

A SEROLOGICAL AND IMMUNOLOGICAL STUDY  
ON THREE LIZARDS OF GREECE (SAURIA : LACERTIDAE)

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The degree of immunological cross-reactions of an antiserum against a protein antigen with the corresponding proteins of other related taxa, reflects evolutionary changes on antigenic determinants of comparing homologous proteins. These changes are the expression of genetic differences existing among compared taxa. On the basis of this kind of immunological analysis of plasma proteins and hemoglobins and with related electrophoretic studies, various problems of taxonomy, phylogeny and evolution of many reptilian taxa have been investigated by many investigators (Lykakis, 1973; Dessauer, 1974 a,b; Higgins and Rand, 1975; Gorman and Kim, 1976, etc). Among these studies some are referred to lizard serum proteins, hemoglobins and enzymes (Gorman et. al., 1974; Higgins, 1973; Dessauer, 1974 a,b; Higgins and Rand, 1975). By these methodological approach, qualitative and quantitative differences were revealed among various genera and congeneric species.

In the present study, an electrophoretic and immunological analysis of three lizards from Greece is reported. All three species belong to the genus *Lacerta*. The results obtained are discussed in connection with zoogeographical and ecological observations.

**Materials and methods**

*Animals and animal sera*

The lizards used are *Lacerta viridis*, *L. trilineata* and *L. taurica* from Peloponnese and two Ionian islands (Zakynthos and Cephalonia). Individual blood samples were collected from the heart of anaesthetized animals. After clotting the sera were separated and stored at -25 °C until use. Two rabbits were immunized with serum of the lizard *L. viridis*. One part of serum and one part of complete freund's adjuvant were emulsified. One ml of the emulsion was subcutaneously distributed in the neck region and 3 weeks later each rabbit was given a similar

injection on the hind footpads. Three weeks later a boosted injection of 0.3 ml serum was injected and 5 days after the third injection of a similar dose of serum was given into rabbits. Rabbits were exsanguinated one week after the last antigen administration. Rabbit blood was al-

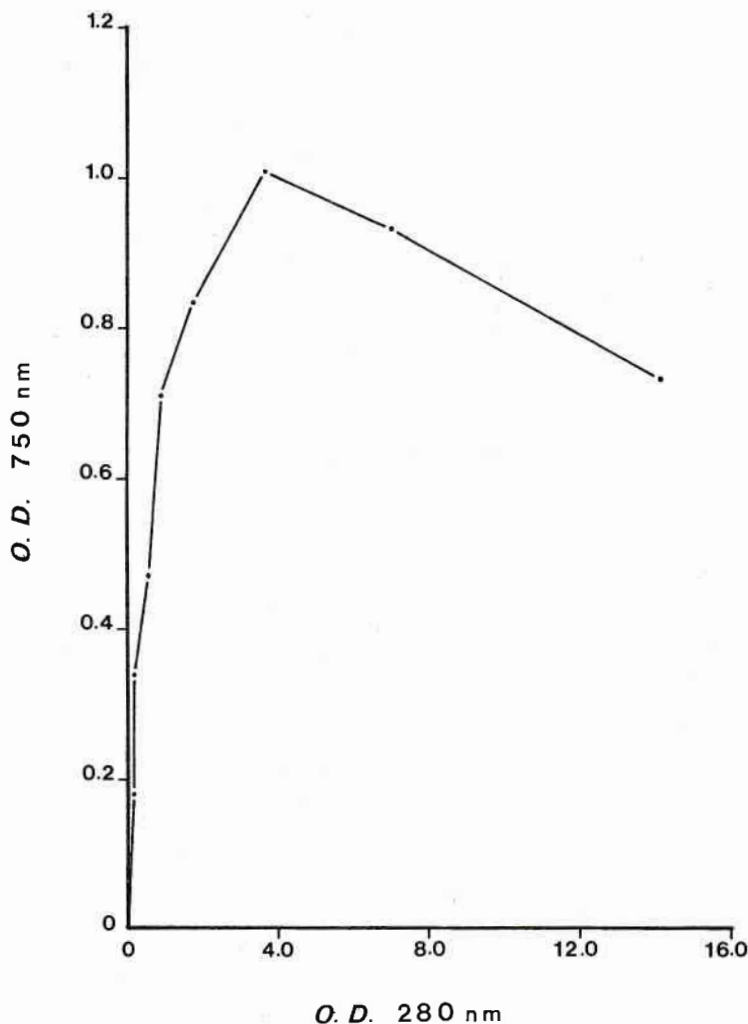


Fig. 1. A typical titration curve of a rabbit antiserum against the serum proteins of the lizard, *L. viridis*. On the horizontal axis the antigen concentrations and on the vertical axis the immune precipitate formed.

lowed to clot, the separated serum clarified by centrifugation (twice) at 8,000 rpm. on a Sorvall refrigerated centrifuge and stored in small aliquots at  $-25^{\circ}\text{C}$ .

