

J. H. LEATHEM, DIRECTOR  
 BUREAU OF BIOLOGICAL RESEARCH  
 RUTGERS UNIVERSITY  
 THE STATE UNIVERSITY OF  
 NEW JERSEY  
 NEW BRUNSWICK, N. J. 08903  
 U. S. A.

# The Serological Museum

Bulletin No. 48

December, 1972

## Archaeobatrachia Versus Neobatrachia: A First Serological Approach

J. M. Cei \*

Since its discovery in *Ceratophrys ornata* (Saez and Brum, 1959), octoploidy of neotropical ceratophryid frogs (*Ceratophrys ornata*, *C. dorsata*) was demonstrated by a number of cytological contributions (Bogart, 1967; Beçak, Beçak and Rabello, 1967). However the peculiar geographical trends of polyploid-diploid populations of *Ceratophrys ornata* were explained by Barrio et al. (1970). On the other hand Morescalchi (1967) and Bogart (1969) point out the striking similarity between the diploid karyotypes of *Ceratophrys* or *Lepidobatrachus* ( $2n = 26$ ) and the karyotypes of the holarctic Pelobatid frogs (*Pelobates*, *Scaphiopus*). Such cytological evidence may suggest some kind of evolutionary and phyletic relationships between a Northern or Laurasian Pelobatid stock and a Southern, probably Gondwanian, Leptodactylid stock of Anurans (Morescalchi, 1967). That assumption could be a really surprising one, because of the past and present disjunctive distribution of Pelobatidae and Leptodactylidae, and the still widely accepted phylogenetic relationships between the ancient Discoglossid and Pelobatid lines (Noble, 1931; Tihen, 1965; Inger, 1967; Zweifel, 1956; Estes, 1970). Moreover the recent systematic arrangements by Laurent (1942; 1967) or Reig (1958) emphasize the subordinal status of Archaeobatrachia (Liopelmoidea, Pipoidea, Discoglossioidea, Pelobatoidea, Microhyloidea: in accordance with Laurent, 1967), opposing them to suborder Neobatrachia, which assembles the so-called "modern" or morphologically more evolved families of frogs, such as Leptodactylids, Ranids, Bufonids, Hylids, etc. The natural placement of Pelobatoidea fits therefore nearer the phyletic step of Discoglossioidea, than that of Leptodactylids, in agreement with the most classical views since Noble's reports, and in discordance with the above mentioned new cytological findings.

It would be interesting to carry on a serological study of the controversial question by means of the precipitin tests,

