

BUREAU OF BIOLOGICAL RESEARCH
J. B. ALLISON, DIRECTOR
J. H. LEATHAM, ASSISTANT DIRECTOR
RUTGERS
THE STATE UNIVERSITY
NEW BRUNSWICK, N. J. 08903
U. S. A.

COLECCION HERPETOLOGICA
Y BIBLIOTECA
Dr. José Miguel Cei

The Serological Museum

Bulletin No. 30

• November, 1963

Electrophoretic Patterns and Systematic Relations in South American Toads¹

by J. M. Cei and R. Cohen
*Instituto de Biología,
Universidad Nacional de Cuyo,
Mendoza, Argentina*

The utility of paper electrophoresis as a discriminating method in systematic studies was pointed out in the papers by Dessauer and Fox (6) and Dessauer, Fox, and Ramirez (7). It may be useless or play only a secondary role in studies of species from different phylogenetic stocks. Many recent studies support its value in taxonomic studies of closely related forms, especially those in their incipient stages of speciation.

Since 1959 our interest has been addressed to the study and control of the specificity and characteristics of the seroproteinic patterns of neotropical batrachians belonging to the local bufonid and leptodactylid stocks. We have observed a remarkable and specific constancy in their electrophoretic patterns, under standard conditions of comparison (*i.e.* Buffer pH

1. Presented to the International Conference on Taxonomic Biochemistry, Physiology and Serology held at Lawrence, Kansas, September 1962.

8.6; 9 v/cm run six hours; ionic strength 0.05; 1.5 ma/cm; Whatman 3mm strips). Sera from adult specimens recently bled by cardiac puncture were used. In our experience, frozen sera are equally useful in most instances. We shall present here some conclusive results that demonstrate a significant agreement between the suggested taxonomic relationship of various neotropical species of the genus *Bufo* and arrangements based on the electrophoretic patterns of their serum proteins. The optical densities were determined by an Elphor H densitometer (Bender and Hobein, Munich) Bromphenol blue.

As indicated by Tihen (8) in his attempt to define a supraspecific grouping of the New World toads, the genus *Bufo* still needs systematic and evolutionary studies. Tihen's arrangement and suggested evolution are based on the available osteological characters.

He assumes that the probable early members of this generic stock were similar in their major features to the paleotropical toads such as *regularis*, *mauritanicus*, and related forms. Five major species groups of New World toads are recognized: the *haematiticus* group, with only a single surviving Central American species, originating from the most ancient American stock; the *valliceps* group, a very large and diversified group; the *spinulosus* group, apparently limited to one Andean form; the *americanus* group, extending throughout North America; the *boreas* group, related to a *Bufo bufo*-like stock and probably of very early penetration in the New World continents.

The *valliceps* group was further divided into a South American section, with the species *marinus*, *arenarum*, *ictericus*, *paracnemis*, *crucifer*, *blombergi*; a Mexican section (*alvareus*, *valliceps*, *coccifer*, etc.); and a Car-

ibbean section, very heterogeneous, including the *debilis* complex and *granulosus*, *guentheri*, *pettocephalus*, *punctatus*, *quercicus*, *retiformis*, and *typhonius*.

Our present report deals with the species of the *marinus* stock in the South American section of the *valliceps* group of Tihen, the *spinulosus* group, and three southern forms of the *granulosus* complex.

In a previous publication (Bertini and Cej, 1), the general features of the electrophoretic seroprotein fractions of the *Bufo marinus* complex were illustrated. Another study (5) describes the population variations of these proteins in the Chilean species *Bufo spinulosus*. A remarkable uniformity was found in the serum protein patterns of the four species *Bufo arenarum*, *B. marinus*, *B. paracnemis*, and *B. ictericus*. These species shared some features and also retained some individual variability.

