT-1 after eight days (Fig. 3). The ciliates could remove 68%, of copper in three and 74% in eight days (Fig. 4).

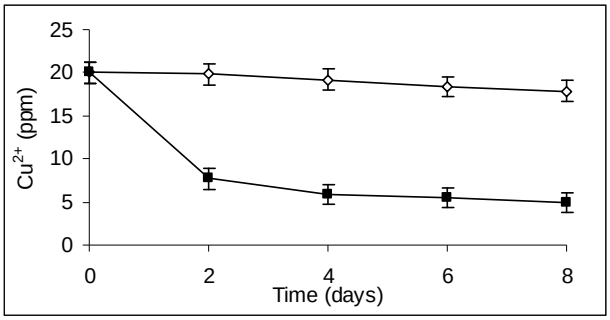

Fig. 4. Uptake of Cu^{2+} by *Tetrahymena* RT-1 growing in Cu^{2+} containing medium. The controls contain medium without cells of the isolate. ◊ Control and ■ Treated.

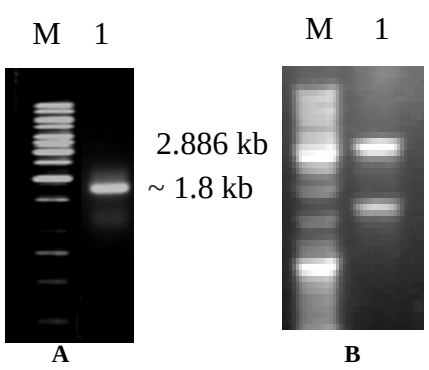

Fig. 5. A, Amplification of SS rRNA gene using primers based on conserved regions in eukaryotic SS rRNA genes. M represents marker lane and lane 1, amplified *Tetrahymana* RT-1 SS rRNA gene; B, Restriction of pTZ57R/T containing SS rRNA gene of *Tetrahymana* RT-1 with *Eco*RI and *Hind*III. M represents marker lane and in lane 1 the upper band represents the restricted vector (2.886 kb), whereas the lower band represents insert *Tetrahymana* RT-1 SS rDNA (1762 bp).

rRNA gene and phylogenetic analysis

Nearly complete SS rDNA gene (~1.8 kb, Fig. 5) was obtained after PCR using primers based on conserved regions in eukaryotic18S rRNA genes. The sequence was deposited in GenBank (accession no. EF428128). *T. tropicalis* and *T. mobilis* were found closely related to *Tetrahymena* RT-1. The homology with both species was 98%.

Nucleotide differences between species were tabulated (Table I) after the alignment of the sequences using CLUSTAL-W. More basepair differences are observed in two regions: from 484 to 756 and from 1325 to 1672. Deletion of A and T (at positions 616 and 539, respectively) while insertion of C (at position 1494) and G (at position 1515 and 1601) was observed in *Tetrahymena* RT-1. Generally only T → A, A → T and G → T transversions were observed among the genus *Tetrahymena* but certain specific transversions (A → C, C → A and G → C) were also observed when sequence of *Tetrahymena* RT-1 was compared with the sequences of other species of the genus. Transitions among A → G and G → A were observed while transversions were of A→ C (at

