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ALBUMIN EVOLUTION AND ITS PHYLOGENETIC IMPLICATIONS IN THE PLETHODONTID SALAMANDER GENERA *PSEUDOEURYCEA* AND *CHIROPTEROTRITON*

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ABSTRACT: The immunological technique of quantitative micro-complement fixation was used to study albumin evolution in the plethodontid salamander genera *Pseudoeurycea* and *Chiropterotriton* from Mexico and Central America. Antisera were prepared for representatives of the five species-groups of *Pseudoeurycea* and for the α and β groups of *Chiropterotriton*. A matrix of reciprocal tests was used to construct a dendrogram illustrating probable relationships. The α and β groups of *Chiropterotriton* are as distant from each other as they are from *Pseudoeurycea*. The *rex* and *gadovii* groups of *Pseudoeurycea* are not well differentiated, and unidirectional tests to 13 additional species of *Pseudoeurycea* indicate that many species have been incorrectly assigned to groups. *Pseudoeurycea cephalica* is especially remote from all other species of *Pseudoeurycea* tested; it is even more remote from the species of *Chiropterotriton* tested. The *cephalica*, *leprosa*, and *bellii* groups are all well distinguished from each other and from the *gadovii* (including *rex*) group. Within *Chiropterotriton* β the *bromeliacia* and *picadoi* groups are sharply distinguished from each other. The great range of immunological distance measured in this study (from 0 to over 100) indicates that both of these genera are as differentiated genetically as is the North American genus *Plethodon*, and that the evolutionary history of each of these genera encompasses most of Cenozoic time.

Key words: Albumin, Amphibia, Caudata, *Chiropterotriton*, Phylogeny, *Pseudoeurycea*

NEARLY half of the living salamanders are presently included in one taxon—the supergenus *Bolitoglossa* of the tribe Bolitoglossini, family Plethodontidae. This exclusively tropical group is found only in the New World; its only close relatives are the genera *Hydromantes* and *Batrachoseps*, both of which are inhabitants of western North America (*Hydromantes* occurs in Europe as well). The supergenus *Bolitoglossa* is considered to be the most derived lineage of salamanders, and has undergone an extensive adaptive radiation (Wake and Lynch, 1976). The relationships of its component lineages are poorly understood, for there is no fossil record and there has been extensive morphological parallelism and convergence (Wake, 1966). Attempts are being made to integrate diverse kinds of data into a coherent picture of relationships; the present report is the first to deal with macromolecular evolution in the group.

Of the eight genera currently recognized in the supergenus *Bolitoglossa*, *Pseudoeurycea* and *Chiropterotriton* are the most generalized in morphology (see review in Wake and Lynch, 1976). We have chosen them for a detailed investigation of albumin evolution, using the quantitative immunological technique of micro-complement fixation (MC'F). In previous work we used this technique in conjunction with starch-gel electrophoresis and morphology to analyze salamander phylogeny (Larson et al., 1981; Maxson and Maxson, 1979; Maxson et al., 1979; Wake et al., 1978). Electrophoretic and morphological analyses relevant to the present study will be reported elsewhere.

The 23 described species of *Pseudoeurycea* are placed in five species-groups, four of which occur only in Mexico. The fifth group is mainly Guatemalan, but two species enter Chiapas, Mexico. We se-

TABLE 1.—Salamanders of the genus *Pseudoeurycea* (after Wake and Lynch, 1976).

Species group	Species	
bellii	*P. bellii*[a,b]	
cephalica	*P. cephalica*[a,b]	*P. conanti*
	P. cochranae[b]	*P. galeanae*
	P. werleri[b]	*P. scandens*[b]
	P. altamontana[b]	
gadovii	*P. gadovii*[b]	*P. unguidentis*[b]
	P. smithi[a,b]	*P. melanomolga*[b]
leprosa	*P. leprosa*[a,b]	*P. robertsi*[b]
	P. anitae	*P. firscheini*
	P. juarezi[b]	*P. mystax*
	P. nigromaculata[b]	
rex	*P. rex*[b]	*P. goebeli*[b]
	P. brunnata[a,b]	*P. exspectata*[b]

[a] Albumin purified and antisera prepared.
[b] Albumin samples extracted.

