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Short Notes

Skeletochronological analysis of age structure in populations of four species of Malagasy poisonous frogs, genus *Mantella*

Olga Jovanovic*, Miguel Vences

Abstract. Age structure of populations of four species of endemic Malagasy frogs of the genus *Mantella* (*M. aurantiaca*, *M. baroni*, *M. bernhardi*, *M. madagascariensis*) was examined by skeletochronology based on 96 specimens from nine different localities. In more than half of these (57%), no lines of arrested growth (LAGs) were found, and the number of LAGs recognized in the remaining specimens was mostly one, and probably two in three specimens. It is generally considered that each LAG corresponds to one year of life; our results therefore confirm that in *Mantella* populations almost all specimens are in their first or second year of life.

Keywords: Amphibia, Madagascar, *Mantella aurantiaca*, *Mantella baroni*, *Mantella bernhardi*, *Mantella madagascariensis*, Mantellidae, skeletochronology.

Malagasy poison frogs of the genus *Mantella* are attractive, small diurnal frogs. They are highly priced in pet trade and several species are threatened. Obtaining data on their life history has been identified as priority to develop strategies of conservation and possible sustainable harvesting of these frogs (Andreone, Mercurio and Mattioli, 2006; Rabemananjara et al., 2008b).

Crucial data to understand longevity, population dynamics and fecundity of a species come from the age structure of natural populations. Because the age of adult amphibians cannot be precisely estimated on the basis of body length or mass (Halliday and Verrell, 1988), several methods are known to assess the age of amphibians and reptiles of which skeletochronology is among the most reliable ones (Castanet and Smirina, 1990). This method identifies lines of arrested growth (LAGs) found in the bones which correspond to resting periods in their hard tissues. During periods of reduced activity such as hibernation and aestivation, bone tissue apposition stops and a LAG is formed; therefore, each LAG represents one year of life, as was confirmed by mark-recapture studies (Tejedo, Reques and Esteban, 1997). LAG formation can reflect not only seasonal, but also intrinsic biological rhythms, such as one might encounter in tropical species that are active year-round without hibernation or estivation (Castanet et al., 1993; Guarino, Andreone and Angelini, 1998; Khonsue, Matsui and Misawa, 2000; Kumbar and Pancharatna, 2001, 2002; Morrison, Hero and Browning, 2004; Lai, Lee and Kam, 2005; Marangoni et al., 2009).

For this study we used samples of preserved *Mantella* specimens from the collections of the Zoological Museum Amsterdam (ZMA), the Université d'Antananarivo, Département de Biologie Animale (UADBA), and the Zoologische Staatssammlung München (ZSM). The vouchers had been collected in 2004 at eight different locations in Madagascar: Anosibe An'ala, Besariaka, Manombo (two sites), Ranomafana-Ranomafanakely, Torotorofotsy (two sites), Vevembe and belong to four species: *Mantella aurantiaca* ($n = 29$), *M. baroni* ($n = 20$), *M. bernhardi* ($n = 46$) and *M. madagascariensis* ($n = 15$).

Individual frogs were sexed and measured for snout to vent length (SVL) to the nearest 0.1 mm. Skeletochronology was performed applying the following procedure: bones of

Zoological Institute, Technical University of Braunschweig, Spielmannstr. 8, 38106 Braunschweig, Germany

*Corresponding author; e-mail: olgajo@tu-bs.de

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the forelimbs (humerus and radio-ulna, and in some cases phalanges) were first decalcified in 3% HCOOH (time of decalcification was variable, from 2-20 minutes depending on the size of the bones). Afterwards, the bones were rinsed in deionised water for 10 minutes. Samples were then mounted in tissue-tek on a cryomicrotome and sectioned (25-30 micrometer thickness since the bones were too small for finer sections). Sections were collected in 1xPBE buffer and spread on the chromalaum/gelatin-coated glass slides. Sections were dried for several hours and stained in 0.05% cresyl-violet, followed by rinsing in deionised water for a few minutes. Afterwards, they were left to dry for ca. half an hour and fixed. Complete sections were checked on a light microscope, and photographed with a Nikon Coolpix 595 digital camera. Some of the sections were checked by an independent observer. Rank Order Correlations, Spearman, of numbers of LAGs with SVL were carried out using STATISTICA 7.1 (data analysis software system; StatSoft, Inc., 2005).

Cross sections of the long bones in adult *Mantella* in the diaphyseal region showed two concentric layers that were mostly not vascularised. The periosteal bone structure that comprises the outermost layer of the bone was more developed than the endosteal bone which encircles the medullar cavity. These two layers were sometimes very similar in texture, hindering their discrimination.

