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Karyotype and Relationships of *Anolis desechensis*

MATTHEW C. BRANDLEY,[1,2] ADDISON WYNN,[3] AND KEVIN DE QUEIROZ[3]

[1]*Museum of Vertebrate Zoology and Department of Integrative Biology, University of California, Berkeley, California 94720-3160, USA; E-mail: brandley@berkeley.edu*
[3]*Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560-0162, USA*

ABSTRACT.—We determined the karyotype of *Anolis desechensis* and compared it with the known karyotypes of other members of the *Anolis cristatellus* series. The diploid (2N) number of chromosomes of two male *A. desechensis* was 27, with six pairs of large metacentric macrochromosomes, six pairs of microchromosomes of gradually decreasing size, and sex chromosome heteromorphism (three sex chromosomes, X_1X_2Y). This chromosome complement is identical to that of *A. cristatellus* and *A. scriptus*, thus providing additional evidence of a close relationship to these taxa. The evolution of chromosome number within the *cristatellus* series appears to have involved minimal homoplasy and therefore contains useful phylogenetic information.

Historically, karyotype data have played a significant role in inferring the phylogenetic relationships of Caribbean *Anolis* lizards (e.g., Gorman et al., 1968, 1983; Gorman and Atkins, 1969; Gorman and Stamm, 1975), and they continue to be used in recent phylogenetic analyses of the entire *Anolis* clade (Poe, 1998, 2004) as well as various subclades (e.g., Creer et al., 2001, for the *roquet* series; Brandley and de Queiroz, 2004, for the *cristatellus* series). Within the *cristatellus* series, a clade of anoles that inhabit the Puerto Rican Island Bank, St. Croix, and the southern Bahamas, several relationships implied by karyotypes were corroborated (or at least not decisively contradicted) by the results of a combined morphological (including karyotypic), allozyme, and DNA phylogenetic analysis (Brandley and de Queiroz, 2004). These are (1) a close relationship between the *bimaculatus*, *distichus*, and *cristatellus* series (all of which exhibit sex chromosome heteromorphism and reductions in chromosome number relative to the ancestral 2N = 36); (2) a sister relationship between the *bimaculatus* and *cristatellus* series (which share a reduction of 2N to < 33/34, though the combined analysis weakly favors a sister relationship between the *bimaculatus* and *distichus* series); and (3) a close relationship between *Anolis scriptus* and *Anolis cristatellus* (2N = 27/28), as well as possibly *Anolis ernestwilliamsi* and *Anolis desechensis* (karyotypes previously unknown). Thus, despite increasing reliance on DNA sequence data, karyotypes remain informative characters for phylogenetic inference.

Karyotype data have been reported for 11 of 13 currently recognized species of the *A. cristatellus* series (Gorman et al., 1983). Data are lacking for *A. ernestwilliamsi* and *A. desechensis*—two species for which Brandley and de Queiroz (2004) inferred close phylogenetic affinities to *A. cristatellus* and *A. scriptus* (the four species together forming the *cristatellus* superspecies). Here we report the karyotype of *A. desechensis* and discuss its implications concerning the phylogenetic relationships of that species and karyotype evolution in the *cristatellus* series.

MATERIALS AND METHODS

Two male specimens of *A. desechensis* (USNM 561839 and 561840), collected in the vicinity of Puerto de los Botes, Isla Desecheo, Puerto Rico, on 28–29 December 1998, were karyotyped. Each specimen was treated with 0.05% colchicine (approximately 0.15 cc/g body weight) four hours before being sacrificed. Tissues (bone marrow, liver, gut, and testes) were minced in 0.8% sodium citrate and allowed to sit for 20 min, strained, and the cell-bearing suspension centrifuged. Thereafter methods followed Patton (1967). Multiple dividing cells in mitosis, and first and second meiosis, were examined. Mitotic chromosome pairs were matched by eye and characterized in terms of the total number of chromosomes and relative size. Karyotypes were characterized in terms of the total number of chromosomes as well as the numbers of chromosomes in different categories based on morphology (e.g., metacentric versus acrocentric) and relative size. Although differential staining of chromosome segments (chromosome banding) provides additional information about chromosomal homologies, it was not employed in the present study. Standard banding techniques have generally proved unsuccessful on the chromosomes of ectothermic reptiles (but see Kasahara et al., 1987; Yonenga-Yassuda et al., 1988) and data on banding patterns are not available for other members of the *A. cristatellus* series. To estimate karyotype evolution in the *cristatellus* series, the chromosome number was coded as a discrete character and optimized using the parsimony criterion implemented by MacClade 4.07 (W. P. Maddison and D. R. Maddison, Sinauer Associates, Sunderland, MA, 2000) on the combined 12S and cytochrome *b* mtDNA phylogeny of Brandley and de Queiroz (2004: fig. 8), except that the relationships among the *bimaculatus*, *cristatellus*, and *distichus* series were left unresolved because of weak support (bootstrap proportion < 50%).

