



Publisher Wildlife Information Liaison Development Society www.wild.zooreach.org

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Cover: Pipistrellus tenuis recorded during the small mammalian fauna study, Manipur, India. © Uttam Saikia.

Journal of Threatened Taxa | www.threatenedtaxa.org | 26 September 2022 | 14(9): 21805-21810

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.7727.14.9.21805-21810

#7727 | Received 26 October 2021 | Final received 10 March 2022 | Finally accepted 14 August 2022





OMMUNICATION

Age estimation of Tiger *Panthera tigris* (Linnaeus, 1758) and Lion *Panthera leo* (Linnaeus, 1758) (Mammalia: Carnivora: Felidae): applicability of cementum annuli analysis method

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Abstract: We describe the applicability of the cementum annuli analysis technique for estimating the age of Tiger *Panthera tigris* and Asiatic Lion *Panthera leo* using incisor teeth. We used I_2 and I_3 incisor teeth from the right mandible of Tiger and I^2 and I^3 from the left premaxilla of the Lion. The longitudinal sections of the teeth were prepared using an economical hand grinding technique with the help of sandpaper, followed by decalcification and staining with hematoxylin. Two cementum layers were observed under the microscope in each of the I_2 and I_3 incisor teeth of the Tiger and six cementum layers were observed in each of the I^2 and I^3 incisor teeth of the Lion. The permanent incisors in Tiger and Lion erupt between 12 and 14 months of age; hence, we added one year to the counted number of cementum layers to estimate the final age of Tiger and Lion incisors. The age of Tiger and Lion incisors were estimated to be of three years and seven years, respectively. This method may be suitable for estimating other carnivores' age and applicable in wildlife forensic studies.

Keywords: Big cats, carnivore, epoxy, grinding, incisor, premolar, teeth, wildlife forensics.

Editor: Angie Appel, Wild Cat Network, Bad Marienberg, Germany.

Date of publication: 26 September 2022 (online & print)

Citation: Vipin, C.P. Sharma, V. Sharma, S.P. Goyal, H. Stevens & S.K. Gupta (2022). Age estimation of Tiger *Panthera tigris* (Linnaeus, 1758) and Lion *Panthera leo* (Linnaeus, 1758) (Mammalia: Carnivora: Felidae): applicability of cementum annuli analysis method. *Journal of Threatened Taxa* 14(9): 21805–21810. https://doi.org/10.11609/jott.7727.14.9.21805-21810

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Funding: The study was funded by the Wildlife Institute of India, Dehradun

Competing interests: The authors declare no competing interests.

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Author contributions: V, CPS, SPG & SKG conceived the study. V, CPS & VS carried out the laboratory work. V & HS did the data analysis. V, VS & SKG wrote the article. CPS, SKG, SPG & HS reviewed the article.

Acknowledgements: We gratefully acknowledge the support of the director and dean, Wildlife Institute of India, Dehradun.









INTRODUCTION

The age of carnivores needs to be estimated in studies about the demography of species and for understanding population dynamics (Skalski et al. 2005; Foresman 2012), age class (Angerbjorn et al. 2004; Creel et al. 2004), population monitoring trends (Barthold et al. 2016), human-wildlife interactions (Conover 2002; Frank et al. 2005), and illegal wildlife trade (Williams et al. 2015). The widely used methods for age estimation of carnivores are assessments of tooth eruption (Slaughter et al. 1974), wearing of a tooth crown (Harris 1978; Stander 1997; Gipson et al. 2000), closure of pulp chamber (Marks & Erickson 1966; Zapata et al. 1997; Binder & Van Valkenburgh 2010), and cementum analysis (Klevezal & Kleinenberg 1967; Matson 1981; White & Belant 2016).

The method to estimate the age of the Tiger *Panthera tigris* has mostly been limited to assessing tooth eruption, wearing (Mazak 1979, 1981; Miles & Grigson 2003), and gum line recession (Fàbregas & Garcés-Narro 2014). The methods described to assess the age of the Lion *Panthera leo* refer to sizes of body and mane, pigmentation on the nose, tooth wear (Schaller 1972; Smuts et al. 1978; Whitman et al. 2004; Whitman & Packer 2007; Ferreira & Funston 2010), closure of the pulp chamber (White & Belant 2016), the ratio of tooth areas (White et al. 2016), tooth eruption (Schneider 1959) and cementum analysis (Spinage 1976; Smuts et al. 1978; White & Belant 2016).

Amongst the various age determination methods available, the cementum analysis method has been recommended for its accuracy (Mundy & Fuller 1964; Marks & Erickson 1966; Klevezal & Kleinenberg 1967; Craighead et al. 1970; Willey 1974, Grue & Jensen 1979; Johnston et al. 1987; Matson et al. 1993; Mbizah et al. 2016; Vipin et al. 2018). The described technique does not need a costly microtome for tooth sectioning, so most of the items required are generally available in a standard lab (Vipin et al. 2018).

