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Comparative longevity and age at sexual maturity in twelve rainforest frogs of the genera *Boophis*, *Gephyromantis*, and *Mantidactylus* (Anura: Mantellidae) from Madagascar

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Abstract

Comparative longevity and age at sexual maturity in twelve rainforest frogs of the genera *Boophis, Gephyromantis,* and *Mantidactylus* (Anura: Mantellidae) from Madagascar. Data on the age at sexual maturity and longevity of some mantellid species of the genera *Boophis, Gephyromantis,* and *Mantidactylus* that inhabit the low altitude rainforest of Masoala (northeastern Madagascar) are presented. Counts of lines of arrested growth (LAGs) were used to calculate longevity in these species; these data contribute to assessment of the threat level of the studied anurans. *Boophis* includes species of medium- to large-sized frogs (SVL = 30–65 mm) that attain sexual maturity in 1–3 years and live 3–9 years (i.e., mid-longevity). *Mantidactylus* and *Gephyromatis* include small- to large-sized species (SVL = 22–107 mm and 35–49 mm, respectively) that attain sexually maturity in 1–3 years and live 1–8 and 3–7 years, respectively.

Keywords: age structure, Masoala, natural history museums, skeletochronology, vertebrate collections.

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Resumo

Longevidade comparada e idade da maturidade sexual em doze anuros dos gêneros *Boophis, Gephyromantis* e *Mantidactylus* (Anura: Mantellidae) da floresta tropical de Madagascar. Apresentamos aqui dados sobre a idade na maturidade sexual e longevidade para algumas espécies de Mantellidae dos gêneros *Boophis, Gephyromantis* e *Mantidactylus* que habitam a floresta tropical de baixa altitude de Masoala (nordeste de Madagascar). Contagens de linhas de crescimento (LAGs) foram utilizadas para calcular a longevidade dessas espécies; esses dados contribuem para avaliações do nível de ameaça desses anuros. *Boophis* inclui espécies de médio a grande porte (SVL = 30–65 mm) que atingem a maturidade sexual em 1–3 anos e vivem de 3–9 anos (i.e., longevidade média). *Mantidactylus* e *Gephyromatis* incluem espécies de pequeno a grande porte (SVL = 22–107 e 35–49 mm, respectivamente) que atingem maturidade sexual em 1–3 anos e vivem de 1–8 e 3–7 anos, respectivamente.

Palavras-chave: coleções de vertebrados, estrutura etária, esqueletocronologia, Masoala, museus de história natural.

Introduction

The ongoing discovery of new amphibian species in Madagascar represents a great conservation challenge for this biodiversity hotspot. Currently, 310 species are formally recognized, with at least another 200 candidate species yet to be described (Glaw and Vences 2007, Vieites *et al.* 2009, Perl *et al.* 2014). Most of these anurans are threatened by a series of factors that include habitat loss and degradation, emergent pathogens, exotic species introductions, and collection for food and pet trade (Andreone *et al.* 2005, 2006, 2016). Despite the paramount importance to assess species vulnerability and to draw conservation redlists (Andreone and Luiselli 2003, Andreone *et al.* 2016), the ecology of most anurans in Madagascar is still understudied. Many species are known only from their types (i.e., holotypes and paratypes), and for several taxa, little or nothing is known about their natural history, such as food and habitat preferences, fecundity, and longevity. This is not surprising given the remote distributions of these anurans and the limited funding available for amphibian ecological studies in contrast to biodiversity assessments and relatively rapid bio-molecular analyses.

