

PRELIMINARY GENETIC ANALYSIS OF MEDITERRANEAN BLUEFIN TUNA CAUGHT IN LIBYAN WATERS

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SUMMARY

The existence of the Eastern and Western Atlantic Bluefin tuna stocks has been generally assumed. Recently several genetic studies seem to confirm this putative stock differentiation. Although, none of the genetic studies found significant differences between the Mediterranean locations a more exhaustive analysis is needed inside this sea. In this sense, in the present study we have analysed the control region sequence variability of ten Libyan Bluefin samples in order to enhance the knowledge of the global Mediterranean population. No differences were detected when the Bluefin from Libya were compared to the data from the rest of the Mediterranean. These preliminary results suggest a genetic homogeneity between this sample and the rest of the Mediterranean.

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FOREWORD

This report concern to the tuna analysis held in the Laboratori d'Ictiologia Genètica at the University of Girona in Spain, during the stay of Dr. El-Tawil from 23rd to 27th of October of 1999. All this reports was achieved in the COMPEMED project framework under the IEO of Málaga co-ordination.

INTRODUCTION

Bluefin tuna (*Thunnus thynnus*, Linnaeus, 1758) belong to the Scombridae family, which includes also bonito, mackerels and other tunas. Bluefin tuna is found in the Eastern Atlantic from South Africa (Talbot & Penrith, 1963) north to the coast of Norway (Gibbs & Collette, 1967) including the Mediterranean Sea, which is recognised as the only spawning area for Bluefin tuna in the Eastern Atlantic (Richards, 1976). Being pelagic and fast swimmer, it has been shown to migrate long distances (Mather III, 1979). In the Atlantic Ocean, the existence of two putative stocks is generally assumed (ICCAT, 1997). This division is based the knowledge of the two putative separated spawning grounds, one in the Mediterranean Sea and a second in the western Atlantic. Recent genetic studies carried out in the Bluefin tuna probably confirm the possible stock differentiation between the western Atlantic and the eastern Atlantic - Mediterranean stock differentiation (Pujolar & Pla, 2000, Alvarado Bremer *et al.*, 1999).

Concerning to the Mediterranean Sea a series of genetics, studies were done in the recent years (Pla *et al.*, 1995; Pla *et al.*, 1998; Pujolar & Pla, 2000). Any of the studies using both protein electrophoresis and mitochondrial DNA sequencing could no reject the hypothesis of a single genetic population of the Mediterranean Bluefin tuna. Although these authors have an exhaustive sampling along the Mediterranean, none of them has used samples from the African coast of the Atlantic Sea.

In the present work, we have obtained the mitochondrial control region sequence of eleven Bluefin tuna from Libya to enhance the knowledge of the genetic population structure of the Mediterranean Sea. Additionally, we present the first complete mitochondrial D-loop sequence in order to compare their sequence variability with our preliminary data from the rest of the Mediterranean.

MATERIAL AND METHODS.

Bluefin samples used for mitochondrial DNA sequence analysis were collected in the Mediterranean coast of Libya during the 1999 fishing season (see Table 1 for more sampling details). The laboratory outlines are described in Viñas *et al.* (1999), with minor modifications. In the present work, a combination of primers were used in order to obtain the complete mtDNA control region sequence: L15998-PRO (Alvarado Bremer *et al.*, 1995) which was complementary to the tRNA^{Phe} flanking D-loop fragment, was used in combination with the FST (Pla *et al.*, 1995), corresponding to tRNA^{Phe} gene adjacent to 3' control region end.

The full length (863 bp) of the Bluefin mtDNA control region were edited by eye with Programs SEQ ED. (version 1.3) and XESEE (Cabot & Beckenbach, 1989) and aligned using *Thunnus thynnus thynnus* (GenBank accession number X82653) sequences as reference. Total of sequence variation was assessed estimating nucleotide diversity (p ; Nei, 1987) and haplotypic diversity (h ; Nei & Tajima, 1981). Gene phylogenia was reconstructed using the Neighbor-Joining (NJ) algorithm (Saitou & Nei, 1987) on a matrix of Kimura two-parameter distance model (Kimura, 1980). A bootstrap test (Felsenstein, 1985) of 1000 replicates was carried out to check the strength of each branch of the tree. All these calculations were performed using the PHYLIP package (Felsenstein, 1993).

