

Longevity and body size in three populations of *Dyscophus antongilii* (Microhylidae, Dyscophinae), the tomato frog from north-eastern Madagascar

GIULIA TESSA¹, FABIO M. GUARINO², CRISTINA GIACOMA³, FABIO MATTIOLI⁴, FRANCO ANDREONE¹

¹ Museo Regionale di Scienze Naturali, Sezione di Zoologia, Via G. Giolitti, 36, I-10123 Torino, Italy. Corresponding author. E-mail: franco.andreone@regione.piemonte.it

² Università di Napoli Federico II, Dipartimento di Biologia Funzionale e Strutturale, Via Cinthia, I-80126 Napoli, Italy

³ Università degli Studi di Torino, Dipartimento di Biologia Animale e dell'Uomo, Via A. Albertina, 13, I-10123 Torino, Italy

⁴ Acquario di Genova, Area Porto Antico, Ponte Spinola, I-16128 Genova, Italy

Abstract. Age profile and body size were studied in three populations of the rare and understudied tomato frog, *Dyscophus antongilii*, from NE Madagascar. For each individual, a phalanx was clipped and the bone used for skeletochronology. Sexual dimorphism is significantly different between all three populations: females are larger and heavier than males, with males also being distinguishable by a more yellowish throat. Age structure analysis was possible on two populations (Antara, Lampirano). The age within the two populations ranged between 3 and 7 years (mean \pm SD = 5.0 \pm 0.2) for males, and 3 to 11 years (mean \pm SD = 5.8 \pm 0.3) for females. Longevity was positively correlated to body size and weight within both sexes and populations. Sexual maturity was reached between 2 and 3 years, with sexual maturity recorded for males significantly lower than for females.

Keywords. Age profile, *Dyscophus*, Madagascar, sexual dimorphism, skeletochronology.

The highly diverse Madagascan amphibian fauna includes three microhylid sub-families: Cophylinae, Scaphiophryininae, and Dyscophinae. While Cophylinae inhabit rainforests, Scaphiophryininae and Dyscophinae usually colonise open habitats and can be found in arid and semi-arid areas. The genus *Dyscophus* is represented by three species, of which the tomato frog *D. antongilii* is one of the best-known frogs from Madagascar. This bright, red-orange coloured species, typical of NE Madagascar, has a stout body and reaches a size of 105 mm (Glaw and Vences, 2007).

The chromatic attractiveness and peculiar morphology has resulted in the tomato frog becoming one of the preferred targets for the international pet-trade. During the

eighties it was exported in high numbers, resulting in its inclusion on CITES Appendix I (Andreone et al., 2006). Currently, it is known from two main distribution areas: first, around Maroantsetra and second, next to the Ambatovaky reserve, which resulted in its classification as “near threatened” (Raxworthy et al., 2004; Andreone et al., 2005a). Like other species of the genus, it breeds in still and slow-moving water, such as swamps, shallow ponds or artificial sewage ditches. During the breeding season the males emit a sound and rhythmic advertisement call that gives the frog its Malagasy vernacular name, “sahongogogno”.

Surprisingly, very little information exists on the biology of *D. antongilii* and data on its life history are patchy, which mainly refer to a few observations made on captive individuals (Pintak, 1987), or reported anecdotally. This is also true for the closely related *D. guineti*, similar in morphology and life history, yet not listed on CITES and exported in high numbers (Andreone et al., 2006).

Considering the importance of life history data, in terms of conservation, this study provides the first data, obtained from skeletochronological methodology (Castanet, 1975), and analysis on the age structure of three populations of *D. antongilii*.

Age structure was studied as part of a wider conservation project on the species, with an individual's age estimated by counting the lines of arrested growth (LAGs). LAGs result from alternating cold-warm and/or wet-dry seasons indicating yearly rhythms and thus breeding seasons. Skeletochronological methods have been used on a variety of species from temperate regions (Guarino et al., 1995, 2003) and more recently it was applied to species from tropical and subtropical regions, including Madagascar (Guarino et al., 1998; Andreone et al., 2002, 2005b).