TABLE I

anti- <i>Pelobates cultripes</i> PORTUGAL	× <i>Pelobates cultripes</i> (PORTUGAL) 553 <i>Pelobates fuscus</i> (RUMANIA) <i>Pelobates syriacus</i> (ISRAEL)	100 % 83.9 80.5
SERUM A	<i>Discoglossus pictus</i> (PORTUGAL) <i>Bombina bombina</i> (RUMANIA)	70.4 66.1
	<i>Ceratophrys ornata</i> (TUCUMAN-ARG.) <i>Ceratophrys ornata</i> (SANTA FE-ARG.) <i>Pyxicephalus adspersus</i> (RHODESIA) <i>Breviceps gibbosus</i> (CAPETOWN) <i>Hyla arborea</i> (ISRAEL)	31.4 30.6 28.4 27.6 23.3
anti- <i>Pelobates cultripes</i> PORTUGAL	× <i>Pelobates cultripes</i> (PORTUGAL) <i>Scaphiopus couchii</i> (ARIZONA) <i>Discoglossus pictus</i> (ALGER) <i>Xenopus gilli</i> (CAPETOWN)	100 % 79.5 73.4 66.2
SERUM B	<i>Rana ridibunda</i> (ALGER) <i>Bufo mauritanicus</i> (ALGER) <i>Ceratophrys ornata</i> (TUCUMAN-ARG.) <i>Lepidobatrachus asper</i> (SANTIAGO DEL ESTERO-ARGENTINA).	36.1 33.7 30.1 25.2
anti- <i>Pelobates cultripes</i> PORTUGAL	× <i>Pelobates cultripes</i> (PORTUGAL) <i>Pelobates syriacus</i> (ISRAEL)	100 % 82.5
SERUM C	<i>Scaphiopus intermontanus</i> (STATE OF WASHINGTON-USA). <i>Discoglossus pictus</i> (PORTUGAL) <i>Xenopus laevis</i> (CAPETOWN) <i>Ceratophrys ornata</i> (SANTA FE-ARG.)	77.9 69.5 64.1 30.00
ANTISERUM 1:1 anti- <i>Pelobates cultripes</i> PORTUGAL	× <i>Pelobates cultripes</i> (PORTUGAL) <i>Pyxicephalus adspersus</i> (RHODESIA) <i>Ceratophrys ornata</i> (BUENOS AIRES-AGR.) <i>Ceratophrys ornata</i> (SANTA FE-ARG.)	100 % 26.6 30.1 31.3
SERUM D anti- <i>Pelobates syriacus</i> ISRAEL	× <i>Pelobates syriacus</i> (ISRAEL) <i>Pelobates cultripes</i> (PORTUGAL) 533 <i>Pelobates cultripes</i> (PORTUGAL) 592 <i>Scaphiopus intermontanus</i> (STATE OF WASHINGTON-USA) <i>Discoglossus pictus</i> (PORTUGAL) <i>Xenopus laevis</i> (CAPETOWN)	100 % 82.2 81.3 74.6 73.7 60.4
	<i>Ceratophrys ornata</i> (TUCUMAN-ARG.) <i>Ceratophrys ornata</i> (SANTA FE-ARG.) <i>Pyxicephalus adspersus</i> (RHODESIA)	32.0 33.3 28.4
anti- <i>Scaphiopus couchii</i> ARIZONA	× <i>Scaphiopus couchii</i> (ARIZONA) <i>Pelobates cultripes</i> (PORTUGAL) 592 <i>Pelobates cultripes</i> (PORTUGAL) 553 <i>Discoglossus pictus</i> (PORTUGAL)	100 % 77.9 71.3 45.5

(Continued on page 2)

\* Instituto Biología Animal, Universidad Nacional de Cuyo, Mendoza, Argentina.

following the careful photronreflectometric techniques introduced since 1943 by Alan Boyden and colleagues, and applied on several occasions to the phyletic and evolutionary problems of Anurans (Cei, 1963; 1965; 1969; 1970a, 1970b, 1972a, 1972b). Extensive information about the characteristics and performance of the Libby's photronreflectometer (AMINCO) used in the present work, may be obtained from many of the former papers (Boyden, Bolton, Gemeroy, 1947; Boyden, 1956, 1967). Sera have been obtained by cardiac puncture and stored at  $-20^{\circ}\text{C}$ , all in a suitable interval of time to avoid the effects of aging on the specific antigenic properties (Frair, 1969; Cei and Castro, 1970). Immune sera were produced in rabbits, by reenforced antigens (Freund's coadjuvant). Antigen dilutions in homo-heterologous tests began from a first 1:5 dilution (Evans Buffer). The following simultaneous samples of frogs have been utilized: 24 species and 21 localities, covering a good bit of the whole distribution of the involved taxa of Pipidae, Discoglossidae, Pelobatidae, Ceratophryidae, Leptodactylidae, Bufonidae, Hylidae, Ranidae, Microhylidae.

