View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by El Servicio de Difusión de la Creación Intelectual

**Reprint from Herpetologica, Vol. 17, Jan. 20, 1962, No. 4**

COLECCION HERPETOLÓGICA
Y BIBLIOTECA
Dr. José Miguel Cei

BIBLIOTE
JORGE D. WIL

# Seroprotein Patterns in the *Bufo marinus* Complex

F. Bertini and J. M. Cei

The systematic relationships of the large species of neotropical toads of the *Bufo marinus* complex have not been defined since the studies of Lutz (1925). On the basis of recent contributions (Lutz and Kloss, 1952; Cochran, 1955; and Vellard, 1959) the following fundamental taxonomic units can be recognized: 1) an Amazonian population extending north to Mexico, corresponding to *Bufo m. marinus* (Linnaeus) with some subspecies bordering its range, as *B. marinus poeppigii* (Tschudi); 2) a well-defined eastern form, *Bufo ictericus* Spix, from the humid coastal Brasilian realm; 3) a central, latitudinally widespread giant form, *Bufo paracnemis* Lutz, adapted to the dry and open uplands or catingas; 4) two closely related southern and southeastern forms, *Bufo arenarum* Hensel extending from Matto Grosso and Rio Grande do Sul to the paragonian meseta of Río Negro, and *Bufo rufus* Garman in the Brasilian states of Matto Grosso and Minas Gerais (see Fig. 1). The specific interrelation of *B. arenarum* and *B. rufus* have not been studied. *Bufo ictericus* and *B. paracnemis* were formerly described as geographical forms of *B. marinus* (Müller, 1927; Mertens, 1930; Müller and Hellmich, 1936; Lutz and Kloss, 1952) but their specific status was recently re-evaluated (Cochran, 1955). *Bufo paracnemis* appears to be sympatric with *B. arenarum*,

*B. rufus* and *B. ictericus*. *B. ictericus* is probably sympatric with *B. arenarum* and *B. rufus* (see Fig. 1).

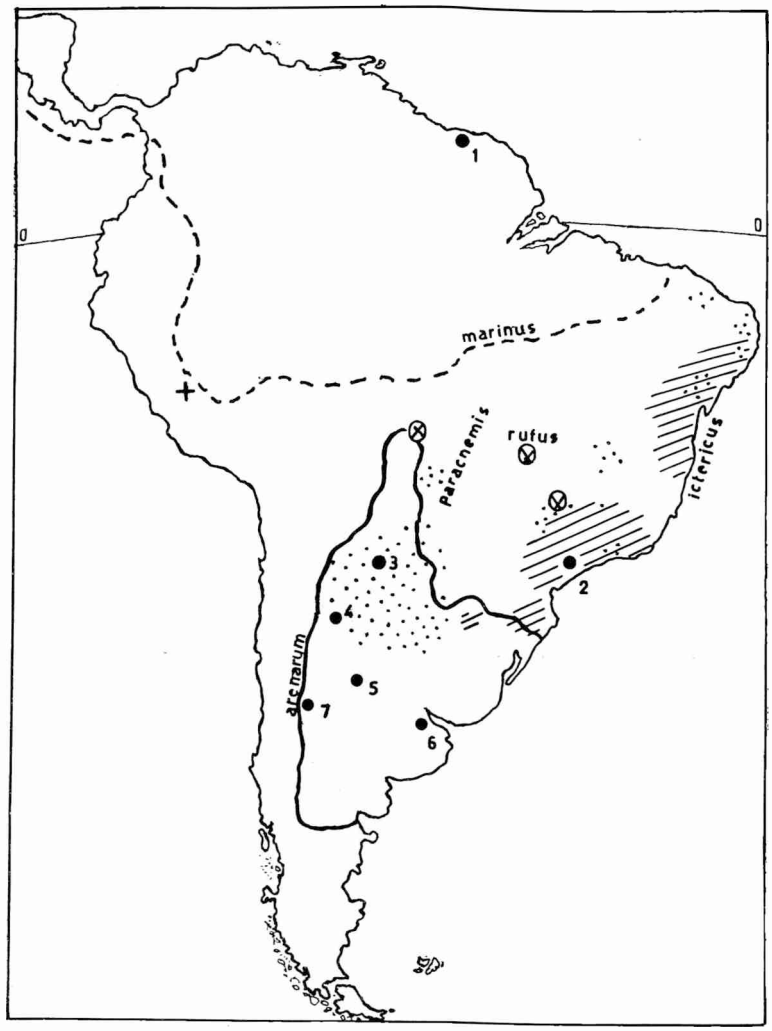


FIG. 1.—Distribution of the toads of the *marinus* complex in South America.

