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THE KARYOTYPE OF *VANZOLINIUS DISCODACTYLUS* AND COMMENTS ON USEFULNESS OF KARYOTYPES IN DETERMINING RELATIONSHIPS IN THE *LEPTODACTYLUS*-COMPLEX (AMPHIBIA, LEPTODACTYLIDAE)

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Information on karyotypes of several species of the frog genera *Adenomera* and *Leptodactylus* recently has become available. Bogart (In press) described and figured the karyotypes of 16 species of *Leptodactylus* and 3 species of *Adenomera* (reported as the *marmoratus* group members of the genus *Leptodactylus*). We have obtained information on the karyotypes of 9 species of *Leptodactylus* and *Vanzolinius discodactylus*. The purpose of this paper is to describe the karyotype for the previously unreported *V. discodactylus* and offer alternatives to the relationships among the *Leptodactylus*-complex to those proposed by Bogart (In press). It should be pointed out that the genera *Adenomera, Leptodactylus,* and *Vanzolinius* have been considered to all belong to the genus *Leptodactylus* until recently.

Methods and Materials

The technique and terminology used in preparation and description of the karyotypes follows Patton (1967). Approximately 50 cells were examined from marrow, spleen, or testis tissue of the specimens. The material was examined

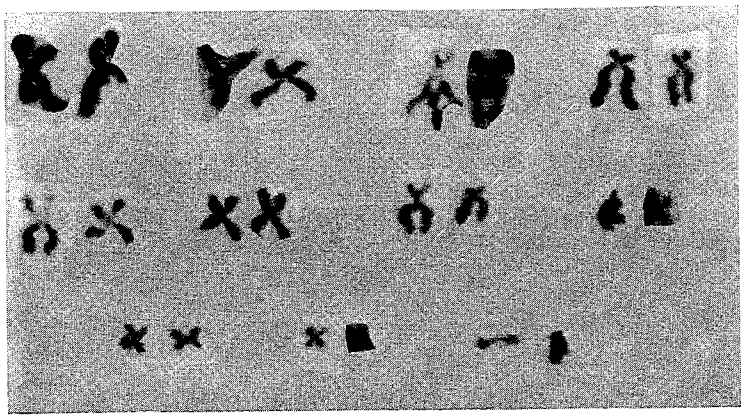

FIG. 1. Karyotype of *Vanzolinius discodactylus*, specimen WRH 464. Chromosome pair number 3 with overlap.

using a phase contrast microscope and the better metaphase figures were photographed. The chromosomal spreads used in the analysis were those with few or no overlapping chromosomes in the condensed state. Each arm of every chromosome was measured and the chromosomes were grouped in pairs according to their size and arm ratio. Our preparations did not allow the location of secondary constrictions.

The specimens were taken from the following localities: *Leptodactylus bolivianus, melanonotus*—Costa Rica. *Leptodactylus bufonius, chaquensis, fuscus, latinasus*—Argentina: Salta; Embarcacion. *Leptodactylus mystaceus, pentadactylus, wagneri, Vanzolinius discodactylus*—Ecuador: Napo; Limoncocha. Specimens and slides will be deposited at the Natural History Museum, Los Angeles County.

The Karyotype of *Vanzolinius discodactylus*

The karyotype of *Vanzolinius discodactylus* is characterized as follows: diploid number = 22; three pair of metacentrics (Fig. 1, chromosome pair numbers 5, 8, 9); four pair of submetacentrics (Fig. 1, chromosome pair numbers 1, 2, 6, 10); three pair of subtelocentrics (Fig. 1, chromosome pair numbers 3, 4, 7); one pair of acrocentrics (Fig. 1, chromosome pair number 11); fundamental number = 42.

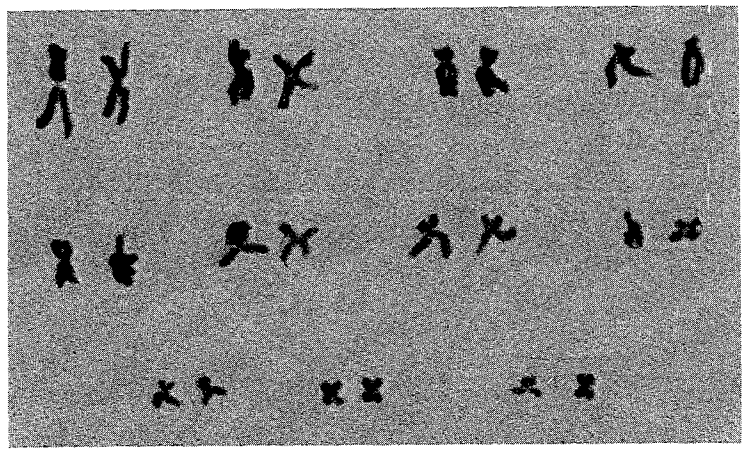

FIG. 2. Karyotype of *Leptodactylus chaquensis*, specimen WRH 1402.

