

Original Article

Bone histology of the Late Pleistocene *Prolagus sardus* (Lagomorpha: Mammalia) provides further insights into life-history strategy of insular giant small mammals

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ABSTRACT

Fossils provide an excellent opportunity to study and understand the evolution of insular environments free of human-made perturbations. Here, we evaluated the life-history traits and strategy of the extinct insular giant *Prolagus sardus* (Mammalia: Lagomorpha) by examining microscopically its fossilized bone tissues (osteohistology, skeletochronology, and quantitative geometry). For this task, a complete ontogenetic series of femora retrieved from the Late Pleistocene Grotta della Medusa (NW Sardinia, Italy) were analysed. Our results reveal that: (i) *P. sardus*' pups were weaned at large size; (ii) the species' maturation was delayed in its life cycle; and (iii) *P. sardus* lived longer than expected for its size. Hence, the giant *P. sardus* should have a slow pace of life triggered by the low levels of extrinsic mortality of the insular habitat. On the other hand, bone tissue differences between *P. sardus* and *Ochotona* were found in the vascularization, slow-growing bone presence, and cortical and medullary growth trajectories. The results obtained in this study concur with the eco-evolutionary responses described to date in extinct insular lagomorphs, as well as provide new empirical evidence about the phenomenon known as 'insular gigantism' as a pattern of evolution of small-sized mammals in genuine insular ecosystems.

Keywords: insular gigantism; Grotta della Medusa; life-history strategy; Lagomorpha; Mammalia; palaeohistology; osteohistology; Sardinia; skeletochronology

INTRODUCTION

Lagomorphs are key species in ecosystems, i.e. they are the basal trophic level of some food webs, affect the hydrology and soil biology, model plant communities, etc. The Order Lagomorpha comprises 91 extant species of two families, Ochotonidae and Leporidae. Ochotonidae consist of a single extant genus, 30 species with a clearly relictual distribution in confined areas of central Asia and North America; whereas Leporidae have 61 species pertaining to 11 genera, some of them monotypic, showing a worldwide distribution (excepting Antarctica) and a more variable range of adaptations and ecological roles (Hoffmann and

Smith 2005, Alves and Hackländer 2008). However, in some cases their dispersal to new regions was not 'natural' and occurred as a consequence of human-caused introductions (accidental or intentional, e.g. *Oryctolagus* and *Lepus* because of their significance for human activities) (Chapman and Flux 1990, Bonino *et al.* 2010 and references therein; Fenner 2010). The success and dominance of leporids in present-day ecosystems was established only a relatively short time after their dispersal from North America to Eurasia, via Berginia, which occurred during the Late Miocene (c. 8 Mya), and later to Africa (Flynn *et al.* 2014). The leporid genera that gave rise to the Eurasian

'invasion' had thrived confined in North America since *c.* 15 Mya. Prior to the Miocene, the taxonomic and palaeobiogeographic scenario was completely different: Ochotonidae and other extinct lagomorph suprageneric, formal or informal, entities (i.e. Prolagidae and stem lagomorphs) were incredibly diverse, abundant, widespread, and, in some cases, extremely long-lasting (López-Martínez 2008, Angelone *et al.* 2019).

Recent studies were dedicated to investigating a possible causal effect of climate change on the palaeodistribution and palaeobiodiversity of some lagomorph taxa with, interestingly, non-univocal results. This re-opened the debate whether the effects can be attributed solely to climate change vs. (or in addition to) palaeogeography plus other biological aspects (Angelone and Zhang 2021, Čermák *et al.* 2021). In this respect, *Prolagus sardus* (Wagner, 1829), the last representative of a 24 Myr long-lasting European lineage and the sole taxon other than Ochotonidae and Leporidae to have survived to historical times (Angelone *et al.* 2019 with references), represents a unique window to the past. Its study can provide clues to understanding the response of lagomorphs to changing environments (particularly to insularity), which is useful in the framework of current natural and anthropically driven changes of the present-day world (Leack *et al.* 2015).

The study of biological hard structures of past species at the tissue level provides meaningful details about their biology (e.g. life-history traits, growth strategies, etc.), which are impossible to achieve with traditional anatomical approaches (de Buffrénil *et al.* 2021a). In the last decade, several investigations using this microscopic technique have successfully provided valuable biological insights into some extinct lagomorphs (Kolb *et al.* 2015, Moncunill-Solé *et al.* 2016). The present research provides a histological analysis of the *P. sardus* remains, considering their ontogenetic variability, thanks to the abundant remains retrieved from Late Pleistocene Grotta della Medusa (NW Sardinia, Italy; 22.390–21.910 ka cal BP; see Palombo *et al.* 2017). Our analyses aim to improve the interpretations and conclusions provided previously (that are limited by samples not covering all the ontogenetic categories) and to give key details to understanding the biological strategies adopted by small mammals under insular selective pressures in habitats that have not been perturbed by human action.

MATERIAL AND METHODS

Material, measurements, and reconstruction of body mass and longevity

The femur is considered the most suitable bone for carrying out skeletochronology techniques in small mammals (Barker *et al.* 2003, García-Martínez *et al.* 2011, Kolb *et al.* 2015, Moncunill-Solé *et al.* 2016). In this regard, the present study involved 15 femora of *Prolagus sardus* of different dimensions and plate fusion status (Table 1; Supporting Information, Table S1), that formed an ontogenetic series ranging from juveniles to adults. These fossil bones were retrieved from the Late Pleistocene fossiliferous red clay layer of the Grotta della Medusa (NW Sardinia, Italy), also known as Dragonara Cave in the palaeontological literature (Palombo and Rozzi 2014, Palombo *et al.* 2017). *Prolagus sardus* material is curated in the 'Laboratorio di Paleontologia dei Vertebrati, Università Rome Tre' (LPV,

Roma, Italy). For comparison purposes, we used 13 femora of three extant ochotonid species (Table 1) that recent studies highlight as the closest extant relatives to *Prolagus*, which probably represents a familiar rank of its own [see Family Prolagidae in Gureev (1960) and Bover *et al.* (2022), with references]: *Ochotona collaris* (Nelson, 1893) (*N* = 5), *Ochotona princeps* (Richardson, 1828) (*N* = 3), and *Ochotona dauurica* (Pallas, 1776) (*N* = 5). The first two species belong to the talus-ecotype (non-burrowing leapers that dwell in rock piles), whereas the latter is typical of the meadow-ecotype (burrowers that inhabit meadow and steppe ecosystems) (Smith 1988). The bones of *Oc. princeps* and *Oc. collaris* are curated at the Museum of the North Mammal Collection of the University of Alaska Museum (UAM, Fairbanks, USA) and those of *Oc. dauurica* belong to the collection of the Museum of Southwestern Biology of the University of New Mexico (MSB, Albuquerque, USA).

Extant lagomorphs show minimal sexual dimorphism (Lu *et al.* 2014, Ferreira *et al.* 2015), so sex is not considered as a discriminant feature in the present study. We categorized the specimens into three age classes, on the basis of growth plate fusion: (1) juvenile (J) (= with unfused epiphyses or one fused epiphysis though with visible suture lines), (2) young adults (Y) (= with fused epiphyses but some of them still show visible suture line); and (3) adults (A) (= with both epiphyses fully fused and suture lines not distinguishable) (Kauhala and Soveri 2001, Moncunill-Solé *et al.* in press). In broken specimens (*N* = 2), the age category was refined according to metrics and histology. The measurements (in mm) were taken using a digital calliper (error \pm 0.05 mm) following standards (Moncunill-Solé *et al.* 2015: fig. 1): anteroposterior diameter at the midshaft (DAPm), transversal diameter at the midshaft (DTm), maximum femoral length (FLmax), femoral proximal transversal diameter (FTDp), femoral distal anteroposterior diameter (FAPDd), and femoral distal transversal diameter (FTDd).