*Bufo marinus* southern limit indicated by dashed line; +, *B. marinus poeppigii;* ×, *B. rufus;* hatched area, *B. ictericus;* stippled areas, *B. paracnemis;* solid line denotes limits of *B. arenarum.*

Numbered localities are the populations of the various species that were studied: 1. Paramaribo, Surinam. 2. Sao Paulo, Brasil. 3. Formosa, Argentine Chaco; 4. Tucuman; 5. Cordoba; 6. Buenos Aires and 7. Mendoza, Argentina.

Recent studies (Buzzati-Traverso and Rechnitzer, 1953; Lanza and Antonini, 1955; Dessauer and Fox, 1956; Boyden and Paulsen, 1957; Zweig and Crenshaw, 1957; Van Sande and Karcher, 1960), indicate the value of biophysical tests in elucidating the relationship of closely related species. The studies here reported are an attempt to check independently the status of the species of the *Bufo marinus* complex by an electrophoretic analysis of the seroproteins.

## Materials and Methods

Sera were analyzed by paper electrophoresis, with paper Whatman 3MM (utilizing Veronal Buffer, 0.05 ionic strength, with 9V per centimeter, run 6 hours at 8° to 10°C. The recognizable bands were cut, eluted and measured by colorimetric methods. Characteristic electrophoretic curves were made by densitometry (Elphor H Densitometer).

All the specimens were received alive in the laboratory, and the sera studied within a week after their extraction. Specimens were from the following localities: *Bufo marinus*, Paramaribo, Surinam; *Bufo ictericus*, Sao Paulo, Brasil;; *Bufo paracnemis*, Formosa, Argentine Chaco, and Tucuman, Argentina; *Bufo arenarum*, Tucuman, Cordoba, Mendoza, and Buenos Aires, Argentina.

## Results and Discussions

As suggested in a preliminary note (Bertini and Cei, (in press), four fundamental fractions of these toads are easily separated in the patterns by their electrophoretic mobility. Such a serological system presents analogies with those observed in other genera of bufonids and leptodactylids (Cei and Bertini, in press *a*). The fast band, which is indicated as A (or albumin-like fraction) is similar in the four species. The relative concentration (Table 1, and Fig. 2) is 27 per cent in *B. marinus*, but increases in *B. arenarum* (31 per cent) and in *B. paracnemis* (33.5 per cent). However, *B. ictericus* presents a strikingly low A-concentration (14 per cent).

*Bufo marinus* probably had an Amazonian origin. It is a very versatile, invasive and adaptative species, as indicated by its secondary colonization of many different regions of the world. *Bufo arenarum* is a typical inhabitant of dry or semi-arid areas, and its variable resistance to desiccation has been reported (Cei, 1959). *Bufo paracnemis* is a xerophilic form, characteristic of the hot and seasonally very differentiated arid centro-neotropical realm, extending from Southern Chaco in Argentine to Bahia, Brasil. In contrast, *Bufo ictericus* is recorded as a toad from the eastern humid forest region. Cochran (1955:29) states: ". . . . It was interesting to note that well-marked individuals of *paracnemis* are found in the same places as *ictericus*, proving the ability of

