

THE RELATIONSHIPS OF SOME CERATOPHRYID AND
LEPTODACTYLID GENERA AS INDICATED BY
PRECIPITIN TESTS

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Ceratophryidae Cope (1863) has been recognized in recent years as a natural taxon by some workers, distinguished from Leptodactylidae, to which it was allocated by previous authors (Noble, 1931). According to Miranda Ribeiro (1926), Leitao de Carvalho (1946), and Reig (1958), the genera *Ceratophrys*, *Lepidobatrachus*, *Macrogenioglottus*, *Odontophrynus*, *Stombus*, *Zachaenus*, and *Proceratophrys* should be included in the family, and added to it is the recently discovered *Amblyphrynus* (Cochran and Goin, 1961). *Stombus*, synonymized with *Ceratophrys* by Cochran, is of doubtful status (Reig, unpublished data). *Lepidobatrachus* Budgett was considered as a synonym of *Ceratophrys* by Boulenger (1919) and Parker (1931), but Nieden (1923) and Vellard (1948) recognized it as a genus of Leptodactylidae. They considered it to be intermediate between *Ceratophrys* and the primitive leptodactylid frog *Calyptocephalella*, based on osteological peculiarities. However, a close relationship between the Chilean frog *Calyptocephalella* and *Lepidobatrachus* was not accepted by Reig (1960a); he proposed to establish a new subfamily for this ceratophryid genus because of its evident apomorphic characteristics.

Considering the many unsolved problems and the diversity of opinion in regard to the proper phyletic and systematic positions of genera and species of Ceratophryidae, an attempt at a careful comparison of their serological reactions with those of other anurans, particularly of the leptodactylid stock, using a quantitative precipitin technique certainly seemed desirable.

The method, currently applied to determine animal and plant relationships, may shed new light on some of the phyletic positions of those genera whose status is still uncertain.¹

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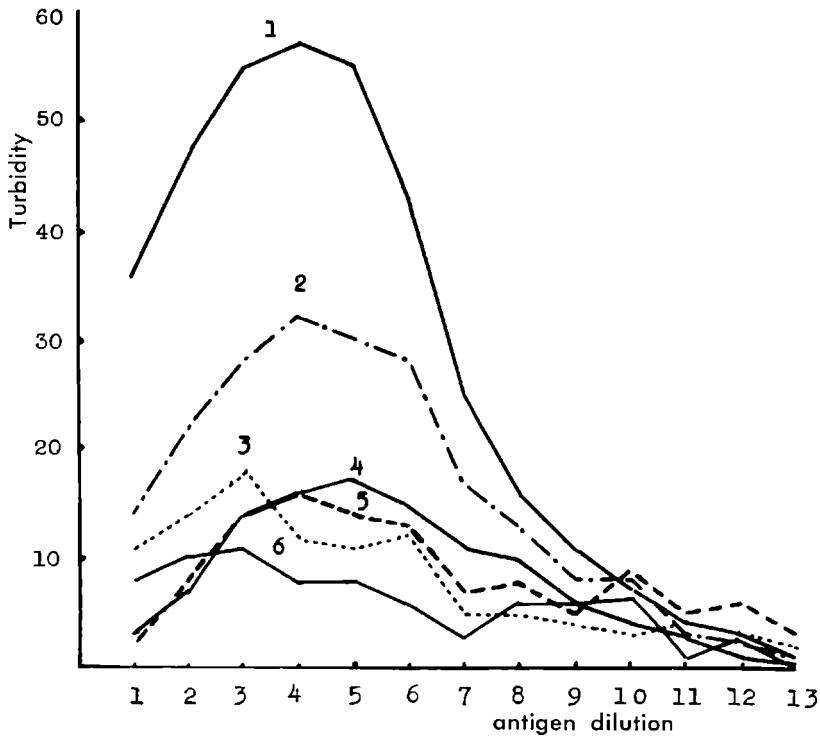


FIG. 1.—Serological relationships between the Ceratophryidae and Leptodactylidae as indicated by use of an anti-*Ceratophrys ornata* serum and the following six antigens: 1, *C. ornata* (CB-18) \times *C. ornata* (A-0109), 100% area; 2, \times *Lepidobatrachus salinicola* (A-0031), 57.2% area; 3, \times *Odontophrynus americanus* (A-0029), 28.8% area; 4, \times *Leptodactylus ocellatus* (A-0194), 30.0% area; 5, \times *Odontophrynus cultripes* (A-0150), 32.5% area; and 6, \times *Calyptocephalella gayi* (A-0041), 21.3% area.

