

MRNA'S SECONDARY STRUCTURE FOR HAPLOTYPES OF THE GENE WHICH DETERMINS THE CYTOCHROME'S B SYNTHESIS, FOR *CARASSIUS AURATUS GIBELIO* BLOCH. 1783 INDIVIDUALS SAMPLED FROM MOVILENI AND IASI POPULATIONS

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Abstract: In present paper, the main purpose was to establish the mRNA's secondary structure for the gene which determines the cytochrome's b synthesis, for *Carassius auratus gibelio* Bloch. 1783 individuals, having the origin in two different populations - Movileni and Iași, as well as those structures' comparison, with similar models of the same gene, taken from the GenBank, for different species and subspecies of *Carassius* genera. Thus, regions from mtDNA were sequenced for 10 individuals of each analyzed population, based on a purified DNA matrix, using a method with phenol : chloroform : izoamyl alcohol (25:24:1). Those genes were subsequently amplified and sequenced using two complementary universal primers, obtaining both complementary chains, which ulterior formed a unique strand. MRNA's secondary structure transcription and modeling was made using the GeneQuest module of LaserGene's program.

INTRODUCTION

RNA is ubiquitous in the cell and is important for many processes. The activity of RNA is determined by its structure, the way it is folded back on itself. Secondary structure modeling of RNA predicts, or otherwise determines, the pattern of Watson-Crick, wobble and other, non-canonical pairings that occur when RNA is folded.

Messenger RNA (mRNA) secondary structures can be used in part to explain translational controls in mRNA (Mac Donald, 1990, Smit and van Duin, 1990), and replication controls in single-stranded RNA viruses (Mills et. al., 1990). Although the vast majority of known mRNAs code for proteins or structural RNAs, some do not (Brannan et. al., 1990, Brown et al., 1991); and it is likely that the secondary structures of these transcripts play an important role in their regulatory function in the cell.

RNA is not just a passive structural element or a regulator. It is also an active component in many situations. Thus RNA acting alone is able to catalyze RNA processing (Cech and Bass, 1986, Cech, 1990). In a protein-RNA complex, the RNA component of ribonuclease P is an active component of tRNA processing (Darr et al., 1992).

The sequence of nucleotides of an RNA molecule carries the information required for its actual conformation in three dimensions. Investigating secondary structure, or folding, of the nucleotide chain may lead to a first sketch of the organization of the molecule. Up to the present, RNA secondary structures have been predicted by applying various topological and thermodynamic rules for finding the energetically most favorable structure for a given sequence (Tinco et. al., 1971).

The structure of RNA molecules can be discussed at an empirically well established level of resolution known as secondary structure. It refers to a topology of binary contacts arising from specific base pairing, rather than geometry cast in terms of coordinates and distances. The driving force behind secondary structure formation is the stacking of base pairs. The formation of an energetically favorable helical region, however, also implies the formation of an energetically unfavorable loop region.

The secondary structure provides both geometrically and thermodynamically a scaffold for the tertiary structure. Its free energy accounts for a large share of the overall free energy of the full structure (Wuchty et. al., 1998).

In the last two decades several important approaches have been developed for the prediction of secondary structure from an RNA sequence. The "mfold" program, developed with dynamic programming algorithms, predicts optimal structure and suboptimal structures through free energy minimization (Zuker, 1989, Zuker and Stiegler, 1981). The partition function approach by McCaskill in 1990, computes base pair probabilities and binding probability for any base. A and C program for this algorithm is available in a suite of RNS secondary structure software known as Vienna RNA package.

MATERIALS AND METHODS

For the experiments, were used 10 individuals of *Carassius gibelio* Bloch. individuals from Movileni population (Iasi district), which were used for cytochrome b molecular marker sequencing.

Researches were developed until the sequences obtaining in University of Vigo, Genetics and Molecular Phylogeny Lab.

We used for gene amplification, DNA template that was extracted using phenol : chloroform : isoamylalcohol (25 : 24 : 1) method (Ausubel et. al., 1992, Ausubel et al., 1995). The technique is used for mitochondrial DNA extraction from muscle which has the origin from fresh, refrigerated or kept in ethanol individuals. Cellular lyses were done using a lyses buffer and K proteinase which were incubated at 37°C for 12 hours. After the incubation period the samples were centrifuged 2 times, each time for separate 1 layer from liquid column. After the final separation, the purified DNA is precipitated in absolute ethanol kept at -20°C and centrifuged for pellet obtaining. All pellets were resuspended in TE buffer (pH=8.0) and kept at -20°C.

