Caspian J. Environ. Sci. 2017, Vol. 15 No. 1 pp. 75~84

CJES
Caspian Journal of Environmental Sciences

[Research]

Skeletochronological assessment of age in the Persian mountain salamander, *Paradactylodon gorganensis* (Clergue-Gazeau and Thorn, 1979) (Caudata: Hynobiidae) from Golestan Province, Iran

S. Zivari, H. Gholi Kami*

Department of Biology, Faculty of Sciences, Golestan University, Gorgan, Golestan Province, Iran.
** Corresponding author's E-mail:hgkami2000@yahoo.com*

(Received: Aug. 07. 2016 Accepted: Jan. 11. 2017)

ABSTRACT

Lines of arrested growth (LAGs) were assessed in the Golestan Province population of the critically - endangered museum specimens of Persian mountain salamander, *Paradactylodon gorganensis* (Clergue-Gazeau and Thorn, 1979) from Northeastern Iran using skeletochronology. LAGs were clearly discernable in the tibia and femur bone cross-sections and could be interpreted for age determination. The number of LAGs in the sampled Golestan Province population (n=29)was found to be 8-11 (9.75 ± 1.13 years) in males (n=12), 7-13 (9.26 ± 1.98 years) in females (n=15) and 7 and 8 (7.50 ± 0.70 years) in two unknown sex specimens for the first time.The snout-vent length (SVL) was 90.20 - 119.38 (103.85 ± 10.48) mm in males, 77.60 - 135.00 (105.96 ± 15.89) in females and 108.00 and 123.00 mm in unknown sex specimens. It was not found significant correlation between body size and age ($P > 0.05$). Although limited in sample size, the data suggest that males with ages over eleven years are absent in the fixed specimens studied. Our study showed that skeletochronological method can be successfully applied to Iranian *P. gorganensis* based on the clear arrest of growth in the hibernation period, as previously observed in other species in subtropical regions.

Key words: LAGs, Northeastern Iran, *Paradactylodon gorganensis*, Persian mountain salamander, Skeletochronology.

INTRODUCTION

Age determination of amphibians and reptiles is important in order to obtain information about mortality, longevity, and other ecological factors. In most cases, it provides accurate estimations of individual ages and therefore overcomes a major difficulty in demographic and life history studies (Yilmaz *et al.* 2005; Uzum & Olgun 2009).Therefore, several methods have been developed and used for estimating age of animals. An alternative tool to get such data for amphibians is skeletochronology which is considered the most reliable method of age estimation by counting the lines of arrested growth (LAGs) recorded in long bones (Castanet *et al.* 1993; Ashkavandi *et al.* 2012).However, although skeletochronology is established as a reliable method to assess age in amphibians, it has been applied mainly to temperate species (Halliday & Verrell 1988; Sinsch 2015), because of hibernation or prolonged dormancy in that region. For amphibians in temperate climates, each year of growth during the warm season, and the subsequent slowing of growth in the cool season (hibernation), commonly called Lines of Arrested Growth (LAGs), can be counted much like the growth rings in many trees (McCreary *et al.* 2008). Bone tissues of tailed amphibians are very simple in structure and LAGs are generally very clear and easily studied (Misawa & Matsui 1999). Because the periosteal cortex is the broadest and the medullar cavity narrowest in the middle part of the diaphysis of limb bones, this section is best-suited for skeletochronological detection of growth marks (Castanet & Smirina 1990; Castanet *et al.* 1993).

The first successful attempts on amphibians were based on the skull bonessuch as parasphenoid in*Necturus maculosus* (Senning 1940) and pterygoid in*Lithobatus* [*Rana*] *catesbianus* (Schroeder & Baskett 1968).Thereafter, round bones have become the material of choice (Sinsch 2015; Castanet & Smirina 1990; Castanet *et al.* 1993). Skeleto-chronology has been used to study variety of anurans (Khonsue *et al.* 2001; Yilmaz *et al.* 2005; Ashkavandi *et al*. 2012) and urodeles (Lima *et al.* 2000; Bovero *et al.* 2006; Eden *et al.* 2007; Uzum 2009; Farasat & Sharifi 2016). Age and body size are two demographic traits or standard characteristics that were used by many researchers for understanding the evolutionary life history and ecology of amphibians (Morrison & Hero 2003; Liao & Lu 2011; Gül *et al.* 2014). Due to the critically - imperiled status of much of salamanders, skeletochronological analysis of amphibian tissues from museum collections may provide some insights into historic demographic constituency of populations, species, and could also assist in planning or management wild populations if reintroduction is attempted in the future (Lindquist *et al.* 2012).Caetano & Leclair (1996) applied this method to red spotted newts (*Notophthalmus viridescens*) using the circumference of the LAGs in cross-sections of the humerus (Snover 2002).