The body mass (BM) of adult individuals of *P. sardus* was reconstructed using predictive regression equations. BM reconstruction models were computed based on a large set of extant lagomorph species (*N* = 44), with worldwide distribution and covering the size diversity of Lagomorpha (for details see Moncunill-Solé *et al.*, 2015). The BM mean was calculated by averaging the weight estimation using each of the epiphyseal dimensions (FAPDd, FTDD, FTDp; for statistical procedures see Moncunill-Solé *et al.* 2018). In the case of extant *Ochotona* individuals, the weight was obtained from specimens' tags and museum records. It is well known that BM is strongly correlated with several life-history traits (Calder 1984, Peters 1986), so the longevity of the assessed *P. sardus* specimens was inferred using their BMs and the allometric regression models proposed by Blueweiss *et al.* (1978). These allometric predictions (Table 1) were compared with the longevity values gained from the histological study, providing a scaling context.

Preparation and imaging of histological slides

Histological thin sections were obtained following standardized protocols (García-Martínez *et al.* 2011, Kolb *et al.* 2015, Moncunill-Solé *et al.* 2016, Orlandi-Oliveras *et al.* 2016, Miszkiewicz *et al.* 2020, Miszkiewicz and van der Geer 2022). Each bone was completely embedded in epoxy resin (EPOFIX Struers) due to its small size. For the correct interpretation of

Table 1. Biological (age category, body mass, and allometrical longevity) and histological details (number of LAGs) relative to the assessed femora of *Prolagus sardus* ($N = 15$), *Oc. collaris* ($N = 5$), *Oc. dauurica* ($N = 5$), and *Oc. princeps* ($N = 3$). Specimens with bone tissues damaged by microorganisms are underlined. Abbreviations: no = no observable/not present, see others in the main text.

Species	Specimen	Age	BM (in g)	LAGs	Allometrical longevity (in years)
<i>P. sardus</i>	R129	J		0	
<i>P. sardus</i>	R77	J		0	
<i>P. sardus</i>	R113	J		0	
<i>P. sardus</i>	<u>R137</u>	J		no	
<i>P. sardus</i>	GD52	J		0	
<i>P. sardus</i>	R000	J		1	
<i>P. sardus</i>	R136	J/Y		2	
<i>P. sardus</i>	R30	J		2	
<i>P. sardus</i>	<u>GD19</u>	J		no	
<i>P. sardus</i>	<u>D19</u>	J		no	
<i>P. sardus</i>	R12	J/Y		2	
<i>P. sardus</i>	A17	Y	662.27	3	5.21
<i>P. sardus</i>	R44	Y	569.11	2	5.07
<i>P. sardus</i>	R5	A	663.09	8	5.21
<i>P. sardus</i>	<u>B045</u>	A	730.28	no	5.29
<i>Oc. collaris</i>	UAM 2929	A	124.10	2	
<i>Oc. collaris</i>	UAM 2947	A	126.90	1-2?	
<i>Oc. collaris</i>	UAM 58315	Y	67.00	0	
<i>Oc. collaris</i>	UAM 63933	A	121.20	0-1?	
<i>Oc. collaris</i>	UAM 63937	A	122.30	1	
<i>Oc. dauurica</i>	MSB 215075	Y	81.00	0	
<i>Oc. dauurica</i>	MSB 215674	J	48.00	0	
<i>Oc. dauurica</i>	MSB 215680	Y	139.00	0	
<i>Oc. dauurica</i>	MSB 215940	J	25.00	0	
<i>Oc. dauurica</i>	MSB 215953	A	141.00	0	
<i>Oc. princeps</i>	UAM 35060	A	130.00	0-2?	
<i>Oc. princeps</i>	UAM 35119	A	-	1	
<i>Oc. princeps</i>	UAM 13936	A	144.00	4	

the section, the orientation of the bone was recorded through the whole process by marking mediolateral and antero-posterior axes. Once embedded, the femoral midshaft (the surface of interest) was exposed using a Struers' Accutom-2 precision saw cutter. The obtained resin block was polished on a glass sheet coated with carborundum powder with decreasing particle size (from 600 up to 1000 grit) and then fixed to a glass slide using the epoxy adhesive EPOTEK 301. The thin histological sections were obtained with the precision saw cutter (0.5 mm cutting disk) to a final thickness of about 100–110 μm , and were polished again with a gradient of carborundum (800–1200 grit) by hand. Finally, they were dehydrated through a graded series of alcohol baths, cleared in Histo-Clear II for 5 min, and covered with coverslips (100 μm) fixed with UV light-cured acrylic adhesive (Loctite 350). Histological slides of *P. sardus* were carried out at the Key Laboratory of Vertebrate Evolution and Human Origins (Chinese Academy of Sciences, China), whereas those of extant *Ochotona* at the 'Servicio de preparación de rocas y materiales duros, impresión y escaneado en 3D' (Universidad de Zaragoza, Spain). All of them are stored at the 'Departamento de Física e Ciencias da Terra, Universidade da Coruña' (UDC, A Coruña, Spain). Histological thin sections were observed under

transmitted light microscope (Nikon Eclipse E600, combined with a digital camera ZEISS AxioCam ICc3) and polarized light ones with lambda filters (Optika B-500POL and Leica Leitz DMR, with digital camera Nikon DS-Fi1). Slides were photographed using the 10 \times lens for full image composition, and regions of interest were captured using 20 \times and 40 \times lenses.

Osteohistology and growth marks

Bone tissues were typologically classified following the criteria of *de Ricqlès et al. (1991)*, *de Margerie et al. (2002)*, and *de Buffrénil et al. (2021a)*, considering mainly bone matrix and vascularization. Primary bone tissues can be remodelled by secondary osteons (= Haversian bone), surrounded by cement lines (*Padian and Lamm 2013*). Besides, growth marks can be identified in bone tissues, which record changes in the bone deposition rate and allow age-related life-history traits to be inferred (*Castanet et al. 1993*). Of particular interest are cyclical lines of arrested growth (LAGs) as they record annual growth cycles in lagomorphs (*Millar and Zwickel 1972*, *Ohtaishi et al. 1976*, *Frylestam and von Schantz 1977*, *Henderon and Bowen 1979*, *Iason 1988*). These cyclical bone growth marks are ever-present in mammals and are related to photoperiod, metabolic rates, and

hormone cues (Castanet *et al.* 2004, Köhler *et al.* 2012). In this regard, the individual's age at death (= longevity) can be inferred by counting the number of cyclical LAGs throughout the whole primary bone (Castanet *et al.* 2004, Castanet 2006, Kolb *et al.* 2015, Moncunill-Solé *et al.* 2016). Concerns associated with this skeletochronological approach, such as secondary bone or medullary resorption, are considered of lesser importance in lagomorphs, mainly as a consequence of their relatively short life and small size. On the other hand, non-cyclical marks can also appear in the bone cortex, reflecting ontogenetic events related to biological stress (e.g. neonatal or weaning lines), remodeling processes (e.g. resorption lines), and fibres' anchorage (e.g. Sharpey's fibres), among others (Castanet *et al.* 2004, Nacarino-Meneses and Köhler 2018, de Buffrénil and Quilhac 2021). A neonatal line marks the beginning of postnatal cortical growth. In mammals, an abrupt transition in the type of bone tissue is observed following birth, documenting acceleration in growth rate (Calderón *et al.* 2021). At present, no neonatal lines have been reported in fossil bones, though they are common in extant squamates and have been identified in other tetrapods, including mammals (Nacarino-Meneses and Köhler 2018, de Buffrénil *et al.* 2021b). A weaning line occurs as a consequence of dietary shift stress, and in large mammals it separates the pre-weaning tissue, with a high growth rate, from post-weaning tissue, which is deposited more slowly. This latter line is strongly marked in regions where the radial bone growth is constrained by the shape of the bone, thereby giving rise to partial lines (Calderón *et al.* 2021). Besides, growth arrest is more pronounced during the weaning stage (lasting 1–2 weeks) compared to birth (3 days), as observed in studies on other mammalian species, such as red deer (Calderón *et al.* 2021).