Pipidae: *Xenopus laevis*, *Xenopus gilli*, from Capetown (South Africa); Discoglossidae: *Discoglossus pictus*, from Algarve (Portugal) and Alger (Algeria), *Bombina bombina* from Bucuresti (Rumania); Pelobatidae: *Pelobates cultripes* from Porto de Mos (Portugal), *Pelobates syriacus* from Israel, *Pelobates fuscus* from Bucuresti (Rumania), *Scaphiopus intermontanus* from the State of Washington (United States), *Scaphiopus couchii* from Arizona (United States); Hylidae: *Hyla arborea* from Israel, *Hyla meridionalis* from Algarve (Portugal); Ceratophryidae: *Ceratophrys ornata* from Tucumán, Santa Fe and Buenos Aires (Argentina), *Lepidobatrachus asper* from Santiago del Estero (Argentina); Leptodactylidae: *Odontophrynus occidentalis* from Córdoba (Argentina), *Leptodactylus ocellatus* from Mendoza (Argentina); Bufonidae: *Bufo mauritanicus* from Alger (Algeria), *Bufo bufo* from Paris (France), *Bufo arenarum* from San Luis (Argentina); Ranidae: *Rana ridibunda* from Alger (Algeria), and Lisboa (Portugal), *Rana grayi* from Capetown (South Africa); *Ptychadaena superciliaris* from Moçamedes (Angola), *Pyxicephalus adspersus* from Salisbury (Rhodesia), *Pyxicephalus delalandi* from Capetown (South Africa); Microhylidae: *Breviceps gibbosus* from Capetown (South Africa).

The results of the crossed precipitin reactions are indicated in the tables I and II. The results of the tests with anti-*Pelobates cultripes* serum (B) are also plotted on the graphs of figure 1. It is quite evident that any crossed test between species of the same genus gives a very high homo-heterologous percent, suggesting a great amount of common

TABLE I (Continued)

	<i>Rana ridibunda</i> (ALGER)	27.6
	<i>Bufo mauritanicus</i> (ALGER)	25.3
	<i>Ceratophrys ornata</i> (TUCUMAN-ARG.)	31.6
	<i>Odontophrynus occidentalis</i> (CORDOBA-ARG.)	20.0
	<i>Leptodactylus ocellatus</i> (MENDOZA-ARG.)	24.6
anti- <i>Discoglossus pictus</i> ALGER	× <i>Discoglossus pictus</i> (ALGER)	100 %
	<i>Pelobates cultripes</i> (PORTUGAL)	70.8
	<i>Pelobates syriacus</i> (ISRAEL)	75.9
	-----	
	<i>Scaphiopus couchii</i> (ARIZONA)	43.3
	-----	
	<i>Rana ridibunda</i> (ALGER)	31.4
	<i>Bufo mauritanicus</i> (ALGER)	25.9
	<i>Ceratophrys ornata</i> (TUCUMAN-ARG.)	26.7
	<i>Ceratophrys ornata</i> (SANTA FE-ARG.)	24.0
	<i>Odontophrynus occidentalis</i> (CORDOBA-ARG.)	23.6