There is little information on skeleton-chronological studies of Iranian amphibians. One of these published papers is on the marsh frog, *Rana ridibunda* (Ashkavandi *et al.* 2012), and the second on green toad, *Bufo viridis* (Ashkavandi *et al.* 2012). Age determination and growth of the endangered Kaiser׳s mountain newt, *Neurergus kaiseri* in the Southern Zagros range is the only published paper on Iranian salamanders (Farasat & Sharifi 2016). This research is the first study on skeletochronology of the Persian mountain salamander, *Paradactylodon gorganensis* in Iran.

Two families, four genera and seven species of salamanders have been reported from Iran (Baloutch & Kami 1995; Rastegar-Pouyani *et al.* 2008, 2015; Safaei- Mahroo *et al.* 2015).The Persian brook salamander, *Batrachuperus persicus* (Eiselt & Steiner 1970) is the first Hynobiid salamander described based on five salamander larvae collected around Asalem in the Talesh Mountains, Guilan Province, Iran (Eiselt & Steiner 1970), and subsequently reported from Weyser in southeast of Chalus City, Mazandaran Province, Iran (Schmidtler & Schmidtler 1971; Ahmadzadeh & Kami 2009). New distribution records (Kami &Vakilpoure 1996) and adult specimens of this species were described for the first time (with photos of *B. gorganensis*) in Guilan and Ardabil provinces, Iran (Kami 1999).A second hynobiid salamander, Persian mountain salamander, *Batrachuperus gorganensis* (Clergue-Gazeau & Thorn 1979) was described from Shirabad cave in Golestan Province (Clergue-Gazeau & Thorn 1979) and was identified as *P. persicus* some years ago (Kami 2004). This species was classified in a new genus, *Paradactylodon* (Risch 1984) and *Iranodon* (Dubios & Raffaelli 2012). Total length of this salamander reaches 282 mm (Kamiunpublished data) (Fig. 1). This species status is categorized as Critically Endangered (CR) in the IUCN Red list (Safaei- Mahroo *et al.* 2015). The important aims of this study were to compare the age structure and body size, to determine the longevity of males and females, to gain the correlation between age and SVL as well as to provide data for species conservation in a population of *Paradactylodon gorganensis*in Golestan Province, Iran.

MATERIALS AND METHODS

A total of 12 adult males and 15 adult females and two unknown sex adult specimens were studied. All specimens were deposited in Zoological Museum of Golestan University (ZMGU). These materials were collected from Shirabad Cave (36°57' N, 55° 03 ' E) between years 1994-2009. This cave is situated in about 70 km east of Gorgan City, southeast of Khanbebain and Shirabad Village at about 420 m above sea level. The snout - vent length (SVL) was measured to the nearest 0.01 mm, using a digital caliper. Sexes of salamanders were determined after dissection byobserving their gonads. Two different long bones were used in this study including the femur and tibia of the left hind limb. Bones were fixed in 10% formalin, the bones were decalcified in 10% (v/v) chloric acid during the period of decalcification, for 48 h. After decalcification, the bones were washed thoroughly for 24 hand dehydrated with 70, 80, 90, 95, 100% Methanol (eachfor 1.5 h). Cleaningthe surrounding tissues with Xylol (for 1.5 h), processed in paraffin block preparation (for 1.5 h), tissues were embedded in small paraffin block. Serial sections (5, 7, 10 µm and for some specimens20µm) were cut using a rotary microtome, stained with Hematoxylin and Eosin, and observed under an Olympus BX51 microscope equipped with digital camera DP12. The camera mounted and linked to a monitor and computer with image software. The number of lines of arrested growth (LAGs) was counted in the periosteal bone.

Age classes and SVL were normally distributed (Kolmogorov-Smirnov D test, P-value > 0.05), thus allowing comparisons using parametric tests (t - test).