The following abbreviations were used to describe histological thin sections: cement line, CL; external fundamental system, EFS; fibrolamellar bone complex, FLC; Haversian system; HS; inner cortical layer, ICL; lamellar bone, LB; lines of arrested growth, LAGs; medullary cavity, MC; neonatal line, NL; non-cyclical growth mark, nonCGM; non-vascular bone tissue, NV; parallel-fibred bone, PFB; primary osteon, PO; resorption line, RL; Sharpey's fibres, SF; secondary osteon, SO; simple vascular canal, SV; weaning line, WL; woven-fibred bone, WB.

Quantitative geometrical analysis

Composition of the femur cross-sections were carried out by aligning and merging several images (10×) using Adobe Photoshop CS6 v.13.0 × 64 (1990-2012 Adobe Systems Incorporated). The images were converted into greyscale, 8-bit format. For calculating cross-sectional geometrical data, we used ImageJ 1.53k and the standardized protocol described by Miszkiewicz and van der Geer (2022). Following the scale setting, the margins were cropped, positioning the femur cross-sections in the centre. The noise points (e.g. resin bubbles), in or outside the medullary cavity, were eliminated using the free-hand selection tool. This selection tool was also used to trace the cortical area, and pixels were painted using black colour. Finally, the image was binarized using the threshold option, which forces greyscale pixels to black or white ones. On the transformed images of the cross-sections of each specimen, we gained the following geometrical data: cortical area (CA), medullary area (MA), and total area (TA); and the indexes CA/TA and CA/

MA (Skedros *et al.* 2003, Miszkiewicz and van der Geer 2022), using the Analyze Particle tool of ImageJ 1.53k software.

Descriptive statistical analyses of the variables were carried out, testing the normality (Shapiro–Wilk test and Q–Q plots) and equality of the variances (Levene's test). The most appropriate test of equality of means (T-test, ANOVA, Kruskal, Wallis, etc.) was used to identify differences in CA, MA, TA, DAPm, and DTm: (i) among age categories (J, Y, and A) of *P. sardus*; and (ii) among species (only adults of *Ochotona* spp. and *P. sardus*). Due to the noticeable size differences among groups, we also analysed the ratios CA/TA and CA/MA, which remove the effect of body size. In addition, ANCOVA tests were conducted to assess statistically the existence of differences in the growth trajectories among species (dependent variables: CA, MA, TA; covariables: DAPm and DTm, proxies of body size; and factor or independent variable: group). Prior to the analyses, the data were logarithmically transformed. The level of significance of all statistical tests was fixed at 0.05 and was conducted using the software jamovi v.2.3.24.0 (The jamovi Project 2022).

RESULTS

Osteohistology and skeletochronology

Four of the *P. sardus* femora were attacked by microorganisms (e.g. bacteria, fungi, parasites, etc.) (Table 1; Supporting Information, Appendix S1), preventing their typological interpretation and reducing the sample size to an ontogenetic series of 11 individuals ensuring representation of all age classes.

Prolagus sardus from the Medusa Cave (Late Pleistocene, Sardinia, Italy)

The youngest specimens (unfused epiphyses; R129, R77, and R113) show an early ontogenetic stage (Fig. 1A, B), characterized by a fast-growing bone formed mainly by FLC without LAGs. Vascularization, consisting of SV and PO, is abundant and mostly oriented longitudinally (parallel to the proximodistal axis). This early stage of rapid growth rate is especially remarkable in the smallest specimen (R129; FLmax = 28 mm), in which slow-growing bone traces have not been recognized (Fig. 1A, B). In R129 it is also possible to identify a partial nonCGM in the posterior region of the bone cortex, which we have related to an event of biological stress (likely a NL or WL) due to its main traits (position, sharpness, etc.) and resemblance to previous reports (Fig. 1B, white arrowhead).

Later in ontogeny, but still during active growth (GD52, R000, and R30, with unfused epiphyses), an early periosteal deposition of poorly vascularized PFB with longitudinally oriented SV is observed (Fig. 1C). PFB marks a decrease in the growth rate and a higher degree of tissue organization. Around the same time as PFB appears, the FLC is known to begin its resorption in the inner region of the cortex, expanding the MC, and depositing there an intermittent ICL of endosteal LB (Fig. 1D). In the next stage, LB is deposited in the outer cortex of the posterior region, before the attainment of skeletal maturity. This condition is illustrated in Fig. 1D (depicting the posterior region of R000, a relatively large-sized juvenile, with unfused epiphyses), where the cortical region of FLC surrounded by a very thin LB can be observed. At this moment, LAGs begin to appear in the outermost cortex (Fig. 1E, F), while the growth