Here we provide new ecological data on three understudied anuran genera of the family Mantellidae based on integration of published literature, field observation, museum specimens, and skeletochronology. We provide data on age at maturity and longevity for 12 species that inhabit the low-elevation Masoala rainforest of northeastern Madagascar, as follow: six species of *Boophis* Tschudi, 1838 (*B. anjanaharibeensis* Andreone, 1996; *B. axelmeyeri* Vences, Andreone, and Vieites, 2005; *B. englaenderi* Glaw and Vences, 1994; *B. roseipalmatus* Glaw, Köhler, De la Riva, Vieites, and Vences, 2010; *B. tephraeomystax* (Duméril, 1853); and *B.* aff. *reticulatus*); two species of *Gephyromantis* Methuen, 1920 (*G. luteus* [Methuen and Hewitt, 1913] and *G. redimitus* [Boulenger, 1889]); and four species of *Mantidactylus* Boulenger, 1895 (*M.* aff. *betsileanus* “Andranofotsy” Ca26; *M.* aff. *femoralis* “Marojejy” Ca43; *M.* aff. *grandidieri* “North”; and *M.* aff. *zipperi*). The candidate species names follow the indication and nomenclature provided by Glaw and Vences (2007) and Vieites *et al.* (2009).

Materials and Methods

This study is based on the skeletochronological analysis of preserved museum specimens

(Appendix I) and is limited to the species for which at least four specimens were available. The taxonomic determination of these species is based on morphology and molecular tools (barcoding). Data presented here were obtained using bones (usually the phalanx of the third toe) or phalanges taken from specimens housed in the Parc Botanique et Zoologique de Tsimbazaza, Antananarivo (Madagascar, PBZT) and in the Museo Regionale di Scienze Naturali, Torino (Italy, MRSN). Franco Andreone and collegues collected the specimens during surveys in the Masoala rainforests in November and December of 1998 and 1999; these are preserved in 65% ethanol. We sexed specimens by comparing secondary sexual characters (i.e., presence of nuptial pad and vocal sacs in *Boophis,* presence of femoral glands and vocal sacs in *Mantidactylus* and *Gephyromantis*) and/or looking at gonads by making a small slit in the venter. We classified individuals that lacked evident secondary sexual characters as juveniles. We measured specimens (snout–vent length, SVL) to the nearest 0.1 mm with a dial calipers.

Skeletochronology was performed following standard protocols already used in studies of other Malagasy species (e.g., Guarino *et al.* 1998, Andreone *et al.* 2002), as follows. We immersed bones in 5% nitric acid for 2 hr and then cross-sectioned the bones at 16 µm with a cryostat and stained them in Ehrlich's haematoxylin for about 15 min. Two independent researchers examined the sections and counted the lines of arrested growth (LAGs) with a light microscope. We estimated age by counting LAGs. These lines are the result of alternating cold-warm and/or wet-dry seasons, and usually indicate yearly rhythms (Castanet 1975). To estimate age at sexual maturity, we adopted the criterion of LAG rapprochement as proposed by Kleineberg and Smirina (1969), where slower growth is related to an adult age. We identified LAGs affected by bone remodelling using osteometrical analysis based on the youngest specimen available (Tessa *et al.* 2007). False lines, intraseasonal, incomplete, and undefined LAGs cause by growth interruption (sensu Castanet *et al.* 1993) were identifed.

Results

The species of *Boophis* examined have a wide range of body sizes; *B. englaenderi* has a maximum SVL of 33.49 mm, whereas that of *B. roseipalmatus* is 64.82 mm. Both species of *Gephyromantis* are medium-sized (maximum SVL = 48.70 mm), *Mantidactylus* contains relatively small species (*M.* aff. *betsileanus* "Andranofotsy" Ca26; maximum SVL = 32.83 mm), as well as large ones (*M.* aff. *grandidieri* "North"; maximum SVL = 107.10 mm; Table 1).