RESULTS AND DISCUSSION

The complete mtDNA control region sequence was obtained for the eleven samples (Figure 1). Every sequence is unique, with a haplotypic diversity of $h = 1$, and a nucleotide diversity of $p = 0,0035$ for the entire sample. Although the high degree of observed DNA variation, these figures are very similar with the results already obtained in our laboratory, with a total Mediterranean haplotypic diversity of $h = 0,096$ and $p = 0,035$ (Pla *et al.*, 1998).

The tree topology clustered the sequences in three divergent clades; only the third clade shows 100% bootstrap values (Figure 2). These three clades were also previously observed in the Mediterranean (Pla *et al.*, 1998) with a high sequence divergence of the third clade. The clade I comprise eight sequences (72%), the clade II

presented two unique sequences (18%) and the remaining belongs to the third clade (9%). Although the low sample size a preliminary analysis of the heterogeneity of the clade distribution could be done, using as reference the frequencies already observed in our laboratory. The results of this analysis showed homogeneity of the clade distribution between the samples from the rest of the Mediterranean Sea and the Libyan samples ($P = 0.3440 \pm 0.0150$), suggesting a great genetic homogeneity between all the Mediterranean Sea.

CONCLUSION

The results obtained in this preliminary study show that the samples analysed are part of the general distribution of tuna among the Mediterranean Sea. In this sense, this data confirm the genetic homogeneity of tuna distribution in the Mediterranean, and the hypothesis of the Mediterranean Bluefin tuna belongs to one single biological stock.

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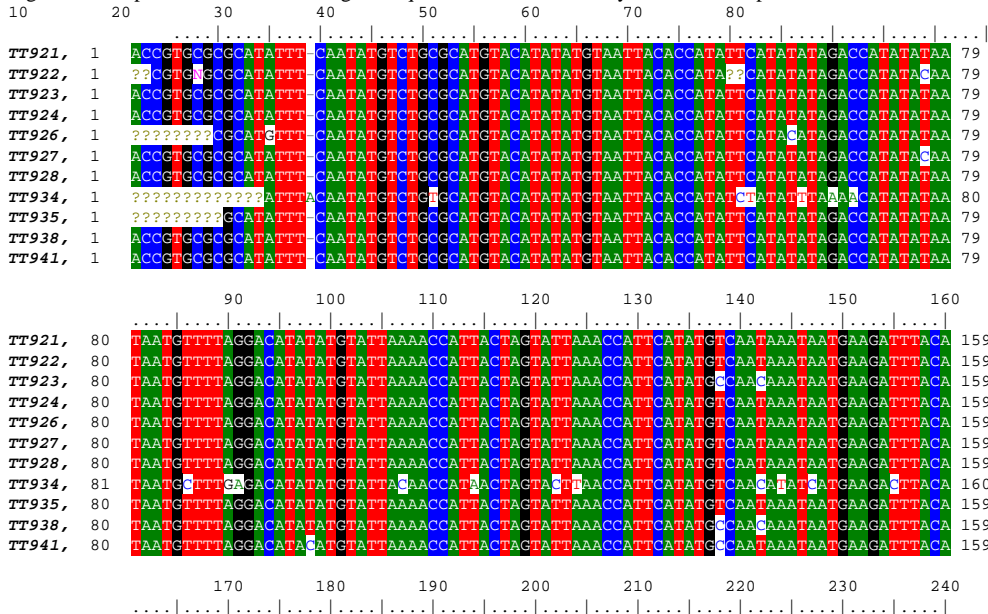
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Table 1. Samples employed in this study

Number	Code	Date	Location	Kg	FL	Sex
TT920	03	8/6/99	Zrig	77	162	male
TT921	05	8/6/99	Zrig	98	167	female
TT922	07	8/6/99	Zrig	45	154	female
TT923	08	8/6/99	Zrig	62	162	male
TT924	09	8/6/99	Zrig	60	159	female
TT926	11	8/6/99	Zrig	63	160	female
TT927	12	8/6/99	Zrig	51	157	male
TT928	13	8/6/99	Zrig	42	154	female
TT934	22	20/6/99	Zrig	26	115	female
TT935	23	--	Gazira	--	223	--
TT938	26	19/6/99	Zrig	78	170	female
TT941	31	20/6/99	Zrig	41	150	male

Figure 1. Complete mtDNA control region sequence of the eleven Libyan bluefin samples.