TABLE II

anti- <i>Ceratophrys ornata</i> TUCUMAN- ARGENTINA	× <i>Ceratophrys ornata</i> (TUCUMAN-ARG.)	100 %
	<i>Ceratophrys ornata</i> (SANTA FE-ARG.)	93.9
	<i>Ceratophrys ornata</i> (BUENOS AIRES-ARG.)	91.6
	-----	
	<i>Odontophrynus occidentalis</i> (CORDOBA-ARG.)	33.9
	<i>Leptodactylus ocellatus</i> (MENDOZA-ARG.)	32.6
	<i>Pelobates cultripes</i> (PORTUGAL)	31.3
	<i>Scaphiopus couchii</i> (PORTUGAL)	30.6
	<i>Discoglossus pictus</i> (ALGER)	25.3
	<i>Rana ridibunda</i> (ALGER)	23.7
	<i>Pyxicephalus adspersus</i> (RHODESIA)	25.8
	<i>Bufo mauritanicus</i> (ALGER)	30.0
anti- <i>Pyxicephalus adspersus</i> RHODESIA	× <i>Pyxicephalus adspersus</i> (RHODESIA)	100 %
	<i>Pyxicephalus delalandi</i> (CAPETOWN)	84.2
	-----	
	<i>Breviceps gibbosus</i> (CAPETOWN)	44.6
	-----	
	<i>Rana grayi</i> (CAPETOWN)	38.5
	<i>Rana ridibunda</i> (ALGER)	32.9
	-----	
	<i>Pelobates cultripes</i> (PORTUGAL)	29.4
	<i>Leptodactylus ocellatus</i> (MENDOZA-ARG.)	30.9
	<i>Ceratophrys ornata</i> (TUCUMAN-ARG.)	26.3
	<i>Bufo mauritanicus</i> (ALGER)	24.3
	<i>Hyla meridionalis</i> (PORTUGAL)	35.5
anti- <i>Rana ridibunda</i> ALGER	× <i>Rana ridibunda</i> (ALGER)	100 %
	<i>Rana ridibunda</i> (PORTUGAL)	88.9
	-----	
	<i>Ptychadaena superciliaris</i> (ANGOLA)	42.4
	-----	
	<i>Pyxicephalus adspersus</i> (RHODESIA)	33.9
SERUM A	-----	
	<i>Bufo mauritanicus</i> (ALGER)	28.1
	<i>Pelobates cultripes</i> (PORTUGAL)	32.9
	<i>Scaphiopus couchii</i> (ARIZONA)	26.4
	<i>Discoglossus pictus</i> (ALGER)	30.3
	<i>Ceratophrys ornata</i> (TUCUMAN-ARG.)	25.5
	<i>Leptodactylus ocellatus</i> (MENDOZA-ARG.)	25.7
anti- <i>Rana ridibunda</i> ALGER	× <i>Rana ridibunda</i> (ALGER)	100 %
	<i>Pyxicephalus adspersus</i> (RHODESIA)	33.7
	-----	
	<i>Breviceps gibbosus</i> (CAPETOWN)	31.4
SERUM B	<i>Bufo bufo</i> (FRANCIA)	26.8
	<i>Ceratophrys ornata</i> (SANTA FE-ARG.)	23.5
	<i>Ceratophrys ornata</i> (TUCUMAN-ARG.)	24.1

(Continued on page 3)

whole albumin-globulin antigens. Between *Pelobates cultripes*, *syriacus* and *fuscus* the percents exceed 80; percents such as 84.2 or 88.9 are given by *Pyxicephalus adspersus* × *P. delalandi*, and *Rana ridibunda* from Portugal × *Rana ridibunda* from Algeria. All the experimental populations of *Ceratophrys ornata* exceed percents of 90 in many reciprocal tests, stressing a remarkable serological homogeneity of the species both in its diploid (Tucumán, Santa Fe) and octoploid populations (Buenos Aires). On the contrary the impressive serological differentiation of the widespread species of the *Bufo* group may be pointed out.

A noticeable high level of serological relationships was then supported by the several crossed tests between Discoglossid, Pipid and Pelobatid frogs. Percents ranging from 71.3 and 79.5 are the rule between *Pelobates* and *Scaphiopus*, but also between *Pelobates* and *Discoglossus*. The amount of common whole seric antigens seems to be considerable (66.1%–75.9%), lowering somewhat in the crossed reactions between *Scaphiopus* and *Discoglossus* (43.3%–45.5%). Likewise percents of the homo-heterologous reactions are significantly high in the tests between Pelobatids and *Xenopus* (60.4%–66.2%). The serological reality of Archaeobatrachia is therefore evident in agreement with the former tentative approaches by Reig or Laurent.

But our attempts failed to make evident any eventual serological tie between Pelobatid and Ceratofryid frogs, to support a re-examination of their phyletic relationships in accordance with the reported caryological similarity. All the performed 15 crossed reactions between *Ceratophrys* or *Lepidobatrachus* and *Pelobates*, *Scaphiopus* or *Discoglossus* lie always at percents ranging from 24.0 to 33.3. They are not dissimilar from percents observed between Pelobatids or Discoglossids and *Leptodactylus* and *Odontophrynus* (20.0%–24.6%), but likewise they do not differ from percents between Pelobatids or Discoglossids and Ranids (*Rana*, *Ptychadaena*, *Pyxicephalus*: 26.6%–36.1%) and between Pelobatids or Discoglossids and Bufonids (24.1%–33.7%), Hylids (23.3%) or Microhylids (25.7%–27.6%). A true breakage seems to separate Archaeobatrachia and Neobatrachia, and serological distances between *Ceratophrys* and *Pelobates* do not support a close evolutive relationship such as could be inferred by their showy caryological convergences.