TABLE 2.—Salamanders of the genus *Chiropterotriton* (after Wake and Lynch, 1976).

Chiropterotriton α	*Chiropterotriton β*
multidentatus species-group	*bromeliacia* species-group
C. multidentatus[a,b]	*C. bromeliacia*[a,b]
C. arboreus	*C. cuchumatanus*[b]
C. magnipes	*C. rabbi*[b]
C. mosauri	*C. xolocalcae*
	C. megarhinus
chiropterus species-group	*picadoi* species-group
C. chiropterus[b]	*C. picadoi*
C. chondrostega[b]	*C. richardi*
C. dimidiatus[b]	*C. nasalis*[b]
C. lavae	*C. barbouri*
	C. veraepacis[b]
priscus species-group	
C. priscus[b]	

[a] Albumin purified and antisera prepared.
[b] Albumin samples extracted.

lected one member of each species-group for preparation of antisera (Table 1). Two groups of essentially generic level are recognized in *Chiropterotriton* (Lynch and Wake, 1978; Wake and Lynch, 1976). The α group is restricted to the mountains of eastern Mexico, north of the Isthmus of Tehuantepec. There are three species-groups and nine species in *Chiropterotriton* α; we prepared antisera to one species (Table 2). *Chiropterotriton* β is mainly Central American in distribution; it occurs from just south of the Isthmus of Tehuantepec to the Meseta Central of Costa Rica. There are two species-groups and 10 species; we prepared antisera to one species (Table 2). Because most species of *Pseudoeurycea* and *Chiropterotriton* are small, rare, or both, it was impractical to produce more antisera. However, the number of antisera available was adequate to investigate the relationships of the genera and the major species-groups. While we emphasize those species for which tests of reciprocity are presently possible, we also report unidirectional tests for a number of other species belonging to the major groups studied.

MATERIALS AND METHODS

Plasma (preserved in phenoxyethanol) from 18 species of the genus *Pseudoeurycea* and 10 species of the genus *Chiropterotriton* was used as a source of albumin. Voucher specimens of all species are in the Museum of Vertebrate Zoology, University of California, Berkeley.

Albumin was purified from plasma of five species of *Pseudoeurycea* (Table 1) and two species of *Chiropterotriton* (Table 2). Due to the small size of the animals, plasma from several members of the same population was pooled. Purification was by single-step, polyacrylamide-gel electrophoresis (Maxson et al., 1979). Albumin was identified by its fluorescence in the presence of 8-anilino-1-napthalene sulfonate (Hartman and Udenfriend, 1969).

Antisera to pure albumins were prepared in male Dutch Belted rabbits by the following procedure. Rabbits received an initial intradermal injection of Freund's complete adjuvant and albumin (1.2:1). This was followed at 7 weeks by an intradermal injection of Freund's in-

TABLE 3.—Matrix of immunological distances among albumins of species of plethodontid salamanders of the genera *Pseudoeurycea* and *Chiropterotriton*.

Species tested	Antisera						
	B	R	C	L	S	CB	CM
P. bellii (B)	0	39	74	52	43	84	88
P. brunnata (R)	53	0	72	48	15	67	77
P. cephalica (C)	78	49	0	64	62	90	107
P. leprosa (L)	72	53	88	0	35	92	88
P. smithi (S)	47	21	71	35	0	60	87
C. bromeliacia (CB)	79	53	90	92	71	0	104
C. multidentatus (CM)	88	66	100	57	87	98	0

complete adjuvant and albumin (1.2:1). Three weeks later, 1 cm^3 of albumin solution was administered intravenously in the marginal ear-vein; this was repeated one week later. The rabbit was bled from the marginal ear-vein or by heart puncture one week after the second intravenous injection. A total of 2–3 mg of albumin was administered per rabbit over the total period. Three rabbits were used for each immunogen, and individual antisera were tested for purity by the criteria described by Wallace et al. (1973). Individual antisera were pooled in inverse proportion to their micro-complement fixation (MC'F) titers, and all reported results were obtained with these pooled antisera.