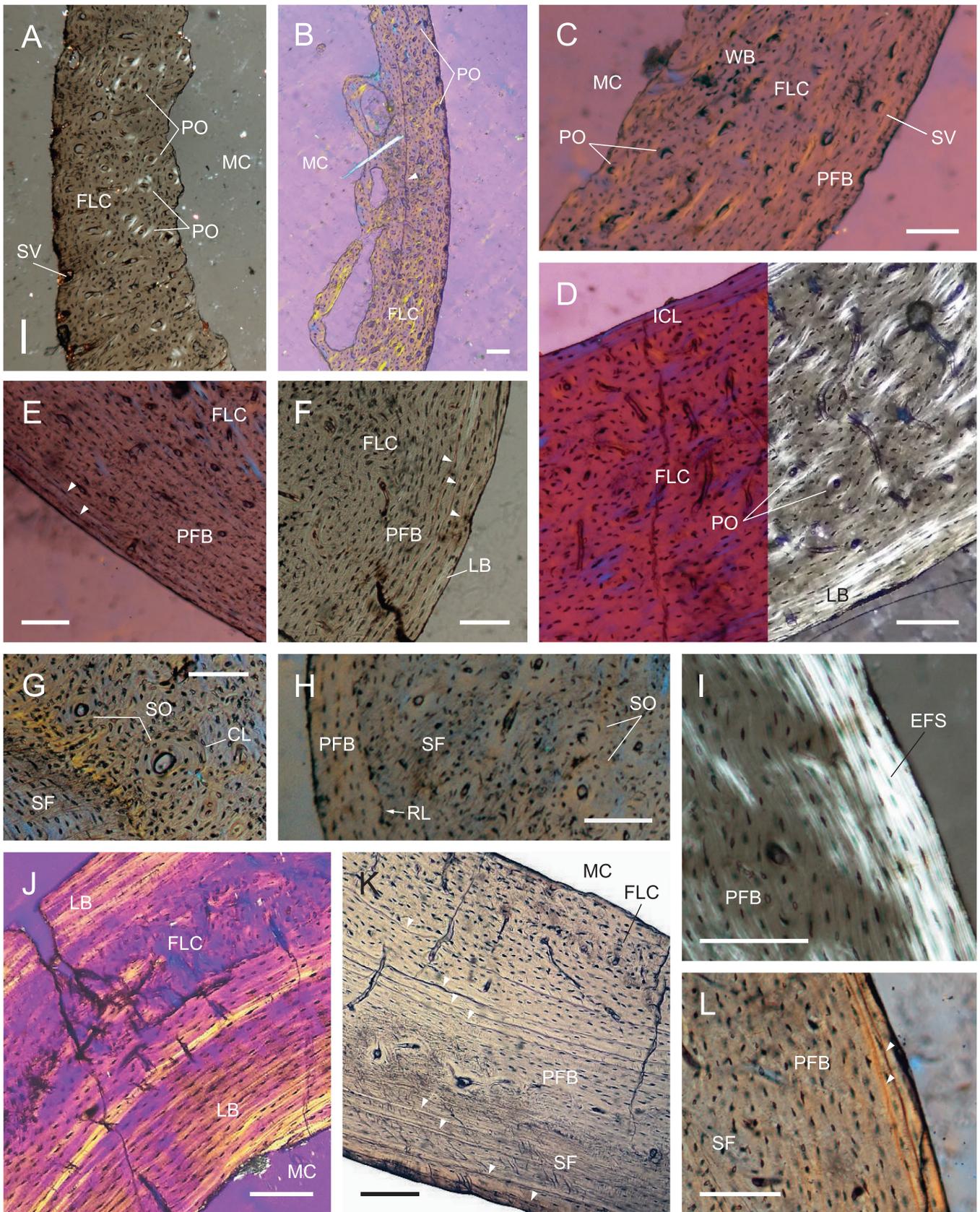


Figure 1. Bone histology of *P. sardus* specimens. A, B, R129 (juvenile, 0 LAG) showing the anterior region (A) formed by FLC with SVs and POs, and posterior one (B) where a nonCGM was identified (arrowhead). C, GD52 (juvenile, 0 LAG), medial region showing early external deposition of PFB with some SVs. In the inner cortex, WB is visible, as well as FLC with POs and SVs. D, R000 (juvenile, one LAG), posterior region with FLC sandwiched between the ICL and the outer cortex of LB (reversed image). E, R136 (young adult) showing two LAGs (arrowheads). F, A17 (young adult) with three LAGs (arrowheads). G, R30 (juvenile, two LAGs), detail of the lateral region with SOs

rate should sensibly decrease around its deposition. In the largest juveniles suitable for the analyses (R000 and R30, with unfused epiphyses; since those that actually were the largest—GD19 and D19—could not be analysed due to tissue damage), one to two LAGs are visible, evidencing late epiphyseal closure compared to extant species (Table 1). In this age category, we observed the highest histological variability among individuals. For example, although R000 and R30 have unfused epiphyses and similar size (FLmax *c.* 40–43 mm), they present differences at the histological level (e.g. R30 is strongly affected by remodelling, with clear RL, etc.). The strong muscular anchorages, noted in the posterior region, increase the intragroup histological variation. These appear along with large SOs in the FLC, pointing out remodelling events (Fig. 1G).

In the young adults (A17 and R44, showing fused epiphyses with suture lines), HSs are recognized in several regions of the cortex. Particularly, they dominate the lateral area, whereas in the posterolateral one an external layer of highly organized PFB appears (Fig. 1H). A clear RL is identified in the outer cortex, separating the PFB or EFS from the rest of bone tissues. These specimens presented two to three LAGs, pointing to an age at death of *c.* 2–3 years (Fig. 1F; Table 1). In the largest young adults (A17, with a minimal count of three LAGs) and in adults (R5; see next paragraph), the EFS is present (Fig. 1I). Accordingly, based on the number of observed LAGs and the moment of appearance of the EFS, maturity is probably attained at *c.* 3 years old. Two broken specimens (R136 and R12) have an intermediate status between juveniles and young adults when considering growth plate fusion and size. At the histological level, they have also an intermediate typology, shifted towards nearing maturity (higher LB presence and >1 LAG) (Fig. 1L; Table 1).

In the adults (R5, with fused epiphyses), PFB and LB are the most common tissues, occurring in most of the outer and inner cortices in the posterolateral and anteromedial regions, respectively (Fig. 1J). A wider EFS is noted externally, and PFB surrounds the SOs situated in the innermost cortex. There is a higher appositional rate of the EFS and ICL at the anterior region, with higher rates of remodelling (Fig. 1J). In the examined sample, a maximum of eight LAGs are identified (Fig. 1K; Table 1), suggesting a minimum age of 8 years at death. Such a specimen (R5) shows an important vascularized growth band between the fourth and fifth LAGs, which we hypothesize to be related to a particular muscular traction in the region. Accordingly, we can also observe SF through the whole of the outer cortex (Fig. 1K), with the highest presence in the medial and lateral regions. The muscular attachment is especially remarkable in the medial side, coinciding with the internal growth band of the posteromedial region.

Extant relatives: Ochotona collaris, Ochotona dauurica, and Ochotona princeps

The five specimens of *Oc. dauurica* show mostly fast-growing bone tissues, though among them we can recognize juveniles,

young adults, and adults considering the fusion status of the growth plates (Table 1; Supporting Information, Appendix S1). The vast majority of the bones of juveniles and young adults are formed by FLC/WB with a high number of osteocytes and vascular canals (Fig. 2A, B). The external periosteal layer is pervaded with abundant SVs, mainly longitudinally and radially oriented (Fig. 2B). None of the femora showed LAGs or growth marks, except RLs. This suggests that all the specimens died before reaching the first year of life. Despite this, we can note an ICL of slow-growing bone and PFB in some cortical regions of the adult specimen (MSB 215953, 0 LAG), as well as scarce and punctual outer deposit of LB in the lateral region (Fig. 2C).

On the other hand, *Oc. collaris* ($N = 5$) and *Oc. princeps* ($N = 3$) show a very distinct histological typology. All of these individuals are adults, except a specimen with suture lines (UAM 58315, *Oc. collaris*). Poorly vascularized PFB is the most common tissue observed in the histological slides—deposited in the external and internal cortices—besides a middle layer of FLC, sandwiched between them (Fig. 2D). The vascularization of this FLC is particularly abundant in some specimens (UAM 35060, *Oc. princeps*), with longitudinal and radial SVs (Fig. 2E). The nearly adult specimen (UAM 58315, *Oc. collaris*), without LAGs, shows a much reduced region of external PFB, signalling the earliest deposition of slow-growing bone before the epiphyseal plate fusion. Most of the other specimens have at least one LAG (Fig. 2D), with a maximum of four recorded in UAM 113936 (*Oc. princeps*) (Fig. 2F). This oldest specimen also shows an ICL in the anteromedial region. The absence of LAGs in some adult femora (fully fused epiphyses) points to an attainment of skeletal maturity before the first year of life. In most of the histological sections, we could observe RLs separating the PFB from the internal layer of FLC. The EFS is not visible in any of them, LB is absent or very rare in all the specimens.

Quantitative geometrical analysis

Geometrical and size data were successfully gained from the assessed specimens (see raw data in Supporting Information, Table S2), albeit BM reconstruction of *P. sardus* was only possible in unbroken fused (or nearly fused) femora, belonging to young adults or adults. The CA values range from 3.39 to 8.86 mm² in *P. sardus*, and from 0.54 to 2.43 mm² in *Ochotona* (Fig. 3A, D), noting a lesser variation in the MA results (from 3.30 to 7.53 mm² in *P. sardus* individuals, and 1.41 to 2.13 mm² in *Ochotona*). The extant and extinct adult specimens show, on average, a CA/MA index of 1.11 and 1.32, respectively, and CA/TA index of 0.52 and 0.57 (Fig. 3B, E). When assessing size variables, the values of DTm are slightly larger and are more clustered around the mean than those of DAPm (Fig. 3C, F). In general, the distribution of the log-transformed variables do not deviate from normality ($P > 0.05$; with the exception of logCA/MA and logCA/TA in *Oc. collaris* adults), though it cannot be calculated in groups of very small sample size (e.g. adult *P. sardus*, $N = 2$) (Supporting

and SFs. H, R44 (young adult, two LAGs), lateral region with SOs and SFs, what prevents to determine the primary bone tissue. A clear RL was also noted. I, A17 (young adult, three LAGs), posteromedial region displaying a clear EFS formed by compact LB. J, K, R5 (adult, eight LAGs) showing the anteromedial region (J) with FLC surrounded by LB in the inner and outer cortex and posteromedial region (K) with eight LAGs (arrowheads). L, R12 (young adult, with broken epiphyses) showing two LAGs (arrowheads). For abbreviations, see the text. Scale bars equals 100 μ m.