We detected sharp and evident LAGs in all species that we examined (Table 1, Figure 1), thereby indicating seasonality in the Masoala rainforest, despite an expected climatic homogeneity throughout the year. We think that this reflects climatic fluctuations and/or food availability. The bone sections of *Boophis* appeared rather homogenous, with a contrasted resorption line (RL) separating the endosteal from periosteal bones. The two layers were formed by lamellar and pseudolamellar bone tissue. Resorption affected the first (29%) and occasionally (6%) the second LAG. LAGs were evident in periosteal bone, and more extensive than in the endosteal bone. False lines were present in 24% of the sections examined. Mean age was around 4 years (range 2–9), only slightly less that of *B. tsilomaro* (Andreone *et al.* 2002) and close to *B. williamsi* (Andreone *et al.* 2014). Species of *Mantidactylus* and *Gephyromantis* species had clearly visible LAGs in the periosteal bone, which is separated from the endosteal bone by RL. The presence of false and double lines was rare (4% of the examined sections). Resorption affected partially the first and innermost LAG (16%). Small- and medium-sized species (SVL = 20–60 mm) had a mean age between 4 and 6 years, similar to that of *Bohemantis microtympanum* (Angel, 1935), a genus recently separated from *Mantidactylus*. *Mantidactylus* aff. *grandidieri* "North," the largest species examined (SVL > 100 mm), had the highest LAGs number (8), with sexual maturity reached at a maximum of 5 years.

Most of the species of *Boophis* reach sexual maturity after 1–2 years; however, in *B. englaenderi,* males seemed to reach sexual maturity in 2 years and females in 3 years, and in *B. tephraeomystax,* females reached sexual maturity in 3 years. In the species of *Mantidactylus*, sexual maturity usually is reached in 3 years in females and in 2 years in males; *M.* aff. *grandidieri* "North," is an exception because some males and females may reach maturity in 4 and 5 years, respectively. In *Gephyromantis,* sexual maturity usually occurs in 2 years in both sexes, but some female *G. redimitus* reach sexual maturity in 3 years and some male *G. redimitus* in 1 year (Table 1).

Discussion

As shown in several other species (Guarino *et al.* 1998, 2003, 2008, Andreone *et al.* 2002, 2011, 2014, Tessa *et al.* 2007, 2011, Jovanovic and Vences 2010), our data demonstrate the value of skeletochronology as a tool in the study of anurans in tropical and subtropical regions such as Madagascar. Because LAGs were detected in all of the species examined (Figure 1), it seems that seasonality characterizes the forests of Masoala Peninsula, despite the low altitude. In other frogs studied, such as the large, iconic, red microhylid, *Dyscophus antongilii* Grandidier, 1877 that also lives in and around Masoala, LAGs were not visible; this absence was thought to indicate a lack of seasonality at the low altitude and suburban studied site (Tessa *et al.* 2007). However, *D. antongilii* usually inhabits open areas, breeding in ponds and ditches in the town of Maroantsetra, which is warm throughout the year; thus, seasonality probably is less marked in this species than in forest-dwelling species.

The varying life spans of the three genera may reflect differing habitat adaptations. All the species of *Mantidactylus* that we sampled are terrestrial or scansorial and diurnal/nocturnal, whereas the species of *Gephyromantis* are nocturnal, and arboreal or semi-arboreal, and the species of *Boophis* are nocturnal and arboreal. However, there is substantial intrageneric variation in size and age data. In general, smaller-sized species do not live as long and reach sexual maturity earlier than larger-sized species of larger sizes. *Mantidactylus* aff. *grandidieri* "North" reaches sexual maturity later than do other species, despite having comparable longevity. *Mantidactylus* aff. *grandidieri* "North" is edible (Andreone *et al.* 2012), and the oldest and largest individuals frequently are collected for consumption. This may have a bearing on our results and requires additional study.