Samples of male, adult specimens of *Bufo marinus* from Costa Rica (S. Jose, April 1962, 7 specimens), from Peru (Iquitos, Amazonia, May 1962, 2 specimens) and Surinam (Paramaribo, May 1962, 4 specimens) were studied again. The specimens represent most of the geographical area of distribution of this species. The electrophoretic trends of *marinus* are illustrated in Figure 1. Previously reported patterns by Bertini and Cej (4) were obtained again in the Surinam and Peru samples. In the Costa Rica specimens, two densitometrically separate fractions of the globulinic G_1 band were frequently obtained. The G_1 fractions are analogous to those obtained in the *ictericus* pattern and are easily separated by elution.

Previous data by Bertini (1) were thus confirmed for the widespread and strongly adaptive *marinus* toad. The specific differences previously found between *paracnemis* and *ictericus* were also repeated. They are the Giant Toad of the xeric "catinga" belt and the Yellowish Toad of the eastern Brazilian tropical forest. Figure 1 shows the seroprotein fractions characteristic for both species. Evidently they originated from a general *marinus*-like type, probably under some specific adaptive condition of plasma equilibrium.

A number of observations by Bertini and Cej (1, 2) support the great stability of the seroprotein pattern of the Argentine Toad *Bufo arenarum*. Quantitative variations of some globulinic fractions were reported in the case of some populations such as those in Cordoba and Mendoza.

Other of our data reaffirm the striking uniformity of the seroprotein constitution of *arenarum*. Our analysis could really support, in quite a different way, Tihen's statement that "... this is a very uniform group osteologi-

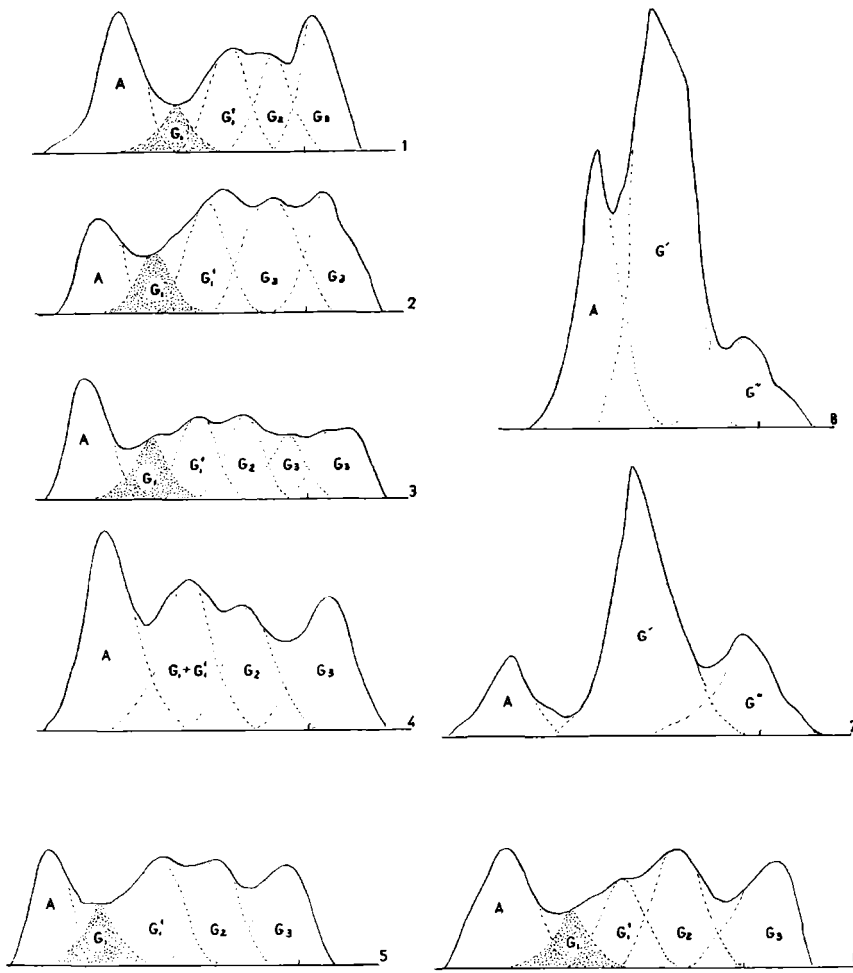


Figure 1. Densitometric curves and electrophoretic patterns in the major species of the *Bufo marinus* group.

