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# A Skeletochronological Study on a Subtropical, Riparian Ranid (*Rana swinhoana*) from Different Elevations in Taiwan

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**ABSTRACT**—We estimated the age, longevity, and growth patterns of a subtropical ranid, *Rana swinhoana* from high (Lishing) and low (Wulai) elevations using skeletochronology. In addition, we also measured body mass and length of frogs from five other localities. Results showed that both snout-vent length and body mass of frogs were significantly correlated with altitudes for both sexes. Frogs of Lishing were significantly larger and older than that of Wulai. We used LAGs to estimate the age and growth of frogs and found that the growth of Wulai frog of both sexes slowed down at an earlier age than that of Lishing frogs. Male and female frogs from Wulai did not exceed 6 and 7 years, respectively, while the maximum age of males and females of Lishing was 7 and 11 years, respectively. Results suggest that the LAGs observed in *R. swinhoana* correspond to low temperature and/or decreased food availability instead of desiccation during the harsh annual period (November to February). Skeletochronological data suggest that the variations of body size of *R. swinhoana* among elevations are likely associated with the growth, age at sexual maturity, and longevity.

**Key words**: age determination, altitude, life history traits, lines of arrested growth, skeletochronology, subtropical anuran

## INTRODUCTION

Skeletochronology in amphibians has been proven a reliable technique for age and growth assessment in many species where the age of animals can be inferred from growth marks (lines of arrested growth or LAG) of phalanges without sacrificing the animals (Kleinenberg and Smirina, 1969; Hemelaar and van Gelder, 1980; Gibbons and McCarthy, 1983; Hemelaar, 1985; Acker *et al.*, 1986; Kusano *et al.*, 1995; Castanet *et al.*, 1996) The development of this technique enables reseachers to collect demograhpic parameters such as age, age at sexual maturity, past growth history, and longevity in a relatively short time, which otherwise can only be collected by a long-term mark-recapture project. Subsequently, these parameters can be used to understand many aspects of ecological and evolutionary aspects of life history traits of anurans (Hemelaar, 1988; Augert and Joly, 1993; Ryser, 1996; Marunouchi *et al.*, 2000; Miaud *et al.*, 2000).

Earlier studies have repeatedly demonstrated that the formation of LAGs can be found in temperate amphibians because of the alternation of seasonal climates (Hemelaar and van Gelder, 1980; Gibbons and McCarthy, 1983; Driscoll, 1999), and skeletochronological works have been reviewed (Halliday and Verrell, 1988; Castanet and Smirina, 1990). Many of these studies have been done to address the life-history characteristics of frog populations over different latitudes or altitudes (Hemelaar, 1988; Caetano and Castanet, 1993; Schabetsberger and Goldschmid, 1994; Leclair and Laurin, 1996; Ryser, 1996; Miaud *et al.*, 1999). To date, however, very few skeletochronological studies have been conducted on tropical and subtropical frogs, partly because it has been thought that frogs in these biogeographical regions do not have LAGs due to the stable climates they experience. Only recently, pioneering skeletochronological works have demonstrated that LAGs can be observed in tropical and subtropical frogs (Guarino *et al.*, 1998; Khonsue *et al.*, 2000; Kumbar and Pancharatna, 2001; Morrison *et al.*, 2004). Nevertheless, due to limited data on the demographic measurements of populations, there is almost no study that adequately looked into the life history traits in this biogeographical region (Morrison *et al.*, 2004). In addition, most studies to date suggest that the

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presence of LAGs in tropical and subtropical anurans is likely caused by unfavorable conditions during the dry season (Guarino *et al.*, 1998; Khonsue *et al.*, 2000; Kumbar and Pancharatna, 2001). However, for subtropical riparian species such as *R. swinhoana,* water is readily available in streams (Kam and Chen, 2000). In theory, if desiccation is not a threat to frogs, we would expect that the LAGs may not be found in *R. swinhoana.*

The purpose of this study was to conduct a skeletochronological study on a ranid, *Rana swinhoana* from a lowland and highland population. Specifically, our aims were (1) to assess the prevalence of LAGs in riparian anuran species from the subtropical region, (2) to determine elevational variations in body size among frog populations, (3) to determine the growth patterns of male and female frogs in lowland and highland populations, and (4) to assess the life history characteristics (longevity and age at sexual maturity) of frog populations over different elevations.