Reactivity was measured by the quantitative MC'F technique (Champion et al., 1974), and the data are reported in immunological distance units (IDU). One IDU is approximately equivalent to one amino acid difference (Maxson and Wilson, 1974).

RESULTS

Antisera were successfully prepared for all seven species of salamanders used in this study. The antibody titers (as defined in Champion et al., 1974) ranged from a low of 1500 (*P. bellii*) to a high of 6300 (*C. multidentatus*). The average titer (2750) was somewhat lower than that typically found in antisera prepared to albumin of diverse amphibians (Maxson et al., 1979); this probably is a function of the relatively small amounts of albumin available. Despite the relatively low titers, the antisera exhibited standard behavior, with an average slope for all seven antisera of 385, similar to slopes reported by other workers for mammalian, anuran, and salamander albumins (Champion et al., 1974; Larson et al., 1981; Maxson et al., 1979).

MC'F tests were conducted in all pairwise combinations and the averages of these reciprocal tests are shown in Table 3. The percent standard deviation from reciprocity (Maxson and Wilson, 1975) was 11%, similar to that reported in other studies (e.g., Maxson and Wilson, 1975) but somewhat higher than in one study of salamanders (Maxson et al., 1979). The data in Table 3 were averaged and a dendrogram (Fig. 1) was constructed using the method described by Farris (1972), but modified so that each monophyletic group of the tree uses only the closest (in a cladistic sense) outside lineage in assigning relative length to limbs. This modification minimizes errors of inference (Maxson and Wilson, 1975). The percent "standard deviation," defined by Fitch and Margoliash (1967) as a measure of "goodness of fit" of the tree to the data, is 11.4%. The *F*-statistic defined by Prager and Wilson (1978) is 6.9%. The method of tree construction that we have used makes no assumption concerning rates of albumin evolution.

In addition to our reciprocal tests, unidirectional tests were made using the 5 antisera of *Pseudoeurycea* with 13 additional species of the genus, and the 2

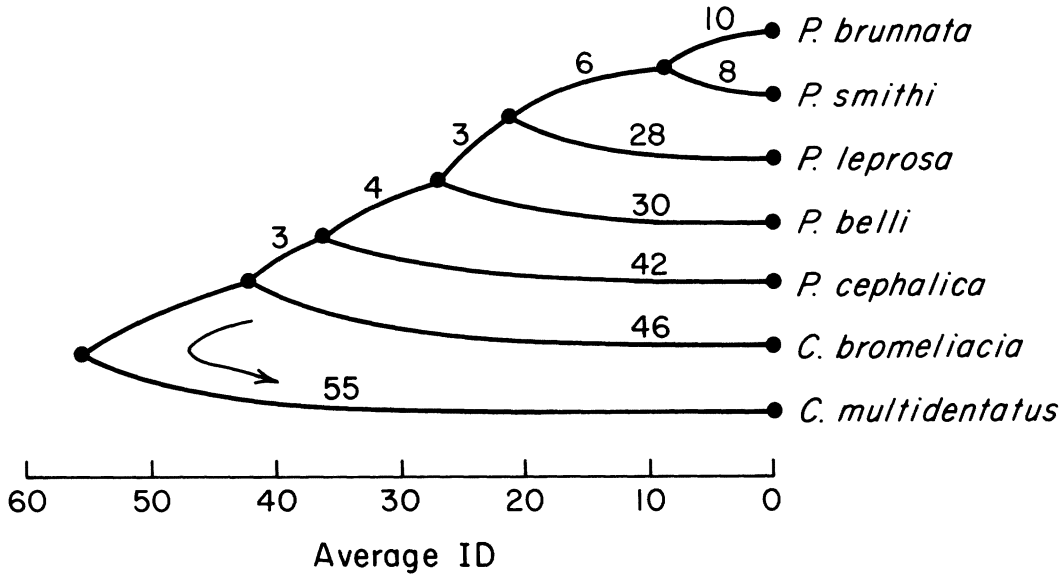

FIG. 1.—Dendrogram constructed from averaged reciprocal data in Table 3 according to method of Farris (1972), modified as outlined in text. This phylogeny gives the branching order of the lineages leading to the modern species. The numbers on the branches are the amounts of albumin change (expressed in immunological distance units) estimated to have occurred along each branch since the species last shared a common ancestor.