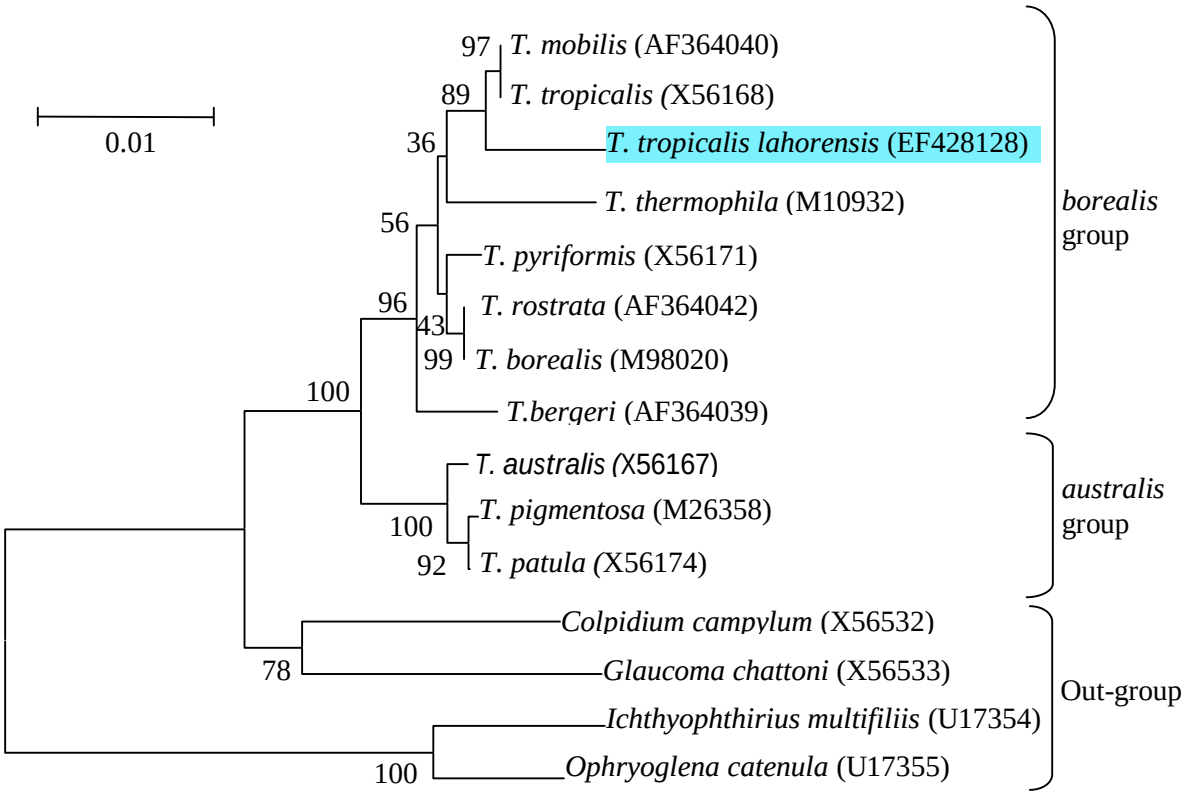

Fig. 6. A distance tree for tetrahymenid ciliates inferred from small subunit ribosomal DNA sequences. The tree was derived from evolutionary distances produced by the Kimura-2- parameter correction model (Kimura, 1980). The numbers at the nodes represent the bootstrap percentages of 1,000 for the least squares method (LS [Fitch and Margoliash, 1967]) followed by the bootstrap values for the neighbor joining method (NJ) of Saitou and Nei (1987). Evolutionary distance is represented by the branch length separating the species. The scale bar corresponds to 5 substitutions per 100 nucleotide positions.

position 4), T → A (at position 573), C → A (at position 1717), G → A (at position 935) and G → C (at position 1743). At position 511, A/C is present in other *Tetrahymena* species while it is a deletion in the case of *Tetrahymena* RT-1.

The general topologies of the trees inferred from least squares, neighbor-joining (Fig. 6); minimum evolution and maximum parsimony (data not shown) were quite similar. *Tetrahymena* RT-1 grouped with other members of the *borealis* group rather than *australis* group, when both main clusters were taken in consideration (Kypke *et al.*, 2001). In all analyses, *T. thermophila* branched basal to *Tetrahymena* RT-1 within the *borealis* group (Fig. 6). The other relationships within the *borealis* group, however, have to be regarded as unresolved. As already stated by Kypke *et al.* (2001) phylogenetic tree (Fig. 6, neighbor-joining) confirmed very small evolutionary distances within *borealis* and *australis* groups, but the distances are larger between the species of their two main clusters. Stable and comparable trees with higher bootstrap support for *australis* group and *borealis* group were computed by both least-squares and neighbor-joining distance analyses.