METHODS AND MATERIALS

The sera for the tests were procured from living adult specimens of the following genera and species: *Ceratophrys ornata* (Bell), Santiago del Estero, Argentina; *Lepidobatrachus salinicola* Reig and Cei, Santiago del Estero, Argentina; *Odontophrynus americanus* (Duméril and Bibron), Tucuman, Argentina; *Odontophrynus cultripes* Reinhardt and Lutken, Belo Horizonte, Brasil; *Odontophrynus occidentalis* (Berg), Neuquen, Argentina; *Odontophrynus occidentalis* (Berg), San Luis, Argentina; *Calyptocephalella gayi* (Guichenot), central Chile; *Leptodactylus ocellatus* (Linnaeus), Mendoza, Argentina; *Pleurodema tucumana* Parker, Tucuman, Argentina.

The animals captured were bled immediately by cardiac puncture; the small quantity of the sera available was sometimes the

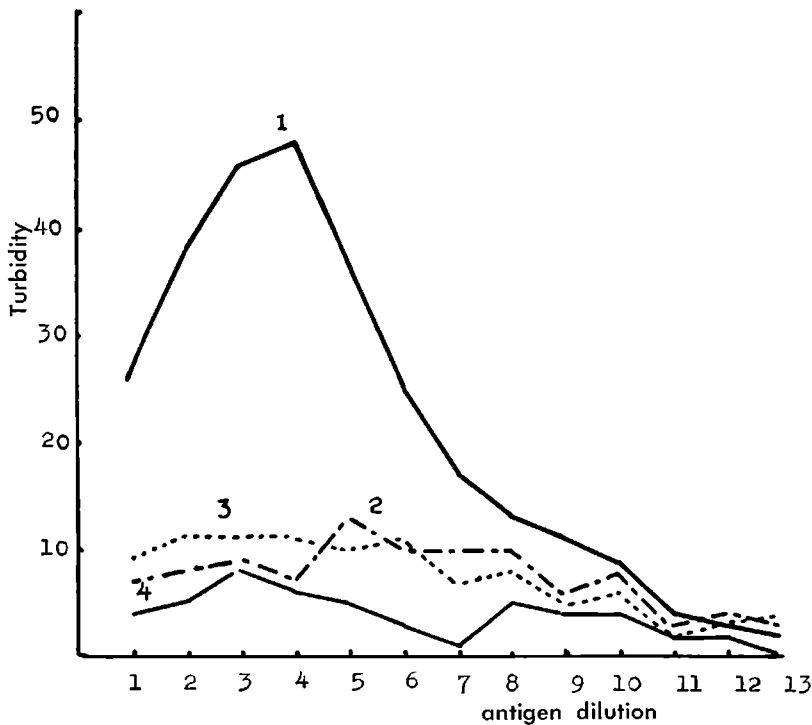


FIG. 2.—The relationships between Ceratophryidae and Leptodactylidae with an anti-*Odontophrynus americanus* serum and the following four antigens: 1, *O. americanus* (CB-20) \times *O. americanus* (A-0029), 100% area; 2, \times *Lepidobatrachus salinicola* (A-0031), 35% area; 3, \times *Ceratophrys ornata* (A-0109), 35% area; 4, \times *Calyptocephalella gayi* (A-0041), 17.5% area.

limiting factor in this research. After clotting, the expressed sera were regularly filtered through Seitz filter, merthiolated, and stored under sterile conditions. Antisera were prepared in rabbits by a short-injection series. A first injection was followed after 2 weeks by a second injection and a subsequent injection was given 8 days later, all the injections being 2 mg. of protein per kg. of body weight of the injected rabbit, mixed with an equivalent quantity of coadjuvant (Tween 80). The specificity of the obtained antisera was sufficient for our purposes, being also sensitive to the antigens of close relatives. The precipitin reactions were analyzed by tests carried out by the Photroner technique, as described by Libby (1938) and Boyden (1942) and co-workers.