We used Pearson correlation to examine the relationship between age and SVL. Data analysis was performed using SPSS 22 and Excel 2013 (Microsoft Office), and interpreted at α = 0.05.

Fig. 1. Adult *P. gorganensis* from Shirabad cave, total length 282 mm (Photo by H.G.K).

RESULTS

The averaged SVL were 103.85± 10.48 mm in males, 105.96 ± 15.89 mm in females and also 108.00 and 123.00 mm in the two unknown sex specimens (Table. 1).

In all examined sections, the hema-toxylinophilic lines, interpreted as LAG, were clearlyobserved in the cross-section (Fig. 2).

Both SVL and age showed normal distribution (Kolmogorov-Smirnov test, P > 0.05).The range of age was 7-13 years in females, 8-11 years in males and also 7 and 8 years in the two unknown sex specimens (Fig. 3). The Pearson correlation showed no significant correlation between SVL and age (P > 0.05, Fig. 4).

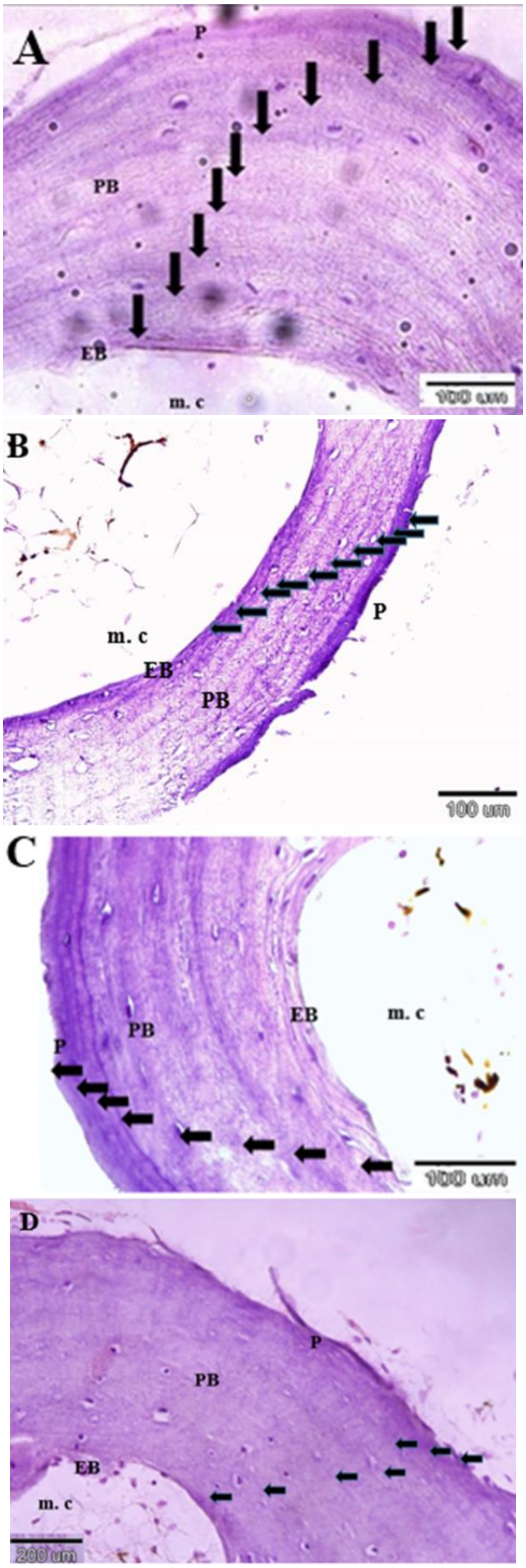

Fig.2. Cross-section of tibia and femur of *P. gorganensis*. Arrows: line of arrested growth (LAGs); EB = Endosteal Bone, m.c. = marrow cavity, PB = Periosteal Bone, P = Periphery. (A) Femural bone cross - section at diaphyseal level, male (SVL= 96.97 mm), 10 LAG, 7µm. (B) Tibia bone cross section at diaphyseal level, Female (SVL= 120.06 mm), 10 LAGs were observed in the periosteal bone, 5 µm. (C) Femural bone cross section at diaphyseal level, male (SVL= 108.58 mm), 8 LAG, 10µm. (D) Femural bone cross section at diaphyseal level, Female (SVL= 104.84 mm), 8 LAG, 10µm. H&E.