The purified DNA was used like template for Cyt b gene amplification. In this way, we used two sets of primers, 1 set for each amplified segment: H15149 5'-AACTGCAGCCCTCAGAATGATATTTGTCCTCA-3', L14724 5'-CGAAGCTTGATATGAAAAACCATCGTTG-3' for tRNA^{Glu}/CytbI and Thr-R16496 5'-ACCTCCRATCTYCGGAGGACA-3', L15138 5'-ATGATGACCGCCTTCGTAGGCTA-3') for CytbII/tRNA^{Thr}.

The amplification process followed the same PCR program for each amplified segment, using an annealing temperature of 45.0°C and 30 replication cycles.

The PCR products were electrophoretically tested using a 1.5% agarose gel with 30ml volume and purified in QIAGEN columns, using a reaction kit and a protocol provided by the same mark. Product quantification was made in electrophoresis using a 3% agarose gel, with 30ml volume and ØX174 – *Hinf*I molecular weight marker.

The sequencing reaction was made using a Beckman Coulter reaction kit and the same primers (L14724 and H10720 for the first segment, L15138 and Thr-R16496 for the second, but with higher concentrations). The aligning temperatures were 45°C for both segments, with 30 replication cycles.

For both segments 2 DNA chains (direct and reverse) were sequenced.

Fluorescence diagrams corresponding to nucleotides sequences were made with a BECKMAN COULTER sequence analyzer and the sequence correcting and interpretation, using CEQ2000 software program.

Sequences alignment that comes from one species individuals was made with The EyeBall Sequence Editor (ESEE 3.1) software.

For a clear differentiation of studied species, we compared our haplotypes sequences with other that come from different species of the same genus. The all other sequences that were used for comparison have the same provenience – NCBI Genbank (National Center for Biotechnology Information). In this way, we used the sequences for *Carassius cuvieri* (Genbank Ref. no. AB045144) and *Carassius carassius* (Genbank Ref. no. AY714387).

RESULTS AND DISCUSSIONS

The mRNA's secondary structure for all analyzed haplotypes was modeled using the minimum binding energy and the binding temperature. After sequences alignment, using Clustal V method (Higgins and Sharp, 1989), 2 different new haplotypes from both populations were characterized. Compared to the sequences from GenBank, was recorded an general haplotype, characteristic for both populations formed by sequences with 100% similarity and a second one haplotype which has a mutation in 258 position – a transition by reason of guanine substitution with adenine.

For general haplotypes from both populations, the minimum energy of folded structure is about -142,4kcal/mol using a folding temperature of 20°C. The folding result, reveal a structure with 65 single stranded regions and 40 double stranded regions (Figure 1 - Figure 5).