The value of specimens housed in natural history collections to conservation assessments cannot be underestimated. There are specimens other than type series housed in major collections, and these can be used to study diet (either through dissection or using non-invasive and non-destructive; e.g., Fortuny *et al.* 2015, Scherz *et al.* 2016), to analyze the pattern of prevalence, intensity, and distribution of some pathogens (e.g., for the occurrence of emerging infective diseases: Zhu *et al.* 2014), for genetic purposes (e.g., Wandeler *et al.* 2007), for anatomical preparations and also for determining age at maturity, and species longevity (Suarez and Tsutsui 2004, Tessa *et al.* 2011). As demonstrated in other studies, the use of preserved specimens for skelectrochronology is possible in amphibians from Madagascar (Guarino *et al.* 2010, Andreone *et al.* 2011). These data, combined with genetic data and data collected in the field, represent a storehouse of information vital to developing sensible conservation measures (e.g., defininition of quotas of collectable individuals and inform researchers about the ecological sensitivities of understudied amphibians (Andreone *et al.* 2016).

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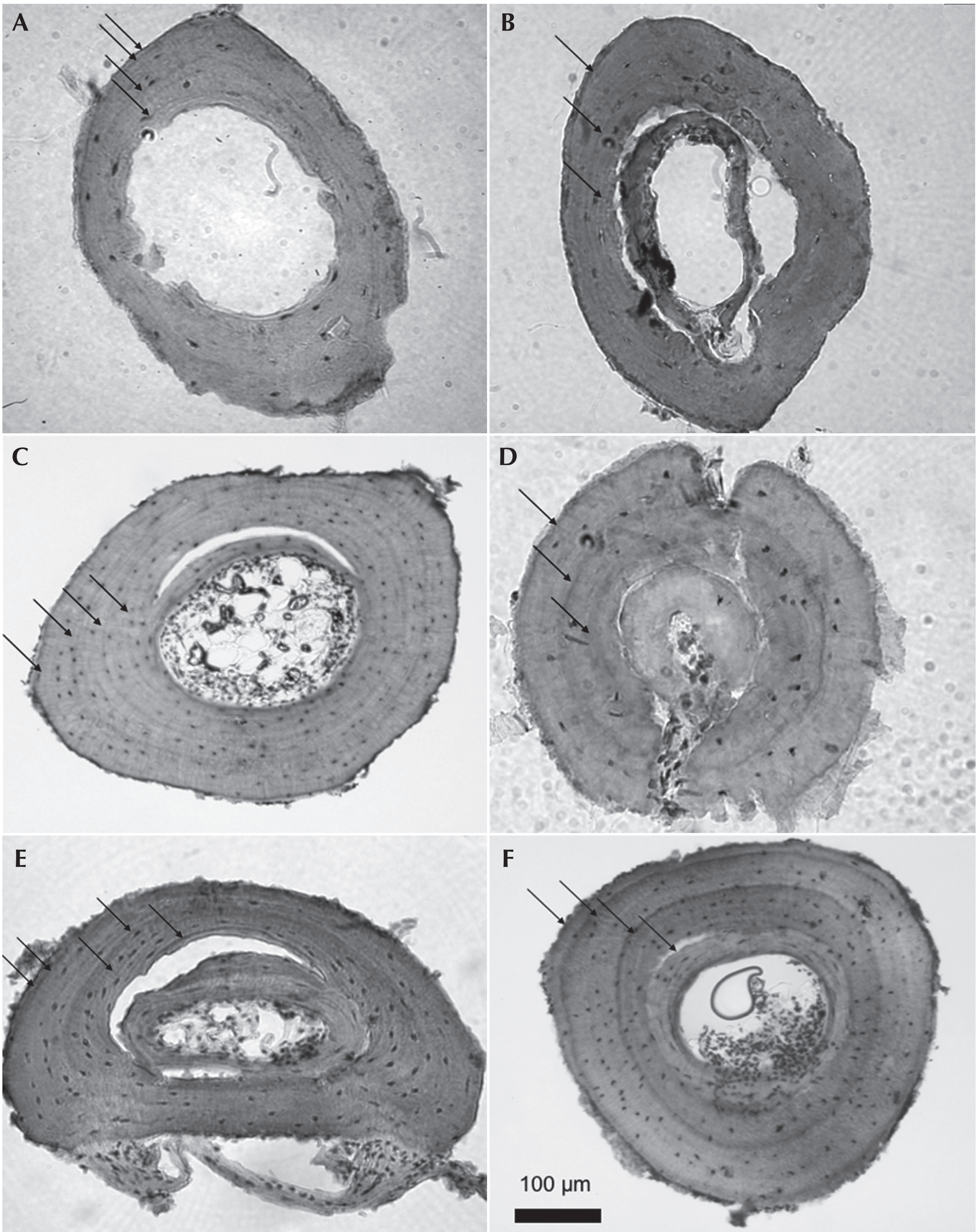