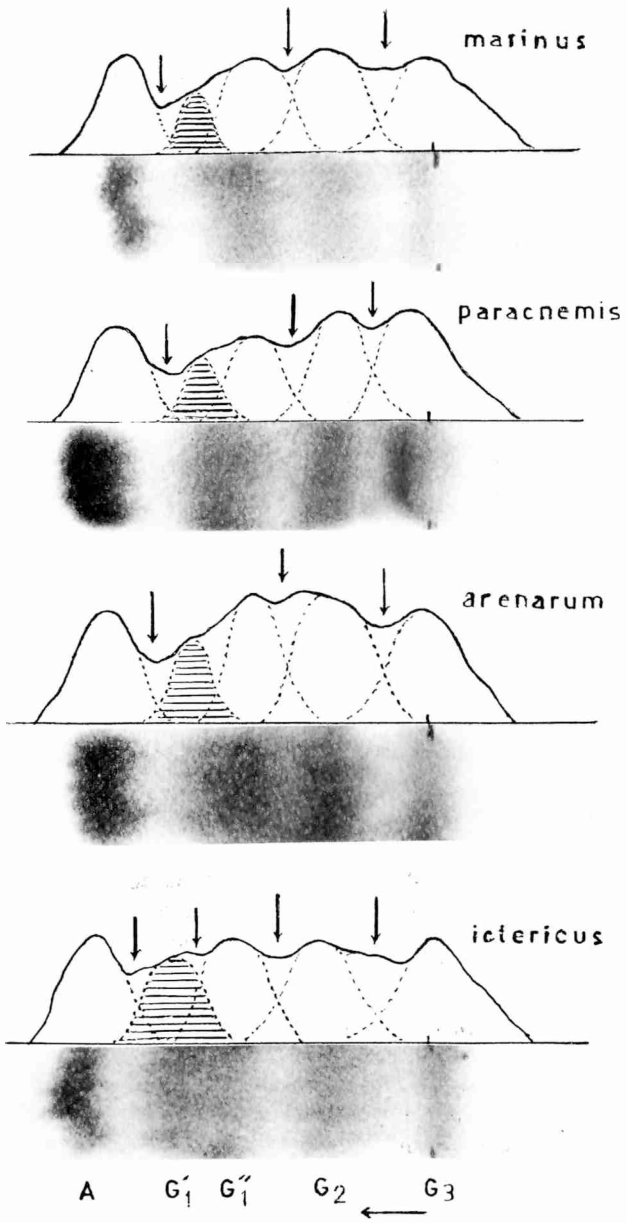


FIG. 2.—Densitometric curves and electrophoretic patterns in the four species studied. Arrows indicate the position of the cuts made for elution.

both to exist as distinct species without intergrading. Apparently *ictericus* frequents streams all of the time, while *paracnemis* roams

widely on the drier parts of the mountain ridges except during the actual breeding season when it also must resort to the water . . . ." ". . . . *Bufo ictericus* seems to be completely divided geographically, at least in eastern Brasil, from the northern *marinus* by the species *paracnemis*, which occupies Minas Gerais, Bahia and Pernambuco, living in dry upland regions which are impossible to the more aquatic *marinus* and *ictericus*."

Thus considering the well-recognized physiological function of albumin as onco-osmotic regulating factor of the body-water, our electrophoretic results fit the ecological position of the toads in the order of their relative water independence. Many authors have demonstrated that the albumin component is lacking or in low concentration in aquatic vertebrates (e.g. Irisawa and Irisawa, 1954). Frieden *et al.* 1957 show electrophoretically that a sudden increase of the albumin peak in anurans is correlated with metamorphosis.

The following globulin fractions, indicated as $G_1$, $G_2$, $G_3$ in our patterns, demonstrate other specific characteristics. In the four species the $G_1$ band can be divided densitometrically into two fractions ($G_1$ and $G_1'$). However, only in *Bufo ictericus* are the two fractions clearly separable so that they can be eluted independently and their relative concentrations determined.

The ratio of relative concentration of bands $G_1$ and $G_2$ reveals some significant specific trends. The mean of the individual ratios is higher in *B. marinus* (1.24-1.41) than in *B. paracnemis* (1.12-1.16), but very low in *arenarum* (0.73-1.10) where some remarkable populational differences are reported (Bertini and Cei, 1960). If both $G_1$ fractions of *ictericus* were considered together the ratio $G_1/G_2$ would be strikingly elevated in this species (1:54).