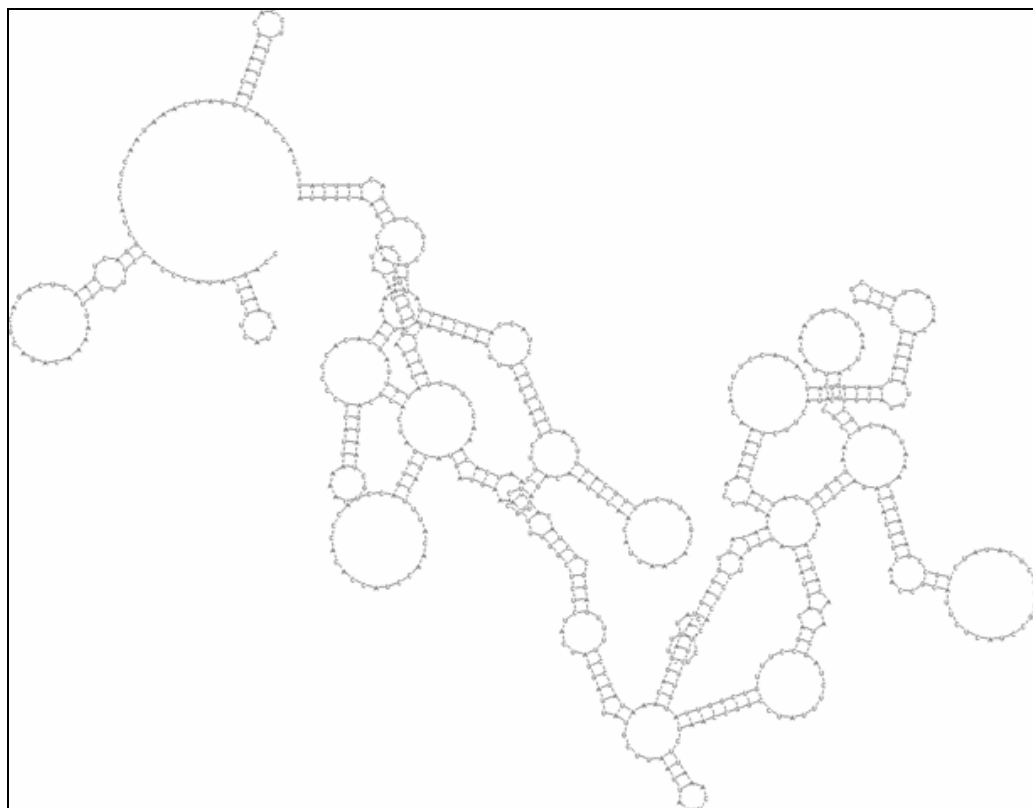


Figure 1 mRNA's secondary structure for *Carassius gibelio* Bloch general haplotype of both analyzed populations

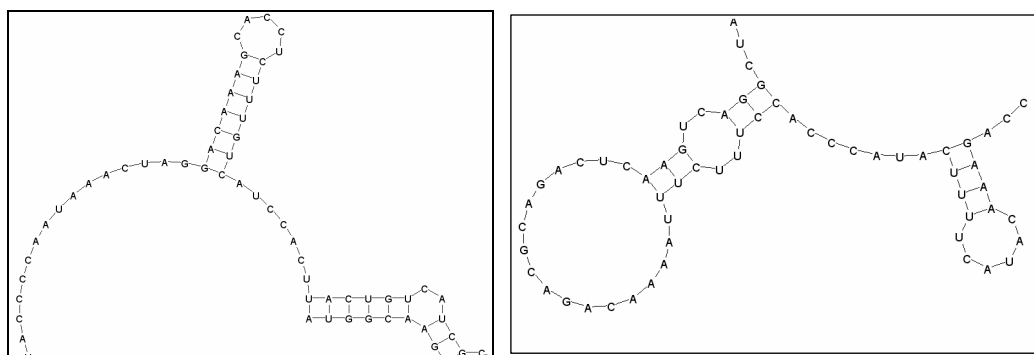


Figure 2 mRNA's secondary structure for *Carassius gibelio* Bloch general haplotype of both analyzed populations – details 1 and 2

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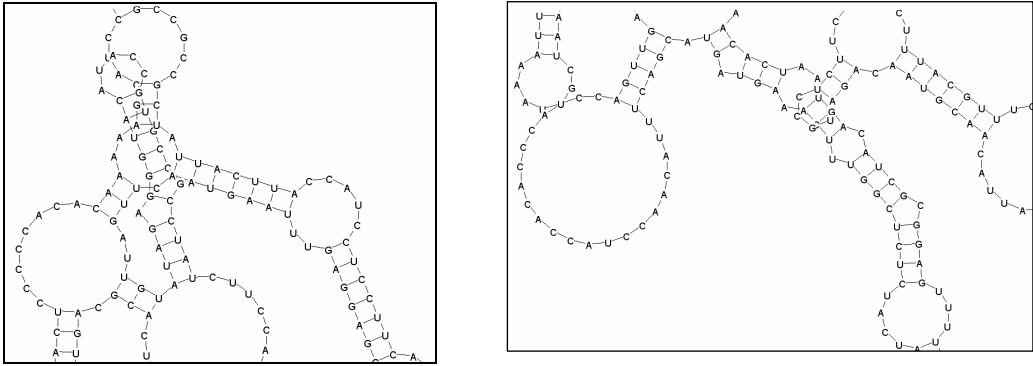


Figure 3 mRNA's secondary structure for *Carassius gibelio* Bloch general haplotype of both analyzed populations – details 3 and 4