It is a noticeable observation that serological distances between Ceratofryids, Leptodactylids, Bufonids, Hylids, Ranids and Microhylids, are very large (percents: 23.5–35.5%) and comparable to the same distances extending between Archaeobatrachia and Neobatrachia. That means a quicker evolutive or genetic differentiation of the probably tachytelic, stocks of "modern" Anurans. In example, Ranids placed some years

TABLE II (Continued)		
anti- <i>Breviceps gibbosus</i> CAPETOWN	× <i>Breviceps gibbosus</i> (CAPETOWN)	100 %
	<i>Pyxicephalus adspersus</i> (RHODESIA)	42.0
	<i>Rana ridibunda</i> (ALGER)	33.5
	-----	
	<i>Bufo mauritanicus</i> (ALGER)	27.5
<i>Pelobates cultripes</i> (PORTUGAL)	25.7	
anti- <i>Bufo mauritanicus</i> ALGER	× <i>Bufo mauritanicus</i> (ALGER)	100 %
	<i>Bufo arenarum</i> (SAN LUIS-ARG.)	54.6
	-----	
	<i>Rana ridibunda</i> (ALGER)	30.4
	<i>Pelobates cultripes</i> (PORTUGAL)	30.6
SERUM A	<i>Scaphiopus couchii</i> (ARIZONA)	24.7
	<i>Discoglossus pictus</i> (ALGER)	24.1
	<i>Ceratophrys ornata</i> (TUCUMAN-ARG.)	33.0
	<i>Leptodactylus ocellatus</i> (MENDOZA-ARG.)	28.1
	anti- <i>Bufo mauritanicus</i> ALGER SERUM B	× <i>Bufo mauritanicus</i> (ALGER)
<i>Pyxicephalus adspersus</i> (RHODESIA)		24.5

ago in the former genus *Rana*, such as *Pyxicephalus* or *Ptychadaena*, demonstrate very low percents of crossed precipitin reactions with *Rana* species (32.9%–42.9%), in like manner to the reported data for the *Bufo* group (Ceï, 1972a, 1972b). Near serological relationships are tentatively inferred by a few preliminary tests between a Ranid (*Pyxicephalus*) and a Microhylid (*Breviceps*), giving as percents 42.0–44.6. This first result agrees with Morescalchi's caryological report (1968) on phyletic relationships between Ranidae and Microhylidae, and with the evidence of the quantitative analysis by Kluge and Farris (1969). Microhylidae have been placed tentatively in Archaeobatrachia by Laur-

ent, but they appear reasonably as Neobatrachia in Reig's arrangement.

Our conclusions, strengthened by the present serological report and discussion indicate Archaeobatrachia and Neobatrachia as independent taxo-genetic or phyletic branches, on the ground of the crossed immunological reactions. Contrarily to the present caryological evidence no direct serological relationships may be assumed between Pelobatid and Ceratofryid frogs, but a very short serological distance seems to lie between Pelobatidae and Discoglossidae. That finding agrees with a number of former morphological, paleontological, paleogeographic and biogeographical studies on the ancestry of primitive Pelobatids,