TABLE 4.—Albumin comparisons involving additional species of *Pseudoeurycea*.

Species	Immunological distance				
	Anti-R	Anti-C	Anti-L	Anti-S	Anti-B
P. rex species-group					
P. brunnata (R)	0	72	48	15	53
P. exspectata	27	92	38	18	53
P. goebeli	15	65	36	18	50
P. rex	24	>130	35	20	>105
P. cephalica species-group					
P. cephalica (C)	49	0	64	62	78
P. altamontana	28	73	54	31	57
P. cochranae	21	74	53	30	68
P. scandens	—	97	50	—	—
P. werleri	61	102	53	65	—
P. leprosa species-group					
P. leprosa (L)	53	88	0	35	72
P. juarezi	27	72	31	17	46
P. nigromaculata	53	—	42	49	—
P. robertsi	40	85	59	29	59
P. gadovii species-group					
P. smithi (S)	21	71	35	0	47
P. gadovii	5	79	46	14	58
P. melanomolga	8	—	—	4	45
P. unguidentis	8	79	34	0	44
P. bellii species-group					
P. bellii (B)	39	74	52	43	0

TABLE 5.—Albumin comparison involving antisera to *Chiropterotriton*.

Species tested	Anti-*multidentatus*	Anti-*bromeliacia*
Chiropterotriton α		
C. multidentatus	0	98
C. chiropterus	53	81
C. chondrostega	29	74
C. dimidiatus	25	—
C. priscus	26	71
Chiropterotriton β		
C. bromeliacia	104	0
C. cuchumatanus	—	21
C. rabbi	90	14
C. nasalis	108	64
C. veraepacis	100	67

antisera of *Chiropterotriton* with 8 additional species of that genus. Results are reported in Tables 4 and 5.

The species of *Pseudoeurycea* display a surprisingly heterogeneous pattern. Immunological distance of *P. bellii* averaged 49 ± 6 units (SD) to the species of the *gadovii* group, 52 ± 2 units to the species of the *rex* group, 59 ± 13 units to the *leprosa* group, and 68 ± 10 units to the *cephalica* group. The *bellii* group contains but a single species; it is essentially equidistant from the *gadovii* and *rex* groups, and somewhat further from the *leprosa* and *cephalica* groups. The members of the *gadovii* and *rex* groups are relatively close, but they display surprising variability considering the relatively small immunological distances (12 ± 8). Further, *P. exspectata* and *P. rex*, of the *rex* group, are less distant from *P. smithi* than they are from *P. brunnata*. Conversely, *P. gadovii*, of the *gadovii* group, is closer to *P. brunnata* than to *P. smithi*. The greatest distance shown by any member of either of these two groups is 27 (*P. exspectata–P. brunnata*), and three species of the *gadovii* group (*P. gadovii, P. melanomolga, P. unguidentis*) have very low distances to both *P. brunnata* and *P. smithi* (all but one measurement less than 10 IDU).

The relatively close similarity of members of the *gadovii* and *rex* groups stands in sharp contrast to the heterogeneous *leprosa* and *cephalica* groups. *Pseudoeurycea juarezi* and *P. robertsi* of the *leprosa* group are closer to *P. brunnata* and *P. smithi* than they are to *P. leprosa*, and the shortest distance of any species to *P. leprosa* is 31 (*P. juarezi*). The next closest species to *P. leprosa* (30–40 IDU) are members of the *gadovii* and *rex* groups: *P. unguidentis, P. smithi, P. rex, P. goebeli, P. exspectata*. All are closer to *P. leprosa* than to any member of the *leprosa* group, save *P. juarezi*.