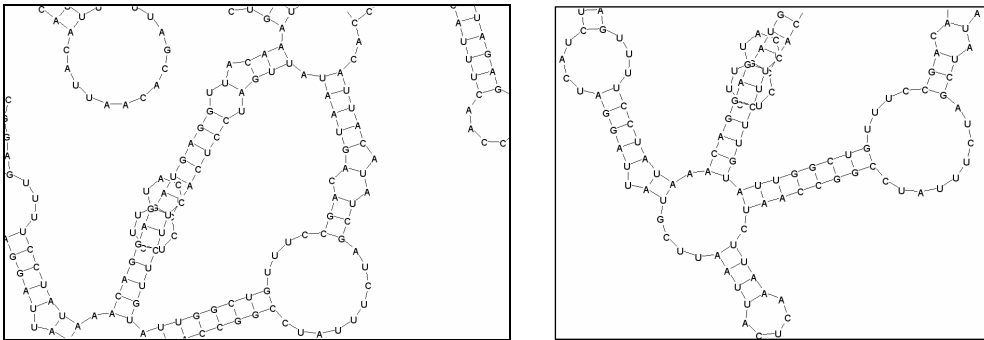


Figure 4 mRNA's secondary structure for *Carassius gibelio* Bloch general haplotype of both analyzed populations – details 5 and 6

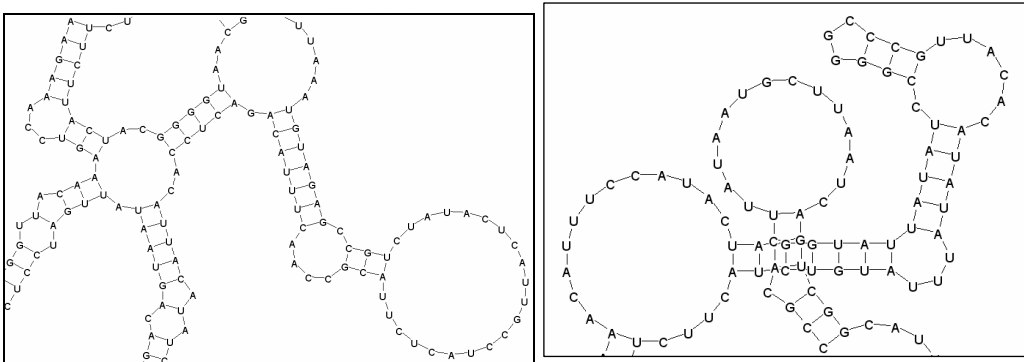


Figure 5 mRNA's secondary structure for *Carassius gibelio* Bloch general haplotype of both analyzed populations – details 7 and 8

For the second haplotype, the minimum energy of folded structure is about -140,01kcal/mol using a folding temperature of 20°C. The folding result, reveal a structure with 64 single stranded regions and 38 double stranded regions (Figure 6 - Figure 11).

