Chapter 6
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The Genetic Diversity of *Triturus vittatus vittatus* (Synonym *Ommatotriton vittatus*) Along the Southern Border of its Distribution

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ABSTRACT

The aim of this chapter is to investigate the genetic diversity of *Triturus vittatus vittatus* (synonym *Ommatotriton vittatus*). This salamander species is found in different breeding sites throughout the southern border of its distribution. Two mitochondrial DNA markers, *12S* and *16S*, and the nuclear rhodopsin gene (RHO) were used to examine the genetic variations in this species. AMOVA analysis was conducted to investigate the distribution of the variation and differential connectivity among populations, regions, and populations within regions. This analysis of the 163-bp RHO DNA sequence did not reveal any significant genetic variation among the different populations in the various habitats according to detection of population differentiation. In contrast, comparison of *12S* rRNA (357 bp) and *16S* rRNA (521 bp) revealed high variation among the regions for the different populations. AMOVA showed high dissimilarity between the breeding sites of newts at the southern border of their distribution.

GRAPHICAL ABSTRACT

Variation of mitochondrial *16S* rRNA in *Triturus vittatus vittatus* in different habitats at the southern limit of its distribution.

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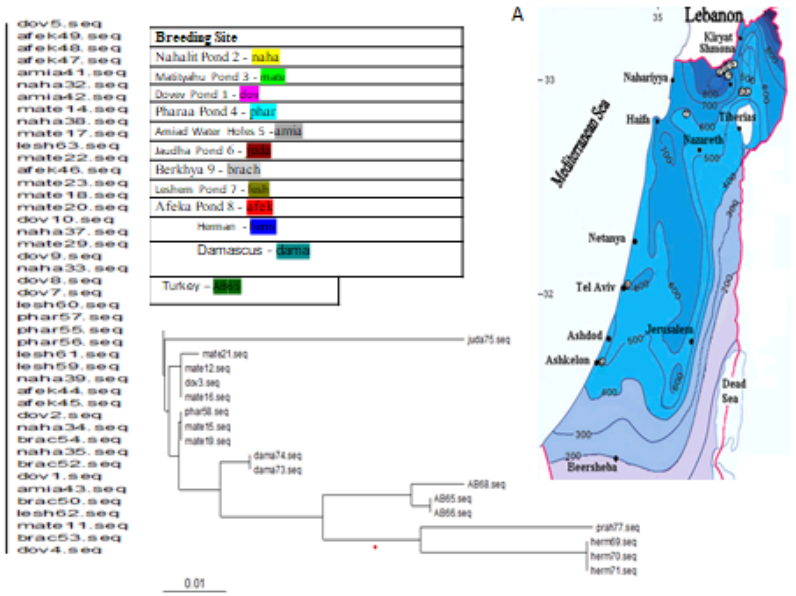

Keywords: Salamander; Triturus vittatus vittatus; DNA markers; AMOVA; genetic variation; population.

1. INTRODUCTION

The banded newt (*Triturus vittatus*) is one of seven newt species found in Israel. It is found at the southern border of their distribution, and is adapted to extremely unstable conditions [1]. The banded newt *Triturus vittatus vittatus* (syn: *Ommatotriton vittatus*) is found in Turkey, Syria, and Israel, and at the southern border of its distribution, it is adapted to extremely unstable conditions (*O. nesterovi, O. ophryticus and O. vittatus*) [2-4]. However, *T. v. vittatus* adaptation at the southern border of its distribution in Israel has not been well-documented [5]. *T. vittatus* in the aquatic phase reproduces mainly in unpredictable habitats such as winter pools that generally contain water only until the beginning of the summer, although occasionally these pools contain water throughout the year [1]. Various genetic markers have been employed to investigate the genetic variation in populations of *T. v. vittatus* in this region, due to the difficulty in explaining the variation in this small area, such as random amplified polymorphic DNA (RAPD) [6] and nucleotide sequences of mitochondrial cytochrome b and D-loop [7]. The current study explored the genetic divergence among populations using mitochondrial rRNA markers *12S* and *16S*, as well as the nuclear rhodopsin gene (*RHO*).