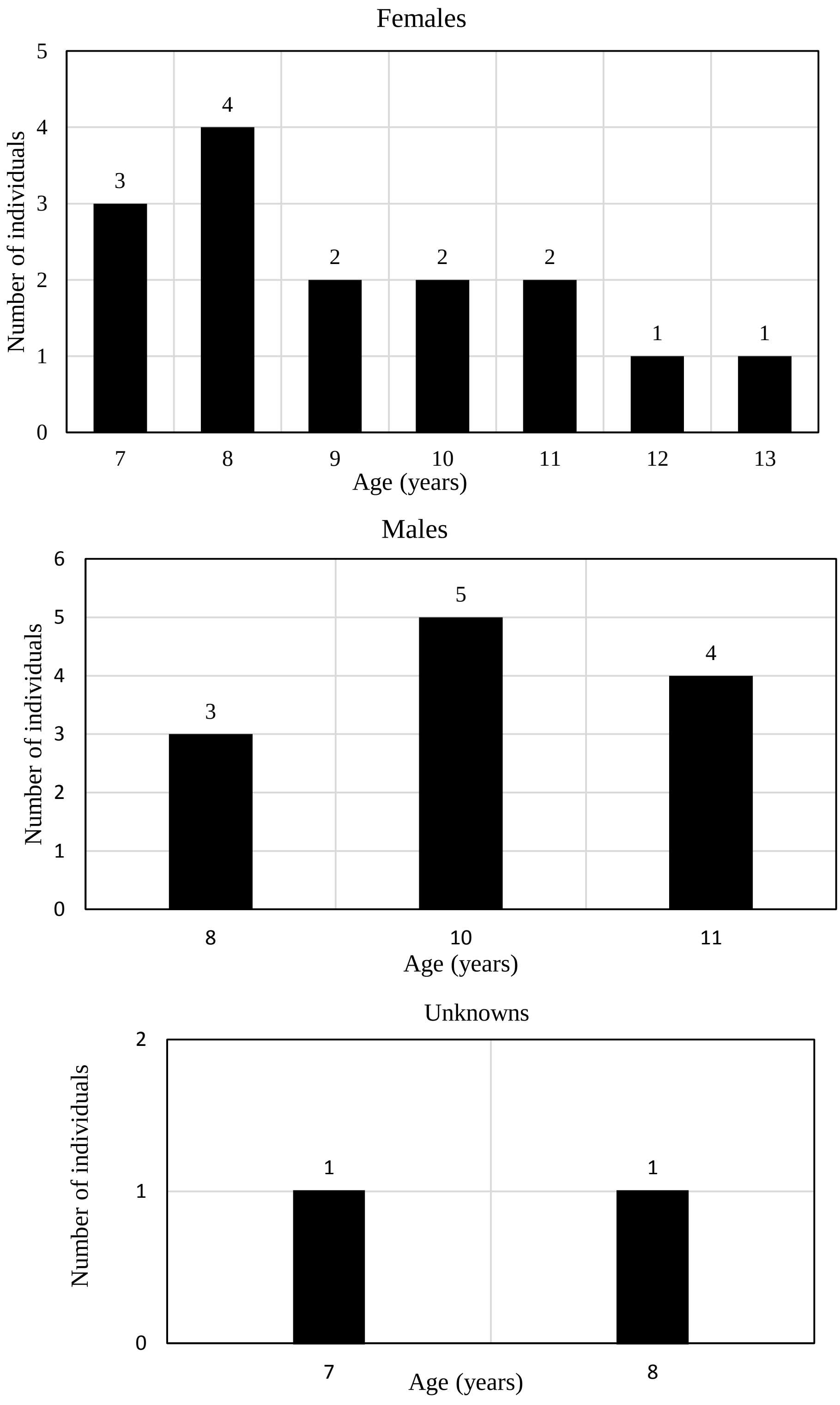

Fig. 3. Age frequency of the *P. gorganensis* (adult specimens).