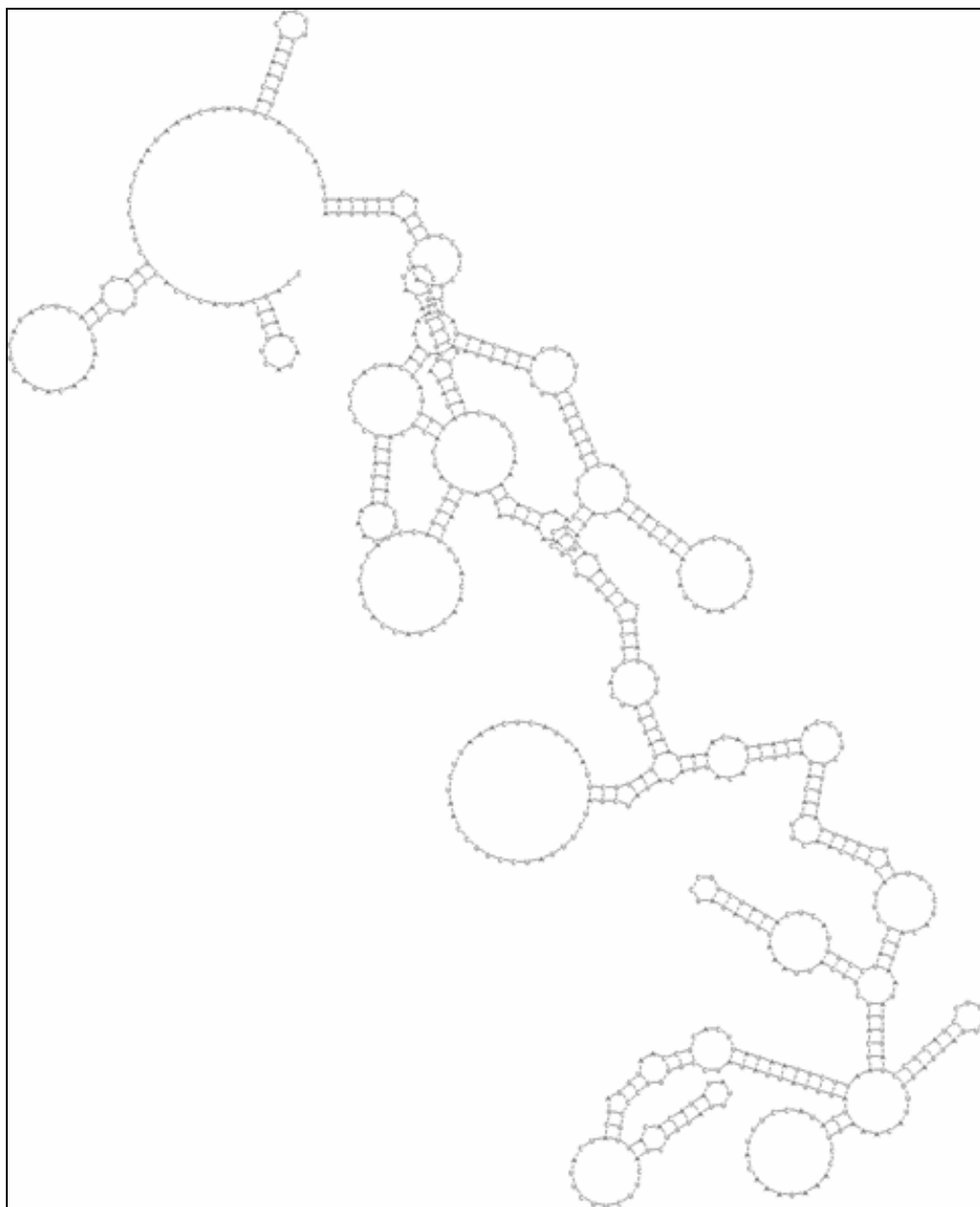


Figure 6 mRNA's secondary structure for *Carassius gibelio* haplotype with a mutation in position 258

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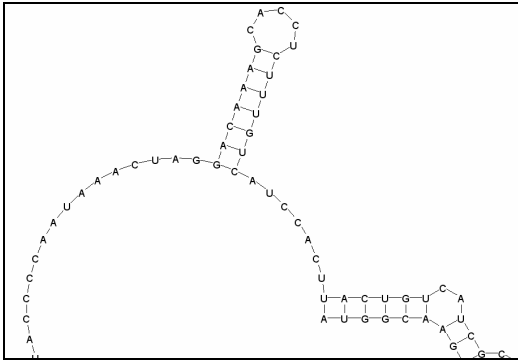


Figure 7 mRNA's secondary structure for *Carassius gibelio* haplotype with a mutation in position 258 – details 1 and 2

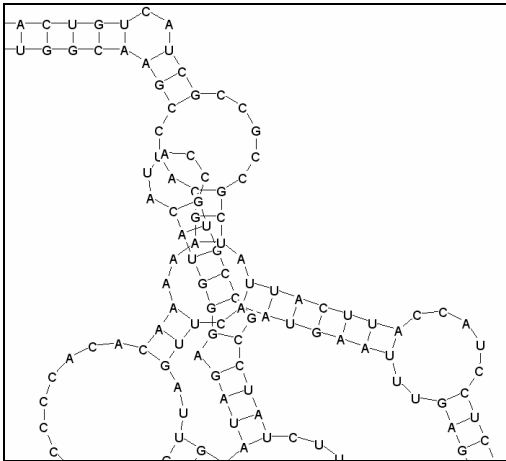
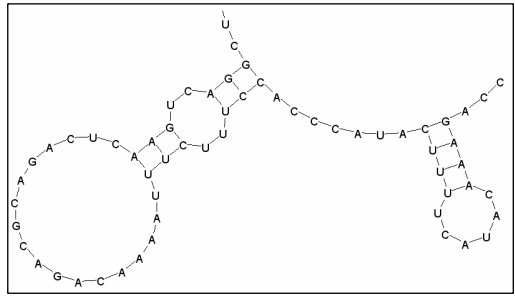