## MATERIALS AND METHODS

### Study animal

*Rana swinhoana* is a mid to large-sized frog, widely distributed from 100 to 1890 m in Taiwan (Wang and Chan, 1978; Kam *et al.*, 1998; Kam and Chen, 2000), and preliminary observations have demonstrated that body size varies greatly among elevations (Kam, unpublished data). This nocturnal, riparian species is found along torrential streams, rivers, and rapids with scattered boulders of various sizes. Frogs retreat into crevices between boulders during the daytime, and are active at night, perching on boulders or rocks in the vicinity of rapids or riffles. *Rana swinhoana* is agile during activity, and it usually produces short, impulsive, high-pitched calls (Wang and Chan, 1978) which are similar to those of many riparian species (Duellman and Trueb, 1986).

### Study sites and collection

We collected *R. swinhoana* from a high (Lishing) and low (Wulai) altitudinal population from June to September of 2000. Lishing is located at an elevation of about 1600 m (approximately 24°8'N, 121°11'E) with mean air temperature of 16.7°C (ranges 12.0– 20.1°C) whereas Wulai is located at an elevation of about 200 m (24°51'N, 121°40'E) with mean air temperature of 19.7°C (ranges 13.1–25.4°C). The rainy seasons at both locations are similar, with wet season in the spring-summer (rainfall: 975 and 1199 mm for Lishing and Wulai, respectively) and dry season in the fall-winter seasons (rainfall roughly half that of wet season). From May 1999 to July 2000, we also collected more *R. swinhoana* from five other locations for body size analysis. The locations are: Huisun (1000–1100 m), Luengmei (720 m), Taiping (640 m), Dili (320–420 m), and Shrtan (230–260 m).

For each field trip, we waded up selected streams and searched for frogs in the stream and along the stream bed. Frogs were easy to spot at night because they usually perched on boulders or rocks, particularly in the vicinity of rapids or riffles. Male frogs called year round and were identified by the presence of vocal sacs and/or nuptial pads (Kam *et al.*, 1998). Frogs showed sexual dimorphism in body size where the SVL of female frogs rarely smaller than 60 mm (Kam *et al.*, 1998). Thus, frogs with SVL larger 60 mm and without vocal sac were categorized into adult females, whereas frogs with SVL smaller than 60 mm and without vocal sac were categorized into subadults or juveniles. We collected 77 individuals (46 adult males, 26 adult females, and 5 juveniles or subadults) from Lishing and 64 individuals (20 adult males, 31 adult females and 13 juveniles) from Wulai. In addition, we collected 30 (26 males and 4 females), 6 (6 males), 5 (5 males), 9 (7 males and 2 females), and 3 (2 males and 1 females) individuals from Huisun, Luengmei, Taiping, Dili, and Shrtan, respectively for cross site comparisons. Snout-vent length was measured to the nearest 0.1 mm with an electronic caliper and body mass was measured to the nearest 0.5 g with a portable electronic balance. We removed the third phalanx of the fourth toe from the right hind foot and stored in 70% alcohol for age determination. Frogs were released on site after we had measured and sampled the body size and the phalanx, respectively.

### Skeletochronological analyses

We conducted a skeletochronological study on 65 frogs (43 males and 22 females) from Lishing and 43 frogs (15 males and 28 females) from Wulai. Methods for skeletochronology are modified from Kusano *et al.* (1995) and Leclair and Laurin (1996). Briefly, the cut phalanges were fixed in 10% formalin for at least 5 days, decalcified in 3% nitric acid for at least 3 hr, placed in distilled water overnight, cleaned of surrounding tissues, and mounted in cryocompound. Cross-sections about 20 μm thick were made with a freezing microtome. All sections were stained with Ehrlich hematoxylin to reveal the lines of arrested growth (LAG) and mounted on glass slides in glycerol- gelatin. One mid-diaphyseal section per individual was photographed under microscope by using a CCD camera and transferred as a digital photo on a computer screen. The LAGs were examined independently by two observers, and the age of each individual was determined by counting the number of LAGs. In cases of disagreement, we reexamined the bone sections and drew a consensus conclusion together.