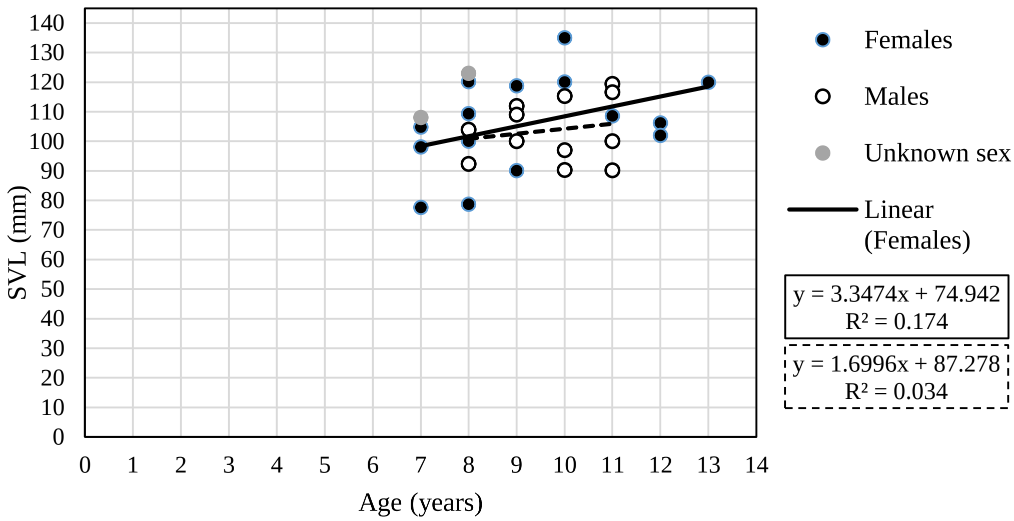

Fig.4. Absence of correlation between age classes and SVL in females, males and unknown sex specimens of *P. gorganensis* from Golestan Province, Iran.

Table 1. Age and SVL† of the *P. gorganensis* in a population from Golestan Province. Values are mean ±standard deviation and sample size.

Individuals	SVL (mm‡)	Age (years)
Males (n§=12)	103.85 ± 10.48	9.75 ± 1.13
Females (n=15)	105.96 ± 15.89	9.26± 1.98
Unknown (n=2)	115.50 ± 10.60	7.50 ± 0.70

The snout-vent length†
Millimeter‡
Number of individuals§

DISCUSSION

Age determination is very important in ecological studies (Gül *et al.* 2014). The use of skeletochronological method allows the determination of individual variation of life history traits and provides data on growth and age at maturity (Morrison & Hero 2003; Yilmaz *et al.* 2005; Liao & Lu 2011; Sinsch 2015). Skeletochronology is arguably the most common tool used to decipher the age of various amphibian taxa (Castanet & Smirina 1990; Eden *et al.* 2007). Age distribution information can be an important part of understanding the biology of any population (McCreary *et al.* 2008). Our study showed that skeletochronology method can be successfully applied to *P. gorganensis* from Iran by observing clear arrest of growth in the hibernation period, as previously observed in other species in subtropical regions. This is the first study using skeletochronology to estimate the age structure of *P. gorganensis* in Iran. Stained LAGs were clearly visible between zones of thicker layers of bone deposited in growth periods (Fig. 2). In general, long bones such as the humerus, femur and phalanges, are the preferred skeletal element for application of the technique (Halliday & Verrell 1988; Castanet *et al.* 1993; Snover 2002). The pattern of LAGs deposited is considered to be genetically controlled with reinforcement by seasonality such as temperature fluctuations (Ashkavandi *et al.* 2012). Presence of periosteal LAGs and annular growth indicated that age could be estimated in the species of genus *Paradactylodon*. In study of skeletochronology in urodeles, femurs (Caeatano & Leclair 1996), digits (Khonsue *et al.*

2001; Uzum 2009; Lindquist *et al.* 2012), and combination of femurs or humeri and digits (Lima *et al.* 2000) have been examined (Ento & Matsui 2002). Likewise, studies on bone growth and age calibration among successful captive breeding programs at various zoos and research facilities could be informative, especially if they are paired with studies on specimens currently held in museums (Lindquist *et al.* 2012).

The maximum observed longevity found in the present study was 13 years for females and 11 years for males. The age of *P. gorganensis* is not significantly correlated with SVL within each sex. Farasat and Sharifi (2016) reported that maximum longevity for *Neurergus kaiseri* in the Southern Zagros range, Iran, was 14 years in males and 12 years in females. SVL and age were positively correlated in both males and females in the *N. kaiseri*.