Figure 8 mRNA's secondary structure for *Carassius gibelio* haplotype with a mutation in position 258 – details 3 and 4

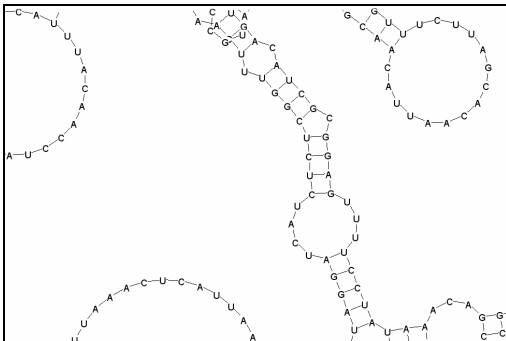
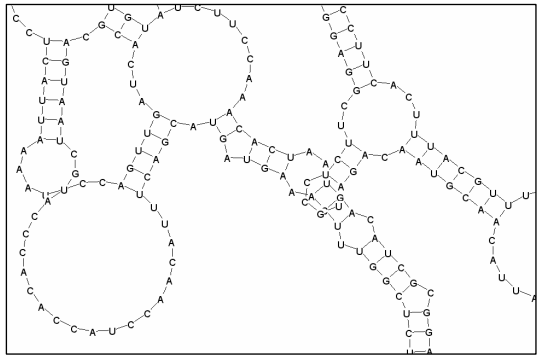
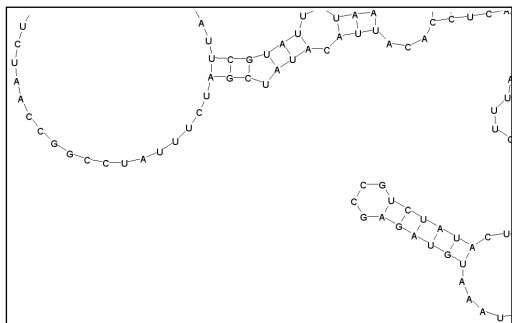


Figure 9 mRNA's secondary structure for *Carassius gibelio* haplotype with a mutation in position 258 – details 5 and 6



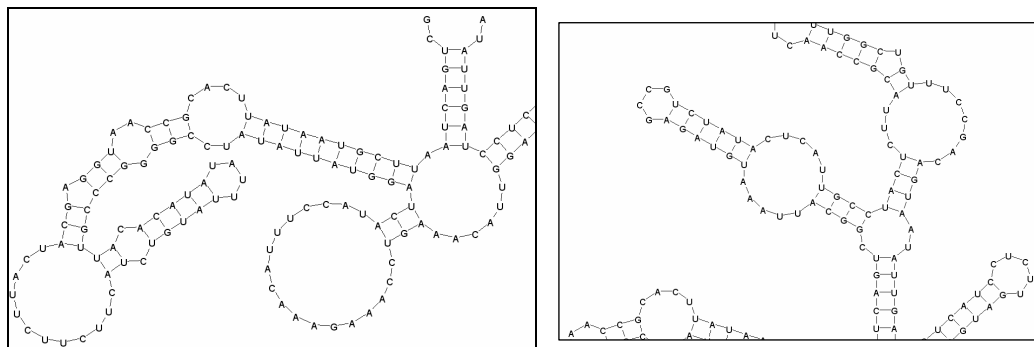


Figure 10 mRNA's secondary structure for *Carassius gibelio* haplotype with a mutation in position 258 – details 7 and 8

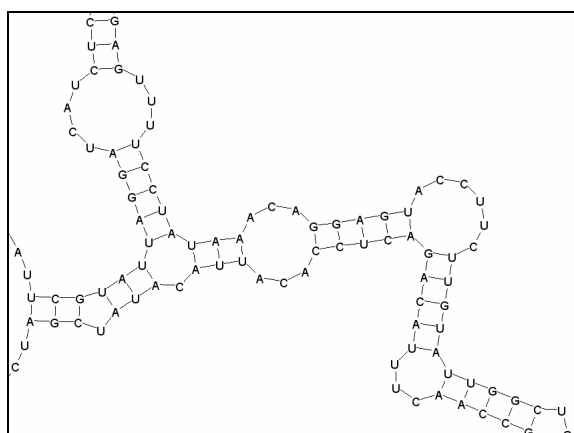


Figure 11 mRNA's secondary structure for *Carassius gibelio* haplotype with a mutation in position 258 – detail 9