Three breeding colonies of tomato frogs were used in this study. The first population was studied at Maroantsetra (15°25.44'S, 49°40.82'E; elevation ~ 10 m a.s.l.), a small town located on the Antongil's Bay, the best-known locality for the species. The breeding site was a small, urban pond used by local people for breeding geese and ducks. The habitat surrounding the pool was represented by ricefields, cultivated fields, meadows and marshes. The other two sites were located more than 100 km south of Maroantsetra, close to the Ambatovaky Special Reserve. The Antara site (16°53.25'S, 49°10.99'E; 486 m a.s.l.) was situated next to the homonymous river. The frogs were located within a swampy area found within a coffee and vanilla cultivation. The third site was at Lampirano (sometimes also quoted as Iampirano) (16°49.31'S, 49°11.06'E; 484 m a.s.l.). Frogs at this site were found along the sandy banks of a slow running river. At all the sites, the climate was warm-humid with an annual average temperature ranging between 20-30 °C, and a total annual rainfall between 3000 and 4000 mm.

At all localities, tomato frogs were searched during the rainy season (January-February 2006), when they were active and in breeding condition. Individuals were captured at night with the aid of torches, sexed and measured snout to posterior vent (SVL, at the nearest 0.1 mm) with dial callipers and weighed using a spring balance (to the nearest 1 g). Males were distinguished from females by their comparatively smaller size, lighter colouration, yellow throat and the presence of deferent ducts that were visible through the belly. The last two phalanges on the second toe of the right foot were removed, for a mark-recapture study, and kept in 90% ethanol for skeletochronology data. They were later decalcified in 5% nitric acid for about 2 h, cross-sectioned at 12 µm with a cryostat and

stained with Ehrlich's haematoxylin for about 10 min (Guarino et al., 1995, 1998). Sections were observed under a Zeiss Axioscop light microscope, and images captured with a Prog Res 3008 colour video camera and KS 300 software interfaced with a personal computer.

At Maroantsetra, toe samples were collected from 35 frogs (22 females and 13 males), at Antara from 37 individuals (30 females and 7 males), and at Lampirano from 39 individuals (14 females and 25 males). Adult males were significantly smaller than females in all three populations (Maroantsetra: males = $61.33 \text{ mm} \pm 5.76$ and females = 88.34 ± 5.06 , $t = 14.01$, $P < 0.01$; Antara: 58.79 ± 3.99 vs 80.98 ± 7.31 , $t = 10.85$, $P < 0.01$; Lampirano: 59.62 ± 2.58 vs 81.28 ± 6.23 , $t = 12.43$, $P < 0.01$). Females from Maroantsetra were significantly larger than females from Antara ($t = 4.19$; $P < 0.01$), and Lampirano ($t = 3.56$; $P < 0.01$). Male weight was consistently lower than female weight across all populations: Maroantsetra: 28.6 ± 8.5 vs 92.4 ± 14.2 , $t = 16.59$; $P < 0.001$; Antara: 24.6 ± 3.4 vs 58.3 ± 15.1 , $t = 11.1$; $P < 0.01$; Lampirano: 26.9 ± 2.5 vs 53.5 ± 19.8 , $t = 4.99$; $P < 0.01$. Also, females from Maroantsetra were consistently heavier in comparison with females from Antara ($t = 8.32$; $P < 0.01$) and Lampirano ($t = 6.37$; $P < 0.01$).

Regarding age structure and determination, the LAG counting was possible for Antara and Lampirano individuals only. In cross sections of their phalanges, a layer of endosteal lamellar bone was present at the edge of medullar cavity, clearly-cut separated from a layer of periosteal bone by a resorption line (Fig. 1). LAGs were visible in both bone layers, but for LAG counting only the periosteal LAGs were used because periosteal bone appeared more developed and less affected by re-adsorption.