Karyotype data for species other than *A. desechensis* were obtained from Gorman and Atkins (1966, 1969), Gorman et al. (1968), Gorman (1973), Gorman and Stamm (1975), T. P. Webster in Williams (1977), and Gorman et al. (1983).

[2] Corresponding Author.

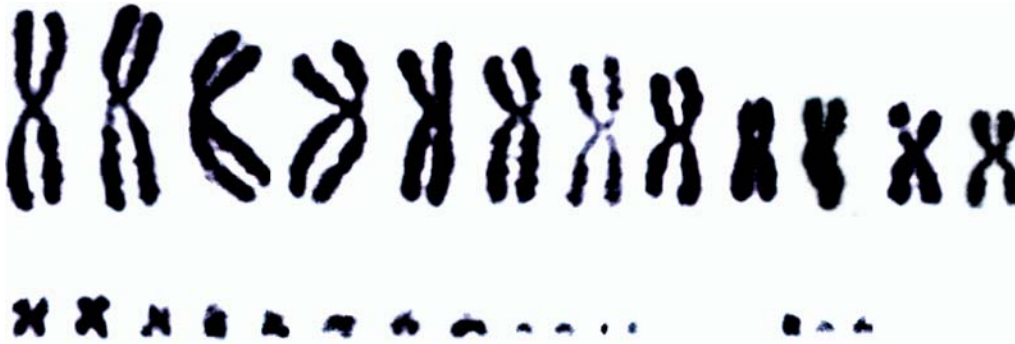

FIG. 1. A karyotype of a male *Anolis desechensis* (USNM 561840), 2N = 27, X_1X_2Y. Autosomal chromosome pairs are arranged in order of decreasing size from upper left to lower right, followed by the sex chromosomes.

RESULTS AND DISCUSSION

Nine mitotic spreads were identified. The karyotypes of the two male specimens of *A. desechensis* were identical, consisting of 2N = 27 chromosomes, with six pairs of metacentric macrochromosomes (the last two pairs of similar size but smaller than the first four pairs), and six pairs of microchromosomes of gradually decreasing size, the largest two pairs of which were identifiably metacentric (Fig. 1). Three chromosomes do not form pairs of equal size and thus presumably represent the male sex chromosomes (X_1X_2Y). This interpretation is supported by 15 first meiotic division spreads in which we observed a trivalent (Fig. 2) and in 17 second meiotic division spreads where we consistently identified either seven or eight microchromosomes as expected with X_1X_2Y sex chromosome heteromorphism. The karyotype of *A. desechensis* is, within the limits of resolution of this study, indistinguishable from that of *A. cristatellus* and *A. scriptus* (Gorman et al., 1968).

A recent phylogenetic analysis of the *cristatellus* series using morphological (including karyotypic), allozyme, and mtDNA data (Brandley and de Queiroz, 2004) inferred a strongly supported (1.00 Bayesian ''posterior probability''; 0.89 likelihood bootstrap proportion) clade composed of *A. desechensis*, *A. ernestwilliamsi*, *A. cristatellus*, and *A. scriptus* (Fig. 3). The karyotype of *A. desechensis* contributes further evidence in support of this close phylogenetic relationship. *Anolis desechensis* exhibits the same derived chromosome number (2N = 27 in males) found in *A. cristatellus* and *A. scriptus* with similar relative chromosome sizes and morphologies, as well as X_1X_2Y male sex chromosomes. No females were examined, but given the species' close relationship to *A. cristatellus* and *A. scriptus*, we can predict that *A. desechensis* females will possess similar karyotypes to those of female *A. cristatellus* and *A. scriptus* (2N = 28, $X_1X_1X_2X_2$).

Sex chromosome heteromorphism occurs in all members of the *bimaculatus*, *cristatellus*, and *distichus* series that have been karyotyped to date. The only other reported case of obvious sex chromosome heteromorphism in anoles is in *Anolis biporcatus* (Gorman, 1973), a distantly related (Nicholson, 2002; Poe, 2004) Central American ''beta'' (*Norops*) anole. This condition can therefore be inferred to have evolved in the ancestor