On the basis of sequence homology (as indicated by phylogenetic tree as well) the local isolate belonged to either *Tetrahymena tropicalis* or *Tetrahymena mobilis*. *Tetrahymena* RT-1 showed 98.6% sequence similarity with both while percentage homology between the two is 99.8%. On the basis of the results of this study *Tetrahymena* RT-1 is suggested to be a new subspecies of *Tetrahymena tropicalis* and named *Tetrahymena*

Table I.- Variation (specific and general mutations) in the nucleotide sequence of SS rDNA of *Tetrahymena tropicalis lahorensis* compared with other *Tetrahymena* species.

Sequence Position 5′ → 3′

Species	3	4	129	189	228	262	267	268	270	273	275	276	484	485	487	511	517
T. tropicalis lahorensis	T	A	G	C	C	A	A	G	C	G	T	T	C	T	_	_	T
T. tropicalis	C	C	.	.	.	.	.	.	.	.	.	.	.	.	_	A	A
T. australis	C	C	.	T	T	.	T	A	.	.	.	A	T	A	_	A	.
T. bergeri	C	C	A	T	.	.	C	A	.	.	.	G	T	.	_	A	.
T. borealis	C	C	.	.	.	.	.	A	.	.	.	.	.	.	_	A	.
T. mobilis	C	C	.	.	.	.	.	.	.	.	.	.	.	.	_	A	A
T. patula	C	C	.	T	.	.	T	A	.	.	.	A	T	A	_	A	.
T. pigmentosa	C	C	.	T	.	.	T	A	.	.	.	A	T	A	_	A	.
T. pyriformis	C	C	.	T	.	.	.	A	.	.	.	.	T	.	_	A	A
T. thermophila	C	C	.	.	.	G	.	.	T	A	C	.	T	.	C	C	A
T. rostrata	C	C	.	.	.	.	.	A	.	.	.	.	.	.	_	A	.

Sequence Position 5′ → 3′

Species	539	567	573	616	644	647	648	652	659	662	665	670	678	721	722	751	756
T. tropicalis lahorensis	_	A	T	_	T	A	A	C	T	A	A	A	T	A	A	C	_
T. tropicalis	T	G	A	A	.	.	.	.	.	.	.	.	.	.	.	.	_
T. australis	T	G	A	A	C	T	T	.	A	G	.	G	C	G	G	T	_
T. bergeri	T	G	A	A	C	.	.	.	.	G	G	.	C	G	.	.	_
T. borealis	T	G	A	A	C	T	T	.	.	G	.	.	.	.	.	.	_
T. mobilis	T	G	A	A	.	.	.	.	.	.	.	.	.	.	.	.	_
T. patula	T	G	A	A	C	T	T	.	A	G	.	G	C	G	G	T	_
T. pigmentosa	T	G	A	A	C	T	T	.	A	G	.	G	C	G	G	T	G
T. pyriformis	T	G	A	A	C	T	T	.	A	G	.	.	.	.	.	.	_
T. thermophila	T	G	A	A	C	T	T	T	.	G	.	.	.	.	.	.	_
T. rostrata	T	G	A	A	C	T	T	.	.	G	.	.	.	.	.	.	_

Sequence Position 5′ → 3′

Species	920	921	935	1033	1267	1325	1326	1330	1333	1339	1340	1370	1454	1459	1479	1497
T. tropicalis lahorensis	C	A	G	A	G	C	T	G	C	G	T	_	G	T	T	C
T. tropicalis	.	.	T	.	A	.	.	.	.	.	.	_	.	.	.	_
T. australis	.	.	T	T	A	.	C	.	.	.	C	_	.	.	.	_
T. bergeri	.	.	T	.	A	.	.	.	.	.	.	_	.	C	A	_
T. borealis	.	.	T	.	A	.	.	.	.	.	.	_	.	.	.	_
T. mobilis	.	.	T	.	A	.	.	.	.	.	.	_	.	.	.	_
T. patula	.	.	T	T	A	T	C	.	.	A	.	_	.	.	.	_
T. pigmentosa	.	.	T	T	A	T	C	.	T	A	.	_	.	.	.	_
T. pyriformis	.	.	T	.	A	.	.	.	.	.	.	_	.	.	.	_
T. thermophila	A	C	T	.	A	.	.	A	T	.	.	_	A	.	.	_
T. rostrata	.	.	T	.	A	.	.	.	.	.	.	G	.	.	.	_