### *Starch gel electrophoresis*

Starch gel for serum electrophoresis was prepared as originally described by Smith (1968). The buffers used for gel preparation and electrophoresis are described by Aston and Bradon (1961), with minor modifications. Electrophoresis were performed with constant current of 2.5 m-A/cm for 2 and 1/2 hours and sliced gels stained in 0.01 % amidoblack solution.

### *Immunodiffusion and immunoelectrophoresis*

Agar gel for immunodiffusion and immunoelectrophoresis analysis was prepared using 1 % of special agar-noble (Difco) in barbital buffer, pH 8,4; Electrophoresis was carried out at 5 m-A per slide for 1 and 1/2 hours and immunoprecipitation lines, were developed in approximately 24 hours. Slides were washed, dried and stained in acid fuchsin solution.

### *Quantitative immunoprecipitation*

A series of dilutions of sera from lizards was prepared in borate-buffered saline and a titration curve obtained by mixing 0,2 ml of antiserum with an equal volume of lizard serum dilution. Tubes were allowed for 2 hours at room temperature and then stored for approximately 24 hours at  $-4^{\circ}\text{C}$ . Immune precipitates were washed three times with cold borate-buffer saline and protein contents determined by Lowry method. A typical titration curve is shown in Fig. 1.

## **Results**

The electrophoretic analysis of serum proteins of the lizards studied, revealed some differences mainly confined on  $\alpha$  and  $\beta$  globulin regions (Fig. 2). The electrophoretic patterns of serum proteins of the lizards *L. viridis* and *L. trilineata* don't show major differences. On the contrary, the serum protein pattern of the lizard *L. taurica* differs more pronounced from the corresponding pattern of the other two lizards.

Electrophoretic mobility of albumins of all three lizards are similar.

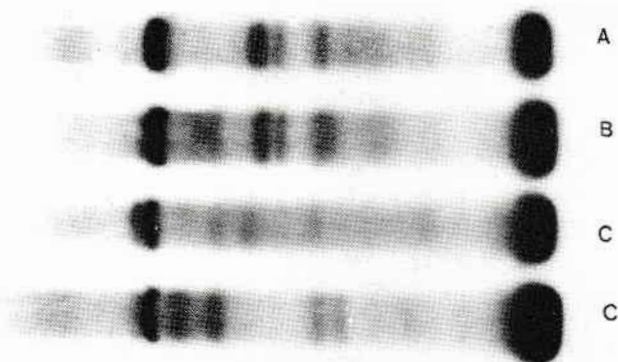


Fig. 2. Electrophoretic patterns of the serum proteins of the lizards: A = *L. trilineata*, B = *L. viridis* and C = *L. taurica*.

The immunoelectrophoretic comparison of serum proteins, using rabbit antisera against serum proteins of the lizard *L. viridis*, reveals strong antigenic differences among the three lizards studied. The differences concern the number of visible precipitin lines and the strength of some of them. At least 12 immunoprecipitation lines were formed

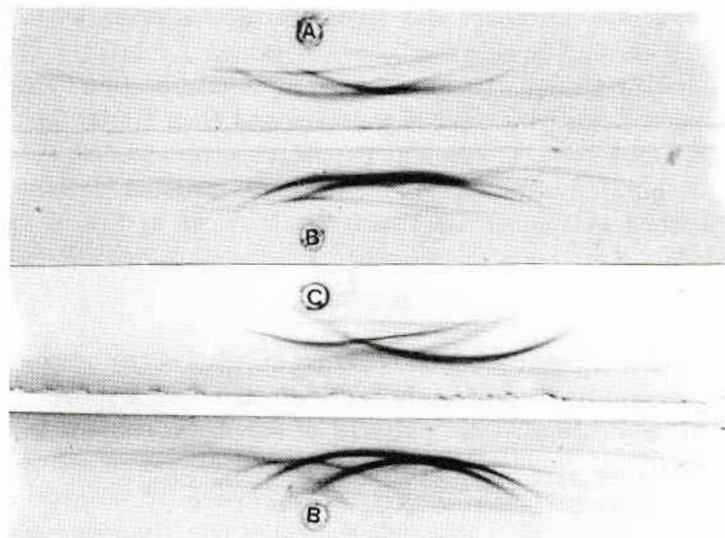


Fig. 3. Immunoelectrophoretic patterns of the serum proteins of the lizards: A = *L. trilineata*, B = *L. viridis* and C = *L. taurica*.