In a study conducted on the females of *Salamandrina perspicillata* in Italy, maximum lifespan was 12 years (Bovero *et al.* 2006). In a hynobiid, *Hynobius kimurae* in two populations (Misawa & Matsui 1999), the age frequencies of the oldest individuals, were 14 years (in males) and 12 years (in females) in Tokyo as well as 20 years (in males) and 17 years (in females) in Kyoto which were over twicethose of *H. nebulosus*: 4.34 years in males and 4.84 years in females (Ento & Matsui 2002). In *Triturus karelinii* from a population in northwest of Turkey, Uzum & Olgun (2009) reported that maximum life spanswere estimated to be 9 years for males and 8 years for females, and no significant difference were found between the age frequenciesof the two sexes. Skeletochronology would seem to provide some promise for successful use in age estimation and population studies and management (Lindquist *et al.* 2012).

The *P. gorganensis* status is categorized as CR in the IUCN red list (Safaei- Mahroo *et al.* 2015). This salamander is an endemic species occurring in Northeastern of Iran (Baloutch & Kami 1995; Rastegar-Pouyani *et al.* 2008; Rastegar-Pouyani *et al.* 2015). Endemic species are important parts of the natural heritage of a country and of global significance (Rastegar-Pouyani *et al.* 2015).

The Persian mountain salamander is very sensitive to environmental change because it lives in a special situation. Furthermore, it seems that this species is on the verge of extinction and will become extinct in Iran if no new conservation attempts are undertaken in the near future. Based on this study, some conservation efforts are offered (Ahmadzadeh & Kami 2009).

REFERENCES

Ahmadzadeh, F & Kami, HG 2009, Distribution and conservation status of the Persianbrook salamander, *Batrachuperus (Paradactylodon) persicus* (Amphibia: Caudata: Hynobiidae) in north-western Iran.*Iranian Journal of Animal Biosystematics*, 5: 9-15.

Ashkavandi, S, Gharzi, A & Abbassi, M 2012, Age determination by skeletochronology in*Rana ridibunda* (Anuran: Amphibia). *Asian Journal of Experimental Biological Sciences* 3: 156-162.

Ashkavandi, S, Gharzi, A & Abbassi, M 2012,A skeletochronological stimation of age structure in a population of the *Bufo viridis* (Anuran: Amphibia) in Central Zagros,Iran*. Asian Journal of Experimental Biological Sciences*, 3: 99-104.

Baloutch, M& Kami, HG 1995, Amphibians of Iran. Tehran University Press, Tehran, Iran, 177.

Bovero, S, Angelini, C& Utzeri, C 2006, Aging *Salamandrina perspicillata* (Savi 1821) by skeletochronology. *Acta Herpetologica*, 1: 153-158.

Caetano, MH & Leclair, JrR 1996, Growth and population structure of Red-spotted newts (*Notophthalmus viridescens*) in permanent lakes of the Laurentian Shield, Quebec. *Copeia*, 4: 866-874.

Castanet, J& Smirina, F1990, Introduction to the skeletochronological method in amphibians and reptiles.*Annales Des*

Sciences Naturelles Comprenant la Zoologie, 13: 191-196.

Castanet, J, Francillon-Vieillot, H, Meunier, FJ& de Ricqles, A 1993, Bone and individual aging. In Bone growth. 7, Hall, BKK (ed.), CRC Press, Boca Raton, Florida, 245–283.

Dubois, A& Raffaëlli, J 2012, A new ergotaxonomy of the order Urodela Duméril, 1805 (*Amphibia, Batrachia*). Alytes, Paris, 28: 77–161.

Clergue-Gazeau, M & R, Thorn, 1979, Une nouvelle espece de salamander du genere*Batrachuperus* in Provence de l'Iranseptentrional (Amphibia, Caudata, Hynobiidae). *BulletinSociétéd'Histoire Naturelle, Toulouse*, 114 (3/4), 455-460.

Ento, K, Matsui, M 2002, Estimation of age structure by skeletochronology of a population of *Hynobius nebulosus* (*Amphibia Urodela*) in a breeding season. *Zoological Science.* 19: 241 247.