In some individual samples of *B. paracnemis* the $G_3$ band is divided into two similar fractions. Due to the variability of such division we have considered both the fractions as one band in all calculations of concentration of $G_3$. The $G_3$ band does not present evident differences between the four species.

The four toads show related electrophoretic patterns and can be clearly distinguished from other species of the genus *Bufo*. For comparison in Fig. 3 the electrophoretic pattern of a Chilean population of *Bufo spinulosus* is presented (Cei and Bertini, in press b). It is obvious that this pattern corresponds to another species of bufonids not in the *marinus* complex.

*Bufo paracnemis* can be defined as a *B. marinus*-like toad with a very high albumin concentration and a lower ratio of $G_1/G_2$ globulin bands. *Bufo arenarum* can be distinguished from *B. marinus* and *B. paracnemis* by a very low $G_1/G_2$ ratio. *Bufo ictericus* presents a very low albumin concentration, an evident division of the

$G_1$ band into two similar fractions, and the highest $G_1/G_2$ ratio. *B. ictericus* is therefore the most differentiated form of the group.

Perhaps *B. arenarum* and *B. paracnemis* are evolutionary lines from a *marinus*-like stock, while the peripheral species *B. ictericus* represents an independent evolution from *B. marinus* of a species adapted to less arid conditions than *B. arenarum* and *B. paracnemis*.

TABLE 1.—Relative concentration of the seroprotein fractions in the toads of the *marinus* complex.

| SPECIES and NO. | | ALBUMIN | GLOBULINS | | | |
|---|---|---|---|---|---|---|
| | | A | $G_1$ | $G_2$ | $G_3$ | Ratio, $G_1/G_2$ |
| *Bufo marinus* | | | | | | |
| SURINAM | ♂ 11 | 268.±3.7 | 24.3±1.4 | 21.5±2.9 | 26.2±4.0 | 1.24 |
| April, 1960 | | (18.9-37.7) | (18.5-31.0) | (17.3-36.1) | (20.7-42.6) | |
| | ♀ 6 | 27.1±8.7 | 26.3±2.6 | 17.3±2.5 | 27.4±5.3 | 1.41 |
| | | (19.7-37.6) | (22.0-31.0) | (14.4-24.0) | (20.3-34.9) | |
| *Bufo ictericus* | | | | | | |
| SAO PAULO | ♂ 11 | 14.2±1.5 | 15.2±1.4* | 23.9±1.3 | 25.8±2.9 | 1.54** |
| Nov., 1959 | | ( 6.0-23.6) | ( 7.6-24.3) | (15.5-31.8) | (13.1-47.6) | |
| *Bufo paracnemis* | | | | | | |
| TUCUMAN | ♂ 6 | 33.5±7.0 | 23.5±0.9 | 20.4±1.7 | 22.8±1.9 | 1.16 |
| Dec., 1959 | | (23.9-43.5) | (20.0-25.7) | (16.9-25.5) | (19.2-27.8) | |
| | ♀ 4 | 25.6±16.5 | 23.5±1.9 | 20.7±1.9 | 30.1±3.9 | 1.14 |
| | | (14.8-33.5) | (20.9-26.8) | (18.8-24.9) | (26.1-35.5) | |
| FORMOSA | ♂ 11 | 23.6±1.3 | 26.7±0.9 | 26.2±6.7 | 24.2±2.6 | 1.12 |
| March, 1960 | | ( 7.1-29.2) | (20.1-32.5) | (16.8-43.9) | (17.3-33.5) | |
| *Bufo arenarum* | | | | | | |
| MENDOZA | ♂ 24 | 31.1±1.0 | 21.6±0.7 | 27.1±1.3 | 27.1±1.3 | 1.07 |
| Aug., 1959 | | (18.2-39.8) | (14.4-26.5) | (15.6-23.7) | (17.2-47.3) | |
| Aug., 1960 | ♂ 10 | 23.9±1.1 | 23.8±0.8 | 21.5±0.7 | 30.5±1.9 | 1.10 |
| | | (20.4-30.1) | (19.0-27.1) | (17.9-24.7) | (21.5-44.2) | |
| CORDOBA | ♂ 11 | 22.5±1.7 | 25.7±0.9 | 25.1±1.4 | 26.3±1.5 | 1.02 |
| Aug., 1960 | | (14.9-33.7) | (18.4-27.7) | (20.4-33.0) | (18.9-36.4) | |
| TUCUMAN | ♂ 10 | 27.3±1.1 | 22.1±0.6 | 26.6±0.6 | 23.7±1.0 | 0.87 |
| Aug., 1959 | | (22.0-31.3) | (19.1-25.0) | (24.0-29.5) | (16.8-27.1) | |
| BUENOS AIRES | ♂ 10 | 30.2±0.8 | 20.6±0.8 | 28.0±0.9 | 21.1±0.7 | 0.73 |
| Aug., 1959 | | (25.8-35.0) | (17.3-24.6) | (22.7-32.9) | (19.0-26.4) | |
| | ♀ 4 | 29.1±1.2 | 22.6±1.1 | 26.8±1.6 | 21.6±2.3 | 0.84 |
| | | (26.3-32.0) | (20.4-25.0) | (22.1-29.2) | (17.0-27.0) | |