when serum of the lizard, *L. viridis*, was developed with rabbit anti-serum against the serum of that lizard. However, with this antiserum, the sera of the lizards *L. trilineata* and *L. taurica* form 10 and 8 immunoprecipitation lines respectively. The intensity of lines are decreasing as following: *L. viridis* → *L. trilineata* → *L. taurica*. The above differences are shown on Fig. 3. In addition, by immunodiffusion antigenic differences of serum proteins were shown to exist among the above three species (Fig. 4).

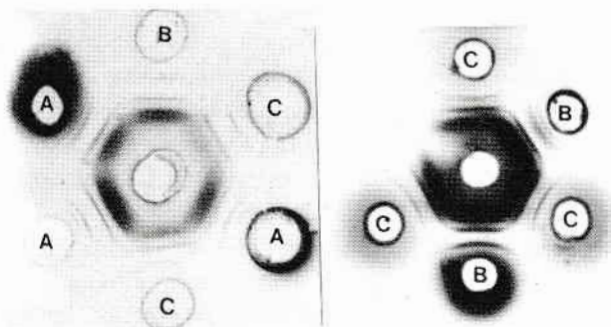


Fig. 4. Comparison of serum proteins of lizards by immunodiffusion: A = *L. trilineata*, B = *L. viridis* and C = *L. taurica*.

The results obtained by quantitative immunoprecipitation are summarized on Table 1. Immune precipitates formed by sera of the three lizards at equivalence zone are expressed as an optical density

Table I. Results of quantitative immunoprecipitation of lizard sera, developed with a rabbit antiserum against serum of *L. viridis*.

Species	Mean O.D. at 750nm	Percentage of precipitation (%)	Immunological distance
<i>L. viridis</i>	1,028 (6)	100	—
<i>L. trilineata</i>	1,012 (7)	98	2
<i>L. taurica</i>	0,620 (6)	60	40

Lizard serum concentration for comparison: 1/30 serum dilution. Numbers in parenthesis show the number of samples tested.

(O.D.) at 750 nm. From the values of O.D. of each species (i.e. 1.028 for *L. viridis*, 1.012 for *L. trilineata* and 0.620 for *L. taurica*), the immunological distances were calculated by abstracting the percentage of each precipitate from 100. The value of 100 corresponds to the immune precipitate of the reference (or homologous) species, the serum of which was used for antiserum preparation.

### Discussion

According to the existing fossil records the modern Squamata and Rhynchocephalia are derived from Lepidosaurian in early permian period and various families of modern lizards appeared in the middle Jurassic (Romer, 1966). In a detailed two-volume monograph of Lacertidae (Boulenger, 1920) this family appear to be a large one. On the above work 22 genera and 145 species belonging to Lacertidae are described. Modern Lacertidae have a palaeartic-ethiopian distribution and in Greece is the most rich in species family among sauria. At least 12 species of Lacertidae are referred to encounter in the mainland and islands of Greece (Ondrias, 1968). However, it seems that serological and immunological studies on *Lacerta* species from Greece are nonexistent.

Both electrophoretic and immunological results obtained in the present study, revealed a great similarity between *L. viridis* and *L. trilineata*. The third lizard *L. taurica* shows more pronounced differences among the three lizards (immunological distance 40 from *L. viridis*).

As far as other morphological characteristics is concern, it can be said very briefly that body size and various body parts measurements and the scale pattern of some body regions, differ strongly in the three lizard species (Table 2). The lizard, *L. taurica* is a small one with a mean

Table II. Some body characteristics of three lizards.

Species	Mean weight (grams)		Mean whole body length (mm)		Mean snout - vent length (mm)	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
<i>L. viridis</i>	40,3 (7)	33,3 (6)	37,2 (7)	32,7 (6)	119 (8)	111 (8)
<i>L. trilineata</i>	—	16,7 (6)	—	29,0 (6)	—	93 (7)
<i>L. taurica</i> *	5,7 (15)	4,1 (7)	18,9 (15)	16,7 (7)	66 (18)	63 (14)

\* From Zakynthos population.