TT921,	160	TAAACCATAC	AAATAAACCTCAACATTCATCTTGAATTCAGGCCGATTAACCGAGATTTAAGACCTAACATAAATCTAAA	238
TT922,	160	NAAACCATAT	AAATAAAGCTCAACATTCATCTTGAATTCAGGCCGATTAACCGAGATTTAAGACCTAACATAAATCTAAA	238
TT923,	160	TAAACCATAC	AAATAAACCTCAACATTCATCTTGAATTCAGGCCGATTAACCGAGATTTAAGACCTAACATAAATCTAAA	238
TT924,	160	TAAACCATAC	AAATAAACCTCAACATTCATCTTGAATTCAGGCCGATTAACCGAGATTTAAGACCTAACATAAATCTAAA	238
TT926,	160	TAAACCATAC	AAATAAACCTCAACATTCATCTTGAATTCAGGCCGATTAACCGAGATTTAAGACCTAACATAAATCTAAA	238
TT927,	160	TAAACCATAT	AAATAAAGCTCAACATTCATCTTGAATTCAGGCCGATTAACCGAGATTTAAGACCTAACATAAATCTAAA	238
TT928,	160	TAAACCATAC	AAATAAACCTCAACATTCATCTTGAATTCAGGCCGATTAACCGAGATTTAAGACCTAACATAAATCTAAA	238
TT934,	161	TAAAGCATGACAA	TTACTTCAACATCTTACCTACATTCAGGATTAATAAACCGAGATTTAAGACCTAACATAAATCTAAA	239
TT935,	160	TAAACCATAC	AAATAAACCTCAACATTCATCTTGAATTCAGGCCGATTAACCGAGATTTAAGACCTAACATAAATCTAAA	238
TT938,	160	TAAACCATAC	AAATAAACCTCAACATTCATCTTGAATTCAGGCCGATTAACCGAGATTTAAGACCTAACATAAATCTAAA	238
TT941,	160	TAAACCATAC	AAATAAACCTCAACATTCATCTTGAATTCAGGCCGATTAACCGAGATTTAAGACCTAACATAAATCTAAA	238

		250	260	270	280	290	300	310	320	
TT921,	239	TCGCTTAAGCCATACCAAGTCTCCCTCATCTCTGACATCTACGTAAACTTAAGCGCAGTAAGAGCCTACCATCCAGTCCAT								318
TT922,	239	TCGCTTAAGCCATACCAAGTCTCCCTCATCTCTGACATCTACGTAAACTTAAGCGCAGTAAGAGCCTACCATCCAGTCCAT								317
TT923,	239	TCGCTTAAGCCATACCAAGTCTCCCTCATCTCTGACATCTACGTAAACTTAAGCGCAGTAAGAGCCTACCATCCAGTCCAT								317
TT924,	239	TCGCTTAAGCCATACCAAGTCTCCCTCATCTCTGACATCTACGTAAACTTAAGCGCAGTAAGAGCCTACCATCCAGTCCAT								317
TT926,	239	TCGCTTAAGCCATACCAAGTCTCCCTCATCTCTGACATCTACGTAAACTTAAGCGCAGTAAGAGCCTACCATCCAGTCCAT								317
TT927,	239	TCGCTTAAGCCATACCAAGTCTCCCTCATCTCTGACATCTACGTAAACTTAAGCGCAGTAAGAGCCTACCATCCAGTCCAT								317
TT928,	239	TCGCTTAAGCCATACCAAGTCTCCCTCATCTCTGACATCTACGTAAACTTAAGCGCAGTAAGAGCCTACCATCCAGTCCAT								317
TT934,	240	TCGCTTAAGCCATACCAAGTCTCCCTCATCTCTGACATCTACGTAAACTTAAGCGCAGTAAGAGCCTACCATCCAGTCCAT								318
TT935,	239	TCGCTTAAGCCATACCAAGTCTCCCTCATCTCTGACATCTACGTAAACTTAAGCGCAGTAAGAGCCTACCATCCAGTCCAT								317
TT938,	239	TCGCTTAAGCCATACCAAGTCTCCCTCATCTCTGACATCTACGTAAACTTAAGCGCAGTAAGAGCCTACCATCCAGTCCAT								317
TT941,	239	TCGCTTAAGCCATACCAAGTCTCCCTCATCTCTGACATCTACGTAAACTTAAGCGCAGTAAGAGCCTACCATCCAGTCCAT								317