Continued

Sequence Position 5′ → 3′

Species	1515	1525	1530	1542	1583	1601	1647	1655	1657	1658	1665	1666	1668	1672	1717	1743
T. tropicalis lahorensis	G	A	G	G	A	G	T	C	G	T	A	T	G	A	C	A
T. tropicalis	_	G	.	C	_	_	_	.	A	.	.	.	.	.	A	T
T. australis	_	G	T	C	_	_	_	T	A	C	G	.	A	.	A	T
T. bergeri	_	G	.	C	_	_	_	.	.	.	.	C	.	.	A	T
T. borealis	_	G	.	C	_	_	_	.	.	.	.	C	.	.	A	T
T. mobilis	_	G	.	C	_	_	_	.	A	.	.	.	.	.	A	T
T. patula	_	G	T	C	_	_	_	T	A	C	G		A	.	A	T
T. pigmentosa	_	G	T	C	_	_	_	T	A	C	G	.	A	.	A	T
T. pyriformis	_	G	.	C	_	_	_	.	.	.	.	C	.	G	A	T
T. thermophila	_	G	.	C	_	_	_	.	A	C	G	.	.	.	A	T
T. rostrata	_	G	.	C	_	_	_	.	.	.	.	C	.	.	A	T

General mutations within the genus *Tetrahymena* are highlighted.

tropicalis lahorensis subsp. nov. It can further be inferred that sequence of SS rRNA gene only is not sufficient and adequate for proper ciliate identification. The identification of animal species based on specific DNA fragment sequence (such as mitochondrial DNA) can additionally be used to discriminate such species. The method is called DNA barcoding (Hebert *et al*., 2003). Based on this approach Barth *et al*. (2006) and Lynn and Kypke (2006) confirmed that species pairs of *Paramecium* and *Tetrahymena*, identical by the SSrRNA gene sequences, can be distinguished using Cytochrome c Oxidase subunit 1 (cox1*;* Chantangsi *et al*., 2007) mitochondrial marker. This *cox1* gene has been anticipated as a DNA barcode to identify animal species.

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Table I.- **Variation (specific and general mutations) in the nucleotide sequence of SS rDNA of *Tetrahymena tropicalis lahorensis* compared with other *Tetrahymena* species.**

Sequence Position 5′ → 3′

Species	3	4	129	189	228	262	267	268	270	273	275	276	484	485	487	511	517
T. tropicalis lahorensis	T	A	G	C	C	A	A	G	C	G	T	G	T	C	T	T	.
T. tropicalis	C	C	.	.	.	.	.	.	.	.	.	G	.	C	.	.	.
T. australis	C	C	.	T	T	.	T	A	.	.	.	A	T	A	.	A	.
T. bergeri	C	C	A	T	.	.	C	A	.	.	.	G	T	.	.	A	.
T. borealis	C	C	.	.	.	.	.	A	.	.	.	.	.	.	.	A	.
T. mobilis	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A
T. patula	C	C	.	T	.	.	T	A	.	.	.	A	T	A	.	A	.
T. pigmentosa	C	C	.	T	.	.	T	A	.	.	.	A	T	A	.	A	.
T. pyriformis	C	C	.	T	.	.	.	A	.	.	.	.	T	.	.	A	A
T. thermophila	C	C	.	.	.	G	.	.	T	A	C	.	T	.	C	C	A
T. rostrata	C	C	.	.	.	.	.	A	.	.	.	.	.	.	.	A	.