*In this species a division of Globulin $G_1$ into two parts has been demonstrated. The second fraction we choose to call $G_1'$. It has the following value:

21.7±0.9
(16.0-26.3)

**$(G_1+G_1'/G_2)$

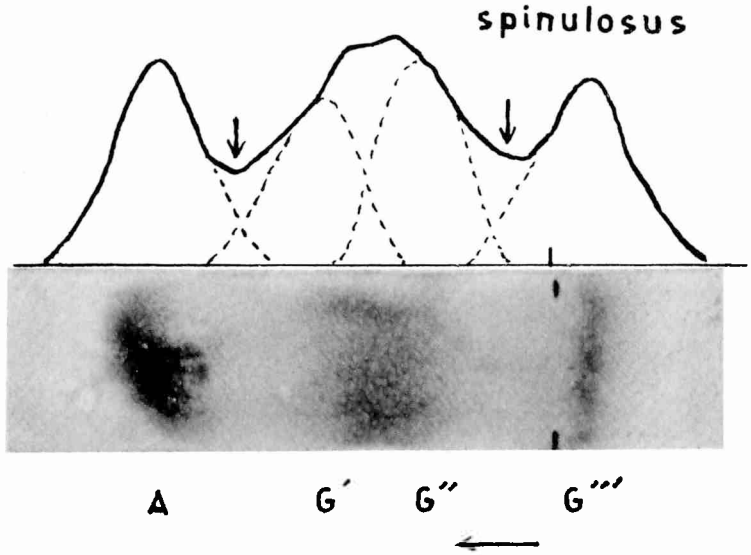


FIG. 3.—Electrophoretic patterns of *Bufo spinulosus* from S. Pedro de Atacama, Chile. This pattern is markedly different from that of the *marinus* complex.

In any case, the seroprotein patterns of *B. paracnemis* and *B. ictericus* support the classification of each form as a distinct species (Cochran, 1955).

The analyzed seroproteins characteristic of these species can be summarized as follows:

| | A-concentration (%) | $G_1$ band | $G_1/G_2$ ratio |
|---|---|---|---|
| *marinus* | high | not well divided | 1.24-1.41 |
| *paracnemis* | high | not well divided | 1.12-1.16 |
| *arenarum* | high | not well divided | 0.73-1.10 |
| *ictericus* | low | divided, two fractions | 1.54 |

ACKNOWLEDGMENTS

We wish to acknowledge for their valuable help in obtaining material Mr. W. Bockerman from Sao Paulo, Brasil and Dr. C. D. Geijskes of the Museum of Surinam, Paramaribo. We wish to thank for her valuable help in the Laboratory Dr. A. M. Paganotto of the Universidad Nacional de Cuyo, San Luis. Many special thanks for the very valuable aid in the preparation of the manuscript to Dr. R. Ruibal of the University of California, Riverside.