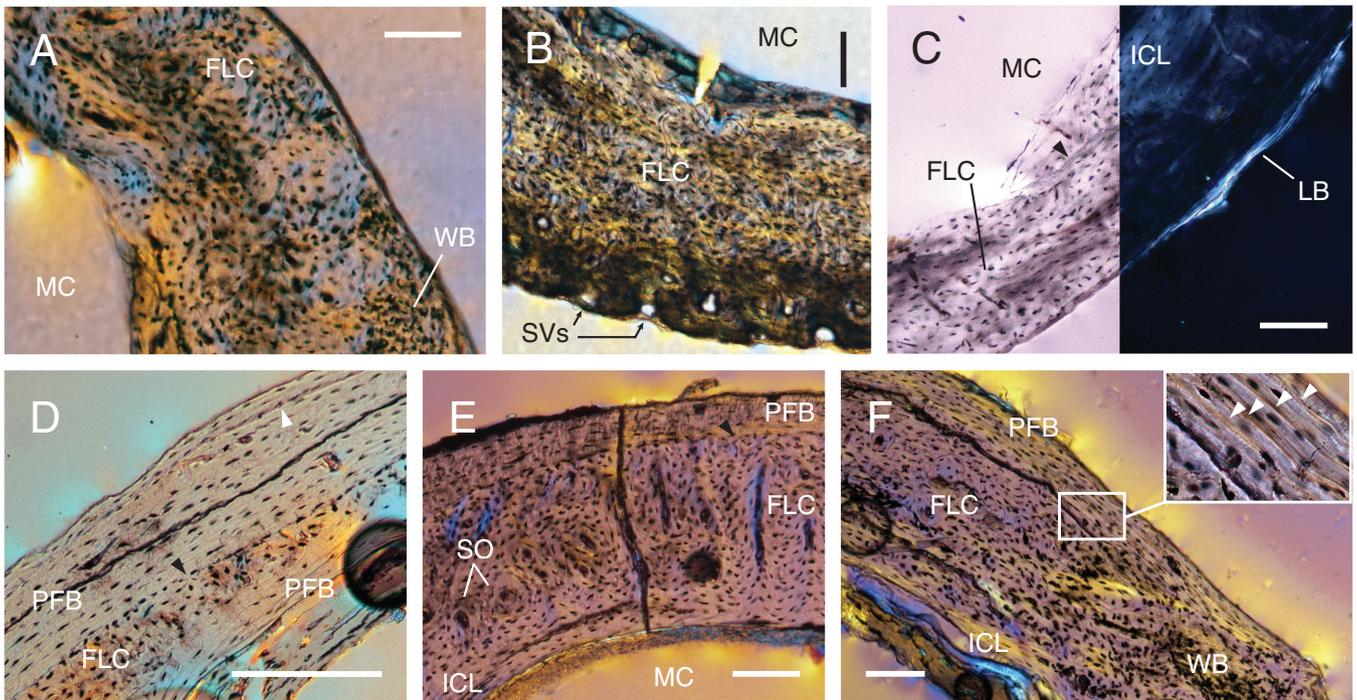


Figure 2. Bone histology of *Ochotona* specimens. A–C, *Oc. dauurica*. A, MSB 215940 (juvenile) showing a cortex formed by FLC and WB. B, MSB 215680 (young adult), with abundant SVs in the outer cortex. Note that microorganisms attacked this region, hiding bone tissues. C, MSB 215953 (adult) with FLC sandwiched between ICL and a scarce LB layer. Note the strong RL (black arrowhead). D, *Oc. collaris* UAM 63937 (adult), with an extensive deposition of PFB and clear RL (black arrowhead) splitting it from FLC. Notice the presence of one LAG (white arrowhead). E, F, *Oc. princeps*. E, UAM 35060 (adult), anterior region with PFB surrounded by a FLC full of SOs. F, UAM 113936 (adult), with detail of the PFB region, showing four LAGs (white arrowheads). For abbreviations, see the text. Scale bars equals 100 μm .

Information, Appendix S1). Hence, the subsequent statistical tests for these variables and groups are non-parametric.

Statistical tests signal significant differences among age categories of *P. sardus* considering MA, TA, and DTm ($P < 0.05$; Supporting Information, Appendix S1). Particularly, juveniles show smaller values than the other groups (Fig. 3A, C), whereas the results of young adults and adults are very close. These differences are related to a size effect, since the ratios CA/TA and CA/MA are indistinguishable among age categories ($P > 0.05$; Fig. 3B). When adults of extant ochotonids and *P. sardus* are considered, differences are observed in MA and TA ($P < 0.05$; Supporting Information, Appendix S1). In this case, *Oc. collaris* shows smaller values than *P. sardus* (Fig. 3A, D). However, considering that there are not statistical differences among adults of the three extant species (excepting TA, $P \approx 0.038$; Fig. 3D–F), we prefer to include all of them in a sole group (*Ochotona*) for running statistical tests with groups of larger sample size. In this latter case, results reveal that extant ochotonids and *P. sardus* have significant differences in all geometrical and size variables ($P < 0.05$; Supporting Information, Appendix S1). However, the absence of differences in the ratios CA/MA and CA/TA indicate that they are probably the result of a size effect between groups (Fig. 3B, E).

The growth trajectories are drawn using the dependent variables CA, MA, and TA, and the groups (fix factor) *P. sardus* and *Ochotona* (all species in a sole group, see previous paragraph). In all of the cases, the ANCOVA model is significant ($P < 0.001$), as well as size proxy (DAPm or DTm) denoting a relationship with fix factor ($P < 0.01$; Supporting Information, Appendix

S1). Overall, the statistical study reveals heterogeneity of the slopes in most of the assessed cases [interaction of the fix factor and covariable (the size), $P < 0.05$; Supporting Information, Appendix S1]. That is, the growth trajectories (relation between the dependent variable and the covariable) of these two groups are different (Fig. 3H, I). In only two cases can we assume homogeneity of slopes (interaction $P > 0.05$), and only in one of them does the ANCOVA test fail to identify significant difference among groups (Fig. 3G). In this regard, results show that *P. sardus* has larger values of MA and TA than an average extant *Ochotona* of similar size (Fig. 3H, I). In the case of CA, the results are more doubtful (being similar in size between groups or a slightly smaller in the case of *P. sardus*) (Fig. 3G; Supporting Information, Appendix S1).

DISCUSSION

Timing of *Prolagus sardus* life-history events: osteohistology and skeletochronology

The evaluation of the *P. sardus* juvenile specimens revealed that during early ontogeny the main bone tissue is a highly vascularized FLC, in line with previous investigations (Kolb *et al.* 2015, Moncunill-Solé *et al.* 2016). At this stage, a nonCGM related to a stress period was recognized in the smallest individual. These kinds of marks, described as incomplete or partial circumferences (Calderón *et al.* 2021), have been previously observed and recorded only in very few extant mammalian taxa, highlighting their singularity (Morris 1970, Castanet *et al.* 2004, Nacarino-Meneses and Köhler 2018). In our case, we did not evidence