Because of the small quantities of ceratophryid sera available, the antigen dilution was begun at 1:5 (Evans Buffer). A dilution of 1:2.5 or 1:1 should be used to obtain a full zone of excess antigen, but as is shown by the accompanying graphs (Figs. 1-4),

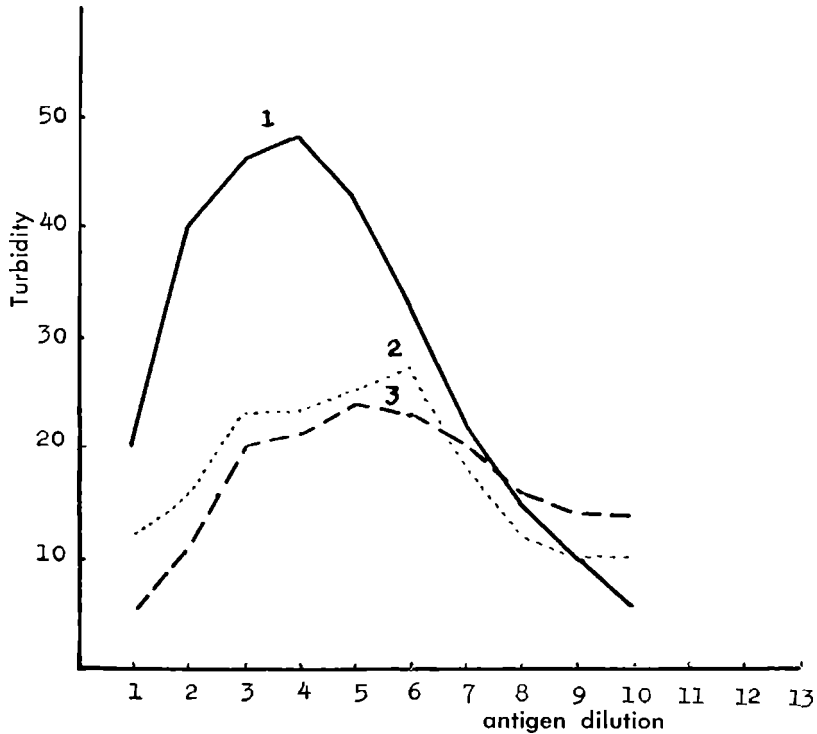


FIG. 3.—The relationships between *Odontophrynus americanus* and *Odontophrynus occidentalis* with an anti-americanus serum and the following three antigens: 1, *americanus* (CB-19) \times *americanus* (A-0029), 100% area; 2, \times *occidentalis* Sierras San Luis population (A-0161), 62.1% area; 3, *occidentalis* Neuquen population (A-0186), 59.3% area.

failure to obtain full antigen excess at 1 : 5 dilution did not change fundamentally the serological value of the obtained curves of turbidities and the significance of their heterologous/homologous area ratios.

RESULTS

Table 1 shows the comparative results of the precipitin tests between anticeratophryid and antileptodactylid sera and homologous and heterologous antigens.

Anti-*Ceratophrys ornata* serum (Fig. 1) gives the highest per cent value (57.2%) with *Lepidobatrachus salinicola* antigen; the lowest, with *Calyptocephalella gayi* and *Odontophrynus*, is at the same serological distance as *Leptodactylus ocellatus*.