To date, studies on age estimation of South Asian mammals through cementum layer analysis are limited to Chital *Axis axis* (Vipin et al. 2018). Here we present the applicability of this method for estimating the age of Tiger and Asiatic Lion.

MATERIAL AND METHODS

We used Tiger mandible and Lion skull from Wildlife Forensic and Conservation Genetics Cell's repository. We tested the applicability of the developed method to estimate the age of an incisor (I_3) from the mandible

of a Tiger seized in the illegal wildlife trade, which was sent to Wildlife Institute of India, Dehradun, for species confirmation.

Sample collection and preparation of longitudinal section of teeth

Canines of Tigers and Lions are in high demand compared to other species' teeth in the illegal wildlife trade. Hence, in comparison to other types, the chances of their availability for determining age are limited. In both species, the permanent incisors number is six times more than premolar (PM²), which is a plus point if some tooth gets damaged during processing for cementum analysis. Therefore, we selected incisors in the current study and based on the availability of their types, the incisors were extracted.

Two permanent incisor teeth (I_2 , I_3) out of three were used from the right mandible of a Tiger (Image 1A, B, C) and two incisors (I^2 , I^3) from the left premaxilla of a Lion. The teeth from the Tiger mandible were extracted by boiling it in water for ten minutes, after which they detached easily from the mandible. From the Lion premaxilla, the teeth were removed with the help of pliers with utmost care so that the periodontal membrane remained intact. We used the protocol described by Vipin et al. (2018) for preparing the longitudinal sections of the incisor teeth with a thickness of around 57 μ m with steps, as shown in Image 2. We then used a Leica DMR microscope to examine the tooth sections.

Calculation of age from cementum annuli

In felids, all permanent incisors except I³ erupt before other teeth (Miles & Grigson 2003). In Tiger, the permanent tooth eruption starts between 8.8-9.5 months and completes at the age of 12-14 months (Mazák 1979, 1981). In Lion, permanent I¹, I₁, I², I₃ fully erupts between 9–11 months and I³ and I₃ start erupting by the end of this period (Smuts et al. 1978). I³ and I₃ completely replace their deciduous counterparts at the age of 12 to 14 months, while P2 starts erupting between this period in Lions (Smuts et al. 1978). Though no published data related to age estimation of Tiger using cementum analysis is available; many researchers have used permanent incisors, canine, and second premolar teeth to develop age estimation methods in Lions utilizing this technique (Smuts et al. 1978; Cheater 2006; White & Belant 2016). The time taken by different tooth types for their permanent eruption has been reported unequal in other species of carnivores and ungulates (Zapata et al. 1995; Azorit et al. 2004). In P2 of African Lions, it is established that the first rest is formed in the second

Age estimation of Tiger and Lion Vipin et al



Image 1. Tiger mandible: A—extracted incisors (I1, I2, I3) | B—side view | C—lingual view

year of age, so we had to add one to the counted number of cementum lines to estimate the final age (White & Belant 2016). We counted the acellular cementum layer in the root portion of the teeth, which is formed annually and stains dark with hematoxylin (Matson et al. 1993). Therefore, we added a minimum of one year in both species' final age estimation. The presence of one cementum layer in a permanent incisor tooth of Tiger and Lion indicates that the animal has lived one year at least. The age of sectioned teeth in years was calculated according to the formula

Age in year = Number of cementum layers + 1 year
The cementum layers in the incisor teeth were
photographed wherever these were seen distinctly and
clearly.

RESULTS

The teeth sections of the Tiger showed two cementum layers for I₂ (Image 3 A, B, C) and I₃ (Image 4 A, B); thus, the Tiger's age was estimated to be three years. The Lion had six cementum layers in I² (Image 5A, B) and I³ (Image 6); therefore, Lion's age was estimated to be seven years

We found two dark cementum layers on the Tiger's incisor seized in the illegal wildlife trade; hence its age was estimated as three years (Image 7). Therefore, the developed method may also be applied to estimate the age of tigers in the illegal wildlife trade.

DISCUSSION

White & Belant (2016) used paired PM² teeth for estimating the age of free-ranging African Lions of unknown age through cementum line count and showed that cementum layer count in PM2 is unsuitable for ageing Lions. Their analysis revealed that in 19 out of 31 PM² pairs, the cementum line count differed by 1-2 lines and even increased to seven lines for other pairs. According to Smut et al. (1978), cementum lines in canine teeth of Lions complied significantly with their known ages. So to compare the results of White & Balent (2016) about Asiatic Lions, a large sample size of the PM² teeth is needed, or more incisors or a different tooth type needs to be analysed for cementum layer count. In ungulates, the accuracy of age estimation through cementum analysis decreases with the age of the specimens (Hamlin et al. 2000). More research is necessary to assess whether this is also true for carnivores.