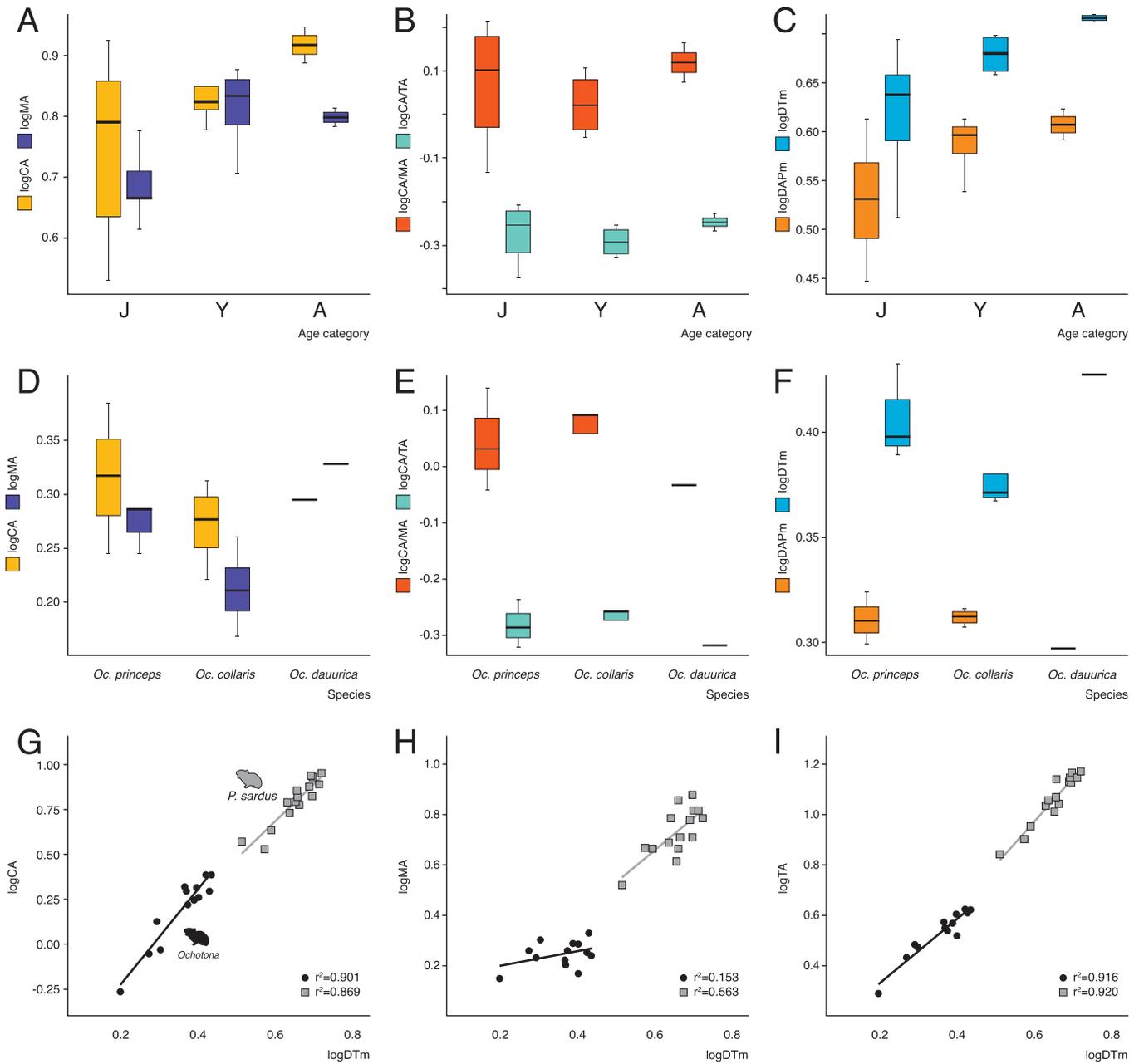


Figure 3. A–F, boxplots of log-transformed geometrical (CA, MA, CA/MA, and CA/TA) and size variables (DAPm and DTm). A–C, *Prolagus sardus* age categories (J, Y, and A). D–F, adults of *Oc. princeps*, *Oc. collaris*, and *Oc. dauurica*. See [Supporting Information, Table S2](#) for the raw data, including mean and standard deviation for species and age category. G–I, growth trajectories of CA, MA, and TA, considering DTm (size proxy), of *P. sardus* ($N = 15$) and *Ochotona* ($N = 13$). See [Supporting Information, Appendix S1](#) for statistical results.

major differences in bone tissue type following the deposition of this nonCGM, ruling out its attribution to a NL (Calderón *et al.* 2021). By contrast, its characteristics and resemblance to previous evidence allow us to tentatively relate it to a WL (Castanet *et al.* 2004). Considering the diameter of this nonCGM (≈ 2.4 mm), and the midshaft diameters of adults, the weaning should occur when the juvenile achieved 55–60% adult size (≈ 375 – 425 g). This value is in agreement with those documented in *Oc. princeps* (adult BM = 157.63 g and BM at weaning = 88.49 g; 56%) but are far from the 79% observed in *Lepus alleni* Mearns, 1890 and *L. californicus* Gray, 1837, and from the 13% and 9% of *Oryctolagus cuniculus* (Linnaeus, 1758) and *Sylvilagus floridanus*

(Allen, 1890), respectively (Jones *et al.* 2009). Large ratios (= larger, more fully developed pups) are related to more precocious offspring (large size at birth, small litters, or monotocous, etc.) (Dobson and Oli 2007), which in general suggest a slow pace of life (Gaillard *et al.* 1989, Bielby *et al.* 2007, Trillmich *et al.* 2019). The presence of a WL is quite exceptional evidence, never described in bones belonging to an extinct small mammal (< 2 kg) so far.

Later in ontogeny, different steps towards a reduction of the growth rate were identified in *P. sardus*. First, in middle-sized juveniles, the tissues become more organized with the deposition of PFB at endosteal and periosteal levels. Later, LB is deposited

forming an intermittent ICL before the occurrence of the first LAG. However, it is not until the approximately first year of life that the first LB is deposited externally and the presence of EFS is only seen in young adults and adults. The decrease in growth rate, marked by deposition of slow-growing tissues, has been related to maturity, though there is a lack of consensus about how this is recorded in bone microstructure. Generally, age at maturity, described as the periosteal apposition fall, is typically recognized by EFS presence (Castanet *et al.* 2004, García-Martínez *et al.* 2011). Whilst some authors have linked its deposition to skeletal maturity (epiphyseal fusion) (Chinsamy-Turan 2005, Klein *et al.* 2012, Nacarino-Meneses *et al.* 2016, Woodward *et al.* 2020 and references therein), others also attributed it to sexual maturity as well (Köhler and Moyà-Solà 2009, Köhler *et al.* 2012, Marín-Moratalla *et al.* 2013). However, this last inference is not confirmed as both maturities can be asynchronous (e.g. sexual preceding skeletal one) (Martínez-Maza *et al.* 2014, Jordana *et al.* 2016). In small mammals, maturity (skeletal and sexual) is a very difficult trait to trace, since, in general, their bones are subjected to rapid growth rates and most life-history events occur within the first year of life (Castanet *et al.* 2004, García-Martínez *et al.* 2011).

Considering the aforementioned, our results suggest that *P. sardus*' skeletal maturity was attained at 3 years, though the first slow-growing tissue appeared at the age of 1 year. Juveniles and most of the young adults (≤ 2 years) were in somatic growth (unfused epiphyses) and EFS was not present. In the case of pikas, there are no records about the pace and time of skeletal growth plate fusion. Our *Ochotona* specimens with LAGs show fully fused epiphyses, pointing to an earlier ending of somatic growth (i.e. earlier than the first year of life) with respect to *P. sardus*, regardless of their ecotype. The sexual maturity of these species, however, is different: *Oc. princeps* and *Oc. collaris* (talus-ecotype) attained it at 1 year after birth, and *Oc. dauurica* (meadow-ecotype) at 4–5 weeks (Smith and Weston 1990, Matsuzaki *et al.* 1998, Lanier and Hik 2016). In the case of *P. sardus*, we do not have enough evidence to determine whether skeletal maturity and sexual maturity were synchronous or not. Recent studies on the age of maturity in the giant insular leporid *Nuralagus rex* Quintana *et al.* (2011) (Pliocene of Menorca, Spain; BM = 8 kg, medium mammal) also signal a delayed maturation on the basis of EFS presence, though they correlate it with sexual maturation (Köhler *et al.* 2022). Based on this, Riyahi *et al.* (2011) determined a sexual maturity of this species at 2–3 years of age, whereas Köhler *et al.* (2022) suggested that females attained it at age of 4 years. If we consider leporids, available data indicate that the distal femoral epiphyseal closure of *Or. cuniculus* (breed New Zealand; BM = 4.5–5.4 kg) occurs at an average age of 5.25–5.50 months (radiographically and histologically) (Kaweblum *et al.* 1994). In the case of *S. floridanus* (BM = 1.2 kg), long bone epiphyses fusion occurs at age of ≈ 4.5 months (133 days) (Young *et al.* 2022). Thus, we can affirm that skeletal maturity was attained quite slowly in *P. sardus* considering its BM (c. 700–750 g) (Moncunill-Solé *et al.* 2021).