Figure 1. Histological sections of the specimens examined. Arrows indicate the lines of arrested growth (LAGs). (**A**) *Mantidactylus* aff. *femoralis* "Marojejy" Ca43, MRSN A3716, male, 4 LAGs, SVL = 35.65 mm. (**B**) *Mantidactylus* aff. *zipperi*, MRSN A3711, male, 3 LAGs, SVL = 29.21 mm. (**C**) *Boophis axelmeyeri,* PBZT, male, 4 LAGs (the two outer lines converge for the most part before to split), SVL = 65.10 mm. (**D**) *B. englaenderi,* MRSN A3060, male, 3 LAGs, SVL = 32.60 mm. (**E**) *B. roseipalmatus,* MRSN A5112, female, 5 LAGs, SVL = 52.18 mm. (**F**) *Gephyromantis redimitus,* MRSN A4061, male, 4 LAGs, SVL = 43.82 mm.

Table 1. Data on sex, body size (SVL, mm), longevity (LAGs number, corresponding to years) and age (years) at sexual maturity in the analysed species.

Species	Sex (number of examined individuals)	SVL	LAGs	Age at sexual maturity
		mean ± SD (range)	mean ± SD (range)	mean ± SD (range)
Boophis anjanaharibeensis	Males (3)	34.34 ± 0.24 (34.08–34.39)	3.67 ± 0.58 (3–4)	1.67 ± 0.58 (1–2)
	Juveniles (1)	20.25 (-)	0 (-)	(-)
Boophis axelmeyeri	Males (4)	47.44 ± 2.30 (45.01–50.55)	6.75 ± 1.26 (5–8)	1 ± 0 (1)
	Females (2)	53.55 ± 7.70 (48.10–59.00)	7.50 ± 2.12 (6–9)	1 ± 0 (1)
Boophis englaenderi	Males (3)	32.02 ± 1.83 (29.96–33.49)	3.00 (-)	2.33 ± 0.57 (2–3)
	Females (1)	31.01 (-)	4.00 (-)	3 (-)
Boophis roseipalmatus	Males (3)	54.70 ± 3.23 (52.18–58.35)	6.00 ± 1.00 (5–7)	1 ± 0 (1)
	Females (3)	64.08 ± 0.65 (63.58–64.82)	7.00 ± 1.00 (6–8)	1.33 ± 0.58 (1–2)
Boophis aff. *reticulatus*	Males (4)	35.46 ± 1.37 (34.08–37.09)	5.50 ± 0.58 (5–6)	1.75 ± 0.50 (1–2)
Boophis tephraeomystax	Males (2)	35.68 ± 4.61 (32.42–38.94)	4.50 ± 0.70 (4–5)	1 ± 0 (1)
	Females (6)	42.84 ± 5.35 (33.86–47.47)	5.33 ± 1.37 (3–7)	2 ± 0 (1–3)
Mantidactylus aff. *betsileanus* “Andranofotsy” Ca26	Males (7)	24.76 ± 2.37 (22.38–28.80)	3.28 ± 0.49 (3–4)	2.14 ± 0.38 (2–3)
	Females (2)	29.66 ± 4.47 (26.50–32.83)	4.50 ± 0.71 (4–5)	3 ± 0 (3)
Mantidactylus aff. *femoralis* “Marojejy” Ca43	Males (5)	34.41 ± 2.06 (31.38–37.16)	3.00 ± 0.71 (2–4)	2 ± 0 (2)
	Females (4)	49.15 ± 7.76 (41.53–57.52)	4.75 ± 0.96 (4–6)	3 ± 0 (3)
Mantidactylus aff. *grandidieri* ‘’North’’	Males (8)	73.66 ± 12.43 (47.00–83.40)	5.75 ± 1.67 (3–8)	2.86 ± 0.26 (2–4)
	Females (2)	97.30 ± 13.86 (87.50–107.10)	5.50 ± 0.71 (5–6)	4 ± 1 (3–5)
	Juveniles (2)	45.45 ± 12.09 (36.90–54.00)	2.00 ± 1.41 (1–3)	- -