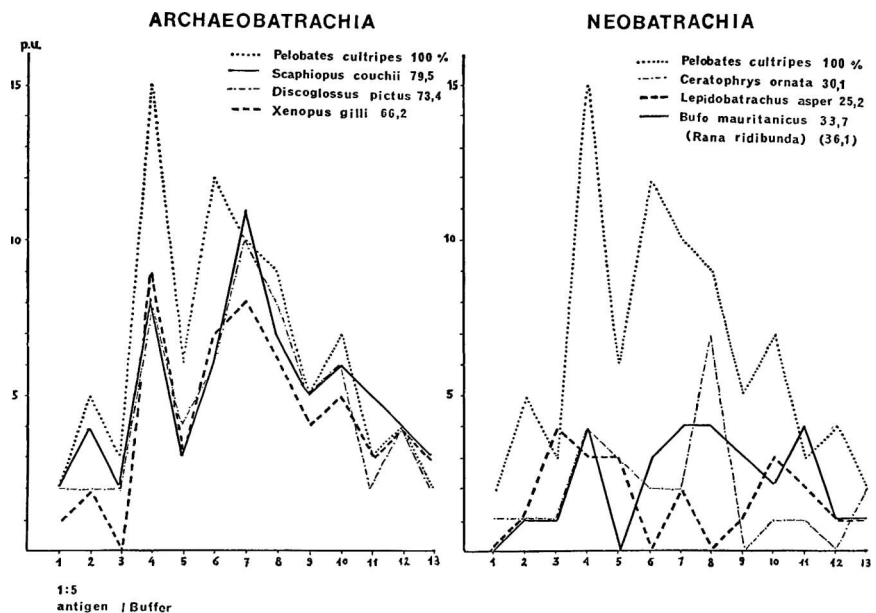


Fig. 1. Serological relationships between Archaeobatrachia and Neobatrachia as indicated by the use of an anti-*Pelobates cultripes* serum (B) and the antigens of *Scaphiopus*, *Discoglossus*, *Xenopus* and *Ceratophrys*, *Lepidobatrachus*, *Bufo* (or *Rana*). The curves are expressed by P.U. = Photronreflectometric Units. The sera are tested in a doubling dilution series, beginning at the left with a dilution of one part of serum to five parts of buffered saline.

