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Growth and Longevity by Skeletochronological Analysis in *Mantidactylus microtympanum*, a Rain-Forest Anuran from Southern Madagascar

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Skeletochronology has proven to be a reliable assessment of individual age in temperate amphibians and reptiles, especially those inhabiting areas with drastic seasonal climatic variation. The alternation of seasons induces discontinous growth throughout life and can be observed at the bone tissue level as an alternation of abundant bone matrix and lines of arrested growth (LAGs). Conversely, amphibians and reptiles from tropical and subtropical areas, and particularly those inhabiting rain forests with relatively constant environments, are expected to have either ill-defined or no LAGs, since these species presumably have no or scarce interruptions and hence continuous growth. Alternatively, if these species undergo annual patterns of inactivity, then a seasonal pattern of LAGs should be detectable. However, despite the great number of skeletochronological papers recently published on amphibians (Castanet et al., 1993, 1996; Guarino et al., 1995), no one has ever investigated amphibians from tropical rain forests.

The aim of this study was to examine skeletochronology in *Mantidactylus microtympanum*, an endemic anuran from the Malagasy rain forest, and to determine whether this technique was appropriate for estimating age structure of tropical herpetofauna. Blommers-Schlösser (1993) includes *M. microtympanum* in the subfamily Mantellinae (Ranidae), which is endemic to Madagascar. This large-sized species (snout–vent length up to 100 mm) inhabits rain-forest streams in the southeast of the Grand'Ile, and it is active and visible mainly at night. Males and females differ in some characters, such as belly convexity, biometric ratios, and peculiar structure of cloaca in the female. Both juveniles and adults have a brownish back, although it is mottled in the former. The large size of the species allowed us to perform skeletochronological analysis on phalanges alone and, therefore to mark and release the individuals on the site of sampling.

Materials and Methods

The Andohahela Strict Nature Reserve is located in southern Madagascar and is composed of three parcels (76,000 ha) with different climatic and vegetational characters. Parcel 1 (63,000 ha), which is covered with one of the southernmost rain forests of Madagascar, ranges in altitude between 100 and 1956 m. Annual average temperatures ranges from 15.6 C (July) to 29 C (February). The total annual rainfall ranges between 1500 and 3000 mm; there are 166 days, distributed throughout the year, on which precipitation falls. Compared with other rain forests, the study area shows a seasonal variability between the rainy warmest months (October–February) and the "dry-coldest" months (March–September; Nicoll and Langrand, 1989; UICN/PNUE/WWF, 1990). Individuals of *M. microtympanum* were captured in Parcel 1 situated at altitudes of 200–700 m between the villages of Isaka-Ivondro and Eminiminy, 30 km north-northwest of Tolagnaro, Toliara Province, (24°45′30″S, 46°51′15″E). Surveys were carried out in two periods, 15 April to 5 May and 5–19 November 1994.

The frogs were found in relatively high concentrations along the Ampasy stream, at an elevation of approximately 500 m. A 100-m linear tract of this stream was chosen for a study of a *M. microtympanum* subpopulation. The frogs were captured at night by hand with the aid of head torches; then they were measured from snout to vent (SVL, to the nearest 0.1 mm) and marked by toe-clipping (Donnelly et al., 1994). The penultimate phalanx of the second digit of the right hind foot of each individual was used for skeletochronological analysis.

The cut phalanges were fixed in 10% formalin and then, after washing in tapwater, were transferred to 70% ethanol. Skeletochronological analysis was performed according to the routine protocol (Guarino et al., 1995): decalcification in 5% nitric acid for at least 2 h, sectioning by cryostat at 12–15 μm of thickness, staining with Ehrlich's haematoxylin. A small sample of nondecalcified phalanges was embedded in resin (LR-White, hard tissue, Resin London Company) and studied with a polarizing microscope. The number of LAGs destroyed because of endosteal resorption was assessed on photos of three different cross-sections of phalanges, at middiaphyseal level, according to Paton et al. (1991). Twenty-seven males, 32 females, and five juveniles were studied. Snout–vent length and LAGs number were expressed as mean ± 2 SD. All numerical data were analyzed by Student's *t*-test. A probability level of $P \leq 0.05$ was considered as significant.