Bufo marinus: 1—Iquitos, Peru; 2—Paramaribo, Surinam; 3—S. Jose de Costa Rica; *Bufo paracnemis*: 4—Formosa, Central Chaco; *Bufo ictericus*: 5—Misiones, Argentina; *Bufo arenarum*: 6—Montevideo, Uruguay; *Bufo granulosus* complex: 7—*B.g. fernandezae* Buenos Aires, Argentina; 8—*Bufo gr. mayor*, Formosa, Central Chaco. (7-8, by Bertini, unpublished)

Table I

Relative Concentration of the Seroprotein Fractions in *Bufo spinulosus*
Cordilleran Populations from Mendoza, between 34° and 37° South Latitude

Samples		No. of Specimens	Albumin	Globulins*			Ratio G ₁ /G ₂	Ratio A/G
Cordilleran Streams				G ₁	G ₂	G ₃		
Arroyo Hondo	3 ♂		30.1	27.0	18.2	24.3	1.52	0.43
Carrizalito	2 ♀		23.8	27.8	14.3	33.8	1.92	0.32
Sosneado	4 ♂		31.2	23.4	17.4	27.5	1.36	0.45
	1 ♀		42.5	18.6	13.5	25.2	1.37	0.74
Malargue	1 ♀		24.7	25.2	22.1	27.8	1.11	0.32
Rio Grande	12 ♂		35.5	26.7	19.4	17.9	1.40	0.55
Ranquil	5 ♂		33.9	25.6	18.8	21.2	1.38	0.51
	1 ♀		28.4	22.9	19.7	28.7	1.16	0.40
Cordilleran								
Streams	24 ♂	33.8±0.7†	25.9±0.6	18.8±0.5	21.0±3.4	1.40±0.06	0.51±0.01	
	5 ♀	28.6±3.6	24.4±1.8	16.7±1.7	29.8±1.7	1.49±0.18	0.41±0.18	
<i>Bufo arenarum</i> (from S. Rafael Mendoza, 34° 30' SL)								
	13 ♂	27.1±1.3	22.2±0.6	19.2±0.2	32.7±2.0	1.16±0.05	0.37±0.02	

Cordilleran *Bufo spinulosus* ♂ / *Bufo arenarum* ♂ :

Ratio G₁/G₂, t = 3.00, P = 0.01

Ratio A/G, t = 4.48, P = 0.001

*Globulins numbered in accordance with their mobilities.

†Standard error.

cally; most species differ from each other only in minor detail . . ." ". . . it presumably is descended from the representatives of the group that reached South America at an early date and has undergone only minor changes since that time . . ."

Quite different trends are shown, on the other hand, by the southern representative forms of the *granulosus* complex, belonging to the "Caribbean section" of Tihen. From the studies of Bertini (4, in press) summarized in Figure 1, *Bufo granulosus fernandezae* and *Bufo granulosus d'orbigny*, morphologically closely related, both differ from the close Chacoan form *Bufo granulosus major*, mostly in the relative mobility of the albumin-like, fastest fraction. We are dealing here with a peculiar genetic system, probably in different stages of evolution in its widespread population. From the

patterns presented in Bertini's papers the striking difference between the characteristic albumin and globulin bands of the *marinus* group and those of the *granulosus* group, strongly support the argument for a separate evolution for the two bufonid branches, as proposed by Tihen.