We recommend validating the current procedure while estimating age based on cementum layer count. Matson et al. (1993) suggested two main tests for validating the cementum analysis for estimating age, namely the "blind" duplicate test when two or more teeth are available and using a tooth of known age but without having the prior knowledge of its age. Teeth of known age were not available for both species; however, all incisor teeth showed clear and distinct cementum layers. The periodontal membrane in all studied teeth



Take longitudinal section of tooth by grinding on a set of sand papers with hand

Take incisor tooth, grind longitudinally on median plane on sand papers of 600, 1200 and 1500 grits, paste on slide, grind from other side

J Decalcification

Leave glass slide having tooth section in decalcification solution for 18 hrs



Remove decalcification solution by washing

Wash 'epoxy-tooth section complex' in 10 ml distilled water for 2 min thrice



Stain 'epoxy-tooth section complex' in 1 ml hematoxylin staining solution for 1 hr at 45°C. Wash in running water for 10 min



Differentiation, mounting and visualisation of tooth section under microscope for counting cementum layers

Separate tooth section from 'epoxy-tooth section complex', differentiate in acid alcohol for 10 sec.

Repeat this step till we get clear and distinct cementum layers. Wash in 10 ml distilled water for 2 min, place in 1 ml saturated lithium carbonate solution for 1 min, wash in distilled water for 10 sec, mount on glass slide and look under microscope

cementum layers



Calculation of ageAge in year = Number of cementum layer + 1

Image 2. Flow chart of the procedure followed for age determination through cementum layer count in incisor tooth.

confirms that all cementum layers were present in the longitudinal sections. Hence, the current method can show all cementum layers clearly and distinctly in incisor teeth.

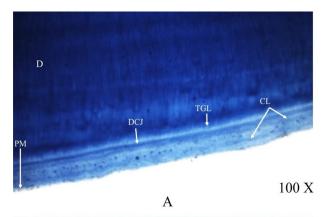
The same protocol may be applied to develop age estimation protocols for other mammal species.

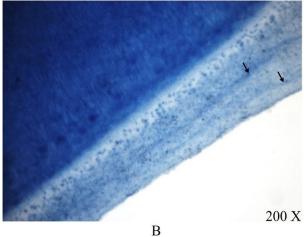
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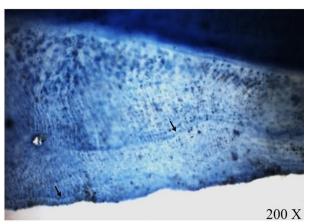


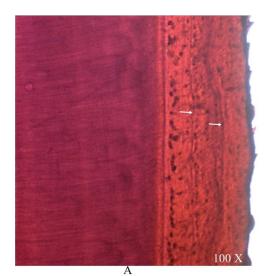
Image 3. The longitudinal section of $\rm I_2$ tooth of Tiger showing two cementum layers in three different regions (A, B and C) under 100X and 200X magnifications. D—dentine | TGM—Tome's Granular Layer | CL—Cementum Layer | DCJ—Dentine Cementum Junction | PM—Periodontal Membrane.

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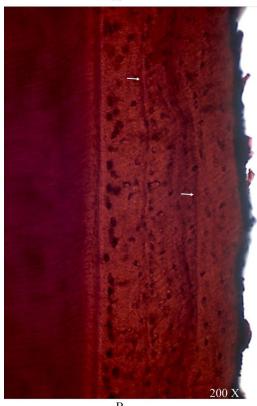


Image 4. The arrows in the longitudinal section of $\rm I_3$ of the Tiger showing two cementum layers at 100X and 200X magnifications.

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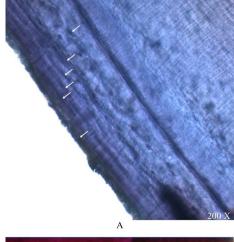




Image 5. The arrows in the longitudinal section of I² of the Lion showing six cementum layers in two different regions (A and B).

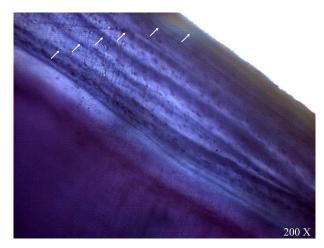


Image 6. The arrows in the longitudinal section of I^3 of the Lion showing six cementum layers.





Image 7. The arrows in the longitudinal section of $\rm I_3$ from seized Tiger skeleton showing two cementum layers.

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ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

September 2022 | Vol. 14 | No. 9 | Pages: 21751–21902 Date of Publication: 26 September 2022 (Online & Print) DOI: 10.11609/jott.2022.14.9.21751-21902

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