Occasionally, hematoxylinophilic lines appeared as double lines or false lines and were counted as one or no LAG, respectively. In order to consider the possibility of resorption of the inner LAGs due to bone restructuring, back calculation were necessary as in *Rana temporaria* (Esteban, 1990) and *Rana pipiens* (Leclair and Castanet, 1987). Because LAGs were nearly oval in *R. swinhoana*, we examined LAG resorption by comparing the major axis of the innermost LAG of each adult with the mean of juveniles LAG with no resorption at all (i.e., comparison of a single observation with the mean of a sample, t-test, a type I error of 0.01; Pagano and Gauvreau, 2000). For example, if the major axis of the innermost LAG of an adult was significantly greater than the mean LAG of juveniles with no resorption, then we would conclude that resorption occurred and a LAG was added for that adult. The total number of LAGs was assessed by counting visible LAGs plus those estimated as totally destroyed by endosteal resorption. The major axes of all LAGs were measured directly on the digital photograph and the data transformed in real values (mm) were used as a “growth indicator” to back calculate individual growth history and sizes patterns (Leclair and Castanet, 1987; Ryser, 1996).

### Statistical analyses

All tests were processed with the SAS statistical program (SAS Institute Inc, 1996). We used the Students t-test to compare SVL, body mass, mean age and growth indexes and Chi-square test to compare age distributions. Pearson correlation coefficient was used to examine the relationship between SVL and elevations (i.e., sampling sites) and body mass and elevations. We designated a sampling site as an experimental unit. We calculated the means of SVL and body mass of male and female frogs from each sampling site before we correlated them with the sites.

## RESULTS

### Body length and mass

Frogs from Lishing were significantly larger and heavier than those from Wulai (Table 1). Many male (76%) and female (85%) frogs from Lishing were over 70mm and 80 mm in SVL, respectively, but none from Wulai attained these sizes. Sexual dimorphism was also observed in SVL and body mass (Table 1). When measurements from individuals from other localities were included in the analysis, we found a significant correlation between SVL and elevations for both sexes (males: SVL=52.79±0.011 [elevation], $r$=0.84, $P$=0.048, $n$=7; females: SVL=72.39±0.008 [elevation], $r$=0.95, $P$=0.014, $n$=6). Similar results were found for the body mass (males: Body mass=11.70±0.018 [elevation], $r$=0.87, $P$=0.026, $n$=7; females: Body mass=28.36±0.026 [elevation], $r$=0.94, $P$=0.016, $n$=6).

### Skeletochronology

In all phalangeal cross-sections, clearly defined hematoxylinophilic lines, interpreted as LAGs, were clear and relatively easy to count but those near the outer bone margin were occasionally difficult to discern (Fig. 1). The distance between LAGs was variable because of an inconstant rate of periosteal bone growth, and the thickness of the growth layers progressively declined from the inside to the outside of the sections, representing a decrease in bone growth. We have observed the presence of double lines and false lines in *R. swinhoana* in which the proportion of frogs that had double lines was 14% (15 of 109) and false lines was 7% (8 of 109) However, the appearance of double lines and false lines did not cause any serious problems in the process of counting. Endosteal resorption was observed in 69 and 77% of individual from Lishing and Wulai populations, respectively (Table 2).

### Age structure

The age distributions were significantly different between Lishing and Wulai populations in both sexes (Fig. 2; Chi-Square test, males: $\chi^2$=9.92, $P$=0.007; females: $\chi^2$=21.43, $P$=0.0003), with individuals from Lishing being older than those from Wulai (Table 1). Male and female frogs from Wulai did not exceed 6 and 7 years, respectively; while the maximum age of males and females from Lishing

**Table 1.** Snout-vent length (SVL), body mass, and estimated age of adult frogs from highland and lowland populations. The sample sizes are in parentheses. M and F represent male and female, respectively. *t* and *P* represent *t* values and significance probability, respectively.