Results

The analysis of phalangeal cross-sections showed two concentric layers. The inner layer (or endosteal bone) was limited to the medullary cavity (in juveniles, the former was lacking or largely reduced; Fig. 1A) and was characterized by few, flattened osteocytes. This layer typically appeared to be constituted by alternating dark and light lines under "crossed prisms" of the polarizing microscope, indicating that it consisted of lamellar bone. The outer layer (or periosteal bone), wider and with numerous osteocytes, was largely made of pseudolamellar bone. In all phalangeal cross-sections, clearly defined hematoxylinophilic lines, interpreted as LAGs, were observed both in the periosteal and endosteal bone: only those of the periosteal bone are considered here (Fig. 1A–E). Generally, the number of visible LAGs could be easily estimated, because of the low incidence of double and supplementary LAGs (Castanet and Smirina, 1990). In some individuals, one LAG was split into numerous hematoxylinophilic lines, each of which was interpreted as a single LAG (Fig. 1D–E). The total number of LAGs was assessed by count of visible LAGs plus those estimated as totally destroyed by endosteal resorption. The morphometric study, performed on photographs of cross-sections of phalanges from juveniles and adult individuals, showed that the first inner LAG was totally reabsorbed in numerous individuals of both sexes. A smaller percentage of specimens showed total or partial resorption of the first two LAGs (Table 1).

Four juveniles (50, 52, 58, and 74 mm in SVL) showed two LAGs and one specimen, 74 mm in SVL, had five LAGs. Two of the three sexually mature males, with a comparatively small SVL (52 and 54 mm, respectively), exhibited two LAGs, the third (56 mm) had three LAGs. Adult males were significantly smaller than females (males: $\bar{x}$ = 74.7 ± 8.2, n = 26; females: $\bar{x}$ = 94.1 ± 3.2, n = 33; $t = 9.68$, $P < 0.001$, df = 8.51). The average LAGs number differed significantly between the sexes (males: $\bar{x}$ = 4.1 ± 1.1, n = 25; females: $\bar{x}$ = 4.9 ± 0.9, n= 30; $t =$ 3.2 , $P < 0.01$ df = 52). The specimen with the highest LAG number was a female with seven LAGs. The distribution of the LAGs number (Fig. 2) indicates that most of the males (65.3%) had two to four LAGs; among females, individuals with five LAGs were the most frequently observed LAG class (about 58.7%). Sexes differed in SVL in all classes of LAGs but class 6 (class with three LAGs, $t = 3.8$, $P < 0.02$, df = 5; class with four LAGs, $t = 5.8$, $P < 0.01$, df = 12; class with five LAGs, $t = 4.75$, $P < 0.01$, df = 22). Only males exhibited a positive correlation between size and number of LAGs (Pearson's product-moment correlation: r = 0.46, $P < 0.02$, n = 23).

Discussion

Though varying in shape and distinctness, clearly defined hematoxylinophilic lines, or LAGs, have been observed at the bone level in all the amphibians studied by the skeletochronological method (Castanet et al., 1993, 1996; Wake and Castanet, 1995). One of the main issues is to ascertain the periodicity by which LAGs are produced, particularly in the absence of experimental data using the mark-release-recapture approach (Gibbons and McCarthy, 1983) or vital fluorescent labeling (Francillon, 1979; Francillon and Castanet, 1985). This study shows that a Malagasy rain-forest species also displays distinct LAGs in its phalanges. Based on the climatic features of the Andohahela rain forest, we hypothesize that the LAGs observed in *M. microtympanum* correspond to the harsh annual period (March–September), during which temperature, rainfall, and food availability decrease. Consequently, in *M. microtympanum*, one LAG is very likely deposited each year. Therefore, for this hypothesis, the mean age of adults would be approximately four years in males and five in females. However, it cannot be excluded that periodic growth in *M. microtympanum* might be also a result of other biological rhythms, such as reproductive activity (Esteban, 1990), which may be synchronized with the endogenous growth rhythm of this species, as suggested by Castanet et al. (1993).