2. MATERIALS AND METHODS

2.1 Sites Studied and Larvae Collected

T. v. vittatus larvae were collected in a hand net from winter ponds where the newts were present [8]. The locations and characteristics of the ponds are presented in Fig. 1 and Table 1, respectively [9]. Tissue samples were extracted from whole tails of larvae and clipped pieces of tails from adults. In addition, two breeding sites outside of Israel, in Syria (Damascus) and the European part of Turkey, were sampled, where two clipped-tail tissue samples of *T. v. vittatus* and four clipped-tail tissue samples of another species were collected. In Israel, samples of 5–10 larval or adult *T. v. vittatus* were collected from the breeding sites (ponds).

2.2 DNA Extraction and Sequencing

DNA extraction involves the isolation of DNA from biological samples, such as cells or tissues, usually followed by amplification of specific regions of the DNA by polymerase chain reaction (PCR). The amplified DNA can then be sequenced by various methods, such as Sanger sequencing or next-generation sequencing [10].

In this study, genomic DNA was extracted from tissue samples using the QIAamp DNA Mini Kit, followed by visualization on a 0.8% agarose gel and measurement of DNA concentration using a NanoDrop 1000 spectrophotometer. PCR amplification of *12S* and *16S* genes was performed using specific primers, and RAPD fragments were obtained. The PCR products were separated by electrophoresis and purified before sequencing at Hy Laboratories. The neighbor-joining method [11] was used to generate phylogenetic trees from distance matrices based on multiple sequence alignments, which were carried out using the MegAlign computer program [12-14].

2.3 Statistical Analysis

To assess genetic similarity among the samples, the neighbor-joining method was used, and the genetic distances were calculated using the maximum composite likelihood method [15]. The results are presented as the number of base substitutions per site. Analysis of molecular variance (AMOVA) was conducted to investigate the distribution of the variation and differential connectivity among populations, regions, and populations within regions. The proportion of the total variance among populations (PhiPT), regions (PhiRT), and populations within regions (PhiPR) was examined using populations nested within regions. GenAlEX was used for the analysis [16].

Table 1. Locations where *T. v. vittatus* breeding in the southern region was found

Breeding site	Area	Latitude	Longitude	Altitude
Nahalit Pond 1	Upper Galilee	776401	243657	665
Dovev Pond 4	Upper Galilee	772801	239158	740
Pharaa Pond 3	Upper Galilee	774580	242784	682
Amiad Water Holes 6	Hula Valley	757994	251721	212
Jaudha Pond 5	Hula Valley	761398	257589	110
Leshem Pond 7	Lower Galilee	750976	225612	300
Afeka Pond 8	Central Coastal	670453	182364	20

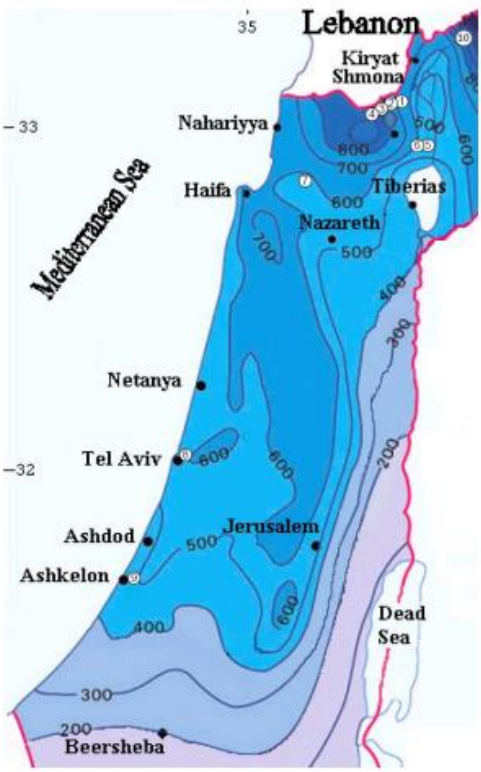

Fig. 1. Map showing locations of *T. v. vittatus* breeding sites (numbered according to Table 1), with an overlay of average annual precipitation (in mm) for each location