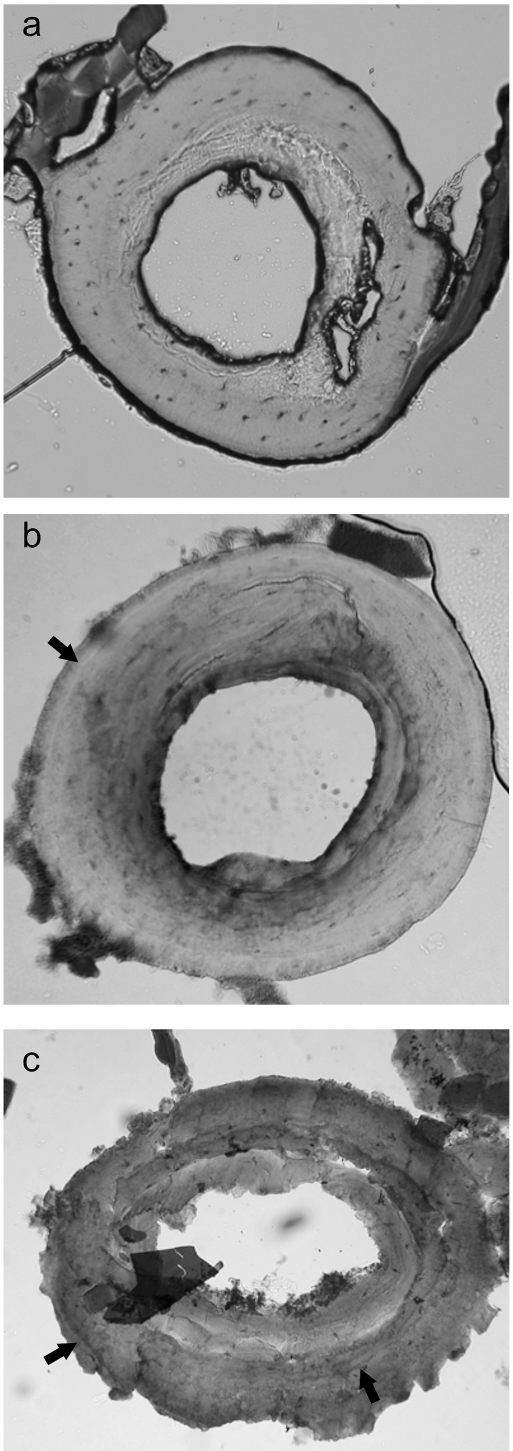
Out of 110 samples that were processed, 96 sections showed recognizable bone structures. Results for each species are shown in table 1. In 55 samples (57%) we could not recognize any LAGs, in 38 samples (40%; at least one individual of each of the four species studied) one LAG was recognized and in only three samples (3%; one specimen each of *M. baroni*, *M. madagascariensis*, *M. aurantiaca*) we observed structures possibly corresponding to two LAGs (fig. 1). Comparisons of SVL and number of LAGs revealed a positive correlation between these two variables only in *M. bernhardi* ($r = 0.38$, $p = 0.01$) (fig. 2).

The small number of LAGs agrees with previous findings in other *Mantella* species. In the study of Guarino et al. (2008), from 41 *M. cowani* specimens, 26 could be analyzed and 0-3 LAGs were found; in *M. baroni*, out of 42 specimens, 24 could be analyzed and also had 0-3 LAGs.

We assume that in *Mantella*, the number of LAGs corresponds to years of age, and that some or all animals without LAGs are thus in their first growth period. This is generally in agreement with captive data that indicate *Mantella* attaining sexual maturity within one year (observed in *M. aurantiaca*; Staniszewski, 1998). However, general differences in LAG formation among populations and species may exist; for example, in two South American subtropical anurans (*Ceratophrys cranwelli* and *Dermatonotus muelleri*) two LAGs per year were formed in specimens reared in captivity (Maragoni et al., 2009). In our study we did not record double-line LAGs which could be indicative of irregular LAG accumulation (Peabody, 2005; Sinsch, Oromi and Sanuy, 2007). Furthermore, LAGs can be absent in populations with constant, year-round activity, as it was found

Table 1. Number of samples (n) for which sections with recognizable bone structures were obtained and observed LAGs.

Species	Locality	n	No LAGs	1 LAG	2 LAGs
M. aurantiaca	Torotorofotsy	18	4	13	1 (?)
M. aurantiaca	Torotorofotsy 2	7	5	2	0
M. baroni	Anosibe An'ala	1	1	0	0
M. baroni	Besariaka	4	2	1	1 (?)
M. baroni	Ranomafanakely	13	13	0	0
M. bernhardi	Manombo camp	2	0	2	0
M. bernhardi	Manombo forest	21	7	14	0
M. bernhardi	Vevembe	20	18	2	0
M. madagascariensis	Anosibe An'ala	4	3	1	0
M. madagascariensis	Besariaka	5	1	3	1 (?)
M. madagascariensis	Ranomafanakely	1	1	0	0
Total		96	55	38	3