Tropical rain forests, although more stable than temperate habitats, are also subject to seasonal climatic change and food availability which may affect the growth of predators (Wolda, 1978; Toft, 1980; Karr and Freemark, 1983). Andreone (1994, 1996) showed that, in a Madagascar rain forest, the community composition and abundance of amphibians change throughout the year. Thus, although *M. microtympanum* is most likely active in every period of the year, its growth probably undergoes fluctuations because of lowered temperatures and food availability during the dry season.

Many aspects of the life history of *M. microtympanum*, including egg type, tadpole morphology, size of froglets at metamorphosis and rates of growth, are unknown. Although data on frog size at metamorphosis and at one year are

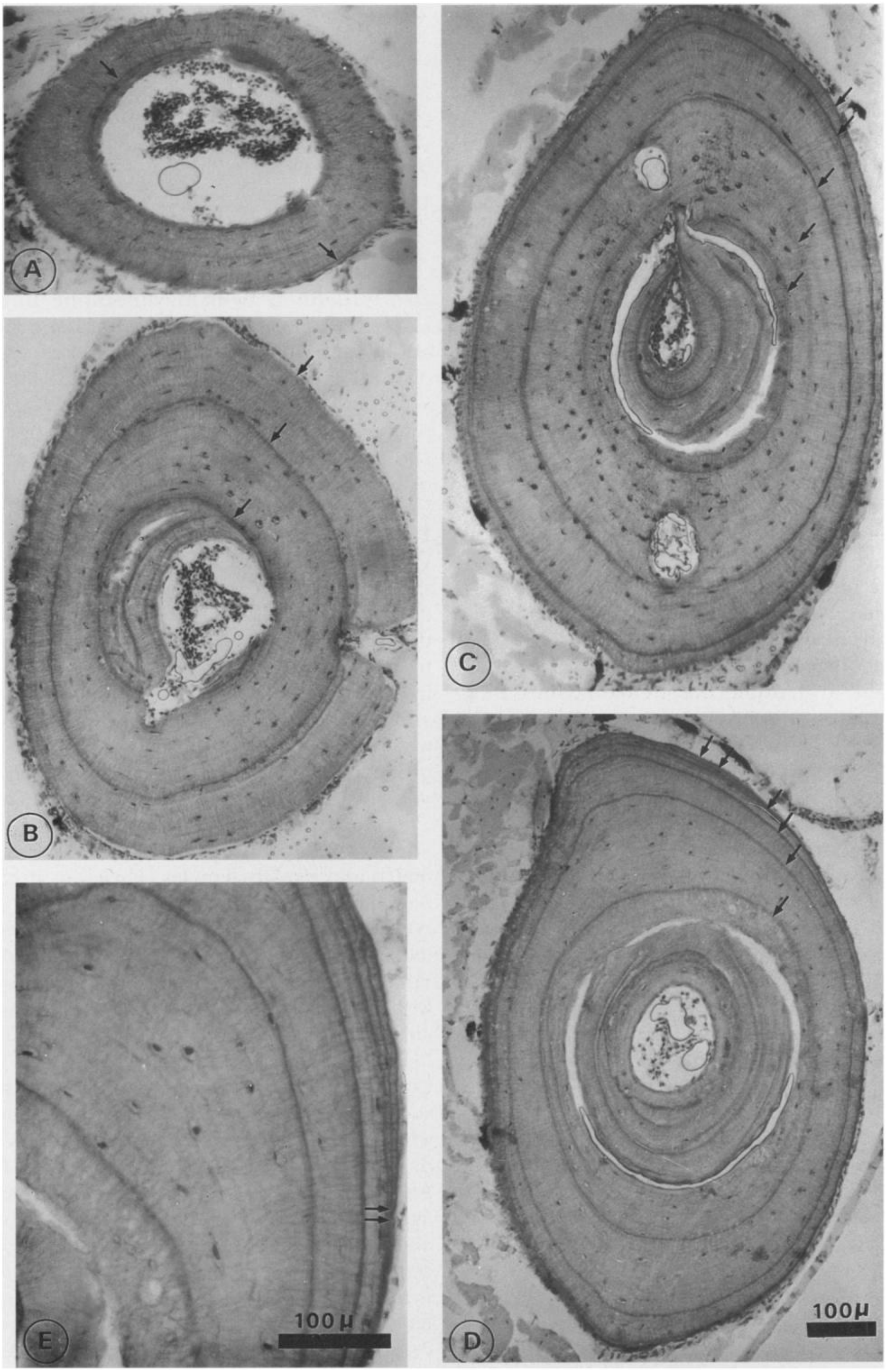

Fig. 1. Cross-sections at the diaphyseal level of phalanx of *Mantidactylus microtympanum.* Arrows indicate LAGs (lines of arrested growth). All figures (except E) are at the same magnification. (A) Young specimen, 52 mm in SVL, with two LAGs. (B) Female, 100 mm in SVL, with three LAGs, the second of which is double. (C) Female, 98 mm in SVL, with five LAGs. (D) Female, 98 mm in SVL, with six visible LAGs plus one totally reabsorbed. (E) Same section as (D) seen at higher magnification. Note the splitting of a peripheral LAG into several (double arrows).

TABLE 1. PERCENTAGE DATA DERIVING FROM THE SKELETOCHRONOLOGICAL ANALYSIS IN *Mantidactylus microtympanum* (LAG = Line of Arrested Growth).

Sex	Sample size	Individuals with defined LAG number	Individuals without reabsorbed LAGs	Individuals with the 1st LAG totally reabsorbed	Individuals with 2nd LAG totally reabsorbed