| | SVL (mm) | | Body mass (g) | | Estimated age | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | means | | Maximum | | Minimum | |
| | M | F | M | F | M | F | M | F | M | F |
| Highland (Lishing) | 72.5±4.5 (46) | 84.6±3.9 (25) | 43.02±7.37 (46) | 72.59±4.80 (25) | 5.12±1.28 (43) | 6.55±1.82 (22) | 7 (43) | 11 (22) | 2 (43) | 4 (22) |
| Lowland (Wulai) | 57.5±2.8 (20) | 72.6±3.8 (31) | 17.64±2.49 (20) | 34.81±6.70 (31) | 3.73±1.10 (15) | 4.71±1.01 (28) | 6 (15) | 7 (28) | 2 (15) | 2 (28) |
| *t* | 16.69 | 11.61 | 20.78 | 11.83 | 4.02 | 4.23 | | | | |
| *P* | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | |

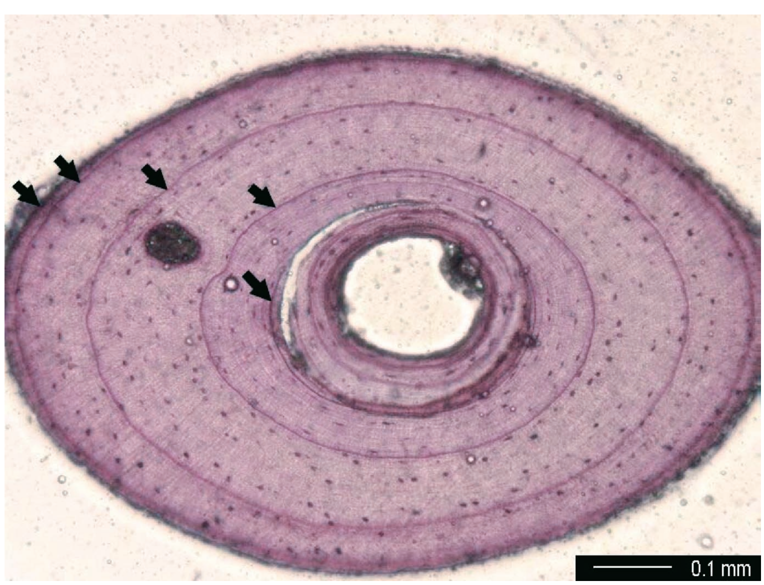


**Fig. 1.** A cross section of the diaphysis of a phalange showing lines of arrested growth (LAGs) of *Rana swinhoana*.

**Table 2.** Percentage of individuals with none, one, or two LAGs removed by endosteal resorption. Sample sizes are in parentheses.

| | 0 LAG (%) | 1 LAG (%) | 2 LAGs (%) |
|---|---|---|---|
| Highland (65) | 31 | 65 | 4 |
| Lowland (43) | 23 | 77 | 0 |