Table 2. Samples of newts (*T. v. vittatus*) taken from different breeding sites for DNA analysis. (Berekhya 9 is missing)

Breeding site	Area	*12S* rRNA	*16S* rRNA
Nahalit Pond 2	Upper Galilee	7	8
Matityahu Pond 3	Upper Galilee	13	14
Dovev Pond 1	Upper Galilee	9	9
Pharaa Pond 4	Upper Galilee	5	4
Amiad Water Holes 5	Hula Valley	3	3
Jaudha Pond 6	Hula Valley	1	1
Leshem Pond 7	Lower Galilee	5	5
Afeka Pond 8	Central Coastal	6	6
Hermon 10	North Israel	3	
Damascus		2	2
Turkey		3	3

Table 3. Primers for *RHO* amplification

Analysis	Primer	Sequence 5'-3'
Rhodopsin	trhodf	TGTCCCCTTCTCCAACAAGA
Rhodopsin	trhodr	GAACCCTCCAAAGACCATGA

3. RESULTS

The difference among the populations was examined by comparing genes *RHO*, *12S* and *16S*. There was no genetic variability between different populations in the *RHO* sequences (Fig. 2), with an AMOVA value of 0 (Table 4). However, there were differences in the *12S* and *16S* sequences between the populations (Figs. 3 and 4).

Fig. 2. The sequence of *RHO* (163 bp)

Table 4. AMOVA of gene sequences across multiple breeding sites

Gene	PhiPT value	P-value	Nm
RHO	0	0.01	
12S rDNA	0.802	0.01	0.124
16S rDNA	0.636	0.01	0.286

Fig. 3 presents nucleotide variations in the mitochondrial 12S rRNA (357 bp) gene fragment among populations of T. v. vittatus. Some DNA sequences from the different newt populations in Israel were similar. However, four populations (Jaudha Pond, Hermon, Turkey, and Damascus) were dissimilar from other breeding site populations.

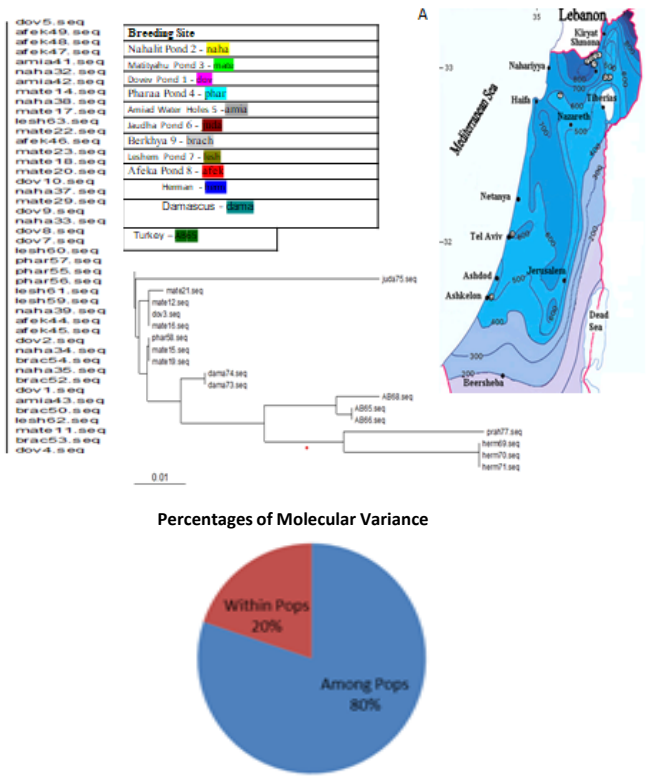

Fig. 3. Nucleotide variation in mitochondrial *12S* rRNA gene of the newt species *T. v. vittatus*. (A) Calculated standard parameters of nucleotide variation within the 357-bp fragment of the gene in a phylogenetic tree that depicts the evolutionary relationships among populations. The tree was constructed using the neighbor-joining method and is scaled at 0.01. (B) Results of the AMOVA

