

Use of Natural Marks to Identify Individual *Bokermannohyla hylax* (Amphibia: Anura)

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Photographs by the senior author except where noted.

Abstract.—Of 16 *Bokermannohyla hylax* (13 males and three females) assessed during a seven-month field study in Blumenau, Santa Catarina, Brazil, five were recognized visually by natural marks when recaptured and the other eleven from photographic records of the flanks and dorsum. This is the first time that identification by natural marks has been used to individually identify frogs. To test this method, waistbands with colored plastic beads were tied to the frogs' waists to verify individual recognition upon recapture. Photographic identification is a scientific tool that consists of cataloging photographs of the animal for individual recognition and

later identification in studies of behavior and population dynamics. Many individuals displayed thanatosis upon handling and some vocalized. Such vocalization is distinct from those associated with territoriality, advertisement, and release calls. The identification of individual *B. hylax* using digital images was an efficient, low-cost, and non-invasive method that might be of benefit in the study of other amphibian species.

Some marking techniques currently used in studies of amphibians are extremely aggressive and are not recommended for certain taxonomic groups due to dermal sensitivity, small size, or the potential for dermal regeneration. Given amphibian physiology, these limitations extend to care in using subcutaneous pigments (Donnelly et al. 1994). Most tags, such as plastic or metallic plates used for some reptiles and amphibians, have



Male *Bokermannohyla hylax* with a waistband bearing a unique combination of beads used for individual identification of frogs.



Bokermannohyla hylax with a waistband in natural habitat.



Male *Bokermannohyla hylax* in a bromeliad.



Individuals were released immediately after waistbands were attached.

been adapted from studies of birds or fish, but the relative size of such tags compromises behavior in small individuals. Their use in amphibian studies is consequently questionable (Raney 1940, Woodbury 1956).

A commonly used and inexpensive method for marking amphibians is toe-clipping. Among reptiles, the natural loss of toes justifies the use of this method (Middelburg and Strijbosch 1988, Hudson 1996); however, the natural loss of toes does not indicate the absence of trauma. Even though the amputation apparently does not affect the overall performance of some lizards (Guttman and Creasey 1973), a study by Clarke (1972) showed that a low recapture index as well as a low survival rate was displayed by anurans of the genus *Anaxyrus* (formerly *Bufo*) that had been toe-clipped. Clarke (1972) also noticed that the recapture index had an inverse relation to the number of clipped toes, and that affected frogs demonstrated lower dexterity while handling prey. With salamanders, the problem is the quick regeneration of amputated parts, making the permanent identification of individuals much more difficult.

Clearly, researchers need to develop a new and efficient marking technique for amphibians, one that will not cause physiological, biological, or behavioral harm, and does not qualitatively or quantitatively affect data. The use of unique natural marks for identification in herpetological research is one alternative. Some limitations exist. In chelid turtles, plastral marks might be vulnerable to ontogenetic variation and care must be taken to mark areas that do not change with age (Cabrera and Colantonio 2001). The objective of our research was to record unique characteristics of individual *Bokermannohyla hylax*, enabling “mark”-recapture population studies of this species.

Methods

The study was conducted in Parque Natural Municipal São Francisco de Assis (26°55'07.71" S, 49°04'33.79" W), a conservation unit in Blumenau, Santa Catarina State, Brazil. The study area consists of steep hillsides cov-

ered by dense mesic forest (Veloso et al. 1971) in different successional stages. The 23-ha park, which is bisected by a stream, is a fragment of the Brazilian Atlantic Forest and located downtown in the city of Blumenau.

Sampling took place from June 2006 to May 2007. A total of 26 bi-weekly assessments were performed at pre-determined sites. Efforts to locate nocturnally active *Bokermannohyla hylax* extended from 2030–0230 h and involved a total of 156 person-hours.

All sites were sampled during each visit, generally in the following order: Lake at the entrance of the park, dock beach, dock stream, lower quadrant, and stream hill. This sequence was occasionally inverted due to active vocalization at various sites. We searched for frogs in trees, bromeliads, shrubs, and water lilies in and in proximity to permanent or temporary bodies of water. All environments are favorable for anuran mating. Individuals were located primarily by their calls. Once found, a flashlight was used to capture vocalizing males.

We recorded weight, tympanum diameter, toe-disk diameter, snout-vent length, and foot length (using a Vernier Caliper 50 x 0.05 mm/6x1/128") for each individual, noted any abnormalities, and documented ambient conditions (temperature, relative humidity, atmospheric pressure). Following measurement, the flanks and dorsum of each frog were digitally photographed to record individual patterns of crossbands and other marks. This process was facilitated by the species' habit of employing thanatosis, an adaptive behavior meant to mimic death. Along with thanatosis some males vocalized in a fashion quite distinct from calls associated with territoriality, advertisement, or release. Subsequently, we tied a waistband of polyester string and numbered colored beads around the inguinal region of each animal. All animals were released at the exact site of capture. Upon recapture, the pattern of crossbands and circles on each individual



An individuals employing thanatosis after release.

was compared to photographs, and the number on the inguinal band was used to confirm identity.

Results and Discussion

The patterns of *B. hylax* are unique. Crossbands can be continuous or fragmented, displaying the same width along the entire stripe or tapering either at the beginning or end of the stripe. The crossbands can be straight or



curved and with or without intervening irregular circular or ovoid shapes along the inguinal flanks. The shapes, number, pattern, and position of the marks vary individually. Combinations of unique characters enabled the accurate identification of 16 individuals in nature and another 11 individuals in the herpetological collection of the Laboratory of Zoology of Fundação Universidade Regional de Blumenau (FURB). When two individuals possessed similar marks on the same side, they could be differentiated by examining the inguinal regions or by using photographs taken with their legs extended. Of the frogs examined in the field, four displayed a combination of crossbands and oval shapes or irregular circular shapes. Of the individuals analyzed from the FURB herpetological collection, six displayed a combination of crossbands and oval or irregular circular or oval shapes and five displayed a pattern consisting solely of crossbands. No two patterns were identical.

In this study, the use of natural marks to recognize individuals was extremely satisfactory, enabling the recognition of individuals in the field and lab. Although nine animals required comparisons of multiple photographs, we quickly learned to identify many individuals without resorting to photographs at all.

The use of natural marks may be limited to only some species, namely those that display distinct and variable patterns. Color photographs worked best (Bradfield 2004), but black-and-white images or even drawings would be adequate for this and at least some other species. Stevens et al. (2007) stressed care when choosing the camera and when manipulating images in order to guarantee consistent representation of patterns.

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Although usually a dark gray or black, some Marine Iguanas (*Amblyrhynchus cristatus*) also have patches of green and red (known as "Christmas tree coloration"). Iguanas pay little attention to what they crawl over; piles of basking lizards can be three or four animals deep.