		330	340	350	360	370	380	390	400	
TT921,	319	TTCTTAATGCATACGGTTATTGAAGGTGAGGGACAATAAATTTGGGGTAACACTTAGTGAATATTCTGGCATTTGGT								398
TT922,	318	TTCTTAATGCATACGGTTATTGAAGGTGAGGGACAATAAATTTGGGGTAACACTTAGTGAATATTCTGGCATTTGGT								397
TT923,	318	TTCTTAATGCATACGGTTATTGAAGGTGAGGGACAATAAATTTGGGGTAACACTTAGTGAATATTCTGGCATTTGGT								397
TT924,	318	TTCTTAATGCATACGGTTATTGAAGGTGAGGGACAATAAATTTGGGGTAACACTTAGTGAATATTCTGGCATTTGGT								397
TT926,	318	TTCTTAATGCATACGGTTATTGAAGGTGAGGGACAATAAATTTGGGGTAACACTTAGTGAATATTCTGGCATTTGGT								397
TT927,	318	TTCTTAATGCATACGGTTATTGAAGGTGAGGGACAATAAATTTGGGGTAACACTTAGTGAATATTCTGGCATTTGGT								397
TT928,	318	TTCTTAATGCATACGGTTATTGAAGGTGAGGGACAATAAATTTGGGGTAACACTTAGTGAATATTCTGGCATTTGGT								397
TT934,	319	TTCTTAATGCATACGGTTATTGAAGGTGAGGGACAATAAATTTGGGGTAACACTTAGTGAATATTCTGGCATTTGGT								398
TT935,	318	TTCTTAATGCATACGGTTATTGAAGGTGAGGGACAATAAATTTGGGGTAACACTTAGTGAATATTCTGGCATTTGGT								397
TT938,	318	TTCTTAATGCATACGGTTATTGAAGGTGAGGGACAATAAATTTGGGGTAACACTTAGTGAATATTCTGGCATTTGGT								397
TT941,	318	TTCTTAATGCATACGGTTATTGAAGGTGAGGGACAATAAATTTGGGGTAACACTTAGTGAATATTCTGGCATTTGGT								397

		410	420	430	440	450	460	470	480	
TT921,	399	TCCTACTTCAGGGCCATAGCTTGGTAAACATCCCCATTCCTTCATCGACGCTTGCCATAAGTTATTGGTGGAGTACATGAG								478
TT922,	398	TCCTACTTCAGGGCCATAGCTTGGTAAACATCCCCATTCCTTCATCGACGCTTGCCATAAGTTATTGGTGGAGTACATGAG								477
TT923,	398	TCCTACTTCAGGGCCATAGCTTGGTAAACATCCCCATTCCTTCATCGACGCTTGCCATAAGTTATTGGTGGAGTACATGAG								477
TT924,	398	TCCTACTTCAGGGCCATAGCTTGGTAAACATCCCCATTCCTTCATCGACGCTTGCCATAAGTTATTGGTGGAGTACATGAG								477
TT926,	398	TCCTACTTCAGGGCCATAGCTTGGTAAACATCCCCATTCCTTCATCGACGCTTGCCATAAGTTATTGGTGGAGTACATGAG								477
TT927,	398	TCCTACTTCAGGGCCATAGCTTGGTAAACATCCCCATTCCTTCATCGACGCTTGCCATAAGTTATTGGTGGAGTACATGAG								477
TT928,	398	TCCTACTTCAGGGCCATAGCTTGGTAAACATCCCCATTCCTTCATCGACGCTTGCCATAAGTTATTGGTGGAGTACATGAG								477
TT934,	399	TCCTACTTCAGGGCCATAGCTTGGTAAACATCCCCATTCCTTCATCGACGCTTGCCATAAGTTATTGGTGGAGTACATGAG								478
TT935,	398	TCCTACTTCAGGGCCATAGCTTGGTAAACATCCCCATTCCTTCATCGACGCTTGCCATAAGTTATTGGTGGAGTACATGAG								477
TT938,	398	TCCTACTTCAGGGCCATAGCTTGGTAAACATCCCCATTCCTTCATCGACGCTTGCCATAAGTTATTGGTGGAGTACATGAG								477
TT941,	398	TCCTACTTCAGGGCCATAGCTTGGTAAACATCCCCATTCCTTCATCGACGCTTGCCATAAGTTATTGGTGGAGTACATGAG								477