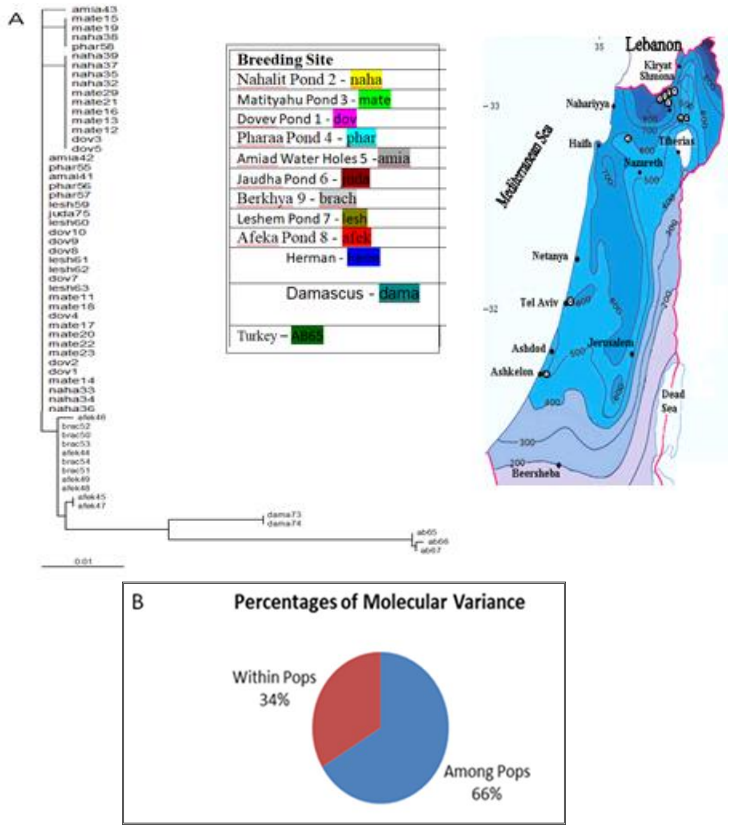

Fig. 4. (A) Calculated standard parameters of nucleotide variation in the mitochondrial *16S* rRNA (521 bp) gene fragment among *T. v. vittatus* populations. (B) AMOVA results

Table 4 shows that there are significant differences between and among populations in the two genes, *12S* and *16S*, by AMOVA. Specifically, the results indicate that there is greater variation among populations than within populations. In addition, when comparing the *16S* rRNA (521 bp) fragment, four populations (Afeka, Berekhya, Turkey, and Damascus) were found to have higher levels of variation compared to the others (Table 4).

4. DISCUSSION

The genetic, biological, and ecological differences among various populations of *T. v. vittatus* at the southern limit of its distribution have been extensively studied [17-22,7,9,13,14,23,24]. Here we found divergence among the populations similar to that reported for *Triturus karelinii*, indicating differences in niche. This

study contributes to the existing research by analyzing three additional genetic markers (*RHO*, *12S*, and *16S*) that diverge among the populations that have adapted to different environmental conditions [5,22]. These results support previous studies on *T. v. vittatus* using RAPD [9] and mitochondrial DNA [7], which also found divergence among populations living in extreme or more humid conditions in northern Israel.

The findings of this study are consistent with research on other Urodela and Anura species, such as *Salamandra infraimmaculata* [25-27] and *Pseudepidalea viridis* [19], which also examined genetic variation among different habitats. Newts have two habitats during their life cycle: aquatic and terrestrial, with adaptation to the terrestrial habitat being more important for their distribution than the aquatic habitat, as suggested by Degani [5] and supported by previous research [22].

5. CONCLUSION

This study's results support this hypothesis by showing that genetic divergence is greater among populations in extreme terrestrial conditions than in populations from more humid sites in northern Israel.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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He has research and academic experience in gene expression mechanisms in hormonal control of fish growth and reproduction, animals in the ecological system and environmental effects on habitat selection and molecular markers in fish. His area of research includes applied scientific research in the fields of animal biotechnology, and environmental and agriculture sciences. He has published more than 250 articles and 6 books.

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