Sequence Position 5′ → 3′

Species	539	567	573	616	644	647	648	652	659	662	665	670	678	721	722	751	756
T. tropicalis lahorensis	_	A	T	_	T	A	A	C	T	A	A	A	T	A	A	C	_
T. tropicalis	T	G	A	A	.	.	.	.	.	.	.	.	.	.	.	.	_
T. australis	T	G	A	A	C	T	T	.	A	G	.	G	C	G	G	T	_
T. bergeri	T	G	A	A	C	.	.	.	.	G	G	.	C	G	.	.	_
T. borealis	T	G	A	A	C	T	T	.	.	G	.	.	.	.	.	.	_
T. mobilis	T	G	A	A	.	.	.	.	.	.	.	.	.	.	.	.	_
T. patula	T	G	A	A	C	T	T	.	A	G	.	G	C	G	G	T	_
T. pigmentosa	T	G	A	A	C	T	T	.	A	G	.	G	C	G	G	T	G
T. pyriformis	T	G	A	A	C	T	T	.	A	G	.	.	.	.	.	.	_
T. thermophila	T	G	A	A	C	T	T	T	.	G	.	.	.	.	.	.	_
T. rostrata	T	G	A	A	C	T	T	.	.	G	.	.	.	.	.	.	_

Sequence Position 5′ → 3′

Species	920	921	935	1033	1267	1325	1326	1330	1333	1339	1340	1370	1454	1459	1479	1497
T. tropicalis lahorensis	C	A	G	A	G	C	T	G	C	G	T	_	G	T	T	C
T. tropicalis	.	.	T	.	A	.	.	.	.	.	.	_	.	.	.	_
T. australis	.	.	T	T	A	.	C	.	.	.	C	_	.	.	.	_
T. bergeri	.	.	T	.	A	.	.	.	.	.	.	_	.	C	A	_
T. borealis	.	.	T	.	A	.	.	.	.	.	.	_	.	.	.	_
T. mobilis	.	.	T	.	A	.	.	.	.	.	.	_	.	.	.	_
T. patula	.	.	T	T	A	T	C	.	.	A	.	_	.	.	.	_
T. pigmentosa	.	.	T	T	A	T	C	.	T	A	.	_	.	.	.	_
T. pyriformis	.	.	T	.	A	.	.	.	.	.	.	_	.	.	.	_
T. thermophila	A	C	T	.	A	.	.	A	T	.	.	_	A	.	.	_
T. rostrata	.	.	T	.	A	.	.	.	.	.	.	G	.	.	.	_

Sequence Position 5′ → 3′

Species	1515	1525	1530	1542	1583	1601	1647	1655	1657	1658	1665	1666	1668	1672	1717	1743
T. tropicalis	G	A	G	G	A	G	T	C	G	T	A	T	G	A	C	A

General mutations within the genus *Tetrahymena* are highlighted.

Table I.- Variation (specific and general mutations) in the nucleotide sequence of SS rDNA of *Tetrahymena tropicalis lahorensis* compared with other *Tetrahymena* species.

Continued…

Sequence Position 5′ → 3′

Species	3	4	129	189	228	262	267	268	270	273	275	276	484	485	487	511	517
T. tropicalis lahorensis	T	A	G	C	C	A	A	G	C	G	T	T	C	T	_	_	T
T. tropicalis	C	C	.	.	.	.	.	.	.	.	.	.	.	.	_	A	A
T. australis	C	C	.	T	T	.	T	A	.	.	.	A	T	A	_	A	.
T. bergeri	C	C	A	T	.	.	C	A	.	.	.	G	T	.	_	A	.
T. borealis	C	C	.	.	.	.	.	A	.	.	.	.	.	.	_	A	.
T. mobilis	C	C	.	.	.	.	.	.	.	.	.	.	.	.	_	A	A
T. patula	C	C	.	T	.	.	T	A	.	.	.	A	T	A	_	A	.
T. pigmentosa	C	C	.	T	.	.	T	A	.	.	.	A	T	A	_	A	.
T. pyriformis	C	C	.	T	.	.	.	A	.	.	.	.	T	.	_	A	A
T. thermophila	C	C	.	.	.	G	.	.	T	A	C	.	T	.	C	C	A
T. rostrata	C	C	.	.	.	.	.	A	.	.	.	.	.	.	_	A	.