in some tropical frogs including one population of a species from Madagascar (*Dyscophus antongili*; Tessa et al., 2007) and two Indonesian species (*Fejervarya limnocharis* and *F. cancrivora*; Kusrini and Alford, 2006). On the first glance, this explanation does not apply to *Mantella* since they are generally known to hibernate between May and August (Rabemananjara et al., 2008a). All our collecting localities are in Madagascar's rainforest belt, with year-round regular precipitation but markedly cooler and dryer conditions during the austral winter. In our study, absence of LAGs characterized in particular all specimens of *M. baroni* and *M. madagascariensis* from Ranomafana National Park. Our collecting sites at Ranomafana have the highest altitude of all sites studied here (table 2), but there is no reason to assume that this would lead to particularly non-seasonal yearly activity. Similar to the other collecting localities, Ranomafana is characterized by a seasonal difference in temperature and rainfall but there are no strictly dry and rainy seasons, and rainfall on average is recorded on 200 days per year (Andreone, 1994).

A further confounding factor could be the resorption of the first LAG. In order to minimize the possibility of such endosteal resorption (which could completely remove the inner LAG) we compared the sections from the whole bone, making sure to include the section at the point of narrowest marrow cavity and thickest cortical bone. Nevertheless we cannot fully exclude such influences because no information of the number of LAGs in *Mantella* specimens of known age, e.g. assessed by mark-recapture, are available.

The average life span for *Mantella* species in captivity is considered to be between five to eight years (Staniszewski, 2001). However,

Figure 1. Representative humerus cross-sections. Arrows indicate lines of arrested growth. a) no LAGs present; *M. crocea*, snout to vent length (SVL) 17.7 mm; b) one LAG present; *M. aurantiaca*, SVL 20.2 mm; c) structures possibly corresponding to two LAGs; *M. madagascariensis*, SVL 24.9 mm.

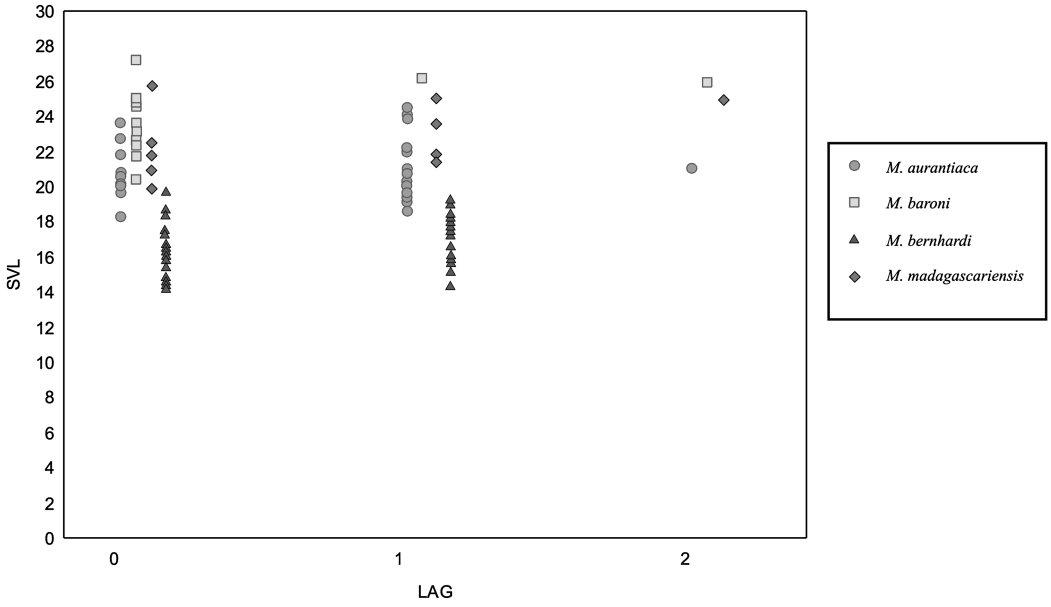


Figure 2. Snout to vent length (SVL) of all specimens compared to the number of LAGs found.

Table 2. Geographical coordinates of studied localities.

Locality	Altitude, m	Coordinates
Anosibe An'ala	940	19°25′S, 48°13′E
Besariaka	976	19°07.718′S, 48°16.838′E
Manombo	44	23°01.699′S, 47°43.892′E
Ranomafanakely	1134	21°14.921′S, 47°22.307′E
Torotorofotsy	960	18°52.483′S, 48°22.350′E
Torotorofotsy 2	941	18°52.573′S, 48°22.243′E
Vevembe	581	22°47.686′S, 47°11.228′E

due to the strong differences between captivity and natural conditions (such as more regular food availability and absence of predation) longevity data from captivity cannot be extrapolated to wild populations. Although we cannot fully exclude that the low number of LAGs in *Mantella* specimens found in this study and by Guarino et al. (2008) could be in part caused by irregular accumulation or resorption of LAGs, we are convinced that the available data are indicative of a short longevity of usually not more than two years in these frogs. This agrees with the observation from other anurans where free-ranging individuals are known to have a maximum longevity of less than half of that observed in captivity (Sinsch, Lehmann and Geiger, 2006).

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