Odontophrynus americanus antiserum (CB-18) (Fig. 2) gave similar per cent values with *Ceratophrys* and *Lepidobatrachus* sera (35%), not too far from the per cent obtained with a *Pleuro-*

TABLE 1.—Anti-ceratophryid and anti-leptodactylid sera \times ceratophryid and leptodactylid sera.

Anti-serum	Species	Homologous area	Heterologous area	Species	Per cent Heterol. area/Homol. area
CB-18	<i>Ceratophrys ornata</i>	360	206	<i>Lepidobatrachus salinicola</i> (A-0031)	57.2
CB-18	<i>Ceratophrys ornata</i>	360	104	<i>Odontophrynus americanus</i> (A-0029)	28.2
CB-18	<i>Ceratophrys ornata</i>	360	110	<i>Odontophrynus cultripes</i> (A-0150)	30.5
CB-18	<i>Ceratophrys ornata</i>	360	108	<i>Leptodactylus ocellatus</i> (A-0194)	30.0
CB-18	<i>Ceratophrys ornata</i>	360	117	<i>Pleurodema tucumana</i> (A-0111)	32.5
CB-18	<i>Ceratophrys ornata</i>	360	77	<i>Calyptocephalella gayi</i> (A-0041)	21.3
CB-20	<i>Odontophrynus americanus</i>	280	98	<i>Ceratophrys ornata</i> (A-0109)	35.0
CB-20	<i>Odontophrynus americanus</i>	280	98	<i>Lepidobatrachus salinicola</i> (A-0031)	35.0
CB-20	<i>Odontophrynus americanus</i>	280	86	<i>Pleurodema tucumana</i> (A-0111)	30.7
CB-20	<i>Odontophrynus americanus</i>	280	49	<i>Calyptocephalella gayi</i> (A-0041)	17.5
CB-12	<i>Leptodactylus ocellatus</i>	171	58	<i>Ceratophrys ornata</i> (A-0109)	33.9
CB-12	<i>Leptodactylus ocellatus</i>	171	46	<i>Odontophrynus americanus</i> (A-0029)	26.9
CB-12	<i>Leptodactylus ocellatus</i>	171	51	<i>Lepidobatrachus salinicola</i> (A-0031)	29.8
CB-12	<i>Leptodactylus ocellatus</i>	171	64	<i>Pleurodema tucumana</i> (A-0111)	37.4
CB-12	<i>Leptodactylus ocellatus</i>	171	27	<i>Calyptocephalella gayi</i> (A-0041)	15.7
CB-19	<i>Odontophrynus americanus</i>	283	176	<i>Odontophrynus occidentalis</i> San Luis (A-0161)	62.1
CB-19	<i>Odontophrynus americanus</i>	283	168	<i>Odontophrynus occidentalis</i> Neuquen (A-0186)	59.3

A) Ceratophryidae versus Leptodactylinae and Leptodactylinae versus Ceratophryidae: average of all tests: 30.6% (range: 26.9–33.9).

B) *Ceratophrys* and *Lepidobatrachus* versus *Odontophrynus*: average of all tests: 32.3% (range: 28.8–35.0).

C) *Ceratophrys* versus *Lepidobatrachus*: 57.2%.

D) *Leptodactylus* versus *Pleurodema*: 37.4%.

E) *Odontophrynus* versus *Odontophrynus*: 60.7% (range: 59.3–62.1).

F) Ceratophryidae and Leptodactylinae versus Calyptocephalellinae: average of all tests: 18.1% (range: 15.7–21.3).

dema tucumana antigen (30.7%). The greatest serological divergence also indicated by the precipitin test with *Calyptocephalella* antigen (17.5%). However, relatively high values of turbidities were obtained between an *Odontophrynus americanus* antiserum (CB-19) and antigens of two populations of a related species of