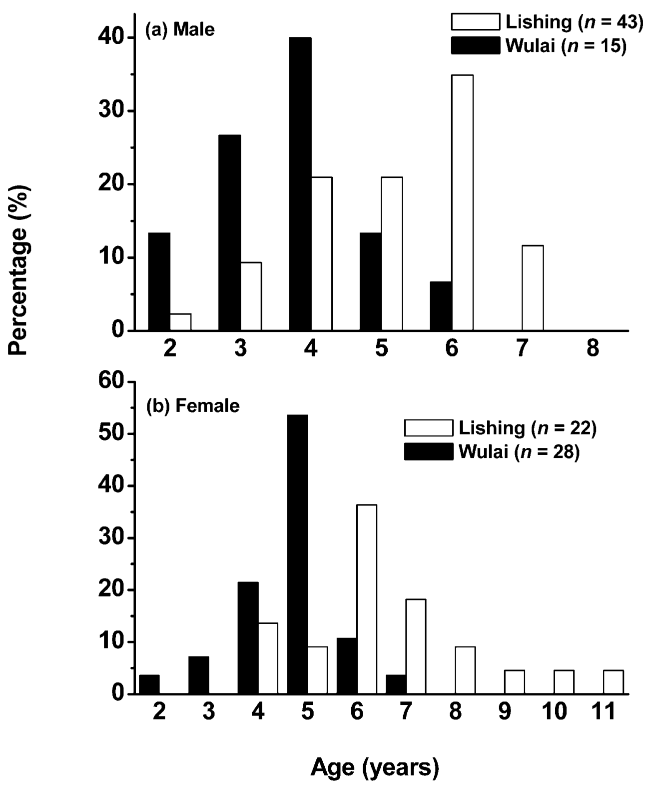


**Fig. 2.** Distribution of estimated age of (a) male and (b) female *Rana swinhoana*.

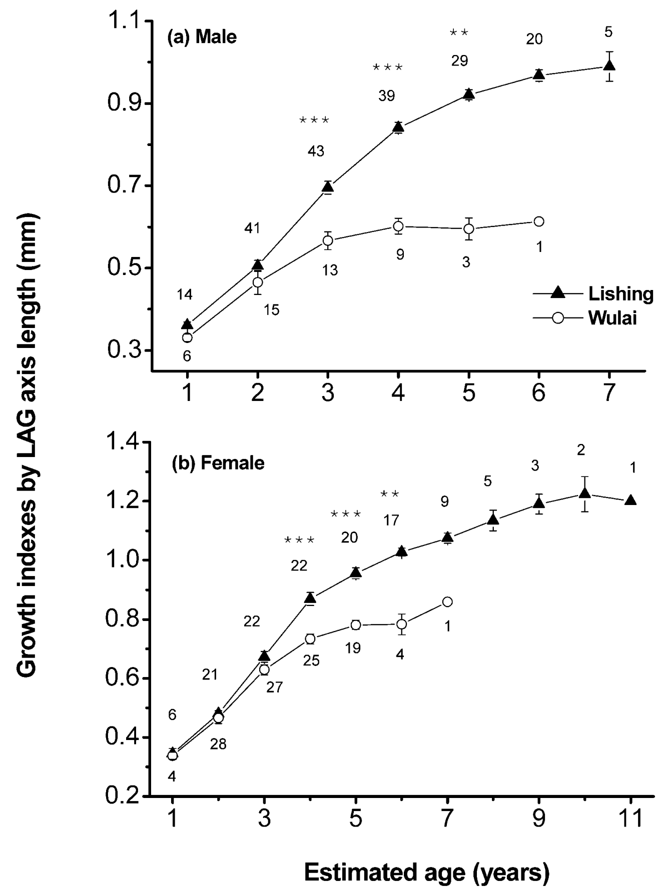


**Fig. 3.** Relationship between the growth indexes and estimated age for (a) male and (b) female *Rana swinhoana*. Growth indexes were compared between highland and lowland populations, and significant differences were labeled. ** and *** represent significant differences at a 0.01 and 0.0001 level, respectively. Sample sizes are labeled next to the symbols. Values are means±SE.

was 7 and 11 years, respectively.

## Growth patterns

The growth rates for males in the two populations were similar up until 3 years of age when growth rates slowed dramatically in males from Wulai (Fig. 3). We compared growth indexes of two populations each year and found significant differences at 3 years of age or older (t test, $P<0.05$; Fig. 3a). Similar results were observed for females, however, the differences in growth indexes between females from the two populations were statistically significant at 4 years of age or older (t test, $P<0.05$; Fig. 3b).

## DISCUSSION

Our histological results revealed that LAGs were present in *R. swinhoana*, as observed in other temperate zone amphibians for which annual periodicity of bone marks have been studied. These results and those of previous studies suggest that the presence of the LAG in tropical and subtropical regions is more common than originally thought. Guarino *et al.* (1998) first conducted a skeletochronological study on *Mantidactylus microtympanum* in a subtropical region (latitude 24°C) on Madagascar where the climate is characterized by warm, wet summers and dry, cool winters. They hypothesized that the relatively harsh climatic winter condition (i.e., low temperature and dry season) couples with reduced food availability, are likely to affect bone growth over seasons. Two studies conducted in tropical regions in Thailand (latitude 13°N; Khosue *et al.*, 2000) and southern India (15°N; Kumbar and Pancharatna, 2001) also found LAGs in most of the species examined. These tropical areas experience relatively warm temperatures with fluctuations of less than 10°C and dry season, thus, researchers contended that the moisture rather than the temperature and/or food availability retarded growth of bone of frogs.

