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The Application of Skeletochronology to Estimate Ages of Three Species of Frogs in West Java, Indonesia

MIRZA DIKARI KUSRINI*
and
ROSS A. ALFORD
School of Tropical Biology, James Cook University
Townsville 4811, Queensland, Australia

** Corresponding author*
Present address: Department of Forest Resources Conservation & Ecotourism
Faculty of Forestry, Institut Pertanian Bogor
Kampus Darmaga, Bogor 16680, Indonesia
e-mail: mirza_kusrini@yahoo.com

Skeletochronology has been used successfully to estimate age in temperate and sub-temperate frogs (Halliday and Verrel 1988; Khonsue et al. 2002; Morrison et al. 2004; Reaser 2000; Ryser 1988). In temperate species, lines of arrested growth (LAGs) in bones are usually distinct and are formed during winter (Halliday and Verrel 1988; Reaser 2000; Ryser 1988). Few attempts have been made to estimate age using this method in tropical frogs (Guarino et al. 1998; Khonsue et al. 2000; Kumbar and Pancharatna 2001; Pancharatna et al. 2000) and it has not previously been applied to Indonesian frogs.

As part of a population study of three species of edible frogs in Indonesia, we examined phalanges taken from toe clips of *Fejervarya limnocharis*, *F. cancrivora*, and *Limnonectes macrodon* to determine whether it is possible to estimate the ages of individuals of these tropical species using skeletochronology. For every frog captured, we recorded sex, body size as measured by SVL (snout–vent length) and mass, and clip code. Since each frog was numbered uniquely, phalanges used for skeletochronological analysis were taken from different toes. *Fejervarya limnocharis* and *F. cancrivora* were encountered in rice fields, and *L. macrodon* was found primarily at streams.

We analysed samples from 103 *F. limnocharis* (SVL = 15.68–53.00 mm), 35 *F. cancrivora* (SVL = 14.50–56.68 mm) and 42 *L. macrodon* (SVL = 47.06–105.22 mm). Samples of the rice field frog species were taken during surveys carried out between September 2001 and June 2002 in West Java province, Indonesia, in the Residencies of Bogor and Sukabumi. Samples from Bogor were taken from rice fields in Situ Gede (06°33'50.8"S, 106°44'10.2"E, elevation 220 m above sea level) and Caringin (06°43'22.4"S, 106°49'22.8"E, elevation 478 m asl) while samples from Sukabumi were taken from Ciptarasa (06°51'07.3"S, 106°30'22.9"E, elevation 810 m asl), and Panguyangan (06°52'13.1"S, 106°31'04.4"E, elevation 760–800 m asl); both are village enclaves within Mount Halimun National Park. Samples of *L. macrodon* were taken during surveys carried out in streams at Cilember (06°39'36.7S, 106°56'42.1"E) and Ciapus Leutik (06°39'48.0"S, 106°44'42.7"E) both in Bogor Residency, from June 2002 to May 2003. Mean monthly temperatures in the area normally vary by less than 10°C over the year. The Bogor and Sukabumi Residencies are one of the wettest regions in Java, with monthly rainfalls greater than 200 mm in most locations throughout the year (Whitten et al. 1997).

Clipped toes were stored in 4% formalin and returned to our laboratory at James Cook University, Australia for processing. Each toe was wrapped in sponge and placed individually in a cassette, then decalcified overnight (approximately 24 h) in 10% formic acid solution. Toes were then embedded in wax in a vacuum infiltrated processor and cut into sections transversely at 10 µm thickness using a rotary microtome. Sections were put on slides and stained with Mayer's Haematoxylin. The best and clearest sections were chosen for observations and were examined using an Olympus BH dual-head microscope at 100x magnification to view marks of skeletal growth. Due to staining problems (sections too dark or/and curled), which make interpretation difficult, sections from 7 *F. limnocharis*, 5 *F. cancrivora*, and 1 *L. macrodon* were not included in the analysis. Lines of arrested growth (LAG) were interpreted from the occurrence of hematoxylinophilic lines in the periosteal layer in sections taken from the central regions of the diaphysis. We examined the results by analyzing the differences of mean SVL between groups that have LAGs and no LAGs (*F. limnocharis* and *F. cancrivora*) and evaluated the relationship between body size and number of LAGs using Spearman's correlation (for *L. macrodon*).