Table 1. *Continued.*

Species	Sex (number of examined individuals)	SVL mean ± SD (range)	LAGs mean ± SD (range)	Age at sexual maturity mean ± SD (range)
Mantidactylus aff. *zipperi*	Males (4)	26.61 ± 2.50 (24.44–29.21)	3.50 ± 1.29 (2–5)	1.25 ± 0.25 (1–2)
	Females (3)	29.86 ± 3.68 (26.19–33.56)	4.00 ± 3.68 (3–5)	2.67 ± 0.33 (2–3)
Gephyromantis luteus	Males (3)	37.22 ± 2.61 (34.56–46.17)	3.67 ± 2.40 (3–5)	2 ± 0 (2)
	Females (2)	44.32 ± 2.61 (42.48–46.17)	4.00 (-)	2 ± 0 (2)
Gephyromantis redimitus	Males (14)	46.44 ± 2.01 (43.80–48.40)	4.50 ± 1.22 (3–7)	1.54 ± 0.14 (1–2)
	Females (3)	47.18 ± 1.37 (46.04–48.70)	4.33 ± 0.58 (4–5)	2.67 ± 0.33 (2–3)
	Juveniles (1)	17.58 (-)	0 -	-

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Appendix I. *List of specimens used for skeletochronological analysis.*

Abbreviations: MRSN, specimens housed in the Museo Regionale di Scienze Naturali, Torino; PBZT, specimens housed in the Parc Botanique et Zoologique de Tsimbazaza, Antananarivo.

Mantellidae Boophinae: *Boophis anjanaharibeensis* Andreone, 1996 (MRSN A4433, A4391, A4373, A4374); *Boophis axelmeyeri* Glaw & Vences, 1997 (MRSN A3117, A3120, A4114, A4115, A4252, A4321); *Boophis englaenderi* Glaw & Vences, 1994 (MRSN A4105, A4106, A4354, A4408); *Boophis roseipalmatus* Glaw *et al.,* 2010 (MRSN A4293–4295, A4400, A4299, A3260); *Boophis* aff. *reticulatus* (MRSN A4308, A4309, A4242, A4243); *Boophis tephraeomystax* (Duméril, 1853) (MRSN A4126, A4127, A4130-4135).

Mantellidae Mantellinae: *Gephyromantis luteus* (Methuen & Hewitt, 1913) (MRSN A3229, A3786, A3787, A3780, A3230); *Gephyromantis redimitus* (Boulenger, 1889) (MRSN A3163, A4010, A3161, A4061, A3829, A4060, A4067, 11 phalanges, PBZT); *Mantidactylus* aff. *betsileanus* "Andranofotsy" Ca26 (MRSN A3680, A3677, A3726, A3727, A3731, A3798, A3734, A3730, A3678); *Mantidactylus* aff. *femoralis* "Marojejy" Ca43 (MRSN A 3713-3716, A3759, A3173–3175); *Mantidactylus* aff. *grandidieri* "North" (12 phalanges, PBZT); *Mantidactylus* aff. *zipperi* (MRSN A3710-3712, A3741, A3745, A3748, A3752).