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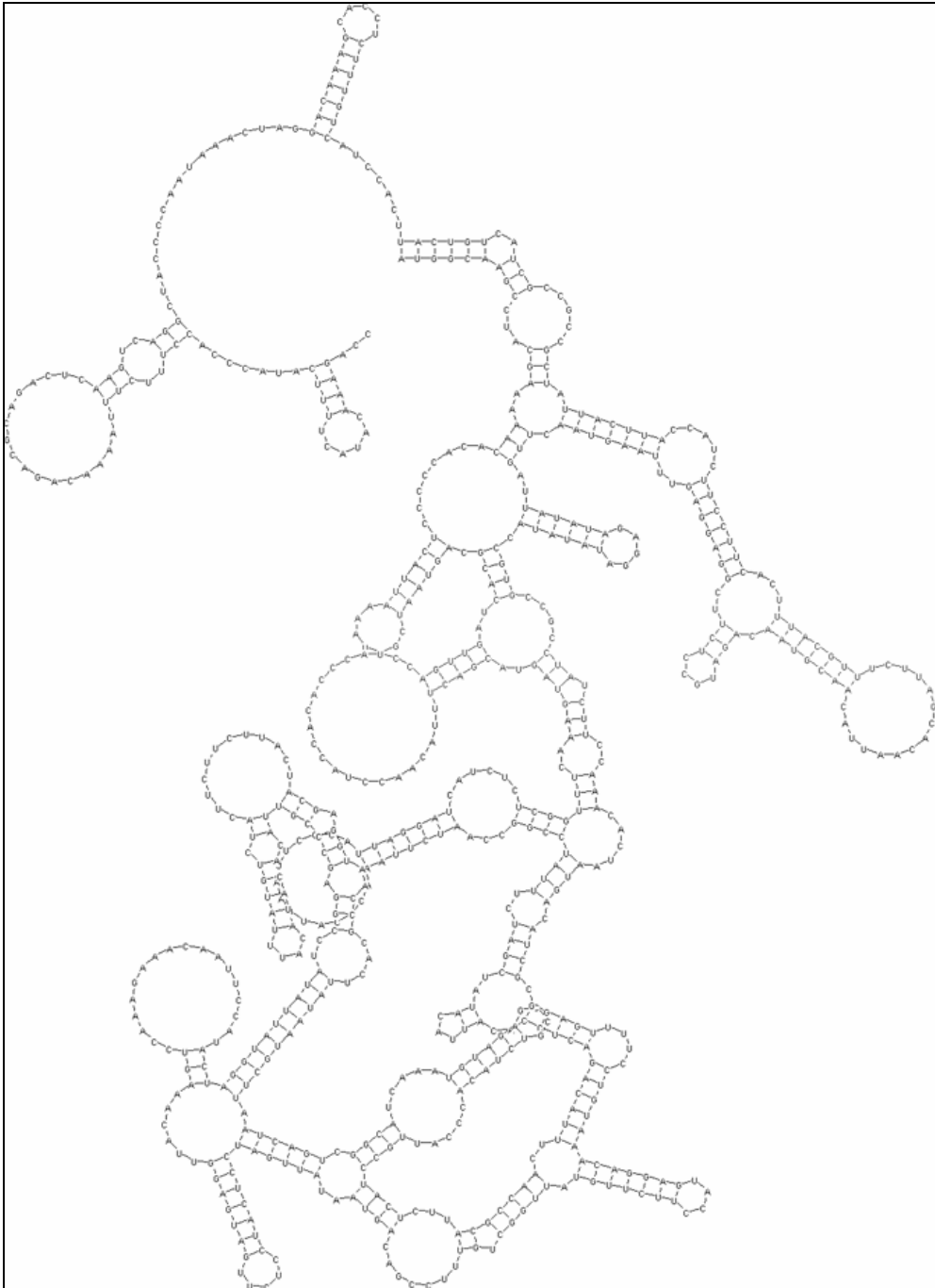