The presence of LAGs in *R. swinhoana* suggests that environmental variables other than moisture regimes is the cause for the formation of LAGs, which may differ from results of earlier studies on tropical and subtropical anurans (Guarino *et al.*, 1998; Khonsue *et al.*, 2000; Kumbar and Pancharatna, 2001). *Rana swinhoana* is a riparian species, and water is readily available in streams, thus, desiccation is unlikely to be a threat to frogs. Kam and Chen (2000) reported that *R. swinhoana* is active year round, however, seasonal abundance fluctuated greatly in that significantly more frogs are found in the warm and wet summer than in the cold and dry winter. Seasonal activity of amphibians is principally regulated by environmental temperature and moisture (Duellman and Trueb, 1986), and activity levels reflect the physiological conditions of organisms (Wiest, 1982; Pough *et al*., 1983). The result of stepwise regression reveals a significant relationship between frog abundance and air temperature, but not precipitation (Kam and Chen, 2000), suggesting that seasonal activity of *R. swinhoana* is associated more with changes in temperature than with changes in rainfall. It is most likely that low temperatures in winter depress physiological functions and reduce activity of frogs, resulting in the formation of LAGs. Due to the limitation of time and accessibility, we were unable to establish a field study to successfully recapture frog to confirm the number of LAG deposited in a year. However, based on the activity pattern of frogs, we believed that the LAGs observed in *R. swinhoana* correspond to the harsh annual period (November to February) during which temperature and/or food availability decrease. Consequently, one LAG is deposited each year as in other ranid frogs (Leclair and Castanet, 1987; Ryser, 1988; Bastien and Leclair, 1992; Esteban *et al*., 1995; Kusano *et al*., 1995; Platz *et al*., 1997; Khonsue *et al*., 2001a, b).

Our findings that the body size of *R. swinhoana* varied among elevations in both sexes are similar to results of earlier studies where life history traits of populations were related to latitudinal or altitudinal variation in habitats (Licht, 1975; Berven, 1982a, b; Hemelaar, 1988; Caetano and Castanet, 1993; Ryser, 1996; Morrison and Hero, 2003; Morrison *et al*., 2004). We contend that the large variations in body size of *R. swinhoana* among elevations are likely associated with the growth, age at sexual maturity and longevity of frogs. Growth can be inferred from LAGs which become closer to each other from the center of the bone toward the periphery, a sign of slowing of the growth rate (Kleinenberg and Smirina, 1969; Gibbons and McCarthy, 1984; Hemelaar, 1988; Caetano and Castanet, 1993). The growth indices of Lishing populations were initially similar to those of the Wulai population. However, growth rate in male and female frogs of the Wulai populations slowed down at age two and three, respectively, whereas growth in individuals from the Lishing population remained relatively constant until four years of age when it slowed down for both sexes. Differences in the timing of the slowing of growth rate may due to the early sexual maturity of Wulai frogs which invest more energy in reproduction instead of growth (Hemelaar, 1988). In contrast, individuals from Lishing populations continue to grow and reach sexual maturity at the later age. This delayed sexual maturity, in turn, enables the frogs to maintain longer periods of high growth rate and therefore reach larger body sizes. It is important to note that even though the Lishing population encounters colder temperature, their initial growth rate did not differ from that of the Wulai population. It is possible that countergradient selection effects may have offset the detrimental effects of colder temperature on growth of Lishing population (Berven *et al*., 1979; Ryser, 1996). In addition, field observation revealed that highland population is sympatric with less species (*Bufo bankorensis* and *Rana sauteri*) when compared to lowland population which coexists with at least eight species (*B. bankorensis, Polypedates megacephalus, Rhacophorus moltrechti, Rana kuhlii, Rana latouchii, Rana limnocharis,* and *R. sauteri*; Kam, unpublished data). Among them, *B. bankorensis*, *R. kuhlii, R. latouchii,* and *R. sauteri* to some extent overlap in microhabitats and food with *R. swinhoana* (Do and Lue, 1980; Kam *et al*., 1998; Lue *et al.,* 1999). Thus, it is also possible that high food availability and less interspecific competition in highland may have resulted in faster growth rate of frogs that offsets the cold temperature effects.