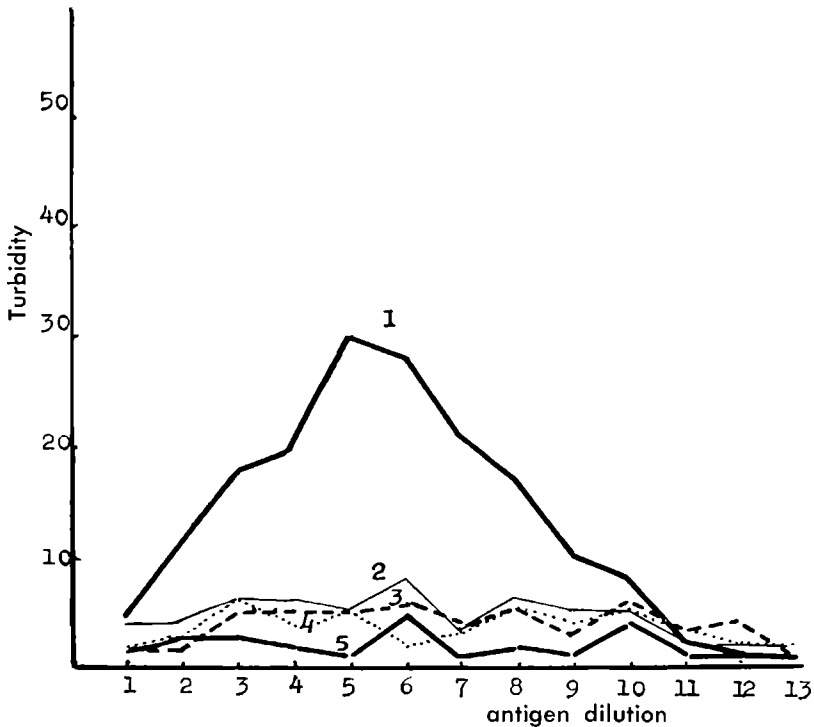


FIG. 4.—The relationships between Ceratophryidae and Leptodactylidae with an anti-*Leptodactylus ocellatus* serum and the following five antigens: 1, *L. ocellatus* (CB-12) × *L. ocellatus* (A-0041), 100.1% area; 2, × *Ceratophrys ornata* (A-0109), 33.9% area; 3, × *Pleurodema tucumana* (A-0111), 37.4% area; 4, *Odontophrynus americanus* (A-0029), 26.9% area; 5, *Calyptocephalella gayi* (A-0041), 15.7% area.

this genus (*Odontophrynus occidentalis*), namely 59.3 and 62.1% (Fig. 3).

On the other hand, a *Leptodactylus ocellatus* antiserum (CB-12) gave the relatively highest reaction with *Pleurodema tucumana* (37.4%), and similar per cent values with ceratophryid antigens, between 26.9 and 33.9%. The weakest reaction is again shown by the test with *Calyptocephalella* serum (15.7%), Fig. 4.

DISCUSSION

The precipitin tests agree well with Reig's opinion that there is no close relationship between the primitive Chilean frog and any of the genera of ceratophryid or leptodactylid frogs considered here. The tests agree with the morphologically evident divergence between *Calyptocephalella* and other actual forms of leptodactylid stock. Subfamily status of this ancient batrachian easily can be supported. Comparison of the reactions given by a *Ceratophrys*

antiserum and the antigen of *Lepidobatrachus* and *Calyptocephalella* suggests the probable absence of any intimate serological relation between the Chacoan and Chilean genera.

Lepidobatrachus is clearly the form most closely related to *Ceratophrys* (Fig. 1); the degree of its affinity, with our serological "yardstick," is of the same order of magnitude as the often-reported values between species of the same genus. This preliminary paper indicates, by the order of magnitude of the reactions, that all the ceratophryid genera here considered may have undergone a long period of independent diverging evolution.

Reactions found between anti-*Odontophrynus* serum and *Ceratophrys* and *Lepidobatrachus* antigens give the same per cent values as did reactions between antisera against these genera and *Leptodactylus* and *Pleurodema* antigens. On this basis, there does not seem to be real serological justification for placing together in the same family *Odontophrynus* and the other ceratophryid genera studied here.