Figure 12 mRNA's secondary structure for *Carassius carassius*

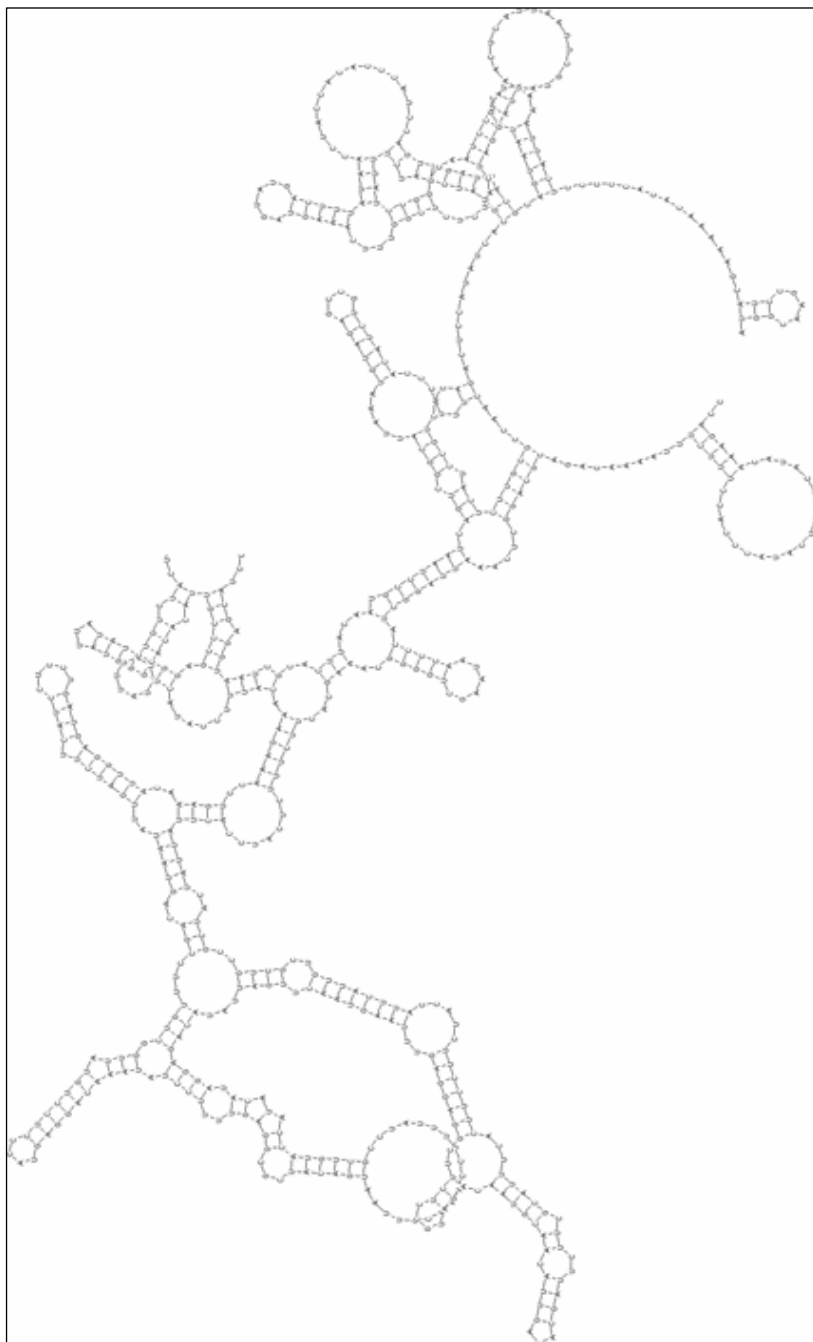


Figure 13 mRNA's secondary structure for *Carassius cuvieri*

CONCLUSIONS

The cytochrome's b lengthiness is about 1141 base pairs for *Carassius gibelio* Bloch.

The existent differences between sequences from all analyzed species are owed to some mutations like transitions and transversions, which emerge inside of speciation process.

We establish two new haplotypes for *Carassius auratus gibelio* Bloch., with a defined number of base pairs and structure.

The mRNA's secondary structure is different for different species and subspecies of the same genera.

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