		490	500	510	520	530	540	550	560	
TT921,	479	ATTCAATTAAGCCACATGCCGGGGTTCTCTAGGGGGTCAGGTTATTTTTTCTCCTTCCCTTCACTTGACATCTCA								558
TT922,	478	ATTCAATTAAGCCACATGCCGGGGTTCTCTAGGGGGTCAGGTTATTTTTTCTCCTTCCCTTCACTTGACATCTCA								557
TT923,	478	ATTCAATTAAGCCACATGCCGGGGTTCTCTAGGGGGTCAGGTTATTTTTTCTCCTTCCCTTCACTTGACATCTCA								557
TT924,	478	ATTCAATTAAGCCACATGCCGGGGTTCTCTAGGGGGTCAGGTTATTTTTTCTCCTTCCCTTCACTTGACATCTCA								557
TT926,	478	ATTCAATTAAGCCACATGCCGGGGTTCTCTAGGGGGTCAGGTTATTTTTTCTCCTTCCCTTCACTTGACATCTCA								557
TT927,	478	ATTCAATTAAGCCACATGCCGGGGTTCTCTAGGGGGTCAGGTTATTTTTTCTCCTTCCCTTCACTTGACATCTCA								557
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TT934,	479	ATTCAATTAAGCCACATGCCGGGGTTCTCTAGGGGGTCAGGTTATTTTTTCTCCTTCCCTTCACTTGACATCTCA								558
TT935,	478	ATTCAATTAAGCCACATGCCGGGGTTCTCTAGGGGGTCAGGTTATTTTTTCTCCTTCCCTTCACTTGACATCTCA								557
TT938,	478	ATTCAATTAAGCCACATGCCGGGGTTCTCTAGGGGGTCAGGTTATTTTTTCTCCTTCCCTTCACTTGACATCTCA								557
TT941,	478	ATTCAATTAAGCCACATGCCGGGGTTCTCTAGGGGGTCAGGTTATTTTTTCTCCTTCCCTTCACTTGACATCTCA								557

		570	580	590	600	610	620	630	640	
TT921,	559	CAGTGCAAATGCAACAATGATCAACAAGGTAGAACAATTTCTTGCTTGAGGGTAAATAGTCTGCATGGCTTAATTCCTA								638
TT922,	558	CAGTGCAAATGCAACAATGATCAACAAGGTAGAACAATTTCTTGCTTGAGGGTAAATAGTCTGCATGGCTTAATTCCTA								637
TT923,	558	CAGTGCAAATGCAACAATGATCAACAAGGTAGAACAATTTCTTGCTTGAGGGTAAATAGTCTGCATGGCTTAATTCCTA								637
TT924,	558	CAGTGCAAATGCAACAATGATCAACAAGGTAGAACAATTTCTTGCTTGAGGGTAAATAGTCTGCATGGCTTAATTCCTA								637
TT926,	558	CAGTGCAAATGCAACAATGATCAACAAGGTAGAACAATTTCTTGCTTGAGGGTAAATAGTCTGCATGGCTTAATTCCTA								637