A separate category for the *spinulosus* group is more difficult to accept. We have studied (5) only a population-chain from the Atacama mountains and the deserts in Northern Chile. However, the Central Chilean form, which is synonymous with the *Bufo chilensis* (sic), is incorporated by Tihen in the South American section of the *valliceps* group. An evident geographical variation has occurred. In the northern populations (the Atacama and Huasco samples) the fastest globulinic fractions are impossible to separate by elution, probably because of protein-protein binding. The populations differ also in albumin concentration; a lower A/G ratio is characteristic of the aquatic population from Huasco River which is surrounded by sterile deserts. In the sample from Santiago, Chile, a clear separation into four globulinic bands was recognized, with a relatively very high G₁/G₂ ratio. In our present studies, a number of *spinulosus* populations from the Mendoza Cordilleras were analyzed. The neighboring and, in some instances, sympatric species, *Bufo arenarum*, was used as a comparative electrophoretic standard. Our results are summarized in Table I. The characteristic trends described

above for the Central Chilean populations (*Bufo chilensis* Tschudi) were repeated here in all the series of Mendoza Cordilleran samples. A high albumin content is typical of all the *spinulosus* geographical forms (with the exception of those from the Atacama rivers). Four clearly divided globulinic fractions are recognized in the Central Chilean and Mendoza forms. The forms have a high G₁/G₂ ratio which is always higher than in the surrounding *Bufo arenarum* populations. An evident division of the G₃ band with a small cataphoretic fraction is generally visible in Argentine populations of the *spinulosus* group.

The electrophoretic pattern of *spinulosus* is clearly distinguishable from all other species of the same genus, including its closely allied *arenarum*. The characteristic seroprotein trends of this toad might indicate its convergence with *arenarum* especially in the sympatric geographical areas. However, the difference is not of sufficient weight to separate the group as a distinct species. It is better considered as a section of the *spinulosus* population complex. In Tihen's view "the propriety of recognizing such a distinct species group should be perhaps questionable." Perhaps it should be as he claims that "it should be considered a fourth or Andean section of the *valliceps* group." His same indecision as to the synonymy of *chilensis* with the *marinus* of the South American section supports such a procedure.

In our present report the peculiarities of the seroprotein pattern could be strikingly reinforced from our comparative serological data. Thus, the accuracy of paper electrophoresis as a simple expression of population differences is reaffirmed.

REFERENCES

- Bertini, F. Metodos no morfologicos en el estudio de la Filogenia y Sistemática. La electroforesis en papel aplicada a un problema de diferenciación de subespecies argentinas de *Bufo granulosus*. Tesis Fac. Bioquim. Univ. Nac. Tucuman, 1961.
- Bertini, F. and Cei, J. M. Electroferogramas de proteínas sericas en el genero *Bufo*. Actas Trabajos del Primer Congr. Sudamericano. Zool. LaPlata 12-23, 161-167, 1959.
- Bertini, F. and Cei, J. M. Ovservaciones electroforeticas en seroproteinas de poblaciones argentinas de *Bufo arenarum*. Rev. Soc. Arg. Biol. 36:355-362, 1960.
- Bertini, F. and Cei, J. M. Seroprotein patterns in the *Bufo marinus* complex. Herpetologica 17(4):231-238, 1962.
- Cei, J. M. and Bertini, F. Proteinas sericas de *Bufo spinulosus*; estudio electroforetico y variación geografica. Arch. Bioqu. Farm. Univ. Nac. Tucuman (in press), 1962.
- Dessauer, H. and Fox, W. Characteristic electrophoretic patterns of plasma proteins of orders of Amphibia and Reptilia. Science 124:225-226, 1956.
- Dessauer, H. C., Fox, W., and Ramirez, J. R. Preliminary attempt to correlate paper electrophoretic migration of hemoglobins with phylogeny in Amphibia and Reptilia. Arch. Biochem. 71:11-16, 1957.
- Tihen, J. A. Osteological observations on New World *Bufo*. The American Midland Naturalist. 67(1):157-183, 1962.

Officers of the Serological Museum

Ralph J. DeFalco
Director

Douglas G. Gemeroy
Secretary

Mabel G. Boyden
Custodian of Collections

Alan Boyden
Editor

Joseph G. Baier and Charles A.
Leone

Associate Editors