



# Species identification of the *Anopheles fluviatilis* complex using phylogenetic analysis PCR-sequencing in southwestern Iran

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## Introduction

*Anopheles fluviatilis* is transmission cause of malaria disease in mountainous areas in some parts of Iran. Some of malaria transmitter species in this country also are known as biological forms. The aim of this study was to identify *An. fluviatilis* complex species using phylogenetic analysis PCR-sequencing.

## Methods

In this research *Anopheles fluviatilis* was caught from different areas of Kohgiluyeh and Boyer-Ahmad province at southwestern Iran at 2013. DNA was taken from 4 *An. fluviatilis* selected samples and PCR tests of 28S- D3 part was done on sample's DNA and then after sequence of obtained results were identified and were compared with similar samples of *An. fluviatilis* based on data's of gene word bank . Phylogenetic tree and individual sequences of samples were calculated.

## Results

Obtained results of PCR product of 28S-D3 part of rDNA gene with the length of 333bp in *An. fluviatilis* of this province is shown in Figure 1.

All four samples of *An. fluviatilis* were sequenced which its results were recorded in Genbank with accession numbers of KJ396263, KJ396264, KJ396265 and KJ396266.

For verification of sequencings, species identification and phylogeny relationships of studied samples and existing samples in gene world bank, sequences were tested by Online BLAST software (available in Pubmed) and were compared with other sequences in gene world bank.

Figure 2 's findings shows that difference of U and T species of *Anopheles fluviatilis* is in number 78 nucleotide.

Obtained phylogeny tree of analysis of 28S-D3 part of rDNA gene, showed 4 samples of *An. fluviatilis* of Yasuj city. Sequence of other complex species (T-U) and also *An. superpictus* were as out group species.