Some *P. sardus* young adults and adults have important regions of LB and SOs (Haversian systems) in the innermost cortex. The largest specimen had eight LAGs in the EFS, pointing to a minimum age at death of 8 years. This minimum longevity is much extended than the 5 years estimated by its adult BM (Table 1).

In addition, it is greater than the largest *P. sardus* minimum age reported in previous studies (Kolb *et al.* 2015), and it is the longest longevity documented in a species of *Prolagus* (considering mainland and insular populations) up to now (Kolb *et al.* 2015, Moncunill-Solé *et al.* 2016).

Histology and life-history strategies of *Ochotona*

Two major groups of extant pikas can be distinguished when considering life-history patterns: (i) the talus group, and (ii) the meadow or steppe group, though they are all remarkably similar at the morphological level (Smith 1988, Smith *et al.* 1990, 2018). Talus pikas are non-burrowing leapers that inhabit rock piles, characterized by low extrinsic mortality (average mortality) and interspecific competition levels in which birth and death rates are in equilibrium (stable low population densities). Under this scenario, selection favours traits that maximize efficiency (*K*-selection strategy) (Pianka 2011). Hence, talus pikas show low fecundity rates (two litters per season of few pups, and only a few juveniles are successfully weaned), later maturation (first reproduction occurs not before reaching the first year of life), and a longer lifespan (Smith 1988). In burrowing pikas, opposite life-history traits are observed (short-lived, fluctuating population densities, fast maturation, large juvenile production, etc.). They grow in habitats characterized by strong environmental perturbations (e.g. flooding or cold temperatures) with high mortality rates, such as meadows, steppes, and forests (Smith 1988). In these environments, the fitness of individuals is increased by traits that maximize productivity (*r*-selection strategy) (Pianka 2011). In view of the above, talus pikas show a slower life history than the meadow ones.

In the present study, we have assessed the bone tissue of specimens from the two groups. Unfortunately, most of the life-history traits detailed above are not recorded in their bones (e.g. number of pups per litter), but we were able to identify some significant differences. In the case of *Oc. dauurica* (meadow group), the absence of LAGs and the fusion of epiphyses point to an early attainment of skeletal maturity (before the first year of life), agreeing with an *r*-strategy. Based on the literature, *Oc. dauurica* females reach sexual maturity very rapidly, being able to produce a litter in the same year of their birth. Their pups are altricial (naked, without fur, closed eyes, etc.) and weaned at 1 month old (Sokolov *et al.* 1994, Ciwko 2014). In the wild, they do not survive more than 1 or 2 years (representing the 14% and 3% of the population, respectively) (Smith *et al.* 2018). The absence of a large amount of slow-growing tissue in their bones also points to a fast growth rate. Conversely, the deposition of slow-growing tissue is common (even this appears in the youngest individual with suture lines and remodelling fast-growing one) in the bones of *Oc. collaris* and *Oc. princeps* (talus group), but the skeletal maturity is attained also before the first year. However, they are longer lived specimens (one to four LAGs in adults). According to our results, *Oc. collaris* reaches the adult size very rapidly (40–50 days) and is sexually reproductive as yearlings. This species has a relatively long lifespan (4–7 years) (Leininger 2009, Smith *et al.* 2018). In the case of *Oc. princeps*, these events occur similarly. Sexual maturity of males and females is documented on average at 1 year (in the summer after birth), though they reach the adult size in only 3 months, and their lifespan ranges from 3 to 8 years (Peri 2012).

When comparing bone tissues of *P. sardus* and long-lived *Ochotona*, clear differences concerning the grade of vascularization and tissue type were observed. In pikas of slow life history (excepting the youngest specimens), vascularization is scarce, whereas in all age categories of *P. sardus*, SVs and POs are very abundant. This distinction could be related to the higher metabolic needs of larger species. Avascular bones are common in skeletons of small vertebrates, because superficial capillaries are enough to supply the vascular demands of tissues (de Buffrénil *et al.* 2021c). The other main difference is the amount of slow-growing bone: LB hardly ever appears in thin sections of adult *Ochotona* but is frequent in those of *P. sardus*. This signals that a slower growth rate probably occurred in *P. sardus* at adult stages (Amprino 1947, de Margerie *et al.* 2002, 2004). The results and comparisons are preliminary, as they are based on a limited sample of pikas not covering their ontogenetic variation. Future studies involving samples with a complete ontogenetic range are needed to describe in detail the variation of bone tissues in pikas and improve the comparisons with their relative *P. sardus*.

Quantitative geometrical analysis

Size (or its proxy, BM) covaries with an extensive set of morphological, behavioural, physiological, life-history, and ecological variables, and when organisms change in size (scaling) they have to face structural, functional, and biological consequences (Calder 1984, Schmidt-Nielsen 1984, Peters 1986). For this reason, it is relevant to consider size in comparative studies (especially with marked size differences, as it is our case) (Glazier 2022). Our results do not identify difference among geometrical variables (CA, MA, TA) when size effect is removed (ratios CA/MA and CA/TA), suggesting that proportions of cortical and medullary cavity remain stable in all groups (ontogenetic groups of *P. sardus*; and interspecific groups of adult *Ochotona* and *P. sardus*). However, significant differences were identified between growth trajectories (considering juveniles, young adults, and adults): *P. sardus* shows slightly thinner cortex and a proportionally larger medullary cavity than ochotonids. Thicker cortices in terrestrial amniotes have been related to more resistance to bending and torsion forces/loads, and cortex % (at mid-diaphysis of limb bones) has been correlated with body mass (weight bearing support), ecology, locomotor activities (swimming, cursorial, etc.), or phylogeny (Currey and Alexander 1985, Francillon-Vieillot *et al.* 1990, Ruff 2000, Cubo *et al.* 2005, Legendre *et al.* 2012, Quemeneur *et al.* 2013, Canoville *et al.* 2022). Overall, terrestrial taxa are characterized by moderate cortical thickness with a well-differentiated medullary cavity (without bony trabeculae), aquatic forms by more compact, dense bones (appendicular long bones, ribs, etc., up to ~90%) without a free medullary cavity, and semi-aquatic or amphibious species by intermediate traits (thicker cortices and more trabecular network, though overlapping with those of terrestrial relatives) (Laurin *et al.* 2011, Canoville *et al.* 2021). By contrast, the cortices of land-dwelling graviportal taxa and large terrestrial birds (extinct) are thicker and the medullary smaller than general tetrapods (to a lesser extent than aquatic taxa) (Houssaye *et al.* 2016, Canoville *et al.* 2022), whereas in the case of flying amniotes, the cortical thickness is lower than terrestrial ones and show expanded medullary cavities (Currey and Alexander 1985, Canoville *et al.* 2021). Researchers testing

the digging specialization in small mammals failed to find a relationship with compactness (Meier *et al.* 2013), though thicker bone walls have been documented (Montoya-Sanhueza and Chinsamy 2016). The lifestyles of *P. sardus* and pikas (particularly those from the talus) are not very different. *Prolagus sardus* was not a good runner, but had skills for digging, climbing, and scrambling (Dawson 1969, Moncunill-Solé 2021), and talus pikas show a general reduction of their cursoriality but are good leapers (Reese *et al.* 2013, Young *et al.* 2014). Our preliminary results do not clarify if the differences in cortical and medullary proportions between these two clades may be related to phylogeny or other factors (small sample, environmental effect, etc.).