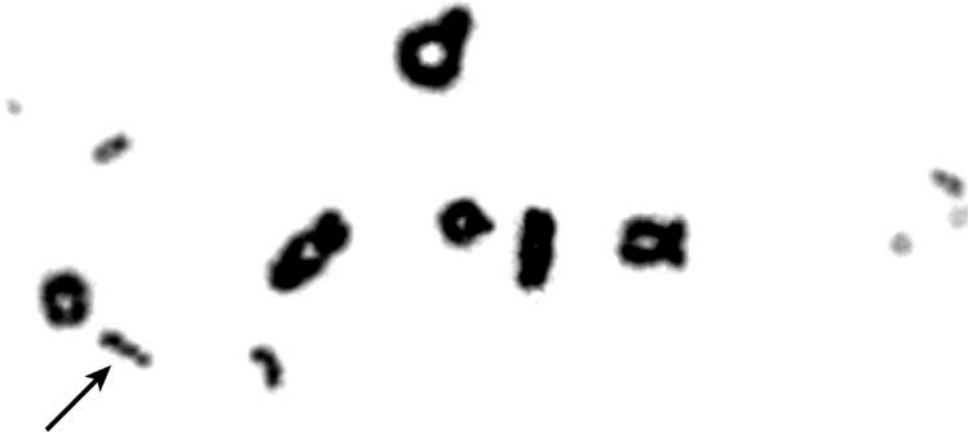

FIG. 2. First meiotic division spread of a male *Anolis desechensis* (USNM 561839). The putative trivalent is indicated by the arrow.