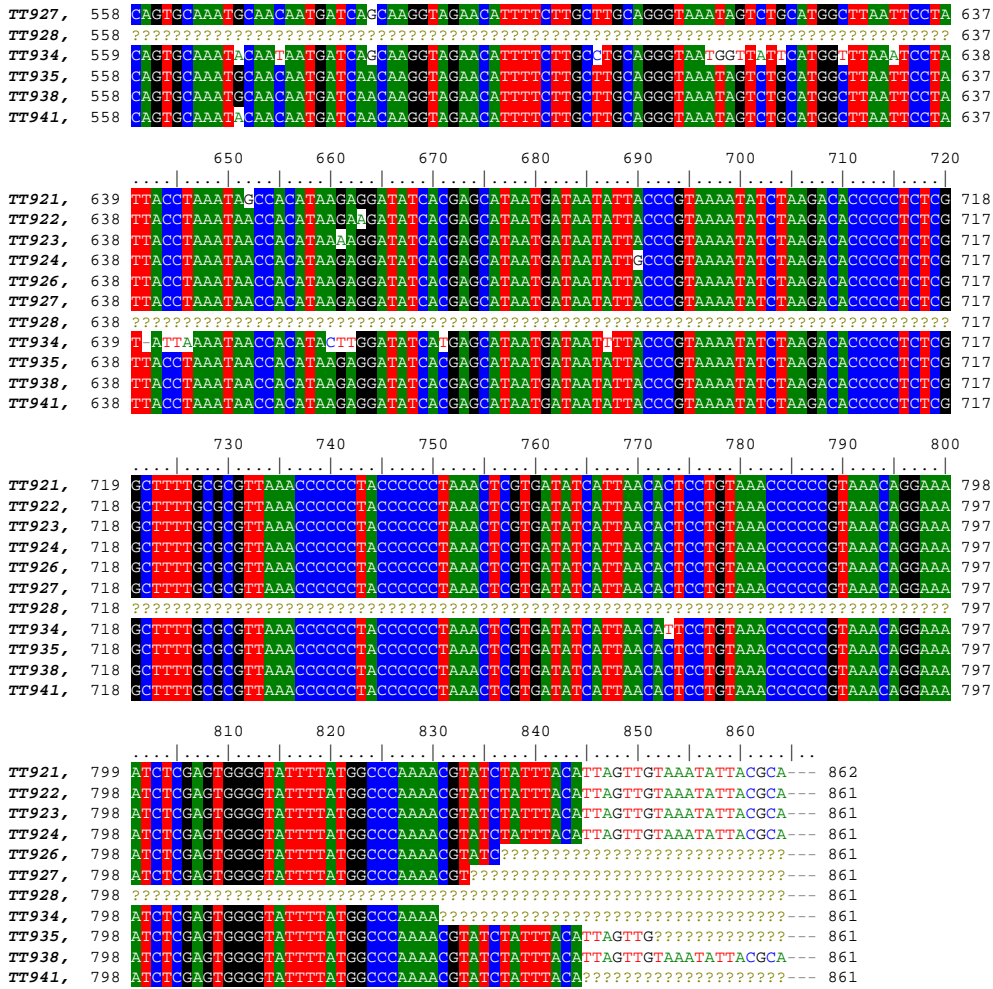
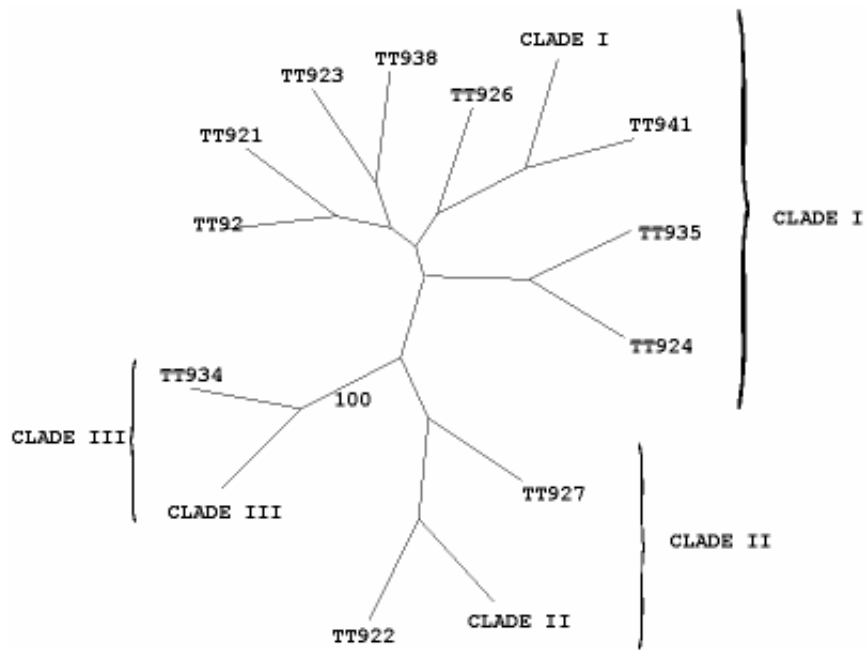


Figure 2. Neighbor-Joining tree using Kimura two-parameters distance of the 11 distinct sequences. Numbers in the internal branches showed bootstrap values higher than 75% after 1000 replicates. Calde I, II and III sequences are used as references for their own clades



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