Males	27	92.0%	65.2%	31.1%	4.7%
Females	32	91.0%	28.0%	69.0%	3.0%
Juveniles	5	100%	80%	20%	0%

lacking, it is clearly evident that, as in other tropical anurans (Duellman and Trueb, 1986), juveniles grow faster than sexually mature frogs. In fact, juveniles showed a body size over 50% of the maximum size reached by adults. Sexual maturity in *M. microtympanum* likely occurs no earlier than the second year of age, because up to that moment all specimens show juvenile characters. Males probably reach maturity one year earlier than do females (two and three years, respectively).

At the predicted age of first reproduction, females are larger than males. This size bias persists within each age class in the following years. Thus, as in other anurans (Ryser, 1988; Bastien and Leclair, 1992), delayed sexual maturity in females may cause larger size and greater longevity than in males. The delayed maturity implies that the individual invests its energy resources mostly in body growth, so as to attain a larger size at the age of first reproduction. Because egg number is generally correlated with female size in anurans (Pettus and Angleton, 1967; Berven, 1982), the larger female size might confer an adaptive advantage (Cherry and Francillon-Vieillot, 1992) by increasing fecundity.

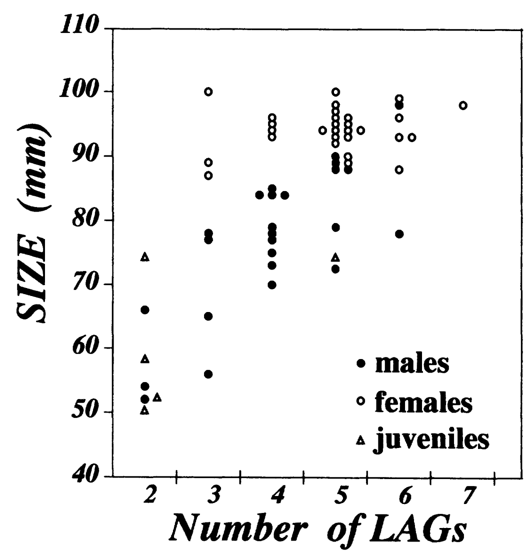

FIG. 2. Plot distribution of LAGs (lines of arrested growth) versus SVL in the studied *Mantidactylus microtympanum* sample from Andohahela rain forest, southern Madagascar. The individuals with a dubious LAG numbers (two males and three females) are not shown.

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