

Serological Relationships of the Patagonian Toad *Bufo variegatus* (Gunther)

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Bufo variegatus is an intriguing South American toad, usually related to the *spinulosus* group, a southwestern representative of the so-called "narrow-skulled" line of the genus (Blair, 1972). On the other hand it shows some peculiar morphological traits fairly characteristic of the Eurasian *calamita* group, e.g. its rounded parotoids, laterally allocated on a line which is internal to the edge of the upper eyelid. Geographical distribution and ecological trends of *Bufo variegatus* are also interesting, this austral anuran being especially related with the Antartandic or Valdivian *Nothofagus* forest, a biocenotic community relic of the Tertiary times. The Antartandic forest is now restricted to the rainy Chilean environments of the Pacific shores, however in the Oligocenic and Miocenic periods it extended through the Patagonian flats, at present a whole deserts area between the Atlantic coast and the early Cordilleran embossments. A remarkable fossil amphibian fauna has been found there (Scarritt Pocket formation, Upper Oligocene: Schaffer, 1949; Chaffee, 1952). This assembles leptodactylid frogs still existing in the Chilean batrachofauna (*Caelyptocephalella* or *Caudiverbera*, *Eupso-phus*) together with some uncertain forms, e.g. *Eophractus*, and a small toad, also reported as a telmatobiid frog (Lynch, 1971) but probably belonging to the bufonid *calamita*-stock from the northern continent (Tihen, 1962; Estes, pers. comm.). The evidence of a Patagonian *calamita*-like toad in the Cenozoic *Nothofagus* biocenosis sounds very suggestive, having in mind the above mentioned morphological similarities between the living *Bufo calamita* from Europe and *Bufo variegatus*. Ancestral relationships between *Bufo variegatus* and the Holarctic and Paleotropical toads have been postulated by Gallardo (1962). Their common origin was discussed and several morpho-ecological and zoogeographical trends were analyzed by this author, however no further discussions cleared up the puzzling problem of the *Bufo variegatus* relationships or strengthened Gallardo's theoretical assumptions.

Whereas the immunological criterion is of unquestionable discriminating value at interspecific levels, as pointed out in a number of contributions by means of the homo-heterologous precipitin tests, using the current photorelectometric methods, crossed reactions between *Bufo*

variegatus, *B. calamita* and other toads were carried out, their results being reported in the present note. Samples of *Bufo variegatus* from the Valdivian forest (near Valdivia, Chile) and from the neighborhood of the Lacar lake (Neuquén, Argentina) were obtained, at the same time as samples of *Bufo calamita* from Portugal (Minho), *Bufo spinulosus* from Mendoza (Argentina) and *Bufo bufo* from Naples (Italy). All the animals were bled simultaneously and their sera maintained in deep freezer before using. Rabbits from a uniform strain were synchronously used as donors of the antisera; serial antigen injections were reinforced by Freund's adjuvant. Homologous reactions, expressed in percentage of turbidimetric units (crf. Libby's technique; Boyden and DeFalso, 1943; Boyden, 1967) have been assumed as 100%. Relative percentages of the heterologous reactions were considered as numerical expression of the relative similarity of the corresponding antigens in the tested species. They stress significant degrees of serological and genetic relationships, in accordance with the high specificity of the synthesis of the albumin-globulins in vertebrate sera.

The results of the crossed precipitin reactions are reported in Table I. Close serological relationships are pointed out between *Bufo calamita* and *Bufo variegatus* from Chilean and Argentine samples (80–75% in homo-heterologous reactions). Heterologous percentages between *Bufo variegatus* and *Bufo spinulosus* are lower (50–52%) and their serological distance is comparable to that shown by the Valdivian toads and *Bufo bufo* from Europe. Thus only a morphological convergence can be suggested by the precipitin tests between *Bufo variegatus* and *Bufo spinulosus* instead of some preterite phyletic and taxonomic relation. Also in the Martin's discussion

(1972) of the osteological characters of the genus, a *variegatus* group is recognized, differing in several traits from the proper members of the *spinulosus* group, as *spinulosus*, *atacamensis*, *trifolium*, *flavolineatus*, *limensis*, *chilensis*.

The tentative Gallardo's assumption concerning a probable ancient relationship between some early Tertiary ancestors of *Bufo variegatus* and the main line of the Holarctic toads does not disagree on the contrary with the immunological evidence. Moreover the extant short serological distance between *Bufo variegatus* and *Bufo calamita* is a very suggestive finding when the probable presence of *calamita*-like toads in the Oligocenic *Nothofagus* forest of Patagonia is considered. The evolutive interest of our results could be greatly improved if the immunological tests should be extended to another species of the *spinulosus* complex, above all *Bufo rubropunctatus* Guichónot, sympatrid with *Bufo variegatus* on a wide area of its austral, forest range.

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Table I
Percentages of homo-heterologous reactions in crossed precipitin tests between *Bufo variegatus* and other species of toads.

ANTIGENS	ANTISERA		
	<i>Bufo variegatus</i> Valdivia	<i>Bufo calamita</i> Portugal	<i>Bufo spinulosus</i> Mendoza
<i>Bufo variegatus</i> Valdivia	100	78	50
<i>Bufo variegatus</i> Lago Lacar	95	75	—
<i>Bufo calamita</i> Portugal	80	100	55
<i>Bufo bufo</i> Napoles	57	58	50
<i>Bufo spinulosus</i> Mendoza	52	57	100