LAGs appeared as faint lines in the periosteal layer of cross sections of phalanges of *F. limnocharis* and *F. cancrivora*, whereas in *L. macrodon* they were darkly stained and distinct lines (Fig. 1). The maximum number of LAGs found in samples from *F. limnocharis* and *F. cancrivora* was one and for *L. macrodon* was five. The most common state for all species was to have no LAGs visible (73% of *F. limnocharis*, 63% of *F. cancrivora*, and 61% of *L. macrodon*). One LAG occurred in 27% of *F. limnocharis*, 37% of *F. cancrivora*, and 20% of *L. macrodon*. Only *L. macrodon* had more than one LAG (10% with 2, 7% with 3, and 2% (1 individual) with 5).

There were no significant differences in mean body size be-

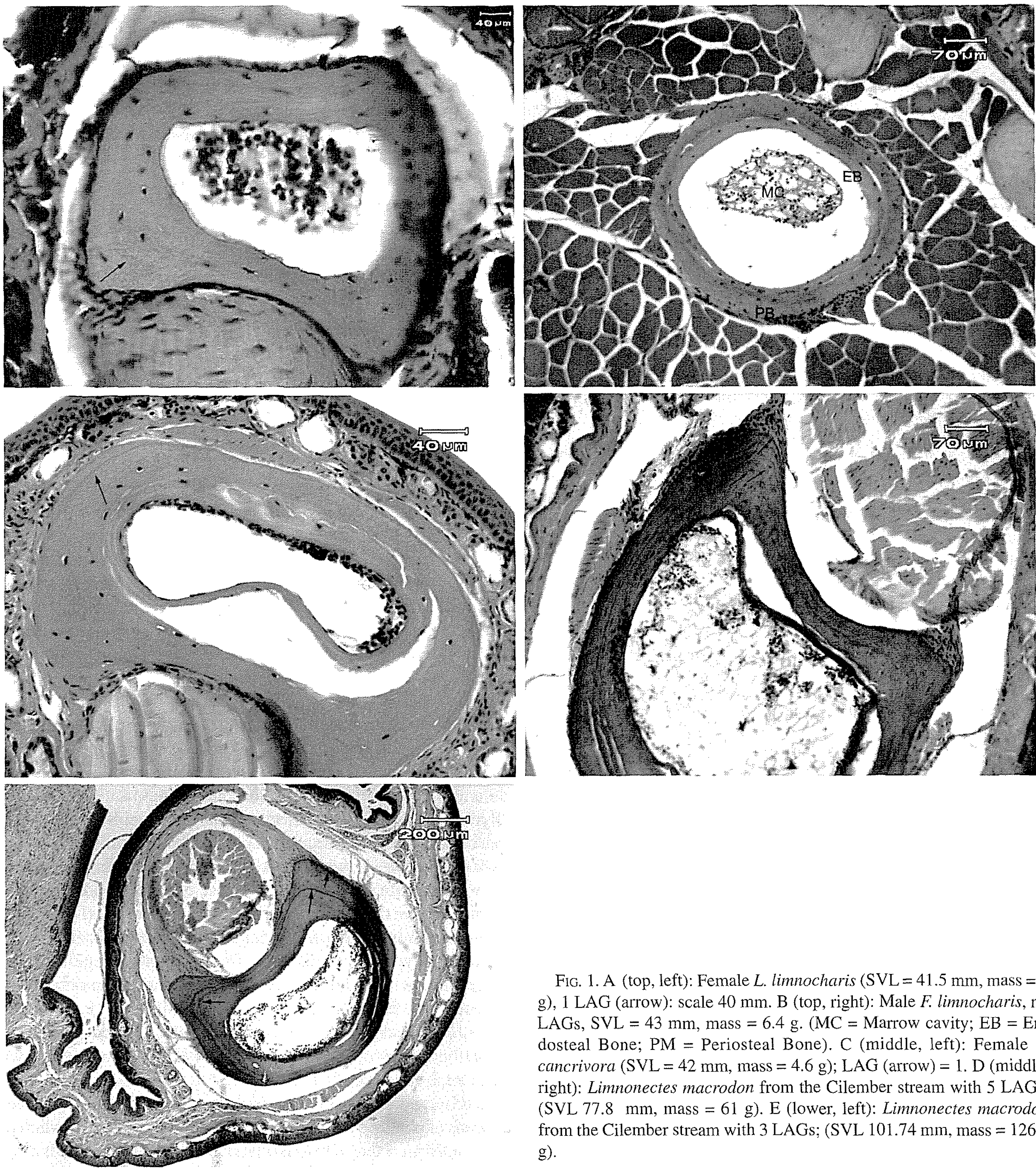

FIG. 1. A (top, left): Female *L. limnocharis* (SVL = 41.5 mm, mass = 5 g), 1 LAG (arrow): scale 40 mm. B (top, right): Male *F. limnocharis*, no LAGs, SVL = 43 mm, mass = 6.4 g. (MC = Marrow cavity; EB = Endosteal Bone; PM = Periosteal Bone). C (middle, left): Female *F. cancrivora* (SVL = 42 mm, mass = 4.6 g); LAG (arrow) = 1. D (middle, right): *Limnonectes macrodon* from the Cilember stream with 5 LAGs; (SVL 77.8 mm, mass = 61 g). E (lower, left): *Limnonectes macrodon* from the Cilember stream with 3 LAGs; (SVL 101.74 mm, mass = 126.5 g).

tween groups that had LAGs and no LAGs, both in *F. limnocharis* (t_{93} = 0.358, P = 0.723) and in *F. cancrivora* (t_{28} = 0.369, P = 0.723). However, there was a significant positive relationship between body size and LAG number in *L. macrodon* (Spearman correlation, r_s = 0.399, P = 0.01); larger *L. macrodon* tended to possess more LAGs than smaller individuals (Fig. 2). This suggests that the number of LAGs may reflect age in this species. However, since each LAG class spanned wide and overlapping ranges of snout-vent lengths, it is impossible to estimate age from the size of frogs.