The *cephalica* group is even more heterogeneous, for none of the members we have tested has an immunological distance less than 70 to *P. cephalica*, and all have lesser immunological distances to all other *Pseudoeurycea* antisera used. *Pseudoeurycea cephalica* appears to have the most differentiated albumin (range of immunological distances 49–130, mean 76) tested in the genus. Two putative members of the *cephalica* group, *P. altamontana* and *P. cochranae*, have albumin closest to *P. brunnata* and *P. smithi*. Our data are incomplete for *P. scandens* and *P. werleri*, but both seem to have distinct albumin (minimal distance of both species to *P. leprosa* is about 50).

If *P. smithi* and *P. brunnata* are considered part of the same lineage, one might expect relatively unrelated species to show similar immunological distances to both species. For some species this is indeed the case, and the difference between the highest and lowest measure to *P. smithi* and *P. brunnata* is 20% or less of the lowest figure (*P. altamontana*, average distance 29.5; *P. werleri*, 63; *P. nigromaculata*, 51; *P. bellii*, 41). The only species that seems markedly less distant from one than from the other is *P. leprosa* (average immunological distance to *P. smithi*, 35; to *P. brunnata*, 50.5).

Immunological distances between the *α* and *β Chiropterotriton* are greater than those within *Pseudoeurycea*. The distance between *C. multidentatus* (*α*) and *C. bromeliacia* (*β*) is large, with very good reciprocity (98–104).

Unidirectional tests using the two *Chiropterotriton* antisera were made to eight other species in the genus (Table 5). Immunological distance of *C. multidentatus* to β species ranges from 90–108 ($\bar{x}$ = 100.5). Immunological distance of *C. bromeliacia* to α species ranges from 71–90 ($\bar{x}$ = 82). Within the α group, *C. multidentatus* is about equally distant from *C. chondrostega, C. dimidiatus,* and *C. priscus* (25–29), but the distance from *C. multidentatus* to *C. chiropterus* is considerably greater (53). The β group is more diverse. The distance of *C. bromeliacia* to other β species ranges from 14 (*C. rabbi*) and 21 (*C. cuchumatanus*) to 64 and 67 (*C. bromeliacia* to *C. nasalis* and *C. veraepacis*, respectively).

The mean of reciprocal immunological distance between *C. bromeliacia* and the five antisera of *Pseudoeurycea* is 77.8 (range 53–92, SD 14.3). The comparable value for *C. multidentatus* is similar (mean 84.5, range 57–107, SD 14.7). Both *C. bromeliacia* and *C. multidentatus* are more similar to *Pseudoeurycea* than to each other (mean IDU = 101).

Discussion

Prior to Taylor (1944), most workers classified plethodontid salamanders from the New World tropics in the genus *Bolitoglossa* (= *Oedipus*). Taylor established both *Pseudoeurycea* and *Chiropterotriton,* which were distinguished mainly on the basis of size (*Pseudoeurycea* is the larger) and foot shape (*Chiropterotriton* has relatively larger, broader feet, with expanded terminal phalanges and a relatively long fifth toe), as well as some osteological characters. To this day the genera are recognized essentially as defined by Taylor, although a number of additional species have been described.

Taylor (1944) recognized (but did not diagnose) five species-groups of *Pseudoeurycea*: *gadovii, smithi, cephalica, bellii,* and *leprosa*. A detailed anatomical study of *Pseudoeurycea* was presented by Baird (1951), who included *P. smithi* (the sole representative of Taylor's *smithi* group) in the *gadovii* group, but otherwise reached systematic conclusions identical to those of Taylor. The only recent systematic account of the entire genus is the provisional annotated list of species groups and species by Wake and Lynch (1976), who observed that *Pseudoeurycea* "is in need of taxonomic revision." They essentially followed Taylor, as modified by Baird, except for assigning Guatemalan species included by Taylor in the *leprosa* group to a separate *rex* group.

The results of the present study support the removal of the Guatemalan species from the *leprosa* group, but the immunological data also indicate a close relationship between the *rex* and *gadovii* groups. In view of the lack of morphological diagnosis for the *rex* group, we suggest that it simply be included within an expanded *gadovii* group.

