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FURTHER IMMUNOLOGICAL EVIDENCE  
FOR THE VALIDITY OF THE FAMILY BOMBINIDAE  
(AMPHIBIA SALIENTIA) \*

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LANZA et al. (1975), using Libby's photoneutron reflectometric technique, demonstrated the existence of a relatively strong intergeneric distance between *Alytes* and *Discoglossus*, which is stronger than that occurring between *Discoglossus* and the Pelobatid genus *Pelobates*. At the same time they pointed out that both *Alytes* and *Bombina* differ from *Discoglossus* in other important characters and concluded that the first two could be located in the family Bombinidae Fitzinger, 1826 and the latter in the family Discoglossidae Günther, 1858. In order to convalidate this hypothesis further immunological research on *Discoglossus*, *Alytes* and *Bombina*, was carried out and the results are reported in the present paper.

MATERIALS AND METHODS

The following samples were studied: *Discoglossus pictus* Otth, 1837 from Lisbon (Portugal); *Alytes obstetricans boscai* Lataste, 1879 from Minho (Portugal); *Bombina variegata pachypus* (Bonaparte, 1838) from the Rio a' Buti stream (Calvana Mountains near Prato, Florence, Italy).

All the specimens were bled in the same period and utilized in the comparative precipitin tests.

Immunological reactions were tested according to Libby's photoneutron reflectometric technique (LIBBY, 1938a; 1938b; 1938c; BOYDEN et al., 1947). As pointed out by one of us in several reports since 1963 (see CEI, 1972, for the bibliography), this technique offers a quantitative expression (in galvanometric units) of the whole antigenic relationship between species and species-groups at a different taxonomic level of physiological and genetic differentiation.

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Rabbits of a uniform strain were injected with collected sera, using microsamples (0.3 ml) reinforced by Freud's adjuvant. As indicated by trial bleedings, a noticeable degree of immunity was obtained after 45 days. Rabbits were bled by cardiac puncture and the immune sera tested with progressive antigen dilutions.

Table 1.

*Homo-heterologous precipitin reaction between anti-Discoglossus, anti-Alytes and anti-Bombina sera and homo-heterologous specific antigens (turbidimetric measurements expressed as % of the homologous reaction: see text). Data obtained in 1974 (LANZA et al., 1975) and in 1975.*

		Antigens							
		<i>Discoglossus pictus</i> Palermo (Sicily), 1974	<i>Discoglossus pictus</i> Minho (Portugal), 1974	<i>Discoglossus pictus</i> Lisbon (Portugal), 1975	<i>Discoglossus sardus</i> Vizzavona (Corsica), 1974	<i>Alytes obstetricans boscai</i> Minho (Portugal), 1974	<i>Alytes obstetricans boscai</i> Minho (Portugal), 1975	<i>Alytes cisternasii</i> Boscá, 1879 Alentejo (Portugal), 1974	<i>Bombina variegata pachypus</i> Prato (Italy), 1975
Antisera	<i>Discoglossus pictus</i> Palermo (Sicily), 1974	100	92.9	—	81.1	—	—	52.7	—
	<i>Discoglossus pictus</i> Minho (Portugal), 1974	92.3	100	—	82.6	61.1	—	—	—
	<i>Discoglossus pictus</i> Lisbon (Portugal), 1975	—	—	100	—	—	61.4	—	60.0
	<i>Discoglossus sardus</i> Tschudi, 1837 Vizzavona (Corsica), 1974	82.7	80.6	—	100	63.4	—	—	—
	<i>Alytes obstetricans boscai</i> Minho (Portugal), 1975	—	—	60.5	—	—	100	—	74.4
	<i>Bombina variegata pachypus</i> Prato (Italy), 1975	—	—	59.7	—	—	71.0	—	100

The whole precipitin reaction with the homologous antigen is indicated as 100% (homologous reaction) and corresponds to the amount of the reactions of the immune serum with the progressive antigen dilutions. Heterologous reactions of the same immune serum with different specific antigens are expressed as percentages of the homologous reaction. Differences in the homo-heterologous precipitating systems (anti-

gens-antibodies) are strengthened by the different scattering of the flocculated molecules as evidenced by the photronic photocell and its galvanometric circuit. The intensity of the precipitin reactions at a different dilution, i.e. at a different antigens/antibodies proportion, can thus be obtained numerically by photronreflectometric units. More than 90% in a homo-heterologous reaction is assumed to correspond to a conspecific or populational relationship (see, for instance, CEI, 1971). Lower percentage may correspond to interspecific or intergeneric relationships in accordance with the genetic or evolutionary affinities of the species tested.

### RESULTS AND CONCLUSIONS

The data obtained in 1974 (LANZA et al., 1975) and 1975 (Table 1) show that the following degree of immunological relationship exists in the genus *Discoglossus* and between *Discoglossus*, *Alytes* and *Bombina*:

- a) *D. pictus* from Sicily versus *D. pictus* from Portugal: 92.3-92.9%
- b) *D. pictus* versus *D. sardus*: 80.6-82.7%
- c) *A. obstetricans boscai* versus *B. variegata pachypus*: 71.0-74.4%
- d) *Discoglossus* versus *Alytes* and *Bombina*: 52.7-63.4%.

In analogy with the data obtained studying other Anurans (see for instance CEI, 1971 and 1972), we may assume that the above-mentioned differences in percentage may correspond respectively to (a) intraspecific (populational or racial), (b) interspecific, (c) intergeneric and (d) family relationships. According to recent studies by CRESPO (in preparation), *Discoglossus pictus* also differs markedly from *Alytes obstetricans boscai* and *Alytes cisternasii* also in haemoglobins and lactic dehydrogenase isozymes pattern (analysed by polyacrylamid and cellulose acetate electrophoresis).

### SUMMARY

New immunological data obtained using Libby's photron-reflectometric technique confirms that *Discoglossus* belongs to a different family (Discoglossidae Günther, 1858) from that of *Alytes* and *Bombina* (Bombinidae Fitzinger, 1826).

### RIASSUNTO

Nuovi dati immunologici ottenuti con la tecnica fottronriflettometrica di Libby confermano che *Discoglossus* appartiene a una famiglia (Discoglossidae Günther, 1858) diversa da quella di *Alytes* e di *Bombina* (Bombinidae Fitzinger, 1826).

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