Sequence Position 5′ → 3′

Species	539	567	573	616	644	647	648	652	659	662	665	670	678	721	722	751	756
T. tropicalis lahorensis	_	A	T	_	T	A	A	C	T	A	A	A	T	A	A	C	_
T. tropicalis	T	G	A	A	.	.	.	.	.	.	.	.	.	.	.	.	_
T. australis	T	G	A	A	C	T	T	.	A	G	.	G	C	G	G	T	_
T. bergeri	T	G	A	A	C	.	.	.	.	G	G	.	C	G	.	.	_
T. borealis	T	G	A	A	C	T	T	.	.	G	.	.	.	.	.	.	_
T. mobilis	T	G	A	A	.	.	.	.	.	.	.	.	.	.	.	.	_
T. patula	T	G	A	A	C	T	T	.	A	G	.	G	C	G	G	T	_
T. pigmentosa	T	G	A	A	C	T	T	.	A	G	.	G	C	G	G	T	G
T. pyriformis	T	G	A	A	C	T	T	.	A	G	.	.	.	.	.	.	_
T. thermophila	T	G	A	A	C	T	T	T	.	G	.	.	.	.	.	.	_
T. rostrata	T	G	A	A	C	T	T	.	.	G	.	.	.	.	.	.	_

Sequence Position 5′ → 3′

Species	920	921	935	1033	1267	1325	1326	1330	1333	1339	1340	1370	1454	1459	1479	1497
T. tropicalis lahorensis	C	A	G	A	G	C	T	G	C	G	T	_	G	T	T	C
T. tropicalis	.	.	T	.	A	.	.	.	.	.	.	_	.	.	.	_
T. australis	.	.	T	T	A	.	C	.	.	.	C	_	.	.	.	_
T. bergeri	.	.	T	.	A	.	.	.	.	.	.	_	.	C	A	_
T. borealis	.	.	T	.	A	.	.	.	.	.	.	_	.	.	.	_
T. mobilis	.	.	T	.	A	.	.	.	.	.	.	_	.	.	.	_
T. patula	.	.	T	T	A	T	C	.	.	A	.	_	.	.	.	_
T. pigmentosa	.	.	T	T	A	T	C	.	T	A	.	_	.	.	.	_
T. pyriformis	.	.	T	.	A	.	.	.	.	.	.	_	.	.	.	_
T. thermophila	A	C	T	.	A	.	.	A	T	.	.	_	A	.	.	_
T. rostrata	.	.	T	.	A	.	.	.	.	.	.	G	.	.	.	_

Sequence Position 5′ → 3′

	1515	1525	1530	1542	1583	1601	1647	1655	1657	1658	1665	1666	1668	1672	1717	1743
is	G	A	G	G	A	G	T	C	G	T	A	T	G	A	C	A
	_	G	.	C	_	_	_	.	A	.	.	.	.	.	A	T
	_	G	T	C	_	_	_	T	A	C	G	.	A	.	A	T
	_	G	.	C	_	_	_	.	.	.	.	C	.	.	A	T
	_	G	.	C	_	_	_	.	.	.	.	C	.	.	A	T
	_	G	.	C	_	_	_	.	A	.	.	.	.	.	A	T
	_	G	T	C	_	_	_	T	A	C	G		A	.	A	T
	_	G	T	C	_	_	_	T	A	C	G	.	A	.	A	T
	_	G	.	C	_	_	_	.	.	.	.	C	.	G	A	T
	_	G	.	C	_	_	_	.	A	C	G	.	.	.	A	T
	_	G	.	C	_	_	_	.	.	.	.	C	.	.	A	T

General mutations within the genus *Tetrahymena* are highlighted.