Insights into the life-history strategy of the ‘giant’ small insular mammals

Insular environments are recognized ecologically by their reduced resource levels and lower extrinsic mortality (Sondaar 1977). In this insular ecological context, and following the theoretical models (based on Life History Theory; Palkovacs 2003), selection promotes a shift in life-history strategy, though the relative influence of these selection pressures depends on the taxon size. In the case of small mammals, the low extrinsic mortality is the primary driver. Their fitness is increased by maturing later (increased age at maturity and longevity) and at a larger adult size, which promotes an increased fecundity (probably in the size of the offspring, being more competitive). That is, selection favours a shift towards the slow end of the life-history continuum, being more efficient in resource acquisition and more competitive, and maximizing lifetime reproduction (allocating less energy to early reproduction). Empirical evidence reinforces these predictions, both in extant and extinct biotas (Adler and Levins 1994, Fons *et al.* 1997, Palkovacs 2003 and references therein). Although this general life-history explanation accounts for the widely observed trends, there is no consensus with other biological disciplines. In this regard, community ecologists work on demonstrating that selection pressures (predation, competition, and resource) operate directly on body size and other biological traits of insular dwellers (Heaney 1978, Lomolino 2005, Durst and Roth 2015; among others).

The timing of *P. sardus* life-history events recorded in our study points to a slow pace of life, which improves the fitness of the species, and agrees with the models proposed by Life History Theory and previous studies (Moncunill-Solé *et al.* 2021, *in press*). Although we were not able to determine the mass at birth or age at weaning, our results suggest that weaning occurred at large size (55–60% of adult size, ~375–425 g), which could point to precocial pups. Accordingly, the Corsican population of the lesser white-toothed shrew produces, statistically, litters of fewer pups but of larger size than mainland relatives (Fons *et al.* 1997). Larger offspring were also observed in the insular bank vole populations (Mappes *et al.* 2008), as well as in the insular Santa Catarina’s guinea pig (19% mass of adult female), concurrently with smaller litter sizes (Salvador and Fernandez 2008). Insular opossums (Austad 1993) and amphibians (Wang *et al.* 2009, Xu *et al.* 2019) pursue this trend too. In this regard, and following present evidence, it is likely that *P. sardus* gave birth to a lower number of pups per litter than what is expected allometrically, but of larger size. This improved its juvenile mortality rate and increased the fitness of the *P. sardus* population, which was near

carrying capacity (higher population densities) (Stearns 1992). The somatic growth of the species was prolonged (3 years), though with present data we cannot affirm that the onset of reproduction time was reached at such a late age. Allometrically, the species should mature sexually at 10 months (Blueweiss *et al.* 1978). The smallest *Sylvilagus*, similar in size to *P. sardus*, reach sexual maturity between the fifth and sixth month of life [*S. bachmani* (Waterhouse, 1839), 610 g, 154 days; *S. audubonii* (Baird, 1858), 756 g, 164 days; Tacutu *et al.* 2018]; whereas in the case of long-lived pikas (130–200 g, from talus ecotype), sexual maturity is reached as yearlings, contrasting with the 7 months expected allometrically (*Oc. princeps*, Moncunill-Solé *et al.* 2016). Even though we cannot affirm the exact moment when maturity occurred, it is likely that *P. sardus* age at maturity was also delayed to that expected allometrically. The later ontogenetic stage (adulthood) of the species was extended (minimum lifespan of 8 years), as occurred in other small and large insular mammals (Austad 1993, Köhler and Moyà-Solà 2009, Riyahi *et al.* 2011, Kolb *et al.* 2015, Moncunill-Solé *et al.* 2016, Orlandi-Oliveras *et al.* 2016, Köhler *et al.* 2021 and references therein, Miszkiewicz and van der Geer 2022).

Contrary to other insular small-sized mammals, *P. sardus* coexisted with some aerial and terrestrial predators in the Late Pleistocene Sardinian ecosystems (birds of prey, small carnivores, etc.), the most noteworthy of which is the Sardinian dhole, *Cynotherium sardous* Studiati, 1857 (Palombo and Rozzi, 2014). Although it was originally described as a specialized *Prolagus* hunter (Malatesta 1970), the most recent studies have considered that the dhole preyed on different small-sized animals (including small mammals, birds, etc.) (Lyras and van der Geer 2006, Lyras *et al.* 2006). The life-history traits of *P. sardus* recorded in the present study (shifting towards the slow end) did not follow the high predation risk mainland strategy and they are similar to those predicted when decreased mortality dominates (Palkovacs 2003). This suggests that the predation pressure of *C. sardous* towards *P. sardus* was not so extreme as some researchers have suggested and is lower than those recorded in mainland environments. In fact, these studies did not show direct evidence that *P. sardus* was the only target of this carnivore (Lyras and van der Geer 2006, Lyras *et al.* 2006). In addition, the species may have adopted several biological or behavioural strategies to face predators (as occurs in talus pikas) (Schoenherr 1992). The preliminary assessments of their bones, and those from their insular sister-taxa, suggest skills for digging and scrambling rough terrains, where the individuals could hide (Dawson 1969, Moncunill-Solé 2021). Such habitats (cliffs, high rocky coasts, etc.) were common in Sardinia during the Last Glacial Maximum (age of the Grotta della Medusa), characterized by a low stand sea-level (c. 130 m depth below to the present-day level) and the emergence of a great part of the present Mediterranean continental shelf (Deiana *et al.* 2021). Future studies on postcranial bones could draw out insights on the ecology and habitat preference of *P. sardus*, as well as provide relevant data about the Sardinian ecological networks.

CONCLUSIONS

In recent years, the study of the eco-evolutionary adaptations of insular small mammals has generated a surge of interest from the

scientific community. The present paper contributes to this debate assessing the life-history traits and strategy of the extinct insular ‘giant’ *Prolagus sardus* with a sample that spanned different ontogenetic stages, retrieved from Grotta della Medusa (Late Pleistocene). The osteohistological and skeletochronological study of fossil femora revealed that: (i) *P. sardus*’ pups were weaned at large size; (ii) species’ maturation was delayed in its life cycle; and (iii) *P. sardus* lived longer than expected for its size. Such biological traits are predicted in those insular species more affected by the decreased mortality (mainly small-sized ones), and are typical of taxa that are more efficient in resource acquirement and allocated less energy to early reproduction (*K*-selection strategy). In this regard, it is probable that the predation pressure exerted by the Sardinian dhole was not so extreme as several researchers has proposed, and/or that *P. sardus* adopted several behavioural strategies to face them. On the other hand, the comparative histological and quantitative geometrical analysis among *Ochotona* spp. and *P. sardus* identified clear differences. Less vascularization and low presence of slow-growing bone tissue characterized the *Ochotona* femoral thin section, which have been tentatively related to its smaller size and faster growth rate. In addition, they also differed in cortical and medullary growth trajectories, which could be the consequence of differences in body mass, ecology, locomotor activities, and phylogeny. The results obtained in the present research concur with the eco-evolutionary responses described in extinct insular lagomorphs and other small mammals to date, and provide new empirical evidence about evolution in insular domains not perturbed by human action.

SUPPLEMENTARY DATA

Supplementary data are available at *Zoological Journal of the Linnean Society* online.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the Supplementary Data of this paper.

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