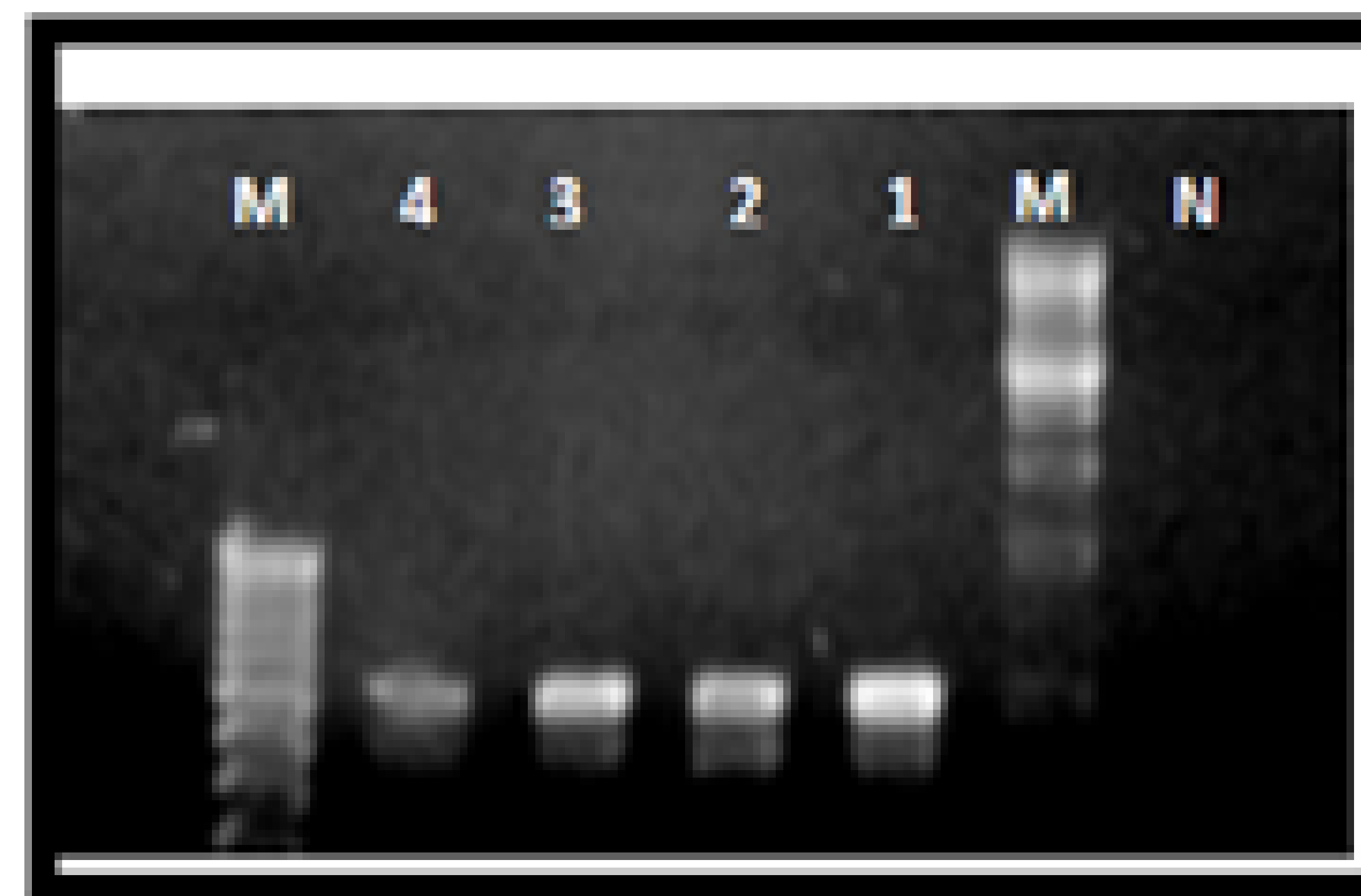


Figure 1: 1,2, 3 and 4 show samples of *Anopheles fluviatilis*; "M" shows marker size 100bp; "N" shows negative control

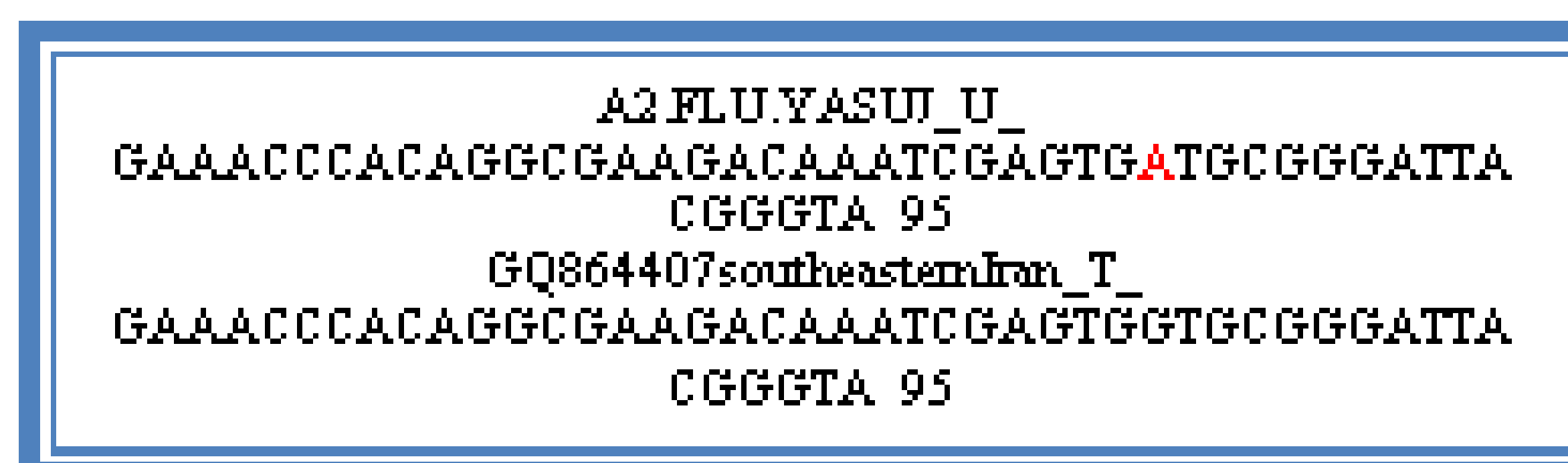


Figure 2: Difference between *Anopheles fluviatilis* U and T is in 78 nucleotide that is "A" in *An. fluviatilis* U and "G" in *An. fluviatilis* T

## Conclusions

This study have been the first genetic diversity study of *An. fluviatilis* in Kohgiluyeh and Boyer-Ahmad province which four samples of *An. fluviatilis* were selected as representative of this anopheles population in area and their DNA were extracted and were used in PCR reactions. it seems that T-U specie in rest of the country be as hybrid species.

This study showed that *An. fluviatilis* has species separate branch in southwestern Iran (with U genotype) which is different with branch of southeastern Iran (Hormozgan, Kerman and Sistaan & Baluchestaaan provinces) with T genotype.

## Further Research

I recommend further research for the other vector species such as *An. superpictus* in this area and other infested areas.

## References and cited literature

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