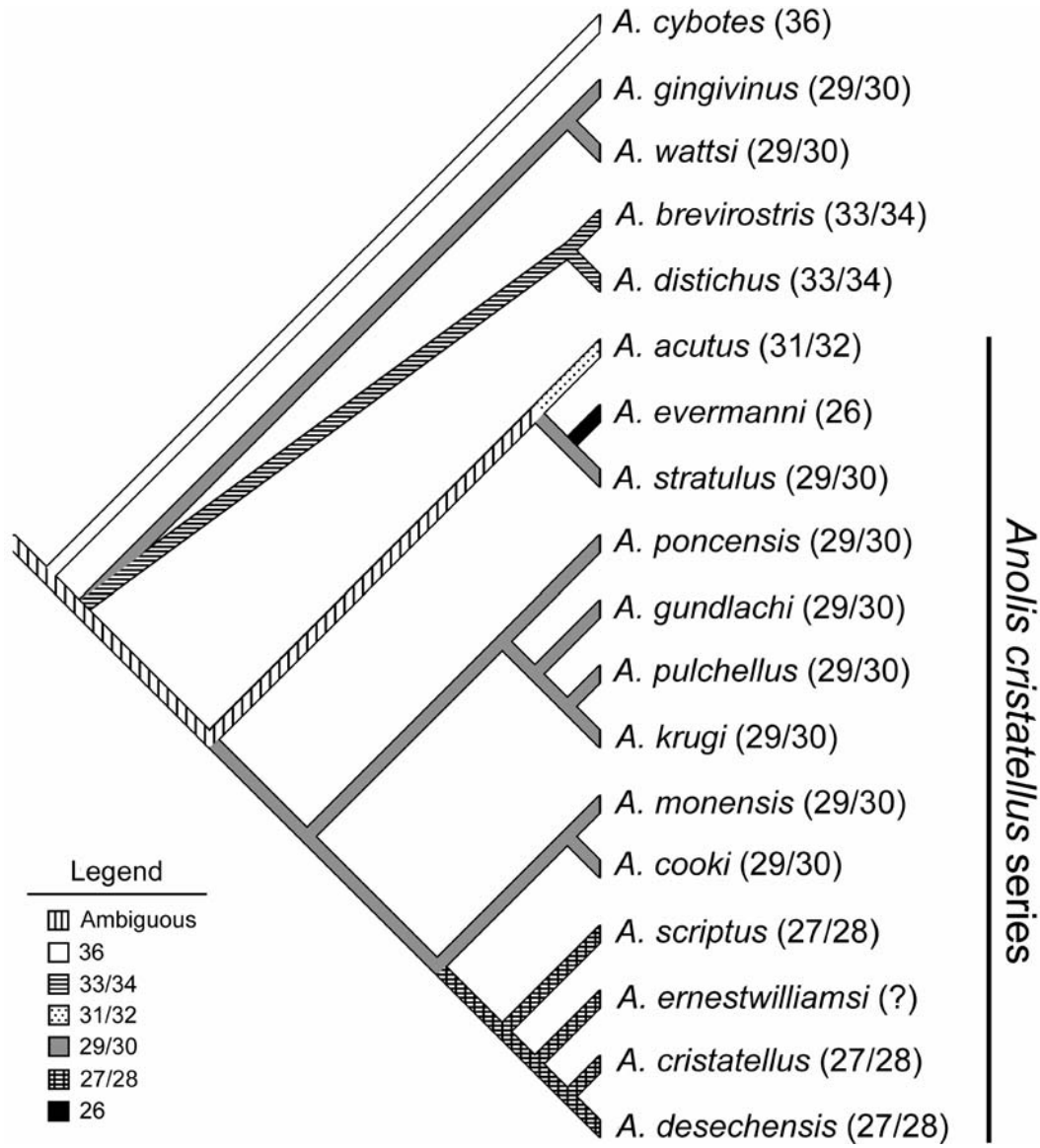

FIG. 3. The evolution of karyotypes within the *Anolis cristatellus* series. Chromosome numbers are given parenthetically after each species name and are also indicated by branch shading. Reconstructions of ancestral chromosome numbers (including ambiguous cases) are indicated by branch shading. An ambiguous state reconstruction was assigned to a branch if the reconstruction was ambiguous for any of the different possible resolutions of the polytomy among the three series (i.e., the reconstructions are not based on the polytomous tree). The tree is from Brandley and de Queiroz (2004:fig. 8), with the sister relationship between the *bimaculatus* series (represented by *Anolis gingivinus* and *Anolis wattsi*) and the *distichus* series (represented by *Anolis distichus* and *Anolis brevirostris*) collapsed because of weak support (bootstrap proportions $< 50\%$).

to the clade composed of those three series (*Ctenonotus* in the taxonomy of Brandley and de Queiroz, 2004). Additionally, these three series all exhibit a reduction in diploid chromosome number from the ancestral 2N = 36 condition found in most other anoles (Gorman, 1973) to 2N $\leq$ 33/34, although the precise ancestral chromosome number for the clade (*Ctenonotus*) is uncertain because of variation within the *cristatellus* series, and weakly supported relationships among the three series. If future phylogenetic analyses find significant support for a sister relationship between the *cristatellus* and *bimaculatus* series, then the most parsimonious optimization of the diploid chromosome number in their common ancestor would be 2N = 29/30.

The ancestral number of chromosomes for the *cristatellus* series is uncertain because of the poorly supported relationships of this series relative to the *distichus* and *bimaculatus* series. Only one resolution (*bimaculatus* + *cristatellus* series) of the polytomy among these three series results in an unambiguous assignment of the ancestral karyotype (2N = 29/30). For the two other possible resolutions, 2N = 29/30 (the condition in the *bimaculatus* series), 2N = 31/32 (the condition in *Anolis acutus*), and 2N = 33/34 (the common condition in the *distichus* series) are equally parsimonious. The karyotype of *A. brevirostris* was coded as unknown in the phylogenetic analysis of Brandley and de Queiroz (2004). However, we recently learned that the karyotype of *A. brevirostris* had been determined by T. Preston Webster to be similar to that of *A. distichus* (see Williams, 1977).

Regardless of the ancestral condition, character optimization infers four total changes in chromosome number within the *cristatellus* series, most of which have resulted from the gain or loss of pairs of microchromosomes. The 2N = 31/32 chromosome number of *A. acutus* may be ancestral for the *cristatellus* series, or it may represent an increase (if 2N = 29/30 is ancestral). An unambiguous reduction to 2N = 27 (males), with 24 autosomes, occurred in the common ancestor of *A. cristatellus*, *A. desechensis*, and *A. scriptus*; *A. evermanni* represents a separate reduction to 24 autosomes. In addition, *A. evermanni* has lost an X (sex) chromosome resulting in XY sex chromosome heteromorphism instead of the ancestral X_1X_2Y and a diploid chromosome number of 2N = 26 (males and females).

It would be useful to determine the karyotype of female *A. desechensis* and of male and female *A. ernestwilliamsi*. Female *A. desechensis* are expected to have a diploid number of 2N = 28, based on that of male *A. desechensis*. Male and female *A. ernestwilliamsi* are expected to have diploid numbers of 2N = 27 (male) and 2N = 28 (female), or a further derivation of this condition, based on the karyotypes of closely related male *A. desechensis*, *A. cristatellus*, and *A. scriptus* and females of other species in the *cristatellus* series. In any case, optimizing the currently available data for the species of the *cristatellus* series demonstrates that karyotypes continue to provide useful phylogenetic information. There exists no homoplasy in sex chromosome hetermorphism (CI = 1.0), and little homoplasy in chromosome number (CI = 0.56–0.71 depending on the resolution of the basal polytomy). Thus, karyotypes can complement phylogenetic studies based on morphological and/or DNA characters and may resolve relationships left unresolved by other more homoplastic data. Moreover, karyotype data provide information relevant to a number of interesting biological phenomena, including hybridization, sex determination, and chromosome evolution. For these reasons, we encourage researchers continue to collect karotype data, especially in clades where an abundance of these data already exist (e.g., anoles).

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