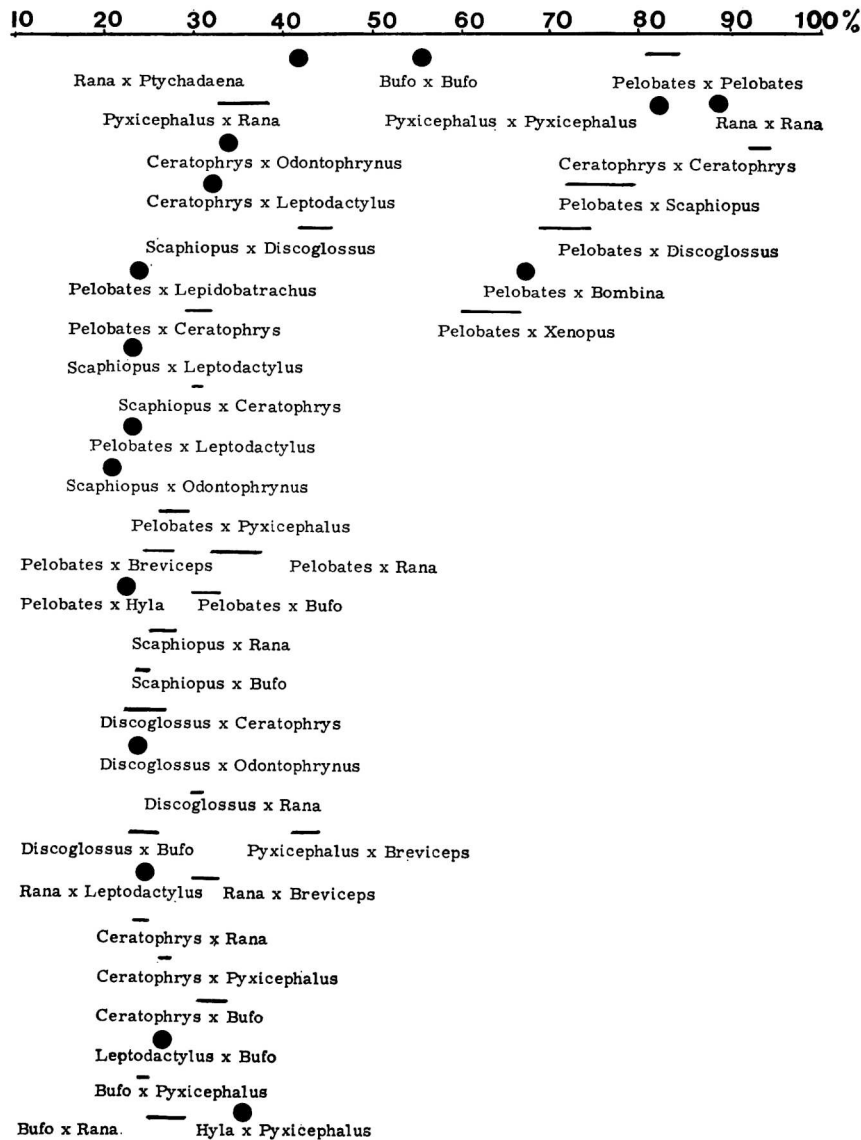


Fig. 2. Serological distances between several representative genera of Archaeobatrachia and Neobatrachia. Relative amounts of serological correspondence are indicated as percents of the crossed homo-heterologous reactions. Single tests are indicated as solid circles. Where more than one test was made between the same taxa, the range of values is represented as a line. In all cases the symbols are placed in the space next above the taxa concerned.

probably related to some Discoglossid-like stock of late Mesozoic Anurans.

The remarkable antigen differentiation of the several families of Neobatrachia must be pointed out. The tachyelic features of some of these families may intervene as a probable factor of disjunctive evolution. That is specially evident in Bufonid and Ranid toads and frogs, such as genus *Bufo* or many forms previously referred to the ancient genus *Rana*, like the African *Pyxicephalus* and *Ptychadaena* species. Some very preliminary serological data seem at last to support the probable phyletic relationships between Ranid and Microhylid frogs. Serological results are now in ac-

cordance with the cytogenetic evidence, such as the findings elucidated by the Morescalchi's recent careful caryological works.

#### Acknowledgments

I wish to acknowledge for their extensive aid and collaboration, Dr. Eviatar Nevo from the University of Jerusalem (Israel), Dr. R. Siboulet from the University of Alger (Algeria), Dr. J. Fuhn from the Institute of Biology of Bucaresti (Rumania), Dr. E. Crespo from the Museu Zoologico of the Faculty of Science of Lizboa (Portugal), Dr. V. G. Roig from the Institute of Animal Biology of the Cuyo University (Argentina), Dr.

A. Barrio from the University of Buenos Aires (Argentina), Dr. J. A. Loveridge from the University of Salisbury (Rhodesia), Mr. J. Visser from Capetown (S. Africa), Dr. R. Ruibal from the University of California, Riverside (U.S.A.). I wish to acknowledge and thank my collaborators Miss M. P. Castro, and L. P. Castro from the Institute of Animal Biology at the Cuyo University, and Prof. Alan Boyden from the Rutgers University, New Brunswick (U.S.A.) for his kind revision of the manuscript.