Numbers in parenthesis show the number of samples tested.

body weight of 5,7 (oo) and 4,1 (oo) grams and a snout-to-vent length of 66 (oo) and 63 (oo) mm. On the other extreme is the lizard, *L. viridis*, with corresponding values 40,3 (oo) and 33,3 (oo) grams and 119 (oo) and 111 (oo) mm. Between those extremes lies the lizard, *L. trilineata*. So, serological and immunological findings are in agreement with morphological characteristics. All these findings might suggest an earlier separation of the lizard, *L. taurica*, from the phylogenetic ancestry of the other two lizards. On the other hand, the lizards, *L. viridis* and *L. trilineata* are grouped to the subgenus *Lacerta* but the lizard, *L. taurica*, belongs to the subgenus *Podarcis* (Cyren, 1941 and Boulenger, 1920).

From zoogeographical and ecological point of view there are some preliminary interesting observations. The lizards, *L. viridis*, which is a common species on the continental parts of Greece is a relatively rare species on the Ionian islands studied. The lizard, *L. taurica*, is a dominant species among lizards community on Ionian islands according to our current study and to an older report (Mahnert, 1973). When this lizard is in sympatry with *L. viridis* their ecological requirements are apparently different. So, the large lizard, *L. viridis* has been confined for its activities to habitats with thick bushes and other unapproachable habitats. The lizard, *L. taurica*, is found in different habitats, mainly covered by grass and low vegetation and its density is approximately 1 individual/10m<sup>2</sup>.

The lizards *L. viridis* and *L. trilineata* were found in most cases in the same habitat, even in an area of few m<sup>2</sup>. On this point we are in agreement with other authors (Klemmer, 1975).

### Summary

The present study concerns with phylogenetic relationship of three lizards belonging to Lacertidae, i.e. *L. viridis*, *L. trilineata* and *L. taurica*. The studied populations are those on western parts of Peloponnese and Ionian islands Cephalonia and Zakynthos. The degree of phylogenetic relationships is conferred from electrophoretic and immunological analysis of the serum proteins. These findings are correlated with some ecological and zoogeographical observations. The lizard, *L. taurica*, which is a dominant species in the lizard community of Ionian islands, shows greater remoteness from the lizard, *L. viridis*, in comparison with

the other lizard (*L. trilineata*). Also, some body measurements are in agreement with the immunological and electrophoretic results obtained. The above findings suggest that the lizard, *L. taurica*, was earlier diverged from the common evolutionary line of the lizards under study.

### Résumé

Les auteurs examinent les affinités phylogéniques entre trois espèces du genre *Lacerta* d'après des spécimens provenant de populations du Peloponnèse occid. et des îles Ioniennes. Ils donnent une estimation du degré de parenté en se fondant sur l'analyse électrophorétique et immunologique des protéines sériques et ils font un rapprochement entre leurs résultats et certaines données écologiques et zoogéographiques.

*Lacerta taurica*, qui domine dans les îles de la Mer Ionienne présente un degré de parenté nettement moindre avec *L. viridis* qu'avec *L. trilineata* qui paraît très proche de la première. Ils considèrent que les données somatométriques renforcent les résultats immunologiques et électrophorétiques et concluent que l'espèce, *L. taurica* s'est détachée de façon précoce de la ligne évolutive commune, au départ, aux trois espèces étudiées.

### Περίληψη

Στήν παρούσα έρευνα ανάλυσται ή φυλογενετική συγγένεια τριών ειδών σαυρών τής οικογενείας Lacertidae. Τα είδη που εξετάζονται είναι τα ακόλουθα *Lacerta viridis*, *L. trilineata* και *L. taurica*. Οί εξεταζόμενοι πληθυσμοί προέρχονται από την Δ. Πελοπόννησο και τα νησιά του 'Ιονίου Ζάκυνθο και Κεφαλλονιά. Τα φυλογενετικά εύρήματα συσχετίζονται με όρισμένα οικολογικά και ζωογεωγραφικά στοιχεία. Ο βαθμός τής φυλογενετικής συγγένειας συνάγεται από την ήλεκτροφορητική και ανοσολογική ανάλυση των πρωτεϊνών του όρου. Η σάυρα *L. taurica*, ή όποία κυριαρχεί στα νησιά του 'Ιονίου, παρουσιάζει μικρότερο βαθμό συγγένειας προς τό είδος *L. viridis* ένω τό είδος *L. trilineata* είναι πολύ συγγενές με τό προηγούμενο. Επίσης τά σωματομετρικά στοιχεία ένισχύουν τά ανοσολογικά και ήλεκτροφορητικά αποτελέσματα. Από τά στοιχεία αυτά συμπεραίνεται ότι τό είδος *L. taurica* άποχωρίστηκε ένωρίτερα από την κοινή εξέλικτική γραμμή των εξεταζομένων σαυρών.



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