A "core" group of species has immunological distances to both *P. smithi* and *P. brunnata* on the order of 20 or less. These species include *P. smithi, P. brunnata, P. gadovii, P. goebeli, P. melanomolga,* and *P. unguidentis*. Another group is slightly more distant, with average immunological distances on the order of 30: *P. exspectata, P. juarezi, P. rex,* and *P. cochranae*. *Pseudoeurycea robertsi* and *P. altamontana* are even more distant (maximum distance = 40). All of the aforementioned species are closer to *P. smithi* and *P. brunnata* than to any other species for which antisera were tested. Some of these species have never been placed in either the *rex* or *gadovii* groups, but all six of the above-listed "core" species have. *Pseudoeurycea cochranae* was placed in the *leprosa* group by Taylor (1944) and in the *cephalica* group by Wake and Lynch (1976), whereas our study suggests that this species is not closely related to either lineage. Based on external appearance and ecological characteristics, *P. robertsi* and *P. altamontana* were placed in the *leprosa* group by Taylor. Wake and Lynch concurred with respect to *P. rob-*

ertsi, but assigned *P. altamontana* to the *cephalica* group. The present study indicates that both species are far closer to *P. smithi* and *P. brunnata* than they are to *P. leprosa*, and both are remotely related (over 70 IDU) to *P. cephalica*. *Pseudoeurycea juarezi* has been thought to be a close relative of *P. firscheini* and *P. nigromaculata* (Regal, 1966), both of which are considered close relatives of *P. leprosa*. Our data indicate that *P. juarezi* is indeed reasonably close to *P. leprosa* (31), but closer still to *P. brunnata* (27) and *P. smithi* (17).

Of the species of the *leprosa* group that we tested, only *P. nigromaculata* is closer to *P. leprosa* than to any other antiserum we prepared, but even this relationship is relatively remote (IDU = 42).

The content of the *cephalica* group is brought into question by our discovery that no member of the *cephalica* group is closer than 73 IDU to *P. cephalica*. Incomplete data for *P. scandens* and *P. werleri* indicate that neither species is closer than 50 IDU to any other antiserum tested.

Pseudoeurycea bellii, the only member of the *bellii* group, is relatively remote from all other *Pseudoeurycea* (smallest IDU = 43, to *P. smithi*).

We conclude that most of the species of *Pseudoeurycea* tested are moderately close relatives that provisionally are to be considered members of the *gadovii* species-group. Our data indicate that the *bellii*, *leprosa*, and *cephalica* groups each contain a single, phyletically distinct species.

Wake and Lynch (1976) recognized three species-groups within *Chiropterotriton* α: *chiropterus*, *multidentatus*, and *priscus*. Our immunological data indicate that two species-groups may be justified. *Chiropterotriton chondrostega*, *C. dimidiatus* (both previously assigned to the *chiropterus* group), and *C. priscus* are all relatively close to *C. multidentatus*, whereas *C. chiropterus* comprises a second group that is relatively remote from *C. multidentatus*.

Wake and Lynch (1976) and Lynch and Wake (1978) previously recognized two very distinct species-groups in *Chiropterotriton* β: *bromeliacia* and *picadoi* (Lynch and Wake, 1978, erroneously referred to the latter as the *nasalis* group). This arrangement is supported by our data. *Chiropterotriton bromeliacia*, *C. cuchumatanus* and *C. rabbi* (*bromeliacia* group) are closely related; the latter species are 21 and 14 IDU from *C. bromeliacia*. However, *C. nasalis* and *C. veraepacis* (*picadoi* group) are remote from *C. bromeliacia* (IDU = 64 and 67 respectively), and presumably from the rest of the group as well. The latter two species are only a little closer to *C. bromeliacia* than are some species of *Chiropterotriton* α, but relative to *C. multidentatus* all members of *Chiropterotriton* β are about equally remote.