#### REFERENCES

- Barrio, A. and Rinaldi de Chieri, P. 1970. Relaciones cariosistematicas de los Ceratophryidae de la Argentina (Amphibia, Anura). *Physis* 30,80:321-329.
- Beçak, M. L., Beçak, W. and Rabello, M. N. 1967. Further studies on polyploid Amphibians (Ceratophryidae). I. Mitotic and meiotic aspects. *Chromosoma* 22: 192-201. Berlin.
- Bogart, J. P. 1967. Chromosomes of the South American amphibian family Ceratophryidae with a reconsideration of the taxonomic status of *Odontophrynus americanus*. *Can. J. Genet. Cytol.* 9:531-542.
- Bogart, J. P. 1969. Los cromosomas de los generos *Chacophrys* y *Scaphiopus*. II *Jorn. Arg. Zool.* 1969, Santa Fe and Paraná. (In press.)
- Boyden, A. 1956. On measuring Serological Correspondence among Antigens. *The Serological Museum Bull.* 16:3-8.
- Boyden, A. 1967. The place of precipitin testing among newer trends in taxonomy (Symposium on Newer Trends in Taxonomy held at New Delhi on January 28-30, 1966). *Bull. Nat. Inst. of Sciences of India* 34:108-117.
- Boyden, A. and De Falco, R. J. 1943. Report on the use of the Photoreflectometer in serological comparisons. *Phys. Zoology*, 16,3:229-241.
- Boyden, A., Bolton, E. and Gerneroy, D. 1947. Precipitin testing with special reference to the photoelectric measurement of turbidity. *The Jour. of Immun.* 57,3:211-227.
- Cei, J. M. 1963. Some Precipitin Tests and Preliminary Remarks on the Systematic Relationships of Four South American Families of Frogs. *The Serological Museum. Bulletin No.* 30:4-6.
- Cei, J. M. 1965. The relationships of some Ceratophryid and Leptodactylid genera as indicated by precipitin tests. *Herpetologica* 20,4:217-224.
- Cei, J. M. 1969. Tests de precipitinas y relaciones serológicas entre especies del género *Lepidobatrachus* Budgett (Anura, Ceratophryidae). *Physis* 28,77:273-277.
- Cei, J. M. 1970. La posición filética de Telmatobiinae, su discusión reciente y significado crítico de algunos inmunotests. *Acta Zool. Lilloana* 27:181-192.
- Cei, J. M. 1970. Relaciones serológicas entre los *Leptodactylus* del grupo *Ocellatus-chaqueensis* de la cuenca Chaco-paranense y la forma *Macrosternum*. *Acta Zool. Lilloana* 27:299-306.
- Cei, J. M. 1972. Precipitin tests and taxo-serological status of some European toads of *Bufo bufo* group. *Arqu. Museu Bocage*, 2ª Serie, V. IV, 1972 (in press).
- Cei, J. M. 1972. Geographical barriers and serological relationships in some African Toads of the *Bufo regularis* complex. *Arqu. Museu Bocage*, 2ª Serie, V. IV, 1972 (in press).
- Cei, J. M. and Castro, M. P. 1970. A correlation between age and reduced properties of specific Amphibian seroproteins, by means of precipitin tests. *The Serological Museum Bull.* 43:3-5.
- Estes, R. 1970. New Fossil Pelobatid Frogs and a review of the Genus *Eopelobates*. *Bull. Mus. of Comparative Zool.* 139,6: 293-339.
- Frair, W. 1969. Aging of serum proteins and serology of marine turtles. *The Serological Museum Bull.* 42:1-3.
- Inger, R. F. 1967. The development of a Phylogeny of Frogs. *Evolution*, 21,2:369-384.
- Kluge, A. G. and Farris, J. S. 1969. Quantitative phyletics and the evolution of Anurans. *Systematic Zoology* 18,1:1-32.
- Laurent, R. 1942. Note sur les procoeliens firmisternes (Batrachia Anura). *Bull. Mus. Roy. Hist. Nat. Belgique* 18,43:1-20.

(Continued on page 8, col. 3)

## Archaeobatrachia Versus Neobatrachia

(Continued from page 4)

- Laurent, R. 1967. Taxonomia de los Anuros. Acta Zool. Lilloana 22:207-210.
- Morescalchi, A. 1967. The close karyological affinities between a *Ceratophrys* and *Pelobates* (Amphibia Salientia). *Experientia* 23(1071):1-4.
- Morescalchi, A. 1968. Some trends of karyological evolution in the Anura. Actas IV Congreso Latinoamericano de Zoología, Caracas (in press).
- Noble, G. K. 1931. Biology of the Amphibia. XII—557 pp. New York, McGraw Hill. Co.
- Reig, O. A. 1958. Proposiciones para una nueva macrosistemática de los anuros. *Physis* 21,60:109-118.
- Saez, F. A. and Brum, N. 1959. Citogenética de anfibios anuros de América del Sur. Los cromosomas de *Odontophrynus americanus* y *Ceratophrys ornata*. Anal. Fac. Med. 44:414-423. Montevideo.
- Tihen, J. A. 1965. Evolutionary Trends in Frogs. Amer. Zoologist 5:309-318.
- Zweifel, R. G. 1956. Two pelobatid frogs from the Tertiary of North America and their relationship to fossil and Recent forms. Amer. Mus. Novit. 1762:1-45.