Eden, CJ, Whiteman, HH, Duobinis-Gray, L& Wissinger, SA 2007, Accuracy assessment of skeletochronology in the Arizona tiger salamander (*Ambystoma tigrinum nebulosum*). *Copeia*, 2: 471–477.

Eiselt, J&Steiner, HM 1970, Erstfundeines hynobiiden Molches in Iran, 1970. *Annalen des Naturhistorischen Museum Wien*, 74: 77-90, (In Germany).

Farasat, H, Sharifi, M 2016, Ageing and growth of the endangered Kaiser's mountain newt, *Neurergus kaiseri* (*Caudata: Salamandridae*), in the Southern Zagros range, Iran. *Journal of Herpetology*, 50: 120-125.

Gül, S, Özdemir, N, Kumlutaş, Y &Ilgaz, Ç 2014, Age structure and body size in three populations of *Darevskia rudis* (Bedriaga, 1886) from different altitudes. *Herpetozoa,* 26: 151–158.

Halliday, TR&Verrell, PA 1988, Body size and age in amphibians and reptiles. *Journal of Herpetology*, 22: 253-265.

Kami, HG1999, Additional specimens of the Persian mountain salamander, *Batrachuperus persicus* (Amphibia: Hynobiidae) from Iran. *Zoology in the Middle East,* 19: 37-42.

Kami, HG, Vakilpoure, E 1996, Geographical distribution of *Batrachuperus persicus*. *Herpetological review*, 27: 147.

Kami, HG2004, The Biology of Persianmountain salamander,*Batrachuperuspersicus* (Amphibia, Caudata, Hynobiidae) in Golestan Province, Iran. *Asian HerpetologicalResearch*, 10: 182-190.

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

Liao, WB & LuX 2011, Variation in body size, age, and growth in the Omeitreefrog (*Rhacophorus omeimontis*) along an altitudinal gradient in western China. *Ethology EcologyEvolutionJournal*. 23: 248–261.

Lima, V, Arntzen, JW, Ferrand, NM 2000, Age structure and growth pattern in two population of the golden-striped Salamander *Chioglossa lusitanica* (Caudata, Salamandridae). *Amphibia- Reptilia,* 22: 55-68.

Lindquist, E, Redmer, Michael & Brantner, E 2012,annular bone growth in phalanges of five Neotropical Harlequin frog (Anura: Bufonidae: *AteloIpus*). *Philomedusa*, 11: 117-124.

McCreary, B, Pearl, CA & Adams, JM 2008, A protocol for aging Anurans using skeletochronology. US Geological Survey Open-File Report, 2008-1209.

Misawa, Y& Matsui, M 1990, Age determination by skeletochronology of the Japanese salamander *Hynobius kimurae* (Amphibia- Urodela). *Zoological Science.* 16: 845–851.

Morrison, C& Hero, JM 2003, Geographic variation in life-history characteristics of amphibians: A review. *Journal of AnimalEcology*, 72: 270-279.

Rastegar-pouyani, N, Gholamifard, A, Karamiani, R, Bahmani, Z, Mobaraki, A, Abtin, E, Faizi, H, Heidari, N, Takesh, M, Sayyadi, F, Ahsani, N, Brown, kr 2015, Sustainable management of the Herpetophauna of the Iranian Plateau and Coastal Iran. *Amphibian and Reptile Conservation,* 9: 1-15.

Rastegar-Pouyani, N, Kami, HG, Rajabzadeh, M, Shafiei, S, Anderson, SC 2008, Annotated checklist of amphibians and reptiles of Iran. *IranianJournalofAnimalBiosystematics*, 4: 43–66.

Risch, JP 1984, Breve diagnose de *Paradactylodon*, genre nouveau durodele de l Iran (Amphibia, Caudata, Hynobiidae). *Alytes,* 3: 44-46.

Safaei-Mahroo, B, Ghaffari, H, Fahimi, H, Broomand, S, Yazdanian, M, NajafiMajd, E Hosseinian, Yousefkhani SS, Rezazadeh, EH, osseinzadeh, MS, Nasrabadi, R, Rajabizadeh, M, Mashayekhi, M, Motesharei, A, Naderi, A& Kazemi, SM 2015, The Herpetofauna of Iran: Checklist of taxonomy, distribution and conservation status. AsianHerpetological Research, 6: 257–290.