Our results suggest that skeletochronology may not be useful on some species such as *F. limnocharis* and *F. cancrivora* that are active all year and live in environments in which temperature does not show major seasonal fluctuations, even when there are dis-

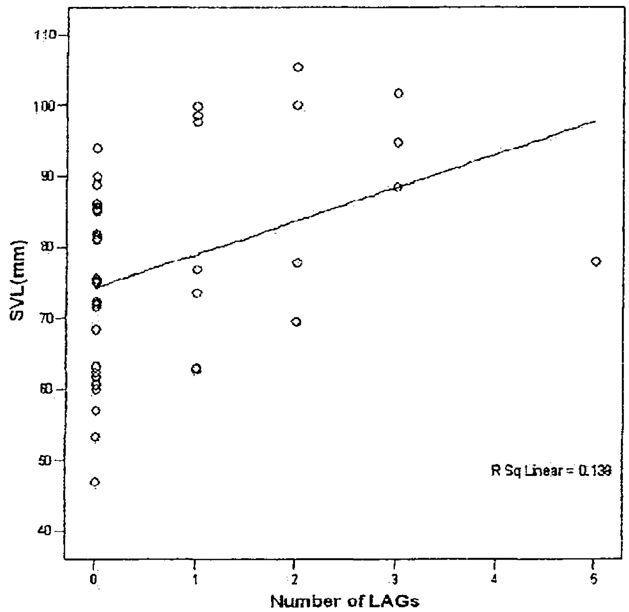

FIG. 2. Snout–vent length (mm) versus number of lines of arrested growth (LAGs) for *Limnonectes macrodon*.

tinct wet and dry seasons. Both species only showed weak LAGs, when any were evident. Population studies (Kusrini 2005) indicated that they remain in an area of habitat while conditions are favourable, but move on when conditions become unfavourable. Because individual paddy fields have varying seasonal patterns of filling and draining, sowing and harvesting, paddy-field systems taken as a whole tend to be largely aseasonal. The lack of seasonal effects on growth in these species could therefore be due to the frogs moving locally to favorable habitats as conditions change. It is also possible, however, that the age structure suggested by the LAGs we detected in these species is correct; both have been suggested to be short lived (Jaafar, 1994; Kusrini 2005).

Our results show that skeletochronology may be applicable to *L. macrodon*. LAGs found in *L. macrodon* were quite distinct, suggesting that growth was slower during certain times. In contrast to the paddy-field species, mark recapture data showed that *L. macrodon* is philopatric, remaining in place during the dry season but reducing its activity levels (Kusrini 2005). This suggests that the pattern of LAGs we found in *L. macrodon* may be due to changes in growth rate caused by seasonal rainfall patterns, with reduced growth during the dry season, which typically occupies three months each year.

Further study is required to validate that LAGs are formed annually in *L. macrodon*, either by clipping recaptured frogs in the future or possibly by examining museum specimens covering a wide range of SVL sizes.

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