This project was partially supported by financial assistance from the Consejo Nacional de Investigaciones Cientificas y Tecnicas, Argentina.

LITERATURE CITED

BERTINI, F. AND J. M. CEI. (in press). Electroferogramas de proteinas séricas en el genero *Bufo*. Acta 1° Congr. Sudamer. Zoologia. La Plata.

———. 1960. Observaciones electroforéticas en seroproteinas de poblaciones argentinas de *Bufo arenarum* Hensel. Rev. Soc. Arg. Biol., 36:355-362.

BOYDEN, A. A. AND E. C. PAULSEN. 1957. Significance of serum electrophoretic patterns in systematic serology. Serol. Mus. Bull., 18:7.

BUZZATI, TRAVERSO A. A. AND A. B. RECHNITZER. 1953. Paper partition Chromatography in taxonomic studies. Science, 117(3029):58.

CEI, J. M. 1959. Ecological and physiological observations on polymorphic populations of the toad *Bufo arenarum* Hensel from Argentine. Evolution, 13(4):532-6.

CEI, J. M. AND F. BERTINI. (in press *a*). Diferencias entre *Leptodactylus ocellatus* y *L. chaquensis* reveladas por via electroforética en suero. Acta 1° Congr. Sudamer. Zoologia, La Plata.

———. (in press *b*). Seroproteinas de poblaciones de *Bufo spinulosus*. estudio electroforético y variación geográfica.

COCHRAN, D. M. 1955. Frogs of Southeastern Brasil. United States Nat. Mus. Bull. 206:1-423.

DESSAUER, H. AND W. FOX. 1956. Characteristic Electrophoretic Patterns of plasma proteins of orders of Amphibia and Reptilia. Science, 124(3214):225-6.

FRIEDEN, E., A. E. HERNER, L. FISH AND E. J. CASSON LEWIS. 1957. Changes in serum proteins in Amphibian Metamorphosis. Science, 126(3273): 559-560.

IRISAWA, H. AND A. F. IRISAWA. 1954. Blood serum protein of the marine Elasmobranchii-. Science, 120:849.

LANZA, B. AND F. M. ANTONINI. 1955. Sulla possibilitá di distinguere specie tra loro diverse per mezzo del protidogramma del siero-Studio su *Rana esculenta* L. e su *Rana dalmatina* Bonap. Monit. Zool. Ital., 63(4):293-9.

LUTZ, A. 1925. Batraciens du Brésil, II. Compt. Rend. Soc. Biol. Paris. 93(22):211-224.

LUTZ, B. AND G. R. KLOSS. 1952. Anfibios anuros do Alto Solimões e Rio Negro. Apontamentos sobre algumas formas e suas vicariantes. Mem. do Inst. Oswaldo Cruz. 50:625-678.

MERTENS, R. 1930. Zoologische Ergebnisse einer Reise von Otto Condé. 2-Amphibien und Reptilien. Folia Zool. Hydrob. Univ. Lettlands, Riga, 1-2:161-6.

MÜLLER, L. 1927. Amphibien and Reptilien der Ausbeute Prof. Bresslau's in Brasilien, 1913-14. Abhandl. Senckerb. Naturf. Gesellsch., 40(3):259-304.

MÜLLER, L. AND W. HELLMICH. 1936. Wisenschaftliche Ergebnisse der Deutschen Gran Chaco-Expedition. Amphibien und Reptilien. 1-Teil: Amphibia, Chelonia, Loricata:1-120.

VAN SANDE, M. AND D. KARCHER. 1960. Species Differentiation of Insects by Haemolymph Electrophoresis. Science, 131(3407):1103-4.

VELLARD, J. 1959. El genero *Bufo*. Mem. Mus. Hist. Nat. Jav. Prado, Lima. 8:1-48.

ZWEIG, G. AND J. W. CRENSHAW. 1957. Differentiation of species by paper Electrophoresis of serum proteins of *Pseudemys* Turtles. Science, 126 (3282):1065-7.

*Instituto de Biologia, Fac. Ciencias Medicas, Universidad Nacional de Cuyo, Mendoza, Argentina.*