Schmidtler, JJ& Schmidtler, JJ 1971, Eine Salamander-Novitataus Persien *Batrachuperus persicus*. Aquar Mag. 5: 443-445.

Schroder, E, Baskett, T 1968, Age estimation, growth rates and population structure in Missouri bullfrogs. *Copeia*, 3: 583-592.

Senning, WC 1940,A study of age determination and growth of *Necturus maculosus* based on the parasphenoid bone. *American Journal of Anatomy,* 66: 483-494.

Sinsch, U 2015, Review: Skeletochronological assessment of demographic life-history traits in amphibians. *Herpetological Journal,* 25: 5-13.

Snover, ML 2002, Growth and ontogeny of sea turtles using skeletochronology: methods, validation and application to conservation. Duke University Marine Laboratory Graduate Program in Ecology Duke University, pp. 1-144.

Üzüm, N, Olgun, K 2009, Age and growth of the southern crested newt. *Triturus karelinii* (Strauch 1870), in a lawland population from Northwest Turkey. *Acta Zoologica Academiae Scientiarum Hungaricae.,* 55: 55-65.

Üzüm, N2009, A skeletochronological study of age, growth and longevity in a population of the Caucasian Salamander, *Mertensiella caucasica* (Waga 1876) (Caudata: Salamandridae) from Turkey. *North-West. Journal 0f Zoology,*5: 74-84.

Yilmaz, N, Kutrup, B, Çobanoğlu, Ü& Özoran, Y 2005, Age determination and some growth parameters of a *Rana ridibunda* in Turkey. *Acta Zoologica Academiae ScientiarumHungaricae*, 51: 67–74.

تعیین سن سمندر کوهستانی *Paradactylodon gorganensis* (Clergue-Gazeau and Thorn, 1979)(Caudata: Hynobiidae) استان گلستان با استفاده از روش اسکلتوکرونولوژی

س. زیوری، ح.ق. کمی*

گروه زیست‌شناسی، دانشکده علوم، دانشگاه گلستان، گرگان، ایران

(تاریخ دریافت: ۹۵/۰۵/۱۷ تاریخ پذیرش: ۹۵/۱۰/۲۲)

چکیده

در این مطالعه با استفاده از روش اسکلتوکرونولوژی، خطوط توقف رشد (LAGs)در جمعیتی از نمونه‌های موزه‌ای گونه به‌شدت در معرض خطر *Paradactylodon gorganensis* شمال شرقی ایران مورد بررسی قرار گرفت. LAGها به‌خوبی و به‌وضوح در برش‌های عرضی استخوان ران و ساق قابل روئیت بوده و امکان شمارش را برای برآورد سن مهیا کرده است. تعداد LAGها در نمونه‌های تثبیت‌شده استان گلستان در ۱۲ نمونه نر، ۱۱-۸ (۱/۱۳ ± ۹/۷۵ سال)، ۱۵ نمونه ماده،۱۳ -۷ (۱/۹۸ ± ۹/۲۶ سال) و در دو نمونه با جنسیت نامشخص ۷ و ۸ (۰/۷۰ ± ۷/۵۰ سال) بود. طول پوزه تا مخرج (SVL) در نمونه‌های نر، ۱۱۹/۳۸-۹۰/۲۰ (۱۵/۸۹ ± ۱۰۵/۹۶) میلی‌متر، در نمونه‌های ماده، ۱۳۵/۰۰-۷۷/۶۰ (۱۵/۸۹ ± ۱۰۵/۹۶) میلی‌متر و در دو نمونه با جنسیت نامشخص ۱۰۸/۰۰ و ۱۲۳/۰۰ میلی‌متر بود. ارتباط معنی‌داری بین اندازه بدن و سن یافت نشد ($P > 0.05$).اگرچه تعداد نمونه‌های تثبیت‌شده که در این مطالعه استفاده شدند، اندک است، اما همین اطلاعات نشان می‌دهد که نرهای بالای ۱۱ سال در بین این نمونه‌ها وجود ندارد. همانند دیگر مطالعات انجام شده در مورد گونه‌های مناطق ساب تروپیکال و براساس وجود خطوط توقف رشد در طی زمستان‌خوابی، مطالعات ما نشان داد که روش اسکلتوکرونولوژی بر روی گونه *P. gorganensis* ایران نیز قابل اجرا و عملیاتی است.

* مولف مسئول