Karyotypic Comparisons of *Leptodactylus* Species

The karyotypes of the species of *Leptodactylus* we examined agree with previously published accounts in that all species have a diploid number of 22.

Although Barbieri (1950) reported the diploid number of *L. chaquensis* as 2n = 22, he did not include a metaphase figure of the karyotype. We include a metaphase figure (Fig. 2) of this species for comparative purposes. There are four pair of metacentrics (Fig. 2, chromosome pair numbers 1, 6, 8, 11); five pair of submetacentrics (Fig. 2, chromosome pair numbers 2, 5, 7, 9, 10); and two pair of subtelocentrics (Fig. 2, chromosome pair numbers 3, 4).

Our analysis of chromosome morphology based on centrometric position differs considerably from previously published accounts (Table 1). These differences could be accounted for in three different ways. First, the different karyotypes could be the result of different techniques. Bogart (In press) used the corneal squash technique while we have used the marrow and spleen cell suspension technique. Second, the differences could be due to geographic variation within species. Third, the differences might be due to different interpretations of morphology and of which pairs are ho-

TABLE 1. Comparison of karyotypes for species of *Leptodactylus*.

Source	Species	Meta-centrics	Submeta-centrics	Subtelo-centrics	Acro-centrics
Bogart[1]	*bolivianus*[2]	8	2	1	0
Present Study	"	4	5	2	0
Bogart	*bufonius*	7	2	2	0
Present Study	"	6	2	3	0
Bogart	*fuscus*	7	3	1	0
Present Study	"	5	3	3	0
Bogart	*melanonotus*	6	3	2	0
Present Study	"	4	4	3	0
Bogart	*mystaceus*	5	3	3	0
Present Study	"	4	4	3	0
Bogart	*pentadactylus*	7	3	1	0
Denaro[3]	"	4	3	4	0
Present Study	"	2	6	3	0
Bogart	*wagneri*	2	4	1	4
Present Study	"	2	2	3	4

[1] Bogart (In press).
[2] Reported as *insularum*.
[3] Denaro (1972).

mologous. We think that the third explanation could very well account for many of the differences noted in the karyotypes of Table 1. Until homologues can be determined objectively, we think that the karyotype data must be interpreted extremely conservatively.

RELATIONSHIPS

There are two aspects of the karyotypes that appear to be conservative in the sense that they are not influenced by interpretations by different workers and also appear to provide information of possible use in predicting relationships. These aspects are the diploid number and the presence or absence of acrocentric chromosomes. Character states of these two karyotypic characters are assigned as follows:

Diploid number of chromosomes: Character state a = diploid number of 26. This is the primitive state of the family (Lynch, 1971, p. 37). State A = diploid number of 24 and is

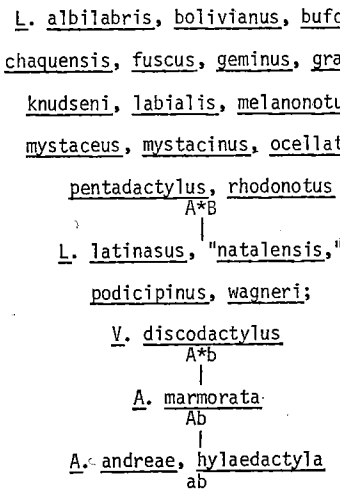

FIG. 3. Proposed relationships based on karyotypes. Lower case letters indicate primitive states, capital letters indicate derived states, asterisks indicate secondarily derived states. Character a is the diploid number, character b is presence or absence of acrocentric chromosomes. Also see text. Based on data from Barrio (1973), Bogart (In press), Denaro (1972), Heyer (1972), and present study.

derived. State A* = diploid number of 22 and is secondarily derived.

Acrocentric chromosomes: Character state b = acrocentric chromosomes present. This is the primitive state, generally recognized by chromosome workers, although there is no information to support this from the primitive members of the family Leptodactylidae. Character state B = acrocentric chromosomes absent and is derived.