An attempt to determine the possible relationship between closely related species of *Odontophrynus* is also presented. When an anti-*Odontophrynus americanus* serum was tested with *Odontophrynus occidentalis* antigens, both from the tested populations (Sierras of San Luis and Neuquen), the resulting reactions show a more evident chemical similarity of proteins as indicated by the heavier turbidities. It is significant that the reciprocal serological positions of both populations of *Odontophrynus occidentalis*, according to their per cent values (62.1 and 59.3%), are so similar.

Further morphological studies will no doubt contribute to the determination of the real degree of taxonomic relationships between the various ceratophryid genera. However, present and future serological data may also be an aid since these genera, based on chemical characters, represent a more conservative genetic stock. Thus, the complicated problem of a true phylogenetic and biological classification of the Neotropical anurans may be reached through the combined study of comparative morphology and systematic serology.

SUMMARY

Preliminary serological studies by Photroner's tests show the long period of independent diverging evolution of the Neotropical anuran family Ceratophryidae. There is some evidence of close affinities between *Ceratophrys* and *Lepidobatrachus*, but *Odontophrynus* is actually as distinct serologically from other ceratophryid genera as the leptodactylid genera *Leptodactylus* and *Pleurodema*. There is a wide serological separation between the very ancient Chilean frog *Calyptocephalella* and any of the other leptodactylid and ceratophryid forms here considered. These results agree with a possible subfamily status of *Calyptocephalella*.

LITERATURE CITED

- BOULENGER, G. A. 1919. On the genus *Lepidobatrachus* Budgett. Ann. Mag. Nat. Hist., Ser. 9, 3:531-533.
- BOYDEN, A. 1942. Systematic serology: a critical appreciation. Physiol. Zool., 15:109-145.
- COPE, E. D. 1865. Sketch of the primary groups of Batrachia Salientia. Nat. Hist. Rev., New Ser., 5:97-120.
- COCHRAN, D. M., AND C. J. GOIN. 1961. A new genus and species of frog (*Leptodactylidae*) from Colombia. Fieldiana, Zoology, 39, 48:543-546.
- LEITAO DE CARVALHO, A. 1946. Um novo genero de Ceratofrideo do Sudeste baiano. Bol. Museu Nacional, Nova Ser. Zoologia, 73:1-5.
- LIBBY, R. L. 1938. The photoneflectometer—an instrument for the measurement of turbid systems. J. Immunol., 34:71-73.
- MIRANDA RIBEIRO, A. DE. 1926. Notes para servirem ao estudo dos Gymnobatrachio (*Anura*) brasileiros. Arch. Mus. Nacional, 27:1-227.
- NIEDEN, F. 1923. *Anura*. I. Subordo *Aglossa* und *Phanaeroglossa*. Sectio I: *Arcifera*. Das Tierreich. Lief. 46:1-584.
- NOBLE, G. K. 1931. The biology of the Amphibia. McGraw-Hill Book Co., New York. xiii + 577 pp.
- PARKER, H. W. 1931. Reports on an expedition to Brasil and Paraguay in 1926-27. III. J. Linn. Soc. London, Zoology, 37:285-289.
- REIG, O. A. 1958. Proposiciones para una nueva macrosistemática de los Anuros. Physis, 21, 60:109-118.
- . 1960a. Las relaciones genericas del anuro chileno *Calyptocephalella gayi* (D. & B.). Actas by Trab. I Congr. Sudameric. Zool. La Plata, 12-24 Oct. 1959, 4:113-130.
- . 1960b. La anatomía esqueletica del genero *Lepidobatrachus* (*Anura*: *Leptodactylidae*) comparada con la de otros Ceratofrinos. *Ibid.*, pp. 133-147.
- SCHAEFFER, B. 1949. Anurans from the early Tertiary of Patagonia. Bull. Amer. Mus. Nat. Hist., 93, 2:41-68.
- VELLARD, J. 1948. Batracios del Chaco argentino. Acta Zool. Lilloana, 5:137-174.

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