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## REFERENCES

Acker PM, Kruse KC, Drehbiel EB (1986) Aging *Bufo americanus* by skeletochronology. J Herpetol 20: 570–574

Augert D, Joly P (1993) Plasticity of age at maturity between two neighbouring populations of the common frog (*Rana temporaria* L.). Can J Zool 71: 26–33

Bastien H, Leclair RJ (1992) Aging wood frogs (*Rana sylvatica*) by skeletochronology. J Herpetol 26: 222–225

Berven KA (1982a) The genetic basis of altitudinal variation in the wood frog *Rana sylvatica*. I. An experimental analysis of life history traits. Evolution 36: 962–983

Berven KA (1982b) The genetic basis of altitudinal variation in the wood frog *Rana sylvatica*. II. An experimental analysis of larval development. Oecologia 52: 360–369

Berven KA, Gill DE, Smith-Gill SJ (1979) Countergradient selection in the green frog, *Rana clamitans*. Evolution 33: 609–623

Caetano MH, Castanet J (1993) Variability and microevolutionary patterns in *Triturus marmoratus* from Portugal: age, size, longevity and individual growth. Amphib-reptil 14: 117–129

Castanet J, Smirina E (1990) Introduction to the skeletochronological method in amphibians and reptiles. Ann Sci Nat Zool 11: 191–196

Castanet J, Francillon-Vieillot H, Bruce RC (1996) Age estimation in Desmognathine salamanders assessed by skeletochronology. Herpetologica 52: 160–171

Do MC, Lue KY (1982) Comparative stomach analysis on the eleven species of amphibians in Taiwan, including endemic species of *Hynobius formos* nus. Taiwan Mus 25: 225–234

Driscoll DA (1999) Skeletochronological assessment of age and population stability for two threatened frog species. Aust J Ecol 24: 182–189

Duellman WE, Trueb L (1986) Biology of amphibians. McGraw-Hill Book Company, New York

Esteban M (1990) Environmental influences on the skeletochronological record among recent and fossil frogs. Ann Sci Nat Zool 11: 201–204

Esteban M, Garcia-Paris M, Castanet J (1995) Use of bone histology in estimating the age of frogs (*Rana perezi)* from a warm temperate climate area. Can J Zool 74: 1914–1921

Gibbons MM, McCarthy TK (1983) Age determination of frogs and toads (Amphibia, Anura) from north-western Europe. Zool Scr 12: 145–151

Gibbons MM, McCarthy TK (1984) Growth, maturation and survival of frogs *Rana temporaria L*. Holarctic Ecol 7**:** 419–427

Guarino FM, Andreone F, Angelini F (1998) Growth and longevity by skeletochronological analysis in *Mantidactylus microtympanum,* a rain-forest anuran from southern Madagascar. Copeia 1998: 194–198

Halliday TR, Verrell PA (1988) Body size and age in amphibians and reptiles. J Herpetol 22: 253–265

Hemelaar A (1985) An improved method to estimate the number of year rings resorbed in phalanges of *Bufo bufo (L.*) and its application to populations from differnet latitudes and altitudes. Amphib-reptil 6: 323–341

Hemelaar A (1988) Age, growth and other population characteristics of *Bufo bufo* from different latitudes and altitudes. J Herpetol 22: 369–388

Hemelaar ASM, van Gelder JJ (1980) Annul growth rings in phalanges of *Bufo bufo* (Anura, Amphibia) from the Netherlands and their use for age determination. Netherland J Zool 30: 129–135

Kam YC, Chen TC (2000) Abundance and movement of a riparian frog (*Rana swinhoana)* in a subtropical forest of Guandau stream, Taiwan. Zool Stud 39: 67–76

Kam YC, Chen TC, Yang JT, Yu FC, Yu KM (1998) Seasonal activity, reproduction, and diet of a riparian frog (*Rana swinhoana)* from a subtropical forest in Taiwan. J Herpetol 32: 447–452

Khonsue W, Matsui M, Misawa Y (2000) Age determination by skeletochronology of *Rana nigrovittata*, a frog from tropical forest of Thailand. Zool Sci 17: 253–257