A phylogeny based on these two characters is presented (Fig. 3). Certain comparisons of this phylogeny with previously proposed species groupings (Heyer, 1968) are of interest. As only two characters are used, the clusterings of species at certain steps of the phylogeny are large, particularly for the last. The most advanced cluster contains all of the members of the *ocellatus* (*L. bolivianus, chaquensis, ocellatus*) and *pentadactylus* (*knudseni, pentadactylus, rhodonotus*) species

groups examined. All species examined of the *fuscus* group (*L. albilabris, bufonius, fuscus, geminus, gracilis, labialis, mystaceus, mystacinus*) except *L. latinasus* are in this group, and one member of the *melanonotus* group (*L. melanonotus*) is also represented in the most advanced group. The rest of the *melanonotus* group members (*"natalensis," podicipinus, wagneri*) are in an ancestral grouping. This generally corresponds to the scheme previously proposed (Heyer, 1969), in which the *melanonotus* species group is among the most primitive species groups in the genus. The two exceptions to this agreement of phylogenetic schemes are *L. melanonotus* and *L. latinasus*. In the case of *L. melanonotus*, the advanced state of the karyotype may indicate that *L. melanonotus* has diverged from *podicipinus* and *wagneri* to a greater degree than expected on the basis of standard morphological analysis. This explanation makes biogeographic sense as *L. melanonotus* has a Middle American distribution pattern, while *podicipinus* and *wagneri* are South American species.

We think that the karyotype evidence gives greater insight to the relationships among the species of the *melanonotus* group, but that the karyotypes should not be emphasized more than standard morphological evidence. For example, *L. melanonotus* should not be removed from its group members *podicipinus* and *wagneri* because it has a more derived karyotype. We are also hesitant in recognizing species within the *melanonotus* group based only on karyotypic evidence. Bogart (In press) and Denaro (1972) found different numbers of acrocentric chromosome pairs in the two geographic samples of *L. podicipinus* they examined. We prefer to give a conservative explanation to this karyotypic variation and consider the two karyotypes to represent the same species. Similarly, until more evidence is gathered, we prefer to consider the karyotype Bogart (In press) reported as *"natalensis"* to be a geographic variant of *wagneri*, differing in acrocentric number. Because *L. podicipinus* and *wagneri* show geographic differences in the number of acrocentrics, both species would appear to be ideal systems for detailed population study by the use of karyotypes to determine evolutionary patterns within the species, as has been successfully used in some species of small mammals (e.g. Patton, 1972) and *Sceloporus* (e.g. Cole, 1972).

Members of the genus *Adenomera* apparently have the most primitive karyotypes of the *Leptodactylus*-complex. The karyotype of *Vanzolinius* is comparable to the more primitive karyotypes of *Leptodactylus* species. Karyotypic information is limited in delineating relationships at the generic level within the *Leptodactylus*-complex.

Bogart (In press), using chromosome morphology based upon centromere position, postulated relationships that crossed many of the species groups lines that were based on other morphological evidence (Heyer, 1968). We think that his analysis is overextended as he is basing homologies on measurements. As Pathak, Hsu, Shirley, and Helm (1973) demonstrated, homologies based on karyotype measurements can be incorrect (in this case banding indicated the errors based on measurements). Atchley (1972) has also demonstrated the pitfalls of predicting relationships from karyotypes when the homologues are not known with certainty. The conservative use of karyotype information as used herein is more consistent with relationships of the taxa based on other data sets.

Bogart (In press) postulated that because *Adenomera* had a primitive karyotype, terrestriality (no free swimming, feeding larva) is primitive within the *Leptodactylus*-complex. We disagree with this hypothesis for two reasons. First, the homologous chromosomes are not known. It may be that the karyotype of members of the genus *Adenomera* is actually derived. Second, there is no reason why a group of frogs cannot retain a primitive karyotype while having a derived life history pattern. The morphological evidence (Heyer, 1969, 1972, 1974) is quite conclusive that terrestriality is a derived condition in the *Leptodactylus*-complex; we see no reason to overthrow this evidence in light of the supposedly primitive karyotypes of *Adenomera* species.

We conclude that until homologous pairs of chromosomes can be determined accurately (banding probably will give this information), 1) the information content of karyotypes in elucidating relationships among *Leptodactylus* species and genera of the *Leptodactylus*-complex is limited, and 2) the

karyotype evidence must be interpreted within the framework of other morphological and ecological evidence and can not profitably be used as an entirely independent set of information in elucidating relationships.

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