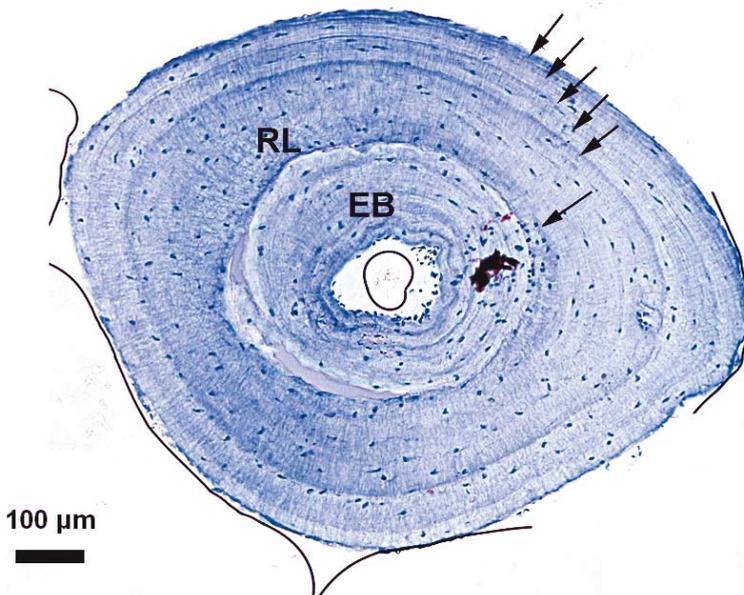


Fig. 1. Phalangeal cross-section ($\times 10$), at the diaphyseal level, of *Dyscophus antongilii* from Lampirano. Female, 817 mm SVL, showing 6 LAGs (arrows), the third of which very faint. EB = endosteal bone; RL = reversal line.

Because all the frogs were collected during the warm season, the outer section of the bone was not externally delimited by a LAG. In such cases, the outer margin was interpreted as an indiscernible LAG, and the number of years was calculated as the number of visible LAGs + 1. Difficulties usually encountered in skeletochronological studies include double lines, recorded in 6.8% of samples in the studies' populations. False lines were more frequent, recorded in 23% of samples. LAGs affected by bone remodelling were identified by osteometrical analysis: the perimeters of the reversal lines of adults were compared with the first visible LAG of young specimens. The first inner LAG was totally reabsorbed in 50% of females and in 34.5% of males, while in only a small percentage of males and females were the first two LAGs totally resorbed (Table 1).

Table 1. Data on intracortical bone remodelling in *Dyscophus antongilii* from Antara and Lampirano populations.

Sex	Number	Number of resorbed LAGs		
		0	1	2
Males	32	19 (59.4%)	11 (34.5%)	2 (6.3%)
Females	42	19 (45.2%)	21 (50.0%)	2 (4.8%)

Cross sections of toes taken from Maroantsetra individuals showed homogeneous histological features in both endosteal and periosteal bones. Over 51% of the analysed samples did not record any LAG, while over 48% of the specimens recorded one to three incomplete or less visible LAGs. These incomplete lines did not correspond to the typical annual LAG nor did they correlate with size (Fig. 2). Therefore, it was assumed that skeletochronology was not an applicable method for age determination of individuals from Maroantsetra. This lack of any discernible LAG was considered a consequence of a uniform, warm climate typical of a low-altitude site, where frogs were active all year without any metabolism fluctuations and thus consequent LAG formations. Differences in the applicability of skeletochronological method between populations of the same species but living under different climatic conditions have been observed in other anurans (Morrison et al., 2004), but need further investigation.