Khonsue W, Matsui M, Hirai T, Misawa Y (2001a) Age determination of wrinkled frog, *Rana rugosa* with special reference to high variation in postmetamorphic body size (Amphibia: Ranidae). Zool Sci 18: 605–612

Khonsue W, Matsui M, Hirai T, Misawa Y (2001b) A comparison of age structures in two populations of a pond frog *Rana nigromaculata* (Amphibia: Anura). Zool Sci 18: 597–603

Kleinenberg SE, Smirina EM (1969) A contribution to the method of age determination in amphibians. Zool Zh 48: 1090–1094

Kumbar SM, Pancharatna K (2001) Occurrence of growth marks in the cross section of phalanges and long bones of limbs in tropical anurans. Herpetol Rev 32: 165–167

Kusano T, Fukuyama K, Miyashita N (1995) Age determination of the stream frog, *Rana sakuraii,* by skeletochronology. J Herpetol 29: 625–628

Leclair RJ, Castanet J (1987) A skeletochronological assessment of age and growth in the frog *Rana pipiens* Schreber (Amphibia, Anura) from southwestern Quebec. Copeia 1987: 361–369

Leclair RJ, Laurin G (1996) Growth and body size in populations of mink frogs *Rana septentrionalis f*rom two latitudes. Ecography 19: 296–304

Licht LE (1975) Comparative life history features of the western spotted frog, *Rana pretiosa,* from low- and high-elevation populations. Can J Zool 53: 1254–1257

Lin YL, Hou PCL (2002) Applicability of skeletochronology to the anurans from a subtropical rainforest of sourthern Taiwan. Acta Zool Taiwanica 13: 21–30

Lue CY, Tu MC, Shang G (1999) A field guide to the amphibians and reptiles of Taiwan. Great Nature Magazine Publisher, Taipei

Marunouchi J, Hiroaki U, Ochi O (2000) Variation in age and size among breeding populations at different altitudes in the Japanese newts, *Cynops pyrrhogaster*. Amphibia-Reptilia 21: 381–396

Miaud C, Guyetant R, Elmberg J (1999) Variations in life-history traits in the common frog *Rana temporaria* (Amphibian: Anura): a literature review and new data from the French Alps. J Zool, London 249: 61–73

Miaud C, Guyetant R, Faber H (2000) Age, size, and growth of the alpine newt, *Triturus alpestris* (Urodela: Salamandridae), at high altitude and a review of life-history trait variation throughout its range. Herpetologica 56: 135–144

Morrison C, Hero JM (2003) Geographic variation in life history characteristics of amphibians: a review. J Anim Ecol 72: 270–279

Morrison C, Hero JM, Browning J (2004) Altitudinal variationi in the age at maturity, longevity, and reproductive lifespan of anuran in subtropical queenland. Herpetologica 60: 34–44

Pagano M, Gauvreau K (2000) Principles of biostatistics. Duxbury Press, Pacific Grove

Platz JE, Lathrop A, Hofbauer L, Vradenburg M (1997) Age distribution and longevity in the Ramsey Canyon leopard frog, *Rana subaquavocalis*. J Herpetol 31: 552–557

Pough FH, Taigen TL, Stewart MM, Brussard PF (1983) Behavioral modification of evaporative water loss by a Puerto Rican frog. Ecology 64: 244–252

Ryser J (1988) Determination of growth and maturation in the common frog, *Rana temporaria,* by skeletochronology. Zool Soc Lond 216: 673–685

Ryser J (1996) Comparative life histories of a low- and a high-elevation population of the common frog *Rana temporaria.* Amphib-reptil 17: 183–195

SAS Institute, Inc (1996) SAS/STAT User's guide. SAS Inst Inc, Gary

Schabetsberger R, Goldschmid A (1994) Age structure and survival rate in alpine newts (*Triturus alpestris)* at high altitude. Alytes 12: 41–47

Wang CS, Chan SK (1978) Ra*na narina swinhoana B*oulenger (Anura: Ranidae) from Taiwan. Quat J Taiwan Mus 30: 329–339

Wiest JA Jr (1982) Anuran succession at temporary ponds in a post oak-savanna region of Texas. US Fish and Wildl Res Rep 13: 39–47

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