The dendrograms presented by Wake and Lynch (1976) indicate that *Chiropterotriton* α is closer to *Pseudoeurycea* than either is to *Chiropterotriton* β. Our data indicate that the two antisera of *Chiropterotriton* are closer to *Pseudoeurycea* than to each other, but we cannot with confidence say which is the closer. The data support the concept of α and β groups, and also support the contention of Lynch and Wake (1978) that the *bromeliacia* and *picadoi* groups are very distinct. The implications of these results will be discussed elsewhere, in the context of a detailed morphological analysis.

Rabb (1956) described *C. priscus*, a species he believed to be somewhat intermediate in anatomical features between *Chiropterotriton* and *Pseudoeurycea*. He specifically compared *C. priscus* with *P. galeanae*, a member of the *cephalica* group. *Chiropterotriton priscus* is immunologically close to *C. multidentatus* (IDU = 26), but remote from *P. cephalica* (IDU = 74). Rabb (1956) and Wake and Lynch (1976) hypothesized that the similarities between *C. priscus* and some *Pseudoeurycea* are based on retained generalized and prim-

itive anatomical features. Our data are consistent with such an interpretation.

As is evident from inspection of the internodal distances in Figure 1, rates of albumin evolution in this assemblage have been reasonably regular. An exception is the lineage containing *P. brunnata* and *P. smithi,* which has accumulated considerably less immunological distance during its evolution than have the other lineages. While the accumulation of immunological distance is mainly a time-dependent phenomenon (Wilson et al., 1977), at least some species of *Pseudoeurycea* have undergone different rates of change. If we accept the estimate that roughly 100 units of immunological distance accumulate between two lineages every 55–60 million years (Maxson and Wilson, 1975; Wilson et al., 1977), the evolutionary history of some of the groups discussed herein may extend over most of the Cenozoic. The species of *Pseudoeurycea* and *Plethodon* show very similar distributions of immunological distances (Maxson and Maxson, 1979; Maxson et al., 1979), indicating that the two genera, neither of which is strongly differentiated in morphology, may be of similar age and have had a long history of cladogenesis.

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IN MEMORIAM: ARTHUR LOVERIDGE

CARL GANS

ON 16 February 1980 the herpetological community lost Arthur Loveridge—professional herpetologist, explorer, popular writer, radio personality, soldier, and long-time curator of amphibians and reptiles at the Museum of Comparative Zoology. He published his first paper in 1913 and was still actively concerned with natural history at the time of his death on the island of St. Helena. In between, he published well over 200 books, research papers, popular articles, and reviews, including the volumes *Many Happy Days I've Squandered* (1944), *Tomorrow's a Holiday* (1944), *Reptiles of the Pacific World* (1945), *I Drank the Zambesi* (1953), and *Forest Safari* (1956).

Mr. Loveridge was born in Penarth, Glamorgan, Wales, on 28 May 1891. He appears to have had an abiding interest in natural history from his earliest years. An enthusiasm for natural history is not uncommon in boys, nor is the trend to assemble masses of natural history objects. The spectacular difference between Loveridge and other enthusiastic boy-naturalists was his total commitment to the pursuit of natural history as a career. In 1914, at the age of 24, he applied for the curatorship of the Nairobi Museum, advising in the application that he had over 300 cases of natural history and anthropological specimens and over 250 jars of "spirit-preserved" reptiles. He noted proudly that he was prepared to handle techniques of mounting and preserving all of his captures, described his complex system of registration and collecting numbers, and referred to an 80-page catalog, with short descriptions of all specimens, all typed by himself. Even his handwriting was small and meticulously formed with little change between top and bottom of pages or successive sheets. Field notes and labels (as well as his many letters) alike were beautifully readable.

Judging from comments in his books and from documents made available from the archives of Harvard University, Loveridge completed school and then was made by his family to serve two years in a business apprenticeship, where he was in charge of the storeroom and associated record keeping. He next obtained a one-year course at the University College of South Wales, Cardiff, under Professor W. N. Parker, and then took a six-month appointment as an assistant in the Manchester University Museum, cataloging, rebottling, and re-identifying the collection. In 1911, he was appointed assistant