Frogs from the Antara and Lampirano sites did not differ significantly between SVL and LAG number (ANCOVA males: $F = 0.02$, $P = 0.90$; ANCOVA females: $F = 1.59$, $P = 0.22$) (Fig. 3). Individuals from these populations showed a LAG number ranging between 3 and 11 (mean LAG number \pm SD: males, 5.03 ± 0.99 ; $n = 32$; females, 5.81 ± 1.95 ; $n = 42$). The oldest specimen recorded was a female with 11 LAGs (Table 2). For this reason data from Antara and Lampirano sites were pooled into one data set for analysis.

Differences in the number of LAGs, SVL, weight and age at sexual maturity were not observed between males and females. LAG number was significantly related to SVL and weight within the two sexes and two populations.

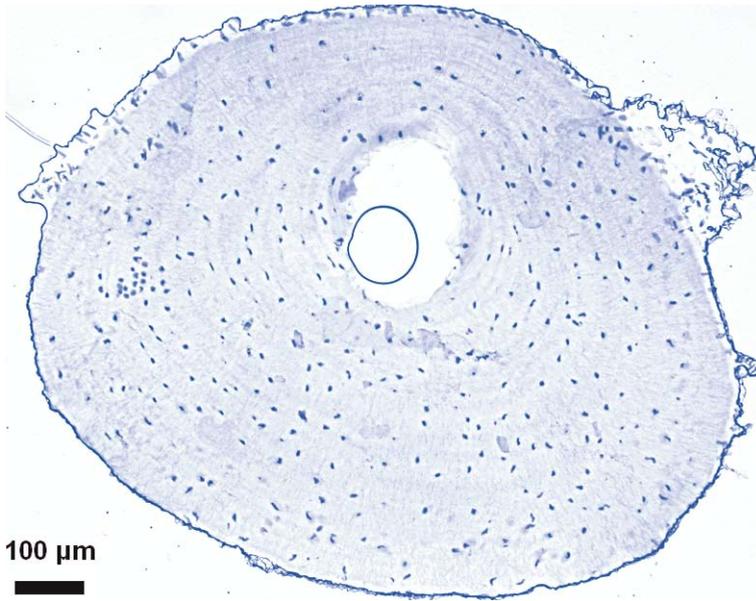


Fig. 2. Phalangeal cross-section ($\times 10$), at the diaphyseal level, of *Dyscophus antongilii* from Maroantsetra. Female, 865 mm SVL, showing no LAGs, and with evident lamellar structure. ($\times 10$).

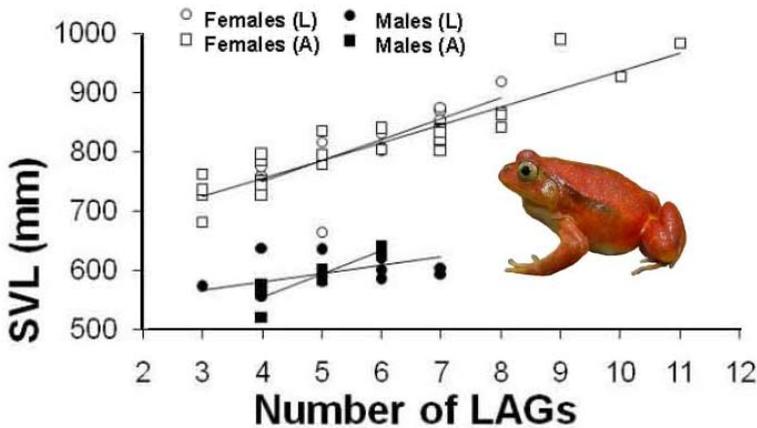


Fig. 3. Correlation between size and number of LAGs (L = Lampirano, A = Antara).

Age at sexual maturity differed significantly between the sexes (2.78 ± 0.20 vs 2.12 ± 0.34 , $t = 3.582$; $P < 0.01$), with females reaching sexual maturity one year later than males. Such a trend has already been observed in another species of Malagasy frog, *Boehmantis microtypanum* (Guarino et al., 1998). Females were known to invest greater ener-

Table 2. Data on age and size of *Dyscophus antongilii* in the studied populations. Values are indicated as mean \pm standard deviation; extreme values are between parentheses, and total number of examined phalanges.

Locality (m a.s.l.)	Habitat	Sex	Snout-vent length (mm)	Weight (g)	LAG count
Maroantsetra (~10 m)	Urban site	Males	61.33 \pm 5.76 (52.5-75.1) n = 13	28.6 \pm 8.5 (20-47) n = 13	Not visible
		Females	88.34 \pm 5.06 (76.3-96.6) n = 22	92.4 \pm 14.2 (50-110) n = 22	Not visible
Antara (486 m)	Coffee plantation	Males	58.79 \pm 3.99 (52.1-64.2) n = 7	24.6 \pm 3.4 (20-31) n = 7	4.86 \pm 0.90 (4-6) n = 7
		Females	80.98 \pm 7.31 (68.2-98.9) n = 30	58.3 \pm 15.1 (41-105) n = 30	5.82 \pm 2.23 (3-11) n = 28
Lampirano (484 m)	River	Males	59.62 \pm 2.58 (55.6-64.0) n = 25	26.9 \pm 2.5 (22-32) n = 25	5.08 \pm 1.04 (3-7) n = 25
		Females	81.28 \pm 6.23 (66.5-91.9) n = 14	53.5 \pm 19.8 (26-82) n = 14	5.79 \pm 1.31 (4-8) n = 14

gy resources into body growth, permitting a higher number of eggs and thus increasing fecundity (Woolbright, 1983).

Individuals recorded at Maroantsetra were both larger and heavier than those from the other two sites (Fig. 4). Such differences could be due to differences in habitats, site elevation and trophic availability. For example, Lampirano and Antara recorded an elevation around 500 m a.s.l. and variances in climate (temperature, humidity) were likely more variable throughout the year. Such variances potentially obligates frogs from these two sites to observe a period of hibernation/aestivation, resulting a regular LAG deposition. Alternatively, at Maroantsetra, the lower elevation site (0-10 m a.s.l.), an increased stability in temperature results in continuous activity of the frogs throughout the year, hence the larger size. Furthermore, at Maroantsetra the tomato frog colonises ponds within the town that were frequented by domestic fowl and possibly resulting in increased availability of nutrients in the water.

Regarding conservation concerns, the populations at Antara and Lampirano (only recently signalled by Raxworthy et al., 2004) appear to be potentially more protected from human disturbance via agricultural and grazing practices. However, tomato frogs at Maroantsetra were likely to experience greater pressure from such disturbances due to the fact that they are located within the a highly modifiable urban environment. Hence this population at Maroantsetra should be considered at greater potential threat of extirpation. The conservation of this population presents several problems because it would be necessary to restrict the use of these ponds by local people. For these reasons, it would be rec-

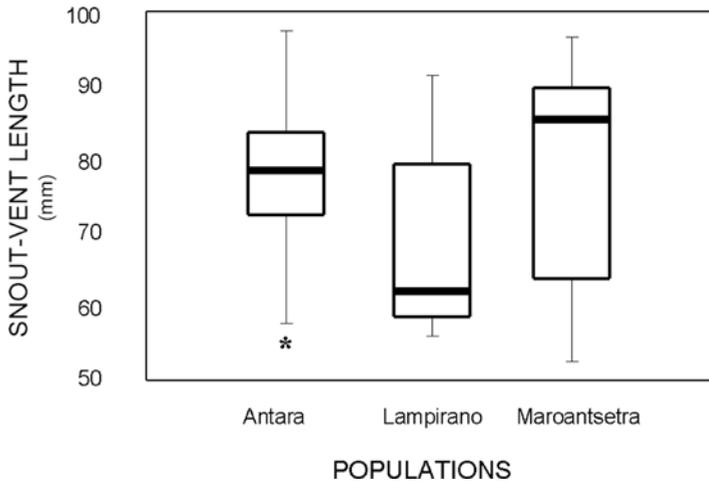


Fig. 4. Comparison of size in the three populations of *Dyscophus antongilii*.

ommended that the current, known populations of *D. antongilii* should be carefully managed, especially considering the species status as an icon of amphibian conservation on Madagascar. The age structure and other biological parameters, when correctly collected, would allow the proposition of a greater detailed management plan to ensure the species conservation.

ACKNOWLEDGEMENTS

The fieldwork was possible upon the help of A. Sarovy and J.E. Randrianirina. Thanks to the Malagasy authorities for providing collecting and export permits. A.I. Carpenter kindly corrected a first draft of this paper. The study was funded by the Zoo Zürich, WAZA, BIOPAT, Gondwana Conservation and Research, and EAZA. Antongil Conservation kindly helped during the permanence at Maroantsetra.

REFERENCES

- Andreone, F., Cadle, J.E., Cox, N., Glaw, F., Nussbaum, R.A., Raxworthy, C.J., Stuart, S.N., Vallan, D., Vences, M. (2005a): Species review of amphibian extinction risks in Madagascar: conclusions from the Global Amphibian Assessment. *Conserv. Biol.* **19**: 1790-1802.
- Andreone, F., Guarino, F.M., Randrianirina, J.E. (2005b): Life history traits, age profile and conservation biology of the panther chameleon, *Furcifer pardalis* (Cuvier 1829) at Nosy Be, NW Madagascar. *Trop. Zool.* **18**: 209-225.

- Andreone, F., Mercurio, V., Mattioli, F. (2006): Between environmental degradation and international pet trade: conservation strategies for the threatened amphibians of Madagascar. *Natura*, **95**: 81-96.
- Andreone, F., Vences, M., Guarino, F.M., Randrianirina, J.E. (2002): Natural history and larval morphology of *Boophis occidentalis* (Anura: Mantellidae: Boophinae) provide new insights into the phylogeny and adaptive radiation of endemic Malagasy frog. *J. Zool., Lond.* **257**: 425-438.
- Castanet, J. (1975): Quelques observations sur la présence et la structure des marques squelettiques de croissance chez les amphibiens. *Bull. Soc. Zool. Fr.* **100**: 603-620.
- Glaw, F., Vences, M. (2007): A fieldguide to the amphibians and reptile of Madagascar. Third edition. Vences u. Glaw Verlag GbR, Cologne.
- Guarino, F.M., Andreone, F., Angelini, F. (1998): Growth and longevity by skeletochronological analysis in *Mantidactylus microtympanum*, a rain-forest anuran of southern Madagascar. *Copeia* **1998**: 194-198.
- Guarino, F.M., Angelini, F., Cammarota, M. (1995): A skeletochronological analysis of three syntopic amphibian species from southern Italy. *Amphibia-Reptilia* **16**: 297-302.
- Guarino, F.M., Lunardi, S., Carlomagno, M., Mazzotti, S. (2003): A skeletochronological study of growth, longevity, and age at sexual maturity in a population of *Rana latastei* (Amphibia, Anura). *J. Biosc.* **28**: 775-782.
- Morrison, C., Hero, J.M., Browning, J. (2004): Altitudinal variation in the age at maturity, longevity, and reproductive life span in the anurans in subtropical Queensland. *Herpetologica* **60**: 34-44.
- Pintak, T. (1987): Zur Kenntnis des Tomatenfrosches *Dyscophus antongili* (Grandidier 1877). *Salamandra* **23**: 106-121.
- Raxworthy, C., Vences, M., Andreone, F., Nussbaum, R. (2004): *Dyscophus antongilii*. In: IUCN 2006. – 2006 IUCN red list of threatened species. www.iucnredlist.org. Downloaded on 06 June 2007.
- Woolbright, L.L. (1983): Sexual selection and size dimorphism in anuran Amphibia. *Am. Nat.* **121**: 110-119.